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Mark K.J. Ooi
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**Comparative ecology of rare and common species in a
fire-prone system**

A thesis submitted in fulfilment of the requirements for the award of the degree

DOCTOR OF PHILOSOPHY

from the

UNIVERSITY OF WOLLONGONG

by

MARK K. J. OOI

B. Env. Sci., MSc (Hons)

SCHOOL OF BIOLOGICAL SCIENCES

2007

Certification

I, Mark Ooi, declare that this thesis, submitted in fulfilment of the requirements for the award of Doctor of Philosophy, in the School of Biological Sciences, University of Wollongong, is wholly my own work unless otherwise referenced or acknowledged. The document has not been submitted for qualifications at any other academic institution.

Mark Ooi

30th May 2007

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Abstract

Due to escalating rates of extinction around the world, it is important to focus research and management on those species most at risk. Factors such as climate change and human population growth are also placing increasing pressure on species that are currently more common and widespread. Understanding the ecology of common species as well as those species that are already threatened is therefore central to conservation biology. In particular, studies targeting species for which little or nothing is currently known can increase our general ecological knowledge and also help to identify species with critical life history traits that could be limiting under future environmental scenarios.

The Ericaceae family in Australia (formerly Epacridaceae) is an example of one such group of species. Our current understanding of the ecology of the Ericaceae in this region is poor, even though species within this family make up a significant proportion of the understorey in temperate, fire-prone plant communities. To address this gap in our knowledge, I selected several obligate-seeding shrub species within the genus *Leucopogon* (Ericaceae) as the focus for this study in south-eastern Australia. Obligate-seeding species such as these, with soil-stored seed banks, have been the subject of far fewer studies than their counterparts that resprout or have canopy-stored seed banks. I used a demographic approach, aimed at providing fundamental ecological data for a rare species, *Leucopogon exolasius*, and some common and more widespread congeneric taxa, in order to explore processes that potentially limit their relative abundance.

Plants of obligate-seeding species are killed by fire, so persistence of populations is dependent primarily on regeneration from stored seed. Fundamental to understanding any aspect of seed ecology is being able to determine whether a seed is viable and dormant or inviable. Taking this primary step in this study was complicated by the fact that the adequacy of the most common method used for checking seed viability, the tetrazolium test, is hard to assess for difficult to germinate species such as *Leucopogon*, and there are no data assessing its applicability for most Australian species. I therefore compared the results of the tetrazolium test with a simple cut test. When estimating the proportion of viable seeds, a strong correlation was found between the two methods for the three species of *Leucopogon* used ($r > 0.9$). The cut test and tetrazolium test both also produced good estimates of viability of seed lots when compared to germination potential of non-dormant seeds retrieved from burial. The results not only supported the use of the less laborious cut test as a reliable method for estimating seed viability throughout this study, but also provided information to assist the accuracy and applicability of the tetrazolium method, previously unavailable for this group of native Australian species.

Dormancy-breaking cues for species within the genus *Leucopogon* are poorly understood and appear to be complex, with laboratory studies often resulting in little or no germination. Due to the difficulties experienced in germinating *Leucopogon* in other studies, I initially established dormancy class in order to identify the mechanisms responsible for controlling dormancy of the three study species. Assessment of seed morphology and preliminary laboratory germination experiments led me to classify the primary dormancy of *L. exolasius*, *L. setiger* and *L. esquamatus* as morphophysiological. Further germination trials revealed that seasonal temperatures overcame primary dormancy and controlled the timing of germination, as has been found for other species with a physiological dormancy component. Despite the fact that the study species display a flush of post-fire seedling emergence in the field, fire cues did not break primary dormancy. Once seasonal temperatures overcame primary dormancy, however, there was a trend for smoke to enhance germination. Knowing if fire is responsible for breaking dormancy, or whether it simply enhances levels of post-fire germination for seeds in which dormancy has been overcome by other factors, is important for a greater understanding of plant population dynamics.

Dormancy had not previously been classified for any species from this fire-prone region, and it was unknown whether physiological dormancy was a common trait, or perhaps a potential cause of rarity. It was also unknown whether a lack of response by *Leucopogon* seeds to fire cues was particular to these species, or a consequence of physiological dormancy mechanisms generally. To investigate this, I estimated the relative proportions of dormancy types for shrub species which occurred in fire-prone habitats in south-eastern Australia. I also assessed the literature for evidence of the effects of fire cues on species with a physiological dormancy component. Representatives of all dormancy classes were found to occur in the region, in proportions similar to that estimated for other fire-prone regions around the world. Over 50% of shrub species had a physiological dormancy component, whilst over 40% had physical dormancy. Additionally, when the assessment was confined to threatened species, the relative proportion of physiologically dormant species represented increased, indicating that dormancy type plays a role in determining rarity. Seasonal temperatures, not fire cues, were the main factors that broke physiologically related dormancy mechanisms. Physiological factors, and therefore seasonal temperatures, are likely to be important in controlling the dormancy and patterns of post-fire germination of many species in fire-prone regions.

Obligate-seeding species are dependent on recruitment from the seed bank for populations to recover after fire. Longevity of seeds stored in the soil is therefore a particularly critical life

history trait of the study species. Two points were addressed in this part of the study. Firstly, I estimated the relative seed bank longevity of the *L. exolasius*, *L. setiger* and *L. esquamatus*. A short-lived seed bank in relation to typical fire return intervals for the region would indicate this as a cause of species rarity. Secondly, I wanted to determine whether primary dormancy was required for seed bank persistence, and whether the distinction between dormancy and persistence affects our understanding of seed bank dynamics in fire-prone regions. Using *in situ* seed burial trials, I found that all three species had persistent seed banks, with estimated half-lives between 3.5 and 5.5 years. Laboratory germination trials and embryo measurements of retrieved seeds showed that primary dormancy was broken during the first year of burial, and I concluded that specific requirements were therefore needed for germination. These results supported the findings from the previous germination Chapter, and supported the conclusion that fire cues that are observed to promote germination *in situ* are not necessarily the ones that break dormancy. Most studies in fire-prone regions have failed to distinguish between these two factors, with attention subsequently diverted away from mechanisms actually controlling dormancy and seed bank dynamics.

Realising the potential importance of seasonal germination cues highlighted by previous Chapters, I formulated the hypothesis that seasonal emergence patterns are more likely for species with a physiological dormancy component than their physically dormant counterparts. In regions like south-eastern Australia, which has no distinct rainfall season, seasonal germination could delay post-fire seedling emergence, and subsequently hinder recruitment, depending on the timing of the fire event. I therefore assessed the impact of seasonally delayed emergence and fire season on recruitment success of the three study species, and ascertained how vulnerable they are to changes to fire season. Post-fire seedling survival and growth were measured after the same fire event, comparing the physiologically dormant *Leucopogon* species (displaying seasonal emergence) with physically dormant or “hard-seeded” species (displaying season-independent emergence). I found that *Leucopogon* emergence was delayed compared to physically dormant species and, as a consequence, both survival and growth were significantly reduced. Intra-specific comparisons of *Leucopogon* species after winter and summer fires, indicated that seasonal germination requirements delayed seedling emergence by 12 months after winter fires, in relation to other co-occurring species, and by 3 to 6 months after summer fires. Seedlings emerging after summer fires grew and matured more quickly than those emerging after winter fires. Because species with physically dormant seeds have quick emergence tied closely to fire, whereas the speed of emergence of physiologically dormant species is dependent on fire season, I concluded that fire might not have been the primary force

selecting for physiologically dormant species. Season of fire could strongly influence the persistence of the study species, and other seasonally emerging species.

The final aim of this study was to assess any potential causes of rarity. In addition to the fundamental ecological data already collected for several key life history traits, data on fecundity, dispersal mechanisms and seedling survival and growth were also analysed and used in a comparative assessment, to establish whether there were any plausible causes of rarity of *L. exolasius*. Compared to the two common congeners, *L. setiger* and *L. esquamatus*, as well as to other obligate-seeding species in the region, *L. exolasius* had a markedly longer primary juvenile period. This raises the likelihood that short inter-fire intervals cause local extinction of populations of this species by killing plants prior to maturation and seed bank replenishment. Seasonal emergence, identified during the analyses of physiological dormancy mechanisms, also had the potential to increase the length of the maturation period. Local extinction of the Emu, a large bird and primary long distance dispersal mechanism for fleshy-fruited species, may have limited opportunities for range expansion and recolonisation.

The investigation of critical life history stages during this study has led to the conclusion that *L. exolasius* persistence appears to be bound to fire frequency. More surprising, however, is the finding that fire season could potentially influence persistence considerably, not only of *L. exolasius*, but of a large proportion of species with physiological dormancy that occur in fire-prone regions. Consequently, the ability of *L. exolasius*, and many other species, to persist in the future would be compromised by changes to the fire regime. The impacts of implemented fires and the effects of climate change are both forecast to promote higher fire frequency and cause changes to peak fire season. These changes could represent significant threats to *L. exolasius* populations, as well as to many other obligate-seeding species in the region with long primary juvenile periods and physiological dormancy. Further research into the effects of seasonal dormancy and germination requirements, particularly in relation to fire season, could help to gain a greater understanding of plant population dynamics and persistence in fire-prone regions. A greater understanding of dormancy mechanisms generally in fire-prone regions could also shed further light onto questions such as the evolution of species in relation to fire.

Acknowledgements

If I were to be completely honest about this PhD, I'd have to say that it has been somewhat of a marathon experience. In fact, in terms of an athletics event, I think a PhD in plant ecology would have to resemble something like the slightly obscure steeplechase, although with less water jumps. It might not be the most popular event on the calendar, but there is a small and passionate support base urging you on from the stands. It can be a lonesome pursuit, with lots of hurdles to negotiate, and it feels like the end is a very, very long way away. But (and I'm determined to take this metaphor all the way to its absolute limit), there's a grand prize at the end which is, hopefully, a trophy cup full of knowledge, as well as fledgling expertise, a couple of publications, new-found friends, a greater understanding of how some parts of this world work, unlimited job prospects, fame, fortune, a new car, smiling people staring at you as you walk down the street, calling out your name and beckoning you into their homes to offer you coffee, cake and a cuddle on the couch. Hopefully. Overall, I think I'm just trying to say that this sometimes difficult experience has felt very worthwhile in the long run. And when I think about the support I've received to get to this point of my education, career and life, I feel very grateful indeed. Tony Auld and Rob Whelan cannot be thanked enough for their ever-helpful encouragement and wisdom. I think that when I first met both of them, I was a much younger, somewhat travel- and surf-addicted person, dressed in boardshorts and a torn t-shirt. How they ever saw potential in me I'll never know, but their supervision has played no small part in helping me along life's path. There has been a lot of intellectual input, in varying shapes and sizes, that has helped brew some of the ideas presented in this study. This input has come in the form of discussions, comments, hallway chats and ponderings over cups of coffee, from many colleagues, friends and ecologists that pass in the night, including Jack Baker, Andrew Denham, David Keith, Belinda Kenny, Todd Minchinton, Charles Morris, John Porter, Mark Tozer, and several folk who remain anonymous in the world of journal referees. Financial support from an Australian Research Council APAI Scholarship made all of this possible. Indispensable practical help was provided in one way or another by Emilie-Jane Ems, Barbara Rice, Mark Robinson, Paul Thomas, Mark Westoby (all who provided access to seed collections), Amelia Martyn from the NSW Seedbank (Mt. Annan Botanic Garden, Botanic Gardens Trust, Sydney), and Natasha

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Chapter 1

General Introduction



1.1 Demographic studies and the rare versus common approach

A thorough knowledge of demographic processes is necessary if we are to gain a full understanding of the ecology of plant populations (Harper 1977). Demographic data are fundamental to identifying critical life history stages and assessing the ability of plant populations to persist in the face of a wide variety of processes limiting growth and reproduction and causing mortality. These data are also required for accurate ecological modelling and for the informed management of plant populations in their natural habitats (Norris 2004).

Due to recent high levels of extinction world-wide (Thomas *et al.* 2004a), there is an immediate need to focus research and management on those species most at risk. Factors such as climate change (Thomas *et al.* 2004b) and increased human population size (Gaston 2005) are placing increasing pressure on species that are currently more common and widespread. This highlights the need for understanding the ecology of common species as well as those species that are already threatened. In particular, studies targeting species for which little or nothing is known can not only increase our general ecological knowledge, but also help to identify those with critical life history traits that could potentially be limiting under future environmental scenarios.

In terms of conservation biology, a rare species is defined as one of limited abundance and distribution (Kunin & Gaston 1993; Gaston 1994). Although rarity alone does not necessarily imply that a species is at risk of extinction, it is one of the main parameters used to assess this (e.g. IUCN 2001). Under schemes that categorise species based on their potential risk of extinction, the majority of plant species classified as threatened are rare (Gaston 1994; Keith 1998; Keith *et al.* 2000). As such, demographic studies, as well as an understanding of the potential causes of rarity and decline, are particularly valuable for the conservation and management of rare and threatened plant species (Schemske *et al.* 1994; Bevill & Louda 1999; Lavergne *et al.* 2004).

In this study, I have chosen a demographic approach, aimed at providing fundamental ecological data for a rare species and some common and more widespread congeneric taxa, in order to explore processes that potentially limit their relative

abundance. Comparative ecological studies between rare species and common congeners can help to detect possible limiting factors within a species (Kunin & Gaston 1993; Schemske *et al.* 1994; Pantone *et al.* 1995; Bevill & Louda 1999). Although it is unlikely that generalisations exist regarding the causes of rarity (Fiedler 1987; Gaston 1994), insights gained from data collected in comparative studies are particularly useful for the conservation of rare species (Lavergne *et al.* 2004). Additionally, comparative studies have contributed to the detection of broader ecological patterns of the taxa involved and the communities in which they inhabit, irrespective of the detection of limiting factors.

1.2 Critical life-history stages in fire-prone regions

The suggested framework for identifying life history stages crucial to population persistence involves initially collecting detailed demographic data on all life history stages (Schemske *et al.* 1994). Comparative analyses can then be used to distinguish critical life history traits and stages. However, in fire prone habitats, natural and recurrent fire regimes have shaped the evolution, and hence the demography and life history traits, of the associated flora (Gill 1981; Keeley 1986; Naveh 1994). Critical life history stages are therefore often linked to fire, for those species that occur in fire prone habitats (Whelan 1995; Bond & van Wilgen 1996).

Fire causes varying levels of mortality of plants and recruitment from seeds is necessary for population persistence. Species that resprout after fire may be relatively resilient to a single fire event but long-term persistence is dependent on some recruitment from seed (Keeley & Zedler 1978). Species that have individuals killed by fire, called ‘fire-sensitive’ or ‘obligate seeder’ species, have adapted to fire intervals that exceed their primary juvenile period the majority of the time (Lamont *et al.* 1991). Obligate seeders are dependent upon a stored seed bank, both for short-term regeneration and long-term persistence, and are therefore more sensitive to changes to the fire regime.

Many species in fire-prone environments around the world, including Australia, the Mediterranean Basin, South Africa and the U.S.A have seed dormancy and subsequent germination cued to fire, such that a flush of seedling emergence occurs in the post-fire environment (Kruger & Bigalke 1984; Keeley 1991; Bell *et al.* 1993; Trabaud 1994; Auld & Tozer 1995; Carrington 1999). The post-fire environment can be beneficial to seedling survival and growth due to increased availability of resources, as well as reduced competition and relative freedom from herbivory (Whelan 1995; Bell 1999). Predictions about the persistence of plant populations in fire-prone regions can be made by examining key factors in relation to fire. These factors are: (1) plant mortality caused by fire, (2) dormancy and germination, and (3) seedling establishment and survival (Whelan 1995; Whelan *et al.* 2002). Aspects of the fire regime, including frequency, intensity and season, are all potentially important determinants of plant population dynamics.

1.3 The study system

The sandstone vegetation of the Sydney region is one of several centres of high species richness and endemism on the Australian continent (Crisp *et al.* 2001). The fire-prone habitats of this relatively small area of south-eastern Australia contain approximately 2000 plant species (Auld *et al.* 2000). Fundamental ecological studies have not been conducted for the majority of these species. As such, there is surprisingly little known about many families, even some that are dominant components of fire-prone vegetation communities across the continent.

The studies described in this thesis have focused primarily on several members of the genus *Leucopogon* in the Ericaceae family, but also include investigation of co-occurring species. *Leucopogon* is the largest genus within the sub-family Styphelioideae, (formerly recognised as Epacridaceae) with approximately 230 species, and is part of the largest tribe, Styphelieae. Despite being a widespread and diverse understorey shrub component of temperate Australian heaths, woodlands and forests, members of the Styphelioideae have been the subject of few ecological studies. Their ecology is poorly understood, particularly in south-eastern Australia. Studies on

members of this large sub-family have mainly been conducted in the Mediterranean-type climates in Australia's south-west (e.g. Meney *et al.* 1994; Dixon *et al.* 1995; Bell & Pate 1996; Keighery 1996; Tieu *et al.* 2001) whilst only a few relatively recent studies have appeared from the non-Mediterranean east coast (Keith 1997; 2002; Gilmour *et al.* 2000; O'Brien & Johnstone 2004; Ooi *et al.* 2004a; 2006a). Dormancy mechanisms for members of the Styphelioideae, and particularly the drupaceous seeds of species within the Styphelieae tribe, appear to be complex. Seeds of species from Styphelioideae have been described as 'difficult to germinate' (Dixon *et al.* 1995).

The three *Leucopogon* species used as the focus for this study include the rare and threatened *L. exolasius*, and two common congeners, *L. setiger* and *L. esquamatus*. More detailed descriptions of each species are given in the methods within subsequent chapters. In two earlier studies (Ooi *et al.* 2004a; 2006a), I identified the basic responses of these species to fire, including plant mortality and seedling emergence patterns. These studies showed that all three species are obligate seeders with soil-stored seed banks and that, unlike many other species within the same vegetation communities, all display a seasonal emergence pattern, with peak densities recorded around autumn or early winter (Ooi *et al.* 2004a; 2006a). However, it is important to understand the processes underlying these observed patterns, and also to assess the potential impacts these patterns may have on population persistence.

1.4 This study

The first main aim of this study was to gain an understanding of the dormancy mechanisms controlling germination of these, as well as other species, in this fire-prone region. This is of particular importance because of the importance of germination for population persistence in fire-prone regions. It is also important as it can clarify the sequence of processes occurring naturally in the seed bank (Baskin & Baskin 2003a). The majority of seed germination studies in fire-prone regions have concentrated on the extent to which dormancy is broken by fire, rather than exploring all the mechanisms that might be responsible for breaking dormancy (Keeley 1995).

To address this significant gap in our knowledge of the ecology of fire-prone systems, I explored the question of dormancy and its effects on population dynamics. I started by clarifying two important concepts. Firstly, dormancy is defined as a characteristic of the seed rather than of its environment (Vleeshouwers *et al.* 1995). As such, the processes that are important in overcoming primary dormancy may be distinct from those stimulating germination from a seed bank. Secondly, the seed dormancy classification system outlined by Baskin & Baskin (2004), distinguishes between physical, morphological and/or physiological mechanisms that control dormancy, and can therefore help to identify the most likely factors involved in overcoming dormancy. These approaches were chosen, in part, due to the limited success of previous approaches made to questions of dormancy and germination in this group of species. Additionally, applying these concepts at a broader scale, to other species in the region, could help to identify the mechanisms that control population dynamics and species coexistence within a community.

The second main aim of this study focussed on the effects of season and associated temperature changes on seed dormancy, seedling recruitment and, subsequently, population persistence. Studies of seasonal effects in fire-prone regions with non-Mediterranean climates have been limited. This may be due to the often observed rapid germination response by many species after fire, where it is assumed that a synchronous flush of emergence occurs for the majority of species once there is sufficient moisture. However, previous research on *Leucopogon* species in south-eastern Australia, has found that regardless of the fact that there is no distinct rainfall season, seedlings emerge at the same time of year. This occurs irrespective of earlier rainfall events or the timing of a fire. Subsequently, the post-fire flush of emergence can be delayed in relation to many other co-occurring species (Ooi *et al.* 2004a). Seasonal temperatures are one of the most important dormancy-breaking factors for plant species around the world (Baskin & Baskin 1998), yet understanding of seasonal affects in fire-prone regions is somewhat limited. A seasonal component, either to dormancy or germination, in a region that has no distinct rainfall season can potentially have an affect on species population persistence.

The final aim of this study was to assess any potential causes of rarity. This aim was focussed particularly on the threatened species *L. exolasius*, using a comparative approach with the more common congeners, *L. setiger* and *L. esquamatus*. However, at a broader scale, there has been little study of the relationship between classes of dormancy and rare and threatened species. The evolution of dormancy types may provide some insights into the relative ability of species to persist in the long-term, under present and future fire regimes.

To address the aims outlined above, I posed the following broad questions:

- (1) Are seeds of *Leucopogon* species dormant at the time of release from the parent plant and, if so, what factors promote loss of dormancy? Understanding dormancy mechanisms of these species may help to identify factors that drive the seasonal emergence pattern displayed by *Leucopogon* species in the field.
- (2) What classification of seed dormancy do the three species have? What are the relative proportions of dormancy classes in this region and do direct fire cues break all forms of dormancy? Some forms of dormancy may pre-date the predominance of fire as a disturbance and therefore give insights into species that have adapted to fire, as compared to those that have persisted through fire.
- (3) What proportion of species from each dormancy class are listed as threatened? Following on from Question 2, this question assesses whether species that have persisted in the face of fire, as opposed to being adapted to it, are more threatened as a result.
- (4) How persistent are the seed banks of the three *Leucopogon* species and what is the relationship between seed dormancy and seed bank longevity? Obligate-seeding species are dependent primarily on recruitment from the seed bank after fire. Understanding this life-history stage is fundamental to predicting population persistence, as well as resilience in the face of increased fire frequency.

- (5) Does delayed emergence reduce seedling survival and/or growth? Seasonal germination in a region with no distinct rainfall season means that emergence can be delayed depending on the timing of the fire event. This question sets out to assess the effect of delayed emergence, and hence determine if the timing of fire can affect recruitment and population persistence.
- (6) Are there any identifiable ecological differences between *L. exolasius*, *L. setiger* and *L. esquamatus*? This comparison will help to determine the potential causes of rarity in *L. exolasius*. Results could lead to a better understanding of how the species persists, as well as provide a platform for more informed management.

1.5 Thesis structure

This thesis has been written as a series of manuscripts prepared for publication. Each chapter therefore stands alone and, as such, their introductions are detailed, quoting relevant literature for the ecological principles being investigated and setting the context for each of the aims addressed. There is a small amount of repetition within the introductions as a result of this approach.

Chapter 2 describes an experiment aimed at assessing an efficient way of estimating seed viability of the three *Leucopogon* species studied. Chapters 3 and 4 describe and interpret experiments investigating dormancy of *Leucopogon* and other species within the Sydney region, addressing Questions 1 to 3 above. An experiment investigating seed bank longevity and its relationship to dormancy is presented in Chapter 5 (addressing Question 4). Chapter 6 reports on seedling survival and growth experiments conducted after several fire events (Question 5). Chapter 7 presents a comparative analysis using data from several of the previous Chapters, as well as presenting new data on factors such as dispersal. This final Chapter explores any plausible causes of rarity in *L. exolasius* (Question 6), and discusses the potential for other species with similar life history traits to persist under future environmental conditions. I have included an Appendix that contains reprints of four papers that have been published as a result of the work conducted during this study.

Chapter 2

Comparison of the cut and tetrazolium tests for assessing seed viability



Preface

Knowing the viability of a seed lot is a fundamental part of germination experiments. The tetrazolium test is one of the most reliable ways to assess viability. However, no guidelines exist for most Australian species, and the adequacy of the test has been hard to prove for species that are difficult to germinate. Species within the genus *Leucopogon* fall into this category. This Chapter compares two methods for testing the viability of *Leucopogon* seeds. The results will be important for interpreting subsequent work throughout the thesis.

Publication

Ooi, M.K.J., Auld, T.D. & Whelan, R.J. (2004) Comparison of the cut and tetrazolium tests for assessing seed viability: a study using Australian native *Leucopogon* species. *Ecological Management & Restoration*, **5**, 141-3.

2.1 Introduction

A viable seed is defined as a seed that has the potential to germinate (Bradbeer 1988). Dormancy mechanisms prevent the germination of viable seeds until suitable conditions arise. However, dormancy-breaking treatments are unknown for many species. Determining whether poor germination is explained by unknown dormancy mechanisms or by low levels of viability is an essential part of understanding how species persist in the landscape (Baskin & Baskin 1998). Viability testing can determine the level of viability of seed lots and is the primary step when assessing the effectiveness of dormancy-breaking treatments. It is also important for practitioners, such as restoration ecologists, who need to know the germination potential of a seed lot.

Two of the most common methods for checking seed viability are the cut and tetrazolium tests. The cut test is one of the simplest methods and is a quick way to estimate seed viability, although its accuracy is rarely assessed. The tetrazolium test is more time consuming but is thought to provide an accurate assessment of viability (Bradbeer 1988; Sawma & Mohler 2002; Thompson *et al.* 2002). However, interpretation of staining patterns from the tetrazolium test can be very species specific and for most Australian species there are no data assessing its applicability or accuracy (Thompson *et al.* 2002).

Dormancy breaking cues for species within the genus *Leucopogon* (Styphelieae: Epacridaceae) are poorly understood and laboratory studies often result in little or no germination (e.g. Bell *et al.* 1995; Roche *et al.* 1997). The aims of this study were to assess and compare both tests to see if they produced reliable viability estimates for these species. In particular I wished to see if the less laborious cut test provided a reliable assessment of seed viability. I did this using several *Leucopogon* species, and assessed how well cut and tetrazolium test results related to germinability. I also examined the level of correlation between results from the cut and tetrazolium tests across a range of likely applications of these tests, including fresh seeds, seeds buried for a year and seeds remaining dormant after germination trials.

2.2 Methods

Freshly matured fruit were collected from populations of *Leucopogon exolasius*, *L. esquamatus* and *L. amplexicaulis* at Royal (34° 03'S, 151° 03'E) and Heathcote (34° 07'S, 150° 58'E) National Parks to the south of Sydney, New South Wales in early 2000. *Leucopogon* fruit are drupes, with a woody pericarp (Figure 2.1), containing a single seed (or occasionally two seeds for *L. esquamatus*). Flesh was removed from the fruits by soaking for several days.

The cut test, on unimbibed seeds, involved using a scalpel to cut the endocarp and expose seed tissue. Seeds were scored as viable if firm, moist and white endosperm was present. Shrivelled dry, black/dark brown or mushy seeds were scored as inviable. To assess viability using tetrazolium, seed tissues were exposed as above and then imbibed in distilled water overnight. They were then placed in an aqueous tetrazolium solution (1% 2,3,5 triphenyl tetrazolium chloride) in a dark cabinet. Viable metabolic tissues of seeds soaked in this solution stain pink or red.

Duration of immersion for staining to occur can vary between species (Gravina & Bellairs 2000). Using between 30 and 120 fresh seeds of each species, depending on availability, seeds were initially immersed for 24 hours at approximately 24° C. A species with a known response to tetrazolium, *Acacia oswaldii*, was included to ensure that the solution was effective. Subsequent seed lots were assessed after immersion for 72 hours, 96 hours or 7 days.

To assess whether estimates of viability from the two tests give an accurate estimate of potential germinability, comparisons were made with results from germination trials (Ooi, unpublished data). Fresh seeds were subjected to stratification or burial treatments. A minimum of three replicates of 20 seeds were then assigned to assessment by the cut test, the tetrazolium test or to germination trials. In germination trials, seeds were placed on filter paper lined petri dishes. These were moistened with distilled water and incubated at approximate seasonal (winter (16/6° C), spring/autumn (20/10° C) and summer (~28/18° C)) alternating temperature and light regimes (12/12 hour). Germination was scored on emergence of the radicle and was checked weekly.

(a)



See print copy for figure 2.1b

Figure 2.1 (a) *Leucopogon exolasius* drupes, with flesh removed to expose the woody endocarp, and (b) the slightly smaller drupes of *L. esquamatus*.

