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Do dormancy-breaking temperature thresholds change as seeds age in the soil seed bank?

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Do dormancy-breaking temperature thresholds change as seeds age in the soil seed bank?

Abstract

In fire-prone ecosystems, many species regenerate after fire from persistent soil seed banks. Species with physically dormant (PY) seeds have dormancy broken by fire-related heat. The magnitude of post-fire recruitment, to predict response to varying fire severity, is commonly estimated by testing dormancy-breaking temperature thresholds of fresh PY seeds. However, seeds spend years in the soil during the inter-fire period, and determining whether dormancy-breaking thresholds change over time is essential to accurately predict population persistence. Germination of four south-eastern Australian PY species from the Fabaceae family (Acacia linifolia, Aotus ericoides, Bossiaea heterophylla and Viminaria juncea) were studied. Dormancy-breaking temperature thresholds vary inter-specifically and the species represented either high or low dormancy-breaking threshold classes. Freshly collected seeds, and seeds that had been buried in the field or stored in dry laboratory conditions for 6 and 18 months were subjected to a fire-related range of heat treatments (40¿100°C). Seed ageing increased germination response to heat treatments, effectively lowering the dormancy-breaking thresholds of three species. The fourth species, A. linifolia, initially had a relatively large non-dormant fraction which was lost as seeds aged, with older seeds then displaying PY broadly similar to the other study species. Patterns of threshold decay were species-specific, with the thresholds and viability of low-threshold species declining more rapidly than high-threshold species. The non-dormant fraction did not increase over time for any of our study species. Instead of increasing their non-dormant fraction, as is common in other vegetation types, these fire-prone PY species displayed a change of dormancy-breaking temperature thresholds. This is an important distinction, as maintaining dormancy during the inter-fire period is essential for population persistence. While changes in sensitivity to dormancy-breaking treatments have previously been reported as seeds age, our study provides the first test of changes to temperature thresholds, which increases the range of germination response from the seed bank under varying fire severity.

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17   **Running title: Dormancy-breaking thresholds change with seed aging**

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24   **ABSTRACT**

25   In fire-prone ecosystems, many species regenerate after fire from persistent soil seed banks.

26   Species with physically dormant (PY) seeds have dormancy broken by fire-related heat. The

27   magnitude of post-fire recruitment, to predict response to varying fire severity, is commonly

28   estimated by testing dormancy-breaking temperature thresholds of fresh PY seeds. However,

29   seeds spend years in the soil during the inter-fire period, and determining whether dormancy-

30   breaking thresholds change over time is essential to accurately predict population persistence.

31   Germination of four south-eastern Australian PY species from the Fabaceae family (*Acacia*

32   *linifolia*, *Aotus ericoides*, *Bossiaea heterophylla* and *Viminaria juncea*) were studied. Dormancy-

33   breaking temperature thresholds vary inter-specifically and the species represented either high or

34   low dormancy-breaking threshold classes. Freshly collected seeds, and seeds that had been

35   buried in the field or stored in dry laboratory conditions for six and 18 months were subjected to

36   a fire-related range of heat treatments (40°C-100°C). Seed ageing increased germination

37   response to heat treatments, affectively lowering the dormancy-breaking thresholds of three

38   species. The fourth species, *A. linifolia*, initially had a relatively large non-dormant fraction

39   which was lost as seeds aged, with older seeds then displaying PY broadly similar to the other

40   study species. Patterns of threshold decay were species-specific, with the thresholds and viability

41   of low threshold species declining more rapidly than high threshold species. The non-dormant

42   fraction did not increase over time for any of our study species. Instead of increasing their non-

43   dormant fraction, as is common in other vegetation types, these fire-prone PY species displayed

44   a change of dormancy-breaking temperature thresholds. This is an important distinction, as

45   maintaining dormancy during the inter-fire period is essential for population persistence. While

46   changes in sensitivity to dormancy-breaking treatments have previously been reported as seeds

47   age, our study provides the first test of changes to temperature thresholds, which increases the

48   range of germination response from the seed bank under varying fire severity.

49

50   **Key words**: Dormancy-breaking temperatures, Fabaceae, fire, physical dormancy, seed aging,

51   seed storage, soil seed banks

52

53   **Introduction**

54   Physical dormancy (PY) is common in fire-prone ecosystems around the world, particularly

55   prevalent in the Fabaceae, Cistaceae, Malvaceae and Rhamnaceae (Ooi, 2007, Turner *et al*.,

56   2013; Baskin and Baskin, 2014), all of which form persistent soil seed banks. The impermeable

57   hard seed coat of PY seeds prevents gas and water exchange which inhibits their germination. In

58   fire-prone ecosystems, PY is broken by fire-related soil heating, with dormancy-breaking

59   temperatures ranging between 40°C and 120°C (Jeffrey *et al*., 1988; Keeley, 1991; Thanos *et al*.,

60   1992; Gonzále-Rabanal and Casal, 1995; Moreira *et al*., 2010; Ooi *et al*., 2014; Liyanage and

61   Ooi, 2015). Dormancy-breaking temperature thresholds maintained by physically dormant

62   species are often used to predict the potential level of post-fire response (e.g. Bradstock and

