School of Molecular and Life Sciences

Fire intensity, seasonal variation and seeds traits may influence seed fates in Banksia woodlands

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This thesis is presented for the Degree of

Doctor of Philosophy

of

Curtin University

December 2018

Declaration

To the best of my knowledge and belief this thesis contains no material previously published by any other person except where due acknowledgment has been made. This thesis contains no material which has been accepted for the award of any other degree or diploma in any university.

Signature:

Date: 7/12/2018

Declaration of candidate contribution

This thesis contains a mixture of sole-authored work, and co-authored published work. Chapters 2 and 3 are presented as manuscripts that have been accepted for publication in peer-reviewed journals. The contributions of co-authors to chapters 2 to 3 are outlined below. Each chapter is self-contained, including tables, figures, references and appendices. The published pdf versions of each of these chapters are attached at the end of the thesis.

Chapter 2: *Seed moisture content as a primary trait regulating the lethal temperature thresholds of seeds.*

Published in Journal of ecology: First published: 11 November 2018

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Authors' contributions

Ryan Tangney, Ben Miller, and David Merritt conceived the ideas and designed methodology; Ryan Tangney collected and analysed the data, supported by Joe Fontaine. Ryan Tangney led the writing of the manuscript and all authors contributed critically to the editing of drafts and gave final approval for publication.

Chapter 3: *A method for extensive spatiotemporal assessment of soil temperatures during an experimental fire using distributed temperature sensing in optical fibre.*

Published in International journal of wildland fire - Received 14 July 2017, accepted 5 December 2017, published online 9 February 2018.

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Authors' contributions

Ryan Tangney, Ben Miller, and David Merritt conceived the idea; Ryan Tangney collected and analysed the data. Nader Issa supported installation of DTS and provided support for the analysis. Ryan Tangney led the writing of the manuscript and all authors contributed critically to the drafts and gave final approval for publication.

Acknowledgements

This thesis would not have been possible without the unwavering support and guidance that I have received throughout from many individuals and organisations. To everyone involved, I am humbly thankful.

To my steadfast supervisors, Dr Ben Miller and Dr David Merritt, I am incredibly fortunate and grateful to have had you both as supervisors. Dr Ben Miller is especially thanked for taking a risk on a new student that had recently swapped disciplines. To David Merritt and Dr Ben Miller, you both have fundamentally shaped my ethos as a research scientist, and for that I am incredibly grateful. Thank you also to Prof Kingsley Dixon and Prof Grant Wardell- Johnson for your administrative support at Curtin University.

To my collaborators , John N Callow, Nader Issa and Joe Fontaine, thank you for providing assistance throughout my thesis. Your support is greatly appreciated. To Nader Issa, in particular, thank you for introducing me to DST technology, and supporting me through a crash course in optical physics.

To the Kings Park Bushland management, Steve Easton, Ryan Glowacki and Martin Brothersøn, I thank you for your support throughout including all the field assistance, seed collections, and for the approvals to conduct so many fires within the park.

I am also thankful to all those who helped me in the field, lab, or glasshouse throughout, thank you: Russell Miller, Anthea Challis, Dmitry Ivanov, Travis Rasmessen, Darcy Robertson, Renae Makin and Cory Bovin. I'd also like to thank Hannah Lloyd-Deely, who was my very first student that I supervised during her summer scholarship at kings park, so thank you Hannah for your work ethic and support while I was working out how to supervise. To all my colleagues and fellow students at Kings park science, I want to thank you for the

encouragement and guidance that you have provided to me and I can hopefully carry working and collaborating with you all.

Finally, I would like to thank Gracie Barrow, without your support throughout my candidacy, I would not be the person I am today. Gracie your support has provided me with so much confidence. I would also like to thank Gracie for her support in the field and in the lab and for all the baking that supported me throughout my candidacy.

Contents

Thesis rationale, outline and aims

This thesis examines the interactions between fire, fuels and seed biology to assess how seeds respond to fire in Banksia woodlands, specifically how they survive through fire.

Interactions between functional plant processes and fire have been fundamental in ecosystems in fire-prone environments around the world (Keeley 2011). Fire regimes differ between ecosystems as influenced by biogeographic interactions between four fundamental factors: the amount of available biomass, how readily biomass will burn, the frequency of weather conducive to fires, and frequency of ignition sources (Bradstock 2010). The relative strength of each of these drivers will influence the prevailing fire regime of the region and changes in the dominant influence of the fire regime may cause a switching of the prevailing fire regime of the region (Bradstock 2010). Fire regimes are defined by the frequency, season, intensity, shape and size of fire that is experienced most commonly within an ecosystem (Bowman et al. 2011). A switching of fire regimes away from historic fire regimes may cause shifts in species abundance and persistence. In fire-prone systems, recruitment from seeds following fire is prolific and provides a pathway for population persistence and expansion. For many species, recruitment from seed is the only means of population recovery following fire events, so seed survival through fire is vital in order to maximize post-fire recruitment. Therefore, understanding how seed survival through fire may be affected by changes in fire regime provides critical evidence that will aid in the management and maintenance of our ecosystems.

The general aim of this thesis was to understand seed survival through fire, and factors that may affect survival, including weather, fuels, and seed and plant traits. Each of the four experimental chapters describe factors that are either directly related to seed survival during fire or relates to a seed's ability to emerge after fire.

The specific aims of the research chapters are:

- Identify the lethal threshold of seeds of Banksia woodland species of differing fire response, seed storage syndromes, and seed morphological traits. Quantify the effect of seed moisture content on lethal temperature thresholds, and examine associations between plant and seed traits and lethal thresholds.
- Develop methods for installation, characterisation, and calibration of distributed temperature sensing (DTS) in optic fibre in order to measure soil temperatures during experimental burns.
- Examine the interactions between seed traits (lethal temperatures and seedling emergence depth) and soil heating under a range of fuels loadings and compositions.
- Predict seed hydration based on readily measureable weather and site variables in order to provide a tool that will aid in minimizing fire impacts on soil seed banks during hazard reduction burns.

The first experimental chapter of this thesis aims to identify the lethal temperature thresholds of seeds from common Banksia woodland species (including non-native species) of differing fire response, seed storage syndromes and seed morphological traits. Further, this first chapter investigates the effects of seed moisture content on lethal temperature thresholds, and examines the associations between plant and seed traits and lethal thresholds. Using novel non-linear modelling methods, this chapter presents a unique method for estimating lethal temperature thresholds, termed T_{50}, the temperature required to render half of the seed batch nonviable.

The second experimental chapter of this thesis describes a new method to measure soil temperatures during fire. Using distributed temperature sensing (DTS) and Raman scattering within optic fibre, this chapter describes a method that allows for continuous measurements of soil temperature during fire, the calibration needed and the associated temperature error of those calibrations. This method vastly increases the amount of data generated per fire, both spatially and temporally. Included in this chapter is a discussion of the limitations of the method and defines the future research direction.

The third experimental chapter examines the interactions between fuel loading, soil heating, emergence depth and lethal temperatures. This chapter assesses how different fine fuel loadings, (i.e. increasing gross weight of the fine fuel loadings and modifying the species composition of the fuel loadings) affect soil heating. In parallel, seed emergence behaviour was assessed (maximum emergence, mean emergence at each depth and emergence rate from each depth), and identification of seed traits that predict the maximum emergence depth of seedlings. Finally, using the soil heating data, emergence data and lethal temperature thresholds, from data in chapter 1, this chapter assessed whether seedling emergence would be restricted or eliminated due to soil temperatures exceeding the T_{50} of seeds and penetrating into the soil deeper than maximum emergence capabilities of seedlings.

The last experimental chapter measured seed moisture throughout 2017. This allowed the development of a predictive model that informs when seeds are more sensitive to elevated soil temperatures resulting from fires. Using additive logistic models to predict when seeds are at their highest moisture content, this chapter assessed which weather and site variables predict seed moisture and to hindcast specific periods of the year when seeds are wet throughout 2017.

These research chapters will be synthesised in a discussion chapter that will highlight the results of each chapter in the context of fire management, while suggesting future directions and unanswered questions that have arisen from this current research.

The remainder of this introductory chapter will present the current understanding of fire, fire regimes, the effects of soil temperatures, and the influence that seed and plant traits impart on seed survival through fire and interactions that drive seed survival through fire.

“In the beginning the Universe was created. This has made a lot of people very angry and been widely regarded as a bad move.”

- *Douglas Adams*

Chapter 1: General Introduction

Part 1: Fire and Fire regimes

Fire impacts every vegetated continent on the planet (Bowman et al. 2009). Driven by global climate patterns, fire has broad impacts on global habitats for people, animals and plants alike (Bowman et al. 2011). Influencing the structure and distribution of vegetation, carbon cycling and climate feedbacks, fire has global influences that extend beyond local ecosystems (Bowman et al. 2009).

Fire requires three key elements in order for combustion: an ignition source, fuels to burn, and oxygen (Quintiere 2006). Frequency of natural ignitions follow historic variations in global atmospheric oxygen concentrations (Bowman et al. 2009). Combustion of vegetation requires a minimum of 13 % atmospheric oxygen, while a concentration of 30 % atmospheric oxygen will lead to combustion of vegetation that is still moist (Bowman et al. 2009). Since the rise of distant *Homo* species, fire has been utilised for a myriad of purposes such as farming (Jones 2012), cooking (Gowlett and Wrangham 2013), and hunting (Welch 2014).

The first appearance of fossil charcoal indicates that fire has been shaping plant responses as far back as 400 million years ago (Bowman et al. 2009). Early *Homo* species started harnessing fire as far back as 690-790 thousand years ago (Goren-Inbar et al. 2004) and the use of fire may have contributed to the early evolution of distant *Homo* species (Wrangham 2009). The frequency of ignitions, scale of management and control has increased steadily over the past century as demands for safety and protection from fire have increased (Bowman et al. 2011).

Interactions between climate and soil feedbacks drive the fire regime of any ecosystem and the relative strength of the interactions between fundamental factors including: frequency of weather conducive to fire; fuel and biomass moisture content; amount and type of biomass; and the frequency of ignition sources, drive fire regimes of global ecosystems (Bradstock 2010). The prevailing climate of a region delimits the activity and frequency of fire, influencing fuel moisture, vegetation and fuel structure (Pausas and Paula 2012). Fuel moisture dictates when fuels are available to burn, while vegetation and fuel structure defines the amount and the connectedness of fuel components throughout the landscape (Santana et al. 2011). The relative influence of each of these elements determine fire activity and are closely related to the productivity of an ecosystem (Pausas and Bradstock 2007). In productive areas with ample rainfall, fire activity is driven by how often flammable conditions are achieved (how frequently fuels dry out enough for fire to carry). Whereas, in arid systems, low productivity means fires are driven by fuel availability more so than flammable conditions (Pausas and Paula 2012). Describing the frequency, intensity, severity and fire season (as well as the type and size of fire) that has historically impacted an ecosystem is termed the fire regime of the region (Krebs et al. 2010).

Plants and ecosystems are not intrinsically adapted to fire, instead ecosystems have adapted to a specific fire regime that has shaped these ecosystems over time (Keeley et al. 2011). As a consequence, human induced modifications of fire regimes may shift species and trait distributions (Keeley et al. 2011). The following sections present descriptions of fire regime elements and examples of how modifications in the frequency, intensity, severity or season of burns may induce changes in species composition and ecosystem function, with specific focus on seed survival through fire.

Frequency is a measure of how often fire affects an ecosystem, ranging from: a yearly cycle of fire, common in savannah grasslands (Wright and Clarke 2008), to several hundred years in some ecosystems, such as wet forests (Cawson et al. 2017) or low productivity forests (Gosper et al. 2013). The frequency of fire in fire prone ecosystems has direct effects on the persistence of functional adaptive traits (Rundel et al. 2018). For example, storage of seeds within the canopy of adult plants is a more favourable strategy when there is an intermediate fire frequency (Rundel et al. 2018). An increased fire frequency may reduce the ability for adult plants to produce sufficient seed crops to persist (Pausas and Keeley 2014), whereas a decreased fire frequency that exceeds the lifespan of the parent plant may reduce the ability for these shorter lived species to persist (Lamont et al. 1991, Enright et al. 1998).

Intensity is a measure of how much energy is produced during fire, measured in KW m^{-2}, but frequently includes measures of temperature (soil, surface or canopy) and residency time (Keeley 2009). Intensity is driven by interactions between fuel loadings and fuel structure (and fuel moisture) as well as weather conditions including ambient temperatures, relative humidity and wind speed (Brooks et al. 2004). Ecologically, high intensity fires have the potential to kill plants and seeds present within any fire site (Keane et al. 2009). High intensity fires are hard to manage and can cause loss of life and property damage (Price and Bradstock 2012). In fire-prone areas, active fuel management aims to reduce the occurrence and impact of high intensity wildfires through the use of hazard reduction burns, which reduce fuels under conditions conducive to lower intensity burns (Agee and Skinner 2005). However, under the most severe fire weather, fuel management practices may not be sufficient to reduce the impacts of catastrophically high intensity fires (Price and Bradstock 2012).

Fire severity is a direct measure of above ground and below ground organic matter consumption (Keeley 2009). Measures of severity were established to provide a quantifiable way to assess ecosystem effects (Keeley 2009). The measures are commonly inclusive of crown damage, branch damage, scorching, consumption of litter and duff layers, and in some cases measures of changes to soil characteristics including soil structure and hydrophobicity (Keeley 2009). Severity is a measure that provides information on the spatial patterns of fuel consumption and of patchiness across the landscape and plant responses to variation in intensity, where direct measurements of intensity are absent (Palmer et al. 2018). Fire severity and fire intensity are correlated (Wade 1993, McCaw et al. 1997), however, spatial patterns of fire severity will be associated more closely with fuel and vegetation moisture than with fire intensity (Bradstock et al. 2010). High fire severity can lead to increased seed germination as a result of elevated soil temperatures (Wright et al. 2016, Palmer et al. 2018). On the contrary, high severity fires may also reduce post fire recruitment if temperatures exceed lethal thresholds of seeds (Busse et al. 2010).

Fire season refers to the historic fire season, that is, the season or seasons of the year when probability of fire is highest. Season of fire is closely linked with the prevailing climate and productivity of the region. In savannah grasslands of Northern Australia, the prevailing season of fire is the dry season, specifically, the late dry season once sufficient fuels have accumulated and cured (Shirai et al. 2003). Whereas in Mediterranean climates (hot summer Mediterranean climates), the prevailing fire season is late summer/early autumn, which also aligns with the period of lowest fuel moisture (Pausas and Paula 2012). Changes in the seasonality of fires away from historic fire regimes may have direct effects on plant responses following fire (Roche et al. 1998, Ooi 2010), including a reduction of seed survival following exposure to high temperatures either in wet soils (Fer and Parker 2005) or when seeds are fully hydrated (Ruprecht et al. 2016).

Fire regimes are relatively stable over geological time periods within climate regions, as long as the fundamental drivers of the regime remain unchanged (Bradstock 2010). These drivers may include biomass accumulation reaching a point when fuel accumulation is sufficient to carry fire (Pausas and Moreira 2012) in the case of arid systems, for example, or the availability of biomass to burn in seasonally dry ecosystems (Cawson et al. 2017).

Disruptions to global fire regimes are being driven by changes in climate as well as fire management practices, both of which contribute to switching the drivers of fire regimes within an ecosystem (Bradstock 2010). Changes in climate is affecting fire regimes in regions where climates are becoming hotter and drier, resulting in a wider seasonal period when weather conditions are conducive to high intensity fires (Flannigan et al. 2013, Jolly et al. 2015). In many ecosystems and governance regions, fire regimes are actively managed to reduce the incidence and damage of high intensity fires. This is achieved through the implantation of hazard reduction burning practices (Australasian Fire and Emergency Services Authorities Council 2016).

Hazard reduction burning (also referred to as prescribed burning or fuel reduction burning) is a management procedure whereby fire is introduced into environments at higher frequency than historic regimes (Bradshaw et al. 2018) in order to reduce hazards associated with high fuel loads and intense wildfires (Agee and Skinner 2005). Intended to create areas of low fuel loading with the aim of reducing the spread and intensity of wildfires (Penman et al. 2011), hazard reduction burns are implemented in and around areas of significant social, economic and cultural value and in some cases for ecological benefit across broad landscapes (Fernandes and Botelho 2003). By design, hazard reduction burns are conducted during seasons and weather conditions that allow for adequate control and management of the fire area (Penman et al. 2011). This may result in changes to the fire regime of the area, away from the primarily natural cycle of ignitions occurring only when fuel and soil moisture is lowest, in favour of ignitions occurring during periods of the year when both fuel moisture and soil moisture are elevated (Agee and Skinner 2005). On average, hazard reduction burns are lower intensity, lower severity and smaller in size, with management use of these procedures frequently encouraging heterogeneous application across the landscape to create a matrix of mixed fuel ages (Fernandes and Botelho 2003). However, the effectiveness of hazard reduction burning as a management tool to reduce fire impacts is an active area of research (Price and Bradstock 2012, Bradshaw et al. 2018, Furlaud et al. 2018).

Substantial enquiry has examined how plants respond following modifications to fire regimes associated with hazard reduction burning, including how plant responses differ with changes in fire intensity (Bradstock and Auld 1995, Knox and Clarke 2006), changes in severity (Wright and Clarke 2008, Palmer et al. 2018), frequency (Peterson and Reich 2001, Andersen et al. 2005), size (Turner et al. 1997, Bird et al. 2012), type (Wagle and Eakle 1979, Cumming 2001) and season (Bowen and Pate 2004, Knox and Clarke 2006). Similarly, extensive investigation of interactions between seeds and hazard reduction burning has yielded great insight to the ecology and persistence of soil and canopy stored seeds. These include examination of germination stimulation of soil stored seeds (Auld and O'Connell 1991, Odion and Davis 2000, Knox and Clarke 2006, Palmer et al. 2018), the potential for seed mortality of soil stored seeds (Auld and O'Connell 1991, Bell and Williams 1998, Luna et al. 2007) and soil temperatures generated during wildfires or hazard reduction burns (Bradstock and Auld 1995, Williams et al. 2003, Thomas et al. 2010).

Part 2: Fire temperatures and fire fuels.

Temperatures generated during fires are the result of the combustion of fuels, which vary spatially throughout the landscape as well as throughout the fuel strata. Despite the frequency of fires and the clear links between plant responses and fire, direct *in-situ* measurements of fire temperatures have proved difficult and technically challenging, with issues including disturbance to the fuel layer and the soil during installation, loss of equipment and data due to fire damage, inability to control environmental variables and large temperature variation even at small spatial scales (Burrows 1999).

Despite the technical issues associated with measuring soil temperatures during fire, attempting to quantify soil heating during fire remains of significant interest in fire ecology literature (Bradstock et al. 1992, Bradstock and Auld 1995, Burrows 1999, Stoof et al. 2011, Savadogo et al. 2012).

Soils are poor conductors of heat, with soil temperatures decreasing rapidly with increasing soil depth. Yet soil temperatures during fire can still exceed 150 °C in the top 3 cm of the soil (Savadogo et al. 2012, Stoof et al. 2013). Soil temperatures during fire are driven by fuel consumption (fire severity) that is in direct contact with the soils, and to a lesser extent fire intensity (Bradstock and Auld 1995).

Fuels in a fire context refer to any combustible material within the burn area (Agee and Skinner 2005) across all fuel strata as characterised by where the fuels reside:

1) Ground layer fuels include: duff, organic soils, roots, rotten logs.
2) Surface fuels include: leaf litter, fallen logs, branches and twigs, grasses and vegetation close to the surface.
3) Canopy fuels include: standing plant of all strata, including the shrub layer and over-story species.

Surface fuels are most influential on soil temperatures, as combustion of surface fuels is in direct contact with the soil profile, and are responsible for delivering thermal energy into the soil. Surface litter fuels can be classified into functional groups based upon the rate of moisture loss in each fuel class (Fosberg and Deeming 1971). Derived from the direct measure of the diameter of the fuel component, fuels are sectioned into two broad groups, fine fuels (1 hour fuels) comprised of leaves, small sticks and bark between 0 and 6 mm in diameter, and coarse fuels comprised of larger wooden fuels including sticks and logs. Coarse fuels are further separated into two functional groups, 10 hour fuels (6-25mm) and 100 hour fuels (25-75mm), which takes into account the longer drying times of larger woody items (Cohen and Deeming 1985). Surface fuels are dynamic and vary spatially and temporally in mass, particle size and packing density (Facelli and Pickett 1991). Depending on the ecosystem, total fuel loading can range from sparse, unconnected fuels of open grass lands of arid regions comprised mostly of fine fuels (Wright and Clarke 2008), up to heavy laden ecosystems with thick leaf and duff layers and large fallen trees, synonymous with fire exclusion policies in some Californian forests (Keeley 2002).

Moreover, combustion of different fuel components may drive differences in heating patterns spatially (Bradstock and Auld 1995). Between the surface layer and two cm into the soil, maximum soil temperatures may be more closely associated with consumption of fine fuels, whereas between two and three cm into the soil, course fuel consumption may have greater influence on the patterns of soil heating (Bradstock and Auld 1995). Consequently, spatial and temporal dynamics of surface fuels may affect soil temperatures, in turn influencing seed survival through fire. However, aside from fuel loadings, soil moisture will also influence soil temperatures during fire (Aston and Gill 1976).

Soil moisture influences maximum soil temperature, buffering against heat penetration into soils. Wetter soils with higher water content could alter both the maximum temperature experienced in soils and rate of heating and cooling in soils (Stoof et al. 2013). Soil moisture quenches some of the thermal energy directed into the soil from the fire, as liquid water is dissipated into steam, buffering temperatures (Aston and Gill 1976). However, the differences between dry and wet soil decreases with depth, with no significant difference in effect below 5 cm (Stoof et al. 2013).

Soil moisture may buffer temperature penetration into soils, but fuel moisture dictates whether fuels are able to ignite. The accumulation of fuels following fire is unique for each ecosystem, influenced by the climate and vegetation of the region (Facelli and Pickett 1991) and similarly, fuel moisture dynamics will change between ecosystems and from season to season directly influencing the fuel consumption and severity of fire.

Fire severity and intensity is intrinsically linked with climate and weather patterns and fuel moisture. Fire intensity is influenced by ambient temperature, relative humidity, and wind speed experienced within the burn area (Keeley 2009), whereas fire severity is influenced by fuel moisture and fire intensity (Keeley 2009). Increased fuel moisture leads to less fuel consumption resulting in patchy, incomplete consumption of the fuel layer (Knapp and Keeley 2006), while fires during periods when fuel moisture is lower, leads to a more complete consumption of the fuel layer, resulting in higher severity fires (Enright and Lamont 1989b). Often, the implementation of low intensity fires is a directive for many land and fire managers as they are easier to manage and control, producing fuel reduction treatments in line with management targets (Agee and Skinner 2005). As a result of lowered intensities and high moistures in fuels and soils, temperatures in the soil will be lower (Aston and Gill 1976) as less fuel is consumed (Knapp and Keeley 2006) and less heat penetrates the soil (Stoof et al. 2013). However, plant responses to fire may be different as fire season changes. Species are adapted to specific fire regimes (Pausas and Bradstock 2007) and modifications to the fire regime by the way of changes in season and intensity may shift species composition, diversity and functional trait assemblages within fire-prone systems (Keeley 2011).

Part 3: Plant adaptations to fire regimes.

While this thesis focuses on seed survival through fire, plants have a suite of traits that confer fitness advantages in fire-prone environments (Keeley et al. 2011). Below are examples of plant traits that increase fitness in some fire regimes and may directly influence seed survival in fire or influence seed survival traits.

There is strong evidence that some functional plant traits are associated with an increased fitness advantage in fire-prone environments (Keeley et al. 2011), including: serotiny (Lamont et al. 1991), resprouting (Lamont et al. 2011) and flammability (Pausas et al. 2017). While the origins of these traits is contentious (Bradshaw et al. 2011 *vs* Keeley et al 2011), each of these traits (or suite of traits) provide fitness advantages in fire prone ecosystems. Adapted to specific fire regimes (not adapted to fire directly), the frequency of occurrence of these functional traits is continuous along the fire regime spectrum, but the probability of occurrence of these traits is closely linked with the fire regime of the region (Pausas and Bradstock 2007, Keeley et al. 2011).

Serotiny refers to the storage of seeds within closed cones or fruit in the canopy of adult plants for longer than one year (Lamont 1991). Serotiny is frequently expressed in fire-prone, nutrient poor and seasonally dry areas, found in families including Proteaceae, Myrtaceae, Cupressaceae, Casuarinaceae, Bruniaceae and Pinaceae (Lamont et al. 1991). Seed release from serotinous cones or fruit is normally triggered by fire, and seeds are short lived once released, germinating as soon as conditions are suitable (Enright et al. 2007). Serotiny provides increased fitness when seed release cues are synchronised with the opening of a wider range of suitable establishment sites (e.g. following fire). Expression of serotiny depends on the fire frequency in relation to the lifespan of the plant, the probability of inter-fire recruitment, and the range of fire frequencies experienced (Lamont et al. 2011). Serotiny is conveyed across a gradient based on the length of time seeds are retained within the canopy of the adult plant. This gradient ranges from strong serotiny, where seeds are held within cones indefinitely until fire cues are received (Lamont et al. 1991), through to weak serotiny, where seeds are released without the need for fire cues (Cowling and Lamont 1985). The degree to which plant species express serotiny is closely linked with the probability of inter-fire recruitment (i.e. abundant resources for successful recruitment), if inter-fire recruitment is probable, then seeds arriving between fire that are released by weakly serotinous species are able to germinate and establish (Cowling and Lamont 1985). If inter-fire recruitment is limited, seeds released inter-fire, when recruitment is less probable (i.e. when resources are limited and new seedlings will not be able to compete with adult plants), then strong serotiny is favoured as seedlings will be best suited for establishment following fire (Lamont et al. 1991). Seeds of species that are serotinous may have higher temperature resilience compared to soil stored seeds. Temperatures experienced in the canopy during fires can far exceed those experienced in the soil (e.g. 600°C in the canopy (Enright and Lamont 1989a) compared to temperatures in soils that reach 120-150 °C (Bradstock and Auld 1995)), as higher amounts of fuel in the canopy, more heat from convection and radiation directed into the canopy, and the insulating effect of soil from fire temperatures (particularly as soil depth and moisture content increase) all influence fire temperature (Stoof et al. 2013). Whether seeds are stored in the canopy or in the soil may influence their ability to withstand fire temperatures, as selection for thermal tolerance may differ between the two seed storage syndromes.

Resprouting is one of the two broad mechanisms that plant populations utilise in order to persist following fire, the other being recruitment from seed following fire. Resprouting is the process of new shoots emerging from existing plant meristems following disturbances including fire (Pausas and Keeley 2014). Resprouting provides fitness advantages when the inter-fire period is short, as short inter-fire intervals provide limited opportunity for plant species to accumulate sufficient seed to self-replicate (Enright et al. 1998). Instead, as resprouting species are able to produce seeds shortly following fire and distribute during the fire free period, resprouting species are favoured in short-interval ecosystems (Pausas 2001). The frequency of resprouting increases with fire frequency (Lamont et al. 2011), however other site factors including site productivity and aridity may also influence the frequency of occurrence (Pausas and Keeley 2014). Species unable to resprout are killed by fire and rely entirely on their seeds to regenerate; seeds of these species may be able to survive higher temperatures compared to seeds from resprouting species, as resprouters can re-grow vegetatively following fire and recruit from seed between fire events.

Flammability is a suite of plant traits that influence fuel dynamics, which in turn dictate the probability and behaviour of fire and can be measured across varied scales (Pausas et al. 2017). Broadly, these plant traits are best exemplified in litter fuels of plants (Pausas and Moreira 2012), and can be grouped into three main categories based on leaf and plant traits associated with three functional flammability types: hot flammable, fast flammable or non-flammable (Pausas and Moreira 2012). Each of these groupings correspond to leaf traits (similarly litter fuel traits) that confer particular influence on fire behaviour and ignition probability (Pausas et al. 2017). Hot flammable traits are found frequently in post-fire seeding species including serotinous species and species with seed dormancy release linked to heat pulses. High in standing biomass, fuel continuity and bulk density, these traits frequently lead to high temperatures and long combustion times, resulting in high mortality of the individual and surrounding plants (Pausas and Moreira 2012). Hot flammable species encourage combustion around individual plants, potentially opening up recruitment niches for successive seedling emergence following fire (kill thy neighbour hypothesis (Bond and Midgley 1995)). Fast flammable species are consumed quickly during fire with fast combustion and relatively low temperatures. Common among resprouting species, fire induced flowering species and grasses, fast flammable species have thin leaves, low bulk density but provide high fuel continuity throughout. Species with fast flammable traits ensure adult plants persist through fire by minimizing combustion temperatures and exposure times (Pausas et al. 2017). Non-flammable species are those species that despite living in fire-prone systems, have traits that limit combustion. Low standing biomass, low bulk density, and sparse unconnected fuels, mean that non-flammable species are able to persist in otherwise highly flammable systems (Pausas et al. 2017). These flammable plant traits may affect fuel loadings and the amount of energy directed into soils, influencing spatial arrangement of soil temperatures and in turn, potentially affecting seed survival in fire.

All three of these broader trait groups have fitness advantages in fire-prone systems where the expression of functional traits increases the chance of reproduction and survival. These same trait groups may affect the ability of seeds to survive fire. In many fire-prone systems, recruitment from seeds within soil seed banks is the prominent method for population persistence and expansion following fire, and seeds have developed an array of dormancy mechanisms and germination strategies that while may not be directly adaptive to fire or fire regimes, are responsive to fire derived cues.

Part 4: Seed, seeds survival and soil stored seed banks

This section of the thesis establishes the importance of seeds in fire-prone ecosystems and the strategies seeds utilise to time germination and emergence, and discusses the relevance of seed survival through fire.

Recruitment from seeds is fundamental for many species in fire-prone systems. Seeds as a means for reproduction allow for population expansion, dispersal and persistence. The importance of seeds in population dynamics of fire-prone species is reflected by the abundance of literature examining how seeds respond to fire, including: the effect of heat on dormancy (Martin et al. 1975, Tarrega et al. 1992, Valbuena et al. 1992, Bell et al. 1993, Herranz et al. 1998, Ferrandis et al. 1999, Keeley and Babr-Keeley 1999, Hanley and Lamont 2000, Baskin 2003, Williams et al. 2003, Zuloaga-Aguilar et al. 2010, Ooi et al. 2014), the effect of smoke and smoke derived chemicals on germination stimulation (Brown 1993, Keeley and Fotheringham 1997, Roche et al. 1997, Keeley and Fotheringham 1998, Staden et al. 2000, Flematti et al. 2004, Çatav et al. 2018), interactions between fire and seedling growth (Roche et al. 1998, Gleadow and Narayan 2007, Ooi 2010, Wright et al. 2016) and lethal heat shock on seeds (Bell and Williams 1998, Habrouk et al. 1999, Odion and Davis 2000, Hanley et al.

2003, Fer and Parker 2005, Gleadow and Narayan 2007, Traba et al. 2007, Ooi et al. 2014, Ruprecht et al. 2015).

Soil stored species from fire-prone systems may include a range of dormant and non-dormant seeds. A seed is classified as dormant if it does not have the capacity to germinate in a specified time period under any combination of normal environmental factors that are otherwise favourable for its germination (Baskin and Baskin 2004). The two dormancy types most frequent in fire-prone ecosystems are physical dormancy (PY) and physiological dormancy (PD), and both of these dormancy types have dormancy release or germination stimulation cues associated with fire (Baskin and Baskin 2001).

Physically dormant seeds have impermeable seed coats which keep internal cellular tissues at lower moisture contents compared to the surrounding soils and environments (Baskin 2003). Heat associated with fire is a fundamental mechanism that leads to the rupturing of the water gap in the seed coats of PY seeds (Dell 1980), thereby allowing hydration of internal tissues (Baskin 2003). In some cases PY seeds can be rendered non dormant by high soil temperatures over summer (Ooi et al. 2014) or by abrasion over time.

Similarly, fire-related germination stimulants are associated with seeds with physiological seed dormancy (PD) or morphophysiological dormancy (MPD). Physiological dormancy is alleviated most commonly by seasonal temperature stratification, but also frequently requires a second set of cues to stimulate germination; this is regularly smoke and smoke derived chemicals (Baskin and Baskin 2001, Merritt et al. 2006, Çatav et al. 2018). Some seeds with PD or MPD may require a combination of smoke and heat to stimulate germination (Tieu et al. 2001). Seeds responsive to smoke-derived germination cues have been documented in a range of seeds including a wide range of species native to Australia (Merritt et al. 2009), agricultural weed species (Adkins and Peters 2001) and agricultural cropping species (Drewes et al. 1995).

Seeds responsive to smoke are highly sensitive to the active compounds (karrikinolide (Merritt et al. 2009)), and germination can be stimulated with concentrations as low as 1 ppt (Flematti et al. 2004).

If all seeds, including dormant seeds, are to proceed through to an adult plant, they must be present, survive, and germinate at a time that is appropriate to seedling establishment. If seeds are to germinate after fire they must: survive elevated soil temperatures; be in the soil deep enough to be insulated from the soil temperatures during fire, but not too deep as to not be able to emerge; or be dispersed into a post-fire area. The majority of seeds are either in the soil or in the canopy of adult plants during fire, thus are subjected to elevated temperatures and must be able to survive the temperatures generated by fire, or be able to emerge from sufficient depth in the soil to avoid lethal soil temperatures. These different strategies that seeds utilise to reach a safe post-fire site may be reflected in seed traits. For example, seeds that have high tolerance to lethal temperatures may employ a suite of traits that provide insulation and protection, like thick seed coats (Bell and Williams 1998) or embryos deep within endospermic tissue. On the other hand, seeds that emerge from deep within the soil may have another suite of traits that may not provide protection and insulation, but rather may facilitate increased burial depth (Majer 1982, Smith et al. 1999) or increased depth from which seedlings can emerge (e.g. a large seed mass (Bond et al. 1999)).

Seed survival through fire has been addressed in a number of studies, with a focus on understanding the effects of high temperatures on seed survival (Tarrega et al. 1992, Valbuena et al. 1992, Rivas et al. 2006, Zuloaga-Aguilar et al. 2010) or how changes in the length of exposure time to elevated temperatures affects seed survival (Bell and Williams 1998, Herranz et al. 1998, Hanley and Lamont 2000). However, only one attempt to reconcile changes in temperature and changes to exposure time in one metric to allow for comparisons has been attempted (Paula and Pausas 2008).

Substantial evidence has been presented to establish that seeds from a wide range of systems are able to survive high temperatures associated with soil temperatures during fire (Keeley 1991, Bell and Williams 1998, Herranz et al. 1998, Hanley and Lamont 2000). For example, seeds of *Templetonia retusa* from southwest Australia can germinate after exposure to 100 °C for 30 minutes (Bell and Williams 1998). Seeds of California sagebrush, *Artemisia californica,* native to western parts of North America, can germinate after exposure to 120 °C for 5 minutes (Keeley 1991), and soil stored seeds of *Psoralea bituminosa* from the Mediterranean basin are able to survive exposure to 150 °C for one minute (Herranz et al. 1998). These examples and others (Tarrega et al. 1992, Valbuena et al. 1992, Rivas et al. 2006, Zuloaga-Aguilar et al. 2010), are based on experimental treatments that examine the survival of seeds during fires under dry conditions representative of historic fire conditions in temperate regions. However, hazard reduction burns are increasingly conducted outside historic fire season, as conditions are more favourable for management and containment burns due to cooler temperatures and elevated fuel and soil moisture (Agee and Skinner 2005). Consequently, soil seed banks may be increasingly exposed to elevated temperatures from fire under conditions of higher soil moisture, when seed moisture content will also be higher for many species (Merritt et. al. 2007).

Seed moisture content fundamentally influences physiological reactions in seeds (Leopold and Vertucci 1989). Directly linked with relative humidity (RH %) of the surrounding environment, seed moisture content (*sensu* the water potential of seeds(MPa)), imparts differing hydration states that define cellular processes within seeds (Walters et al. 2005). Seed moisture content varies based upon the vapour pressure of surrounding medium (Wuest 2007). At a water potential of ≤-15 MPa (≤85 % RH) seeds enter into a dry state, where metabolism is hardly measurable. Seed longevity increases as water potential decreases below -15 MPa, and particularly so as seed water potential decreases to below -200 MPa (≤22% RH) (Walters et al. 2005, Leprince et al. 2017). While in a dry state, internal chemical reactions within seeds are slowed as cytosolic water assumes a glassy state, restricting reactions; and of those reactions that do take place while in a dry state (e.g. oxidative reactions), almost all are exclusively at the detriment to seed viability (Walters et al. 2005).

Seed moisture will fluctuate throughout the lifetime of a seed, oscillating between hydration states, influenced by the prevailing weather and seasonal patterns that the seeds are subjected to (Turner et al. 2006). In ecosystems with well-defined seasons, like those within Mediterranean climates (Aschmann 1984), elevated seed moisture will be restricted to distinct seasons associated with frequent rainfall. An example of an ecosystem that experiences strong seasonal climates is the Banksia woodlands situated in southwest Australia. In Banksia woodlands, modifications to fire regimes (i.e. season, frequency and intensity) as a consequence of fire management and prescribed burning may affect seed survival through fire, particularly as prescribed burning is increasingly being implemented during periods of elevated fuel and soil moisture (Bradshaw et al. 2018).

Part 5: Banksia woodlands

The focal species for this thesis are all common to Banksia woodlands, an ecological community of southwest Western Australia. Banksia woodlands are a global biodiversity hotspot and were recently classified as a threatened ecological community by the Federal Government of Australia (Department of the Environment 2018). This threat status is due to increasing threats to biodiversity from fragmentation, reduction in extent of extant patches, and reduced community integrity driven by the additive effects of disease, invasive species and changes to fire regimes. These threats have led to reduced native species abundance and local extinctions of species from Banksia woodland patches (Department of the Environment 2018). This community presents unique opportunities for research into understanding the direct effects of each of the threatening processes.

Occurring on well drained, low nutrient sandy soils, Banksia woodlands are characterised by the presence of key *Banksia* species, including at least one of the following: *Banksia attenuata, B. menziesii, B. prionotes* or *B. ilicifolia*. Other mid-story and canopy forming species common to Banksia woodlands include: *Eucalyptus gomphocephala, E. marginata, Corymbia calophylla*, *Nuytsia floribunda* and *Allocasuarina fraseriana.* Highly diverse shrub and herb layers characterise Banksia woodlands with representation from families including Orchidaceae, Restionaceae, Haemodoraceae, Droseraceae, Fabaceae, Asteraceae, Myrtaceae, Ericaceae and Proteaceae. The high species diversity of Banksia woodlands makes the remaining remnants ecologically valuable, providing habitats for up to 20 nationally threatened species (Stevens et al. 2016). Banksia woodlands are in close proximity to many urban and peri-urban environments, and clearing for development is a key driver of their fragmentation.

Banksia woodlands are fire-prone ecosystems, influenced by the hot-summer Mediterranean climate (Aschmann 1984) which drives the vegetation complex and fuel accumulation of the region. Banksia woodlands experience strong seasonal weather patterns with high temperatures extending December through to March and restricted rainfall (average maximum temperature > 29 °C, rainfall < 25 mm, Bureau of Meteorology (2018)). Most of the rain is deposited in the winter months, June through to the end of August (Department of the Environment 2018). Average yearly rainfall ranges from 560 mm in the northern range of Banksia woodlands, to 830 mm in the southern extremes of the range (Bureau of Meteorology 2018).

These strong seasonal patterns encompassing frequent hot-summer droughts coupled with flammable vegetation traits mark a distinct wildfire season for Banksia woodlands, which extends from November through March (Hobbs and Atkins 1990). The proximity and biodiversity of Banksia woodlands highlight the importance of fire management in this ecosystem, as minimizing loss of lives and damage to infrastructure is vital in order to ensure the safety of people that share the urban-Banksia woodland interface. As a management option, hazard reduction burning is practiced within Banksia woodlands with the aim to reduce fuel loading and thereby minimizing the risk of wildfire impacting people and infrastructure (Burrows and McCaw 1990).

Part 6: Conclusion

This chapter has identified the fundamental elements that are associated with seed survival through fire, such as the influence of fire regimes, and seed and plant traits. Identification of these interacting elements provide the central context for which the following experimental chapters are placed. The four experimental chapters that follow, attempt to extend the current understanding of the interactions that drive seed survival through fire.

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Chapter 2 was first published in Journal of Ecology in November 2018. It is presented below in the same format as published in the journal.

Chapter 2: Seed moisture content as a primary trait regulating the lethal temperature thresholds of seeds.

Abstract

Fire has long shaped biological responses of plants and plant communities in many ecosystems; yet, uncontrolled wildfire frequently puts people and infrastructure at risk. Fuel or hazard-reduction burning outside of the historic fire season is a common and widespread practice aimed at reducing the risk of high-severity fires, which ideally also considers biodiversity values. Within fire-prone systems, seed banks are critical for plant species' regeneration, and seeds are typically adapted to survive the passage of fire and to regenerate in response to cues associated with historic fire regimes. However, species-specific tolerances to the heat from fire exist; likely influenced by a range of physical, physiological, and morphological seed traits, which may differ between seasons. Identification of these tolerances and associated seed traits may inform fire and species-management.

We determined the lethal temperatures for seeds in relation to their moisture content, and other key traits that we hypothesised may be associated with survival. Seeds from 14 native species, and 4 species non-native to fire-prone Mediterranean climate Banksia woodlands of southwest Western Australia, were exposed to temperatures between 50 °C and 180 °C for 3 mins, at three different moisture contents. The temperature at which half the seeds were killed (T_{50}) was estimated using non-linear modelling. Seed mass, seed shape, embryo type, plant resprouting ability, seed storage syndrome, and native/non-native status, were quantified and modelled for their relationship with T_{50}.

Increased moisture content was a significant predictor of elevated seed mortality. Seeds with higher moisture (95% RH) content perished at much lower temperatures. Seeds with low

moisture content (15 or 50% RH), were able to survive significantly higher temperatures (median increase of 38 °C and 31°C higher respectively). Seeds with basal embryos showed significantly lower T_{50} than other embryo types.

Synthesis: Seeds with elevated moisture contents have lower lethal temperature thresholds, leading to increased seed mortality during fire events when seeds (and soils) are moist. Thermal tolerance varied among co-existing species within this fire-prone system. These data suggest potential concern for the impacts of aseasonal burning practices (i.e. cool/wet season burning), and highlight the importance of taking seed moisture content into account when planning and implementing prescribed burning.

Introduction

Fire has shaped biological responses of plants and plant communities and is linked to many ecological processes in fire-prone ecosystems (Miller and Murphy 2017). Key plant reproductive processes influenced by fire include: stimulation of flowering (Bowen and Pate 2004); the breaking of seed dormancy of soil-stored seeds via heat (Ooi et al. 2014) or germination stimulation by smoked-derived chemicals (Dixon et al. 1995, Çatav et al. 2018); and the initiation of seed release from serotinous cones (Lamont et al. 1991, Schwilk and Ackerly 2001, Huss et al. 2017). Globally, the frequency of high intensity fires is increasing (Bowman et al. 2009, Flannigan et al. 2013). Hazard reduction (prescribed) burning is principally used to reduce fuel loads and fire hazard (Fernandes and Botelho 2003), and to a lesser extent to stimulate soil seed banks (Bell et al. 1993), control invasive species (Keeley 2006) and promote heterogeneity in managed landscapes (Mallik and Gimingham 1983). Hazard reduction burning practices aim to mitigate risks and minimize the consequences of high-intensity wildfires by limiting the total amount of fuel available in landscapes (Fernandes and Botelho 2003). However, the link between fire and biological processes in fire-prone systems means that altered fire regimes may have detrimental impacts on plant communities.