Proportional data of viable seeds assessed by the viability tests and final germination data were checked for normality and arcsine transformed prior to analysis using ANOVA.

To assess correlation between results from the cut and tetrazolium tests, seed lots of each species were subjected to both types of viability test. Seed lots had undergone different treatments, including stratification and burial and therefore potentially had a range of viabilities. This allowed an assessment of the applicability of tests to seeds subjected to different treatments, as well as ascertaining the consistency of correlation over a range of viabilities. Slight adjustments interpreting the cut test were necessary when testing buried seeds. Light brown but still firm and moist seeds were assessed as viable from the cut test, with discolouration assumed to be a result of burial. Proportions of viable seeds from each test type were compared. Data were arcsine transformed after testing for normality as above. Correlation coefficients between cut and tetrazolium test results were calculated with the intercept fixed at the origin and tested against the value 1, the expected value if each viability test produced the same result. To do this, coefficients were transformed to Fisher's z and the normal deviate calculated. Critical values could then be obtained from the t table.

2.3 Results

No *Leucopogon* seeds stained after 24 hours immersion in tetrazolium. *Acacia oswaldii* seeds stained dark red during this time in the same solution. *Leucopogon exolasius* and *L. esquamatus* seeds immersed for 72 and 96 hours similarly produced almost no staining, while *L. amplexicaulis* stained well after 72 hours. Immersion for 7 days produced complete staining for all species though of varying intensity.

Substantial mean proportions of germination (± 1 standard error (SE)) were recorded for *L. exolasius* ($76.7 \pm 6.0\%$), *L. esquamatus* ($68.3 \pm 7.3\%$) and *L. amplexicaulis* ($77.8 \pm 4.5\%$). The tetrazolium test uniformly produced lower viability assessments, however, there were no significant differences between results from the cut test, tetrazolium test or proportion of seeds germinated for *L. exolasius* (ANOVA, P

= 0.1406), *L. esquamatus* (ANOVA, $P = 0.0999$) or *L. amplexicaulis* (ANOVA, $P = 0.5276$).

A positive tetrazolium result was concluded if seeds stained light pink as well as red. The proportion of stained seeds that were light pink after 7 days immersion varied greatly between seed lots for *L. exolasius* (0% - 60%) and *L. esquamatus* (0% - 100%). For *L. amplexicaulis*, the proportion of light pink stained seeds decreased from 31% after 96 hours immersion, to 0% after 7 days. When using only red stained seeds in the analysis, tetrazolium results were significantly lower for *L. exolasius* (ANOVA, $P = 0.0001$) and *L. esquamatus* (ANOVA $P = 0.0003$). Due to higher proportions of red stained *L. amplexicaulis* seeds, no significant differences were found (ANOVA, $P = 0.203$).

Highly significant correlations were found between the cut test and tetrazolium for *L. exolasius* ($r = 0.947$), *L. esquamatus* ($r = 0.934$) and *L. amplexicaulis* ($r = 0.955$) (Figure 2.2). This pattern appeared consistent between fresh seeds, seeds from burial trials or seeds from germination trials. Correlation coefficients did not differ significantly from a value of one.

2.4 Discussion

The cut test and tetrazolium test both produced good estimates of viability of seed lots when compared to germination potential for all three study species. The cut test is used in many studies as it is quicker and less laborious than the tetrazolium test, however, its accuracy has rarely been assessed. It is concluded from this study that the cut test using unimbibed seeds provides a reliable estimate of viability in the *Leucopogon* species examined.

This study has also provided information to assist the accuracy and applicability of the tetrazolium method, previously unavailable for this group of native Australian species. Tetrazolium testing provides an accurate assessment of viability for

(a)

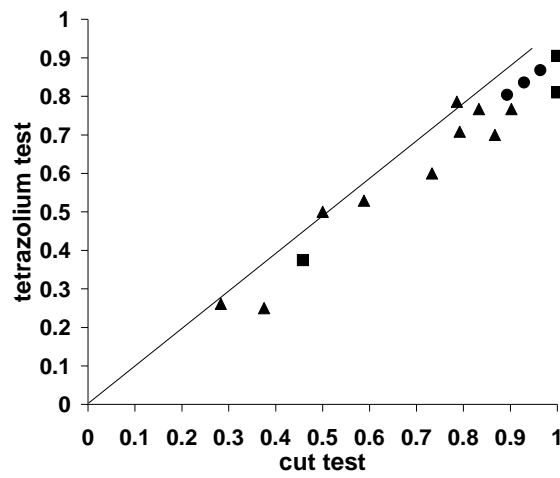
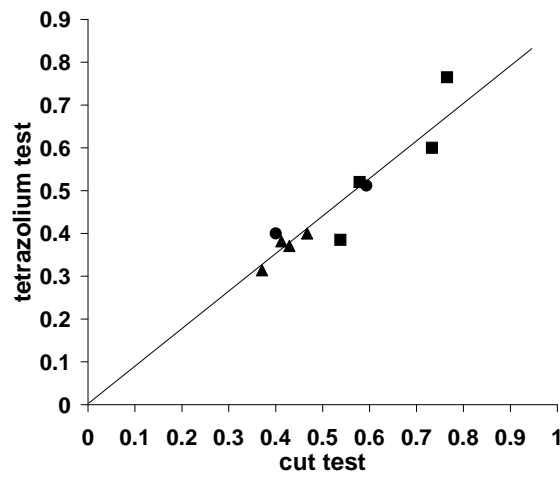
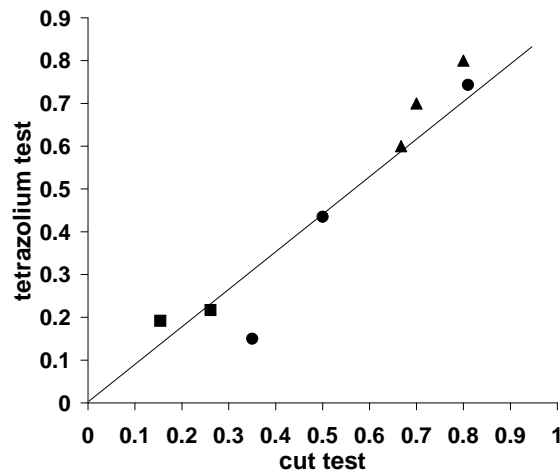


Figure 2.1 Proportion of viable seeds assessed by the tetrazolium test plotted against viable seeds assessed by the cut test for (a) *Leucopogon exolasius* (b) *Leucopogon esquamatus* and (c) *Leucopogon amplexicaulis*. Plots are of untransformed data. The expected linear relationship of $r = 1$ is plotted on each graph. Symbols represent seeds subjected to different treatments (● = fresh, ▲ = buried, ■ = post germination trials)

(b)



(c)



Leucopogon species if light pink as well as dark red staining is assessed as viable, and the amount of time for staining can take up to 7 days.

Leucopogon seeds took a longer amount of time to stain in tetrazolium compared to the *Acacia* species used in this study and compared to results from many other germination studies. Staining occurs for many species after approximately 24 hours, and sometimes up to 48 hours imbibing in solution (Moore 1985; Bradbeer 1988). Time of imbibition in viability studies of numerous Australian species using the tetrazolium test conducted at room temperatures, also falls within a 24 to 48 hour period (e.g. Gravina & Bellairs 2000; Moles *et al.* 2003). Increasing the temperature during imbibition can reduce the length of time to staining (Moore 1985; Gravina & Bellairs 2000) and this may need to be considered when checking viability of *Leucopogon* seeds.

Although all viable seeds of the study species stained completely, intensity of staining was variable, with many staining light pink rather than red. Thompson *et al.* (2002) also found that seeds of a number of Australian species stained only faintly but still produced healthy germinants. International Seed Testing Association guidelines (Moore 1985) state that the position and size of the necrotic areas in the embryo or endosperm, and not necessarily the intensity of the colour, determine whether seeds are classified as viable or non-viable. Germinants in this study all appeared healthy and the low intensity of staining may be due to factors related to dormancy, such as low levels of respiration.

Due to the long period of imbibition and the low intensity of staining, previous studies that have used tetrazolium to check viability of *Leucopogon* seeds may have erroneously attributed a lack of germination to high proportions of inviable seeds. For example, Bell *et al.* (1995) reported 0% viability in a *L. verticillatus* seed lot and Clarke *et al.* (2000) reported only 6.2% viability in a *L. muticus* seed lot. No germination was recorded in either study. Although it is possible that viability levels were low, the lack of germination could also be due to the complex dormancy mechanisms of *Leucopogon*. The erroneous assumption that a seed lot has low levels or no viability may lead to a loss of potentially important information regarding seed dormancy.

Chapter 3

Dormancy and the fire-centric focus: investigating the germination of three *Leucopogon* species



See print copy for figure

Preface

Although previous studies of the Ericaceae, including *Leucopogon*, provide evidence that fire promotes a flush of germination in the field, a failure to break dormancy in germination trials has resulted in little understanding of the dormancy mechanisms operating. As such, this Chapter outlines work classifying dormancy for three obligate-seeding *Leucopogon* species, to gain insights into factors controlling germination of this group. This approach had not previously been used in fire-prone regions but is considered to be of particular use for understanding population dynamics of species with complex dormancy mechanisms. Many germination studies from fire-prone regions have concentrated on fire only, rather than exploring other mechanisms that might be responsible for breaking dormancy.

Publication

Ooi, M.K.J., Auld, T.D. & Whelan, R.J. (2006) Dormancy and the fire-centric focus: germination of three *Leucopogon* species (Ericaceae) from south-eastern Australia. *Annals of Botany*, **98**, 421-430.

3.1 Introduction

Dormancy in plant propagules can restrict germination and seedling emergence to times when there are suitable environmental conditions (Baskin & Baskin 1998; Bell 1999). The germination of many species from fire-prone habitats is related, either directly or indirectly, to fire. Germination cues that are directly related to fire include heat shock (Keeley 1987; Auld & O'Connell 1991; Bell *et al.* 1993), smoke or smoke products (de Lange & Boucher 1990; Dixon *et al.* 1995; Keeley & Fotheringham 1997) and combinations of these (Keith 1997; Kenny 2000; Thomas *et al.* 2003). Indirect cues include light and ambient temperature conditions (Bond & van Wilgen 1996) that are altered as a result of the canopy and leaf litter being burned and removed. These cues can lead to a pulse of seedling emergence in the post-fire environment, where there are increased resources available as well as reduced competition (Gill 1981; Keeley 1991; Whelan 1995; Bell 1999).

There is still limited understanding of the range of dormancy mechanisms operating in fire prone regions around the world. This is partly due to the large number of species that display obvious and rapid germination responses to fire characteristics, such as heat and smoke (Auld & O'Connell 1991; Keeley 1991, Brown 1993; Dixon *et al.* 1995; Whelan 1995; Bond & van Wilgen 1996). As a result, the majority of seed germination studies have concentrated on the extent to which dormancy is broken by fire rather than exploring all the mechanisms that might be responsible for breaking dormancy (Keeley 1995). Seed dormancy is defined as a characteristic of the seed rather than of its environment (Vleeshouwers *et al.* 1995). Overcoming primary dormancy is therefore distinct from stimulating germination from a seed bank (Thompson *et al.* 2003). Factors required to break primary dormancy may be completely unrelated to fire, however, fire may provide subsequent germination cues and suitable environmental conditions (Bell 1999).

There is growing evidence that many species in fire-prone environments do not have primary dormancy broken by fire. Germination can be enhanced after fire in some species, but in others it can be diminished or unchanged (Keeley 1987; Davis *et al.* 1989). Laboratory studies support this, because a significant number of species show no

response to heat or smoke treatments (Dixon *et al.* 1995; Roche *et al.* 1997; Keeley & Fotheringham 1998; Clarke *et al.* 2000; van Staden *et al.* 2000). Deciphering which cues are controlling primary dormancy in these species may be more difficult than with species that respond directly to fire cues.

Seed dormancy classification, using a system such as that outlined by Baskin & Baskin (2004), distinguishes between physical, morphological and/or physiological mechanisms as controls on dormancy and can help to identify the most likely factors involved in overcoming dormancy. Stratification at seasonal temperatures is the main known requirement for overcoming both morphological and physiological factors, whereas heat shock or scarification can overcome physical dormancy. To classify seed dormancy, information on permeability of seeds, embryo morphology and germination response at different seasonal temperatures, among other things, is required (Baskin & Baskin 2003a). Classification can help to gain an understanding of a species' dormancy by enabling comparisons with other species within the same dormancy class.

Several studies have suggested that morphological and/or physiological factors play a role in controlling dormancy of species from fire-prone regions (Schatral *et al.* 1997; Keeley & Fotheringham 1998; Bell 1999; Tieu & Egerton-Warburton 2000; Allan *et al.* 2004). However, the role of fire as a cue for breaking primary dormancy involving morphological and physiological mechanisms is yet to be clarified.

In Australia's fire prone habitats, the predominance of fire as a disturbance appears to focus most study effort on the role that fire plays in breaking dormancy, and less on other mechanisms. Many common species from temperate understorey vegetation, including several members of the Ericaceae, Rutaceae and Dilleniaceae, have unknown dormancy mechanisms (Fox *et al.* 1987; Dixon *et al.* 1995; Auld 2001; Baskin & Baskin 2003a; Allan *et al.* 2004), although it is likely that many of these species have some form of physiological dormancy (Ooi 2007). Some progress has been made in unravelling dormancy cues of individual species from these groups, including *Leucopogon conostephioides* and *L. melaleuroides* (Tieu *et al.* 2001; O'Brien & Johnston 2004) and several *Hibbertia* species (Schatral *et al.* 1997; Allan *et al.* 2004). Nevertheless, freshly dispersed seeds of the majority of species from these groups tested

in laboratory germination trials tend not to respond to direct fire cues (Dixon *et al.* 1995; Roche *et al.* 1997; Clarke *et al.* 2000; Tieu *et al.* 2001), and in many cases do not germinate at all. Considerable germination in response to fire has been observed for the same species either *in situ* or after burial (Dixon *et al.* 1995; Enright *et al.* 1997; Roche *et al.* 1997; Tieu *et al.* 2001; Wills & Read 2002; Rokich *et al.* 2002; Ooi *et al.* 2004a). During burial, seeds experience daily and seasonal temperature fluctuations, physical deterioration of seed structures and physiological changes over time (Baskin & Baskin 1998). This suggests that a sequence of processes may be required. The first set of processes are required to break primary dormancy, with subsequent processes then promoting increased levels of germination post-fire.

In this study I used three *Leucopogon* (Ericaceae) species from south-eastern Australia to investigate dormancy mechanisms and timing of germination, and the potential role that burial, seasonal temperature regimes, dark and fire cues play in controlling these mechanisms. *Leucopogon* is the largest genus within the sub-family Styphelioideae (formerly recognised as Epacridaceae), with approximately 230 species, and is part of the largest tribe, Styphelieae. Dormancy mechanisms for this genus (and tribe) appear complex and seeds have been described as difficult to germinate (Dixon *et al.* 1995). Unlike many other species within the same plant communities, *Leucopogon* displays a seasonal emergence pattern from a soil-stored seed bank, with peak densities recorded in autumn and early winter (Ooi *et al.* 2004a). There is no distinct rainfall season, but seedlings emerge at the same time of year, irrespective of rainfall or the timing of a fire event. Nevertheless, there is increased germination post-fire. Subsequently, the post-fire flush of emergence can be delayed in relation to many other co-occurring species (Ooi *et al.* 2004a).

My specific aims are to answer the following questions:

- (1) Are seeds of the study species dormant at the time of release from the parent plant?
- (2) What classification of seed dormancy do the three *Leucopogon* species have?
- (3) Is dormancy broken during burial? What factors promote loss of dormancy?

- (4) Do fire cues break primary dormancy? What are the relative effects of fire cues on both fresh seeds and buried seeds?
- (5) What drives the seasonal emergence pattern displayed by *Leucopogon* species in the field?

3.2 Methods

3.2.1 STUDY SPECIES AND SEED COLLECTION

The three study species are all obligate seeders (Ooi *et al.* 2006a) with soil-stored seed banks (Ooi *et al.* 2004a). *Leucopogon exolasius* is an erect shrub that grows to approximately 2 metres, and is listed as ‘vulnerable’ both under the national Australian *Environment Protection and Biodiversity Conservation Act* (1999), and under the state of New South Wales (NSW) *Threatened Species Conservation Act* (1995). The species is endemic to the southern Sydney region of NSW, Australia and occurs in woodlands. *Leucopogon setiger*, also an erect shrub to approximately 2 metres, is more widespread, extending from the central western slopes of NSW to the coast (distributional range > 450km). It is found in woodlands and open forest. *Leucopogon esquamatus* is a slender shrub, which grows to approximately 1 metre. It is also widespread, occurring from the coast to the mountains (as for *L. setiger*), but extending south into the states of Victoria and Tasmania (distributional range > 1000km). It occurs in heath, woodlands and open forests.

Leucopogon fruit are drupes. Fresh exocarps of both *L. exolasius* and *L. setiger* are fleshy, while the endocarp is hard and lignified. *Leucopogon esquamatus* exocarp is papery and the endocarp comparatively more fibrous. Seeds are held inside the fruit. Drupes ripen in summer (generally November-December) and were collected between 1999/2000 and 2003/04 from within Royal (34° 03’S, 151° 03’E), and Heathcote (34° 07’S, 150° 58’E) National Parks in the southern Sydney region, and at Garigal (33° 46’S, 151° 14’E) National Park in northern Sydney. Before the commencement of experiments, flesh was removed from the collected drupes of each species by soaking in water for several days. After drying, a minimum of 40 drupes were used to estimate

mean weight. Drupes were stored in envelopes at room temperature in the lab (~ 22° C) prior to commencement of experiments.

The climate in the Sydney region is temperate with no dry season (using the Köppen classification system) (Stern *et al.* 2000). Average annual rainfall for the area is approximately 1050 mm. It is distributed relatively evenly throughout the year, with the four highest monthly averages occurring in January, March, June and November. Average temperatures (maximum/minimum) are approximately 27/18° C and 16/6° C in summer and winter respectively. Climate data were obtained from the Lucas Heights weather station, a few kilometres from the southern collection sites.

3.2.2 SEED EMBRYO MORPHOLOGY AND GROWTH

Twenty-five embryos from freshly dispersed seeds were used for each of the three *Leucopogon* species to investigate embryo morphology. Drupes were scarified using a scalpel to nick the pericarp at the bract end, exposing seed tissues. After soaking for at least 48 hours, seeds were removed from the drupes by cutting away the pericarp with a scalpel. Once removed, embryos were separated from endosperm using a scalpel and fine forceps. Embryos could often be squeezed from the seed by applying a small amount of pressure with the forceps. Seed and embryo lengths were measured using a dissecting microscope fitted with a stage micrometer. Embryo length to seed length ratios (E:S) were calculated.

To ascertain whether embryos grow prior to germination, the same methods of measurement were employed. Twenty fresh seeds of each species were placed in petri dishes lined with filter paper and moistened with distilled water. These were then stratified for 24 weeks in incubators at warm (28/18° C), cold (16/6° C) or cold followed by warm temperatures, before embryos were excised and measured. Seeds subjected to burial treatments (described below in Experiment 3) were also used to measure embryo growth. A minimum of 20 embryos were measured immediately after retrieval from the soil. A similar number were measured after the buried seeds had been subsequently incubated for 24 weeks at warm temperatures.

3.2.3 IMBIBITION AND VIABILITY

To see whether freshly matured drupes could imbibe water, three replicates of five fruits for each species were placed on moist filter paper. At six time periods (0, 1, 3, 5, 12 and 96 hours), each replicate batch was weighed after excess water was removed from the drupe surface with blotting paper. Further observations of excised seeds were made before and after imbibition to assess the relative softness of the endosperm. This was evaluated to indicate whether water was penetrating through the endocarp and into the seed.

For each species, collected drupes were pooled across sites. To test the viability of each pooled lot, a cut test was conducted using a minimum of three replicates of 20 drupes. Seeds that were firm and contained healthy looking white endosperm were considered viable. Viability was high (60% – 92%) for all three species. The cut test provides an accurate assessment of viability for these species (Ooi *et al.* 2004b). Each drupe usually contained a single viable seed, although approximately 20% of *L. esquamatus* drupes contained two viable seeds. However, the term “seed” will be used from here on to describe the whole dispersal unit.

3.2.4 GERMINATION EXPERIMENTS

Between 60 and 120 seeds, depending on availability, divided into three replicates were used for each treatment during all germination experiments. During experiments, seeds were placed on three layers of moistened filter paper in 9cm petri dishes in light and temperature controlled germination cabinets. Incubators were set at 12 hour/12 hour light/dark and maximum/minimum temperature cycles. Seeds were checked weekly and watered with distilled water as required. Germination was scored on the emergence of the radicle and expressed as a proportion of total viable seed.

Experiment 1: Assessing seed dormancy. The definition of a dormant seed is one that does not have the capacity to germinate under combinations of normal environmental factors (such as temperature and light/dark) that are otherwise favourable for its germination (Baskin & Baskin 2004). To assess whether seeds of the study species have a primary dormancy (i.e. dormant at the time of primary dispersal), I used freshly dispersed seeds and tested for their germination response to seasonal temperatures (3 levels; winter, spring/autumn and summer) and light (2 levels; light, dark). For each species, seeds were equally divided into three groups of six replicate dishes. Half of the six dishes were assigned to a dark treatment and wrapped in two layers of aluminium foil. Each group of six dishes was then placed at each of three seasonal temperature settings in three incubators. The incubators were set at temperatures approximating either (i) winter (16/6° C), (ii) spring/autumn (20/10° C) and (iii) summer (28/18° C) conditions. Germination was monitored for 30 weeks. When checking germination for the dark treatment, seeds were exposed to dim lighting for less than 20 seconds.

Experiment 2: Single season temperatures – fire cues and scarification. To assess the effect of fire cues on primary dormancy at any one of the three separate season temperatures, factorial combinations of smoke (2 levels; smoked, unsmoked) and heat shock (2 levels; heated, unheated) were applied to replicate dishes of seeds in experiments conducted soon after seed collection. Incubator settings were the same as described above. Additionally, when sufficient seed was available, scarification was included as an independent treatment to assess whether there was a physical component to primary seed dormancy (Table 3.1). Smoke was applied to both the seeds and filter paper (used as the substrate during the experiments) for approximately 10 minutes, using a beekeeper smoker, burning a combination of dry and fresh vegetation material collected from the study sites. This period of time is reported to enhance germination in a number of species in the region (Morris 2000; Thomas *et al.* 2003). Smoked filter paper maintains good levels of smoke derivatives (Keith 1997) and smoke cues have been reported as being able to persist in the soil for long periods (van Staden *et al.* 2000). Heat treatments were set at 90° C for 10 minutes in an oven. This duration and temperature has been reported to enhance germination in a wide range of species (Auld & O'Connell 1991; Keith 1997). For each species, each replicate smoke and heat

Table 3.1 Treatments used for the initial dormancy experiments on fresh seeds at the three different incubation temperatures for 30 weeks. * denotes that these treatments were only followed for 15 weeks.

Treatment	<i>L. exolasius</i>			<i>L. setiger</i>			<i>L. esquamatus</i>		
	16/6°	20/10 °	28/18 °	16/6°	20/10 °	28/18 °	16/6°	20/10 °	28/18 °
control	x	x	x	x	x	x	x	x	x
smoke	x	x	x	x	x	x	x		
heat	x	x	x	x	x	x	x		
smoke + heat	x	x	x	x	x	x	x	x	
dark	x*	x	x*	x*	x	x*	x*		x
scarified	x*		x*	x*		x*	x*		x*

Table 3.2 Treatments applied for experiments on fresh seeds conducted at the seasonal temperature regime. Changes between each temperature level in the incubators followed a time line similar to natural changes occurring in the field, from summer then autumn, winter and then onto the following summer, over a 60 week period. Experiments run at 20/10° were used as a control.

Treatment	<i>L. exolasius</i>		<i>L. setiger</i>		<i>L. esquamatus</i>	
	Seasonal	20/10°	Seasonal	20/10°	Seasonal	20/10°
control	x	x	x	x	x	x
smoke	x	x	x	x	x	x
heat	x	x	x	x	x	x
dark	x				x	
smoke + heat	x	x	x	x	x	x
smoke + dark	x				x	
heat + dark	x				x	
smoke+heat+dark	x				x	
scarified	x	x	x	x	x	x

treatment was applied separately to avoid pseudoreplication (Morrison & Morris 2000). For smoke X heat treatments, the heat treatment was applied first. Seeds were scarified by nicking the seed coat with a scalpel.

Experiment 3: Single season temperatures – burial. To assess whether primary dormancy is broken during burial, experiments were conducted at spring/autumn (20/10° C) and summer (28/18° C) alternating temperatures, using seeds retrieved after 18 months burial *in situ*. Seeds stored dry in envelopes in the lab for 18 months were used as a control. Insufficient seeds were available to also run the experiment at winter temperatures (16/6° C). Buried seeds used in the experiments had been placed in soil-filled mesh bags and buried within the top 5cm of the soil at the seed collection sites soon after seed release. Mesh size retained the seeds but allowed moisture to pass through. Bags were retrieved approximately 18 months after burial and had experienced the late summer period, followed by a winter, summer, winter cycle and retrieved as the next summer approached. Seeds were sifted out, washed in distilled water and viability was assessed prior to the experiments.

Experiment 4: Seasonal temperature regimes – germination phenology and fire cues. These experiments were designed to study the germination phenology of the study species and enable comparisons with temporal emergence patterns observed in the field (Ooi *et al.* 2004a). I also wanted to determine the effects of fire cues and darkness, when combined with a seasonal temperature regime, on dormancy and germination timing. The design included temperature variation (2 levels; unvarying control at 20/10 °C and varying temperatures following the seasonal pattern), factorial combinations of smoke, heat and dark (*L. exolasius* and *L. esquamatus*), or smoke and heat only (*L. setiger*), and scarification (Table 3.2).

Seasonal temperature pattern incubators were set to mimic seasonal changes in the study region. Four temperature levels were chosen, reflecting monthly averages in the field. These temperatures were 28/18° C (December, January, February), 25/15° C (October and March), 20/10° C (April, May, September) and 16/6° C (June, July, August). Changes between each temperature level in the incubator followed a time line similar to natural changes. For example, incubators set at 28/18° C for 3 months, then

changed to 25/15° C for 1 month, 20/10° C for the next 2 months and 16/6° C for 3 months, mimicked a seasonal pattern in the field from summer through to winter. Fire cues were applied as described earlier. Each experiment was started at warm temperatures, with seeds therefore undergoing a warm, cold, warm, cold cycle (i.e following a pattern of summer, autumn, winter, spring, summer temperatures and so on). The time period applied to mimic the first summer were shorter than subsequent summer temperature periods, reflecting the shorter time following seed maturation during the first summer in the field. Smoke and heat shock were applied to seeds after this first summer treatment, which consisted of 4 weeks at 25° C followed by 6 weeks at 20/10° C. All species placed in the incubator at a constant temperature regime of 20/10° C (equivalent to April/May or September) for 60 weeks, were treated with factorial combinations of smoke and heat only (Table 3.2).

Experiment 5: Seed ageing processes. This experiment aimed to determine which aspect of the ageing process was responsible for breaking primary dormancy by comparing seeds previously buried (therefore undergoing seasonal changes, as well as the physical deterioration and leaching that occurs *in situ*) with seeds aged in an incubator set at a seasonal regime (therefore undergoing a seasonal temperature treatment only). Dry-stored seeds were used as a control for all species. For *L. exolasius*, it was also possible to test whether there were any different ageing effects between a single season temperature and an annual seasonal temperature regime, by including seeds previously incubated for 60 weeks at 20/10° C in the comparison.

Seeds from the burial treatment had been buried for 15 months *in situ* using methods described earlier. Previously incubated seeds (seeds from the above germination timing experiment) had undergone 60 weeks (approximately 15 months) at temperatures mimicking seasonal changes. Dry-stored seeds were aged for 18 months in envelopes at room temperature (~22° C). It was assumed that the effect of an extra 3 months storage for dry-stored seeds was minimal. After the ageing treatments were completed, replicate dishes for all three species were placed in the incubator. Incubator temperatures were initially set at 28/18° C and moved through the seasonal regime over 30 weeks.

