School of Molecular and Life Sciences

Investigation of the Impact of Hydrological and Thermal Stress on Germination Response to Identify Ecological Filters of Rarity and Endemism

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Declaration

To the best of my knowledge and belief this thesis contains no material previously published by any other person except where due acknowledgment has been made.

This thesis contains no material which has been accepted for the award of any other degree or diploma in any university.

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R. P. V. G. Subhashi Wasanthi Rajapakshe

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All authors conceived the ideas and designed the methodology; I collected and analysed the data; I wrote the manuscript; all authors contributed to the revisions of the manuscript.

The study presented in Chapter 3 has been submitted to the journal 'Restoration Ecology' for peer review and is currently in the stage of 'minor revisions'.

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Abstract

Seed germination is a critical stage in the life cycle of most plants. Previous studies suggest that widespread species tend to germinate over a broader range of temperatures, whereas short-range taxa are more likely to be restricted to a narrower germination window. Within Western Australia, rocky outcrop habitats host unique plant communities that are floristically distinct from the deep-soil vegetation of the surrounding habitats. These geographically isolated, island-like, shallow-soil ecosystems harbour well over 100 unique range-restricted species, many of which are classified as threatened, as well as many other taxa that are broadly distributed across the larger landscape. The ecological filters behind these patterns of plant diversity are unclear at present. The objective of this thesis was to assess the thermal stress and water stress responses of seeds of a broad range of species associated with outcrop habitats. This was undertaken in order to better understand the role of these environmental variables in shaping the germination niche of outcrop endemic taxa, and investigate the ecological and evolutionary implications of these interactions as a way to inform future conservation actions.

As the first step of this project the responses of two key traits, maximum germination (G_{max}), and time required to reach 50 % germination (t_{50}) in response to thermal stress (5 – 35 °C) and water stress (-1.5 – 0 MPa) were assessed in two range-restricted non-dormant *Eucalyptus* species (*Eucalyptus caesia* subsp. *caesia* and *E. ornata*) associated with granite outcrop habitats, and two widely distributed and congeneric species (*E. salmonophloia* and *E. salubris*). A non-linear model fitting approach based on established theories was used to quantify germination responses. In terms of t_{50}, the germination response of the two range restricted species were constrained to a

narrower temperature range, but they were more tolerant of water stress compared to the two widely distributed taxa. There was no consistent pattern in the response of G_{max}. This experiment revealed that the two range-restricted taxa are adapted to germinate rapidly during irregular rainfall episodes on shallow-soil outcrop habitats. Conversely, the two broadly distributed taxa are adapted to synchronize germination with the rainfall season, a strategy followed by many taxa living in deeper-soils surrounding outcrops.

For the next step of this project, the germination responses in of five physically dormant *Acacia* species of varying distributional ranges were assessed in order to investigate whether range-restricted taxa (*Acacia woodmaniorum* and *A. karina*) associated with Banded Ironstone Formations (BIFs) are more tolerant of hydrothermal stress compared to widely distributed congeneric species (*A. assimilis*, *A. exocarpoides* and *A. ramulosa*). The modelling approach used in the previous experiment was further developed to quantify germination responses (in terms of G_{max} and t_{50}) to a range of hydrothermal regimes (10 – 30 °C and -0.8 – 0 MPa). The seeds of range-restricted taxa were more tolerant of hydrothermal stress compared to their widely distributed congenerics. This experiment revealed that higher levels of hydrothermal stress in the two range-restricted species is likely to facilitate rapid seedling establishment during irregular rainfall events, and enable those species to access uncontested niche space on outcrops. Complex interactions between thermal stress and water stress were revealed, suggesting that in order to gain a comprehensive understanding of niche dynamics on outcrop habitats, interactions between these niche axes must be quantified.

As the final step of this study, the hydrothermal performance niche of seeds (in terms of G_{max} and t_{50}) of three threatened physiologically dormant *Tetratheca* species

endemic to BIF outcrops were compared with their edaphic occurrence niches, to assess the role of hydrothermal stress in shaping their germination niche. The modelling approach developed in the previous steps were utilized to quantify germination responses to a range of hydrothermal regimes (10 – 25 °C and -1.2 – 0 MPa). Compared to the outcrop endemic *Eucalyptus* and *Acacia* species that were studied, BIF specialist *Tetratheca* species were slow to germinate and their germination responses were constrained to narrow niches at cooler temperatures. These observations suggest that outcrop specialist *Tetrathecas* may have evolved under more mesic conditions, and due to aridfication of the broader landscape, their distribution may have become constrained to microhabitats on outcrops that can facilitate their persistence.

This study highlights the significant role of hydrothermal stress in shaping the germination niche of flora in Mediterranean climatic ecosystem, and demonstrates how mechanistic models based on a sound theoretical context can be used to identify critical stress tolerance thresholds of rare and range-restricted taxa. The methods used in this study can be employed to generate research data that is critical for the seed-based conservation of threatened flora.

Table of Contents

List of Figures

List of Tables

List of abbreviations

ACLEP: Australian Collaborative Land Evaluation Program

AIC: Akaike Information Criterion

AOO: Area of Occupancy

BIF: Banded Ironstone Formation

EOO: Extent of Occurrence

Eqn: Equation

DF: Degree of Freedom

DRF: Declared Rare Flora

GAM: Generalized Additive Models

G_{max}: Maximum germination

GPS: Global Positioning System

g_0: The base value of t_{50} prior to the beginning of its exponential increase

IBRA: Interim Biogeographic Regionalisation for Australia

KAR_1: Karrikinolide

NCST: National Committee for Soil and Terrain

PD: Physiological Dormancy

PEG: Polyethylene Glycol

PY: Physical Dormancy

P1: Priority 1

p: Final germination percentage

RH: Relative humidity

R_{max}: The upper limit of the germination response curve at T_{opt}

r_{max}: Maximum germination rate at a given temperature $(1/t_{50})$

SE: Standard error

SWAFR: South Western Australia Floristic Region

T_{max}: The upper limit of thermal stress tolerance

T_{opt}: Optimum temperature for germination

t_{50}: The time that is required for 50 % of seeds (as a proportion of G_{max}) to germinate

WA: Western Australia

w_c :The critical water stress level at which t_{50} begins to escalate exponentially

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Chapter 1

General introduction and literature review

1.1 Introduction

1.1.1 Niche dynamics

According to niche theory proposed by Hutchinson (1978), the fundamental niche of species refers to the entire range of conditions in which it can potentially survive and reproduce. The fundamental niche comprises multiple biotic and abiotic variables (i.e., it is *n* dimensional; Fig. 1.1), such as climatic variables like temperature or rainfall patterns, the availability of a particular essential mineral in the soil habitat of a plant, or the rate of pollen dispersal which may depend on the availability of pollination vectors and the conditions that support pollen tube growth (Hutchinson 1978, Soberón and Nakamura 2009). In contrast, the realised niche/occurrence niche refers to the set of conditions under which a species currently exists (Elith and Leathwick 2009). The fundamental niche of a species is rarely fully realized (Fig. 1.1), largely as a result of ecological and spatial filters (Soberón and Nakamura 2009), and therefore the occurrence niche of a species usually constitutes a subset of its fundamental niche (Pironon, Villellas et al. 2018). Measurements of species' performance across various environmental gradients/dimensions (e.g. metabolic rate and resource consumption rate at different stages of the life cycle) constitute the species performance niche, which is also a subset of the fundamental niche (Tomlinson, Dixon et al. 2015). There is usually a high level of overlap between the occurrence niche and performance niches of species, meaning that species can only occur in environments that can facilitate their development, survival and reproduction (Martínez-Meyer, Díaz-Porras et al. 2013, Van Couwenberghe, Collet et al. 2013, Pironon, Villellas et al. 2018).

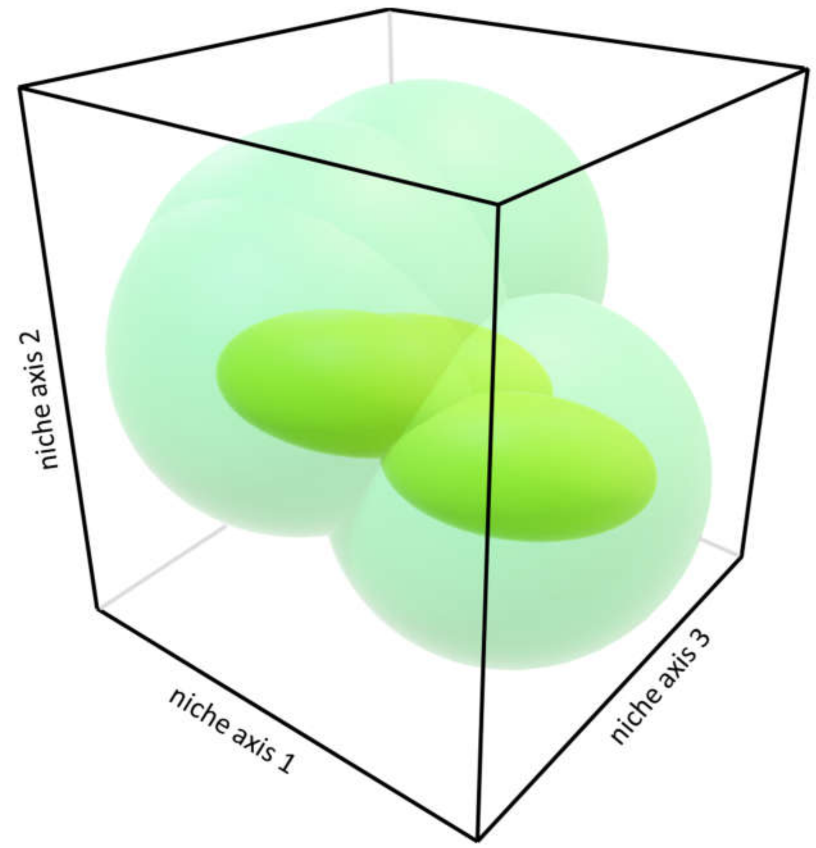


Fig. 1.1. Schematic depiction of the fundamental niche and the realised niche of a hypothetical species in an *n* dimensional hypervolume. Each axis represents an environmental gradient that defines a limiting factor on the species' population. The dark green cloud represents the fundamental niche, consisting of conditions that the species can tolerate, while the brighter green cloud indicates the realised niche, consisting of niche space where the species actually occurs.

Of the two filters identified by Soberón and Nakamura (2009), ecological filters are probably the most pervasive, because very few species live in isolation. In diverse ecosystems, the interactions between different species can be drivers to constrain the realised niche, either through competition, or through the absence of critical commensal species (Phillips, Peakall et al. 2014). In natural systems, co-occurring species usually exhibit a high degree of overlap along several niche axes (Fig. 1.2), while the level of overlap is minimal for other niche axes (Lasky, Bachelot et al. 2015, Guderle, Bachmann et al. 2018). This phenomenon is referred to as niche partitioning (Fig. 1.2), and it contributes to diffuse competition (Monterroso, Rebelo et al. 2016, Mori, Ferretti et al. 2019). In natural systems, interactions between niche axes are common, but research data on the implications of such interactions are scarce (Costa-Pereira, Araújo et al. 2019).

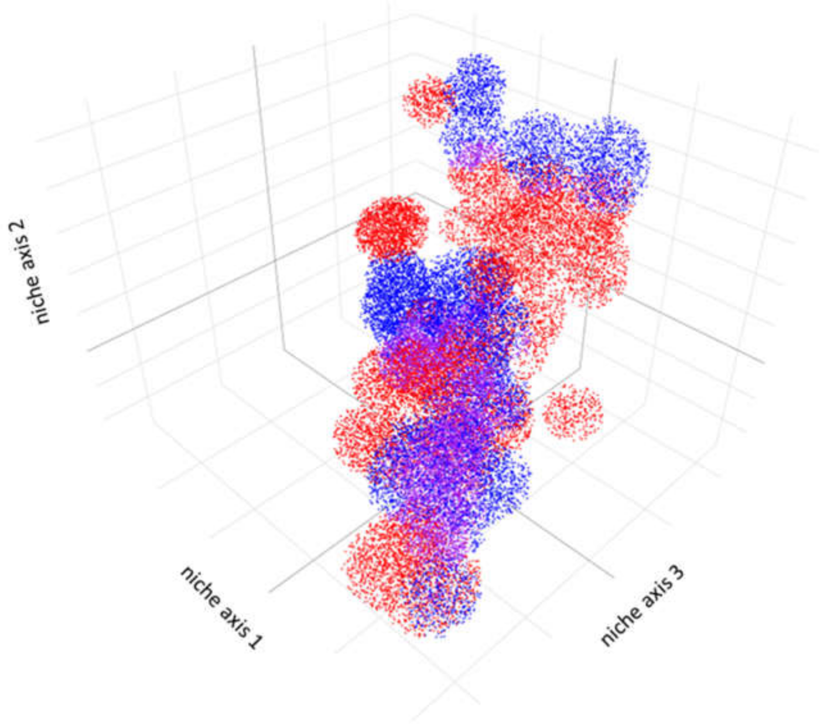


Fig. 1.2. Three dimensional occurrence niche of two hypothetical congeneric and co-occurring species depicted by blue and red points respectively. Purple points indicate the space in which the species co-occur, while red and blue points indicate regions of niche separation. The niche axes along which the species separate could be microclimatic (e.g. number of degree days), or they could be ecological (e.g. time of day for optimal foraging).

In order to gain a comprehensive knowledge of the niche dynamics of a species, it is essential to investigate the performance response of species along different environmental gradients during different stages of the life cycle (Warren, Wright et al. 2011, Merow, Latimer et al. 2014). However, all stages of the life cycle do not contribute equally to overall population dynamics (de Kroon, Plaisier et al. 1986, Pironon, Villellas et al. 2018), and species' population growth rates are primarily determined by the performance rates of several vital stages of the life cycle (Butterfield and Briggs 2011, Del Vecchio, Fantinato et al. 2020). For animals, these life stages may include fertilization of eggs and early developmental stages (Olson 1996, Kimirei, Nagelkerken et al. 2013, Lima, Ángeles-González et al. 2020). For plants, the most important life history stages may include pollination (Fig. 1.3), seed germination and seed dispersal (Yates and Ladd 2004, James, Sheley et al. 2013, Fernández-Pascual, Pérez-Arcoiza et al. 2017, Ladd, Yates et al. 2019). There is a general shortage of research in the literature that has assessed the performance niches of the critical life stages of taxa such as seed germination and the implications for overall population dynamics (Ehrlén and Morris 2015, Pironon, Villellas et al. 2018).

A core population consists of individuals that exhibit the highest rates of physiological performance (reflects optimum environmental conditions) along a given environmental gradient (Abeli, Ghitti et al. 2020). Conversely, a marginal population comprises of organisms that exist under extreme environmental conditions (close to one end of an environmental gradient), and therefore such individuals exhibit the lowest vital performance rates (Abeli, Ghitti et al. 2020). At a given life history stage, an individual may be part of a core population with regards to performance along one environmental axis, and represent a marginal population with regards to performance along another environmental gradient (Pironon, Villellas et al. 2018). During different stages of the life cycle, the physiological performance rates that are critical for overall population growth rate may vary (Sexton, McIntyre et al. 2009). Therefore, individuals of a population may exhibit optimum physiological performance along one environmental axis at a given life history stage to compensate for low performance rates across another environmental gradient at a different life history stage (Pironon, Villellas et al. 2015). Variability in vital performance rates at population level is referred to as demographic compensation, a strategy by which stable populations can be sustained (niche conservatism) via buffering against extreme changes in the environment (Pironon, Villellas et al. 2018).

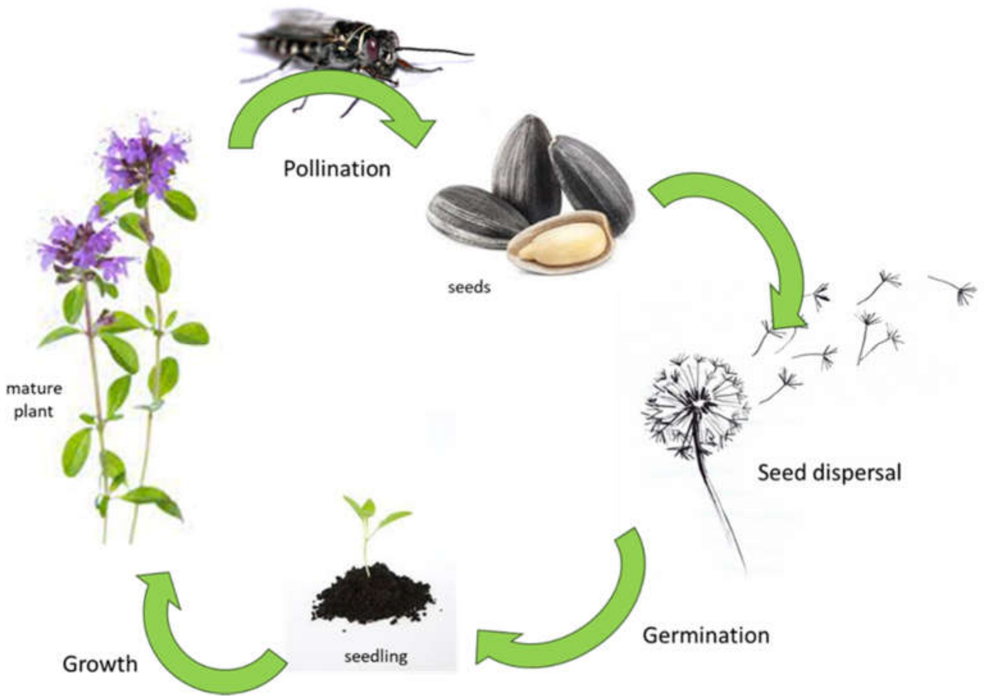


Fig. 1.3. Vital stages of the life cycle of a plant. Following pollination, an embryo develops inside the fertilized egg. Seeds containing embryos are dispersed. Seedlings emerge from germinated seeds and subsequently become mature plants.

1.1.2 Characterizing performance niches of species

The breadth of the performance niche of a species along any environmental gradient is limited to the range of environmental conditions that are conductive (tolerance thresholds) to its metabolic activities (Tomlinson 2019). Rates of chemical reactions generally increase with increasing temperature (Gates 2016). However, metabolic reactions in the tissues of organisms are catalysed by enzymes (Huang, Ran et al. 2020). Since enzymes are proteins, they have a specific thermal threshold beyond which they rapidly denature and cease to function (Peterson, Daniel et al. 2007). The combination of these two processes causes a rapid increase in physiological performance of organisms up to a critical thermal threshold (Fig. 1.4), beyond which performance declines rapidly as a result of enzyme denaturation (Angilletta Jr 2006). In the past, there have been attempts to quantify physiological tolerance thresholds of organisms using linear regression approaches (Bradford 2002). However, linear regression models tend to compress the natural variability pattern of the performance of organisms (Ashford and Sowden 1970, Yates, Hobbs et al. 1996). Furthermore, the physiological underpinnings of these models are unclear, and the symmetrical hump-shaped curves that have been employed to describe thermal performance of organisms do not conform to established principles of thermal biology that suggest thermal performance curves of ectotherms should be unimodal and asymmetric (Angilletta Jr 2006, Peterson, Daniel et al. 2007). As well, linear regression models do not account for interspecific variability in responses among taxa that may have ecological and evolutionary implications (Huey and Kingsolver 1989, Tomlinson, Dixon et al. 2015, Tomlinson 2019). Thus the parameters derived from these models may be inaccurate and difficult to interpret in a biological context (Angilletta Jr and Angilletta 2009). Recently, non-linear performance models based on a sound theoretical context have been employed to characterize physiological responses of various ectothermic species of animals and plants including germinating seeds (Lewandrowski, Erickson et al. 2017, Onofri, Benincasa et al. 2018, Turner, Lewandrowski et al. 2018, Tomlinson 2019). The main advantage of this non-linear model fitting approach is that it only fits parameters that define the model, which in turn can be interpreted in a biological context (Yan and Hunt 1999, Tomlinson, Dixon et al. 2015). This method minimizes overfitting of data and the parameters derived from the model can be directly compared to gain insights into interspecific variability in the germination response when applied to seeds (Tomlinson 2019).

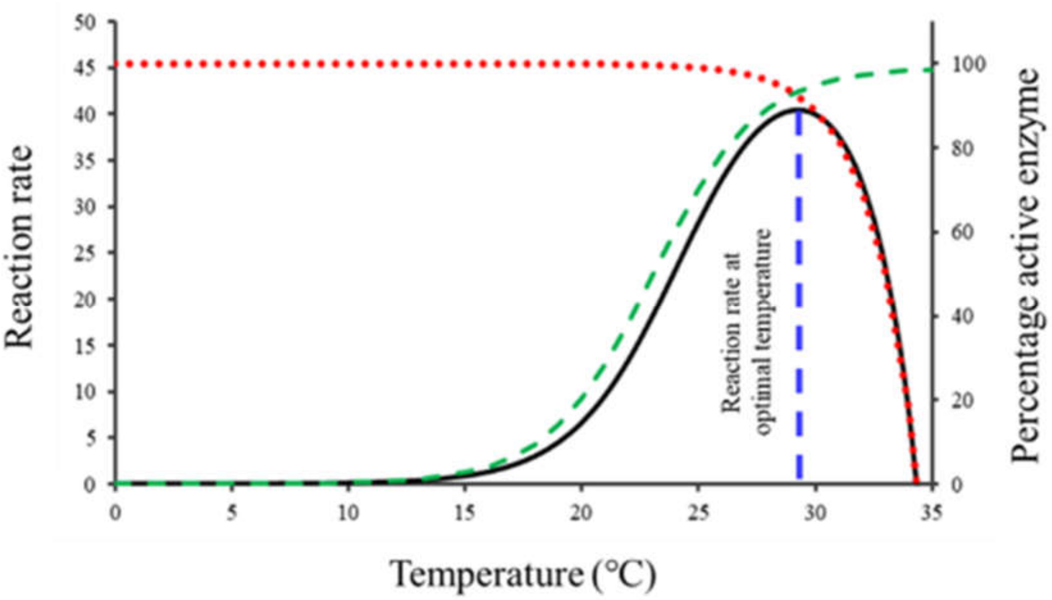


Fig. 1.4. Thermal performance of an enzyme; Dotted green line depicts reaction rate of any chemical reaction, the dotted red line represents the decline of reaction rate from a specific threshold due to thermal denaturation of the enzyme, and the black hump-shaped curve represents the resulting thermal performance model.

Seed germination is one of the critical stages of the life cycle of plants during which they are potentially highly vulnerable to environmental stresses as they transition from quiescent drought resistant seed to metabolically active drought sensitive seedling (Baskin and Baskin 2003). Consequently, the germination niche represents a vital demographic stage of the fundamental niche of plants as it cannot be reversed once seeds commit to the germination process (Donohue, Rubio de Casas et al. 2010, Fernández-Pascual, Pérez-Arcoiza et al. 2017). Therefore, quantifying the performance niche of seeds is essential to gain a comprehensive understanding of population dynamics (Cochrane 2018, Del Vecchio, Fantinato et al. 2020).

1.1.3 Seed germination

Seeds possess adaptations to germinate during windows of opportunity when environmental conditions align with their innate requirements and are likely to maximize seedling recruitment success (Fig. 1.5, Merritt, Turner et al. 2007). Seed germination generally occurs as a response to specific environmental cues such as changes in soil temperature and moisture content, and exposure to light or smoke (Merritt, Turner et al. 2007, Kildisheva, Erickson et al. 2019). Therefore, performance rates of seeds across multiple environmental gradients constitute the performance niche of seed germination (Bell, Rokich et al. 1995).

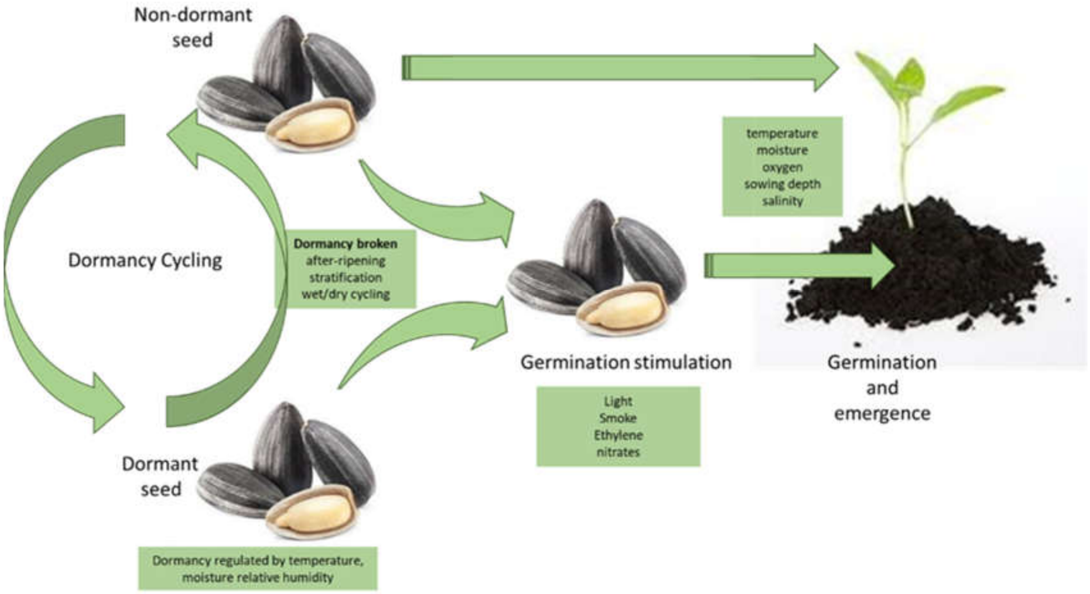


Fig. 1.5. Critical factors and main germination pathways that regulate seed dormancy, and germination as seeds transition from seed to seedling. Seeds at the time of dispersal can be either dormant or non-dormant, and may cycle (i.e. dormancy cycling) between both states over different seasons as they enter and transit the optimal recruitment period if conditions are not completely suitable to promote and sustain germination. To germinate, dormant seeds need to lose dormancy first via exposure to various species-specific combinations of moisture, and temperature. Once seeds are non-dormant, these can either germinate/emerge when the right conditions are present, or at this point may require a stimulant (i.e. smoke, light or ethylene) to promote germination. Stimulants act as a secondary layer of ecological filters signalling to the seed that it may be an appropriate time to germinate, i.e. after a fire (smoke), near the soil surface (light), or in waterlogged (ethylene) soil (adapted from Turner, Lewandrowski et al. 2018).

Seed dormancy is a mechanism that optimizes germination with the window of opportunity that is most suitable for seedling establishment (Baskin and Baskin 2003). Seed dormancy traits are diverse and mainly determined by phylogenetic and/or ecological factors with five main classes currently recognised based on a set of seed attributes (Wang, Baskin et al. 2009, Dayrell, Garcia et al. 2017). Non-dormant seeds germinate to > 70 % within 28 days under favourable environmental conditions without any treatment (Baskin and Baskin 2004). Physically dormant seeds (PY) are characterized by the presence of a water impermeable seed coat thus cannot imbibe water. (Baskin, Baskin et al. 2000). Under natural conditions, alleviation of PY may occur with disturbance events such as brief exposure to high (> 60 °C) soil temperatures during seasonal fires or extreme heat wave events, or more gradually in response to alternating temperatures or high humidity (Bell, Plummer et al. 1993, Hudson, Ayre et al. 2015). Physiological seed dormancy (PD) is characterized by a physiological inhibition within the embryo that supresses and prevents the emergence of the radicle until an appropriate stimulus is applied that overcomes dormancy (Baskin and Baskin 2003). According to previous studies on physiologically dormant species, regulation of seed dormancy and germination are two processes that occur in response to the interplay between soil temperature, moisture content and time (hydrothermal time) during different parts of the year (Baskin and Baskin 2003, Steadman and Pritchard 2004). Essentially, ‘hydrothermal time’ is a temporally informed version of the hydrothermal germination niche discussed above. However, the interplay between soil temperature, moisture content and time is a dynamic continuum, and shifts in hydrothermal time can cause seeds of some species to continually shift between dormant and non-dormant states (Finch-Savage and Footitt 2017, Miller, Symons et al. 2019). Compared to PY, dormancy alleviation requirements of physiologically dormant seeds tend to be more complex and gradual, and may include several steps such as after-ripening, stratification, cycles of wetting and drying, or dry heat that are aligned with various soil based environmental processes that seeds are exposed to (Fig. 1.5) while in the soil seed bank (Merritt, Turner et al. 2007, Hoyle, Steadman et al. 2008, Long, Gorecki et al. 2015). In order to gain a comprehensive knowledge of the dynamics of the germination niche, it is essential to identify the key environmental variables that affect seed dormancy regulation and germination (Fig. 1.5), and also quantify performance of seeds across each environmental gradient (Cochrane 2018, Del Vecchio, Fantinato et al. 2020).

1.1.4 The impact of thermal stress and water stress on the seed germination niche

Thermal stress and water stress have been identified as two critical environmental variables that affect the germination response of seeds, especially in arid ecosystems, as moisture in particular is a very limited and ephemeral resource (Bell, Plummer et al. 1993, Merino-Martín, Courtauld et al. 2017, Cochrane 2020). Since the metabolic processes central to seed germination are mediated by enzymes, thermal performance of seeds (during germination) as a consequence should resemble a unimodal, asymmetric hump-shaped curve (Fig. 1.4) characteristic of all enzyme mediated reactions seen right across biology (Yan and Hunt 1999, Angilletta Jr 2006). In most plant species, absorption of water from the external environment is essential to initiate the incipient metabolic processes of seed germination (Tweddle, Dickie et al. 2003, Kaya, Okçu et al. 2006). Therefore, metabolic reactions associated with seed germination should be impeded by reduced water availability (Muscolo, Sidari et al. 2014, Huang, Ran et al. 2020). Numerous studies have investigated the role of thermal stress and water stress in shaping the germination niche of plants, as well as ecological and evolutionary implications of these responses (Carta, Bedini et al. 2013, Walder and Erschbamer 2015, Fernández-Pascual, Pérez-Arcoiza et al. 2017, Del Vecchio, Fantinato et al. 2020). However, there is a shortage of studies that have incorporated established principles of chemical kinetics to quantify how thermal stress and water stress affects seed germination, particularly for species with distributions restricted to specific habitats (Turner, Lewandrowski et al. 2018). The concept of ‘hydrothermal germination niche’ is well established in the field of agriculture (Onofri, Benincasa et al. 2018). However, as a result of artificial selection over the last 12,000 years (Baskin and Baskin 2003), germination responses of crop species are likely to be much less variable compared to wild species, but there remains a shortage of studies assessing the thermal and hydrological responses of seeds of wild species on a theoretical basis (Duncan, Schultz et al. 2019). The role of these two environmental gradients in shaping patterns of plant distribution based on seed ecology are not well understood (Luna, Pérez et al. 2012, Robinson, Di Virgilio et al. 2019). Quantifying thermal and hydrological stress responses of seeds in an established theoretical context is essential to gain a comprehensive understanding of niche dynamics (Turner, Lewandrowski et al. 2018). Non-linear germination models have already been used to describe and explore the effects of environmental factors on germination success (Tarszisz, Tomlinson et al. 2017, Lewandrowski, Erickson et al. 2018), and could potentially be used to quantify germination responses of seeds in response to water and temperature stress (Yan and Hunt 1999). Employing a non-linear model fitting approach to quantify thermal and hydrological germination responses could lead to a comprehensive understanding of how these environmental gradients affect the germination niche as well as ecological and evolutionary implications.

1.1.5 Inselberg environnents

Specialised environments tend to result in unique communities with species and adaptations that are distinct from surrounding, more widespread, habitats (MacArthur and Wilson 2016). Among other adaptations that plants make to isolated or specialised habitats, the germination niche is likely to be strongly adapted to local environmental conditions (Cross, Turner et al. 2015). Inselberg such as granite outcrops (Fig. 1.6A) and Banded Ironstone Formations (BIFs; Fig. 1.6B) comprise of fine-scale mosaics of niche habitats that are characterised by unique and highly localised environmental conditions (Porembski and Barthlott 2000, Jacobi and Fonseca do Carmo 2008, Gibson, Yates et al. 2010). Inselbergs harbour edaphically isolated island-like shallow-soil plant communities that are floristically distinct from the vegetation in the deeper-soil low-lying plains surrounding these outcrops (Withers 2000, Jacobi, Do Carmo et al. 2007). Inselbergs are found in a wide range of ecosystems, and their distribution reflect underlying geological features and weathering processes (Byrne and Hopper 2008, Porembski and Barthlott 2012). Opportunities for evolution in rocky outcrop habitats are largely driven by edaphic isolation from the surrounding vegetation matrices, local topographic elements and climatic variables (Jacobi, Do Carmo et al. 2007, Gibson, Yates et al. 2010, Porembski and Barthlott 2012, Do Carmo and Jacobi 2016). Consequently, plant communities on outcrop habitats host combinations of taxa that are regionally broadly distributed, as well as range-restricted species that are highly adapted to localised microhabitats (Gibson, Yates et al. 2010, Porembski and Barthlott 2012, Do Carmo and Jacobi 2016). Therefore, inselberg communities tend to be high in species richness compared to deeper-soil habitats in the low-lying environments surrounding outcrops (Main 1997, Mares 1997, Withers 2000, Yates, Hopper et al. 2003, Schut, Wardell-Johnson et al. 2014), yet the mechanisms driving this species turnover, such as adaptations to specific germination niches (among others), aren’t fully understood.

Fig. 1.6. Images of representative inselberg habitats from Western Australia. **A)** The summit of Castle Rock, a granite emergent in the Porogurups range near the south coast; **B)** The Banded Iron Formations of the Juna Range in the Pilbara Region of the northwest. Images both provided by S. Tomlinson

In Western Australia Granite outcrops and BIFs are primarily located in low to moderate rainfall zones (Fig. 1.7A, Fig. 1.8) such as the Yilgarn region (Main 1997, Withers 2000, Gibson, Yates et al. 2010). The climate of southern parts of Western Australia is Mediterranean (Hopper and Gioia 2004). In this region rainfall mainly occurs from late autumn to early spring (Fig. 1.8), and aridity increases in a north-easterly direction due to low frequency and intensity of rainfall episodes (Bell, Plummer et al. 1993, Gibson, Coates et al. 2007, Ladd, Yates et al. 2019). Compared to vegetation matrices in the surrounding low-lying environments, outcrop habitats in Western Australia are generally water-limited due to high soil water evaporation associated with the lack of vegetation cover, generation of high surface temperatures on rocky surfaces (especially in summer) and low water retention capacity of shallow-soils (Elliott, Lewandrowski et al. 2019). However, weathering of outcrops has produced various shaded microhabitats such as slopes, gullies, cracks and fissures that can concentrate and retain water for long periods much of which can seep and pool within the underlying porous subsurface rock strata (Yates, Gibson et al. 2011, Di Virgilio, Wardell-Johnson et al. 2018, Miller, Symons et al. 2019). Flora that utilize these diverse niche habitats include range-restricted outcrop specialist (Fig. 1.7B) as well as taxa that are broadly distributed in surrounding deep-soil habitats often across large ranges (Withers 2000, Byrne 2019).

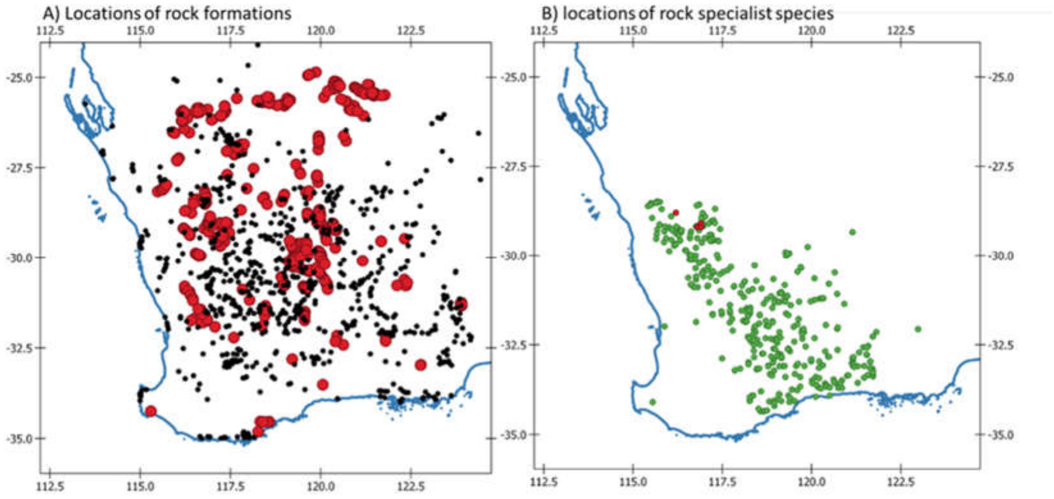


Fig. 1.7. A) Distribution of banded ironstone formations (red dots) and other rocky outcrops (black dots) in the south west of Western Australia. **B)** Distributional records of a rare rock outcrop specialist species (*Acacia woodmaniorum*; red dots) and a widespread dominant congeneric species (*Acacia assimilis*; green dots) in Western Australia based on Atlas of Living Australia (2021).

