

Clarifying the role of fire heat and daily temperature fluctuations as germination cues for Mediterranean Basin obligate seeders

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- **Background and Aims** This study aims to determine the role that both direct effects of fire and subsequent daily temperature fluctuations play in the seed bank dynamics of obligate seeders from the Mediterranean Basin. The short yet high soil temperatures experienced due to passage of fire are conflated with the lower, but longer, temperatures experienced by daily fluctuations which occur after removing vegetation. These germination cues are able to break seed dormancy, but it is difficult to assess their specific level of influence because they occur consecutively after summer fires, just before the flush of germination in the wet season (autumn).
- **Methods** By applying experimental fires, seed treatments were imposed that combined fire exposure/non-fire exposure with exposure to microhabitats under a gradient of disturbance (i.e. gaps opened by fire, mechanical brushing and intact vegetation). The seeds used were representative of the main families of obligate seeders (*Ulex parviflorus*, *Cistus albidus* and *Rosmarinus officinalis*). Specifically, an assessment was made of (1) the proportion of seeds killed by fire, (2) seedling emergence under field conditions and (3) seeds which remained ungerminated in soil.
- **Key Results** For the three species studied, the factors that most influenced seedling emergence and seeds remaining ungerminated were microhabitats with higher temperature fluctuations after fire (gaps opened by fire and brushing treatments). The direct effect of fire decreased the seedling emergence of *U. parviflorus* and reduced the proportion of seeds of *R. officinalis* remaining ungerminated.
- **Conclusions** The relevance of depleting vegetation (and subsequent daily temperature fluctuation in summer) suggests that studies focusing on lower temperature thresholds for breaking seed dormancy are required. This fact also supports the hypothesis that the seeding capacity in Mediterranean Basin obligate seeders may have evolved as a response to a wide range of disturbances, and not exclusively to fire.

Key words: Adaptation, *Cistus albidus*, exaptation, fire-adaptative trait, fire heat, post-fire germination, *Rosmarinus officinalis*, seed bank dynamics, seedling emergence, temperature fluctuation, *Ulex parviflorus*.

INTRODUCTION

Fire is one of the most recurrent disturbances in Mediterranean Basin (MB) ecosystems (Di Castri *et al.*, 1981) and, consequently, plant species should present strategies that enable them to persist. Species often resprout from the ground biomass that survives the passage of fire (resprouters), recruit new individuals from a fire-resistant seed bank (seeders) or combine both strategies (facultative species, *sensu* Keeley, 1986). Traditionally, the appearance of the different strategies in MB ecosystems has been associated with their lineage age (Herrera, 1992; Verdú, 2000). Normally, resprouters are taxa that evolved during the Tertiary period, before the Mediterranean climate appeared (Pausas *et al.*, 2006). In contrast, seeding capacity evolved later during the Quaternary period and concomitantly with the Mediterranean climate and frequent fires (Pausas and Verdú, 2005; Pausas *et al.*, 2006; Saura-Mas and Lloret, 2007). In fact, it is postulated that, in addition to climate and soil, fire has been an important

selective pressure in the evolution of the post-fire seeding trait (high germination and establishment ability immediately after fire; Keeley *et al.*, 2012).

In MB ecosystems, shrub species with an ‘obligate seeder’ strategy are limited to three families: Cistaceae, Fabaceae and Lamiaceae (Pausas and Verdú, 2005). Nevertheless, these families are a major component of fire-prone shrublands and they successfully regenerate from soil seed banks (Quintana *et al.*, 2004; Santana *et al.*, 2012). Cistaceae and Fabaceae seeds possess a hard coat that can allow them to persist in the soil seed bank over an extended period of time. In addition, this coat establishes a physical dormancy that heat can break and, consequently, trigger germination (Thanos *et al.*, 1992; Baeza and Vallejo, 2006; Paula and Pausas, 2008). Lamiaceae species are not hard coated, but some exhibit enhanced germination when seeds are exposed to heat and/or smoke (Moreira *et al.*, 2010). This directly fire-stimulated germination may enhance plant fitness by different mechanisms and may, thus, be under positive selection

(Keeley *et al.*, 2012). For example, seedlings can rapidly access more resources and grow more rapidly, time to maturity is shortened and the amount of seeds stored before the next fire can increase (Bond and van Wilgen, 1996; Ladd *et al.*, 2005; Verdú and Traveset, 2005).

