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Intra-population level variation in thresholds for physical dormancy-breaking temperature

Ganesha S. Liyanage* and Mark K. J. Ooi

*Centre for Sustainable Ecosystem Solutions, School of Biological Sciences, University
of Wollongong, Wollongong, NSW 2522, Australia*
* For correspondence. E-mail gslbl998@uowmail.edu.au

● **Background and Aims** Intra-population variation in seed dormancy is an advantage for population persistence in unpredictable environments. The important role played by physically dormant species in these habitats makes understanding the level of variation in their dormancy a key ecological question. Heat produced in the soil is the major dormancy-breaking stimulus and, in fire prone ecosystems, soil temperatures generated by fire may vary spatially and over time. While many studies have investigated variation in initial dormancy, a measure that is of little value in fire-prone ecosystems, where initial dormancy levels are uniformly high, intra-population variation in dormancy-breaking temperature thresholds has never been quantified. This study predicted that species would display variation in dormancy-breaking temperature thresholds within populations, and investigated whether this variation occurred between individual plants from the same maternal environment.
● **Methods** The intra-population variation in dormancy-breaking thresholds of five common physically dormant shrub species (family Fabaceae) from fire-prone vegetation in south-eastern Australia was assessed using heat treatments and germination trials. Replicate batches of seeds from each of four maternal plants of *Dillwynia floribunda*, *Viminaria juncea*, *Bossiaea heterophylla*, *Aotus ericoides* and *Acacia linifolia* were treated at 40, 60, 80, 100 and 120 °C.
● **Key Results** Dormancy-breaking response to heat treatments varied significantly among individual plants for all species, with some individuals able to germinate after heating at low temperatures and others restricting germination to temperatures that only occur as a result of high-severity fires. Germination rate (T_{50}) varied among individuals of three species.
● **Conclusions** Variation detected among individuals that were in close proximity to each other indicates that strong differences in dormancy-breaking temperature thresholds occur throughout the broader population. Differences found at the individual plant level could contribute to subsequent variation within the seed bank, providing a bet-hedging strategy, and represent a mechanism for increasing the probability of population persistence in the face of fire regime variability.

Key words: Seed germination, bet-hedging strategy, coexistence, dormancy-breaking thresholds, Fabaceae, fire, intra-population, germination, hard seeds, population persistence, seed bank.

INTRODUCTION

Within plant species, intra-population variation in seed dormancy has been described as an advantage for the survival of populations in unpredictable environments (Fenner, 1991; Beckstead *et al.*, 1996; Andersson and Milberg, 1998; Gutterman, 2000). Examples of such variation in seed dormancy and germination response have been presented primarily from arid or water-limited ecosystems (e.g. Quinlivan, 1971; Benech Arnold *et al.*, 1992; Baskin and Baskin 1998; Baloch *et al.*, 2001). However, fire-driven vegetation is one of the primary disturbance-prone environments in the world, where recruitment from seed is a critical life-history stage (Keeley, 1991; Bond and van Wilgen, 1996), yet very little is known about how dormancy, and more specifically the degree of dormancy, varies in these ecosystems (Ooi, 2007; Ooi *et al.*, 2014).

For physically dormant species, where germination is prevented by an impermeable seed coat, the majority of studies investigating variation in dormancy have focused primarily on measuring the proportion of dormant seeds that occurs among populations, although several studies have also looked within populations, including both between and within individual plants (e.g. Pérez-García, 1997; Norman *et al.*, 2002; Lacerda *et al.*, 2004; Masaka and Yamada, 2009). However, in fire-prone regions, dormancy of seed lots of such species usually exceeds 75 %, and can often be 100 % (e.g. Jeffery *et al.*, 1988; Auld and O'Connell, 1991; Herranz *et al.*, 1998; Ooi *et al.*, 2014). Assessing the proportion of dormant seeds produced in these habitats therefore provides little useful information when trying to determine variation among or within populations (Hudson *et al.*, 2015). Heat produced by the passage of fire provides the main dormancy-breaking cue and the levels of response of dormant seeds to different fire-related temperature treatments would provide a useful and much more relevant measure of dormancy variation in fire-prone flora (Auld and

O'Connell, 1991; Ooi *et al.*, 2009, 2012). The important role played by physically dormant species in these regions, where they are often the dominant component of the flora, as well as the increase in anthropogenic fire management (Ooi, 2007; Moreira and Pausas, 2012; Ooi *et al.*, 2014), makes understanding the variation in their germination response a key ecological question to be addressed.