63   Auld, 1995; Williams *et al*., 2004; Santana *et al*., 2010; Ooi *et al*., 2014; Wright *et al*., 2015).

64

65   Fire-related dormancy-breaking temperature thresholds vary between PY species (Trabaud &

66   Oustric, 1989; Auld and O'Connell, 1991) and within populations of single species (Liyanage

67   and Ooi, 2015). It has been proposed that such variation has been selected for because of the

68   inherent variability of fire (Trabaud and Oustric, 1989; Ooi *et al*., 2014; Liyanage *et al*., 2016),

69   which results in variation in soil heating over the area burnt (Bradstock *et al*., 1992; Penman and

70   Towerton, 2008). Having different dormancy-breaking temperature thresholds among species

71   can therefore contribute to species coexistence, by distributing germination over space (Trabaud

72   and Oustric, 1989; Ooi *et al*., 2014). It has also been suggested that within-species variation can

73   operate as a bet-hedging mechanism, ensuring that at least some germination is possible across a

74   range of fire severities, and that PY thresholds are maintained by covarying with seedling

75   characteristics, which ensure that seedling performance matches the post-fire conditions

76   (Liyanage and Ooi, 2015; Liyanage *et al*., 2016). Dormancy-breaking threshold temperatures

77   have been used to estimate the potential magnitude of post-fire seedling establishment,

78   depending on the fire severity and/or the amount of soil heating that occurs (Wright *et al*., 2015).

79   Species with high dormancy-breaking temperature thresholds ($\geq 80°C$) require a relatively hot

80   fire to produce suitable temperatures for breaking dormancy in the soil, whereas species with low

81   thresholds may respond to lower severity fires or even soil heating generated during summer in

82   canopy gaps (Auld and Bradstock, 1996; Santana *et al*., 2010; 2013; Ooi *et al*., 2014; Liyanage

83   and Ooi, 2015).

84

85   Predicting the germination response of species which form persistent seed banks is made

86   complicated by the changes that potentially occur within the soil or over time. In most studies

87   investigating fire-related dormancy-breaking thresholds of PY species, freshly collected seeds

88   are used (e.g. Jeffrey *et al*., 1988; Trabaud and Oustric, 1989; Auld and O'Connell, 1991;

89   Keeley, 1991; Moreira *et al*., 2010; Ooi *et al*., 2012; Liyanage and Ooi, 2015). However, during

90   burial, daily and seasonal temperature fluctuations cause physiological changes to seeds and

91   physical deterioration of seed structures, which could change their dormancy and germination

92   characteristics compared to those of fresh seeds, thereby changing the way they respond to fire-

93   related dormancy-breaking cues (van Staden *et al*., 1994; Schatral 1996; Roche *et al*., 1997;

94   Zeng *et al*., 2005).

95

96   Alteration of dormancy-breaking responses in stored seeds has been identified for a number of

97   physiologically dormant species (e.g. Roche *et al.*, 1997; Tieu *et al.*, 2001; Baker *et al*., 2005;

98   Turner *et al*., 2013), with the proportion of seeds that respond to smoke cues (but also to heat for

99   some species) generally reported to increase after storage in laboratory or field conditions,

100   presumably due to dry after-ripening. For PY species, changes to the dormant fraction with

101   storage and a general pattern of increased germination with longer storage have been reported

102   (e.g. Morrison *et al*., 1992; Van Assche and Vandelook, 2006; Galíndez *et al*., 2010; Orscheg

103   and Enright, 2011; Hudson *et al*., 2015). However, in fire-prone systems, variation in the non-

104   dormant fraction is usually small for PY species, and of little value for predicting post-fire

105   response (Ooi *et al*., 2012, 2014). While there are other examples of studies of PY species, where

106   the effects of storage on germination response to a single 'heat' treatment (representing fire) has

107   been tested (e.g. Baker *et al*., 2005; Turner *et al*., 2013), no studies have investigated the effects

108   of storage on dormancy-breaking temperature thresholds. These thresholds are the key seed-

109   related characteristic that contributes to determining recruitment for PY species, and

110   understanding such changes are therefore important for robustly predicting population

111   persistence.

112

113   In this study, we investigated the effects of seed ageing on dormancy-breaking temperature

114   thresholds of four PY species from south eastern Australia, all within the family Fabaceae. Two

115   of the species are known to have a high temperature threshold for breaking dormancy (*Acacia*

116 *linifolia* and *Viminaria juncea*) while the other two have low temperature thresholds (*Aotus*

117 *ericoides* and *Bossiaea heterophylla*). Physical dormancy is represented in around 45% of

118 dormant shrub species in this region (Ooi, 2007), and previous work suggests that some species

119 within the physically dormant Fabaceae family display lower levels of initial dormancy after dry

120 storage (Morrison *et al*., 1992). Additionally, other studies have shown that the germination

121 response of fresh seeds of a number of species is negligible, even at the highest soil temperatures

122 likely to occur during fire (e.g. Auld and O'Connell, 1991; Ooi *et al*., 2014; Liyanage and Ooi,

123 2015). These findings suggest that changes in dormancy over time are not only likely, but for

124 some species are essential if a germination response is to occur. We therefore hypothesise that

125 dormancy-breaking temperature thresholds of PY species change with time within the seed bank,

126 and that both the storage conditions and duration can affect the magnitude of change. More

127 specifically, we addressed the following questions:

128

129 (i) Do dormancy-breaking temperature thresholds change as seeds age in the soil seed bank?