For example, fires implemented at longer or shorter intervals than the historic regime (Enright et al. 2014), or fires in seasons when plants are less able to respond to fire (Roche et al. 1998), may contribute to a reduction in species persistence and eventual loss of some species (Enright et al. 2015), or the modification of ecosystem structure (Brooks et al. 2004) and function (Enright 2011).

Seeds are fundamental to the natural regenerative success of many plant species in fire-prone regions. For the majority of species, if seeds are to enable successful regeneration, they must be present, survive fire, and germinate at a time that is appropriate to seedling establishment. If seeds are to germinate after fire, they must be able to survive the temperatures generated by fire. The ability of seeds to tolerate high temperatures has been well documented across fire-prone systems (Tarrega et al. 1992, Valbuena et al. 1992, Rivas et al. 2006, Zuloaga-Aguilar et al. 2010). For example, soil stored seeds of *Cytisus* species from the Mediterranean basin are able to survive exposure to 150 °C for one minute (Herranz et al. 1998), seeds of *Daviesia cordata* from southwest Western Australia can germinate after exposure to 100 °C for 10 minutes (Bell and Williams 1998), and seeds of *Rhus trilobata*, native to western parts of North America, can germinate after exposure to 120 °C for 5 minutes (Keeley 1991). Seeds of *Hakea trifurcata*, a species native to southwest Western Australia, normally stored in the canopy (i.e. serotiny), can survive exposure to 100 °C for 10 minutes while out of their protective cones and still germinate (Hanley and Lamont 2000). These examples, and others (Tarrega et al. 1992, Valbuena et al. 1992, Rivas et al. 2006, Zuloaga-Aguilar et al. 2010), are based on experimental treatments to examine the survival of seeds during fires under dry conditions representative of historic fire conditions in temperate regions. However, hazard reduction burns are increasingly conducted outside of the historic fire season, as conditions are more favourable for management and containment of the burn due to cooler temperatures and elevated fuel and soil moisture (Agee and Skinner 2005). Soil temperatures during fires conducted under favourable management conditions are often lower than during wildfires due to reduced fuel consumption and thermal energy dissipating quickly in wet soils (Maia et al. 2012). These interactions between fuel and soil moisture and fire severity are complex, both spatially and temporally, as fuel and soil moisture vary throughout seasons and throughout landscapes (Bradstock et al. 2010, Palmer et al. 2018). Similarly, plant and seeds responses also vary through seasons (Roche et al. 1998, Fer and Parker 2005). Nevertheless, soil seed banks increasingly are exposed to elevated temperatures from fire under conditions of higher soil moisture, when seed moisture content will also be higher for many species(Merritt et al. 2007). Seed moisture content fundamentally influences physiological reactions in seeds (Leopold and Vertucci 1989), and it follows that the tolerance of seeds to fire may differ during periods of higher or lower soil/seed moisture.

In unsaturated soils, seeds with permeable seed coats passively absorb water vapour from the soil (Wuest 2007), and, provided sufficient moisture is available and temperatures are suitable, germination of non-dormant seeds may occur. However, if seeds are dormant, they will remain in the soil irrespective of their moisture status until specific environmental cues release dormancy and allow germination to occur. Some environmental cues that release dormancy and induce germination are derivatives of fire, and include smoke and heat (Baskin and Baskin 2001). Smoke acts to promote germination of seeds that possess physiological dormancy (PD) once PD is first alleviated (Merritt et al. 2007), and heat can act on seeds with PD, or on seeds with physical dormancy (PY). Seeds with PY possess a water impermeable seed coat and may be rendered permeable (non dormant) via heat. Without such cues, PY seeds remain dormant, and their impermeable seed coat ensures internal seed tissues remain dry regardless of soil moisture (Baskin 2003). With the exception of seeds with PY, during periods of high soil moisture seed moisture content also increases(Turner et al. 2006, Merritt et al. 2007), and seeds exposed to high temperatures while in wet soils or while fully hydrated become increasingly sensitive to high temperatures (Fer and Parker 2005, Ruprecht et al. 2016). Consequently, fires that occur when seed moisture is high may result in increased seed mortality.

Other traits of plants from fire-prone systems that aid in persistence and post fire regeneration include the ability to resprout following fire, and the storage of seeds in the canopy of adult plants (serotiny) (Keeley et al. 2011). The frequency of occurrence of these plant traits amongst the vegetation in fire prone systems is influenced by climate patterns and fire regimes (Cowling and Lamont 1985, Pausas 2001, Enright et al. 2015), and the tolerance of seeds to fire may in turn be influenced by these parent plant traits. For example, non-sprouting species are killed by fire and rely entirely on their seeds to regenerate; seeds of these species may be able to survive higher temperatures compared to seeds from resprouting species, as resprouters can re-grow vegetatively following fire and recruit from seed between fire events. Likewise, seeds of species that are serotinous may have higher temperature resilience compared to soil stored seeds. During fires, temperatures experienced in the canopy can far exceed those experienced in the soil (e.g. 600°C in the canopy (Enright and Lamont 1989a) compared to 120-150 °C in soils (Bradstock and Auld 1995)), as higher amounts of fuel in the canopy, more heat from convection and radiation directed into the canopy, and the insulating effect of soil from fire temperatures (particularly as soil depth and moisture content increase) all influence fire temperature (Stoof et al. 2013). Yet seeds of serotinous species are not always present in the canopy during fire, but rather are within the soil. In some ecosystems, including our study site of southwest Western Australia, seeds are released spontaneously each year between fire events (Cowling and Lamont 1985). Also, in some serotinous species seeds are released from cones gradually after 5-7 years, which is potentially shorter than the fire interval of the area (Lamont et al. 1991). Thus, consideration of how seed moisture content affects seed survival also relevant to (weakly) serotinous species that shed seeds into the pre-fire soil environment.

The ability of seeds to survive fire may also be associated with physical and physiological seed traits (Bell and Williams 1998). Surviving the high temperatures associated with fire has historically been linked with hard-seededness; meaning those seeds that possess physical dormancy (Rolston 1978, Dell 1980). However, many seeds within fire-prone systems do not possess this trait, yet must still survive fire temperatures. Therefore, other traits may be associated with seed survivorship. These may include specific morphological features – some embryo types may be more insulated within the seed, for example. Further, evidence from Chile suggests that seed shape is influenced by fire frequency (Gómez-González et al. 2011), which may lead to some seed shapes being more resilient to elevated fire temperatures. Lastly, seeds with lower mass have been found to increased germination after fire compared to heavier seeds in one Western Australian study (Hanley et al. 2003).

Banksia woodlands are fire-prone ecosystems of the biodiverse southwest Western Australia situated in a Mediterranean climate region. These woodlands are in close proximity to urban and peri-urban environments and have recently been listed as a threatened ecological community (Department of the Environment 2018) due to threats from land clearing, climate change and changes in historic fire regime. For these highly diverse woodlands, hazard reduction burns are conducted in spring (i.e. September - December) and autumn (i.e. March-May). However, due to decreasing autumn rainfall over recent years which is forecast to continue (Andrys et al. 2017) there has been a reduction in safe weather conditions for hazard reduction burning in autumn. A narrowed window of opportunity increases pressure on land managers to conduct hazard reduction burns in spring, and potentially winter (i.e. June-August), in attempts to reduce wildfire risk. However, seeds may be more susceptible to fire temperatures when seeds have high moisture content, which may ultimately result in reduced biodiversity and localized species loss.

This study aimed to identify the lethal temperature threshold of seeds of Banksia woodland species of differing fire response, seed storage syndromes, and seed morphological traits. We quantified the effect of seed moisture content, and examined associations between plant and seed traits and lethal thresholds. By quantifying patterns in seed mortality, functional trait based responses to aseasonal burning can be used to inform management, further contributing to decision processes for managing fire-prone landscapes.

Methods

Species selection

Mature seeds of fourteen native species were collected from wild plant populations of remnant Banksia woodland within the Perth region during 2015 (Table 1). Four non-native species were also collected from Kings Park woodland (an urban Banksia woodland situated in Perth, Western Australia) between October 2015 and April 2016. All species are common in this fire-prone ecosystem and were selected as being representative of both soil and canopy stored species, resprouting and nonsprouting species, and native and non-native species that produce seeds that range in size and shape. After collection, seeds were stored in a controlled environment room at 15% relative humidity (RH) and 15 °C until experiments commenced in 2016.

Seed and plant traits

A range of traits were measured to assess their relationship to seed lethal temperature. For plant fire-response, all species capable of resprouting, whether epicormically, basally, or from underground storage organs, were classified as resprouters, whereas species unable to resprout and that rely solely on recruitment from seed were classified as non-sprouters (Paula and Pausas 2008). Seed embryo classification was limited to upper level classification (to division and subdivision) as outlined in Martin (1946). In particular, embryos of seeds were classified into one of the following classes: embryos in foliate subdivision of the axile division (hereafter foliate); embryos in the linear subdivision of the axile division (hereafter linear); embryos in the basal division (hereafter basal); and embryos in the miniature subdivision of the axile division (hereafter miniature). Seed mass was determined by weighing ten replicates of 100 seeds, data presented as mean single seed mass (mg) and standard error. Seed width to length ratio was measured using a Epson flatbed scanner (Epson Australia, Sydney, NSW, Australia) on a sample of 100 seeds and analysed using WinSEEDLE™ (Régent Instruments Inc., Québec, QC, Canada) and calculated using the ratio of straight width divided by straight length. Classification of field seed storage syndrome used two classes: species that release their seeds directly into the soil seed bank upon maturation were classed as soil stored; and species that hold seeds within cones or capsules were classed as canopy stored (serotinous) regardless of duration of canopy storage (serotiny strength). Species were classified as native or non-native to Banksia woodlands according to Barrett and Tay (2016) (Table 1).

Modelling lethal temperature

To quantify lethal temperatures of seeds, a number of steps were followed to ensure that seeds were viable. To ensure filled seeds were used for experiments (i.e. had intact embryonic and endosperm tissue), seeds from each species were X-rayed using a Faxitron Specimen Radiography System (MX-20 Cabinet X-ray Unit) (Faxitron, Wheeling, Illinois, USA) and any non-filled seeds were removed.

Following seed fill assessment, and prior to implementing heat treatments to determine lethal temperatures, seeds of some species required dormancy-break pre-treatments to induce germination (Table 1). The application of these pre-treatments allowed for the maximum germination response after the heat treatment, in order to accurately determine seed survival. For species known to possess water-impermeable seed coats (i.e. physical dormancy, PY), experiments were conducted on both dormant and non-dormant seeds, to examine the potential protective impact of this type of dormancy to high temperature (i.e. through excluding water uptake). For seeds with PY (those of *Acacia pulchella*, *Acacia saligna*, *Bossiaea eriocarpa*, and *Kennedia prostrata*), immersion in hot water (Table 1) was used to break dormancy through the rupturing of the water gap – a specialised section of the seed coat that forms the primary pathway for water uptake (Turner et al. 2009, Gama-Arachchige et al. 2010). To obtain a sample of dormant (PY) seeds, non-treated seeds were soaked for 24 hours in deionised water, and any seeds that imbibed during this period were removed from the sample. All seeds were subsequently separated into four replicate batches of 25 seeds per replicate.

Prior to exposure to heat treatments, sub-samples of seeds were pre-conditioned to each of three different moisture contents. To achieve desired seed moisture content, replicate batches of non-dormant seeds of all species were placed inside small paper envelopes in air-tight polycarbonate electrical enclosure boxes (28 × 28 × 14 cm; NHP Fibox, Australia), above a non-saturated solution of LiCl (anhydrous, Sigma®, Australia). The concentration of LiCl was adjusted to achieve the desired relative humidity of 15% RH, 50% RH, or 95% RH (740 g L^{-1}, 364 g L^{-1} and 48 g L^{-1} of LiCl, respectively). The boxes were then stored at 20 °C (Hay et al. 2008) for two weeks (seed moisture content data for each species at each of the storage conditions is included in Table S1). The use of 15% RH, 50% RH, and 95% RH provided seeds with moisture contents within each of three distinct regions of seed hydration known to confer differing levels of physiological activity within seeds (Walters et al. 2005).

Following the adjustment of seed moisture content, seeds from all species were exposed to heat pulses between 80 °C to 160 °C for three minutes (temperatures were shifted slightly for some species, inclusive of 50-180 °C to improve precision of species-specific thresholds; full temperature exposures are included in supplementary Table S1). The heat treatments applied were selected as representative of soil temperatures measured during experimental fires conducted in Banksia woodlands, with a treatment time of 3 minutes corresponding to a typical duration over which soil temperatures are at their maximum (Tangney et al. 2018a). To ensure a uniform and consistent heating environment between replications of heating, seeds were placed inside stainless steel mesh bags (2 µm diameter mesh holes) and the bags were inserted into a bed of dry, bleached white silica sand that had been pre-heated to the required temperature in a laboratory oven (Contherm, Korokoro, New Zealand). Replicates were heat-treated separately. Sand temperature was monitored using a K-type thermocouple attached to a digital thermometer, independent of the oven settings. After heat treatment, bags were removed from the sand, and the seeds were removed from these and allowed to cool at room temperature and humidity.

Following heat treatment, seed survival was assessed via germination testing. Seeds were surface sterilized using 3% calcium hypochlorite ($CaOCl_2$) for 20 minutes under vacuum, and each replicate was plated on a Petri dish containing 0.7% (w/v) water agar. Dishes were incubated at 15°C under a 12/12 hour light/dark regime (30 µM m^{-2} s^{-1}, 400 – 700 nM) for a minimum of six weeks, or until no more germination was recorded for at least two weeks. Once germination had ceased, a cut test was performed on non-germinated seeds to determine viability. Seeds were assessed as alive if they had germinated or if their embryo and endosperm tissues was firm, white and moist, and dead if no germination had occurred and the endosperm and embryo were chalky, grey and dry. For seeds of *Anigozanthos manglesii* that did not germinate, a 1% triphenyl tetrazolium chloride stain was used to assess seed viability (Ooi et al. 2004), as a cut test alone was less conclusive than for other species due to their underdeveloped, tiny embryo.

Table 1: Species used in this analysis, including seed dormancy type and pre-treatments (if required), plant and seed traits. Family names are presented without the –aceae for brevity. Abbreviations: Physical dormancy - PY, non-dormant - ND, morphophysiological dormancy – MPD, physiological dormancy – PD, morphological dormancy - MD. Hot water - HW, smoke water - SW. Non sprouter - NS, resprouter - R. Soil stored seed - S, canopy stored seed - C. Seeds with embryos in the foliate subdivision of the axile division – F, seeds with embryos in the linear subdivision of axile – L, seeds with embryos in the basal division – B, seeds with embryos in the miniature subdivision of the axile division – M. Species that are non-native to Banksia woodland are indicated by an asterisk.

Species	Family (-aceae)	Dormancy type	Pre-treatment	Weight (mg)	Width :Length	Fire Response	Storage Type	Embryo Type
Acacia pulchella R.Br	Fab	PY	HW 2 mins	7.46	0.45	NS	S	F
Acacia saligna (Labill.) H.L.Wendl.	Fab	PY	HW 2 mins	21.48	0.52	NS	S	F
Allocasuarina fraseriana (Miq.) L.A.S.Johnson	Casuarin	ND	Nil	4.03	0.52	R	C	F
Anigozanthos manglesii D.Don	Haemodor	MPD	Nil	1.00	0.79	R	S	M
*Asparagus asparagoides** (L.) Druce	Asparag	PD	Nil	6.59	0.90	R	S	L
Austrostipa compressa (R.Br.) S.W.L.Jacobs & J.Everett	Po	PD	SW	1.59	0.23	NS	S	B
Austrostipa elegantissima (Labill.) S.W.L.Jacobs & J.Everett	Po	PD	SW	0.61	0.11	NS	S	B
Banksia prionotes Lindl.	Protea	ND	Nil	23.47	0.64	NS	C	F
Banksia sessilis (Knight) A.R.Mast & K.R.Thiele	Protea	ND	Nil	6.17	0.46	NS	C	F
Bossiaea eriocarpa Benth.	Fab	PY	HW 30 secs	1.99	0.63	R	S	F
Conostylis candicans Endl.	Haemodor	PD	SW	0.27	0.52	R	S	M
*Ehrharta calycina** Sm.	Po	PD	Nil	1.48	0.40	R	S	B
Eucalyptus gomphocephala DC.	Myrt	ND	Nil	2.51	0.67	R	C	F
*Freesia alba x leichtlinii**	Irid	MD	Nil	7.29	0.70	R	S	L
*Gladiolus caryophyllaceus** (Burm.f.) Poir.	Irid	PD	Nil	3.53	0.85	R	S	L
Haemodorum spicatum R.Br.	Haemodor	ND	Nil	3.68	0.82	R	S	M
Kennedia prostrata R.Br	Fab	PY	HW 1 mins	31.14	0.76	NS	S	F
Melaleuca systena Craven	Myrt	ND	Nil	0.28	0.83	R	C	L

Data analysis

To establish both species-specific trends and functional trait patterns in relation to lethal temperatures, we first analysed seed responses to heat exposure for individual species and used modelling to identify lethal temperatures (T_{50}). Second, we used an all subsets approach to identify the predictive strength of an *a priori* set of functional traits hypothesised to describe the variance in lethal temperatures and supplemented this analysis with pairwise comparisons of literature-identified traits of importance. Lastly, we used a commonality analysis to decompose the effects of significant variables appearing in the top ranked model and partition their predictive effects into common or unique effects based upon their explanatory strength on T_{50} variance.

Species-specific seed survival data were modelled using non-linear functions in the 'DRC' package (Ritz et al. 2016) within the statistical program R (R Development Core Team 2017). Implementation of non-linear functions enabled prediction of the temperature required to render half of the seed lot dead under a range of moisture conditions (effective dose or ED %), here referred to as T_{50} (temperature at which 50% of the seeds were dead), in a similar manner to other measures of lethality such as LD_{50} in toxicology (LeBeau 1983). Species-specific curve fitting was based upon log-likelihood estimations and Akaike Information Criterion (AIC). Curve fitting identified either a three-parameter non-linear Weibull (Equation 1) or a three-parameter log-logistic function (Equation 2), as outlined in Ritz et al. (2016); (d) is the parameter for maximum survival, (b) the *slope* of the curve $F(x)$, and (e) the 50% point of the curve relative to the control (T_{50}), (for model selection see supplementary materials Table S2).

Equation 1: Three-parameter Weibull function

$$f(x) = 0 + (d - 0)\,exp(-exp(b(log(x) - e)))$$

Equation 2: Three-parameter log-logistic function.

$$f(x) = 0 + \frac{d - 0}{1 + \exp\left(b(\log(x) - \log(e))\right)}$$

T-tests were used to establish evidence of significant differences between treatment groups; we report 95% confidence intervals for each treatment alongside t-values and p-values. We used non-heat treated seeds as controls, which form the baseline of the analysis. For brevity, seeds preconditioned at 15%, 50% or 95% RH are referred to as ‘seeds at 15% RH’ etc. Full response curves for seeds of each species are featured in supplementary materials (Fig. S1 and S2, model selection Table S2).

To establish predictive links between traits and T_{50}, multi-factorial generalized linear regression models with Gaussian link functions were used to explain variation within T_{50}. Using a set of biologically relevant seed and plant traits we created a global multi-factorial generalized linear regression model set containing the variables: seed mass, seed width : length ratio, life form, storage type, native or non-native, embryo type, and relative humidity. Relative humidity was fixed in all candidate models. Interactions between relative humidity and other predictive variables were included in the global model (Full script included in supplementary materials Fig S3). Continuous variables were centred and scaled. An all subset regression was carried out using MuMin package (Burnham and Anderson 2003) which ranked subsets of generalised linear models by Akaike’s information criterion (AIC). A model set comprising the top ranked subset of candidate models with a $\Delta AIC < 2$ was retained. Ranking of candidate models was based on AICc, ΔAIC, and AIC model weights (ω_i; Table S3 supplementary materials).

Because we were interested identifying the effects of seed moisture content on lethal temperatures among differing functional seed and plant traits, generalised linear regression models were used to investigate patterns of T_{50} between functional traits, inclusive of relative humidity. For each of the categorical traits, we employed post-hoc Tukey HSD pairwise comparisons to understand which groups differed from one another.

Lastly, to determine how much variation was derived from each seed trait, commonality analysis was conducted on the best performing model using the *yhat* package (Nimon Kim et al. 2013). Commonality analysis decomposes the multiple R^2 from the regression analysis into its unique and common effects (Ray-Mukherjee et al. 2014), such that the analysis can identify how much variance in the dependent variable (i.e. T_{50}) is uniquely explained by one or multiple independent variables (Nimon and Oswald 2013). This allows for inspection of seed traits that most influence T_{50}.

Results

Lethal temperature

Seed moisture content significantly influenced the T_{50} of seeds of each species (Fig. 1). In all cases, the highest T_{50} was observed in seeds of lowest moisture content, and the lowest T_{50} was observed in seeds of the highest moisture content (15% RH *v* 95% RH; Fig. 1 and Fig .2). Seeds at 15% RH had a mean T_{50} 9.5 °C higher than seeds at 50% RH (124 *v*. 114 °C, t= -2.75, P=0.008, Table 2), and 34 °C higher than that of seeds at 95 % RH (89 °C, t=-9.92, P=<0.001, Table 2). The T_{50} of seeds at 50 % RH was 25 °C higher than that of seeds at 95 % RH (Table 2).

The T_{50} of non-dormant seeds at 15% RH ranged from 93 °C up to 152 °C (for *Ehrharta calycina* and *Banksia sessilis* seeds, respectively) – a range of 59 °C. The T_{50} of non-dormant seeds at 50% RH spanned a range of 69 °C, from 75 °C to 144 °C (for *Asparagus asparagoides* and *B. sessilis* seeds, respectively). The T_{50} of non-dormant seeds at 95% RH ranged from 56 °C up to 114 °C – a range of 58 °C (for *E. calycina and Conostylis candicans* seeds, respectively) (Fig. 1).

Of those species with PY seeds (*Acacia pulchella*, *Acacia saligna*, *Bossiaea eriocarpa* and *Kennedia prostrata*), for which both non-dormant and dormant seeds were examined, T_{50} was in most cases equal in dormant seeds and in those non-dormant seeds at 50% and/or 15% RH (Fig. 1). Only *K. prostrata* had a significantly higher T_{50} for dormant seeds (148 °C dormant seeds vs. 134 °C seeds at 15% RH, t = 4.61, P <0.01).

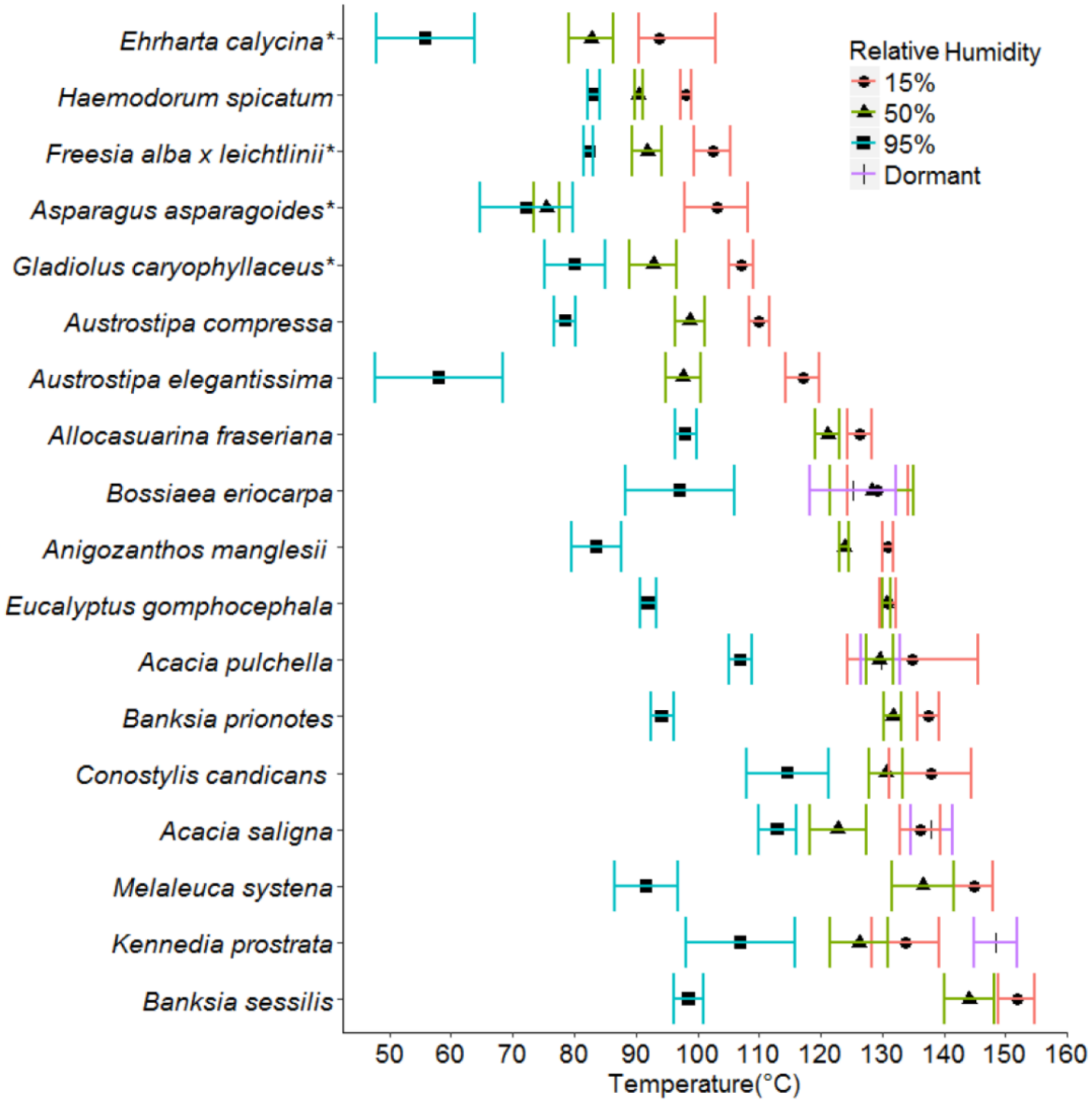

*Figure 1: Modelled T_{50} (mean and 95% confidence intervals) for 18 species of native and non-native (indicated *) species in Banksia woodland communities for seeds pre-conditioned at indicated relative humidity (RH). Pre-conditioned seeds were exposed to a range of temperatures for three minutes in a laboratory oven. Seeds of all species were pre-conditioned and exposed to heat in a non-dormant state; for seeds with physical dormancy (*Acacia pulchella, A. saligna, Bossiaea eriocarpa, Kennedia prostrata*) seeds were also treated in the dormant state (no pre-conditioning). Species in the figure are arranged from lowest mean T_{50} (across all RH) to highest mean T_{50}.*

Seeds of only four species had a T_{50} greater than 100°C at 95% RH (*A. pulchella* 107 °C, *K. prostrata* 107 °C, *A. saligna* 113 °C and *C. candicans* 115 °C). Overall, seeds at 50% RH had a lower T_{50} compared to seeds at 15% RH; the only exceptions being for seeds of *Eucalyptus gomphocephala* and *C. candicans*, where there was no significant difference in T_{50} of seeds at 15% and 50% RH (Fig. 1).

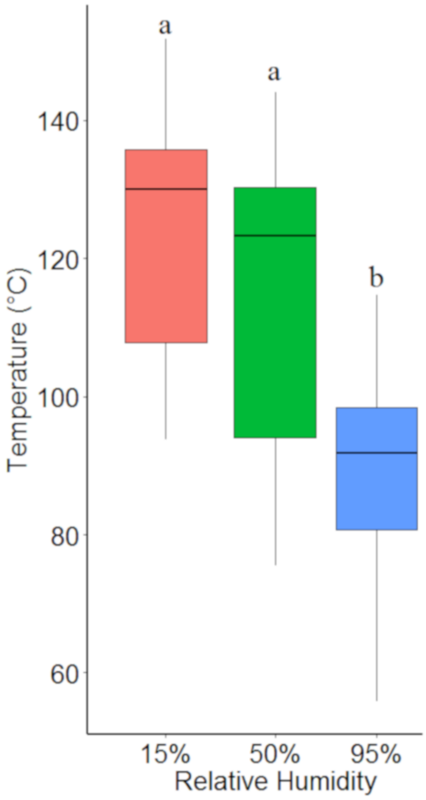

Figure 2: Modelled T_{50} for seeds of all 18 species grouped by relative humidity (RH) treatments, excluding dormant seeds of the four species with physically dormant seeds (Acacia pulchella, A. saligna, Bossiaea eriocarpa, Kennedia prostrata). Horizontal lines represent the median T_{50} for seeds of each RH treatment, and boxes extend to 25th-75th percentiles and whiskers span 10th and 90th percentiles, Treatments with the same letter are not significantly different resulting from post-hoc pairwise Tukey HSD test.

Seed and plant traits

The top ranked candidate generalized linear regression model accounted for 80% of the variation observed in T_{50} across species and treatments (Multiple R-squared: 0.83 and adj R-squared: 0.80, $F_{8, 49}$=30.27, P = <0.001). The predictive variables that remained after model selection were relative humidity, embryo type, seed width to length ratio, and whether the species is native or non-native (Table 2). The top three candidate models resulted in good explanation of the data (> adj. R^2 = 75), with relative humidity, embryo type and whether the species is native or non-native, being retained in each of the candidate models. No interactions between relative humidity and any of the other predictive variables were significant in any of the top ranked models (supplementary materials Table S2).

Seed embryo type was included in the top model and had a significant influence on T_{50} ($F_{3,45}$ = 16.94, P= <0.01). Seeds with basal embryos (n=3) had the lowest T_{50} of any embryo class, regardless of RH. Mean T_{50} of seeds with basal embryos was 34 °C lower than the mean T_{50} of seeds with foliate embryos (t = 5.15, P <0.01), 34 °C lower than seeds with linear embryos (t = 3.913, P <0.01) and 25 °C lower than seeds with miniature embryos (t = 3.058, P <0.01) (Table 2, Fig 3A).

The seed storage syndrome (soil vs. canopy stored) had a significant influence on T_{50} ($F_{1,51}$= 13.054, P=<0.001), but storage syndrome was not included in top ranked model (Table 2). Seeds of serotinous species (n= 5) had consistently higher T_{50} values than those of species with soil stored seeds (n= 13), with the average T_{50} values differing amongst serotinous and soil stored seeds at 15, 50%, and 95% RH. (Fig. 3B).

Plant fire-response had a significant influence on the T_{50} of seeds ($F_{1,50}$= 5.052, p=0.029), but was not included in the top ranked candidate. The T_{50} values of seeds from non-sprouting species, while higher than seeds from resprouting species (mean T_{50}, 118.3 *vs* 105.4, respectively), were not significantly higher (Fig. 3C).

Whether or not a species was native to Banksia woodlands had significant influence on average T_{50} of seeds ($F_{1,51}$= 40.810, P = <0.001). Seeds of non-native species (n = 14) had a mean T_{50} that was 23°C lower than seeds of species native to Banksia woodlands (t = -4.420, P <0.01, n= 4) (Table 2) and comparisons were significantly different at all RH levels (Fig. 3D).

Neither seed width to length ratio, or single seed weight had any relationship with T_{50} across any treatment groups, nor any interactions with RH. Only seed width to length ratio was included in the top ranked model, but this was not a significant variable in the top ranked model.

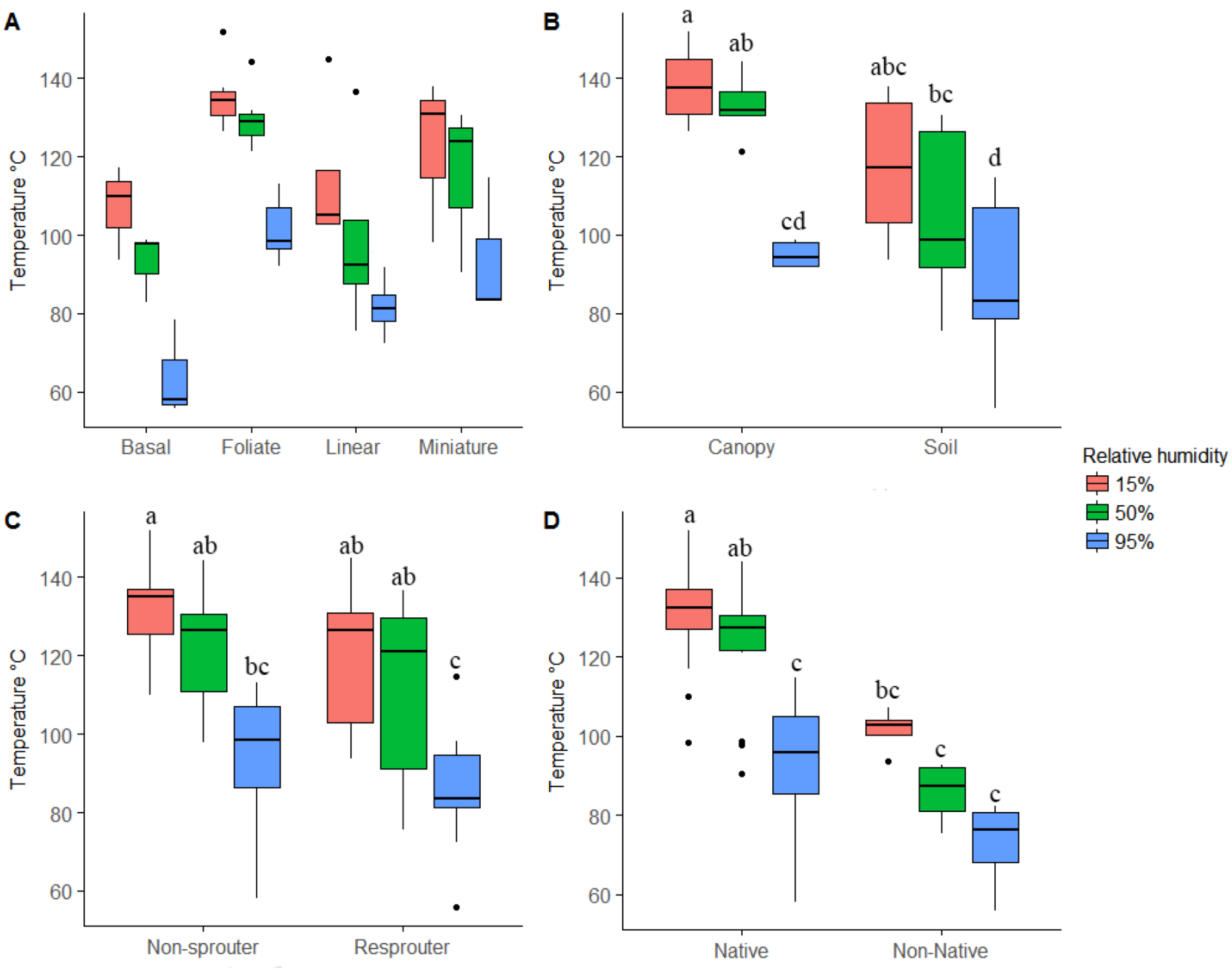

Figure 3: Modelled and pooled T_{50} following preconditioning at indicated RH and across A) embryo type; B) seed storage syndrome; C) fire response, and; D) native and non-native species. Boxplots show 25th-75th percentiles and whisker span is inclusive of 10^{th} and 90^{th} percentiles. Dots indicate outliers outside the 10^{th} or 90^{th} percentile. Results from post-hoc pairwise Tukey HSD included, treatments with the same letter are not significantly different.

Table 2: Analysis of variance table of the optimised model based on AIC for the effects of seed and plant traits on T_{50} of seeds constructed using MuMin. Variables that are significant (<0.05) are bolded. For full model selection, including weights and code *(Fig. S3, S4 and Table S2 in supplementary materials)*

Dependent variable: T_{50}	Estimate	Standard error	t value	Pr(>|t|)
(Intercept)*	101.7992	6.766	14.964	<**0.001**
50% RH	-9.472	3.475	-2.748	**0.008**
95% RH	-34.352	3.475	-9.924	<**0.001**
Dormant RH	-0.832	5.980	-0.152	0.880
Native : Non-native	-23.212	5.252	-4.357	<**0.001**
Seed Width: Length ratio	-5.074	2.786	-1.827	0.074
Embryo Type: Foliate subdivision	34.095	6.614	5.241	<**0.001**
Embryo Type: Linear subdivision	34.300	8.673	3.953	<**0.001**
Embryo Type: Miniature subdivision	25.951	8.490	3.126	**0.003**

Residual Std. Error: 10.42 (df = 49)
F Statistic: 30.27 (df = 8; 49)
P-value: <0.0001
Multiple R-squared: 0.83
Adjusted R-squared: 0.80

*Intercept represents native species with basal embryos preconditioned at 15% RH

Commonality analysis identified the relative effect of each of the traits associated with variations in T_{50}, by degrading the R^2 of the optimised model into either unique or common effects. ‘Unique effects’ identify variation explained by variables on their own, while ‘common effects’ identify the influence of correlated variables on the overall R^2 (Fig. 4).

Relative humidity had the largest unique effect, accounting for 0.379 of overall R^2, followed by seed embryo type, uniquely accounting for 0.120 of R^2. Classification of native or non-native seeds uniquely accounted for 0.065 of the R^2, and seed width to length ratio uniquely accounted for 0.011 of the R^2 (Fig. 4).

Seed embryo type had the largest common effect at 0.234 of the overall R^2. Classification of native or non-native species had a common effect of 0.212, suggesting that these two variables may be interactive with other variables, instead of conferring unique effects. Relatively humidity had a 0.06 common effect on overall R^2, and width to length ratio of the seed had a negative common effect of -0.010. The negative common effect of width to length ratio essentially removes irrelevant variance in other predictor variables (Fig. 4). Including seed width to length ratio increased the overall model fit and the overall adjusted R^2 and decreased AIC values relative to models without seed width to length ratio (See supplementary information for extended model analysis). On its own, seed width to length ratio was not significant (Table 2), but acted as an additive variable that increased the predictive power of other variables (Fig. 4).

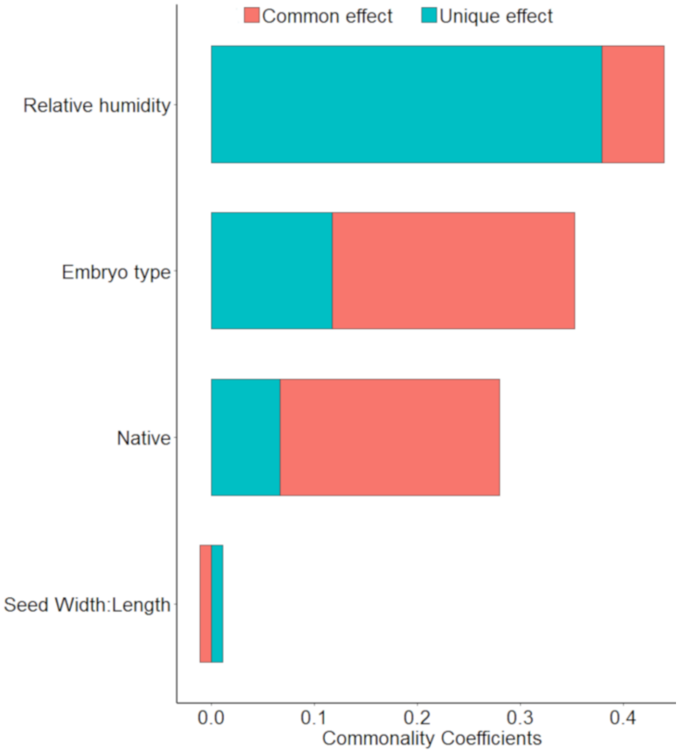

Figure 4: Results from the commonality analysis, whereby total R^2 is partitioned into common and unique effects among the variables. Unique effects refer to how much influence a single variable had on the overall R^2, whereas common effects refer to how much combined influence correlated variables have on the overall R^2.

Discussion

Our data indicate that the moisture content of seeds has a significant influence on the maximum temperatures at which they can survive. Across all species tested, higher moisture content reduced seed survival at temperatures representative of those experienced in soils during fire. Variation in lethal temperatures between species can be partly explained by seed traits; in particular, variation in seed embryo type was strongly associated with varying temperature sensitivity. Seeds with foliate and miniature embryos had increased survivability compared to seeds with other embryo types.

At the lowest moisture content tested, seeds of all but one of the 18 species studied could survive temperatures over 100°C. The ability of seeds to survive this threshold has been previously reported in Australian plants (Hanley et al. 2003). It has been suggested that the ability to survive temperatures >100°C is particularly a trait of seeds of Australian species (Bell and Williams (1998), potentially due the high intensity fires commonly experienced in Australia. However, Herranz et al. (1998) found that seeds of some legume species of the Mediterranean basin were able to germinate after exposure to 150°C, and Valbuena et al. (1992) found that two species of Cistaceae from the south of France were able to survive 200°C. Thus, the ability of seeds to survive high temperatures may be a common trait among species from fire-prone environments. Nonetheless, temperatures generated by fire can far exceed the lethal temperature of seeds, particularly in the upper soil levels where temperatures are highest (e.g. >150 °C, Tangney et al. 2018). Spatially diverse fuel and duff layers and the resultant variations in soil temperatures in fire, whereby high fuel and duff accumulation can increase soil temperatures in the upper soil layers (Marino et al. 2012) while combusting, will contribute strongly to seed survival.

Comparing seeds preconditioned at 95% RH versus at 15% RH shows that on average, seed moisture content (across this range) contributed to a 34°C difference in lethal temperatures.

Seeds of our study species are killed by much cooler temperatures when they are hydrated, consistent with results found for species of other ecosystems (Fer and Parker 2005, Ruprecht et al. 2016). Moisture content is known to strongly influence physiological reactions in seeds (Bewley et al. 2013). At very low moisture contents (i.e. for seeds pre-conditioned at 15% RH), physiological reactions in seeds are constrained due the extreme viscosity (i.e. glass state) of the water (Walters 2005). For seeds hydrated at RH > 70%, water becomes increasingly available and enzymatic processes commence. Seed respiration rates increase and the synthesis of new proteins commences above 90 - 95% RH as free water becomes readily available within cells (Walters et al. 2005). Seed mortality induced by exposure to extremely high temperatures at such moisture contents may be a result of the available free water becoming heated to a point where it damages cellular machinery. In dry seeds, in addition to the strongly bound nature of the water, protection against extremely high temperatures may arise from small Heat Shock Proteins (sHSP). These proteins accumulate within the seed embryo during the maturation drying that occurs in late development stages, around the same time as seeds acquire dormancy and desiccation tolerance (Wehmeyer et al. 1996). These sHSP's may provide general protection to key cellular machinery while seeds are in a dry state, but they become less abundant and will confer less protection as seeds hydrate (Leprince et al. 2017)

The relationship between seed hydration status and lethal temperature has important consequences for seed survival during fire: exposure to fire while seeds are hydrated may result in higher mortality. Seeds rapidly hydrate when soil moisture is increased (Turner et al. 2006), so burning during periods of elevated soil moisture potentially increases the risks of exceeding lethal temperatures of seeds. However, it is also the case that soil temperatures during fire may be lower when the soil is wet, compared to when the soil is dry. Soil moisture quenches some of the thermal energy directed into the soil from the fire, as liquid water while present, is dissipated into steam, buffering temperatures (Stoof et al. 2011). An increased level of moisture in fuel also reduces fuel consumption, which will also lead to a reduction in heat penetrating into the soil (Marino et al. 2012). These phenomena aside, our data demonstrate temperatures experienced in wet soils can still exceed the lethal temperatures of wet seeds: in our study 14 out of the 18 species had a T_{50} under 100 °C, which is well below the soil temperatures measured under heavy fuels while soils are wet (Aston and Gill 1976). Therefore, during wetter seasons (i.e. winter and spring in Mediterranean-type climate regions including our Banksia woodlands), high soil moisture during hazard reduction burns may increase the risk of seed mortality, potentially reducing post-fire recruitment from seed for many species. Although, for seeds with physical dormancy, provided they are dormant (and thus dry), our results demonstrate their tolerance to fire will not be influenced by soil moisture. Clearly, PY is a seed trait that might strongly influence the composition of the post-fire species community. Fires that occur during periods of higher soil moisture, when seeds with other dormancy types are hydrated (i.e. seeds with permeable seed coats), and therefore more likely to be killed, may shift community composition towards a greater abundance of species that produce seeds with PY, in turn resulting in reduced diversity of the post-fire plant community.