Experiment 6: Fire response of aged seeds. This experiment aimed to assess the effects of fire treatments on aged seeds. Seeds aged by 15 months burial (as described above), were used and treated with factorial combinations of smoke and heat shock cues (*L. setiger* and *L. esquamatus*), or with smoke treatments only applied to *L. exolasius*. Replicate dishes for all three species were then placed in the incubator, as described above, and monitored for 30 weeks.

3.2.5 DATA ANALYSES

Final percent germination was expressed as a proportion of seeds assessed as viable at the beginning of the trials. All data were assessed for homogeneity of variance using Levene's test and arcsine transformed if required, to meet the assumptions for parametric analysis. For each species, experiments were designed to analyse germination data using a three-factor orthogonal ANOVA, with smoke, heat and dark as factors. For experiments where a dark treatment was not applied, data were analysed using a two-factor ANOVA and a one-factor ANOVA used with smoke only experiments. Effects of seed ageing experiments were also analysed using a one-factor ANOVA, with ageing as the factor. Multiple comparisons were made using the Student-Newman-Keuls (SNK) test, with appropriate adjustments made for multiple factor ANOVAs with significant or non-significant interactions (Underwood 1997). All graphs are presented using untransformed data.

Temporal germination patterns for all experiments using the seasonal temperature regime were plotted. For each species, total numbers of germinants were pooled and the proportions germinating at each time period calculated. For comparisons, proportional data were standardised by categorising into four time periods, which were equivalent to temperatures occurring December – February (summer), March – May (autumn), June – August (winter) and September – November (spring).

3.3 Results

3.3.1 EMBRYO MORPHOLOGY AND GROWTH AND SEED IMBIBITION

All species contained differentiated embryos that are underdeveloped and linear. These were positioned at the base of the seed, surrounded by endosperm. Embryo:seed ratios were approximately one-third for each species (Table 3.3). Embryos of freshly dispersed *L. exolasius* and *L. setiger* seeds had not grown after any of the stratification treatments. *Leucopogon esquamatus* mean embryo length increased from 0.76 mm (± 0.021 (1 SE)) to 1.03 mm (± 0.025) after 24 weeks warm stratification. A total of 23% had germinated and several embryos had grown to the full length of the seed. Measurements of embryos after 18 months burial revealed that embryo lengths had not changed for any species. Too few of the previously buried *L. esquamatus* seeds remained ungerminated for assessment after 24 weeks warm incubation. However, *L. exolasius* mean embryo length had increased from 0.95 mm (± 0.034) to 1.40 mm (± 0.162). Several *L. exolasius* embryos had grown to the full length of the seed.

Fresh drupes of all species were able to imbibe water. *Leucopogon exolasius*, *L. setiger* and *L. esquamatus* total weights increased by 15%, 17% and 33% respectively. Approximately 50% of this weight increase occurred within 3 hours for all species. Endosperm structure had weakened considerably after imbibition, indicating that water had penetrated past the pericarp and into the endosperm.

Table 3.3 Morphological details of the three study species (mean \pm SE).

Species	Fruit weight (mg)	Seed length (mm)	Embryo length (mm)	E:S ratio	Embryo type
<i>Leucopogon exolasius</i>	13.1 \pm 0.37	3.07 \pm 0.034	0.95 \pm 0.034	0.31	Underdeveloped linear
<i>Leucopogon setiger</i>	10.9 \pm 1.70	3.00 \pm 0.033	1.14 \pm 0.029	0.38	Underdeveloped linear
<i>Leucopogon esquamatus</i>	4.9 \pm 0.16	2.41 \pm 0.035	0.76 \pm 0.021	0.32	Underdeveloped linear

3.3.2 EXPERIMENT 1 - ASSESSING SEED DORMANCY

Leucopogon exolasius and *L. setiger* seeds did not germinate within 30 weeks at any of the single diurnal temperatures. Fresh seeds of these species therefore have a primary dormancy at release. No germination was recorded for fresh *L. esquamatus* seeds during 30 weeks at 20/10° C or 16/6° C. However, approximately 14% germinated at 28/18° C (12 hour/12 hour light/dark) and the majority of these germinated between 8 and 12 weeks into the experiment (data not shown). The majority of fresh *L. esquamatus* seeds are therefore dormant at seed release but a small proportion can germinate after several months at warm temperatures. None of the seeds kept in darkness germinated.

3.3.3 EXPERIMENTS 2 AND 3: SINGLE SEASON TEMPERATURES – FIRE CUES, SCARIFICATION AND BURIAL

No seeds treated with fire cues or scarification germinated at single season temperatures (Experiment 2). Seeds that had been previously buried for 18 months germinated to relatively high proportions, with more seeds germinating recorded at 28/18° C than at 20/10° C (Experiment 3, Figure 3.1). No seeds dry-stored for 18 months in the lab germinated during Experiment 3 (data not shown).

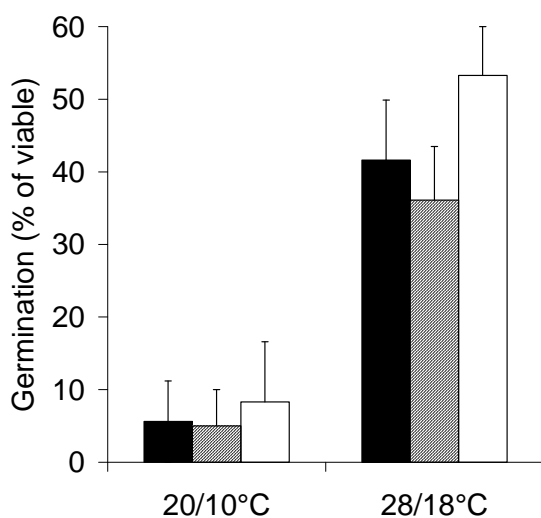


Figure 3.1 Experiment 3: Mean percentage germination of previously buried seeds after 30 weeks at two different temperatures for *Leucopogon exolasius* (■), *L. setiger* (▨) and *L. esquamatus* (□). Error bars represent standard errors of means.

3.3.4 EXPERIMENT 4: SEASONAL TEMPERATURE REGIMES – GERMINATION TIMING AND FIRE CUES

Fire cues. Seeds kept in the unvarying temperature regime (20/10° C) for 60 weeks did not germinate. At the seasonal temperature regime, too few *L. setiger* seeds germinated to conduct any statistical analyses. There was a significant, and negative effect of heat as a main effect on germination of *L. exolasius* ($F_{1,16} = 5.56$, $P = 0.031$) and *L. esquamatus* ($F_{1,16} = 11.19$, $P = 0.004$). Total *L. exolasius* germination was quite low (<10%) whilst *L. esquamatus* germination was relatively high, with over 30% germination recorded for untreated and smoke treated seeds (data not shown). Scarification did not significantly increase germination for any species.

Germination phenology. Due to relatively small numbers of germinants, analyses of germination phenology for each species were conducted using pooled data from all treatments. No germination of *L. exolasius* or *L. setiger* occurred during the first cycle of warm temperatures. For all seeds that germinated, approximately 89% of *L. exolasius* and 100% of *L. setiger* did so in the autumn equivalent period, between weeks 38 and 48, after the second cycle of warm temperatures (Figure 3.2). The rest germinated at times equivalent to late summer. *Leucopogon esquamatus* seeds germinated in two pulses, after both warm cycle periods (Figure 3.2). Of the total, 32% germinated in the first pulse and 68% in the second. No germination was recorded for 25 weeks between the two pulses (equivalent to the end of winter, spring and summer periods). Approximately 85% of seeds that germinated did so in the autumn equivalent period. For all three species, almost no germination occurred after the onset of 16/6° C winter equivalent temperatures, around week 50 (Figure 3.2), even though a large number of viable seeds were still present.

3.3.5 EXPERIMENT 5: SEED AGEING PROCESSES

Treatments significantly affected germination for *L. exolasius* ($F_{3,8} = 23.34$, $P < 0.001$), *L. setiger* ($F_{2,6} = 107.48$, $P < 0.001$) and *L. esquamatus* ($F_{2,6} = 6.91$, $P = 0.028$). Seasonal incubation resulted in similar (two species) or greater (one species) germination than burial, whilst dry storage resulted in either zero or slight germination

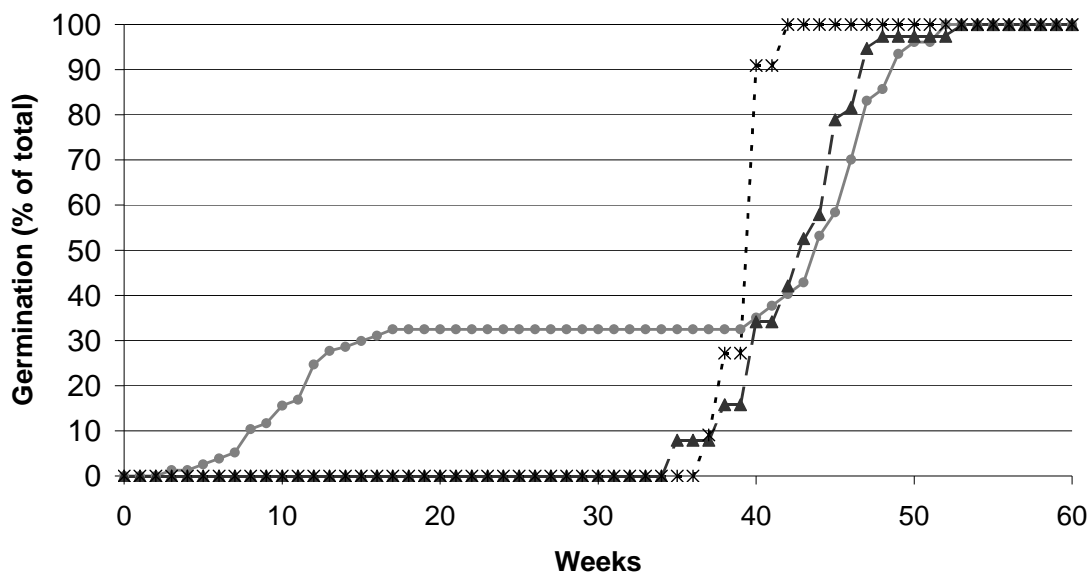


Figure 3.2 Experiment 4: Temporal patterns of germination of fresh seeds during 60 weeks at the seasonal temperature regime for (a) *Leucopogon exolasius* (▲ and dashed line), (b) *L. setiger* (✱ and dotted line) and (c) *L. esquamatus* (● and solid line). Data are the cumulative percentage, each week, of the total number of seeds germinated.

(Figure 3.3). Seasonally incubation and burial also resulted in greater germination than incubation at a single season temperature for *L. exolasius* (Figure 3.3a).

3.3.6 EXPERIMENT 6: FIRE RESPONSE OF AGED SEEDS

The effects of applied fire treatments on seeds previously aged by 15 months burial varied considerably. Smoke, the only treatment applied to *L. exolasius*, increased germination totals from 41% to 60%, but this increase was not significant (Figure 3.4a). For *L. setiger*, there was a significant interaction between smoke and heat ($F_{1,8} = 13.22$, $P = 0.007$). Smoke increased germination for unheated seeds, but this effect disappeared for heated seeds (Figure 3.4b). For all treatments, *L. esquamatus* germinated to between 72% and 87% (Figure 3.4c).

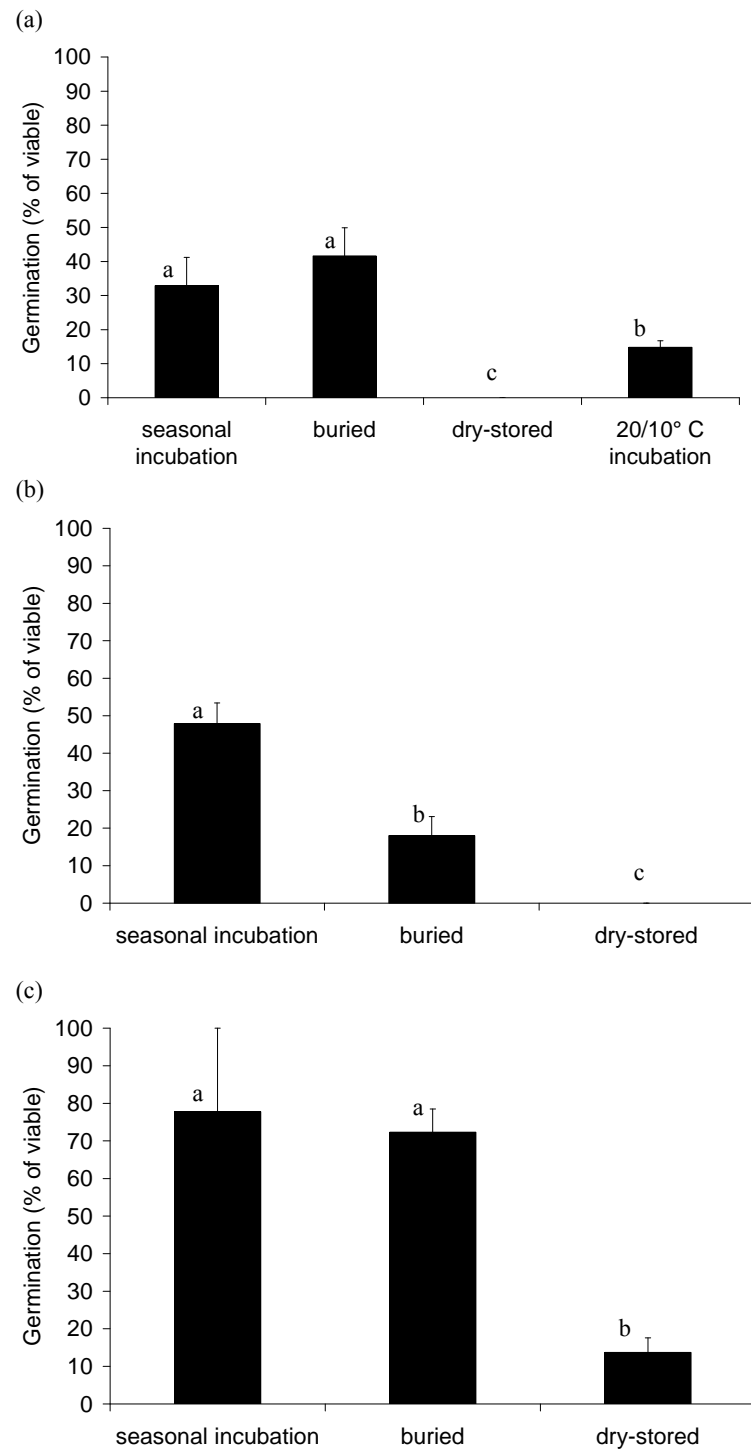


Figure 3.3 Experiment 5: Effect of different ageing treatments on mean percentage germination for (a) *Leucopogon exolasius* (b) *L. setiger* and (c) *L. esquamatus* after 30 weeks at the seasonal temperature regime, starting at warm temperatures. Error bars represent standard errors of means. Means with the same letter are not significantly different (SNK test, $P > 0.05$).

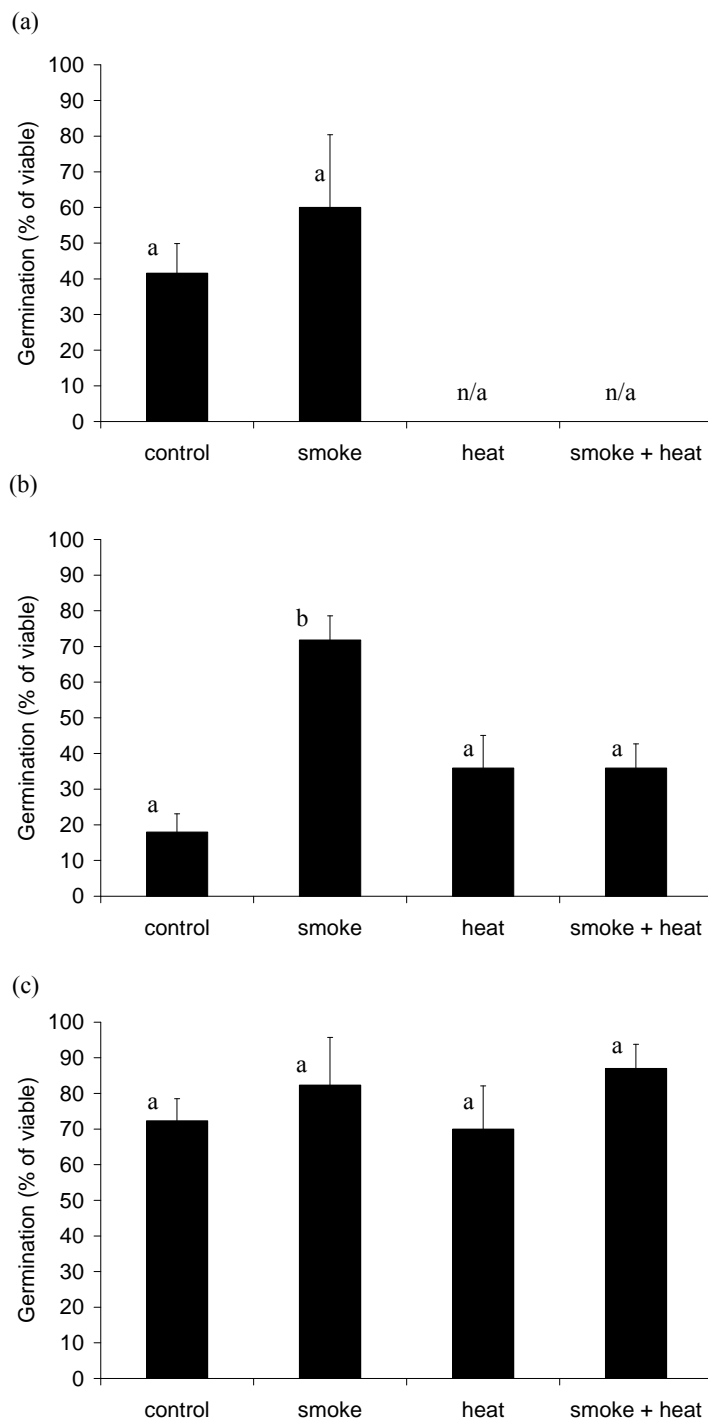


Figure 3.4 Experiment 6: Treatment effects on seeds, previously aged by 18 months burial, then treated with smoke and/or heat. Columns represent mean percentage germination after 30 weeks at the seasonal temperature regime, starting at warm temperatures for (a) *Leucopogon exolasius* (b) *L. setiger* and (c) *L. esquamatus* (n/a denotes treatment not applied).

3.4 Discussion

Fresh seeds of the *Leucopogon* species studied are dormant at release and have underdeveloped linear embryos. Seeds with underdeveloped embryos that have the ability to grow, which do not germinate after testing for several weeks at temperatures that typically occur in their environment, have a primary dormancy that is a combination of morphological and physiological factors (Baskin & Baskin 1998). The primary dormancy of all three species investigated is therefore classified as morphophysiological (Baskin & Baskin 2004). Morphological dormancy is imposed by the need for embryos to grow to a species-specific length before germination can take place. This growth occurs at particular seasonal temperatures (Baskin & Baskin 1998). In the case of the *Leucopogon* species, embryos of two of the species were able to grow at warm temperatures and several seeds observed after incubation had embryos that had extended to the full length of the seed. Embryos must therefore triple in size before germination occurs. Physiological dormancy is caused by physiological inhibition mechanisms within the embryo or structures surrounding the embryo. Physiological dormancy can be broken before, after or concurrently with morphological dormancy and is dependent on stratification at warm or cold temperatures, or some combination of these, experienced within the habitat (Baskin & Baskin 1998). In the field stratification occurs at seasonal temperatures.

This study supports the conventional concept that stratification at seasonal temperatures is the principal mechanism for breaking morphophysiological dormancy (Baskin & Baskin 1998). It has been hypothesised that other factors that occur during burial, such as physical deterioration of the seed coat or general ageing, is an important requirement for *Leucopogon* germination (Roche *et al.* 1997; Tieu & Egerton-Warburton 2000). However, in this study, seeds subjected to temperatures, equivalent to seasonal changes, germinated to at least the same levels as the buried seeds (Experiment 5). Incubator aged seeds are subjected to temperature changes only and do not undergo the same physical deterioration. Results from other assessments, such as the ability of fresh seeds to imbibe water and the lack of increase in germination from scarified treatments, also suggest that seasonal temperatures are more important than reducing mechanical constraints for breaking dormancy. In addition, seeds dry-stored at room

temperature for 18 months, or incubated at a single temperature setting (Experiment 5), produced much lower levels of germination, emphasising that fluctuating temperatures are the cue rather than simply an ageing process.

The distinction between whether fire cues break primary dormancy or increase subsequent germination after primary dormancy is broken is an important one, as it clarifies the sequence of processes occurring naturally in the seed bank (Baskin & Baskin 2003a). The fire cues, smoke and heat shock, did not break primary morphophysiological dormancy of freshly dispersed seeds of the *Leucopogon* species studied, and very low levels of germination were recorded both at single-season temperatures (Experiment 2) and seasonally varying temperatures (Experiment 4). Combined with evidence from other studies (Keeley 1987; Dixon *et al.* 1995; Keeley & Fotheringham 1998; Clarke *et al.* 2000), it is considered unlikely that fire cues overcome this type of dormancy in species from other fire-prone habitats. However, once primary dormancy was broken in my study (during burial), there was a trend for smoke to enhance germination for two of the study species. Although not dependent entirely upon smoke (untreated seeds from all experiments germinated to between 20% and 80%), germination of *L. exolasius* and *L. setiger* seeds was increased by between 10% and 55% above control treatments.

One possible explanation for these results is that smoke may have a positive effect on seeds that remain morphologically dormant, after physiological dormancy has been removed during burial or stratification. Embryo lengths of the study species remained unchanged after burial but grew in length and germinated once placed at warm incubation temperatures in light. Smoke increases the sensitivity of seeds to the hormones that promote embryo growth (and therefore overcome morphological dormancy) (van Staden *et al.* 2000; Schwachtje & Baldwin 2004) and this could increase the proportion of seeds within a seed lot that germinate. Identifying the order that morphophysiological factors are broken would help to validate this hypothesis.

Factors other than smoke may also promote embryo growth and germination from a persistent seed bank. *Leucopogon* germination pulses significantly in the first germination season after fire and observations suggest that they are possibly gap

recruiters (Ooi *et al.* 2004a). Gaps are created after fire by the removal of canopies and litter (Keeley 1995; Bond & van Wilgen 1996). Light and increased temperature amplitude both provide mechanisms that could promote a flush of seedlings, either post-fire or in gaps. A light requirement is an important mechanism adopted to detect gaps, particularly by small-seeded species, from a variety of habitats (Pons 1992), however, its importance in regulating dormancy and germination in Australian fire-prone habitats is not well understood (Bell *et al.* 1993; Clarke *et al.* 2000). Daily temperature amplitude can increase significantly after the removal of above-ground canopy and litter (Auld & Bradstock 1996). Further investigations into the effect of smoke, light and daily temperature amplitude on *Leucopogon* germination are needed to gain a fuller understanding of recruitment dynamics.

In the field, seasonal emergence from a persistent soil seed bank has been recorded for all three species, even though the region does not have seasonal rainfall patterns (Ooi *et al.* 2004a; 2006a). These patterns impress the importance of seasonal temperatures, not only for breaking primary dormancy, but also for determining germination timing via subsequent conditional dormancy. The majority of germination of seasonally incubated seeds was timed to the period equivalent to autumn, possibly because immature embryos required a long period of time at warm temperatures for growth, after physiological dormancy was overcome. *Leucopogon* seedling emergence occurs primarily in autumn and winter in the field (Ooi *et al.* 2004a). Allowing for the lag time between radicle protrusion and seedling emergence of 3 to 4 weeks (M.K.J. Ooi, pers. obs.), results from the germination trials and seedling emergence patterns in the field correlate well.

Although the three *Leucopogon* species studied are the first in south-eastern Australia to be classified as having a physiological dormancy component, there is strong biogeographical evidence to suggest that morphological and physiological factors, and therefore seasonal temperatures, are likely to control the dormancy of many species from this fire-prone region (Baskin & Baskin 1998; Ooi 2007). It is therefore likely that a suite of dormancy mechanisms exists in fire prone regions generally (Bell 1999), and a greater understanding of all of these can contribute to a better understanding and management of these ecosystems. A relatively recent increase in the practise and

scrutiny of practical management applications, such as habitat restoration and native species horticulture, has focussed the need for this understanding.

Chapter 4

Dormancy classification and potential dormancy-breaking cues for shrub species from fire-prone south-eastern Australia



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Preface

Having classified *Leucopogon* species as morphophysiological dormant (MPD), as well as highlighted the distinction between dormancy and germination cues, this Chapter has two broad aims. The first is to assess the effects of fire cues on other species with MPD, to see whether results from Chapter 3 are specific to *Leucopogon* or a potential consequence of dormancy mechanisms generally. The second is to estimate the relative proportions of dormancy types over a broader geographical range.

Publication

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4.1 Introduction

In fire-prone regions the post-fire environment is characterised by a flush of seedling emergence. It is considered advantageous for seedlings to emerge quickly after fire as there are increased levels of nutrients, decreased levels of competition and consequently a greater chance of survival (Whelan 1995; Bond & van Wilgen 1996). This is particularly important for obligate-seeding species, as population persistence after fire is dependent primarily on germination and recruitment from propagules stored in a seed bank (Keith 1996). Seeds of species with a canopy-stored seed bank are mostly non-dormant, however, those with a soil-stored seed bank often have some type of dormancy (Bell *et al.* 1993).

For a species to take advantage of post-fire conditions, and because fire is the predominant disturbance, many ecologists assume that seed dormancy is broken by fire cues. This is indeed true for a large number of species, namely the dormancy-breaking effect of heat shock on hard-seeded legumes (e.g. Auld & O'Connell 1991). However, freshly dispersed seeds of many other species from fire-prone regions do not germinate during laboratory trials, even after being treated with fire cues (for examples, see Dixon *et al.* 1995; Roche *et al.* 1997; Keeley & Fotheringham 1998; Clarke *et al.* 2000; van Staden *et al.* 2000; Ooi *et al.* 2006b), and their dormancy mechanisms remain unknown. These same species may still display a flush of emergence after fire *in situ* or after burial (Dixon *et al.* 1995; Enright *et al.* 1997; Roche *et al.* 1997; Wills & Read 2002; Rokich *et al.* 2002; Ooi *et al.* 2004a). Observations of seedling emergence *en masse* can therefore indicate that the passage of fire has provided a germination cue, but it does not necessarily indicate that primary dormancy has been broken by fire.

This apparent dichotomy highlights the need for a clearer understanding of the distinction between the processes of dormancy release and germination. Implying and defining seeds as being dormant because of a lack of germination from a soil-stored seed bank is a concept that may not necessarily hinder plant ecology research (Thompson *et al.* 2003). For example, a number of studies use this concept of dormancy and provide clear descriptions of seed bank dynamics (Enright *et al.* 1997; Roche *et al.* 1997). However, it can cause problems when trying to define certain research questions

(Vleeshouwers *et al.* 1995; Thompson *et al.* 2003), and as is the case here, particularly those aimed at identifying the dormancy mechanisms of species in fire-prone environments. A relatively recent increase in the practise of practical ecological management applications, and species-specific management, has focussed on the need for a greater understanding of dormancy mechanisms in fire-prone regions (Merritt & Dixon 2003; Ooi *et al.* 2006b).