130 Similarly, do initial levels of dormancy also change?

131 (ii) Is there an interaction between the effects of aging and type of storage condition?

132 (iii) Does the changing pattern of dormancy-breaking temperature thresholds vary among

133 species? Particularly, do high threshold species maintain the requirement for high temperatures

134 over time?

135 (iv) How can the changes observed potentially affect recruitment and population persistence?

136

137 **Methods**

138 ***Study species and region***

139 The dormancy-breaking temperature thresholds of four common native shrub species from the

140 fire-prone sclerophyll vegetation of the Sydney region (Royal 34°03′ S, 151°03′ E and Heathcote

141 34°07′ S, 150°58′ E National Parks) in south eastern Australia were examined to assess their

142 change in dormancy response with ageing. Rainfall in the study region is aseasonal, with

143 approximately 1100 mm falling annually and peak monthly means occurring in January, March

144 and June. Average monthly maximum/minimum temperatures are 26/18 °C and 16/8 °C in

145 summer and winter respectively (Australian Government Bureau of Meteorology, 2016). All

146 study species occur within the Fabaceae family and produce physically dormant seeds (PY).

147 Seeds were collected from 15-20 randomly selected mother plants of *A. linifolia, B. heterophylla*,

148 *A. ericoides* and *V. juncea*, from single populations during the summer of 2013 (November –

149 December). Among these species, previous work by Liyanage and Ooi (2015) has shown that

150 freshly collected dormant seeds of *A. linifolia* and *V. juncea* had high dormancy-breaking

151 temperature thresholds, requiring at least a 100 °C treatment to reach 50% germination. *Bossiaea*

152 *heterophylla* and *A. ericoides* represented low dormancy-breaking temperature threshold species,

153 requiring a 60 °C treatment or less to reach 50% germination. The temperature treatment ranges

154 applied for this study were therefore relevant to each threshold group.

155

156 *Dormancy assessment of fresh seeds*

157 A proportion of the collected seeds were used to assess initial dormancy-breaking temperature

158 thresholds. Dry oven 10 minute temperature treatments of 40, 60, 80 and 100 ºC were applied to

159 three replicates of 15-20 fresh seeds (depending on seed availability) for the high threshold

160 species *V. juncea* and *A. linifolia.* For the low threshold species *B. heterophylla,* and *A. ericoides*,

161 40 and 60 °C treatments only were applied, as these were high enough to promote maximum

162   germination. Treatment levels were based on the range of temperatures experienced in the soil

163   during fire (Ooi *et al*., 2014). Temperature treated seeds were allowed to cool and then placed on

164   moistened filter paper in replicate petri dishes to germinate under a 25/18 °C and 12/12 hour

165   light/dark regime in a temperature-controlled incubator. This temperature regime was used to

166   mimic summer mean maximum and minimum temperatures. Summer is the time that most

167   natural fires occur (McLoughlin 1998), and seeds that have their dormancy broken during fire

168   respond to the next rainfall event, which in this region is most likely to occur in summer due to

169   an aseasonal rainfall pattern. Three untreated replicates were used as the control for each species.

170   Germination was recorded for six weeks at two day intervals and scored on emergence of the

171   radicle. To assess viability at the end of each germination trial, ungerminated seeds were

172   scarified and placed back in the incubator for up to four week. All seeds had imbibed by this

173   point and had either germinated or become soft and mushy. Seeds germinating after scarification

174   represented those that had remained dormant from the treatment but were still viable. Viability of

175   each replicate was calculated as the total number of seeds germinating before and after

176   scarification.

177

178   ***Seed ageing and dormancy assessment***

179   The remaining seeds from each species were divided into two sets, with one used for a field

180   burial trial and the other for dry storage in laboratory conditions. For the burial treatment, seeds

181   from each species were equally divided into sand-filled nylon mesh bags (10 x 20 cm) with a

182   mesh size of 2 mm. For the smaller seeded species *A. ericoides* and *V. juncea*, bags containing

183   50 seeds each were used, while for the larger seeded *A. linifolia* and *B. heterophylla* each bag

184   contained 25 seeds (Table 1). This ensured that the seed to sand proportions were similar across

185   species. In December 2013, the bags were buried within the top 2 cm of the soil profile at the

186   same field sites where seeds of each species were originally collected from. The bags were

187   randomly assigned to one of three plots within each site, to account for within-site spatial

188   variation, and their locations recorded with a GPS for later retrieval. After six and 18 months, a

189   minimum of three bags for each species were retrieved and air dried. Intact seeds were extracted

190   from the sand by sieving. The number of seeds damaged during burial were recorded and seeds

191   from each plot then pooled to randomise any microclimatic effects. Heat treatments were then

192   applied as described above, with the same range of temperatures and the same conditions for

193   germination that were used for fresh seeds. For the laboratory-stored seeds, replicates were

194   placed in paper bags and stored at ambient laboratory conditions (~20 to 23 °C) prior to use in

195   germination trials after six and 18 months.

