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Germination ecology of the endangered species *Asterolasia buxifolia* (Rutaceae): smoke response depends on season and light

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Germination of fire-prone plant species can be complex. Understanding this key component of the regeneration niche can help elucidate mechanisms for persistence and inform conservation. Like many threatened species in fire-prone south-eastern Australia, *Asterolasia buxifolia* is restricted to riparian habitats. Germination ecology of this physiologically dormant family (Rutaceae) is not well understood. We found that seeds germinated only with smoke at winter temperatures. Darkness inhibited germination. These results help us understand why such species may be limited to riparian habitat.

Abstract

In fire-prone regions, many plant species rely on persistent seed banks for post-fire recovery. Understanding dormancy and germination cues is therefore important to predict population response. However, the germination ecology of species with physiologically dormant seeds in fire-prone regions is complex. We used the endangered species *Asterolasia buxifolia,* from riparian habitat in fire-prone south-eastern Australia, to investigate physiologically dormant seeds and their response to fire. We assessed whether fire cues alone promoted germination, or whether seasonal factors and light also played a role. Additionally, we tested the resilience of seeds to heat shock temperatures produced in soil during fire, in order to identify potential factors that restrict such species to fire refugia. Seeds only germinated at winter seasonal temperatures, and had an obligate smoke and light requirement. Heat shock treatments above 80˚C slowed the germination rate. Smoke-related germination and the tolerance of *A. buxifolia* seeds to high fire-related temperatures demonstrated that recruitment dynamics can be driven by fire, however, germination is restricted to winter temperatures. This highlights the potential that changes to fire season may have on population persistence. The slow germination rate caused by heat, and a light requirement, may contribute to restricting this species to riparian habitat.

Introduction

In fire-prone regions, many plant species rely on seeds stored within seed banks for post-fire recovery and population persistence (Whelan 1995). Dormancy and/or specific germination requirements allow the seeds to persist through the inter-fire period, with seeds germinating when conditions are favourable in the post-fire environment, where there is less competition and a necessity to recruit to replace individuals killed by the fire (Whelan 1995; Baskin and Baskin 2014). Fire cues such as heat and smoke can overcome dormancy and therefore allow germination from the seed bank (Keeley 1991; Ooi 2007; Merritt *et al*. 2007; **Invalid source specified.**. Hence, understanding dormancy mechanisms and germination cues is important for gaining a clear picture of seed bank and plant population dynamics in fire-prone regions.

In a study from the fire-prone heath and woodlands of south-eastern Australia, Ooi (2007) estimated that approximately 50% of all dormant shrub species had seeds with physical dormancy, while the rest had either physiological or morphophysiological dormant seeds, both of which have a physiological component (Merritt *et al*, 2007; Ooi 2007; Baskin and Baskin 2014). However, when classifying only those shrub species listed as threatened (i.e. at some risk of extinction), the distribution of dormancy classes skewed heavily towards species with a physiological component to their dormancy (> 70%) (Ooi, unpubl. data). It is therefore possible that dormancy and germination cues play a role in determining why some species are rare or threatened in this, and other, fire-prone regions.

Physiological dormancy is the most common dormancy type throughout the world and is primarily broken by stratification of the seed at seasonal temperatures or via dry after-ripening (Baskin and Baskin 2014). While our knowledge of physiological dormancy is well developed in a number of climate regions, such as in temperate and arid vegetation types (and particularly for many annual species), the understanding of how physiological dormancy is controlled in fire-prone vegetation is still developing. From the studies that have been conducted, however, and from the behaviour of species in other vegetation types, it is likely that season and smoke may be important for physiologically dormant fire-prone species (Roche *et al.* 1998; Baker *et al.* 2005; Ooi *et al.* 2006; Thompson and Ooi 2010; Cross *et al.* 2013; Mackenzie *et al.* 2016). This relationship between smoke and season means that the timing of the fire event can have significant effects on recruitment success (Ooi 2010). Additionally, light may play a role in germination, although whether it is likely to have a positive, neutral or negative effect on Australian fire-prone species is largely unknown (Merritt *et al*. 2006; Downes *et al*. 2015).