Fire also leads to indirect effects that may strengthen the flush of germination and seedling establishment. The low red/far-red ratio experienced under canopies inhibits the germination of many species (Gorsky *et al.*, 1978; Rees, 1997), including Fabaceae and Cistaceae species (Roy and Sonié, 1992; Baeza and Roy, 2008). Therefore, microhabitat modification by canopy consumption may change the light spectrum and promote germination. Indirectly, the incidence of solar radiation promotes a shift in the range of daily soil temperatures by sustaining high temperatures over significant periods of time (Bradstock and Auld, 1995; Baeza and Roy, 2008). Recent studies have indicated that these fluctuations may also act as germination cues for species with physical dormancy (Baeza and Roy, 2008; Santana *et al.*, 2010b, 2012; Ooi *et al.*, 2012). Specifically, thresholds for breaking physical dormancy would be exceeded in summer, when daily soil temperatures reach the highest values and fluctuate more widely. In line with this, it has been suggested that, alternatively to fire heat, the seasonal climatic patterns of the Mediterranean climate (i.e. dry summer periods with high temperatures followed by wet, mild autumns) could also have participated in the selection of hard seed dormancy (Pausas *et al.*, 2006; Baeza and Roy, 2008). Seasonal dryness is the most limiting factor for seedling establishment in Mediterranean species (Baskin and Baskin, 1998). Thus, dormancy breakage by summer temperatures could avoid germination and seedling establishment failures before and during dry periods (Baeza and Roy, 2008).

One point that has been the object of little study is the specific influence that direct (heat shock produced by fire passage) and indirect (microhabitat modification and subsequent daily soil temperature fluctuations) effects of fire have on the breakage of seed dormancy. The short yet high soil temperatures experienced due to passage of fire are conflated with the lower, but longer, temperatures experienced by the daily fluctuations. These two germination cues occur consecutively after summer fires, just before the flush of germination in the wet season (i.e. autumn; Quintana *et al.*, 2004; Santana *et al.*, 2012). Thus, it is difficult to assess their specific influence independently by direct field observations. Many laboratory experiments have attempted to identify the optimal and lethal temperature thresholds for a wide range of species (see Paula and Pausas, 2008, and references therein); however, these experiments have mostly focused on fire temperatures and have neglected daily soil temperature fluctuations. Furthermore, laboratory experiments obviate the possible complex interactions between the direct effects of fire and the subsequent microhabitat modification. To answer the question of which factor is more important in determining seed bank dynamics (i.e. direct effects of fire vs. microhabitat modification) may throw light on which selective pressure has predominated in shaping the post-disturbance seeding trait.

This study first aims to determine the temperature patterns that can potentially affect seed bank dynamics during three experimental fires and subsequent daily fluctuations in summer. Its second aim is to determine the role that both direct effects

of fire and microhabitat modification play in the seed bank dynamics of obligate seeders from the MB. Specifically, we assessed (1) the proportion of seeds killed by fire, (2) seedling emergence under field conditions and (3) the seeds which remained ungerminated in soil. For this purpose, we placed containers with a controlled content of seeds in conditions combining fire exposure/non-fire exposure with exposure to microhabitats under a gradient of disturbance (i.e. gaps opened by fire, mechanical brushing and intact vegetation). The seeds used were representative of the species of the three families of obligate seeders: *Ulex parviflorus* (Fabaceae), *Cistus albidus* (Cistaceae) and *Rosmarinus officinalis* (Lamiaceae).