196

197   *Analysis*

198   The percentages for initial viability (based on the number of viable seeds in the controls),

199   mortality (the percentage of seeds killed) at each temperature treatment, as well as germination

200   and the non-dormant fraction were calculated for fresh, field-stored and laboratory-stored seeds

201   for each retrieval period. The non-dormant fraction over time was estimated using the number of

202   viable seeds germinating in the untreated controls at 0, 6 and 18 months. Before each calculation,

203   viable seed number per replicate was corrected using the mean viability of the control replicates.

204   Data were analysed using Generalised Linear Models (GLMs) with a binomial error structure

205   and logit link function for each species separately. Analyses were conducted using the R

206   statistical platform (R Core Development Team, 2014).

207

208 *Germination response*

209 To analyse germination data, storage time, type of storage (soil or laboratory) and temperature

210 treatments were assigned as the predictor variables. We used model selection and determined the

211 best fitting model from all possible subsets of three predictor variables using Akaike's

212 Information Criterion (AIC) (Akaike, 1973).

213

214 Based on germination results from the heating experiments, the lowest mean temperatures

215 required to produce at least 20% and 50% germination of initially dormant seeds were calculated

216 ($G_{20\%}$ and $G_{50\%}$). A minimum of 20% increased seed germination is considered to be a high

217 enough response to produce a noticeable flush of seedling emergence in the post-fire

218 environment (Ooi *et al*., 2014). 'Obligate pyrogenic dormancy class' species (Ooi *et al*., 2014)

219 are defined as high threshold species, and require at least an 80°C heat shock to reach 20%

220 germination. We therefore used the $G_{20\%}$ index to identify whether dormancy-breaking

221 temperature thresholds changed during storage in the soil. A lowering of the $G_{20\%}$ index would

222 indicate that thresholds for producing a post-fire germination response were being reduced as the

223 seed bank aged.

224

225 *Decay pattern of high threshold seeds*

226 Both low and high threshold species have at least some proportion of seeds at the dispersal stage

227 that have high dormancy-breaking thresholds. To identify how quickly the seed banks reduce

228 from high to low threshold, we calculated the half-life of the high threshold fraction for each

229 species. This was done by plotting the percentage of seeds germinating at the next treatment

230 temperature below 80°C for each replicate tray (i.e. the 60°C response) against time (duration of

231 burial). The decay rate was then estimated by fitting exponential curves to each plot. The half-

232 life of the initial high dormancy-breaking threshold seed fraction was then calculated from the

233 exponential equation,

234 $$y = ae^{-bt}$$

235 where, *a* is the initial high dormancy-breaking threshold seed percentage, *b* is the decay rate and

236 *y* is the percentage of high dormancy-breaking threshold seeds remain in the soil seed bank at

237 time *t* (Auld *et al*, 2000). Replicates of some species showed a total loss of the high threshold

238 seed fraction after the first retrieval (6 months), and in this case a linear regression was used to

239 estimate the decay rate. For both model types, half-life was calculated by solving the equation

240 for half of the initial percentage of high threshold seeds. For *B. heterophylla*, the 60°C treatments

241 couldn't be conducted due to a lack of seeds in the field burial treatments. In this case, there were

242 little differences between field and lab-stored results, and so the decay rate was based on lab-

243 stored seed data. The half-life of high threshold seeds were not calculated for *A. linifolia* because

244 this species displayed no change in threshold over the time period used in our study.

245

246 *Change in viability and dormancy over time*

247 We assessed how viability and the non-dormant fraction changed, and whether resilience to fire-

248 related temperatures was maintained over time. Change in viability and initial dormancy after

249 each storage time period of the laboratory-stored seeds was assessed using a one-factor GLM

250 with time as the predictor. Due to viability of most retrieved field-buried seed lots being at or

251 close to 100%, we investigated loss of laboratory-stored viability and the number of lost seeds

252 from each of the retrieved bags. We plotted the mean for each over time, as well as comparing

253 the percentage of lost seeds per replicate bag with the percentage of seeds becoming inviable

254   during laboratory storage using regression. This allowed assessment of whether seed loss in the

255   field could potentially be related to loss of non-dormant or inviable seeds.