Due to their topography, soil type and moisture, riparian zones can buffer against fire and therefore act as a refuge for fire sensitive species (Pettit and Naiman 2007).This is highlighted by the number of rare and threatened species that are restricted to riparian habitats (Naiman *et al.* 1993; Pickup *et al.* 2003; Ooi *et al.* 2006a; Pettit and Naiman 2007). One hypothesis for this is that species restricted to such areas do not maintain traits that are as tightly bound to fire, or the fire regime of the surrounding vegetation, and retract to riparian areas where they can persist via other drivers in addition to fire**Invalid source specified.**. For example, Pickup *et al.* (2003) found that dormancy mechanisms and seed bank dynamics of the endangered species *Grevillea rivularis* were related to disturbance more generally, and could therefore respond to both flooding and fire. Additionally, other studies have found that high soil moisture can be critical for successful recruitment of species in these habitats (Dwire and Kauffman 2003; Jackson and Sullivan 2009). Identifying the drivers of seed dormancy and germination can therefore help to develop an understanding of the reasons for rarity and the mechanisms for persistence within riparian fire-prone systems. In particular, the magnitude of germination response with or without the presence of fire cues can inform how tightly bound species are to fire-related dynamics (Ooi *et al.* 2014), while the tolerance of seeds to high temperatures can ascertain whether seeds respond to fire or simply tolerate it (Ramos *et al.* 2016). Riparian habitat fires are often less intense and soil moisture is high (DeBano 2000; Dwire and

Kauffman 2003), which translates to lower soil temperatures than the surrounding vegetation matrix. Species may therefore show a lower resilience to heat shock treatments than similar species from a dry habitat.

In our study, we investigated the germination ecology of *Asterolasia buxifolia* (Rutaceae) a threatened species from south-eastern Australia, a highly fire-prone environment with an aseasonal rainfall climate. This species is representative of numerous other threatened species from fire-prone habitats, many of which are restricted to riparian areas. It is likely to have physiological dormancy (Martyn *et al.* 2009), and therefore may have some type of seasonal germination requirement. Little is currently known of the ecology of *A. buxifolia*, or indeed of a number of threatened physiologically dormant species from fire-prone regions, limiting the potential for implementing effective management and conservation initiatives.

To gain a better understanding of the recruitment dynamics of physiologically dormant species in riparian systems, and how they are related to fire, the germination response and resilience of seeds to heat shock temperatures generated by fire were investigated for *A. buxifolia*. The main aims of this study were to address the following questions:

(i) What type of dormancy does *Asterolasia buxifolia* exhibit?
(ii) Are there seasonal temperature requirements of germination for this species and, if so, which seasonal temperatures are important and how do they interact with fire cues such as heat and smoke? Is there any evidence suggesting fire is necessary for recruitment or that seeds are resilient to heat?
(iii) What conditions produce the highest levels of germination and can this be utilised for *ex situ* conservation collections?

Methods

Study species and site

Asterolasia buxifolia (Rutaceae) is a tall woody shrub, known from only one population in the Blue Mountains region of New South Wales (NSW), in south-eastern Australia. The species is listed as endangered under the NSW Threatened Species Conservation Act 1995. First discovered in the 1830's it was later thought to be extinct, but was rediscovered in 2000 in a riparian zone (a transitional area between a water body and upland terrestrial area), with plants occurring no more than 20m from the river's edge (Makinson 2002). Current estimates suggest there are around 2200 individuals occurring along a 2-3km stretch of river (Collette 2016). The seeds of *Asterolasia buxifolia* are small and held in a soil-stored seed bank. As with other species from the family, seeds are initially ballistically dispersed and, based on observations of new seedlings occurring near ant nests, then undergo secondary dispersal via myrmecochory. Little is known of the phenology of the plant, although it has a short-term flowering season in late summer-early spring where small yellow flowers are produced and seeds formed shortly after pollination (Mackinson pers comm.).

The region where *A. buxifolia* occurs is highly fire-prone and has an aseasonal rainfall climate. However, the site of the population is long unburnt (> 40 years), and several seedlings have been observed emerging during the inter-fire period suggesting that multiple recruitment mechanism or strategies may be employed (Collette 2016). Additionally, anecdotal evidence suggests that the species may be an obligate seeder (i.e. standing plants are killed by fire), highlighting the importance of seed germination for population persistence post fire.