Many seed physiologists define seed dormancy as a characteristic of the seed and not the environment (Vleeshouwers *et al.* 1995; Fenner & Thompson 2005). As such, dormancy-breaking cues may overcome primary dormancy, but germination can still be prevented until favourable environmental conditions occur. Environmental conditions that can control germination include seasonal temperatures and light conditions. Seeds may also enter into a secondary dormancy (Baskin & Baskin 1998, 2004). By taking the physiological view of dormancy, a clearer hypothesis for more complex dormancy mechanisms in fire-prone environments may be suggested. Factors required to break primary dormancy may be completely unrelated to fire, however, fire may provide subsequent cues and suitable environmental conditions to promote germination (Bell 1999).

Classifying dormancy type is a fundamental step for understanding the germination ecology of a species (Baskin & Baskin 2004). Placing species into even the broadest dormancy classes can help to identify likely dormancy-breaking cues, by enabling comparisons with other species within the same class. Seeds classified as non-dormant (ND) germinate in the first few weeks under some combination of normal environmental conditions experienced in their natural habitat (Baskin & Baskin 1998). Seeds with physical dormancy (PY) have an impermeable seed coat that needs to be broken before germination can occur. Physiological dormancy (PD) prevents germination until a chemical change occurs within the seed (Fenner & Thompson 2005) and this is generally brought about by stratification at some combination of seasonal temperatures (Baskin & Baskin 1998). Morphological dormancy (MD), where the embryo is immature or not fully developed, is very rarely recorded in temperate regions, as it is usually coupled with PD (Baskin & Baskin 1998). Freshly dispersed seeds that

have a combination of PD and MD are classified as having morphophysiological dormancy (MPD).

Other than the many hard-seeded species with PY, there is a considerable gap in our knowledge about the dormancy classes that species from fire-prone regions belong to. In this study, I aimed to classify the dormancy of a number of shrub species from the fire-prone heaths and woodlands of south-eastern Australia, and subsequently highlight the types of dormancy represented in these systems. Because of the lack of studies classifying dormancy in fire-prone regions, the effects of fire cues on dormancy types, other than heat shock on PY, have never explicitly been tested. Therefore, I also aimed to examine whether the primary dormancy of species, particularly those with MPD, can be overcome by cues directly related to fire, such as smoke and heat shock.

Additionally, by focussing in on a more discrete geographical range, the proportions of different dormancy types were estimated using shrub species from the east coast of Australia. These were compared to dormancy-class distribution compiled using threatened species only. The role that seasonal temperatures play in controlling dormancy and germination in the region were also discussed. Unlike Mediterranean-type climates, south-eastern Australia has no distinct rainfall season (Stern *et al.* 2000) and is climatically more similar to non-Mediterranean fire-prone regions such as Florida (Carrington & Keeley 1999) or the south-eastern cape regions of South Africa (Deacon *et al.* 1992; Cowling & Lombard 2002). Throughout the world, seasonal temperature is the primary factor that overcomes seed dormancy, and plays a dual role by also providing a germination cue (Fenner & Thompson 2005). This has rarely been acknowledged in the south-east Australian region. A clearer understanding of seasonal temperature effects on dormancy and germination mechanisms may therefore highlight possible effects of season on post-fire population recovery and vegetation dynamics for the many thousands of species, including a number of threatened ones, from fire-prone habitats in this region.

4.2 Methods

4.2.1 CLASSIFICATION OF DORMANCY TYPES

A dormant seed is one that does not have the capacity to germinate under combinations of normal environmental factors that are otherwise favourable for its germination (Baskin & Baskin 2004). To assess what type of dormancy seeds have, it is therefore necessary to test for germination at temperature regimes similar to those that occur naturally in the field. Additionally, embryo morphology needs to be investigated to identify whether or not there is a morphological component to dormancy and seed imbibition has to be assessed to identify physical dormancy. Several species were chosen, ranging from families that are considered to have more complex dormancy, including members of the Ericaceae (formerly Epacridaceae), Rutaceae and Santalaceae, to species that are likely to have no dormancy (Table 4.1).

4.2.2 SEED EMBRYO MORPHOLOGY AND IMBIBITION

Seeds were sectioned using either a scalpel and dissecting microscope or a microtome. *Astroloma*, *Persoonia* and *Styphelia* fruit were first cracked open using a bench vice. Seed and embryo lengths were measured. To see whether freshly matured seeds could imbibe water, 20 dry seeds for each species were weighed and then placed on moist filter paper for 5 min. Excess water was then removed from the seed surface with blotting paper before being weighed again. This was used as the starting weight. Seeds were then placed on plain agar (10 g/L) and weighed every hour for at least 6 hours and subsequently every 24 hours for a further 4 days. Additionally, to ensure water was actually passing through the seed coat, several seeds of each species were soaked in a solution of methylene blue for 4 days. At the end of this period, seeds were dissected to assess where staining had occurred.

4.2.3 GERMINATION EXPERIMENTS

To assess whether seeds of the study species have a primary dormancy (i.e. are dormant at the time of dispersal), freshly dispersed seeds were used where possible. Either 60 or 75 seeds, depending on availability, were divided into three replicates and used for each treatment. Seeds were placed on plain agar (10 g/L) in 9 cm Petri dishes. Three groups of three replicate dishes were then placed at each of three seasonal temperature settings in incubators. A dark treatment was also included for species with sufficient seed numbers (Table 4.1). Dishes assigned to a dark treatment were wrapped in two layers of aluminium foil. Incubators were set at 8/16 hour light/dark and maximum/minimum temperature cycles. Temperatures approximating either (i) winter (15/5°C), (ii) spring/autumn (20/10°C) and (iii) summer (25/15°C) conditions were used. Seeds were checked weekly and monitored for 6 weeks. Checking for germination in the dark treatments was conducted under green safe lights. Germination was scored on the emergence of the radicle and expressed as a proportion of total viable seed.

4.2.4 THE EFFECTS OF FIRE CUES ON MORPHOPHYSIOLOGICAL DORMANCY

It is important to know whether fire cues break primary dormancy or provide germination cues once primary dormancy is overcome (Baskin & Baskin 2003a). The effect of heat shock on breaking PY is well known in this region and is excluded from this study. Smoke is also known to increase levels of germination for many species from fire-prone environments (Brown 1993; Dixon *et al.* 1995; Bell 1999). However, the effects of smoke or heat shock on breaking PD or MPD is not so clear. To test this, the effects of charcoal, smoke and/or heat shock on seeds of species with MPD were investigated by reviewing germination studies. Morphophysiological dormancy was chosen as the test dormancy type because a larger number of species could be identified as having MPD with a greater degree of confidence, compared to species with PD, by inference from embryo morphology data. Shrub species from temperate regions that have seeds with underdeveloped embryos are very likely to have MPD (Baskin & Baskin 1998). Also, MPD is overcome by similar mechanisms as PD in many regions around the world.

Morphophysiologically dormant species within Australia were identified by reviewing studies targeting dormancy classification or embryo morphology (Martin 1946; Corner 1976; Schatral 1996; Schatral *et al.* 1997; Baskin & Baskin 1998, 2003a; Tieu & Egerton-Warburton 2000; Ooi *et al.* 2006b; M.K.J. Ooi, unpublished data). If no specific data were available, embryo morphology, and subsequently dormancy type, was inferred from closely related species. Germination studies that had used species with MPD as the subject, and included charcoal, smoke and/or heat shock treatments applied to fresh seeds, were then identified and the results collated (Bell *et al.* 1987; Dixon *et al.* 1995; Roche *et al.* 1997; Campbell 1999; Clarke *et al.* 2000; Ooi *et al.* 2006b). If possible, germination totals at the end of 30 days were used, as this is the period by which germination should have occurred if dormancy is broken (Baskin & Baskin 2004). Studies were only selected if it was possible to identify that seeds had not received pre-treatments, such as long storage or cold stratification.

4.2.5 PROPORTION OF DORMANCY TYPES IN THE SYDNEY FLORA SHRUB LAYER

A literature review was also used to identify and estimate the distribution of dormancy types of shrub species from the east coast Australian region. Distributions for threatened species were also separated out and graphed. Only shrub species found in fire-prone heath, woodland and forest habitats, occurring in the Central Coast Botanical Subdivision of the state of New South Wales (NSW) (Harden 1992), were used for this part of the study. Species lists were obtained using the PlantNET database (<http://plantnet.rbgsyd.nsw.gov.au>) and other references and herbaria data (e.g. Benson & McDougall 1995). This subdivision includes the Sydney Basin. As well as the references mentioned above, dormancy types were then identified or inferred from Auld & O'Connell (1991), Pannell (1995), Campbell (1999), Kenny (2000), Morris (2000), Auld *et al.* (2000), Auld (2001), Thomas *et al.* (2003) and Bhatia *et al.* (2005), as well as a number of databases and unpublished data.

4.3 Results

4.3.1 CLASSIFICATION OF DORMANCY TYPES

All species imbibed water within the first few hours. Methylene blue stain had passed through the seed coats of all species after 4 days. Embryo morphologies ranged from underdeveloped rudimentary types to those that were fully developed and non-endospermic (Table 4.1). Of the five species of Ericaceae tested, only one, *Leucopogon amplexicaulis* (Rudge) R. Br., did not have an underdeveloped embryo. Only seeds of *Petrophile pulchella* (Schrader & J.C. Wendl.) germinated, a species with a canopy-stored seed bank from the family Proteaceae, with total germination reaching 100% at all three temperature regimes. Rates of germination were slower, however, at low temperatures (data not shown). For the species used in this study, MPD and PD were the only dormancy types identified (Table 4.1).

Table 4.1. Species used and results from experiments assessing dormancy class. Germination percentages for each species are for all three temperature treatments. The abbreviation n/t denotes species not tested using dark treatment. Dormancy classes are described as physiological dormancy (PD), morphophysiological dormancy (MPD) and non-dormant (ND).

Species	Family	Embryo type	Imbibes water	% germination light	% germination dark	Dormancy class
<i>Astroloma pinifolium</i>	Ericaceae	Underdeveloped linear	Yes	0	n/t	MPD
<i>Leucopogon amplexicaulis</i>	Ericaceae	Linear	Yes	0	0	PD
<i>Leucopogon juniperinus</i>	Ericaceae	Underdeveloped linear	Yes	0	0	MPD
<i>Leucopogon parviflorus</i>	Ericaceae	Underdeveloped linear	Yes	0	0	MPD
<i>Styphelia viridis</i>	Ericaceae	Underdeveloped linear	Yes	0	0	MPD
<i>Persoonia laurina</i>	Proteaceae	Non-endospermic	Yes	0	n/t	PD
<i>Petrophile pulchella</i>	Proteaceae	Non-endospermic	Yes	100	n/t	ND
<i>Correa alba</i>	Rutaceae	Linear	Yes	0	n/t	PD
<i>Leptomeria acida</i>	Santalaceae	Underdeveloped rudimentary	Yes	0	0	MPD

4.3.2 THE EFFECT OF FIRE CUES ON MORPHOPHYSIOLOGICAL DORMANCY

Sixty-two species from around Australia that were likely to have MPD were identified from studies that applied fire treatments to fresh seeds. Twenty-four genera within the families Antheriaceae, Apiaceae, Dasypogonaceae, Dilleniaceae, Ericaceae, Iridaceae, Ranunculaceae and Santalaceae were represented. Of the 62 species identified, 96% failed to germinate with or without fire cue treatments or, occasionally, produced minimal (< 5%) germination, at the end of 30 days.

4.3.3 PROPORTION OF DORMANCY TYPES IN THE SYDNEY FLORA SHRUB LAYER

A total of 700 shrub species from fire-prone heath and forest communities within the Central Coast Botanical Subdivision were identified. Nearly half of these species were members of the Fabaceae, Proteaceae and Myrtaceae. A further third of all shrub species came from the Rutaceae, Ericaceae, Asteraceae and Lamiaceae families, all of which are known to have many species with a physiological component to their dormancy. Because little information was available on germination requirements or dormancy type for most of the species identified, inferences were made, sometimes at a family level. For example, data from other fire-prone regions show that the majority of species in the Rutaceae have PD (Bell *et al.* 1993; Baskin & Baskin 1998). Most Rutaceae in this study were therefore assigned to PD unless available research suggested otherwise. Most Sapindaceae and Rhamnaceae were classified as PY using the same logic. Many Asteraceae were left as unknown due to the variability of dormancy types shown by species within this family. Although it is likely that species have either ND or PD, neither classification could confidently be assigned. Dormancy type was identified, or more usually confidently inferred, for 608 shrub species (see Appendix 1). Of these, 28.6% were classified as non-dormant and 71.4% classified as dormant. Expressed as a proportion of dormant species only, the largest class of dormancy represented was PY (42.6%) followed by PD (39.8%) and MPD (16.9%) (Figure 4.1a). For the 54 threatened species identified within this group, 76% were classified as having some form of dormancy. Expressed as a proportion of dormant

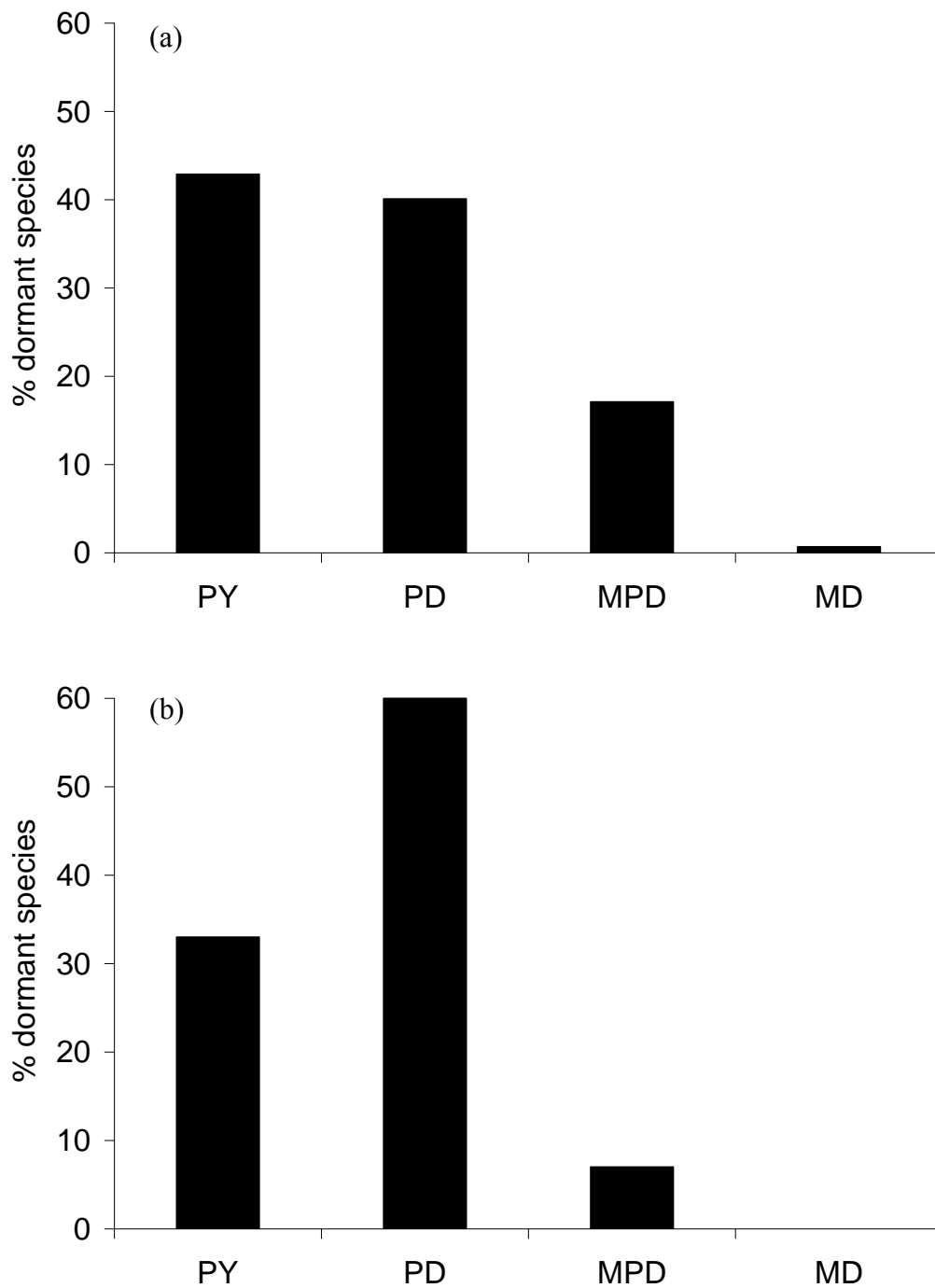


Figure 4.1. Shrub species from the Central Coast Botanical Subdivision (New South Wales) in south-eastern Australia in each dormancy class. Graphs show the proportion of (a) all species ($n = 434$) and (b) threatened species ($n = 54$), known or inferred to be dormant (i.e. excluding species that are non-dormant or have unknown dormancy). Dormancy types are PY (physical), PD (physiological), MPD (morphophysiological) and MD (morphological).

threatened species, PD became the largest class of dormancy (60%), followed by PY (33.2%) and MPD (6.8%) (Figure 4.1b).

4.4 Discussion

All five classes of dormancy occur in fire-prone Australian vegetation. Germination trials conducted in this study found representative species of two dormancy types, PD and MPD. Most species within the tribe Styphelieae (Ericaceae) (Ooi *et al.* 2006b; M.K.J. Ooi, unpublished data) and the Dilleniaceae (Schatral 1996) have MPD, whilst those within the Rutaceae, Lamiaceae (Baskin & Baskin 1998) and also *Persoonia*, seem to have PD. Numerous other studies have found many species with PY, including most legumes and many Rhamnaceae (e.g. Auld & O'Connell 1991; Turner *et al.* 2005). Combinational dormancy (PY and PD) occurs in several *Hibbertia* species (Bell 1999; Allan *et al.* 2004) and MD is likely to occur in some mistletoes (Loranthaceae) (Baskin & Baskin 1998) and several species of *Trachymene* (Apiaceae) (McKenzie & Ooi, in review). These results are not surprising, however, they highlight the fact that a lack of previous study on dormancy classification is not due to a lack of dormancy types in fire-prone regions.

From the NSW Central Coast Botanical Subdivision on the east coast of Australia, the proportion of shrub species classified as PD and PY was similar to that for similar fire-prone vegetation types (described as 'matorral' in Baskin & Baskin (1998)). This vegetation type has the highest proportion of shrub species with PY in the temperate climate zone. It is quite possible that this dormancy class has an advantage in fire-prone vegetation, because the heat shock dormancy cue translates to fast post-fire recruitment. Interestingly, there was nearly double the proportion of species with MPD in this region compared to other matorral vegetation (Baskin & Baskin 1998). This could be due to the relatively high proportion of Ericaceous species with this class of dormancy in Australia. Ericaceae from the Mediterranean and South Africa tend to have PD (Baskin & Baskin 1998), whilst many species within the Ericaceae in Australia, at least within the Styphelieae tribe, have underdeveloped embryos (Ooi *et al.* 2006b; M.K.J. Ooi, unpublished data).

Classifying dormancy can help to decipher the requirements for breaking dormancy. In various climatic regions around the world, MPD (and PD) is broken by stratification of seeds at seasonal temperatures (Baskin & Baskin 1998). Species with MPD that are from fire-prone regions also appear to have dormancy broken by seasonal temperatures. Even though fire is the predominant disturbance, fires themselves do not provide the dormancy-breaking cue. Nearly all species identified during this study as having MPD, which were the subject of experimental treatments using fire cues such as charcoal, smoke or heat shock, did not germinate. In a recent study, Ooi *et al.* (2006b) found that several *Leucopogon* (Ericaceae) species also did not have MPD broken by the fire cues, smoke and heat shock. Furthermore, in that study, stratification at seasonal temperatures was identified as the main dormancy-breaking factor overcoming MPD during burial. Once MPD was broken by seasonal temperatures, smoke could enhance subsequent levels of germination. There is evidence from other studies showing that burial can break primary dormancy of species with MPD and that subsequent smoke treatments can increase germination (Roche *et al.* 1997; Keeley & Fotheringham 1998; Tieu *et al.* 2001). Taxa include *Leucopogon*, *Astroloma*, *Conostephium*, *Achrotriche* (all genera within the Styphelieae (Ericaceae)) and *Hibbertia* (Dilleniaceae) species from Australia and the Californian chaparral species *Dendromecon rigida* and *Dicentra chrysantha*.

The distinction between whether fire cues break primary dormancy or increase subsequent germination after dormancy is broken is an important one, as it clarifies the sequence of processes occurring naturally in the seed bank (Baskin & Baskin 2003a). Acknowledging this distinction can subsequently enable a more structured approach when trying to germinate species with more complex dormancy and germination mechanisms, and this is particularly important when using fresh seeds, such as for restoration or horticultural purposes. Species with MPD or PD need to have primary dormancy broken by stratification at some combination of seasonal temperatures (Vleeshouwers *et al.* 1995; Baskin & Baskin 1998; Fenner & Thompson 2005) before other factors can have an effect.

Seasonal temperatures can play a dual role, both breaking dormancy and providing a germination cue (Fenner & Thompson 2005). This can subsequently affect the timing or season of emergence. It appears that the great majority of species with a physiological component to their dormancy have seasonal temperatures acting in both roles (Baskin & Baskin 1998). For example, species with non-deep simple MPD require a warm or cold stratification period to break dormancy, before embryo growth and germination occur at warm temperatures (Baskin & Baskin 2004).

In a study conducted in the Sydney region, Ooi *et al.* (2004a) found that emergence of several *Leucopogon* (Ericaceae) species occurred after fire during the late autumn or winter period, irrespective of earlier significant rainfall. These species have MPD, as well as temperature dependent germination (Ooi *et al.* 2006b). Emergence of these species can therefore be delayed, with the magnitude of delay dependent upon the season of the fire event. In the same study, the post-fire seedling emergence of many species, with either PY or ND (e.g. family Fabaceae and species of *Banksia*, *Hakea* and *Grevillea* from the family Proteaceae), occurred much more quickly. Many studies have found that for species with PY in south-eastern Australia, germination will occur rapidly after fire, as soon as sufficient moisture is available (Auld & O'Connell 1991; Auld & Tozer 1995; Ooi *et al.* 2004a).

Seasonal emergence is a common phenomenon in many Mediterranean-type habitats, where it is considered that germination coincides with a distinct winter rainfall period (Whelan 1995; Bond & van Wilgen 1996; Bell 1999). For example, in the Mediterranean-type climate of the Californian chaparral, the fire season is June to October (i.e. summer to early autumn) and the majority of seedling emergence occurs in spring (Carrington & Keeley 1999). As resprout growth is limited by drought immediately after the June to October fire season, and then by winter temperatures, delayed post-fire emergence occurs concurrently with resprouting. The seasonal delay in seedling emergence in these habitats is therefore not expected to be disadvantageous (Carrington & Keeley 1999).

However, seasonal emergence in regions where there is no distinct rainfall season, such as south-eastern Australia and in the east of the Cape Floristic Region in South

Africa, could cause enough delay to disadvantage subsequent survival and growth (Jones *et al.* 1997; Verdú & Traveset 2005) of species with a seasonal requirement to their germination. Depending on the season of the fire event, seedling emergence of these species could lag behind that of species that emerge more rapidly, as well as resprouters, by up to 1 year. Although the sample size was relatively small, the dormancy-class distribution for threatened species in this study, showed a distinct shift away from PY as the dominant class. This suggests that PD, and consequently seasonal emergence, may not be the optimal dormancy type under current environmental conditions (e.g. Jurado & Flores 2005).

It is therefore possible that in these environments, species with PD or MPD (more seasonal emergers) could be more sensitive than species with PY (more rapid emergers) to any seasonal shift in the fire regime. In this study, 42.6% of the east coast shrub flora of Australia had some level of PY, whilst 56.7% of species had either PD or MPD. There is some evidence that fire regimes within the region are shifting from predominantly spring/summer to autumn/winter, due particularly to an increase in implemented burning (McLoughlin, 1998). Could this lead to a shift in species composition in these habitats? And does this place threatened species with PD or MPD at an even greater risk? These questions highlight the need for more research into dormancy classification, dormancy mechanisms and subsequent recruitment in fire-prone vegetation, particularly of species with PD or MPD. This includes members of the Ericaceae, Rutaceae, Apiaceae, Lamiaceae and Santalaceae.

Chapter 5

Assessment of *Leucopogon* seed bank longevity: distinguishing between seed bank persistence and seed dormancy



See print copy for figure

Preface

Fundamental ecological data are required to explore the relative abilities of rare and common species to persist. For obligate-seeding species, one of the most important ecological traits to consider is seed bank longevity. This can determine how much dependence is placed on regular seed input, as well as the relative abilities of species to recover after fire. In fire-prone regions, a long-lived seed bank is beneficial as it can ensure that seeds are available for germination after temporally variable fire events. In this Chapter, I investigate seed bank longevity of the three study species. There is currently no information regarding soil seed bank dynamics of species within the Ericaceae in the south-east Australian region.

Publication

Ooi, M.K.J., Auld, T.D. & Whelan, R.J. (2007) Distinguishing between persistence and dormancy in soil seed banks of three shrub species from fire-prone southeastern Australia. *Journal of Vegetation Science*, **18**, 405-412.

5.1 Introduction

Habitats subjected to variable disturbance regimes tend to select for species with persistent seed banks (Fenner & Thompson 2005). Germination from a seed bank has the potential to be delayed such that seedling emergence coincides with the post-disturbance environment, at a time when seedling establishment may be facilitated and recruitment is most needed for population persistence. In fire-prone regions recruitment is often linked to the occurrence of fire (Whelan 1995). Although many species have seed banks held in the plant canopy, most fire-prone communities including Australia, California, the Mediterranean basin, South America and South Africa are dominated by species with soil-stored seed banks (Parker & Kelly 1989; Jiménez & Armesto 1992; Auld *et al.* 2000; Keeley & Fotheringham 2000; Holmes & Newton 2004).

Recruitment from seed banks is necessary for population persistence because fire causes varying levels of mortality of plants. Species that resprout after fire are relatively resilient to a single fire event but long-term persistence is still dependent on some recruitment from seed (Keeley & Zedler 1978). Species that have individuals killed by fire, called fire-sensitive or obligate seeders, have adapted to fire intervals that exceed their primary juvenile period the majority of the time (Lamont *et al.* 1991). Obligate seeders are dependent primarily upon stored seed, both for short-term regeneration and long-term persistence, and are therefore more sensitive to seed bank dynamics.

The magnitude of the post-fire flush of seedling emergence is dependent on several factors, including seed bank longevity, seed dormancy and germination cues (Auld *et al.* 2000). Dormancy is defined as a characteristic of the seed rather than of its environment (Vleeshouwers *et al.* 1995). Seed dormancy classification, using a system such as that outlined by Baskin & Baskin (2004), distinguishes between physical, morphological and/or physiological mechanisms as controls on dormancy. Except for physically dormant seeds that germinate as a result of heat, recent studies postulate that fire cues do not overcome other classes of primary dormancy (Ooi *et al.* 2006b; Ooi 2007).

Physiological dormancy is caused by physiological inhibition mechanisms within the embryo or structures surrounding the embryo, and can be broken before, after or

concurrently with embryo growth (Baskin & Baskin 1998). Overcoming physiological dormancy is dependent on stratification at warm or cold seasonal temperatures, or some combination of these, experienced within the habitat (Baskin & Baskin 1998). For many species from fire-prone regions, incorporation into the soil seed bank itself has been found to overcome primary physiological dormancy (Tieu *et al.* 2001; Baker *et al.* 2005a; Keeley *et al.* 2005; Ooi *et al.* 2006b). Germination cues directly related to fire (e.g. smoke, heat), or indirectly related to fire (e.g. increased light, changes in daily temperature range), can then produce a post-fire flush of seedling emergence (Whelan 1995; Bond & van Wilgen 1996), but only after a period of burial.

Persistent seed banks are often thought to be dependent on primary seed dormancy, and in fact many ecological studies assume that these two factors are indistinguishable (Thompson *et al.* 2003). However, the failure to make a distinction between the processes that break dormancy and those that promote germination from a persistent seed bank can hamper our understanding of seed dormancy mechanisms and ecological processes that drive population dynamics (Vleeshouwers *et al.* 1995; Thompson *et al.* 2003). To find out what stimulates seeds to germinate from a persistent seed bank, information about the type of dormancy, as well as how and when each type of dormancy is broken, is required (Baskin & Baskin 1998).

