The Hitchhiker’s Guide to Post-Heatwave Seed Germination: Don't Panic!

A thesis submitted to the University of Technology Sydney in completion of the degree of Master of Science (Research)

Philippa Roslyn Alvarez

BSc (Environmental Sciences), University of Technology Sydney

Faculty of Science, School of Life Sciences
University of Technology Sydney
October 2020

Supervisors
Dr Brad Murray (UTS)
Dr Megan Murray (UTS)
Dr Cathy Offord (ABG)

Certificate of original authorship

I, Philippa Roslyn Alvarez, declare that this thesis, is submitted in fulfilment of the requirements for the award of Master of Science (Research), in the School of Life Sciences, Science at the University of Technology Sydney.

This thesis is wholly my own work unless otherwise reference or acknowledged. In addition, I certify that all information sources and literature used are indicated in the thesis.

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This research is supported by the Australian Government Research Training Program.

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Date: 25/10/2020

Acknowledgements

I have so very many people to thank for helping me during this experience, so I think I shall start from the beginning. To my parents: Mum, Papa, and Dad (I have a wonderfully confusing family). I wish to thank you all for being so very supportive of my journey through this chaos we call life. You've each inspired me to follow my dreams and to approach any and everything with passion and intense curiosity. Mum, you were always my creative inspiration, and my Excel and stationery nerd with whom I could bond for hours with over my colourful spreadsheets. Papa, you've always read through my work and helped with my grammar and spelling since day one – I'd like to think I've improved since the early high school days but whooze too no… Dad, you've shown me how to approach work with a meticulous level of detail, a very handy skill to have in science. Thank you all. Along similar lines I should thank all my families: the Batemanses, Alvarezes, Williamses, Griesserses and Wheelerses. But with mention of the matriarch of each (in no particular grandmotherly order): to Roslyn – for sharing your strength, to Carol – for sharing your passion, to Beth – for sharing your belief, to Carmen – for sharing your smile, and to Gill – for sharing your humour.

To my wonderful colleagues, and friends, at the Australian Botanic Garden Mount Annan, without all of you I would have floundered in a bottomless pit of seeds. Lotte, our wonderful go-to woman, you have mentored me through my entire time at ABG – volunteer to lab assistant to student and everything in-between. Karen, Nathan, Zoe-Joy, and Ganesha, you've each helped me numerous times with questions and ideas I have had and answered them so thoughtfully and kindly. So too have you, Peter, Graeme, Jess, and Kat, you were all instrumental in helping me prepare my experiments, making sure my seeds were well equipped to handle all things I threw at them. Amelia, my longevity chapter was improved so much thanks to your input and notes. Amanda, Veronica, and Maureen, you helped me along the way with friendship and help when I needed it. Lyndle, you have been saving grace throughout this process. Our endless discussions on the joy of being students have motivated me and kept me sane these past few years. Suz, your help with R in the early days was so beneficial and you have been so kind with the questions I asked of you. Katharine, you gave me the platform to leap into the world of seeds with those initial days of volunteering. Without you, I wouldn't have found such a wonderful second home at ABG.

To all at UTS, you have also been a great help getting me to the finish line. Annie, Thom, and Kieran, our journey through this process together and our discussions about all things postgraduate life related have kept me grounded when it would have been so easy to float to the roof of building 7. Dan, your endless help with R has been a god send. It would have taken me twice as long to finish if you hadn't have helped in the kind and understanding way that you did. To the volunteers I had during my rather intense experimental phase - Laura, Elise, Kimberly, Erin, Sarah, Jordan, Kate, and Lauren - thank you all. Without all of you, I would have floundered even more. I hope you each achieve the goals you set for yourselves, as we discussed many of them in your time volunteering. I also hope you have your zombie apocalypse plans updated after this manic year.

To my friends, Kahleah and Jasmine, both best friends and volunteers combined, you helped me physically, mentally, and emotionally through this process. There isn't enough room in this entire document for me to explain how much I have needed and loved you both. I shall try to make it up to you with more trips to HJs, Maccas and GJs – a lifetimes worth of trips. To all of Panda, you guys were the chaotic distraction of food, parties, and laughter I needed to get through these past few years. And to Laurence, in the final 6 months of my thesis, and during coronavirus lockdown, you have been a key source of happiness and joy in my life, thank you.

Last, but very certainly not least, to my supervisors. I'm unsure I can explain my gratitude in words but I'm going to sure as hell try. Brad, without you there would be no thesis. Really. Not only have you been an amazingly patient editor, you have been a mentor, friend, and fellow nerd (the title of this thesis is a prime example). You helped guide me through the tough, anxiety-fuelled times with wisdom and patience. Thank you. Cathy, you have been a part of my career-driven journey since I started at ABG. As a woman in science, you have so powerfully forged a career for yourself that I hope to emulate one day, thank you for being my role model and mentor. To my co-supervisor Megan, thank you for your guidance over the years in both academic and tattoo-driven pursuits.

Arbitrarily, I would like to thank coronavirus for throwing a plot twist into the last few months of my thesis. Thank you, I probably needed to be completely locked down to finish this, plus the bonus of a seemingly imminent implosion of the planet was a real morale booster.

I would like to acknowledge that these acknowledgements are rather long for a master's thesis. I am a closeted sentimentalist and had to address as many people as I could, they've all been so helpful throughout this entire process. I even needed to thank a virus.

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Abstract

Human-induced climate change is the primary source of a global increase in temperature and extreme weather events such as heatwaves. Heatwaves are increasing in intensity and frequency causing detrimental changes to plant communities worldwide, with the temperate woodlands in south-eastern Australia as no exception. The frequency and intensity of heatwaves in the Cumberland Plain Woodland (CPW) are expected to increase, exposing seeds, the most vulnerable stage of the plant lifecycle, to new conditions. This could alter the composition and biodiversity of this threatened ecological community. In this thesis, I aim to understand the link between the physiological mechanisms behind seed germination and the ecological context of these species to understand the future plant community composition of the CPW region.

I first established the effect of experimental heatwaves on seed germination attributes in native plant species, with focus on intensity and and frequency. Each species' response to each treatment differed with no consistent pattern. A few species were driving the species x treatment interaction so, I considered the underlying mechanisms causing this interaction by studying the life-history traits of species. I found that life-history traits were related to the interspecific patterns of variation in all three germination attributes, with life form having the greatest influence over seed germination. Seed mass and dormancy also influenced germination attributes but to a lesser degree than life form, and fire response only partially influenced germination attributes.

Considering the findings outlined above, I focused on seed longevity of a small subset of species . I found that exposure to a single, experimental, post-fire heatwave had no

significant effect on seed longevity compared to a control group not exposed to heatwave conditions. However, the responses of species varied differently depending on the time exposed to heatwaves. I did find a species x time interaction. There was also a link between taxonomic group and germination attributes. Asteraceae were short-lived and Myrtaceae were long-lived, however, Fabaceae did not have the same kind of longevity, instead *A. decurrens* was short-lived and *H. violacea* was long-lived.

The work presented in this thesis provides information regarding: the effect of intense and frequent heatwaves on seed germination attributes, the role of life-history traits in contributing to seed germination attributes over and above the idiosyncratic levels found after a heatwave event, and the impact of a single intense heatwave on species' longevity, from native plant species found in the Cumberland Plain Woodland.

Chapter 1

Introduction

"In the beginning the Universe was created. This had made many people very angry and has been widely regarded as a bad move." – Douglas Adams, The Hitchhiker's Guide to the Galaxy

1.1 Climate change and plants

Forecasts project that the global mean temperature will increase between 2°C to 4.5°C by the end of this century as a direct result of human-induced climate change (Meehl *et al.* 2007). The term 'human-induced climate change' refers to the impact of released greenhouse gas emissions on the planet's natural systems from human activities (Pachauri and Reisinger 2007; Rosenzweig *et al.* 2008). Since the industrial revolution, burning fossil fuels, mass agriculture, and deforestation have all contributed to increases in methane and CO_2 levels (Pachauri and Reisinger 2007). These atmospheric changes alter weather systems causing global warming and an increase in more extreme weather events (Sun and Wang 1996). Climate variability in Australia has three naturally occurring sources: the El Nino-Southern Oscillation (ENSO), the Indian Ocean Dipole (IOD), and the Southern Annular Mode (SAM; Nicholls 1988; Chiew *et al.* 1998; Ashcroft *et al.* 2014; Reisinger *et al.* 2014). Each of these phenomena dictate the climate of Australia and when they interact can cause intense periods of drought or flooding (Chiew *et al.* 1998; Suppiah *et al.* 2007; Ashcroft *et al.* 2014; Karoly 2014). However, it is the influence of human-induced climate change on these naturally occurring climatic events that drives devastating weather events with greater intensity, frequency, and duration than ever before (Timmermann *et al.* 1999; Wang *et al.* 2017).

Destructive climate can drastically alter terrestrial ecosystems worldwide (Dale *et al.* 2001; Thomas *et al.* 2004; Giam *et al.* 2010; Gilman *et al.* 2010). An increase in more intense weather events can pose a risk to biodiversity and ecological functioning of plant communities on a global scale (Thuiller *et al.* 2008). More specifically, under these conditions ENSOs are predicted to affect the dynamics of terrestrial ecosystems causing irreversible change (Holmgren *et al.* 2001), especially to the Australian landscape. As an already arid country, Australian plant species are well adapted to cope with the constant changes associated with the ENSO cycles (Nicholls 1991; Hoffmann *et al.* 2019; Santoso *et al.* 2019), however, as these cycles become more intense over time, the changes may occur too quickly for suitable adaptation to occur in plant species (Santoso *et al.* 2019). These changes can subject species to conditions beyond their physiological limitations (Parmesan and Yohe 2003; Kelly and Goulden 2008; Thuiller *et al.* 2008; Ooi *et al.* 2009; Marchin *et al.* 2015; Parmesan and Hanley 2015). Periods of intense heat, as seen in extreme weather events such as heatwaves, also put pressure on species to adapt rapidly.

1.2 Heatwaves and plants

Along with increased global mean temperature, extreme weather events associated with a warming climate are also expected to increase in intensity, frequency, and duration (Easterling *et al.* 2000; Meehl and Tebaldi 2004; Meehl *et al.* 2007; Perkins *et al.* 2012). Heatwaves are no exception. In the past, Australia has seen an increase in the number and frequency of heatwaves as a direct result of human-induced climate change (Karoly 2014; Steffen *et al.* 2014; Perkins-Kirkpatrick *et al.* 2016). The definition of a heatwave, “when the maximum and the minimum temperatures are unusually hot over a three-day period at a location” (Bureau of Meteorology 2020b), broadly encompasses multiple interpretations in the literature. A range of studies looking at the effect of different types and lengths of heatwaves on plant species use multiple interpretations of the heatwave definition (Perkins *et al.* 2012; Perkins and Alexander 2013; Hancock and Hughes 2014; Maher *et al.* 2018; French *et al.* 2019). These studies then focus on a range of different ecosystem types from around the world.

Over the past two decades, extreme heatwave events have been recorded from around the world with the European heatwaves of 2003, 2006 and 2007, the North American heatwaves of 2006 and 2011, and the Australian heatwaves of 2013 and 2017 currently the most widely studied in the literature (García-Herrera *et al.* 2010; Lau and Nath 2012; Perkins-Kirkpatrick *et al.* 2016). These circumstances allowed scientists to study the effect of heatwaves in ecological communities such as forests (Pichler and Oberhuber 2007; Lamers *et al.* 2009; Matusick *et al.* 2016), grasslands (Teuling *et al.* 2010; Cremonese *et al.* 2017) and woodlands (Van Gorsel *et al.* 2016). An Australian example by Van Gorsel *et al.* (2016) studied water use in southern Australian woodlands during the 2013 summer heatwave. They found that, ultimately, woodlands changed from a carbon sink into a carbon source during the heatwave, a detrimental change to the role of the woodland in mitigating climate change. This study is one of many demonstrating that heatwaves can drastically shift global plant communities from beneficial to harmful ecosystems.

Studies focusing primarily on south-eastern Australia address the concern for ecological systems during and after intense heatwaves (Deo *et al.* 2007; Cowan *et al.*

2014; Perkins-Kirkpatrick *et al.* 2016). There is evidence that species in this region are tolerant of heatwaves and are able to withstand harsh, dry conditions for extended periods of time (Hill and French 2003; French *et al.* 2019). However, in the past there has always been some form of respite once a harsh, dry summer has occurred. In contrast to previous conditions, forecasts predict species will need to endure longer, drier summers, with more intense and frequent heatwaves, with no respite (Steffen *et al.* 2014). Threatened ecological communities are among some of the main communities fighting against the increase in heatwave intensity and frequency, an example of one such community is the Cumberland Plain Woodland (CPW). For the CPW, the effects of intense and frequent heatwaves may prove devastating on plant development and growth (Chapin III *et al.* 2000; Walther *et al.* 2002; Hatfield and Prueger 2015). While these studies focus on plant communities and the response of large-scale systems to extreme weather events like heatwaves, there is a paucity of information about the response of the seed stage of the plant life cycle. Very simply, if seeds are more susceptible to heatwaves than whole plants, then plant community composition may change drastically.

1.3 Heatwaves and seeds

Arguably, the most vulnerable stage in the plant's lifecycle is the seed stage (Baskin and Baskin 1998; Donohue *et al.* 2010). For plant species to mitigate the challenges posed by climate change such as more frequent and intense heatwaves, seed survival and successful germination within the soil seed bank is vital (Read *et al.* 1997; Hulme 1998; Tang *et al.* 2003). A seed incapable of germination due to nonviability, damage or dormancy (Bewley and Black 1994; Vleeshouwers *et al.* 1995; Offord and Meagher 2009; Bradbeer 2013) is unlikely to be successful evolutionarily. Exposure to adverse environmental conditions can cause physiological and biochemical damage to a seed, preventing germination from occurring (Bewley and Black 1982; Thomas *et al.* 2010; Mackenzie *et al.* 2016). As such, the changing environment poses a detrimental risk to ongoing seed survival in the soil seedbank and therefore the continuation of native plant species in a region. If the seeds of some species are comparatively more susceptible to changes in the environment than others, then overall plant community composition may be altered.

Temperature-based experiments on seed germination have a long history (Auld and Bradstock 1996; Wang *et al.* 2003; Gleadow and Narayan 2007; Orsenigo *et al.* 2015; Maikano *et al.* 2018). High temperatures have been shown to cause irreversible damage to a seed's physiological structure (Baskin and Baskin 1988; Roberts 1988). However, studies into the response of seed germination to a range of heatwave scenarios are not common. Predominantly, the literature focuses on the biological and physiological aspect of temperature influence on seed germination but not on the ecological implications. Fire ecology provides a bridge between physiology and ecology (Keeley 1987; Baker *et al.* 2005; Ooi *et al.* 2006; Fidelis *et al.* 2016; Mackenzie *et al.* 2016; Palmer *et al.* 2018; Hodges *et al.* 2019). Studies in seed dormancy found high temperature thresholds in species growing in a fire-prone region (Ooi *et al.* 2014), linking the biological mechanisms of the seed to seed ecology (Collette and Ooi 2020). Incorporating both the physiological and ecological aspects in seed germination can amplify its relevance and broaden knowledge more cohesively (Vleeshouwers *et al.* 1995; Jiménez-Alfaro *et al.* 2016).

Bridging the gap between physiology and ecology within a plant community has been investigated to some extent in plants of the CPW. Restoration has been the primary impetus for research in the CPW (Wilkins *et al.* 2003). One reason for concern based on the issues surrounding restoration in this region can be seen in the encroachment of African olive over areas widely covered with native plant species, threatening plant diversity (Cuneo and Leishman 2013; Nguyen et al. 2016). Without monitoring, this issue has been unresolved for over a decade (von Richter *et al.* 2005). To better understand the native plant species in this region, *in situ* testing of species' composition post-fire has provided detailed information about the soil seedbank and the seed germination attributes of species in the CPW (Hill and French 2003). Seed germination attributes to heat and smoke testing have occurred in native Poaceae species from the CPW with the results establishing role of heat on species seed recruitment and the overall composition of species after an event involving a heat stimulus (Clarke and French 2005). The importance of understanding this response to heat in seed germination attributes could drastically alter the management strategies used to conserve the CPW in future.

1.4 Native plant species of the Cumberland Plain Woodland

The CPW resides in the Sydney Basin with National Parks acting as ecological boundaries to the north, south and west. The Bureau of Meteorology (2001) describes the climate as temperate with a warm/hot summer and no dry season. Found on Bringelly shale, the plant species that grow in this bioregion are adapted to clay and loam soil types, which become waterlogged after high rainfall events (Bannerman and Hazelton 1989; Watson 2005). The main source of biodiversity for the region is found in the ground layer, with perennials such as *Themeda triandra, Einadia nutans,* and

Microlaena stipoides (Benson 1992). In the understory layer, herbaceous shrubs like *Bursaria spinosa* and *Indigofera australis* form a dense thicket (Tozer 2003; Watson 2005). The dominant tree species include *Eucalyptus moluccana, E. crebra,* and *E. tereticornis* (Myrtaceae) and *Acacia decurrens* (Fabaceae).

1.5 Significance and research context

The goal of this thesis is to understand the germination responses of a range of native Australian plant species to a range of heatwave conditions brought about by our changing climate, thus bridging the gap between the physiological response of seeds to heat treatments and the ecological implications of heatwaves. I chose to focus on the CPW, as a region of interest and a threatened ecological community. Gaining an insight into how the species in the CPW respond to these future projections could provide valuable insight for future management strategies and to maintain biodiversity of the region. This thesis approaches this endeavour from multiple angles to cover a broad area of understanding, to be enhanced and replicated for other important ecological communities.

1.6 Thesis aims and structure

I address four research aims in my thesis:

1. To determine the resilience of seeds from the CPW to intense and frequent heatwaves using laboratory experiments.
2. To understand how a range of heatwave scenarios affect (1) the time taken for seeds to begin germination, (2) the duration of time over which seeds germinate, and (3) the total proportion of seeds that germinate.

3. To determine whether plant life-history traits are related to interspecific patterns of variation in germination onset, duration, and the total proportion of seed germination.
4. To explore the effect of a single post-fire heatwave on the long-term longevity of six native plant species of the CPW.

This thesis addresses these aims regarding the effect of intense and frequent heatwaves on native plant seeds from the CPW in the following Chapters.

Chapter 2 uses a germination experiment to test the resilience of seed germination attributes against intense and frequent heatwaves. An experimental heatwave is expected to delay germination onset, prolong germination duration, and reduce total germination proportion across species and beyond the idiosyncratic levels typically expected within a species.

In Chapter 3, a study into the influence of plant life-history traits on germination attributes expands on the information from Chapter 2, providing an in-depth analysis into the underlying mechanisms that govern seed germination. I ask, do life-history traits such as seed mass, dormancy, fire response, or life form, directly influence the germination attributes of native plant species from the CPW?

While Chapters 2 and 3 focus on the direct and immediate response of germination attributes to heatwaves, Chapter 4 aims to understand the long-term effect of heatwaves by examining seed survival in a longevity experiment. After exposure to a single, intense post-fire heatwave, I determine the effect of an experimental heatwave on the ageing process of seeds. It is expected that the post-fire heatwave conditions will cause early seed death when compared to the survival of seeds not exposed to heatwave conditions. I will also gain valuable insight into the longevity of species previously untested in a longevity experiment, to add to the global database of information regarding seed longevity.

In Chapter 5, I synthesise all concepts from Chapters 2, 3, and 4, discussing them in relation to a broader context within current literature.

Chapter 2

Effects of experimental heatwaves on seed germination in native plant species of the Cumberland Plain Woodland

“For a moment, nothing happened. Then, after a second or so, nothing continued to happen.” – Douglas Adams, The Hitchhiker’s Guide to the Galaxy

2.1 Introduction

Projections by the Intergovernmental Panel on Climate Change (IPCC) place the Earth’s increase in global temperatures somewhere between 2.6°C and 4.8°C by the end of the 21st century (Meehl *et al.* 2007). Such changes in climatic conditions have the capacity to increase the frequency, duration, and intensity of heatwaves around the world (Easterling *et al.* 2000; Meehl and Tebaldi 2004; Meehl *et al.* 2007; Perkins *et al.* 2012). These increases have the potential to impact plant seed germination, with serious implications for the structure and function of ecosystems around the world (Milbau *et al.* 2009; Walck *et al.* 2011; Mondoni *et al.* 2012; Orsenigo *et al.* 2015). It is the aim of this Chapter to focus on two of these heatwave conditions: intensity and frequency.

An intense heatwave is synonymous with an increase in temperature; the more intense a heatwave is, the greater the likelihood of a noticeable impact on the day-to-day function of a species (Teskey *et al.* 2015). Increases in the intensity of future heatwaves may therefore be problematic for plant communities (Dale *et al.* 2001; Mondoni *et al.* 2012; Wolkovich *et al.* 2012; Parmesan and Hanley 2015; Cochrane 2017; Maher *et al.* 2018). The effect of this is likely to be noticeable in south-eastern Australia (Deo *et al.* 2007; Suppiah *et al.* 2007; Laurance *et al.* 2011), which has experienced some of the hottest days on record for the region this past summer (Bureau of Meteorology 2020a). Extreme weather events, like intense heatwaves, are becoming more frequent and are predicted to worsen in the next few decades (Bureau of Meteorology 2020b).

The frequency of intense heatwaves is also set to increase as the global effects of climate change intensify (Karl and Trenberth 2003; Meehl and Tebaldi 2004; Meehl *et al.* 2007). What was once a rare event has now become associated with a typical Australian summer. A rapidly changing landscape is quickly becoming exposed to more intense bouts of heat, but also in quick and unrelenting succession (Meehl *et al.* 2007). A recent study identified that focusing on a single heatwave event may not convey accurate information about the responses of plant communities to heatwaves (French *et al.* 2019), instead only scratching the surface of what underlying processes may actually occur. Instead, an examination of the effects of multiple heatwave scenarios in laboratory-based experiments therefore provide a foundation for more realistic ecological research focusing on the effects of these events in an ecological community.

Some ecosystems may cope with increases in the intensity and frequency of heatwaves. However, it is not known which ecosystems may be more susceptible than others. In the present study, the focus is centred on the Cumberland Plain Woodland (CPW). This threatened ecological community lies within the Sydney Basin Bioregion on the south-east coast of Australia, its main threats deriving from land development and urbanization (Davies and Christie 2001). Understanding the influence of climate-induced heatwaves on a well-known, ‘hardy’ community (Benson and Howell 2002) will provide a baseline for exactly how severe these climatic changes may be to other

Australian plant communities, especially in terms of the most vulnerable aspect of a plant’s life cycle, the seed stage (Baskin and Baskin 1998; Donohue *et al.* 2010).

Plant seeds are highly reliant on temperature for successful germination (Bewley and Black 1994; Bewley 1997; Baskin and Baskin 1998). Each species has a temperature range in which they are more likely to succeed in germination, known as an optimum temperature. As an important trigger for seeds, once they find themselves in their optimum temperature range, they are better equipped to prepare for growth and establishment (Ooi *et al.* 2014; Ruiz-Talonia *et al.* 2018). Outside their optimum temperature, seeds are found to be less likely to germinate and are less successful in the seedling stage (Gleadow and Narayan 2007; Ooi *et al.* 2014; Hatfield and Prueger 2015; Cochrane 2017). Evidence supporting seed success in high intensity heatwaves is currently ambiguous, equally so with more frequent heatwaves. One study found resilience in native plants in the face of high temperatures (French *et al.* 2019), while another found adverse effects on seed germination (Gleadow and Narayan 2007). With uncertainty in this field, independent studies such as this one can be tailored to individual ecosystems.

The aims of this Chapter are to understand the resilience of plant species in the CPW to intense and frequent heatwaves using laboratory experiments focusing on seed germination. To do this, I investigated the germination attributes of 15 native plant species to three different heatwave scenarios with the focus on either: heatwave intensity – ‘Experiment 1’, or heatwave frequency – ‘Experiment 2’. For the intensity experiment, these scenarios (described in more detail below in the Methods) were selected to be ecologically relevant to my study species, and importantly, were established using temperature data collected from the Bureau of Meteorology and

IPCC projections. For the frequency experiment, the highest temperature from the intensity experiment was coupled with information from a study using frequency simulations about heatwaves (French *et al.* 2019), to provide three heatwave scenarios. It is expected that as heatwaves increased in intensity and frequency, seed germination attributes would be adversely affected, thus proving a significant treatment effect of experimental heatwaves Each experiment was designed to answer the following questions: do the heatwave scenarios affect (1) the time taken for seeds to begin germination, (2) the duration of time over which seeds germinate, and (3) the total proportion of seeds that germinate?