The population is located at approximately 750m above sea level, in the western region of the Blue Mountains. This altitude, along with distance from the coast, provides a climate that is cold in the winter months and potentially very hot in the summer months. The closest Australian Government Bureau of Meteorology weather station is located at Mt. Boyce, approximately seven kilometres away. The mean winter maximum/minimum temperatures are 10.2 /3.1°C, while the corresponding mean summer maximum/minimum is 23.1/12.7°C. Rainfall occurs relatively evenly throughout the year (Fig. 1).

Seed collection

Seed collection was carried out in September 2015, by bagging flowers on at least 30 mature individuals with high flower densities, spread throughout the population, after they had been pollinated and just before fruit set (a period of around 2 weeks). This allowed the seeds to reach maturity and then be captured in the bags prior to natural ballistic dispersal. A high seed fill was observed from initial assessment of the collected seed lot (~ 85%) (Frith *et al.* 2009). Seeds were then sorted via their weight using a Selecta zigzag gravity seed separator and unfilled seeds discarded, leaving mainly viable seeds in the seed lot used for experiments. Seeds were stored in a temperature and humidity controlled room set at 15°C and 15% humidity for six months before commencement of the experiments

Imbibition test and seed morphology

To assess whether seeds were able to imbibe water, and therefore test if the seed coat was impermeable and played a role in controlling germination, an imbibition test was performed (Baskin and Baskin 2014). Twenty *A. buxifolia* seeds were chosen randomly from the collected seed lot, and were individually weighed with a set of A&D GR-202 scales to the nearest 0.01 mg. Seeds were then placed onto allocated areas of moistened filter paper. Every hour for 6 hours, seeds were removed and excess moisture dried off. Seeds were weighed and then returned to the filter paper. After the first 6 hours, seeds were left overnight to imbibe further and weighed again the next day, 24 hours after the experiment had commenced. At the end of the experiment, the change in seed weight over time was calculated. Imbibed seeds were also dissected under a dissection microscope to investigate their embryo type which helps to inform the type of dormancy that seed are likely to exhibit (Baskin and Baskin 2014).

Germination experiments

Experiment 1: the effects of seasonal temperatures and fire cues on germination

The seasonal requirements for germination, and how these interacted with fire cues, were investigated using a fully factorial experiment with the factors season, heat and smoke. Incubators were used to mimic seasonal differences, set at temperature cycles of 11/3°C, 18/6°C or 25/11°C, approximating winter, spring/autumn and summer conditions respectively for the study region. One incubator only was used per seasonal temperature treatment, and temperature cycles set within each incubator were monitored with Thermoline ibuttons. Temperatures were based on the average minimum and maximum temperatures for in winter, spring/autumn and summer based on Bureau of Meteorology data from the Mt. Boyce weather station (Fig. 1), which is within 10 km of the study site (although at slightly higher elevation than the study site). The experiment was established with four treatments at each seasonal temperature (control, smoke, heat, smoke + heat). Treatments were applied to four replicates (dishes) with 20 seeds in each dish. Smoke treatments consisted of irrigating dishes with smoke water, which had been made by burning leaf litter and passing the smoke produced through water for three hours via a vacuum pump. This solution was diluted to 2% before being applied to seeds, as this concentration had been shown to produce the highest response in a pilot study on species with a known smoke response (Catelotti unpubl. data). For the heat shock treatment, dry seeds were transferred to a 90 mm glass petri dish and placed in an oven set at 60°C, for a period of 10 minutes. Each replicate was heated separately (Thomas *et al.* 2003; Ainsley *et al.* 2008). Seeds were then transferred to plastic 90mm petri dishes lined with qualitative filter paper, where they were irrigated with distilled water (or smoke water) until the filter paper was consistently moist. Heat shock was applied prior to smoke treatments.