In fire prone regions, physical dormancy has been relatively well studied (e.g. Auld & O'Connell 1991; Bell 1999; Keeley & Fotheringham 2000). However, it is only recently that physiological dormancy factors have been explored (Schatral *et al.* 1997; Keeley & Fotheringham 1998; Bell 1999; Tieu *et al.* 2001; Baker *et al.* 2005a, b; Ooi *et al.* 2006b; Ooi 2007). Understanding the relationship between dormancy mechanisms with a physiological component and seed bank persistence in fire-prone regions is therefore still at a relatively early stage.

To my knowledge, there are no examples from fire-prone regions explicitly describing continued seed bank persistence after primary dormancy loss. In this study, I investigated the longevity patterns of experimentally buried seeds of three obligate-seeding species of *Leucopogon* (Ericaceae, formerly Epacridaceae) from the fire-prone sandstone communities of south-eastern Australia. Seed burial studies of species within

the Ericaceae from the Mediterranean climates of south-western Australia have suggested that seed bank longevity is considerably variable, even between closely related species (Roche *et al.* 1997; Tieu *et al.* 2001). Understanding soil seed bank dynamics at a species level is therefore important, and is essential for plant population management and accurate modelling. There is currently no information regarding soil seed bank dynamics of ericaceous species in the non-Mediterranean climate region of south-eastern Australia.

I also examined the role that dormancy plays in maintaining seed bank persistence. The study species have a deep morphophysiological dormancy (Ooi *et al.* 2006b), and therefore have a physiological dormancy component combined with a small, underdeveloped embryo (morphological dormancy). This makes them ideal candidates for studying the sequence in which dormancy is overcome during burial, and to test whether or not the seed banks can persist once primary dormancy is broken. To do this, I examined germination responses and embryo growth over time, during burial. The main questions posed in this study therefore were:

- (1) What are the longevity patterns of experimentally buried *Leucopogon* seeds?
- (2) In what order are the morphophysiological dormancy factors broken during burial?
- (3) Does seed germinability change over time?
- (4) Is primary dormancy a requirement for seed bank persistence?

5.2 Methods

5.2.1 STUDY SPECIES AND SEED COLLECTION

Leucopogon (Ericaceae) is a large genus consisting of about 230 species. The Ericaceae make up a significant part of the understorey in fire-prone communities in Australia. The three study species are all obligate seeders with soil-stored seed banks (Ooi *et al.* 2006a). Emergence in the field is seasonal, with seedlings appearing at the same time of year (peaking in early winter, ca. May – June), even if there is significant rainfall at

other times of the year. Recruitment mainly occurs after fire with seedling emergence still restricted to the same emergence season (Ooi *et al.* 2004a). Embryos are underdeveloped and need to grow to the full length of the seed prior to germination. All three species are classified as morphophysiological dormant and therefore require stratification at either warm and/or cold temperatures to overcome dormancy (Ooi *et al.* 2006b). In the field stratification occurs at seasonal temperatures.

Leucopogon exolasius is an erect shrub that grows in woodlands to approximately 2 m, and is a threatened species restricted to the southern Sydney region of New South Wales (NSW), Australia. *Leucopogon setiger*, also an erect shrub to approximately 2 m, is more widespread, extending from the central western slopes of NSW to the coast (distributional range > 450 km). It is found in woodlands and open forest. *Leucopogon esquamatus* is a slender shrub, which grows to approximately 1 m. It is also widespread, occurring from the coast to the mountains (as for *L. setiger*), but extending south into the states of Victoria and Tasmania (distributional range > 1000 km). It occurs in heath, woodlands and open forests.

The climate in the Sydney region is temperate with no dry season (using the Köppen classification system) (Stern *et al.* 2000). Average annual rainfall is approximately 1050 mm, distributed relatively evenly throughout the year, with the four highest monthly averages occurring in January, March, June and November. Average temperatures (maximum/minimum) are approximately 27/18° C and 16/6° C in summer and winter respectively.

Fruits of all study species ripen in summer (November-December) and were collected in 2000/01 from within Royal (34° 03'S, 151° 03'E) and Heathcote (34° 07'S, 150° 58'E) National Parks in southern Sydney. *Leucopogon* fruit are drupes. Fresh exocarps of both *L. exolasius* and *L. setiger* are fleshy, while the endocarp is hard and lignified. *Leucopogon esquamatus* exocarp is papery and the endocarp comparatively more fibrous than hard. Seeds are held inside the fruit. All three species contain seeds with underdeveloped linear embryos surrounded by endosperm (Ooi *et al.* 2006b). Before the commencement of experiments, flesh was removed from the collected fruits of each species by soaking in water for several days.

5.2.2 INITIAL SEED DORMANCY, VIABILITY AND EMBRYO LENGTH

For each of the species, fruits were collected and then tested for viability. A cut test was conducted using three replicates of 20 fruits. The cut test provides an accurate assessment of viability for these species (Ooi *et al.* 2004b). Seeds that were firm and contained healthy looking white endosperm were considered viable and the proportion of viable seeds was calculated. Embryo length was recorded by excising the embryos of between 20 and 30 seeds with a scalpel and fine tweezers and measuring them using an ocular micrometre. Each fruit usually contained a single seed, so the term “seed” is used from here on in this paper.

Initial levels of germinability of the seed lots were assessed by germination trials. For each species, 20 seeds were placed into three replicate 9 cm diameter petri dishes on filter paper moistened with distilled water. Each group of three dishes was then placed in an incubator set at 12 hour/12 hour light/dark (using 30 W cool white fluorescent tubes) and maximum/minimum temperature cycles. Optimum germination of these species occurs at warm summer-type temperatures (Ooi *et al.* 2006b), therefore I tested for their germination response in incubators set at temperatures approximating summer (25/15° C) conditions. Germination was scored on emergence of the radicle and monitored at weekly intervals for 18 weeks. Although the experiment was formally completed after 18 weeks, germination from several dishes of each species continued to be recorded for 30 weeks.

5.2.3 SEED BANK LONGEVITY IN UNBURNT VEGETATION

Seed burial trials were conducted in the field at sites where seed collections had been made. Seeds of all three species were buried just after the time of natural seed fall, at the end of summer (March) 2001. Species did not co-occur, so each of the three sites contained only a single species. At each site, three plots were randomly located within the vicinity of mature plants. At each plot, three bags made from 2 mm nylon mesh, containing a mixture of soil from the plot and 40 seeds of the study species were buried in the top 5 cm of the soil.

For each species, one bag from each plot was retrieved each year, during the summer (December – February), over the 3-year period of the study. During retrieval, any emerged seedlings were recorded. Recovered bags were taken to the laboratory and the contents air-dried. The soil was then sifted and the numbers of intact and split seeds were recorded. Previous observations in the laboratory had shown that germinating seeds created a distinctive split in the endocarp. Split seeds, as well as seeds with radicles already emerged, were therefore described as germinating *in situ*. Viability, embryo length and germinability of retrieved seeds was assessed as above, using 3×20 seeds. After the second and third year retrievals, viability and embryo lengths were also assessed at the end of the germination trials. Numbers of seeds per replicate used for germinability trials were occasionally lower, due to the small proportion of seeds already germinated *in situ*. As a control, seeds of each species were also tested for germinability and viability after one and 3 years dry-storage in the laboratory. Tests on dry-stored seeds were not done after 2 years due to limited seed numbers. To determine the length embryos needed to grow to before germination occurred, data on embryo lengths were collected during germinability trials. For each species, embryos of several seeds that had split but not yet germinated (i.e. prior to radicle emergence) were excised and measured as described earlier.

5.2.4 DATA ANALYSES

Regressions were fitted to proportional data of the intact viable seed fraction over time. To allow a comparison of seed viability of intact seeds across all sampling times, the viable seed fraction was estimated using the equation from Auld *et al.* (2000):

$$IV_t = (N_t/N_0) \times V_t$$

where IV_t is the intact viable seed fraction at time t ; N_t is the number of intact seeds at time t ; N_0 is the number of seeds buried per replicate at time $t = 0$ and V_t is the viable seed fraction at time t . Both linear and exponential regressions were fitted to the data,

with exponential curves the best fit for all three species. Seed bank half-life was estimated by substituting the decay rate into the equation. To enable a comparison of seed bank longevity between species, percentage decline was analysed by two-factor ANOVA, with initial viability of all species adjusted to 100%. Species and time were orthogonal and treated as fixed factors.

Comparisons of germinability and proportions of seeds germinating *in situ* were also made by two-factor ANOVA with species and time as orthogonal factors. For comparisons after germination trials, final percent germination was expressed as a proportion of seeds assessed as viable at the beginning of each trial. Although not designed to test for small-scale spatial variation between plots, observations suggested some between plot differences. Therefore, for each species, comparisons of possible plot effects were made by two-factor ANOVA without replication, with time and plot as factors. This approach does not allow for a test for the significance of the interaction and a cautious approach is recommended for testing the significance of main effects (Zar 1984). The significance levels were therefore increased to 0.01. For each species, comparisons of embryo length before and after burial, as well as proportion of viable seeds before and after dry-storage, were made by a one-factor ANOVA. Multiple comparisons were made using the Student-Newman-Keuls (SNK) test. For all ANOVAs data were assessed for homogeneity of variance using Levene's test and arcsine transformed if required to comply with the assumptions for parametric testing.

5.3 Results

Both initial dormancy and viability of freshly collected seeds were relatively high for all species (Table 5.1). The proportions of intact viable seeds declined over time (Figure 5.1), and the regressions fitted to the relationship between time and the intact viable seed fractions were significant for all three species (Table 5.1). The regressions explained a large amount of the variation of seed decay for *L. exolasius* and *L. esquamatus*, but less for *L. setiger*, where there was greater variation between replicates (Table 5.1). Half-lives of the seed banks calculated from these data suggested that all three species have persistent seed banks (Table 5.1). There were no significant

Table 5.1. Results from the regression analysis of seed bank longevity for the three study species. Initial seed germinability and viability data are means, with standard errors in parentheses. Estimated seed bank half-lives are means, with a range of one standard error above and below the mean decay rate.

Species	Initial germinability (%)	Initial viability (%)	Predicted decay rate (SE)	R ²	Estimated half-life (years)	Significance
<i>Leucopogon exolasius</i>	0	92.33 (1.33)	0.127 (0.037)	0.592	5.45 (4.22 – 7.73)	0.007
<i>Leucopogon setiger</i>	0	83.33 (4.91)	0.156 (0.070)	0.366	4.44 (3.07 – 8.06)	0.049
<i>Leucopogon esquamatus</i>	13.50 (5.40)	61.67 (2.33)	0.200 (0.060)	0.565	3.47 (2.67 – 4.95)	0.008

differences between species, but there were significant differences found between retrieval time periods ($F_{3,24} = 10.561$; $P \leq 0.001$). Post-hoc tests revealed that initial viability differed from viability at all other time periods. Seed viability did not differ between freshly collected seeds and seeds stored in the laboratory for 1 or 3 years.

Losses from the seed bank due to germination *in situ* were quite small, and there were no increases over time for any of the three species (Figure 5.2). A plot effect was found for *L. exolasius*, with significantly higher *in situ* germination at Plot 2 ($P = 0.007$).

There were relatively high levels of germination of seeds recovered from burial and tested for germinability in the laboratory trials (Figure 5.3). Onset of germination occurred after six to 10 weeks. Anecdotal monitoring recorded germination occurring up until week 26. Significant differences were found between species, with greater proportions of *L. esquamatus* germinating than the other two species at each of the time periods ($F_{2,18} = 11.325$; $P = 0.001$). For each species, there were no significant differences in germinability between seeds retrieved at different time periods, except for *L. setiger*, which had no germination after retrieval in the third year (Figure 5.3). There were no significant differences between germinability of freshly collected seeds and seeds stored in the laboratory for 1 or 3 years.

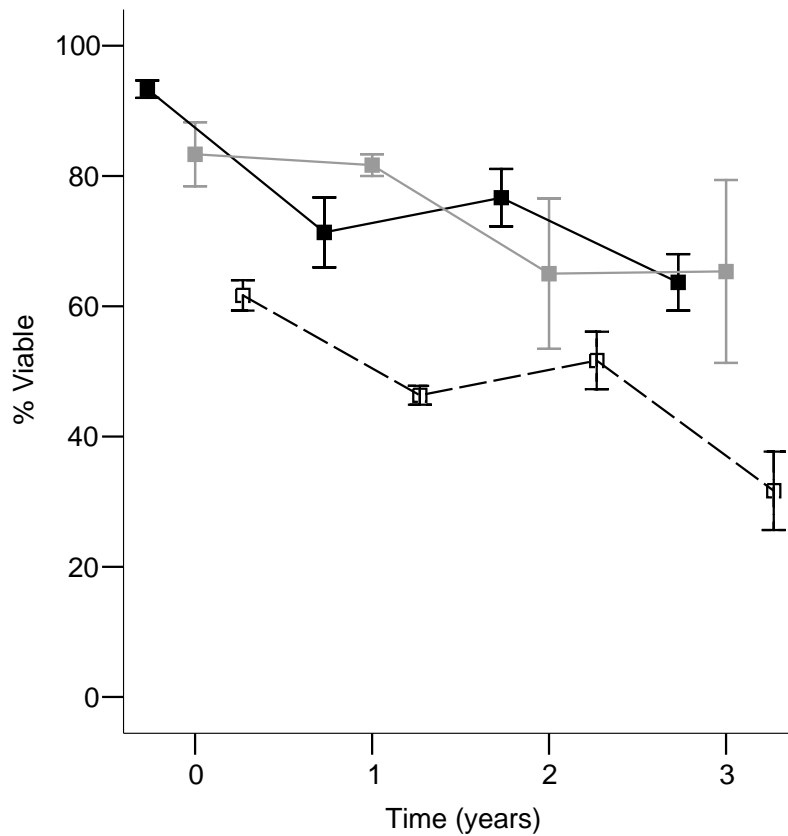


Figure 5.1. Seed bank decay over time for *Leucopogon exolasius* (■), *L. setiger* (■) and *L. esquamatus* (□). Data are mean proportion of viable intact seeds remaining in the soil at each time period. Bars represent 1 standard error. NB. Points for each species have been staggered along the time axis for the purpose of clarity only.

There was no significant change in embryo length for *L. setiger* or *L. esquamatus* over 3 years of burial. A significant difference was detected for *L. exolasius*, with mean embryo lengths of buried seeds slightly longer after the first retrieval period than initial lengths ($F_{3,128} = 7.175$; $P \leq 0.001$). However, mean embryo lengths at all time periods still indicated that embryos of most seeds were considerably underdeveloped (Figure 5.4). The difference was attributed to several *L. exolasius* seeds that had begun to germinate, with embryo lengths of the majority of seeds remaining unchanged. Seeds that were split but not yet germinated had embryos approximately triple the size of initial lengths (Figure 5.4).

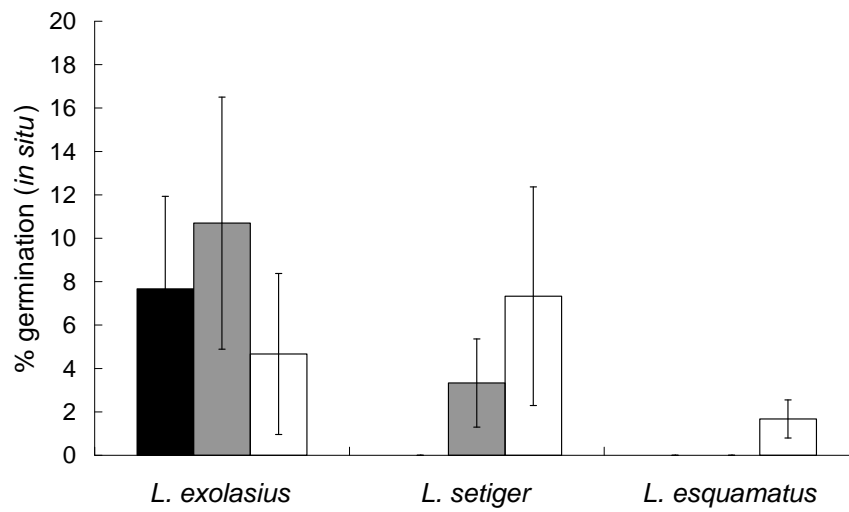


Figure 5.2 Germination of buried seeds *in situ* after burial for 1 year (■), 2 years (■) and 3 years (□). Data are mean proportions of viable seeds germinated. Bars represent 1 standard error.

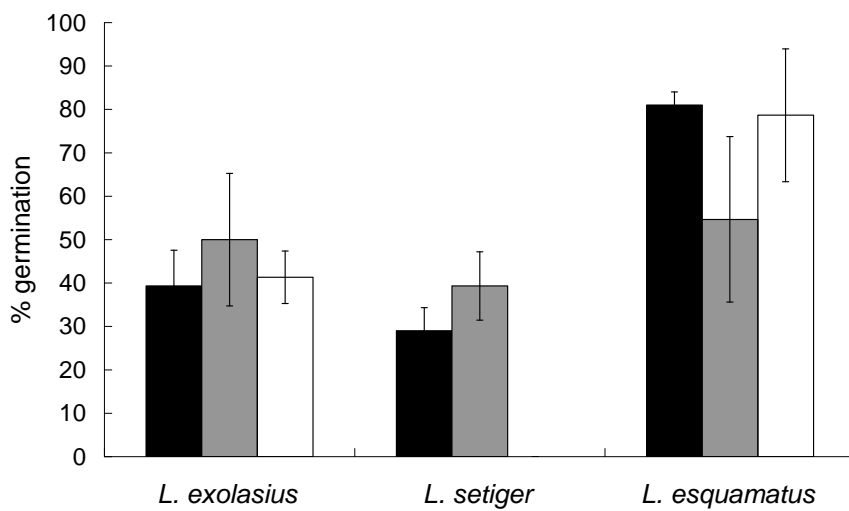


Figure 5.3 Germination of seeds in the laboratory at the end 18 weeks at 25/15° C after burial for 1 year (■), 2 years (■) and 3 years (□). Data are mean proportions of viable seeds germinated. Bars represent 1 standard error.

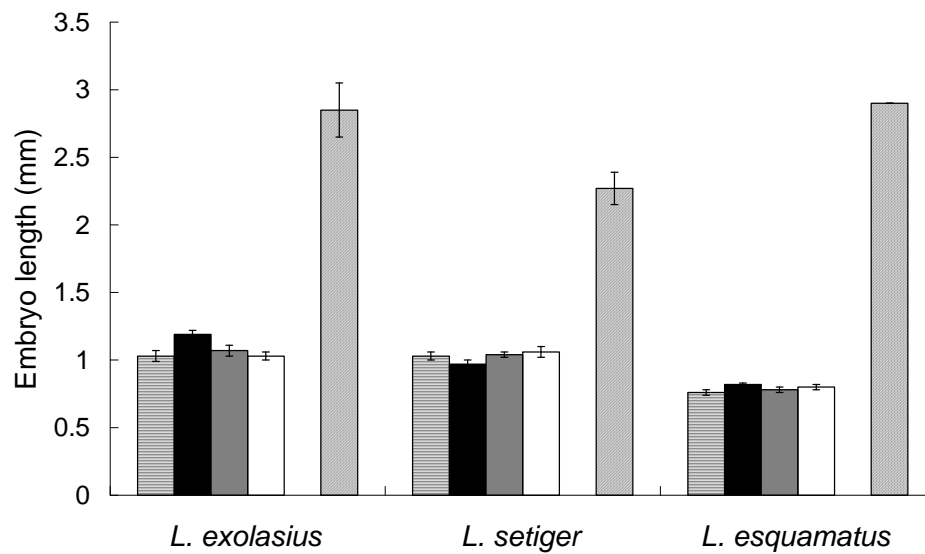


Figure 5.4 Embryo lengths of seeds when first collected at dispersal (▨), and then after burial for 1 year (■), 2 years (■) and 3 years (□). Mean lengths of split seeds, just prior to germination are also shown (▨). Data are mean lengths of at least 20 embryos. Bars represent 1 standard error.

5.4 Discussion

This study has confirmed firstly, that all three species had potentially long-lived seed banks in the absence of fire. The regressions were relatively good predictors of seed decay over time and predicted half-lives were well in excess of 3 years. All species are therefore broadly classified as having long-term persistent seed banks (total seed bank persistence > 5 years, Thompson *et al.* 1997). Secondly, it has provided an outline of the sequence of events seeds undergo; from being dormant at dispersal, to losing primary dormancy during burial, and finally to germination.

Seed bank losses in unburnt habitat occurred mainly due to loss of seed viability, rather than germination. Viability at all retrieval time periods differed significantly from initial viability. This was a result of a relatively rapid decay upon burial for both *L. exolasius* and *L. esquamatus*, followed by a more gradual decline. *In situ* germination contributed only slightly to overall seed bank decline and there was no increase in the

level of *in situ* germination over time. However, significantly higher *in situ* germination for *L. exolasius*, coupled with a plot effect, suggested that levels could change as a result of small-scale spatial variation. The *L. exolasius* plot with higher germination occurred where a recent gap in the canopy had been created, and it is likely that gap creation could promote germination from the seed bank, even in unburnt habitat. This is supported from another recent study of these species, where small but regular pulses of germination in unburnt habitat were recorded, particularly in areas where recent gaps had formed (Ooi *et al.* 2006a).

Long-lived seed banks are considered to be an important trait of plants from fire-prone landscapes (Bell *et al.* 1993; Tyler 1996; Pausas *et al.* 2004). Research investigating 14 species from the same region as this study, found that 13 had long-lived seed banks (Auld *et al.* 2000), with all of these considered likely to be persistent over long time scales. Plants from habitats susceptible to temporal variability in disturbances benefit from maintaining persistent seed banks because seeds are available over a wide time period. This increases the chance of successful recruitment whenever the disturbance does occur. Fire is a temporally variable disturbance and would therefore provide a selective impetus for long-lived seed banks. Such selection would not be as strong in habitats with more predictable or regular disturbances, such as seasonal drought (Thompson *et al.* 1998; Fenner & Thompson 2005).

Results indicated that the physiological dormancy was broken prior to morphological dormancy, for at least a proportion of the seed bank, during the first year of burial. All viable *L. exolasius* and *L. setiger* seeds, and the majority of *L. esquamatus* seeds, were dormant at the time of dispersal. Embryo lengths for each of the species did not increase, or increased only marginally, over time during burial. However, once seeds were retrieved from burial and placed in germination trials, embryos grew and seeds germinated. Although some germination did not occur until week 26 during laboratory trials, suggesting that processes other than embryo growth were occurring for some seeds during incubation, there was no germination from the control group, which had not been buried. This contributes to a growing body of evidence, showing that species from fire-prone habitats can overcome physiological dormancy during burial

(Roche *et al.* 1997; Keeley & Fotheringham 1998; Tieu *et al.* 2001; Baker *et al.* 2005a, b; Keeley *et al.* 2005; Ooi *et al.* 2006b).

Like many other species, stratification at seasonal temperatures overcomes physiological dormancy of *Leucopogon* seeds during burial (Ooi *et al.* 2006b). Because this occurred within the first year of burial, I was able to narrow down the combination of temperatures required. Results from earlier work, where experimental seed burial had been delayed until autumn and retrieved the following summer (M. Ooi, unpubl. data), showed that the initial warm stratification during the first summer was not necessary for overcoming dormancy. As such, physiological dormancy in these species is broken either by cold only, warm only, or cold followed by warm, stratification. Baskin *et al.* (2005) found that another species within the Ericaceae, *Leptecophylla tameiameiae*, required an extensive period of warm stratification to overcome deep physiological dormancy.

Results from the embryo measurements taken in this study indicated that seeds are committed to germinating once embryo growth is initiated, and therefore seeds with fully developed embryos are not maintained in the soil. Embryo growth and germination occurred at temperatures typical of summer, however, the proportion germinating did not peak until between weeks 10 and 14. This peak correlated well with the autumn seasonal flush of emergence displayed in the field (Ooi *et al.* 2004a).

Opportunistic evidence from the germinability trials suggested that seeds can re-enter dormancy, undergoing cyclical dormant and non-dormant periods. Both *L. exolasius* and *L. esquamatus* seeds germinated to similar levels after each of the three retrieval periods. However, *L. setiger* seeds did not germinate after the third year retrieval, even though a large proportion remained viable. While seeds were retrieved routinely during summer, the third year retrieval occurred several weeks earlier in the season than either the first or second year retrievals. A lack of any germination could suggest that reimposed dormancy had not been overcome so early in the season for the third year *L. setiger* seeds. Cyclical dormancy has been recorded for several other morphophysiologically dormant species from fire-prone habitats (Tieu *et al.* 2001; Baker *et al.* 2005b).

Although my germinability trials showed that dormancy is broken during burial, there was no increase in levels of *in situ* germination over time. Specific germination cues are therefore required and are distinct from dormancy-breaking cues. Subsequently seed banks have the potential to persist in a non-dormant state for many years. The post-fire flush of germination displayed by these species, as well as the increased levels of germination resulting from tree-fall (Ooi *et al.* 2006a, b), indicate that germination cues are likely to be related to gap creation. Increased daily temperature amplitudes and light levels, both of which were experienced by seeds during germinability trials, can be created by gaps produced as a result of fire (Whelan 1995; Auld & Bradstock 1996). Due to the depth of burial of the seeds in my experiments, I suggest that increased temperature amplitude, rather than light levels, may be more important for promoting germination of *Leucopogon*.

The much higher levels of *Leucopogon* emergence observed post-fire, compared to those resulting from tree-fall gaps, may result from smoke providing a germination cue, as it does for many fire-prone species (van Staden *et al.* 2000). In a previous study, smoke significantly increased germination of *L. setiger* seeds retrieved from burial and, although not significant, there was a similar trend for *L. exolasius* (Ooi *et al.* 2006b). There is now considerable evidence suggesting that physiological dormancy has to be broken before smoke becomes affective (Roche *et al.* 1997; Keeley & Fotheringham 1998; Tieu *et al.* 2001; Ooi *et al.* 2006b; Ooi 2007).

Seeds were able to persist ungerminated in the soil seed bank over several years, even after physiological dormancy was overcome. Primary dormancy was therefore not necessary for seed bank persistence. These results provide direct evidence that overcoming primary dormancy is distinct from stimulating germination from a seed bank in fire-prone vegetation. Physiologically dormant species in other fire-prone regions also appear to have dormancy broken during burial, with seeds remaining either non-dormant, or cycling in and out of dormancy, until suitable germination conditions arise (e.g. Roche *et al.* 1997; Tieu *et al.* 2001; Keeley *et al.* 2005). The distinction between a dormant seed bank and a persistent one is, in part, a matter of definition (Vleeshouwers *et al.* 1995; Thompson *et al.* 2003). However, there is the potential that

not distinguishing between these states could hamper our understanding of dormancy mechanisms, particularly in fire-prone communities.

In fire-prone regions, it is likely that the lack of distinction made between primary dormancy and seed bank persistence arose partly due to the large number of species that are hard-seeded. This primary physical dormancy is broken by heat during the passage of fire and subsequently has a direct effect on seed bank persistence. Physical dormancy is not reversible and the seed bank declines due to a germination pulse as soon as there is sufficient moisture. However, many species in fire-prone regions have a physiological component to their dormancy (Baskin & Baskin 1998; Ooi 2007). Dormancy-breaking cues are likely to be unrelated to the germination cues that promote a post-fire flush of emergence from the seed bank of these species. Cues that are observed to promote germination from the seed bank *in situ*, are not necessarily the ones that break primary dormancy of freshly dispersed seeds. Identifying them as such may divert attention away from other mechanisms actually controlling primary dormancy.