MATERIALS AND METHODS

Study area and site selection

The study was carried out inland in the Valencian Community (SE Spain) at three sites: Onil (38°39'N-0°39'W), Pardines (38°40'N-0°39'W) and Ayora (39°07'N-0°57'W). In all cases, study sites were shrublands with a history of recurrent fires (two fires in the last 25 years; Santana *et al.*, 2010a). At the onset of this study, vegetation consisted of shrublands (approx. 1–1.5 m in height) dominated by the obligate seeders *Cistus albidus*, *Rosmarinus officinalis* and *Ulex parviflorus*. Resprouting shrubs, such as *Quercus coccifera* and *Juniperus oxycedrus*, were scarce, and the grass *Brachypodium retusum* was the main herbaceous species. Altitude ranges between 900 and 1050 m a.s.l., and climate is typically Mediterranean. Mean annual rainfall ranges between 466 (Onil) and 537 mm (Ayora). There is a pronounced summer drought from June to August, with no more than 65 mm of rain at any site. The mean annual temperature is approx. 14 °C, and the mean maximum temperature for the hottest month (July) is 30 °C. To minimize environmental variability between sites, all the sites were northwardly oriented, located on marls and their soils were Regosols (FAO, 1988).

Monitoring of soil temperature during experimental fires

We selected one plot of approx. 30 m × 20 m at each site, where we set up an experimental fire. The areas to be burned were previously delimited by a 5 m wide fire break in which vegetation was eliminated through mechanical brushing. All three sites were burned in June 2006, and there was a 1 week interval between each experimental fire. Fires were ignited as a line encompassing the entire upwind flank of the experimental plot (head-fires). As a safety measure, fire fighters and forest rangers were present for each experimental fire. In these ecosystems dominated by obligate seeders, the maximum density of seeds stored in the seed bank was found in the first centimetre of the soil profile (2–5 times greater than in deeper layers; Ferrandis *et al.*, 1999; Baeza, 2001; Traba *et al.*, 2004; Clemente *et al.*, 2007). Following this premise, we measured the soil temperature at a 1 cm depth. For this purpose, 15 insulated chrome–alumel thermocouples (K-type) were distributed throughout each plot. Thermocouples were equally distributed following a stratified design under main species patches, i.e. *B. retusum*, *C. albidus*, *R. officinalis* and *U. parviflorus* (Santana *et al.*, 2011).

Thermocouples were protected from fire by a stainless steel sheath and were connected to a data logger (CR1000; Campbell Scientific, North Logan, UT, USA). Records were taken from 20 min before the experimental fire was initiated until 2 h after the fire had been extinguished. Soil temperature was recorded every 10 s. For more details of fire performance and behaviour, see [Santana et al. \(2011\)](#). It is worth noting that fire temperatures in experimental fires are expected to be lower and of shorter duration than in natural fires, where environmental conditions are more severe.

Monitoring of daily soil temperature throughout summer

After the experimental fires, we discerned three different microhabitats under a gradient of disturbance for assessing summer soil temperatures: (1) the canopy gaps deriving from the complete consumption of vegetation by fire (gap hereafter); (2) mechanical brushing located in the 5 m wide fire break contiguous to the experimental fires (here, all woody vegetation was eliminated through mechanical brushing to be left lying on the soil surface; brushing hereafter); and (3) intact vegetation contiguous to the experimental burning plots (vegetation hereafter). Treatments represented a gradient in soil cover, from 0 % for gaps to 100 % in vegetation. Brushing had intermediate values (approx. 60 %).

Soil temperatures were recorded in all three microhabitats over a 2 month period in summer 2006 (from July 21 to September 21). They were recorded every hour with a temperature probe (Hobo[®] Event, Onset Computer Corporation, Bourne, MA, USA). Similarly to fire temperatures, soil temperatures were also measured at a 1 cm depth. A narrow hole (3 cm wide, 2 cm deep approximately) was excavated and sensors were inserted horizontally at the desired depth. Then, soil was restored as closely as possible to its original state. If there was any litter present, it was removed and replaced after setting up the sensors.