Maintaining variation in seed germination response in fire-prone regions is important because of the inherent variability of heat produced by fire in the soil (Auld, 1986; Bradstock *et al.*, 1992; Govender *et al.*, 2006; Penman and Towerton, 2008; Ruprecht *et al.*, 2013). Soil temperatures during any particular fire incident can vary over space due to available fuel moisture and distribution of fuel load, and by the subsequent varying fire intensity produced (Govender *et al.*, 2006). Additionally, fire-induced heating of the soil decreases with depth (Auld, 1986). As a result, species would ideally need to maintain seed germination strategies that allow some portion of seeds to germinate under either high or low soil temperatures. This would ensure that at least some seedling emergence, and potentially recruitment, will occur in response to naturally variable components of the fire regime. For physically dormant species in fire-prone regions, it is likely that temperature thresholds have been shaped in response to the selective pressure of fire (Keeley *et al.*, 2011), and that variation in dormancy breaking temperature thresholds at both the inter- and the intra-specific scale is a key driver of population persistence and community coexistence (Ooi *et al.*, 2014).

Variation in the degree of dormancy within a population can provide species with a mechanism to overcome the risk of failed recruitment. In fire-prone regions, two consecutive fires within a short period of time can negatively affect population persistence because the second fire kills the seedlings produced after the first fire before they reach maturity and contribute to replenishment of the seed bank (Whelan, 1995; Odion and Tyler, 2002). Germination variability within a population would ensure that germination of some portion of seeds from the seed bank could occur after a single fire, while a portion remains in the seed bank and is able to germinate after subsequent fires (Baskin and Baskin, 1998; Ayre *et al.*, 2009; Ooi *et al.*, 2014). Variation in the dormancy loss provides a bet-hedging mechanism to buffer populations against such losses (Thanos and Georghiou, 1988; Gutterman, 2000; Ooi *et al.*, 2009). Furthermore, differences found in dormancy and germination responses within and between individuals, especially where maternal plants have grown in similar microclimates and the seeds produced during similar time periods, may suggest that it is not only parent plant environmental effects but also genetic factors that can play a role in driving variation both within and between plants (Baloch *et al.*, 2001; Donohue *et al.*, 2005; Narbona *et al.*, 2006).

Several studies have identified variation in dormancy-breaking thresholds between populations by seeds subjected to the same heat treatments (Auld and O'Connell, 1991; Ooi *et al.*, 2012), but variation among individuals within the same population, using a range of temperatures to ascertain temperature thresholds required to break dormancy, has not previously been the subject of any study. In this study, we predicted that species would display a range of fire-related dormancy-breaking temperature thresholds within populations, which can result from seeds produced by different neighbouring individual plants. We

also set out to investigate whether the range of variation in dormancy-breaking requirements among individuals differed between species. This would allow us to predict whether some species would have a greater ability to respond to variation in dormancy-breaking cues than other species, and therefore potentially have a greater ability for population persistence in unpredictable environments under both current conditions and future climatic conditions. More specifically, we addressed the following main questions: (1) Do seed characteristics, including weight, initial viability, initial dormancy, dormancy-breaking temperature thresholds and germination rate, vary among individuals within populations in physically dormant species from fire-prone environments? (2) Does the level of dormancy broken differ between individuals in response to the same temperature treatment? (3) Do some species maintain greater variation in dormancy-breaking thresholds among individuals than others, and how could this affect their ability to persist?

METHODS

Study species and site

We selected a group of common species from coastal fire-prone heath and woodland to gain both an understanding of variation in dormancy between individuals within each species, and how this variation differed among the species themselves. The five species studied were all from the Fabaceae: *Dillwynia floribunda*, *Viminaria juncea*, *Bossiaea heterophylla*, *Aotus ericoides* and *Acacia linifolia*. All species are native perennial shrubs from south-eastern Australia, which have physical dormancy (Ooi *et al.*, 2014). The physically dormant Fabaceae family is a major component of the understorey vegetation in Australia (Auld, 1986). The majority of adult plants of *D. floribunda*, *A. linifolia* and *V. juncea* are killed by fire, while *B. heterophylla* and *A. ericoides* adults are often killed by fire but maintain some capacity to resprout (Auld and O'Connell, 1991; Kubiak, 2009). The study sites were located in fire-prone vegetation in the Heathcote (34°07′ S, 150°58′ E) and Royal (34°03′ S, 151°03′ E) national parks, both at the southern end of the Sydney sandstone basin in south-eastern Australia. All species produce mature fruits between October and January.