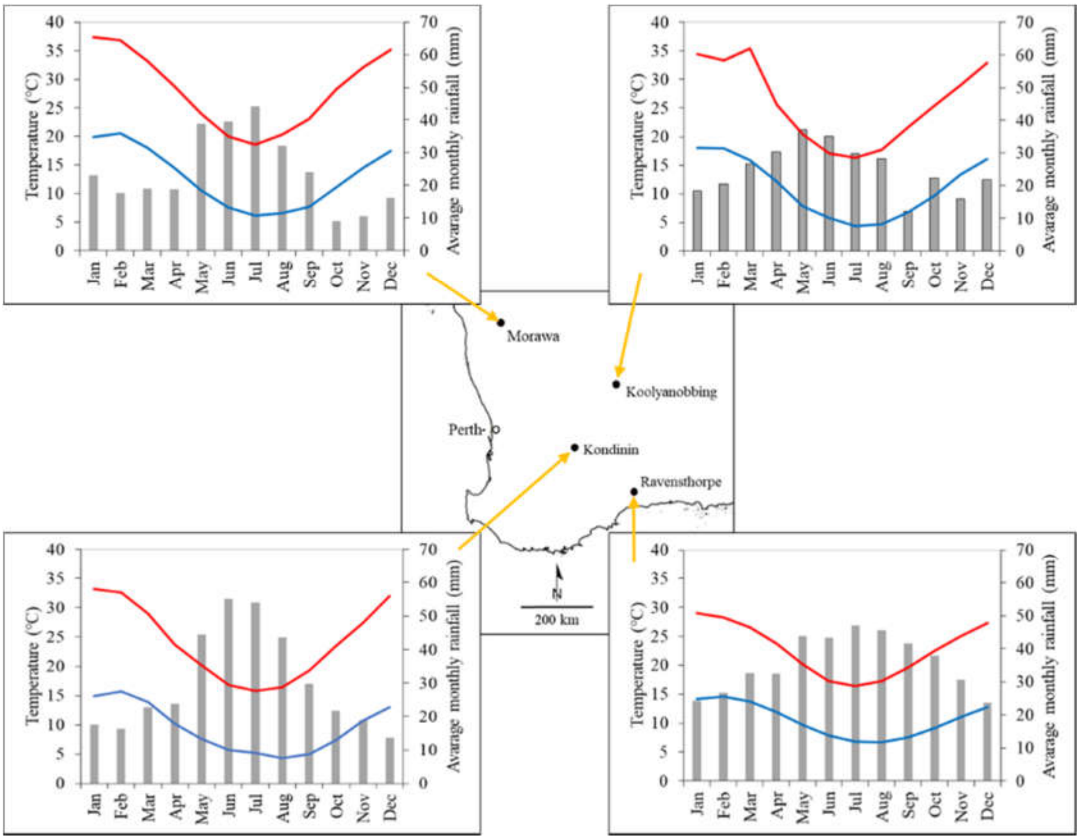
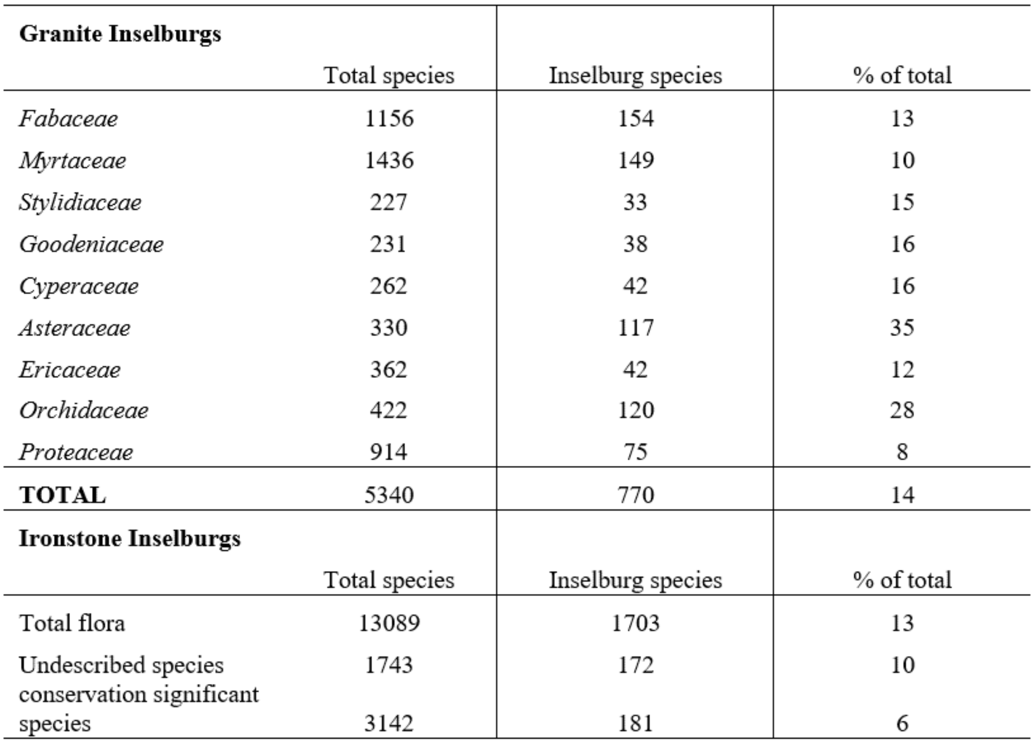


Fig. 1.8. Long term climatic data for four outcrop habitats in different regions of Western Australia, encompassing mean monthly maximum (red line), mean minimum temperatures (blue line) and mean monthly rainfall represented by grey bars (Bureau of Meteorology 2018).

1.1.6 Narrow range endemic species and unique ecological communities on outcrops

Granite and BIF outcrop habitats of Western Australia harbour significant regional biodiversity (Table. 1.1, Hopper and Gioia 2004, Gibson, Yates et al. 2010). Species that are restricted to outcrop habitats constitute about 10 % of all threatened flora species native to Western Australia, yet rock outcrops occupy only a fraction (< 1 %) of the total land area (Western Australian Herbarium, 1998). Many species of plants confined to granite outcrops are endangered because of activities related to agricultural development such as the introduction of invasive species, altered fire regimes, livestock grazing, dieback and salinity as a large number of these outcrops are found across the highly modified and developed Western Australia wheatbelt region (Hopper, Brown et al. 1997, Porembski 2000, Yates, Hopper et al. 2003). In comparison, BIF specialists are largely concentrated within landscapes that are very prospective for iron ore mining (Gibson, Meissner et al. 2012). A recent study on 25 BIF outcrops that have high iron ore content and potential targets for mining has identified 44 BIF specialist taxa (Table. 1.1) of which 36 are of conservation concern, including nine species of declared rare flora (Gibson, Yates et al. 2010, Wege, Thiele et al. 2015). In order to implement cost-efficient sustainable management protocols for outcrop endemics, it is essential to gain a comprehensive knowledge of their ecology (Yates and Ladd 2004, Yates, Gibson et al. 2011). Since seed-based restoration represents a major component of *in-situ* and *ex-situ* conservation approaches for range-restricted taxa, is it important to understand how various environmental factors affect the niche dynamics of germination (Clemente, Müller et al. 2017, Cochrane 2018, Cochrane 2020). Even though thermal stress and water stress are two major environmental variables that drive niche dynamics in outcrop ecosystems (Turner, Lewandrowski et al. 2018, Elliott, Lewandrowski et al. 2019), there is a general shortage of research data on how outcrop endemic species respond to these stress factors (Robinson, Di Virgilio et al. 2019). Given that seed germination is largely underpinned by metabolic processes, it should be possible to quantify physiological niches of germination following general principles of thermal performance (Yan and Hunt 1999, Angilletta Jr 2006). Quantifying thermal and hydrological tolerance thresholds of seeds is essential to construct conservation strategies for range-restricted taxa associated with outcrops (Paul, Dixon et al. 2014, Turner, Lewandrowski et al. 2018). It could also provide important insights into the role of thermal stress and water stress in shaping the patterns of rarity and endemism observed on outcrop ecosystems and perhaps provides some guidance as to which taxa may be more prone to drought and climate changes and direct the selection of suitable habitat for establishing new translocated populations in future (Di Virgilio, Wardell-Johnson et al. 2018, Duncan, Schultz et al. 2019, Robinson, Di Virgilio et al. 2019).

Table. 1.1. The proportional representation of inselberg species of the ten largest plant families of the South Western Australian Floristic Region (SWAFR), and the proportion of undescribed species or species of conservation concern associated with inselbergs in the SWAFR. Granite outcrops are home to between 8 and 35 % of plant species in the SWAFR (adapted from Hopper, Fiedler et al. 2021), while BIF ranges harbour 10 % of the known, but undescribed plants species, and nearly 6 % of all plants of conservation concern (adapted from Gibson, Coates et al. 2007). BIF ranges represent approximately 1.4 % of the land area in the SWAFR (adapted from Gibson, Coates et al. 2007).



Granite Inselburgs	Total species	Inselburg species	% of total
Fabaceae	1156	154	13
Myrtaceae	1436	149	10
Stylidiaceae	227	33	15
Goodeniaceae	231	38	16
Cyperaceae	262	42	16
Asteraceae	330	117	35
Ericaceae	362	42	12
Orchidaceae	422	120	28
Proteaceae	914	75	8
TOTAL	5340	770	14
Ironstone Inselburgs	Total species	Inselburg species	% of total
Total flora	13089	1703	13
Undescribed species	1743	172	10
conservation significant species	3142	181	6

1.2 Research objectives

1.2.1 General objective

The aim of this thesis was to quantify the thermal and hydrological germination responses of a broad range of species associated with outcrop habitats of Western Australia and to compare them to closely related, widespread species in order to understand the role and relative importance of environmental variables in shaping the germination niche of outcrop endemic taxa, and assess the ecological and evolutionary implications as a consequence of these interactions.

1.2.2 Specific objectives

1. Thermal stress and water stress tolerance in seeds of non-dormant species (*Eucalyptus* species) endemic to granite outcrops

   To investigate whether the thermal stress and water stress response of seeds of several non-dormant species associated with outcrops conform to established theoretical principles, assess whether broadly distributed taxa have broader thermal and hydrological performance niches compared to range-restricted species, and identify ecological and evolutionary implications.

2. Hydrothermal stress tolerance in seeds of physically dormant species (*Acacia* species) endemic to BIF outcrops

   To assess the thermal stress and water stress responses of seeds of several physically dormant species associated with BIF outcrops, quantify potential interactions between these two environmental variables, investigates whether broadly distributed taxa have broader hydrothermal performance niches compared to range-restricted species, and identify ecological and evolutionary implications.

3. Hydrothermal stress tolerance in seeds of physiologically dormant species (*Tetratheca* species) endemic to BIF outcrops

   To quantify hydrothermal germination responses of seeds of several physiologically dormant species restricted to BIF outcrops, assess potential interactions between these two environmental variables, compare hydrothermal occurrence niches and performance niches of test species during the stage of germination, and identify ecological and evolutionary implications.

Chapter 2

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

2.1 Abstract

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 localised 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 congenerics *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 congenerics. 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.

2.2 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 and Fonseca do Carmo 2008, Gibson, Yates et al. 2010). Opportunities for evolution in these habitats largely result from edaphic isolation from the surrounding vegetation matrices and the unique and highly localised environmental conditions commonly found in these niche landscapes (Porembski and Barthlott 2000, Withers 2000, Jacobi and Fonseca do Carmo 2008, Gibson, Yates 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, Yates 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, Hopper et al. 2003, Schut, Wardell-Johnson et al. 2014), and contribute significantly to regional biodiversity (Hopper and Gioia 2004, Safford, Viers 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 SWAFR (Byrne and Hopper 2008, Wege, Thiele 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 and Ladd 2004, Carta, Bedini et al. 2013, Tapper, Byrne et al. 2014, Cross, Turner et al. 2015). 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 (Carta, Bedini et al. 2013, Cross, Turner et al. 2015, Cross, Turner et al. 2015, Cross, Barrett 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, Pérez et al. 2012, Turner, Lewandrowski 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, Do Carmo et al. 2007, Gibson, Yates 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, Byrne et al. 2014). 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, Brown et al. 1997, Withers 2000). Exposed granite surfaces are characterised 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 localised 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, Yu 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, Whittall et al. 2011, Tapper, Byrne et al. 2014), and these warrant further investigation.

The transition from seed to seedling represents one of the most critical stages of the plant life cycle (Lloret, Peñuelas et al. 2004, James, Sheley 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, Baskin et al. 1997, Tweddle, Dickie et al. 2003, Luna, Pérez et al. 2012). Consequently, the environmental requirements for dormancy alleviation and seed germination are usually both definable, highly nuanced and species-specific (Turner, Lewandrowski 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, Rokich et al. 1995, Merritt, Turner 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 the habitats provide environments that are likely to differ markedly from other parts of the landscape (Turner, Lewandrowski et al. 2018, Elliott, Lewandrowski et al. 2019). Consequently, investigation of the germination ecology of seeds from range-restricted and ecologically specialised flora should be a principle area of research to better understand their demographic limitations which may assist with their ongoing conservation and management (Luna, Pérez et al. 2012, Clemente, Müller et al. 2017). Furthermore, identifying some unifying theoretical constraints to seed germination is essential for constructing *a priori*, mechanistic hypotheses underpinning these demographic limitations.

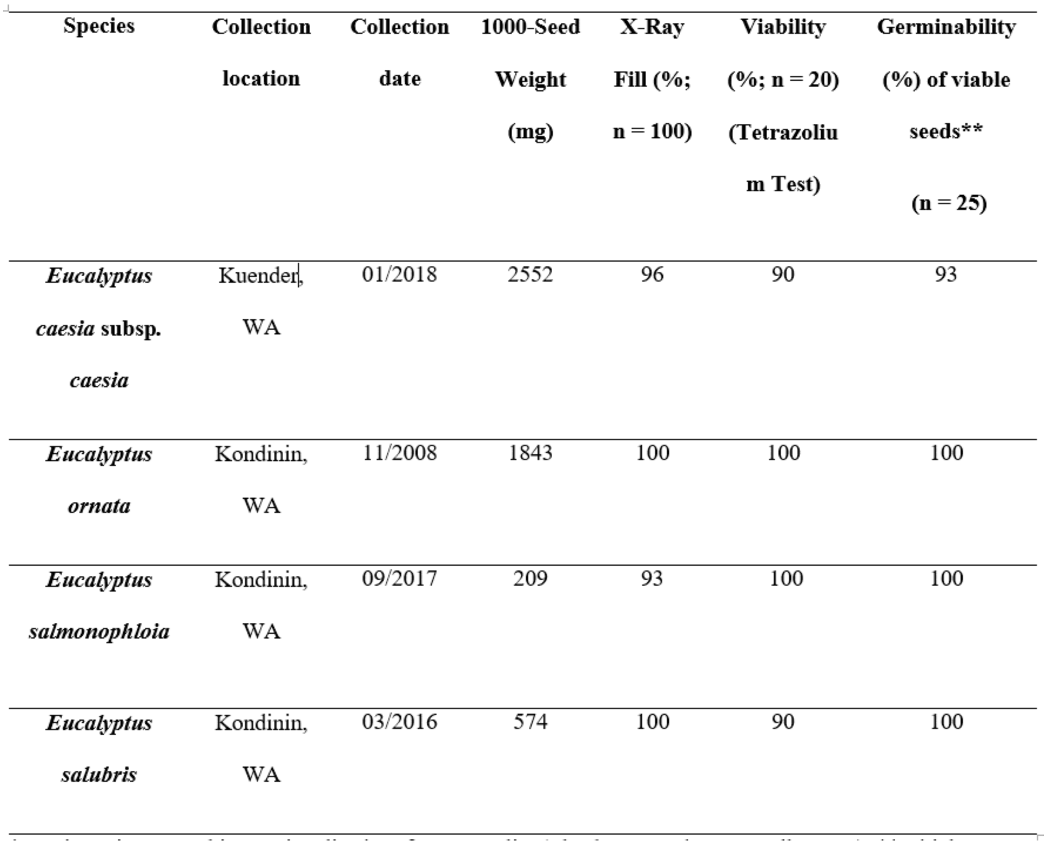
There have been efforts made to develop models of seed germination in relation to temperature and water stress (Bradford 2002), but these have been heavily data-referential, and have not been consistent with the theoretical underpinnings of the wider thermal performance literature (Angilletta Jr and Angilletta 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 catalysed by enzymes that have a specific thermal threshold beyond which they denature (Peterson, Daniel 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 catalysed by the denaturing enzymes. Therefore thermal performance curves of enzymes are hump shaped and distinctly asymmetrical (Angilletta 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 and Kingsolver 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 specialised (stenothermy; Debat and David 2001, Ghalambor, McKay 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 species distributional range 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, Byrne et al. 2014). 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: (1) Are seeds of non-dormant range-restricted species more sensitive to incubation temperature compared to common congeneric taxa? And (2) Are seeds of non-dormant range-restricted species more sensitive to water stress compared to common congeneric taxa?

2.3 Materials and methods

2.3.1 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 response. 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., Appendix. 1.1). 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. 2.1). *Eucalyptus caesia* Benth. subsp. *caesia* and *E. ornata* are range-restricted mallees that are gazetted as priority 3 and 4 respectively (Western Auastralian Herbarium 2018) so are of some conservation concern (Coates and Atkins 2001). *Eucalyptus. caesia* subsp. *ceasia* is distributed across 25 populations in the Avon Wheatbelt, Coolgardie and Mallee (Bezemer, Krauss 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, Hobbs 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 mesic Mediterranean SWAFR (South West Australia Floristic Region) to the semi-arid IBRA regions such as Coolgardie (Yates, Hobbs 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. 2.1). Seeds from all species were stored under cool, dry conditions until utilised in this study.

Table. 2.1. Seed traits of selected *Eucalyptus* species used in this study. *E. caesia* subsp. *caesia* is the most tightly-distributed species, occurring in only 3 IBRA regions*, followed *by E. ornata* (2 IBRA regions), *E. salmonophloia* (8 IBRA regions) and *E. salubris* (9 IBRA regions).



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.

2.3.2 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 and Merritt 2016).

For seeds that were filled, seed viability was also investigated using Tetrazolium staining (Lakon 1949). Reduction of 2, 3, 5 triphenyltetrazolium chloride ($C_{19}H_{15}N_4Cl$) 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 and Mohler 2002). Samples of 20 seeds per species were horizontally dissected and exposed to 1 % tetrazolium for a period of four hours at 25 °C. We used stained seeds to calculate percentage viability of seed lots (Table. 2.1).

2.3.3 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 (1,400 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. 2.1). Seeds were surface sterilized with 2 % (w/v) calcium hypochlorite ($Ca[OCl]_2$) under vacuum (-70 kPa) for 30 minutes 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, Carr 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 (Appendix. 1.2). We scored germination as radicle emergence greater than 2 mm, and plates were scored four days a week for a period of 28 days.

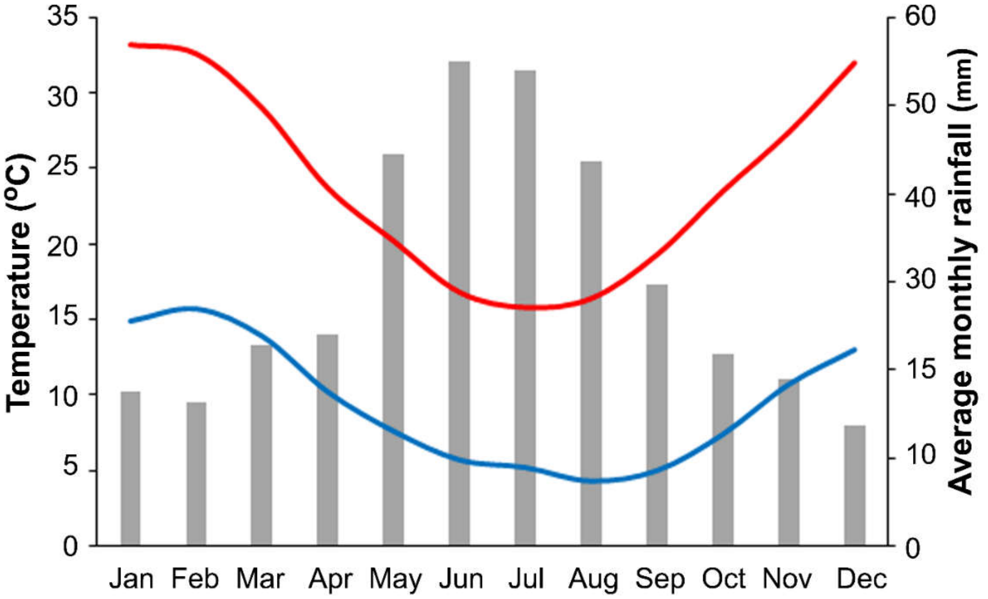


Fig. 2.1. Long-term climatic data for the Kondinin meteorological station, encompassing average monthly maximum (red line) and minimum (blue line) temperatures, and average monthly rainfall (grey bars).The temperature range of the experimental trials conducted here span the full temperature range in the region, from 5 °C to 35 °C.

2.3.4 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 (1,400 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.

2.4 Statistical analysis

2.4.1 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 and Sowden 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 generalised 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, Erickson et al. 2017, Tarszisz, Tomlinson et al. 2017). The *drc* package (Ritz and Streibig 2012) was used to fit a three-parameter log-logistic function to germination data in the *R* statistical environment (R Core Team 2013);

$$germination = \frac{G_{max}}{1+\frac{time}{t_{50}}^{b}} \qquad (1)$$

where, G_{max} is the upper limit for the germination rate, and the lower limit of germination rate is assumed to be 0 (Lewandrowski, Erickson 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_{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 of 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 *AICcmodavg* 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_{max}) by comparing each permutation with the common curve using the Akaike Information Criterion (Burnham and Anderson 2002). The log-logistic model grouped into unique species and temperature categories was utilized to estimate t_{50} and G_{max} values for each replicate of all species incubated under different treatment regimes. We used model estimates for b, G_{max}, and t_{50} to calculate time (in days) to reach G_{max} and 10 % of G_{max} (t_{10}) for all replicates exposed to different treatment regimes.

2.4.2 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, Daniel et al. 2007), appropriate non-linear thermal performance functions are yet to be described for seeds (Yan and Hunt 1999). Therefore, we estimated unimodal asymmetrical model fits for the $1/t_{50}$ estimates for our thermal response data using a thermal performance function which has been described by Yan and Hunt (1999) for the temperature response of maximum rate of growth in plants;

$$r_{max} = R_{max} \left(\frac{T_{max}-T}{T_{\max} - T_{opt}}\right)\left(\frac{T}{T_{opt}}\right)^{\frac{T_{opt}}{T_{max}-T_{opt}}} \qquad (2)$$

where r_{max} is the maximum germination rate at any temperature (T), T_{opt} is the optimum temperature for germination at the peak of the performance function, T_{max} is the limit of thermal tolerance, where germination ceases and R_{max} is the asymptotic maximum germination rate at T_{opt}. Henceforth, $1/t_{50}$ will be referred to as the thermal performance of maximum germination rate, r_{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 characterised as R_{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.

2.4.3 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;

$$t_{50} = g_0 + e^{\{k \times (w + w_c)\}} \tag{3}$$

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 w_c is the critical water stress level at which t_{50} begins to escalate exponentially.

2.4.4 Unique parametrisation

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 parameterise unique values of R_{max}, T_{opt}, T_{max}, g_0 and w_c for each species in terms of t_{50} and G_{max}.

2.5 Results

2.5.1 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. 2.2a). Both *Eucalyptus caesia* subsp. *caesia* and *E. ornata* exhibited relatively constant high final germination percentages (> 80 %) from 10 - 30 °C, while final germination percentages of *E. salmonophloia* and *E. salubris* decreased from 98 % to < 76 % at 25 °C (Fig. 2.2a). 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. 2.2a). For all taxa except *E. caesia* subsp. *caesia* the maximum temperature at which germination occurred was 35 °C (Fig. 2.2a). Within the range of 15 - 25 °C, estimated time to reach G_{max} was ≤ 30 days for most replicates of the four species (Fig. 2.3a). For *E. caesia* subsp. *caesia* and *E. ornata*, deviation from favourable temperatures increased variability in G_{max} and lengthened the time required to reach G_{max} (Fig. 2.3a). However, for *E. salubris* the time to reach G_{max} was relatively consistent across 10 - 30 °C (Fig. 2.3a).

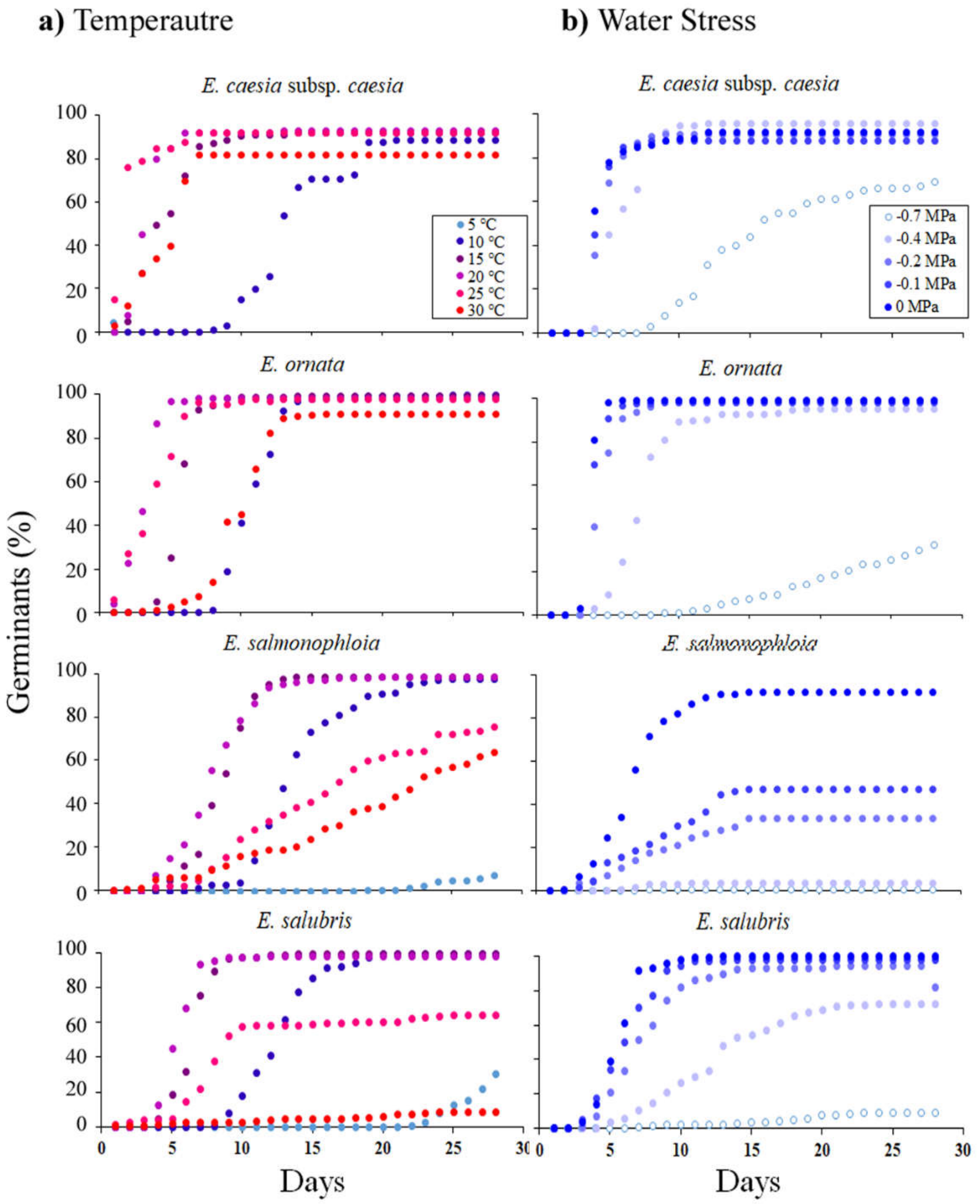


Fig. 2.2. Mean cumulative germination percentage for replicates of seeds of four Western Australian *Eucalyptus* species following exposure to an increasing range of temperatures **(a)** and water stress levels **(b)** incubated in constant darkness for 28 days. Eight replicates of 25 seeds were used for each treatment. Error bars depicting standard error of the mean have been omitted for clarity.

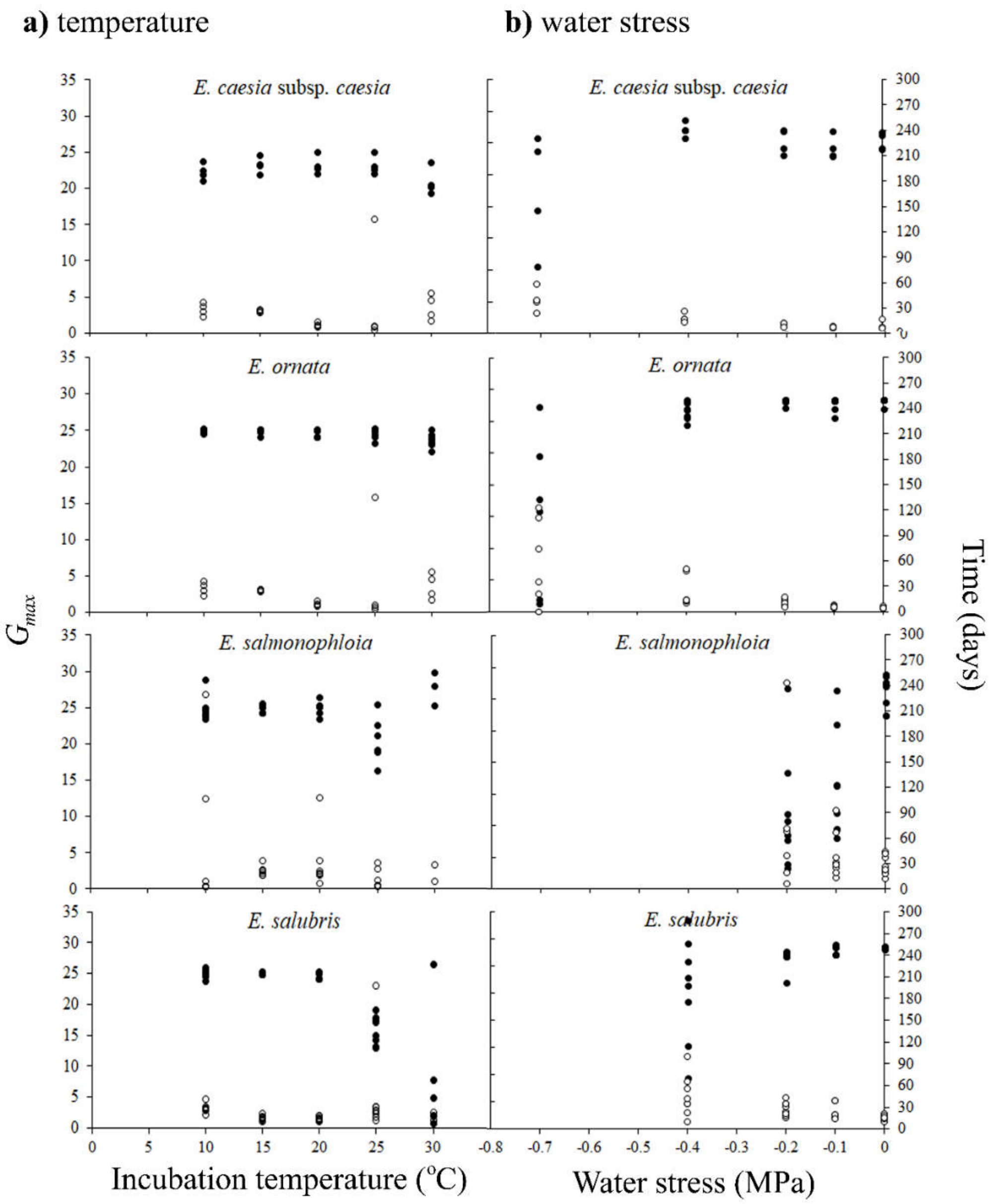


Fig. 2.3. Estimates for maximum germination (G_{max}) and time to reach G_{max} predicted by a three-parameter log-logistic function for the germination responses of four Western Australian *Eucalyptus* species following incubation in constant darkness at varying temperature regimes **(a)** and water 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.

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. 2.2b). Conversely, the final germination of *E. salmonophloia* and *E. salubris* seeds decreased to < 80 % at -0.1 MPa and -0.4 MPa, respectively (Fig. 2.2b). 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. 2.2b). For all tested species estimates for time to reach G_{max} and variability of these estimates increased with rising water stress (Fig. 2.3b).

2.5.2 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; Appendix. 1.3) indicating that both 'species' and 'incubation temperature' were factors that contributed to variability in germination response (Appendix. 1.3). The log-logistic curve could not be fitted to the germination response data for 5 °C and 35 °C since final germination percentages were very low (< 31 %) at these temperatures (Fig. 2.2a). The distribution of the r_{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. 2.4a). The most parsimonious model resolved unique R_{max}, T_{opt} and T_{max} values defining the thermal performance of r_{max} for each species (Eqn. 2, Fig. 2.4a). For *E. caesia* subsp. *caesia* and *E. ornata* estimated T_{opt} values were 25.4 ± 0.25 °C and 23.0 ± 0.37 °C respectively, whereas for *E. salmonophloia* and *E. salubris* estimates for T_{opt} were 17.7 ± 1.94 °C and 20.1 ± 0.97 °C respectively (Fig. 2.4a). 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. 2.4a). For all tested species estimated T_{max} was within the range of 30.5 - 32 °C. A thermal performance function could not be resolved for the G_{max} estimates of the log-logistic model (Fig. 2.3a) because they were highly conserved across all experimental temperatures.

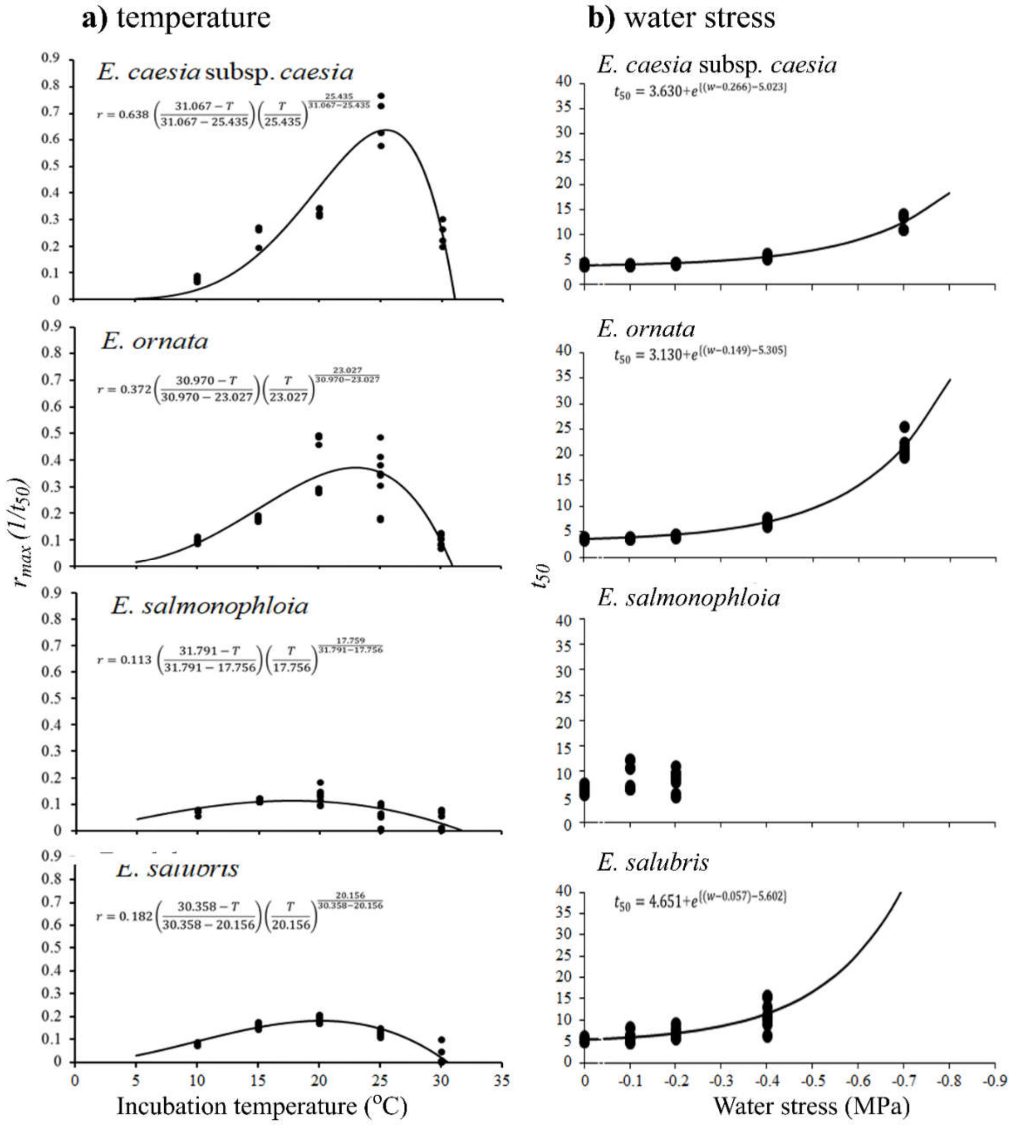


Fig. 2.4. Thermal performance and water stress tolerance in four Western Australian *Eucalyptus* species in terms of time to reach 50% germination (t_{50}); **(a)** Dots represent $1/t_{50}$ estimates for each replicate of seeds after exposure to different temperature regimes and the smooth lines represent the permutations of the thermal performance curve fitted to the $1/t_{50}$ estimates (r_{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.