Aseasonal burning (i.e. the implementation of fire outside of a natural fire season, as a way to manage fire intensity during hazard reduction burning) has a number of potential impacts. Previous studies have recognised a reduction in post-fire seedling recruitment success (Ooi 2010), a more limited opportunity window of environmental conditions suitable for germination following winter and spring burns (Baker et al. 2005), and a reduction in seedling emergence and survival from spring fires (Roche et al. 1998). Based on the results of the present study, we propose there is an increased risk of seed mortality if aseasonal burning is implemented during those periods when seeds are hydrated. In Banksia woodlands, prescriptions for managed burning in autumn are dictated by fuel and soil dryness, with most burns requiring at least some prior rainfall to increase soil and fuel moisture levels above those of the dry summer prior to implementation. However, the results presented here suggest that increases in seed moisture content reduce the ability of seeds to survive fire. If land and fire mangers are using fuel reduction burning to minimise risk of intense, high severity fires, there is an increasingly limited window where fire can be implemented that minimise negative effects to soil stored seeds while remaining safe for land managers to implement, as conditions for conducive to wildfires increase globally (Flannigan et al. 2013).

Aside from seed moisture content, the next most influential seed trait in relation to temperature sensitivity was embryo type. Seeds with foliate and miniature embryos, which had higher lethal temperatures than the other species in this study, have their embryos protected within the seed either by the surrounding endosperm and/or by a thicker seed coat (Martin 1946). In seeds with foliate embryos, some level of insulation against high temperatures may be provided by the fully developed cotyledons that encapsulate the embryonic axis, as present in seeds of the *Fabaceae* (Martin 1946). Seeds with basal embryos had the lowest lethal temperature at all seed moisture contents tested. Basal embryos, common to grasses (including the three studied here) are located within seeds at the base of the endosperm (Martin 1946), and in grasses these are covered only by thin tissues that comprise the palea and lemma. Insulation of the embryo from heat is therefore likely limited, leading to a higher chance of heat-induced damage. For these seeds, other protection mechanisms may come in to play. For example, seeds of both *Austrostipa* species may minimise exposure to high temperatures via their self-burial mechanism, whereby the hygroscopic properties of the awn create a drilling action that may increase the depth at which the seeds reside in the soil (Smith et al. 1999).

We found seeds of species that are native to Banksia woodlands to have significantly higher lethal temperature thresholds than seeds of non-native species in this community. Seeds of the native Banksia woodlands species have similar thermal tolerances to seeds of species from different bioregions of Australia; reports of lethal temperatures of around 120°C are common for Australian seeds (Auld and O'Connell 1991, Judd 1993, Bell and Williams 1998, Herranz et al. 1998, Ooi et al. 2014). The four non-native species in our study are all common invasive species to Banksia woodlands, and are native to South Africa (Barrett and Tay 2016). Lower lethal temperatures in these non-native species could be driven by fire characteristics of their native range, or, more likely, plants of these species possess other functional traits that make seed survival during fire less important for persistence. For example, three of these species possess below-ground vegetative structures that allow for resprouting after fire; a common trait among invasive species (Raymond 1996). Despite being previously identified that seeds from nonsprouting species had an increased resilience to fire temperatures (Paula and Pausas 2008), our results did not find a significant difference in lethal temperatures of seeds from either resprouting or nonsprouting species. It is possible that the lower temperatures more generally experienced in a managed fire regime might support expansion of resprouting species, relative to a wildfire driven fire regime.

Canopy stored seeds had higher lethal temperatures compared to soil stored seeds, even when dry. This could potentially result from selection: the insulative properties of soil provides increased temperature buffering as soil depth increases, which in turn reduces the maximum heat seeds will experience in the soil, with soils rarely exceeding 200 °C (Mercer et al. 1994, Bradstock and Auld 1995). On the other hand, fruits that contain (and may somewhat insulate) seeds stored in the canopy can experience higher temperatures (> 600 °C) for short periods of time, ranging from 0.5 to 3 minutes (Judd 1993). The lethal temperature of canopy-stored seeds was 37 °C less, on average, when these seeds were wet (i.e. pre-conditioned at 95% RH); a much greater difference than was found between dry and wet soil-stored seeds (20 °C). Serotinous species hold seeds in capsules or cones for extended periods, often many years, presumably maintaining low moisture conditions within to extend the longevity of the stored seeds. The combination of insulation provided by cones or capsules and the apparent dry storage conditions may increase the protection to seeds during a fire. In some canopy-stored species, seeds are released between fires, as opposed to being triggered by fire to release seeds (Cowling and Lamont 1985). Seed release into unburnt areas may put these species at an increased risk of seed mortality if burning coincides with moist conditions.

This study demonstrates that seed moisture content is a key determinant of seed survival under elevated temperatures. Other seed and plant traits are also influential, including seed morphology and dormancy status, seed storage and plant fire-response syndromes. Future research and management should consider the potential effects of altered intensity and season of burning on seed survival, particularly burns that are implemented under conditions of increased soil moisture.

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Chapter 2: Supplementary materials

Appendix S1 : Seed moisture and T_{50} across all relative humidty treatments.

Table S2: Average moisture content (± SE) of seeds after storage for two weeks under the storage conditions of 15 %, 50 % and 95 % relative humidity, stored at 20°C. Species-specific temperature exposures also included.

Species	MC % at 15% RH	T_{50}°C at 15% RH	MC% 50% RH	T_{50} °C at 50% RH	MC% at 95 % RH	T_{50}°C at 95% RH	heat exposures for 3 minutes (°C)
Acacia pulchella	2.7 ± 0.2	134.8	7.1 ± 0.1	129.5	130.9 ± 1.7	106.9	25-90-100-110-120-130-140-150-180
Acacia saligna	4.4 ± 0.2	136.1	9.2 ± 0.1	122.7	16.7 ± 1.6	113.0	25-90-100-110-120-130-140-150-180
Allocasuarina fraseriana	5.3 ± 0.6	126.3	7.6 ± 0.5	121.1	16.0 ± 0.9	98.8	25-80-90-100-110-120-130-140-150
Anigozanthos manglesii	9.2 ± 2.1	130.9	10.4 ± 1.4	123.8	18.3 ± 2.6	83.5	25-80-90-100-110-120-130-140-150
Asparagus asparagoides	4.9 ± 0.4	103.0	9.7 ± 0.4	75.5	32.4 ± 1.1	72.2	25-50-70-80-90-100-110-120-140-150-180
Austrostipa compressa	5.4 ± 1.0	109.9	11.1 ± 0.4	97.3	24.7 ± 0.8	75.9	25-50-70-80-90-100-110-120-140-150
Austrostipa elegantissima	4.9 ± 0.4	117.0	12.5 ± 1.2	97.6	22.3 ± 1.4	58.0	25-50-70-80-90-100-110-120-140-150-180
Banksia prionotes	6.3 ± 0.3	137.4	9.7 ± 0.1	131.6	23.7 ± 0.3	94.2	25-90-100-110-120-130-140-150
Banksia sessilis	4.9 ± 0.4	151.8	9.6 ± 0.3	144.0	29.6 ± 0.4	98.5	25-90-100-110-120-140-150-160-180
Bossiaea eriocarpa	3.7 ± 0.3	129.1	7.7 ± 0.9	128.3	30.6 ± 5.9	97.1	25-90-100-110-120-130-140-150-180
Conostylis candicans	5.6 ± 0.5	137.8	9.2 ± 0.8	130.5	22.1 ± 1.6	114.6	25-90-100-120-130-140-150-180
*Ehrharta calycina**	7.4 ± 1.6	93.7	11.1 ± 1.3	82.7	25.4 ± 1.1	55.8	25-50-70-80-90-100-110-120-130-140-150-180
Eucalyptus gomphocephala	5.3 ± 1.4	130.9	13.6 ± 1.2	130.6	17.2 ± 1.1	92.0	25-80-90-100-110-120-130-140-150-160
*Freesia alba**	6.1 ± 0.4	102.4	11.5 ± 0.4	91.8	34.4 ± 0.6	82.3	25-50-70-80-90-100-110-120-130-140-150-180
*Gladiolus caryophyllaceus**	4.9 ± 0.6	107.1	9.3 ± 0.9	92.8	31.8 ± 0.8	80.1	25-80-90-100-110-120-140-150-180
Haemodorum spicatum	5.2 ± 0.7	98.1	9.7 ± 0.8	90.4	25.0 ± 1.6	83.1	25-80-90-100-110-120-140-150-180
Kennedia prostrata	4.9 ± 0.1	133.7	9.9 ± 0.2	126.2	19.6 ± 1.3	106.9	25-90-100-110-120-130-140-150-180
Melaleuca systena	3.8 ± 1.0	144.7	12.6 ± 1.6	136.5	20.8 ± 2.0	91.6	25-80-90-100-110-120-130-140-150

Appendix S2 : Model set and model selection for T_{50} estimates and global model

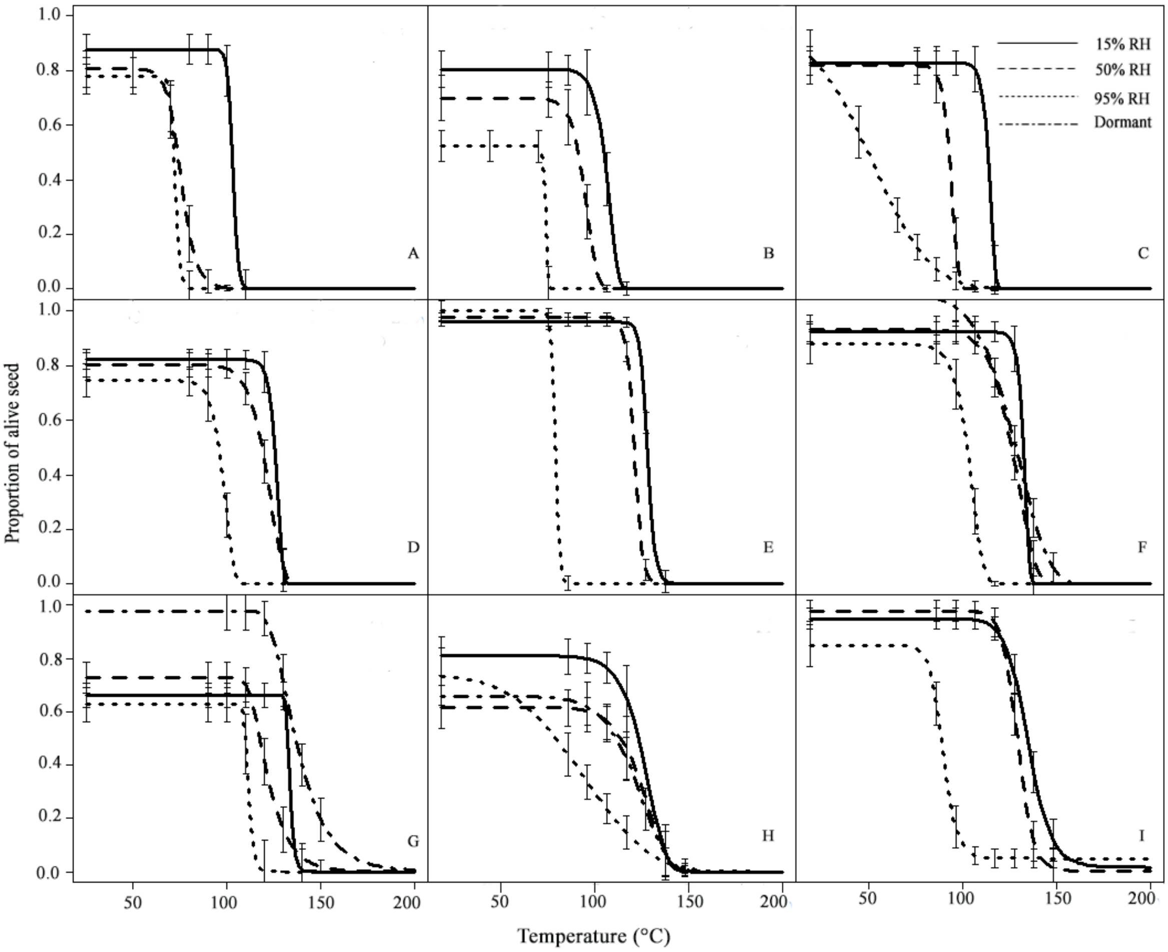


Figure S1: Modelled response curves of nine of the 18 species including the effects of RH on lethal temperature. T^{50} is calculated as the temperature required to reduce number of alive seeds by 50%. Mean T^{50} and ± standard error; where the standard error = 1-(0.05/2) quantile in the t distribution with degrees of freedom equal to the residual degrees of freedom for each model. A: *Asparagus asparagoides,* B: *Austrostipa compressa,* C: *Austrostipa elegantissima,* D: *Allocasuarina fraseriana,* E: *Anigozanthos manglesii,* F: *Acacia pulchella,* G: *Acacia saligna,* H: *Bossiaea eriocarpa,* I: *Banksia prionotes.*

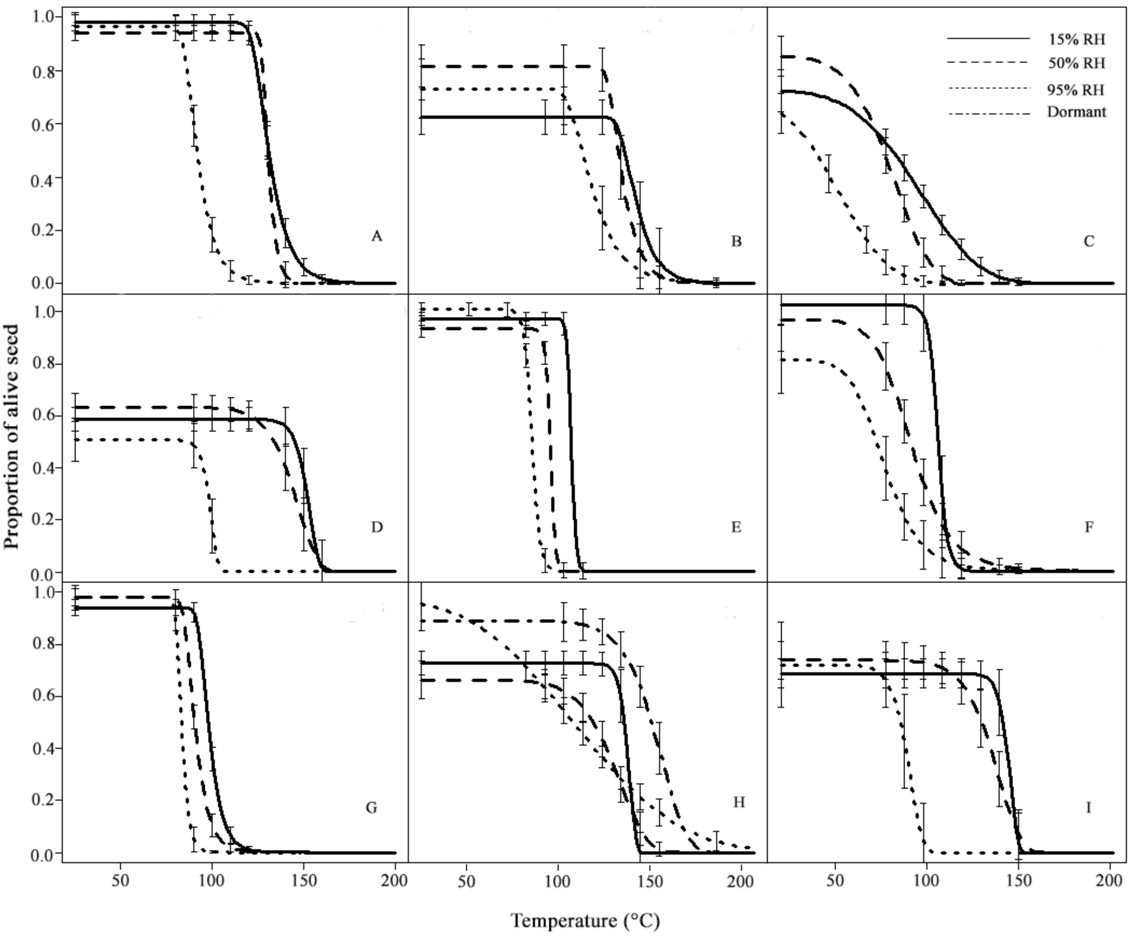
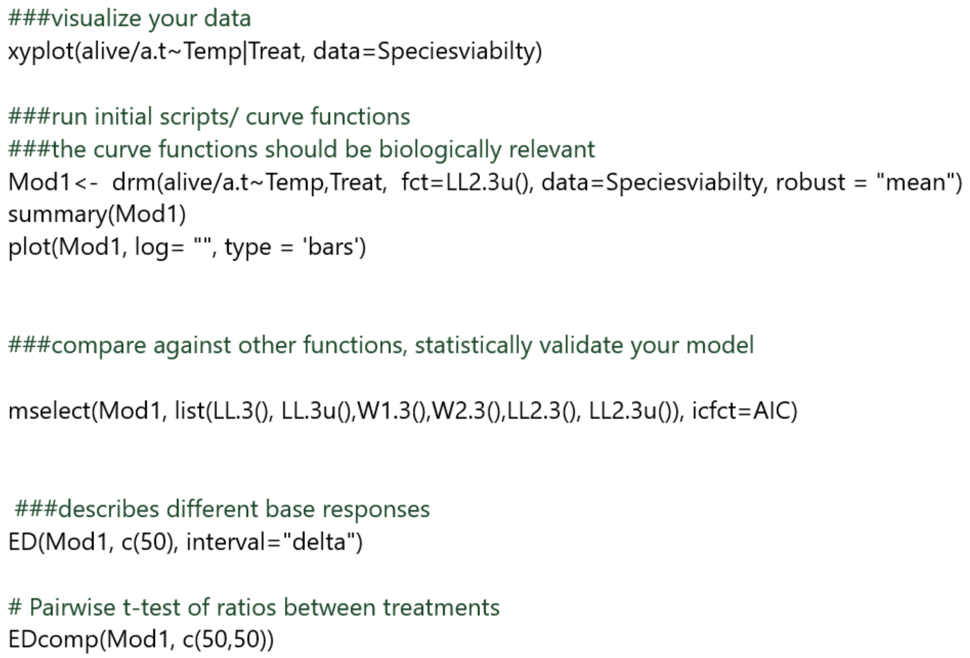


Figure S2: Modelled response curves of nine of the 18 species including the effects of RH on lethal temperature. T^{50} is calculated as the temperature required to reduce number of alive seeds by 50%. Mean T^{50} and ± standard error; where the standard error = 1-(0.05/2) quantile in the t distribution with degrees of freedom equal to the residual degrees of freedom for each mode. A: *Eucalyptus gomphocephala,* B: *Conostylis candicans,* C: *Ehrharta calycina,* D: *Banksia sessilis,* E: *Freesia alba x leichtlinii,* F: *Gladiolus caryophyllaceus,* G: *Haemodorum spicatum,* H: *Kennedia prostrata,* I: *Melaleuca systena.*



```
###visualize your data
xyplot(alive/a.t~Temp|Treat, data=Speciesviabilty)

###run initial scripts/ curve functions
###the curve functions should be biologically relevant
Mod1<-  drm(alive/a.t~Temp,Treat,  fct=LL2.3u(), data=Speciesviabilty, robust = "mean")
summary(Mod1)
plot(Mod1, log= "", type = 'bars')


###compare against other functions, statistically validate your model

mselect(Mod1, list(LL.3(), LL.3u(),W1.3(),W2.3(),LL2.3(), LL2.3u()), icfct=AIC)


 ###describes different base responses
ED(Mod1, c(50), interval="delta")

# Pairwise t-test of ratios between treatments
EDcomp(Mod1, c(50,50))
```

Figure S3: Full script for model selection of species-specific lethal temperature curves. Identification of the most parsimonious model used mselect within DRC to select the model with the lowest AIC and highest Log- likelihood, limited to a set of three parameter non-linear functions, model selection in Table S2.

Table S2: Full model description following model selection in DRC for each species. Model selection used AIC and Log- likelihood to identify the most parsimonious model for each species. Final model selection for each species is bolded. Model selection was either a three parameter Log-logistic model (LL.3, LL2.3, LL.3u or LL2.3u) or a three parameter Weibull model (W1.3 or W2.3)

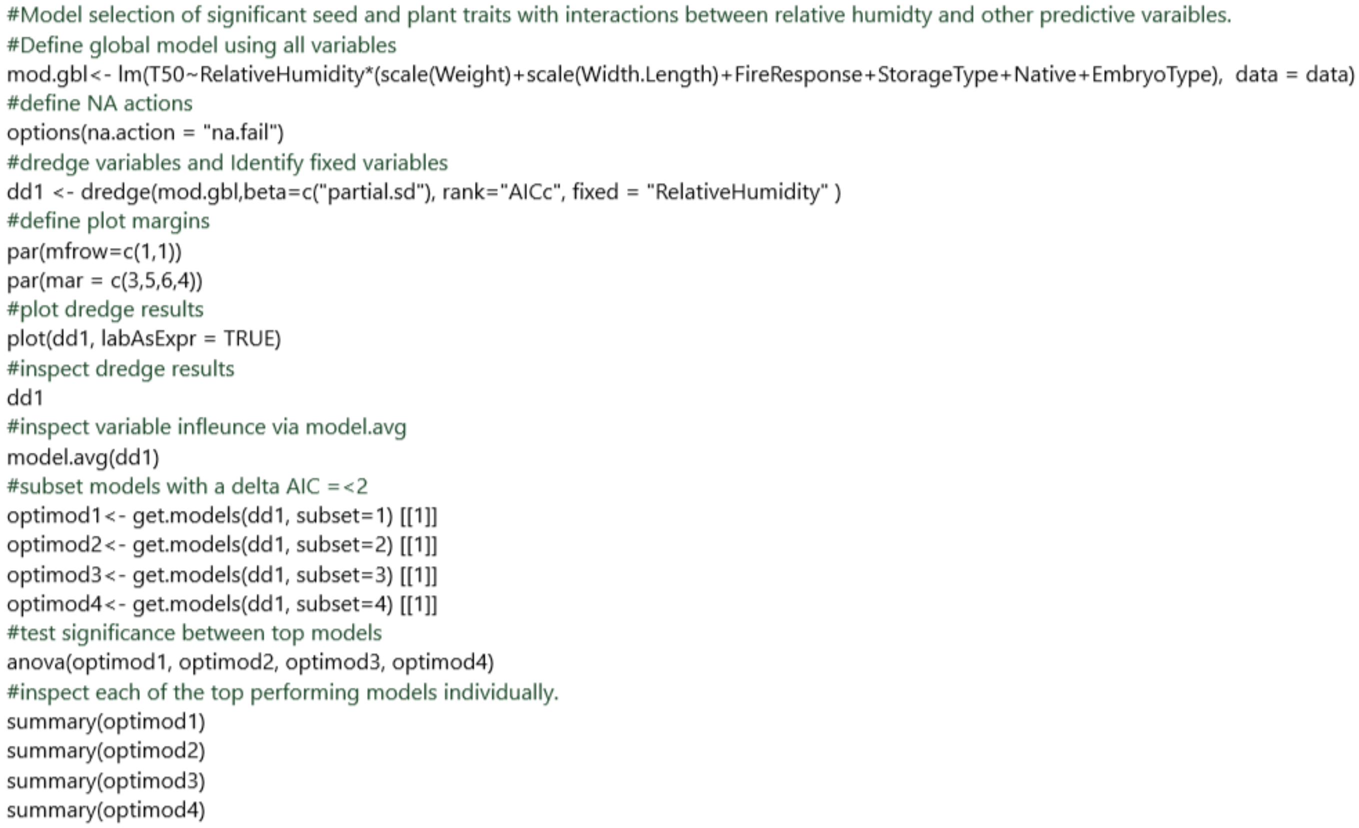
Species	Model	LogLik	AIC	Lack of fit	Res var
Asparagus asparagoides	**LL.3**	**120.496**	**-220.99**	**5.07^{-17}**	**0.00904**
	LL2.3	120.4722	-220.944	4.98^{-17}	0.009044
	W1.3	119.1128	-218.226	1.95^{-17}	0.009245
	W2.3	106.1178	-192.236	2.25^{-21}	0.0114
	LL.3u	85.66898	-151.338	1.05^{-27}	0.015855
	LL2.3u	83.31912	-146.638	1.94^{-28}	0.016467
Anigozanthos manglesii	**LL.3**	**203.94**	**-387.88**	**1.42^{-05}**	**0.00146**
	LL2.3	203.8799	-387.76	1.37^{-05}	0.001464
	W2.3	203.2006	-386.401	9.19^{-06}	0.001483
	W1.3	202.9436	-385.887	7.89^{-06}	0.00149

	LL.3u	187.9875	-355.975	6.56^{-10}	0.001965
	LL2.3u	187.6879	-355.376	5.39^{-10}	0.001976
Allocasuarina fraseriana	**W1.3**	**122.541**	**-225.08**	**1.92^{-02}**	**0.00647**
	LL.3	119.0072	-218.014	3.58^{-03}	0.006912
	LL2.3	118.945	-217.89	3.47^{-03}	0.00692
	W2.3	118.5846	-217.169	2.89^{-03}	0.006967
	LL.3u	58.89721	-97.7944	4.69^{-20}	0.021261
	LL2.3u	57.11076	-94.2215	1.32^{-20}	0.021983
Austrostipa compressa	**W1.3**	**131.195**	**-242.39**	**7.06^{-01}**	**0.00514**
	LL.3	130.6211	-241.242	6.39^{-01}	0.005199
	LL2.3	130.4311	-240.862	6.17^{-01}	0.005218
	W2.3	128.891	-237.782	4.40^{-01}	0.005375
	LL2.3u	43.7805	-67.561	2.67^{-24}	0.027618
	LL.3u	42.00937	-64.0187	7.40^{-25}	0.028575
Austrostipa elegantissima	**W1.3**	**118.308**	**-216.62**	**1.17^{-17}**	**0.00826**
	LL.3	113.0943	-206.189	3.05^{-19}	0.009032
	LL2.3	113.0181	-206.036	2.89^{-19}	0.009044
	W2.3	110.9295	-201.859	6.64^{-20}	0.009375
	LL.3u	83.05375	-146.108	1.42^{-28}	0.01516
	LL2.3u	80.70786	-141.416	2.58^{-29}	0.015786
Acacia pulchella	**W1.3**	**147.39**	**-268.78**	**1.46^{-06}**	**0.0069**
	LL.3	145.4188	-264.838	4.53^{-07}	0.007113
	LL2.3	142.2597	-258.519	6.70^{-08}	0.007461
	LL.3u	131.2259	-236.452	6.32^{-11}	0.008819
	LL2.3u	129.3322	-232.664	1.84^{-11}	0.009076
	W2.3	48.45016	-70.9003	3.40^{-36}	0.03091
Acacia saligna	**W2.3**	**107.55**	**-189.1**	**5.36^{-07}**	**0.01262**
	W1.3	105.8035	-185.607	1.88^{-07}	0.012963
	LL2.3	105.7824	-185.565	1.85^{-07}	0.012967
	LL.3	104.9739	-183.948	1.14^{-07}	0.013127

	LL.3u	56.68277	-87.3655	1.07^{-21}	0.027286
	LL2.3u	53.87022	-81.7404	1.48^{-22}	0.028473
Bossiaea eriocarpa	**W1.3**	**103.627**	**-181.25**	**1.16^{-06}**	**0.01157**
	LL.3	100.4372	-174.874	1.63^{-07}	0.012198
	LL.3	100.4372	-174.874	1.63^{-07}	0.012198
	LL2.3	100.4363	-174.873	1.63^{-07}	0.012198
	W2.3	98.94143	-171.883	6.42^{-08}	0.012506
	LL.3u	64.84779	-103.696	7.45^{-18}	0.022075
	LL2.3u	58.04629	-90.0926	6.22^{-20}	0.024725
Banksia prionotes	**LL.3**	**108.468**	**-196.94**	**1.32^{-03}**	**0.00674**
	LL2.3	108.459	-196.918	1.32^{-03}	0.006745
	W2.3	106.9965	-193.993	5.88^{-04}	0.006953
	W1.3	106.2201	-192.44	3.80^{-04}	0.007067
	LL.3u	102.0338	-184.068	3.30^{-05}	0.007711
	LL2.3u	98.48198	-176.964	3.79^{-06}	0.008303
Banksia sessilis	**W1.3**	**86.9976**	**-154**	**1.82^{-01}**	**0.00979**
	LL.3	86.91021	-153.82	1.76^{-01}	0.009811
	LL2.3	86.89939	-153.799	1.76^{-01}	0.009813
	W2.3	86.84806	-153.696	1.72^{-01}	0.009824
	LL2.3u	36.22876	-52.4575	4.05^{-15}	0.029525
	LL.3u	34.00765	-48.0153	8.40^{-16}	0.030986
Conostylis candicans	**W2.3**	**58.7628**	**-97.526**	**8.32^{-01}**	**0.01518**
	LL.3	57.56395	-95.1279	6.67^{-01}	0.015645
	LL2.3	57.2362	-94.4724	6.18^{-01}	0.015773
	W1.3	55.86018	-91.7204	4.23^{-01}	0.016325
	LL.3u	24.11405	-28.2281	3.86^{-09}	0.036103
	LL2.3u	20.87958	-21.7592	4.16^{-10}	0.039144
Ehrharta calycina	**W1.3**	**151.096**	**-282.19**	**2.02^{-02}**	**0.00594**
	LL.3	145.2351	-270.47	1.40^{-03}	0.006511
	LL2.3	145.2351	-270.47	1.40^{-03}	0.006511

	W2.3	140.9651	-261.93	1.59^{-04}	0.00696
	LL2.3u	121.608	-223.216	1.91^{-09}	0.009418
	LL.3u	120.0558	-220.112	7.17^{-10}	0.00965
Eucalyptus gomphocephala	**W2.3**	**137.754**	**-255.51**	$\mathbf{2.24^{-02}}$	**0.00498**
	LL.3	132.631	-245.262	1.89^{-03}	0.005478
	LL2.3	132.4118	-244.824	1.69^{-03}	0.0055
	LL.3u	128.306	-236.612	1.86^{-04}	0.005935
	LL2.3u	126.1329	-232.266	5.47^{-05}	0.006179
	W1.3	123.8618	-227.724	1.47^{-05}	0.006444
Freesia alba	**LL.3**	**195.162**	**-370.32**	$\mathbf{1.66^{-06}}$	**0.00195**
	W2.3	195.1478	-370.296	1.64^{-06}	0.001952
	W1.3	195.0756	-370.151	1.57^{-06}	0.001955
	LL2.3	193.5298	-367.06	6.16^{-07}	0.002009
	LL2.3u	171.1993	-322.399	3.12^{-13}	0.002994
	LL.3u	171.1736	-322.347	3.06^{-13}	0.002995
Gladiolus caryophyllaceus	**W2.3**	**77.2399**	**-134.48**	$\mathbf{6.51^{-06}}$	**0.01528**
	LL.3	72.79967	-125.599	4.39^{-07}	0.016589
	LL2.3	72.79863	-125.597	4.39^{-07}	0.016589
	LL.3u	71.10342	-122.207	1.53^{-07}	0.017119
	W1.3	69.74201	-119.484	6.50^{-08}	0.017556
	LL2.3u	67.01224	-114.025	1.14^{-08}	0.018466
Haemodorum spicatum	**W2.3**	**181.949**	**-343.9**	$\mathbf{3.73^{-04}}$	**0.0022**
	LL.3	176.939	-333.878	2.20^{-05}	0.002411
	LL.3u	175.1468	-330.294	7.67^{-06}	0.002493
	LL2.3	174.9085	-329.817	6.66^{-06}	0.002504
	LL2.3u	171.2689	-322.538	7.36^{-07}	0.002678
	W1.3	167.7159	-315.432	8.06^{-08}	0.002861
Kennedia prostrata	**W1.3**	**122.262**	**-218.52**	$\mathbf{2.38^{-08}}$	**0.01064**
	LL.3	118.581	-211.162	2.42^{-09}	0.011228
	LL2.3	90.22592	-154.452	1.74^{-17}	0.017037

	W2.3	89.05575	-152.112	7.76^{-18}	0.017333
	LL.3u	68.47551	-110.951	4.34^{-24}	0.023459
	LL2.3u	63.3971	-100.794	1.18^{-25}	0.025278
Melaleuca systena	**W1.3**	**55.3343**	**-90.669**	**1.83^{-02}**	**0.02292**
	LL2.3	54.24474	-88.4895	1.11^{-02}	0.023391
	LL.3	54.2223	-88.4446	1.10^{-02}	0.023401
	W2.3	53.63727	-87.2745	8.30^{-03}	0.023656
	LL.3u	18.47676	-16.9535	4.76^{-12}	0.045364
	LL2.3u	17.36148	-14.723	2.24^{-12}	0.046311



```
#Model selection of significant seed and plant traits with interactions between relative humidty and other predictive varaibles.
#Define global model using all variables
mod.gbl<- lm(T50~RelativeHumidity*(scale(Weight)+scale(Width.Length)+FireResponse+StorageType+Native+EmbryoType),  data = data)
#define NA actions
options(na.action = "na.fail")
#dredge variables and Identify fixed variables
dd1 <- dredge(mod.gbl,beta=c("partial.sd"), rank="AICc", fixed = "RelativeHumidity" )
#define plot margins
par(mfrow=c(1,1))
par(mar = c(3,5,6,4))
#plot dredge results
plot(dd1, labAsExpr = TRUE)
#inspect dredge results
dd1
#inspect variable infleunce via model.avg
model.avg(dd1)
#subset models with a delta AIC =<2
optimod1<- get.models(dd1, subset=1) [[1]]
optimod2<- get.models(dd1, subset=2) [[1]]
optimod3<- get.models(dd1, subset=3) [[1]]
optimod4<- get.models(dd1, subset=4) [[1]]
#test significance between top models
anova(optimod1, optimod2, optimod3, optimod4)
#inspect each of the top performing models individually.
summary(optimod1)
summary(optimod2)
summary(optimod3)
summary(optimod4)
```

Figure S4: Full code for model selection of most parsimonious model for correlations between T_{50} seed trait correlates. Inclusions of relative humidity and other predictive variables is included and relative humidity is fixed within all subsets of this generalized linear model. Generated using and MuMIn package (Burnham and Anderson 2003) respectively, all within R statistical package.

*Table S3: Model set of top ranked models following AIC ranking, top four models are presented with cut off =< 2.0Δ AIC. Final model selection focused on comparing individual AIC values, Δ AIC and weight. Estimate and Standard errors included, * represent significance level*

	Dependent variable: T^{50}			
	(Mod1)	(Mod2)	(Mod3)	Mod4
Intercept	101.79*** (6.77)	111.24*** (4.45)	101.43*** (6.74)	105.47***(7.75)
Embryo Type: Foliate	34.09*** (6.61)	25.12*** (4.51)	33.29*** (6.62)	33.44***(6.45)
Embryo Type: Linear	33.94*** (8.67)	21.11*** (5.17)	36.26*** (8.86)	32.99***(8.47)
Embryo Type: Miniature	25.95*** (8.48)	13.72** (5.31)	28.24*** (8.67)	26.76**(8.26)
Native: Non-Native	-23.21*** (5.25)	-25.80*** (5.17)	-23.35*** (5.23)	-21.69***(5.49)
scale(Weight)			2.10 (1.77)	
scale(Width/Length)	-5.07 (2.79)		-6.16** (2.92)	-5.21(2.69)
Storage Type: Soil				-5.05(5.75)
Relative Humidity: 50%	-9.47*** (3.47)	-9.47** (3.55)	-9.47*** (3.46)	-5.45(6.36)
Relative Humidity: 95%	-34.35*** (3.47)	-34.35*** (3.55)	-34.35*** (3.46)	-43.35***(6.36)
Relative Humidity: Dormant	-0.83 (5.97)	-1.06 (6.11)	-1.51 (5.98)	1.18(6.19)
Storage Type-Soil: Relative Humidity 50 %				-5.57(7.49)
Storage Type-Soil: Relative Humidity 95 %				12.46(7.49)
Storage Type-Soil: Relative Humidity Dormant				NA(NA)
Observations	58	58	58	58
R^{2}	0.832	0.820	0.836	0.852
Adjusted R^{2}	0.804	0.795	0.806	0.817
Residual Std. Error	10.425 (df = 49)	10.663 (df = 50)	10.382 (df = 48)	10.06(df = 46)
F Statistic	30.26(df =8; 49)	32.60(df =7; 50)	27.28(df =9; 48)	24.24(df= 11;46)
Log likelihood	-213.370	-215.270	-212.533	-209.477
AIC	451.4	452.3	452.8	453.2
Δ AIC	0.00	0.87	1.38	1.81
Weight	0.136	0.088	0.068	0.055

* <0.05, **<0.01, ***<0.005

Chapter 3 was first published in February 2018 in the International Journal of Wildland Fire as a research note and is presented in the same format as it appears in the journal.

Chapter 3: A method for extensive spatiotemporal assessment of soil temperatures during an experimental fire using distributed temperature sensing in optical fibre.

Abstract

The use of distributed temperature sensing (DTS) for ecological applications has increased rapidly in the last 6 years. Here we demonstrate the first use of DTS to measure soil temperatures during a fuel reduction burn – in an urban grassy Tuart-Banksia woodland remnant near Perth, Western Australia. Optical fibre with an acrylate material coating (diameter 242 µm), but no other jacketing or cabling, was buried in the soil at depths between 0 and 5 centimetres. Measurements were recorded over 316 m of optical fibre using a DTS measurement unit, providing data over a 5.5 hour period at 20 second intervals; resulting in 1,243 temporal measurements at 60 cm spatial resolution. Soil temperatures were calibrated to an error of ±6.8% at 250°C. Methods for installation, calibration and data visualization are presented. Issues associated with assessment of DTS in a fire ecology context are discussed.

Introduction

Soil heating due to fire has long been a research focus in ecology; including seed ecology (Roche et al. 1998, Luna et al. 2007, Paula and Pausas 2008), to forestry (Bradstock and Auld 1995, Burrows 1999), to soil chemistry (DeBano et al. 1998, Neary et al. 1999). In seed science and fire management, particular interest lies in understanding how fires induce seed dormancy loss or seed death in soil seed banks. (Tarrega et al. 1992, Ferrandis et al. 1999, Hanley and Lamont 2000, Williams et al. 2005, Auld and Denham 2006, Luna et al. 2007, Zuloaga-Aguilar et al. 2010). Historically, three approaches have been used to understand fire thermal characteristics in soil: thermocouples, pyrometers and calorimeters. Of these, only thermocouples provide data on temporal variation in temperature. Thermocouples are the most widely used technology, but also the most expensive. Installation of thermocouples is labour intensive as probes, cables and loggers require specific protective equipment to persist through fire (Burrows 1999). Most notably, thermocouples, pyrometers and calorimeters all measure temperature at a single point, but, as fire behaviour and soil attributes are heterogeneous at a range of scales, multiple point measurements are required to properly assess soil temperatures in fire. Making the leap from single point measurements to spatially continuous measurements will allow for finer scale mapping of fire characteristics and soil heating.

Distributed temperature sensing (DTS) has been used since the 1970's for continuous measurement of temperatures in diverse environments for fire incidence monitoring, hydrology, oceanography and mining (Selker et al. 2006). The key advantage of DTS over other approaches is that temperature can be measured continuously at sub-meter resolution over potentially thousands of metres of fibre, and at time and temperature resolution comparable to thermocouples.

The DTS system we use for this study utilizes the temperature-dependence of Raman scattering in the glass matrix of the fibre to measure the temperature of the glass fibre-core along the length of the fibre (Ukil et al. 2012), without the need to attach any temperature transducers to the fibre. Scattered light contains three spectral components, the Rayleigh component which has the same wavelength as the laser source, the Stokes component, which has higher wavelength than the source laser in which photons are generated, and the anti-Stokes component with a lower wavelength than the source laser, the intensity of the anti-Stokes component is temperature dependant, while the Stoke component is not. Calculating the ratio between these two components, local temperature measurements can be obtained (Dakin et al. 1985).

Location of the temperature measurement is determined by sorting the returning Raman signals into time-delay bins and computing distance along the fibre using the known speed of light in the fibre, thereby allowing examination of temperature from small sections along the continuous length of optical fibre (Hausner et al. 2011, Mandal et al. 2015). The size of these sections, limited by the resolution of the time-delay bins, is the limiting factor for spatial resolution.

Here we present the first use of DTS in optical fibres to measure soil temperatures during a prescribed burn. We use an uncoated, off-the-shelf cable, which has minimal thermal lag as cladding mass is avoided by using bare fibre. This cable solution is cheaper, and allows flexible deployment with a small spool and fibre size. We demonstrate applicability for understanding of fire and soil heating, and to ecological processes including soil hydrophobicity, seed dormancy, and post-fire regeneration from seed. We identify technical and practical challenges associated with this type of DTS installation, and possible solutions or areas where future research is required.

Methods

Study site

This study was carried out in conjunction with a fuel reduction burn of a 6 hectare area in a remnant urban woodland (Bold Park) on the Swan Coastal Plain of Western Australia (31° 57' 23.4108'S', 115° 46' 4.5048" E). The site comprises grey calcareous sandy soils predominately derived from young Spearwood and Quindalup dune systems (Barrett 2005). Vegetation is characterized as Banksia-Eucalypt woodland, with a high proportion of exotic species (predominately the perennial grass *Ehrharta calycina*).

The fire was implemented on 24 April 2016, as a low-moderate intensity burn, ignited at approximately 1314 hours Australian Western Standard Time (AWST). Pre burn available ground fuels comprising mixed Banksia–Eucalypt woodland fine and woody fuels and *Ehrharta calycina* grass, were estimated to be 10.3 Mg/ ha^{-1}, the burn treated an estimated 90% of the burn area leaving approximately 1-2 Mg/ ha^{-1} of ground fuels post fire. Estimated rate of spread of the fire during the experiment was between 0.48 and 1.08 km/hr.

Optic fibre installation and distributed temperature sensing technology.