2.2 Materials and Methods

2.2.1 Study Region

The CPW is a threatened ecological community (Environmental Protection Biodiversity Conservation Act 1999; Threatened Species Conservation Act 1995) that lies within the Sydney Basin in south-eastern Australia (Fig. 2.1). Extending from the ocean in the east to the Blue Mountains in the west, and from the Hornsby Plateau in the north to the Woronora Plateau in the south, this ecological community consists of both urbanised land and untouched woodland. The natural areas of the CPW, which formerly covered approximately 107 000 ha, have decreased to 13.1% of their original size due to land clearing associated with urbanisation (Tozer 2003). The woodlands and forests of the CPW are dominated by *Eucalyptus* canopy species, a shrubby understorey consisting typically of species from families including Fabaceae, Pittosporaceae and Asteraceae, and a rich diversity of species from the Poaceae in the grassy understorey (Benson and Howell 2002). Between 2006 and 2019, average minimum and maximum summer temperatures were 16.5°C and 29.2°C respectively, and the lowest and highest summer temperatures were 11.1°C and 38.2°C respectively (Bureau of Meteorology station no. 68257). Rainfall mostly occurs in the wetter summer/autumn months, with annual rainfall averaging around 700 ml throughout the CPW. Wildfires are an important component of the ecology of the region, with fire intervals of no less than four years and no more than 15 years essential to maintaining the integrity of the structure and function of native ecosystems (Watson 2005).

2.2.2 Study species and seed collection

The selection of species for these germination experiments was based on ensuring representation of a diversity of native plant species from a range of plant growth forms in the CPW bioregion. The species that were used in the experiments captured the broad taxonomic range found in the study region, which included 15 species from 13 genera spread across eight families (Table 2.1). Seeds for these species were sourced from either fresh field collections or from a seedbank collection. Seeds of four species were collected from stands of CPW at the Australian Botanic Garden Mount Annan (ABG) between February and March 2018 and seeds of 11 species from the same area were obtained from the ABG PlantBank storage facilities (Table 2.1).

2.2.3 Experimental design

All seeds were initially left to acclimatise to room temperature and humidity for approximately a fortnight. I then compared germination attributes among and within the 15 study species across three temperature treatments for Experiment 1. In an oven (Labec G701; Marrickville, Australia), dry seeds were exposed to a heatwave scenario for a period of five days, this length of time being representative of a typical heatwave in this region (Bureau of Meteorology 2020b), at a prescribed temperature for each treatment (Table 2.2). For Experiment 2, seeds were either exposed to a control temperature (22°C) or the highest temperature used in Experiment 1, 60/22°C (Table 2.2), with each time-period represented as a treatment group. A time-period refers to a heatwave + non-heatwave event occurring in succession, a five-day heatwave + a six-day gap, and so on, depending on treatment.

Following each treatment, and once species had been pre-treated using appropriate germination requirements (such as physical scarification or removal of external coverings), 10 seeds were placed on 9 cm petri dishes, each dish containing 8 g/L of agar. Experiment 1 had five replicates of ten seeds; Experiment 2 had six replicates of ten seeds. After each corresponding experiment, dishes were placed into species-specific optimum temperatures for germination (based on initial trials and information provided by the Seed Information Database, Royal Botanic Gardens Kew 2018; Appendix 2.1). While some species were placed into incubators with diurnal temperatures (30/15°C and 15/5°C), others were placed in constant temperatures (20°C). The light settings for all incubators were diurnal on 12 hr/12 hr light/dark rotation.

Germination checks commenced two days after seeds were placed into the incubators. No germination occurred before placement in the incubators. Each group was checked every second day for the first 14 days. From day 14 to day 30, the plates were checked every four days, and then weekly until no further germination was observed for 14 days in a row. Germination was defined as emergence of a radicle of more than 2 mm. After this time, a cut test was used to reveal the condition of those seeds which had not germinated (Ooi *et al.* 2005). Germinability was assessed to be the most ecologically relevant measure of seed germination success, not viability. In this study, germination total is represented by those seeds that germinated out of the total number of seeds with the potential to germinate, i.e., viable seeds, not empty or seeds destroyed by predation.

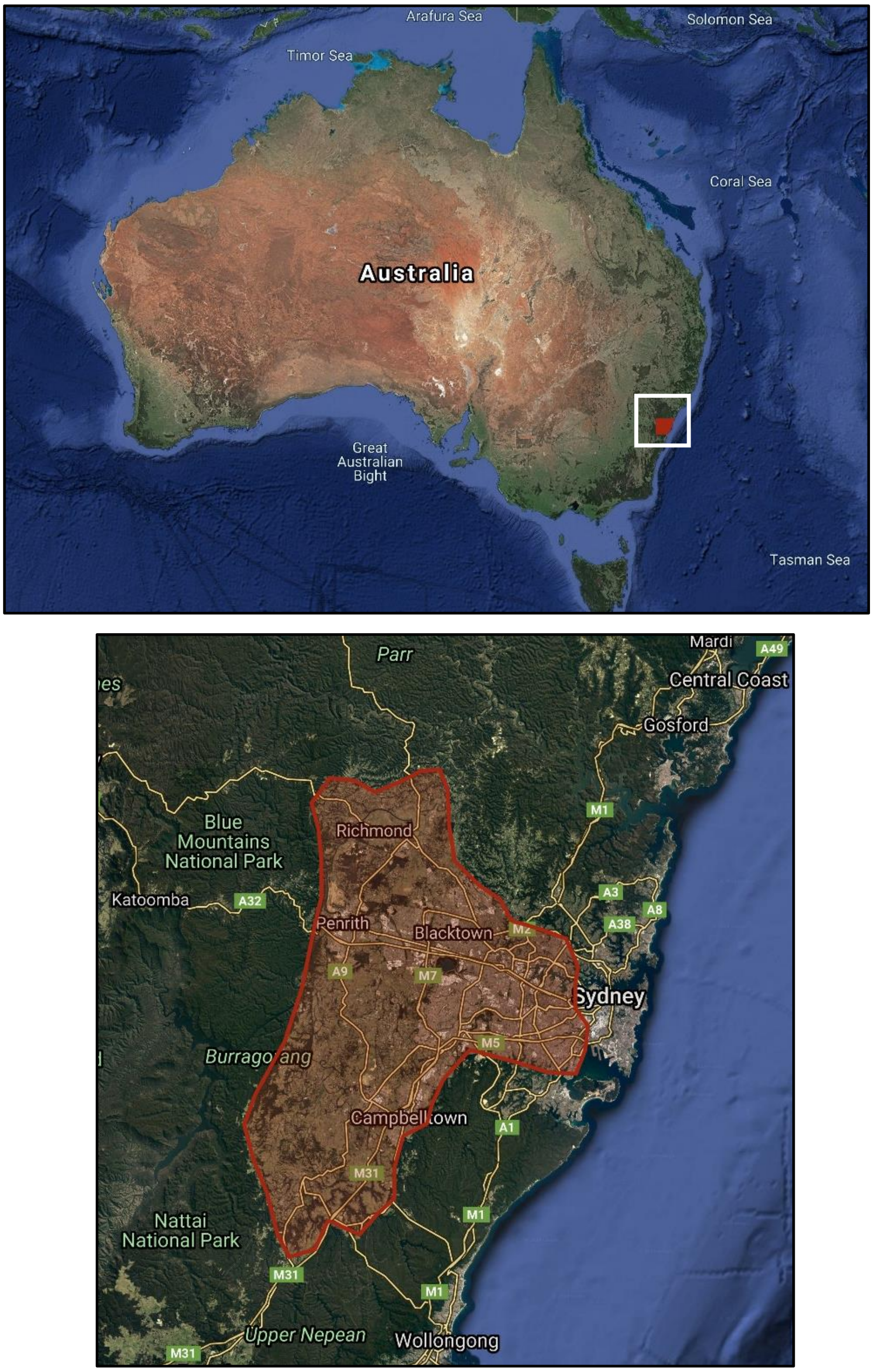


Figure 2.1. A map showing the boundary in relation to the Cumberland Plain Woodland (Google Maps 2020) in relation to Australia. Only 13% of the land within the red boundary line is comprised of untouched land, the remainder is urbanised.

Table 2.1. Study species used in the germination experiment with taxonomic family and growth form information. Collection refers to either field collection of seeds or PlantBank accessed seeds. Pre-treatment refers to any standard treatments used to promote seed germination or break seed dormancy.

Family	Species	Collection	Pre-treatment	Growth form
Asteraceae	*Calotis lappulacea*	Field	N/A	Herb
	Cassinia aculeata	PlantBank	N/A	Shrub
Chenopodiaceae	*Einadia nutans* subsp. *nutans*	Field	Removed from fruit	Groundcover
Fabaceae	*Acacia decurrens*	PlantBank	Scarification	Shrub
	Acacia falcata	PlantBank	Scarification	Shrub
	Hardenbergia violacea	PlantBank	Scarification	Woody climber
	Indigofera australis	PlantBank	Scarification	Shrub
Lamiaceae	*Plectranthus parviflorus*	Field	N/A	Herb
Myrtaceae	*Eucalyptus crebra*	PlantBank	Removal of frass	Tree
	Eucalyptus tereticornis	PlantBank	Removal of frass	Tree
Poaceae	*Dichanthium sericeum*	PlantBank	Removal of floret	Grass
	Microlaena stipoides	PlantBank	Removal of floret	Grass
	Themeda triandra	PlantBank	Removal of floret	Grass
Ranunculaceae	*Clematis glycinoides* var. *glycinoides*	PlantBank	N/A	Woody climber
Sapindaceae	*Dodonaea viscosa* subsp. *cuneata*	PlantBank	Scarification	Shrub

Table 2.2. Summary of each heatwave scenario within each experiment: Experiment 1 – Intensity and Experiment 2 – Frequency. All temperatures have been sourced from the Bureau of Meteorology (BOM) and have been provided for Experiment 1 and Experiment 2. Number of heatwaves in Experiment 2 were sourced from (French *et al.* 2019) and temperatures based on Experiment 1 temperatures.

Experiment 1 – Intensity	Experiment 2 – Frequency
Control group – BOM	No heatwave
29/17°C	22°C
Recent heatwave – BOM	One heatwave
39/21°C	60/22°C
Projected heatwave – BOM + IPCC	Three heatwaves
43/25°C	60/22°C
Post-fire heatwave* – (von Richter, unpublished data) 60/22°C	Five heatwaves 60/22°C

* this temperature was obtained using a data logger placed on recently burnt ground in the CPW woodland, no canopy cover was present.

To establish the range of temperatures for Experiment 1, I gathered meteorological data from the Bureau of Meteorology for the summers between 2013 and 2018 inclusive (Bureau of Meteorology 2020b) for four locations spanning the distribution of the CPW. Diurnal temperature data were obtained for the four sites. After initial on-site testing, it was established that topsoil temperature had little to no difference compared with ambient air temperature. It was assumed that the seeds were present within the top 5 cm of soil and in the shade of the above canopy, only in the post-fire scenario was it assumed no canopy cover would be present. All the selected sites experienced heatwave events between 2013 and 2018. The definition of a heatwave was based on the Bureau of Meteorology’s description of three or more days of high minimum and maximum temperatures that are unusual for that location (Bureau of

Meteorology 2020b). The four sites were located near the boundary lines of the CPW and positioned more than 17 km from each other. The sites included the weather stations at Richmond RAAF (northern boundary, -33°36’S, 150°47’E), Camden airport (southern boundary, -34°2’S, 150°41’E), Bankstown airport (eastern boundary, -33°55’S, 150°59E) and Penrith Lakes AWS (western boundary, -33°43’S, 150°41’E).

2.2.4 Statistical analyses

Three separate analyses were performed for both experiments, one for each response variable: onset of germination (the time taken for seeds to begin to germinate); germination period (duration of time over which seeds germinated); and total germination (the total proportion of seeds that germinated). A general linear model was used to determine the effects of temperature and species (each a fixed explanatory variable) on onset of germination. A similar model design was used to determine the effects of temperature and species on germination period. All model assumptions for normality and homogeneity of residuals were met. To investigate the effects of temperature and species on total germination, a generalised linear model with a binomial error structure and a logit link was used. If the model was found to have overdispersion, a quasibinomial error structure was used. In the case that a significant species x treatment interaction was observed in any of the models, single-predictor models were used to unpack the interaction. These were performed within each species to detect whether there was a species-specific response in germination across the four treatments (treatment, with four levels, was the single predictor variable in these 15 models); and within each of the four treatments to detect differences among species within a given treatment (species, with 15 levels, was the single predictor variable in these four models). All statistical analyses were performed using R statistical software

(R core team 2019) through R Studio (Version 3.5.1) and the emmeans package post-hoc tests (Lenth 2019).

2.3 Results

2.3.1 Experiment 1 - Onset of germination

There was a significant effect of species ($F_{14,\ 540} = 613.084$, $P < 0.0001$) and a significant species x treatment interaction ($F_{42,\ 540} = 2.705$, $P < 0.0001$) on onset of seed germination (Fig. 2.2; Appendix 2.2). There was a marginally significant effect of treatment on onset of germination ($F_{3,\ 540} = 2.753$, $P = 0.042$). Breaking down the significant interaction term in the model, germination onset differed significantly among treatments within only four of the 15 species (Fig. 2.3; Appendix 2.3). Significant treatment effects were observed in *Calotis lappulacea, Clematis glycinoides, Einadia nutans* and *Hardenbergia violacea*, however, no consistent direction of pattern emerged across these species. I found that *Calotis lappulacea* took significantly longer to begin germination after treatment at recent heatwave temperatures compared to recent average temperatures. *Clematis glycinoides* took significantly longer to begin germination in both the projected heatwave and the post-fire heatwave temperatures, and *Einadia nutans* took less time to begin germination in the recent heatwave temperature when compared to the other three treatment temperatures. *Hardenbergia violacea* had delayed onset in the post-fire heatwave treatment. Most species had no discernible difference between treatments in their onset of seed germination.

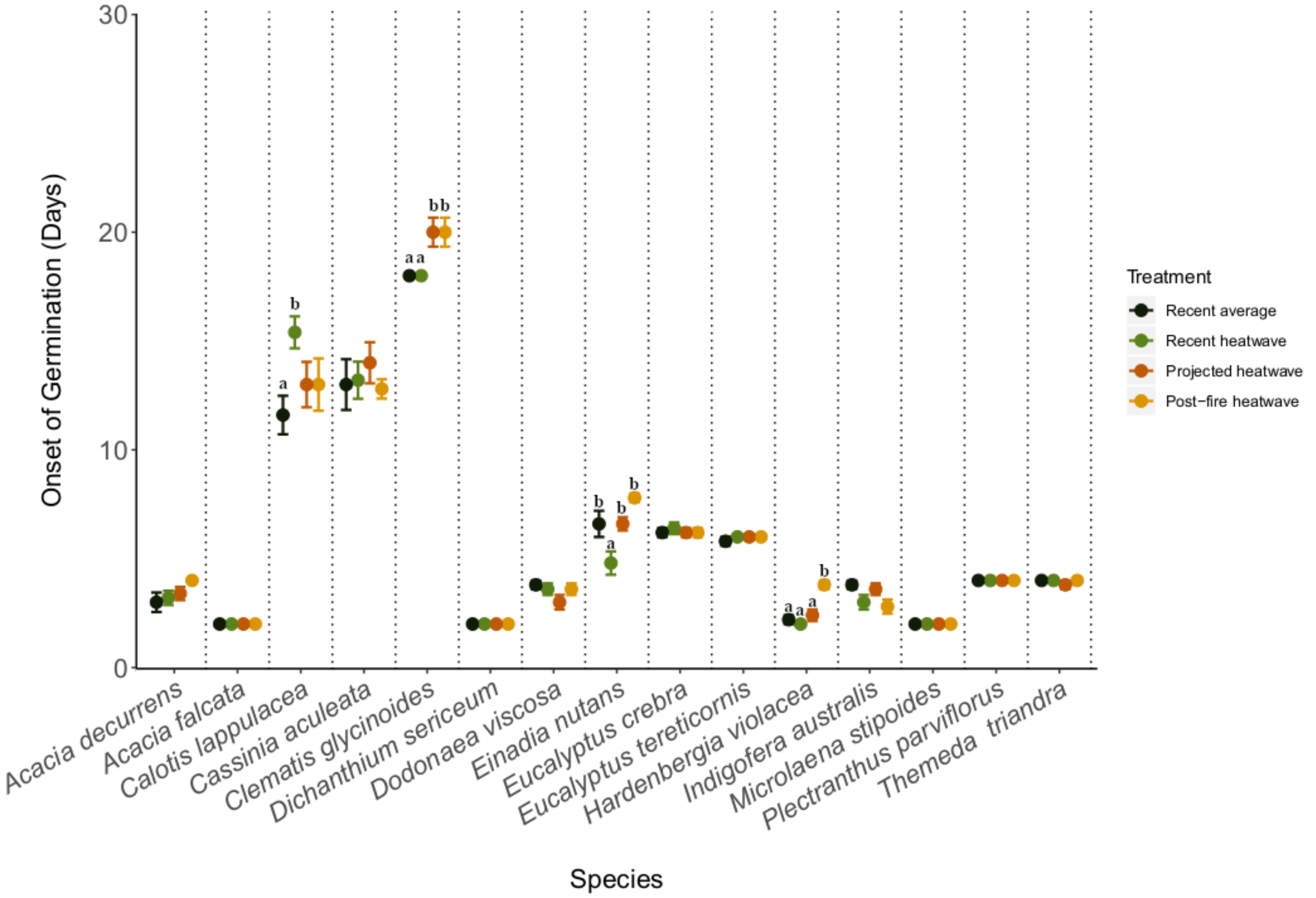


Figure 2.2. The onset of seed germination (mean, ± SE) for each species (separated by the dotted lines) across each of the four experimental treatments (colour coding on the right of the graph). These graphs focus on significant differences among treatments within each species. Recent average 29/17°C, recent heatwave 39/21°C, projected heatwave 43/25°C, and post-fire heatwave 60/22°C. Significant differences (Tukey's post-hoc $P < 0.05$) among treatments within each species are shown by different letters.

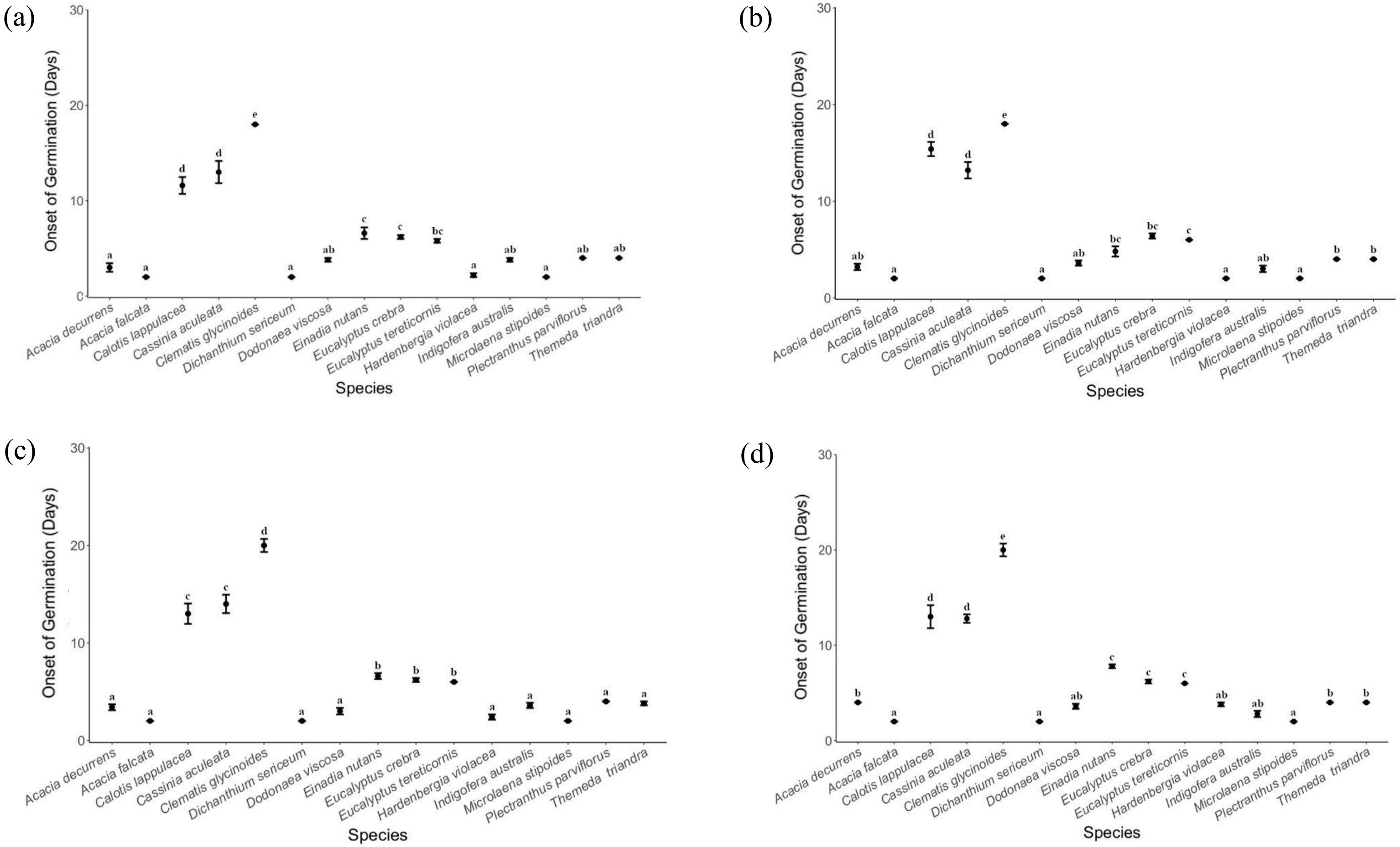


Figure 2.3. The onset of seed germination (mean, ± SE) in the four experimental treatments: (a) recent average, (b) recent heatwave, (c) projected heatwave, and (d) post-fire heatwave for each species. Recent average 29/17°C, recent heatwave 39/21°C, projected heatwave 43/25°C, and post-fire heatwave 60/22°C. Significant differences (Tukey's post-hoc $P < 0.05$) among species within each treatment are shown by different letters.

Significant differences were found across species within each of the four treatment groups (Fig. 2.3; Appendix 2.4). Most notable across all four treatments were two groups that consistently stood out with slower onset of germination regardless of temperature treatment. First, *Calotis lappulacea, Cassinia aculeata,* and *Clematis glycinoides* formed a group that was more prone to much slower onset of germination, and second, *Einadia nutans, Eucalyptus crebra,* and *Eucalyptus tereticornis* formed a group demonstrating marginally slower germination onset (Fig. 2.3). All other species started to germinate within the first five days regardless of temperature treatment.

2.3.2 Experiment 1 - Duration of germination

There was a significant effect of species ($F_{14, 540} = 76.902$, $P < 0.0001$) on germination duration as well as a significant species x treatment interaction ($F_{42, 540} = 2.036$, $P < 0.0001$; Fig. 2.4; Appendix 2.5). There was no significant effect of heatwave treatment on germination duration ($F_{3, 540} = 1.112$, $P = 0.344$). Breaking down the significant interaction term in the model, germination duration differed significantly among treatments within only two of the 15 species (Fig. 2.4; Appendix 2.6). Significant treatment effects were observed in *Cassinia aculeata* and *Einadia nutans*. *Cassinia aculeata* spent significantly more time germinating in the recent heatwave temperature than the post-fire heatwave temperature. *Einadia nutans* spent a significantly longer period germinating in the recent heatwave temperature compared to the other three treatment temperatures. Among the rest of the species, there was considerable variation in the way each treatment affected duration of germination with no overall pattern observed across treatments.