2.5.3 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; Appendix 1.3). 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. 2.4b). The exponential function fitted to the t_{50} estimates resolved g_0, k and w_c 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. 2.4b). For *E. caesia* subsp. *caesia*, *E. ornata* and *E. salubris* estimated w_c values were -0.266 ± 0.098, -0.149 ± 0.049 and -0.057 ± 0.250 MPa respectively (Fig. 2.4b). 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 (Fig. 2b, Fig. 2.4b). Furthermore, the exponential function could not be fitted to the G_{max} estimates of the log-logistic model (Fig. 2.3b).

2.6 Discussion

The results of this study demonstrates 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 stenothermy 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}.

2.6.1 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, Rokich et al. 1995, Ruiz-Talonia, Carr 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, Rokich et al. 1995, Jiménez-Alfaro, Silveira et al. 2016). Bell, Rokich 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 final germination percentage in all species tested (Fig. 2.2a), more substantial influences could be seen on germination rate (t_{50}). Deviations from favourable temperature ranges for germination increased time to reach G_{max} and variability in estimates for time to reach G_{max} in all four taxa (Fig. 2.3a). It is possible that, at least insofar as understanding thermal constraints, maximum germination is a less informative functional trait (Saatkamp, Cochrane 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_{opt} estimates for all four species were within a range of 17 - 26 °C, and T_{max} values were between 29 - 32 °C (Fig. 2.4a). Locations from which seeds for this study were collected are in a Mediterranean climate, characterized by hot dry summers and mild wet winters (Bell, Plummer et al. 1993; Fig. 2.1). 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, Plummer et al. 1993). The T_{opt} and T_{max} estimates for the four taxa clearly reflect a preference for synchronizing germination between late autumn to early spring (Fig. 2.4a), 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, Rokich 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, Pérez et al. 2012, Clemente, Müller et al. 2017), and the data that we present here indicate that it can be parameterised 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 percentage in all species tested in this study (Fig. 2.2b), consistent with previous studies of *Eucalyptus* species (Pearce, Van Der Moezel et al. 1990), and the broader Western Australia flora (Cochrane 2018, Turner, Lewandrowski et al. 2018).

2.6.2 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_{opt}, and water stress, represented by lower w_c, compared to their widespread congenerics (Fig. 2.2), but had narrower ranges of thermal tolerance in terms of r_{max} (Fig. 2.4a). Of the four species, the broadly-distributed *E. salmonophloia* and *E. salubris* were the most drought-sensitive, with critical thresholds at -0.1 MPa and -0.4 MPa respectively, compared to the critical threshold for *E. caesia* subsp. *caesia* and *E. ornata* at -0.7MPa (Fig. 2.3b). We suggest that these adaptations to water stress relate to the below-ground environments that characterise 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 are generally lower than the surrounding environment because the soils in these habitats are shallower compared to that 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, Turner 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_{max}, combined with high drought tolerance in terms of G_{max}, time to reach G_{max} and t_{10} 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, Hobbs et al. 1994). Low thermal and drought tolerance in terms of final germination percentage and the relatively low r_{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 (Fig. 2.2, Fig. 2.4a). 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, Plummer 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, Plummer 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 congenerics living in less restrictive habitats is less plastic (Graves and Taylor 1988, Giménez-Benavides, Escudero et al. 2005, Giménez-Benavides and Milla 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 specialisation in other taxa (Seebacher and Franklin 2005).

2.6.3 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, Müller et al. 2017). However, in order to get deeper insights in to 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 and Billings 1961, Felsenstein 1985, Luna, Pérez et al. 2012). Nevertheless, our findings are broadly consistent with results reported on the basis of larger numbers of species (Cochrane 2017, Ruiz-Talonia, Carr 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 characterised 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, Lewandrowski et al. 2018). More correctly, seed germination responds to a dynamic hydro-thermal niche (Hardegree, Walters 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, Turner 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, Lewandrowski et al. 2018).

2.6.4 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 (t_{50}), and that at least some seed germination traits are consistent with broader theories of thermal biology. Water stress, however, caused an exponential increase in t_{50},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 congenerics. 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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Chapter 3

The impact of hydrothermal stress on germination; a study on five *Acacia* species associated with Banded Ironstone Formations of Western Australia

3.1 Abstract

Seeds are highly adapted to germination windows focussed upon specific hydrothermal thresholds in the external environment, which maximize the likelihood of seedling survival, where, according to niche theory, generalist species should have broader hydrothermal performance niches than specialist taxa. To test this assumption the impact of hydrothermal stress (10 - 30 °C and -0.8 - 0 MPa) on the time to 50 % germination (t_{50}) and on maximum germination (G_{max}) was assessed in two narrow-range species (*Acacia woodmaniorum* and *A. karina*) restricted to Banded Ironstone Formations and three broadly distributed and dominant congenerics *A. assimilis*, *A. exocarpoides* and *A. ramulosa*. The hydrothermal germination niches of the study species were broadly congruent with hydrothermal conditions of their habitats. The two range-restricted taxa were more tolerant of hydrothermal stress (in terms of G_{max} and t_{50}) compared to the three widely distributed taxa, suggesting that tolerance of greater hydrothermal stress by both range-restricted *Acacia* species is likely to be adaptive to establishment in uncontested niche space. Additionally, complex interactions between thermal stress and water stress were identified, signifying the role of these environmental gradients in shaping the germination niche as well as patterns of plant diversity in BIF ecosystems. This study highlights the importance of

quantifying interactions between niche dimensions and their implications for species performance which will aid future restoration efforts for micro-endemic species impacted by mining.

3.2 Introduction

The occurrence niche is defined as the set of conditions under which a species currently exists, and it constitutes a subset of the fundamental niche (Elith and Leathwick 2009). The performance niche of a species refers to its performance rates across various environmental gradients (Sánchez-Gómez, Valladares et al. 2006, Rosbakh, Pacini et al. 2018). Niche theory predicts that generalist species usually (but not always) have broader niches compared to specialist species (Carboni, Zelený et al. 2016, Ainsworth and Drake 2020), thus perform moderately well in many conditions (the "jack of all trades" strategy; Huey and Hertz 1984). Specialist taxa by comparison, generally outperform generalists in their uncontested niche space (Wilson, Burgess et al. 2008, Boulangeat, Lavergne et al. 2012, Peers, Thornton et al. 2012).

There is often a good concordance between species occurrence niche and performance niches (Martínez-Meyer, Díaz-Porras et al. 2013, Van Couwenberghe, Collet et al. 2013, Pironon, Villellas et al. 2018). In order to fully understand the niche dynamics of a species, it is important to assess the impact of various environmental variables on species performance during all stages of its life cycle (Warren, Wright et al. 2011, Merow, Latimer et al. 2014). However, not all life stages contribute equally to overall population growth rate (de Kroon, Plaisier et al. 1986, Pironon, Villellas et al. 2018); species' niche structures are often determined by the performance of critical demographic stages (Sánchez-Gómez, Valladares et al. 2006, Fernández-Pascual, Pérez-Arcoiza et al. 2017, Del Vecchio, Fantinato et al. 2020). There is a shortage of studies in the literature that have accounted for the niches of vital demographic stages of taxa as organisms transition from juvenility to reproductively active adults (Pironon, Villellas et al. 2018).

Several past studies have highlighted the significance of the regeneration niche of plants and its pivotal role in shaping the overall niche structure of species (Donohue, Rubio de Casas et al. 2010, Fernández-Pascual, Pérez-Arcoiza et al. 2017, Del Vecchio, Fantinato et al. 2020). Investigation of the impact of various environmental factors on germination response is essential to identify the ecological constrains that limit the distribution of range-restricted taxa (Jiménez-Alfaro, Silveira et al. 2016, Cochrane 2018, Cochrane 2020), yet the influence and interaction of various environmental factors on the vital performance rates of seeds and the geographic range of plants is poorly quantified (Pironon, Villellas et al. 2018). Temperature and water stress have been identified as critical environmental dimensions that determine the success of seed germination, especially in arid ecosystems (Cochrane, Hoyle et al. 2014, Krichen, Vilagrosa et al. 2017, Zogas, Kosman et al. 2020). The concept of the "hydrothermal" niche is now a well-established concept defining seed germination (Bradford 2002, Hardegree, Roundy et al. 2018, Onofri, Benincasa et al. 2018). However, studies that have quantified hydrothermal responses of seeds within a broader theoretical context are scare in the restoration space (Lewandrowski, Erickson et al. 2017, Turner, Lewandrowski et al. 2018). Investigating the impact of various environmental axes on vital performance rates of closely related sympatric species with varying niche breadths may provide important insights into the role of the hydrothermal niche in structuring populations and distributions of narrow-range endemic plants.

Inselbergs such as Banded Ironstone Formations (BIFs) and granite outcrops harbour unique shallow-soil ecosystems across the earth (Byrne and Hopper 2008, Porembski and Barthlott 2012, Do Carmo and Jacobi 2016). Plant communities on rocky outcrops tend to be highly biodiverse and floristically distinct from surrounding deep-soil flora (Porembski and Barthlott 2000, Jacobi, Do Carmo et al. 2007, Poot, Hopper et al. 2012), often hosting both range-restricted, edaphically specialised taxa and those that are regionally widely distributed (Wyatt 1997, Withers 2000, Gibson, Yates et al. 2010). While the regional biodiversity values of these flora are well established, (Porembski 2007, Jacobi and Fonseca do Carmo 2008, Gibson, Meissner et al. 2012), the ecological filters responsible for these distinctive patterns are yet to be fully investigated and explained (Gibson, Yates et al. 2010, Poot, Hopper et al. 2012, Tapper, Byrne et al. 2014, Di Virgilio, Wardell-Johnson et al. 2018).

Banded Ironstone Formations are found in different regions throughout the world and are particularly prevalent in Western Australia with many distributed across the arid to semi-arid Yilgarn craton (Gibson, Meissner et al. 2012). The exposed, skeletal soils of BIF outcrops (Yates, Gibson et al. 2011) tend to be generally water-limited compared to those of neighbouring deep-soil vegetation matrices (Elliott, Lewandrowski et al. 2019), but they are also highly spatially heterogeneous, where weathering has produced shaded slopes, crevices and gullies that can retain rain water for longer periods (Yates, Gibson et al. 2011, Di Virgilio, Wardell-Johnson et al. 2018). These diverse niche environments are utilized by plant assemblages that exhibit high species richness and endemism. Over 90 % of Western Australian BIF specialist taxa are of conservation concern (Gibson, Meissner et al. 2012), and many BIF specialist species are restricted to small numbers of ranges (or parts thereof), or even a single outcrop (Gibson, Meissner et al. 2012). According to recent molecular phylogenetic studies, these outcrops function as refugia during periods of environmental stochasticity for both BIF specialists and their widely distributed co-occurring congenerics (Byrne, Krauss et al. 2019). However, there is a major shortage of research data on how environmental variables affect the patterns of plant diversity in BIF habitats (Yates, Gibson et al. 2011, Di Virgilio, Wardell-Johnson et al. 2018).

Western Australian BIF ranges are located in moderate to low rainfall zones (Gibson, Meissner et al. 2012), and are further characterised by rapid evaporation from the skeletal BIF soils due to high surface temperatures, low water-retention capacity of soil and lack of vegetation cover (Elliott, Lewandrowski et al. 2019). Thus the combined effect of thermal and water stresses represents a major selective force in these habitats (Yates, Gibson et al. 2011), yet the extent to which thermal stress and water stress affect the germination niche of BIF associated species and their impact on patterns of species distribution is yet to be broadly investigated (Turner, Lewandrowski et al. 2018). The major objective of this study was to compare the germination responses of two range-restricted BIF specialist taxa with those of three widely distributed co-occurring species to address the following research questions;

1. Does hydrothermal performance of physically dormant seeds conform to established mechanistic principles?
2. What are the ecological and evolutionary implications of the hydrothermal germination response of BIF associated congeneric flora?
3. Does hydrothermal performance of seeds of BIF associated congeneric species conform to niche theory?

3.3 Materials and methods

3.3.1 Species selection and sourcing

Five co-occurring species of *Acacia* (Fabaceae) found within the same vegetation communities with physical-seed dormancy (PY) that are endemic to Western Australia (Western Australian Herbarium 1998), but with widely differing area of occupancy

(AOO) and extent of occurrence were used for this study (EOO; Appendix. 2.1; Fig. 3.1). Seeds of two range-restricted species restricted to BIF habitats (*A. woodmaniorum* Maslin & Buscumb and *A. karina* Maslin & Buscumb) and three broadly distributed congeneric species were tested (*A. assimilis* S. Moore, *A. exocarpoides* W. Fitzg. and *A. ramulosa* W.Fitzg. Pedley). Seeds of the two range-restricted species were freshly harvested from wild populations, and seeds of the widely distributed taxa were purchased from a commercial seed supplier (Nindethana Seed Company, King River, Western Australia). The two range-restricted species *Acacia woodmaniorum,* and *A. karina* are gazetted as Priority 1 and Declared Rare Flora (DRF) under Western Australian conservation legislation respectively (Western Australian Herbarium 1998), so are of major conservation concern (Nevill, Anthony et al. 2010, Millar, Coates et al. 2013). Populations of *A. woodmaniorum* are limited to several adjacent BIFs in the Mungada/Mt Karara area of the arid to semi-arid Yalgoo IBRA (Interim Biogeographic Regionalisation for Australia) region (AOO ~ 12 km2; Thackway and Cresswell 1997). The habitats of this species include gullies, crevices and BIF slopes (Millar, Coates et al. 2013). *Acacia. karina* is restricted the Blue Hills range of the Yalgoo region (AOO ~ 44 km^2). Scattered populations of *A. karina* occur on BIFs, BIF slopes and adjacent flat plains of Mt Karara and Mt Jackson (Byrne 2019). Conversely, *A. assimilis, A. exocarpoides* and *A. ramulosa* are common broadly distributed dominant shrub or tree species native to southern parts of Western Australia (Meissner and Caruso 2008), though *A. ramulosa* is also widely distributed across other Australian states as well (Atlas of Living Australia 2020). The habitats of *A. assimilis* include granite outcrops, sandplains and slopes of rocky hills, whereas, *A. exocarpoides* inhabits rocky sites and hills (Western Australian Herbarium 1998, Meissner and Caruso 2008). *Acacia ramulosa* mostly occur on dunes and sandplains

(Ward, Bragg et al. 2018). *Acacia assimilis* is widely distributed across the more mesic Mediterranean SWAFR (South West Australia Floristic Region; Hopper and Gioia 2004) and to a lesser extent in the semi-arid Coolgardie region (Meissner and Caruso 2008). In contrast, the distributional ranges of *A. exocarpoides* and *A. ramulosa* extend from these semi-arid regions to arid IBRA regions, such as Murchison and Great Victoria Desert (Western Australian Herbarium 1998). The AOO of all three common species ranges from 560 to > 2,000 km^2 (Appendix. 2.1).

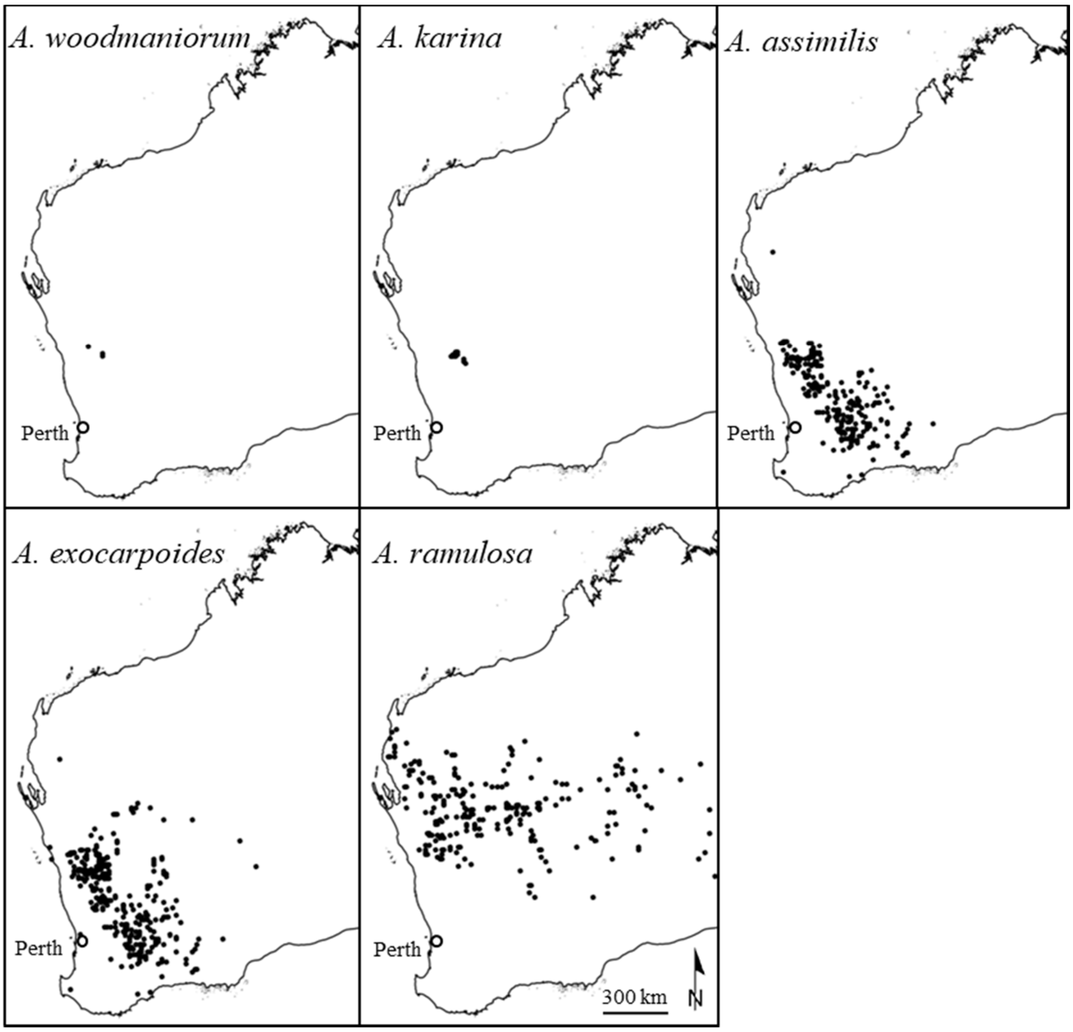


Fig. 3.1. Distribution of the five study species in Western Australia (black dots) based on Atlas of Living Australia (2020).

3.3.2 Seed quality

Before commencing this study, seed material was stored in a controlled environment of 15 °C and 15 % relative humidity at the Biodiversity Conservation Centre, Kings Park, Western Australia. Seeds were separated from chaff using a vacuum aspirator (SELECTA BV Gravity Seed Separator, the Netherlands). Percentage seed fill of each test species was quantified by X-ray analysis of 100 seeds (MX-20 digital X-Ray cabinet, Faxitron, USA). A seed that contains a mature fully-formed embryo and endosperm was considered to be a filled seed and identified by uniform white/grey coloration, whereas the absence of these tissues signified a lack of seed fill and was scored as non-viable (Erickson and Merritt 2016).

3.3.3 Germination requirements

Prior to experimentation, PY of the test species was alleviated by either exposure to boiling water or acid scarification to select the most effective way to render seeds water permeable (i.e. non dormant). For each species, samples of 20 seeds were initially exposed to boiling water for 2 min, as this approach has been previously reported to be highly effective in rapidly alleviating PY in multiple *Acacia* species (Bell 1999, Baskin, Baskin et al. 2000). Hot water-treated seeds and control seeds (samples of 20 non-treated seeds per species) were placed on a moist (9 ml of water per Petri dish) 84 mm germination paper (Advantec, Dublin, CA, USA) in 90 mm plastic Petri dishes. Petri dishes were sealed with plastic wrap to prevent desiccation and covered with aluminium foil to minimise the impact of light on germination. Samples were incubated in darkness at 20 °C for 28 days.

For species that exhibited < 80 % germination (i.e. *A. woodmaniorum*, *A. karina*, and *A. ramulosa*) following hot water treatment (Appendix. 2.1), the efficacy of acid scarification for dormancy alleviation was subsequently tested (Rincón-Rosales, Culebro-Espinosa et al. 2003, González-Castañeda, Angoa-Pérez et al. 2004). Samples of 20 seeds each were exposed to 98 % (v/v) sulphuric acid (H_2SO_4) for one hour and rinsed with distilled water. Rinsed seeds were then exposed to sodium hydrogen carbonate (100 mM $NaHCO_3$) solution for 10 mins to neutralize residual acid, and rinsed again with distilled water. Acid treated seeds were incubated at 20 °C in darkness for 28 days as previously described. Percentage germinability was calculated at the end of the test period and used to identify the most appropriate dormancy alleviation method for each species to obtain high (> 80 %) germination (Appendix. 2.1).

3.3.4 Hydrothermal tolerance

To quantify the germination response of seeds to hydrothermal stress, three to five replicates of 20 seeds per species (depending on seed availability) were exposed to 30 hydrothermal stress regimes (water stress levels of 0.0, -0.2, -0.4, -0.6 and -0.8 MPa at 10, 15, 20, 25 and 30 °C). Seeds were placed on 84 mm germination paper (Advantec, Dublin, CA, USA) infused with either distilled water or different concentrations of polyethylene glycol 8000 (PEG) solution to generate the range of water potentials outlined above for each incubation temperature. Nine ml of distilled water or PEG solution was administered per 90 mm plastic Petri dish following the methodology described by (Michel 1983). The temperature regimes used in this study encompass a wide range of the hydrothermal conditions reported for the locations of the test species across different seasons (Bureau of Meteorology 2018). Prior to plating seeds were surface sterilized using 2 % (w/v) calcium hypochlorite (Ca $[OCl]_2$) under vacuum (-70 kPa) for 30 minutes followed by rinsing thrice with sterile deionized water for several minutes per wash. Seed plating was conducted in a laminar flow cabinet under sterile conditions. Following the plating of seeds, Petri dishes were tightly sealed with plastic wrap to prevent desiccation during the incubation period. Petri dishes were also wrapped with aluminium foil to minimize exposure of seeds to light which could potentially cause a confounding effect on germination (Bell 1994, Ruiz-Talonia, Carr et al. 2018). To accurately record the temperature that seeds were exposed to during incubation iButton data loggers (Maxim Integrated™, San Jose, USA) were placed in the middle of each stack of six Petri dishes (Appendix. 2.2). Radicle emergence greater than 2 mm was scored as germination, and plates were scored four days per week for a duration of up to 28 days.

3.4 Statistical analysis

3.4.1 Germination modelling

A non-linear regression approach was used to estimate the performance characteristics of each species, because unlike linear regression methods it does not compress the natural variance pattern of germination response data (Ritz and Streibig 2008). Nonlinear regression requires a theoretically-rooted hypothetical function around which to fit the patterns in the response parameter (in this case germination), and so minimizes overfitting by restricting model fits only to the parameters that define the model (Wood 2011, Tomlinson 2019). First, the relationship between time and germination response was investigated for each hydrothermal stress regime by fitting a non-linear log-logistic function to the germination data (Tarszisz, Tomlinson et al. 2017, Saatkamp, Cochrane et al. 2019). A three parameter logistic model was fitted to the data using the *drc* package (Ritz and Streibig 2012) in the *R* statistical software environment (R Core Team 2013);

$$germination = \frac{G_{max}}{1+\frac{time^{b}}{t_{50}}} \tag{1}$$

where, G_{max} is maximum germination, t_{50} is the time that is required for 50 % of seeds (as a proportion of G_{max}) to germinate and b represents the slope of the equation at t_{50}. This equation assumes that the lower limit of germination is 0 (Lewandrowski, Erickson et al. 2017). First, for each species a common convergent curve was fitted to the number of germinants proportional to the number of incubated seeds for all hydrothermal regimes. Next the data were grouped by hydrothermal stress regime and produced multiple permutations of the primary model by fitting distinct values to the parameters of the equation. The logistic function categorized in unique stress categories was used to estimate t_{50} and G_{max} for each replicate of seeds exposed to each hydrothermal stress regime.

3.4.2 Hydrothermal tolerance

Thermal performance of organisms generally conform to the established principals of chemical kinetics, and thus can be characterized by a unimodal asymmetric curve (Angilletta Jr 2006). The thermal performance function which has been described by Yan and Hunt (1999) to assess the thermal performance of plants is based on these principles, and therefore is appropriate to describe the thermal stress responses of seeds in terms of t_{50} (Rajapakshe, Turner et al. 2020);

$$r_{max} = R_{max} \left(\frac{T_{max}-T}{T_{\max} - T_{opt}}\right)\left(\frac{T}{T_{opt}}\right)^{\frac{T_{opt}}{T_{max}- T_{opt}}} \tag{2}$$

where r_{max} $(1/t_{50})$ is the maximum germination rate at a given temperature (T), T_{opt} is the optimum temperature for germination where germination is at its most rapid, T_{max} is the upper limit of thermal stress tolerance, at which germination ceases and R_{max} is the upper limit of the germination response curve at T_{opt}.

In terms of G_{max}, germination response to temperature should begin at the lowest temperature that is conductive to metabolic reactions of seed germination, increase with rising temperature up to a point, and decrease with escalating heat stress, until germination ceases at T_{max} due to thermal denaturation of enzymes (Peterson, Daniel et al. 2007, Rosental, Nonogaki et al. 2014, Singh, Singh et al. 2019). Thus, a unimodal symmetrical quadratic function was selected to characterize the germination response of seeds in terms of (G_{max}) to thermal stress;

$$G_{max} = hT^2 + iT + j \qquad (3)$$

where (G_{max}) represents maximum germination, T is any temperature, h and i are numerical coefficients and j is the Y-intercept.

In the same way that thermal stress impedes germination, water stress is also detrimental to the seed germination process (Kaya, Okçu et al. 2006, Muscolo, Sidari et al. 2014, Turner, Lewandrowski et al. 2018). Water stress in the external environment reduces water availability which is essential to initiate the metabolic reactions of seed germination (Huang, Ran et al. 2020). Increasing water stress in the external environment of a seed causes a logistic decrease in G_{max} (Mesgaran, Onofri et al. 2017, Turner, Lewandrowski et al. 2018, Duncan, Schultz et al. 2019).

On the basis of the above principles, a hydrothermal model that synchronously defines the two dimensional hydrothermal germination niche of a given species, that is characterized by thermal stress and water stress was constructed;

$$G_{max} = \frac{p}{1+e^{\{q\times[log(w)-log(t50)]\}}} \qquad (4)$$

where G_{max} is the predicted maximum germination for a test species at any hydrothermal regime, p is maximum germination at any hydrothermal regime as estimated by Eqn. 3 (by fitting Eqn. 3 to the final germination percentages of a species at a given water stress regime at different temperatures), q is a germination constant, w is the water stress level and t_{50} is time required for 50 % of the seeds at a specific hydrothermal regime (as estimated by 1/Eqn. 2; Fig. 3.2).

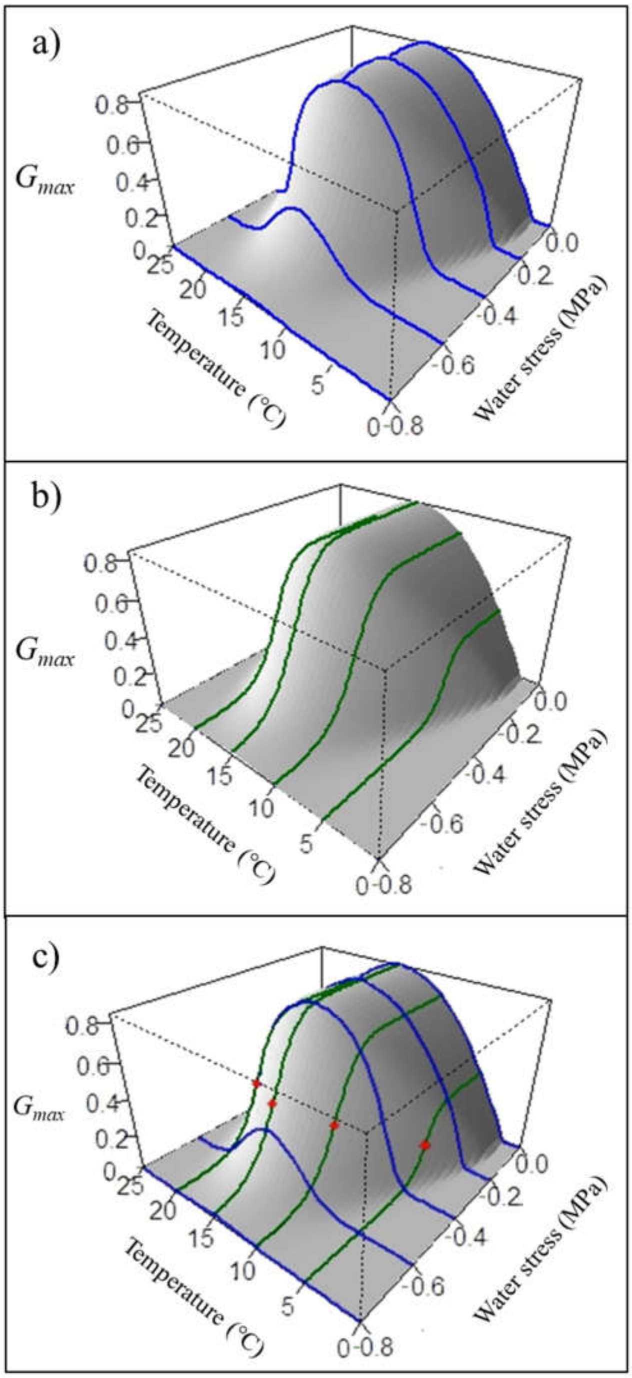


Fig. 3.2. Schematic representation of the hydrothermal germination model described by Eqn. 4; **a)** Blue lines depict the parameter p of Eqn. 4; G_{max} estimates predicted by fitting a quadratic function (Eqn. 3) to the final germination percentages of a taxon at 0.0, -0.2, -0.4, -0.6 and -0.8 MPa across a thermal spectrum of 0 – 25 °C. **b)** Green lines depict the logistic function fitted to the final germination percentages of the taxon at 5, 10, 15, 20 and 25 °C across a water stress spectrum of -0.8 – 0 MPa. **c)** Red dots depict estimates for G_{max} as predicted by Eqn. 4 at 5, 10, 15, 20 °C and -0.30, -0.50, -0.57, -0.48 MPa respectively.

3.4.3 Unique parametrisation

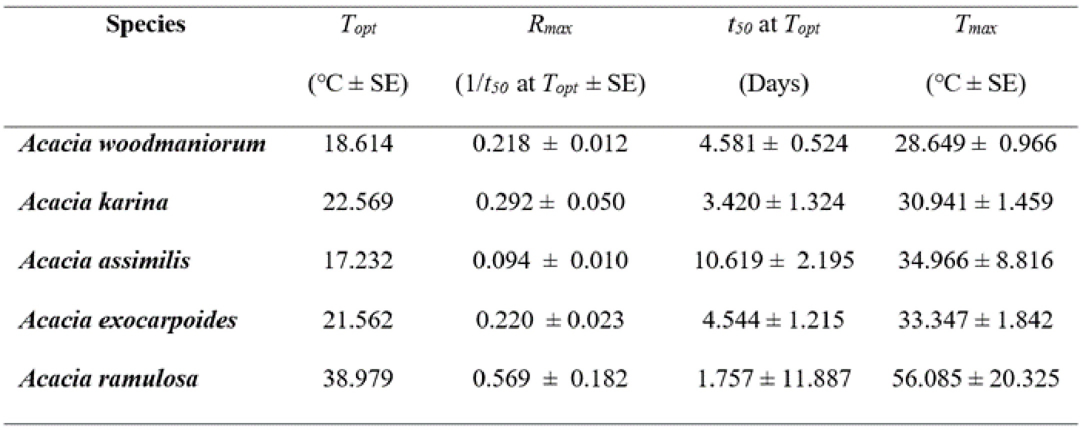
The *nls* function was used to fit distinct values to the parameters of Eqn. 2 and 3 to parametrise unique R_{max}, T_{opt}, T_{max}, and G_{max} estimates across a wide range of temperatures at each different water stress regime (Ritz and Streibig 2008). In order to construct a hydrothermal germination niche for each test species, Eqn. 4 was fitted to the observed final germination percentage values (p). The hydrothermal germination niche for each species was constructed in an *R* software environment using the packages *ggplot* and *Lattice* (Wickham and Wickham 2007, Sarkar 2008).

3.5 Results

3.5.1 Germination modelling

In all test species the response of r_{max} ($1/t_{50}$) to temperature at each water stress level was a unimodal asymmetric hump-shaped curve (Fig. 3.3a). In the absence of water stress, time to reach 50 % germination (t_{50}) at T_{opt} was longest in *A. assimilis* (10.6 days, Table. 3.1), whereas the estimate for t_{50} at T_{opt} was shortest in *A. ramulosa* (1.8 days). In *A. exocarpoides*, *A. karina and A. woodmaniorum* estimates for t_{50} at T_{opt} and 0 MPa were 4.6, 3.4 and 4.6 days respectively (Table. 3.1). Increasing water stress decreased the breadth of the thermal performance niche of r_{max} in all taxa (Fig. 3.3a). In the absence of water stress the T_{opt} estimates for all test species except *A.ramulosa* were between 17 - 23 °C (Fig. 3.3a, Table. 3.1). The T_{opt} estimate for *A. ramulosa* at 0 MPa was higher than that of any other species (39.0 ± 11.9 °C; Fig. 3.3a, Table. 3.1).

Table. 3.1. Coefficients for the thermal performance function (Eqn. 2; (± 1 SE) of effects of thermal stress on time to reach 50 % germination (t_{50}) at 0 MPa (absence of water stress) for the five *Acacia* species, where T_{opt} is the optimum incubation temperature for germination, R_{max} is the upper limit of germination response curve (in terms of r_{max}) at T_{opt} and T_{max} is the highest temperature at which germination was observed.



Species	T_{opt} (°C ± SE)	R_{max} ($1/t_{50}$ at T_{opt} ± SE)	t_{50} at T_{opt} (Days)	T_{max} (°C ± SE)
Acacia woodmaniorum	18.614	0.218 ± 0.012	4.581 ± 0.524	28.649 ± 0.966
Acacia karina	22.569	0.292 ± 0.050	3.420 ± 1.324	30.941 ± 1.459
Acacia assimilis	17.232	0.094 ± 0.010	10.619 ± 2.195	34.966 ± 8.816
Acacia exocarpoides	21.562	0.220 ± 0.023	4.544 ± 1.215	33.347 ± 1.842
Acacia ramulosa	38.979	0.569 ± 0.182	1.757 ± 11.887	56.085 ± 20.325

At 0 MPa the estimated T_{max} of *A. ramulosa* was 56.1 ± 20.3 °C indicating a higher level of thermal stress tolerance compared to the other four species (Fig. 3.3a, Table. 3.1). In contrast, T_{max} at 0 MPa was the lowest in *A. woodmaniorum* (28.6 ± 1.0 °C) whereas, in *A. assimilis, A. exocarpoides* and *A. karina* T_{max} values at 0 MPa were between 30 – 35 °C (Fig. 3.3a, Table. 3.1). The thermal performance function (Eqn. 2) could not be fitted to the three widely distributed taxa at -0.6 MPa due to low germination percentages at those treatment regimes (Fig. 3.3a).