We used non-cabled LaserWave *FLEX* Fibre (OFS, Norcross, Georgia, USA) with 50 µm graded index glass core, 125 µm glass cladding, and acrylate coating to 242 µm diameter. The fibre was installed 6 days prior to the burn. Litter was removed from the soil surface and the fibre was buried manually using a hand trowel at approximately 1 cm depth for a total length of 525 m. Heavy rain occurred immediately after installation with some resulting soil movement. The fibre was rechecked for burial along its length and litter cover returned to mimic pre-disturbance conditions. To increase redundancy (in the event of fibre breakages), the fibre was installed in a loop (loop diameter approximately 10m), with both forward and reverse directions of the fibre installed in parallel, 5 cm apart, to minimise disturbance of the site. Before the burn, the fibre sustained six breaks along the forward length of fibre (the last at 209 m), but no breaks occurred in the reverse direction. Although fibre breaks can be identified and repaired on site, we report on one channel, and results presented are those from the 316 m unbroken section.

Each end of the fibre loop was aligned to a single channel in a portable generator powered Silixa XT DTS (Silixa Ltd, Elstree, Hertfordshire, UK.) via an E2000 connector (Silixa Ltd, Elstree, Hertfordshire, UK). A water bath at ambient temperature served as a reference for calibration. A coil of approximately 20 m of cable was placed in the water bath, the temperature of which was logged simultaneously with the fibre temperature by platinum-resistance thermistors (PT-100) (Silixa Ltd, Elstree, Hertfordshire, UK) temperature probes rated to

±0.1°C connected directly to the DTS system, the length of this reference section was significantly greater than the sampling interval which meets the criteria set out by Tyler et al. (2009). A sampling interval of 25.4 cm with an acquisition time of five seconds per channel was used; we used all four channels on the Silixa XT (Silixa Ltd, Elstree, Hertfordshire, UK). Each channel was measured every 20 seconds on rotation (5 seconds each channel), with 0 seconds idle between channels, while all four channels were used, results presented are from the one unbroken continuous fibre length.

To ensure the DTS system could accurately measure temperatures up to 300°C, a post- hoc calibration procedure was developed to identify the three parameters needed to calibrate temperatures using DTS (see supplementary materials S1, Fig S1 and Fig S2 and (Hausner et al. 2011) for fundamental understanding of DTS calibration). The post- hoc calibration procedure was performed using a laboratory oven (Contherm, Korokoro, Lower Hutt, New Zealand) and encompassed the upper limit of temperatures experienced during this fire, from 190°C to 300°C. This procedure confirmed that the DTS was capable of measuring accurately up to 300°C, with an estimated error of ± 6.8% at 250°C (see Supplementary material in formation Fig. S2). γ (K), temperature scaling factor is fixed during the manufacture of fixed in the DTS system to 481.5° K and this was confirmed during the oven test. Differential attenuation along the fibre ($\Delta\alpha$) was also confirmed in the laboratory; over 2000 m of fibre, the attenuation was measured to be 5.40 × 10□5 Db m–1. This was assumed to be similar to that of the field deployment, as attenuation over 316 m will not be significant. C was determined to be between 1.509 and 1.567 calculated dynamically using a 20-m reference section within a water bath on site, calculated for each measurement during the measurement period to account for local fluctuations in the measurement equipment (Hausner et al. 2011). This *post-hoc* lab based protocol meant that only one bath was required in the field, and was also necessary as calibration over the experimental temperature range (<300°C) was not feasible in the field.

Results

Pre-fire soil temperatures were relatively reasonably consistent across the length of the fibre, ranging between 25°C and 39°C. The mean pre-fire temperature across the full length of the fibre was 30°C, with a maximum of 39°C measured between 262 m and 263 m. Comparison of these temperatures with those recorded during the last measurement showed hotspots remaining after 5 h (fig. 1a).

Soil temperatures were monitored for 332 min, with the fire heating at least part of the cable for 250 min. The raw trace of the first measurement showed a consistent intensity, with no obvious step losses, indicating that the fibre was unbroken in the 316 m examined (fig. 1b). The raw trace for the last measurement showed no breaks or step losses, although hot spots remained (fig. 1c).

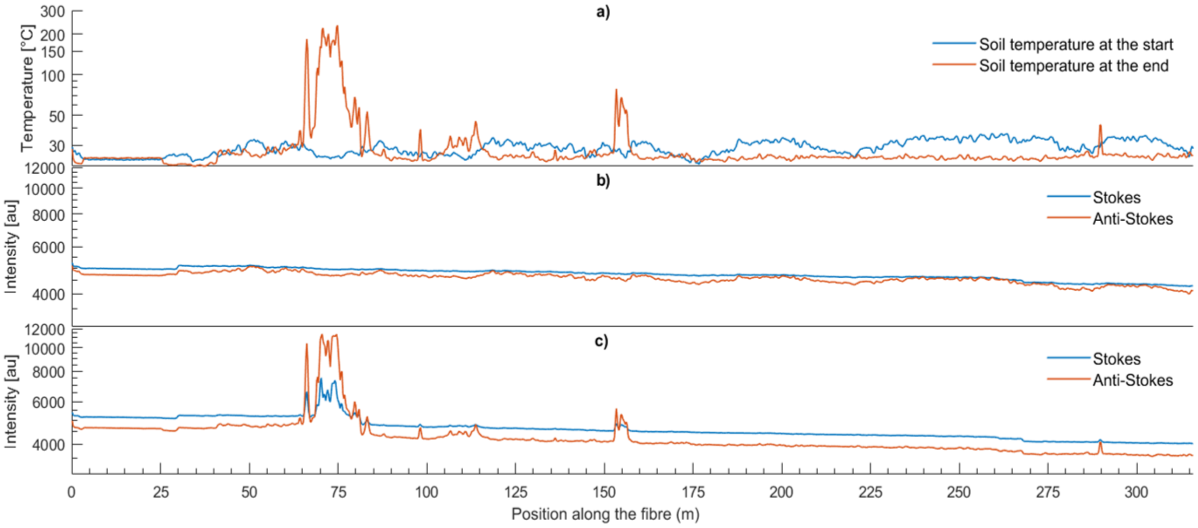


Figure 1: a) Soil Temperature (°C) measured along the 316m length of fibre at the start of the measurement period (1:15pm) and at the end of the measurement period. Log scale. b) Raw trace of stokes and antistokes at the start of the measurement period. Log scale.c) Raw trace of stokes and antistokes at the end of the measurement period.

Maximum soil temperature reached 283°C, at 82 m. A section approximately 20m in length experienced both high temperatures and prolonged heating - temperatures remained >250°C for 20 min in small areas of this section. By contrast, a 33m length of fibre, between 230 and 263 m, measured very limited soil heating during the fire (temperatures remained below 40^{o}C) (fig. 2b).

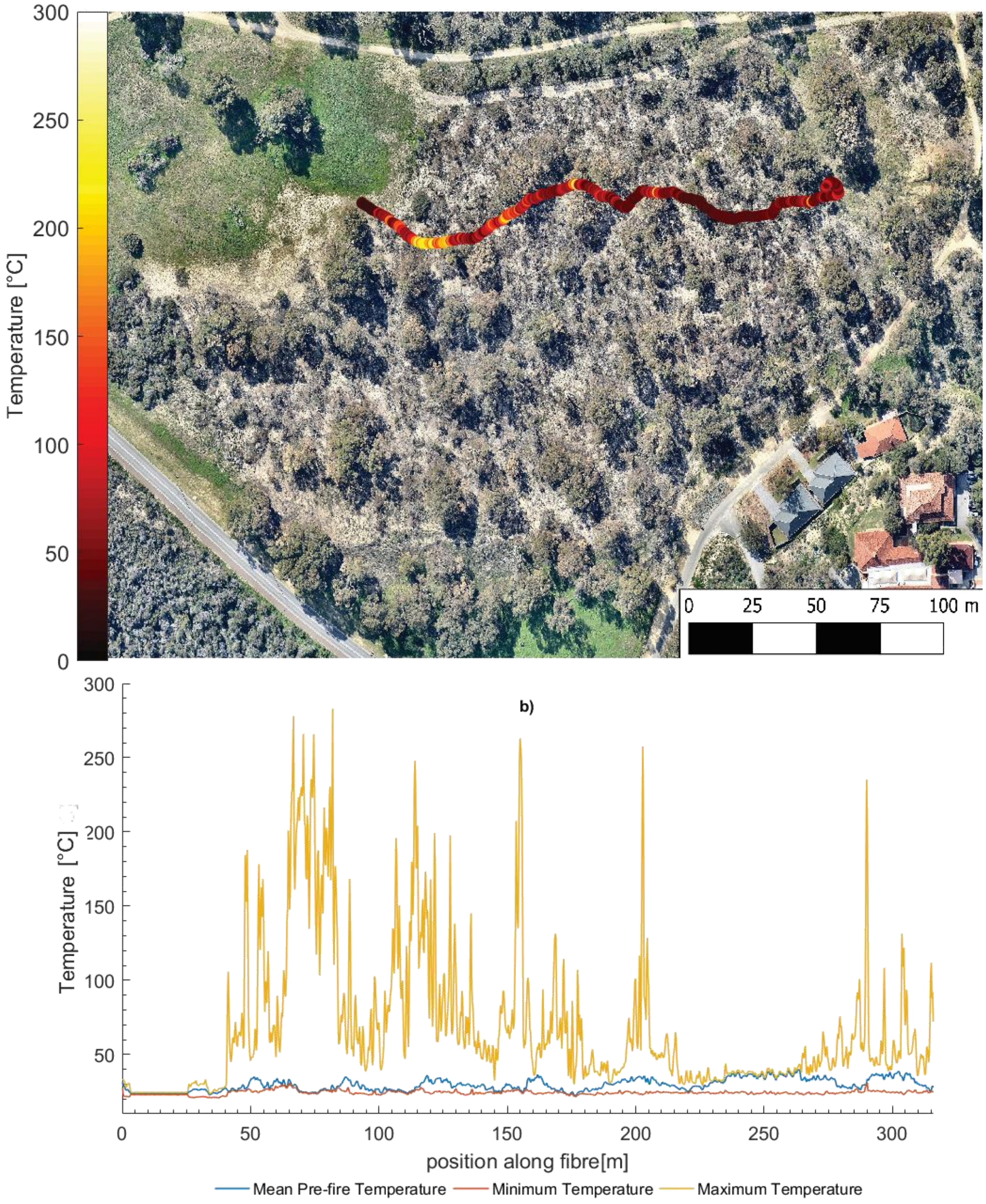


Figure 2: a) Heat map overlaying the fire site, showing the spatial distribution of maximum soil temperatures along the 316m length of fibre. The fire was lit on the right edge (eastern edge of the fire site) and propagated westward, encountering the fibre first at 316m along the fibre. B) Mean Pre-fire soil temperatures, minimum soil temperatures and maximum soil temperatures along the 316m length.

Measured temperatures also displayed temporal heterogeneity along the fibre (fig. 3), with different sections of the fibre heated for different periods. This temporal heterogeneity in soil heating can be examined in the context of biologically relevant temperatures, for example: 250°C (representing a temperature at which organic material will start to break down in soils (Certini 2005); 100°C (representing a temperature that is lethal to many seeds (Bell and Williams 1998), and; 60°C (a temperature that can alleviate physical dormancy in seeds (Ooi et al. 2014) (fig. 3b). The longest period that soil temperatures remained above 60°C was 172 min, measured at 153 m along the fibre. Notably, soil temperatures at that point didn't exceed 250°C, whereas immediately adjacent, at 155 m, soil temperatures remained over 250°C for approximately 8 min. The section of fibre that remained hottest for longest was at 66 m, where temperatures remained above 250 °C for 27 min. The section that recorded the highest temperature (283 °C at 82 m) only remained above 250°C for 1.6 min. Large parts of the fibre reached over 100°C, with temperatures at hotspots remaining over 100°C for up to 168 minutes (at 66m). Outside of the major hotspots, temperatures over 100°C were recorded for periods shorter than 50 minutes (Fig. 3b).

Spatial heterogeneity of temperature was further examined at four sections, selected to display different heating and cooling rates during the fire (fig. 3a). Rates of cooling were different between two hotspots, with the area around 66m taking a longer time to cool, compared to the area ~ 113 m (fig. 3a). Further, at 110m soil heating was low, in contrast to measurements taken 2m further along the fibre (at 113m) where temperatures rose to above 200°C. At 202m along the fibre, temperatures heated to approximately 124°C quickly, before cooling quickly, demonstrating comparatively different heating and cool pattern. At 235m no soil heating as a result of fire was observed and soil temperatures remained fairly consistent (fig. 3a).

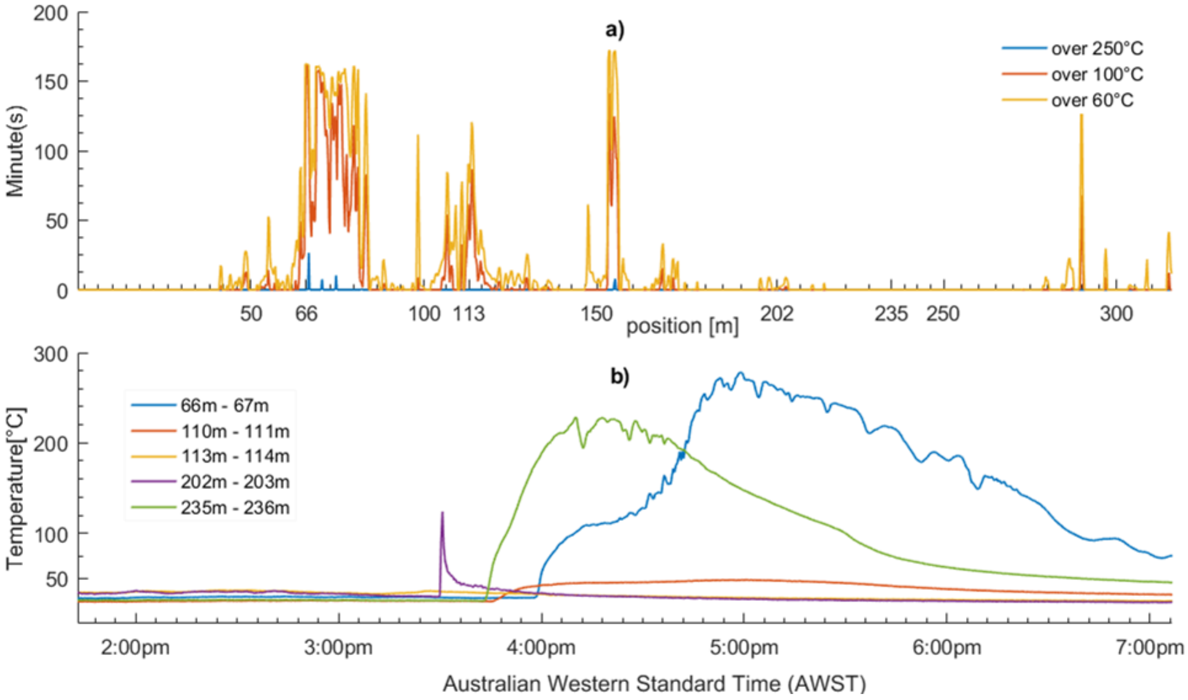


Figure 3: a) Duration of soil heating above biologically relevant temperatures measured along the fibre, presented as minutes above each threshold temperature of 250°C, 100°C and 60°C. b) Five sections of the fibre that display different maximum soil temperatures and different heating and cooling rates. .

Discussion

Here we have demonstrated the first use of distributed temperature sensing to measure soil temperatures during a prescribed fire. The data shows the potential of DTS as a research tool to understand the effects of fire at landscape-scales, particularly variation in soil temperature at both broad spatial and temporal scales. DTS can provide data applicable to a range of applications, including: modelling of the effect of fuel type and composition on soil heating, or the effects of fire on soil seedbanks. Such data can increase our knowledge of the effects of fire on soils during out of season burning; and how fires affect landscape scale changes to soil chemistry and soil structure. Our present understanding of these questions is largely limited by the number, measurement range and survival of conventional point temperature measurement probes that can be deployed, relative to the massive spatial and temporal heterogeneity of soil temperature during fire events that we demonstrate here with spatially-continuous measurements.

One of the most attractive aspects of DTS technology is the inexpensive cost of the fibre. We used an off-the-shelf fibre that reduced costs associated with customization, as well as lead time for production. This option allows for large-scale collection of data points for approximately the same cost as a single, high temperature rated thermocouple (excluding labour costs and DTS unit rental or purchase). The fibre used in this experiment was chosen for two reasons, 1) for the absence of an external sheath to allow for a more responsive measurement able to capture temperature fluctuations with minimal thermal lag, and; 2) its availability off-the-shelf at very low cost. The present installation using uncoated fibre was intended to be single-use because of anticipated breakages during the fire, or increased fragility from scorching. Nonetheless, the fibre was fully retrievable after the fire – a concern in this installation as we did not wish to leave fibre fragments in the soil.

No dramatic changes in Stokes or anti-Stokes amplitude are evident between the first and last trace (Fig. 1b and 1c). This demonstrates that fibre darkening mechanisms, such as hydrogen darkening, which results from hydrogen build-up within the silica lattice of the fibre, and which leads to reduced intensity of back scatted light, is not significant in these data (Bonnell et al. 2015). Therefore, specialized custom cables may be unnecessary in order to avoid such effects when used under similar temperatures and exposure periods. Further, the fibre used here had minimal thermal mass and minimal coatings, allowing the highest response to thermal fluctuations. We did not observe the potential problem highlighted by Cram et al. (2016), whereby their use of a fibre armour to heavily reinforce the cable may have led to thermal lag in measured temperatures.

We chose to calibrate temperatures post-hoc in controlled laboratory conditions for two reasons: (1) *ex-situ* calibration, allows for calibration beyond the range measured to fully encompass measured temperatures- ; and (2) greater control of calibration equipment; in this case an oven and a water bath – both were monitored using thermocouples rated to ±0.5°C, and together were able to be scaled to reach the range experienced during the fire. Using the equations from Hausner et al. (2011) and a three section calibration method, we successfully calibrated to ± 6.8% at 250°C which is the upper limit of the measurement capabilities of non-modified DTS equipment (Mandal et al. 2015). An on-site water bath at ambient temperature allowed us to use the first 20m of cable as a reference and to cross check the calibration with temperatures measured on the day. Using the techniques outlined here provides a simple, low risk method of calibrating fire temperatures without using point data gathered from single points along the fibre and exposing expensive equipment to fire conditions.

Future users of this technology need to be aware of potential short comings of this method. Firstly, fibre placement is awkward in relation to obstacles such as rocks, trees and logs. Secondly, precise placement of the fibre at a known depth is difficult. The 242µm diameter fibre used here is easily shifted by soil settling, rain and wind. Although it can withstand significant strain down the fibre, micro bends (with a bend radius smaller than 2mm) will cause the fibre to snap or induce attenuation, requiring a fusion splicer to fix the break. This can be difficult in remote installations and may result in a step-loss error that requires specific processing and in-situ calibration to correct (Hausner et al. 2011). During fibre installation the focus was to limit soil and litter disturbance. In this system, fuels are not heavily compacted and soils are predominately sandy and settle quickly, in this case we buried the fibre a week in advance of the fire to allow for soil settling and fuels were treated as carefully as possible to replicate original fuel structuring. Consideration as to whether this method is suitable should be taken on a case by case basis. Soil and litter structure should also be considered and taken in into account during installation of the fibre.

Future efforts should focus on establishing a procedure that allows for precise burial of the fibre at known depths, such as the use of a cable plough. Precise and accurate burial depth has also been recognised in other work as a major limitation (Steele-Dunne et al. 2010). The ability to control burial depth and the potential for multiple concurrent depths combined with the spatial and temporal coverage provided by DTS systems, will allow for greatly expanded measurement of fire conditions, beyond the point source data historically employed in this area of study. This DTS approach allows for new insight and to quantitatively revisit and revaluate known soil temperature thresholds for seed and fire ecology.

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Chapter 3: Supplementary material

Table S1: Weather conditions during the burn taken from Bureau of meteorology Swanbourne weather station, 500m directly west of the burn area

Date	Time AWST	Wind direction	Wind speed km/h	Temp °C	Dewpoint °C	Relative humidity %
24/04/2016	20:00	NW	11	21.1	20.8	99
24/04/2016	19:30	NW	11	21.2	20.9	98
24/04/2016	19:00	NNW	9	21.3	20.8	97
24/04/2016	18:30	NNW	9	21.7	20	90
24/04/2016	18:00	N	13	21.9	19.6	87
24/04/2016	17:30	N	11	21.9	19.8	88
24/04/2016	17:00	N	13	22.1	20.2	89
24/04/2016	16:30	NNW	15	22.3	20	87
24/04/2016	16:00	NNW	20	22.9	19.7	82
24/04/2016	15:30	NNW	17	22.9	19.3	80
24/04/2016	15:00	NW	17	23.9	20.6	82
24/04/2016	14:30	NW	17	23.6	19.9	80
24/04/2016	14:00	NW	15	24.2	20.1	78
24/04/2016	13:30	NNW	19	22.9	18.2	75
24/04/2016	13:00	NNW	19	23.5	17.9	71

24/04/2016	12:30	NNW	22	23.3	16.6	66
24/04/2016	12:00	NNW	20	24.3	15.5	58

Calibration equation

Temperature (T measured in Kelvin) at any particular position along the fibre (z) was estimated by using Equation 1 which is outlined in Hausner et al. (2011).

$$T(z) = \frac{\gamma}{\ln \frac{P_S(z)}{P_{aS}(z)} + C - \Delta\alpha z} \qquad \text{Equation 1}$$

Whereby: γ in °Kelvin represents a shift in energy between a photon at the wavelength of the incident laser and the scattered Raman photon; $\Delta\alpha(m^{-1})$ is the differential attenuation coefficient for the Raman Stokes and anti-Stokes wavelengths within the fibre; PS (z) and PaS (z) are the Stokes and anti-Stokes signals at position z along the fibre, respectively; and C is a dimensionless calibration parameter that accounts for the incident laser and the DTS instrument (Hausner et al. 2011). Temperature in Kelvin is converted to Celsius.

Post-hoc calibration results

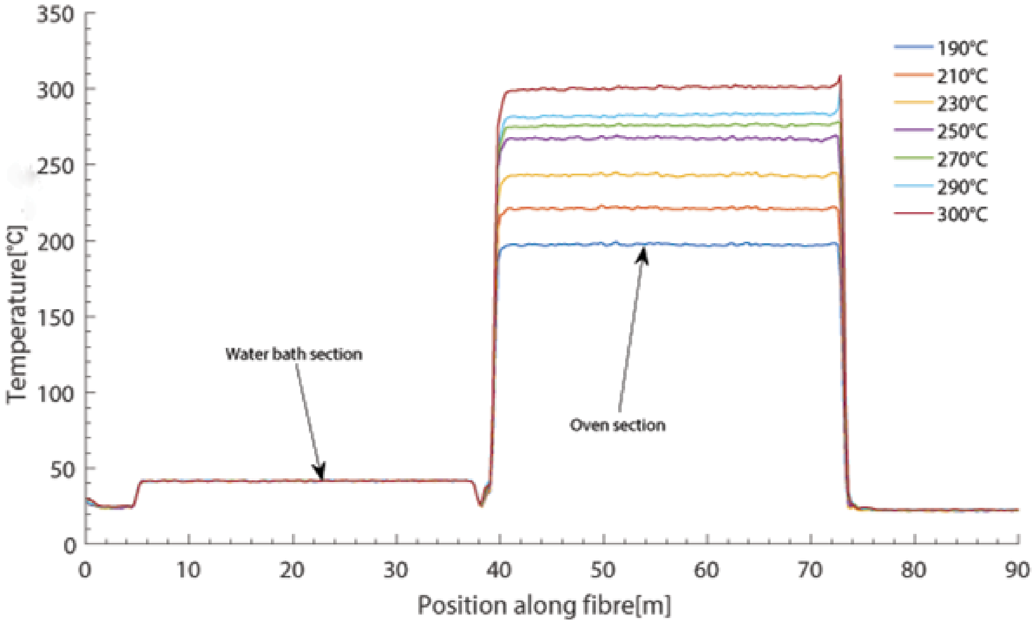


Figure S1: image of calibration procedure, using an oven and water bath and room temperature section. Water bath was set to 40°C while oven was set to a range of temperatures between 190°C and 300°C. The bath and Oven were measured independently using k-type thermocouples. The range of temps chosen were to examine how the DTS preformed at extremely high temperatures as well as in an attempt to test temperatures high than those generated in the fire. This setup also allowed us to post-hoc calibrate our field measurements by calculating the three independent calibration co-constants required as set up by (Hausner et al. 2011).

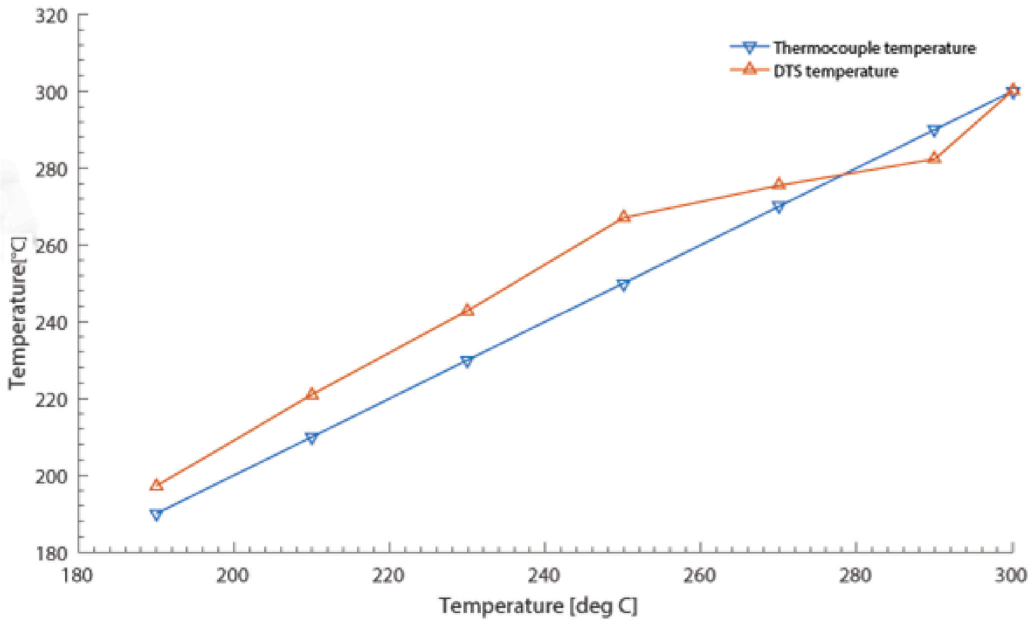


Figure S2: The results from the oven calibration show an error of 6.8% at 250°C. Taking the mean temperature of the oven section and comparing it to the independent thermocouple allows for an estimation of temperature deviation. This shows that the largest error in temperature estimates occurs at 250°C, where DTS temperatures over estimate temperature by 6.8%. This occurs as a function of the error in our C estimations, whereby changes of 0.1 in C greatly accuracy of temperature estimations at high temperatures.

Chapter 4 builds on the methods presented in chapter 3 by using DTS technology to measure soil temperatures during small experimental fires where the fuel loadings have been modified.

Chapter 4: Soil heating from the combustion of fine fuels has the potential to restrict seedling emergence.

Abstract

Seedling emergence following fire is vitally important for plant population persistence and expansion in fire-prone environments, providing an advantage to those seedlings emerging as competition for resources is lowest. However, seeds stored *in-situ* throughout fire events are most are risk to elevated temperatures that may lead to seed mortality. Seeds present in the soil seed bank can be insulated from extreme temperatures during fire; in this context, burial at depth is advantageous to seed survival. For seeds to emerge from soil seedbanks, energy reserves in the seed are harnessed within the seed are consumed to produce a hypocotyl/coleoptile, and if those stores are depleted before seeds are able to convert to autotrophic energy production, pre-emergent mortality will occur. To investigate the relationships between emergence behaviour, seed survival, and soil temperatures during fire, we set out to examine the emergence behaviour (e.g. mean emergence across a range of soil depths, maximum emergence depth and emergence rate) of 17 species, inclusive of fourteen native species and four invasive species at seven depths within soil, comprising seeds with a range of seed and plant traits. Further, we assessed soil temperatures from five experimental fires using exclusively fine fuels ranging in weight from 8 Mg/Ha^{-1} to 20 Mg/Ha^{-1}. Using distributed temperature sensing in optic fibre, measurements were recorded between 1 and 10 cm into the soil. Combining the measured soil temperatures of the experimental fires with emergence behaviour we were able to assess if seedling emergence would be impacted by soil temperatures generated by fine fuel combustion. Maximum emergence depth was strongly linked to seed mass: heavier seeds able to emerge from deeper within the soil profile, with some seeds able to emerge from up to 10 cm within the soil. Soil temperatures from the two of the five experimental fires exceeded the lethal thresholds of seeds from five species, under 15 Mg/Ha^{-1} and seven species under 20 Mg/Ha^{-1}. However, temperatures did not exceed the lethal thresholds of 12 species under any of the fire conditions tested. Seeds with lower lethal tolerances were more at risk to increasing soil temperatures than seeds with shallower maximum emergence. These results suggest that spatial arrangement of fuels, which drive soil heating, may be partially responsible for the spatial arrangement of seedling emergence following fire.

Introduction

In many fire-prone ecosystems around the globe, a pulse of seedling recruitment is common following fire (Auld and Denham 2006, Traba et al. 2007). Emergence of seedlings post-fire is advantageous to colonisation as there is reduced competition between individuals, increased nutrient availability, and lower herbivory pressures (Whelan 1995, Bell 1999). Seedlings that emerge at the first suitable opportunity following fire also have an increased likelihood of reaching maturity before the arrival of the next fire event, thus maximizing reproductive potential (Miller and Dixon 2014).

Seedlings that emerge following fire stem from two distinct sources of seeds: (1) those present in soil or canopy seed banks within the fire site that survive, and (2) those that arrive shortly following the fire via dispersal from surrounding sites or from post-fire flowering species. Seeds present in soil or canopy seed banks provide the greatest contribution to post-fire seedling recruitment, due their higher numbers as compared to newly dispersed seeds (Enright and Lamont 1989b, Auld and Denham 2006, Bradbury et al. 2016). However, seeds present within the soil of the fire site are most at risk to the elevated temperatures experienced during fire events. To capitalize on the passing of fire and successfully regenerate, seeds must possess traits that confer survival of high temperatures and that ensure germination occurs during periods that are most suitable for successful germination, emergence, and establishment. An example of seed traits associated with survival to high temperature exposure are certain seed embryo types (e.g. seeds with embryos in the foliate subdivision of the axile division - as defined in Martin (1946))(Tangney et al. 2018b). Nevertheless, seed survival through fire does not necessarily mean seeds can emerge following fire, as seedling emergence is also dependent upon burial depth.

Seeds present in the soil seed bank can be insulated from extreme temperatures during fire; in this context burial at depth is advantageous to seed survival (Auld and Denham 2006), as soil temperatures decrease rapidly as soil depth increases, rarely exceeding 150 °C at a depth of 5 cm, even under the most intense fire conditions (DeBano 2000). However, a trade off exists as seedling emergence from soil requires harnessing of energy reserves within the seed, and if those stores are depleted before seeds reach the surface and are able to convert to autotrophic energy production, pre-emergent mortality will occur (Forcella et al. 2000). It follows that seeds that have a large reserve mass have an increased ability to emerge from deeper in the soil profile (Kitajima and Fenner 2000), which may prove advantageous for recruitment post-fire, as they can emerge from deeper in the soil, where temperatures are lower (Riba et al. 2002).

Seeds can also be insulated from fire via storage in protective woody cones and capsules within the canopy of mature adults (Lamont et al. 1991). This plant trait (serotiny) is common within fire-prone ecosystems, particularly those with strong seasonal fire weather (e.g. Mediterranean climates (Enright et al. 1998, Causley et al. 2016)). The release of seeds from within cones and capsules is commonly triggered by the high temperatures associated with fire (Lamont 1991) dispersing seeds into an environment favourable for recruitment. However, seeds can also be released from cones between fire events, resulting in seeds being deposited into the soil

(Cowling and Lamont 1985). Serotinous seeds that are released during the inter-fire period are resultantly exposed to the passage of fire.

Soil temperatures during fire are driven primarily by the combustion of surface fuels, with higher soil temperatures resulting from higher fuel consumption, or higher fire severity (Keeley 2009). The spatial arrangement of surface fuels contributes to spatially diverse temperatures within the soil (Santana et al. 2011) and a mosaic of temperatures can be present within a single fire site (Tangney et al. 2018a).

Variation in soil temperatures during fire is produced by the properties of surface fuels including weight, particle size, and species composition (Pausas and Moreira 2012). The accumulation of fuels following fire is unique in each ecosystem, influenced by the climate and vegetation of the region (Facelli and Pickett 1991). Generally, fuels accumulate faster than they decompose, particularly in dry environments where the breakdown of fuels is slow. However, productivity in arid areas is also low; as such, fuel production rates are also slow and fuels accumulate over long periods. Conversely, in hot and wet ecosystems, decomposition of forest litter fuels is faster than production (Witkamp and Van der Drift 1961). Surface fuels can be classified into functional groups based upon the rate of moisture loss of each fuel class (Fosberg and Deeming 1971). Derived from the direct measure of the diameter of the fuel component, fuels are sectioned into two broad groups: fine fuels (1 hour fuels), comprised of leaves, small sticks and bark (0-6 mm diameter), and; coarse fuels, comprised of larger sticks and logs. Coarse fuels are further separated into two functional groups: 10 hour (6-25 mm) fuels, and 100 hour (25-75 mm) fuels, these groupings taking into account the longer drying times of larger woody debris (Cohen and Deeming 1985). Spatially and temporally dynamic surface fuels vary in mass (Mg/Ha^{-1}), particle size, and density (Facelli and Pickett 1991). Depending on the system, landscape fuel loading can range from sparse, unconnected fuels of open grass lands of arid regions comprised mostly of fine fuels (Wright and Clarke 2008), to heavily laden systems with thick leaf and duff layers and large fallen trees synonymous with fire exclusion policy in some Californian forests (Keeley 2002). In some locations, temperatures will far exceed the lethal temperature thresholds of seeds (Tangney et al. 2018b) while in other locations temperatures will be cooler, and in fact suitable to alleviate seed dormancy (Tieu et al. 2001, Ooi et al. 2014).

Species-specific compositions of surface fuel can also influence soil temperatures (Pausas et al. 2017) and produce spatial diversity in soil temperature patterns (Ganteaume 2018), driven by the flammability of leaf and plant traits. The flammability of individual species, and the relative abundance of flammable traits have direct effects on the broader fire regime of the ecosystem (Pausas et al. 2017). Flammability consists of three major dimensions, heat release, ignitability and fire spread rate; together these dimensions define the flammability of a species (Pausas and Moreira 2012). Through the ensemble of flammable traits, different plant species influence fire behaviour and the resultant soil temperatures, creating a spatial mosaic of soil temperatures, which may influence patterns of seed survival and seed mortality within the soil.

Banksia woodlands are a biodiverse fire prone ecosystem situated in the south west of Western Australia (Stevens et al. 2016), within a dry summer, Mediterranean climate. Coinciding with a concentration of population and urban development, *Banksia* woodlands are situated predominately in and around high value infrastructure and population centres, making it top priority of land managers to reduce the impacts of fires. Recently described as a threatened ecology community (Department of the Environment 2018), *Banksia* woodlands are frequently highly disturbed systems with a high number of invasive species that modify species composition and fuel structure (Brown et al. 2016). The fuel dynamics of undisturbed *Banksia* woodlands are discontinuous, aerated, and highly variable throughout the landscape, with spatial variation in fuel accumulation within and among woodland communities (Burrows and McCaw 1990). However, fine fuels stabilise at 3 – 8 Mg/Ha^{-1}, six to seven years post-fire

(Burrows and McCaw 1990). Throughout *Banksia* woodlands, controlled use of fire is applied to reduce fuel loadings, with the aim of lowering the likelihood of intense wildfires (Agee and Skinner 2005). Termed hazard reduction burns (also called prescribed burns) there primary purpose is to reduce fire risk through the combustion of surface and near surface fuels, subsequently reducing total fuel availability throughout the landscape (Agee and Skinner 2005). Identifying how fuel loadings, particularly fine surface fuels can influence soil heating in Banksia woodlands, coupled with seedling emergence traits of Banksia woodland species, will provide insights into the relationship between fuel dynamics and post fire recruitment in Banksia woodlands.

If the variation in soil temperatures exhibited in *Banksia* woodland during fire (Tangney et al. 2018a) can be explained by localised fuel dynamics, then understanding the relationship between soil heating and emergence depth would provide evidence to suggest that post fire emergence patterns are driven in part by spatial fuel arrangement. Emergence behaviour, including maximum emergence depth and the speed of emergence are influenced by morphological and physiological seed traits that are unique to each species (Bond et al. 1999). Therefore, interactions between emergence patterns and other seed traits (e.g. those conferring survival of fire temperatures) may define species-specific strategies that seeds possess to survive through, and regenerate after fire. For example, seeds that are capable of emerging from deeper in the soil profile may have lower lethal temperature thresholds than seeds that can only emerge from shallow depths, as selection for higher temperature tolerance may act strongest on seeds that have limited emergence depths.

In this study, we set out to establish seedling emergence patterns, namely the rate and proportion of emergence, of 17 species from eight soil depths. Species were selected based on their seeds representing a range of traits, including mass, shape, resprouting ability, embryo type, seed storage syndrome, and lethal temperature. We subsequently compared emergence patterns to fire temperatures derived from experimental fires under modified fuel loadings to assess whether temperatures measured within soils were higher than what seeds can survive. This allowed us to explore the relationship between soil temperature, seed survival, and emergence depth and infer the capacity of different seeds with different seed traits to emerge under varying fuel loading regimes. This provided insight to the potential impacts of burning under a wide range of fuel regimes and the potential effect on post-fire species composition, including examining differences between native and invasive species strategies for persistence through fire.

Methods

Species selection and seed sourcing

Seeds of thirteen species native to *Banksia* woodlands and four non-native were sourced from wild plant populations, collected from within the Perth region (31.9505° S, 115.8605° E) between October 2015 and April 2016. All study species are common in *Banksia* woodlands and were selected to represent those that produce seeds with a range of physical and physiological traits, storage syndromes (i.e. soil vs. canopy stored seeds), and adult plant fire-responses (i.e. seeder vs resprouter). After collection, seeds were cleaned and stored in a controlled environment room at 15% RH and 15°C, until the use in June 2017.

Seedling emergence depth and emergence rate

Seeds from each of the 17 species were first x-rayed using a Faxitron Specimen Radiography System (MX-20 Cabinet X-ray Unit) (Faxitron, Wheeling, Illinois, USA), and only filled seeds (i.e. those with intact embryonic tissue) were selected for further treatment. Seeds were treated to release dormancy, using published methods (Table S1 in supplementary information) and subsequently separated into 4 replicates per treatment, and 25 seeds per replicate.

To determine the emergence behaviour of seeds from each species, seven centimetres of bleached white silica sand was first placed within each of 32 polystyrene boxes (60 cm wide x 40.5 cm long x 26 cm high). Bleached silica white sand was used in this experiment to replicate the texture and organic properties of *Banksia* woodlands soils. In each box, 25 seeds from each of the 17 species were then sown in separate rows on top of this sand layer. Once seeds had been sown, sand was added accordingly in order to achieve the required experimental depth (no sand added (surface: 0 cm), 1 cm, 2 cm, 3 cm, 4 cm, 5 cm, 7 cm and 10 cm) and softly tamped down to ensure depth within the box was equal. Each soil depth had four replicate boxes. The boxes were then placed in a glasshouse during the annual growing season (July – October 2017), with equal light distributed to each box, and watering was carried out to ensure sand was kept moist but not wet, with adequate drainage in each box. Seedling emergence was scored three times a week, starting from the day after sowing (day 1). A seedling was considered to have emerged when the cotyledon or coleoptile had emerged from the seed coat (in regards to surface sown seeds), or had emerged from within the sand (for seeds sown below the surface).

Three measures of emergence were calculated; maximum emergence depth, mean emergence at each depth (± standard error), and number of days required for 75% emergence (emergence rate, ± standard error). Maximum emergence depth was defined as the deepest depth at which at least one seedling was recorded. Mean emergence (± standard error) was calculated for each depth from the four replicates of 25 seeds, and one way –ANOVA were generated for each species (emergence ~ depth) were used to tested differences between depths tested using post-hoc pairwise Tukey HSD.

To calculate the emergence rate of seedlings from each depth, the number of seedlings were counted three times a week, proving a cumulative count of seedling emergence over time. Emergence rate was modelled using non-linear functions using the ‘drm’ function available within ‘DRC’ package (Ritz et al. 2016) within R statistical program (R Development Core Team 2017), fitting three-parameter non-linear functions as outlined in Ritz et al. (2016): (*d*) is the parameter for maximum emergence, (*b*) the *slope* of the curve $F(x)$ and (*e*) is the 75% point of the curve, in this case the number of days it took for 75% of the seeds to emerge from each depth. As this analysis requires a minimum of three seedlings per depth, any depth with three or less seedlings was excluded from the analysis. Each model fit was tested to establish best fit based upon log-likelihood estimations and Akaike Information Criterion index and the best fit was selected for each species (full model selection results and code presented in supplementary material Fig S1 and Table S2). Differences in rate of emergence between each of the depths were analysed with a pairwise t-test of ratios within DRC (Ritz et al. 2016).

Seed and plant traits

A range of seed and plant traits were measured to assess their relationship with maximum emergence depth. The lethal temperature (T_{50}) of seeds of each species was considered as an explanatory variable and defined as determined in Tangney et al. (2018b) as the temperature required to kill 50% of a seed batch after exposure for 3 minutes. The T_{50} for seeds pre-hydrated to 50% RH was chosen as it represents the most frequent hydration status of soil-stored seeds throughout the year (Turner et al. 2006).

Plant responses to fire was considered as two broad classes: species that are capable of resprouting in any manner were classified as resprouters, whereas species unable to resprout and that rely solely on recruitment from seed were classed as non-sprouters (Paula and Pausas 2008). Seed embryo classification was limited to upper level classification (to division and subdivision) as outlined in Martin (1946). In particular, embryos of seeds were classified into one of the following classes: embryos in foliate subdivision of the axile division (hereafter foliate); embryos in the linear subdivision of the axile division (hereafter linear); embryos in the basal division (hereafter basal); and embryos in the miniature subdivision of the axile division (hereafter miniature).. Seed mass was determined by weighing ten replicates of 100 seeds and presented as single seed mass (mg). Seed width to length ratio was measured for 100 seeds using a Epson flatbed scanner (Epson Australia, Sydney, NSW, Australia) and analysed using WinSEEDLE™ (Régent Instruments Inc., Québec, QC, Canada) by calculating using the ratio of straight width divided by straight length. Seed storage syndrome was classed as soil stored (i.e. species that release mature seeds directly into the soil seed bank) or canopy stored (i.e. species that hold mature seeds within cones or capsules). Species were classified as native or non-native to *Banksia* woodlands according to Barrett and Tay (2016), full list of seed and plant traits used are included in supplementary materials (Table S1).

To establish predictive influences of seed traits on maximum seedling emergence depth, multi-factorial generalized linear regression models with Gaussian link functions were used to test significant relationships (full code for model ranking provided in supplementary materials Fig. S2). Continuous variables were centred and scaled. All variables were included in the base model. Model optimisation was carried out using MuMin package (Burnham & Anderson 2003), which ranks subsets of generalised linear models, with each candidate model ranked by AICc, within R statistical program (R Development Core Team 2017) a limit of two predictor variables was implemented to avoid overfitting the data.