Monitoring of seed bank dynamics

To explore the role that both direct effects of fire and microhabitat modification have on seed bank dynamics, we employed a set of containers with a controlled content of seeds. These containers were distributed within an experimental design which combined the fire exposure/non-fire exposure with a later exposure to different microhabitats. Containers were rectangular and handmade, with a 0.5 mm stainless steel mesh that was open at the top (11 × 7 × 7 cm; L × W × H). The mesh was big enough to allow water permeability, but avoided loss of seeds. We used seeds of the dominant species: *C. albidus* (*Cistus* hereafter), *R. officinalis* (*Rosmarinus* hereafter) and *U. parviflorus* (*Ulex* hereafter). *Cistus* and *Ulex* are obligate seeders with hard-coated seeds broken by heat, for example they undergo enhanced germination if exposed to dry heat (80–120 °C for 5–10 min; [Baeza and Vallejo, 2006](#); [Moreira et al., 2010](#)). *Rosmarinus* is a soft-seeded species in which germination is stimulated by high temperatures (80–120 °C, 5–10 min) and smoke (liquid smoke solution; [Moreira et al., 2010](#)). The seeds of *Rosmarinus* and *Ulex* were collected by hand early in summer 2006 from at least 20 plants at the Pardines site. We collected seeds when fruits

ripened, but just before release from the parent plant. For *Cistus*, seeds were collected directly from plant capsules in late August 2005. All the seeds were stored under laboratory conditions (dry; <22 °C) until they were processed for treatment applications in June 2006. In order to check the initial degree of physical dormancy in hard-coated species (*Ulex* and *Cistus*), we submerged 100 seeds of each species in distilled water for 24 h. Imbibed seeds gave us an estimation of non-dormant seeds (8 % for *Cistus* and 1 % for *Ulex*). Containers were first filled with soil with similar site characteristics, but which was extracted from a semi-arid zone (>35 km away from the experimental sites) where the studied species were absent. We placed 30 seeds of each species at a 1 cm depth. Then, the container was filled completely and buried in each microhabitat (see below for more details). After burying the containers, the microhabitat was restored as closely as possible to its original state.

In order to arrange the containers exposed directly to fire effects, 20 containers were buried within each burned plot the day before the experimental fires. Since fire intensity was likely to increase as the flame front moved upslope, containers were distributed as uniformly as possible within a 10 × 10 m square in the centre of the plots. The day after the fire, these containers were extracted and divided randomly into sets of five. Then, they were placed in the three different microhabitats: gap, brushing and vegetation. To do this, a 5 × 5 m plot was laid out within each microhabitat, and five points within this plot were randomly chosen from a 1 × 1 m grid (Fig. 1). The remaining five containers were taken to the laboratory to check the proportion of seeds killed by fire. Additionally, we arranged a second set of 20 containers that had not been exposed to the direct effect of fire. They were placed the day after the fire together with the other containers at each point and microhabitat (Fig. 1). In summary, the experimental design allowed us to provide six treatments that experienced summer conditions in different microhabitats, and which were exposed or not exposed to fire. Specifically: (1) seeds exposed only to the gap environment after fire; (2) seeds exposed to the effects of fire plus the gap environment; (3) seeds exposed to brushing; (4) seeds exposed to fire plus brushing; (5) seeds exposed to vegetation; and (6) seeds exposed to fire plus vegetation (Fig. 1).