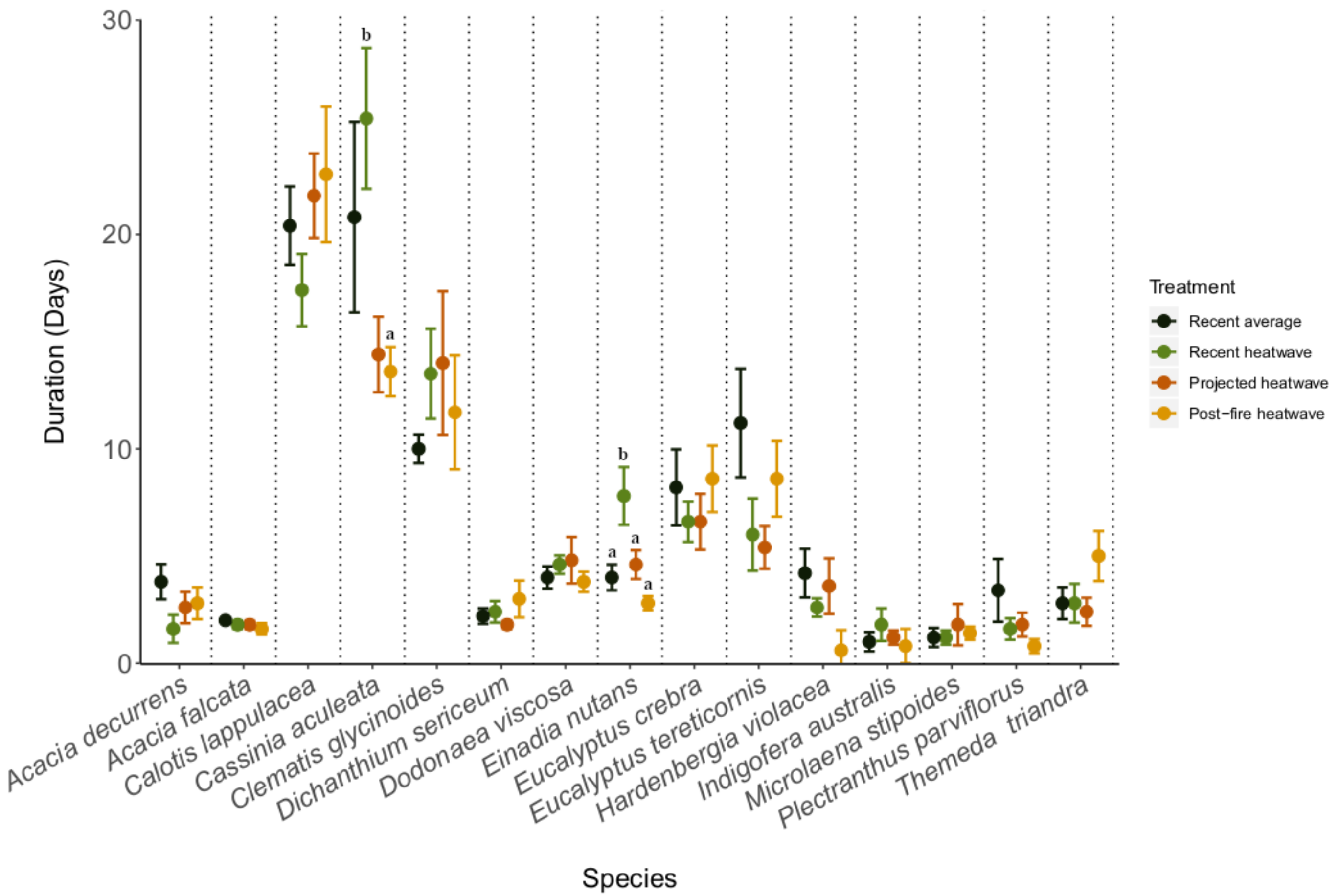


Figure 2.4. Duration time of germination (mean, ± SE) for each species (separated by the dotted lines) across each of the four experimental treatments (colour coding on the right of the graph). These graphs focus on significant differences among treatments within each species. Recent average 29/17°C, recent heatwave 39/21°C, projected heatwave 43/25°C, and post-fire heatwave 60/22°C. Significant differences (Tukey's post-hoc $P < 0.05$) among treatments within each species are shown by different letters.

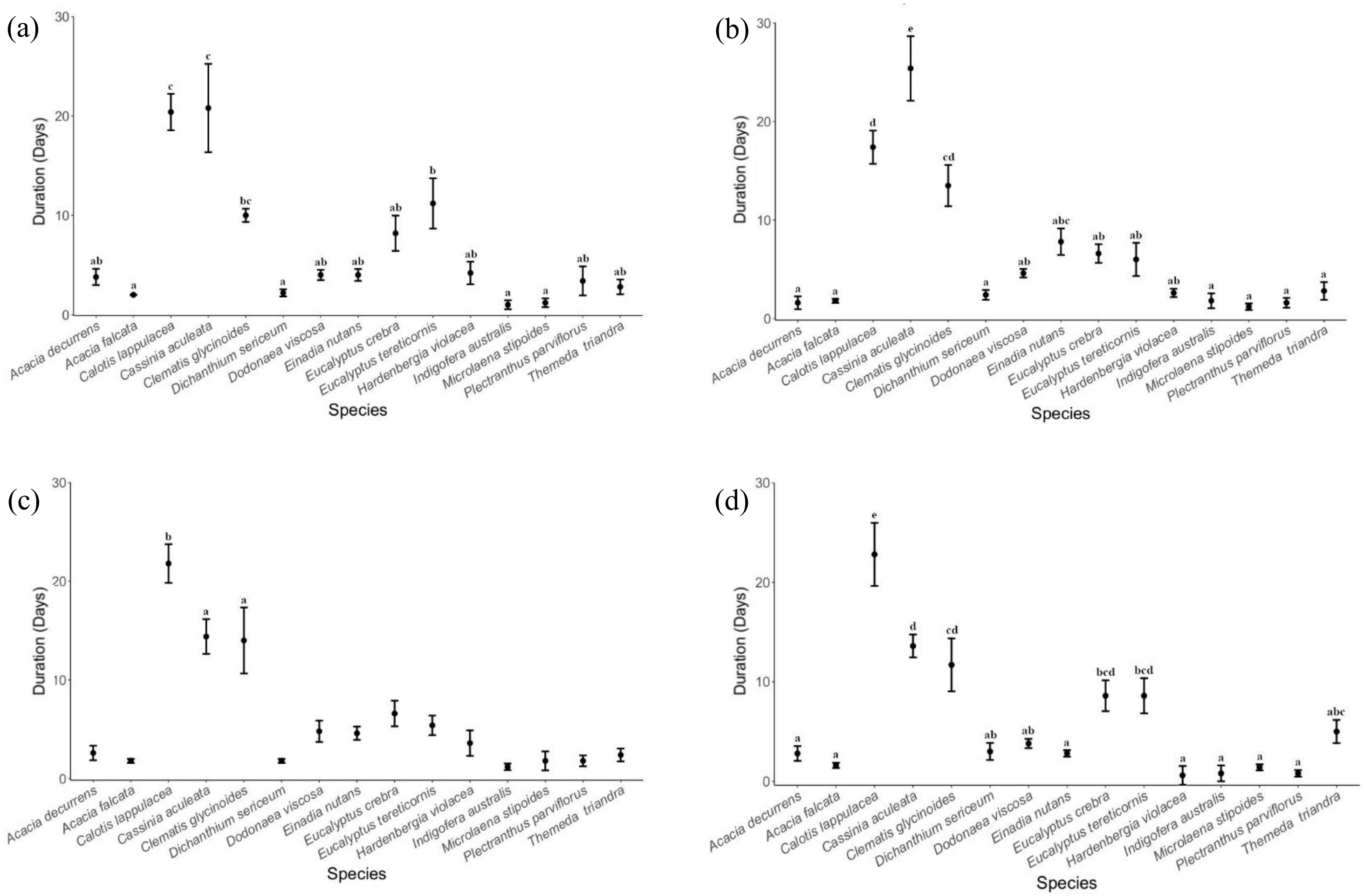


Figure 2.5. Duration of seed germination (mean, ± SE) in the four experimental treatments: (a) recent average, (b) recent heatwave, (c) projected heatwave, and (d) post-fire heatwave for each species. Recent average 29/17°C, recent heatwave 39/21°C, projected heatwave 43/25°C, and post-fire heatwave 60/22°C. Significant differences (Tukey's post-hoc $P < 0.05$) among species within each treatment are shown by different letters.

Significant differences were found across species within each of the four treatment groups (Fig. 2.5; Appendix 2.7). Overall, most species spent less than a week in the process of germinating, with *C. lappulacea, C. aculeata,* and *C. glycinoides* taking significantly longer, anywhere between 10 to 20 days to complete germination. *Eucalyptus crebra* and *Eucalyptus tereticornis* took approximately 10 days to complete germination in the recent average heatwave and post-fire heatwave. Unlike onset of seed germination, there were no other distinct groups present.

2.3.3 Experiment 1 - Total proportion of germination

There was a significant effect of species ($\chi^2 = 506.3$, df = 14, $P < 0.0001$) on the total proportion of seed germination as well as a significant species x treatment interaction ($\chi^2 = 100.8$, df = 42, $P < 0.0001$; Fig. 2.6; Appendix 2.8). There was no significant effect of heatwave on total seed germination ($\chi^2 = 0.44$, df = 3, $P < 0.933$). Breaking down the significant interaction, total proportion of seed germination differed significantly among treatments within only one of the 15 species (Appendix 2.9). Significant treatment effects were observed only in *Hardenbergia violacea*. *Hardenbergia violacea* had significantly fewer seeds germinate in the recent average and post-fire heatwave than the other two treatment temperatures. All other species had considerable variation in the way each treatment affected total proportion of seed germination with no overall pattern observed across treatments.

Significant differences were found across species within each of the four treatment groups (Fig. 2.7; Appendix 2.10). Overall, each species varied in the total proportion of seed germination, with *Eucalyptus tereticornis* reported to have the lowest germination proportion across all four treatments. *Dichanthium sericeum* and

Indigofera australis had 100% germination with all other species ranging from 55% to 95% germination.

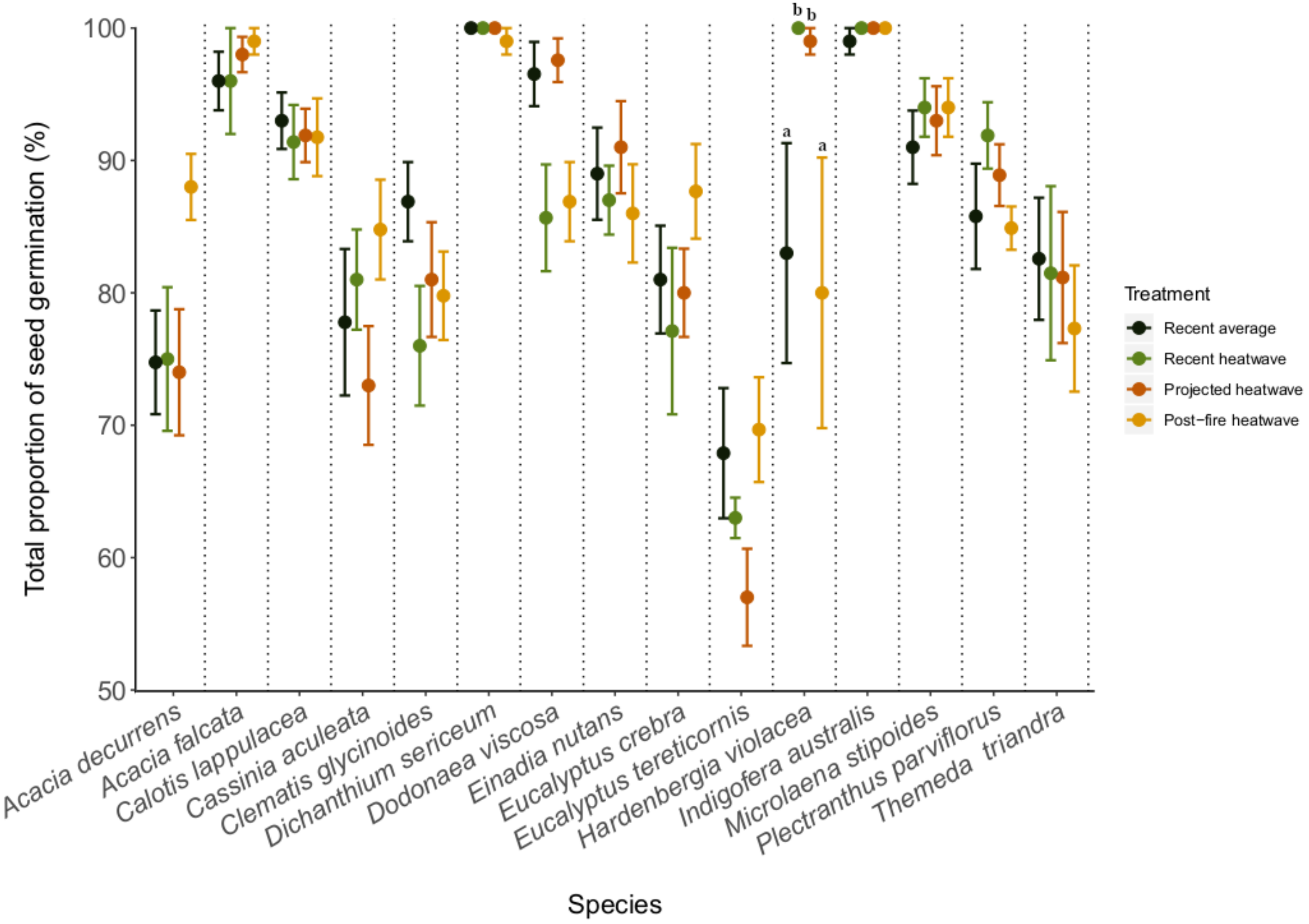


Figure 2.6. Total proportion of germination (mean, ± SE) for each species (separated by the dotted lines) across each of the four experimental treatments (colour coding on the right of the graph). These graphs focus on significant differences among treatments within each species. Recent average 29/17°C, recent heatwave 39/21°C, projected heatwave 43/25°C, and post-fire heatwave 60/22°C. Significant differences (Tukey's post-hoc $P < 0.05$) among treatments within each species are shown by different letters.

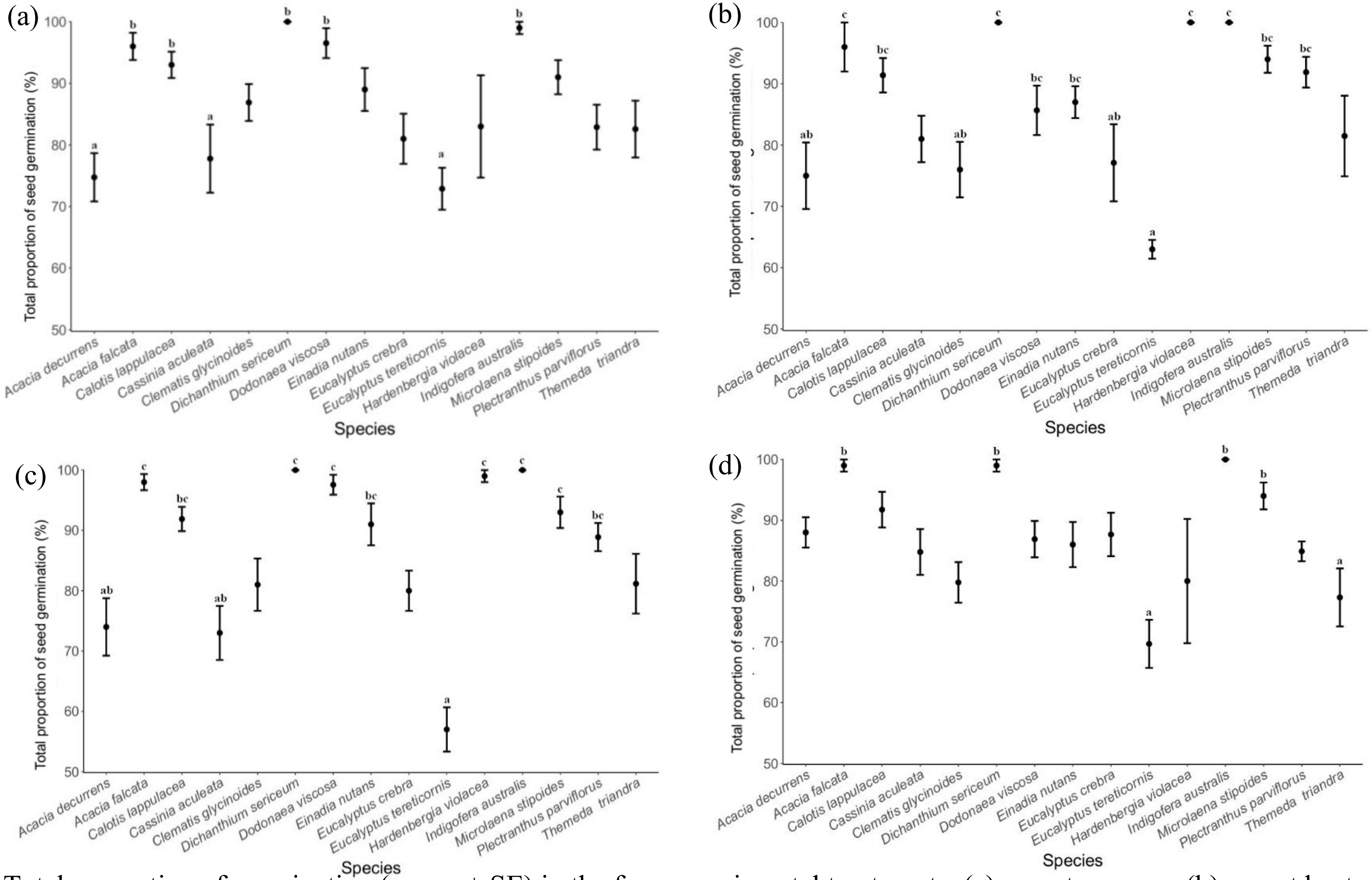


Figure 2.7. Total proportion of germination (mean, ± SE) in the four experimental treatments: (a) recent average, (b) recent heatwave, (c) projected heatwave, and (d) post-fire heatwave for each species. Recent average 29/17°C, recent heatwave 39/21°C, projected heatwave 43/25°C, and post-fire heatwave 60/22°C. Significant differences (Tukey's post-hoc $P < 0.05$) among species within each treatment are shown by different letters.

2.3.4 Experiment 2 - Onset of germination

There was a significant effect of species ($F_{14, 300} = 226.30$, $P < 0.000$) and a significant species x treatment interaction ($F_{42, 300} = 2.14$, $P < 0.000$) on onset of seed germination (Fig. 2.8; Appendix 2.11). There was no significant effect of treatment on onset of germination ($F_{3, 300} = 1.39$, $P = 0.246$). Expanding on the species x treatment interaction, germination onset differed significantly among treatments within only one of the 15 species, *Clematis glycinoides* (Fig. 3.2; Appendix 2.12). *Clematis glycinoides* took significantly longer to begin germination after one heatwave scenario when compared with the five heatwaves scenario. All other species had similar onset of seed germination across the four heatwave scenarios.

Significant differences were found across species within each of the four treatment groups (Fig. 2.9; Appendix 2.13). Three species took between 10 to 22 days to begin germination, *Calotis lappulacea, Cassinia aculeata* and *Clematis glycinoides*. *Calotis lappulacea* and *Clematis glycinoides* were significantly more delayed in their onset when compared with all other species, across all treatments. *Cassinia aculeata* was only significantly more delayed than other species in the no heatwave and one heatwave treatments. Most species began germination within the first eight days after placement into agar and incubation.

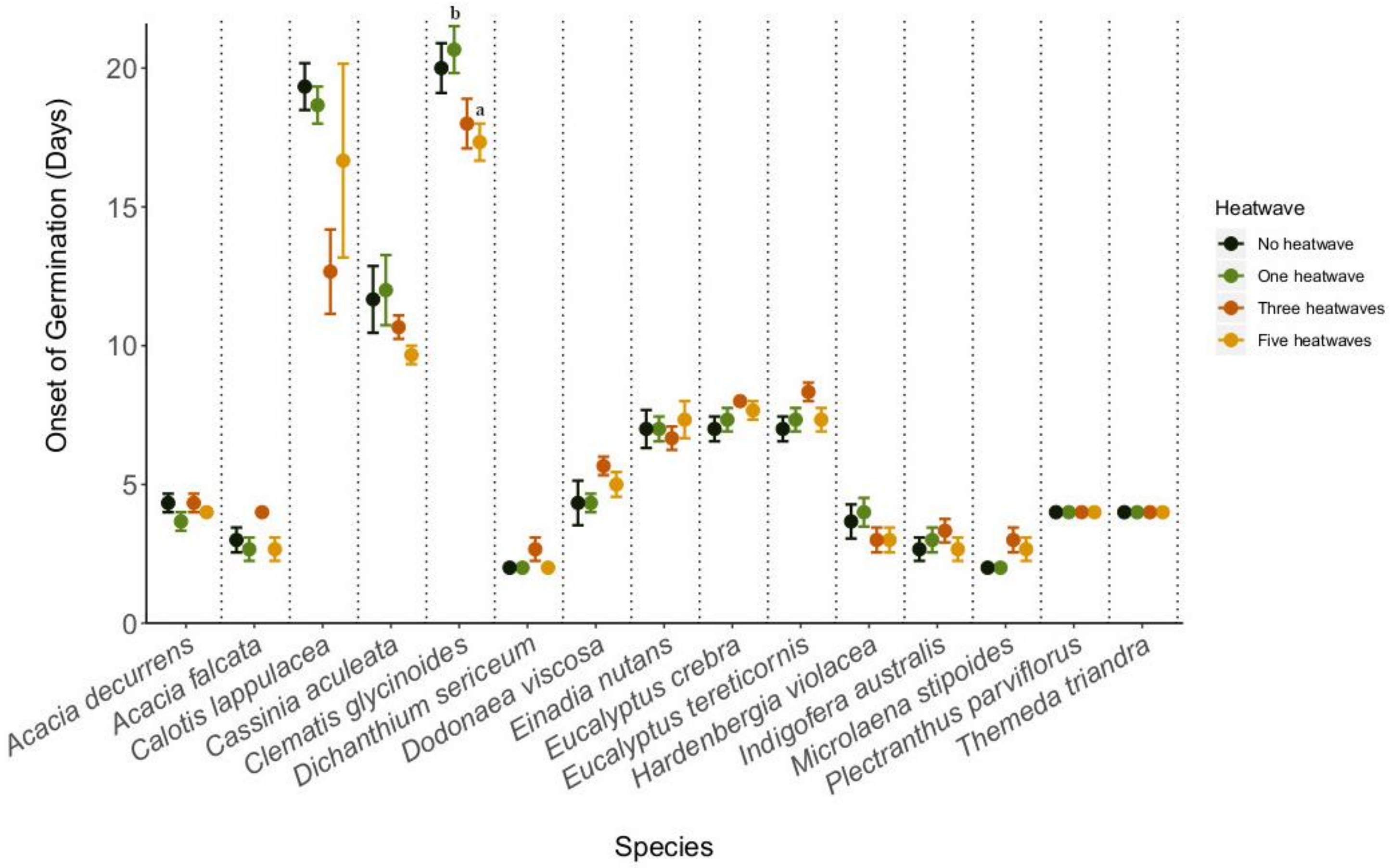


Figure 2.8. The onset of seed germination (mean, ± SE) for each species (separated by the dotted lines) across each of the four experimental treatments (colour coding on the right of the graph). These graphs focus on significant differences among treatments within each species. No heatwave, one heatwave, three heatwaves, and five heatwaves. Significant differences (Tukey's post-hoc $P < 0.05$) among treatments within each species are shown by different letters.