For seeds that were initially irrigated with smoke water, all subsequent irrigation was done with distilled water so they were only exposed to the smoke treatment once. Germination trials were conducted in three light, humidity and temperature controlled Thermoline incubators running on

12/12-hour day/night cycles at each of the seasonal temperatures. Temperatures were additionally monitored by Thermochron ibuttons placed in each incubator to ensure temperatures were maintained. Replicate dishes were checked every week for new germination and moistened with distilled water as required. Germination was scored on emergence of the radicle. The germination experiments continued for a total of 11 weeks, until the rate of germination had slowed, with less than one seed germinating per week. Germination percent was calculated based on the assumption of 100% viability, as an initial cut test revealed that seeds had a very high initial viability close to this level. This and all subsequent cut tests were performed using hydrated seeds under a dissecting microscope. Seeds were scored as inviable if there was no embryo present, or the embryo had lost structural integrity and/or had become brown in colour. Inversely, they were scored as viable if the embryo and endosperm was intact, and a white colour was maintained. Cut tests provide a quick and accurate assessment of seed viability (Ooi *et al.* 2004a).

Experiment 2: resilience to increasing heat shock and germination under dark conditions

The optimal seasonal temperature for germination was established from Experiment 1 (winter temperature cycles of 11/3˚C) and there was an obligate requirement for smoke. Therefore, to assess the effect of heat and dark treatments, two experiments were conducted at these winter temperatures. Firstly, to investigate the effect of heat, seeds were subjected to treatments ranging from 40˚C, increasing by 20˚C increments to 120˚C. The high temperatures are towards the upper limit of those experienced beyond 1 cm depth in the soil during fire (Auld and Bradstock 1996; Ooi *et al.* 2014). As before, four replicates of 20 seeds were used for each treatment level and heat shock was applied for 10 minutes. Smoke treatments were then applied to all seeds. After treatments, replicates were placed in the incubator at 11/3˚C. The control treatment for this experiment consisted of replicates which had the smoke treatment applied but no heat. The impact of heat was assessed by quantifying increased or decreased germination and mortality in comparison to the control seeds.

Viability of all remaining seeds following germination trials was tested by cut tests as described above. To confirm that our assessment of viability was robust, a tetrazolium test was performed on 100°C treated seeds. The ungerminated seeds from each of the four replicate dishes were cut, and if they appeared viable, then placed into one of four sample jars filled with a 1% tetrazolium solution. After four days in a dark cupboard at laboratory temperatures (approximately 22°C), seeds were extracted from the solution and cut open under a dissection microscope where their colour was assessed. Viable seed tissue stains pink or red in tetrazolium solution (Ooi *et al.* 2004a). The results from the tetrazolium test were consistent with the cut tests.

Secondly, to investigate the effect of light on germination, we established a factorial experiment also at the 11/3°C temperature cycle, using the factors smoke (+/-) and light (+/-). Due to limited remaining seeds, we used three replicates of 20 seeds for treated seeds, and three replicates of 14 seeds for the control in this experiment. Smoke treatments were applied as above, while dishes assigned to dark treatments were wrapped in two layers of aluminium foil.

Statistical analysis

For the imbibition test, the percentage of weight change was plotted against time. The final average (n=20) weight after 24 hours was then compared to the initial average weight using a paired T test. The data were tested for normality using a Shapiro-Wilk W test and was found to be normally distributed. For Experiment 1, there was an obvious effect of winter temperature observed (< 2% germination at any other seasonal temperature). The germination data were analysed within season using a 2 factor (heat and smoke) GLM with a binomial distribution and logit link function. For Experiment 2, both germination and final viability data were analysed using a 1 factor (heat) GLM with a binomial distribution and a logit link function. For both germination experiments there was no evidence for overdispersion of data. Post-hoc Tukey’s HSD tests were performed in order to assess differences between treatment levels.

To test for the rate of germination in Experiment 2, t50 (time to reach 50% of total seeds germinated) values were calculated by plotting the percentage of seeds that germinated in each replicate against time, then fitting a linear model and solving the equation of the line to calculate the x intercept when y=50. A 1 factor (heat) ANOVA was then performed on the t50 data. Prior to analysis, the data were tested for normality and no transformations were required. All analyses were performed using the JMP pro 11.0.0.0 (SAS Institution Inc.) statistical package.

Results

Imbibition test and seed morphology

Mean seed weight significantly increased after 24 hours by 0.25 mg (~ 11% increase) from 2.26 mg to 2.51 mg (t=2.44, df=19, p=0.001). Seeds were endospermic and contained a linear embryo, which extended along the full length of the seed.