256

257   **Results**

258   All four species showed an increase of germination in response to heat treatments over storage

259   time (Fig. 1). In three of the study species, there was a significant interaction between

260   temperature and storage time (*V. juncea df* =4, χ^2 = 139.21, *P* < 0.001; *A. ericoides df* = 2, χ^2 =

261   111.51, *P* < 0.001; *B. heterophylla df* = 2, χ^2 = 111.53, *P* < 0.001), showing that germination

262   response to heat treatments increased as seeds aged (Table 2). Although there was no significant

263   effect of type of storage for these three species, examination of the data indicated that *B.*

264   *heterophylla* and *A. ericoides* displayed differences at the 40 °C treatment between the lab- and

265   soil-stored seeds. For both of these species, a decline in germination after 18 months lab-storage

266   appeared to be related to a large decline in viability (Table 3). Additionally for *B. heterophylla*,

267   there is a much sharper lowering of threshold obvious at 40 °C for lab-stored seeds compared to

268   field buried seeds. For *A. linifolia* however, there was a similar significant interaction between

269   storage time and temperature (*df* =4, χ^2 = 165.22, *P* < 0.001), as well as a significant difference

270   between laboratory and field stored seeds (*df* = 1, χ^2 = 250.95, *P* = 0.003) (Table 2). *Acacia*

271   *linifolia* seeds increased germination to 80% at the 80 °C treatment after six months field burial,

272   whereas maximum germination of laboratory stored seeds was less than 30%, even after 18

273   months storage (Fig. 1). There was little decrease in initial dormancy levels (i.e. no significant

274   increase in the non-dormant fraction) observed as a result of storage time (Fig. 1).

275   Counterintuitively, *A. linifolia* displayed a significant increase in initial dormancy levels in

276   laboratory stored seeds as they aged (df =2, χ^2 =5.32, P < 0.001) (Table 3). There was a similar

277   increase in initial dormancy levels for field buried seeds for all species (Table 3).

278

279   Dormancy-breaking temperature thresholds measured by $G_{20\%}$ and $G_{50\%}$ were higher for freshly

280   collected seeds, ranging from 80-100 °C in high threshold and 60 °C in low threshold species,

281   than after storage (Table 3). The $G_{20\%}$ displayed a dramatic decrease over time for three of the

282   study species, with both low threshold species, *B. heterophylla* and *A. ericoides*, dropping their

283   threshold to the lowest fire-related temperature treatment of 40°C after only six months. The

284   high threshold *V. juncea* also moved from a high to low threshold classification (Ooi *et al.*,

285   2014). However, the $G_{50\%}$ showed that a large proportion of *V. juncea* seeds maintained a high

286   threshold for at least six months (Table 3).

287

288   The estimated half-life of the high threshold seed fraction varied considerably between species

289   (Table 4). The fractions of initial high threshold seeds were less than 50% for both low threshold

290   species, which was reduced to almost 0% at the first retrieval (6 months). The estimated half-

291   lives of their high threshold fractions were therefore below six months for both *Aotus ericoides*

292   and *B. heterophylla* (Table 3). For the high threshold *V. juncea* the half-life of the high threshold

293   fraction of seeds was relatively long, taking over 21 months. The half-life for *A. linifolia* could

294   not be robustly calculated dormancy-breaking thresholds did not decay over the 18 months of

295   burial.

296

297   Viability of the high threshold species *A. linifolia* did not decline over time. However it did

298   decline significantly for both of the low threshold species *B. heterophylla* (df = 2, χ^2 =7.196, P <

299   0.001) and *A. ericoides* (*df* =2, χ^2 =11.095, *P* = 0.029), and the high threshold species *V. juncea*

300   (*df* = 2, χ^2 =2.54, *P* =0.002) by approximately 35%, 25% and 10% respectively, after laboratory

301   storage for 18 months (Table 3) (Fig. 2A). For buried seeds, there was a significant decline in the

302   number of whole seeds retrieved over time for all species except *A. ericoides* (Fig. 2B). The

303   relationship between the number of seeds missing from bags at retrieval and the proportion of

304   seeds losing viability was highlighted by a strong positive correlation for *B. heterophylla* (R^2 =

305   0.506) and *V. juncea* (R^2 = 0.785) (data not shown). However, no clear relationship was found

306   for *A. ericoides* or *A. linifolia*.

307

308   **Discussion**

309   We observed a clear lowering of dormancy-breaking temperatures over storage time for three of

310   our physically dormant study species. The effects of ageing differed between species however,

311   with the low threshold species *B. heterophylla* and *A. ericoides* losing viability and dropping

312   threshold levels more quickly than their high threshold counterparts. For the high threshold

313   species, a proportion of *V. juncea* seeds were reduced to lower threshold levels over time,

314   whereas all *A. linifolia* seeds maintained high thresholds over time. This provides an insight into

315   the dynamics of dormancy-breaking temperature thresholds within persistence soil seed banks.

316   These results have implications for allowing more sophisticated predictions for post-fire

317   regeneration in response to fire (Keith *et al*., 2002; Wills and Read, 2002; Hudson *et al*., 2015)

318   which would not be possible from experiments that have tested the germination of stored seeds

319   using non-dormant fractions or single fire-related temperature treatments.

320

321   Variation in dormancy-breaking temperature thresholds of fire-following species is directly

322   related to understanding both the response to variation in fire severity (and the related levels of

323   soil heating) and the impacts of changes to the fire regime associated with fire management and

324   climate change. For example, species with high dormancy-breaking temperature thresholds

325   would likely fail to recruit after less severe burns, such as those produced during cool season

326   prescribed fires that result in low soil temperatures (Auld and O'Connell, 1991; Auld and

327   Bradstock, 1996; Penman and Towerton, 2008; Ooi *et al*., 2014; Liyanage and Ooi, 2015). The

328   results from our study suggest that some high threshold species, such as *V. juncea*, have a

329   mechanism that provides some level of seed germination response under low severity conditions.

