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**Short communication**

# The effect of simulated heat-shock and daily temperature fluctuations on seed germination of four species from fire-prone ecosystems

**Talita Zupo[1]*, M. Jaime Baeza[2,3] and Alessandra Fidelis[1]**

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## ABSTRACT

Seed germination in many species from fire-prone ecosystems may be triggered by heat shock and/or temperature fluctuation, and how species respond to such fire-related cues is important to understand post-fire regeneration strategies. Thus, we tested how heat shock and daily temperature fluctuations affect the germination of four species from fire-prone ecosystems; two from the Cerrado and two from the Mediterranean Basin. Seeds of all four species were subjected to four treatments: Fire (F), temperature fluctuations (TF), fire+temperature fluctuations (F+TF) and control (C). After treatments, seeds were put to germinate for 60 days at 25°C (dark). Responses differed according to species and native ecosystem. Germination percentage for the Cerrado species did not increase with any of the treatments, while germination of one Mediterranean species increased with all treatments and the other only with treatments that included fire. Although the Cerrado species did not respond to the treatments used in this study, their seeds survived the exposure to heat shock, which suggests they possess tolerance to fire. Fire frequency in the Cerrado is higher than that in Mediterranean ecosystems, thus traits related to fire-resistance would be more advantageous than traits related to post-fire recruitment, which are widespread among Mediterranean species.

**Keywords:** Cerrado, Mediterranean Basin, mean germination time, physical dormancy, post-fire germination

Fire is a natural disturbance present in many ecosystems worldwide where plant species can persist by resprouting, recruiting from seeds, or use both strategies (Bond & Midgley 2001; Pausas *et al.* 2004; Bond & Keeley 2005). Consequently, in fire-prone ecosystems, plant species can show different seed germination strategies that optimize plant survival in face of recurrent fires, where germination may be triggered by different mechanisms, such as heat shocks, temperature fluctuations and smoke (Williams *et al.* 2005; Moreira *et al.* 2010; Santana *et al.* 2010; 2013; Moreira & Pausas 2012).

Intense short-term heating effects caused by fire have been reported to enhance germination in several species of fire-prone ecosystems, mainly in hard-seeded species

[1] Departamento de Botânica, Instituto de Biociências, Universidade Estadual Paulista, Av. 24A, 1515, 13506-900, Rio Claro, SP, Brazil
[2] Fundación Centro de Estudios Ambientales del Mediterráneo, C/ Charles Darwin, 14. 46980, Paterna, Valencia, Spain
[3] Departamento de Ecologia, Universidad de Alicante, Ap. 99. E-03080. Alicante, Spain
* Corresponding author: talita.zupo@gmail.com

where high temperatures may break physical dormancy enabling germination (Williams *et al*. 2003; Reyes & Trabaud 2009; Moreira *et al*. 2010; Moreira & Pausas 2012). Other studies have found no evidence that heat shocks effectively trigger germination in species with both permeable and impermeable seeds (Luna *et al*. 2007; Dayamba *et al*. 2010; Jaureguiberry & Diaz 2015; Le Stradic *et al*. 2015; Fichino *et al*. 2016; Fidelis *et al*. 2016). Although some species from fire-prone ecosystems showed to be unaffected by high temperatures produced during fire, their seeds survived the exposure to high temperatures, and could be considered as fire-resistant (Luna *et al*. 2007; Jaureguiberry & Diaz 2015; Le Stradic *et al*. 2015; Fichino *et al*. 2016; Fidelis *et al*. 2016).

After fire, gaps open within the vegetation and lead to an increase in the incidence of solar radiation, altering soil temperatures (Auld & Bradstock 1996; Santana *et al*. 2013), which results in seeds being exposed to high temperatures (e.g. 50-55°C) and wide temperature fluctuations. Recent studies have indicated that these temperature fluctuations also act as germination cues in hard-seeded species, breaking physical dormancy and enabling germination (Baeza & Roy 2008; Santana *et al*. 2010; 2013; Jaganathan 2015). Moreover, in non-dormant seeds, daily temperature fluctuations may affect the rate of germination (Musso *et al*. 2015; Le Stradic *et al*. 2015). However, some hard-seeded species require higher temperature thresholds for dormancy release, and physical dormancy is broken with temperatures reached only with fire (Moreira & Pausas 2012; Ooi *et al*. 2014). Ooi *et al*. (2014) suggested that species whose physical dormancy is broken with lower temperature thresholds, e.g. temperatures reached in vegetation gaps, have facultative pyrogenic dormancy, while those species that need higher temperatures, reached only with fire, have obligate pyrogenic dormancy.