Individual correlations were also carried out to assess single variable effects. Categorical variables were assessed using one – way ANOVA and post-hoc Tukey HSD tests (R Development Core Team 2017); continuous variables were directly assessed via generalised linear models. Means, standard errors, test statistics, and p-value are reported where appropriate.

Measuring soil temperatures under fine fuel loadings.

To assess soil temperatures within the soil profile, five experimental fires were conducted under a range of fuel loadings and fuel compositions. The fires were conducted in May and June of 2017 and the effect of increasing fuel loading on soil temperatures was assessed under field conditions (Table 1). Soil temperature was measured using distributed temperature sensing (DTS). A 50m length of 250 µm diameter optical fibre was laid (non-cabled LaserWave FLEX Fibre (OFS, Norcross, Georgia, USA)) in a pit measuring 2m x 2m with a sloped floor from 0 cm to 10 cm deep (Fig. 1a). The pit was constructed in a highly degraded site adjacent to native *Banksia* woodlands within Kings Park (Fig. 1b). Soils within the pit were grey sandy soils with little to no organic material; similar properties to well drained, low nutrient soils typical of *Banksia* woodlands (Barrett and Tay 2016). The fibre was laid in a double ended configuration, with both ends connected to a Silixa XT DTS (Silixa Ltd, Elstree, Hertfordshire, UK) by an E2000 connector (Silixa Ltd, Elstree, Hertfordshire, UK). The fibre was laid in parallel lines, 10cm apart, across the slope of the pit (Fig. 1), and the pit was backfilled and smoothed, providing measurements ranging from 1 cm -10 cm below the surface, maximum depth was at 10cm. Surface (0cm) measurements were avoided to minimise potential damage to the fibre. Temperature measurements are generated each 25.4cm length along the fibre, providing the equivalent to 15 linearly adjacent measurement locations within each depth category (i.e. 1cm-1.9cm) and 150 measurement locations in total. Measurements were recorded every 5 seconds for a minimum of 100 minutes following ignition. Each direction of the fibre was treated as a single ended trace and temperature was calibrated following Tangney et al. (2018a).

Fuels were collected, sorted to exclude fuels > 6mm in diameter, dried for 48 hours at 105 °C in an oven (Contherm, Korokoro, New Zealand), and weighed to fit the desired amount of fuel for each experimental fire. Fuels ranged from 8 Mg/Ha^{-1} to 20 Mg/Ha^{-1} comprising exclusively of fine fuels (0-6mm diameter), with the composition of the fuels selected to represent fuel bed variation experienced in *Banksia* woodlands (Table 1). Fuels were arranged as evenly as possible over the pit a minimum of two hours before ignition. Fuels were extended beyond the edge of the measurement array by approximately 0.5 m to ensure equal fuel loading on the edges of the array. Fuel moisture measurements were not taken before ignition as fuels were removed from the oven 2 hours before ignition, and assumed dry. Ignition of each of the five experimental fires was initiated at approximately 11am on different days. Wind speed, relative humidity, and ambient temperatures (Table 1) were recorded each day by a department of agriculture (DAFWA) weather station situated 50 m away from the burn area. Soil moisture was determined by taking five soil samples from within the pit area 15 minutes before ignition, sampled using an 8 cm long by 2 cm diameter clear plastic cylinder. Each soil sample was capped and placed in an airtight bag before being taken directly for weighing. Soils were weighed in the cylinder and then oven dried at 103°C for a minimum of 17 hours (Contherm, Korokoro, New Zealand). Soil moisture was calculated as [(dry soil weight – wet soil weight)/ dry soil weight] x 100 with cylinder weight subtracted from both dry and wet soil weights (Table 1). Fuel consumption was estimated visually once all combustion had ceased, and recorded as percentage fuel remaining (Table 1).

Defining species-specific seedling emergence and mortality zones.

For each species, seedling emergence and mortality zones were compiled by combining the maximum emergence depth and the lethal temperature data. This allowed for a visualization of seedling emergence zones under the range of fuels tested: Zone one: too hot - If soil temperatures exceeded the T_{50} of seeds, then seeds were considered to have been killed as dead (i.e. soil temperature > species-specific T_{50}). Temperatures had to be greater than the T_{50} for a minimum of 3 minutes for the seeds to be classed as residing within this zone. Zone two - safe zone. This zone comprised soil temperatures lower than T_{50} and soil depths from which seeds were able to emerge (i.e. soil temperature < species-specific T_{50} and depth < maximum emergence). Zone three: too deep - This zone comprised soil depths that exceeded the maximum seedling emergence depth (i.e. depth > maximum emergence).

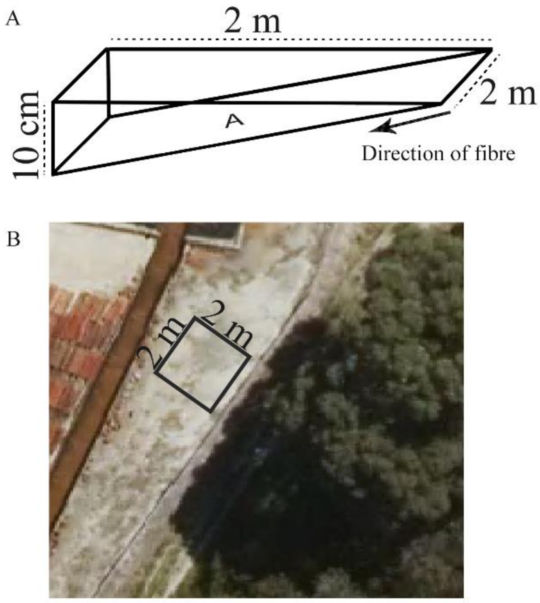


Figure 1: a) Experimental set up of the soil array to measure soil temperatures under fires using modified fuel loading. Fibre was laid along the bottom (labelled A) of the pit and soil backfilled and levelled off to be equal with surrounding area to create a profile from 1cm to 10cm below the fuel surface. Diagram is not to scale. b) Position pit in a heavily degraded site adjacent to Banksia woodlands within Kings Park, Perth WA. Diagram is not to scale.

Table 1: Weather and fuel conditions of the five experimental burns. All ignitions took place at 11am local time and fuels were dry at time of ignition. Soil moisture was unmodified from field conditions. Banksia woodland fuels comprised of a mix of sticks and leaves < 6mm in diameter from predominantly Banksia over story trees.

Date of burn	Mg/Ha^{-1}	Fuel components	Fuel consumption	Soil moisture (%DW)	Relative humidity 11am	Wind direction 11am	Wind speed (km/h) 11am	Temperature (°C) 11am
25/05/17	8	*Ehrharta calycina* grass (20%): *Banksia* woodland fuels (80%)	>95%	2.7	62.0	N	11.7	19.8
6/06/17	10	*Banksia* woodland fuels (100%)	>95%	2.2	30.1	NNE	20.9	20.5
8/06/17	12	*Banksia* woodland fuels (100%)	>95%	1.9	58.3	ESE	17.6	18.1
12/06/17	15	*Banksia* woodland fuels (66%): *Allocasuarina fraseriana* cladodes (33%)	>95%	1.2	27.2	N	13.1	19.4
15/06/17	20	*Allocasuarina fraseriana* cladodes (100%)	>95%	1.0	74.5	SE	2.6	17.1

Results

Seedling emergence depth and emergence rate

Seeds of all 17 species had highest emergence from the surface, or mean emergence from deeper depths was not significantly different from surface emergence (Table2).

Seeds of *C. candicans, A. compressa, A. elegantissima, E. gomphocephala,* and *M. systena* all had a maximum emergence depth of 2 cm. Seeds of *A. fraseriana* and *A. manglesii* emerged from up to 3 cm depth. Seeds of *E. calycina, B. prionotes,* and *H. spicatum* emerged from a maximum of 5 cm. *Bossiaea eriocarpa,* and *G. caryophyllaceus* have a maximum emergence of 7 cm. Seeds of the remaining species *A. pulchella, A. saligna, A. asparagoides, F. alba* × *leichtlinii* and *K. prostrata*, all emerged from 10 cm or deeper (Table 2).

*Table 2: Maximum emergence depth and mean emergence percentage (± SE) at each depth. Maximum emergence depth was defined as the depth from at which at least one seedling emerged. Cells with the same lettering are not significantly different from each other. Grey cells identify highest emergence depth. * denotes non-native species. Full tukey post-host analysis results are included in supplementary materials*

Species	Maximum emergence depth (cm)	Surface	1cm	2cm	3cm	4cm	5cm	7cm	10cm
Acacia pulchella	≥10	66 ± 1.2^{ab}	83 ± 3.6^{a}	88 ± 1.0^{a}	88 ± 1.4^{a}	91 ± 1.4^{a}	89 ± 0.7^{a}	85 ± 2.0^{a}	43 ± 1.9^{b}
Acacia saligna	≥10	64 ± 0.8^{a}	67 ± 1.9^{a}	82 ± 0.8^{a}	81 ± 2.2^{a}	51 ± 2.0^{a}	55 ± 1.3^{a}	57 ± 3.7^{a}	46 ± 4.6^{a}
Allocasuarina fraseriana	3	76 ± 1.4^{b}	21 ± 1.8^{a}	3 ± 0.75^{a}	1 ± 0.2^{a}	0	0	0	0
Anigozanthos manglesii	3	45 ± 4.2^{ab}	56 ± 3.8^{b}	6 ± 0.5^{a}	2 ± 0.2^{a}	0	0	0	0
*Asparagus asparagoides**	≥10	43 ± 2.0^{ab}	41 ± 1.4^{ab}	70 ± 1.8^{b}	51 ± 2.6^{b}	59 ± 1.9^{b}	59 ± 2.5^{b}	24 ± 1.6^{ab}	13 ± 1.6^{a}
Austrostipa compressa	2	25 ± 0.4^{a}	20 ± 1.8^{a}	4 ± 0.4^{a}	0	0	0	0	0
Austrostipa elegantissima	2	77 ± 0.8^{b}	65 ± 3.1^{b}	2 ± 0.2^{a}	0	0	0	0	0
Banksia prionotes	5	71 ± 1.1^{c}	49 ± 4.1^{bc}	31 ± 2.4^{ac}	23 ± 3.3^{ab}	30 ± 0.6^{ac}	21 ± 1.9^{a}	0	0
Bossiaea eriocarpa	7	61 ± 1.8^{b}	58 ± 2.7^{b}	56 ± 0.8^{b}	64 ± 0.3^{b}	61 ± 1.9^{b}	47 ± 1.8^{b}	1 ± 0.2^{a}	0
Conostylis candicans	2	36 ± 0.9^{b}	7 ± 0.2^{a}	2 ± 0.5^{a}	0	0	0	0	0
*Ehrharta calycina**	5	30 ± 0.6^{ab}	43 ± 1.4^{b}	38 ± 1.8^{ab}	32 ± 2.1^{ab}	25 ± 1.4^{ab}	12 ± 1.3^{a}	0	0
Eucalyptus gomphocephala	2	83 ± 0.8^{b}	57 ± 4.4^{ab}	27 ± 1.7^{a}	0	0	0	0	0
*Freesia alba × leichtlinii **	≥10	96 ± 0.7^{b}	88 ± 2.6^{b}	94 ± 0.9^{b}	86 ± 1.1^{b}	93 ± 1.7^{b}	89 ± 1.1^{b}	11 ± 0.2^{a}	2 ± 0.2^{a}
*Gladiolus caryophyllaceus**	7	83 ± 2.1^{b}	98 ± 0.5^{b}	89 ± 1.0^{b}	73 ± 3.9^{b}	90 ± 2.5^{b}	64 ± 2.1^{b}	3 ± 0.2^{a}	0

Haemodorum spicatum	5	89±1.4^{c}	75±2.9^{bc}	69±2.2^{bc}	20±2.3^{a}	38±3.5^{ab}	1±0.2^{a}	0	0
Kennedia prostrata	≥10	62±0.9^{a}	69±0.6^{a}	72±1.0^{a}	53±2.4^{a}	62±1.4^{a}	74±1.6^{a}	47±1.1^{a}	60±2.2^{a}
Melaleuca systena	2	87±1.3^{c}	42±2.5^{b}	1±0.2^{a}	0	0	0	0	0

Seeds of *E. calycina* had the quickest emergence rate of any species, requiring 14 days for 75% emergence on the soil surface (Table 3). *Austrostipa compressa* and *A. elegantissima* were also quick to emerge, requiring 16 days to emerge from surface sown seeds. The species that required the longest time to emerge on the surface was *A. pulchella*, requiring 41 days to reach 75% emergence (Table 3). For 12 out of 17 species, the quickest time to 75% emergence was recorded for those seeds sown on the soil surface. However, seeds from all members of the Fabaceae tested (*A. pulchella, A. saligna, B. eriocarpa and K. prostrata*) emerged more quickly when sown below the soil surface. Seeds of *A. pulchella* (c. 22 d)*, A. saligna* (18 days) and *K. prostrata* (20 days) emerged fastest at 1 cm, 1-2 cm, and 1-5 cm, respectively and seeds of *B. eriocarpa* emerged fastest at between 1 – 4cm (Table 3). The only other species that was quick to emerge from below the soil surface was *G. caryophyllaceus*, requiring 26 days to reach 75% emergence at 1cm.

*Table 3: Mean time (days) required for 75% emergence ± SE of seeds from each depth. Fastest average time to emergence is highlighted in dark grey cells; more than one grey cell indicates that emergence rates are not significantly different between depths. Cells with the same lettering are not significantly different from each other. Grey cells identify quickest emergence depth. Denotes species invasive to Banksia woodlands**

Species	0cm	1cm	2cm	3cm	4cm	5cm	7cm	10cm
Acacia pulchella	41.4±1.1^{a}	22.5±0.5^{b}	24.6±0.4^{c}	24.8±0.4^{c}	24.6±0.7^{c}	27.3±0.5^{c}	30.6±0.6^{c}	35.2±1.2^{a}
Acacia saligna	36.6±1.5^{a}	18.2±0.4^{b}	19.3±0.5^{b}	21.0±0.7^{c}	22.4±0.7^{c}	20.5±0.7^{b}	23.1±0.9^{c}	27.4±1.3^{c}
Allocasuarina fraseriana	38.6±1.0^{a}	52.5±15.3^{a}	0	0	0	0	0	0
Anigozanthos manglesii	30.2±1.6^{a}	34.4±1.5^{b}	0	0	0	0	0	0
*Asparagus asparagoides**	40.5±1.1^{a}	58.9±7.0^{b}	44.4±1.7^{bc}	49.7±1.9^{bc}	44.7±1.5^{bc}	49.6±1.6^{bc}	58.1±2.5^{b}	65.5±10.1^{b}
Austrostipa compressa	16.2±1.1^{a}	20.2±1.9^{b}	22.7±3.2^{b}	0	0^{c}	0	0	0
Austrostipa elegantissima	16.8±0.3^{a}	22.7±1.3^{b}	0	0	0	0	0	0
Banksia prionotes	38.6±1.5^{a}	45.6±4.3^{ab}	43.6±2.2^{b}	48.7±8.3^{b}	44.8±2.0^{b}	53.8±10.2^{b}	0	0
Bossiaea eriocarpa	34.0±1.8^{a}	31.1±2.3^{ab}	25.8±0.9^{b}	27.1±0.8^{b}	27.3±0.8^{b}	28.8±0.9^{b}	0	0
Conostylis candicans	27.3±0.4^{a}	36.3±4.5^{b}	33.7±9.2^{b}	0	0	0	0	0
*Ehrharta calycina**	14.7±1.2^{a}	16.3±1.2^{ab}	19.6±1.8^{b}	18.6±1.5^{b}	18.6±2.2^{b}	19.0±3.0^{b}	0	0
Eucalyptus gomphocephala	19.7±0.7^{a}	21.9±1.3^{ab}	26.8±3.0^{b}	0	0	0	0	0
Freesia alba × leichtlinii *	28.6±0.4^{a}	30.2±0.5^{b}	34.5±0.6^{c}	38.3±0.8^{d}	35.4±0.5^{c}	39.7±0.5^{d}	50.2±6.8^{e}	0
*Gladiolus caryophyllaceus**	29.4±0.8^{a}	26.5±0.5^{b}	33.4±0.9^{c}	36.7±1.0^{d}	32.5±0.6^{c}	39.4±1.4^{d}	0	0
Haemodorum spicatum	32.0±0.8^{a}	38.0±1.2^{b}	47.6±1.8^{c}	50.9±4.6^{c}	46.5±2.1^{c}	0	0	0
Kennedia prostrata	33.9±1.2^{a}	20.0±0.5^{b}	20.1±0.4^{b}	20.5±0.5^{b}	20.4±0.4^{b}	21.8±0.4^{b}	24.6±0.7^{c}	26.3±0.5^{c}
Melaleuca systena	19.0±0.4^{a}	27.4±1.9^{b}	0	0	0	0	0	0

Seed and plant traits

Using seed and plants traits, a list of working models was compiled using MuMin, inclusive of all combinations of seed and plant traits, to identify traits predictive of maximum emergence depth. Model optimisation and minimisation identified two models that provided high levels of model fit (Table 4). The model most suitable was model one, selected based upon AICc, F-statistic (Table 4), and this model explained approximately 65% of the variation (Multiple R-squared: 0.640, Adjusted R-squared: 0.0.590 (Table 5)). Model one included, log transformed seed mass and whether seeds were native or non-native. (Table 5).

Table 4: Comparison of the two most suitable models from the optimisation and minimization process. Estimated responses ± SE, Bolded variables represent significant <0.05. df = degrees of freedom.

Model comparison

	Maximum Emergence	
	(model one)	(model two)
(Intercept)	**- 4.742 ± 0.613**	2.783 ±1.615
Embryo Type: Foliate		**6.478 ±1.394**
Embryo Type: Linear		**5.696 ± 1.466**
Embryo Type: Miniature		3.931^{-16} ± 1.534
Log transformed seed mass	**2.430 ± 0.557**	**1.382** ± 0.419
Non-native	3.848 ± 0.127	
Storage Type: Soil		**5.783 ± 1.197**
Observations	17	17
AICc	83.21	84.16
Multiple R^2	0.640	0.777
Adjusted R^2	0.590	0.7026
Residual Standard Error	2.208 (df = 14)	1.879 (df = 12)
F-statistic	**12.48** (df = 2 on 14)	**10.45** (df = 4 on 12**)**

Intercept refers to canopy stored seed with basal embryo.

Seed mass was included in the optimised model (Table 5) and was the only variable that displayed significant correlation with maximum emergence depth, with heavier seeds able to emerge from a significantly greater depth than lighter seeds ($R^2 = 0.81$, p= <0.0001) (Fig. 2A). Fig. There were no significant differences in emergence depth between fire-responses, or native and non-native species, and there was no correlation between seed shape and maximum emergence depth, however, whether seeds were native or non-native was included as a categorical factor in the optimised model (Table 5).

Table 5: Analysis of variance table of the optimised multiple regression model for the effects of seed and plant traits on maximum emergence of seeds, constructed using MuMin. Variables that are significant (<0.05) are bolded.

Optimised model

	Maximum emergence			
Variable	Estimate	Std. Error	T value	p-value
(Intercept)	4.741	0.6138	7.725	**<0.0001**
Non-native seeds	3.848	1.275	3.019	**0.009**
Log transformed mass	2.430	0.557	4.360	**0.0006**
Residual standard error: 2.208 on 14 degrees of freedom				
Multiple R-squared: 0.640, Adjusted R-squared: 0.5862				
F-statistic: 12.48 on 2 and 14 Degrees of freedom **, p-value: 0.0007**				

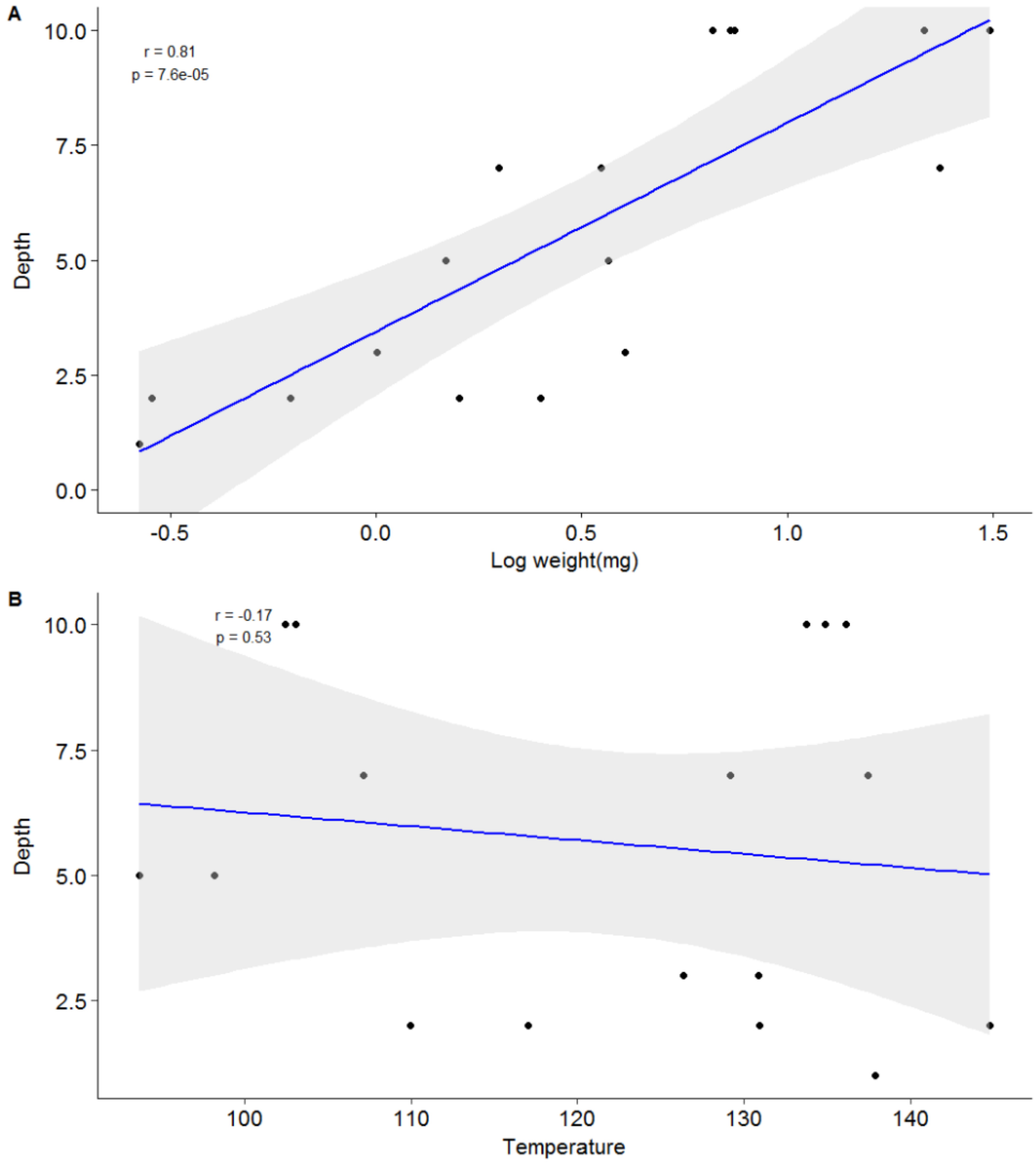


Figure 2: Maximum emergence depth of all 17 species compared to A) seed mass, log transformed measured in mg; B) T_{50}- Lethal temperature (Tangney et al. In Prep). Plots show fitted linear models with shaded 95% confidence intervals around the modelled mean; r^2value and p-value indicate goodness of fit and significance of the linear fit.

Measuring soil temperatures under fine fuel loadings.

In all cases, the mean soil temperatures were highest at 1cm depth, and mean temperatures increased as fuel loadings increased under all experimental fires (Fig. 3). However, maximum temperatures did not follow the same trend, with some maximum temperatures recorded at depths deeper than 1cm.

Under fuels comprising of a mix of Ehrharta grass and fine Banksia fuels, distributed at 8 Mg/Ha^{-1}, soil temperatures reached a maximum of 108 °C at 1 cm below the surface. Soil temperatures did not exceed 100 °C below 1cm, yet, maximum soil temperatures above 60°C were measured down to 5 cm (Fig. 3A). Fuels weighing 10 Mg/Ha^{-1} and comprising of 100% Banksia leaves generated soil temperatures not exceeding 100 °C at any depth, with a maximum soil temperature of 74.7 °C recorded at 5 cm deep. The highest mean soil temperature of 53.9 °C was recorded at 1cm, and maximum soil temperatures exceeded 60 °C down to 5 cm into the soil. (Fig. 3B). Soil temperatures generated under fine fuels comprising of 100% *Banksia* leaves distributed at 12 Mg/Ha^{-1} did not exceed 100 °C at any depth, with a maximum temperature of 87.4 °C recorded at 5cm, and the highest mean soil temperature of 60 °C recorded at 1 cm. Soil temperatures exceeded 60 °C down to 6cm (Fig. 3C). Soil temperatures from the consumption of fuels comprised of a mix of *Banksia* fuels and *Allocasuarina* cladodes weighing 15 Mg/Ha^{-1} reached a maximum soil temperature of 98.2 °C at a depth of 1 cm and the highest mean soil temperature of 75.5 °C also recorded 1 cm. Soil temperatures exceeded 60 °C down to 8 cm (Fig. 3D). Soil temperatures recorded under 20 Ma/Ha^{-1} of *Allocasuarina* cladodes were the highest of any of the experimental burns, with a recoreded maximum of 111.0 °C at 1 cm, a mean of 78.9 °C at 1 cm, and temperatures exceeding 60 °C recorded down to 7 cm (Fig. 3E).

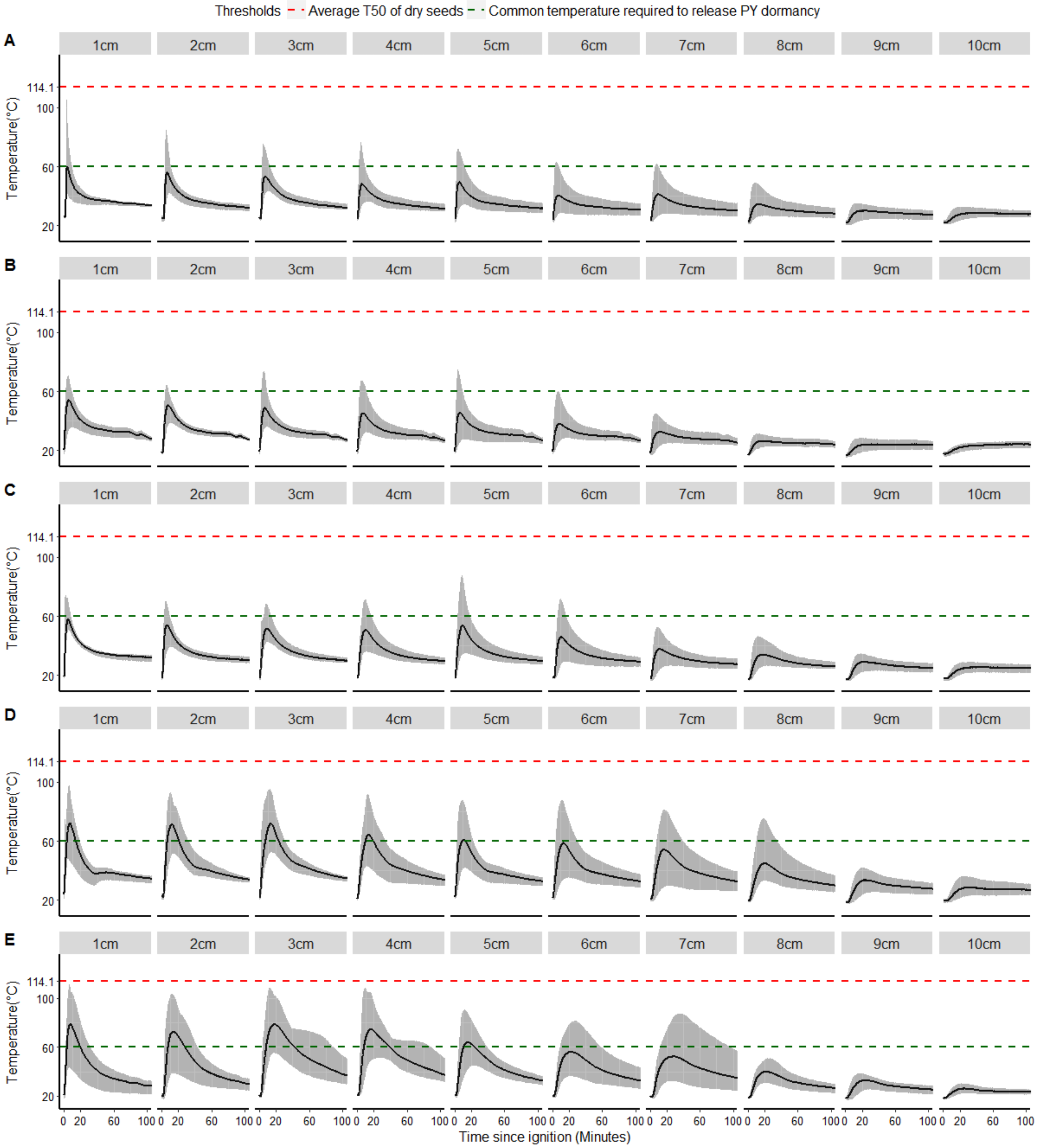


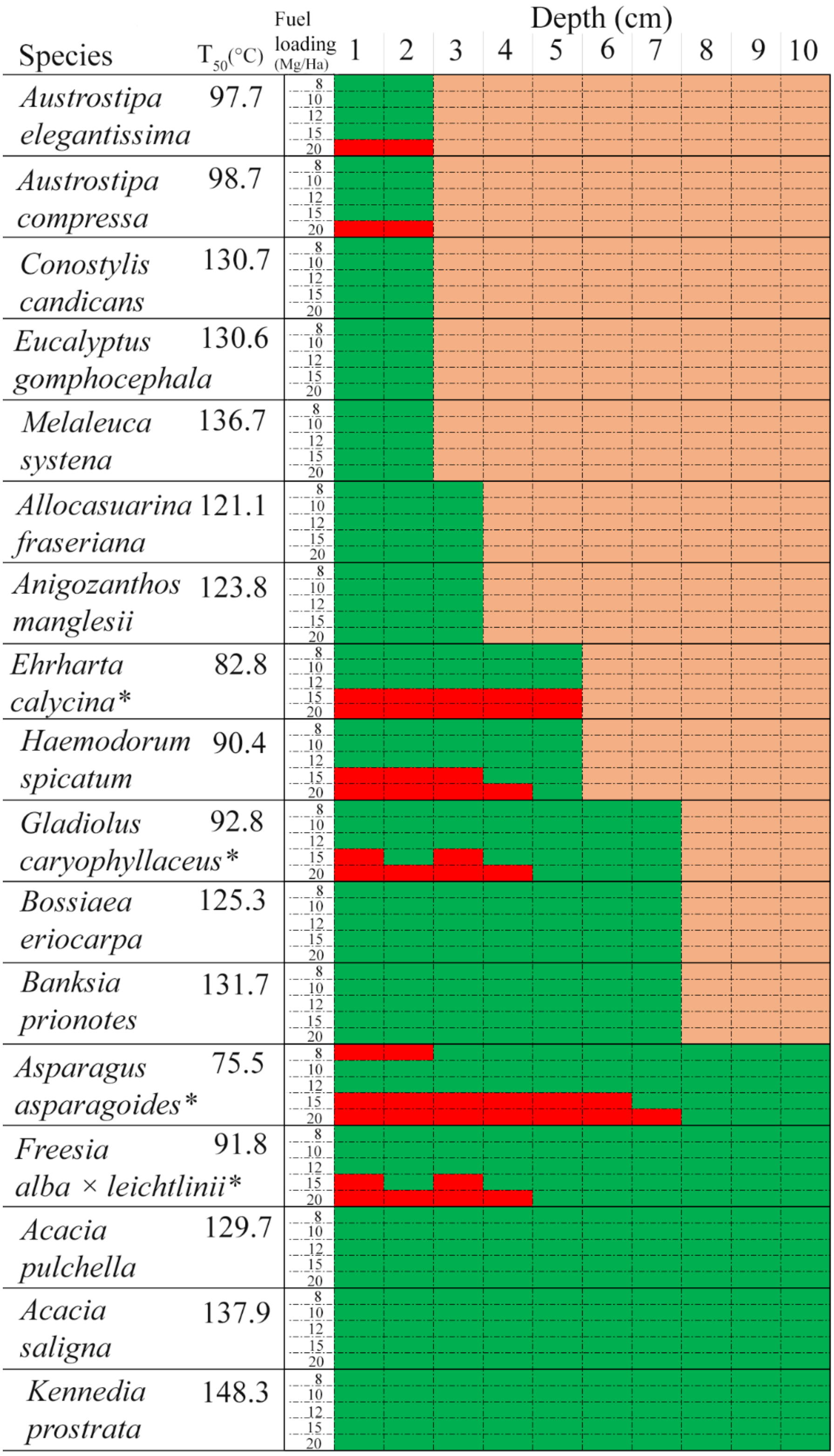
Figure 3: Soil temperatures from experimental burns measured using Distributed temperature sensing at indicated soil depth. Mean temperatures are represented by the solid black line, the range of temperatures are depicted by the grey shaded area. The dotted lines represent key biological thresholds that represent temperatures linked to dormancy break of physically dormant (PY) seeds (Ooi et al. 2014) and seed mortality(Hopper and Gioia 2004). Fuels

comprised A) 8 Mg/Ha^{-1} *of 20%* Ehrharta *grass and 80%* Banksia *fuels; B) 10 Mg/Ha*$^{-1}$ *of 100%* Banksia *woodland leaf and small sticks; C) 12 Mg/Ha*$^{-1}$ *of 100%* Banksia *woodland leaf and small sticks; D) 15 Mg/Ha*$^{-1}$ *of 66%* Banksia *woodland leaf and small sticks and 33%* Allocasuarina *cladodes; E) 20 Mg/Ha*$^{-1}$ *of 100% Allocasuarina cladodes.* The *diameter of all fuels was between 0 mm and 6 mm.*

Defining species-specific seedling emergence and mortality zones.

Soil temperatures exceeded the T_{50} of seven out of 17 species, including all four non-native species (*Asparagus asparagoides, Gladiolus caryophyllaceus, Freesia alba x leichtlinii* and *Ehrharta calycina*), both native grasses (*Austrostipa compressa* and *Austrostipa elegantissima*) and the native resprouting *Haemodorum spicatum* (Fig. 4).

Under 8 Mg/Ha^{-1} of mixed Ehrharta/Banksia fuel, soil temperatures greater than lethal threshold of *Asparagus asparagoides* seeds were recored down to 2 cm below the surface (Fig. 4). Soil temperatures did not exceed any species specific T_{50} thresholds under either 10 Mg/Ha^{-1} or 12 Mg/Ha^{-1} of fuel. However, under 15 Mg/Ha^{-1} of a mix of *Banksia* fuels and Allocasuarina cladodes, temperatures exceeded the T_{50} of five species, *A.aspragoides, Haemodorum spicatum, Freesia alba x leichtlinii*, *Gladiolus caryophyllaceus* and *Ehrharta calycina* (Fig. 4). Under the highest fuel loading of 20 Mg/Ha^{-1} of Allocasuarina cladodes, soil temperatures exceeded the T_{50} of seeds of seven species including *A.aspragoides, Haemodorum spicatum, Freesia alba x leichtlinii*, *Gladiolus caryophyllaceus* and *Ehrharta calycina* as well as seeds of *Austrostipa elegantissima* and *Austrostipa compressa* (Fig. 4). Soil temperatures were below T_{50} thresholds for seeds of all other species regardless of depth.



Species	T50(°C)	Fuel loading (Mg/Ha)	Depth (cm)									
			1	2	3	4	5	6	7	8	9	10
Austrostipa elegantissima	97.7	8	🟩	🟩	🟧	🟧	🟧	🟧	🟧	🟧	🟧	🟧
		10	🟩	🟩	🟧	🟧	🟧	🟧	🟧	🟧	🟧	🟧
		12	🟩	🟩	🟧	🟧	🟧	🟧	🟧	🟧	🟧	🟧
		15	🟩	🟩	🟧	🟧	🟧	🟧	🟧	🟧	🟧	🟧
		20	🟥	🟥	🟧	🟧	🟧	🟧	🟧	🟧	🟧	🟧
Austrostipa compressa	98.7	8	🟩	🟩	🟧	🟧	🟧	🟧	🟧	🟧	🟧	🟧
		10	🟩	🟩	🟧	🟧	🟧	🟧	🟧	🟧	🟧	🟧
		12	🟩	🟩	🟧	🟧	🟧	🟧	🟧	🟧	🟧	🟧
		15	🟩	🟩	🟧	🟧	🟧	🟧	🟧	🟧	🟧	🟧
		20	🟥	🟥	🟧	🟧	🟧	🟧	🟧	🟧	🟧	🟧
Conostylis candicans	130.7	8	🟩	🟩	🟧	🟧	🟧	🟧	🟧	🟧	🟧	🟧
		10	🟩	🟩	🟧	🟧	🟧	🟧	🟧	🟧	🟧	🟧
		12	🟩	🟩	🟧	🟧	🟧	🟧	🟧	🟧	🟧	🟧
		15	🟩	🟩	🟧	🟧	🟧	🟧	🟧	🟧	🟧	🟧
		20	🟩	🟩	🟧	🟧	🟧	🟧	🟧	🟧	🟧	🟧
Eucalyptus gomphocephala	130.6	8	🟩	🟩	🟧	🟧	🟧	🟧	🟧	🟧	🟧	🟧
		10	🟩	🟩	🟧	🟧	🟧	🟧	🟧	🟧	🟧	🟧
		12	🟩	🟩	🟧	🟧	🟧	🟧	🟧	🟧	🟧	🟧
		15	🟩	🟩	🟧	🟧	🟧	🟧	🟧	🟧	🟧	🟧
		20	🟩	🟩	🟧	🟧	🟧	🟧	🟧	🟧	🟧	🟧
Melaleuca systena	136.7	8	🟩	🟩	🟧	🟧	🟧	🟧	🟧	🟧	🟧	🟧
		10	🟩	🟩	🟧	🟧	🟧	🟧	🟧	🟧	🟧	🟧
		12	🟩	🟩	🟧	🟧	🟧	🟧	🟧	🟧	🟧	🟧
		15	🟩	🟩	🟧	🟧	🟧	🟧	🟧	🟧	🟧	🟧
		20	🟩	🟩	🟧	🟧	🟧	🟧	🟧	🟧	🟧	🟧
Allocasuarina fraseriana	121.1	8	🟩	🟩	🟩	🟧	🟧	🟧	🟧	🟧	🟧	🟧
		10	🟩	🟩	🟩	🟧	🟧	🟧	🟧	🟧	🟧	🟧
		12	🟩	🟩	🟩	🟧	🟧	🟧	🟧	🟧	🟧	🟧
		15	🟩	🟩	🟩	🟧	🟧	🟧	🟧	🟧	🟧	🟧
		20	🟩	🟩	🟩	🟧	🟧	🟧	🟧	🟧	🟧	🟧
Anigozanthos manglesii	123.8	8	🟩	🟩	🟩	🟧	🟧	🟧	🟧	🟧	🟧	🟧
		10	🟩	🟩	🟩	🟧	🟧	🟧	🟧	🟧	🟧	🟧
		12	🟩	🟩	🟩	🟧	🟧	🟧	🟧	🟧	🟧	🟧
		15	🟩	🟩	🟩	🟧	🟧	🟧	🟧	🟧	🟧	🟧
		20	🟩	🟩	🟩	🟧	🟧	🟧	🟧	🟧	🟧	🟧
*Ehrharta calycina**	82.8	8	🟩	🟩	🟩	🟩	🟩	🟧	🟧	🟧	🟧	🟧
		10	🟩	🟩	🟩	🟩	🟩	🟧	🟧	🟧	🟧	🟧
		12	🟩	🟩	🟩	🟩	🟩	🟧	🟧	🟧	🟧	🟧
		15	🟥	🟥	🟥	🟥	🟥	🟧	🟧	🟧	🟧	🟧
		20	🟥	🟥	🟥	🟥	🟥	🟧	🟧	🟧	🟧	🟧
Haemodorum spicatum	90.4	8	🟩	🟩	🟩	🟩	🟩	🟧	🟧	🟧	🟧	🟧
		10	🟩	🟩	🟩	🟩	🟩	🟧	🟧	🟧	🟧	🟧
		12	🟩	🟩	🟩	🟩	🟩	🟧	🟧	🟧	🟧	🟧
		15	🟥	🟥	🟥	🟩	🟩	🟧	🟧	🟧	🟧	🟧
		20	🟥	🟥	🟥	🟥	🟩	🟧	🟧	🟧	🟧	🟧
*Gladiolus caryophyllaceus**	92.8	8	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟧	🟧	🟧
		10	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟧	🟧	🟧
		12	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟧	🟧	🟧
		15	🟥	🟩	🟥	🟩	🟩	🟩	🟩	🟧	🟧	🟧
		20	🟥	🟥	🟥	🟥	🟩	🟩	🟩	🟧	🟧	🟧
Bossiaea eriocarpa	125.3	8	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟧	🟧	🟧
		10	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟧	🟧	🟧
		12	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟧	🟧	🟧
		15	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟧	🟧	🟧
		20	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟧	🟧	🟧
Banksia prionotes	131.7	8	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟧	🟧	🟧
		10	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟧	🟧	🟧
		12	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟧	🟧	🟧
		15	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟧	🟧	🟧
		20	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟧	🟧	🟧
*Asparagus asparagoides**	75.5	8	🟥	🟥	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩
		10	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩
		12	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩
		15	🟥	🟥	🟥	🟥	🟥	🟥	🟩	🟩	🟩	🟩
		20	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟩	🟩	🟩
*Freesia alba × leichtlinii**	91.8	8	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩
		10	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩
		12	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩
		15	🟥	🟩	🟥	🟩	🟩	🟩	🟩	🟩	🟩	🟩
		20	🟥	🟥	🟥	🟥	🟩	🟩	🟩	🟩	🟩	🟩
Acacia pulchella	129.7	8	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩
		10	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩
		12	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩
		15	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩
		20	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩
Acacia saligna	137.9	8	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩
		10	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩
		12	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩
		15	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩
		20	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩
Kennedia prostrata	148.3	8	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩
		10	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩
		12	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩
		15	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩
		20	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩

🟩 Temperatures did not exceed lethal thresholds and seeds can emerge

🟥 Temperatures exceed lethal temperatures of the seeds

🟧 Seeds unable to emerge from this depth

Figure 4: Species-specific seedling emergence zones based on fire temperatures in relation to T_{50} and maximum emergence. Seeds were classified into three categories, if fire temperatures exceeded T_{50} of the seed for a minimum of three minutes seeds were classified as dead. If soil temperatures are below T_{50} thresholds and seeds are able to emerge from this depth, seeds can emerge. If depth is greater than seeds are able to emerge from than seeds will die before emerging. Species ordered from shallowest emergence depth to deepest emergence depth.

Discussion

The interaction between maximum seedling emergence depth and lethal temperatures of the seeds defines the species-specific emergence niches following fire, compounded by soil temperatures that are driven by fuel loading and composition, which ultimately dictates seed survival. Maximum seedling emergence depth is closely associated with seed mass, with heavier seeds generally able to emerge from deeper within the soil profile (Bond et al. 1999, Forcella et al. 2000). However, under the heaviest fuel loadings soil temperatures exceeded lethal thresholds of seven species in this study.