Chapter 6

Delayed emergence and post-fire recruitment success of shrubs in relation to dormancy type and fire season



See print copy for figure

Preface

In work from previous Chapters, I have classified dormancy and identified several factors controlling germination, including the interaction between season and fire cues. To assess the possible effects that dormancy type can have on population and species persistence, in particular the effects of season, this Chapter reports on the relative recruitment success of physiologically and physically dormant species. Understanding post-fire recruitment dynamics of the physiologically dormant *Leucopogon* species will contribute to comparisons made in the final Chapter. Because physiological and physical dormancy are the two most common dormancy types in the world, the results from this Chapter may also have broader implications for fire-prone communities throughout the world.

Publication

Ooi, M.K.J. (in review) Delayed emergence and post-fire recruitment success of shrubs in relation to dormancy type and fire season. *Journal of Applied Ecology*

6.1 Introduction

Fire causes plant mortality and, as a result, successful post-fire recruitment from seed is necessary for both short-term regeneration and long-term persistence (Whelan 1995; Bond & van Wilgen 1996; Keeley *et al.* 2006). Plants that do not resprout, known as obligate-seeding species, are particularly dependent upon recruitment from stored seed. Species that have their dormancy or germination response tightly connected to direct fire cues, such as heat and smoke, are able to time seedling emergence to the immediate post-fire environment, leading to a rapid flush of emergence following the first significant post-fire rainfall.

Rapid seedling emergence is considered to be advantageous, and has been shown to be beneficial for seedling survival and growth (Jones *et al.* 1997; Verdú & Traveset 2005). This benefit is gained from a number of factors in fire-prone regions, including an increased availability of resources and reduced competition (Whelan 1995; Bell 1999; Daskalakou & Thanos 2004; Moles & Westoby 2004a), but is lost to seedlings that have a delayed emergence. Conversely, some delay of emergence is considered to provide an advantage, by spreading risk associated with unpredictable post-fire climate such as drought (Zammit & Westoby 1987; Verdú & Traveset 2005).

Direct fire cues are not the only factors that control seed dormancy and germination. There are a variety of dormancy types among species within any plant community, and therefore a number of factors that can break seed dormancy and promote germination (Keeley 1995; Baskin & Baskin 1998; Fenner & Thompson 2005). In fire-prone regions, a full understanding of the range of dormancy and germination mechanisms operating is still somewhat limited. This is partly due to the large number of species that display obvious and rapid germination responses to fire characteristics, such as heat and smoke (Auld & O'Connell 1991; Keeley 1991, Dixon *et al.* 1995; van Staden *et al.* 2000).

Species with physiological seed dormancy are the most common throughout the world, whilst physically dormant or “hard-seeded” species are the next most common (Baskin & Baskin 2003b, 2004; Fenner & Thompson 2005). Breaking physiological

dormancy is dependent on stratification of seeds at warm or cold temperatures, or some combination of these (Baskin & Baskin 1998). In the field, stratification occurs at temperatures associated with particular seasons. In addition to breaking dormancy, seasonal temperatures also act as a germination cue for physiologically dormant species (Fenner & Thompson 2005), and ensure that seedling emergence occurs during a particular season.

In fire-prone regions, many physiologically dormant species display a seasonal flush of post-fire seedling emergence, because seeds need both a seasonal cue and a fire cue (as well as sufficient moisture) to germinate (Roche *et al.* 1998; Tieu *et al.* 2001; Ooi *et al.* 2004a, 2006b; Baker *et al.* 2005a; Merritt *et al.* 2007). However, many co-occurring species, particularly those that are physically dormant or “hard-seeded”, require only a fire cue, and post-fire seedling emergence occurs independently of season. Germination cues, and potentially dormancy type, can therefore affect the timing of the post-fire flush of seedling emergence.

Species that require a seasonal cue in addition to a fire cue for germination may have seedling emergence considerably delayed in relation to fire (Ooi *et al.* 2004a). For example, the innate seasonal germination requirement of many species in Florida means that seedling establishment occurs during spring. Seedling emergence after autumn fires is subsequently delayed by at least 6 months (Carrington & Keeley 1999). The seasonal delay in seedling emergence can increase the level of competition faced by young seedlings, from both vigorous resprouters and seedlings of physically dormant species that emerge rapidly after fire (Ooi *et al.* 2004a).

The effects of delayed emergence from soil-stored seed banks on recruitment success, caused by germination responses tightly connected to seasonality, have yet to be investigated in fire-prone regions. Nevertheless, approximately half the shrub species that occur in fire-prone regions are likely to have some form of physiological dormancy and therefore have a seasonal requirement for breaking dormancy (Baskin & Baskin 1998; Ooi 2007). As such, they potentially have a seasonal component to their germination and emergence, superimposed onto the germination cue provided by fire. Several studies have identified species from fire-prone regions in which the precise

timing of post-fire emergence appears to be determined by season (Kruger 1984; Midgley 1989; Carrington 1999; Carrington & Keeley 1999; Keith 2002; Ooi *et al.* 2004a; Quintana *et al.* 2004), a pattern that has subsequently resulted in delayed post-fire emergence.

Like a number of other fire-prone habitats in non-Mediterranean climate zones, including Florida and the eastern Cape area of South Africa, rainfall in south-eastern Australia is distributed relatively evenly throughout the year (Figure 6.1). It is therefore possible to separate the effects of seasonal germination and seasonal rainfall on recruitment success in such regions, two factors that are less easily distinguishable in Mediterranean-type climates which have strongly seasonal rainfall patterns. Uncoupling the effects of rainfall and fire season may also enable a clearer examination of assumptions made about plant trait evolution in response to fire regimes (Whelan 1985; Whelan 1995; Bond & van Wilgen 1996; Whelan *et al.* 2002).

This study, conducted in south-eastern Australia, compared the post-fire recruitment success of species that have physiologically dormant seeds (and display seasonal post-fire emergence) with species that have physically dormant seeds (and display rapid post-fire emergence), after the same fire event. All study species were shrubs with soil-stored seed banks. The mechanisms underlying seasonal variation in recruitment success of such species are currently poorly known (Bond & van Wilgen 1996). Several species with a physiological dormancy component, and subsequently a seasonal post-fire germination pattern, have previously been identified in south-eastern Australia, including the threatened species *Leucopogon exolasius*. Co-occurring species, particularly resprouters or physically dormant species that respond to a heat cue, generally emerge with less dependence on season (Ooi *et al.* 2004a, 2006b).

Seasonally emerging species are also the most likely to be affected by changes to fire season. Out-of-season fires (i.e. those occurring outside of the peak hot-season fire period) can have a negative impact on populations of species with an innate seasonal requirement for germination (Kruger 1984, Bond & van Wilgen 1996). The study region is situated on an urban interface where the numbers of implemented out-of-season fires are increasing (McLoughlin 1998). Similarly, climate change is projected to

drive changes to fire season, but over a much broader scale (Williams *et al.* 2001; Cary *et al.* 2006). I therefore also assessed the effects of fire season on the recruitment of physiologically dormant species. Specifically, the main questions posed in this study were:

- (1) Is seedling survival and growth affected by the timing of post-fire emergence related to seed dormancy type?
- (2) How does the timing of fire affect the recruitment success of species with seasonal emergence patterns?
- (3) What does an innate seasonal requirement for germination indicate about the evolution of plant reproductive characteristics in this, and other, fire-prone regions?

6.2 Methods

6.2.1 STUDY REGION

The study was conducted at sites within Royal (34° 03'S, 151° 03'E), and Heathcote (34° 07'S, 150° 58'E) National Parks (NP) in the southern Sydney region of New South Wales (NSW), south-eastern Australia, in fire-prone heath and woodland communities on low-nutrient sandstone derived soils. These National Parks are adjacent and have similar climates, soil types and sclerophyllous vegetation. Experimental fires were implemented, coordinated with hazard reduction activities, in Heathcote NP in August 1999 (winter) and April 2001 (autumn). A wildfire burnt sections of both parks in December/January 2001/02 (summer).

The climate in the Sydney region is temperate with no dry season (using the Köppen classification system) (Stern *et al.* 2000). Average annual rainfall is approximately 1050 mm, distributed relatively evenly throughout the year, with the four highest monthly averages occurring in January, March, June and November (Figure 6.1). Average temperatures (maximum/minimum) are approximately 27/18° C and 16/6° C in summer and winter respectively. Climate data from 1999 to 2006, including

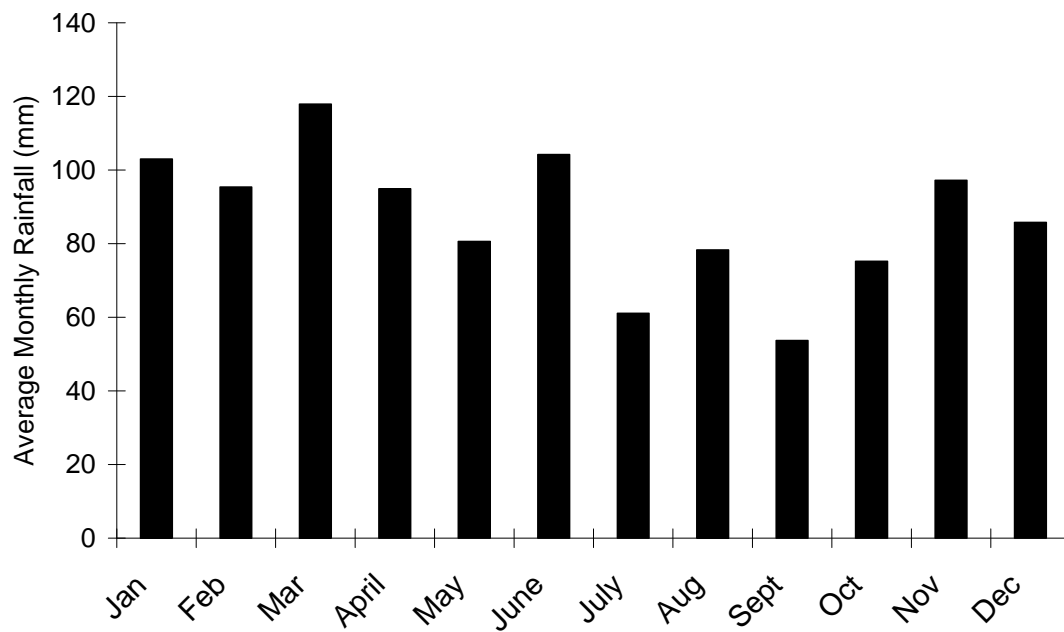


Figure 6.1 Average monthly rainfall from the Lucas Heights weather station.

Table 6.1 Details of the species selected for study. For dormancy class, MPD indicates morphophysiological dormancy. Seed masses are approximate and represent the seed only (i.e. embryo plus endosperm). Seed mass data were either calculated or obtained from Westoby *et al.* (1990). Under fire response, S indicates obligate-seeding species. All species are perennial woody shrubs, with approximate adult plant heights shown under Habit.

Family	Species	Dormancy class	Seed mass (mg)	Fire response	Habit (height) (m)
Ericaceae	<i>Leucopogon esquamatus</i>	MPD	0.9	S	Shrub (0.6-1 m)
	<i>Leucopogon exolasius</i>	MPD	1.5	S	Shrub (1-2 m)
	<i>Leucopogon setiger</i>	MPD	1.4	S	Shrub (1-2 m)
Fabaceae	<i>Dillwynia floribunda</i>	Physical	0.9	S	Shrub (1 m)
	<i>Dillwynia retorta</i>	Physical	2.2	S	Shrub (1-2 m)
	<i>Pultenaea daphnoides</i>	Physical	1.5	S	Shrub (2-3 m)
	<i>Pultenaea tuberculata</i>	Physical	1.3	Variable	Shrub (0.5-1 m)
Sapindaceae	<i>Dodonaea triquetra</i>	Physical	2.3	S	Shrub (1-3 m)

temperature, rainfall and numbers of days that rain fell, were collected from Lucas Heights weather station, within 10 km of the study sites.

6.2.2 STUDY SPECIES

Three species with a physiological dormancy component were selected for study, all within the genus *Leucopogon* (Ericaceae) (Table 6.1). Seeds of all three species are classified as morphophysiologically dormant and current information suggests that they require either a long period of warm stratification, or cold followed by warm stratification, to overcome dormancy (Ooi *et al.* 2007). They display seasonal emergence in the field, with the first seedlings appearing in autumn (March – May). Peak emergence occurs during late autumn or winter, even though significant rainfall is likely to have occurred through summer. Recruitment mainly occurs after fire with seedling emergence still restricted to same emergence season (Ooi *et al.* 2004a). All five physically dormant species selected for study, with season-independent emergence, were representatives of the Fabaceae and Sapindaceae (Table 6.1). For physically dormant species in this region, impermeable seed coats are usually rendered permeable as a result of heat from the passage of fire (e.g. Auld & O’Connell 1991; Morrison *et al.* 1992).

6.2.3 EFFECTS OF DORMANCY TYPE ON RECRUITMENT

To assess the effects that seasonally induced delayed emergence can have on recruitment, seedling survival and growth comparisons were made between species with physical dormancy, which had emerged rapidly (within 2 months) post-fire, and species with physiological dormancy, in which post-fire emergence was delayed by a further 3-9 months (Table 6.1). Seedlings of species sharing the same dormancy type were therefore pooled (136 with physical dormancy and 389 with a physiological dormancy component). The experiment was set up as a randomised block design, using four sites after the 2001/02 fires. Dormancy type, used to group species for analysis, was treated

as a fixed factor (two levels, physical and physiological), with site treated as a random factor (four levels). All species selected were obligate-seeding woody shrubs with persistent soil-stored seed banks. They also had comparable seed size (approximately between 1 and 2.5 mg; Table 6.1), as this was considered to be the most important seed trait potentially affecting early seedling survival and growth (Moles & Westoby 2004b). Seedling height comparisons between the two dormancy groups were made at the end of each summer to standardise time since fire, and also at each year post-emergence to standardise the time available for seedling growth.

6.2.4 FIRE SEASON

To assess whether the season of fire could affect recruitment success of seasonally emerging seedlings, intra-specific comparisons of survival and growth were made between seedlings that had emerged after a winter fire and those emerged after summer fires. Only *L. exolasius* and *L. esquamatus* were used for this part of the study. For each of the species, two sites were established prior to an experimental fire in August 1999 (winter), and a further two sites established prior to a fire in December/January 2001/02 (mid-summer). Sites were nested within fire season. Seedlings resulting from the winter fire experienced a long delay to emergence (up to 12 months), both in relation to the fire and other co-occurring species, whilst seedlings resulting from the summer fire had emerged after a comparatively short delay to emergence (3 to 6 months) (Figure 6.2). Seedling heights were compared at annual intervals post-emergence after the two fire seasons so that time available for growth was standardised. For each species, sites had similar topography and floristic composition. For *L. esquamatus*, however, one set of sites occurred in heath, whereas the other occurred at the woodland/heath interface.

6.2.5 FIELD SAMPLING

At each of the sites mentioned in 6.2.3 and 6.2.4, between 10 and 25 permanent 1 m x 1 m quadrats were located under or near canopies of mature individuals. Seedling emergence had previously been observed occurring primarily within a few metres of

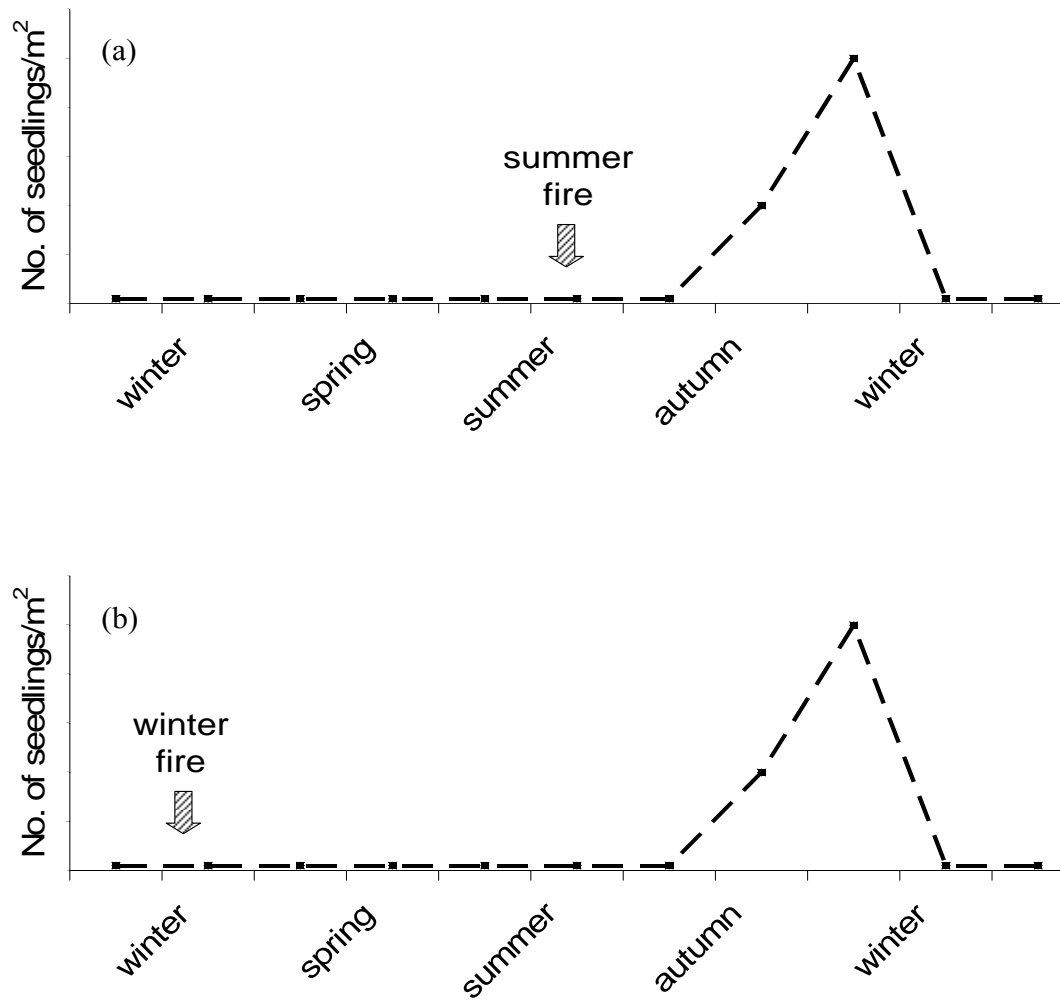


Figure 6.2 Schematic diagram showing the timing of the post-fire seedling flush of physiologically dormant *Leucopogon* species after different fire seasons. Even though rainfall occurs throughout the year, seasonal germination requirements ensure that peak emergence post-fire is still restricted to the same late autumn period. As such, seedling emergence is relatively rapid after summer fires (a), but is delayed by up to 12 months after winter fires (b).

adult canopies. After fire, quadrats were inspected regularly for signs of seedling emergence. Once emerged, all seedlings within quadrats were tagged using steel stakes and brass numbered tags. Seedling densities varied between species, but were generally low (usually between 2 and 8 seedlings/m²). The experiments were designed so that a minimum of 30 seedlings were tagged for each species. If necessary, some seedlings were located outside quadrats via haphazard searching, to increase numbers, although for most species used the minimum number was exceeded considerably. A total of 1428 seedlings were used in the study across all species. Time elapsed between the fire and emergence was recorded upon tagging and survival and height data were subsequently recorded at the end of each summer and each winter for between 3 and 5 years, depending on the year of emergence. For those seedlings that reached maturity during the study, time to first flowering was also recorded. Causes of seedling mortality were identified where possible, and divided into categories. Seedlings that were dried out and withered were assumed to have died from desiccation. Seedlings that were completely removed (where the seedling tag was still in position), or dead as a result of obvious signs of having been eaten (such as chewed remnant stems), were assigned to “herbivory”, either by vertebrates or invertebrates.

6.2.6 ANALYSES

Survival data were analysed using a failure-time approach to compare survival curves, and therefore survival through time (Fox 2001). Comparisons were made by means of a Generalised Wilcoxon (Peto-Prentice) test, which allows for data that have some observations censored. It also allows comparisons between the different groups of seedlings (i.e. sites, dormancy types or fire season in our study). In survival analysis of seedling data, time to failure is the time to the death of each individual seedling. If the experiment ends before all seedlings have died (failed), then the surviving seedlings are right censored. Similarly, if a seedling is lost during the experiment, without a failure being observed, then this data point is also right censored. Additionally, cumulative survival data were analysed at the end of each summer for up to 4 years, depending on year of emergence. The proportion of seedlings surviving at each time period were analysed using a two-factor analysis of variance (ANOVA), with dormancy type (or fire

season) treated as fixed factors and site treated as a random factor. Seedling growth was analysed by repeated measures ANOVA, using a split plot design. Individual seedlings were treated as a random factor and were considered as replicates (plots), allowing a test of between-plot effects (dormancy type or fire season in this study).

6.3 Results

6.3.1 EFFECTS OF DORMANCY TYPE

The proportion of seedlings surviving at the end of 4 years for the physically dormant species ranged across the four study sites from 32% to 71%, whilst survival of physiologically dormant species ranged from 12% to 49%. A two-factor ANOVA showed that the proportion of surviving seedlings of physically dormant species was just significantly higher than those of physiologically dormant species by the end of the fourth summer ($F_{1,3} = 10.810$, $P = 0.046$). There were no significant differences between the four sites ($F_{1,3} = 1.511$, $P = 0.371$). Failure-time analysis for physical versus physiologically dormant species across the four sites differed significantly ($\chi^2 = 86.291$, $P < 0.001$), with physically dormant species (i.e. those that emerged earliest) having a greater chance of survival than later emerging, physiologically dormant species (Figure 6.3).

More than 85% of all dead seedlings, irrespective of dormancy type, had died by the end of the first full summer period. However, there were differences in the relative proportions of cause of death. For physically dormant species, approximately 70% of deaths (at all but one site) were attributed to herbivory, with the remaining 30% present but desiccated. For the physiologically dormant species, these proportions were reversed, with approximately 80% of deaths associated with desiccation.

Too few seedlings remained to analyse height data from one site. However, the repeated-measures ANOVAs uncovered significant differences between the two dormancy types at each of the remaining three sites, both 4 years post-fire and after 4 years of post-emergence growth ($P < 0.001$ at all sites). Two-factor ANOVA assessing

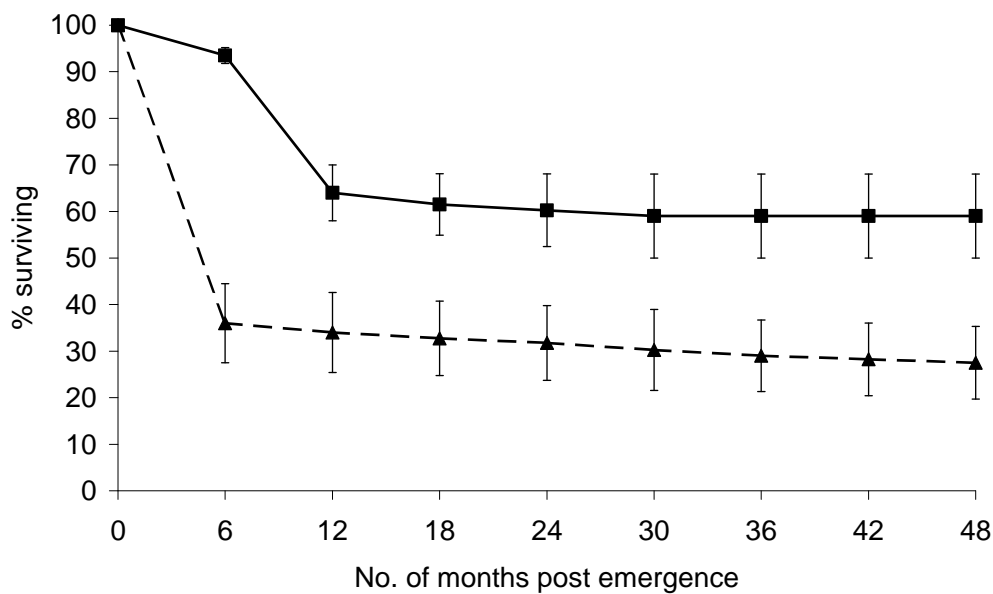


Figure 6.3 Seedling survival through time after a summer fire for physically dormant (solid line (■)) and physiologically dormant (dashed line (▲)) species, across four sites. Data are mean proportions of seedlings surviving at each census. Bars represent 1 standard error each side of the mean.

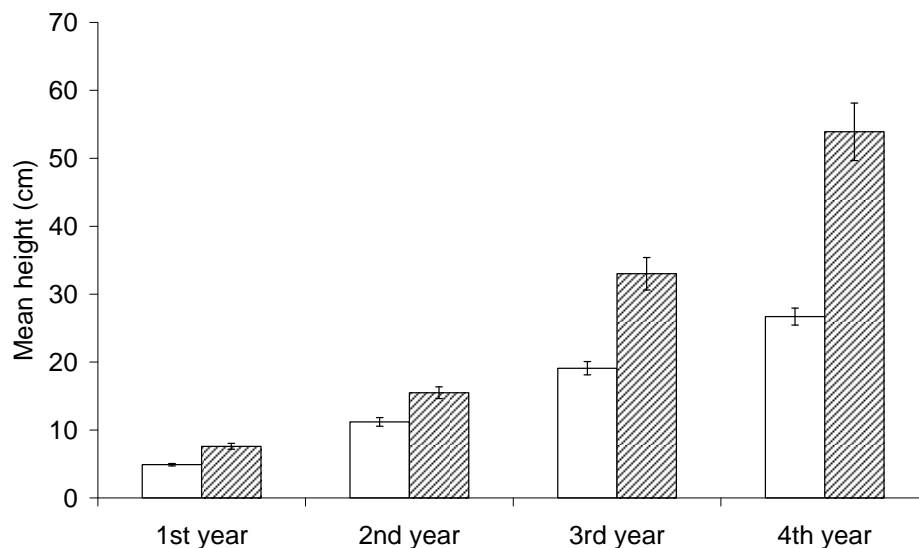


Figure 6.4 Seedling heights after a summer fire for physiologically dormant (□) and physically dormant (▨) species, across four sites. Data presented are mean seedling heights (cm) at each year post emergence, so that the amount of growing time for each group of seedlings has been standardised. Bars represent 1 standard error each side of the mean.

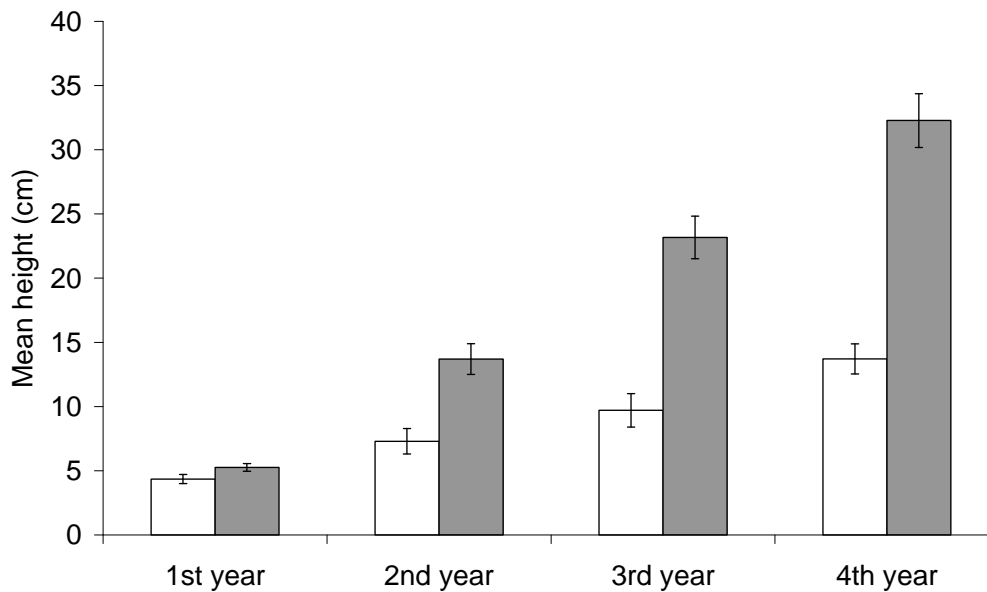


Figure 6.5 Seedling heights of the morphophysiologicaly dormant *Leucopogon exolasius* at the end of each year after a winter fire (□) and a summer fire (■). Due to the species innate seasonal germination cues, emergence after the winter fire was relatively delayed, whilst emergence after the summer fire was more rapid. Data are mean seedling heights (cm) at each year post emergence. Bars represent 1 standard error each side of the mean.