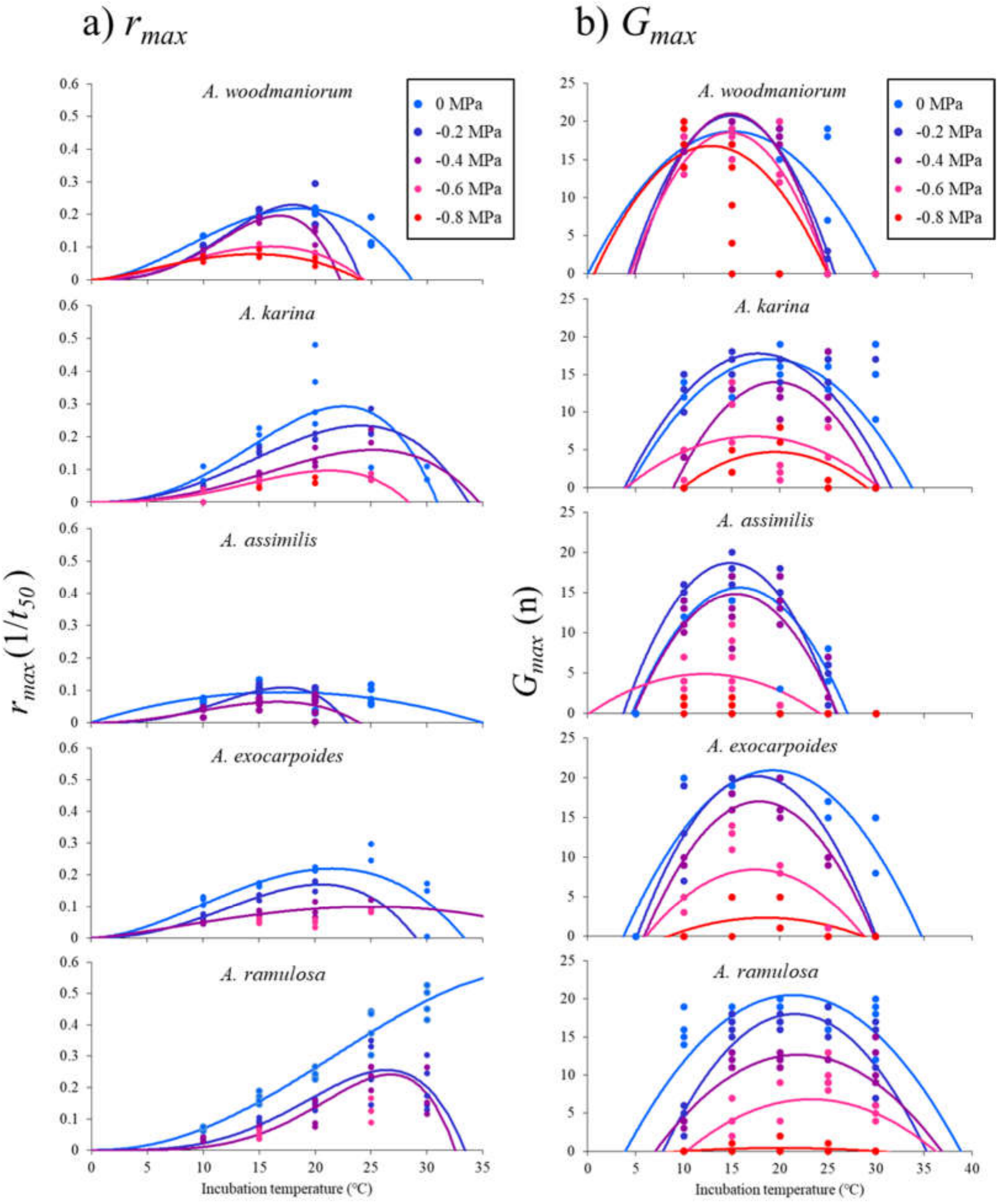


Fig. 3.3. Hydrothermal tolerance in five Western Australian *Acacia* species in terms of time to reach 50 % germination (t_{50}) and maximum germination (G_{max}); **a)** Dots represent $1/t_{50}$ (r_{max}) estimates for each replicate of seeds following exposure to different hydrothermal regimes (as predicted by Eqn. 1), and the smooth lines depict the thermal performance function (Eqn. 2) fitted to the $1/t_{50}$ estimates (r_{max}) of each taxa. **b)** Dots represent G_{max} estimates for each replicate of seeds after exposure to different hydrothermal regimes (as predicted by Eqn. 1), and the smooth lines depict the quadratic model (Eqn. 3) fits for the G_{max} estimates of each species. Three to five replicates of 20 seeds were used for each treatment.

Germination response of G_{max} was a unimodal symmetrical hump shaped curve in all species (Fig. 3.3b). Within favourable temperature ranges for germination and in the absence of water stress estimates for G_{max} were > 80 % in all species after 28 days of incubation (Fig. 3.3b). Estimates for G_{max} in *A. woodmaniorum* were relatively high (> 80 %) and consistent throughout a broad range of water stress regimes (0 - 0.8 MPa) within favourable thermal conditions (~ 10 - 16 °C; Fig. 3.3b). Conversely, in all other test species G_{max} at all thermal regimes declined to < 50 % at -0.8 MPa (Fig. 3.3b). Eqn. 3 could not be fitted to germination responses of *A. assimilis* at -0.8 MPa due to low final germination percentage values (Fig. 3.3b). When seed were not subjected to water stress, a temperature range of ~ 15 - 20 °C was conductive to germination in all taxa (Fig. 3.3b). In the absence of water stress, A. *ramulosa* exhibited the broadest thermal tolerance range (~ 4 - 40 °C), whereas the narrowest thermal tolerance range was observed in *A. assimilis* (~ 5 - 27 °C, Fig. 3.3b). Increasing water stress constrained the breadth of the thermal performance niche in terms of G_{max}. According to the model predictions, the thermal response ranges of *A. assimilis* and *A. karina* were broader at -0.6 MPa compared to that of -0.4 MPa (Fig. 3.3b). Similarly, the thermal response range of *A. woodmaniorum* was broader at -0.8 MPa than that of -0.6 MPa (Fig. 3.3b). Furthermore, estimates of G_{max} for *A. assimilis* at -0.2 MPa were higher than that at 0 MPa within a range of ~ 4 – 25 °C (Fig. 3.3b). These observations could be due to errors in the model fitting process caused by low final germination percentages and high variability in germination response observed at high hydrothermal stress regimes.

3.5.2 Hydrothermal tolerance

In terms of G_{max}, germination response to temperature at any water stress level was a unimodal symmetric and hump shaped curve in all species (Fig. 3.4). At a given temperature G_{max} decreased logistically with increasing water stress (Fig. 3.4). Under favourable thermal conditions and in the absence of water stress, germination response of all taxa exceeded 80 % (Fig. 3.4). In all species increasing water stress decreased the breadth of the thermal performance niche (Fig. 3.4). Similarly, deviation from favourable thermal regimes constrained the breadth of the hydrological performance niche (Fig. 3.4). The hydrothermal germination niche of each species was unique (Fig. 3.4). The hydrothermal germination niches of the two range-restricted taxa were broad and generally comparable to those of *A. exocarpoides* and *A. ramulosa* (Fig. 3.4). *Acacia assimilis* was less tolerant of hydrothermal stress than any other test species (Fig. 3.4). In all test species except *A. exocarpoides* G_{max} declined to < 50 % at -0.6 MPa at all thermal regimes (Fig. 3.4). When exposed to low water stress conditions (0 to -0.4 MPa) the broadest thermal tolerance range was observed in *A. karina*, whereas under favourable thermal conditions *A. exocarpoides* was more tolerant of water stress than any other species (Fig. 3.4).

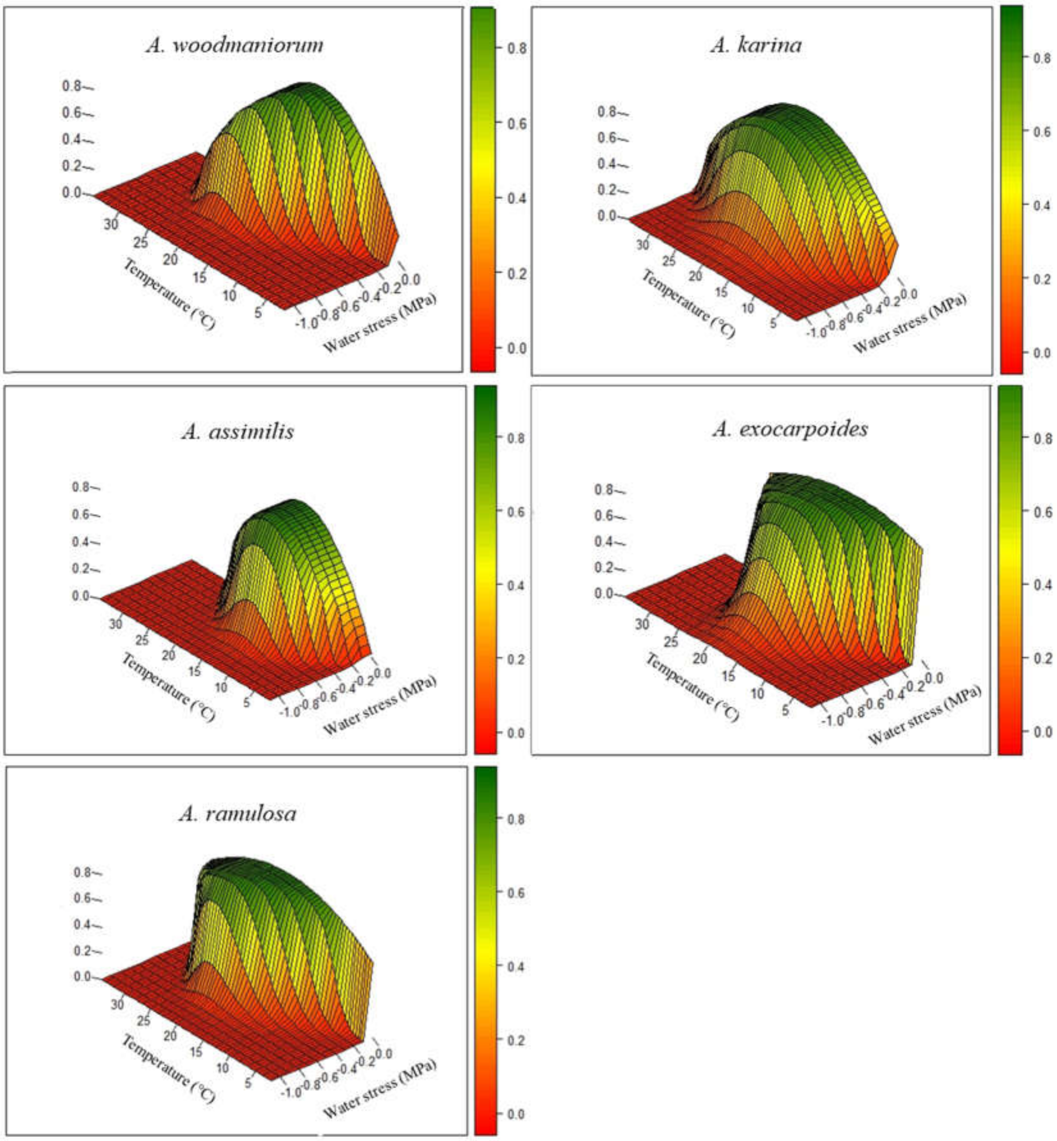


Fig. 3.4. Hydrothermal germination niche for five Western Australian *Acacia* species in terms of maximum germination (G_{max}) estimated by a hydrothermal germination function following incubation of seeds in constant darkness at different hydrothermal regimes for 28 days. The two horizontal axes represent incubation temperature (°C) and water stress level (MPa). The vertical axis represents G_{max} (as a proportion) as estimated by Eqn. 4. Three to five replicates of 20 seeds were used for each treatment regime.

3.6 Discussion

The expectation based on niche theory was that range-restricted species should be defined by a narrower niche than broadly distributed species, but in the case of two-dimensional hydrothermal germination niches, this was not the case. In terms of t_{50}

and G_{max} the two range-restricted taxa were more tolerant of hydrothermal stress compared to the three widely distributed congenerics. Broadly speaking, the results of this study conform with previous controlled experimental findings of seed germination in response to univariate examination of thermal or hydrological stressors (Bell, Rokich et al. 1995, Cochrane 2018, Turner, Lewandrowski et al. 2018). Yet despite the general support for the mechanistic hypotheses underpinning this series of experiments, the interaction between hydrological and thermal drivers of the germination niche are complex, and do not conform to the initial expectations dependent upon niche theory. Consequently, given the impact of these results this has substantial ecological and management implications.

3.6.1 Hydrothermal tolerance

The high seed viability percentages observed in the test species are comparable to those of many *Acacia* species native to Western Australia (Bell, Rokich et al. 1995). The results of this study are in line with most previous studies reporting high final germination percentages of *Acacia* seeds generally reached within 30 days when incubated under favourable hydrothermal conditions following dormancy alleviation (Bell 1994, Reichman, Bellairs et al. 2006, Burrows, Virgona et al. 2009, Cochrane 2020). The high final germination percentages observed in all test species within favourable temperatures in the absence of water stress strongly suggest that seed germination is related to metabolic processes that are optimized when the hydrothermal conditions in the external environment are conductive to successful establishment of seedlings (Huang, Ran et al. 2020). However, in natural environments successful establishment of seedlings is dependent upon the duration of the window of opportunity for germination (Elliott, Lewandrowski et al. 2019). Selection pressure in terms of hydrothermal stress constrains the length of that window of opportunity (Duncan, Schultz et al. 2019).

The findings of this study support the hypothesis that the speed of germination; r_{max} ($1/\,t_{50}$) should follow a unimodal asymmetric hump-shaped curve similar to that of all enzyme mediated reactions (Fig. 3.3a) and, therefore is in accordance with the established principles of thermal biology (Rajapakshe, Turner et al. 2020). In addition, the results of this study support the hypothesis that maximum germination response of seeds (G_{max}) to temperature can be characterized by a quadratic function (Fig. 3.3b, Fig. 3.4), in accordance with previous studies (Singh, Singh et al. 2019). Furthermore, the experimental data are in line with previous research that suggest that at a given temperature, estimates for G_{max} should decrease logistically with increasing water stress (Lewandrowski, Erickson et al. 2017, Turner, Lewandrowski et al. 2018). Increasing water stress constrained the breadth of the thermal performance niche, and suboptimal thermal regimes constrained the breadth of the hydrological performance niche (Fig. 3.4). These observations suggest that thermal stress and water stress are inter-dependent niche dimensions, and they interact to produce a detrimental effect on germination in terms of both r_{max} and G_{max} (Fig. 3.4). The findings of this study suggest that not all niche dimensions can be assumed to be independent, and demonstrates the significance of quantifying the complex interactions between niche axes and their effect on species performance in order to gain a comprehensive understanding of population dynamics (Huang, Zhou et al. 2020).

The findings of this research project suggest that prediction of hydrothermal germination niche by fitting final germination percentages to Eqn. 4 may be an efficient method to quantify and compare stress tolerance thresholds of taxa (Fig. 3.4). However, a major limitation of this method is that time is not incorporated to Eqn. 4

as a parameter beyond the coarse thermal performance estimates of t_{50}, as it is in other, more complex hydrothermal models (Onofri, Benincasa et al. 2018). Nevertheless, simpler models have facilitated the examination of patterns of ecology and evolution in wild species, whereas more complex models have mostly been applied to less phylogenetically variable domestic cultivars that are often highly inbred so possess a high level of germination uniformity (Onofri, Benincasa et al. 2018). Comparing model performance on variable wild species is clearly a research priority.

3.6.2 Patterns of species range and endemism

According to the estimates of G_{max}, *Acacia assimilis* was less tolerant of hydrothermal stress compared to the other four test species (Fig. 3.4). This species inhabits the relatively deep skeletal soils on low-lying areas such as woodlands (Western Australian Herbarium 1998), rocky plains and sand slopes extending from the more arid region in the north east of its range to relatively more cooler mesic environments along parts of the southern coast of Western Australia (Meissner and Caruso 2008). Estimated t_{50} for *Acacia assimilis* at 0 MPa is similar to those of other *Acacia* species native to southwest Western Australia (Cochrane 2020). At 0 MPa, thermal performance of *A. assimilis* in terms of r_{max} and G_{max} is comparable to that of other widespread species native to southwest Western Australia (Rajapakshe, Turner et al. 2020). The results of this study suggest that all test species except *A. ramulosa* are adapted to synchronise germination across the austral winter and spring, a strategy that is common among *Acacia* species as well as many other species native to this region (Bell, Plummer et al. 1993, Bell, Rokich et al. 1995), where high soil moisture levels persist in deep-soil environments for an extended period of time during the cooler months of the year ensuring successful establishment of seedlings (Yates, Hobbs et al. 2000, Merritt, Turner et al. 2007). This observation is in line with previous studies that suggest that flora native to Mediterranean climatic regions generally follow a strategy of summer drought avoidance (Luna, Pérez et al. 2012, Clemente, Müller et al. 2017).

In all test species except *A. assimilis* the germination speed (in terms of t_{50}) was higher at 0 MPa than those of many species native to southwest Western Australia (Yates, Hobbs et al. 2000, Cochrane 2018, Cochrane 2020), and rapid germination persisted for these four species across broad thermal tolerance ranges (Fig. 3.3a). The two range-restricted taxa live on shallow stony metamorphic soils of BIF habitats in the arid to semi-arid Yalgoo region of mid-west Western Australia (Meissner and Caruso 2008). In contrast, the distribution range of *A. exocarpoides* and *A. ramulosa* extend from Yalgoo to the more arid regions of Western Australia such as Murchison and Great Victoria Desert (Western Australia Herbarium 1998, Markey 2008). These arid regions are characterised by high summer temperatures, and annual evaporation rates that significantly exceed the irregular annual rainfall (Markey 2008, Markey and Dillon 2011), such that the window of opportunity for successful establishment of seedlings is often short, unpredictable and may occur when temperatures are high. High thermal performance in terms of G_{max} and r_{max} at low water stress regimes suggest that *A. exocarpoides*, *A. ramulosa* and the two range-restricted taxa are adapted to maximize recruitment success within shorter periods of high soil moisture availability following sporadic rainfall events (Debat and David 2001). These observations are in line with previous studies that have reported species living in water-stressed habitats germinate rapidly whenever soil moisture availability is high, even if the thermal environment is suboptimal (Mott 1974, Jurado and Westoby 1992, Rajapakshe, Turner et al. 2020).

In terms of r_{max} and G_{max}, the two range-restricted taxa were more tolerant of hydrothermal stress compared to *A. exocarpoides* and *A. ramulosa* (Fig. 3.4). *Acacia* *woodmaniorum* generally inhabits the ridgetops of BIF outcrops (Byrne 2019), but this species has also been recently reported from disturbed overburden of mine sites (Western Australian Herbarium 1998). *Acacia karina* lives on both BIFs and the surrounding relatively deep soil slopes and plains (Byrne 2019). The results of this study suggest that higher levels of hydrothermal tolerance in terms of r_{max} and G_{max} may have preadapted these species to persistence in the relatively harsh microclimates of BIF outcrops (uncontested niche space), since these habitats are regularly subjected to higher, levels of hydrothermal stress compared to the surrounding deep-soil environments inhabited by *A. exocarpoides* and *A. ramulosa* (Hopper 2009). The general concordance between the occurrence niches and the hydrothermal performance niche of seeds of the study species highlight the significance of the germination niche in shaping and filtering the overall niche structure of plants, in accordance with previous studies (Cochrane, Hoyle et al. 2014, Marques, Atman et al. 2014). The findings of this study are also in agreement with past studies that have reported that the overall niche structure of a species is mostly determined by species performance rates that influence population growth rate to a large extent, such as r_{max} and G_{max} (Butterfield and Briggs 2011, Pironon, Villellas et al. 2018). The link between the distribution range of the study species and hydrothermal performance of their seeds highlights the role of hydrothermal stress shaping the germination niche of taxa in Mediterranean ecosystems, in accordance with the findings of past research (Cochrane, Hoyle et al. 2014, Krichen, Vilagrosa et al. 2017, Zogas, Kosman et al. 2020). The breadth of the hydrothermal germination niche in the range-restricted species is not consistent with niche theory, which generally predicts that such species should have specialist, narrow niches (Hutchinson 1978, Elith and Leathwick 2009). It was assumed that the germination niche of the species would be strongly indicative of the biogeographical patterns because germination is an important demographic filter in plants (Fernández-Pascual, Pérez-Arcoiza et al. 2017, Del Vecchio, Fantinato et al. 2020). However, the hydrothermal stresses imposed on germination in these habitats actually select for a broad tolerance, suggesting that there must be other filters post-germination that substantially narrow and define the niche of the range restricted species. Quantifying the impact of various environmental dimensions on the vital performance rates of *A. karina* and *A. woodmaniorum* during other life stages may provide important insights into the niche shifts that may have caused these two species to become range-restricted (Pironon, Villellas et al. 2018).

3.6.3 Limitations to interpretation

The research method utilized in this study can be employed to characterize the dynamic hydrothermal germination niche in other threatened plant species (Turner, Lewandrowski et al. 2018). A limitation of this method is that it predicts the hydrothermal germination niche of taxa only on the basis of final germination percentage (p) and therefore time is not incorporated to the equation (Onofri, Benincasa et al. 2018). However previous hydrothermal time models simplify the performance functions in ways that overlook important parameters such as T_{opt}. (Bradford 2002, Onofri, Benincasa et al. 2018). The results of this study are generally consistent with the findings of previous studies on many other taxa (Bell, Rokich et al. 1995, Elliott, Lewandrowski et al. 2019, Cochrane 2020). Hydrothermal performance rates of seeds of the selected taxa were only compared at species level for this study. Vital performance rates of taxa may vary among populations depending on genetic and environmental factors (Sexton, McIntyre et al. 2009, Tudela-Isanta, Ladouceur et al. 2018). The modelling approach utilized in this study can potentially be used to compare rates of hydrothermal performance at population level. The above hypotheses should be tested at population level and a broader range of species should be compared in order to get more insights in to how hydrothermal stress on germination response affects patterns of rarity and endemism (Byrne 2019, Funnekotter, Millar et al. 2019). The impact other environmental variables (e.g. salinity stress, soil acidity) on critical performance rates of the seed germination niche should also be investigated (Seal, Barwell et al. 2018, Tudela-Isanta, Ladouceur et al. 2018).

Since the study species are physically dormant, seed dormancy was readily overcome by simple mechanical means, but dormancy also has a critical role in synchronising germination in many species with optimal soil conditions that promote PY dormancy loss that is not reflected by the methods utilized in this study i.e. hot water and acid treatment (Bell, Plummer et al. 1993). Under natural conditions, dormancy alleviation of PY species generally occurs following brief exposure to high temperatures associated with seasonal fires, soil disturbance or extreme summer temperatures (Bell, Plummer et al. 1993, Bell, Rokich et al. 1995). Nevertheless, unlike many physiologically dormant seeds which can gradually cycle in and out of dormancy in response to changing soil conditions driven in large part by shifts in temperature and moisture, the loss of PY is largely irreversible, thus seeds will readily germinate once provided with suitable conditions. Consequently, for species that exhibit physiological seed dormancy, hydrothermal thresholds for dormancy alleviation and germination may be more dynamic and complex, and therefore likely to be more challenging to characterize (Baskin and Baskin 2003).

3.6.4 Conclusions

The results of this experiment suggest that thermal and hydrological performance of the seeds of physically dormant species adhere to established mechanistic models. This study demonstrates that thermal stress and water stress are inter-dependent environmental dimensions that interact to produce a negative effect on the germination niche of seeds in terms of both the time required to reach 50 % germination (t_{50}) and maximum germination (G_{max}). The experimental data do not conform to a simple interpretation of niche theory where specialist taxa have a narrowly defined niche in which they outperform generalist species. Instead, the hydrothermal stresses of these specialised habitats select for a broad germination niche that facilitates rapid germination under a range of conditions, and it can be speculated that the two range restricted species outperform more widespread species and gain access to uncontested niche space. In other habitats it can be speculated that that the two range restricted species must be outcompeted by some other aspects of demographic filtering or niche partitioning, but this remains untested. Reaching this conclusion was only possible by integrating hydrological and thermal stressors to characterise the germination niche, demonstrating that not all environmental dimensions can be assumed to be independent. Quantifying complex interactions between niche axes as well as their impact on species performance is essential to gain a comprehensive understanding of population dynamics.

Chapter 4

The impact of hydrothermal stress on germination; a study on three endangered *Tetratheca* species restricted to Banded Ironstone Formations of Western Australia

4.1 Abstract

In semi-arid ecosystems, water availability and variable and extreme temperatures constitute major selection pressures on seedling recruitment. Seeds are adapted to synchronize germination with optimal environmental conditions through specific tolerance thresholds of thermal and hydrological stress, which maximize the probability of seedling survival. The extent to which hydrothermal stress affects germination in species with complex dormancy traits and its role in shaping the patterns of rarity and endemism in semi-arid ecosystems, are poorly understood. To explore the interplay between germinating seeds and their environment we studied the impact of thermal and hydrological stress (10 – 25 °C and -1.2 – 0 MPa) on germination performance by assessing germination speed quantified through time to 50 % germination (t_{50}), and on maximum germination (G_{max}) in three threatened physiologically dormant species (*Tetratheca erubescens, T. harperi* and *T. paynterae* subsp. *paynterae*) that are restricted to Banded Ironstone Formations of Western Australia. All test species were slow to germinate ($t_{50} < 18$ days). Complex interactions between thermal stress and water stress were identified, highlighting the importance of quantifying interactions between niche axes and their implications for species performance. Their hydrothermal niches were narrow and constrained to cooler

temperatures that reflect the hydrothermal conditions in the soil during their germination period in the field. These observations suggest that *Tetratheca* species endemic to outcrop habitats are likely to be susceptible to effects of climate change. The findings of this research suggest that the study species are likely to be allopatric, and they may have been broadly distributed in the wider geographic region in the past under wetter and cooler conditions.

4.2 Introduction

The fundamental niche of a species refers to the total set of conditions under which it can potentially survive and reproduce, whereas the occurrence niche, or the realised niche, refers to the conditions under which that species currently exists (Hutchinson 1978, Elith and Leathwick 2009). The fundamental niche of a species is rarely fully realised, and therefore the occurrence niche of a species is usually a subset of its fundamental niche (Pironon, Villellas et al. 2018). While the fundamental niche is theoretically characterised by a number of different parameters (Hutchinson 1978), it can be estimated in a small number of key axes by measuring species performance (e.g. metabolic rate, locomotory capacity or germination rate) across various environmental gradients (Tomlinson, Dixon et al. 2015, Onofri, Benincasa et al. 2018). In order to gain a comprehensive understanding of population dynamics, it is important to assess the impact of different environmental gradients on species performance for all stages of the life cycle (Warren, Wright et al. 2011, Merow, Latimer et al. 2014). However, the relative contribution of different species performance niches to overall population growth rate may be variable (de Kroon, Plaisier et al. 1986, Pironon, Villellas et al. 2018). Therefore, overall population growth rate is primarily determined by the performance niches of several critically important demographic stages of the life cycle (Bykova, Chuine et al. 2012, Liu, Chang et al. 2018). Investigating the link between the occurrence niches and the critical performance niches of taxa could reveal important insights to population dynamics of rare and range-restricted species (Pironon, Villellas et al. 2018).

Seeds germination is a critical stage of the life cycle of plants during which they are most susceptible to environmental stressors (Baskin and Baskin 2003). Therefore, the germination niche represents one of the main components of the overall niche of plants (Donohue, Rubio de Casas et al. 2010, Fernández-Pascual, Pérez-Arcoiza et al. 2017, Del Vecchio, Fantinato et al. 2020). Several studies have highlighted the necessity of assessing the role of different environmental variables on the germination response of range-restricted taxa and the implications for their distributional extent (Vincent, Barrett et al. 2015, Jiménez-Alfaro, Silveira et al. 2016, Cochrane 2018, Cochrane 2020).

Thermal stress and water stress are the two major environmental gradients that determine the success of seed germination in water-limited ecosystems (Cochrane, Hoyle et al. 2014, Krichen, Vilagrosa et al. 2017, Zogas, Kosman et al. 2020). Since seed germination is underpinned by metabolic processes that are mediated by enzymes (Huang, Ran et al. 2020), the germination performance of seeds across a thermal gradient should resemble a unimodal asymmetric hump-shaped curve characteristic of all enzyme mediated reactions (Yan and Hunt 1999, Angilletta Jr 2006, Tomlinson, Dixon et al. 2015). Increasing temperature should increase the thermal performance of seeds up to a critical threshold, beyond which performance should decrease rapidly due to enzyme denaturation (Tomlinson, Dixon et al. 2015). Water stress is also detrimental to seed germination because water stress in the external environment impedes the metabolic reactions of seed germination (Kaya, Okçu et al. 2006, Muscolo, Sidari et al. 2014, Turner, Lewandrowski et al. 2018). Therefore, increasing water stress in the external environment of a seed should cause an exponential increase in time to reach 50 % germination (t_{50}; Bradford 2002, Cochrane 2018, Rajapakshe, Turner et al. 2020) and a logistic decrease in maximum germination (G_{max}; Lewandrowski, Erickson et al. 2017, Turner, Lewandrowski et al. 2018, Rajapakshe, Turner et al. 2020).

Theoretically, both water stress and thermal stress interact to define a multivariate space within which seeds can germinate (Bradford 2002, Onofri, Benincasa et al. 2018). Such hydrothermal stress tolerance thresholds of seeds are determined by both genetic factors and ecological variables (Giménez-Benavides, Escudero et al. 2005, Giménez-Benavides and Milla 2013, Murru, Santo et al. 2017). There is a general shortage of studies that have assessed thermal stress and water stress responses of seeds in an established theoretical context, and accounted for potential interactions between these two niche axes (Hardegree, Roundy et al. 2018, Frischie, Fernández-Pascual et al. 2019, Huang, Zhou et al. 2020). The concept of 'hydrothermal germination niche' is well-established in the literature (Hardegree, Roundy et al. 2018, Onofri, Benincasa et al. 2018), but studies that have incorporated these principles are scarce in the context of restoration (Larson, Sheley et al. 2015, Huang, Liu et al. 2016, Turner, Lewandrowski et al. 2018). Quantifying hydrothermal germination responses of seeds on a sound theoretical basis may provide important insights into the roles of these environmental variables in structuring populations and shaping patterns of rarity and endemism (Clemente, Müller et al. 2017, Duncan, Schultz et al. 2019, Rajapakshe, Turner et al. 2020).

Rocky outcrop habitats such as Banded Ironstone Formations (BIFs) and granite outcrops, host geographically isolated, unique, shallow-soil plant communities in "islands" surrounded by a matrix of contrasting deep soil ecosystems (Byrne and

Hopper 2008, Porembski and Barthlott 2012, Do Carmo and Jacobi 2016). Rocky outcrops harbour a significant component of regional biodiversity, but the environmental variables that have shaped these patterns of plant diversity are not well understood (Gibson, Yates et al. 2010, Poot, Hopper et al. 2012, Tapper, Byrne et al. 2014, Di Virgilio, Wardell-Johnson et al. 2018). Banded Ironstone Formations are widely distributed in the arid to semi-arid Yilgarn region of Western Australia (Gibson, Meissner et al. 2012), and are generally considered to impose greater water stress on plants compared to the low-lying deep-soil habitats surrounding these outcrops (Elliott, Lewandrowski et al. 2019). However, BIFs are also characterised by highly heterogenous habitats such as fissures, gullies and slopes that can store water for long periods following rainfall (Yates, Gibson et al. 2011). Research quantifying the effects of various environmental gradients on distribution patterns as well as the soil germination environment on BIF habitats is scarce (Yates, Gibson et al. 2011, Di Virgilio, Wardell-Johnson et al. 2018).

The seeds of range-restricted taxa living on shallow-soil habitats such as outcrop surfaces tend to have broader hydrothermal performance niches compared to their generalist competitors (Rajapakshe, Turner et al. 2020). Consequently, they have adapted to germinate rapidly when soil moisture content increases for short periods above critical thresholds during irregular rainfall episodes (Rajapakshe, Turner et al. 2020). However, there is a shortage of data on how closely related short-range endemic species vary in their adaptation to hydrothermal stress, especially in the Western Australian context, where non-overlapping distributions can be quite close together though are separated by different landforms and vegetation communities (Gibson et al. 2010; Yates et al. 2011).

Germination performance is, however, also moderated by seed dormancy regulation, which synchronizes germination with the window of opportunity that is optimum for seedling establishment (Baskin and Baskin 2003). BIF endemic taxa are quite diverse in terms of their seed dormancy traits and germination requirements (Elliott, Lewandrowski et al. 2019, Miller, Symons et al. 2019) and there is very little data reporting how hydrothermal stress affects the germination response of range-restricted BIF endemics with complex seed dormancy traits. One of the most complex patterns of seed dormancy is physiological dormancy (PD), which is characterized by a physiological block within the embryo that prevents radicle emergence until dormancy is overcome (Baskin and Baskin 2004). Thermal stress, water stress and exposure to smoke have been identified as abiotic factors that influence dormancy regulation and the germination response of several physiologically dormant species restricted to BIF outcrops of Western Australia (Turner, Lewandrowski et al. 2018, Elliott, Lewandrowski et al. 2019). This raises interesting questions around whether the presence of complex seed dormancy can optimise germination to such specific conditions that the performance of the germination process is not heavily selected, and that interspecific variation in the germination performance niche might be minimal. The major objective of this study was to compare the germination responses of four closely related, but distributionally isolated, physiologically dormant BIF specialist taxa to address the following research questions;

1. Does the hydrothermal germination response of physiologically dormant seeds adhere to established mechanistic principles?
2. Do the hydrothermal performance niches of seeds of BIF specialist taxa reflect their occurrence niches, and what are the ecological and evolutionary implications?

3. Does the hydrothermal germination response of seeds of BIF specialists conform to niche theory?

4.3 Materials and methods

4.3.1 Species selection and sourcing

The distributions of *Tetratheca* species in the semi-arid and arid regions of Western Australia represents an intriguing display of short-range endemism and diversification, where a number of unique species are confined to rocky habitats for reasons that aren't obvious (Di Virgilio, Wardell-Johnson et al. 2018, Byrne, Krauss et al. 2019). Furthermore, they very rarely inhabit more than one outcrop, and neighbouring outcrops often support unique species of *Tetratheca* that have apparently been separated for substantial periods of time (Butcher, Byrne et al. 2007). I used four species of *Tetratheca* (Elaeocarpaceae) that are geographically restricted to BIF habitats in the Yilgarn region of Western Australia in this study (Fig. 4.1, Appendix. 3.1). The distribution of each species is restricted to one of four adjacent BIF ranges (Fig. 4.1) that are approximately within 100 km of each other (Butcher, Byrne et al. 2007, Gibson, Yates et al. 2010). *Tetratheca aphylla* F.Muell. subsp. *aphylla* inhabits the relatively deep skeletal soils of lower slopes, hill crests and cliffs of the Helena and Aurora Range (Yates, Gibson et al. 2011). *Tetratheca erubescens* J.P.Bull is found in rock fissures and crevices associated with cliffs, hill crests and steep slopes of the Koolyanobbing range (Krauss and Anthony 2019). *Tetratheca harperi* F.Muell. inhabits hill crests, cliffs and cliff slopes of the Mt. Jackson Range (Yates, Gibson et al. 2011). *Tetratheca paynterae* Alford subsp. *paynterae* is restricted to fissures on steep cliffs and tors of the Windarling Range (Ladd, Yates et al. 2019). All four species are of high conservation concern as they have been gazetted as Declared Rare Flora

(Western Australian Herbarium 1998). Seeds were harvested from wild populations of these species in 2019 and once collected were cleaned and processed (Appendix. 3.2).

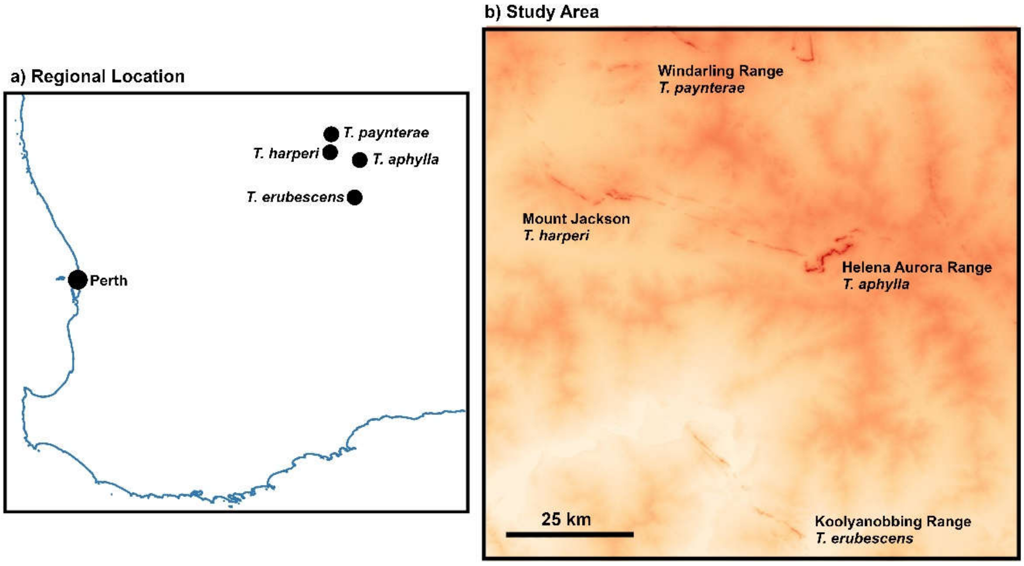


Fig. 4.1. Location of the only known populations of the four study species; a) in the arid region of Western Australia, ~ 400 km north east of Perth. b) A region defined by four unique ironstone ridges. While each of these ridges is only separated by relatively short distances (25 - 50 km), the intervening habitat is characterized by sandplains between 300 and 450 m elevation above sea level (white to yellow), while the ranges are characterized by emergent banded ironstone ranging between 500 m elevation (Koolyanobbing) to 675 m elevation (Helena Aurora), indicated in red.