Seed collection

Mature fruits were collected from four individual plants for each species within around 10 m proximity, with seeds of each individual kept in separate seed lots throughout the experiments. *Viminaria juncea*, *B. heterophylla*, *A. ericoides* and *A. linifolia* fruits were collected between October and November 2013, while *D. floribunda* fruits were collected in October 2012. The collected fruits were opened and seeds were extracted in the laboratory. Healthy and intact seeds were identified visually and stored in sealed paper bags at room temperature until temperature treatments were commenced in December of the same year for each seed collection.

Initial seed weight, viability and dormancy levels for individual plants

To compare initial seed characteristics among individuals within each species, seed mass, viability and dormancy level

were assessed within 30 d of seed collection. Fifty seeds from each individual plant were weighed individually to the nearest 0·01 mg and mean seed weight was calculated. Three replicates of 15–20 seeds were placed on moistened filter paper in 9 cm Petri dishes and incubated at 25/18 °C temperature and 12/12 h light/dark conditions. During incubation, Petri dishes were sealed with plastic to prevent water loss and filter paper was remoistened whenever necessary. Seeds were checked every second day for germination, which was scored on radicle emergence. After 6 weeks of observation, the seeds that did not germinate were checked for viability. Imbibed seeds were tested for viability by pressing them gently using forceps, while the remaining hard seeds were manually scarified using a scalpel and allowed to germinate in the same incubation conditions to determine the total viable seed number. The number of seeds that remained ungerminated prior to scarification was used to determine the dormancy level for each individual.

Dormancy-breaking temperature thresholds of seeds from individual plants

Temperature treatments ranging from 40 to 120 °C were applied for 10 minutes to determine the dormancy-breaking temperature thresholds for individual plants from each species. These treatments represent the soil temperatures and durations that commonly occur in these and other fire-prone habitats (Auld, 1986; Bradstock *et al.*, 1992; Penman and Towerton, 2008; Ooi *et al.*, 2014). Lower temperatures are indicative of cooler fires with low amounts of fine leaf litter (Bradstock and Auld, 1995), while higher temperatures can result from higher-severity fires. Four sets of three replicates of between 15 and 20 seeds were treated for each individual plant for *V. juncea*, *A. ericoides* and *A. linifolia* at 40, 60, 80 and 100 °C in an oven for 10 min. Seeds of individuals from *D. floribunda* and *B. heterophylla* were treated at 40, 60 and 80 °C only, due to limited seed numbers. Sufficient seeds for *V. juncea* and *A. linifolia* allowed an additional treatment of 120 °C to be applied. Following the heat treatments, seeds were allowed to cool and then placed on moistened filter papers in 9-cm Petri dishes. Seeds were incubated at 25/18 °C and light/dark conditions on a 12/12-h cycle. Three replicates of untreated seeds for each individual were included as the control. Germination and viability were assessed following the same procedure as for the initial viability test.

To calculate the mean germination percentages for each individual, we divided the number of germinated seeds by the corrected total viable seed number determined after each treatment, for each replicate. However, when mortality was >90 % as a result of the experimental treatments, data were excluded due to insufficient surviving numbers of seeds. The mean percentage mortality was calculated for each individual for each temperature treatment. The number of days to reach 50 % of the final seed germination (T_{50}) was calculated after the 80 °C treatment, which is the temperature found to produce the most germination across species (Auld and O'Connell, 1991; Ooi *et al.*, 2014). To calculate T_{50}, cumulative germination was plotted against the number of days for each replicate and a polynomial model fitted to the data. The time taken to reach 50 % of the final germination was calculated from the

regression equation and the mean T_{50} taken from the three replicates. Note that the T_{50} values were calculated at 100 °C for *V. juncea* individuals due to insufficient germination after the 80 °C treatment. Finally, we also calculated the minimum temperatures required to achieve 20 % (G_{20}), 50 % (G_{50}) and maximum (G_{max}) germination of viable seeds for each individual, and compared the level of variation in germination response between species using the coefficient of variation (CV). The CV was calculated using germination data recorded from the maximum temperature tested for each species, except *A. linifolia*, where we used data from the 80 °C treatment due to high seed mortality at 100 °C.

Data analysis

Generalized linear models with a binomial error structure and logit link function were used to compare final germination percentages of individuals of each species at each temperature separately. When the final germination proportions among individuals were significant, Tukey's test was used for multiple comparisons (Hothorn *et al.*, 2008). The same analyses were applied to final mortality and viability for each individual. A generalized linear model with a Poisson error structure and log link function were used to compare seed mass and T_{50} at 80 °C among individuals, followed by Tukey's test when differences were significant. All graphs were plotted using untransformed data. All analyses were conduct using the R statistical platform (R Core Development Team, 2014).