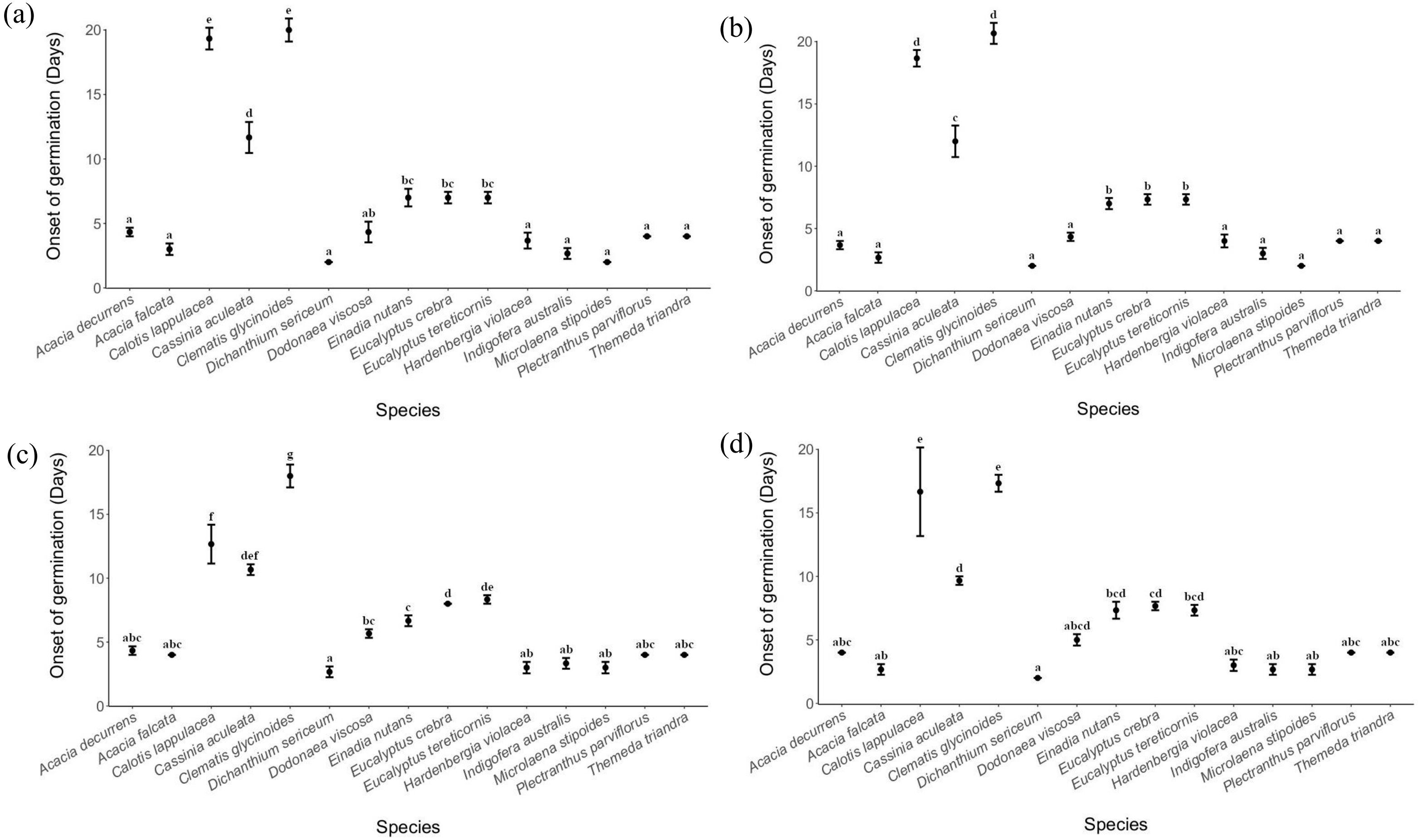


Figure 2.9. The onset of seed germination (mean, ± SE) in the four experimental treatments: (a) no heatwave, (b) one heatwave, (c) three heatwaves, and (d) five heatwaves for each species. Significant differences (Tukey's post-hoc $P < 0.05$) among species within each treatment are shown by different letters.

2.3.5 Experiment 2 – Duration of germination

There was a significant effect of species ($F_{14, 300} = 61.15$, $P < 0.000$) on germination duration as well as a significant species x treatment interaction ($F_{42, 300} = 2.91$, $P < 0.000$; Fig. 2.10; Appendix 2.14). There was no significant effect of multiple heatwaves on germination duration ($F_{3, 300} = 1.91$, $P = 0.128$). The significant interaction between species and treatment was driven by four of the 15 species (Fig. 3.4; Appendix 2.15). Significant treatment effects were observed in *Cassinia aculeata, Dodonaea viscosa, Eucalyptus tereticornis* and *Microlaena stipoides. Cassinia aculeata* spent significantly more time germinating after exposure to three heatwaves than to all other heatwave scenarios; the least amount of time germinating occurred after five heatwaves. *Dodonaea viscosa* spent a significantly longer amount of time germinating after no heatwave than five heatwaves. An increase in the period taken for germination in *Eucalyptus tereticornis* occurred as the number of treatment heatwaves increased, with a significant difference found between the no heatwave scenario and the five heatwaves scenario. *Microlaena stipoides* varied significantly in length of time taken to germinate between one heatwave and three heatwaves. All other species spent a similar amount of time germinating across treatment heatwaves.

A significant difference was observed across species within each of the four heatwave treatment groups (Fig. 2.11; Appendix 2.16). Between treatments, *Calotis lappulacea, Cassinia aculeata* and *Clematis glycinoides* spent anywhere from 10 to 40 days to complete the germination process. Some species that varied between treatments, *Dodonaea viscosa, Eucalyptus crebra* and *Eucalyptus tereticornis,* sometimes took longer than 10 days to complete germination. Most other species took less than 10 day to complete this process.

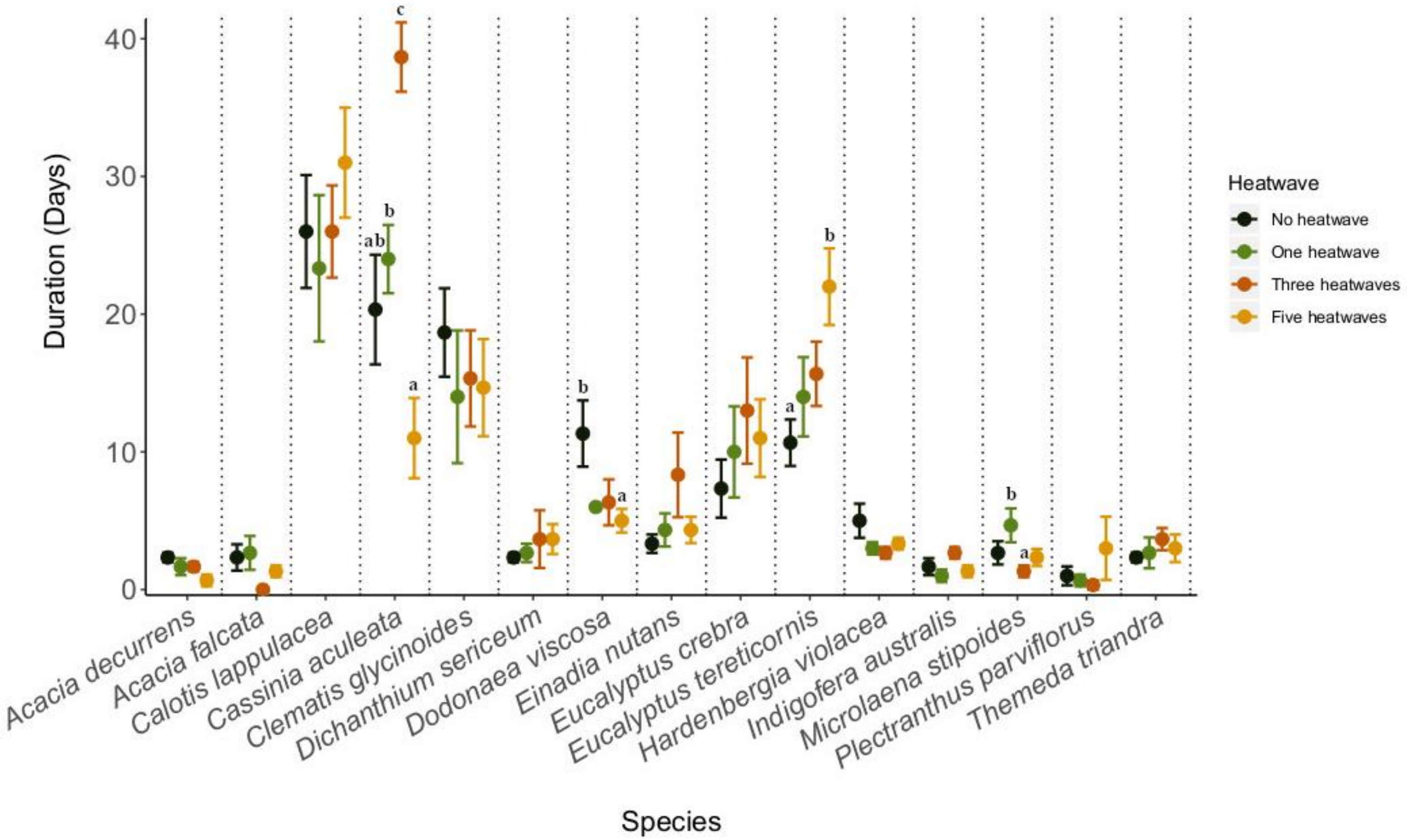


Figure 2.10. Duration time of germination (mean, ± SE) for each species (separated by the dotted lines) across each of the four experimental treatments (colour coding on the right of the graph). These graphs focus on significant differences among treatments within each species. No heatwave, one heatwave, three heatwaves, and five heatwaves. Significant differences (Tukey’s post-hoc $P < 0.05$) among treatments within each species are shown by different letters.

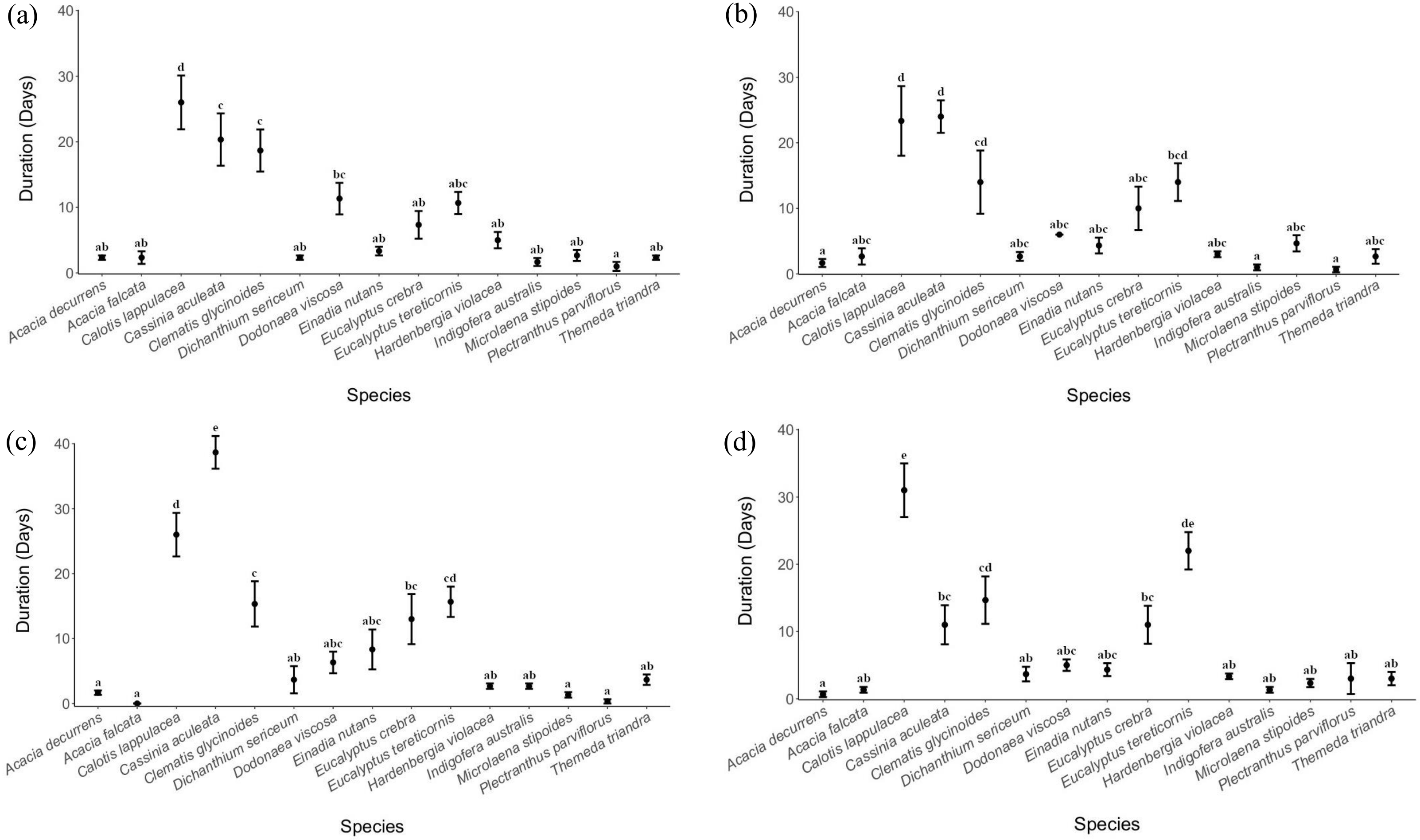


Figure 2.11. Duration time of germination (mean, ± SE) in the four experimental treatments: (a) no heatwave, (b) one heatwave, (c) three heatwaves, and (d) five heatwaves for each species. Significant differences (Tukey's post-hoc $P < 0.05$) among species within each treatment are shown by different letters.

2.3.6 Experiment 2 - Total proportion of germination

There was a significant effect of species ($\chi^2 = 349.7$, df = 14, $P < 0.000$) on the total proportion of seed germination and a significant species x treatment interaction ($\chi^2 = 112.07$, df = 42, $P < 0.000$; Fig. 2.12; Appendix 2.17). There was no significant effect of multiple heatwaves on total seed germination ($\chi^2 = 5.6$, df = 3, $P < 0.133$). Breaking this down further, the significant interaction of total proportion of seed germination was driven by three of the 15 species (Appendix 2.18). These significant treatment effects were observed in *Calotis lappulacea, Cassinia aculeata* and *Hardenbergia violacea*. *Calotis lappulacea* had significantly higher proportions of germination in the no heatwave and one heatwave treatments compared with the five-heatwave treatment. Comparatively, *Cassinia aculeata* was significantly different between no heatwave and five heatwaves, with a steady increase in germination as the heatwaves increased. *Hardenbergia violacea* had lower proportions of germination after no heatwave when compared with one heatwave. The three heatwave and five heatwave treatments had lower germination than the one heatwave treatment but not by a significant amount. All other species varied considerably between treatments but with no other significant effects found.

Significant differences were found across species within each of the four treatment groups (Fig. 2.13; Appendix 2.19). Across all treatments *Acacia decurrens* had significantly low total proportion of seed germination when compared with most other species. After one heatwave *Eucalyptus tereticornis* also had significantly low levels of germination when compared with six of the 15 species. Unlike ‘Experiment 1’, no species consistently had 100% germination; of the 15 species, four species had 100%

germination in at least one of the treatments. The rest of the species ranged from 57% to 98% germination after exposure to the treatments.

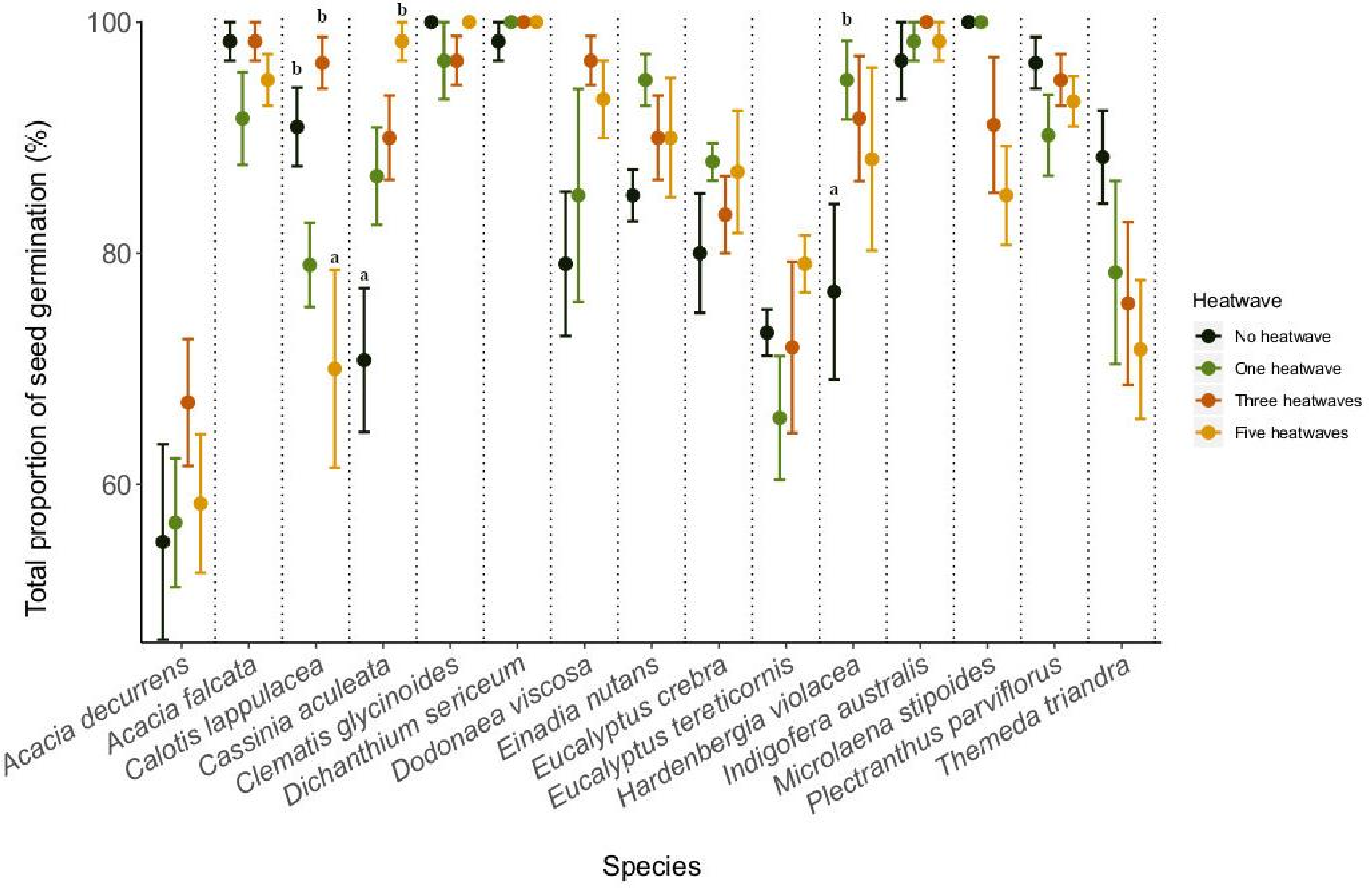


Figure 2.12. Total proportion of seed germination (mean, ± SE) for each species (separated by the dotted lines) across each of the four experimental treatments (colour coding on the right of the graph). These graphs focus on significant differences among treatments within each species. No heatwave, one heatwave, three heatwaves, and five heatwaves. Significant differences (Tukey's post-hoc $P < 0.05$) among treatments within each species are shown by different letters.

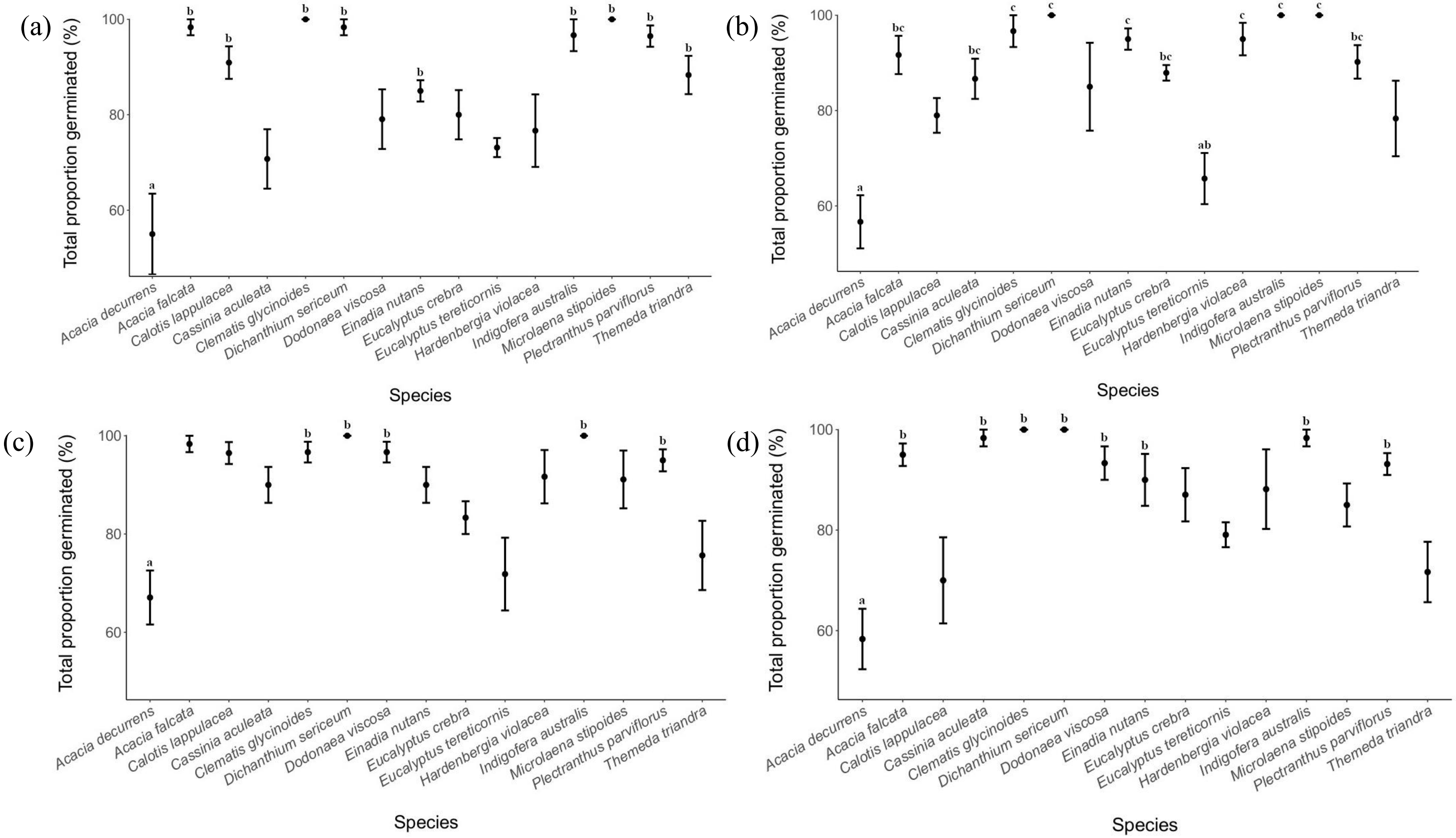


Figure 2.13. Total proportion of seed germination (mean, ± SE) in the four experimental treatments: (a) no heatwave, (b) one heatwave, (c) three heatwaves, and (d) five heatwaves for each species. Significant differences (Tukey's post-hoc $P < 0.05$) among species within each treatment are shown by different letters.

2.4 Discussion

2.4.1 Overall findings

The results of this study showed that experimental heatwave treatments only affected seed germination in a small number of species. Overwhelmingly, germination attributes did not change significantly under the various heatwave treatments, which demonstrates that most native plant species from the CPW were tolerant of a range of heatwave scenarios. This is a reassuring outcome, proving that future projections of climate change induced heatwaves do not have detrimental effects on seed germination in initial laboratory-based trials. Understandably, it was expected that as heatwaves increased in intensity and frequency, seed germination attributes would be adversely affected, thus proving a significant treatment effect of experimental heatwaves. Previous studies allude to this phenomenon, identifying the detrimental effects of high temperatures on basic seed physiology (Ellis *et al.* 1987; Roberts 1988). Conversely, seeds have also been known to favour higher temperatures, more so than cooler temperature, during germination (Milbau *et al.* 2009; Mondoni *et al.* 2012; Orsenigo *et al.* 2014). In this study, seed germination attributes to the experimental heatwaves were inconsistent – neither positively nor negatively affecting germination in a consistent manner. The curious nature of this outcome warrants further investigation, particularly into the species x treatment interaction.