330   This could be interpreted as a bet-hedging mechanism in response to variation in fire severity,

331   which is only developed as seeds age within the seed bank.

332

333   Both the extent to which thresholds drop and the amount of time taken to decrease would be

334   important in determining the ability of a species' seed bank to respond to lower temperatures

335   during fire. While there is an obvious decline in threshold temperatures, particularly when

336   determined by the $G_{20\%}$ index, both high threshold species differ. *Acacia linifolia* maintained its

337   high threshold throughout the duration of the experiment, indicating that the seed bank would

338   retain a high threshold requirement over a typical fire return interval for the region of 7 - 17

339   years (Bradstock and Kenny, 2003). *Viminaria juncea* was more plastic, indicating that a greater

340   proportion of seeds would be able to respond to lower temperatures. However, a relatively long

341   half-life of the high threshold fraction meant that a large proportion of the seed bank would still

342   require an 80°C treatment to produce a germination flush during the next fire event, due to fresh

343   seed input and the slow rate of decline. Identifying both rapid and slow threshold changing

344   species could help to further develop dormancy-breaking threshold groups and understand the

345   population dynamics of fire-prone species associated with seed age and variation in the fire

346   regime.

347

348   The results from our study lead to the question of whether a decline in temperature thresholds is

349   simply a sign of seed decay or a trait which provides some benefit to species persistence. In other

350   regions, where fire is not a driver of population dynamics, a decline in dormancy over time is

351   essential for many PY species. For example, winter annuals lose dormancy over the hot summer

352   months to promote germination during cooler months (Van Assche and Vandelook, 2006), while

353   arid species experience a gradual decline in the dormant fraction to take advantage of sporadic

354   rainfall events (Ooi *et al*., 2009). In fire-prone regions, it is beneficial that seed banks maintain a

355   high proportion of dormant seeds between fires, an assumption supported by our findings of little

356   increase in the non-dormant fraction. However, this differs to a reduction in temperature

357   thresholds. Our study species also displayed a relative increase in the number of dormant seeds,

358   particularly after field burial, a result likely to be related to germination of the non-dormant

359   fraction in the treatment bags (Zalamea *et al*., 2015), and the disintegration of inviable seeds.

360   This hypothesis was supported for at least two of our species, *B. heterophylla* and *V. juncea*,

361   where we found a strong correlation between viability decline during laboratory storage and seed

362   loss from bags during field burial.

363

364   One hypothesis for the role of threshold reduction is related to the dynamics of seed bank

365   formation. Upon reaching the soil surface, seeds can move vertically down the soil profile over

366   time via both biotic and abiotic agents. Rainfall is the key abiotic cause, with the greatest rates of

367   burial occurring in sandy soils (Benvenuti, 2007; Marthews *et al*., 2008). The main biotic vector

368   in our study region is ants, with one study reporting up to 38% of *Acacia suaveolens* seeds

369   moved to nests (Auld, 1986), resulting in a large proportion buried at depths over 5 cm (> 20%

370   of seeds). While some cycling of seeds within the depth profile is possible (Chambers and

371   MacMahon, 1994), it is likely that on average older seeds are buried at greater depth than

372   younger seeds. Soil is an effective insulator and temperatures experienced by the seed bank

373   during fire decrease with increasing soil depth (Auld, 1986; Auld and Bradstock, 1996). The

374   lowering of thresholds over time may therefore be a mechanism for maintaining a post-fire

375   germination response, with older seeds responding to the lower temperatures experienced at

376   depth and younger seeds germinating in response to hotter temperatures closer to the soil surface.

377   This ability would be mediated by seed size (Bond *et al*., 1999, Hanley *et al*., 2003), meaning

378   that larger-seeded species may have less selective pressure to maintain higher thresholds. Future

379   studies looking at dormancy-breaking temperature thresholds as seeds age, across a number of

380   species with a range of seed sizes, would contribute to understanding the basis of this

381   mechanism.

382

383   A striking finding from our experiments provides an answer for conflicting results reported in a

384   number of previous studies. These have shown that dormancy of some PY species is not broken

385   by fire-related temperatures in the laboratory, even though mass seedling emergence has been

386   observed in the post-fire environment. Temperature treatments of up to 110°C produce no or

387   little germination response, yet mortality occurs at 120°C. Examples come mainly from the

388   Fabaceae, and include *Acacia longifolia*, *Mirbelia platylobium* and one of our study species, *A.*

389   *linifolia* (Auld and O'Connell, 1991; Ooi *et al*., 2014) but are also found in the Malvaceae,

390 including *Alyogyne hakeifolia* and *A. huegelii* (Baker *et al*., 2005). This has led to some

391 confusion over the drivers of germination response for these species. All of these studies have

392 been conducted using fresh seeds. The increased levels of seed germination that we observed for

393 aged *A. linifolia* seeds therefore provides an explanation for such observations.