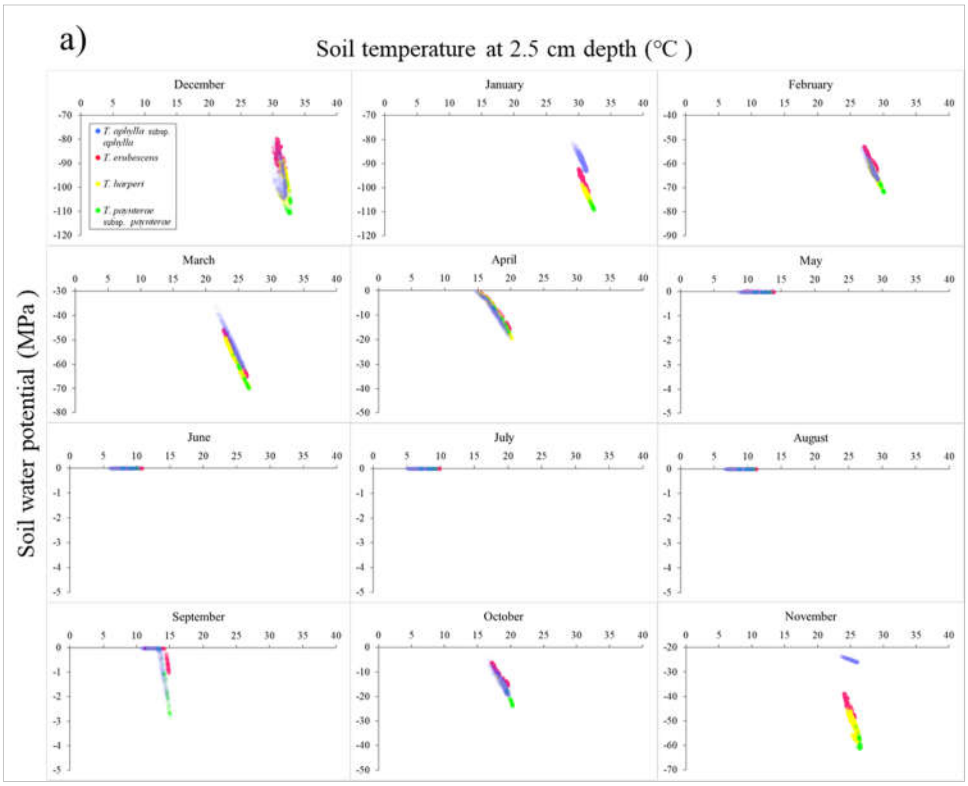
4.3.2 Seed quality

Prior to starting the experiments, seed material was stored at 15 °C and 15 % relative humidity (controlled environment room) at the Kings Park Science laboratories, Western Australia for 15 months. For each species, the percentage of seed fill was assessed by X-ray analysis of five replicates of 50 seeds (MX-20 digital X-Ray cabinet, Faxitron, USA). Seeds containing fully-formed embryos and endosperm were regarded as filled seeds and identified by uniform white shading of these tissues in the

X-Ray images. Seeds without these internal features were scored as non-viable (Erickson and Merritt 2016).

4.3.3 Mapping the hydrothermal occurrence niche

The average monthly hydrothermal occurrence niche of each study species was mapped using unpublished survey data. The known locations were intersected with spatial data describing elevation, aspect and slope, extracted from (Gallant, Wilson et al. 2011, Gallant and Austin 2012, Gallant and Austin 2012), respectively, and edaphic data describing clay, sand and silt percentage composition, extracted from (Viscarra Rossel, Chen et al. 2014, Viscarra Rossel, Chen et al. 2014, Viscarra Rossel, Chen et al. 2014). Soil depth was interpolated from national soil data provided by the Australian Collaborative Land Evaluation Program (ACLEP), endorsed through the National Committee on Soil and Terrain (NCST; www.clw.csiro.au/aclep). Incorporating all of these details to categorise the landscape into different soil types using a soil textural triangle allowed me to apply the *"micro global*" algorithm in the "NicheMapR" package (Kearney and Porter 2017) to estimate microclimatic details that are most relevant to seed germination at the precise locations where each species is known to occur, following methods described by Tomlinson, Lewandrowski et al. (2020). Daily soil temperature and water stress level at midday (11.30 a.m.) were estimated for the GPS location of each recorded plant of the four test species for 2.5 cm and 15 cm soil depth. These two soil depths were selected for the experiment because seedling establishment of the study species are generally constrained to this zone of the soil profile (Yates, Gibson et al. 2011). Estimated average monthly soil hydrothermal conditions at the location of each plant were then used to map the occurrence niches of the four taxa (Fig. 4.2). The seeds of the test species were exposed to a range of hydrothermal treatment regimes based on their occurrence niches during the rainfall season.



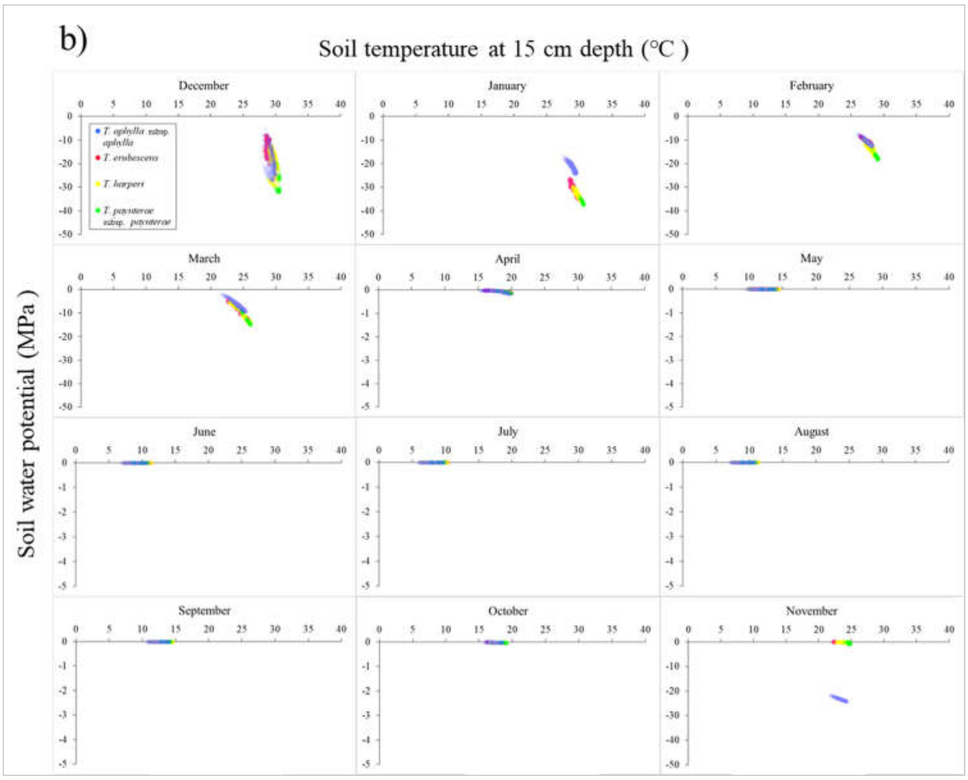


Fig. 4.2. Average monthly hydrothermal conditions of the soil environment (occurrence niche) for all recorded individuals (depicted by coloured dots) of the four *Tetratheca* species used in this study at; a) 2.5 cm soil depth and (b) 15 cm soil depth, estimated using the "*micro_global*" algorithm of the NicheMapR package (Kearney and Porter 2017) .

4.3.4 Germination requirements

Prior to incubation, seeds were surface sterilized with a solution of 2 % (w/v) calcium hypochlorite ($Ca[OCl]_2$) infused with a few drops of a detergent (Tween 80) under vacuum (-70 kPa) for 30 minutes followed by rinsing with sterile deionized water three times for several minutes per wash. Seeds were placed inside 90 mm plastic Petri dishes that were lined with moist (9 ml of water per Petri dish) 84 mm germination papers (Advantec, Dublin, CA, USA). The Petri dishes were sealed with plastic wrap to prevent desiccation and covered with aluminium foil to minimise the impact of light, which is a potential confounding effect on germination (Bell 1994, Ruiz-Talonia, Carr et al. 2018). Before commencing germination experiments, seeds were initially exposed to a warm stratification treatment that, based on previous research, was assumed to be appropriate for dormancy alleviation in all test species (Elliott, Lewandrowski et al. 2019). Seeds were initially stratified at 30 °C for 28 days followed by drying at 15 °C and 15 % RH (cool dry storage) for one week, prior to starting experiments. As well, a random subsample of seeds were also immediately moved from 30 °C to 15 °C while maintaining their hydration status to confirm the effectiveness of the stratification approach used and maintained for four weeks at 15°C to assess their overall germination capacity once physiological dormancy has been alleviated (Appendix. 3.2).

4.3.5 Hydrothermal tolerance

To assess the germination response of seeds to hydrothermal stress, three replicates of 15 seeds per species were incubated on one of 30 hydrothermal stress regimes (water stress levels of 0.0, -0.2, -0.4, -0.6, -0.8 and -1.2 MPa at temperatures of 10, 15, 20, 22 and 25 °C). The hydrothermal regimes used in this experiment encompass a broad range of the hydrothermal conditions reported for the habitats of the test species from autumn to spring (Fig. 4.2). Seeds were placed on 84 mm germination paper (Advantec, Dublin, CA, USA) moistened with either distilled water or polyethylene glycol 8000 (PEG) solution (of different concentrations) to generate the range of water potentials described above for each incubation temperature. Nine ml of distilled water/PEG solution was added per 90 mm Petri dish according the methodology described by (Michel 1983). The Petri dishes were supplemented with 2 % PPM (Plant Preservation Mixture, Austratec Pty Ltd, Bayswater, Vic) to minimize microbial contamination during stratification and incubation treatments. All Petri dishes containing seeds of *T. aphylla* subsp. *aphylla* were irrigated with 1µM karrikinolide (KAR_1) for the duration of the incubation period as an earlier pilot study found an improved germination response for this species following the application of KAR_1 (Appendix. 3.2). The Petri dishes were covered with plastic wrap and aluminium foil as described above. During the incubation period, iButton data loggers (Maxim Integrated™, San Jose, USA) were placed in the middle of each set of Petri dishes that were subjected to the same treatment regime to record the temperature that seeds were exposed to while the germination trial was underway (Appendix. 3.3). Germination was defined as when the radicle emerged out of the testa of > 2 mm was scored three days per week for 56 days. For each test species the soil hydrothermal occurrence niche (generated using the "*micro global*" algorithm as described above) was compared to the hydrothermal performance niche of seeds.

4.4 Statistical analysis

4.4.1 Mapping the occurrence niches

The '*micro global'* algorithm in the *NicheMapR R* package (Kearney and Porter 2017) was employed to estimate the average monthly soil temperature and water stress level at 2.5 cm and 15 cm soil depths for the GPS locations for all recorded individuals of the four test species (based on unpublished survey data). The "*micro global*" algorithm uses a quasi-mechanistic downscaling approach to estimate microclimatic conditions for every hour of the day (Kearney and Porter 2017, Tomlinson, Lewandrowski et al. 2020). These were initially averaged between sunrise and sunset at each location to generate daily temperatures and water stress levels (at 11.30 a.m.) at the GPS locations of all individuals of the test species at the selected soil depth levels. The estimates for daily soil temperature and water stress levels were then used to calculate average monthly values for soil temperature and water stress for the GPS location of each individual plant.

4.4.2 Germination modelling

The germination response of each study species was characterised using a non-linear regression method. As the first step, the relationship between time and cumulative germination rate for each hydrothermal stress regime was assessed by fitting a non-linear log-logistic function to the germination data (Ritz and Streibig 2012). The *drm* function of the *drc* package (Ritz and Streibig 2012) was utilized to fit a three parameter logistic model in the *R* statistical software environment (R Core Team 2013);

$$germination = \frac{G_{max}}{1+\frac{time^{b}}{t_{50}}} \qquad (1)$$

where, G_{max} represents maximum germination, t_{50} represents the time required for 50 % of seeds (as a fraction of G_{max}) to germinate and b is the slope of the function at t_{50}. For each study species, a common convergent curve was fitted to the number of germinants as a proportion of the number of incubated seeds for all hydrothermal stress regimes. Next, the data were grouped on the basis of hydrothermal regime by fitting distinct values to the parameters of the model in order to produce multiple permutations of the primary model. The t_{50} and G_{max} values for each replicate of seeds exposed to each hydrothermal stress regime were estimated by fitting permutations of the logistic function to the data (Tomlinson 2019, Rajapakshe, Turner et al. 2020).

4.4.3 Hydrothermal tolerance

Thermal performance characteristics of organisms generally adhere to the established theories of chemical kinetics and thus can be described by unimodal asymmetric curves (Angilletta Jr 2006). The thermal performance model developed by Yan and Hunt (1999) has previously been found to be the most broadly appropriate to describe whole-organism thermal performance (Tomlinson 2019), and therefore is appropriate to characterize the thermal responses of seeds in terms of germination speed ($1/t_{50}$) and G_{max};

$$r_{max}, G_{max} = R_{max} \left(\frac{T_{max} - T}{T_{\max} - T_{opt}}\right)\left(\frac{T}{T_{opt}}\right)^{\frac{T_{opt}}{T_{max} - T_{opt}}} \quad (2)$$

where r_{max} ($1/t_{50}$) represents the speed of germination as quantified by germination rate at any temperature for 50 % of seeds to germinate over time through Eqn. 1, G_{max} is maximum germination response at any temperature (T), T_{max} represents the maximum temperature threshold for germination, T_{opt} represents the optimum temperature for germination where germination is most rapid or when germination is maximised, and R_{max} is the upper limit of the germination response curve (in terms of r_{max} or G_{max}) at T_{opt}. The *nls* function was used to fit distinct values to the parameters of Eqn. 2 to parametrise unique R_{max}, T_{opt}, T_{max}, and G_{max} estimates across the hydrothermal stress regimes described above (Ritz and Streibig 2008).

4.5 Results

4.5.1 Mapping the occurrence niches

The estimates of the soil hydrothermal conditions in the habitats of the four *Tetratheca* species were generally more variable at 2.5 cm soil depth compared to 15 cm depth. From February to April, there was considerable overlap between the occurrence niches of the four taxa across the different ranges (Fig. 4.2). The highest concordance between the occurrence niches, however, was observed during the cooler months of the years (May to September). During this period, the water pressure deficit of the habitat (at 2.5 and 15 cm soil depth), indicative of water stress to the plant, ranged from 0 MPa to -3.25 MPa, whereas soil thermal regimes varied between 8 and 16 °C (Fig. 4.2). At 2.5 cm soil depth, the water pressure deficit remained higher than -1.5 MPa (theoretical permanent wilting point of most plants) from May to August in the habitats of all test species (Fig. 4.2). However, at 15 cm soil depth, the soil water pressure deficit in the occurrence niches of all four species remained higher than -1.5 MPa from April to October (Fig. 4.2). From November to January, the level of overlap between the occurrence niches of the four-study species decreased (Fig. 4.2).

Separation of the hydrothermal occurrence niches of the four taxa was most pronounced during the warmer months of the year (November to March). During summer (December to February) the thermal conditions of the soil environment (at 2.5 and 15 cm soil depth) of the study species ranged from 25.5 to 33 °C, whereas soil water pressure deficits remained lower than -1.5 MPa (Fig. 4.2). The occurrence niches of *T. aphylla* subsp. *aphylla* and *T. erubescens* were constrained to a thermal regime of 25.5 to 32.5 °C during summer (Fig. 4.2), whereas the thermal niche of *T. harperi* was between 26.5 to 33.0 °C. There was a high degree of overlap between the thermal occurrence niches of these three species (Fig. 4.2). Conversely, the occurrence niche of *T. paynterae* subsp. *paynterae* was constrained to the upper end of the above temperature spectrum (27.5 to 33.0 °C), and exhibited comparatively low levels of overlap with those of the other three taxa during summer (Fig. 4.2).

4.5.2 Germination modelling

The response of r_{max} ($1/t_{50}$) to thermal stress at each water stress regime was a unimodal asymmetric hump-shaped curve for all species, except *T. aphylla* subsp. *aphylla* (Fig. 4.3a). In *T. aphylla* subsp. *aphylla* seeds, germination following stratification were ≤ 20 %, and therefore this species was excluded in further experimentation. In terms of r_{max}, the T_{opt} values (at 0 MPa) for the remaining three species were between 15.2 to 19.9 °C, whereas T_{max} estimates were between 23.9 to 38.2 °C (Table. 4.1). In the absence of water stress, time required to reach 50 % germination (t_{50}) at T_{opt} was longest in *T. harperi* seeds (24.9 days; Table. 4.1), whereas estimated t_{50} at T_{opt} was shortest in *T. erubescens* seeds (18.5 days). In terms of r_{max}, the seeds of *T. erubescens* were more tolerant of hydrothermal stress compared to the two other species (Fig. 4.3). In *T. erubescens* and *T. harperi*, the performance curve of r_{max} became narrower with increasing water stress (Fig. 4.3a). This thermal performance function could not be fitted to the germination data at some hydrothermal regimes due to low germination percentages (Fig. 4.3a).

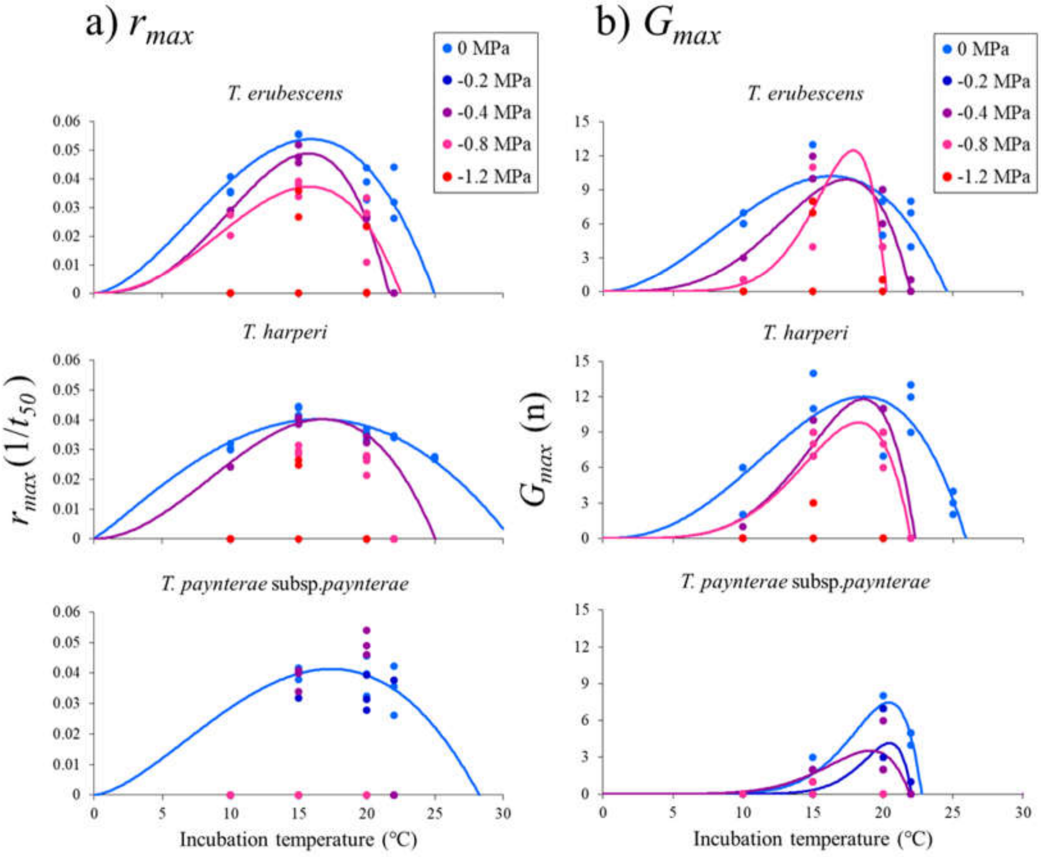


Fig. 4.3. Hydrothermal tolerance in three Western Australian *Tetratheca* species in terms of the time to reach 50% germination (t_{50}) and maximum germination (G_{max}); **a)** Dots depict r_{max} (1/t_{50}) estimates for each replicate of seeds following incubation under different hydrothermal regimes. The lines represent the thermal performance function fitted to the 1/t_{50} (r_{max}) estimates of each species. **b)** Dots depict G_{max} estimates for each replicate of seeds following exposure to different hydrothermal stress regimes. The lines represent the thermal performance function fitted to the G_{max} estimates of each taxa. Three replicates of 15 seeds were used for each treatment combination.

Table. 4.1. Coefficients for the thermal performance model (Eqn. 2; ± SE) of time to reach 50 % germination (t_{50}) and maximum germination (G_{max}) in the absence of water stress (0 MPa) for three *Tetratheca* species, where T_{opt} is the optimum temperature for germination, R_{max} is the upper limit of the germination response curve (in terms of r_{max} or G_{max}) at T_{opt} and T_{max} is the highest temperature at which germination occurred.

Species	***Germination speed, r_{max} (1/t_{50})***				***Maximum germination, G_{max}***		
	T_{opt} (°C ± SE)	R_{max} (1/t_{50} at T_{opt} ± SE)	t_{50} at T_{opt} (Days)	T_{max} (°C ± SE)	T_{opt} (°C ± SE)	R_{max} (G_{max} at T_{opt} ± SE)	T_{max} (°C ± SE)
Tetratheca erubescens	15.9 ± 0.7	0.054 ± 0.004	18.5	25.0 ± 1.1	16.3 ± 0.8	10.2 ± 1.0	24.6 ± 1.2
Tetratheca harperi	16.6 ± 0.5	0.040 ± 0.001	24.9	30.7 ± 7.5	18.6 ± 0.6	12.0 ± 0.9	25.9 ± 0.5
Tetratheca paynterae* subsp. *paynterae	17.4 ± 2.5	0.041 ± 0.004	24.2	28.2 ± 8.8	20.4 ± 0.2	7.5 ± 0.5	22.8 ± 0.2

In terms of G_{max}, the T_{opt} estimates for the three taxa at 0 MPa were between 15.5 to 20.6 °C, whereas T_{max} values ranged from 22.6 to 26.4 °C (Table. 4.1). In all study species estimated G_{max} at T_{opt} and 0 MPa were lower than the final germination rates of the samples that were directly incubated following stratification at 30 °C during the initial pilot study (Appendix. 3.2, Table. 4.1). However, G_{max} estimates at T_{opt} and 0 MPa were higher than the final germination of the samples that were incubated at 15 °C for eight weeks without exposure to 1 μM KAR_1 during the pilot study (Appendix. 3.2, Table. 4.1).

In all test species the curve representing the thermal performance of G_{max} became narrower with increasing water stress (Fig. 4.3b). For *T. erubescens* and *T. harperi*, the estimates of G_{max} were > 60 % at -0.8 MPa under favourable thermal conditions, whereas for *T. paynterae* subsp. *paynterae* G_{max} declined to < 20 % at -0.4 MPa. In the absence of water stress *T. harperi* seeds exhibited the broadest thermal tolerance range (Fig. 4.3, Table. 4.1).

A pattern could not be identified for the response of t_{50} ($1/r_{max}$) to water stress (at a given temperature regime) for any of the study species. In all test species estimates for t_{50} were > 15 days for all hydrothermal regimes that were tested (Fig. 4.4a).

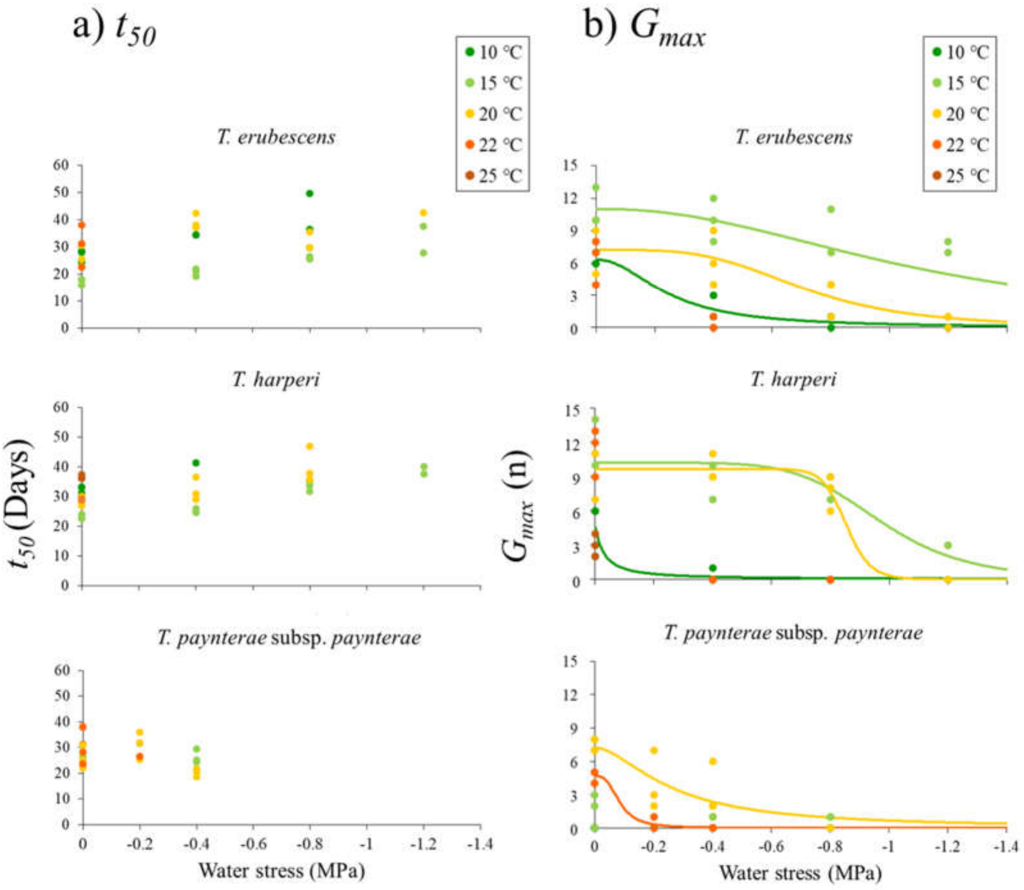


Fig. 4.4. Hydrothermal tolerance in three Western Australian *Tetratheca* species in terms of the time to reach 50% germination (t_{50}) and maximum germination (G_{max}); **a)** Dots depict t_{50} estimates for each replicate of seeds following incubation under different hydrothermal regimes. **b)** Dots depict G_{max} estimates for each replicate of seeds following exposure to different hydrothermal stress regimes. The lines represent the logistic function fitted to the G_{max} estimates of each taxa. Three replicates of 15 seeds were used for each treatment combination.

The response of G_{max} (at a given thermal regime) decreased logistically with increasing water stress for all species (Fig. 4.4b). In all test species the curve representing the hydrological performance of G_{max} was narrower at suboptimal thermal regimes (Fig. 4.4b). For *T. erubescens* and *T. harperi*, the estimates of G_{max} were > 40 % at -0.4 MPa under favourable thermal regimes, whereas for *T. paynterae* subsp. *paynterae* G_{max} declined to < 20 % at -0.2 MPa for all thermal regimes (Fig. 4.4b). For *T. erubescens* and *T. harperi*, the hydrological performance niche (in terms of G_{max}) was broadest at 15 °C, whereas for *T. paynterae* subsp. *paynterae*, the hydrological performance niche was broadest at 20 °C.

The thermal performance function and the logistic function could not be fitted to the G_{max} data at some treatment regimes due to low final germination (Fig. 4.3b, Fig. 4.4b). According to the model predictions, the estimates of G_{max} for *T. erubescens* at -0.8 MPa were higher than that of -0.4 MPa within a range of ~ 15-20 °C (Fig. 4.3b). This observation could be due to errors in the model fitting process caused by low final germination and high variability in germination response observed at high hydrothermal stress regimes.

4.6 Discussion

Our expectation that the germination response (upon dormancy alleviation) of species with physiological seed dormancy to thermal and water stresses should conform to general mechanistic principles was supported by the results of this study. However, the findings of this series of experiments suggest that the germination niche is defined by complex interactions between thermal stress and water stress. As well, our second expectation that, based on the similarity of the occurrence niches of the three *Tetrathca* species, there should be a high degree of overlap between the hydrothermal performance niches of seeds (in terms of t_{50} and G_{max}) was also supported by our data. The study species were generally slow to germinate, and their hydrothermal responses were constrained to narrow niches at cooler temperatures. These observations suggest that outcrop specialist *Tetratheca* species are likely to be allopatric congenerics that may have evolved under more mesic conditions, and, due to aridification of the broader geographic region, their distribution has become constrained to microhabitats on BIF outcrops.

4.6.1 Hydrothermal tolerance

In all test species, high germination responses were observed within favourable temperatures in the absence of water stress, compared to suboptimal conditions. This observation indicated that seed germination is optimized when the hydrothermal conditions in the external environment are suitable to promote the metabolic process of seed germination, which in turn facilitates the establishment of seedlings (Huang, Ran et al. 2020). The findings of this study are consistent with current literature showing that thermal performance of seeds in terms of both maximum germination rate (r_{max}) and maximum germination (G_{max}) conform to unimodal asymmetric hump-shaped curves (Fig. 4.2) that are characteristic of all enzyme mediated reactions (Peterson, Daniel et al. 2007, Abeli, Ghitti et al. 2020, Rajapakshe, Turner et al. 2020). Furthermore, our results are in accordance with previous studies that suggest that at a given temperature, increasing water stress should cause a logistic decrease in the performance of G_{max} (Lewandrowski, Erickson et al. 2017, Mesgaran, Onofri et al. 2017, Turner, Lewandrowski et al. 2018, Duncan, Schultz et al. 2019). It was expected that the performance of t_{50} would increase exponentially with increasing water stress at a given temperature (Rajapakshe, Turner et al. 2020), but an exponential function could not be fitted to the germination response data (Fig. 4.4a). This could be due to the fact that (due to the low number of seeds available for the experiments due to extreme rarity of the study species) the number of replicates used within the germination response range of the study species (along the selected environmental gradients) was insufficient. In all species, increasing water stress constrained the breadth of the thermal performance niche of r_{max} and G_{max}. Similarly, incubation of seeds at suboptimal thermal regimes constrained the breadth of the hydrological performance niche in terms of G_{max} (Fig. 4.4b). In this way, both temperature and water stress work together to limit the germination niche to a two-dimensional optimum. For all the species of *Tetratheca* studied here, that optimum is generally cool (15.2 to 19.9 °C), with relatively high water pressure deficits (Fig. 4.4). Furthermore, although there were subtle differences in the estimates of germination speed and tolerance thresholds, the errors of estimation associated with the non-linear model fits all overlapped, which is strongly suggestive that they models are statistically indistinguishable between species.

The results of this study are consistent with previous research, and suggest that interactions between niche axes, and the implications for species performance must be quantified (Chapter 3, Mesgaran, Onofri et al. 2017, Huang, Zhou et al. 2020). The results of this study suggest that exposure of seeds to suboptimal hydrothermal regimes during the stage of germination could constrain the breadth of the hydrothermal performance niche. Since species performance during the stage of germination is fundamental to shaping the overall niche of plants, this could reduce population growth rate (Giménez-Benavides, Escudero et al. 2005, Cochrane 2017). Given the high concordance between the *Tetratheca* species that we studied, and their apparent preference for cool and moist germination niches, it would be interesting to test these responses in a broader range of taxa, especially those from other rock types (such as *T. deltoidia* from the granite inselberg at Mt Caroline or *T. spenceri* from the sandstone ridges of the Simpson Desert), and BIF specialists from other climate regions (such as *T. fordiana* and *T. butcheri*) should be tested (Western Australian Herbarium 1998), and the implication of genetic factors on germination response should be assessed (Dayrell, Garcia et al. 2017, Dayrell, Fuzessy et al. 2021).

4.6.2 Patterns of species range and endemism

In natural environments, successful seedling establishment is dependent on the duration of the window of opportunity for germination (Yates, Hobbs et al. 1996, Vincent, Barrett et al. 2015, Miller, Symons et al. 2019). Hydrothermal stress, which is a major selective pressure in semi-arid ecosystems, limits the length of that window of opportunity (Cochrane, Hoyle et al. 2014, Cochrane 2017, Elliott, Lewandrowski et al. 2019). There was a high concordance between the soil occurrence niches of the test species (during the rainfall season) and their hydrothermal performance niches (Fig. 4.2, Fig. 4.3, Fig. 4.4) For example, according to the occurrence niche of the study species, soil water pressure deficits remain higher than -1.2 MPa at 15 cm soil depth from April to November (spring to late autumn) in the habitats of *T. erubescens, T. harperi* and *T. paynterae* subsp. *paynterae*, and soil temperature ranges from 5 to 25.5 ℃ (Fig. 4.2). Basically, these locations are minimally stressful during that time, remaining both cool and moist. The T_{opt} estimates for the seeds of the three species at 0 MPa are within this temperature range (Table. 4.1). The high concordance between the occurrence and hydrothermal performance niches of the study species reflect the role of hydrothermal stress in shaping the overall niche of these range-restricted flora in semi-arid ecosystems. Even though the geographical distribution of each study species is restricted to one of several neighbouring BIF outcrops, there was a high level of overlap between their hydrothermal performance niches (Fig. 4.3, Fig. 4.4). This observation provides support to suggestions that the common ancestor of the study species may have been subjected to allopatric speciation (Butcher, Byrne et al. 2007, Byrne, Krauss et al. 2019).

Following stratification at 30 °C for four weeks, the time required to reach 50 % germination under favourable hydrothermal conditions was longer than 18 days for *T. erubescens, T. harperi* and *T. paynterae subsp. paynterae* (Table. 4.1). Compared to *Eucalyptus* and *Acacia* species restricted to outcrop habitats of Western Australia, the three *Tetratheca* species required longer periods of high soil moisture availability to germinate (in terms of t_{50}), and the hydrothermal performance niches were narrower and constrained to cooler temperatures (Chapter 2, Chapter 3). These observations suggest that outcrop endemic *Tetratheca* species may have evolved under cooler and wetter conditions in the past (Crayn, Rossetto et al. 2006), and further suggests that there has been little selection on the species to diversify their germination niche since that speciation event. It can be speculated that, due to the subsequent aridification of the broader landscape, the geographical distribution of the study species may have become limited to microhabitats on outcrops that can retain high soil moisture levels for long periods (Byrne, Yeates et al. 2008). Essentially, all three *Tetratheca* species are apparently restricted to microclimates that closely match their ancestral germination niches. This claim is supported by the observation that, the average monthly soil water pressure deficits of the occurrence niches of the four taxa remained higher than -1.5 MPa for at least four months at 2.5 - 15 cm soil depth (Fig. 4.2). Slow germination has been reported in several taxa endemic to various mesic habitats of Western Australia (Vincent, Barrett et al. 2015, Cochrane 2017, Cochrane 2020), and it could potentially increase seedling survival rates in microhabitats on outcrops that can retain rainwater for several days/weeks following rainfall episodes (Vincent, Barrett et al. 2015, Turner, Lewandrowski et al. 2018, Elliott, Lewandrowski et al. 2019). However, the implications of slow germination in terms of species persistence on BIF outcrops requires further study.

Physiological dormancy is also likely to be due to the evolutionary heritage of the study species (Dayrell, Garcia et al. 2017, Byrne, Krauss et al. 2019). Previous studies on several BIF endemic species suggest that in physiologically dormant range-restricted species, such as *Tetratheca*, the seedling recruitment rate is likely to increase in pulses during the wettest winters, and therefore windows of opportunity for seedling establishment are rare and unpredictable (Yates, Gibson et al. 2011). Groups with other dormancy patterns may not be so specifically optimised in their germination windows, since non-dormant canopy seed banks, and physically dormant species gradually release non-dormant seeds almost continuously throughout the year. As a result, other groups that have become restricted to shallow soil environments seem to bet-hedge, with broad germination niches (Chapters 2, Chapter 3). The physiologically dormant *Tetratheca* species appear to be sheltered from such pressures by a potentially more explicit set of dormancy release requirements, allowing them to retain a slow germination pattern in cool moist conditions (Vincent, Barrett et al. 2015). Slow germination and having comparatively narrow hydrothermal performance niches that restricted to cooler thermal regimes, is likely to make BIF endemic *Tetratheca* species vulnerable to environmental stochasticity, especially in the context of climate change (Cochrane 2017, Elliott, Lewandrowski et al. 2019, Cochrane 2020).

The results of this study suggest that dormancy regulation and germination of the three *Tetratheca* species are complex processes that occur in response to the interplay between thermal stress, water stress and time, during different parts of the year, in line with previous studies (Vincent, Barrett et al. 2015, Turner, Lewandrowski et al. 2018, Elliott, Lewandrowski et al. 2019, Miller, Symons et al. 2019). Physiological dormancy and slow germination speed could potentially facilitate the persistence of a soil seed bank consisting of seeds at different stages of dormancy and germination; so that any given hydrothermal window stimulates the germination of just a fraction of the soil seed bank; a strategy of bet-hedging that facilitates species persistence (Miller, Symons et al. 2019). However, the potential of a population to withstand environmental stochasticity via maintaining a soil seed bank depends on how long the seeds can remain viable in the soil (Baskin and Baskin 2003), as well as the seasonal addition of fresh seeds into the soil seed bank, but there's a shortage of research data on how long the seeds of the test species can persist in the soil .

Compared to the seed samples that were directly incubated at 20 °C (as opposed to drying seeds at 15 °C and 15 % RH for a week) following stratification at 30 °C for four weeks (pilot study; Appendix. 3.2), the G_{max} estimates for the seeds incubated under favourable hydrothermal regimes were lower in all test species (Fig. 4.3). This observation suggests that drying of seeds (at 15 °C and 15% RH for seven days) after stratification treatment, which differed to the process taken for the pilot study, may have induced dormancy or reduced viability in a portion of the seed samples. However, the potential role of hydrothermal stress in regulating dormancy in BIF specialist *Tetratheca* species is yet to be investigated (Miller, Symons et al. 2019).