With these containers, we determined different seed bank dynamics parameters. First, we assessed the proportion of seeds killed by fire by means of containers extracted immediately after fire. Secondly, we assessed seedling emergence under field conditions over the next 2 years (approximately every 10 d in the first year and every month in the second year). Once seedlings had been identified, they were removed, and the depth of emergence was carefully checked. Thirdly, we assessed the proportion of seeds which remained ungerminated in soil. In summer 2008, 2 years after beginning treatments, all the containers were extracted and taken to the laboratory. There, the soil from each container was removed from the container, disaggregated and sieved (0.5 mm mesh). This allowed us to recover the seeds that remained ungerminated directly. The viability of the recovered seeds was checked by cutting and soaking them in tetrazolium solution (1 % concentration) for 24 h. Only those embryos which stained completely red or pink were considered viable. As

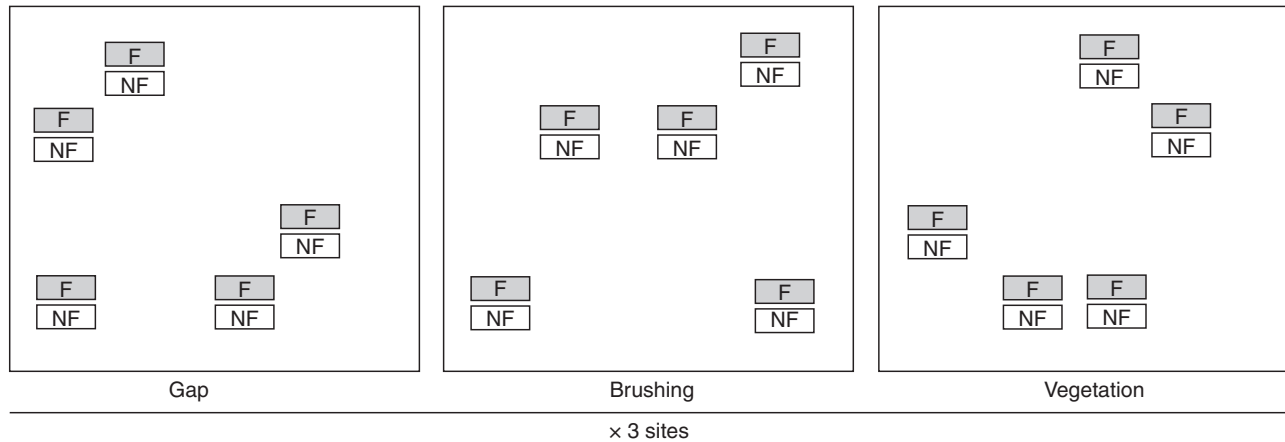


FIG. 1. Arrangement of the containers within the experimental design (F = containers exposed to fire, NF = containers not exposed to fire).

we carefully checked the depth of seedling emergence and the seeds clearly seen on the soil surface were discarded, we assumed that there were no losses or inputs of seeds as regards the initial pool.

Statistical analysis

The proportion of seeds killed by fire was analysed by means of *t*-tests. The seeds of those containers from the same site were pooled and then compared with three similar sets from the initial pool of seeds.

The roles that direct effects of fire and microhabitat modification play in seedling emergence and ungerminated seeds were analysed, in a first step, using Generalized Linear Models (GLMs). In model construction, we considered fire exposure (fire and no fire) and microhabitat (gap, brushing and vegetation) as fixed effects. The interaction between microhabitats and fire exposure was also considered in the GLM. Starting from the full model, the minimal adequate GLM was obtained by sequential removal of non-significant model terms (analysis of deviance, *F*-tests, $P > 0.005$). Since we knew the initial number of seeds, the data showed a binary response (i.e. a seed either emerged or did not, or remained or did not). Thus, we assumed a binomial distribution with a logit-link function. The logits in the baseline were defined by vegetation in microhabitats and the non-fire exposure. Because random effects are not included in GLM, in a second step, we used Generalized Linear Mixed Models (GLMMs) to include these effects (i.e. sites and points where containers were laid out) in the previously selected models (Bolker *et al.*, 2009).

The GLMs and GLMMs were performed using the MASS package in the R software environment (2.14.2; R Development Core Team, Vienna, Austria, <http://r-project.org/>). We used the glm and glmmPQL (the penalized quasi-likelihood approach) functions to fit the data (Bolker *et al.*, 2009).