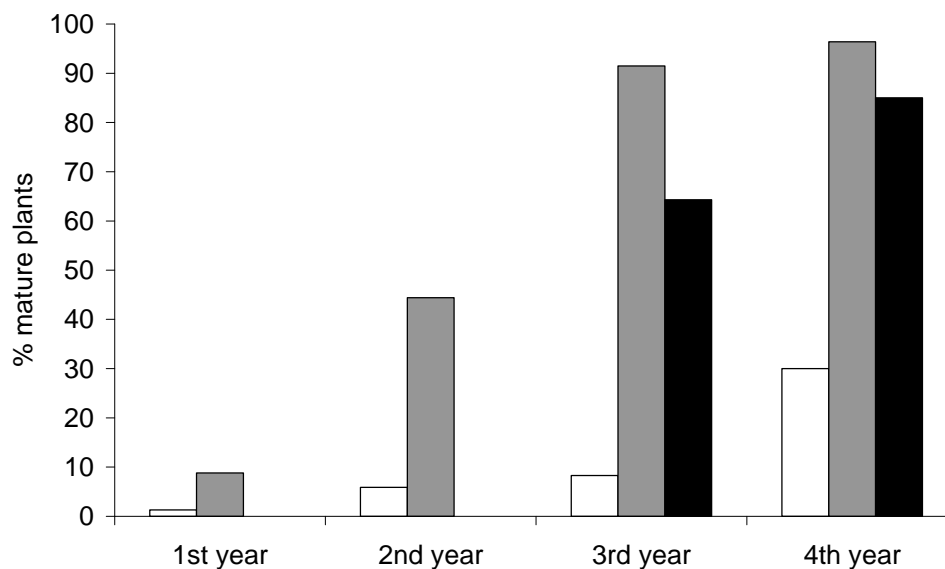


Figure 6.6 Proportion of tagged *Leucopogon esquamatus* seedlings at the heath sites that had flowered by the end of each year after a winter fire (□) or summer fire (■), and proportion of those tagged at the more marginal woodland/heath site that flowered after summer fire (■). NB. Seedlings that emerged at the woodland/heath site after a winter fire were lost to a subsequent unplanned wildfire before maturity.

seedling heights at the end of each year and after each year of growth also revealed significant differences between the two dormancy types ($P < 0.001$ at the end of each year), but not sites ($P > 0.05$ for each year). After 4 years of growth, heights of the early emerging physically dormant species were greater than the physiologically dormant species (Figure 6.4).

6.3.2 EFFECTS OF FIRE SEASON

Seedlings of the two species used in this part of the study, *L. exolasius* and *L. esquamatus*, either grew or matured more slowly when emerging after winter fires, than after summer fires. Comparing seedlings with time available for growth standardised, post winter-fire *L. exolasius* seedlings were significantly smaller than the seedlings appearing after the summer fire by the second summer post-emergence onwards ($F_{1,58} = 8.193$, $P = 0.006$) (Figure 6.5). There were no significant differences found between different seasons of fire for *L. esquamatus* seedling growth ($F_{1,63} = 0.592$, $P = 0.444$). However, only *L. esquamatus* reached maturity during the study period and there were large differences found between the proportions of plants flowering after each of the different fire seasons. At the heath sites, less than 10% of post winter-fire plants had flowered by the third year, whilst approximately 90% of post summer-fire plants had flowered at both the heath and woodland/heath sites (Figure 6.6).

For *L. exolasius*, the winter fire missed one of the replicate sites and comparisons of seedling survival data were therefore made using the Generalised Wilcoxon test only. Survival through time did not differ significantly between delayed emerging post winter-fire and rapidly emerging post summer-fire *L. exolasius* seedlings ($\chi^2 = 0.244$, $P = 0.621$). For *L. esquamatus*, lower levels of survival of seedlings were found after the winter fire at the heath sites ($\chi^2 = 15.117$, $P < 0.001$), but not at the more marginal woodland/heath sites ($\chi^2 = 2.404$, $P = 0.121$). Results from the ANOVA revealed no significant differences between the survival of post winter-fire and summer-fire *L. esquamatus* seedlings by the second year post-fire ($F_{1,3} = 0.290$, $P = 0.686$). No further analyses were possible, due to the loss of the winter burned woodland/heath site to a subsequent wildfire after the second year.

6.4 Discussion

Seedling emergence of shrub species that have physiological dormancy, with germination cues tightly bound to particular seasons, can be delayed in relation to co-occurring physically dormant species that have no seasonal germination requirements (Ooi *et al.* 2004). This study has revealed that this delay can negatively affect post-fire seedling recruitment. Both inter- and intra-specific comparisons showed that seasonally delayed emergence could reduce seedling survival and growth, and increase the time taken for plants to reach maturity, subsequently slowing the recovery of seed bank stocks. It therefore appears that seasonal emergence patterns, associated with seed dormancy and germination cues, can provide a mechanism that determines the variation of recruitment success after fires in different seasons.

The importance of early emergence on seedling growth and recruitment success was highlighted by intra-specific comparisons. Because of the seasonal germination requirements of the physiologically dormant *Leucopogon* species, emergence occurs during the same seasonal period (autumn), irrespective of earlier rainfall, and delay is in relation to the passage of fire. Winter fires delayed *Leucopogon* emergence by nearly 12 months, making post summer fire emergence comparatively rapid (6 months) (Figure 6.2). Earlier emerging, post summer-fire *L. exolasius* seedlings, subsequently had a faster growth rate than delayed, post winter-fire emerging *L. exolasius* seedlings. Climate data collected over the period of study showed that monthly rainfall averages after each fire were very similar and therefore comparable.

Previous observations have shown that seed production is directly related to plant height and canopy size, and slower growth would therefore extend the time taken for plants to reach maturity (Ooi *et al.* 2004a). Maturity of *L. esquamatus* seedlings was also achieved much more quickly after early emergence. It therefore appears that winter fires may have increased the relative levels of competition faced by *Leucopogon* seedlings from rapidly emerging co-occurring seedlings and resprouters, as has been suggested in other fire-prone regions (e.g. Tyler & D'Antonio 1995).

Further support for the importance of early emergence was provided by comparisons between physically and physiologically dormant species. Emergence of the physiologically dormant species studied was delayed in relation to the rapidly emerging physically dormant species by between 3 and 6 months after a summer fire, with the levels of mortality for physiologically dormant species significantly increased. Negative impacts on seedling survival have been found after similar, or much shorter periods of delay, in other fire-prone regions (e.g. Daskalakou & Thanos 2004; Quintana *et al.* 2004). A potential cause of this relative decrease in seedling survival is slower growth, and the reduced amount of time for growth, of delayed emerging seedlings before they experience a full season of summer conditions. Rapid emergence enabled seedlings to grow for almost a year before the first full summer season, whereas seedlings of seasonally emerging species had less than 6 months to establish and grow, and were therefore smaller at the onset of summer.

Although rainfall readily occurs year round in this region, there are higher levels of surface evaporation in the summer months (Bradstock & Bedward 1992). Seedlings of the physically dormant species were comparatively larger, and it is likely that they were better able to access deeper water reserves and resist moisture stress (Richards & Lamont 1996; Dunne & Parker 1999; Keith 2002). Causes of mortality identified during the study supported this hypothesis. Relatively few seedlings (~30%) of the physically dormant species were found desiccated, whereas desiccation accounted for the majority (~80%) of dead seedlings of the seasonally emerging physiologically dormant species.

The effects of seasonally delayed emergence on recruitment success may be more pronounced in aseasonal rainfall regions, like the one studied, but this does not mean that effects are limited to these climate regions. A number of other fire-prone regions around the world have Mediterranean-type climates, where rainfall is strongly seasonal. Because dormancy induced seasonal emergence is closely synchronised with moisture availability, post-fire seedling emergence and resprouting occurs more or less concurrently for the majority of species in Mediterranean-climate regions (Carrington & Keeley 1999). However, seedling emergence delayed even by only a few weeks can negatively affect survival and growth (Jones *et al.* 1997; Verdú & Traveset 2005), and this amount of delay can quite plausibly occur for species with seasonal germination

requirements in Mediterranean climate regions. For example, in a study of three obligate-seeding shrubs in the Mediterranean Basin, Quintana *et al.* (2004) found that the majority of seedlings of the physiologically dormant species *Erica umbellata* emerged from a soil-stored seed bank 1 or 2 months later post-fire, than the physically dormant *Cistus ladanifer*. Earlier emergence of *C. ladanifer* enabled the population to persist through the early onset of drought, whereas the later emerging *E. umbellata* became all but locally extinct.

The species pooled into the physiologically dormant group in this study all occur within the same genus, whereas the pooled physically dormant species are from several genera. As such, seedling survival and growth of the physiologically dormant group may represent within-genus performance only. However, whilst phylogenetic constraints may limit the generalisations that can be made about physiologically dormant species, examples from other studies, such as Quintana *et al.* (2004) mentioned above, suggests that a broader pattern of differences exists between species with different dormancy types.

Physically and physiologically dormant shrub species coexist in approximately equal proportions in this, and other, fire-prone regions (Baskin & Baskin 1998; Ooi 2007). However, the considerable negative effects of the winter, out-of-season fires studied, suggest that fire may not have been the primary force selecting for seasonally emerging physiologically dormant species. Either type of dormancy is conducive to the formation of long-lived seed banks, which provides an initial advantage for species in fire-prone regions. Persistent seed banks are important in habitats susceptible to temporal variability in disturbances, such as fire, because seeds are available over a wide time period (Fenner & Thompson 2005). However, for dormancy mechanisms to play an advantageous role, the timing of germination has to occur during the optimal period for recruitment success (Baskin *et al.* 2000). In fire-prone regions, that time is during the immediate post-fire environment (Whelan 1995; Bond & van Wilgen 1996).

Physically dormant species take advantage of the post-fire environment because heat breaks dormancy (e.g. Auld & O'Connell 1991), and seedlings are quick to emerge into the post-fire environment. This timing of emergence occurs irrespective of the

season of fire. Several other studies assessing recruitment success of woody shrubs in south-eastern Australia have also shown that physically dormant species can germinate quickly (less than 2 months) and maintain high rates of survival after either spring or autumn fires (Moles & Westoby 2004a; Knox & Clarke 2006). This is not to say that there are no differences between seasons, but that significant levels of emergence of physically dormant species are still able to take place at any particular time of year. For physiologically dormant species, however, the timing of emergence is restricted to a particular season. Seedling emergence during the optimal post-fire period is not dependent simply on fire occurrence, but on the timing of fire in relation to each species' particular seasonal cue. Fire *per se* is therefore not the driving force that determines the timing of seedling emergence. I interpret this as a preadaptation of these species to seasonality, as has similarly been suggested for several resprouting species (Keeley *et al.* 2006). Previous environmental conditions in the evolutionary history of these species may have provided a selective force that favoured physiological dormancy and seasonal emergence (see Jurado & Flores 2005).

The large number of physiologically dormant species that occur in fire-prone habitats (Baskin & Baskin 1998; Ooi 2007), suggests that they can ecologically fit well enough to persist, at least under current fire regimes. If we consider dormancy type as a functional trait, it is quite likely that the size of the seasonal emergence window will vary between physiologically dormant species, in the same way that post-fire resprouting success can vary between species and fires. Subsequently, some physiologically dormant species may be more resilient to out-of-season fires than others. For example, in the above-mentioned study by Quintana *et al.* (2004), a significant proportion of seedlings of one physiologically dormant species, *Rosmarinus officinalis*, germinated relatively rapidly in relation to another, *Erica umbellata*. Members of the mostly physiologically dormant Ericaceae family seem particularly tightly bound to a small seasonal emergence window. In addition to *E. umbellata* and the Ericaceae from our study, other studies reporting (often unexplained) delayed emergence after out-of-season fires include *Epacris stuartii* in Tasmania (Keith 2002) and *Arctostaphylos morroensis* in California (Odion & Tyler 2002). All of these ericaceous species are obligate seeders, and three (*L. exolasius* from this study, *E. stuartii* and *A. morroensis*) are listed as threatened.

Knowledge of the factors that affect post-fire recruitment success is not only important for understanding plant population dynamics, but also for informed management. As this study has shown, the season of fire is important for determining the relative extent of delay of seasonally emerging species. For many of these species, a shift from the natural fire season could pose a potential threat, and it would be expected that negative effects would be accentuated as the number of out-of-season fires increased. Climate change is projected to drive changes to fire season and, in areas close to the urban interface, an increasing number of out-of-season managed fires are being implemented (McLoughlin 1998; Cary *et al.* 2006). Populations of threatened species would be of particular concern and the timing of implemented fires requires careful consideration. I found negative effects after a single out-of-season fire on the recruitment success of the threatened *L. exolasius*. Taking a precautionary approach, I suggest that managed fires conducted in areas containing threatened species with a physiological dormancy component, or (if known) a seasonal emergence pattern, should be implemented as close as possible to the natural fire season.

Chapter 7

Comparative analyses of the ecology of the rare shrub *Leucopogon exolasius* and two common congeners



See print copy for figure

Preface

As outlined at the beginning of this thesis, the final aim of this study is to establish whether or not there are any identifiable differences between a rare species and some common congeneric taxa. Much of the data presented in previous Chapters has provided fundamental knowledge for understanding important ecological traits of the three study species. Prior to this study, there was little information or understanding of life-history traits of *Leucopogon*, or of Ericaceae generally, in Australia. In this Chapter, I draw this information together in a comparative analysis to determine potential causes of rarity of *Leucopogon exolasius*. Both intrinsic and anthropogenic factors are explored and the relative abilities of rare and common species to persist are assessed.

Publication

Ooi, M.K.J., Auld, T.D. & Whelan, R.J. (in review) Limiting traits of rare and common plant species: impacts of the fire regime and persistence of ecologically fitting factors. *Biological Conservation*

7.1 Introduction

There is growing evidence that rates of extinction of plant species are increasing, across local and regional scales, throughout the world (Thomas *et al.* 2004a). This rate is influenced by human population growth, and the associated escalating demands on resources and climate-driven changes (Gaston 2005). Species most at risk by the threat of extinction are those that are already rare, and therefore already limited in abundance and/or distribution (Gaston 1994; Wilson *et al.* 2004). Understanding the possible anthropogenic and ecological causes of rarity are therefore crucial to designing and implementing conservation actions.

Demographic studies that lead to an understanding of the potential causes of rarity and decline are particularly valuable for the conservation and management of rare and threatened plant species (Schemske *et al.* 1994). Additionally, comparative studies that investigate the biology of ecologically similar rare species and common congeners can help to detect possible limiting factors within a rare species (Kunin & Gaston 1993; Bevill & Louda 1999). Although it is unlikely that generalisations exist regarding the causes of rarity (Fiedler 1987; Gaston 1994), insights gained from data collected in comparative studies are particularly useful, both for the conservation of rare species and the detection of broader ecological patterns (Bevill & Louda 1999; Lavergne *et al.* 2004). In particular, these broader patterns could help to identify traits that are limiting, for both rare and common species, in the face of changing environmental conditions.

In fire-prone regions, fire causes plant mortality and, as a result, successful post-fire recruitment from seed is necessary for short-term regeneration and long-term persistence (Whelan 1995; Bond & van Wilgen 1996; Keeley *et al.* 2006). Plants that do not resprout, known as obligate-seeding species, are particularly dependent upon recruitment from stored seed. As such, critical factors for the persistence of these species, and potential causes of rarity, are often related to seed ecology and recruitment. Additionally, fire is the predominant influence on plant population dynamics in these regions and may itself need to be considered as a determinant of species rarity (Burne *et al.* 2003).

Climate change and an increased use of managed fires are forecast to cause a shift from current peak fire seasons, and to increase the frequency of fire (Whelan 1995; Bond & van Wilgen 1996; McLoughlin 1998; Williams *et al.* 2001; Cary *et al.* 2006). The threats caused by altered fire regimes and climate change may not only affect the persistence of those plant species that are already threatened with extinction, but also of many common species. Such common species may be at risk because they possess traits that have little flexibility in relation to predicted changes. Recent evidence suggests that species composition within ecological communities may be strongly influenced by the relative abilities of species to cope with climate-driven changes (Suttle *et al.* 2006).

Because limited abundance or distribution of plant species are unlikely to be attributed to a single factor, investigations into several critical biological traits and life-history stages are the most likely to highlight limiting processes (Rabinowitz *et al.* 1984; Bevill & Louda 1999). Part of the benefit of using a small number of species in a comparative study, is that a larger number of factors may be examined with relative ease. Species can also be more easily selected to control for phylogenetic relatedness (Kunin & Gaston 1993; Walck *et al.* 2001). In this study, seed and germination ecology, and the associated post-fire regeneration responses of *Leucopogon exolasius* and the closely related *L. setiger* and *L. esquamatus*, were compared in order to determine any potential causes of rarity for one of them, the threatened *L. exolasius*.

All three species inhabit the sandstone vegetation around Sydney, which is one of several centres of high species richness and endemism on the Australian continent (Crisp *et al.* 2001). This fire-prone region of south-eastern Australia contains approximately 2000 plant species (Auld *et al.* 2000). Fundamental ecological studies have not been conducted for the majority of these species. As a result, there is surprisingly little known about many families, even some that are dominant components of fire-prone vegetation communities across the continent. The genus *Leucopogon* occurs within the Ericaceae (formerly Epacridaceae), which is one such family. The study species selected are all obligate-seeding woody shrubs with soil-stored seed banks (Ooi *et al.* 2006a,b). Although they do not co-occur at the same sites, they inhabit adjoining heath and woodland vegetation types, and have overlapping ranges.

Several reproductive attributes, including fecundity, seed viability, seed dormancy, germination and seedling establishment were investigated for all three species in an attempt to identify limiting factors. Seed dormancy appears to be complex for this group of species, with germination dependent on interactions between fire cues and seasonal temperatures (Ooi *et al.* 2004a, 2006b). Unlike its Mediterranean-type climate counterparts, this fire-prone study region has an aseasonal rainfall climate with no pronounced dry season, which may potentially have consequences for the post-fire recovery of species with innate seasonal germination requirements (see Chapter 6). Demographic responses, including seed bank longevity, seedling survival and growth, and primary juvenile period, were assessed to ascertain the relative competitive abilities of each of the three species. Seed dispersal mechanisms were also examined to determine the potential for population spread for each the study species. Understanding demographic responses to environmental disturbance is crucial in these habitats. Because the study region is situated on an urban interface where the numbers of implemented fires are increasing (McLoughlin 1998) and climate change is projected to drive changes to fire season and frequency (Williams *et al.* 2001; Cary *et al.* 2006), I therefore assessed the effects of fire season on the above demographic responses. Specifically, I wanted to address the following questions:

- (1) Are there any identifiable factors that potentially cause rarity of *Leucopogon exolasius*?
- (2) Can predicted changes to the fire regime potentially affect the persistence of the rare or common species studied?
- (3) Can any insights be gained into the evolution of traits that allow these species to persist in a fire-prone environment?

7.2 Methods

7.2.1 STUDY SPECIES AND SITES

Leucopogon (Ericaceae) is a large genus consisting of about 230 species. The Ericaceae make up a significant part of the understorey in fire-prone communities in Australia.

The three study species are all obligate-seeding woody shrubs with soil-stored seed banks (Ooi *et al.* 2006a, 2007). *Leucopogon* fruit are drupes and fresh exocarps of both *L. exolasius* and *L. setiger* are fleshy, while the endocarp is hard and lignified. *Leucopogon esquamatus* exocarp is papery and the endocarp comparatively more fibrous than hard. Usually one, but occasionally two seeds are held inside each fruit. All three species have seeds with underdeveloped linear embryos surrounded by endosperm and are classified as morphophysiological dormant (Ooi *et al.* 2006b).

Leucopogon exolasius is an erect shrub that grows to approximately 2 metres, and is a threatened species listed as ‘vulnerable’ both under the national Australian *Environment Protection and Biodiversity Conservation Act* (1999), and under the state of New South Wales (NSW) *Threatened Species Conservation Act* (1995). The species is endemic to the southern Sydney region of NSW and is restricted to approximately 14 known populations. It occurs in woodlands, particularly on riverbanks in areas with outcropping sandstone. *Leucopogon setiger*, morphologically very similar to *L. exolasius*, is an erect shrub to approximately 2 metres, and is more widespread, extending from the central western slopes of NSW to the coast (distributional range > 450 km). It is found in woodlands and open forest. *Leucopogon esquamatus* is a slender shrub, which grows to approximately 1 metre. It is also widespread, occurring from the coast to the mountains (as for *L. setiger*), but extending south into the states of Victoria and Tasmania (distributional range > 1000 km). It occurs in heath, woodlands and open forests. Geographic distribution of each species were calculated using IUCN (2001) criteria for extent of occurrence, which is defined as the area contained within the shortest continuous imaginary boundary which can be drawn to encompass all known sites of occurrence of a species.

As much information as possible was collected on the habitat and biology of all three species, and included data from previous studies, observations and the wider literature. This included information on the habitat and substrate preferences of the study species, population structure, seed dormancy, seedling emergence phenology, seed bank longevity and seed viability.

Experimental data were collected from study sites located in the southern Sydney region, within Royal (34° 03'S, 151° 03'E) and Heathcote (34° 07'S, 150° 58'E) National Parks. The climate is temperate with no dry season (using the Köppen classification system) (Stern *et al.* 2000). Average annual rainfall for the area is approximately 1050 mm distributed relatively evenly throughout the year, with the four highest monthly averages occurring in January, March, June and November. Average temperatures (maximum/minimum) are approximately 27/18° C and 16/6° C in summer and winter respectively.

7.2.2 FLOWERING PHENOLOGY AND FECUNDITY

The occurrence of flowering and fruiting was scored over repeated visits to the study sites. To estimate fecundity, a single plot was marked out at two study sites for each species. At each plot, the type of vegetation, slope and aspect were recorded. All individuals within the plots, covering a range of size classes, were tagged using stainless steel wire stakes with numbered brass tags. For each tagged plant, height (or stem length if not vertical), canopy area (calculated as an ellipse from two measurements at right angles) and the presence or absence of buds or flowers were recorded. Eight to ten mature individuals were randomly selected at each plot and the total number of fruit counted on each plant during late spring. To enable comparisons between sites and species, the numbers of fruit per 0.1 m² of canopy were calculated for each plant. Data were tested for homogeneity of variance using Levene's test. Comparisons were conducted using a two-factor nested analysis of variance (ANOVA), with species treated as a fixed factor and site as a random factor.

7.2.3 POST-FIRE SEEDLING SURVIVAL AND GROWTH

For each species, seedling survival and growth experiments were designed so that two replicate plots at each of two sites were burnt during a summer fire in 2001/02. Seedling emergence had previously been observed occurring primarily within a few metres of adult canopies. Between 10 and 25 permanent 1 m x 1 m quadrats were therefore

located under or near canopies of mature individuals within each plot. After fire, quadrats were inspected regularly for signs of seedling emergence. Once emerged, all seedlings within quadrats were tagged. Time of emergence and seedling height were recorded upon tagging. Survival and height were subsequently recorded at the end of each summer and each winter for 4 years. The time taken for tagged individuals to flower was recorded, with the range of number of years after fire for 10% and 50% of the population to reach maturity calculated. The proportions of mature plants were also recorded opportunistically when visiting sites of known age.

The proportion of seedlings surviving and seedling heights were analysed at the end of the fourth summer using a two-factor ANOVA, with species treated as a fixed factor and sites nested within species. Previously presented work investigating the effect of different fire seasons on seedling survival and growth were also used in the interpretation of the data (see Chapter 6).

7.2.4 SEED DISPERSAL MECHANISMS

Observations were made during field visits to identify the main agents of dispersal. *Leucopogon exolasius* and *L. setiger* both have yellow fleshy fruits (drupes) and were therefore thought likely to attract frugivorous vertebrates, particularly birds (e.g. Howe 1986; Keighery 1996). Observations, as well as literature reviews, identified likely seed-dispersing species. It was considered highly probable that estimations of the proportion of fruit taken from plants by vertebrates would be too variable, either for any meaningful estimation of the proportion of fruit taken by birds or for comparisons between species. As such, only a snapshot estimate of the proportion of fruit-fall was made for the two fleshy-fruited species, to ascertain whether or not a significant proportion of fruit reached the ground. This was done by counting the total number of fruit fallen within a 0.1 m² quadrat, placed under canopies of five plants, and compared to mean numbers of fruit produced by the adult plants. However, because *L. esquamatus* does not have fleshy fruit and plants are much smaller, more precise attempts were made to identify the number of fruits reaching the ground, to ascertain whether or not there was any potential for bird dispersal or predation. At two sites, the total number of

seeds on each of ten plants was counted. Sticky seed traps were then placed under each canopy, with each trap covering an area larger than that of the canopy. Each week for a period of 6 weeks, traps were cleared and the numbers of fruit remaining on each plant were counted.

To examine the main mechanisms for the removal of fruit that reached the ground, an exclusion experiment was established using a randomised block design. Five replicate plots were randomly placed within two sites for each species. Each replicate consisted of four treatments, using 10 seeds placed in the centre of a 9 cm petri dish per treatment. Treatments were ants and vertebrates excluded (ALL), ants only excluded (ANT), vertebrates only excluded (VER) and a control with no exclusions (CON). Ants (and other crawling insects) were excluded by application of a non-toxic sticky substance (Tanglefoot™) around the edge of the petri dish. Vertebrates were excluded by 15 x 15 cm cages, made with 1 x 1 cm wire mesh. Data were collected twice daily for 4 days. To compare the number of seeds remaining at the end of 4 days, initial analyses were carried out on raw data using a randomised block design with no replication. Treatments were fixed whilst blocks were treated as a random factor in a two-factor ANOVA. Significance of plot effect (blocks) was tested with caution, by setting the threshold for rejection of the null hypothesis at $P > 0.25$. With no significant difference found between plots, data were pooled and analysed using species and treatment in a two-factor ANOVA. Individual means were tested using the Student-Newman-Keuls test. All data were first tested for homogeneity of variances and transformed when necessary.

7.3 Results

All species occur within the temperate climate zone, with no seasonal rainfall pattern (Table 7.1). The two common species expand into regions with warmer (north) and cooler (south and/or higher altitude) overall temperatures. All species occur on similar low nutrient soils. They also occur in similar woodland habitats, although *L. esquamatus* is more dominant in heath than woodland and *L. exolasius* is restricted

mainly to rocky areas near creeks and rivers (Table 7.1). *Leucopogon exolasius* and *L. setiger* are morphologically very similar. The fleshy yellow drupes are almost

Table 7.1. Comparison of species distribution, habitat and morphological traits for the rare species *L. exolasius*, and the two common congeners *L. setiger* and *L. esquamatus*. Data are based on observations and other literature sources (where references are cited).

See print copy for figure 7.1

indistinguishable, and also similar to a number of other *Leucopogon* species.

Leucopogon esquamatus is more typical of a heath shrub, and is shorter and has a smaller canopy area (Table 7.1).

Although diaspore weights differed amongst species, seed weights (embryo and endosperm) were very similar, as were seed embryo morphologies (Table 7.1). All species displayed high levels of fecundity. *Leucopogon esquamatus* produced significantly more fruit per canopy area than the other two species ($F_{2,4} = 32.624$, $P = 0.004$), however, mean canopy area of mature *L. esquamatus* individuals was also smallest by at least an order of magnitude (Table 7.1). As a result, the overall production of seeds per plant varied little between the three study species. For each species, there were no significant differences found between study sites ($F_{3,47} = 0.398$, $P < 0.755$). Flowering phenology and likely pollinators were similar, as were the seed maturation times. Seed viability was also high for all species (Table 7.2), and consistently highest for the rare *L. exolasius*.