Germination experiments

Experiment 1: the effects of seasonal temperatures and fire cues on germination

Results from Experiment 1, investigating seasonal temperature response, showed that high levels of germination only occurred at the winter temperature treatments (11/3°C). Within the winter temperature treatment there was an interaction between smoke and heat (χ^2 = 6.12, df = 1, p = 0.013), with smoke increasing germination well above non-smoked seeds. The interaction appears to be from a small (5%) difference in germination for the smoke and smoke x heat treatments. Negligible germination occurred at all other seasonal temperature cycles (Fig. 2).

Experiment 2: resilience to increasing heat shock and germination under dark conditions

The heat shock treatments significantly affected germination response (χ^2 = 153.72, df = 4, p < 0.001). Response to temperature treatments of 40˚C and 60˚C did not differ significantly from the control, with approximately 80% of seeds germinating. Germination was negatively affected by heat shock treatments above 80°C, where it dropped to 66% and 6% after the 80˚C and 100°C treatments respectively. No germination occurred after the 120°C treatment (Fig. 3). The rate at which germination occurred was also affected by the heat shock treatments. Time to 50% germination (t50) was able to be calculated for germination of seeds from the control, 40˚C, 60˚C and 80˚C heat shock treatments only, due to negligible germination at the higher temperature treatments. The average t50 value (n=4) was 26 days for the control which progressively increased with temperature treatment to 34 days at 40 ˚C, 32 days at 60˚C, and 55 days at 80˚C. The t50 value for the 80˚C treatment was significantly higher than all lower temperature rates, meaning germination took longer to occur ($F = 19.50$, $df = 3$, $p < 0.001$) (Fig. 4).

Cut tests indicate that seed viability was significantly affected by heat shock treatments ($\chi^2 = 47.04$, $df = 6$, $p < 0.001$). Control seeds had a high viability of 98% and there was no significant negative affect until the 120˚C treatment, where it dropped to an average of 40% (Fig. 3).

Seeds from the 100˚C heat shock treatment that were placed in the tetrazolium solution stained well. Of the 22 seeds that were subjected to this test, 17 (77%) stained dark red indicating viability, and five (23%) had either no stain or pink uneven colouring, indicating inviability, which was consistent with the results from the cut tests (32/40 seeds (80%) viable).

Constant darkness completely inhibited germination. Smoke treatments did not overcome any inhibitory factors imposed by dark conditions. Smoke treated seeds in light conditions germinated to significantly higher levels than any of the other treatments ($\chi^2 =7.327$, $df = 8$, $p < 0.007$) (Fig. 5).

Discussion

The strong requirement for a smoke cue to promote germination, and the tolerance of *Asterolasia buxifolia* seeds to high fire-related temperatures, both clearly demonstrated that recruitment dynamics could be driven by fire. There was negligible germination without smoke, however seeds only germinated when incubated at winter temperature cycles that are typical of the Blue Mountains region where the population is found. These results suggest that the species maintains fire-adaptive traits. Additionally, germination was relatively slow and seeds had a strong light requirement, meaning they would need to be imbibed at or near the soil surface for an extended period. Few areas within the landscape would maintain such conditions, and this, along with other factors such as fire frequency, may contribute to limiting the species ability to expand beyond the riparian zone.

The identification of a linear embryo, along with evidence suggesting that seeds could imbibe water without scarification, confirmed that *A. buxifolia* is a physiologically dormant species. The 11% increase in weight due to water up-take aligns with results from imbibition tests performed on other species from the Rutaceae family (Martyn *et al.* 2009). Very few past studies of physiologically dormant species have identified such a strong preference to a single seasonal temperature as a requirement for germination in Australian fire-prone systems. Almost no germination occurred at warmer temperatures, suggesting that emergence in the field would be highly restricted to cold temperature conditions. Mackenzie *et al.* (2016) found a similar, albeit less constrained preference for winter germination temperatures for a number of common *Boronia* species (also Rutaceae), whilst Ooi *et al.* (2006) found that three *Leucopogon* species (Ericaceae) required long periods of warm stratification to overcome physiological dormancy. The long period of warm stratification resulted in seedling emergence in late autumn or winter post-fire, regardless of earlier rainfall events (Ooi *et al.* 2004).