RESULTS

Initial seed weight, viability and dormancy levels for individual plants

The seed characteristics tested did not vary significantly among individual plants for any of the study species, except for mean seed weight. Dormancy levels and initial viabilities were close to 100 % for all individual plants except *A. linifolia*, which displayed considerable variation in dormancy level, and *B. heterophylla* and *D. floribunda*, which displayed variation in viability (Table 1). Seeds of one *A. linifolia* individual plant displayed an almost 65 % non-dormant seed portion. Mean seed weights differed significantly among individuals for all the tested species (Table 1), and varied the most among individuals of *A. linifolia* and the least for *B. heterophylla*, with the percentage difference between the lowest and highest individuals calculated as 48·2 and 15·5 % for *A. linifolia* and *B. heterophylla*, respectively.

Dormancy-breaking temperature thresholds of seeds from individual plants

All species produced different levels of germination among individuals in response to one or several temperature treatments after 6 weeks of incubation (Fig. 1), meaning that dormancy-breaking thresholds differed between individuals. Germination significantly increased above that of the control after heat treatments for all individuals within all species except for *A. linifolia* (Fig. 1A). In contrast to the germination responses of the

TABLE 1. *Mean seed weight (W), initial viability (V), initial dormancy (D), time taken to 50 % seed germination (T_{50}) and temperatures required for 20 % (G_{20}), 50 % (G_{50}) and maximum (G_{max}) germination within the temperature ranges used for individuals of all studied species. The dash (–) indicates insufficient germinations to represent temperatures. Different letters between individuals within each species section indicate significant differences between means*

Species	Individual	W (mg)	V (%)	D (%)	T_{50} (days)	G_{20} (°C)	G_{50} (°C)	G_{max} (°C)
Acacia linifolia	Individual 1	26·88[a]	93·15[a]	70·73[a]	16·66 ± 2·39[a]	Control	–	40
	Individual 2	35·58[b]	100[a]	60·0[ab]	42·13 ± 13·36[b]	Control	–	Control
	Individual 3	31·46[c]	100[a]	61·67[ab]	18·56 ± 0·6[a]	Control	100	100
	Individual 4	39·84[d]	100[a]	35·65[b]	21·38 ± 2·54[a]	Control	Control	80
Viminaria juncea	Individual 1	7·09[a]	100[a]	93·33[a]	10·83 ± 1·52[a]	100	100	100
	Individual 2	5·89[b]	100[a]	95·0[a]	11·74 ± 0·93[a]	100	100	100
	Individual 3	5·92[b]	100[a]	98·33[a]	12·75 ± 0·98[a]	80	100	100
	Individual 4	5·36[c]	100[a]	86·67[a]	11·12 ± 0·94[a]	80	100	100
Aotus ericoides	Individual 1	4·20[a]	95·56[a]	97·62[a]	6·83 ± 0·20[a]	60	60	100
	Individual 2	4·94[b]	95·24[a]	80·20[a]	10·11 ± 1·23[b]	40	60	100
	Individual 3	4·05[a]	100[a]	97·78[a]	9·50 ± 0·86[b]	60	60	100
	Individual 4	4·23[a]	100[a]	100[a]	5·88 ± 0·20[a]	60	60	100
Dillwynia floribunda	Individual 1	1·54[a]	48[a]	100[a]	15·42 ± 0·37[a]	60	60	80
	Individual 2	1·89[b]	78[b]	94·12[a]	27·29 ± 3·04[b]	60	80	80
	Individual 3	2·10[cd]	87[bc]	90·68[a]	20·48 ± 1·22[c]	60	80	80
	Individual 4	1·96[bd]	98·3[dc]	100[a]	18·80 ± 1·31[ac]	60	60	80
Bossiaea heterophylla	Individual 1	14·64[a]	72·22[a]	82·23[a]	6·70 ± 1·27[a]	40	–	80
	Individual 2	15·57[a]	85·93[a]	83·07[a]	5·86 ± 0·45[a]	60	60	60
	Individual 3	14·73[a]	65·08[a]	86·60[a]	8·96 ± 1·62[a]	60	–	60
	Individual 4	16·91[b]	100[a]	86·67[a]	11·73 ± 3·74[a]	–	–	80