RESULTS

Soil temperatures during and after summer fires

During the experimental fires, the maximum temperatures recorded at a 1 cm depth ranged from 81 to 99 °C (Table 1).

TABLE 1. Maximum temperature, mean maxima and temperature residence times observed for the different experimental fires

Site	Maximum observed (°C)	Mean maxima (°C, mean ± s.d.)	Residence time (h)	
			>40 °C (mean ± s.d.)	>50 °C (mean ± s.d.)
Onil	99.1	42.7 ± 17.1	0.4 ± 1.1	0.1 ± 0.6
Pardines	80.7	45.6 ± 14.5	1.1 ± 1.5	0.1 ± 0.1
Ayora	94.8	47.8 ± 15.1	0.2 ± 0.4	0.1 ± 0.1

Temperatures were recorded by means of thermocouples ($n = 15$) at a 1 cm depth of the soil profile.

The time at which the temperature was >40 °C (temperature residence time) varied, ranging between 0.4 and 1.1 h for the different fires. Temperatures >50 °C lasted a relatively short time and did not exceed 0.1 h in any case (Table 1).

Soil temperatures during the subsequent summer were variable, depending on microhabitats. Gap experienced the highest soil temperature values, with mean daily maxima ranging from 49 to 53 °C at all three sites. A maximum of 68 °C was observed at the Pardines site (Table 2) and the residence times at temperatures >40 °C fell within the 5.8–2.1 h range per day. These residence times decreased at higher temperatures to 0.3–0.6 h d⁻¹ at temperatures of >60 °C (Table 2). Vegetation, in contrast, presented the lowest temperature values; the mean daily maximum was approx. 22 °C and the absolute maxima observed did not exceed 29 °C in any case (Table 2). Brushing had intermediate values; the mean daily maximum ranged from 32 to 39 °C, and the maximum temperature recorded was 56 °C (Table 2). The residence times >40 °C were low and did not exceed 2 h d⁻¹ (Table 2).

Seed bank dynamics

Passage of fire marginally reduced seed viability in *Ulex* from 99.3 ± 1.2 % (mean ± s.d.) to 92.7 ± 4.8 %, but not significantly (Student's *t*-test, $n = 3$, $F = 2.79$, $P = 0.081$). In

TABLE 2. Maximum temperature, mean daily maxima and temperature residence times observed for different microhabitats in summer 2006

Site	Microhabitat	Maximum observed (°C)	Mean daily maxima (°C, mean \pm s.d.)	Residence time (h)		
				>40 °C (mean \pm s.d.)	>50 °C (mean \pm s.d.)	>60 °C (mean \pm s.d.)
Onil	Gap	65.8	49.0 \pm 9.9	4.9 \pm 3.1	2.1 \pm 2.3	0.3 \pm 0.8
	Brushing	41.1	31.7 \pm 5.9	0.1 \pm 0.3	0	0
	Vegetation	28.7	22.7 \pm 3.1	0	0	0
Pardines	Gap	68.3	52.9 \pm 11.0	5.8 \pm 3.3	2.9 \pm 2.5	0.6 \pm 1.3
	Brushing	53.3	38.7 \pm 8.3	1.9 \pm 2.5	0.2 \pm 0.6	0
	Vegetation	27.5	22.1 \pm 2.9	0	0	0
Ayora	Gap	65.0	51.9 \pm 10.9	2.1 \pm 2.5	1.1 \pm 1.6	0
	Brushing	56.0	33.9 \pm 7.7	0.6 \pm 1.7	0.2 \pm 1.1	0
	Vegetation	25.9	21.5 \pm 2.8	0	0	0

Temperatures were recorded over 63 d (from 21 July to 21 September) at a 1 cm depth of the soil profile.