Under dry soils and manipulated fuels, an increasing weight of fine fuel loadings lead to an increase in soil temperatures and, in cases where seeds have low lethal thresholds (T_{50}) and shallow emergence depths, the zone of potential emergence is diminished. Fine fuels (0-6mm diameter fuels), between 10 Mg/Ha^{-1} and 12 Mg/Ha^{-1}, did not generate temperatures greater than the average lethal temperature of dry seeds at any depth. Under the two heaviest fuel loadings, maximum temperatures recorded over a three-minute period were sufficient to exceed the T_{50} of seeds from five and seven species, respectively (Fig. 4) - including all three-grass species, all four non-native species, and one native resprouting species (*Haemodorum spicatum*). Seeds of the other 12 native species all had lethal temperatures above soil temperatures measured during these experimental fires. Soil temperatures recorded were above the T_{50} of three species (*E. calycina, A. elegantissima,* and *A. compressa*) and were recorded down to depths beyond the seeds maximum emergence depth, potentially reducing the ability for these three grass species to emerge following fire in similar fuel regimes.

These data presented here do not support the hypothesis that seeds with lower mass are most at risk to increased soil temperatures (i.e. Bond et al. (1999)), despite being restricted to shallow emergence depths. For example, three out of the four invasive species (*A. asparagoides*, *G. caryophyllaceus,* and *F. alba × leichtlinii*) all have heavy seeds (6.5 mg, 7.2 mg and 3.5 mg, respectively) relative to those of other species, but equally all have low lethal tolerances, making them more at risk to higher soil temperatures. Seeds of *M. systena* and *C. candicans* were the smallest seeds tested (0.28 mg and 0.26 mg, respectively) and were amongst those seeds with the highest lethal tolerances (Tangney et al. 2018b) and for these species; soil temperatures did not exceed T_{50} under any fuel-loading scenario. This suggests that while large seeded species have an increased ability to emerge from depths, they are not necessarily better protected from the soil temperatures generated during fire.

These data suggests that the combustion of fine fuels may generate soil temperatures hot enough to kill seeds under weather and fire conditions similar to those experienced during these experimental burns, potentially reducing seeds of some species from emerging while having minimum negative impacts on others species. However, a landscape scale assessment of fuels indicates that surface fuel accumulation in *Banksia* woodlands rarely exceeds an average of 7 Mg/Ha^{-1} (Burrows and McCaw 1990). Nonetheless, localised fuel accumulation, specifically under the canopies of mature Allocasuarina and Banksia trees, the two dominant over storey tree species in this system, may approach fuel loadings tested here (Burrows and McCaw 1990). These data on soil temperatures reported here are in contrast to soil temperatures measured and predicted in similar Western Australian ecosystems (Burrows 1999). Despite testing fuel loadings (ranging from 4 Mg/Ha^{-1} up to 20 Mg/Ha^{-1}) and fuel moisture similar to those tested here, Burrows (1999) found soil temperatures did not exceed 100 °C at a depth of 1 cm.

Most fuel beds are comprised of a mix of fuel components both fine fuels and coarse fuels (i.e. fuels > 6.0 mm in diameter) (Ottmar et al. 2007). Fine fuels are sufficient to generate temperatures lethal to seeds only under the highest fuel loadings tested, however, combustion of coarse fuel components may generate lethal temperatures at lower loading rates and are most likely responsible for driving high soil temperatures (Santana et al. 2011). Coarse fuels are generally much less abundant compared to fine fuels (Agee and Skinner 2005), resulting in spatial arrangement of fuels both in biomass, and in the size of individual fuel elements, that may drive the mosaic of soil temperatures, similar to those described in Tangney et al. (2018a). Therefore, fine fuels may lead to a mosaic of soil temperatures that are lethal for some species while promoting temperatures inducive to dormancy elevation in physically dormant species (Penman and Towerton 2008, Daibes et al. 2018), simultaneously providing a continuous fuel bed linking fuel elements together. Whereas heavy accumulation of fine fuels and coarse fuels, while limited spatially, may lead to soil heating above that which some seeds can survive (Busse et al. 2010).

Along with structural fuel components, individual species composition of the fuel bed may affect soil heating patterns (Saura-Mas et al. 2010). For example, under 8 Mg/Ha^{-1}, the maximum temperature surpassed maximum temperatures recorded under both 10 Mg/Ha^{-1} and 12 Mg/Ha^{-1}. Fuels used in the 8 Mg/Ha^{-1} fuel layer comprised fine fuels made up of 20% *E. calycina* grass and 80% *Banksia* leaves. Including fuels from *E. calycina* – a dominant invasive grass in *Banksia* woodlands – increased soil temperatures compared to similar fuel loadings without *E. calycina*, however, residence time above 100 °C was around 2 minutes, which is insufficient to induce seed mortality using the current definition of T_{50} as used in this experiment. Soil temperatures under the 8 Mg/Ha^{-1} fuel layer were similar to that measured under 12 Mg/Ha^{-1}, and greater than that measured under 10 Mg/Ha^{-1}. These fuel loadings of 10 and 12 Mg/Ha^{-1} excluded *E. calycina* fuel, suggesting that *E. calycina* may have a strong influence on soil temperatures, creating areas of high temperatures, with fast rate of spread, leading to short residence times. Without *E. calycina*, fuel loadings 10 Mg/Ha^{-1} and 12 Mg/Ha^{-1} had lower temperatures, but soils remained at their maximum temperatures for longer. Increases in soil temperatures during fires in grassy fuels, may be a function of leaf length of species within the fuel bed, as longer leaves are associated with increases in fire severity (Schwilk and Caprio 2011). However, fuel bed composition and loading are not the only determining factors that drives soil heating, as soil moisture will affect how much thermal energy penetrates into the soil (DeBano 2000).

Soil moisture can have a direct effect on soil heating during fire (Aston and Gill 1976, Stoof et al. 2013), as moisture will be converted into steam and released from the soil, reducing the amount of thermal energy absorbed by surrounding soils (Enninful and Torvi 2008). In this study, soil moisture was not controlled during experimental fires; rather we were replicating seasonal soil moisture conditions through the timing of the experimental fires. Throughout the experimental fire period, soil moisture decreased over the period during which we conducted our soil heating experiments, but there was no evidence to suggest that soil moisture was high enough to inhibit heat penetration (as characterised by a slow temperature increase and temperature plateau at 100 °C, e.g. Aston and Gill (1976) and Valette et al. (1994)). While an increase in soil moisture will decrease soil temperatures during fire (Stoof et al. 2013), high soil moisture is associated with increase in seed moisture (Turner et al. 2006) and increases in seed moisture reduce lethal thresholds of seeds(Tangney et al. 2018b). Consequently, fire events during periods when seed moisture is high may lead to increased seed mortality, under a wider range of fuel loadings if fuel consumption is comparable.

Once seeds survive through the passing of fire, quick seedling emergence can be advantageous as a strategy to out-compete neighbours (Dyer et al. 2000). Ultimately, the quicker plants can transition from heterotrophic energy use to autotrophic energy production the sooner the seedling will move away from being limited to the available energy provided by the seed (Forcella et al. 2000). The fastest seedlings to emerge was of *E. calycina*, however, fast seedling emergence was also evident in the other two grass species tested, *A. compressa*, and *A. elegantissima*. These native grasses (*A. compressa*, and *A. elegantissima*.) are also non-sprouting (fire-killed) species that rely entirely on seedling recruitment following fire, so early emergence may provide a competitive advantage over other species (Grundy 2003), as individuals transition to photosynthesis earlier than surrounding seedlings post fire, allowing individuals of these species to establish in recently opened gaps (Verdú and Traveset 2005). In opposition, *E. calycina* is able to resprout following fire and returns to seed production within the first year after fire. Thus, quick emergence of new seedlings following fire may perpetuate the expansion of *E. calycina* populations, increasing species cover, and perpetuate the fire-weed cycle, common among many invasive grasses (D'Antonio and Vitousek 1992, Brown et al. 2016).

Seeds from all four of the Fabaceae species tested (*A. saligna, A. pulchella, B. eriocarpa* and *K. prostrata*) were slower to emerge from the surface (being delayed by 10-20 days) than at any depth tested. Slower emergence may reflect an inability of the radicle to penetrate the soil surface (Forcella et al. 2000). Alternatively and more probable, these larger, round seeds may have a poorer contact with the soil leading to a slower uptake of water from the soil, resulting in seed mortality due to desiccation on the soil surface (Liu et al. 2011). Another possibility is that the germination process simply takes longer, as is common among large seeded species (Buhler 1995). As seeds from all Fabaceae species tested had some form of elaiosome that encourage burial by ants (Majer 1982), these seeds may be adapted to emerging from within the soil compared to emergence from the soil surface.

Conclusion

The interaction between maximum seedling emergence depth and the lethal temperature threshold of the seeds defines the species-specific emergence niches following fire, with soil temperatures driven by fuel loading and composition ultimately dictating seed survivability. Soil temperatures generated from the combustion of fine fuels have the potential to reduce seedling emergence following fire. While heavy seeds were able to emerge from deeper in the soils, fuel loadings of 15 Mg/Ha^{-1} and 20 Mg/Ha^{-1} exceeded the lethal thresholds of seven species, with temperatures being measured deeper within the soil than seeds of three species can emerge. These data suggests that the combustion of fine fuels may generate soil temperatures hot enough to kill seeds under weather and fire conditions similar to those experienced during these experimental burns, potentially reducing seeds of some species from emerging while having minimum negative impacts on others species.

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Chapter 4: Supplementary materials

Table S3: Species used in this analysis, including seed dormancy type and pre-treatments (if required), plant and seed traits. Family names are presented without the –aceae for brevity. Abbreviations: Physical dormancy - PY, non-dormant - ND, morphophysiological dormancy – MPD, physiological dormancy – PD, morphological dormancy - MD. Hot water - HW, smoke water - SW. Non sprouter - NS, resprouter - R. Soil stored seed - S, canopy stored seed - C. Seeds with embryos in the foliate subdivision of the axile division – F, seeds with embryos in the linear subdivision of axile – L, seeds with embryos in the basal division – B, seeds with embryos in the miniature subdivision of the axile division – M. Species that are non-native to Banksia woodland are indicated by an asterisk.

Species	Family (-aceae)	Dormancy type	Pre-treatment	T_{50} at 50% RH (°C)	Weight (mg)	Width: Length	Fire Response	Storage Type	Embryo Type
Acacia pulchella R.Br	Fab	PY	HW 2 mins	129.5	7.46	0.45	NS	S	F
Acacia saligna (Labill.) H.L.Wendl.	Fab	PY	HW 2 mins	122.7	21.48	0.52	NS	S	F
Allocasuarina fraseriana (Miq.) L.A.S.Johnson	Casuarin	ND	Nil	121.1	4.03	0.52	R	C	F
Anigozanthos manglesii D.Don	Haemodor	MPD	Nil	123.8	1.00	0.79	R	S	M
*Asparagus asparagoides** (L.) Druce	Asparag	PD	Nil	75.5	6.59	0.90	R	S	L
Austrostipa compressa	Po	PD	SW	98.7	1.59	0.23	NS	S	B

(R.Br.) S.W.L.Jacobs & J.Everett									
Austrostipa elegantissima (Labill.) S.W.L.Jacobs & J.Everett	Po	PD	SW	97.6	0.61	0.11	NS	S	B
Banksia prionotes Lindl.	Protea	ND	Nil	131.1	23.47	0.64	NS	C	F
Bossiaea eriocarpa Benth.	Fab	PY	HW 30 secs	128.3	1.99	0.63	R	S	F
Conostylis candicans Endl.	Haemodor	PD	SW	130.5	0.27	0.52	R	S	M
*Ehrharta calycina** Sm.	Po	PD	Nil	82.7	1.48	0.40	R	S	B
Eucalyptus gomphocephala DC.	Myrt	ND	Nil	130.6	2.51	0.67	R	C	F
*Freesia alba x leichtlinii**	Irid	MD	Nil	91.8	7.29	0.70	R	S	L
*Gladiolus caryophyllaceus** (Burm.f.) Poir.	Irid	PD	Nil	92.8	3.53	0.85	R	S	L
Haemodorum spicatum R.Br.	Haemodor	ND	Nil	90.4	3.68	0.82	R	S	M
Kennedia prostrata R.Br	Fab	PY	HW 1 mins	126.2	31.14	0.76	NS	S	F
Melaleuca systena Craven	Myrt	ND	Nil	136.5	0.28	0.83	R	C	L

```
#read in emergence data
TM<- read.csv('Ems.csv')
#check data headings
head(TM)
#apply any model
drmTM<-  drm(Seed/25~Day,Depth, fct=LL.3(), data=TM )
#summarize
summary(drmTM)
#plot
plot(drmTM, log= "", type = 'none', normal = TRUE, lty = 1,lwd=2, col= TRUE, ylim =c(0, 1.1), ylab=
"proportion of emerged seedlings", xlab = "days since sowing", legendPos= c(10 , 1))
#compare and rank
mselect(drmTM, list(LL.3(), LL.3u(), W1.3(), W2.3(), LL2.3(), LL2.3u()), icfct=AIC)
#apply top ranked model
drmTM<-  drm(Seed/25~Day,Depth, fct=W1.3(), data=TM )
```

```
#re plot

plot(drmTM, log= "", type = 'none', normal = TRUE, lty = 1,lwd=2, col= TRUE, ylim =c(0, 1.1), ylab=
"proportion of emerged seedlings", xlab = "days since sowing", legendPos= c(10 , 1))

#check model fit

modelFit(drmTM)

#estimates of rate at each depth

ED(drmTM, c(75), interval="delta")

#compare rate of each depth

EDcomp(drmTM, c(75,75))
```

Figure S1: Full script for model selection of species-specific emergence rate curves. Identification of the most parsimonious model used mselect within DRC to select the model with the lowest AIC and highest Log- likelihood, limited to a set of three parameter non-linear functions, model selection in Table S1.

Table S2: Full model description following model selection in DRC for each species. Model selection used AIC and Log- likelihood to identify the most parsimonious model for each species. Final model selection for each species is bolded. Model selection was either a three parameter Log-logistic model (LL.3, LL2.3, LL.3u or LL2.3u) or a three parameter Weibull model (W1.3 or W2.3)

Species	Model	LogLik	IC	Lack of fit	Res var
Asparagus asparagoides	**W1.3**	**995.486**	**-1941**	**1.00**	**0.00806**
	LL2.3	984.923	-1919.8	1.00	0.00824
	LL.3	984.842	-1919.7	1.00	0.00824
	W2.3	966.081	-1882.2	1.00	0.00856
	LL2.3u	890.915	-1731.8	0.69	0.00996
	LL.3u	890.225	-1730.4	0.67	0.00997
Austrostipa compressa	**W1.3**	**318.521**	**-617.04**	**1.00**	**0.01083**
	LL.3	318.247	-616.49	1.00	0.01084
	LL2.3	318.223	-616.45	1.00	0.01084
	W2.3	317.453	-614.91	1.00	0.01089
	LL.3u	259.666	-499.33	0.10	0.01485
	LL2.3u	241.093	-462.19	0.00	0.01641
Austrostipa elegantissima	**W1.3**	**262.029**	**-504.06**	**1.00**	**0.01467**
	LL2.3	260.719	-501.44	1.00	0.01477

	LL.3	260.705	-501.41	1.00	0.01477
	W2.3	258.272	-496.54	1.00	0.01497
	LL.3u	193.293	-366.58	0.01	0.02122
	LL2.3u	187.217	-354.43	0.00	0.02193
Acacia pulchella	**LL2.3**	**740.38**	**-1430.8**	**1.00**	**0.01349**
	LL.3	740.379	-1430.8	1.00	0.01349
	W1.3	739.466	-1428.9	1.00	0.01351
	W2.3	735.948	-1421.9	1.00	0.01361
	LL.3u	515.371	-980.74	0.00	0.02123
	LL2.3u	515.242	-980.48	0.00	0.02123
Acacia saligna	**W1.3**	**709.615**	**-1369.2**	**1.00**	**0.01435**
	LL.3	705.231	-1360.5	1.00	0.01448
	LL2.3	705.224	-1360.4	1.00	0.01448
	W2.3	697.856	-1345.7	1.00	0.01469
	LL.3u	446.244	-842.49	0.00	0.0244
	LL2.3u	445.112	-840.22	0.00	0.02446
Allocasuarina fraseriana	**W1.3**	**221.396**	**-428.79**	**1.00**	**0.00999**
	LL.3	220.354	-426.71	1.00	0.01008
	LL2.3	220.352	-426.7	1.00	0.01008
	W2.3	217.424	-420.85	1.00	0.01032
	LL.3u	186.827	-359.65	0.20	0.01322
	LL2.3u	186.808	-359.62	0.20	0.01322
Anigozanthos manglesii	**W1.3**	**209.596**	**-393.19**	**1.00**	**0.02573**
	LL.3	209.104	-392.21	1.00	0.02578
	LL2.3	209.064	-392.13	1.00	0.02578
	W2.3	208.147	-390.29	1.00	0.02588
	LL.3u	174.757	-323.51	1.00	0.02962
	LL2.3u	171.576	-317.15	1.00	0.03
Banksia prionotes	**W1.3**	**477.225**	**-916.45**	**1.00**	**0.01663**
	LL.3	476.086	-914.17	1.00	0.01669
	LL2.3	476.024	-914.05	1.00	0.01669
	W2.3	472.778	-907.56	1.00	0.01684
	LL2.3u	420.091	-802.18	1.00	0.0194
	LL.3u	419.89	-801.78	1.00	0.01941
Bossiaea eriocarpa	**W1.3**	**658.178**	**-1272.4**	**1.00**	**0.01317**
	LL2.3	656.404	-1268.8	1.00	0.01322
	LL.3	656.183	-1268.4	1.00	0.01323
	W2.3	650.815	-1257.6	1.00	0.01339
	LL.3u	470.12	-896.24	0.00	0.02031
	LL2.3u	470.002	-896	0.00	0.02032
Conostylis candicans	**W1.3**	**723.618**	**-1427.2**	**1.00**	**0.00123**
	LL2.3	721.27	-1422.5	1.00	0.00124

	LL.3	720.771	-1421.5	1.00	0.00125
	W2.3	717.454	-1414.9	1.00	0.00127
	LL.3u	560.387	-1100.8	0.00	0.00295
	LL2.3u	326.669	-633.34	0.00	0.01036
Ehrharta calycina	**W1.3**	**696.896**	**-1355.8**	**1.00**	**0.00922**
	LL.3	695.875	-1353.7	1.00	0.00924
	LL2.3	695.836	-1353.7	1.00	0.00924
	W2.3	694.163	-1350.3	1.00	0.00928
	LL.3u	580.563	-1123.1	0.03	0.0126
	LL2.3u	575.203	-1112.4	0.01	0.01278
Eucalyptus gomphocephala	**W1.3**	**150.581**	**-281.16**	**1.00**	**0.0267**
	LL.3	150.015	-280.03	1.00	0.02678
	LL2.3	150.012	-280.02	1.00	0.02679
	W2.3	148.742	-277.48	1.00	0.02697
	LL2.3u	99.9888	-179.98	0.38	0.03505
	LL.3u	99.5719	-179.14	0.36	0.03513
Freesia alba	**W1.3**	**953.085**	**-1856.2**	**1.00**	**0.00878**
	LL2.3	942.995	-1836	1.00	0.00896
	LL.3	942.506	-1835	1.00	0.00897
	W2.3	920.332	-1790.7	1.00	0.00938
	LL.3u	818.027	-1586.1	0.03	0.01153
	LL2.3u	817.591	-1585.2	0.03	0.01154
Gladiolus caryophyllaceus	**W1.3**	**534.362**	**-1024.7**	**1.00**	**0.01752**
	LL2.3	532.202	-1020.4	1.00	0.0176
	LL.3	531.112	-1018.2	1.00	0.01765
	W2.3	525.91	-1007.8	1.00	0.01786
	LL.3u	421.499	-799	0.34	0.02272
	LL2.3u	421.403	-798.81	0.33	0.02272
Haemodorum spicatum	**LL2.3**	**403.651**	**-769.3**	**0.39**	**0.02027**
	LL.3	403.584	-769.17	0.39	0.02028
	W1.3	402.395	-766.79	0.35	0.02034
	W2.3	401.816	-765.63	0.33	0.02037
	LL2.3u	350.149	-662.3	0.00	0.02341
	LL.3u	350.008	-662.02	0.00	0.02342
Kennedia prostrata	**W1.3**	**907.808**	**-1765.6**	**1.00**	**0.00962**
	LL.3	904.947	-1759.9	1.00	0.00968
	LL2.3	904.939	-1759.9	1.00	0.00968
	W2.3	898.944	-1747.9	1.00	0.0098
	LL.3u	505.04	-960.08	0.00	0.02167
	LL2.3u	503.717	-957.43	0.00	0.02173
Melaleuca systena	**W1.3**	**315.107**	**-610.21**	**1.00**	**0.01103**
	LL.3	313.064	-606.13	1.00	0.01115

	LL2.3	313.048	-606.1	1.00	0.01115
	W2.3	309.671	-599.34	1.00	0.01135
	LL.3u	245.327	-470.65	0.00	0.01605
	LL2.3u	241.43	-462.86	0.00	0.01638

```
#set global model
mod.gbl <- lm(MaxE~T50+scale(Weight)+
scale(Width.Length)+Native+FireResponse+StorageType+ EmbryoType, x= TRUE,
data=Data)
#set na options
options(na.action = "na.fail")
#dredge
dd1 <- dredge(mod.gbl ,rank="AICc")
#set up image parameters
par(mfrow=c(1,1))
par(mar = c(0,5,9,4))
#plot with labels
plot(dd1, labAsExpr = TRUE)
#examine full dredge results
dd1
#get model average
summary(model.avg(dd1))
#get top ranked model
optimodMAXE1<- get.models(dd1, subset=1) [[1]]
#get second top ranked model
optimodMAXE2<- get.models(dd1, subset=2) [[1]]
#examine model summaries
summary(optimodMAXE1)
summary(optimodMAXE2)
#compare models
```

```
anova(optimodMAXE1, optimodMAXE2)
```

Figure S2: Full code for model selection of most parsimonious model for correlations between maximum emergence (MaxE) and seed traits. Generated using and MuMIn package (Burnham and Anderson 2003) respectively, all within R statistical package.

Chapter 4: Supplementary materials Part 2: Tukey post-host results.

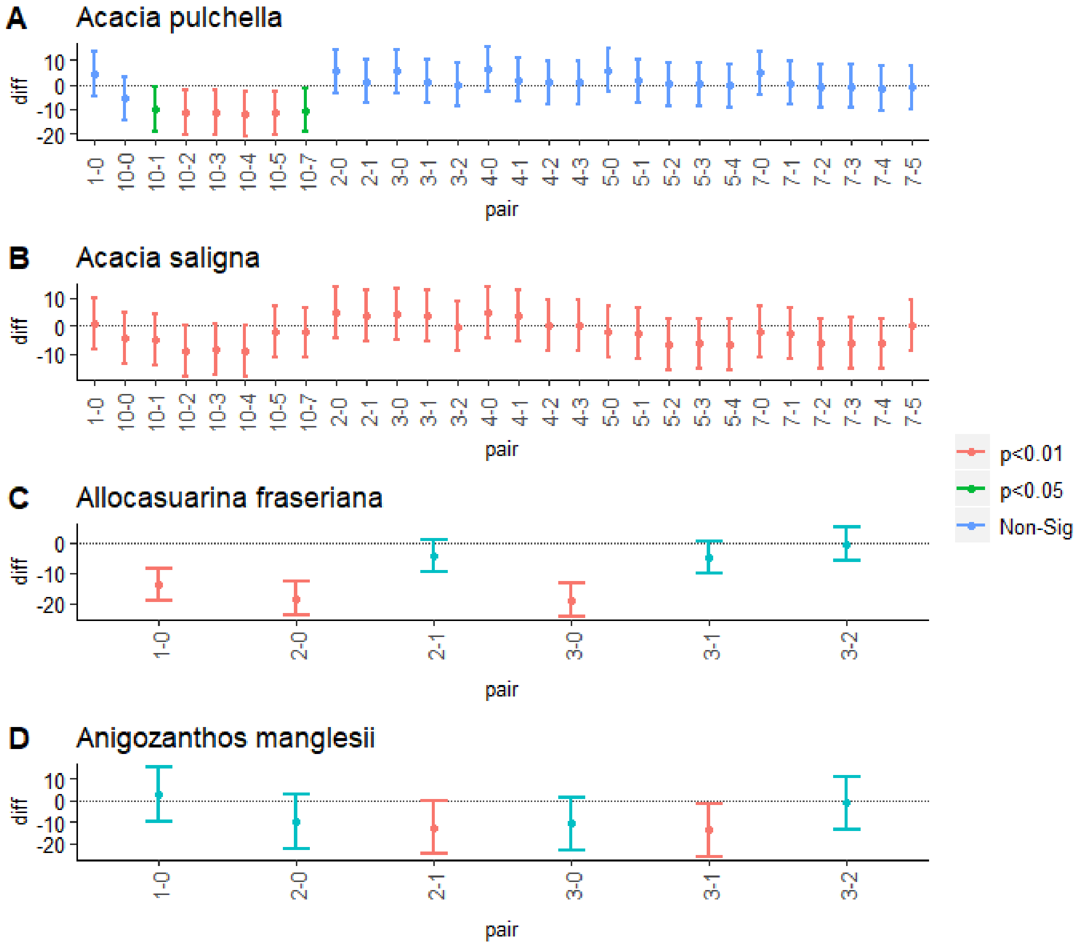


Figure S2:2: Tukey post-hoc test for comparing maximum emergence depth at each depth, for species Acacia pulchella, Acacia saligna, Allocasuarina fraseriana and Anigozanthos manglesii. Bars overlapping the horizontal line deemed not significantly different from each other.

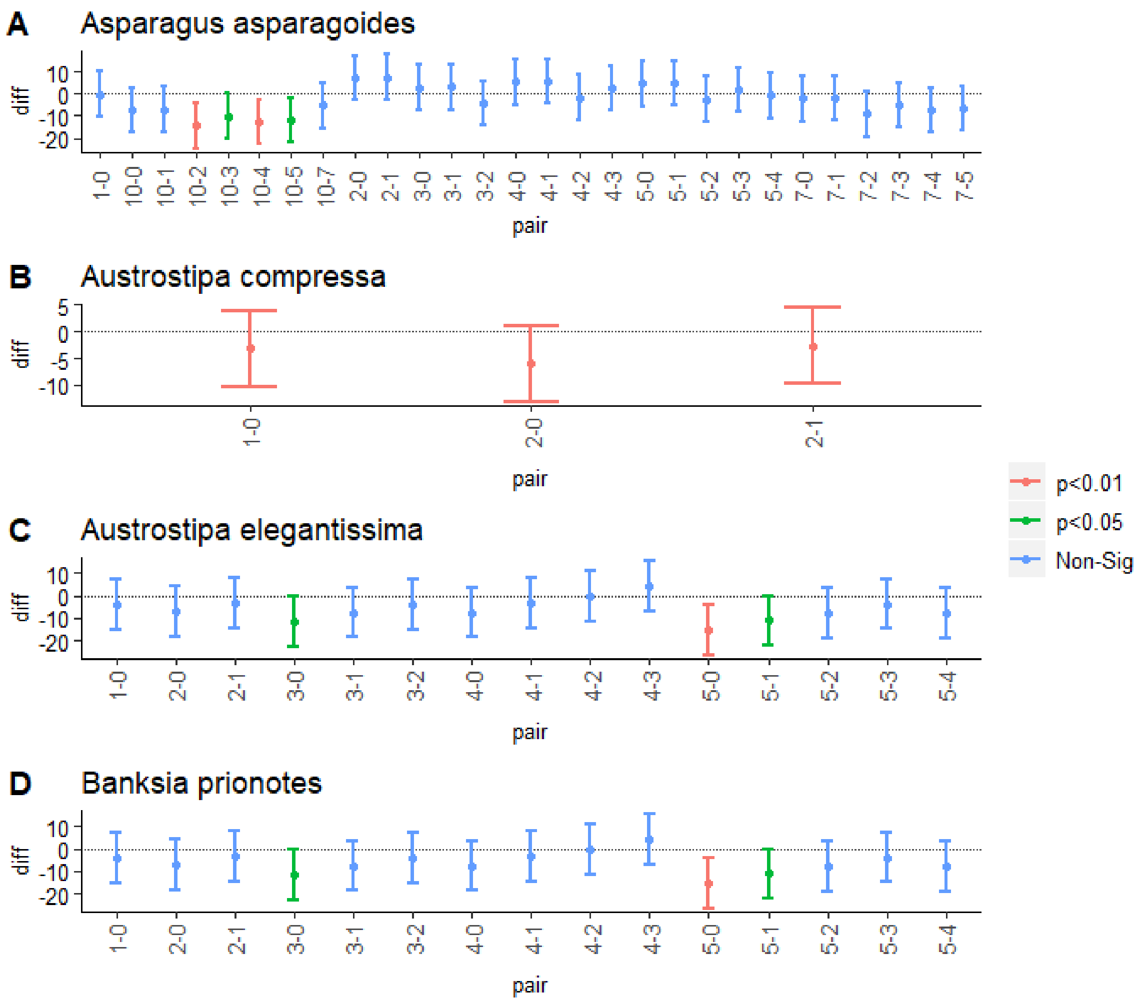


Figure S2:3 Tukey post-hoc test for comparing maximum emergence depth at each depth, for species Asparagus asparagoides, Austrostipa compressa, Austrostipa elegantissima and Banksia prionotes. Bars overlapping the horizontal line deemed not significantly different from each other.

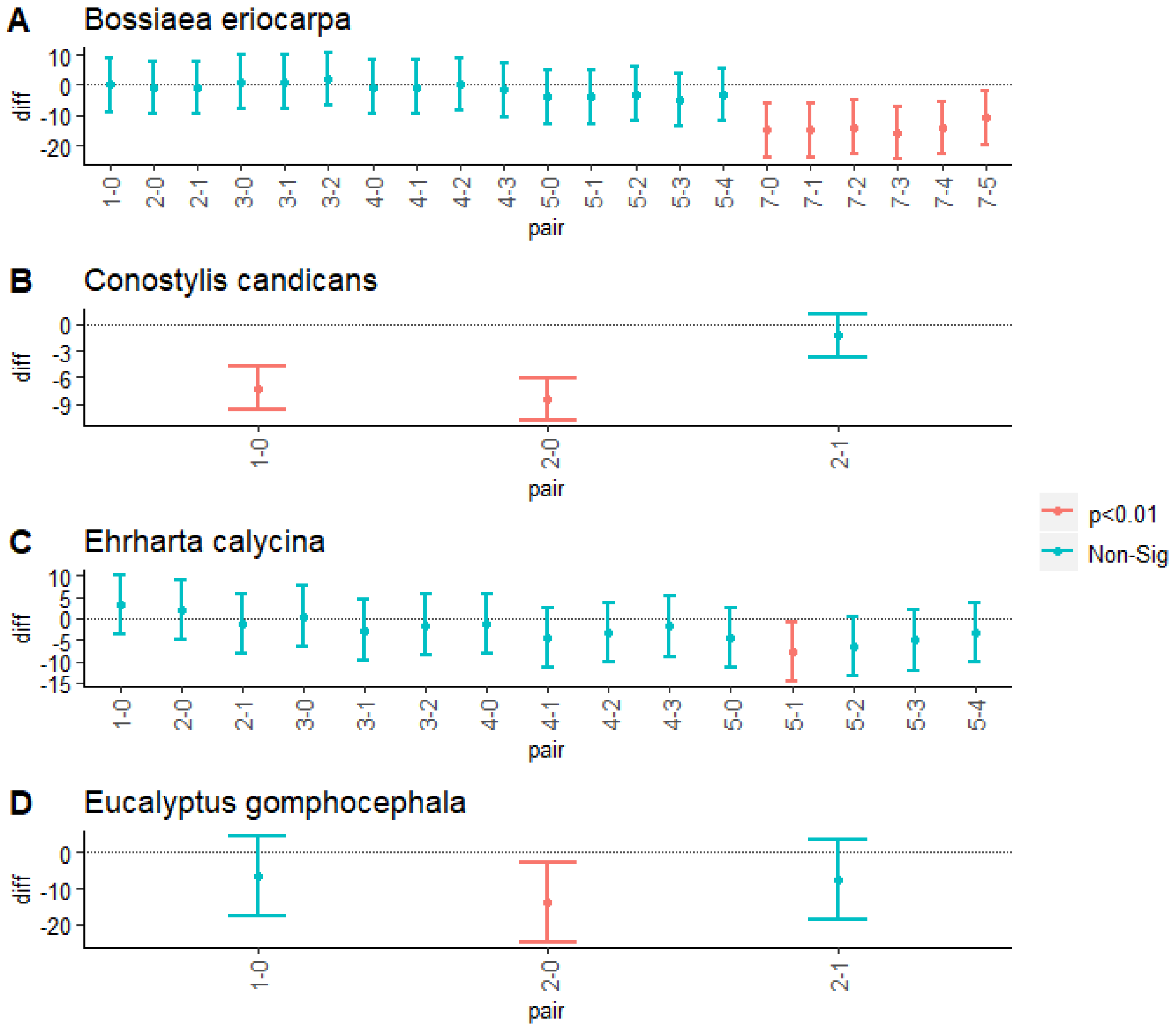


Figure S2:4 Tukey post-hoc test for comparing maximum emergence depth at each depth, for species Bossiaea eriocarpa, Conostylis candicans, Ehrharta calycina and Eucalyptus gomphocephala. Bars overlapping the horizontal line deemed not significantly different from each other.

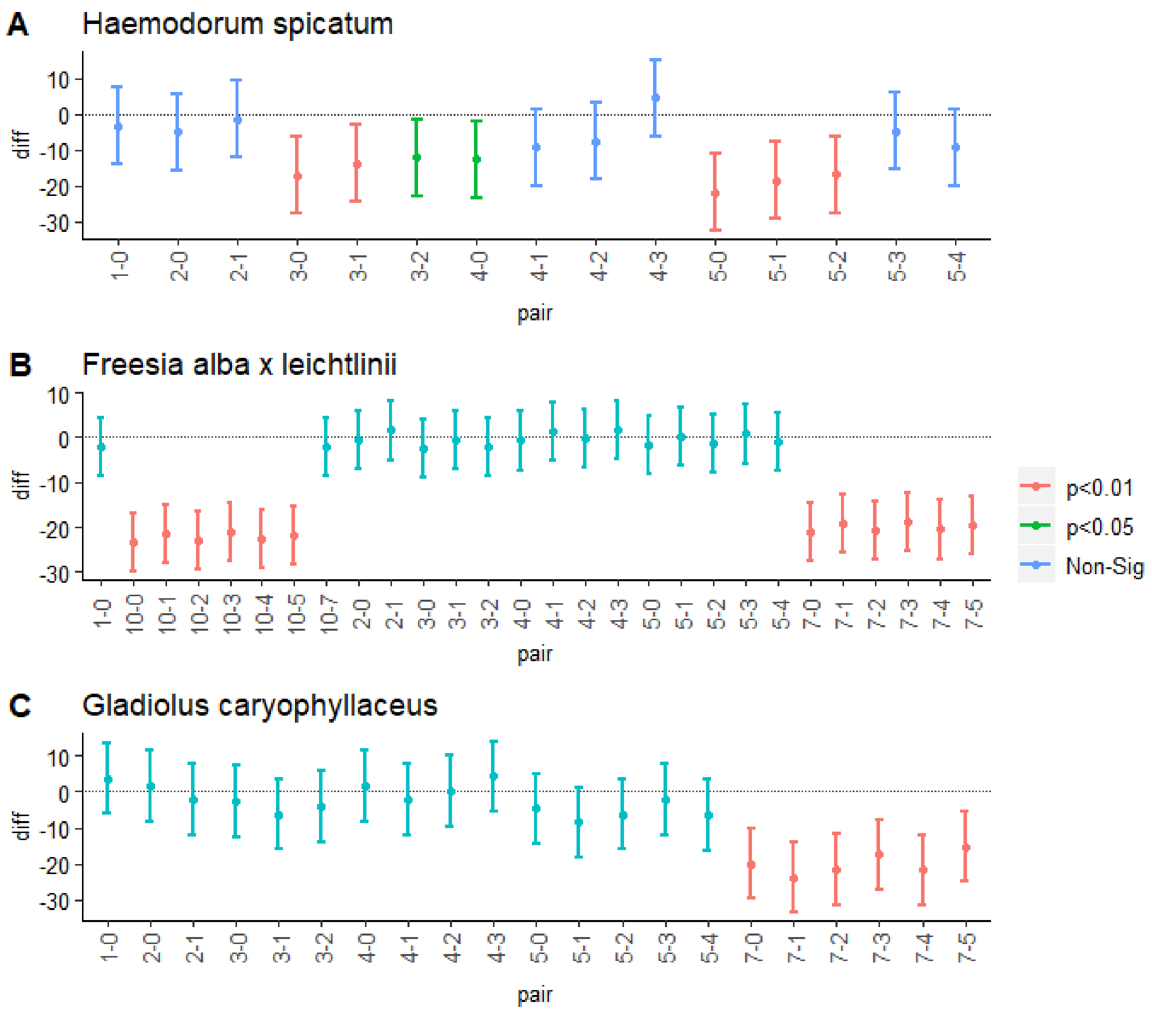


Figure S2:5 Tukey post-hoc test for comparing maximum emergence depth at each depth, for species Haemodorum spicatum, Freesia alba x leichtlinii and Gladiolus caryophyllaceus. Bars overlapping the horizontal line deemed not significantly different from each other.

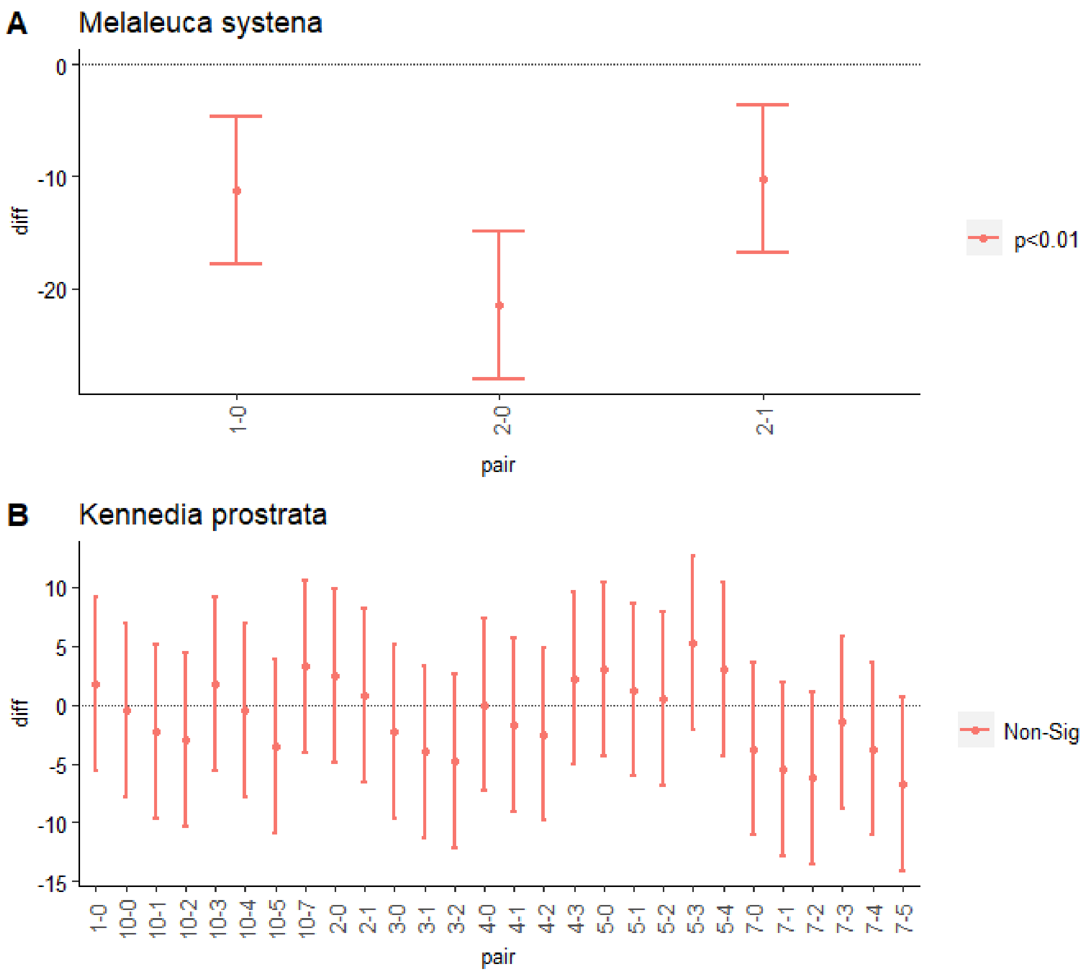


Figure S2:6 Tukey post-hoc test for comparing maximum emergence depth at each depth, for species Melaleuca systena and Kennedia prostrata. Bars overlapping the horizontal line deemed not significantly different from each other.

In chapter 5, I use weather and site variables to predict seed hydration. This is placed in the context of hindcasting specific periods of 2017 where seed hydration would be lowest, and thus seed survival would be maximized.

Chapter 5: Predicting seed hydration using additive logistic regression.

Abstract

Globally, risk of high intensity fires is increasing leading to increased economic costs. To combat this increasing fire risk, hazard reduction burns are regularly conducted to reduce fuel loads, minimising the probability of intensive wildfires. Hazard reduction burns are conducted during seasons and weather conditions that are predicted to yield manageable fire behaviour and characteristics. Consideration of plant response following fire can aid the management of fire intervals, severity and fuel accumulation. However, species responses and tolerances to fire differ from season to season, in particular, the ability of seeds to tolerate extreme temperatures associated with fire is directly linked with seed moisture at the time of exposure. To understand seasonal hydration patterns of Banksia woodland seeds and how it is influenced by weather conditions throughout the year, we measured seed moisture of four common native species and one invasive species to Banksia woodlands, throughout 2017. Additionally, soil moisture and weather conditions were also measured throughout 2017. Using machine learning and predictive binomial classification methods we were able to create a model to predict seed hydration status based on readily available weather and site data. This characterisation allowed for the identification of periods of the year when seeds may be at increased risk to fire temperatures. Seeds from all five species were buried 2 cm in the soil, and a subsample was retrieved every week for the duration of 2017. Each sub-sample was weighed and dried to determine seed moisture of that collection, along with weekly soil samples. Controlled isotherms were created for each species to correlate seed moisture with relative humidity (RH).

Using the isothermic functions, seed moisture from each retrieval was transformed into RH, seeds were classed as wet if modelled RH was above 85% and dry if modelled RH was below 85%. Using a boosted logistic regression classifier, our model was able to predict whether a seed collection was either wet or dry successfully 92% of the time, regardless of species. Major factors that were significant in predicting seed hydration were soil moisture and the number of days since a rain event above 1mm, less. We were able to identify two distinct periods during 2017 where implementation of hazard reduction burns within these periods may have potentially had minimal impacts on soil stored seeds. These data presented here and the associated analysis provides an opportunity to focus on identifying the optimum fire implementation window that will minimize potential impacts on soil stored seeds.