Considering that responses of seeds to fire-related cues can be different according to species and the fire-prone ecosystems where they occur, the aim of our study was to evaluate whether high temperatures caused by fire and/or the daily temperature fluctuations would affect the germination and the mean germination time of four species present in fire-prone ecosystems.

We selected species (three Fabaceae and one Cistaceae) that are very common in the fire-prone ecosystems they occur. *Mimosa leiocephala* Benth. and *Bauhinia dumosa* Benth. are two shrub species (resprouters) found in Cerrado *campo sujo* – a grassland type with a continuous herbaceous layer and scattered shrubs. *Ulex parviflorus* Pourr. and *Cistus albidus* L. are shrub species (seeders) found along the Mediterranean coasts of Southwestern Europe (Tutin *et al*. 1964-1980).

Fires in Cerrado are surface fires of relatively low intensity that rapidly consume the herbaceous biomass (Miranda *et al*. 2002) and can occur every 3-4 years (Coutinho 1982). During fires, temperatures at soil surface can range from 74°C to 768°C in open savanna-like Cerrado (Miranda *et al*. 1993; Pivello *et al*. 2010), while maximum temperatures 1cm belowground usually increase up to around 50°C (Miranda *et al*. 1993), but can occasionally reach over 80°C (T Zupo unpubl. res.). Post-fire temperatures registered in vegetation gaps ranged from 16 to 50°C at soil surface (A Fidelis unpubl. res.). Mediterranean fires are crown fires, which consume canopy biomass of shrub species, and are considered high intensity fires that occur once in several decades (Keeley *et al*. 2011). Fire temperatures in such shrublands can reach up to 400°C at soil surface (Baeza *et al*. 2002) and between 80 and 99°C 1cm belowground, with maximum post-fire temperature in vegetation gaps between 49 and 53°C (Santana *et al*. 2013).

Seeds from the Cerrado species were collected in July and October 2013 in a *campo sujo* area located at the Serra do Tombador Nature Reserve, Goiás, Central Brazil. They were stored in glass recipients at 5°C until the beginning of the experiment in December 2013. Seeds of *U. parviflorus* and *C. albidus* were collected in July and November 2013 in a shrubland with *Pinus halepensis* Mill. located near Alicante (Spain), and stored in plastic bags at controlled temperatures (20°C), until the beginning of the experiment in December 2013. Seeds of all species have orthodox seed storage behavior thus the viability is not affected by storage under low temperatures (Royal Botanic Gardens Kew 2016).

Seeds of all species were subjected to four different treatments prior to germination tests: Fire (F), temperature fluctuations (TF), fire + temperature fluctuations (F+TF) and a control (C). For each species, we used four replicates containing 25 seeds in each treatment. We replicated the experiment three times in order to obtain temporal replications since we only had one alternating temperature incubator (25 seeds x 4 replicates x 4 treatments x 3 temporal replications = 1200 seeds/species). Thus, the experiment is considered a block design with each temporal replicate being a block.

In the F treatment, seeds were put in a regular incubator with an initial temperature of 25°C, rising up to 80°C in ten minutes and then continuously decreasing its temperature back to 25°C in one hour, simulating the passage of fire 1cm belowground (Fig. 1A). The TF treatment consisted of exposing the seeds to daily cycles of alternating temperatures (15°C to 50°C – Fig. 1B) during 30 days in an alternating temperature incubator (Binder KB E5.1) to simulate the daily temperature fluctuation that occurs in vegetation gaps, both in post-fire conditions and in natural conditions during the summers in the Mediterranean Basin and the dry seasons in the Cerrado. For treatment F + TF, both treatments were combined with seeds being first exposed to the high temperatures simulating the passage of fire and subsequently being exposed to the alternating temperatures for 30 days. Finally, Control seeds were not exposed to any of the treatments mentioned above, and seeds were stored at a constant temperature of 20°C until put to germinate. This procedure was applied for each temporal replicate.