2.4.2 Deconstructing the species x treatment interaction

I found a significant species x treatment interaction in all measured germination attributes, across both experiments. A species x treatment interaction is described as a difference between treatments – dependent on species – and vice versa. In terms of this study, the species x treatment interaction is largely driven by specific species' response to all treatments. However, for further interpretation, I also compare all species within each treatment; in essence, the response of treatment is species dependent. From both experiments, and across the response variables, these species – *Calotis lappulacea, Cassinia aculeata, Clematis glycinoides, Dodonaea viscosa, Einadia nutans, Eucalyptus crebra, Eucalyptus tereticornis* and *Hardenbergia violacea* – drove the species x treatment interaction. Six of these species also represent some of the smallest seeds in this study.

2.4.3 Ecological context

Recognising a link between seed mass and germination could result in a discernable pattern being observed. While a study into this link has been previously conducted, no significant effect was found (Moles and Westoby 2006). In contrast, another study revealed a positive relationship between seed mass and germination time, with small seeds germinating more readily than large seeds (Norden *et al.* 2009). These studies contradict my initial observations. Further analysis of seed traits could reveal seed coat as another potential source of variation. Evolutionarily speaking, the seed coat provides a physical defence mechanism against harmful environmental conditions (Mohamed-Yaseen *et al.* 1994).

Among the species recognised as contributors toward the species x treatment interaction, *Acacia* spp., *Hardenbergia violacea* and *Dodonaea viscosa* all have hard-coated seeds. While these were scarified prior to incubation, the seed coat remained intact during the heatwave treatment, preventing internal damage from occurring. Following heat exposure in a natural context, such as a drought, previous results indicate an increase is seed coat hardness (Argel and Humphreys 1983; Noodén *et al.*

1985; Fenner and Thompson 2005). Had my study been conducted in an *in situ* setting, these seeds could have been protected against higher temperatures, making their reactions more stable than noted here. Comparatively, seeds from Asteraceae and Ranunculaceae have minimal protection against heat, leaving them exposed and vulnerable to heat stress. While they did not exhibit a consistent decrease in germination resulting from heat stress, they were more affected than other species. Small seeds on the other hand are more susceptible to external stressors, with lower life expectancy and viability than larger seeds (Corner 1976; Mohamed-Yaseen *et al.* 1994). Another source of species variation in germination attributes could lie within species dormancy.

Each species in this study required individual treatment regarding dormancy: examples include Fabaceae (physical dormancy) and Myrtaceae and Poaceae (physiological dormancy). Delay in germination from some species may be as a result of some form of dormancy (Baskin and Baskin 1985; Offord and Meagher 2009). Crucially, it is important not to confuse dormancy with persistence, as a previous study describes (Ooi *et al.* 2007). Heatwaves might effect seedbank persistence and, with this kind of study, it is difficult to determine whether this was an influencing factor (Thompson *et al.* 1993).

The very nature of conducting laboratory-based experiments such as these two experiments prove that while some findings are relevant, ecologically speaking, these species' reactions may be different in an *in situ* situation. To account for this, Chapter 3 focuses on the role of these seed traits to determine a possible relationship between the germination attributes and the species-specific traits. A study by Saatkamp *et al.* (2018) highlights the necessity of studying seed traits in an ecological context to enhance the global understanding guiding restoration protocol and increase the success of ecosystem conservation.

2.4.4 Concluding remarks

In response to the initial aims of this Chapter, heatwave scenarios did affect the germination attributes of Cumberland Plain Woodland native plant species. However, and in contrast to expectation, intensity and frequency did not directly influence germination. Rather, species were affected by some but not all treatments and vice versa. Resilience to intense and prolonged heatwave events prove that these species are hardier than previously thought, a comforting prospect in the face of a rapidly changing climate. To provide further explanation into these interactions, Chapter 3 establishes seed traits as another source of variation, from an ecological perspective, to these laboratory-based experiments.

Chapter 3

Relationships between plant life-history traits and seed germination in native plant species of the Cumberland Plain Woodland

“Oh no, not again” – Douglas Adams, The Hitchhiker’s Guide to the Galaxy

3.1 Introduction

In the previous Chapter, I found that experimental heatwaves did not consistently affect seed germination across species as I expected. There was no overall reduction in time to germination onset, increase in duration of germination, or reduction in the total proportion of seeds that germinated. Instead, I found that germination attributes varied considerably among species for each treatment, such that there was no consistent interspecific pattern in germination response to increasing intensity or frequency of heatwaves. Under one experimental heatwave treatment, for instance, some species germinated quickly, had short germination periods, and high total germination (e.g. *Acacia decurrens*), while other species had the opposite response (e.g. *Eucalyptus tereticornis*). In this Chapter, I seek to further understand the mechanisms underpinning variation in species’ germination attributes by examining how four key plant life-history traits, including seed mass, seed dormancy, a species’ fire response and plant life form relate to patterns of seed germination. The conceptual approach I adopt here is to ask how much interspecific variation in germination response is explained by these four life-history traits over and above the observed idiosyncratic effects of the heatwave treatments in the previous Chapter.

Comparative studies of seed germination in relation to seed mass typically focus on how seed size affects germination based on the fundamental trade-off between seed size and seed production (i.e. large-seeded vs small-seeded species: Gross and Smith 1991; Kahmen and Poschlod 2008; Norden *et al.* 2009; Barak *et al.* 2018). Generally, large-seeded species germinate faster and into more successful seedlings (Moles and Westoby 2004, 2006). However, depending on the study species and the study system, this is not always the case (Norden *et al.* 2009; Barak *et al.* 2018). Similarly, there are contradictions in the literature for the effect of seed mass on germination duration, with some studies showing that species with large seeds have short germination duration (Verdú and Traveset 2005) while other studies have shown that large-seeded species have long germination duration (Norden *et al.* 2009). In the context of the proportion of seeds that germinate from a pool of viable seeds, some studies have shown that species with large seeds have a low germination proportion (Bu *et al.* 2007) while others have shown that large-seeded species have a high germination proportion (Stanton 1984). The contradictions evident in the literature therefore make it difficult to determine unequivocally just how seed mass variation is likely to affect individual species' germination attributes. Thus, there is an imperative to determine the role of seed mass in explaining variation in seed germination among the native plant species of the Cumberland Plain Woodland (CPW).

Species with dormant seeds require specific environmental conditions for germination to occur (Finch-Savage and Leubner-Metzger 2006; Offord and Meagher 2009). Dormant seeds remain inactive until a thermal, chemical, or physical stimulus begins the metabolic processes (Cochrane and Probert 2006; Ooi *et al.* 2014; Barak *et al.* 2018). Non-dormant species, in contrast, are metabolically prepared to germinate as soon as they become a free-standing seed (Bewley 1997). Once the basic germination requirements are met, e.g. optimum light, temperature, and water availability are present, non-dormant seeds can begin to germinate. Here, I test the hypothesis that the inherent physiological mechanism that has evolved to ensure dormancy in species with dormant seeds continues to have an effect to delay germination onset, as well as prolong germination duration and decrease the total proportion of seeds that germinate, even after dormancy has been broken. It is therefore expected that non-dormant seeds will have quicker germination onset, shorter germination duration and higher germination proportions, than the dormant seeds.

The fire response of a species describes its capacity to regenerate post-fire using one of two strategies: obligate seeding or resprouting. A resprouter has evolved to survive fires and regrow regeneratively from specialized buds such as lignotubers or epicormic buds after fire, while in contrast an obligate seeder cannot generally do this and must rely on seeds alone as its primary survival mechanism in response to fire (Gill 1981; Bradstock *et al.* 1997). Consistent with this mechanism, obligate seeders typically dedicate more resources to reproductive structures than resprouters (Knox and Morrison 2005). In relation to germination properties, the literature presents conflicting results with some studies finding obligate seeders quicker to germinate than resprouters (Knox and Morrison 2005) and others finding the opposite (Zammit and Westoby 1987). Obligate seeders are expected to germinate more readily than resprouters as their investment in seed-based adaptations relating to fire promote quick germination onset and establishment post-fire (Pausas *et al.* 2004). Along similar lines, total proportion of seed germination is also expected to be higher in obligate seeders compared to resprouters (Knox and Morrison 2005). On the other hand, the literature does not provide any definitive evidence demonstrating the effects of fire response on the duration period of germination. This study will provide an insight into the effects of fire response type, specifically for CPW native plant species, to determine whether obligate seeders have a quicker or slower germination onset, longer or short germination duration, and higher or lower germination proportion than resprouters.

As with the other three life-history traits in this study, life form shows contradictory relationships with germination in the literature. Life form describes the habit of a plant, and its size and shape (e.g. tree, shrub, climber, grass). Some studies have found that certain life forms have quicker germination onset, duration, and higher total proportion of germination than other life form types. Grime *et al.* (1981) found forbs and grasses had quicker germination onset and duration than sedges, shrubs and trees, and a higher total proportion of germination in grasses than forbs. However, Bu *et al.* (2008) found woody plants had quicker germination and duration, and higher proportion of germination than grasses and forbs. Other studies found no correlation between life form and germination attributes (Garwood 1983; Morgan 1998). This leads to inconclusive results of how life-history traits may affect germination attributes, especially in a region-specific study. To address these contradictions, this study investigates the role of life form on seed germination attributes in native plant species from the CPW.

In this Chapter, I seek to determine whether the four life-history traits described above are related to interspecific patterns of variation in germination onset, duration, and the total proportion of seed germination. As such, a focus is placed on whether there is a direct influence of life-history traits on the germination attributes above the idiosyncratic levels found in the previous Chapter. This Chapter therefore aims to understand the context-specific role of life-history traits on seed germination attributes in the Cumberland Plain Woodland.

3.2 Materials and Methods

3.2.1 Study species

This Chapter builds on the experimental research performed in Chapter 2 of the thesis and the same set of study species are the focus of this work (see Chapter 2, section 2.X).

3.2.2 Collection of trait information for each species

Table 3.1 presents summary trait information for each species. Seed mass was measured as the weight of 1000 seeds for each species using an analytical balance (Mettler Toledo AG204; Mumbai, India). Seeds were left to equilibrate at room temperature and humidity and all dispersal structures were removed before weighing. Seeds for seed mass measurements were sourced from either fresh field collections or from a seedbank collection (see Chapter 2).

Species were classified as dormant (species which require a germination stimulant if no germination occurs within 6 weeks under suitable conditions) or non-dormant (species which readily germinate within 6 weeks under suitable conditions) based on observations within the study region (noting that dormancy can vary within a species across geographic ranges; Baskin and Baskin 2004). Dormancy mechanisms of species were obtained from Offord and Meagher (2009).

Each species was classified as either an obligate seeder (species that die when exposed to fire and rely on seedbank for regeneration) or a resprouter (species that regenerate after fire through epicormic shoots or lignotubers) based on observations within the study region (noting that fire response can vary within a species across geographic ranges; Gill 1981; Gill and Bradstock 1992). Fire response data were obtained from fire ecology research performed in the Cumberland Plain Woodland (CPW) (Watson 2005; Penman *et al.* 2008; Kubiak 2009).

Information on species' life form was sourced from PlantNET (2019), Tozer (2003), Watson (2005) and (Fairley and Moore 2010). Each species was classified as either a tree (a single-stemmed woody plant > 5m), shrub (a multi-stemmed woody plant < 5m), herb (a non-woody plant < 2m), climber (a small multi-branched woody plant < 1m) or grass (species in Poaceae).

Table 3.1. Study species used in the analysis with taxonomic family and life-history trait information. Seed mass is the weight of 1000 seeds (g). Life form: grass, herb, shrub, tree, or climber. Fire response: obligate seeder or resprouter. Dormancy: dormant or non-dormant.

Family	Species	Seed mass (g)	Life form	Fire response	Dormancy
Asteraceae	*Cassinia aculeata*	0.05	shrub	obligate seeder	dormant
	Calotis lappulacea	0.46	herb	resprouter	dormant
Chenopodiaceae	*Einadia nutans*	0.68	herb	obligate seeder	non-dormant
Fabaceae	*Indigofera australis*	3.71	shrub	resprouter	dormant
	Acacia decurrens	12.99	tree	obligate seeder	dormant
	Acacia falcata	14.09	shrub	obligate seeder	dormant
	Hardenbergia violacea	17.15	climber	resprouter	dormant
Lamiaceae	*Plectranthus parviflorus*	0.24	herb	obligate seeder	non-dormant
Myrtaceae	*Eucalyptus crebra*	0.15	tree	resprouter	non-dormant
	Eucalyptus tereticornis	0.29	tree	resprouter	non-dormant
Poaceae	*Dichanthium sericeum*	0.61	grass	resprouter	dormant
	Microlaena stipoides	5.03	grass	resprouter	dormant
	Themeda triandra	3.77	grass	resprouter	dormant
Ranunculaceae	*Clematis glycinoides*	1.94	climber	resprouter	dormant
Sapindaceae	*Dodonaea viscosa*	3.20	shrub	obligate seeder	dormant

3.2.3 Statistical analysis

Generalized linear models with a Poisson error structure and a log link were used to model the relationships between life-history traits (as explanatory variables) and both the onset of germination and length of germination period (separate response variables in one of two models). To account for overdispersion, a generalized linear model with a quasi-binomial error structure and a logit link related germination proportion (response variable) to the life-history traits (as explanatory variables). Seed mass was log transformed and entered in models as a continuous variable, and life form (five levels), fire response (two levels) and dormancy (two levels) as fixed categorical variables. Two sets of analyses were performed using these models, one set for each of the germination experiments – heatwave intensity and heatwave frequency – described in Chapter 2. A fifth explanatory variable was included in the models that accounted for the treatments in the germination experiments. For the heatwave intensity experiment, treatment was a four-level factor (control, recent heatwave, projected heatwave, post-fire heatwave). Treatment was also a four-level factor for the heatwave frequency experiment (control, one heatwave, three heatwaves, five heatwaves). Post-hoc contrasts with a Tukey correction for multiple comparisons were run for life form where a significant difference was observed. All analyses were conducted using R statistical software (R core team 2019) through R Studio (Version 3.5.1).

3.3 Results

3.3.1 Trait relationships with germination: heatwave intensity

Onset of germination was significantly related to life form ($F_{4,49} = 15.25$, $P < 0.0001$), dormancy ($F_{1,49} = 28.35$, $P < 0.0001$) and seed mass ($F_{1,49} = 66.06$, $P < 0.0001$) (Fig.

3.1), but was not significantly related to fire response ($F_{1,49} = 0.04$, $P = 0.80$) or experimental treatment ($F_{3,49} = 0.03$, $P = 0.97$). Later onset of germination was found in smaller, dormant seeds, with climbers and herbs significantly delayed compared with grasses and shrubs. Comparatively, trees were significantly delayed in their onset compared with the grasses, but faster when compared with climbers.

Germination duration was significantly related to life form ($F_{4,49} = 14.36$, $P < 0.0001$), dormancy ($F_{1,49} = 69.14$, $P < 0.0001$) and seed mass ($F_{1,49} = 105.30$, $P < 0.0001$) (Fig. 3.2), but was not significantly related to fire response ($F_{1,49} = 1.95$, $P = 0.20$) or experimental treatment ($F_{3,49} = 0.18$, $P = 0.90$). Long germination duration was found in smaller, dormant seeds, with grasses significantly shorter in germination duration than any other life form. Shrubs had significantly shorter germination duration than herbs and trees but were not significantly shorter than climbers.

Germination proportion was significantly related to fire response ($\chi^2 = 7.17$, d.f. = 1, $P = 0.007$), life form ($\chi^2 = 53.00$, d.f. = 4, $P < 0.0001$) and seed mass ($\chi^2 = 9.50$, d.f. = 1, $P = 0.002$) (Fig. 3.3) but was not significantly related to dormancy ($\chi^2 = 0.22$, d.f. = 1, $P = 0.60$) or experimental treatment ($\chi^2 = 0.66$, d.f. = 3, $P = 0.90$). High germination proportions were found in larger, resprouting seeds. Trees were significantly lower in germination proportions than any other life form and climbers had significantly higher germination proportions than herbs.

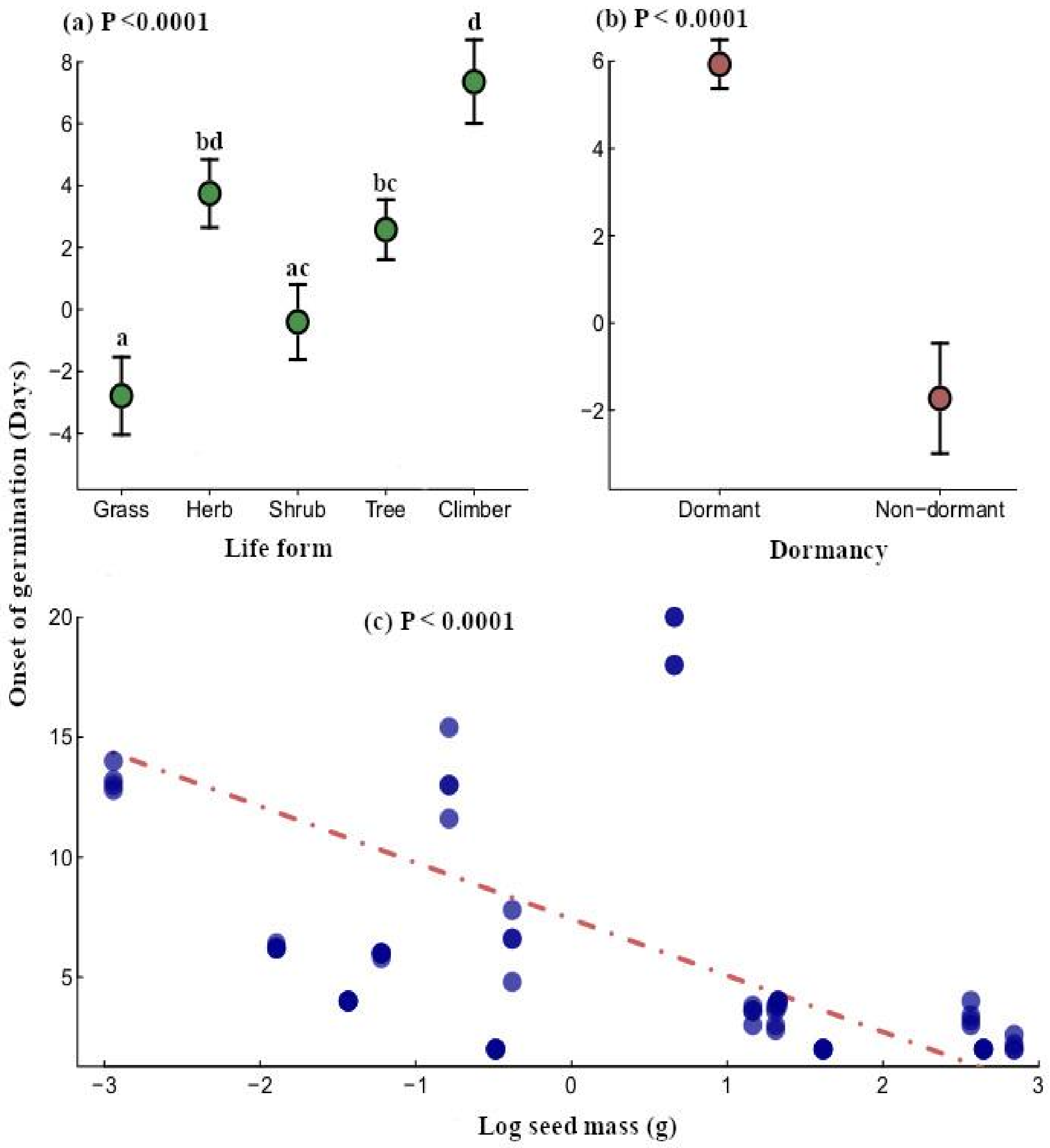


Figure 3.1. The onset of seed germination (mean, ± SE) for (a) life form, (b) dormancy, and (c) seed mass, in the heatwave intensity experiment. Within life form, significant differences (Tukey's post-hoc $P < 0.05$) between groups are displayed with different letters. As a result of modelling techniques, life form and dormancy are plotted across both negative and positive values on the y-axis. Red trend line added showing linear relationship between seed mass and onset of germination.

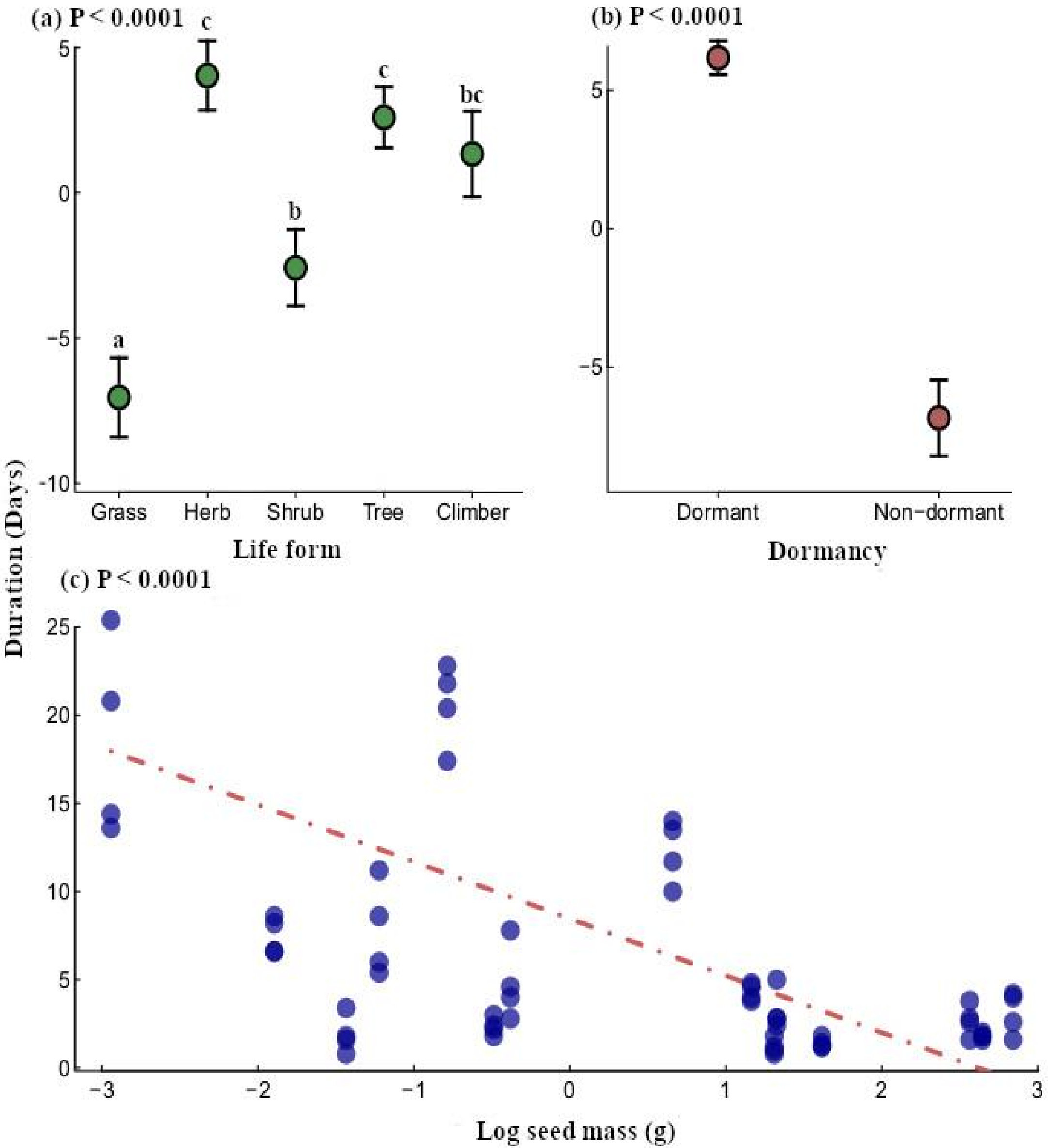


Figure 3.2. Duration of seed germination (mean, ± SE) for (a) life form, (b) dormancy, and (c) seed mass, in the heatwave intensity experiment. Within life form, significant differences (Tukey's post-hoc $P < 0.05$) between groups are displayed with different letters. As a result of modelling techniques, life form and dormancy are plotted across both negative and positive values on the y-axis. Red trend line added showing linear relationship between seed mass and duration of germination.