394

395 For *A. linifolia* we also observed relatively high germination in untreated fresh seeds, compared

396 to other PY species in the study region, suggesting that dormancy may not have been fully

397 developed at dispersal. In our system, there is little adaptive benefit for having a non-dormant

398 seed bank fraction, such as the hypothesised reward for dispersal to safe sites suggested by others

399 (e.g. Paulsen *et al*., 2013; Zalamea *et al*., 2015), because successful recruitment is restricted to

400 the post-fire environment and inter-fire germination is highly likely to fail, irrespective of the site

401 reached (Whelan, 1995; Ooi *et al*., 2012). Additionally, dispersal by mammals is extremely rare

402 for this species, and instead is primarily carried out by ants which are rewarded by the elaiosome

403 (Auld, 1986). We suggest that the lack of dormancy observed in *A. linifolia* is an artefact of

404 using freshly collected seeds, and the development into hard seeds, while relatively slow, is

405 within a time frame which ensures minimal loss to germination. Other studies have reported

406 several PY species that have non-dormant seeds at dispersal, with PY developing on exposure to

407 low humidity levels (Pukittayacamee and Hellum, 1988; Tozer and Ooi, 2014). Tozer and Ooi

408 (2014) found that this occurred rapidly for *Acacia saligna* once humidity dropped below 20%,

409 meaning that initially non-dormant seeds of this PY species were likely to 'harden' within a few

410 days of dispersal in their native habitat, and still be incorporated into the seed bank. In our study,

411 it is difficult to identify the mechanism by which fresh *A. linifolia* seeds become dormant,

412 however, germination response after the lower heat treatments (40°C and 60°C) was significantly

413   lower than the controls, without any increase in seed mortality. This suggests that such

414   temperatures, which can be reached during hot summer days in the soil (Ooi *et al*., 2012), may

415   have a role in the development of PY in the non-dormant fraction, potentially in addition to low

416   humidity. This again highlights the need for further studies assessing both fresh and aged seeds

417   to understand PY variation and response.

418

419   For both low threshold species *B. heterophylla* and *A. ericoides*, the vast majority of seeds

420   required only a 40°C treatment to overcome dormancy after relatively short burial periods. This

421   increases the likelihood of a large germination response of such species to cooler lower severity

422   burns, and also potentially to have dormancy broken by the temperatures produced during the

423   inter-fire period. Ooi *et al*. (2014) found that such temperatures had the potential to promote

424   germination, a response that could significantly increase under predicted future summer soil

425   temperatures. Seedling recruitment during the inter-fire period is rarely successful in these fire-

426   prone systems, meaning that increased levels of dormancy loss could cause significant soil seed

427   bank decay (Ooi *et al*., 2012, 2014). Our study therefore highlights that the risks to population

428   persistence from seed bank decay is higher than previously estimated, due to dormancy-breaking

429   thresholds changing over time, with up to 90% of seeds from the facultative pyrogenic species *B.*

430   *heterophylla* and *A. ericoides* requiring only the lowest temperature treatment of 40°C.

431   Incorporating such data could improve predictions of population dynamics under predicted soil

432   temperatures, particularly in the important but understudied area of seed persistence and climate

433   change (Ooi *et al*., 2014; Hudson *et al*., 2015; Ooi, 2015; Parmesan and Hanley, 2015).

434

435   Most studies of persistent seed banks describe composition, density or germination response at a

436   particular point in time, but far less often describe how these characteristics change over time

437   (Fenner and Thompson, 2005). Instead of losing dormancy, it is clear that our study species show

438   a changing pattern of dormancy-breaking temperature thresholds. While several studies have

439   described a change in sensitivity to dormancy-breaking cues after ageing (Galíndez *et al*., 2010;

440   Turner *et al*., 2013), our study highlights this important distinction in fire-prone regions, where

441   maintaining dormancy is essential for the population, and therefore species, to persist in the long

442   term. A change in thresholds provides a different outcome, and the mechanism underlying these

443   changes appears to differ between species. The gradual decrease in dormancy-breaking

444   temperature thresholds with increasing storage time provides a mechanism for increasing the

445   range of thresholds present within the seed bank at any particular point in time, contributing to

446   the maintenance of a bet-hedging capability or germination from depth. Understanding how

447   widespread, and to what extent, such changes to PY occur could help to improve predictions of

448   seed bank and recruitment dynamics in the post-fire environment, and help to robustly model

449   population persistence. Studies need to be conducted across a larger range of species to draw

450   more general conclusions.

451

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458

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461

Conflicts of interest

None.

464

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611

612 **Figure Legends**

613 **Figure 1:** Mean seed germination (± s.e.) of laboratory stored and field buried seeds, expressed

614 as a percentage of the total initial viable seeds, after heat shock treatments of 40, 60, 80 and 100

615 °C. Different coloured bars represent seeds stored for 0 (■), six (▧) or 18 (□) months. Graphs in

616 the left column represent lab-stored seeds and those on the right represent field-buried seeds of

617 *Acacia linifolia* (A, B); *Viminaria juncea* (C, D); *Bossiaea heterophylla* (E, F) and *Aotus*

618 *ericoides* (G, H).