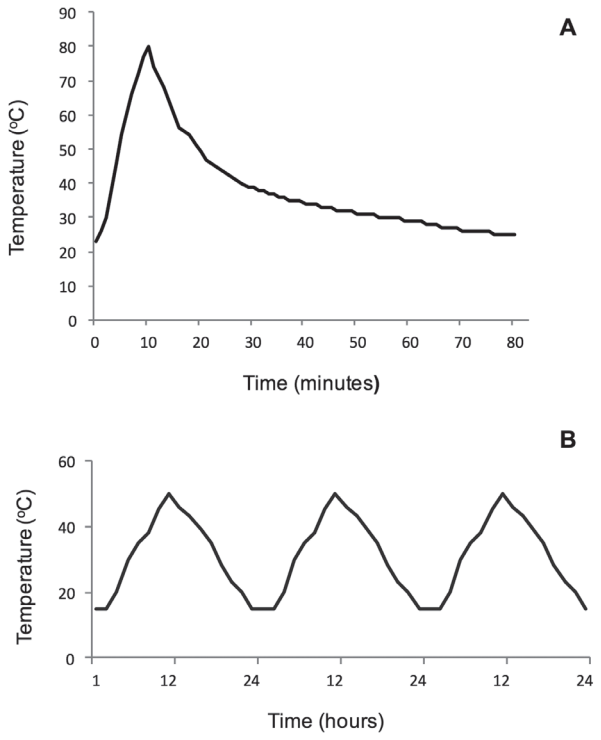


**Figure 1.** Temperature curves that simulate (A) the passage of fire at 1 cm deep soil, F treatment and (B) daily temperature fluctuations after the removal of vegetation, TF treatment.

Seeds were then placed in Petri dishes with one layer of filter paper saturated with distillated water, and put in germination chambers at 25ºC (dark). Seed germination was recorded every two days for 60 days and germination was determined by radicle emergence (Bewley *et al*. 2013). Mean germination time was calculated according to Ranal & Santana (2006). Viability of non-germinated seeds was analyzed at the end of each experiment by means of the Tetrazolium test at 1% solution (AOSA 2005).

Assuming a block design with each temporal replicate being a block, one-way analysis of variance applied to randomization tests (Euclidean distance between sampling units, 10000 iterations) were carried out to evaluate differences in germination percentage and mean germination time between the different treatments (fixed factor) for each species. All statistical analyses were performed using MULTIV (Pillar 2008).

Seeds of *Bauhinia dumosa* showed high germination percentages (> 80%) in all treatments, including the control, whereas seeds of *Mimosa leiocephala* showed low germination (≤ 30%) in all treatments. *B. dumosa* showed lower germination percentage in treatments where fluctuating temperatures were involved (84.2±4.5% and 86.7±6.1% in F+TF and TF treatments, respectively) in relation to both F treatment (97.3±2.8%, both $p \leq 0.001$) and to control (98.2±1.6%, both $p < 0.001$; Fig. 2A). For this species, however, F treatment did not differ from the control ($p = 0.13$; Fig. 2A). Germination percentage of *M. leiocephala* was not affected by F, F+TF and TF treatments ($p > 0.05$; Fig. 2B).

While *Ulex parviflorus* responded to all treatments, *Cistus albidus* responded only to F treatments. Germination percentage of *U. parviflorus* was over 65% in all treatments, except for the control, where germination percentage was 43.7±3.7% (Fig. 2C). Even though there were no significant differences between F, F+TF and TF treatments, they showed greater germination percentage in relation to the control (all $p \leq 0.001$; Fig. 2C). *Cistus albidus* showed higher germination in F treatment in relation to all other treatments (23.8±18%, all $p \leq 0.001$), where germination percentage was < 5% (Fig. 2D).

Seed viability (germinated + stained seeds) remained high after treatments for all species (>70%). Even though *M. leiocephala* and *C. albidus* showed low germination percentages, non-germinated seeds were viable.