Introduction

Globally, the risk of high intensity fires is increasing (Bowman et al. 2009). More frequent and more intense fires have detrimental ecological and economic effects. For example, the increased frequency of wildfires contributed to 17% of the total economic losses from natural disasters in Australia between 2002 and 2013, a doubling from a previous report in 2001 (Sharples et al. 2016, Handmer et al. 2018). To combat this increasing fire risk, hazard reduction burns are regularly conducted to moderate fuel loads, with the aim of reducing the probability of intensive wildfires (Fernandes and Botelho 2003). In order for land managers to implement hazard reduction burns safely, burns are conducted during seasons and weather conditions that are predicted to yield manageable fire behaviour; including periods of lower temperatures and higher soil and litter moisture contents (compared to dry season maximums), and low wind speeds (Agee and Skinner 2005). These abiotic conditions allow for the implementation of hazard reduction burns that, on average, have lower fire intensity, slower rate of spread, and higher heterogeneity in fuel consumption compared to wildfires, ensuring reduced incidences of out of control fires (Agee and Skinner 2005). Management of the intervals between burns is informed by consideration of plant responses following fire (Peterson and Reich 2001), fire severity (Knapp and Keeley 2006), and fuel accumulation following fire (Pausas and Paula 2012). The goals of managed fire regimes often include the protection or enhancement of species diversity or ecosystem function (Agee and Skinner 2005) through the stimulation of soil seed banks (Bradstock and Auld 1995), stimulation of post-fire flowering (Bowen and Pate 2004), or a reduction of invasive species (Keeley 2006). But the primary aim of hazard reduction burning is to reduce the fire risk through the reductions in fuel loadings (Agee and Skinner 2005), albeit the efficacy of these burns remains contentious(Penman et al. 2011).

Plants are not adapted to fire *per se*, but rather to certain fire regimes and their properties with respect to fire intensity, frequency, and season of occurrence (Keeley et al. 2011). The responses and tolerances of plant species to fire change through seasons (Savadogo et al. 2012), and are directly influenced by local environmental conditions and the seasonal phenological states of the various species within the ecosystem. Modifications to historical fire regimes may alter the ability of individual plants, plant populations, and ecosystems to respond to fire (Bradstock 2010). For example, in south west Western Australia, with a historical fire season extending through summer and autumn, spring burns result in significantly reduced seedling recruitment compared to autumn burns (Roche et al. 1998), and those seedlings that do emerge following spring burns are less likely to survive through the following summer, as compared to seedlings that emerge after autumn burns of the same year (Ooi 2010). Seeds have also been shown to have a lower lethal temperature threshold when their moisture content is high during fire (Tangney et al. 2018b), increasing the risk of mortality of soil-stored seeds during out of season burning conducted under higher soil moisture conditions (Aston and Gill 1976).

For many plant species, recruitment from seeds stored in the soil is the primary mechanism for population persistence, regeneration, and expansion following fire. Seeds close to the soil surface experience frequent fluctuations in the amount of moisture, light, and temperature they receive. The ability of seeds to sense their environment is fundamental to regeneration and allows seeds to time germination and consequently emerge in periods of the year when conditions are most suitable for seedling survival (Baskin and Baskin 2001). Seeds close to the soil surface have the highest chance of germination and emergence, yet they are also most exposed to the high temperatures generated by fire, where soil temperatures can exceed 200 °C (Tangney et al. 2018a). While soils provide some insulation from fire temperatures, the ability of seeds to tolerate fire temperatures is directly related to the moisture content of the seed at the time of exposure (Tangney et al. 2018b).

Seeds take up water from soils in a passive manner due the high water potential gradient between dry seeds and the surrounding soil (Hegarty 1978, Wuest 2007). Seeds can cycle between hydrated and dry states, as the surrounding soil moisture conditions fluctuate seasonally (Turner et al. 2006). If environmental cues for seed dormancy break and germination are not met, seeds will remain in a non-germinated, but viable state, before drying down to lower moisture states, remaining in the soil until conditions are suitable, or until seeds lose viability. What drives seeds to lose moisture while in the soil may be influenced by a range of weather (amount of rain, temperature, evaporation), climate and soil conditions that seeds will experience.

Seed moisture content drives physiological reactions within seeds, as seed moisture increases, different physiological processes become possible as a range of metabolic and cellular processes engage (Walters et al. 2005). Five levels of hydration within seeds have been described (Walters et al. 2005) that reflect differing properties of water and the influence of these properties of seed physiology with the boundaries of zone 1: 0-15% relative humidity

(RH); zone 2 15-85% RH; zone 3: 85-95% RH; zone 4: 95-98% RH, and; zone 5: 98->99% RH. In the context of seed survival through the passage of fire, lethal temperature thresholds of seeds are highest when seed moisture content is within zone 1 or zone 2; lethal temperatures of seeds exceed 120 °C and as high as 150 °C (Tangney et al. 2018b). At moisture contents within zone 3, equivalent to >85% RH, seeds have significantly reduced tolerance to high temperatures, and death can occur at temperatures of < 100 °C, and experience on average a 31-38 °C reduction in lethal temperature thresholds whilst in zone 3 compared to zone 1 or 2 (Tangney et al. 2018b). Once seeds enter into this high moisture state (water activity zone 3) lethal temperature thresholds reduce to between 50-100 °C for most seeds (Tangney et al. 2018b), increasing seed sensitivity to elevated temperatures. Thus, the implementation of hazard reduction burns while seed moisture is high may increase the risk of mortality to soil stored seeds.

This study set out to investigate how seed moisture varies through the year and to identify the drivers of seed hydration throughout 2017 in Banksia woodlands. By ascertaining the drivers of seed hydration we were able to create predictions of seed hydration and hindcast periods during 2017 when seed hydration was highest during 2017 and therefore seeds were most vulnerable to elevated soil temperatures.

Methods

The rationale for the methods was to measure seed moisture throughout 2017 and identify weather and site variables that could predict high seed moisture. However, to compare seed moisture and physiological water activity within the seed, we first must create water sorption isotherms, which can be used to convert gravimetric water content to relative humidity, which is a direct measure of seed water activity. Once we had species specific isotherms, we could proceed to identifying weather and site variables that were able to predict when seeds entered into elevated seed water activity zones. The method rationale is outlined below in Fig. 1.

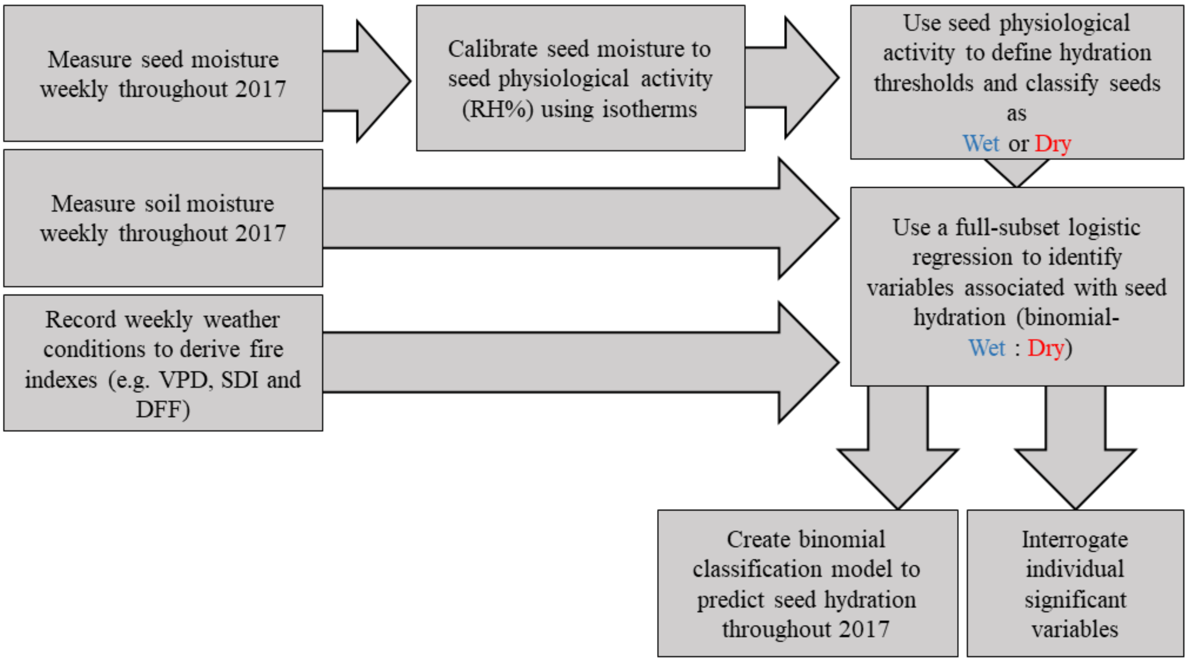


Figure 1: Methodical flow used in this chapter which aims to identify the drivers of seed moisture through 2017.

Measuring seed moisture content of seeds buried in soil

To assess variation in seed moisture content in the soil over a one year period, five species were selected for use: *Anigozanthos manglesii, Asparagus asparagoides, Banksia prionotes*, *Banksia sessilis* and *Conostylis candicans*. All species, except A*sparagus asparagoides*, are native to Banksia woodlands. Mature seeds of each of the four native species were collected from wild plant populations of remnant Banksia woodland within the Perth region during 2015. *Asparagus asparagoides* seeds, were collected from Kings Park woodland (an urban Banksia woodland situated in Perth, Western Australia) between October 2015 and April 2016. These species were selected as representative of common Banksia woodlands species that release mature seeds into the soil seed bank, and seeds of all species readily imbibe water. The inclusion of a non-native species was to allow for comparisons between seeds from native species and seeds from non-native species.

Seeds from each species were partitioned into 153 cotton mesh bags (holes 2µm in size), in order to retrieve three replicates bags for each species each week, for 51 weeks. The number of seeds placed into each bag varied according to seed weight. For *Asparagus asparagoides, Banksia prionotes* and *Banksia sessilis* there was 17 seeds per bag, for *Anigozanthos manglesii* 34 seeds per bag, and for *Conostylis candicans* 167 seeds per bag.

Bags were placed into shallow black seedling trays, with three replicate trays per species. Trays with perforated bottoms to ensure water can flow out, were placed next to each other along the edge of a sandy un-sealed track within bushland of Kings Park, Perth Western Australia. A shallow hole was dug to ensure there was soil contact with the base of the tray. Two centimetres of soil was placed on top of each of the trays, as well as a thin layer of leaf litter, similar to the surrounding undisturbed soil. The 15 trays were buried in groups of five, with each group within a 5 m of each other.

Each week for 51 weeks, one bag from each species from each replicate tray was removed and placed into an air tight resealable plastic bag, and taken immediately back to the laboratory at Kings Park for weighing. Seeds were removed from the cotton mesh bags and each replicate from each species was weighed separately, before being transferred to a clear plastic container for drying. Seeds were dried at 103°C for 17±1 hours before seeds were weighed again. Seed moisture was calculated using the following formula (wet seed weight-dry seed weight)/ dry seed weight x 100).

Measuring soil moisture content

Soil samples were taken from directly in front of each replicate of seed trays using an 8 cm x 2 cm clear plastic cylinder at the same time as seeds were collected. Each soil sample was placed in an airtight bag before being taken directly for weighing. Soils were weighed in the cylinder and then oven (Contherm, Korokoro, New Zealand) dried at 103°C for a minimum of

17 hours. Soil moisture was calculated following (wet soil weight-dry soil weight)/ dry soil weight x 100). Cylinder weight was removed from both wet and dry samples.

Weather data

Weather data for each day of 2017 was collected from the Bureau of Meteorology (Meteorology 2018) Perth Metro weather station, (Lat: -31.92, Lon: 115.87, height: 24.9 m); the closest Bureau of Meteorology weather station to the seed burial area. The weather data used included rain, maximum temperature, relative humidity and evaporation. For each seed collection date, data of weather variables were averaged over the week leading up to seed collection. From these weather variables, a range of weather and fire indexes were created, including, drought factor (DFF), Time since last rain event above 1mm (TSLR), soil dryness index (SDI) and vapour pressure deficient (VPD)

Construction of isotherms for seeds of each species

Water sorption isotherms, which depict the relationship between seed moisture content and equilibrium relative humidity were determined for each species. Three replicate samples of seeds from each of the five species were placed inside small paper envelops. The number of seeds per replicate within each envelope was 50 for *Anigozanthos manglesii*, 20 for *Asparagus asparagoides*, 20 for *Banksia prionotes*, 20 for *Banksia sessilis* and 200 for *Conostylis candicans.* Envelopes were placed inside an air-tight polycarbonate electrical enclosure box (28 x 28 x 14 cm; NHP Fibox, Australia), above a non-saturated solution of LiCl to achieve the desired relative humidity conditions; 15% , 20%, 30% , 50% , 70%, 80%, 90% and 95% RH (741.0 g L^{-1}, 640 g L^{-1}, 520 g L^{-1}, 364 g L^{-1} , 237 g L^{-1}, 171 g L^{-1} , 94 g L^{-1} , 48 g L^{-1} of LiCl, respectively; anhydrous, Sigma®, Australia) (Hay et al. 2008). To achieve 10% RH, a saturated LiCl solution was used and to achieve 5% RH a saturated $ZnCl_2$ solution was used (Vertucci and Roos 1990). All boxes were placed inside an incubator at 20°C.

After three weeks at respective storage conditions, seeds were retrieved and weighed, before being dried in an oven (Contherm, Korokoro, New Zealand) for 17 ± 1 hours at 103°C. Seed moisture content was determined gravimetrically on a dry weight basis calculated using the following formula (wet seed weight-dry seed weight)/ dry seed weight x 100). Plotted isothermal functions were derived from fitted 3rd degree polynomials. This allowed identification of seed hydration state by converting of seed moisture content to relative humidity using 3rd degree polynomials functions.

Model creation, optimisation and training.

This analysis is broken into four major elements. First, using the isothermic functions, seed moisture from each collection was converted to relative humidity and classified as either wet or dry. Second, we set out to identify weather indexes and site variables that can predict seed hydration state. Third, using the previously identified variables, construct a model to predict seed hydration throughout 2017. Forth, examine individual variables for their predictive ability in isolation.

Using the development of the species specific isotherms, seeds from each species for each of the 51 collection dates had their seed moisture converted to relative humidity using the isothermic function. Once each collection had a relative humidity associated with it: each collection was characterised based upon whether the relative humidity was >85% (wet) or below 85% (dry), termed throughout this chapter as seed Hydration statuses.

To model multiple variables that predict hydration status of seeds, multi-factorial generalized logistic regression models were used to test significant predictive effects with the dependent variable ’hydration status’ – the binomial characterisation of dry (< 85% RH) or wet (≥ 85% RH) seeds based on the isothermic relationships. The full predictive variable set used included, average soil moisture at time of collection, time since last rain event >1mm, ,Soil dryness index, Drought factor (DFF) and Vapour pressure deficit (VPD) all calculated from the preceding week before collection. All variables were scaled and centred. Soil dryness index (SDI), Drought factor and Vapour pressure deficit are all measures of soil dryness or low moisture availability and are commonly used by land managers to inform whether conditions are suitable for implementing managed burns.

Model ranking was carried out using MuMin package (Burnham and Anderson 2003) within R statistical program (R Development Core Team 2017). Model selection ranks all subsets of generalised logistic models including interactions between all predictor variables, with each candidate model ranked by Akaike information criterion (AIC), specifically AICc which compensates for small sample sizes. This provided comparisons to the global model and provided a set of top ranked models that were identified using a Δ AIC threshold ≥ 1. From the top ranked model set, final model selection was based upon three metrics: Akaike information criterion, log-likelihood, and residual deviance of the computed model. After optimisation, the top ranked model was subjected to a stepwise additive ANOVA, where each variable in the optimum model was added sequentially to determine additive effect on residual deviance.

Once optimum variables were identified, a step-wise addition ANOVA was conducted to examine the discrete effects of each variable added into the optimised model, as measured by the overall reduction in residual deviance.

a predictive model was constructed using the caret package (Kuhn 2008). A boosted logistic regression, 'LogitBoost' (Friedman et al. 2000) model was used to predict when seeds will be either dry or wet using optimised variable configurations, all of which were centred and scaled. Boosted logistic regression analysis is a machine learning approach where the combination of weak and inaccurate predictor variables are combined to provide highly accurate predictions for classification problems. In this predictive model, similar to the last model, seeds were classed as either dry or wet. All 255 measurements of seed hydration were passed in and separated by a 70/30 split into a training set and a testing set, comprising of 179 measurements in the training set and 76 measurements in the testing set. Using a boosted logistic regression model, comprising of the previously identified variables and cross validated 10 times before the entire cross validation procedure was repeated 300 times. Cross validation allowed for model tuning, specifically, tuning of the number of boosting iterations (nIter) used. The number of boosting iterations was selected based upon three metrics: lowest overall receiver operating characteristic (ROC), highest sensitivity, and highest specificity. After tuning, a confusion matrix was generated using the testing data to assess overall strength of the predictions made by the optimised and tuned model, and the model's ability to correctly predict seed hydration (Full analysis code including full subset regression and model training is included in supplementary materials).

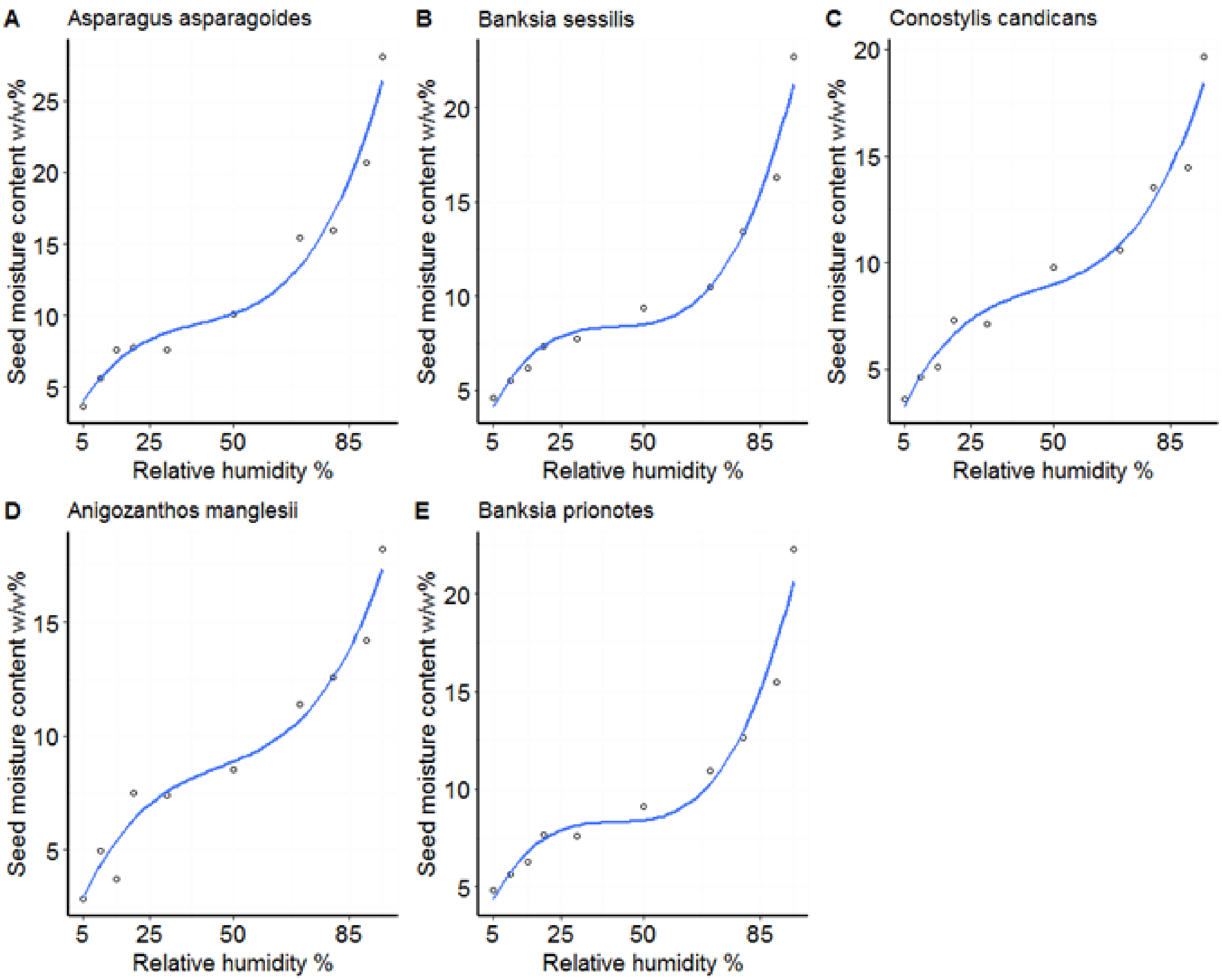
Individual variable assessment

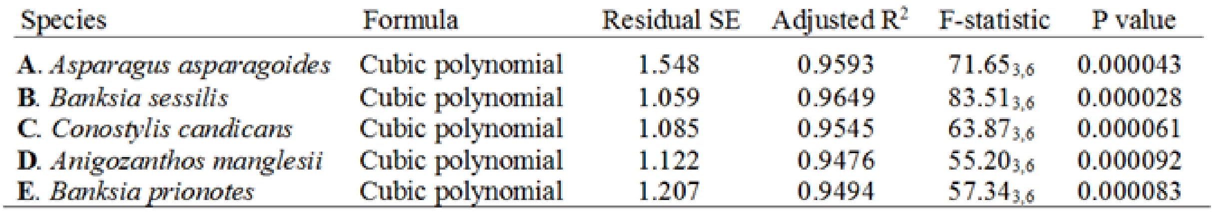
All the optimised variables identified in the earlier steps were assessed for their ability to explain variation in seed moisture content. Soil moisture content was compared directly to seed moisture content using linear models and assessed using r^2 values, allowing for the identification of soil moisture thresholds which may directly identify when seeds are wet. Days since last rain was modelled using curve-linear functions using 'DRC' package (Ritz et al. 2016). Curve fitting for identification of the most suitable function to describe seed moisture loss, was based upon log-likelihood estimations and Akaike Information Criterion (AIC). Curve fitting was limited to either a four-parameter non-linear Weibull (Equation 1) or a four-parameter log-logistic function (Equation 2), as outlined in Ritz et al. (2016); (d) is the parameter for maximum hydration, (b) the *slope* of the curve $F(x)$, and (e) the 50% point of the curve relative to the wettest part of the curve (e.g. Day zero) and (c) is the parameter for least hydrated state (for model selection see supplementary materials Table S2). Using this non-linear estimation of seed moisture loss, allows for an estimate of how many day are required for seeds to lose 95% seed moisture as explained by only one explanatory variable. A 95% decrease in seed moisture was chosen to represent seed hydration decreasing to the driest state, approximately 10% gravimetric moisture content. Weather variables were examined using single variable logistic regression analysis which allowed for estimations of strength and direction of correlated influences.

Results

Construction of isotherms for seeds of each species

The fitting of 3rd polynomials functions to the isotherms allowed for the description of the relationship between seed moisture and relative humidity for each of the collections undertaken in 2017 (Fig 2.). Based on the measured seed moisture content from the three replicate samples of seeds recovered each week, the average relative humidity of the seeds was calculated. Constructed isotherms conform to the typical sigmoidal shape. Each phase of the curve is approximately indicative of the physiological hydration state of the seed, water activity zone 1(≤ 15% RH), water activity zone 2 (> 15% but ≤ 85% RH) and water activity zone 3 (> 85% RH) (Fig 2.) Using the calculated relative humidity, hydration status of seeds was assigned using a binomial categorization: seeds were classed as wet if the modelled relative humidity was greater than 85% RH, and as dry if the modelled relative humidity was less than 85% RH.





Species	Formula	Residual SE	Adjusted R^2	F-statistic	P value
A. *Asparagus asparagoides*	Cubic polynomial	1.548	0.9593	$71.65_{3,6}$	0.000043
B. *Banksia sessilis*	Cubic polynomial	1.059	0.9649	$83.51_{3,6}$	0.000028
C. *Conostylis candicans*	Cubic polynomial	1.085	0.9545	$63.87_{3,6}$	0.000061
D. *Anigozanthos manglesii*	Cubic polynomial	1.122	0.9476	$55.20_{3,6}$	0.000092
E. *Banksia prionotes*	Cubic polynomial	1.207	0.9494	$57.34_{3,6}$	0.000083

Figure 2: Fitted isotherms using 3rd degree polynomial models of relationships between seed moisture content and relative humdity of seeds as drived from standardised storage conditions, each species was modeled indiviually and models were chosen based on goodness of fit. A) Asparagus asparagodies B) Banksia sessillis C) Conostylis candicans D) Anigozanthos manglesii D) Banksia prionotes. Table depics formula used in each isotherm and goodness of fit statistics including: residual standard error(SE), adjusted r^2 , F-statstic and P-value for each model. Subscripted numbers represent degres of freedom on the f-statistic.

Seed moisture of soil stored seeds

Regardless of species, changes in seed moisture content were uniformly rapid in response to rainfall events. Whilst there were differences in the measured seed moisture content between species at any particular retrieval time, patterns of hydration or desiccation were similar across all species. For example, during the last week of January seed moisture content increased rapidly, from 8-10% across all species, to 40 – 60% across all species (Fig. 3A) Similarly, following rainfall in the week of 13th of February, seed moisture content was between 20 – 40% across all species, driven by a large spike in positive net moisture that extended through the start of February (Fig. 3B). In between these rainfall events in January and February, seed moisture content dropped rapidly, to approximately 9 – 11%. A similar rapid wetting and drying event was evident during and immediately following a rainfall even in March (Fig. 3A and 3B). After June 12th, seed moisture remained relatively high (17 - 50% across all species) until October 30th (Fig. 3A). This period coincided with consistent rainfall, lower temperatures and high relative humidity (Fig. 3B). Seeds of *Conostylis candicans* and *Anigozanthos manglesii* had a maximum moisture content lower than *Banksia prionotes, Banksia sessilis and asparagus asparagoides*, which is particularly evident between June and September when seeds remained consistently hydrated (Fig. 3A) coinciding with an extended period of positive net moisture (Fig. 3B) .

Fig.

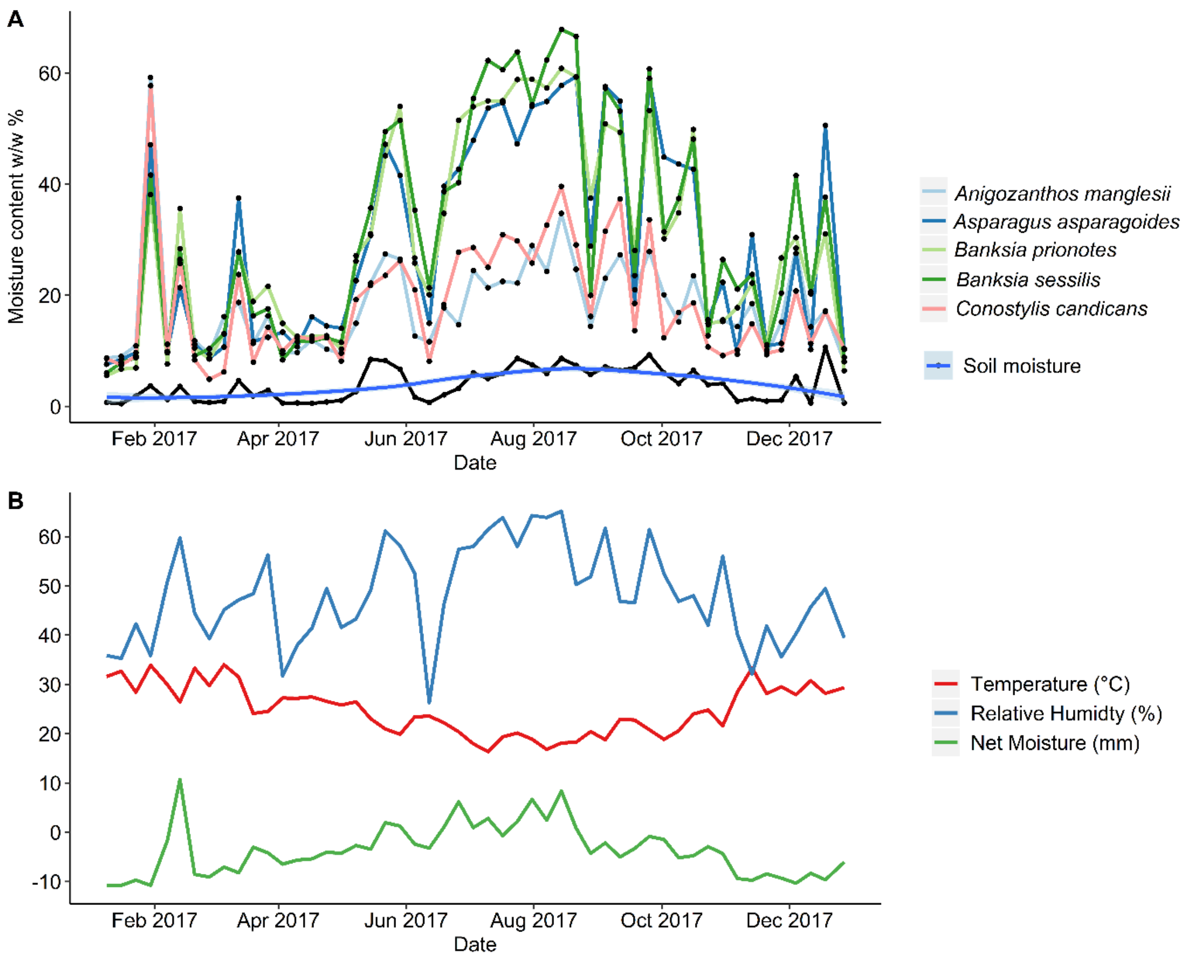


Fig. 3: Seed and weather data visulised weekly. A) Plotted mean seed and soil moisture content, averaged from three collections from each collection date. For each of the five species collections were taken every Monday for the year of 2017. B) Plotted mean air temperature and relative humidty averaged for the week preceeding collection dates,. Net moisture is the sum of rain and evaporation averaged for the week preceeding seed collection dates. All weather data taken from Perth metro weather station(Meteorology 2018)

Using the yearlong average seed moisture for each collection date (average seed moisture for each collection week - Fig. 3A) and combining the yearlong weather data (Fig. 3B), with hydration classification (derived from isotherms - Fig. 2), we were able to identify of two distinct periods of the year were seed moisture content was, on average the lowest (Fig 4). The first dry seed period extended from January 2017 through to May 2017, during which time the average seed moisture content ranged between 9 – 12% across all species, albeit seeds were measured to have high seed moisture content (>16 – 60%) four times during this period (Fig 4). The second dry seed period extended from 23rd of October 2017 through to the end of December 2017, when seed moisture content averaged 9 –14 % across all species. During this second period, there were five instances when seed moisture was higher (ranging from 16 – 41 %). Outside of these two periods seed moisture remained high, approximately 35 % seed moisture on average. (Fig 4).. Using this upper SDI boundary, reduces the autumn burning window to the first two weeks of April, while the spring window remains unchanged, albeit the spring period is not a consistently dry period as three of the six weeks during the spring window experience elevated seed moisture (Fig 4.).

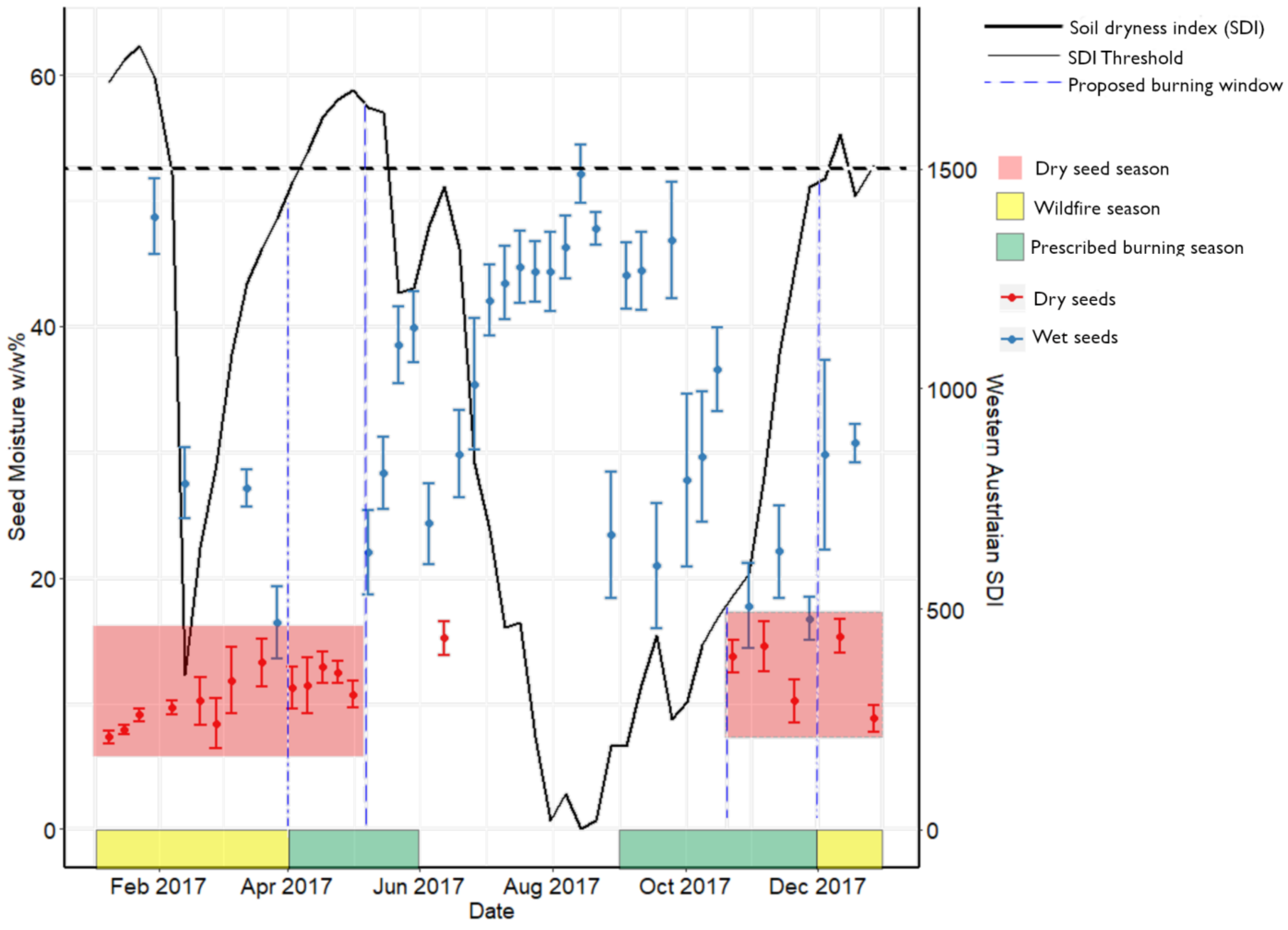


Figure 4: Pooled seed moisture for each collection date including seeds from all species from 2017. Hydration status of each collection date is included, seeds less than 85% RH classified as dry, seeds higher than 85% RH were classifed as wet. Red rectangle highlights are used to designate periods where seeds were dry more freqently than wet. Wildfire season extends from December 1 until March 31st every year, during this time no prescription burning is permitted Prescription burning season starts April first and extends until May 31st, before starting again on Sepetermber first and extending until November 31st. Using the SDI here, we were able to further define a burn window. The upper bound of 1500 SDI is a common metric that is used in Western Australia that defines conditions that are not suitable for managed burns. As a SDI >1500 is indicative of weather conditions and fuel moistures that increase fire intensity and spread, SDI threshold represents an upper boundry on prescription burning opperations, when

SDI exceeds 1500, prescription fire opperations cease. The proposed burning window idenetified in this figure is when prescirbed burning in 2017 would have had the least impact on soil stored seeds. Fire index data from (Meteorology 2018). SDI threshold information from (Council 2016).

Model creation, optimisation and training.

Following the classification of each seed moisture collection based on seed hydration, we built a model to identify predictive variables associated with seed hydration, and specifically what weather variables or site variables were most influential. Using a ΔAIC threshold of ≤ 1, two top ranked models where chosen for manual selection. (Table 1).

Table 1: Model selection table of the three highest preforming multi-factorial linear models derived from MuMin package (Burnham and Anderson 2003). Selection of the optimum model was based upon, log- likelihood, AICc and residual deviance. Model 2 was selected for further use. Estimates and Standard errors are reported, significant variables are bolded (<0.05).

Dependent variable: Hydration status

Model: binomial, link: logit	Model 1	Model 2
(Intercept)	- -0.574 (0.531)	-1.543 (1.037)
Soil Dryness index		0.007 (0.006)
Soil Moisture Content w/w%	**0.857 (0.171)**	**0.953 (0.193)**
Days since last rain event above 1mm	**-0.133 (0.042)**	**-0.145 (0.043)**
Observations	255	255
Log Likelihood	-72.986	-72.344
Akaike Inf. Crit.	152.1	152.8
Weight	0.305	0.207
Residual Deviance	145.97	144.69

Manual model selection, used Log-likelihood, AIC and residual deviance to select the most suitable model. Model 2 was chosen and contained the variables:; Average soil dryness index, averaged across the week preceding seed retrieval, soil moisture content w/w% taken on the day of retrieval and number of days since last rain (DSR) (Table 1). Both ranked models were not significantly different from each other (p = 0.2574), but model 2 had a marginally higher logliklihood, and residual deviance compared to the other three top ranked model (Table 1.)

Table 2: Analysis of variance table of the optimised model used to identify variables associated with characterising seeds into a binomial classification of hydration status. Identified using MuMin package (Burnham and Anderson 2003). Variables that are significant (<0.05) are bolded.

Dependent variable: Hydration status

Model: binomial, link: logit	Estimate	Standard Error	z value	P(>|z|)
(Intercept)	-1.543	1.037	-1.488	0.137
Soil dryness index (SDI)	0.007	0.006	1.100	0.271
Soil Moisture Content w/w%	0.953	0.193	4.926	**<0.0001**
Days since last rain event above 1mm	-0.145	0.043	--3.339	**<0.0001**

Akaike Inf. Crit : 152.69

Null deviance : 324.62 On 254degrees of freedom

Residual Deviance: 144.69 On 251 degrees of freedom

Once we had identified the top ranked model, we examined the individual variable effects (Table 2). The top ranked model, shows a 186 point reduction in residual deviance compared to the null model deviance (Table 2). Soil moisture content, DSR and average evaporation were all significant variables within the top ranked model (Table 2), whereas average maximum temperature and average relative humidity, while included in the top ranked model, were not significant. The largest decrease in residual deviance was observed after the addition of soil moisture, resulting in a 79 point reduction in residual deviance, followed by a 38 point drop after the inclusion of average maximum temperature (Table 3).

*Table 3**:** Additive analysis of sequential stepwise addition of variables contained in the top ranked model to estimate additive effect of each variable on total deviance. Null represents a base model containing no variables. D.f refers to degrees of freedom.*

Dependent variable: Hydration status

Model: binomial, link: logit	D.f	Deviance	Residual D.f.	Residual Deviance
Null	**1**		**254**	**324.62**
Soil dryness index	1	65.01	253	259.61
Soil Moisture Content w/w %	1	97.43	252	162.18
Days since last rain event above 1mm	1	17.48	251	144.69

Once we had identified the most useful predictive variables, we package the variables together within a boosted logistic model (Logitboost) in order to predict seed hydration. The boosted logistic model yielded high predictive accuracy > 90% accuracy (Table 4) and was able to successfully predict whether a seed was dry (i.e. seed relative humidity <85%) or wet (i.e. seed relative humidity >85%) with 92% accuracy (95% CI: 83—97% accuracy). The boosted model was able to assign true wet results 96% of the time (specificity) and true dry results 84% of the time (sensitivity) which results in a balanced accuracy of 90%, significantly higher than a random guess model (i.e. Accuracy > no Information rate) (Table 4).

*Table 4**:** Result in the form of a confusion matrix and statistics of tuned boosted logistic predictive model to classify seed hydration in wet and dry seeds. Cross validation and tuning was conducted in caret (Kuhn 2008).*

		Prediction	***Reference***
Dependent variable: Hydration status, binomial “wet” vs “dry”		Dry	Wet
Model: Boosted Logistic Regression,	Dry	21	2
Binomial ‘LogitBoost’ with repeated cross validation.	Wet	4	49
Accuracy	0.9211		
95% CI	0.836-0.9705		
Sensitivity	0.8400		
Specificity	0.9608		
Balance Accuracy	0.9004		
P-Value (is Accuracy > no Information rate)	**>0.0001**		

Individual variable assessment

Using the variables identified as being most significant at predicting seed hydration, we analysised their effects to assess predictive strength in isolation. Examination of the most significant predictive varibles provided insight into the patterns most influential to driving seed hydration. DSR was a significant predictor in all four top models (Table 2), and displayed a distinct non-linear relationship with seed moisture; requiring approximately 9.3 ± 3.1 days (95% CI: 3.4 -15.1 days, Fig. 5) for seeds to lose 95% of their moisture. Soil moisture was a significant predictor in all four top models (Table 2) and was able to explain 64% of the variation in avgerage seed moisture content (Adjusted r^2=0.64, p-value:<0.0001, Fig. 6).

Soil dryness index was significanly correlated with average seed moisture trhoughout 2017 (Table 2), displaying a strong negative correlation, as SDI increases, seed moisture decreases (Adjusted r^2=0.36, p-value:<0.0001 (Fig. 7).

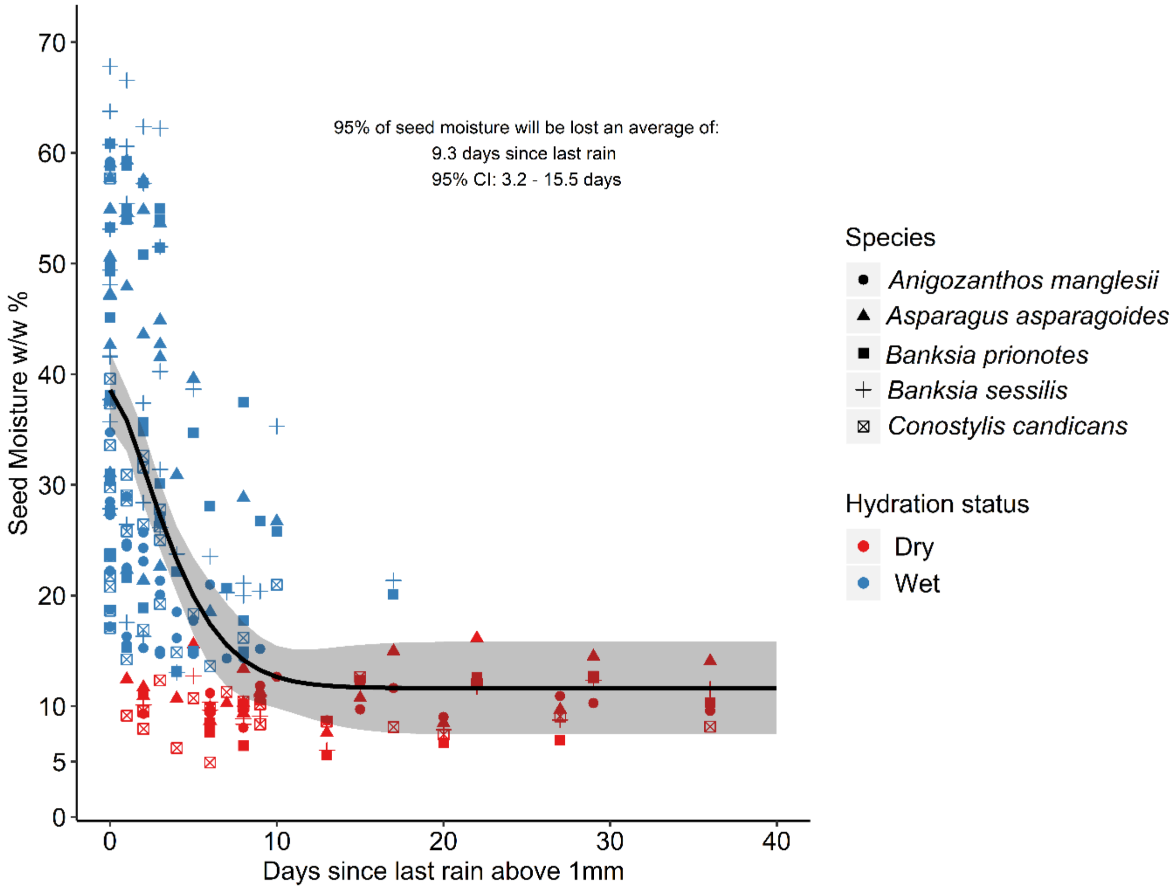


Figure 5: Modelled response between seed moisture content and days since last rain above 1mm, seed moisture reduces as the number days since last rain above 1mm increases. Used to estimate the number of days required for seeds to lose 95% of water down from their highest hydrated state. Individual species represented by unique shapes and mean response, with 95% confidence interval around the mean for pooled species response is plotted. Hydration status of individual seed collections shown in colour to highlight how the hydration status of the seed changes with number of days since last rain event above 1mm.