other species tested, three *A. linifolia* individuals did not show heat-dependent germination (Fig. 1A). Non-dormancy produced high levels of germination in the control seeds, and a lack of germination after heat treatments was only partially explained by treatment-induced mortality (Fig. 2A). Only Individual 3 of *A. linifolia* produced a notably high germination response after heating compared with the control, with an increase in germination of ~59 % after the 100 °C treatment. The highest heat treatment (120 °C) caused 100 % mortality for all *A. linifolia* individuals. Three of the individuals treated at 100 °C suffered >90 % mortality, whilst the remaining Individual 3 produced its highest germination (97 %). There was also large variation between *A. linifolia* individuals in T_{50} values. Individual 2 took almost 3 weeks longer than the other three individuals to reach 50 % of its final maximum germination (Table 1).

Germination response of *V. juncea* at lower temperatures was relatively small (<20 % at 40 and 60 °C). Nevertheless, seeds from individuals displayed two broadly different response patterns. Seed germination percentages of Individuals 3 and 4 gradually increased with increasing temperature treatments, whereas Individuals 1 and 2 showed a sudden increase in germination (100 %) after the 100 °C treatment, compared with low (<10 %) germination after lower temperature treatments (Fig. 1B). This showed a strong high-temperature threshold requirement to break dormancy and therefore a strong bond to fire-related dormancy-breaking cues for all individuals; this was particularly strong for Individuals 1 and 2. Increasing mortality was initiated at the 100 °C treatment for all the individuals of *V. juncea*.(Fig. 2B). For all the individuals of *V. juncea*, the 120 °C treatment caused 100 % seed mortality.

Germination percentages for all *A. ericoides* individuals gradually increased with increasing temperature treatments (Fig. 1C), although Individual 2 significantly increased

germination to 40 % after the low-temperature 40 °C treatment, while the other three individuals needed a 60 °C treatment for a significant germination increase. Maximum germination of the seeds from the latter three individuals was clearly reached at 100 °C, whereas the peak germination produced by Individual 2 did not differ significantly for seeds treated at 60, 80 or 100 °C. Mortality for all individuals gradually increased with increasing temperature treatments (Fig. 2C). The T_{50} value was significantly slower only for Individuals 1 and 4 compared with Individuals 2 and 3 (Table 1).

The seeds of individuals of *D. floribunda* showed a gradual increase in germination with increasing temperature treatment, except for Individual 2, which did not significantly increase germination after the 60 °C treatment. Almost half (48 %) of Individual 2's viable seed lot remained dormant after treatment at 80 °C (Fig. 1D). Even though mortality differed significantly among individuals for each of the temperature treatments, there was no trend of increased mortality with increasing temperature for any of the four individuals (Fig. 2D). The T_{50} values varied among all individuals at 80 °C, and Individual 2 took ~1 week or longer to germinate than other three (Table 1).