4.6.3 Limitations to model interpretations

The results of this study are generally in accordance with the findings of previous research on physiologically dormant species associated with BIF outcrops of Western Australia (Elliott, Lewandrowski et al. 2019). During the pilot test, exposure to KAR_1 enhanced the response of G_{max} in *T. aphylla* subsp. *aphylla* and *T. paynterae* subsp. *paynterae*, potentially indicating a critical environmental gradient (smoke response) that shapes the dormancy alleviation niche of those taxa, particularly under conditions where disturbance events (eg: fire) may occur (Turner, Lewandrowski et al. 2018). The results of this experiment suggest that in order to gain a comprehensive understanding of the germination niche of physiologically dormant species, the critical performance rates of both the dormancy alleviation niche and the germination niche must be quantified. Furthermore, in order to implement effective management and translocation protocols for range-restricted taxa, interactions between critical niche dimensions and the implications for the population demographics must be assessed (Pironon, Villellas et al. 2018). The extent to which climate change can affect the germination niche of range-restricted taxa and the implications for population growth rate must also be quantified (Cochrane, Hoyle et al. 2014, Cochrane 2017). However, a wider range of species should be compared in order to get more insights in to how hydrothermal stress on germination affects patterns of plant distribution in rocky outcrop habitats (Byrne 2019).

4.6.4 Conclusions

This study highlights the significant role of hydrothermal stress on germination in driving population dynamics and distribution patterns of BIF specialist *Tetratheca* species. The results of this experiment suggest that thermal stress and water stress are inter-dependent niche axes, and highlights the importance of quantifying the interactions between niche axes and implications for species performance. The high level of overlap between the hydrothermal germination niches of the study species suggest that they are likely to be allopatric. The slow germination speed of the three *Tetratheca* species, and the observation that their hydrothermal niches are restricted to narrow niches at cooler thermal regimes, indicate that they may have evolved under more mesic conditions, but due to subsequent acidification of the broader geographic region, their distribution may have become restricted to microhabitats on outcrops. The findings of this study suggest that *Tetratheca* species endemic to BIFs are likely to be susceptible to environmental changes.

Chapter 5

General Discussion

5.1 Introduction

Throughout my PhD, I sought to quantify the germination responses of species associated with, and restricted to, rocky outcrops in Western Australia to thermal and hydrological stress. Through comparative study, I aimed to understand the role and relative importance of environmental variables in shaping the germination niche of outcrop endemic taxa. The objectives of my PhD were

1. To investigate whether the thermal stress and water stress response of seeds of several non-dormant, physically dormant, and physiologically dormant species associated with outcrops conform to established theoretical principles;
2. To assess whether broadly distributed taxa of comparable dormancy types have broader thermal and hydrological performance niches compared to range-restricted species, and identify ecological and evolutionary implications;
3. To assess potential interactions between thermal and hydrological stressors to explore the compare hydrothermal occurrence niches and performance niches of closely related outcrop specialist species during the stage of germination, and identify ecological and evolutionary implications.

The findings of this project highlight the role of hydrothermal stress in shaping the germination niches of outcrop plant communities, especially in the unique ways that species endemic to these habitats differ to broadly distributed conspecifics, and to each other. Throughout this study I explored how emerging techniques in non-linear model fitting can be utilized to parameterise germination responses of seeds in an established theoretical context, and also compare stress tolerance thresholds of different taxa. This approach revealed important ecological and evolutionary implications with regards to patterns of plant diversity observed on these habitats. The results of this study suggest that in order to gain a comprehensive understanding of niche dynamics, species performance niches that underpin population growth rates must be quantified. Furthermore, interactions between niche axes and the implications for species performance must be accounted for. Here, I explore the wider implications of my research along four themes: *Germination modelling, Patterns of rarity and endemism, Seed dormancy and persistence,* and *Implications for management and restoration of native flora.*

5.2 Germination modelling

5.2.1 Responses to thermal stress

A persistent pattern that emerged in all of my data was that the germination response of $1/t_{50}$ to thermal stress (at a given water stress level) conforms to an asymmetric hump-shaped curve. This observation is in line with theoretical expectations concerning thermal performance (Yan and Hunt 1999, Angilletta Jr 2006), and lends substantial support to the contention that seed germination is a metabolic process that is mediated by enzymes, and therefore the germination response of seeds is constrained to specific thermal thresholds that are conducive to enzyme activity (Galland, Huguet et al. 2014, Huang, Ran et al. 2020). I also found that an asymmetric hump-shaped model was appropriate to characterize the thermal performance of G_{max} for the three *Tetratheca* species that were tested (Chapter 4 Fig. 3). However, my evidence for this relationship remains equivocal, because an asymmetric hump-shaped curve could not be fitted to the data for the thermal stress response of G_{max} for the five *Acacia* species that were studied. Therefore, a quadratic model was fitted to the data for the thermal performance of G_{max} in the *Acacia* species (Chapter 3 Fig. 3b). As the breadth of the thermal performance niche increased, the shape of an asymmetric hump-shaped model became increasingly similar to the shape of a quadratic model. Therefore, for species that exhibit comparatively broad thermal performance niches, such as the five *Acacia* species that were studied, a quadratic model is likely to be appropriate to characterize the natural variance pattern of G_{max}. The argument could be made that the quadratic is merely an approximation of the hump shaped curve that may have emerged with to advent of a larger or more nuanced data set. However, resolving a consistent pattern in the thermal performance of G_{max} proved to also be impossible for the *Eucalyptus* species that were tested (Chapter 2 Fig. 3), further limiting my general interpretations around this trait. It may be that the selected incubation regimes applied to the *Eucalyptus* species that were tested did not encompass the entire thermal tolerance range of the test species, and that the lack of consistent pattern relates more to the naïve experimental design that I developed early in my doctoral research. Given recent interest in developing functional trait libraries applicable to seed biology (Saatkamp, Cochrane et al. 2019), this particular trait may require greater research focus.

5.2.2 Responses to water stress

In three of the *Eucalyptus* species that were studied, estimates for t_{50} increased exponentially with increasing water stress, in line with the theoretical expectation that the germination response of t_{50} should increase exponentially with increasing water stress (Rajapakshe, Turner et al. 2020). However, in *E. salmonophloia* and the three

Tetratheca species that were tested, there was no consistent pattern in the response of t_{50} to water stress (Chapter 2 Fig. 4b, Chapter 4 Fig. 4a), and therefore an exponential model could not be fitted to the data. It was observed that mechanistic models could not be fitted to the germination data when final germination percentages were low, and/or the variability in germination responses was high due to incubation of seed at suboptimal hydrothermal regimes. Increasing the number of replicates used for germination experiments could potentially increase the precision of the model fitting process. However, the methods used in this thesis may not be suitable to characterise the performance of taxa that exhibit low final germination percentages across the entire thermal stress gradient and water stress gradient.

In all *Acacia* and *Tetratheca* species that were tested, the germination response of G_{max} (at a given temperature regime) decreased logistically with increasing water stress (Chapter 3 Fig. 4, Chapter 4 Fig. 4). This was generally in accordance with the theoretical expectation that the response of G_{max} should decrease logistically with increasing water stress (Lewandrowski, Erickson et al. 2017, Turner, Lewandrowski et al. 2018). In the four *Eucalyptus* species that were tested, there was no consistent pattern in the response of G_{max} (Chapter 2 Fig. 3), but the variability in the estimates for G_{max} increased with increasing water stress. It may be that the number of replicates that were used for this experiment (Chapter 2) were insufficient to encompass the entire germination response range of the four *Eucalyptus* species across the thermal stress gradient and water stress gradient.

The greater difficulty in identifying patterns of response to water stress may relate to the second-hand nature of the influence of water stress of germination. If water stress inhibits germination by inhibiting imbibition (Baskin and Baskin 2003), then there is not only a degree of variability in how the population responses to the stressor, there is also a degree of variability in the susceptibility of the population to the stressor that is less apparent in the direct influence of temperature on chemical kinetics and metabolic processes. Nevertheless, responses to water stress are critical to seed germination (Bradford 2002, Onofri, Benincasa et al. 2018, Lewandrowski, Stevens et al. 2021), and greater focus should be made in quantifying the variability of these responses in natural populations.

5.2.3 Responses to combined hydrothermal stress

In all *Acacia* and *Tetratheca* species that were tested, increasing water stress constrained the breadth of the thermal performance niche of seeds (Chapter 3 Fig. 3, Chapter 4 Fig. 3). Similarly, incubation of seeds of these species at suboptimal temperatures constrained the breadth of their hydrological performance niche (Chapter 3 Fig. 4, Chapter 4 Fig. 4). Therefore, the findings of my research demonstrate that thermal stress and water stress are inter-dependent niche axes, and interactions between these niche axes produce a detrimental effect on germination in terms of both t_{50} and G_{max} at suboptimal hydrothermal regimes (Chapter 3 Fig. 4, Chapter 4 Fig. 3, Chapter 4 Fig. 4). The results of this study demonstrate that quantifying the interactions between thermal stress and water stress, provide an avenue to assess the impact of these stress factors on the germination niche of taxa and, by extension, their overall population growth rate. This is especially valuable, as will be discussed later, in the conservation management of unique species.

Understanding the hydrothermal niche of seed germination has a long-recognised importance (Bradford 2002, Onofri, Benincasa et al. 2018), but an intuitive way to represent and interrogate these patterns hasn't been forthcoming. The three-dimensional plots of the germination hyperspace developed for the *Acacia* species demonstrate that a non-linear model fitting approach based on a sound theoretical context can be employed to quantify and compare stress tolerance thresholds of seeds. The experimental approach used in this study can be utilized to identify stress tolerance thresholds of other rare and range-restricted taxa. Furthermore, there are ecological insights to be gained from this that show that the hydrothermal fitness of a species doesn't necessarily follow the theoretical expectations driven by temperature or water stressors alone. However, a limitation of this model fitting approach is that it does not incorporate time as a variable. Recently, hydrothermal time models have been developed to characterize the performance of seeds in the field of agriculture (Onofri, Benincasa et al. 2018). However, models that do not compress the natural variance pattern of germination data are currently unavailable.

The 3D germination landscapes that were used to predict the performance of the seeds of the five *Acacia* species could not be fitted to the germination data for the three *Tetratheca* species (Chapter 3 Fig. 3). Several factors may have contributed to this, such as comparatively low final germination percentages across a broad range of treatment regimes and the high a level of variability in germination responses was observed at suboptimal hydrothermal regimes (Chapter 4 Fig. 3). However, it should be recognised that physiological dormancy, which characterises *Tetratheca* species, is complicated to alleviate, and may itself be differently hydrothermally optimised in different, closely related species (Vincent, Barrett et al. 2015, Elliott, Lewandrowski et al. 2019). This is somewhat evident in my data, given that my standardised dormancy alleviation protocol failed to release dormancy in *T. aphylla*. Furthermore, due to the limited supply of *Tetratheca* seeds, the number of replicates that were exposed to each treatment regime was low, and may not have offset this innate population-level variability. Increasing the number of replicates used for germination experiments (especially at suboptimal hydrothermal regimes) to encompass the entire response of seeds across the thermal stress gradient and water stress gradient could potentially increase the accuracy of the model fitting process. However, it is also broadly recognised that increased focus on the ecological and evolutionary drivers of complex dormancy states is required to further a conservation agenda in seed biology (Saatkamp, Cochrane et al. 2019).

5.3 Patterns of rarity and endemism

A major objective of my doctoral research was to investigate the role of thermal stress and water stress in shaping the germination niche in a diverse assemblage of plant species associated with outcrop habitats of Western Australia. By developing and applying a consistent analysis protocol using non-linear regressions of germination performance to a wide range of species, my research highlighted the role of hydrothermal stress in shaping the germination niche of taxa native to arid and semi-arid habitats of Western Australia. This revealed important ecological and evolutionary implications with regards to the unique patterns of plant diversity endemic to outcrop habitats in the region. The data for thermal stress and water stress tolerance thresholds of the study species (in terms of G_{max} and t_{50}) were generally in line with previous research (Yates, Hobbs et al. 1996, Cochrane 2017, Elliott, Lewandrowski et al. 2019, Cochrane 2020).

Seeds of *E. salubris*, *E. salmonophloia* and *A. assimilis*, all of which are predominantly found on the deeper-soil habitats species surrounding rocky outcrops and occurring in comparatively higher-rainfall regions (Western Australian Herbarium 1998), were less tolerant of thermal stress and water stress compared to their congenerics living on shallower-soil environments (Chapter 2. Fig. 4, Chapter 3 Fig. 4). *Eucalyptus.*

salubris, *E. salmonophloia* and *A. assimilis* exhibit summer drought avoidance, similar to many other flora native to the deeper-soil habitats of South Western Australia (Bell, Plummer et al. 1993). Slow germination speed and restriction of germination response to cooler temperature regimes may enable *E. salubris*, *E. salmonophloia* and *A. assimilis* to synchronize seedling emergence with the persistently high soil moisture levels and cooler environmental temperatures associated with the rainfall season (early spring to late autumn) in this region (Bell, Rokich et al. 1995).

Conversely, the four *Acacia* species that are native to shallow-soil habitats of Western Australia had comparatively broad hydrothermal performance niches (Chapter 3 Fig. 3). These results suggest that, similar to many other species native to the Western Australian arid zone, these four *Acacia* species have adapted to opportunistically germinate rapidly across a broad range of temperatures when soil moisture content increases for short periods during irregular rainfall episodes (Jurado and Westoby 1992, Cross, Turner et al. 2015, Erickson, Barrett et al. 2016). The two *Eucalyptus* species associated with shallow-soil rock outcrop habitats exhibited similar adaptations (Chapter 2 Fig. 4). It can be speculated that, since outcrop habitats are subjected to higher levels of hydrothermal stress compared to surrounding deeper-soil habitats, these species (*E. ornata* and *E. caesia*) likely also benefit from a strategy of rapid germination in response to soil moisture pulses (Main 1997).

The experimental data for the performance of seeds of *Eucalyptus* and *Acacia* species did not conform to the expectation based on niche theory that broadly distributed species should have broader thermal and hydrological stress tolerance ranges compared to range-restricted taxa (Hutchinson 1978). The experimental data for the *Eucalyptus* and *Acacia* species suggested that range-restricted taxa associated with shallow-soil outcrop habitats (*E. ornata*, *E. caesia* subsp. *caesia*, *A. karina*, *A.*

woodmaniorum) had broader thermal tolerance ranges compared to their congenerics occurring on deeper-soil (less water stressed) environments surrounding outcrops (e.g. *E. salubris*, *E. salmonophloia*, *A. assimillis*). Furthermore, seeds of range-restricted outcrop endemic taxa germinated rapidly under low water stress levels. Adaptations for rapid germination during short periods of high soil moisture availability, even under suboptimal temperatures, is likely to be a bet-hedging strategy by which range-restricted taxa gain access to uncontested niche space on the shallow, skeletal soils typical of rock outcrops (inaccessible to their congenerics inhabiting deeper-soil environments due to comparatively lower levels of hydrothermal stress tolerance) during short and unpredictable rainfall events (Cross, Barrett et al. 2018).

Compared to the *Eucalyptus* and *Acacia* species that were tested, the three *Tetratheca* species were slow to germinate (in terms of t_{50}), their performance niches were narrower and were constrained to cooler thermal regimes (Chapter 4 Fig. 3, Fig. 4). These observations suggest that outcrop specialist *Tetratheca* species are may have been regionally broadly distributed under cooler and wetter climatic conditions in the past (Crayn, Rossetto et al. 2006). It can be speculated that, due to aridification of the broader geographical landscape (White 1994), the distribution of these species may have become constrained to microhabitats on outcrops that can retain rainwater for long periods of time (Byrne, Yeates et al. 2008). This claim is supported by the fact that according to the climatic model used in this study, the average monthly soil water pressure deficits at the GPS locations of the four *Tetratheca* species remained higher than -1.5 MPa for at least four months at 2.5 - 15 cm soil depth (Chapter 4 Fig. 2). There was a high level of overlap between the hydrothermal germination niches of the three *Tetratheca* species, which lends support to suggestions that they may have been subjected to allopatric speciation in the past (Butcher, Byrne et al. 2007).

Physiological dormancy may also be a part of the evolutionary heritage of these species, as other *Tetratheca* such as *T. juncea* and *T. hirsuta* are also reportedly physiologically dormant (Roche, Dixon et al. 1997, Bellairs, Bartier et al. 2006, Byrne, Krauss et al. 2019). Although my research suggests that *Tetratheca* species may largely conform to a pleisiomorphic germination niche, the interspecific differences in dormancy alleviation cues suggested in my data raise the possibility that selection has not operated at the level of the germination niche, but more at the level of the dormancy alleviation niche. However, my data are far from conclusive on the matter, and I suggest that further research into this prospect will be valuable, potentially across a range of speciose, physiologically dormant genera such as *Tetratheca* and *Stylidium*.

Slow germination has been observed in numerous species native to various mesic habitats of southwest Western Australia (Kullmann 1981, Vincent, Barrett et al. 2015, Cochrane 2020). Slow germination could potentially increase seedling survival rates in environments where high moisture levels occur consistently at certain times (i.e., winter) of the year, and where soil moisture persists for several days/weeks between intermittent rainfall episodes due to the seasonally low temperatures and rates of evaporation (Turner, Lewandrowski et al. 2018). Slow germination rates observed for the three *Tetratheca* species may reflect adaptation to cooler and wetter environmental conditions in the past (Crayn, Rossetto et al. 2006). The role of slow germination in facilitating species persistence on outcrops, or whether outcrops in fact provide refugia for species with this slow germination syndrome, requires further investigation.

Previous studies on several BIF endemic taxa suggest physiologically dormant range-restricted species exhibit pulse recruitment during winters that experience the highest levels of rainfall, and therefore windows of opportunity for plant establishment are scarce and unpredictable (Yates, Gibson et al. 2011). Taxa with other dormancy types (e.g. non-dormant and physically dormant taxa) may not be similarly constrained in their germination windows, and their seed banks generally release non-dormant seeds throughout the year (Bell, Rokich et al. 1995, Yates, Hobbs et al. 1996, Booth 2017). The findings of this study suggest that range-restricted *Eucalyptus* and *Acacia* species follow a strategy of bet-hedging, which is facilitated by broad performance niches of seeds across thermal stress and water stress gradients (Chapters 2, Chapter 3). Conversely, germination windows of physiologically dormant *Tetratheca* species are apparently constrained by narrower set of germination requirements, which is facilitated by a pattern of slow germination under cooler and wetter conditions (Vincent, Barrett et al. 2015). The findings of this thesis suggest that spatial heterogeneity in outcrop habitats has produced hydrothermally diverse microhabitats that can facilitate the persistence of a diverse assemblage of plants that are specially segregated, and depending on phylogenetic factors, various strategies may be viable, and that this may be substantially responsible for their high levels of alpha and beta diversity (Gibson, Yates et al. 2010). This observation is in accordance with previous studies on similar habitats (Dayrell, Garcia et al. 2017, Dayrell, Fuzessy et al. 2021).

Slow germination and the possession of comparatively narrow hydrothermal performance niches is likely to make BIF-specialist *Tetratheca* species potentially more vulnerable to future environmental changes, particularly as a consequence of climatic change. As context, southwest Western Australia is predicted to experience a further marked reduction in rainfall on top of the 10 – 20 % reduction already observed since the 1970's (Elliott, Lewandrowski et al. 2019, Cochrane 2020, Scanlon and Doncon 2020).

5.4 Seed dormancy and persistence

The four *Eucalyptus* species that were studied do not form persistent soil seed banks in their natural environment, similar to many other bradysporous Myrtaceae species native to Western Australia (Groom 2015, Booth 2017). Seeds of the *Eucalyptus* species that were studied are retained inside capsules in canopy seed banks (rather than being released into the soil), and seed dispersal can occur throughout the year in response to various environmental factors (Bell, Plummer et al. 1993). According to previous studies on Australian *Eucalyptus* species, rates of seed release from the canopy seed bank decrease during wet periods (Booth 2017). However, seed release rates significantly increase in response to disturbance events such as fires, droughts and heatwaves in *Eucalyptus* forests and woodlands which result in the elimination of large parts of the above ground vegetation (Bell, Plummer et al. 1993). Increased resource availability following disturbance events such as fire has been found to result in increased seedling recruitment success in multiple *Eucalyptus* species native to Western Australia, because the increase in resource availability allows for greater investment in fruit development and improved seed set (Bell 1994). Absence of seed dormancy in the four *Eucalyptus* species may increase the rates of seedling establishment during episodes of high resource availability once the seeds have been released into the soil and favourable hydrothermal conditions that support germination occur (Yates, Hobbs et al. 1996).

In *A. assimilis* and *A. exocarpoides*, PY was alleviated by exposure to boiling water for 2 mins. However, the two range-restricted *Acacia* species and *A. ramulosa* did not respond well to the initial hot water treatment (< 37 % germination), and therefore required acid scarification for dormancy alleviation in order to achieve high germination (> 80 %). This observation indicates, that under natural conditions, the dormancy alleviation requirements for these five species are likely different. This observation is in line with previous studies that suggest dormancy alleviation requirements of *Acacia* species can be species-specific, with many species requiring quite different dormancy alleviating treatments (Bell, Plummer et al. 1993, Bell, Rokich et al. 1995, Steadman 2004, Steadman and Pritchard 2004, Turner, Steadman et al. 2013).

Unlike the *Eucalyptus* and *Acacia* species previously described, the results for the three *Tetratheca* species suggest that dormancy regulation and germination are likely to occur in response to the complex interplay between thermal stress, water stress and time (Vincent, Barrett et al. 2015). Recent research on several BIF associated species suggest that both physiological dormancy and slow germination in the three *Tetratheca* species could potentially contribute towards species persistence via a strategy of bet-hedging which allows some (but not all) seeds to germinate in response to favourable soil conditions as these cycle in and out of physiological seed dormancy (Miller, Symons et al. 2019). However, the persistence of a soil seed bank ultimately depends on how long seeds can survive in the soil (Baskin and Baskin 2003), and currently there is no research data available as to the likely longevity of seeds of the three *Tetratheca* species within the soil seed bank. Nevertheless, preliminary evidence suggests that some *Tetratheca* seeds can persist and remain viable for longer than 12 months, though this remains to be verified (S. Turner personal communication).

The percentage of seeds that responded to the selected dormancy alleviation treatment differed among the three *Tetratheca* species. These observations suggest that, in order gain a comprehensive understanding of population dynamics, all performance niches that are critical for population growth rate, such as the germination niche as well as the dormancy alleviation niche, must be adequately characterized (Pironon, Villellas et al. 2018).

5.5 Implications for management and restoration of native flora

My research suggests that local extinction of species that are range-restricted to rocky outcrops may lead to the global extinction of these taxa. This is especially the case where some outcrops (such as BIF) represent high economic value as mineral resources (Yates, Gibson et al. 2011, Krauss and Anthony 2019). Mitigation translocations and introductions are often proposed as a way to resolve this implied threat, although the success of such translocations, at best, is highly variable (Silcock, Simmons et al. 2019). Recently it has been suggested that high resolution spatial modelling could identify suitable recipient landscapes to increase the likelihood of translocation success (Lannuzel, Balmot et al. 2021), and it has been noted that such models often identify large areas of vacant potential habitat (Tomlinson, Lewandrowski et al. 2020). These vacant spaces have previously been put down to stochastic extinction events, followed by a lack of recruitment or reinvasion (Tomlinson, Lewandrowski et al. 2020). My research suggests that a deeper knowledge of the germination niche, coupled with highly resolved microclimatic data, could indicate that this "vacant" habitat may not be as suitable as the modelling processes suggest. I strongly suggest that more mechanistic aspects of the seed germination niche be incorporated into the management of rare and range restricted species, especially in the identification of prospective recipient landscapes to translocation or introduction.

My doctoral research sought to assess the role of hydrothermal stress in shaping the germination niche of a diverse set of plant species associated with outcrop habitats of

Western Australia. The findings of my research suggest that the special heterogeneity of outcrop surfaces has produced hydrothermally diverse microhabitats that facilitate special segregation of occurrence niches. These microhabitats are utilized by a phylogenetically diverse assemblage of plants, which suggests that a wide array of strategies may be viable in outcrop ecosystems. The germination responses of the test species were generally in accordance with theoretical expectations and previous research. However, the results of this research demonstrate that in order to gain a comprehensive understanding of the dynamics interactions between thermal stress and water stress should be quantified.

References

Abeli, T., M. Ghitti and R. Sacchi (2020). "Does ecological marginality reflect physiological marginality in plants?" Plant Biosystems-An International Journal Dealing with all Aspects of Plant Biology **154**(2): 149-157.

Ainsworth, A. and D. R. Drake (2020). "Classifying Hawaiian plant species along a habitat generalist-specialist continuum: Implications for species conservation under climate change." PloS one **15**(2): e0228573.

Anacker, B. L., J. B. Whittall, E. E. Goldberg and S. P. Harrison (2011). "Origins and consequences of serpentine endemism in the California flora." Evolution **65**(2): 365-376.

Angilletta Jr, M. J. (2006). "Estimating and comparing thermal performance curves." Journal of Thermal Biology **31**(7): 541-545.

Angilletta Jr, M. J. and M. J. Angilletta (2009). Thermal adaptation: a theoretical and empirical synthesis. Oxford, Oxford University Press.

Ashford, J. R. and R. R. Sowden (1970). "Multi-variate probit analysis." Biometrics **26**(3): 535-546.

Baskin, J. M. and C. C. Baskin (2003). Classification, biogeography, and phylogenetic relationships of seed dormancy. In ‘Seed conservation: turning science into practice’. (Eds RD Smith, JB Dickie, SH Linington, HW Pritchard, RJ Probert) London, The Royal Botanic Gardens, Kew.

Baskin, J. M. and C. C. Baskin (2004). "A classification system for seed dormancy." Seed science research **14**(1): 1-16.

Baskin, J. M., C. C. Baskin and X. Li (2000). "Taxonomy, anatomy and evolution of physical dormancy in seeds." Plant species biology **15**(2): 139-152.

Bell, D. T. (1994). "Interaction of Fire, Temperature and Light in the Germination Response of 16 Species From the Eucalyptus marginata Forest of South-Western Australia." Australian Journal of Botany **42**(5): 501-509.

Bell, D. T. (1999). "The process of germination in Australian species." Australian Journal of Botany **47**(4): 475-517.

Bell, D. T., J. A. Plummer and S. K. Taylor (1993). "Seed germination ecology in southwestern Western Australia." The Botanical Review **59**(1): 24-73.

Bell, D. T., D. P. Rokich, C. J. McChesney and J. A. Plummer (1995). "Effects of Temperature, Light and Gibberellic Acid on the Germination of Seeds of 43 Species Native to Western Australia." Journal of Vegetation Science **6**(6): 797-806.

Bellairs, S. M., F. V. Bartier, A. J. Gravina and K. Baker (2006). "Seed biology implications for the maintenance and establishment of Tetratheca juncea (Tremandraceae), a vulnerable Australian species." Australian journal of botany **54**(1): 35-41.

Bezemer, N., S. L. Krauss, D. G. Roberts and S. D. Hopper (2019). "Conservation of old individual trees and small populations is integral to maintain species' genetic diversity of a historically fragmented woody perennial." Molecular Ecology **28**(14): 3339-3357.

Booth, T. H. (2017). "Going nowhere fast: a review of seed dispersal in eucalypts." Australian Journal of Botany **65**(5): 401-410.

Boulangeat, I., S. Lavergne, J. Van Es, L. Garraud and W. Thuiller (2012). "Niche breadth, rarity and ecological characteristics within a regional flora spanning large environmental gradients." Journal of Biogeography **39**(1): 204-214.

Bradford, K. J. (2002). "Applications of hydrothermal time to quantifying and modeling seed germination and dormancy." Weed Science **50**(2): 248-260.

Bruneaux, M. (2017). thermPerf: model fitting for thermal performance curves: R package version 0.0.1.

Burnham, K. P. and D. R. Anderson (2002). Model Selection and Multimodel Inference: a Practical Information-theoretic Approach Springer, New York

Burrows, G. E., J. M. Virgona and R. D. Heady (2009). "Effect of boiling water, seed coat structure and provenance on the germination of Acacia melanoxylon seeds." Australian Journal of Botany **57**(2): 139-147.

Butcher, R., M. Byrne and D. M. Crayn (2007). "Evidence for convergent evolution among phylogenetically distant rare species of Tetratheca (Elaeocarpaceae, formerly Tremandraceae) from Western Australia." Australian Systematic Botany **20**(2): 126-138.

Butterfield, B. J. and J. M. Briggs (2011). "Regeneration niche differentiates functional strategies of desert woody plant species." Oecologia **165**(2): 477-487.

Bykova, O., I. Chuine, X. Morin and S. I. Higgins (2012). "Temperature dependence of the reproduction niche and its relevance for plant species distributions." Journal of Biogeography **39**(12): 2191-2200.

Byrne, M. (2019). "Genetics and ecology of plant species occurring on the Banded Iron Formations in the Yilgarn, Western Australia." Australian Journal of Botany **67**(3): 165-171.

Byrne, M. and S. D. Hopper (2008). "Granite outcrops as ancient islands in old landscapes: evidence from the phylogeography and population genetics of *Eucalyptus caesia* (Myrtaceae) in Western Australia." Biological Journal of the Linnean Society **93**(1): 177-188.

Byrne, M., S. L. Krauss, M. A. Millar, C. P. Elliott, D. J. Coates, C. Yates, R. M. Binks, P. Nevill, H. Nistelberger, G. Wardell-Johnson, T. Robinson, R. Butcher, M. Barrett and N. Gibson (2019). "Persistence and stochasticity are key determinants of genetic diversity in plants associated with banded iron formation inselbergs." Biological Reviews **94**(3): 753-772.

Byrne, M., D. Yeates, L. Joseph, M. Kearney, J. Bowler, M. Williams, S. Cooper, S. Donnellan, J. Keogh and R. Leys (2008). "Birth of a biome: insights into the assembly and maintenance of the Australian arid zone biota." Molecular ecology **17**(20): 4398.

Carboni, M., D. Zelený and A. T. Acosta (2016). "Measuring ecological specialization along a natural stress gradient using a set of complementary niche breadth indices." Journal of Vegetation Science **27**(5): 892-903.

Carta, A., G. Bedini, J. Müller and R. Probert (2013). "Comparative seed dormancy and germination of eight annual species of ephemeral wetland vegetation in a Mediterranean climate." Plant Ecology **214**(2): 339-349.

Clemente, A. S., J. V. Müller, E. Almeida, C. A. Costa, S. L. Dias, J. M. Brehm, R. Rebelo and M. A. Martins-Loução (2017). "What can routine germination tests in seed banks tell us about the germination ecology of endemic and protected species?" Botany **95**(7): 673-684.

Coates, D. J. and K. A. Atkins (2001). "Priority setting and the conservation of Western Australia's diverse and highly endemic flora." Biological Conservation **97**(2): 251-263.

Cochrane, A. (2017). "Modelling seed germination response to temperature in Eucalyptus L'Her. (Myrtaceae) species in the context of global warming." Seed Science Research **27**(2): 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 Ecology **219**(6): 633-647.

Cochrane, A. (2020). "Temperature thresholds for germination in 20 short-range endemic plant species from a Greenstone Belt in southern Western Australia." Plant Biology **22**(S1): 103-112.

Cochrane, A., G. L. Hoyle, C. J. Yates, J. Wood and A. B. Nicotra (2014). "Predicting the impact of increasing temperatures on seed germination among populations of Western Australian Banksia (Proteaceae)." Seed Science Research **24**(3): 195-205.

W. E. Consulting (2012). Regional Flora and Vegetation Survey of the Karara to Minjar Block. Unpublished report prepared for Karara Mining Limited.

Costa-Pereira, R., M. S. Araújo, F. L. Souza and T. Ingram (2019). "Competition and resource breadth shape niche variation and overlap in multiple trophic dimensions." Proceedings of the Royal Society B **286**(1902): 20190369.

Crayn, D. M., M. Rossetto and D. J. Maynard (2006). "Molecular phylogeny and dating reveals an Oligo-Miocene radiation of dry-adapted shrubs (former Tremandraceae) from rainforest tree progenitors (Elaeocarpaceae) in Australia." American journal of botany **93**(9): 1328-1342.

Cross, A. T., M. D. Barrett, S. R. Turner, K. W. Dixon and D. J. Merritt (2018). "Seed-dormancy depth is partitioned more strongly among habitats than among species in tropical ephemerals." Australian Journal of Botany **66**(3): 230-242.

Cross, A. T., S. R. Turner, D. J. Merritt, A. Niekerk, M. Renton, K. W. Dixon and L. Mucina (2015). "Vegetation patterns and hydro-geological drivers of freshwater rock pool communities in the monsoon-tropical Kimberley region, Western Australia." Journal of Vegetation Science **26**(6): 1184-1197.

Cross, A. T., S. R. Turner, M. Renton, J. M. Baskin, K. W. Dixon and D. J. Merritt (2015). "Seed dormancy and persistent sediment seed banks of ephemeral freshwater rock pools in the Australian monsoon tropics." Annals of Botany **115**(5): 847-859.

Dayrell, R. L., L. F. Fuzessy, L. O. Azevedo, H. Lambers, E. J. Veneklaas and F. A. Silveira (2021). "How does spatial micro-environmental heterogeneity influence seedling recruitment in ironstone outcrops?" Journal of Vegetation Science **32**(2): e13010.

Dayrell, R. L., Q. S. Garcia, D. Negreiros, C. C. Baskin, J. M. Baskin and F. A. Silveira (2017). "Phylogeny strongly drives seed dormancy and quality in a climatically buffered hotspot for plant endemism." Annals of Botany **119**(2): 267-277.

de Kroon, H., A. Plaisier, J. van Groenendael and H. Caswell (1986). "Elasticity: the relative contribution of demographic parameters to population growth rate." Ecology **67**(5): 1427-1431.

Debat, V. and P. David (2001). "Mapping phenotypes: canalization, plasticity and developmental stability." Trends in Ecology & Evolution **16**(10): 555-561.

Del Vecchio, S., E. Fantinato, M. Roscini, A. T. Acosta, G. Bacchetta and G. Buffa (2020). "The germination niche of coastal dune species as related to their occurrence along a sea–inland gradient." Journal of Vegetation Science.

Di Virgilio, G., G. Wardell-Johnson, T. Robinson, D. Temple-Smith and J. Hesford (2018). "Characterising fine-scale variation in plant species richness and endemism across topographically complex, semi-arid landscapes." **156**: 59-68.

Do Carmo, F. F. and C. M. Jacobi (2016). "Diversity and plant trait-soil relationships among rock outcrops in the Brazilian Atlantic rainforest." Plant and soil **403**(1-2): 7-20.

Donohue, K., R. Rubio de Casas, L. Burghardt, K. Kovach and C. G. Willis (2010). "Germination, postgermination adaptation, and species ecological ranges." Annual review of ecology, evolution, and systematics **41**: 293-319.

Duncan, C., N. L. Schultz, M. K. Good, W. Lewandrowski and S. Cook (2019). "The risk-takers and-avoiders: germination sensitivity to water stress in an arid zone with unpredictable rainfall." AoB Plants **11**(6): plz066.

Ehrlén, J. and W. F. Morris (2015). "Predicting changes in the distribution and abundance of species under environmental change." Ecology letters **18**(3): 303-314.

Elith, J. and J. R. Leathwick (2009). "Species distribution models: ecological explanation and prediction across space and time." Annual review of ecology, evolution, and systematics **40**: 677-697.

Elliott, C. P., W. Lewandrowski, B. P. Miller, M. Barrett and S. R. Turner (2019). "Identifying germination opportunities for threatened plant species in episodic ecosystems by linking germination profiles with historic rainfall events." Australian Journal of Botany **67**(3): 256-267.

Erickson, T. E. and D. J. Merritt (2016). Seed collection, cleaning, and storage procedures. In ‘Pilbara Seed Atlas and Field Guide: Plant Restoration in Australia's Arid Northwest'. Melbourne, CSIRO Publishing.

Erickson, T. E. e., R. L. e. Barrett, D. J. e. Merritt, K. W. e. Dixon and i. b. Csiro (2016). Pilbara seed atlas and field guide : plant restoration in Australia's arid northwest /

editors: Todd E Erickson, Russell L Barrett, David J Merritt and Kingsley W Dixon, Clayton, Vic. CSIRO Publishing.

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

Fernández-Pascual, E., A. Pérez-Arcoiza, J. A. Prieto and T. E. Díaz (2017). "Environmental filtering drives the shape and breadth of the seed germination niche in coastal plant communities." Annals of Botany **119**(7): 1169-1177.

Finch-Savage, W. E. and S. Footitt (2017). "Seed dormancy cycling and the regulation of dormancy mechanisms to time germination in variable field environments." Journal of experimental botany **68**(4): 843-856.

Frischie, S., E. Fernández-Pascual, C. Ramirez, P. Toorop, M. González and B. Jiménez-Alfaro (2019). "Hydrothermal thresholds for seed germination in winter annual forbs from old-field Mediterranean landscapes." Plant Biology **21**(3): 449-457.

Funnekotter, A. V., M. Millar, S. L. Krauss and P. G. Nevill (2019). "Phylogeographic analyses of (Fabaceae) support long term persistence of populations both on and off banded iron formations." Australian Journal of Botany **67**(3): 194-204.