For species like *A. buxifolia*, with a strong seasonal preference and obligate smoke requirement occurring in an aseasonal rainfall climate, the season that a fire occurs in may be an extremely important determinant of first germination, then recruitment success (Ooi 2010). Cunningham (1984) reported that the natural fire season in the Blue Mountains study region occurs primarily in spring/summer, with very occasional late winter fires. The strong seasonal requirement, and the relatively long period of incubation (8 weeks) to produce germination, could mean that a fire occurring in mid to late winter or spring could have a negative impact on *A. buxifolia* recruitment. Emergence would be delayed for up to a year before the winter temperatures required to promote germination occur (Mackenzie *et al.* 2016), and such species would face higher levels of competition from species with no seasonal requirement, such as resprouting plants and those with physical dormancy (Ooi 2010). Delayed emergence of physiologically dormant species due to fire season has been shown to cause a decrease in seedling emergence, survival and growth resulting from a presumable decrease in competitive ability, in fire-prone aseasonal rainfall regions (Ooi *et al.* 2004; Ooi 2010).

Seed mortality of *A. buxifolia* in relation to heat shock treatments was similar to most species from fire-prone regions around the world, with high levels of seed mortality not occurring until treatments reached 120°C (Auld and O'Connell 1991; Keeley 1991; Tierney 2006; Moreira *et al.* 2010; Ooi *et al.* 2014). This further suggests that *A. buxifolia* maintains characteristics indicating some level of adaptation to fire. Interestingly, germination reduction at 100˚C for *A. buxifolia* was not related to seed mortality, with around 80% of seeds that didn’t germinate maintaining viability. There is a building body of evidence showing that heat shock can increase germination of some physiologically dormant species in fire-prone regions. For example, a 70 - 80°C treatment can improve germination of the Australian species *Prostanthera eurybioides* (Lamiaceae), *Codonocarpus cotinifolius* (Gyrostemonaceae), *Boronia anemonifolia* (Rutaceae), *Epacris micropylla and E. paludosa* (Ericaceae) and several *Darwinia* (Myrtaceae), either alone or by interacting with smoke (Thomas *et al.* 2003; Baker *et al.* 2005; Ainsley *et al.* 2008; Auld and Ooi 2009; Mackenzie *et al.*, 2016). Dormant seeds from other continents continue this trend (e.g. Ribeiro et al. 2012), however, world-wide, few have reported a suppression of germination without loss of viability with heat shock, as we have found in this study.

It is difficult to assess the viability of deeply dormant seeds as cut tests have a level of subjectivity (Ooi *et al.* 2004a). It is therefore possible that rather than our heat shock treated seeds remaining viable and their germination supressed, seeds were damaged, lowering the amount and rate of germination. However, if our viability results are accurate, as the results from our cut versus tetrazolium test data support, heat shock could have suppressed or delayed germination (i.e. germination could have occurred if seeds were given a longer period to do so). Heat shock can result in changes to seed proteins which play an important role in germination (Lu *et al.* 1995; Wood *et al.* 2000; Finkelstein *et al.* 2008). The production of heat shock proteins (HSPs) (which act as molecular chaperones) increases dramatically under stressful temperatures, and these can prevent protein aggregation or potentially participate in refolding of denatured proteins after heat stress (Wang *et al*. 2004; Tripp *et al*. 2009), potentially delaying germination response. While HSPs are found in seeds, our understanding of their role in native species is limited (Feder and Hofmann 1999).

The relationship between fire refugia, such as that provided by riparian habitat, and threatened species, has often brought up the suggestion that life history traits of riparian species are not as closely bound to the fire regime of the broader environment. The obligate requirement for smoke and the resilience to fire-related temperatures displayed by *A. buxifolia* seeds suggests that recruitment of this species is fire-adapted, and that at this life-history stage fire is not limiting. However, the slow rate of germination, which declines even further after heat shock temperatures above 80˚C, means that successful germination and seedling establishment would require very long periods of high soil moisture post-fire, of between four and ten weeks. In the study region, which consists of well-drained sandy soils, it is unlikely that soil moisture would regularly be retained for such periods away from watercourses, particularly at the shallow depths where most of the small-seeded *A. buxifolia* would be able to emerge from. The high levels of moisture associated with riparian habitat could therefore assist germination and the success seedling establishment and survival (Bhattacharjee et al. 2008) and provide part of the explanation of the species restriction to the riparian zone.