For *B. heterophylla*, germination was highly variable among individuals across all temperature treatments (Fig. 1E). Individual 1 displayed a general increase in germination response with increasing temperature (albeit with a decline at the 60 °C treatment), peaking after the 80 °C treatment, the highest temperature used for this species. Individuals 2 and 3 reached their maximum germination after treatment at 60 °C, with Individual 2 showing a particularly strong response, reaching 80 % germination (significantly greater than any other treatment). The subsequent decline in germination after the 80 °C treatment was not caused by increasing mortality (Fig. 2E). Individuals 3 and 4 both maintained a high threshold temperature requirement (>80 °C) to break dormancy, with >66 and
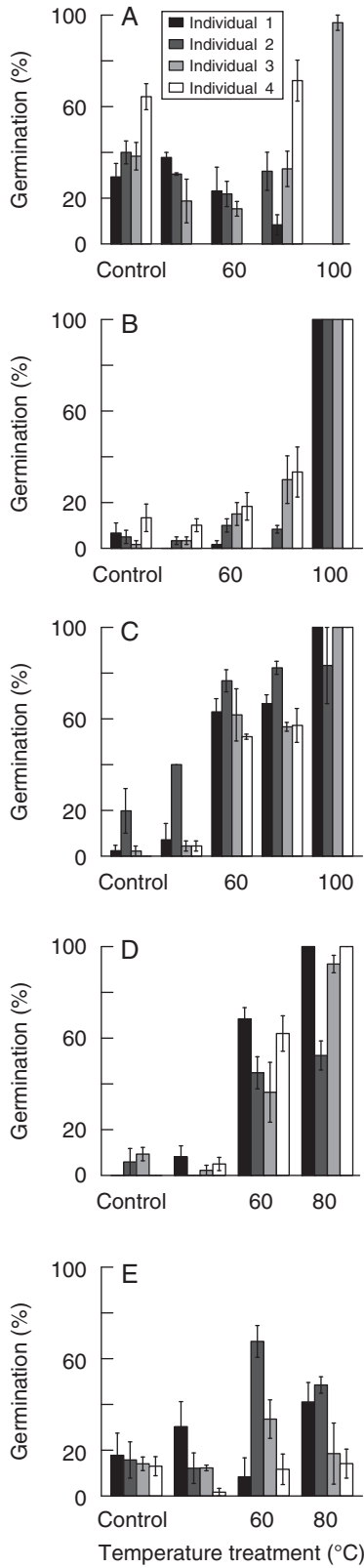
Fig. 1. Mean (± s.e.) germination percentages of seeds from four individuals after different temperature treatments. (A) *Acacia linifolia*; (B) *Viminaria juncea*; (C) *Aotus ericoides*; (D) *Dillwynia floribunda*; (E) *Bossiaea heterophylla*.
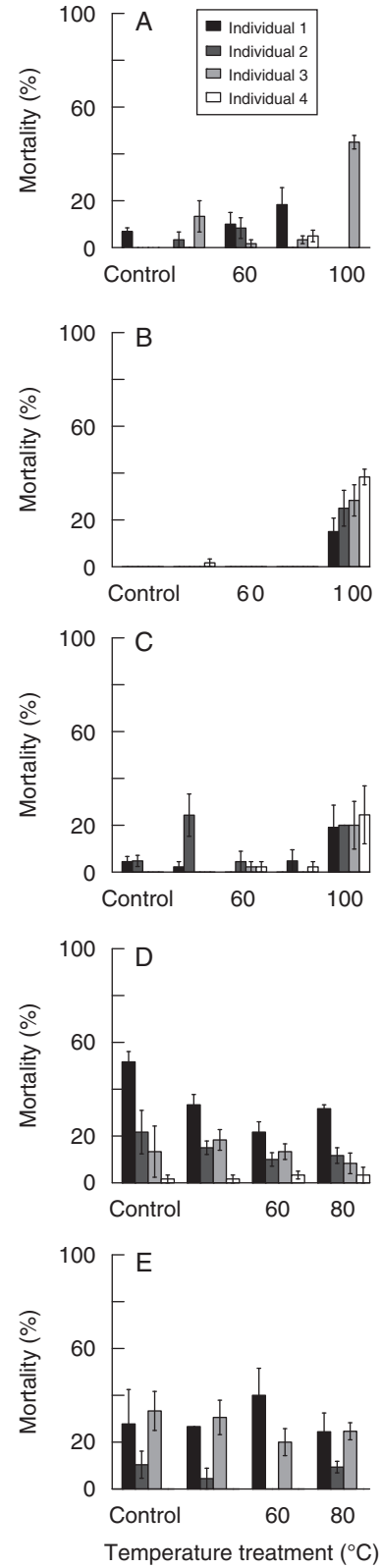
Fig. 2. Mean (± s.e.) mortality percentages of the seeds from four individuals after different temperature treatments. (A) *Acacia linifolia*; (B) *Viminaria juncea*; (C) *Aotus ericoides*; (D) *Dillwynia floribunda*; (E) *Bossiaea heterophylla*.

88 % of their viable seed lots, respectively, remaining dormant after the 80 °C treatment.

Overall comparison between species showed that the dormancy-breaking threshold variation was relatively high for all species except *V. juncea*. All four *V. juncea* individuals reached 50 % (G_{50}) or maximum (G_{max}) seed germination at the same temperature treatment of 100 °C (Table 1), with no individual producing germination >30 % at lower temperatures. This is reflected by the CV values, measuring the level of variability in germination response between species, where *V. juncea* had the lowest CV. Ranking of species by CV showed *A. linifolia* (CV = 0·69) > *B. heterophylla* (CV = 0·58) > *D. floribunda* (CV = 0·26) > *A. ericoides* (CV = 0·08) > *V. juncea* (CV = 0).