Treatments with temperature fluctuations increased the mean germination time of *B. dumosa* (32.4±5.1 days for F+TF and 31.2±7.6 days for TF) in relation to F treatment (16.4±2.2 days, both $p \leq 0.001$) and to the control (12.5±1.4 days, both $p < 0.001$; Fig. 3A). For *M. leiocephala*, mean germination time was greater in TF treatments (16.4±2.8 days) in relation to treatments that involved high temperatures simulating the passage of fire (F, 12.9±5.2 days, $p = 0.048$ and F+TF, 13.4±2.6 days, $p = 0.01$), but did not differ from the control (14.5±1.4 days, $p > 0.05$; Fig. 3B). On the other hand, mean germination time of *U. parviflorus* seeds was not affected by F, F+TF and TF treatments ($p > 0.05$, Fig. 3C). Mean germination time of *C. albidus* was not calculated given that few seeds germinated in the control, F+TF and TF treatments.

Several studies have reported an increase in germination percentage after heat shocks for many species of fire-prone ecosystems, especially for those with physically dormant seeds (Williams *et al*. 2003; Reyes & Trabaud 2009; Moreira *et al*. 2010; Moreira & Pausas 2012). In our study, germination responses to heat shocks were variable: heat shocks did not enhance germination of *Mimosa leiocephala* and *Bauhinia dumosa,* but effectively broke dormancy of *Ulex parviflorus* and *Cistus albidus,* enabling germination.

Seeds of *M. leiocephala* had low germination percentages (≤ 30%) in all treatments with seeds maintaining its viability at the end of the experiment (> 90% viable), confirming the presence of physical dormancy and indicating no fire-dependency regarding dormancy break. Hard-seeded species might have different heat tolerance and different temperature thresholds for dormancy release (Ooi *et al.* 2014). *M. leiocephala* did not have their dormancy broken by the heat shocks of 80ºC used in this experiment, but they did not die when exposed to this temperature, confirming previous studies that seeds of Cerrado are not stimulated by fire, but might be tolerant (Ribeiro & Borghetti 2014; Fichino *et al*. 2016). However, seeds of *U. parviflorus* and *C. albidus*, known to be physically dormant (Baeza