619 **Figure 2**: Comparison of seed viability decline and seed loss during laboratory storage and field

620 burial respectively. Bars represent mean values (± s.e.) for each of the study species for (A) the

621 proportion of inviable seeds after laboratory storage and (B) the number of whole seeds lost

622 during field burial, for six (■) and 18 months (□). (* denotes significant differences between

623 storage time).

624

625 **Table Legends**

626 **Table 1:** Dormancy-breaking threshold group (see Ooi *et al*., 2014), mean seed mass (± s.e.),

627 number of mesh bags buried in the field and number of seeds stored in each mesh bag for fresh

628 seeds of all four studied species.

629 **Table 2:** Results from the binomial GLM for germination of four species in response to

630 temperature treatment (temperature), type of storage (type) and storage time (time) (* represents

631 significant differences at $P < 0.05$).

632   **Table 3:** Initial viability ($\pm$ s.e.), dormancy ($\pm$ s.e.), temperature required to break 20% ($G_{20\%}$)

633   and 50% ($G_{50\%}$) of dormant seeds, and dormancy-breaking temperature threshold group based on

634   the $G_{20\%}$ for different time periods. The dash (-) indicates insufficient germination reached to

635   determine dormancy-breaking threshold group, even in response to the highest temperature

636   treatments.

637   **Table 4:** Some fraction of the seed lot for each species has a high threshold. The estimated mean

638   decay rate ($\pm$ s.e.) and half-life ($\pm$ s.e.) of the high threshold fraction is shown for each study

639   species. NB No decay was recorded for *Acacia linifolia* and so a half-life was not calculated.

640

641 **Table 1**

Species	Dormancy-breaking threshold group	Seed mass (mg)	Number of buried bags	Number of seeds per bag
Acacia linifolia	High	33.23 ± 0.85	24	25
Viminaria juncea	High	6.21 ± 0.13	12	50
Bossiaea heterophylla	Low	15.43 ± 1.84	12	25
Aotus ericoides	Low	4.40 ± 0.84	6	50

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653 **Table 2**

Species	Predictor	*Df*	Deviance (χ^2)	*P-value*
Acacia linifolia	Temperature	4	280.19	<0.001 *
	Time	1	279.68	0.687
	Type	1	250.95	0.003 *
	Time x Type	1	242.63	0.104
	Temperature x Time	4	165.22	<0.001 *
Viminaria juncea	Temperature	4	310.73	<0.001 *
	Time	1	247.92	<0.001 *
	Type	1	246.76	0.605
	Time x Type	1	245.87	0.651
	Temperature x Time	4	139.21	<0.001 *
Aotus ericoides	Temperature	2	276.65	< 0.001 *
	Time	1	184.11	<0.001 *
	Type	1	181.89	0.367
	Time x Type	1	176.51	0.160
	Temperature x Time	2	111.51	<0.001 *
Bossiaea heterophylla	Temperature	2	225.36	<0.001 *
	Time	1	171.59	<0.001 *
	Type	1	166.76	0.259
	Time x Type	1	165.89	0.634
	Temperature x Time	2	111.53	<0.001 *

Table 3

Species	Storage time (months)	Viability		Dormancy		$G_{20\%}$ (°C)	$G_{50\%}$ (°C)	Dormancy-breaking threshold group
		Laboratory	Field	Laboratory	Field			
Acacia linifolia	0	93.33 ± 1.67		76.80 ± 1.59		-	-	High
	6	95 ± 5	92.73 ± 1.38	98.33 ± 1.67	98.03 ± 1.96	80	80	High
	18	95 ± 5	97.92 ± 2.08	95 ± 2.88	94.77 ± 2.62	80	80	High
Viminaria juncea	0	98.33 ± 1.67		91.49 ± 4.44		100	100	High
	6	100 ± 0	100 ± 0	91.67 ± 4.40	98.14 ± 1.85	60	80	Low
	18	88.33 ± 1.67	100 ± 1.67	90.30 ± 7.10	100 ± 0	60	60	Low
Bossiaea heterophylla	0	93.33 ± 1.67		85.57 ± 7.22		60	-	Low
	6	82.61 ± 3.15	97.77 ± 2.22	80.55 ± 7.82	100 ± 0	40	60	Low
	18	56.67 ± 10.29	87.08 ± 6.46	85.53 ± 7.05	100 ± 0	40	40	Low
Aotus ericoides	0	80.69 ± 5.97		92.31 ± 4.44		60	-	Low
	6	83.33 ± 4.41	100 ± 0	96.08 ± 3.92	100 ± 0	40	40	Low
	18	57.67 ± 11.02	100 ± 0	87.61 ± 6.57	100 ± 0	40	40	Low

654

655

656 **Table 4**

Species	Mean decay rate	Mean half-life (months)
Aotus ericoides	-9.373 ± 1.59	2.97 ± 0.03
Bossiaea heterophylla	-4.363 ± 3.14	4.69 ± 0.85
Viminaria juncea	-0.032 ± 0.00	21.13 ± 2.88

657