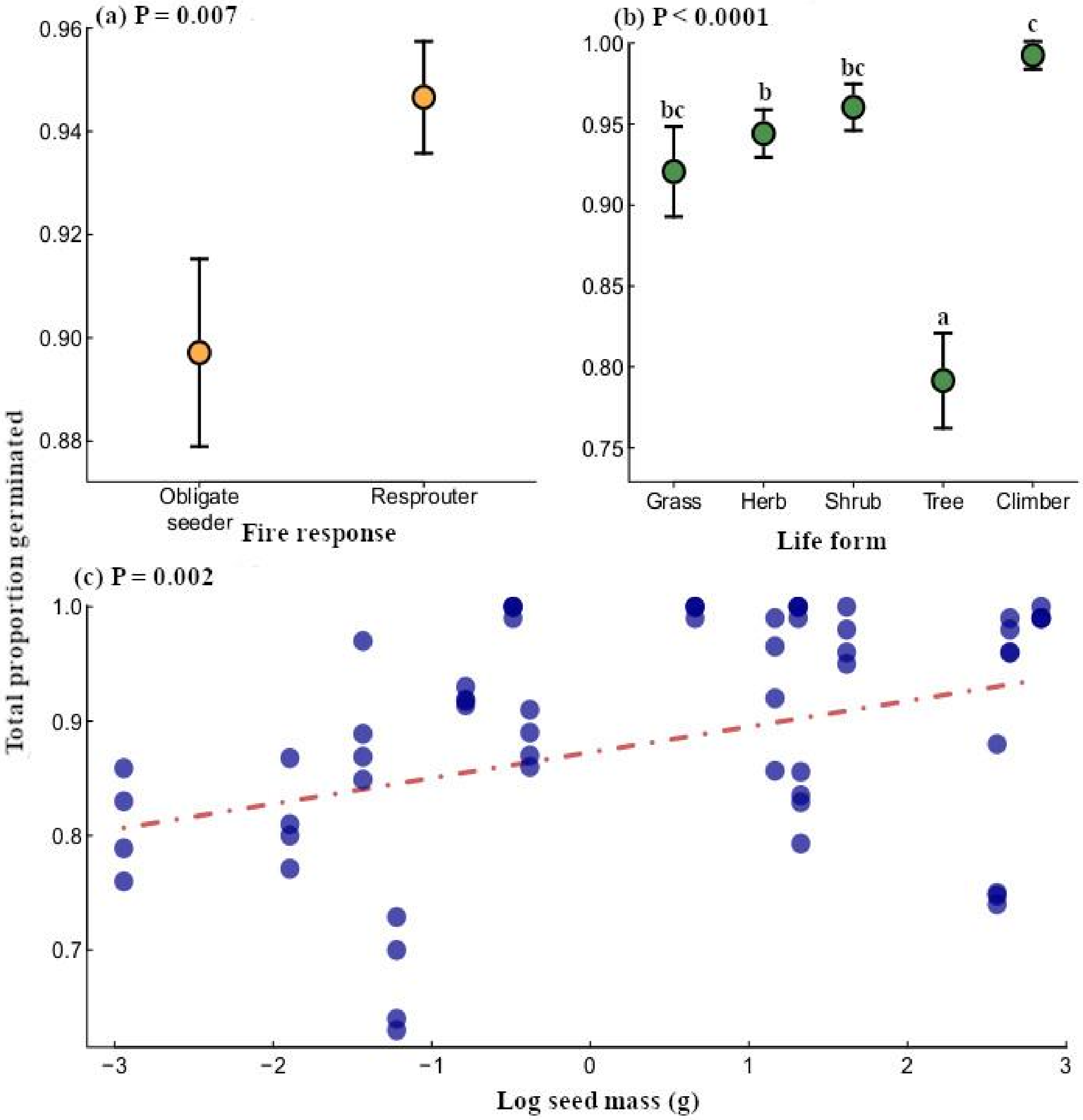


Figure 3.3. The total proportion of seed germination (mean, ± SE) for (a) fire response, (b) life form, and (c) seed mass, in the heatwave intensity experiment. Within life form, significant differences (Tukey's post-hoc $P < 0.05$) between groups are displayed with different letters. As a result of modelling techniques, life form and dormancy are plotted across both negative and positive values on the y-axis. Red trend line added showing linear relationship between seed mass and total proportion of germination.

3.3.2 Trait relationships with germination: heatwave frequency

Onset of germination was significantly related to life form ($F_{4,49} = 17.07$, $P < 0.0001$), dormancy ($F_{1,49} = 29.90$, $P < 0.0001$) and seed mass ($F_{1,49} = 43.99$, $P < 0.0001$) (Fig. 3.4), but was not significantly related to fire response ($F_{1,49} = 2.22$, $P = 0.10$) or experimental treatment ($F_{3,49} = 0.07$, $P = 0.98$). Later onset of germination was found in smaller, dormant seeds, with herbs and climbers significantly delayed compared with grasses and shrubs. Trees were significantly more delayed in onset of germination when compared to grasses.

Germination duration was significantly related to life form ($F_{4,49} = 8.50$, $P < 0.0001$), dormancy ($F_{1,49} = 34.79$, $P < 0.0001$), seed mass ($F_{1,49} = 68.49$, $P < 0.0001$) and fire response ($F_{1,49} = 4.40$, $P = 0.04$) (Fig. 3.5) but was not significantly related to experimental treatment ($F_{3,49} = 0.31$, $P = 0.80$). Long germination duration was found in smaller, resprouting seeds that were dormant. Grasses had a significantly shorter germination period when compared to all other life forms.

Germination proportion was significantly related to life form ($\chi^2 = 39.63$, d.f. = 4, $P < 0.0001$) and only marginally to dormancy ($\chi^2 = 3.99$, d.f. = 1, $P = 0.046$) (Fig. 3.6), but was not significantly related to seed mass ($\chi^2 = 0.06$, df = 1, $P = 0.80$), fire response ($\chi^2 = 1.17$, df = 1, $P = 0.30$) or experimental treatment ($\chi^2 = 1.32$, df = 3, $P = 0.70$). High germination proportions were found in non-dormant seeds, with trees significantly lower in germination proportions than any other life form.

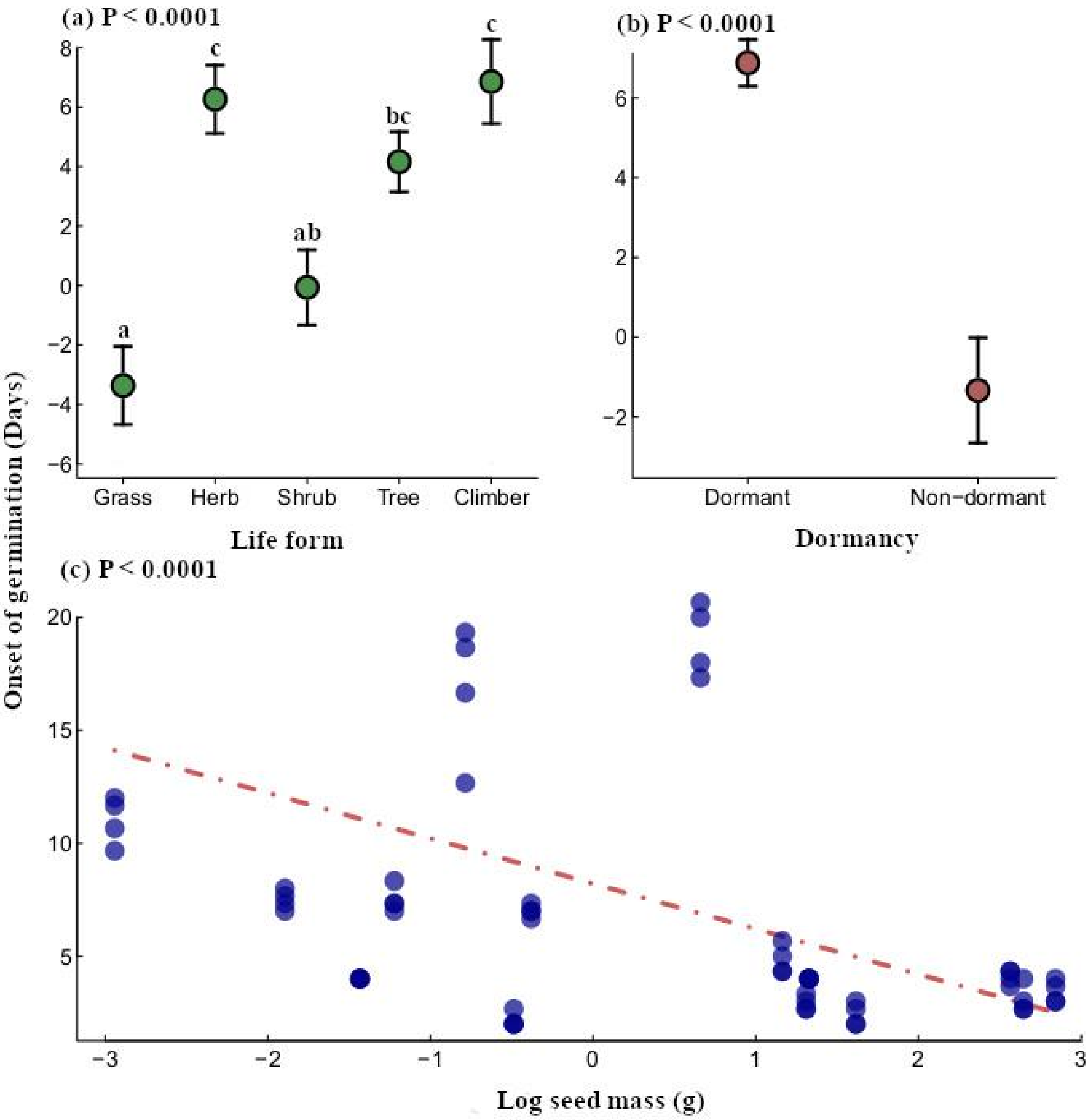


Figure 3.4. The onset of seed germination (mean, ± SE) for (a) life form, (b) dormancy, and (c) seed mass, in the heatwave frequency experiment. Within life form, significant differences (Tukey's post-hoc $P < 0.05$) between groups are displayed with different letters. As a result of modelling techniques, life form and dormancy are plotted across both negative and positive values on the y-axis. Red trend line added showing linear relationship between seed mass and onset of germination.

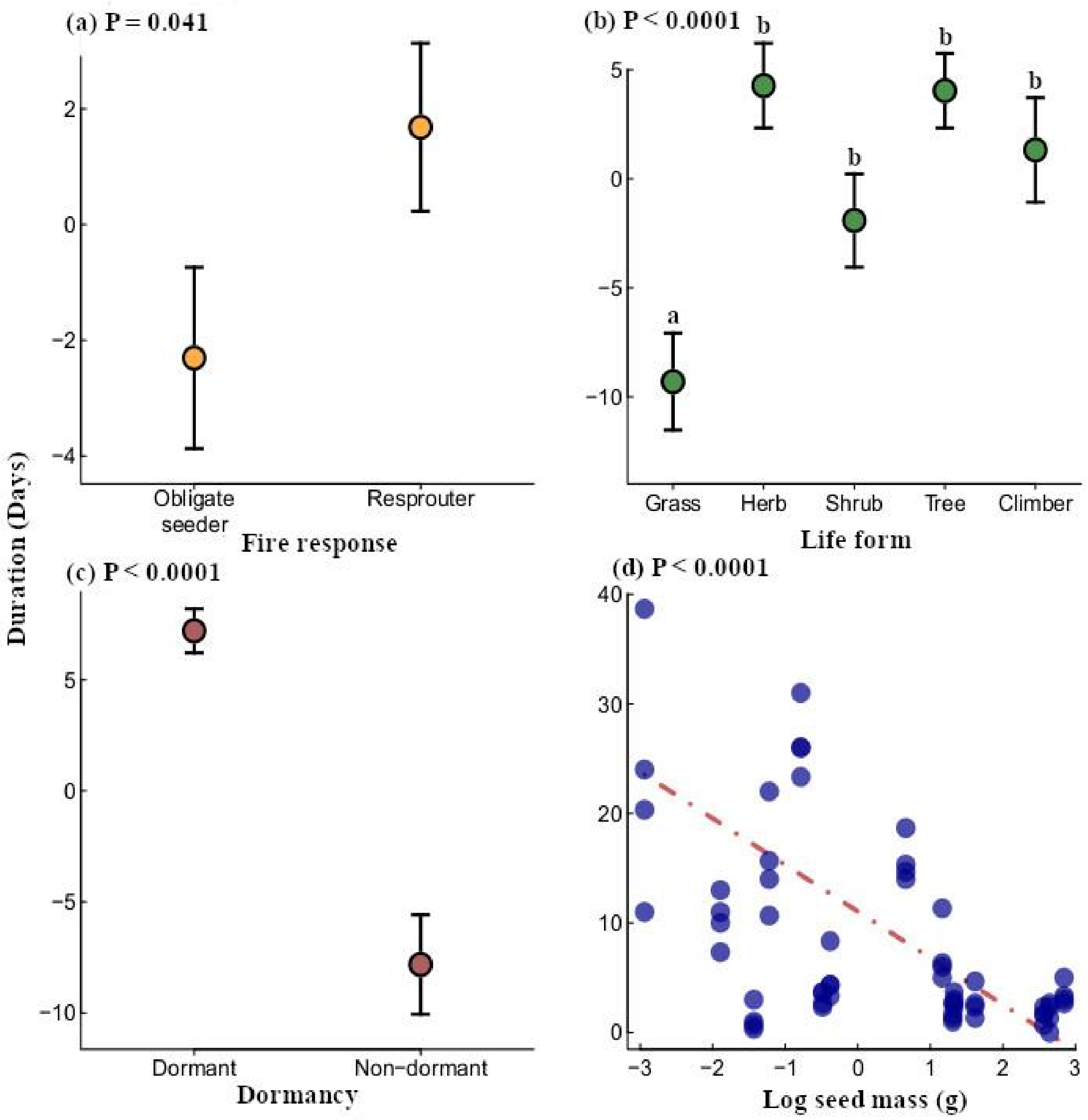


Figure 3.5. Duration of germination (mean, ± SE) for (a) fire response, (b) life form, (c) dormancy and (c) seed mass, in the heatwave frequency experiment. Within life form, significant differences (Tukey's post-hoc $P < 0.05$) between groups are displayed with different letters. As a result of modelling techniques, life form and dormancy are plotted across both negative and positive values on the y-axis. Red trend line added showing linear relationship between seed mass and duration of germination.

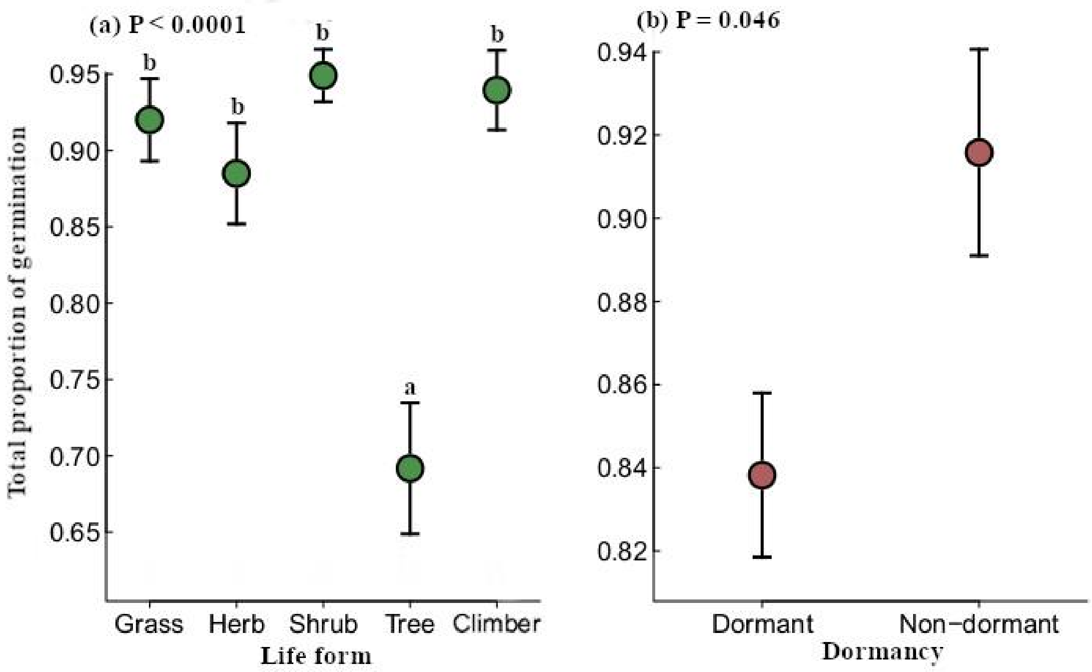


Figure 3.6. Total proportion of germination (mean, ± SE) for (a) life form, (b) dormancy, in the heatwave frequency experiment. Within life form, significant differences (Tukey's post-hoc $P < 0.05$) between groups are displayed with different letters.

3.4 Discussion

3.4.1 Overall findings

This study found that life-history traits are related to interspecific patterns of variation in all three germination attributes, over and above the idiosyncratic patterns observed in the previous Chapter. Life form significantly influenced all three germination attributes across both the intensity and frequency experiments; dormancy influenced onset of germination and duration in both the intensity and frequency experiment, while only significantly influencing total proportion of germination in the frequency experiment; seed mass influenced all three germination attributes within the intensity experiment but did not significantly influence total proportion of germination in the frequency experiment; and fire response influenced total proportion of germination in the intensity experiment and duration of germination within the frequency experiment (Table 3.2; Table 3.3).

In Chapter 2, germination attributes significantly changed under the various heatwave treatments but with each species responding differently to each treatment. It was expected that as a heatwave increased in either intensity or frequency, seed germination attributes would be adversely affected, represented by a significant overall treatment effect of experimental heatwaves without a significant interaction effect between treatment and species. Such an outcome did not emerge for the heatwaves when considered separately in Chapter 2. This Chapter, however, has identified an intriguing pattern for the effects of heatwaves on germination when both heatwave experiments are considered together along a continuum of heatwave severity.

Table 3.2. Summary of seed trait patterns with germination across the intensity and frequency experiments to determine similarity in significance level ($P > 0.05$) when comparing between life-history traits within germination attributes; ns = not significant.

	Onset of germination		**Duration of germination**		**Germination proportion**	
	Intensity	Frequency	Intensity	Frequency	Intensity	Frequency
Treatment	ns	ns	ns	ns	ns	ns
Fire response	ns	ns	ns	0.041	0.007	ns
Life form	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.0001
Dormancy	< 0.0001	< 0.0001	< 0.0001	< 0.0001	ns	0.046
Seed mass	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.002	ns

Table 3.3. Summary of the direction of response by life-history traits in relation to germination attributes. Each life-history trait fire response (resprouter, obligate seeder), life form (grass, shrub, herb, tree, climber), dormancy (non-dormant, dormant) and seed mass (smaller seeds, larger seeds) was placed into one of two categories for each germination attribute based on patterns in Table 3.1. Onset of germination was split into quick and delayed reaction, duration of germination was split into short and long time periods, and germination proportion was split into low and high proportions of germination.

	Onset of germination		**Duration of germination**		**Germination proportion**	
Effect	Quick	Delayed	Short	Long	Low	High
Fire response	-	-	Resprouter*	Obligate seeder*	Obligate seeder	Resprouter
Life form	Grass Shrub	Herb Tree Climber	Grass Shrub	Herb Tree Climber	Tree	Grass Herb Shrub Climber
Dormancy	Non-dormant	Dormant	Non-dormant	Dormant	Dormant^	Non-dormant^
Seed mass	Larger seeds	Smaller seeds	Larger seeds	Smaller seeds	Smaller seeds*	Larger seeds *

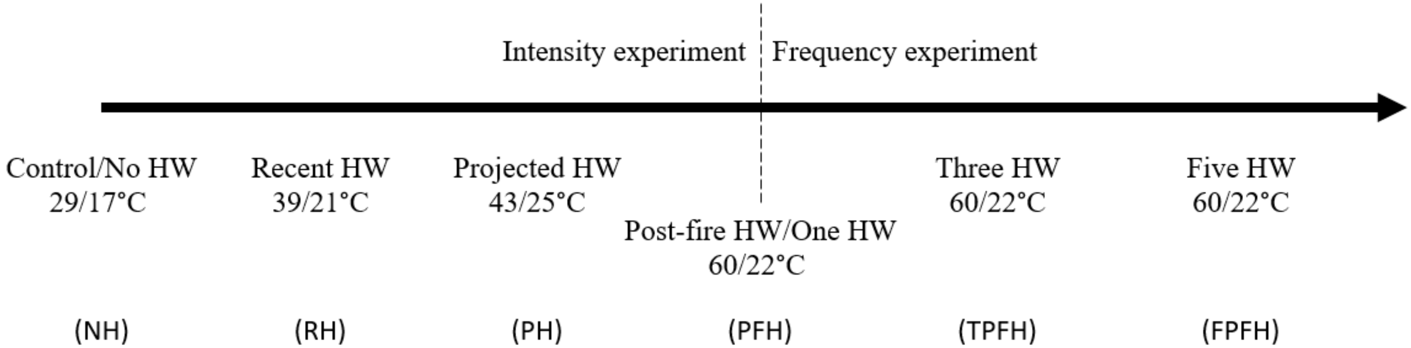
^ Only significant in intensity experiment, not frequency
* Only significant in frequency experiment, not intensity

In the heatwave frequency experiment, seeds were subjected to either one, three or five heatwaves, all at the highest temperature that was used in the intensity experiment (i.e. the post-fire heatwave treatment). Given the nature of these two experiments, the frequency experiment therefore naturally lies along a 'heatwave severity' continuum moving to higher severity from the frequency experiment (Fig. 3.7). As the responses of the life-history traits move along the continuum to the more frequent end, significant relationships change. In total seed germination proportion, fire response and seed mass disappeared as they became non-significant whereas dormancy became significant. Similarly, in seed germination duration, fire response became significant on the frequent end of the continuum. For each trait, I will discuss the patterns found and the level of contribution they have toward seed germination attribute in CPW native plant species.

3.4.2 Seed mass

Seed mass was significantly related to all three germination attributes in the intensity experiment. Larger seeds were quicker to germinate, had shorter germination periods and lower germination proportions than smaller seeds. These results were similar to a few studies in the literature (Moles and Westoby 2004, 2006), but opposed other studies (Verdú and Traveset 2005; Norden *et al.* 2009; Barak *et al.* 2018). In the frequency experiment, a similar pattern emerged where larger seeds had quicker germination onset and shorter germination periods than smaller seeds. However, while seed mass significantly influenced the total germination proportion in the intensity experiment – high germination proportions were seen in larger seeds than smaller seeds – it did not influence germination in the frequency experiment.

Figure 3.7. A continuum of the experimental heatwaves from Chapter 2 ranging from moderate to extreme heatwave conditions. Treatments from the intensity experiment occupy the moderate end of the continuum while the treatments from the frequency experiment occupy the more extreme end of the continuum. Below the line with the arrows are the treatments from both experiments. The dashed line represents the division between the two experiments.



Referring to the experimental heatwave continuum, the significant influence of seed mass on seed germination in the intensity experiment would be placed on the no heatwave (NH) end of the continuum with seeds from the CPW exhibiting a higher proportion of germination in large-seeded species. Working towards the five post-fire heatwave's (FPFH) end of the continuum, the difference between small and large seeds shifts with a reduction in total proportion of germination in the larger seeds resulting in a nonsignificant result (Fig. 2.6). Seemingly, once larger seeds are affected by multiple heatwaves, germination decreases. This can be explained as a bet-hedging strategy. Bet-hedging refers to a species' capacity to produce many offspring to increase its population size (r- selection) or to delay offspring production to wait for the most opportune time to grow (K- selection; MacArthur 1962; Pianka 1970; Reznick *et al.* 2002). In this case, the larger seeds follow the latter pattern, reducing the total proportion of germination after multiple heatwaves until the environmental conditions return to a stable state. For the CPW, if multiple heatwaves become a regular occurrence, large-seeded species may have reduced population numbers, effecting plant community dynamics.