DISCUSSION

Variation in dormancy-breaking temperature thresholds is rarely studied within populations but can contribute to our understanding of the strategies involved in plant population dynamics (Ooi *et al.*, 2014). In this study, we found highly significant variation in dormancy-breaking temperature thresholds among individual plants for all five of the study species from fire-prone vegetation, while T_{50} for most of the species also varied. This variation is likely to occur throughout the broader populations of the species studied because strong differences were detectable, even among four individuals that were in relatively close proximity to each other. These findings help to explain how differences in dormancy and germination characteristics at the individual plant level can contribute to subsequent variation within the seed bank. This finding is novel, highlighting a level of variation that cannot be obtained from studies focused on dormancy variation among species or populations (e.g. Auld and O'Connell, 1991; Ooi *et al.*, 2012). This study has implications both for understanding the strategies that species have developed for population persistence and for how well species may respond to variation in fire severity, a key aspect of the fire regime, which is subject to change as a result of increased management and climatic changes (Ooi *et al.*, 2014).

The type of variation usually considered in germination strategies, allowing species to regenerate in a range of conditions to overcome the unpredictability of disturbances, is the difference in initial levels of dormancy of the seed lot (Cohen, 1966; Philippi and Seger, 1989; Philippi, 1993; Venable, 2007). However, variation of initial dormancy among individuals is not characteristic of our study species, and indeed is not characteristic of many species from fire-prone environments generally (Jeffery *et al.*, 1988; Auld and O'Connell, 1991; Herranz *et al.*, 1998; Ooi *et al.*, 2014). Instead of having variation in initial dormancy, it is clear that these species maintain variation in germination within a population by having different dormancy-breaking temperature thresholds. Differences in seed dormancy thresholds between species have been proposed as a mechanism by which species co-exist (Trabaud and Oustric, 1989; Moreno and Oechel, 1991; Herranz *et al.*, 1998; Ooi *et al.*, 2014). We suggest that intra-population variation in dormancy-breaking threshold temperature is also a mechanism for ensuring population persistence, a strategy likely to be maintained by many species from fire-prone environments.

The variation in dormancy-breaking temperature thresholds within a population can be considered as a form of bet-hedging, although rather than betting against failed recruitment it provides insurance against variability in soil heating. For example, seeds entering the seed bank from plants with a low temperature dormancy-breaking threshold ensure that a portion of seeds germinate, even after a low-severity fire (which potentially produces low levels of soil heating), while the remaining portion of higher temperature-threshold seeds can contribute to a residual soil seed bank. Seeds are then available to germinate after subsequent fires, and supplement seeds produced by newly recruited individuals.

Variation of the fire regime can affect recruitment within a population due to the related variability of the resulting dormancy-breaking or germination cues (Thanos and Georghiou, 1988; Whelan, 1995). For example, variation in the level of heat produced in the soil depends on the fuel type and decreases with soil depth (Bradstock *et al.*, 1992; Auld and Bradstock, 1996). Maintenance of only a single dormancy-breaking threshold for any species within a population would increase risk, particularly for obligate seeders, by limiting the capacity to produce a sufficient germination response for subsequent regeneration. However, having dormancy-breaking temperature thresholds ranging from low to high within a population ensures that a portion of seeds can respond, even with variation in fire-related cues. In our study, all species except *V. juncea* displayed a significant germination response to a range of dormancy-breaking temperatures. *Viminaria juncea* only responded to very high temperature treatments, suggesting it would have the least ability to recruit in the absence of high-severity fires (and subsequent high soil temperatures) or cope with a series of low-severity burns, unless seeds undergo a change in dormancy-breaking thresholds over time. Species that maintain similar dormancy profiles may be more susceptible to decline as a result of variation in the current fire regime, particularly to an increase in low-intensity fires. Identifying such species would ensure that increasing levels of fire management, often consisting of low-severity controlled burns with corresponding low soil temperatures (Penman and Towerton, 2008), do not have a negative impact on recruitment and subsequent biodiversity.

Another key finding from our study is the level of response shown by some individuals at relatively low dormancy-breaking temperatures. Individuals with high dormancy-breaking temperature thresholds (>80 °C) show a strong pyrogenic dormancy-breaking relationship, because such temperatures occur only during the passage of fire in these environments (Auld and Bradstock, 1996; Penman and Towerton 2008; Ooi *et al.*, 2014). However, while lower temperatures of 40 or 60 °C regularly occur during a fire, they can also occur in vegetation gaps during hot months in the inter-fire period (Santana *et al.*, 2013). Individuals with lower dormancy-breaking temperature thresholds can therefore potentially respond to vegetation gaps, providing an opportunity for plant regeneration both after fire and during the inter-fire period (Hanley *et al.*, 2003; Tavsanoglu and atav, 2012; Ooi *et al.*, 2012, 2014). While it is understood that the primary recruitment period occurs after fire, some inter-fire recruitment may contribute to persistence, particularly in long unburnt vegetation, and our results show that this could be related to differences in seeds produced at the individual plant level (Ooi *et al.*, 2012, 2014).