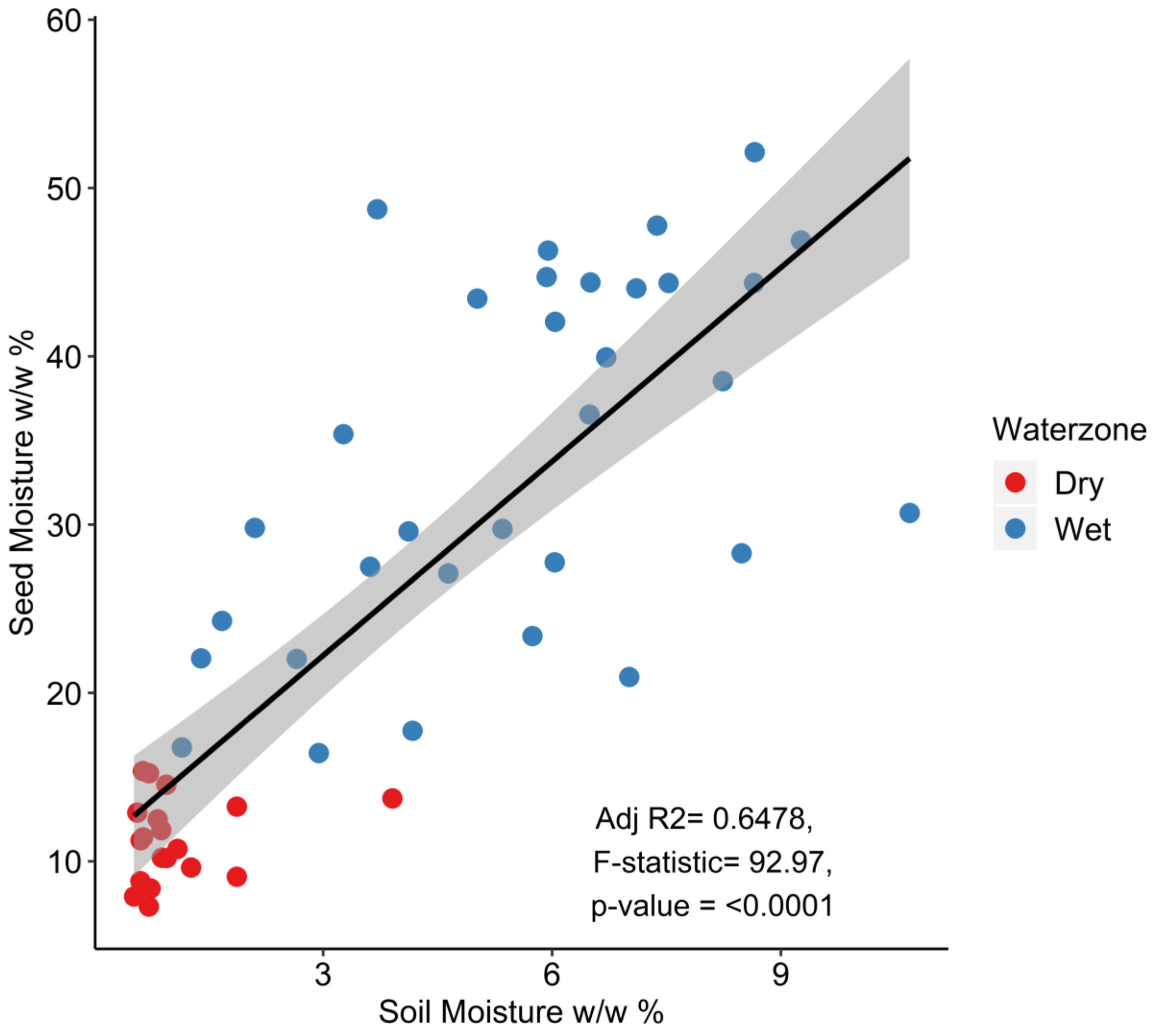


Figure 6: Modelled relationship between average soil moisture and average seed moisture, 95% CI around the mean plotted. Hydration status of average seed collections shown in colour to highlight the range of soil moistures that are associated with dry and wet seeds respectively.

Figure 6:

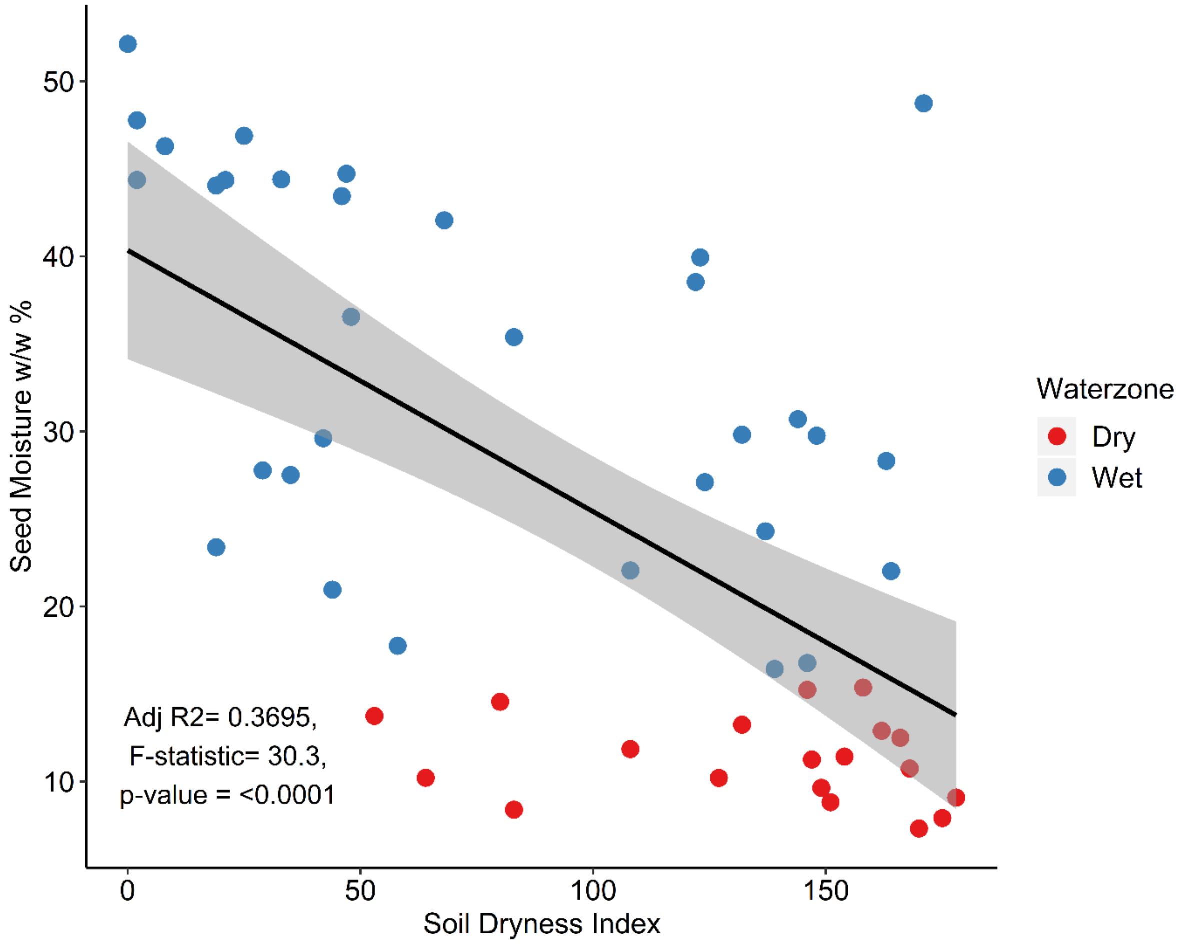


Figure 7: Average Soil dryness index as it relates to with average seed moisture of each of the 51 collections taken through 2017. All index values calculated from weather measurements were taken from Perth Metro weather station, Latitude -31.92 Longitude 115.87 and a height of 24.9m.

Discussion

The model presented here identifies a set of variables that when combined can predict seed hydration throughout 2017. Our model successfully predicted seed hydration correctly 92% of the time and provides clarity on what drives seed hydration and periods of the year when seeds are most vulnerable to elevated temperatures in soil.. These data presented here, and the associated analyses, provides an opportunity to focus on identifying a fire implementation window, that includes consideration of the risk posed to soil seed banks as a result of increased seed moisture.

Two distinct time periods during 2017 were identified that coincided with low seed moisture content, one during late summer and autumn (January- May) extending 17 weeks, and again in spring and early summer (October – December), extending 9 weeks. During these times, seed moisture contents were equivalent to 10-75% RH, except for the 9 weeks where seed moisture exceeded 85% RH, four in the first half of the year (January- May) and five in the later half (October – December). While seeds are dry, the likelihood of seeds surviving the temperatures associated with fire is much greater, with lethal temperatures of seeds exceeding 120 °C for the study species (Tangney et al 2018). However, there was more frequent periods of high seed moisture during the prescribed burning season that extended from September to November compared to prescribed burning season during autumn that extended from March to May. During the spring period (September to November) seeds were determined to be wet (> 85 RH) five times. The lethal temperature of seeds at these elevated moisture contents is much lower, for these species c. 31-38 °C lower than when seeds from the same species are dry (Tangney et al 2018), which may result in higher seed mortality from burns conducted between September and November. The elevated risk posed in spring indicates that if hazard reduction burns had biodiversity outcomes (e.g. stimulating soil stored seeds (Bell et al. 1993)) associated with their prescription, the focus of activities should have been conducted between March and May in 2017. This is in line with evidence that suggest that plants have a reduced response to spring fires, including reduced seedling survival through summer following spring burns due to a reduced growth period before the summer drought (Ooi 2010), delayed or reduced emergence due to a mismatch in germination cues following spring burns (Roche et al. 1998) and reduced post-fire flowering following spring burns (Bowen and Pate 2004). Further, burning activities while seeds are high in moisture, may reduce seed survival, favouring those species that remain dry throughout the year, such as seeds with water impermeable seed coats (e.g. seeds with physical dormancy)

Currently, implementation of hazard reduction burns uses a combination of local weather variables including temperature, relative humidity, wind speed and direction, local site variables like fuel moisture as well as forecast fire metrics including, soil dryness index (SDI) and forest fire danger index (FFDI) in order to assess the risks of implementing burning practices and decide whether conditions are manageable (Anderson 1982, Sullivan et al. 2012). By combining some of these weather variables with known fire season information and fire indexes, we were able to further define periods of the year when prescribed burning activities could be used to avoid periods with elevated seed moisture (Fig. 3). Using the two periods identified, 1) Autumn burning period - March to May and 2) Spring burning period - September to November, we were able to show that in the spring period, for three out of the six week period seeds were wet. Whereas throughout the autumn period there were five weeks where seeds were consistently dry, this would have been the period in 2017 when fire impacts on soil seed banks would have been minimized. However, during this same autumn period the SDI in the Perth region exceeded 1500 which is the upper limit of fire implementation in Western Australia (Council 2016) as conditions are conducive to adverse fire behaviour, resulting in the cessation of burning activities. Incorporating the SDI threshold reduces the possible burning window in autumn to approximately two weeks, while the spring period remained unchanged. This emphasises that only a small window of the year is available to fire managers for burning programs if the implementation of fire programs is for the benefit of soil seed banks.

Seed moisture decreased 95% over an average of nine days following a rain event greater than 1mm (DSR - 95% confidence interval of 12 days, 3-15 days following rain). The use of 95 % moisture loss as a metric was to estimate how many days without rain was required for seeds to return to a dry state. Using DSR as a metric, we were able to demonstrate that there was eight weeks when the time since last rain exceeded nine days, all of which occurred in the first half of the year (Fig 4.). Despite five occurrences in the second half of the year where seeds were dry, each occurrence was followed by rain, interrupting the proposed burning window. By identifying a nine day window, we can be confident that seed moisture is low. Using DSR as a single metric may provide a simple estimation of seed hydration that could be used in other years, particularly if combined with the other variables included in this analysis, such as weather variables and soil moisture.

In this study, seeds were buried under 2 cm of loose soil and a shallow layer of leaf litter. As such, soil moisture may be lost more quickly compared to the rate of moisture loss deeper within the soils, or under heavier litter (Tromp-van Meerveld and McDonnell 2006). Deeper burial, or more leaf litter, may potentially extend the number of days required for seeds to lose sufficient moisture to enter a dry state. Moisture loss from soils is reduced further by the presence of ground cover, including shrub and canopy cover, leading to a reduction in evaporation of moisture from soils (Lamb and Chapman 1943). Consequently, seeds within soils under shrub or canopy cover may remain hydrated for periods significantly longer than seeds measured in this study.

Through the use of machine learning, we were able to group together a set of variables that individually did not provide strong predictive capacity and package them into a robust logistic classification model, through the use of additive boosting. Additive boosting increases the predictive power of classification models by sequentially applying reweighted versions of the training data variables and taking a majority vote of the sequence of classifiers that are produced (Friedman et al. 2000). By reapplying and reweighting the training variables this method, in cases like the one presented here, lead to large increases in predictive power (Friedman et al. 2000).

The model presented here is a single metric and should not be the only piece of information that is used to define potential burning windows. This metric does not attempt to quantify fire risk, or risk of high intensity fires, which have wide ranging implications for fire managers and ecosystem response.

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Chapter 5: Supplementary materials

```
#global model for variable identification used in predicting Water zone
seed.gbl<- glm(Waterzone~ SoilMC*TSLRB+SDIP+DFF+VPD,  family = binomial, data=data3)
options(na.action = "na.fail")
dd1 <- dredge(seed.gbl,rank="AICc")
par(mfrow=c(1,1))
par(mar = c(3,5,6,4))
plot(dd1, labAsExpr = TRUE)
dd1
#identifying Models =< 1 Delta AIC
opti<-(model.avg(dd1, subset = delta < 1))
summary(opti)
#Sub-setting those models
optimod1<- get.models(dd1, subset=1) [[1]]
optimod2<- get.models(dd1, subset=2) [[1]]
optimod3<- get.models(dd1, subset=3) [[1]]
optimod4<- get.models(dd1, subset=4) [[1]]
#investigating each model
summary(optimod1)
summary(optimod2)
summary(optimod3)
summary(optimod4)
#testing significance between each top ranked model
anova(optimod1, optimod2, optimod3, optimod4, test= "Chisq")
#step-wise addition anovoa to identify individual variable effect
anova(optmod2)
#Data partition of testing and training data 70/30 spilt
inTrain = createDataPartition(
  y = data3$Waterzone,
  p=.70,
  list = FALSE)
training = data3[inTrain,]
testing = data3[-inTrain,]
nrow(training)
```

```
nrow(testing)
#setting seed
set.seed(123)
#setting control options
ctrl <- trainControl(method = "repeatedcv", number = 10, repeats = 300,  classProbs = TRUE,
summaryFunction = twoClassSummary, savePredictions = TRUE)
#defining training model, variables, method, data pre-treatments, tune length and metric.
seedFit<- train(
  Waterzone~ ((SoilMC)+(TSLRB)+(SDIP))+(TSLRB),
  data = training,
  method ="LogitBoost",
  preProc= c("center", "scale"),
  tuneLength=20,
  trControl = ctrl,
  metric = "ROC",
  maximize = FALSE )
#visualizing model output for each tune length
seedFit
#visualizing variable importance
varImp(object = seedFit)
#plotting variable importance
plot(varImp(object= seedFit))
#plotting ROC variation across tune length
ggplot(seedFit , xlab="Bootsting iterations" , ylab= "Repeated cross-validation" )
#Predictions of new classes and probabilities based on SeedFit model
seedFitClasses<- predict(seedFit, newdata= testing)
seedFitProbs <- predict(seedFit, newdata = testing, type = "prob")
#output of confusion matrix using class fits and testing data
confusionMatrix(seedFitClasses, testing$Waterzone)
```

Figure S1. Full model code of model ranking and predictive model fitting for the binomial predictive model of “waterzone”

Table S2: Full model description following model selection in DRC for seed moisture. Model selection used AIC and Log- likelihood to identify the most parsimonious model for each species. Final model selection for each species is bolded. Model selection was either a three *parameter Log-logistic model (LL.3, LL2.3, LL.3u or LL2.3u) or a three parameter Weibull model (W1.3 or W2.3)*

Species	Model	LogLik	AIC	Lack of fit	Res var
Seed Moisture	**W1.4**	**-1201.05**	**2412.11**	**0.059**	**152.21**
	LL.4	-1201.41	2412.82	0.050	152.57
	LL2.4	-1201.41	2412.82	0.050	152.57
	W2.4	-1202.48	2414.97	0.028	153.65

“I may not have gone where I intended to go, but I think I have ended up where I needed to be”

- Douglas Adams

Chapter 6: General Discussion

Seed survival through fire is complex, as interactions between soil temperatures, seed moisture content, fuel loadings, weather conditions and species-specific seed traits all play a significant role in defining whether an individual seed will make it through the rigors of exposure to fire. Nevertheless, post-fire recruitment from seed is a major contributor to population persistence and growth in fire-prone systems. This thesis examined the capacity for seeds to survive through fire and identified a range of species-specific responses which contribute to defining the emergence niche of seedlings following fire.

For the duration of this thesis, the aims have concentrated on defining fundamental seed processes associated with seed survival and seedling emergence in fire prone ecosystems.

1. *Seed survival through fire*
    - Identifying lethal temperature thresholds of seeds from species of Banksia woodlands and the effects of seed moisture content on lethal temperature thresholds;
    - Identifying the range of soil temperatures experienced during fire in Banksia woodlands;
    - Identifying fuel loadings that are conducive to soil temperatures exceeding lethal temperature thresholds of seeds; and
    - Identifying weather conditions that predict elevated seed moisture content.
2. *Seedling emergence*
    - Identifying the maximum emergence depth of seeds from Banksia woodlands species;
    - Identifying the proportion of seedling emergence between 0 and 10 cm; and
    - Measuring the rate of emergence of seedlings between 0 and 10 cm.

Each of the four experimental chapters in this thesis encapsulate complex interactions between seeds and their environments, whilst addressing an individual element of seed survival through fire and the seedling emergence process in fire-prone ecosystems. This final chapter will synthesize the research presented in those chapters, highlighting the significance of the findings and identifying areas of potential future research opportunities.

The factors that influence seed survival through fire and emergence are multifaceted and interrelated (Fig. 1, Table 1). While this thesis attempted to address many of the processes involved in understanding seed survival through fire, and subsequent emergence of seedlings, understanding the interactions that ultimately drive seed survival requires understanding the entirety of the system.

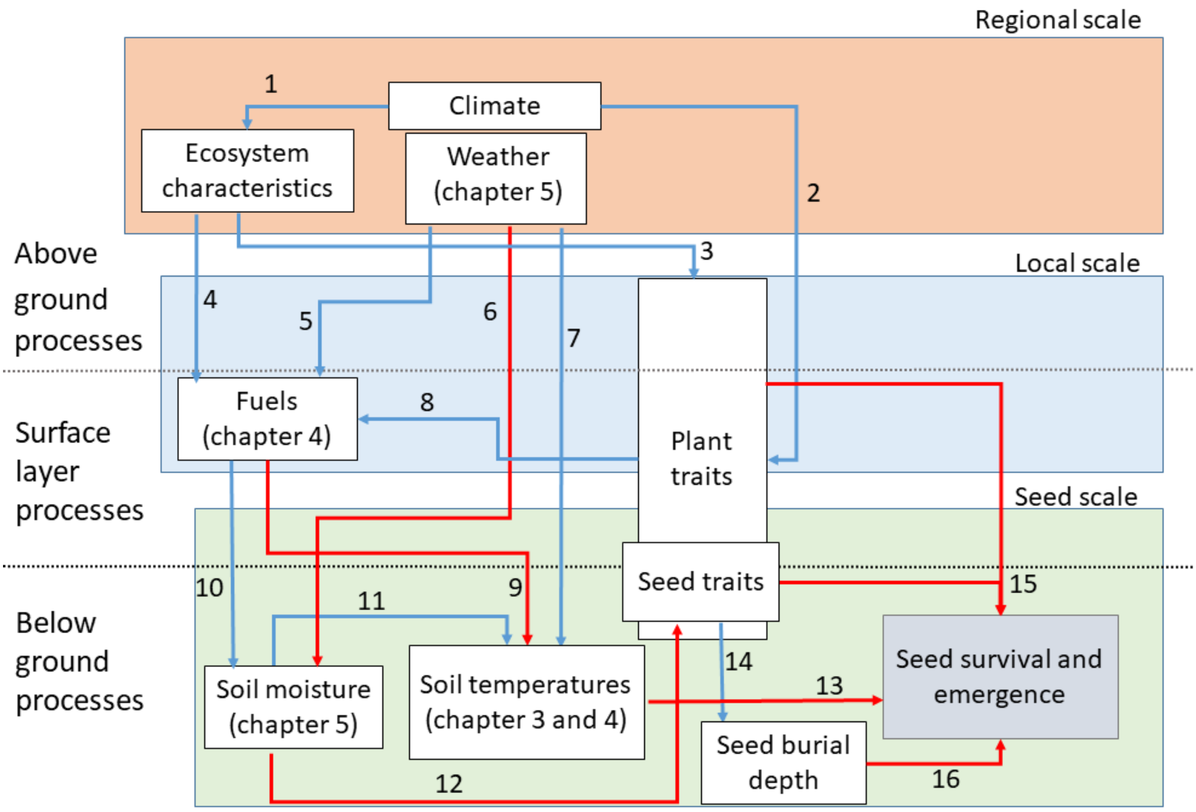

Figure 14: Conceptual diagram of the interactions between factors that affect seed survival through fire and seedling emergence. Boxes depict individual elements that directly or indirectly affect seed survival, arrows between groups depict interactions. Red arrows are those interactions that were explored in this thesis, while blue arrows are those interactions

that were not directly explored in this thesis, but that have been identified in existing literature (Table 1). The inclusion of scale boxes is to indicate the spatial scale that those factors are acting at and the interactions between these scales.

Table 1: Interactions between factors affecting seed survival though fire and seedling emergence as depicted in Figure 1.

Interaction	**Factors**		**Observations on Interactions Between Factors**	**Reference**
1	Climate	Ecosystem characteristics	The climate of the region defines the type of ecosystem in the region, including the type of vegetation and historic fire regime.	(DeBano et al. 1998, Enright 2011, Keeley 2011)
2	Climate	Plant traits	The climate of the region will dictate the range of seed and plant traits expressed by vegetation in the region.	(Keeley et al. 2011, Lamont et al. 2011, Pausas and Keeley 2014)
3	Ecosystem characteristics	Plant traits	The ecosystem characteristics define the expression of plant traits within local regions. (e.g. serotiny or resprouting)	(Lamont et al. 1991, Lamont et al. 2011)
4	Ecosystem characteristics	Fuels	The ecosystem of the region defines the rate of fuel accumulation, composition of the fuel bed and influences the moisture of the fuel bed.	(Grant et al. 1997, Dimitrakopoulos 2002, Andersen et al. 2005, Cawson et al. 2017)
5	Weather	Fuels	Localized weather conditions will influence the fuel moisture and rate of moisture loss or gain.	(Fosberg and Deeming 1971, Eltahir 1998)
6	Weather	Soil moisture	Localized weather conditions will influence the soil moisture and rate of moisture loss or gain from the soils.	Chapter 5 of this thesis and Turner et al. (2006)
7	Weather	Soil temperatures	Localized weather conditions directly impact fire behaviour including fire severity and intensity.	(Bradstock et al. 2010, Flannigan et al. 2013)
8	Plant traits	Fuels	Plant traits effect the rate of fuel accumulation, the composition and	(Schwilk and Ackerly 2001, Schwilk and

			flammability of the fuel bed.	Caprio 2011, Pausas and Moreira 2012)
9	Fuel loading	Soil temperatures	Weight, composition and moisture of the fuel bed directly effects soil heating.	Chapter 4 and (Busse et al. 2010, Saura-Mas et al. 2010, Santana et al. 2011, Wright et al. 2016)
10	Fuel loading	Soil moisture	Fuel loading may influence how much moisture is received by the soil and the rate at which soils absorb and desorb moisture.	(Williams et al. 1990)
11	Soil moisture	Soil temperatures	Soil moisture directly influences soil temperatures: as soil moisture increases, more thermal energy is required to heat soils.	(Aston and Gill 1976, Stoof et al. 2013)
12	Soil moisture	Seed traits	As demonstrated in chapter 5, soil moisture has direct influence on seed moisture content, which in turn effects the lethal threshold of seeds during fire.	Chapter 5 and chapter 2, as well as (Fer and Parker 2005, Turner et al. 2006, Ruprecht et al. 2015)
13	Soil temperatures	Seed survival and emergence	As demonstrated in chapters 3 and 4, soil temperatures can exceed lethal temperatures of seeds and in some cases this may reduce seedling emergence.	Chapter 3 and chapter 4 as well as (Busse et al. 2010)
14	Seed traits	Seed burial depth	Seed traits influence the depth of burial of seeds, for example, the hydroscopic awn of Austrostipa seeds drive seed burial.	(Smith et al. 1999, Saatkamp et al. 2014)
15	Seed and plant traits	Seed survival and emergence	As demonstrated in chapter 2, seed and plant traits directly influence a seeds ability to survive through fire.	Chapter 2
16	Seed burial depth	Seed survival and emergence	The depth at which seeds are buried will directly influence a seeds ability to survive or to emerge.	(Saatkamp et al. 2014) How deep seeds can emerge from was addressed in Chapter 4.

The data presented in this thesis furthers our understanding of seed survival through fire and the seed, plant, and environmental traits that influence seed sensitivity to fire, that drive survival of canopy and soil-stored seed banks and post-fire regenerative capacity. Including a consideration of seed sensitivity to fire will increase our ability to generate meaningful data and to implement fire regimes that consider the consequences on biodiversity. The collective goal of future research into seed survival through fire should be to enable landscape-scale modelling of seed survival by linking seed physiological traits, fire dynamics, and the influence of local weather conditions to accurately predict seed survival.
Ecologically, this thesis addressed interactions between fire, soil heating, and fuels; and addressed significant variables that influence seed survival. Most significantly, this thesis demonstrated that elevated seed moisture content fundamentally changes seed survival and consideration of seed moisture content when implementing fire into environments may lead to increased post-fire response from soil stored seeds. Further, this thesis demonstrated that seed traits influence seed survival through fire and emergence from soils. For example, embryo class significantly influenced seed lethal temperature thresholds, while increasing seed weight led to deeper maximum emergence. Coupled with the ability to predict seed hydration status, defining interactions between seed traits, seed survival and emergence may allow for directed management to focus on biodiversity impacts during fire implementation.

The following sections of this thesis set out a pathway of future work that will aid in understanding the interactions that drive seed survival through fire.

Seed moisture content as a primary trait regulating the lethal temperature thresholds of seeds – Chapter 2.

In Chapter 2, the aims were to define the lethal temperatures required to kill seeds from Banksia woodland species, to establish if seed moisture content influenced lethal temperature thresholds, and to determine whether any particular seed trait or suite of seed traits could aid in predicting the lethal temperature requirements of seeds. The results of chapter 2 definitively showed that seed moisture content at the time of exposure dictates the lethal temperature thresholds of seeds. Increased seed moisture content had an overwhelming effect on seed lethal temperature thresholds, with 17 out of 18 species displaying a significantly lower T_{50} when seed moisture content was ≥ 95% RH. For seeds at this level of hydration, across all species the average decline in T_{50} was between 31-38 °C, compared to seeds at low moisture content (median decline 50% RH and 15% RH respectively).

The ability of dry seeds to tolerate exposure to significantly higher temperatures compared to wet seeds has its roots in fundamental seed physiological processes, potentially extending beyond simply wet seeds having high water content which in turn may damage seed tissues when heated rapidly. During maturation and drying, seeds utilise several mechanisms to protect cellular machinery and architecture that is vital to seed viability, including the synthesis of large macromolecules like proteins and membranes (Bewley et al. 2013). One class of proteins that may confer protection during high temperature exposures are heat shock proteins (HSP) and small heat shock proteins (sHSP) (Wehmeyer et al. 1996). Expressed during the late maturation stage of seed development, sHSP act as chaperones, wrapping around other proteins and molecules to guide them around the cell (Wehmeyer and Vierling 2000). These molecules are increasingly expressed following exposure to thermal shock, driven by the stimulation of heat shock transcription factors (Wehmeyer et al. 1996). This upregulation of sHSP (and HSPs) to a higher expression level following heat shocks may confer increased temperature tolerance in dry seeds. As seed moisture content increases, HSPs and sHSPs are down regulated and become less abundant as seeds transition into a hydrated state (Wehmeyer and Vierling 2000). To test whether sHSPs or HSPs are conducive to higher lethal temperature thresholds, future research should attempt to identify HSPs and sHSPs in native seeds and examine their expression at different hydration states. Linking their expressions at different hydration states to the seeds ability to survive heat shock will aid in the description of their functional role in protection during heat shock.

Despite the results I presented in this chapter, unanswered questions still remain. Specifically, there is uncertainty around the importance of exposure time on seed survival (Paula and Pausas 2008) and whether length of exposure, cumulative exposure, or peak temperature, is more important for seed survival through fire. The analysis presented in this chapter provides a pathway for direct comparisons of the effects on exposure time on seed survival. By using time as the treatment instead of relative humidity (or inclusive of relative humidity), direct comparisons could be made between many different exposure times on the same axis, providing a way to synthesize the effects of length of exposure and temperature together. However, selecting a single exposure time as I did in this chapter made for simple comparisons between species under the same treatment effects, which allowed clear estimates of the effect of seed moisture on lethal temperatures.

A method for extensive spatiotemporal assessment of soil temperatures during an experimental fire using distributed temperature sensing in optical fibre – Chapter 3.

This chapter described a new method for measuring soil temperatures during fire using Distributed Temperature Sensing (DTS) in optic fibre. DTS provided an increased spatial and temporal resolution, as well as increasing data generated from an individual fire. Taking a step beyond point based measurements to continuous measurements throughout a burn area, chapter 3 described significant variation in soil temperatures at spatial resolutions < 1 m, capturing areas of intense soil heating > 200 °C and areas that experienced no soil heating from fire effects.

The use of DTS to measure soil temperatures in fire has great potential, as it provides an opportunity to capture spatial and temporal measurements beyond what has been possible in the recent past. Despite being first described in the mid 1980's (Hartog et al. 1985), DTS is an emerging technology that greatly improves measurement capabilities across a wide range of applications, including, measurement of deep ocean infrastructure (Hartog et al. 2018), oil and gas monitoring (Smolen and van der Spek 2003) hydrogeological monitoring (Simon et al. 2017) and a growing use in measuring fire temperatures including measurement of surface temperatures during fire (Cram et al. 2016).

Other developing applications for DTS include soil moisture sensing (Steele-Dunne et al. 2010) which, when combined with temperature sensing, would transform the ability for ecologists to understand patterns of soil hydration and soil temperature dynamics at vastly increased scales and would be a fundamental step change in seed ecology. Two prominent forms of soil moisture monitoring are currently used. The first is Active-Distributed Temperature Sensing (A-DTS, Simon et al. (2017)), where a metal wire is run alongside the measurement fibre, the metal wire is then heated, and the rate at which the temperature of the measurement fibre heats up is contingent on the soil moisture. The second form of soil moisture monitoring is passive distributed temperature sensing (P-DTS, Steele-Dunne et al. (2010)), where two fibres are laid at different known depths within the soil, parallel to each other, and temperature measurements taken from both fibres. The difference in rate of heating between the upper fibre and the lower fibre can be converted into an estimation of soil moisture (Lu et al. 2016). Both forms of soil moisture monitoring have their advantages, yet both applications are in their infancy and require significant development.

The use of DTS to answer ecological questions is increasing (Tyler et al. 2009) and emerging research is further expanding the usefulness of DTS in ecological studies. However, current technical limitations constrain the further development of DTS in soil temperature measurements during fire. For example, minor inaccuracies in burial depth of the fibre, which may be consequential for measured results, and the amount of labour required to install the fibre, restricts further adoption of the current method presented in chapter 3. One solution to this may be to develop a plough system that directly feeds the fibre into the soil at a known depth, while ploughing a path for the fibre to lay in and simultaneously burying the fibre as the body of the plough moves along, which would provide increased burial accuracy and reduce the labour required to bury the fibre throughout the study area, equally resulting in reduced disturbance of soil and litter.

Nonetheless, the use of DTS in monitoring soil temperatures during fires provided a wealth of data that was not achievable previously. This method could greatly increase our ability to understand fire processes, including direct measurements of fire spread, fire behaviour and fire intensity, as well as capturing the heterogeneity of a burn area, which provides insight into the ecological outcomes of fire and allows for further development of fire implementation methods.

Soil heating from the combustion of fine fuels has the potential to restrict seedling emergence – Chapter 4.

Chapter 4 utilised the DTS method outlined in chapter 3 to: measure soil heating under varying fuel loads; tested whether combustion of a wide range of fine fuel loadings produced temperatures that exceed lethal thresholds of seeds (i.e. those temperature thresholds identified in chapter 2), and; tested whether lethal temperatures penetrated deep enough in the soil to limit seedling emergence. This chapter established that, for successful emergence following fire, seeds need to be deep enough in the soil to avoid lethal temperatures while shallow enough to allow for seedling emergence. This window of suitable depths for post-fire emergence is species-specific, with seeds possessing either a higher lethal temperature tolerance or a deeper maximum emergence depth.

These data on soil heating and seedling emergence depth presented in this chapter can be used to categorise the seeds of different species into four broad groups:

1) Fire sensitive seeds, defined by having a low lethal temperature and a shallow maximum emergence depth (e.g. *Austrostipa elegantissima*). These seeds exist in fire prone systems, but are sensitive to elevated temperatures, and thus can only be successful in areas of low soil heating.
2) Fire tolerant seeds, characterized by a high lethal temperatures and a shallow maximum emergence (e.g. *Conostylis candicans*). These seeds have the ability to survive elevated temperatures that are frequently experienced in the upper layers of soils during fire, and may readily emerge post-fire.
3) Fire avoiding seeds, defined by having a low lethal temperature and a deep maximum emergence (e.g. *Asparagus asparagoides*). These seeds survive by emerging from deeper within the soil where temperatures are less severe, however, seeds near the soil surface will be exposed to the risk due to elevated soil temperatures.
4) Fire recolonising seeds, which are defined by a high lethal temperature and a deep maximum emergence (e.g. *Kennedia prostrata*). These traits enable emergence in areas where soil temperatures are extremely hot, in areas where heat penetrates deep into the soil.

These categories align closely to those proposed in recent research by Pausas (2018), who developed the idea of defining a range of generalised fire response strategies that are frequently expressed in both plants and animals. Using functional trait based groupings can inform predictions of species responses, which may lead to an increased ability to forecast post-fire recruitment.

In this chapter, fuel moisture and soil moisture were not experimentally evaluated, but it is important to recognise that both of these factors have a significant influence on soil temperatures during fire (Busse et al. 2010, Marino et al. 2012). Fuel moisture reduces fuel consumption, reducing thermal energy generated, whereas soil moisture reduces heat penetration into the soil, quenching soil heating to below 100°C until all soil moisture is evaporated. Future research should compare soil heating in a similar manner as was conducted in this chapter, however the experimental conditions should be manipulated to include a range of fuel and soil moisture contents. The inclusion of data generated during the experiments outlined above, would allow for a direct test of whether soil moisture protects against seed mortality, which will need to take into consideration reduced lethal temperature thresholds of seeds with high moisture content. Large temperature data sets generated from fires under wet fuel and soil conditions would directly test the assertion that moist soil temperatures do not reach lethal temperatures of seeds (Burrows 1999), despite seeds dying at much lower temperatures when wet.

Predicting seed hydration using additive logistic regression. – Chapter 5.

Chapter 5 used the principles developed in chapter 2, (i.e. that seeds with a higher moisture content have lower lethal temperature thresholds than seeds with lower moisture content) and set out to identify weather and site variables that influence seed hydration. The goal of this chapter was to predict those periods of the year during which seed moisture content would be elevated to a point that lethal temperature thresholds of seeds are reduced, potentially increasing the risk of seed mortality from fire temperatures. Two distinct periods during 2017 were identified where seed hydration was low, one extending from summer through the end of autumn, and another from the middle of spring to the summer (intuitively these are a continuation of the same dry seed season, extending from late spring through to the middle of autumn). This led initially to the identification of a period of six continuous weeks where hazard reduction burns may be implemented while the risk of fire is reduced and the impacts on soil seed banks are likely to be low (note, each year will have different periods of low seed moisture, based upon the weather conditions experienced). However, integrating fire indexes into this analysis reduced this window to just two weeks in autumn, highlighting the tremendous trade-off that fire managers are faced with, partially as seasonal patterns are changing and weather conditions are reducing fire implementation windows.

Chapter 5 showed the rate at which seed re-dried following rainfall is significantly influenced by prevailing weather conditions. Higher maximum temperatures and evaporation rates reduced the likelihood of seeds having high moisture content, whereas increased relative humidity increases the chances of seeds remaining wet. These environmental factors will strongly influence the number and duration of periods during which seeds are more at risk to fire. The experimental component of chapter 5 was conducted throughout 2017 – a wetter year than recent years in Perth. During January and February, Perth experienced 192 mm of rain, making 2017 the wettest summer on record, and overall 2017 was the wettest year in the past six years (albeit in the months following (April –June), Perth recorded very low rain for the season). Rain fell on 117 days in 2017, and 73 of those days occurred between July and October; the highest number of rainy days during that period for 62 years (Meteorology 2018). This level of rainfall was in contrast to the long term drying trend, and was wetter than the long term average (Andrys et al. 2017). Temperatures also departed from recent averages, with Perth recording the fewest days above 37°C for the last 11 years, while average daytime temperatures were 0.3°C higher than the long term average for the area (Meteorology 2018). These conditions likely lead to seeds remaining hydrated for longer periods than average. As a result, 2017 may have had fewer days during which seeds were in a dry state compared to the historic trend, potentially increasing impacts of prescribed burning on soil stored seeds during 2017. Perth's climate is predicted to become warmer and drier (Andrys et al. 2017), which will potentially lead to an increase in number of days that seeds are dry, and provide an increased window of opportunity for hazard reduction burns that limit soil seed bank impacts. Yet, long term drying and warming trends are linked with weather conducive to increases in high and very high fire danger (Jolly et al. 2015). Future predicted long term drying trends may reduce opportunities for prescribed burning, as suitable and safe conditions are decreasing in frequency.

The analysis and model presented in this chapter could be developed into a digital application for fire and land managers to utilize during the planning and implementation phase of hazard reduction burning. Packing the predictive model into an digital application suitable for a smart phone would allow for automatic calculation of 4 out of 5 required variables (i.e. average rain, average relative humidity, average evaporation and days since last rain > 1mm), with only soil moisture remaining – a variable which would have to be measured on the morning of ignition, and can be done in the field using a hand held water moisture probe (e.g. Theta probe, Delta-T devices, Burwell, Cambridge, United Kingdom.). An application for land and fire managers would not be limited to this predictive model, and could also include other tools to assess if weather and site conditions are suitable for the implementation of hazard reduction burning. As well as including the predictive model outlined in chapter 5, a fire managers toolbox could potentially contain fire behaviour predictions (e.g. project vesta), weather forecasts for the duration of the burn (Bureau of Meteorology), landscape scale fuel assessments (satellite derived data), predictions of smoke movements (Bureau of Meteorology), and all could be shared among fire managers on the fire ground and a broader network of land and department managers. Incorporating all these tools into a digital application would provide fire and land managers detailed site specific resources directly to a digital device, further allowing the delivery of industry leading fire management.

However, the modelling procedure presented here, increases its predictive strength by repeatedly reweighting weak variables. Thus, expanding the model beyond local site predictions may prove to be less effective, particularly as soil moisture measurements will be highly variable within burn sites. To overcome this, soil moisture measurements should be taken from multiple sites within a burn area and coupled with regional weather data representative of an area larger than the burn area.

Conclusion

This thesis demonstrated fundamental interactions between seed hydration and seed lethal temperatures, demonstrated a new and novel method for measuring soil temperatures during fire, examined the interactions between fuel loads and soil temperatures during fire and developed a predictive framework to understand seed hydration and therefore seed sensitivity to elevated soil temperatures. The research included in this thesis provides a strong foundation for understanding potential impacts of aseasonal fires, the effects of elevated fuels on seed survival and demonstrates that despite the harsh conditions seeds are exposed to during fire, their ability to survive and germinate following is tightly aligned with seed and plant traits, providing a wide range of possible combinations to define their niche response.

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Conclusion

This thesis demonstrated the influence of seed traits on seed survival during fire, contributing to the understanding of seed biology and fire ecology. This thesis has also described a new method for measuring soil temperatures during fire, which greatly increases data generated, both temporally and spatially. By developing models that have identified significant relationships between seed survival and seed moisture, this research encourages the consideration of seed moisture content and outlines methods for predicting seed moisture content with the aim of reducing seed mortality during hazard reduction burning.

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Attribution statements

A method for extensive spatiotemporal assessment of soil temperatures during an experimental fire using distributed temperature sensing in optical fibre.

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Published in International journal of wildland fire - Received 14 July 2017, accepted 5 December 2017, published online 9 February 2018

Authors’ contributions

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Seed moisture content as a primary trait regulating the lethal temperature thresholds of seeds.

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Published in Journal of Ecology: First published: 11 November 2018

Authors' contributions

Ryan Tangney, Ben Miller, and David Merritt conceived the ideas and designed methodology; Ryan Tangney collected and analysed the data, supported by Joe Fontaine. Ryan Tangney led the writing of the manuscript and all authors contributed critically to the drafts and gave final approval for publication.

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(Tangney et al. 2018a, Tangney et al. 2018b)

Tangney, R., N. A. Issa, D. J. Merritt, J. N. Callow, and B. P. Miller. 2018a. A method for extensive spatiotemporal assessment of soil temperatures during an experimental fire using distributed temperature sensing in optical fibre. International Journal of Wildland Fire **27**:135-140.

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