3.4.3 Seed dormancy

In the intensity experiment, dormancy significantly affected germination onset and duration, but not the total proportion of seeds that germinated. Non-dormant seeds were considerably quicker to germinate and had a shorter germination period than dormant seeds. This supports the original hypothesis of inherent physiological mechanisms delaying germination onset and duration, even once dormancy has been broken (Cochrane and Probert 2006; Ooi *et al.* 2014; Barak *et al.* 2018). In the frequency experiment, dormancy significantly influenced all three germination attributes. Non-dormant seeds had quicker germination onset, shorter germination periods, and a higher proportion of germination when compared to dormant seeds.

While the difference between dormant and non-dormant seeds was non-significant in the intensity experiment, both had approximately the same proportion of germination as the non-dormant seeds from the frequency experiment. Dormant seeds in the frequency experiment showed around an 8% decline in germination compared with the non-dormant seeds, and the dormant and non-dormant seeds from the intensity experiment. Previously, it has been argued that dormant seeds are more likely to survive higher temperatures than non-dormant seeds due to the physiological differences between dormant and non-dormant seeds (Ramos *et al.* 2017). However, it was also plausible that the physiological mechanisms effecting dormant seeds are not the cause of the reduced germination, rather less germination was the result of a bet-hedging strategy. Dormant seeds reducing germination rates may be a strategic way of preventing premature germination, allowing for optimum germination to occur, as with seed mass. Dormant seeds were previously shown to minimise germination when external circumstances alter the optimum conditions for growth (Murray 1998; Ooi *et al.* 2004). Species composition in the CPW would therefore be significantly altered if multiple heatwaves became prevalent in the region.

3.4.4 Fire response

Fire response was the most variable life-history trait across germination attributes and between the intensity and frequency experiments. Fire response had no effect on germination onset in either experiment. Obligate seeders had shorter germination periods than resprouters in the frequency experiment but did not significantly affect germination duration in the intensity experiment. Conversely, there was no significant effect of total germination proportion in the frequency experiment, but obligate seeders had lower germination proportions than resprouters in the intensity experiment. This contradicts what other studies have found. Throughout the literature, obligate seeders are recognised as more prolific germinators than (Hansen *et al.* 1991; Bell *et al.* 1993; Hunter 2003) as they dedicate more resources to seeds than resprouters (Knox and Morrison 2005). This study used a small subset of species from the CPW and as such, the generalisation of resprouters having higher germination proportions than obligate seeders cannot be made robustly. It is interesting to note, however, that the total proportion of germination in resprouters across the two experiments decreases, resulting in a nonsignificant difference between obligate seeders and resprouters in the frequency experiment. As with the other life-history traits, this can be traced back to bet-hedging strategies, preventing resprouting species from germinating outside of optimum conditions.

Unlike the other life-history traits, fire response was the only trait to have a nonsignificant result in the duration germination response. Obligate seeders had shorter germination periods than resprouters in the frequency experiment, but this was not seen in the intensity experiment. In the total germination response, groups usually had one aspect of the trait increase or decrease, thus causing a nonsignificant result. However, in this case, the obligate seeder had a longer germination period and the resprouter had a shorter germination period in the intensity experiment compared to the frequency experiment, causing the gap between them to become smaller and less significant. Reducing the gap in germination duration between obligate seeders and resprouters would have both groups germinate around the same time, causing a shift in the community dynamics of the CPW.

3.4.5 Plant life-form

Across both experiments, life form exhibited similarities within each germination response. Life form was significantly associated with all three seed germination attributes. Within germination onset, grasses were the quickest to germinate, and climbers and herbs were the slowest to germinate. For germination duration, grasses had the shortest germination period compared to all other life forms. In terms of evolutionary strategies, it is understandable that grasses have quick germination onset and duration compared to other life form types as they are well-known as early colonisers in ecosystems (Lunt and Morgan 2002; Everingham *et al.* 2019), providing groundcover and soil stabilization for other species. For the Cumberland Plain Woodland, this is vital for post-fire regeneration (Hill and French 2003; Clarke and French 2005).

In regard to total germination proportion, all life forms had a high proportion of seeds germinating with the exception of trees, which had a much lower proportion of seeds germinating. While bet-hedging strategies provide some answer (Pianka 1970; Reznick *et al.* 2002; Olofsson *et al.* 2009), the seed mass of the trees in this experiment varied considerably, from some species with very small seeds to other species with comparatively large seeds. So, while this may form some part of the explanation surrounding these results, it is not the whole story. In terms of the experimental heatwave continuum theory, the role of life form on germination attributes was strong enough to be maintained after multiple heatwave events. However, there was a decline in germination performance of almost 15% in some life form types in the total proportion of seeds that germinated in the frequency experiment compared to the intensity experiment. While this life-history trait may be able to maintain the same pattern across both experiments, the overall response mimics the other life-history traits.

3.4.6 Concluding remarks

I have demonstrated that plant life-history traits influence seed germination attributes above and beyond the idiosyncratic effects of the heatwave treatments observed in Chapter 2. In the present Chapter, I have provided a new conceptual approach for combining the outcomes of the intensity experiment with the frequency experiment to demonstrate novel interpretations of germination performances across the germination attributes in relation to the life-history traits. I suggest that in the future further studies are needed using large numbers of species to robustly explore the consistency or not of trait-germination relationships moving along the heatwave intensity spectrum. This will help to better understand population dynamics in ecosystems like the Cumberland Plain Woodland.

Chapter 4

Assessment of seed longevity resilience against an intense heatwave in Cumberland Plain Woodland native plant species

"Would it save you a lot of time if I just gave up and went mad now?" – Douglas Adams, The Hitchhiker's Guide to the Galaxy

4.1 Introduction

In Chapter 2, I examined the effects of intense and frequent heatwaves on seed germination. That Chapter investigated the effects of heatwaves on seed germination immediately after the heatwaves had finished, as opposed to any longer-term effects. In Chapter 3, I studied the relationship between patterns in the germination attributes explored in Chapter 2 and the life-history traits of each species. I demonstrated that plant life-history traits have an important influence on seed germination attributes across the study species, an influence above and beyond the idiosyncratic effects of the heatwave treatments observed in Chapter 2. In this Chapter, I focus on seed longevity patterns in Cumberland Plain Woodland (CPW) and the effect of an intense heatwave on seed longevity over time to explore the longer-term effects of heatwaves on seed germination.

Studying the effects of time on seed longevity in a laboratory, over tens to hundreds of years, could be considered logistically impractical in most cases (Walters *et al.* 2005; but see Leino and Edqvist 2010). In the face of this long-time frame, artificial ageing techniques have been developed to examine patterns in seed longevity with only a year or two of experimental work (Hay *et al.* 2008; Probert *et al.* 2009; Newton *et al.* 2014). These new techniques rank species using a p50 value, which is the time taken for seed viability to fall 50% in a hotter, higher humidity environment than seeds would usually experience in an *ex situ* seedbank. Typically, controlled laboratory experiments simulate a hot, moist environment using LiCl chambers which promote accelerated ageing in seeds (Hay *et al.* 2008). For example, Merritt *et al.* (2014) conducted a large-scale study into the longevity of species from across Australia. They found species longevity under these conditions ranged from a week to three years with species from Myrtaceae and Fabaceae among the longest-lived and species from Asteraceae and Poaceae among the shortest-lived families. Long *et al.* (2008) discussed the potential of using controlled ageing techniques for *in situ* longevity and found it successfully mimicked field trials, supporting its viability as a method of assessing seed longevity.

This Chapter aims to determine the effect of a single post-fire heatwave on the longevity of six native plant species of the CPW. Initially, seeds were subjected to a post-fire heatwave (as performed in Chapter 2) prior to longevity testing using the LiCl technique. The p50 values obtained from the longevity experiment were used to determine which species have short- or long-lived seeds. While artificial ageing does not aim to emulate natural circumstances, it can provide an estimate for how long-lived a species may be in comparison to other species after a post-fire heatwave scenario occurs. As well as this, some species used in this study have not been tested for seed longevity prior to this study. Thus, I will be contributing to a global dataset with new data. The addition of an experimental heatwave before controlled ageing makes this study novel as little work has been done to compare species' seed longevity against a climate change scenario such as an extreme heatwave event.

The information obtained in this study will provide a basic understanding of the longevity of select species in the CPW and determine how hardy these species are in the long term, with the aim to best depict how seeds respond to ageing using a manageable timescale. This information provides an insight into the future biodiversity of the region with a greater chance of success in long- lived seeds from the soil seedbank than short-lived seeds if a disturbance alters the natural system. In most cases, this disturbance may come from a fire, drought, or flood (Dale *et al.* 2001; Benson and Howell 2002; Prieto *et al*. 2009). However, with an increase in extreme climatic events, heatwaves are also a threat to future biodiversity, especially in a threatened ecological community like the Cumberland Plain Woodland (Perkins *et al*. 2012; Maher *et al*. 2018; French *et al.* 2019).

4.2 Materials and Methods

4.2.1 Study Region

See Chapter 2 for details of the CPW, the study region for this Chapter.

4.2.2 Study species

One of the main goals of this experiment was to compare longevity patterns between species within the same taxonomic family in a replicated way. Thus, the six study species were selected from three taxonomic families with two representative species from each family (Table 4.1). Species selection was also based on the availability of a large quantity of seeds necessary for the longevity technique.

4.2.3 Experimental design

This experiment occurred in three sequential stages: the exposure stage, the pre-treatment stage, and the longevity experiment stage.

First, the exposure stage subjected half of the seeds to a single, post-fire heatwave lasting for 5 days (PFH) and the other half of the seeds to a control treatment (as described in Chapter 2). The PFH group was exposed to 60/22°C for five days in an oven (Labec G701; Marrickville, Australia) with a wall timer set on 12/12 hr and the control group were placed in the same conditions but at a constant 22°C. This was considered a control group as all variables were the same except for the treatment temperature. Second, the pre-treatment stage provided time for pre-treatments such as scarification to occur before the longevity experiment began (Table 4.1) to ensure that seeds were exposed to the relative humidity set in each chamber (non-scarified seeds are impermeable). Third, seeds were subjected to the longevity experiment.

The longevity experiment was split into two stages: rehydration and ageing. Rehydration occurred in an air-tight electrical box (28 x 28 x 14 cm; NHP Fibox, Australia) with a non-saturated LiCl solution (385 g/L; anhydrous, Sigma®, Australia) at 47% relative humidity (RH) and 20°C for 14 days. For the ageing stage, seeds were then transferred into another air-tight electrical box, with a non-saturated LiCl solution (280 g/L) at 60% RH and 45°C. Seeds were taken out at pre-determined intervals, in this case 0, 1, 2, 5, 9, 20, 30, 50, 75, 120 days. This experiment was known as group 1. Group 1 consisted of *Acacia decurrens, Calotis lappulacea,* and *Cassinia aculeata* (Table 4.1). For group 2, it was deemed necessary to have a second longevity experiment run for a longer period of time (1, 2, 5, 9, 30, 65, 110, 160, 210, 270 days) as the species involved were determined to be longer lived (Table 4.2; Merritt *et al.* 2014). The species used in group 2 were *Acacia decurrens, Hardenbergia violacea, Eucalyptus crebra,* and *Eucalyptus tereticornis. Acacia decurrens* was used in both experiments as a measure of comparison between both groups. This technique was used by Merritt *et al.* (2014).

Table 4.1. Study species used in the longevity experiment with taxonomic family and growth form information. Group 1 and group 2 refer to the timeframe the seeds were exposed to in the LiCl chambers. Collection refers to either field collection of seeds or PlantBank seed accessions. Pre-treatment refers to any standard treatments used to promote seed germination or break seed dormancy (scarification) or make them easier to handle as pure samples (removal of frass).

Table 4.1	Family	Species	Collection	Pre-treatment	Growth form
Group 1	Asteraceae	*Calotis lappulacea*	Field	N/A	Herb
	Asteraceae	*Cassinia aculeata*	PlantBank	N/A	Shrub
	Fabaceae	*Acacia decurrens**	PlantBank	Scarification	Tree
Group 2	Fabaceae	*Acacia decurrens**	PlantBank	Scarification	Tree
	Fabaceae	*Hardenbergia violacea*	PlantBank	Scarification	Woody climber
	Myrtaceae	*Eucalyptus crebra*	PlantBank	Removal of frass	Tree
	Myrtaceae	*Eucalyptus tereticornis*	PlantBank	Removal of frass	Tree

* *Acacia decurrens* was tested in both group 1 and group 2 but there was no difference found between groups and was therefore analysed together.

Table 4.2. Summary of the conditions present in LiCl chambers for both the rehydration and ageing phases in the longevity experiment. Group 1 and group 2 both had the same temperature/humidity and duration in LiCl chamber for the rehydration, and the same temperature/humidity for ageing. Group 1 had a shorter number of days of removal from LiCl chamber than group 2 which was more spread out.

Table 4.2		Group 1	Group 2
Rehydration	Temperature	20°C	
	Humidity	47%	
	Duration in LiCl chamber	14 days	
Ageing	Temperature	45°C	
	Humidity	60%	
	Days removed from LiCl chamber	0, 1, 2, 5, 9, 20, 30, 50, 75, 120	1, 2, 5, 9, 30, 65, 110, 160, 210, 270

Throughout each stage, seeds remained in 25 ml open glass vials, with 10 samples of 50 seeds (per group) separated by species, treatment, and group. On the assigned day (0, 1, 2, 5, etc. – depending on group), one vial per species, treatment (PFH or control) and group (group 1 or 2) was extracted from the LiCl chamber and sown onto 9 cm petri dishes containing 8 g/L of agar. They were then placed into species-specific optimum temperatures for germination, as described in Chapter 2. Germination checks occurred once a week for at least 42 days and concluded once no germination had occurred after 2 weeks. Germination was defined as emergence of a radicle of more than 2 mm. After this time, a cut test was used to determine the condition of the ungerminated seeds (empty, mushy, predated, etc.).

4.2.4 Statistical analyses

Seed longevity was tested using the seed viability equation (Ellis and Roberts 1980) which tests for a 50% drop in seed viability using a probit analysis.

$$v = K_i - p \, / \, \sigma$$

A quasibinomial logistic regression model with a probit link was used to model the proportion of total seed germination (response variable) as a function of species, time, and treatment (as explanatory variables). Group was removed from statistical analysis as both groups were deemed directly comparable with *A. decurrens* responding in the same manner in both groups. Species was a six-level factor, time was a 10-level factor (10 selected days over a period of 250 days), and treatment was a two-level factor (control and the experimental heatwave). All two-way and three-way interactions were included in the model. If a significant species x time interaction was observed in the model, a pairwise comparison was used to unpack the interaction. All analyses were conducted using R statistical software (R core team 2019) through R Studio (Version 3.5.1).

4.3 Results

Seed longevity varied significantly across all species ($F_{5,676} = 235.052$, $P < 0.0001$). There was also a significant relationship between a decline in the proportion of seeds germinating and increasing time in the LiCL chamber ($F_{1,676} = 1262.251$, $P < 0.0001$; Fig. 4.1). However, there was no signifcant effect in the model of treatment (i.e. control vs treatment) on seed longevity across all species ($F_{1,676} = 1.789$, $P = 0.2$; Fig. 4.1). There was a significant species x time interaction indicating that seed longevity of each species declined over time in different ways ($F_{5,676} = 130.551$, $P < 0.0001$; Fig. 4.2a). As a consequence, all species were ranked according to the p50 value and steepness of the seed survival curve (Table 4.3). Two distinct groups emerged (Figure 4.2b). *Acacia decurrens, Calotis lappulacea*, and *Cassinia aculeata* formed one group (dot-dash group) and *Eucalyptus crebra, Eucalyptus tereticornis,* and *Hardenbergia violacea* formed the other group (consistant dash). The dot-dash group had a much steeper slope, representing sudden decrease in seed survival compared to the consistant dash group, which had a gradual decrease in seed survival. The species x treatment interaction ($F_{5,676} = 0.325$, $P = 1$), treatment x time interaction ($F_{1,676} = 1.621$, $P = 0.2$), and species x time x treatment interaction ($F_{5,676} = 5.843$, $P = 0.3$) were all nonsignificant.

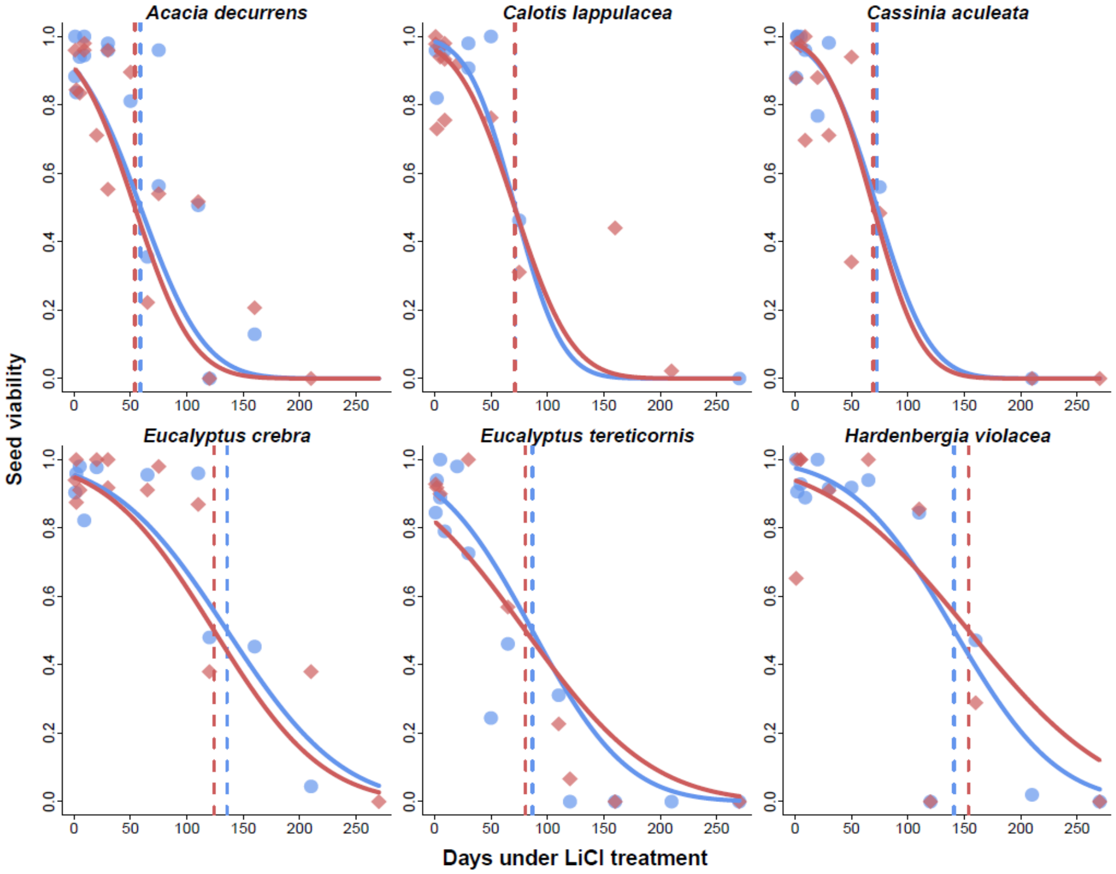


Figure 4.1. Each species x treatment interaction shown as seed viability as a proportion on the y axis and the number of days spent in the LiCl treatment on the x axis. The control group (blue) is directly compared to the experimental heatwave treatment group (red). Both data points (dots) and seed survival curves (solid lines) are included. The vertical dotted line represents p50 in the control group (blue) and the experimental heatwave group (red).

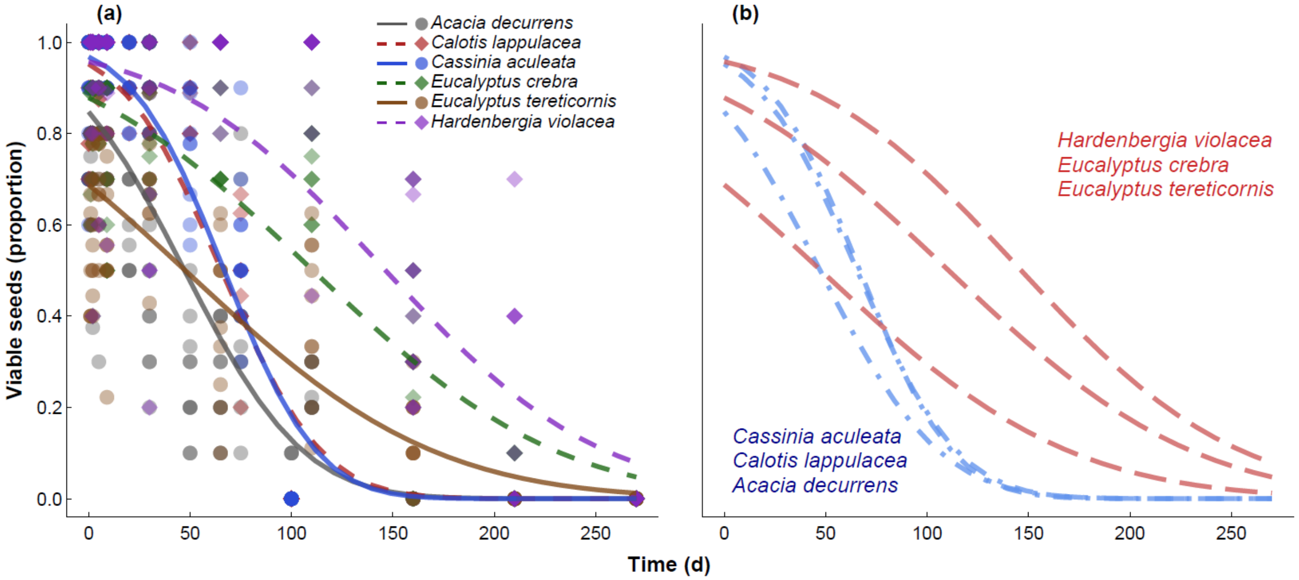


Figure 4.2. A visual summary of all species' seed survival curves with proportion of viable seeds plotted against time (a) all species data points represented as single data points (dots, diamonds) and as a seed survival curve (dotted lines, solid lines) (b) a direct comparison of the two significant groups (blue vs. red) with species listed in the decending order of steepness (most steep to least steep).

Table 4.3 A rank of species according to the steepness of the survival curve and p50. Rank 1-6 of p50 value and steepness of curve.

			Rank	
Species	Family	p50 value	p50	Steepness of curve
Cassinia aculeata	Asteraceae	70	2	1
Calotis lappulacea	Asteraceae	70	2	2
Acacia decurrens	Fabaceae	55	1	3
Hardenbergia violacea	Fabaceae	145	6	4
Eucalyptus crebra	Myrtaceae	130	5	5
Eucalyptus tereticornis	Myrtaceae	80	4	6

4.4 Discussion

4.4.1 Overall findings

This study found that exposure to a single experimental, post-fire heatwave had no significant effect on seed longevity in the study species when compared with a control group not exposed to heatwave conditions. There was also no difference in the rate of decline in seed longevity between the control group and the group subjected to the heatwave. As discussed in Chapter 2, this is a reassuring outcome. Seeds from the CPW were found to be tolerant of this post-fire heatwave scenario supporting the idea that climate change induced heatwaves may not have adverse effects on seed longevity. Instead, the comparative longevity between species provided insight into the similarities and differences of species within taxonomic family groups. This comparison was best reflected in the species x time interaction.

4.4.2 Species x time interaction

In this study, I found a significant species x time interaction when analysing viable seed proportions. When analysed further, the species x time interaction revealed two distinct groups (Figure 4.2b). The first group contained *Acacia decurrens, Calotis lappulacea*, and *Cassinia aculeata* and had a steep seed survival curve, suggesting these species were short-lived (i.e. p50 values 55-70 days). The second group, *Eucalyptus crebra, E. tereticornis,* and *Hardenbergia violacea,* were long-lived and therefore had a gradual seed survival curve (i.e. p50 values 80-145 days). While the differentiation between 70 and 80 days is minimal, the shape of the curve clearly separated the two groups in the way they aged (i.e. gradually or quickly).