All three species had been classified as morphophysiologically dormant and require stratification at seasonal temperatures to overcome dormancy (Ooi *et al.* 2006b). Emergence in the field was therefore also seasonal, with seedlings appearing at the same time of year (peaking in early winter), even if there was significant rainfall at other times of the year. Recruitment mainly occurred after fire with seedling emergence still restricted to same emergence season (Table 7.2). Some inter-fire recruitment had also been recorded (Ooi *et al.* 2006a).

7.3.1 SEEDLING SURVIVAL AND GROWTH

The fire failed to burn all plots established for the seedling survival and growth experiments. As a result, the design was reduced to a one-factor ANOVA comparing the proportions of seedlings surviving at the end of each summer over 4 years. No differences were found between species (all years $F_{2,3} < 0.06$, $P > 0.940$) but there appeared to be large variation between sites for each species. To test for possible site effects, a two-factor ANOVA without replication, with year and site as factors, was

Table 7.2. Comparison of phenological and life-history traits, and demographic responses for the rare species *L. exolasius*, and the two common congeners *L. setiger* and *L. esquamatus*. Data are based on observations and other literature sources (where references are cited).

	<i>Leucopogon exolasius</i>	<i>Leucopogon setiger</i>	<i>Leucopogon esquamatus</i>
Fire response ₁	obligate seeder	obligate seeder	obligate seeder
Flowering times	July – October	July – October	August – September
Flowers (pollinators)	unspecialised (bees, flies, butterflies)*	unspecialised (bees, flies, butterflies)*	unspecialised (bees, flies, butterflies)*
Seed maturation	October – December	October – December	November – December
Seed viability: 2000 ₂	88.90 ± 2.20	59.50 ± 2.42	56.00 ± 2.30
2002	92.33 ± 1.33	83.33 ± 4.91	61.67 ± 2.33
Dispersal mechanisms	vertebrate & ant	vertebrate & ant	ant
Field germination ₃	May – September	May – September	April – August
Primary juvenile period (range given indicates time taken for 10% up to > 50% of population to flower)			
- post summer fire	> 7 years	5 – 6+ years	2 – 3 years
- post winter fire	> 7 years	-	4 – 6 years
Estimated mean seed bank half-life (years) ₃ (range of 1 S.E in brackets)	5.45 (4.22 – 7.73)	4.44 (3.07 – 8.06)	3.47 (2.67 – 4.95)
% seedlings surviving 4 years post-fire (range across two sites)	30 – 33%	23 – 43%	15 – 43%

References used: ₁Ooi *et al.* 2006a; ₂Ooi *et al.* 2007; ₃Ooi *et al.* 2004a. NB. * denotes inferred from observations and from literature reporting on species with similar flower morphology (Keighery 1996).

used. This approach does not allow testing of significance of the interaction and a cautious approach is recommended for testing the significance of main effects (Zar 1984). The significance levels were therefore increased to 0.01. Results indicated large differences between sites for *L. setiger* and *L. esquamatus* ($P < 0.001$), but not for *L. exolasius*. *Leucopogon esquamatus* was significantly smaller than the other two species after 4 years growth ($F_{2,164} = 7.062$, $P = 0.001$). Data from a previous study also showed that growth of *L. exolasius*, but not *L. esquamatus*, was significantly slower for seedlings emerging after a winter fire, than those that emerged after this summer fire (Figure 6.5). Winter fire, however, significantly increased the time taken for *L. esquamatus* to flower (Table 7.2).

7.3.2 SEED DISPERSAL

Two bird species, the Yellow-tufted Honeyeater (*Lichenostomus melanops*) and the Pied Currawong (*Strepera graculina*), were observed taking fruit from plants of the two fleshy-fruited species, *L. exolasius* and *L. setiger*. No birds were observed taking *L. esquamatus* fruit from plants. Emus (*Dromaius novaehollandiae*) were identified from the literature as a species that had a preference for drupaceous fruit of *Leucopogon* and other Ericaceae (Keighery 1996; Quin 1996; McGrath & Bass 1999; Calviño-Cancela *et al.* 2006). Numbers of ericaceous fruit found in emu scats, studied in both eastern and south-western Australia, were orders of magnitude greater than that of any other species. Silvereyes (*Zosterops lateralis*), Currawongs (*Strepera* spp.) and Honeyeater species were also identified from the literature as *Leucopogon* fruit-consumers (Keighery 1996; Stansbury 2001).

The seed trap experiment for *L. esquamatus* was affected by bad weather, with several traps destroyed during the experiment. However, the proportions of fruit from each plant that reached the ground were estimated from the remaining traps, and found to be high at both sites ($97.50\% \pm 1.89$ and $92.8\% \pm 1.99$). These proportions did not differ significantly between sites ($F_{1,11} = 2.701$, $P = 0.129$). The number of seeds counted below *L. exolasius* and *L. setiger* canopies ranged from 8 to 63 and 16 to 44

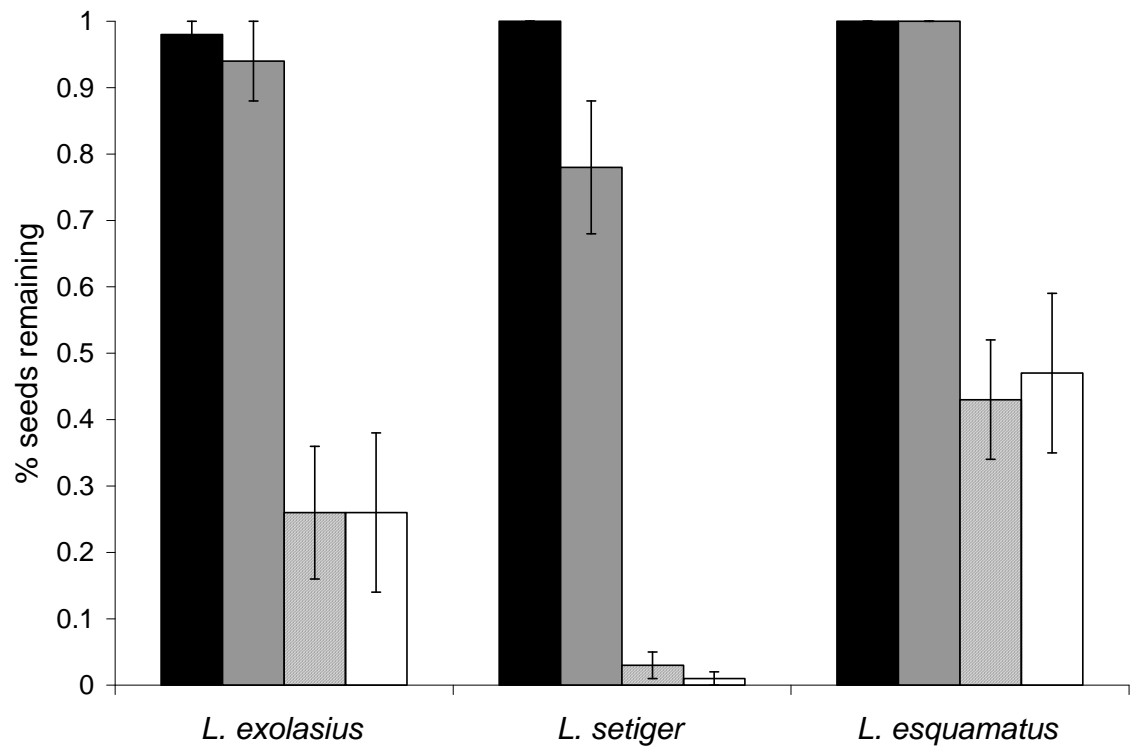


Figure 7.1 Proportion of seeds remaining for all three species at completion of the exclusion experiment. Treatments were ALL (■) (ants and vertebrates excluded), ANT (■) (ants only excluded), VER (▨) (vertebrates only excluded) and CON (□) (control, nothing excluded).

seeds per 0.1 m² respectively, which was very high when compared to the number of fruit produced per canopy area. Split and empty endocarps were often found at the base of plants of all three species, suggesting some level of predation prior to primary seed dispersal.

Seed removal was rapid for both treatments open to ants in the exclusion experiment for all species. There was a significant interaction between species and treatment for the exclusion experiments ($F_{6,88} = 10.873$, $P = 0.018$), indicating that the treatment effects differed between species. *Post hoc* comparisons found that both treatments giving access to ants (CON and VER), had significantly fewer seeds remaining by day 4 for all species (Figure 7.1). For *L. setiger* only, the treatment excluding ants but allowing entry to vertebrates (ANT) also differed significantly from the other three treatments (Figure 7.1). Fruit from the ANT treatment were removed more slowly than the CON and VER treatments, but still showed some removal of seed over the 4 day period for *L. setiger* and *L. exolasius*, indicating that either birds or mammals were attracted to the fruit. A few *L. setiger* seeds were found with damage indicative of rodent predation, identified because the side of the endocarp was chewed and the seed gone. The treatment excluding both ants and vertebrates (ALL) had zero removal in the majority of cases.

7.4 Discussion

Ecological comparisons of the three study species highlighted two factors that could have potentially caused rarity, or may adversely affect population persistence in the future. The first of these factors, a long primary juvenile period, was specific to the threatened species *L. exolasius*. The second factor, the innate seasonal requirement of dormancy and germination mechanisms, may also have contributed to limiting *L. exolasius* recruitment success, particularly when combined with a long primary juvenile period. However, it may have a limiting effect on the persistence of the more common species *L. setiger* and *L. esquamatus* as well, especially under future changes to the fire regime. Two aspects of the fire regime, frequency and season, were both identified as possible influences that could drive population decline.

In fire-prone regions, the time taken for seedlings to reach maturity is critical for obligate-seeding species. Adult plants are killed by fire and seed bank replenishment is dependent primarily on the post-fire cohort producing seeds prior to the next fire (Auld 1987; Bradstock & Kenny 2003). In this study, the majority of *L. esquamatus* and *L. setiger* plants had reached maturity within 2 to 3 years and 5 to 6 years respectively, after several different summer wildfires. No *L. exolasius* plants had reached maturity 6 to 8 years post-fire. Results from studies of over 40 other obligate-seeding shrub species in the region, found only three with primary juvenile periods longer than 6 years, and all three had matured in less than 6 years at some sites (Benson 1985; Keith 1996; Bradstock & Kenny 2003). The primary juvenile period of *L. exolasius* is therefore particularly long by regional standards. Obligate seeders with long primary juvenile periods are particularly at risk to high frequency fires because post-fire recruits may be killed before they reach maturity (Bond 1997; Bradstock & Kenny 2003).

Because there was still no sign of maturation of any *L. exolasius* individuals after any of the fire events studied, it is quite plausible that the species' primary juvenile period well exceeds 8 years. As observed during my study, both the *L. setiger* sites and one of the *L. esquamatus* sites were burnt by wildfires after only 7 years, which is at the lower limit of fire-free intervals for this region (Bradstock *et al.* 1998). I suggest that occasional short interval fires, as part of the natural regime in the past, have limited the extent of *L. exolasius* populations. Projected increases brought about by increasing numbers of managed fires, and also climate change (e.g. McLoughlin 1998; Williams *et al.* 2001; Hughes 2003; Cary *et al.* 2006), are likely to place obligate-seeding species with long primary juvenile periods such as *L. exolasius*, at even greater risk.

The habitat of extant *L. exolasius* populations provides further evidence that slow maturation and a lack of resilience to shorter interval fires may have limited this species. All three study species occur on similar low nutrient soils and are not restricted to any particular aspect or slope. However, *L. exolasius* occurs almost exclusively on rocky riparian sites (Fairley & Moore 1989; Benson & McDougall 1995; Ooi *et al.* 2006a). This type of habitat can provide areas of fire refugia, as has been found for *L. exolasius* (Ooi *et al.* 2006a) and a number of other south-east Australian species (e.g.

Clarke 2002; Keith *et al.* 2002), affectively reducing the frequency of fire faced by resident plant populations. Gill & Bradstock (1995) suggested that rare obligate-seeding plant species are concentrated in parts of the landscape where fires are less common. Although the common congeners *L. setiger* and *L. esquamatus* can occur in rocky habitats, they are not restricted to them.

A lack of identifiable difference between species for the vast majority of factors studied not only enabled us to highlight the primary juvenile period as a potential limiting trait, but emphasised the similar life histories of the three *Leucopogon* species as well. However, similarities were also found for characteristics where differences were expected. Seed dispersal mechanisms, predicted to be different because the rare *L. exolasius* and *L. setiger* had fleshy-fruited drupes whilst *L. esquamatus* did not, were surprisingly similar. Although some seeds of the fleshy-fruited species were obviously taken by birds, the amount appeared to be extremely small and variable. The estimated numbers of fruit reaching the ground was very high for all three species. Although *L. esquamatus* is the only study species previously identified as being ant-dispersed, ants were the principal removal agent for all three species. In fact, seed removal was more complete and fastest for the two fleshy-fruited species. Seed morphological traits may not always accurately predict dispersal mechanisms (Jordano 1995). Whilst fleshy fruits may have evolved to attract frugivores (Howe 1986), current habitat conditions mean that other mechanisms, in this case ants, may dominate.

The extinction of emus from the study region (Auld 1996) may have had a particular impact on *L. exolasius*. Emus have been found to target *Leucopogon* and other similar fleshy-fruited Ericaceae in regions where both coexist, and the number of ericaceous fruit found in scats are often orders of magnitude greater than other species (Keighery 1996; Quin 1996; McGrath & Bass 1999). Because of their considerable mobility, feeding habits and long gut retention times, this large bird could provide significant opportunity for long-distance dispersal of these species, whereas ants and small bird species generally enable only short-distance dispersal and are likely to mainly influence local recruitment patterns (Hughes & Westoby 1992; Calviño-Cancela *et al.* 2006). The presence of a long-distance dispersal agent within the landscape may be particularly important for rare species, as it provides increased opportunities for

range expansion and recolonisation after local population extinction (McGrath & Bass 1999; Calviño-Cancela *et al.* 2006). Conversely, the loss of a long-distance dispersal mechanism, such as found for *L. exolasius* in this study, could limit rare species and provide further cause for rarity.

Morphophysiological dormancy and a seasonal germination requirement were also common to all study species, but could represent a limiting factor for *L. exolasius*. An innate seasonal requirement for germination meant that seedling emergence in the field is seasonal, and seedlings appear at the same time of year (peaking in early winter) even if significant rainfall occurs at other times of the year. Recruitment mainly takes place after fire but seedling emergence is still restricted to the same season (Ooi *et al.* 2004a). *Leucopogon* emergence can therefore be delayed in relation to fire, depending on the season of the fire event. After a winter fire, Ooi (see Chapter 6) found that emergence of *L. exolasius* and *L. esquamatus* was delayed by almost 12 months. This maximum possible length of delay caused slower growth of *L. exolasius* seedlings and increased the two to three year primary juvenile period of *L. esquamatus* up to 4 to 6 years.

An increase of primary juvenile period would raise the chances of fire killing populations prior to maturation and seed bank replenishment. However, whilst significant, the amount of increase for the common species *L. esquamatus* still meant that significant proportions of the populations were likely to reach maturity prior to subsequent fire, even in the event of a short fire-free period such as the seven year interval experienced during this study. Slower seedling growth for the rare species *L. exolasius* could also potentially increase the primary juvenile period, and increase the likelihood that immature populations would be killed, even by more common 10 year fire intervals, which occur regularly under current natural fire regimes. The potential negative effects of seasonal emergence on the common species studied could therefore be offset by the shorter primary juvenile period of *L. esquamatus*, and to a lesser extent *L. setiger*, whereas seasonal germination of the rare *L. exolasius* could exacerbate problems caused by an already long primary juvenile period.

Emergence of all three study species was also delayed after peak-season summer fires, by 3 to 6 months. Co-occurring shrub species without seasonal germination

requirements germinated rapidly after fire (within 1 month), and grew more quickly and survived to higher proportions than their seasonally emerging counterparts (Ooi *et al.* 2004a; Chapter 6). In fire-prone regions, the optimal time to ensure recruitment success is during the immediate post-fire environment (Whelan 1995; Bond & van Wilgen 1996). There is no seasonal pattern to rainfall in the study region and it is unlikely that any benefit could be gained by maintaining a seasonal emergence pattern. The negative effects of winter fires in particular, as well as those of the more typical summer fires, therefore suggest that fire may not have been the primary force selecting for *Leucopogon* species.

I interpret these findings as indicative of a preadaptation of *Leucopogon* germination to seasonality, as has similarly been hypothesised for several resprouting species (Keeley *et al.* 2006). Other evidence, including current primary dispersal mechanisms and considerable inter-fire gap recruitment (Ooi *et al.* 2006a), suggests that previous environmental conditions in the evolutionary history of these species may have been more favourable than the current environment (see Jurado & Flores 2005). Whilst perhaps not optimal, it is possible that all three *Leucopogon* species studied ecologically fit well enough in a fire-prone environment to persist (e.g. see Janzen 1985; Ackerly 2004). However, differences in particular traits, such as the length of the primary juvenile period, have given each species varying abilities to persist under current fire regimes.

Because *L. exolasius* persistence appears to be bound to fire frequency and season, it is likely that its ability to persist in the future will be strongly tested by changes to the fire regime. The impacts of implemented fires and the effects of climate change are both forecast to promote more frequent fires (Williams *et al.* 2001; Cary *et al.* 2006). These changes represent significant threats to *L. exolasius* populations, as well as to many other obligate-seeding species in the region with long primary juvenile periods. The effects of changes to the peak fire season, which has already begun particularly near the urban interface (McLoughlin 1998), could also increase the level of threat faced by *Leucopogon* species, as well as other species with innate seasonal germination requirements. This potential threat needs to be assessed further for a range of species with seasonal dormancy and germination requirements in fire-prone regions.

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Appendices

Appendix 1

This Appendix shows the list of species assigned with a dormancy type (see Chapter 4). For most species, dormancy type was inferred from the literature. A large amount of data was obtained from several main sources including: Baskin & Baskin (1998), which provided reviews of germination studies conducted around the world for a range of genera; Benson & McDougall (1995), which provided similar information, but for species within the study region only; and the PlantNET database, maintained by the National Herbarium of NSW (<http://plantnet.rbgsyd.nsw.gov.au>). Inferences were also sometimes made at the generic level, especially where large numbers of studies have shown a particular germination response for congeneric species. For example, *Banksia* and *Hakea* species within the Proteaceae (and, in fact, many other shrub species with canopy-stored seed banks) have been the subject of numerous studies where germination data has been collected (e.g. Bradstock & Myerscough 1981; Cowling & Lamont 1987; Bell 1994), and all have shown these species to have non-dormant seeds. This is supported by more recent review papers (e.g. Merritt *et al.* 2007). Species such as these were therefore assigned to the non-dormant (ND) class.

The Table on the following page lists the dormancy types assigned to each shrub species from the Central Coast Botanical Subdivision (New South Wales) in south-eastern Australia. If there are no (or few) differences in dormancy types at the generic level, then genus only is listed. Where only one species within a genus has a known dormancy type, or a particular species differs from its congeners in dormancy type, the species name is also given. The number of species within each genus, with the listed type of dormancy, is given in brackets after the genus name. Dormancy types are ND (non-dormant), PY (physical), PD (physiological), MPD (morphophysiological) and MD (morphological). Where references are listed, dormancy type has been inferred using specific information from the cited study. An asterisk next to the reference indicates that direct evidence was available for a species within the listed genera.

Family	Genus or species	Dormancy Class	Reference sources
APIACEAE	<i>Platysace</i> (4 species)	MPD	
	<i>Xanthosia</i> (2 species)	MPD	
ARALIACEAE	<i>Astrotricha</i> (5 species)	MPD	
ASTERACEAE	<i>Cassinia</i> (9 species)	ND	1
	<i>Helichrysum elatum</i>	PD	
	<i>Olearia cordata</i>	ND	
	<i>Vittadinia gracilis</i>	ND	2
CASUARINACEAE	<i>Allocasuarina</i> (7 species)	ND	3*
	<i>Casuarina</i> (2 species)	ND	3
CELASTRACEAE	<i>Cassine australis</i>	PD	4*
	<i>Maytenus silvestris</i>	ND	4*
CUNONIACEAE	<i>Acrophyllum australe</i>	ND	
	<i>Bauera</i> (2 species)	ND	
	<i>Ceratopetalum gummiferum</i>	ND	
DILLENACEAE	<i>Adrastaea salicifolia</i>	MPD	
	<i>Hibbertia</i> (21 species)	MPD	
TREMANDRACEAE	<i>Tetratheca</i> (8 species)	PD	5
ERICACEAE	<i>Acrotriche</i> (2 species)	MPD	
	<i>Astroloma</i> (2 species)	MPD	6
	<i>Brachyloma daphnoides</i>	MPD	
	<i>Dracophyllum secundum</i>	PD	
	<i>Epacris</i> (11 species)	All PD except <i>E. coriacea</i> & <i>E. obtusifolia</i> (ND)	7*
	<i>Leucopogon</i> (16 species)	All MPD except <i>L. amplexicaulis</i> (PD)	6*
	<i>Lissanthe</i> (2 species)	MPD	6*
	<i>Melichrus</i> (2 species)	MPD	
	<i>Monotoca</i> (3 species)	MPD	
	<i>Rupicola</i> (2 species)	PD	
	<i>Sprengelia</i> (2 species)	PD	
	<i>Styphelia</i> (6 species)	MPD	6*
	<i>Woollisia pungens</i>	PD	
EUPHORBIACEAE	<i>Breynia oblongifolia</i>	ND	4*
	<i>Phyllanthus</i> (4 species)	ND	4*
	<i>Ricinocarpos</i> (2 species)	PD	4*
FABACEAE	<i>Acacia</i> (49 species)	PY	8*, 9*
	<i>Almaleea</i> (2 species)	PY	
	<i>Aotus</i> (2 species)	PY	9*
	<i>Bossiaea</i> (12 species)	PY	9*
	<i>Chorizema parviflorum</i>	PY	
	<i>Daviesia</i> (9 species)	PY	9*
	<i>Desmodium rhytidophyllum</i>	PY	
	<i>Dillwynia</i> (10 species)	PY	9*
	<i>Gompholobium</i> (8 species)	PY	9*
	<i>Hovea</i> (4 species)	PY	
	<i>Indigofera australis</i>	PY	
	<i>Lespedeza juncea</i> subsp <i>sericea</i>	PY	
	<i>Mirbelia</i> (3 species)	PY	9*
	<i>Neptunia gracilis</i>	PY	
	<i>Oxylobium</i> (3 species)	PY	
	<i>Pararchidendron pruinosum</i>	PY	
	<i>Paraserianthes lophantha</i>	PY	
	<i>Phyllota</i> (3 species)	PY	9*

Family	Genus or species	Dormancy Class	Reference sources
	<i>Platylobium formosum</i>	PY	9*
	<i>Podolobium</i> (3 species)	PY	
	<i>Pultenaea</i> (27 species)	All PY except <i>P. polifolia</i> (ND)	9*
	<i>Senna</i> (3 species)	PY	4
	<i>Sphaerolobium</i> (2 species)	PY	9*
	<i>Swainsona galegifolia</i>	PY	
	<i>Viminaria juncea</i>	PY	9*
GOODENIACEAE	<i>Dampiera</i> (3 species)	PD	
	<i>Goodenia</i> (3 species)	ND	
	<i>Scaevola albida</i>	PD	
HALORAGACEAE	<i>Haloragis exalata</i> subsp <i>exalata</i>	PD	10
LAMIACEAE	<i>Hemigenia</i> (2 species)	PD	11
	<i>Plectranthus parviflorus</i>	PD	
	<i>Prostanthera</i> (22 species)	PD	4, 10
	<i>Spartothamnella juncea</i>	PD	
	<i>Westringia</i> (3 species)	PD	
LOGANIACEAE	<i>Logania</i> (2 species)	PD	12
LORANTHACEAE	<i>Amyema</i> (3 species)	MD	13
	<i>Dendrophthoe vitellina</i>	MPD	10
MALVACEAE	<i>Hibiscus</i> (3 species)	PY	
	<i>Howittia trilocularis</i>	PY	
MYOPORACEAE	<i>Myoporum</i> (4 species)	PD	
MYRTACEAE	<i>Austromyrtus tenuifolia</i>	ND	
	<i>Baeckea</i> (5 species)	ND	
	<i>Callistemon</i> (9 species)	ND	
	<i>Darwinia</i> (9 species)	PD	14
	<i>Kunzea</i> (5 species)	All ND except <i>K. capitata</i> (PD?)	7, 15
	<i>Leptospermum</i> (21 species)	ND	4*
	<i>Melaleuca</i> (14 species)	ND	
	<i>Triplarina</i> (2 species)	ND	
PITTOSPORACEAE	<i>Bursaria</i> (2 species)	ND	
	<i>Pittosporum</i> (3 species)	ND	16
POLYGALACEAE	<i>Comesperma</i> (3 species)	ND	
PROTEACEAE	<i>Banksia</i> (12 species)	ND	
	<i>Conospermum</i> (6 species)	PD	17*
	<i>Grevillea</i> (27 species)	All ND except <i>G. speciosa</i> (PD?)	18, 19
	<i>Hakea</i> (11 species)	ND	
	<i>Isopogon</i> (3 species)	All ND except <i>I. anemonifolius</i> (PD?)	4*
	<i>Lambertia formosa</i>	ND	
	<i>Lomatia</i> (3 species)	ND	
	<i>Persoonia</i> (24 species)	PD	6, 20
	<i>Petrophile</i> (4 species)	ND	6*
	<i>Symphionema</i> (2 species)	PD	
	<i>Telopea speciosissima</i>	ND	21*
	<i>Xylomelum pyriforme</i>	ND	
RHAMNACEAE	<i>Cryptandra</i> (6 species)	PY	22
	<i>Pomaderris</i> (17 species)	PY	
RUTACEAE	<i>Asterolasia</i> (5 species)	PD	6*
	<i>Boronia</i> (18 species)	PD	6*
	<i>Correa</i> (3 species)	PD	
	<i>Crowea</i> (2 species)	PD	
	<i>Eriostemon australasius</i>	PD	
	<i>Leionema</i> (3 species)	PD	
	<i>Phebalium squamulosum</i>	PD	

Family	Genus or species	Dormancy Class	Reference sources
	<i>Philotheca</i> (7 species)	PD	
	<i>Zieria</i> (13 species)	All PD except <i>Z. involucrata</i> (ND)	17*
SANTALACEAE	<i>Choretrum</i> (3 species)	MPD	
	<i>Exocarpus strictus</i>	MPD	
	<i>Leptomeria acida</i>	MPD	
	<i>Omphacomeria acerba</i>	MPD	
	<i>Santalum obtusifolium</i>	MPD	
SAPINDACEAE	<i>Dodonaea</i> (10 species)	PY	
SOLANACEAE	<i>Cyphanthera</i> (2 species)	ND	11
STACKHOUSIACEAE	<i>Stackhousia viminea</i>	PD	17
STERCULIACEAE	<i>Rulingia</i> (4 species)	ND	4*
STYLIDIACEAE	<i>Stylidium laricifolium</i>	PD	
THYMELAEACEAE	<i>Pimelea</i> (6 species)	PD	1

References cited: ¹Clarke *et al.* 2000; ²Willis & Groves 1991; ³Pannell 1995; ⁴Benson & McDougall 1995; ⁵Bell *et al.* 1995; ⁶Ooi, unpubl. data; ⁷Thomas *et al.* 2003; ⁸Auld 1996; ⁹Auld & O'Connell 1991; ¹⁰Baskin & Baskin 1998; ¹¹Cochrane *et al.* 2002; ¹²Bell *et al.* 1993; ¹³Lamont & Perry 1977; ¹⁴Auld & Ooi, in prep.; ¹⁵Tierney & Wardle 2005; ¹⁶Gleadlow 1982; ¹⁷Auld *et al.* 2000; ¹⁸Morris 2000; ¹⁹Briggs *et al.* 2005; ²⁰Mullins *et al.* 2002; ²¹A.J. Denham, pers. comm.; ²²Turner *et al.* 2005.

Appendix 2

Publications resulting from this work