The strong requirement for light may also provide a reason for restriction to moister riparian habitats. A light requirement is often associated with small-seeded species, to ensure that germination does not occur too deep within the soil profile (Bell 1999). However, soil moisture evaporates more quickly from near the surface (Huang *et al.* 2016), and the long periods with a moist top soil substrate required for germination would most likely be found close to stable watercourses in these habitats. This is one of few studies from eastern fire-prone Australia that have identified a light and smoke requirement for germination, which suggests that further study of other riparian species should include dark treatments.

Despite the obligate requirement for a smoke cue, seedling emergence has been observed in the field even though the *A. buxifolia* population has not been burnt for over 40 years (Collette 2016). This shows that some, albeit small numbers, of seeds can germinate in the absence of fire. A possible reason for this is that physiologically dormant seeds often decrease their levels of dormancy over time via after-ripening, and are subsequently able to germinate under a broader range of conditions as they age (Finch-Savage and Leubner-Metzger 2006; Turner *et al.* 2009; Baskin and Baskin 2014). Although it is likely that a large proportion of the seed bank will maintain the requirement for a fire cue, the ageing process may allow some leakage from the seed bank via germination. This may contribute to maintaining a basal population presence in the absence of fire as older plants senesce, and other disturbance mechanisms, such as flooding, may contribute to recruitment opportunities (e.g. see Pickup *et al.* 2003). However, further long-term study is required to clearly demonstrate whether such recruitment alone is sufficient for viable population persistence.

Management of endangered species such as *A. buxifolia* is extremely important because of their restriction to a single population (Childs *et al.* 2010). Implementing prescribed fires is a key management tool in fire-prone areas, and understanding the outcomes after fire is critical. Obligate seeders are particularly sensitive to changes to the fire regime (Keith 1996; Bowman *et al.* 2014). Results from our germination experiments indicate that it is not only fire frequency, often the usual focus of fire management plans, but also the season of fire which can have consequences for the persistence of physiologically dormant species like *A. buxifolia* because of the strong seasonal temperature requirement. Fire implemented in the late winter or spring period would potentially have a negative impact on *A. buxifolia*, due to the relative delay in emergence, however fires occurring naturally in summer or implemented in autumn would potentially reduce the relative delay of emergence. The majority of managed fires currently occur in cooler seasons to enable better control for safety purposes (McLoughlin 1998), but the timing within this prescribed fire season may have significantly different outcomes.

Ex situ conservation is an important platform for many endangered species across the world, with the goal of supporting the survival of species in the wild by having a back-up 'insurance policy' by storing their seeds (Cochrane *et al.* 2007). For physiologically dormant species in fire-prone regions such as *A. buxifolia,* germination can be complicated, and limit the utility of *ex situ* collections. A critical finding from this study is therefore identification that germination requires remarkably low winter season temperatures typical of the study region and a smoke cue, and that germination in the laboratory environment requires several weeks at such temperatures. This finding can be incorporated into germination protocols of *A. buxifolia ex situ* seed collections.

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Figure captions

Figure 1. Climate data from 1991-2017 from the Australian Government Bureau of Meteorology weather station at Mt. Boyce. Mean monthly minimum (–■–) and maximum (–◆–) temperatures represented by lines, and mean monthly rainfall is represented by bars.

Figure 2. The mean percentage germination of *Asterolasia buxifolia* seeds incubated at three different seasonal temperatures, using factorial applications of smoke and heat. Error bars represent 1 standard error.

Figure 3. The mean percentage germination (□), viable but not germinated (■), and inviable (■) *Asterolasia buxifolia* seeds incubated at the winter temperature cycle. All temperature treatments have smoke applied. Control treatment has smoke but no heat-shock treatment.

Figure 4. Germination rates over the duration of the experiment, represented by the average percentage germination plotted against time (weeks) for each of the heat shock treatment temperatures applied. The 120°C heat shock treatment was excluded because no germination was recorded. Error bars represent 1 standard error.

Figure 5. The average percentage germination of *Asterolasia buxifolia* seeds incubated at winter temperature cycles (11°C day 3°C night), with factorial applications of light and smoke. Error bars represent 1 standard error.