Species displayed similar longevity to other species from the same taxonomic families (Probert *et al.* 2009). Asteraceae were short-lived (*C. lappulacea* and *C. aculeata*) and

Myrtaceae were long-lived (*E. crebra* and *E. tereticornis*), however, Fabaceae did not have the same kind of longevity, instead *A. decurrens* was short-lived and *H. violacea* was long-lived. In a previous study, species from Fabaceae had large variation in total seed longevity (Merritt *et al.* 2014) with the longevity of *Acacia* sp. alone varying from a few years to over 150 years (Leino and Edqvist 2010). While these discrepancies are notable, they can be attributed to a normal level of variation within the family.

4.4.3 Ecological context

Artificially ageing seeds in a LiCl chamber does not truly emulate what occurs *in situ* in the soil seedbank (Hay *et al.* 2008; Newton *et al.* 2014). Other external factors such as microbes, humidity and constantly changing weather all impact the extended viability of a seed thus making seed longevity experiments a guide for what may occur *in situ*, not a prediction (Probert *et al.* 2009). The goal was to best depict how seeds respond to ageing using a manageable timescale. A more accurate representation of post-fire ageing would occur in seed burial trials, however, due to the restricted timeframe, this was not possible. In future, this may prove more conclusively what may occur naturally *in situ*.

This study was able to determine that each of these species age differently over time and that taxonomic family groups age in a similar way. Evidently, species from the same family typically have similar physiological attributes used as a form of protection against the environmental deterioration associated with ageing (Probert *et al.* 2009; Merritt *et al.* 2014; Davies *et al.* 2016). While this was not seen in Fabaceae in the species x treatment interaction, it was clearly observed in Table 4.3, the ranking of species according to the steepness of the seed survival curve, and the p50 value.

Taxonomic family group were clearly linked to seed survival after artificial ageing (Colville and Pritchard 2019).

Various physiological attributes such as seed mass, seed coat and embryo type all contribute to the way a seed ages artificially (Bekker *et al.* 2003; Merritt *et al.* 2014). While not directly tested in this study, these attributes do not appear to have influenced the species x treatment interaction as strongly as taxonomic family group with both large and small seeded species with different seed coat types found with a similar ageing type (short vs long lived). Admittedly, the small sample size in this study cannot conclusively determine a correlation between these attributes and longevity whereas in studies with larger sample sizes, correlations were found (Probert *et al.* 2009; Merritt *et al.* 2014).

In this region, long-lived species have survived in CPW remnants over the past century in spite of continued destruction of land for development (Benson and Howell 1990; Benson and Howell 2002). These seeds have historically persisted through periods of intense drought and fire (Benson and Howell 2002), proving their capacity to withstand extreme environmental conditions like the extreme heatwave scenario tested in this study. The hardiness of these species provides a reassuring outcome for the conservation of this deteriorating threatened ecological community. Maintaining these species in protected land remnants will therefore be easier compared to plant communities highly susceptible to the effects of climate change.

4.4.4 Concluding remarks

This study determined a single, intense heatwave had no effect on the seed longevity of species from the CPW. Instead, a species x time interaction proved the longevity of a small sample of species from this region respond to artificial ageing of seeds distinctly as either a short-lived species or a long-lived species. A taxonomic link to longevity was found with two of three families ageing similarly. In an artificial ageing environment this does not directly translate to an *in situ* scenario but can provide an insight into how species may respond over time. As described in Chapter 2, seeds of these CPW species can survive the devastating effects of a single climate change induced heatwave.

Chapter 5

Discussion

“The Answer to the Great Question... Of Life, the Universe and Everything... Is... Forty-two,' said Deep Thought, with infinite majesty and calm.” – Douglas Adams, The Hitchhiker’s Guide to the Galaxy

5.1 Thesis overview

Human-induced climate change is the driving force behind an increase in global atmospheric temperatures (Meehl *et al.* 2007; Pachauri and Reisinger 2007; Rosenzweig *et al.* 2008). Increased temperatures began during the industrial revolution and are now projected to worsen by the end of this century (Pachauri and Reisinger 2007). These changes to the atmosphere are slowly influencing the climate variables that govern the day to day weather conditions of regions around the world. In south-eastern Australia, the interaction between the El Nino-Southern Oscillation, the Indian Ocean Dipole, and the Southern Annular Mode have the capacity to adversely affect the plant communities in this region through an increase in extreme weather events such as heatwaves (Nicholls 1988; Chiew *et al.* 1998; Holmgren *et al.* 2001; Ashcroft *et al.* 2014; Reisinger *et al.* 2014).

Heatwaves are increasing in intensity, frequency, and duration (Easterling *et al.* 2000; Meehl and Tebaldi 2004; Meehl *et al.* 2007; Perkins *et al.* 2012) and are contributing to a shift in plant community composition (Deo *et al.* 2007; Cowan *et al.* 2014; Perkins-Kirkpatrick *et al.* 2016). This is especially true for woodland communities in south-eastern Australia (Van Gorsel *et al.* 2016). For threatened ecological communities such as the Cumberland Plain Woodland (CPW), the effects of intense and frequent heatwaves may prove devastating on plant development and growth (Chapin III *et al.* 2000; Walther *et al.* 2002; Hatfield and Prueger 2015). However, these effects are relatively understudied, especially when addressing the most vulnerable life stage in a plant's lifecycle, the seed.

Exposure to adverse environmental conditions can cause physiological and biochemical damage to a seed, preventing germination from occurring (Bewley and Black 1982; Thomas *et al.* 2010; Mackenzie *et al.* 2016). While studies into the effect of high temperatures on seed germination are numerous (Bewley and Black 1982; Thomas *et al.* 2010; Mackenzie *et al.* 2016), the direct effect of heatwaves on seed germination are minimal. This thesis addressed this gap in the literature, focusing on connecting seed physiology to the ecology of a region under the influence of intense and frequent heatwaves. I developed laboratory-based experiments to identify the response of seed germination attributes to simulated heatwaves in native plant species from the CPW.

5.2 Were the aims of this thesis met?

My thesis looked broadly at the effect of heatwaves on seed germination attributes, the influence of life-history traits on those germination attributes and the role of an intense heatwave on seed longevity. This thesis successfully addressed these aims by illustrating the resilience of species to intense and frequent heatwaves while also proving the complexity behind seed germination. Through understanding the influence of life-history traits and taxonomy on seed germination, I provided a foundation for further research into these relationships and the connection physiological traits have on the ecology plant communities. To understand these broad concepts in more detail, the following four aims were addressed:

1. *To determine the resilience of seeds from the CPW to intense and frequent heatwaves using laboratory experiments.*

In Chapter 2, I studied the effect of heatwaves on the seed germination attributes of seeds from 15 CPW native plant species. I found that there was no significant effect of treatment on species, proving they were highly resilient to the adverse effects of laboratory-simulated heatwaves. Instead, I found a species x treatment interaction, reflecting an underlying complexity in the seed physiology with some species responding to the treatment but in varied and inconsistent ways.

Similarly, Chapter 4 of this thesis looked at the resilience of seeds from six species in terms of seed longevity. I found they were highly resilient to a single, intense heatwave. As with the experiment in Chapter 2, there was no variation among treatments but instead a species x time interaction, proving the longevity of a small sample of species from this region respond to artificial ageing of seeds distinctly as either a short-lived species or a long-lived species. There was also an underlying link to taxonomy, reflecting the complexity behind the driving forces behind seed germination. Therefore, this aim was met by both Chapter's 2 and 3.

2. *To understand how a range of heatwave scenarios affect (1) the time taken for seeds to begin germination, (2) the duration of time over which seeds germinate, and (3) the total proportion of seeds that germinate.*

In all three Chapters, I address the response of these three seed germination attributes in various heatwave scenarios.

In Chapter 2, the heatwaves were divided into two scenarios, those exposed to increased heatwave intensity and those exposed to increased heatwave frequency. Heatwave intensity was further split into four treatments: recent average, recent heatwave, projected heatwave and post-fire heatwave temperatures. Heatwave intensity was also split into four treatments using the post-fire heatwave temperature from the intensity experiment: no heatwave, one heatwave, three heatwaves and five heatwaves. All heatwaves ran for five days with a six-day gap between heatwaves where necessary. I found that each seed germination attribute had the same response to these treatments, significant variation among species, nonsignificant variation among treatments and a significant species x treatment interaction. The species x treatment interaction revealed a possible underlying mechanism governing seed germination under these scenarios and so I expanded the research into Chapter 3 with a focus of life-history traits.

In Chapter 3, I included the heatwave scenarios in my analysis to prove that life-history traits were influencing seed germination attributes above and beyond the treatment of heatwaves. The life-history traits – seed mass, life form, dormancy, and fire response – each influenced the germination attributes differently but showed definitively that heatwave severity had an overarching impact on the degree of influence these life-history traits had on germination attributes.

Lastly, the experimental heatwaves in Chapter 4 mimicked the single, post-fire heatwave scenario seen in Chapter 2. While the seed germination attributes were not measured in the same way as the other two Chapters, p50 values use the same values – onset, duration, and total proportion of germination – to determine the longevity of species. I found a similar response to the previous Chapters with significant variation among species, nonsignificant variation among treatments, and a species x time interaction. The information from all three of these Chapters prove this aim was met.

3. *To determine whether plant life-history traits are related to interspecific patterns of variation in germination onset, duration, and the total proportion of seed germination.*

Chapter 3 addressed this aim through careful study of the relationship between life-history traits in 15 native plant species from the CPW after the seeds were exposed to adverse environmental conditions: intense and frequent heatwaves. I found that plant life-history traits influenced seed germination attributes above and beyond the idiosyncratic effects of the heatwave treatments observed in Chapter 2. I have provided a new conceptual approach for combining the outcomes of the intensity experiment with the frequency experiment to demonstrate novel interpretations of germination performances across the germination attributes in relation to the life-history traits. Therefore, this research aim was also met.

4. *To explore the effect of a single post-fire heatwave on the long-term longevity of six native plant species of the CPW.*

In Chapter 4, I used a sample of six species from Chapter 2 to further understand the effect of heatwaves on seed germination. With three experimental stages – the exposure stage, the pre-treatment stage, and the longevity experiment stage – I exposed seeds to a single, post-fire heatwave and found significant variation among species, nonsignificant variation among treatments, and a species x time interaction. In this exploration I proved that, similar to the other experiments, seed germination is more complexly influenced by physiology than I had previously thought. Heatwaves did not directly influence germination, further proving the theory that these species would be more resilient than originally thought. Instead, the comparative longevity between species provided insight into the similarities and differences of species within taxonomic family groups. This research confirms the aims were met.

5.3 Future research directions

This thesis focused on the CPW and seed germination of native plant species in response to heatwave events. Thermal tolerance plays an important role in the physiological responses associated with germination, however, as discussed by Walder and Erschbamer (2015) and Orsenigo *et al.* (2014), heat and water stress both impact the seed germination attributes. Most studies combining these environmental stressors are based in agricultural studies (Smith *et al.* 1989; Heatherly 1993; Rahimi 2013). Moving these types of studies into seed ecology with a focus in native Australian plants would provide valuable information for conservation management. While this study successfully established the complex ways heatwaves influence germination attributes, future studies combining heatwaves with water stress would better predict the changes in plant community composition of regions like the CPW.

Further expansion into thermal tolerance of CPW native plant species should include extreme cold weather events in conjunction with extreme heat events. Recently, there has been increased awareness regarding the impact of extreme heat-based studies (Gleadow and Narayan 2007; Ooi *et al.* 2014; Orsenigo *et al.* 2015) and extreme cold-based studies (Bradford 2005; Marcante *et al.* 2012) on seed germination attributes, but not both (Abeli *et al.* 2014a; Orsenigo *et al.* 2014). A current project from the Department of Planning, Industry and Environment (2018-2021) is looking specifically at the thermal tolerance of threatened ecological communities in south-eastern Australia (Department of Planning, Industry and Environment 2018). Encompassing both hot and cold extreme weather events into a single study more accurately portrays the ecology within a system than a study of one extreme or the other. As a temperate region, the CPW experiences both extremes and so species must endure either kind of extreme weather event (Tozer 2003).

This study focused on germination attributes from species based in the CPW. An alternative approach would look at the same measures but in the context of a different region. Alpine regions are currently at the forefront of climate change research (Marcante *et al.* 2012; Orsenigo *et al.* 2014), especially regarding seed germination attributes (Walck *et al.* 2011; Briceño *et al.* 2015) and the influence of heatwaves on those attributes (Orsenigo *et al.* 2015). The continued effects of climate change are seeing alpine regions slowly diminish over time with these regions slowly creeping up in altitude and out of existence. The experimental work addressed in this thesis, coupled with the study by Orsenigo *et al.* (2015), could provide valuable information on the germination attributes of species impacted by intense or frequent heatwave events. Further expansion of this work could include regions of different plant community composition such as those found in other countries with plants not so well adapted to adverse environmental conditions. An example region of interest could be the forests or grasslands of Europe. After experiencing heatwaves, studies looking at plant responses found negative effects on plant physiology during and after a heatwave event (García-Herrera *et al.* 2010; Teuling *et al.* 2010; Abeli *et al.* 2014b). Implementing a study similar to the one seen in Chapter 2 of this thesis could illuminate the need for increased management if seed germination attributes are detrimentally affected by heatwaves.

Trait-based ecology provides a greater understanding of the underlying mechanisms that govern seed germination attributes. Throughout the literature, a large number of studies have focused on life-history traits such as seed mass (Gross and Smith 1991; Kahmen and Poschlod 2008; Norden *et al.* 2009; Barak *et al.* 2018), life form (Grime *et al.* 1981; Garwood 1983; Morgan 1998; Bu *et al.* 2008), fire response (Zammit and Westoby 1987; Pausas *et al.* 2004; Knox and Morrison 2005), and dormancy (Cochrane and Probert 2006; Ooi *et al.* 2014; Barak *et al.* 2018). However, there are studies that also include seed shape (Barak *et al.* 2018), life span (Dalgleish *et al.* 2010), and diaspore mass (Pausas *et al.* 2004), to name a few. This thesis identified four main life-history traits as the drivers of seed germination attributes, however, expanding this to include more traits could unlock a greater understanding of seed biology and therefore determine the influence of all life-history traits on seed germination attributes (Jiménez-Alfaro *et al.* 2016; Saatkamp *et al.* 2018), especially under adverse environmental conditions.

In Chapter 4, a novel approach to species' longevity addresses the impact of an intense heatwave on seed germination after artificial ageing. Seed longevity is quickly becoming a focus in seed ecology as the need for species conservation directs research into ensuring the future of biodiversity is preserved (Merritt *et al.* 2014). Expanding on the study conducted in Chapter 4 to include frequent heatwave events could provide more ecologically relevant information for researchers to better understand the influence of heatwave events on species' longevity. Also, increasing the number of species studied in the CPW would allow for a more comprehensive understanding of species longevity in this region.

5.4 Conclusion

The work presented in this thesis provides information regarding: the effect of intense and frequent heatwaves on seed germination attributes, the role of life-history traits in contributing to seed germination attributes over and above the idiosyncratic levels found after a heatwave event, and the impact of a single intense heatwave on species' longevity, from species found in the Cumberland Plain Woodland. Each study within this thesis was based on extensive research and the current knowledge already established in the seed ecology field. It is with this understanding that I believe my thesis successfully contributes to the field of seed ecology through the ideas developed surrounding the effect of heatwaves on seed germination attributes.

Appendices

Appendix 2.1

Incubator chambers			
Incubator model	Thermoline L + M, Model no. TLMRIL195-1-DS, Manufactured in Smithfield, NSW, Australia.	UAP Cooling, Model no. NEXT DX Os 007, Manufactured in Burwood, VIC, Australia	Labec Temperature Cycling Chamber, Model no. ICC24-2, Manufactured in Marrickville, NSW, Australia.
Temperature settings	15/5°C	20°C (Constant)	30/15°C
Species	*Calotis lappulacea* *Einadia nutans*	*Cassinia aculeata* *Clematis glycinoides* *Dichanthium sericeum* *Eucalyptus crebra* *Eucalyptus tereticornis* *Microlaena stipoides* *Plectranthus parviflorus* *Themeda triandra*	*Acacia decurrens* *Acacia falcata* *Hardenbergia violacea* *Indigofera australis* *Dodonaea viscosa*

Experiment 1

Appendix 2.2

Onset of seed germination – species x treatment

	df	*F* value	*P* value
Treatment	3	2.753	**0.042**
Species	14	613.084	**0.000**
Species x Treatment	42	2.705	**0.000**
Residuals	540		

Appendix 2.3

Onset of seed germination – among treatment – all species

Species	df	*F* value	*P* value
Acacia decurrens	3	1.867	0.153
Acacia falcata		1.000	0.404
Calotis lappulacea		2.585	0.068
Cassinia aculeata		0.349	0.790
Clematis glycinoides		6.000	**0.002**
Dichanthium sericeum		1.000	0.404
Dodonaea viscosa		1.636	0.198
Einadia nutans		7.869	**0.000**
Eucalyptus crebra		0.209	0.889
Eucalyptus tereticornis		1.000	0.404
Hardenbergia violacea		17.65	**0.000**
Indigofera australis		2.757	0.056
Microlaena stipoides		1.000	0.404
Plectranthus parviflorus		1.000	0.404
Themeda triandra		1.000	0.404

Appendix 2.4

Onset of seed germination – within treatment

Treatment	df	*F* value	*P* value
Recent average	14	116.0	**0.000**
Recent heatwave		213.3	**0.000**
Projected heatwave		145.8	**0.000**
Post-fire heatwave		168.3	**0.000**

Appendix 2.5

Duration of seed germination – species x treatment

	df	*F* value	*P* value
Treatment	3	1.112	0.344
Species	14	76.902	**0.000**
Species x Treatment	42	2.036	**0.000**
Residuals	540		

Appendix 2.6

Duration of seed germination – among treatment – all species

Species	df	*F* value	*P* value
Acacia decurrens	3	1.494	0.233
Acacia falcata		0.706	0.555
Calotis lappulacea		1.098	0.363
Cassinia aculeata		3.58	**0.023**
Clematis glycinoides		0.576	0.634
Dichanthium sericeum		0.869	0.466
Dodonaea viscosa		0.493	0.690
Einadia nutans		6.684	**0.001**
Eucalyptus crebra		0.544	0.656
Eucalyptus tereticornis		2.116	0.115
Hardenbergia violacea		2.471	0.078
Indigofera australis		0.491	0.691
Microlaena stipoides		0.242	0.867
Plectranthus parviflorus		1.695	0.185
Themeda triandra		1.773	0.170

Appendix 2.7

Duration of seed germination – within treatment

Treatment	df	*F* value	*P* value
Recent average	14	15.98	**0.000**
Recent heatwave		28.32	**0.000**
Projected heatwave		20.70	**0.000**
Post-fire heatwave		20.33	**0.000**

Appendix 2.8

Total proportion of germination – species x treatment

	df	χ^2	*P* value
Treatment	3	0.44	0.933
Species	14	506.3	**0.000**
Species x Treatment	42	100.8	**0.000**

Appendix 2.9

Total proportion of germination – among treatment – all species

Species	df	χ^2	*P* value
Acacia decurrens	3	8.764	**0.033**
Acacia falcata		2.722	0.437
Calotis lappulacea		0.164	0.983
Cassinia aculeata		4.472	0.215
Clematis glycinoides		4.044	0.257
Dichanthium sericeum		2.780	0.427
Dodonaea viscosa		16.61	**0.000**
Einadia nutans		1.451	0.694
Eucalyptus crebra		3.651	0.302
Eucalyptus tereticornis		4.224	0.238
Hardenbergia violacea		48.51	**0.000**
Indigofera australis		2.765	0.429
Microlaena stipoides		0.848	0.838
Plectranthus parviflorus		4.617	0.202
Themeda triandra		3.350	0.341

Appendix 2.10

Total proportion of germination – within treatment

Treatment	df	χ^2	*P* value
Recent average	14	112.68	**0.000**
Recent heatwave		170.43	**0.000**
Projected heatwave		173.09	**0.000**
Post-fire heatwave		120.73	**0.000**

Experiment 2

Appendix 2.11

Onset of seed germination – species x treatment

	df	*F* value	*P* value
Treatment	3	1.388	0.246
Species	14	226.298	**0.000**
Species x Treatment	42	2.135	**0.000**
Residuals	300		

Appendix 2.12

Onset of seed germination – among treatment – all species

Species	df	*F* value	*P* value
Acacia decurrens	3	1.222	0.328
Acacia falcata		2.867	0.062
Calotis lappulacea		2.301	0.108
Cassinia aculeata		1.333	0.292
Clematis glycinoides		3.656	**0.03**
Dichanthium sericeum		2.5	0.089
Dodonaea viscosa		1.528	0.238
Einadia nutans		0.233	0.874
Eucalyptus crebra		1.515	0.241
Eucalyptus tereticornis		2	0.146
Hardenbergia violacea		0.957	0.432
Indigofera australis		0.556	0.65
Microlaena stipoides		2.647	0.077
Plectranthus parviflorus		1	0.413
Themeda triandra		1	0.413

Appendix 2.13

Onset of seed germination – within treatment

Treatment	df	*F* value	*P* value
No heatwave	14	94.5	**0.000**
One heatwave		122.5	**0.000**
Three heatwaves		62.7	**0.000**
Five heatwaves		24.6	**0.000**

Appendix 2.14

Duration of seed germination – species x treatment

	df	*F* value	*P* value
Treatment	3	1.911	0.128
Species	14	61.148	**0.000**
Species x Treatment	42	2.908	**0.000**
Residuals	300		

Appendix 2.15

Duration of seed germination – among treatment – all species

Species	df	*F* value	*P* value
Acacia decurrens	3	2.429	0.095
Acacia falcata		2.208	0.119
Calotis lappulacea		0.569	0.642
Cassinia aculeata		14.38	**0.000**
Clematis glycinoides		0.296	0.828
Dichanthium sericeum		0.309	0.819
Dodonaea viscosa		3.461	**0.036**
Einadia nutans		1.606	0.219
Eucalyptus crebra		0.582	0.634
Eucalyptus tereticornis		3.725	**0.028**
Hardenbergia violacea		2.057	0.138
Indigofera australis		2.222	0.117
Microlaena stipoides		2.813	0.066
Plectranthus parviflorus		0.953	0.434
Themeda triandra		0.432	0.732

Appendix 2.16

Duration of seed germination – within treatment

Treatment	df	*F* value	*P* value
No heatwave	14	15.59	**0.000**
One heatwave		10.83	**0.000**
Three heatwaves		26.5	**0.000**
Five heatwaves		18.37	**0.000**

Appendix 2.17

Total proportion of germination – species x treatment

	df	χ^2	*P* value
Treatment	3	5.6	0.133
Species	14	349.72	**0.000**
Species x Treatment	42	112.07	**0.000**

Appendix 2.18

Total proportion of germination – among treatment – all species

Species	df	χ^2	*P* value
Acacia decurrens	3	2.204	0.531
Acacia falcata		4.552	0.208
Calotis lappulacea		19.80	**0.000**
Cassinia aculeata		21.44	**0.000**
Clematis glycinoides		5.613	0.132
Dichanthium sericeum		2.777	0.427
Dodonaea viscosa		11.55	**0.009**
Einadia nutans		3.473	0.324
Eucalyptus crebra		1.934	0.586
Eucalyptus tereticornis		2.423	0.489
Hardenbergia violacea		10.24	**0.017**
Indigofera australis		2.807	0.422
Microlaena stipoides		21.76	**0.000**
Plectranthus parviflorus		2.054	0.561
Themeda triandra		5.810	0.121

Appendix 2.19

Total proportion of germination – within treatment

Treatment	df	χ^2	*P* value
No heatwave	14	131.54	**0.000**
One heatwave		123.07	**0.000**
Three heatwaves		94.578	**0.000**
Five heatwaves		118.65	**0.000**

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So long, and thanks for all the fish.