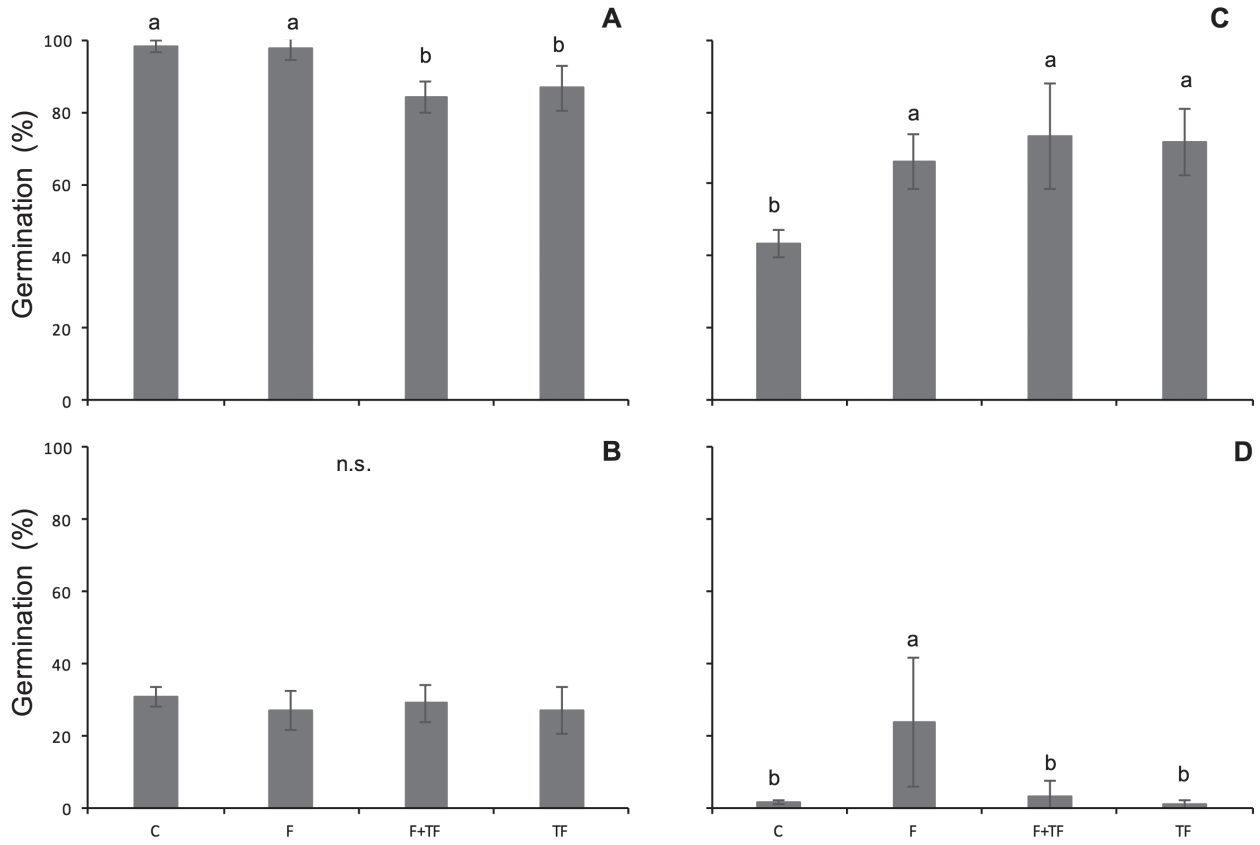


**Figure 2.** Germination percentage (mean ± SD) for A) *Bauhinia dumosa*, B) *Mimosa leiocephala*, C) *Ulex parviflorus* and D) *Cistus albidus* subjected to the treatments C = Control; F = Fire; F+TF = fire followed by temperature fluctuation and TF = temperature fluctuation. Different letters mean significant differences, $p \leq 0.05$.

& Vallejo 2006; Moreira *et al.* 2010), showed an increase in germination after being exposed to such temperature, indicating that the heat shocks were effective in releasing their seeds from dormancy, as has been reported for many hard-seeded species of fire-prone ecosystems (Williams *et al.* 2003; 2005; Reyes & Trabaud 2009; Moreira *et al.* 2010; Moreira & Pausas 2012; Ooi *et al.* 2014).

Such as *M. leiocephala*, other hard-seeded species of subtropical grasslands (Fidelis *et al.* 2016) and also from Cerrado (Fichino *et al.* 2016) did not have their dormancy broken by one-minute heat shocks ranging between 60 and 200°C, suggesting that seeds are resistant to high temperatures and some other mechanism, such as fluctuating temperatures, might be involved in releasing seeds of these species from dormancy. Nonetheless, treatments involving fluctuating temperatures in this study were also unable to break dormancy for *M. leiocephala*, and the mechanism for dormancy release remains unclear. Additional combinations of temperature and time of exposure or exposing seeds to temperature fluctuations for longer periods of time might be effective in breaking dormancy. On the other hand, temperature fluctuations broke physical dormancy of seeds of *U. parviflorus*, suggesting that this species might have lower temperature thresholds for dormancy release (Ooi *et al.* 2014). Given that the germination of *U. parviflorus* is stimulated by both fire and fluctuating temperatures, *U. parviflorus* could be considered as having facultative pyrogenic dormancy release, as proposed by Ooi *et al.* (2014), where germination occurs both with temperatures related to fire and to seasonal temperature changes (Santana *et al.* 2013; Jaganathan 2015).

Alternatively, *B. dumosa* showed high germination percentages (>80%) in all treatments, indicating that seeds from this species are water permeable and do not have dormancy. Cerrado woody species with permeable seed coats (Ribeiro *et al.* 2013; Ribeiro & Borghetti 2014), and herbaceous species with permeable seeds of *campo rupestre* (Le Stradic *et al.* 2015) and campo sujo (Fichino *et al.* 2016) were also unaffected by heat shocks of temperatures > 80°C. On the other hand, fluctuating temperatures led to a decrease in germination percentage of *B. dumosa* and an increase in mean germination time, which might mean that seeds from this species recognize high temperature amplitudes as an unfavorable environmental condition adopting a strategy of not betting all of its seeds at once. This could also be explained by dispersal timing (Salazar *et al.* 2011), given that this species has no dormancy and shed its seeds at the end of the dry season (personal observation),