Galland, M., R. Huguet, E. Arc, G. Cueff, D. Job and L. Rajjou (2014). "Dynamic Proteomics Emphasizes the Importance of Selective mRNA Translation and Protein Turnover during Arabidopsis Seed Germination." Mol Cell Proteomics **13**(1): 252-268.

Gallant, J. and J. Austin (2012). Aspect derived from 1" SRTM DEM-S., v6. CSIRO Data Collection. http://doi.org/10.4225/08/56D778315A62B.

Gallant, J. and J. Austin (2012). Slope derived from 1" SRTM DEM-S. , v4. CSIRO. Data Collection. https://doi.org/10.4225/08/5689DA774564A.

Gallant, J., N. Wilson, T. Dowling, A. Read and C. Inskeep (2011). 1 second SRTM derived digital elevation models user guide, Canberra, Australia, Geoscience Australia.

Gates, S. (2016). Chemical Kinetics, Willford Press.

Ghalambor, C. K., J. K. McKay, S. P. Carroll and D. N. Reznick (2007). "Adaptive versus non-adaptive phenotypic plasticity and the potential for contemporary adaptation in new environments." Functional ecology **21**(3): 394-407.

Gibson, N., D. Coates and K. Thiele (2007). "Taxonomic research and the conservation status of flora in the Yilgarn Banded Iron Formation ranges." Nuytsia **17**(1): 1-12.

Gibson, N., R. Meissner, A. S. Markey and W. A. Thompson (2012). "Patterns of plant diversity in ironstone ranges in arid south western Australia." Journal of Arid Environments **77**(1): 25-31.

Gibson, N., C. J. Yates and R. Dillon (2010). "Plant communities of the ironstone ranges of South Western Australia: hotspots for plant diversity and mineral deposits." Biodiversity and Conservation **19**(14): 3951-3962.

Giménez-Benavides, L., A. Escudero and F. Pérez-García (2005). "Seed germination of high mountain Mediterranean species: altitudinal, interpopulation and interannual variability." Ecological Research **20**(4): 433-444.

Giménez-Benavides, L. and R. Milla (2013). "Comparative germination ecology of two altitudinal vicariant Saxifraga species endemic to the north of Spain." Plant Biology **15**(3): 593-600.

González-Castañeda, J., M. Angoa-Pérez, J. Frías-Hernández, V. Olalde-Portugal, E. Flores-Ancira, T. Terrones-Rincon, O. Van Cleemput and L. Dendooven (2004). "Germination of seeds of huisache (Acacia schaffneri) and catclaw (Mimosa monancistra) as affected by sulphuric acid and mechanical scarification and subsequent growth and survival in a greenhouse and field experiment." Seed Science and Technology **32**(3): 727-738.

Graves, J. and K. Taylor (1988). "A comparative study of Geum rivale L. and G. urbanum L. to determine those factors controlling their altitudinal distribution." New Phytologist **110**(3): 391-397.

Groom, P. K. a. (2015). Plant life of Southwestern Australia : adaptations for survival / Philip K. Groom, Byron B. Lamont managing editor, Katarzyna Michalczyk, Berlin De Gruyter Open Ltd.

Guderle, M., D. Bachmann, A. Milcu, A. Gockele, M. Bechmann, C. Fischer, C. Roscher, D. Landais, O. Ravel and S. Devidal (2018). "Dynamic niche partitioning in root water uptake facilitates efficient water use in more diverse grassland plant communities." Functional Ecology **32**(1): 214-227.

Hardegree, S. P., B. A. Roundy, C. T. Walters, P. A. Reeves, C. M. Richards, C. A. Moffet, R. L. Sheley and G. N. Flerchinger (2018). "Hydrothermal Germination Models: Assessment of the Wet-Thermal Approximation of Potential Field Response." Crop Science **58**(5): 2042-2049.

Hardegree, S. P., C. T. Walters, A. R. Boehm, P. J. Olsoy, P. E. Clark and F. B. Pierson (2015). "Hydrothermal germination models: comparison of two data-fitting approaches with probit optimization." Crop Science **55**(5): 2276-2290.

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

Hopper, S., A. Brown and N. Marchant (1997). "Plants of Western Australian granite outcrops." Journal of the Royal Society of Western Australia **80**: 141-158.

Hopper, S. D. (2009). "OCBIL theory: towards an integrated understanding of the evolution, ecology and conservation of biodiversity on old, climatically buffered, infertile landscapes." Plant and soil **322**(1): 49-86.

Hopper, S. D., P. L. Fiedler and C. J. Yates (2021). "Inselberg floristics exemplify the coast to inland OCBIL transition in a global biodiversity hotspot." Biological Journal of the Linnean Society **133**(2): 624-644.

Hopper, S. D. and P. Gioia (2004). "The southwest Australian floristic region: evolution and conservation of a global hot spot of biodiversity." Annu. Rev. Ecol. Evol. Syst. **35**: 623-650.

Hoyle, G., K. Steadman, M. Daws and S. Adkins (2008). "Physiological dormancy in forbs native to south–west Queensland: Diagnosis and classification." South African Journal of Botany **74**(2): 208-213.

Huang, H., J. Ran, X. Li, Z. Wang, R. Chen, F. Wu, M. Ye, F. Jia, K. J. Niklas and J. Deng (2020). "A General Model for Seed and Seedling Respiratory Metabolism." The American Naturalist **195**(3): 534-546.

Huang, S., Q. Zhou, L. Gu, H. Wang and G. Zhang (2020). "Modeling and simulation study of the stoichiometric niche space and niche overlap based on the copula method." Environmental and Ecological Statistics **27**(1): 115-149.

Huang, Z., S. Liu, K. J. Bradford, T. E. Huxman and D. L. Venable (2016). "The contribution of germination functional traits to population dynamics of a desert plant community." Ecology **97**(1): 250-261.

Hudson, A. R., D. J. Ayre and M. K. J. Ooi (2015). "Physical dormancy in a changing climate." Seed Sci. Res **25**(2): 66-81.

Huey, R. B. and P. E. Hertz (1984). "Is a jack-of-all-temperatures a master of none?" Evolution **38**(2): 441-444.

Huey, R. B. and J. G. Kingsolver (1989). "Evolution of thermal sensitivity of ectotherm performance." Trends in Ecology & Evolution **4**(5): 131-135.

Hutchinson, G. E. (1978). "An introduction to population ecology Yale Univ." Press, New Haven-London.

Jacobi, C. M., F. F. Do Carmo, R. C. Vincent and J. R. Stehmann (2007). "Plant communities on ironstone outcrops: a diverse and endangered Brazilian ecosystem." Biodiversity and Conservation **16**(7): 2185-2200.

Jacobi, C. M. and F. Fonseca do Carmo (2008). "The contribution of ironstone outcrops to plant diversity in the Iron Quadrangle, a threatened Brazilian landscape." AMBIO: A Journal of the Human Environment **37**(4): 324-326.

James, J. J., R. L. Sheley, T. Erickson, K. S. Rollins, M. H. Taylor and K. W. Dixon (2013). "A systems approach to restoring degraded drylands " Journal of Applied Ecology **50**: 730-739.

Jeremiah, T. S. and C. L. Mohler (2002). "Evaluating Seed Viability by an Unimbibed Seed Crush Test in Comparison with the Tetrazolium Test." Weed Technology **16**(4): 781-786.

Jiménez-Alfaro, B., F. A. Silveira, A. Fidelis, P. Poschlod and L. E. Commander (2016). "Seed germination traits can contribute better to plant community ecology." Journal of Vegetation Science **27**(3): 637-645.

Jurado, E. and M. Westoby (1992). "Germination biology of selected central Australian plants." Australian Journal of Ecology **17**(3): 341-348.

Kaya, M. D., G. Okçu, M. Atak, Y. Çıkılı and Ö. Kolsarıcı (2006). "Seed treatments to overcome salt and drought stress during germination in sunflower (Helianthus annuus L.)." European Journal of Agronomy **24**(4): 291-295.

Kearney, M. R. and W. P. Porter (2017). "NicheMapR–an R package for biophysical modelling: the microclimate model." Ecography **40**(5): 664-674.

Kildisheva, O. A., T. Erickson, M. Madsen, K. Dixon and D. Merritt (2019). "Seed germination and dormancy traits of forbs and shrubs important for restoration of North American dryland ecosystems." Plant Biology **21**(3): 458-469.

Kimirei, I., I. Nagelkerken, M. Trommelen, P. Blankers, N. Van Hoytema, D. Hoeijmakers, C. M. Huijbers, Y. Mgaya and A. Rypel (2013). "What drives ontogenetic niche shifts of fishes in coral reef ecosystems?" Ecosystems **16**(5): 783-796.

Körner, C. (2003). Alpine plant life: functional plant ecology of high mountain ecosystems; with 47 tables, Springer-Verlag Berlin Heidelberg.

Krauss, S. L. and J. M. Anthony (2019). "The potential impact of mining on population genetic variation in the Banded Ironstone Formation endemic Tetratheca erubescens (Elaeocarpaceae)." Australian journal of botany **67**(3): 172.

Krichen, K., A. Vilagrosa and M. Chaieb (2017). "Environmental factors that limit Stipa tenacissima L. germination and establishment in Mediterranean arid ecosystems in a climate variability context." Acta Physiologiae Plantarum **39**(8): 175.

Kullmann, W. (1981). Seed germination records of Western Australian plants, Kings Park and Botanic Garden.

Ladd, P. G., C. J. Yates, R. Dillon and R. Palmer (2019). "Pollination ecology of Tetratheca species from isolated, arid habitats (Banded Iron Formations) in Western Australia." Australian journal of botany **67**(3): 248.

Lakon, G. (1949). "The Topographical Tetrazolium Method for Determining the Germinating Capacity of Seeds." Plant Physiology **24**(3): 389-394.

Lannuzel, G., J. Balmot, N. Dubos, M. Thibault and B. Fogliani (2021). "High-resolution topographic variables accurately predict the distribution of rare plant species for conservation area selection in a narrow-endemism hotspot in New Caledonia." Biodiversity and Conservation **30**(4): 963-990.

Larson, J. E., R. L. Sheley, S. P. Hardegree, P. S. Doescher and J. J. James (2015). "Seed and seedling traits affecting critical life stage transitions and recruitment outcomes in dryland grasses." Journal of Applied Ecology **52**(1): 199-209.

Lasky, J. R., B. Bachelot, R. Muscarella, N. Schwartz, J. Forero-Montaña, C. J. Nytch, N. G. Swenson, J. Thompson, J. K. Zimmerman and M. Uriarte (2015). "Ontogenetic shifts in trait-mediated mechanisms of plant community assembly." Ecology **96**(8): 2157-2169.

Lewandrowski, W., T. E. Erickson, E. L. Dalziell and J. C. Stevens (2018). "Ecological niche and bet-hedging strategies for Triodia (R. Br.) seed germination." Annals of Botany **121**(2): 367-375.

Lewandrowski, W., T. E. Erickson, K. W. Dixon and J. C. Stevens (2017). "Increasing the germination envelope under water stress improves seedling emergence in two dominant grass species across different pulse rainfall events." Journal of Applied Ecology **54**(3): 997-1007.

Lewandrowski, W., J. C. Stevens, B. L. Webber, E. L Dalziell, M. S. Trudgen, A. M. Bateman and T. E. Erickson (2021). "Global change impacts on arid zone ecosystems: Seedling establishment processes are threatened by temperature and water stress." Ecology and Evolution.

Lima, F. D., L. E. Ángeles-González, T. S. Leite and S. M. Lima (2020). "Global climate changes over time shape the environmental niche distribution of Octopus insularis in the Atlantic Ocean." Marine Ecology Progress Series **652**: 111-121.

Liu, F.-H., F.-H. Yu, W.-S. Liu, B. O. Krüsi, X.-H. Cai, J. J. Schneller and M. Dong (2007). "Large clones on cliff faces: expanding by rhizomes through crevices." Annals of Botany **100**(1): 51-54.

Liu, R. H., B. Chang, C. Y. Rong, Y. Jiang, R. A. Yang, X. T. Liu, H. F. Zeng and G. H. Fu (2018). "Niche of main woody plant populations of Pterocarya stenoptera community in riparian zone of Lijiang River, China." Ying yong sheng tai xue bao= The journal of applied ecology **29**(12): 3917-3926.

Lloret, F., J. Peñuelas and M. Estiarte (2004). "Experimental evidence of reduced diversity of seedlings due to climate modification in a Mediterranean-type community." Global Change Biology **10**(2): 248-258.

Long, R. L., M. J. Gorecki, M. Renton, J. K. Scott, L. Colville, D. E. Goggin, L. E. Commander, D. A. Westcott, H. Cherry and W. E. Finch-Savage (2015). "The ecophysiology of seed persistence: a mechanistic view of the journey to germination or demise." Biological Reviews **90**(1): 31-59.

Luna, B., B. Pérez, I. Torres and J. Moreno (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**(1): 17-27.

MacArthur, R. H. and E. O. Wilson (2016). The theory of island biogeography, Princeton university press.

Main, B. (1997). "Granite outcrops: A collective ecosystem." Journal of the Royal Society of Western Australia **80**: 113-122.

Mares, M. (1997). "The geobiological interface: Granitic outcrops as a selective force in mammalian evolution." Journal of the Royal Society of Western Australia **80**: 131-139.

Markey, A. S. (2008). "Flora and vegetation of the banded iron formations of the Yilgarn Craton: The central Tallering Land System." Conservation Science Western Australia **7**(1): 121-149.

Markey, A. S. and S. J. Dillon (2011). "Flora and vegetation of the banded iron formations of the Yilgarn Craton: Yalgoo." Conservation Science Western Australia **8**(1): 113-136.

Marques, A. R., A. P. Atman, F. A. Silveira and J. P. de Lemos-Filho (2014). "Are seed germination and ecological breadth associated? Testing the regeneration niche hypothesis with bromeliads in a heterogeneous neotropical montane vegetation." Plant Ecology **215**(5): 517-529.

Martínez-Meyer, E., D. Díaz-Porras, A. T. Peterson and C. Yáñez-Arenas (2013). "Ecological niche structure and rangewide abundance patterns of species." Biology letters **9**(1): 20120637.

Mazerolle, M. (2013). AICcmodavg: Model selection and multimodel inference based on (Q) AICc. R package version 1.32.

Meissner, R. and Y. Caruso (2008). "Flora and vegetation of banded iron formations of the Yilgarn Craton: Mount Gibson and surrounding area." Conservation Science Western Australia **7**(1): 105-120.

Merino-Martín, L., C. Courtauld, L. Commander, S. Turner, W. Lewandrowski and J. Stevens (2017). "Interactions between seed functional traits and burial depth regulate germination and seedling emergence under water stress in species from semi-arid environments." Journal of Arid Environments **147**: 25-33.

Merow, C., A. M. Latimer, A. M. Wilson, S. M. McMahon, A. G. Rebelo and J. A. Silander Jr (2014). "On using integral projection models to generate demographically driven predictions of species' distributions: development and validation using sparse data." Ecography **37**(12): 1167-1183.

Merritt, D., S. Turner, K. Clarke and D. Dixon (2007). "Seed dormancy and germination stimulation syndromes for Australian temperate species." Australian Journal of Botany **55**(3): 336-344.

Mesgaran, M. B., A. Onofri, H. R. Mashhadi and R. D. Cousens (2017). "Water availability shifts the optimal temperatures for seed germination: A modelling approach." Ecological Modelling **351**: 87-95.

Michel, B. E. (1983). "Evaluation of the water potentials of solutions of polyethylene glycol 8000 both in the absence and presence of other solutes." Plant physiology **72**(1): 66-70.

Millar, M., D. Coates and M. Byrne (2013). "Genetic connectivity and diversity in inselberg populations of Acacia woodmaniorum, a rare endemic of the Yilgarn Craton banded iron formations." Heredity **111**(5): 437-444.

Miller, B. P., D. R. Symons and M. D. Barrett (2019). "Persistence of rare species depends on rare events: demography, fire response and phenology of two plant species endemic to a semiarid Banded Iron Formation range." Australian Journal of Botany **67**(3): 268-280.

Monterroso, P., P. Rebelo, P. C. Alves and P. Ferreras (2016). "Niche partitioning at the edge of the range: a multidimensional analysis with sympatric martens." Journal of Mammalogy **97**(3): 928-939.

Mooney, H. A. and W. Billings (1961). "Comparative physiological ecology of arctic and alpine populations of Oxyria digyna." Ecological Monographs **31**(1): 1-29.

Mori, E., F. Ferretti and N. Fattorini (2019). "Alien war: ectoparasite load, diet and temporal niche partitioning in a multi-species assembly of small rodents." Biological Invasions **21**(11): 3305-3318.

Mott, J. J. (1974). "Factors Affecting Seed Germination in Three Annual Species from an Arid Region of Western Australia." Journal of Ecology **62**(3): 699-709.

Murru, V., A. Santo, M. Gallo, C. Cardona, M. Boi and G. Bacchetta (2017). "Comparative germination ecology and seedling growth of two Ibero-Levantine endemic species belonging to the Silene mollissima aggregate (Caryophyllaceae)." Flora **227**: 10-17.

Muscolo, A., M. Sidari, U. Anastasi, C. Santonoceto and A. Maggio (2014). "Effect of PEG-induced drought stress on seed germination of four lentil genotypes." Journal of Plant Interactions **9**(1): 354-363.

Nevill, P., J. Anthony and S. Krauss (2010). "Isolation and characterization of microsatellite markers for the banded ironstone endemic Acacia karina (Leguminosae: Mimosaceae) and cross-species amplification with A. stanleyi and A. jibberdingensis." Conservation Genetics Resources **2**(S1): 321-323.

Olson, M. H. (1996). "Ontogenetic niche shifts in largemouth bass: variability and consequences for first-year growth." Ecology **77**(1): 179-190.

Onofri, A., P. Benincasa, M. B. Mesgaran and C. Ritz (2018). "Hydrothermal-time-to-event models for seed germination." European Journal of Agronomy **101**: 129-139.

Paul, S. K., K. W. Dixon and B. P. Miller (2014). "The persistence and germination of fern spores in fire-prone, semi-arid environments." Australian journal of botany **62**(6): 518.

Pearce, P., P. Van Der Moezel and D. Bell (1990). "Seed germination under salinity stress in Western Australian species of Eucalyptus." Seed Science and Technology (Switzerland) **18**(1): 113-118.

Peers, M. J., D. H. Thornton and D. L. Murray (2012). "Reconsidering the specialist-generalist paradigm in niche breadth dynamics: resource gradient selection by Canada lynx and bobcat." PloS one **7**(12): e51488.

Peterson, M. E., R. M. Daniel, M. J. Danson and R. Eisenthal (2007). "The dependence of enzyme activity on temperature: determination and validation of parameters." Biochemical Journal **402**(2): 331-337.

Phillips, R. D., R. Peakall, M. F. Hutchinson, C. C. Linde, T. Xu, K. W. Dixon and S. D. Hopper (2014). "Specialized ecological interactions and plant species rarity: the role of pollinators and mycorrhizal fungi across multiple spatial scales." Biological Conservation **169**: 285-295.

Pironon, S., J. Villellas, W. F. Morris, D. F. Doak and M. B. García (2015). "Do geographic, climatic or historical ranges differentiate the performance of central versus peripheral populations?" Global Ecology and Biogeography **24**(6): 611-620.

Pironon, S., J. Villellas, W. Thuiller, V. M. Eckhart, M. A. Geber, D. A. Moeller and M. B. García (2018). "The 'Hutchinsonian niche'as an assemblage of demographic niches: implications for species geographic ranges." Ecography **41**(7): 1103-1113.

Poot, P., S. D. Hopper and J. M. van Diggelen (2012). "Exploring rock fissures: does a specialized root morphology explain endemism on granite outcrops?" Annals of Botany **110**(2): 291-300.

Porembski, S. (2000). "The invasibility of tropical granite outcrops ('inselbergs') by exotic weeds." Journal of the Royal Society of Western Australia **83**(3): 131-134.

Porembski, S. (2007). "Tropical inselbergs: habitat types, adaptive strategies and diversity patterns." Brazilian Journal of Botany **30**(4): 579-586.

Porembski, S. and W. Barthlott (2000). "Granitic and gneissic outcrops (inselbergs) as centers of diversity for desiccation-tolerant vascular plants." Plant Ecology **151**(1): 19-28.

Porembski, S. and W. Barthlott (2012). Inselbergs: biotic diversity of isolated rock outcrops in tropical and temperate regions. Berlin, Springer.

Rajapakshe, R. P., S. R. Turner, A. T. Cross and S. Tomlinson (2020). "Hydrological and thermal responses of seeds from four co-occurring tree species from southwest Western Australia." Conservation Physiology **8**(1): coaa021.

Reichman, S. M., S. M. Bellairs and D. R. Mulligan (2006). "effects of temperature and salinity on Acacia harpophylla (brigalow) (Mimosaceae) germination." effects of temperature and salinity on Acacia harpophylla (brigalow) (Mimosaceae) germination **28**(2): 175-178.

Rincón-Rosales, R., N. Culebro-Espinosa, F. Gutierrez-Miceli and L. Dendooven (2003). "Scarification of seeds of Acacia angustissima (Mill.) Kuntze and its effect on germination." Seed Science and Technology **31**(2): 301-307.

Ritz, C. and J. C. Streibig (2008). Nonlinear regression with R. New York, Springer

Ritz, C. and J. C. Streibig (2012). "Dose response curves and other nonlinear curves in Weed Science and Ecotoxicology with the add-on package drc in R ".

Robinson, T. P., G. Di Virgilio, D. Temple-Smith, J. Hesford and G. W. Wardell-Johnson (2019). "Characterisation of range restriction amongst the rare flora of Banded Ironstone Formation ranges in semiarid south-western Australia." Australian journal of botany **67**(3): 234.

Roche, S., K. W. Dixon and J. S. Pate (1997). "Seed Ageing and Smoke: Partner Cuesin the Amelioration of Seed Dormancyin Selected Australian Native Species." Australian journal of botany **45**(5): 783-815.

Rosbakh, S., E. Pacini, M. Nepi and P. Poschlod (2018). "An unexplored side of regeneration niche: seed quantity and quality are determined by the effect of temperature on pollen performance." Frontiers in Plant Science **9**: 1036.

Rosental, L., H. Nonogaki and A. Fait (2014). "Activation and regulation of primary metabolism during seed germination." Seed science research **24**(1): 1-15.

Ruiz-Talonia, L., D. Carr, R. Smith, R. D. B. Whalley and N. Reid (2018). "Effect of temperature and light on germination of 10 species of *Eucalyptus* from north-western NSW." Australian Journal of Botany **66**(8): 657-666.

Saatkamp, A., A. Cochrane, L. Commander, L. K. Guja, B. Jimenez-Alfaro, J. Larson, A. Nicotra, P. Poschlod, F. A. O. Silveira, A. T. Cross, E. L. Dalziell, J. Dickie, T. E. Erickson, A. Fidelis, A. Fuchs, P. J. Golos, M. Hope, W. Lewandrowski, D. J. Merritt, B. P. Miller, R. G. Miller, C. A. Offord, M. K. J. Ooi, A. Satyanti, K. D. Sommerville, R. Tangney, S. Tomlinson, S. Turner and J. L. Walck (2019). "A research agenda for seed-trait functional ecology." The New phytologist **221**(4): 1764-1775.

Safford, H., J. Viers and S. Harrison (2005). "Serpentine endemism in the California flora: a database of serpentine affinity." Madrono **52**(4): 222-257.

Sánchez-Gómez, D., F. Valladares and M. A. Zavala (2006). "Performance of seedlings of Mediterranean woody species under experimental gradients of irradiance and water availability: trade-offs and evidence for niche differentiation." New phytologist **170**(4): 795-806.

Sarkar, D. (2008). Lattice: multivariate data visualization with R, Springer Science & Business Media.

Scanlon, T. T. and G. Doncon (2020). "Rain, rain, gone away: decreased growing-season rainfall for the dryland cropping region of the south-west of Western Australia." Crop and Pasture Science **71**(2): 128-133.

Schut, A. G., G. W. Wardell-Johnson, C. J. Yates, G. Keppel, I. Baran, S. E. Franklin, S. D. Hopper, K. P. Van Niel, L. Mucina and M. Byrne (2014). "Rapid characterisation of vegetation structure to predict refugia and climate change impacts across a global biodiversity hotspot." PLoS One **9**(1): e82778.

Seal, C. E., L. J. Barwell, T. J. Flowers, E. M. Wade and H. W. Pritchard (2018). "Seed germination niche of the halophyte Suaeda maritima to combined salinity and temperature is characterised by a halothermal time model." Environmental and experimental botany **155**: 177-184.

Seebacher, F. and C. E. Franklin (2005). "Physiological mechanisms of thermoregulation in reptiles: a review." Journal of Comparative Physiology B **175**(8): 533-541.

Sexton, J. P., P. J. McIntyre, A. L. Angert and K. J. Rice (2009). "Evolution and ecology of species range limits." Annu. Rev. Ecol. Evol. Syst. **40**: 415-436.

Silcock, J., C. Simmons, L. Monks, R. Dillon, N. Reiter, M. Jusaitis, P. A. Vesk, M. Byrne and D. Coates (2019). "Threatened plant translocation in Australia: A review." Biological Conservation **236**: 211-222.

Singh, B., S. K. Singh, S. K. Matcha, V. G. Kakani, C. Wijewardana, D. Chastain, W. Gao and K. R. Reddy (2019). "Parental Environmental Effects on Seed Quality and

Germination Response to Temperature of Andropogon gerardii." Agronomy **9**(6): 304.

Soberón, J. and M. Nakamura (2009). "Niches and distributional areas: concepts, methods, and assumptions." Proceedings of the National Academy of Sciences **106**(Supplement 2): 19644-19650.

Steadman, K. J. (2004). "Dormancy release during hydrated storage in Lolium rigidum seeds is dependent on temperature, light quality, and hydration status." Journal of Experimental Botany **55**(398): 929-937.

Steadman, K. J. and H. W. Pritchard (2004). "Germination of Aesculus hippocastanum seeds following cold-induced dormancy loss can be described in relation to a temperature-dependent reduction in base temperature (Tb) and thermal time." New Phytologist **161**(2): 415-425.

Tapper, S. L., M. Byrne, C. Yates, G. Keppel, S. Hopper, K. Van Niel, A. Schut, L. Mucina and G. Wardell-Johnson (2014). "Isolated with persistence or dynamically connected? Genetic patterns in a common granite outcrop endemic." Diversity and Distributions **20**(9): 987-1001.

Tapper, S. L., M. Byrne, C. J. Yates, G. Keppel, S. D. Hopper, V. K. P. Niel, A. G. T. Schut, L. Mucina and G. W. Wardell-Johnson (2014). "Prolonged isolation and persistence of a common endemic on granite outcrops in both mesic and semi-arid environments." Journal of Biogeography **41**(11): 2032-2044.

Tarszisz, E., S. Tomlinson, M. E. Harrison, H. C. Morrough-Bernard and A. J. Munn (2017). "Gardeners of the forest: effects of seed handling and ingestion by orangutans on germination success of peat forest plants." Biological Journal of the Linnean Society **123**(1): 125-134.

R Core Team (2013). R: A language and environment for statistical computing. Vienna, R Foundation for Statistical Computing.

Thackway, R. and I. D. Cresswell (1997). "A bioregional framework for planning the national system of protected areas in Australia." Natural Areas Journal **17**(3): 241-247.

Tomlinson, S. (2019). "The mathematics of thermal sub-optimality: Nonlinear regression characterization of thermal performance of reptile metabolic rates." Journal of Thermal Biology **81**: 49-58.

Tomlinson, S., K. W. Dixon, R. K. Didham and S. D. Bradshaw (2015). "Physiological plasticity of metabolic rates in the invasive honey bee and an endemic Australian bee species." Journal of Comparative Physiology B **185**(8): 835-844.

Tomlinson, S., W. Lewandrowski, C. P. Elliott, B. P. Miller and S. R. Turner (2020). "High-resolution distribution modeling of a threatened short-range endemic plant informed by edaphic factors." Ecology and evolution **10**(2): 763-777.

Tudela-Isanta, M., E. Ladouceur, M. Wijayasinghe, H. W. Pritchard and A. Mondoni (2018). "The seed germination niche limits the distribution of some plant species in calcareous or siliceous alpine bedrocks." Alpine Botany **128**(1): 83-95.

Turner, S., K. Steadman, S. Vlahos, J. Koch and K. Dixon (2013). "Seed Treatment Optimizes Benefits of Seed Bank Storage for Restoration-Ready Seeds: The Feasibility of Prestorage Dormancy Alleviation for Mine-Site Revegetation."

Turner, S. R., W. Lewandrowski, C. P. Elliott, L. Merino-Martín, B. P. Miller, J. C. Stevens, T. E. Erickson and D. J. Merritt (2018). "Seed ecology informs restoration approaches for threatened species in water-limited environments: a case study on the short-range Banded Ironstone endemic Ricinocarpos brevis (Euphorbiaceae)." Australian Journal of Botany **65**(8): 661-677.

Tweddle, J. C., J. B. Dickie, C. C. Baskin and J. M. Baskin (2003). "Ecological aspects of seed desiccation sensitivity." Journal of Ecology **91**(2): 294-304.

Van Couwenberghe, R., C. Collet, J. C. Pierrat, K. Verheyen and J. C. Gégout (2013). "Can species distribution models be used to describe plant abundance patterns?" Ecography **36**(6): 665-674.

Vincent, B. J., S. Barrett, A. Cochrane, J. A. Plummer and M. Renton (2015). "Conservation biology of two endemic Beyeria species (Euphorbiaceae) from southern Western Australia." Australian Journal of Botany **63**(6): 484-496.

Viscarra Rossel, R., C. Chen, M. Grundy, R. Searle, D. Clifford, N. Odgers, K. Holmes, T. Griffin, C. Liddicoat and D. Kidd (2014). Soil and Landscape Grid National Soil Attribute Maps - Clay (3" resolution), Release 1. v5. CSIRO. Data Collection. https://doi.org/10.4225/08/546EEE35164BF.

Viscarra Rossel, R., C. Chen, M. Grundy, R. Searle, D. Clifford, N. Odgers, K. Holmes, T. Griffin, C. Liddicoat and D. Kidd (2014). Soil and Landscape Grid National Soil Attribute Maps - Sand (3" resolution), Release 1. v4. CSIRO Data Collection. http://doi.org/10.4225/08/546F29646877E.

Viscarra Rossel, R., C. Chen, M. Grundy, R. Searle, D. Clifford, N. Odgers, K. Holmes, T. Griffin, C. Liddicoat and D. Kidd (2014). Soil and Landscape Grid National Soil Attribute Maps - Silt (3" resolution), Release 1. v5. CSIRO Data Collection. https://doi.org/10.4225/08/546F48D6A6D48.

Walck, J. L., J. M. Baskin and C. C. Baskin (1997). "A comparative study of the seed germination biology of a narrow endemic and two geographically-widespread species of Solidago (Asteraceae). 1. Germination phenology and effect of cold stratification on germination." Seed Science Research **7**(1): 47-58.

Walder, T. and B. Erschbamer (2015). "Temperature and drought drive differences in germination responses between congeneric species along altitudinal gradients." Plant Ecology **216**(9): 1297-1309.

Wang, J. H., C. C. Baskin, X. L. Cui and G. Z. Du (2009). "Effect of phylogeny, life history and habitat correlates on seed germination of 69 arid and semi-arid zone species from northwest China." Evolutionary Ecology **23**(6): 827-846.

Ward, B. G., T. B. Bragg and B. A. Hayes (2018). "Effects of chaining and burning in shrublands of the Peron Peninsula, Shark Bay, Western Australia." International Journal of Wildland Fire **27**(9): 623-635.

Warren, R. J., J. P. Wright and M. A. Bradford (2011). "The putative niche requirements and landscape dynamics of Microstegium vimineum: an invasive Asian grass." Biological Invasions **13**(2): 471-483.

Wege, J., K. Thiele, K. Shepherd, R. Butcher, T. Macfarlane and D. Coates (2015). "Strategic taxonomy in a biodiverse landscape: a novel approach to maximizing conservation outcomes for rare and poorly known flora." Biodivers Conserv **24**(1): 17-32.

White, M. E. (1994). After the greening : the browning of Australia / Mary E. White. Kenthurst, NSW, Kenthurst, NSW : Kangaroo Press.

Wickham, H. and M. H. Wickham (2007). The ggplot package.

Wilson, S. K., S. C. Burgess, A. J. Cheal, M. Emslie, R. Fisher, I. Miller, N. V. Polunin and H. P. Sweatman (2008). "Habitat utilization by coral reef fish: implications for specialists vs. generalists in a changing environment." Journal of Animal Ecology: 220-228.

Withers, P. (2000). "Overview of granite outcrops in Western Australia." Journal of the Royal Society of Western Australia **83**: 103-108.

Wood, S. N. (2011). "Fast stable restricted maximum likelihood and marginal likelihood estimation of semiparametric generalized linear models." Journal of the Royal Statistical Society: Series B (Statistical Methodology) **73**(1): 3-36.

Wyatt, R. (1997). "Reproductive ecology of granite outcrop plants from the south-eastern United States." Journal of the Royal Society of Western Australia **80**: 123-129.

Yan, W. and L. Hunt (1999). "An equation for modelling the temperature response of plants using only the cardinal temperatures." Annals of Botany **84**(5): 607-614.

Yates, C. J., N. Gibson, N. E. Pettit, R. Dillon and R. Palmer (2011). "The ecological relationships and demography of restricted ironstone endemic plant species: implications for conservation." Australian Journal of Botany **59**(7): 691-699.

Yates, C. J., R. J. Hobbs and L. Atkins (2000). "Establishment of Perennial Shrub and Tree Species in Degraded Eucalyptus salmonophloia (Salmon Gum) Remnant Woodlands: Effects of Restoration Treatments." Restoration Ecology **8**(2): 135-143.

Yates, C. J., R. J. Hobbs and R. W. Bell (1994). "Landscape-scale disturbances and regeneration in semi-arid woodlands of southwestern Australia." Pacific Conservation Biology **1**(3): 214-221.

Yates, C. J., R. J. Hobbs and R. W. Bell (1996). "Factors Limiting the Recruitment of Eucalyptus salmonophloia in Remnant Woodlands. III. Conditions Necessary for Seed Germination." Australian Journal of Botany **44**(3): 283-296.

Yates, C. J., S. D. Hopper, A. Brown and S. Leeuwen (2003). "Impact of two wildfires on endemic granite outcrop vegetation in Western Australia." Journal of Vegetation Science **14**(2): 185-194.

Yates, C. J. and P. G. Ladd (2004). "Breeding system, pollination and demography in the rare granite endemic shrub Verticordia staminosa ssp. staminosa in south-west Western Australia." Austral Ecology **29**(2): 189-200.

Zogas, A., E. Kosman and M. Sternberg (2020). "Germination strategies under climate change scenarios along an aridity gradient." Journal of Plant Ecology **13**(4): 470-477.

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Appendix 1 Supplementary data for Chapter 2

Appendix. 1.1 Distributional extent of the study species according to Australian Virtual Herbarium (https://avh.chah.org.au).

Species	Area of occupancy in Western Australia (km^2)	Extent of occurrence in Western Australia (km^2)
Eucalyptus caesia* subsp. *caesia	176	67,607
Eucalyptus ornata	68	7,420
Eucalyptus salmonophloia	1,988	332,027
Eucalyptus salubris	2,072	436,090

Appendix. 1.2 Mean temperatures (± SE in parentheses) inside seed incubators for the duration of experiments.

Experiment	**Species**	**Treatment regime**	**No. of replicates**	**Temperature (°C)**
Thermal performance		°C		
	E. caesia subsp. *caesia*	5	8	5.339 (0.042)
		10	8	10.925 (0.001)
		15	8	14.935 (0.027)
		20	8	18.665 (0.019)
		25	8	25.197 (0.007)
		30	8	30.197 (0.02)
		35	8	34.102 (0.036)
	E. ornata	5	8	5.302 (0.042)
		10	8	11.011 (0.044)
		15	8	14.788 (0.021)
		20	8	18.817 (0.019)
		25	8	25.054 (0.01)
		30	8	30.131 (0.023)
		35	8	34.209 (0.033)

	E. salmonophloia	5	8	6.267 (0.099)
		10	8	11.180 (0.044)
		15	8	15.673 (0.026)
		20	8	18.508 (0.039)
		25	8	24.895 (0.007)
		30	8	30.116 (0.026)
		35	8	33.783 (0.041)
	E. salubris	5	8	6.237 (0.094)
		10	8	10.975 (0.037)
		15	8	15.576 (0.029)
		20	8	18.089 (0.029)
		25	8	24.934 (0.007)
		30	8	30.137 (0.019)
		35	8	34.064 (0.028)

Water stress tolerance at 20 °C		MPa		
	E. caesia subsp. *caesia*	0	8	18.427 (0.018)
		-0.1	8	18.549 (0.02)
		-0.2	8	18.559 (0.018)
		-0.4	8	18.334 (0.033)
		-0.7	8	18.290 (0.03)
	E. ornata	0	8	18.354 (0.024)
		-0.1	8	18.523 (0.009)
		-0.2	8	18.424 (0.024)
		-0.4	8	18.521 (0.009)
		-0.7	8	18.532 (0.032)
	E. salmonophloia	0	8	18.424 (0.014)
		-0.1	8	18.532 (0.009)
		-0.2	8	18.426 (0.009)
		-0.4	8	18.537 (0.006)
		-0.7	8	18.243 (0.028)
	E. salubris	0	8	18.437 (0.01)
		-0.1	8	18.386 (0.028)
		-0.2	8	18.267 (0.024)
		-0.4	8	18.236 (0.014)
		-0.7	8	18.752 (0.032)

Appendix. 1.3 Akaike information criterion comparison (AICc) of the unique parameter permutations of the curvilinear log-logistic model for the number of days since incubation and daily cumulative germination rate of four native western Australian *Eucalyptus* species at all temperature regimes **(a)** and water stress regimes **(b)** assessed as part of this study.