Germination rates varied significantly among individuals in our study, a strategy maintained by species as a form of temporal bet-hedging (Venable, 1989; Fenner and Thompson, 2005; Wang *et al*., 2009). Differences in germination rate spread germination over time, ensuring that some seeds can respond quickly to a rainfall event after dormancy has broken, while others require longer to initiate germination and therefore enable maintenance of a seed bank that can respond to subsequent rainfall events, even if the initial cohort has failed (Afan *et al*., 2013; Chamorro *et al*., 2013). While all individuals of some study species, such as *D. floribunda* and *A. ericoides*, showed similar patterns of higher germination at higher temperature treatments, variation in germination rates among individuals were maintained. This form of bet-hedging is rarely acknowledged in fire-prone environments, even though low levels of recruitment have been observed during 9 months after fire (e.g. Auld and Tozer, 1995).

Variation in germination rate is also a mechanism that can reduce intra-specific seedling competition (Venable, 1989; Fenner and Thompson, 2005). The flush of post-fire seedling emergence shows that conditions immediately after fire are favourable for seedling establishment (van Wilgen and Forsyth, 1992; Carrington and Keeley, 1999; Fenner and Thompson, 2005). However, competition caused by the high density of seedlings is a major reason for seedling mortality after disturbance (Taylor and Aarssen, 1989; Fenner and Thompson, 2005). The differences in germination rates that we found among individuals provides evidence for a mechanism contributing to successful seedling recruitment within a population after fire, by staggering the timing of emergence and reducing seedling–seedling competition.

Treatment-induced seed mortality may also enable a reduction of seedling–seedling competition, and potentially contribute to the persistence of a mixture of high- and low-threshold seeds within a population. For two of our study species, seeds produced from individuals with a low dormancy-breaking temperature threshold suffered greater mortality than their high-threshold counterparts. Individual plants that produce seeds with low thresholds have seeds that can germinate after low-severity fires or, potentially, within canopy gaps during the inter-fire period due to the relatively low soil temperatures generated under these conditions. On the other hand, high-severity fires generate soil temperatures that could promote germination of both low- and high-threshold seeds. This means that there would be an increase in intra-specific seedling competition after high-severity fires. However, the high temperature treatment-induced mortality of seeds from low-threshold *V. juncea* and *A. ericoides* individuals suggests that the level of intra-specific competition after high-severity fires can be offset by seed mortality. The inherent small-scale patchiness of many fires (Ooi *et al*., 2006; Penman and Towerton, 2008) would mean that areas within the same fire event could be dominated by individuals with either high- or low-threshold seeds, depending on the soil temperatures reached.

While it is clear that there is strong selective pressure for maintaining dormancy threshold variation within a plant population, or even within individuals, it is much less clear whether this variation is a result of genetic differences among individuals or of variation in the microclimatic environments that maternal plants experience during seed development. The impacts of environmental factors, including temperature, moisture, light level, nutrients and grazing, on flowering, seed development, dormancy and germination have been observed in many species from a wide range of environments (Taylor and Palmer, 1979; Lacey *et al*., 1997; Gutterman, 2000; Galloway, 2001; Norman *et al*., 2002; Bischoff and Müller-Schärer, 2010; Hernandez-Verdugo *et al*., 2010), highlighting that maternal temperature can influence seed dormancy, even within the same maternal plant. For example, a meta-analysis of such studies has identified a general trend that increasing maternal temperature decreases levels of (physiological) dormancy (Wood *et al*., 1980; Fenner, 1991; Fenner and Thompson, 2005), and that drought can produce seeds with thicker seed coats and increased dormancy in physically dormant seeds (Nooden *el al*., 1985; Fenner and Thompson, 2005). However, a number of studies have also shown variation in dormancy of seeds produced from plants grown under similar environmental conditions, suggesting that there could be a genetic influence in determining variation in seed and dormancy characters (Galloway, 2001; Donohue *et al*., 2005; Narbona *et al*., 2006; Bischoff and Müller-Schärer, 2010). The results from our study, where individuals grown under very similar maternal environment conditions produced seeds with different responses, suggest that genetic differentiation can, in part, play a role in determining the dormancy-breaking thresholds of physically dormant seeds. However, the relative influence of the mother plant environment and the mother plant genotype is yet to be fully understood.

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