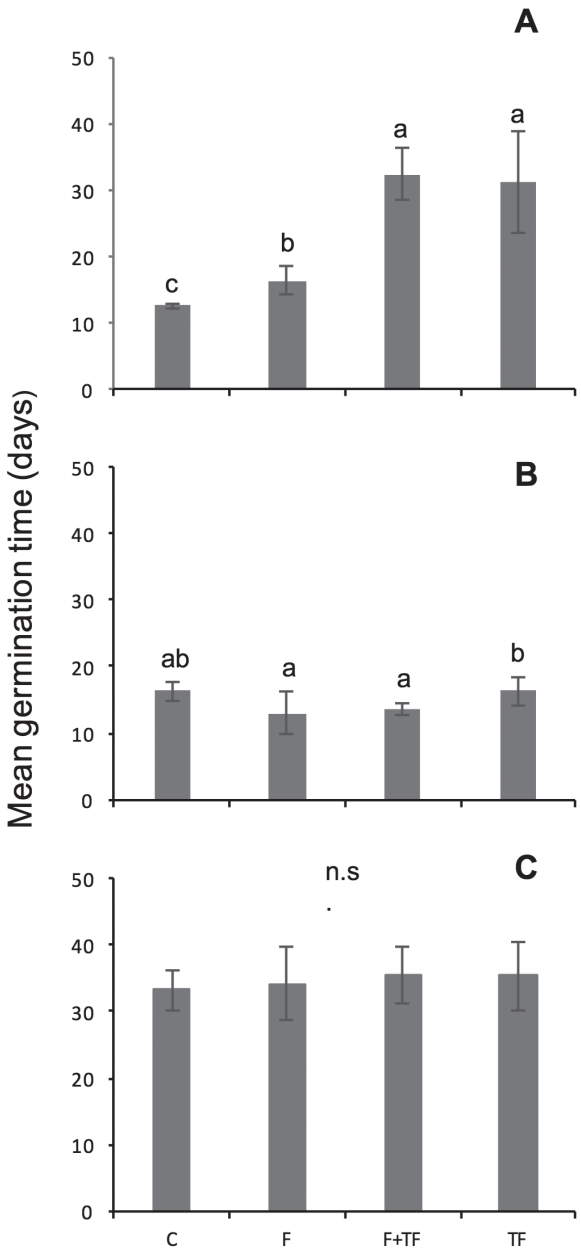


**Figure 3.** Germination time (mean ± SD) for A) *Bauhinia dumosa*, B) *Mimosa leiocephala* and C) *Ulex parviflorus* subjected to the treatments C = Control; F = Fire; F+TF = fire followed by temperature fluctuation and TF = temperature fluctuation. Different letters mean significant differences, $p \leq 0.05$.

allowing them to germinate with the onset of the rainy season that follows. Such response is not a direct effect of fire, but could be seen as an indirect effect given that the amount of gaps increases after the passage of fire. Furthermore, since many natural fires in Cerrado occur at the end of the dry season (Ramos-Neto & Pivello 2000), during the dispersion time of this species, being able to resist high temperatures during fire events should be important for germination during the rainy season.

Even though we found no direct response to fire-related cues in the germination of *M. leiocephala* and *B. dumosa*, seeds of both species tolerated the heat shocks they were exposed to, which could be seen as an adaptation to frequent burning (Overbeck *et al*. 2006). Moreover, both *M. leiocephala* and *B. dumosa* are resprouters (T Zupo unpubl. res.), suggesting that resprouting may be the dominant mechanism for persistence after fire in these species, as in many Cerrado species (Simon *et al*. 2009; Simon & Pennington 2012). On the other hand, germination percentage of *U. parviflorus* and *C. albidus* seeds were enhanced with F treatments, suggesting that temperatures reached with the passage of fire are effective in breaking seed dormancy and enabling germination in these species, which is particularly important for obligate seeder species, whose regenerating strategy after fire relays exclusively on seed germination and establishment (Bond & Midgley 2001; Pausas *et al*. 2004), as is the case for both these species (Baeza & Vallejo 2006; Santana *et al*. 2010).

This study has shown that germination response to fire-related cues may vary according to species and to the fire-prone ecosystems where they occur, which could be due to different fire regimes. Fire frequency in Cerrado is higher than in Mediterranean ecosystems, and thus, traits related to fire-resistance would be more advantageous in such ecosystems than traits related to post-fire recruitment, which are widespread among Mediterranean species. Moreover, Mediterranean species may present germination traits for both heat requirements and temperature fluctuations, enabling regeneration by seeds in post-fire environments, but also in fire free intervals.

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