	Model	Residual Deviance	AICc	df	Weight
a					
	Convergent common curve	8.894	37950.87	4	0
	Species	7.635	36352.77	13	0
	Temperature regime	5.630	33154.56	22	0
	Species + temperature regime	2.608	25106.87	76	1
b					
	Convergent common curve	9.273	28589.64	4	0
	Species	7.981	27422.31	13	0
	Water stress regime	2.664	25819.59	16	0
	Species + water stress regime	6.503	18869.09	61	1

Appendix. 1.4 Preparation of PEG solutions of different concentrations in order to subject seeds of the test species to different water stress regimes. Following the method described by Michel (1983) for each treatment regime, the mass (g) of PEG (solute) required to be dissolved in a known volume of distilled water (ml) in order to induce a known level of water stress (at the selected temperature regime; 20 °C) was calculated as follows:

Required water stress level at 20 °C (MPa)	Mass of PEG (g/ml H_2O)
0	0
-0.1	0.7777
-0.2	0.1160
-0.4	0.1705
-0.7	0.2307
-1.0	0.2789
-1.5	0.3453

Appendix. 2 Supplementary data for Chapter 3

Appendix. 2.1 Distributional extent (Based on Atlas of living Australia) and seed traits of the *Acacia* species used in this study. Shading indicates the two range-restricted species of conservation concern, classified as either Priority 1 (P1) or declared rare flora (DRF) in Western Australia, found in close association with BIF habitats (Western Australian Herbarium 1998).

Species	**Area of occupancy in Western Australia (km^2)**	**Extent of occurrence in Western Australia (km^2)**	**100-Seed Weight (g)**	**Seed fill (%) (n = 100)**	**Viability (%) of filled seeds (n = 10)**		**Germinability (%) of viable seeds** (n = 20)**		
					Uncut	**Cut**	**Non-treated**	**Heat treated****	**Acid scarified*****
Acacia karina	44	1,515	2.873	90	0	100	0	10	95
Acacia woodmaniorum	12	380	1.662	93	30	100	0	10	90
Acacia assimilis	1,200	435,903	0.245	99	10	100	0	85	-
Acacia exocarpoides	564	343,950	3.508	98	0	100	0	100	-
Acacia ramulosa	1,128	984,371	9.910	77	0	80	20	36	100

*Initial germination success of filled seeds was quantified by incubating 20 seeds of each taxa on moist germination paper in Petri dishes under constant darkness at 20 °C followed by regular scoring of germination rate.

**Exposed to hot water (> 90 °C) for 2 mins.

***Exposed to 98 % (v/v) H_2SO_4 for 60 minutes prior to rinsing in running water and washing in 100 mM sodium bicarbonate neutralising solution.

Appendix. 2.2 Mean temperatures (± SE in parentheses) inside seed incubators for the duration of experiments.

Water stress regime (MPa)	**Thermal regime (°C)**	**Temperature (°C)**
0, -0.4, -0.8	10	10.620 (0.018)
	15	14.844 (0.015)
	20	17.766 (0.014)
	25	25.297 (0.006)
	30	30.491 (0.012)
-0.2, -0.6, -1.0	10	10.415 (0.33)
	15	15.125 (0.015)
	20	17.671 (0.042)
	25	25.532 (0.006)
	30	29.789 (0.009)

Appendix. 3 Supplementary data for Chapter 4

Appendix. 3.1 Distributional extent of the study species according to Australian Virtual Herbarium (https://avh.chah.org.au/).

Species	Area of occupancy in Western Australia (km^2)	Extent of occurrence in Western Australia (km^2)
Tetratheca aphylla **subsp. F.Muell.** ***aphylla***	16	107
Tetratheca erubescens **J.P.Bull**	0.035	< 2
Tetratheca harperi **F.Muell.**	12	2,412
Tetratheca paynterae **Alford subsp.** ***paynterae***	40	9,473

Appendix. 3.2 Collection details (location and date), seed traits (mass, fill and viability) and initial germination following the application of several dormancy alleviation treatments of the four *Tetratheca* species used in this study.

Species	**Collection location**	**Collection date**	**Seed Weight (mg)**	**Seed fill (%)**	**Viability (%) of filled seeds (n=15)**	**Germinability (%) of filled seeds* following dormancy alleviation treatments** (n = 15)**				
						Incubation on H_2O for 8 weeks at 15°C.	Incubation on 1µM KAR_1 for 8 weeks at 15°C.	Stratification at 30°C for 4 weeks followed by incubation at 15°C on H_2O for 4 weeks	Stratification at 30°C for 4 weeks followed by incubation at 15°C on 1µM KAR_1 for 4 weeks	Stratification at 30°C for 4 weeks followed by incubation at 20°C on 1µM KAR_1 for 4 weeks
Tetratheca aphylla* subsp. F.Muell. *aphylla	Helena and Aurora Range	Feb. 2019	3.0	79.8 ± 0.1	93.3	0.0	20.0	7.1	26.7	6.5
***Tetratheca erubescens* J.P.Bull**	Koolyanobbing Range	Feb. 2019	5.1	88.3 ± 1.2	100.0	57.1	46.2	92.9	92.9	80.0
***Tetratheca harperi* F.Muell.**	Mt Jackson Range	Feb. 2019	3.3	96.4 ± 1.7	93.3	57.1	66.7	100.0	78.6	93.3
Tetratheca paynterae* Alford subsp. *paynterae	Windarling Range	Feb. 2019	1.6	83.9 ± 0.2	93.3	42.9	53.8	76.9	92.9	73.3

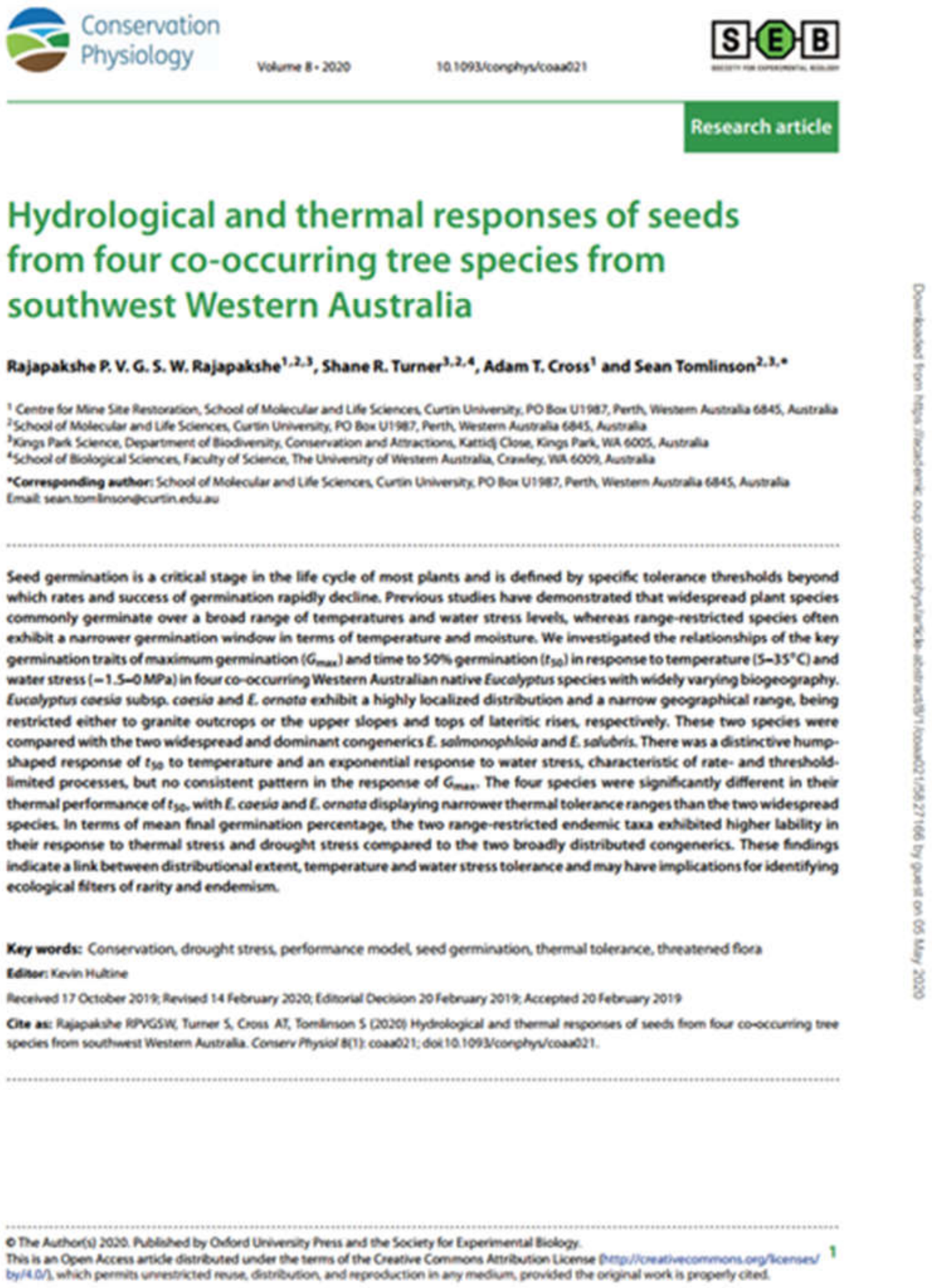
*Initial germination success of filled seeds was quantified by incubating 15 treated seeds of each taxa on moist germination paper in Petri dishes followed by regular scoring of germination.

**Treatments imposed to alleviate seed dormancy and promote germination (warm stratification and karrikinolide (KAR_1).

Appendix. 3.3 Mean temperatures (± SE in parentheses) inside seed incubators for the duration of experiments.

Thermal regime (°C)	Temperature (°C)
10	9.4 (0.02)
15	13.8 (0.01)
20	20.7 (0.03)
22	21.1 (0.02)
25	25.5 (0.01)

Appendix. 4 The publication that has resulted from this research



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Research article

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 congenerics *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 congenerics. 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.

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

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Research article

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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

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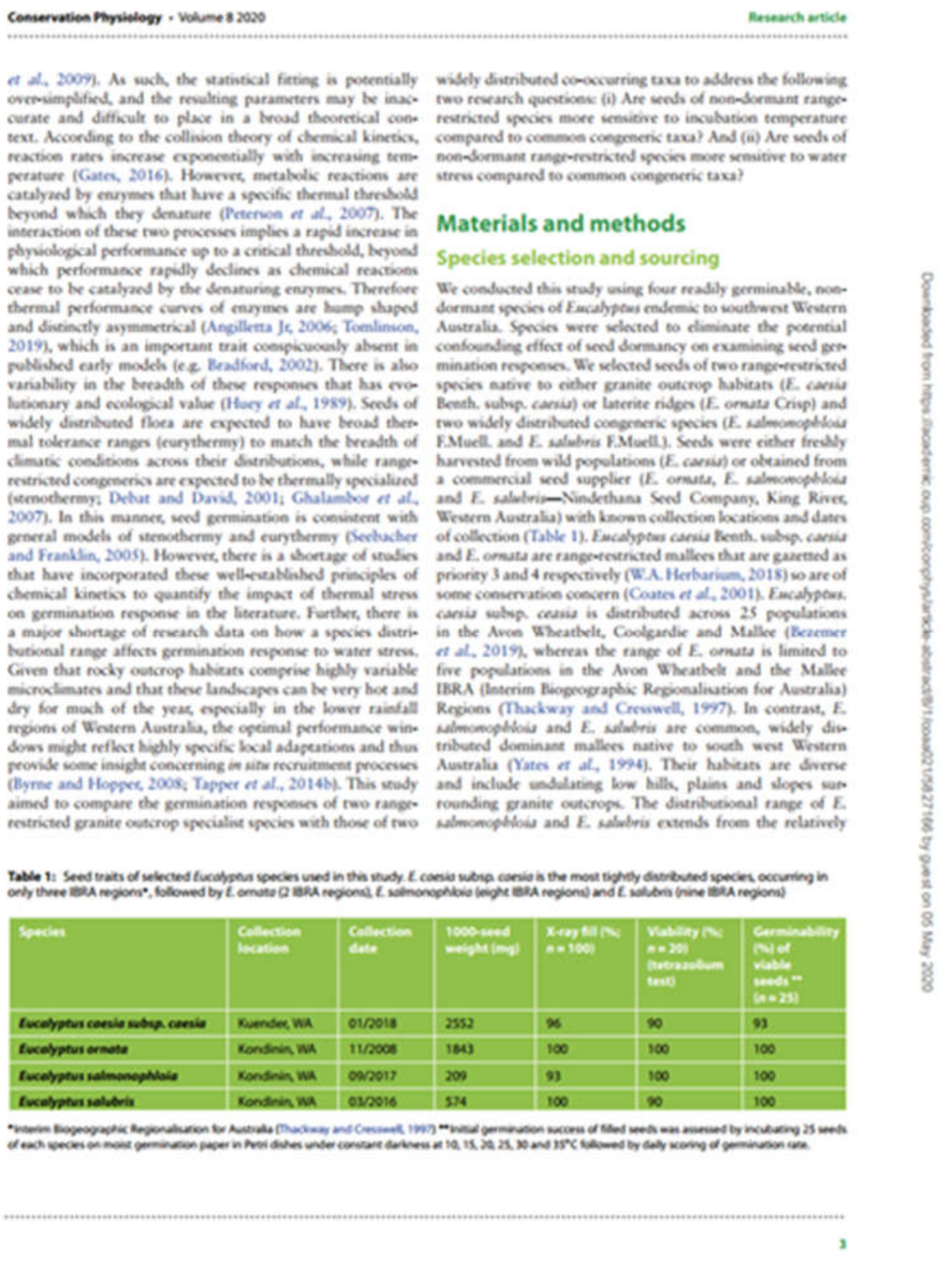


et al., 2009). As such, the statistical fitting is potentially over-simplified, and the resulting parameters may be inaccurate and difficult to place in a broad theoretical context. According to the collision theory of chemical kinetics, reaction rates increase exponentially with increasing temperature (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 (Angilletta 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 distributional range 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. *ceasia* 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 Creswell, 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

Table 1: Seed traits of selected *Eucalyptus* species used in this study. *E. caesia* subsp. *caesia* is the most tightly distributed species, occurring in only three IBRA regions*, followed by *E. ornata* (2 IBRA regions), *E. salmonophloia* (eight IBRA regions) and *E. salubris* (nine IBRA regions)

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 ($C_{19}H_{15}N_4Cl$) 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.

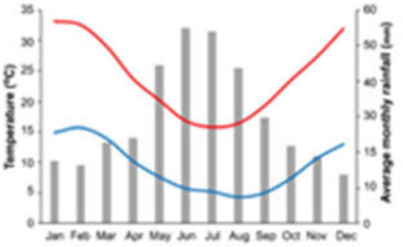


Figure 1: Long-term climatic data for the Kondinin meteorological station, encompassing average monthly maximum (red line) and minimum (blue line) temperatures and average monthly rainfall (grey bars).The temperature range of the experimental trials conducted here span the full temperature range in the region, from 5 to 35°C

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);

$$\text{germination} = \frac{G_{max}}{1 + \frac{time}{t_{50}}^{b}} \tag{1}$$

where G_{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_{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 of 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 *AICmodavg* 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_{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_{max} values for each replicate of all species incubated under different treatment regimes. We used model estimates for b, G_{max} and t_{50} to calculate time (in days) to reach G_{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 and Hunt, 1999). Therefore, we estimated unimodal asymmetrical model fits for the $1/t_{50}$ estimates for our thermal response data using a thermal performance function which has been described by Yan and Hunt (1999) for the temperature response of maximum rate of growth in plants;

$$r_{max} = R_{max}\left(\frac{T_{max} - T}{T_{max} - T_{opt}}\right)\left(\frac{T}{T_{opt}}\right)^{\frac{T_{opt}}{T_{max} - T_{opt}}} \tag{2}$$

where r_{max} is the maximum germination rate at any temperature (T), T_{opt} is the optimum temperature for germination at the peak of the performance function, T_{max} is the limit of thermal tolerance, where germination ceases, and R_{max} is the asymptotic maximum germination rate at T_{opt}. Henceforth, $1/t_{50}$ will be referred to as the thermal performance of maximum germination rate, r_{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_{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;

$$t_{50} = g_0 + e^{\{k*(w+w_c)\}} \tag{3}$$

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 w_c 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_{max}, T_{opt}, T_{max}, g_0 and w_c for each species in terms of t_{50} and G_{max}.

Results

Germination modelling

The two range-restricted species displayed higher final germination percentages over a wider range of temperatures

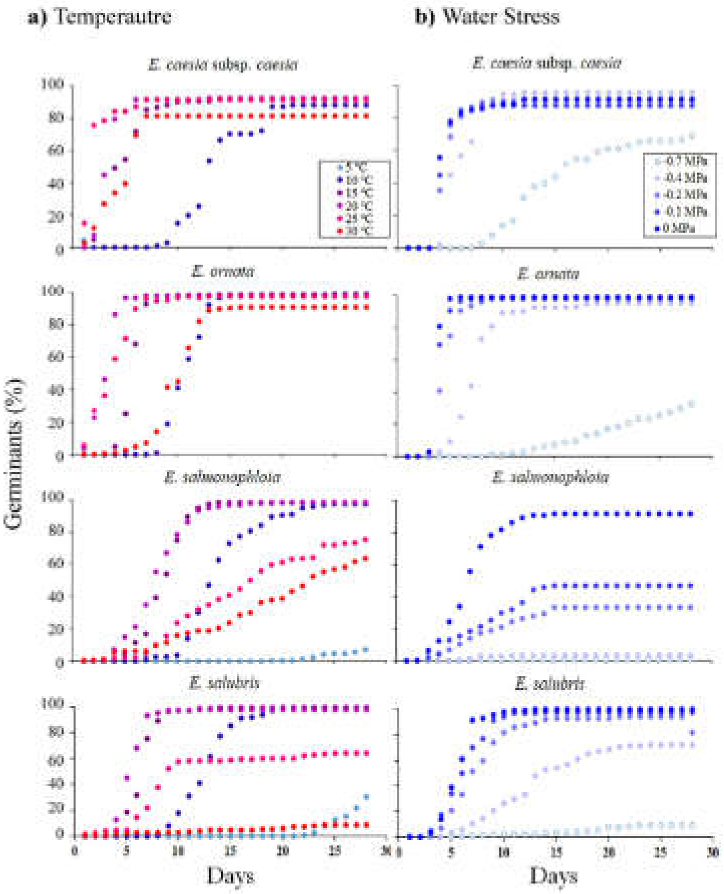


Figure 2: Mean cumulative germination percentage for replicates of seeds of four Western Australian *Eucalyptus* species following exposure to an increasing range of temperatures (a) and water stress levels (b) incubated in constant darkness for 28 days. Eight replicates of 25 seeds were used for each treatment. Error bars depicting standard error of the mean have been omitted for clarity

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

germination occurred was 35°C (Fig. 2a). Within the range of 15–25°C, estimated time to reach G_{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_{max} and lengthened the time required to reach G_{max} (Fig. 3a). However, for *E. salubris* the time to reach G_{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_{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_{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_{max}, T_{opt} and T_{max} values defining the thermal performance of r_{max} for each species (Equation 2, Fig. 4a). For *E. caesia* subsp. *caesia* and *E. ornata*, estimated T_{opt} values were 25.4 ± 0.25 and 23.0 ± 0.37°C respectively, whereas for *E. salmonophloia* and *E. salubris* estimates for T_{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_{max} was within the range of 30.5–32°C. A thermal performance function could not be resolved for the G_{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_c 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_c values were −0.266 ± 0.098, −0.149 ± 0.049 and − 0.057 ± 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_{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 stenothermy 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-Talonia *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

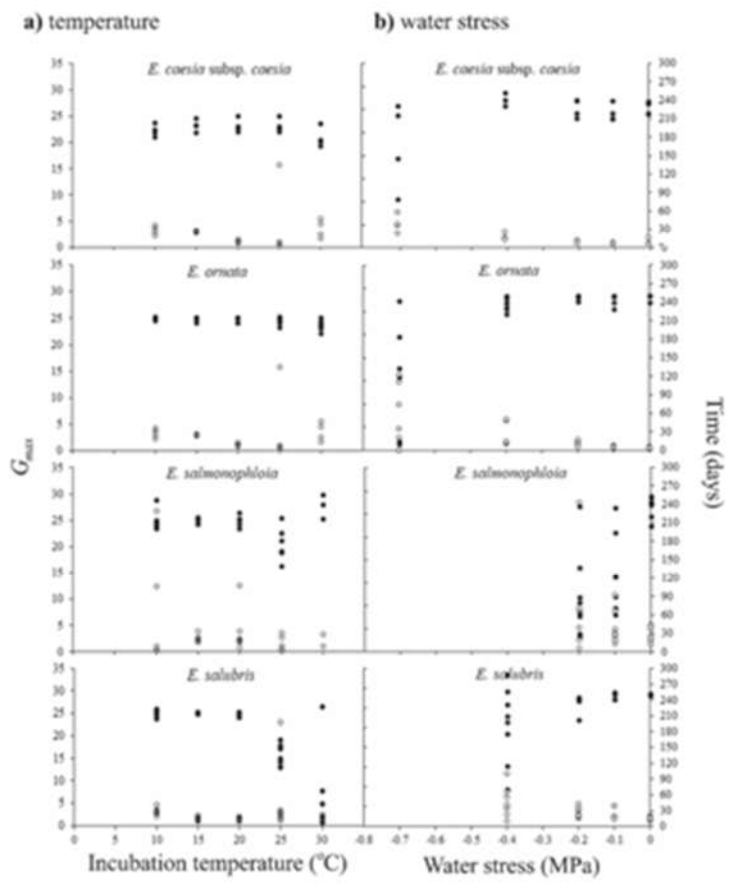


Figure 3: Estimates for maximum germination (G_{max}) and time to reach G_{max} predicted by a three-parameter log-logistic function for the germination responses of four Western Australian *Eucalyptus* species following incubation in constant darkness at varying temperature regimes (**a**) and water 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

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

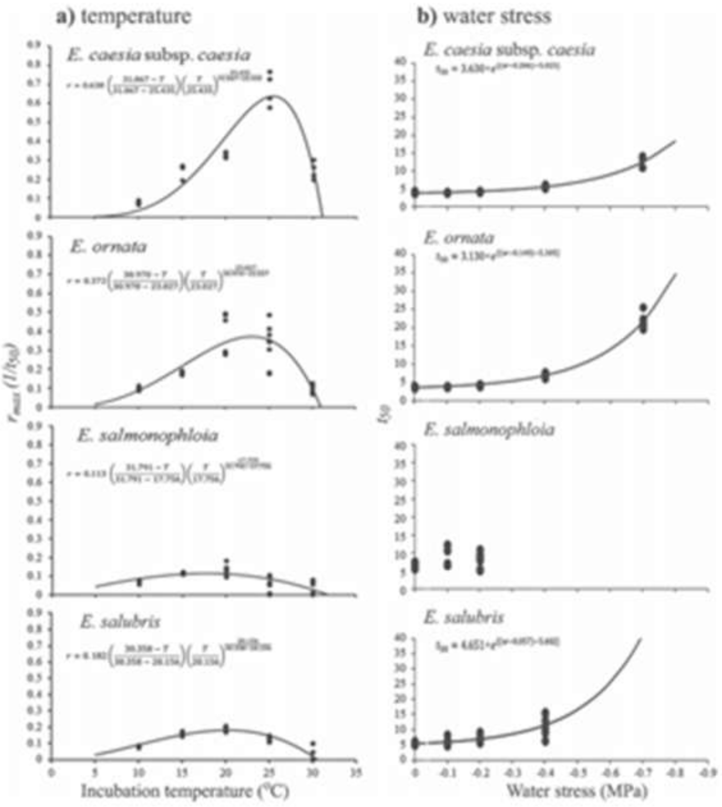


Figure 4: Thermal performance and water stress tolerance in four Western Australian *Eucalyptus* species in terms of time to reach 50% germination (t_{50}). **(a)** Dots represent 1/t_{50} estimates for each replicate of seeds after exposure to different temperature regimes and the smooth lines represent the permutations of the thermal performance curve fitted to the 1/t_{50} estimates (r_{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_{50}). Deviations from favourable temperature ranges for germination increased time to reach G_{max} and variability in estimates for time to reach G_{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_{opt} estimates for all four species were within a range of 17–26°C, and T_{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_{opt} and T_{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).

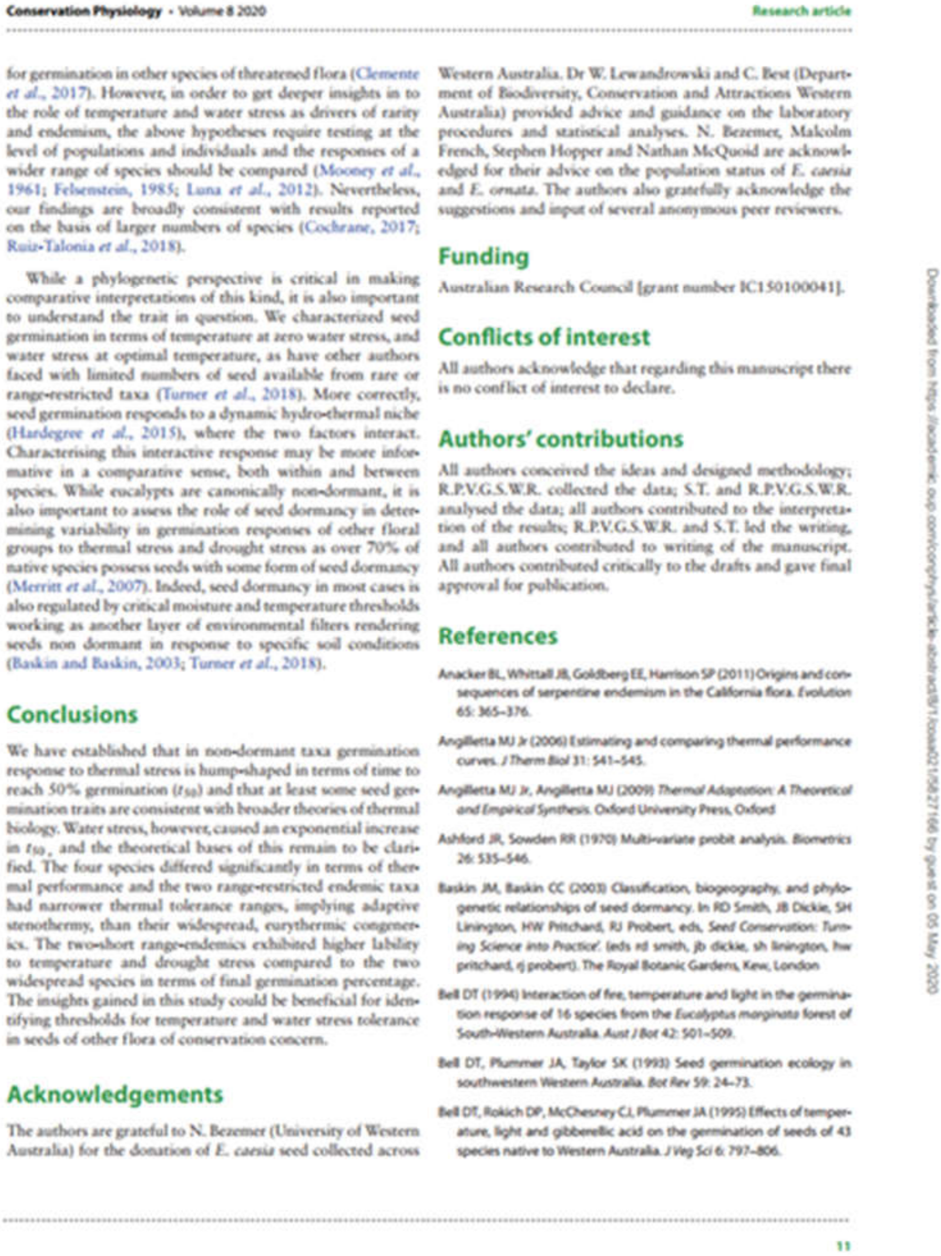
Patterns of distribution size and endemism

In terms of final germination percentage, the two range-restricted endemic taxa were more tolerant of both thermal stress, represented by higher T_{opt}, and water stress, represented by lower ψ_c, compared to their widespread congenerics (Fig. 2), but had narrower ranges of thermal tolerance in terms of r_{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_{max}, combined with high drought tolerance in terms of G_{max}, and time to reach G_{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_{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 congenerics 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 in to 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 (t_{50}) and that at least some seed germination traits are consistent with broader theories of thermal biology. Water stress, however, caused an exponential increase in t_{50}, 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 congenerics. 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). Thermperf: 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 phylogeography and population genetics of *Eucalyptus caesia* (Myrtaceae) in Western Australia. *Biol J Linn Soc* 93: 177–188.

Carta 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 *Saxifraga* 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.

Hardegree SP, Walters CT, Boehm AR, Olsoy 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. *J R Soc West Aust* 80: 141–158.

Hopper SD, Gioia P (2004) The southwest Australian floristic region: evolution and conservation of a global hot spot of biodiversity. *Annu. Rev. Ecol. Evol. Syst.* 35: 623–650.

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. *AMBIO: 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 BO, 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: granitic outcrops as a selective force in mammalian evolution. *J R Soc West Aust* 80: 131–139.

Mazerolle M (2013) *Aiccmodavg*: 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

R Core Team (2013) *R: A Language and Environment for Statistical Computing.* R Foundation for Statistical Computing, Vienna.

Ritz C, Streibig JC (2008) *Nonlinear Regression with R*, Springer, New York.

Ritz C, Streibig JC (2012) Dose response curves and other nonlinear curves in weed science and ecotoxicology with the add-on package drc in R.

Ruiz-Talonia L, Carr D, Smith R, Whalley RDB, Reid N (2018) Effect of temperature and light on germination of 10 species of *Eucalyptus* from north-western NSW. *Aust J Bot* 66: 657–666.

Saatkamp A *et al.* (2019) A research agenda for seed-trait functional ecology. *New Phytol* 221: 1764.

Safford H, Viers J, Harrison S (2005) Serpentine endemism in the California flora: a database of serpentine affinity. *Madrono* 52: 222–257.

Schut AG, Wardell-Johnson GW, Yates CJ, Keppel G, Baran I, Franklin SE, Hopper SD, Van Niel KP, Mucina L, Byrne M (2014) Rapid characterisation of vegetation structure to predict refugia and climate change impacts across a global biodiversity hotspot. *PLoS One* 9: e82778.

Seebacher F, Franklin CE (2005) Physiological mechanisms of thermoregulation in reptiles: a review. *J Comp Physiol B* 175: 533–541.

Tapper SL, Byrne M, Yates C, Keppel G, Hopper S, Van Niel K, Schut A, Mucina L, Wardell-Johnson G (2014a) Isolated with persistence or dynamically connected? Genetic patterns in a common granite outcrop endemic. *Divers Distrib* 20: 987–1001.

Tapper SL, Byrne M, Yates CJ, Keppel G, Hopper SD, Niel VKP, Schut AGT, Mucina L, Wardell-Johnson GW (2014b) Prolonged isolation and persistence of a common endemic on granite outcrops in both Mesic and semi-arid environments. *J Biogeogr* 41: 2032–2044.

Tarszisz E, Tomlinson S, Harrison ME, Morrough-Bernard HC, Munn AJ (2017) Gardeners of the forest: effects of seed handling and ingestion by orangutans on germination success of peat forest plants. *Biol J Linn Soc* 123: 125–134.

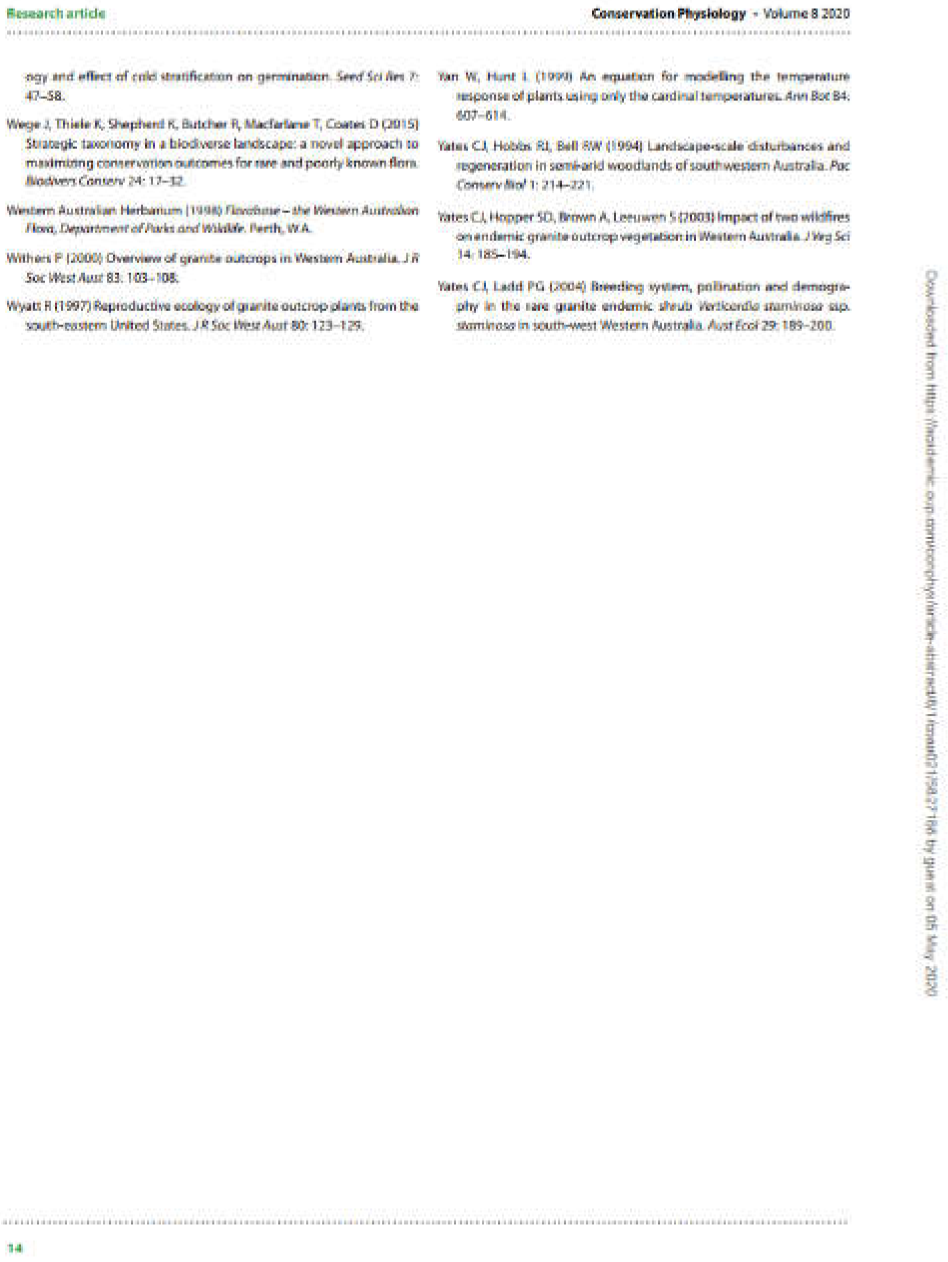
Thackway R, Cresswell ID (1997) A bioregional framework for planning the national system of protected areas in Australia. *Nat Areas J* 17: 241–247.

Tomlinson S (2019) The mathematics of thermal sub-optimality: nonlinear regression characterization of thermal performance of reptile metabolic rates. *J Therm Biol* 81: 49–58.

Turner SR, Lewandrowski W, Elliott CP, Merino-Martin L, Miller BP, Stevens JC, Erickson TE, Merritt DJ (2018) Seed ecology informs restoration approaches for threatened species in water-limited environments: a case study on the short-range banded ironstone endemic *Ricinocarpos brevis* (Euphorbiaceae). *Aust J Bot* 65: 661–677.

Tweddle JC, Dickie JB, Baskin CC, Baskin JM (2003) Ecological aspects of seed desiccation sensitivity. *J Ecol* 91: 294–304.

Walck JL, Baskin JM, Baskin CC (1997) A comparative study of the seed germination biology of a narrow endemic and two geographically-widespread species of *Solidago* (Asteraceae). 1. Germination phenol-



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. *J R Soc West Aust* 83: 103–108.

Wyatt R (1997) Reproductive ecology of granite outcrop plants from the south-eastern United States. *J R 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. *J Veg 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.

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Rajapakshe, R. P., S. R. Turner, A. T. Cross and S. Tomlinson (2020). "Hydrological and thermal responses of seeds from four co-occurring tree species from southwest Western Australia." Conservation Physiology 8(1): coaa021.

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