Rosmarinus, fire significantly reduced viability from 90.2 ± 1.1 to 40.2 ± 24.6 % ($F = 14.69$, $P = 0.025$). *Cistus* seeds showed no significant changes ($F = 2.62$, $P = 0.399$), and the initial viability of 94.2 ± 1.6 % changed to 91.4 ± 4.9 %.

Seedling emergence occurred mainly in the first year after fire. Of the total emergences, 77 % for *Ulex*, 74 % for *Rosmarinus* and 93 % for *Cistus* occurred in the first year. In addition, this emergence varied in accordance with treatments. For *Ulex*, gap and brushing had a significant effect on increasing seedling emergence (regard vegetation treatment as the baseline; Table 3, Fig. 2). Fire exposure also had a significant effect ($P = 0.022$, Table 3) and reduced emergence by approx. 5 % compared with treatments without fire (Fig. 2). For *Cistus*, only gap had a significant effect (Table 3, Fig. 2), while for *Rosmarinus* gap and brushing affected seedling emergence (Table 3).

Persistence of the soil seed bank also varied in accordance with treatment. For *Ulex*, gap and brushing negatively affected the percentage of remaining seeds (Table 3). There was also an interaction between fire and gap that increased the seeds remaining (Table 3, Fig. 2). For *Cistus*, gap and brushing had a significant effect (Table 3), whereas for *Rosmarinus* gap, brushing and fire negatively affected the percentage of remaining seeds (Table 3, Fig. 2).

DISCUSSION

Microhabitat disturbance by vegetation depletion may induce significant soil heating during summer periods. In the gaps which opened by fire, or even in brushing treatments, daily temperature fluctuations may exceed the thresholds for stimulating seed germination in some MB species. Very few studies have identified the lower thresholds for breaking seed dormancy; however, in some 'obligate seeders' (i.e. species of the Cistaceae, Lamiaceae and Leguminosae families), germination can be seen to be enhanced after being exposed to sustained temperatures of between 40 and 60 °C (for some examples, see Trabaud and Casal, 1989; Baeza and Roy, 2008). In addition, this syndrome has also been observed for Leguminosae species from Australian fire-prone areas (Auld and O'Connell, 1991; Tieu et al., 2001; Santana et al., 2010b). Consequently,

germination in heat-stimulated species cannot be understood by taking into account only fire temperatures. It is worth noting that although soil experiences its maximum temperatures during passage of fire (80–100 °C), durations above the stimulating temperature thresholds (40–60 °C) were short (a few minutes) if compared with those experienced in the subsequent summer (a few hours every day).

The relevance of microhabitat modification is reflected in the seedbank dynamics observed in this study. The gaps opened by fire and, to a lesser extent, the brushing treatment were the most significant factors in determining both seedling emergence and the amount of seeds remaining in soil. Direct exposure to fire did not enhance seedling emergence in any species; seedling emergence was even reduced by fire in *U. parviflorus*. These results indicate that daily temperature fluctuation could, in some cases, have a greater influence than fire temperatures during the germination and emergence of heat-stimulated species. In line with this, Trabaud and Casal (1989) observed for *R. officinalis* that, while treatments mimicking fire temperatures gave negligible results, the highest germination rates were noted in those exposed to 40 and 60 °C for 24 h. For *U. parviflorus*, Baeza and Roy (2008) reported that daily exposures (12 h/12 h) to alternating temperatures of 15/45 °C resulted in similar germination rates (approx. 80 %) to treatments mimicking fire temperatures. It should be taken into account, however, that the intensity of fire temperatures may modulate the specific influence of daily temperature fluctuations. In this study, the emergence of *C. albidus* seedlings was low and was mainly determined by the opening of gaps, but fire temperatures were low and of short duration [in comparison with other experimental fires in similar shrublands (Baeza et al., 2002a; De Luis et al., 2004) or natural fires with more severe environmental conditions]. In contrast, Céspedes et al. (2012) observed that germination in *C. albidus* was clearly stimulated by fire after an experimental fire. After a wildfire, Ferrandis et al. (1999) found that for three species of Cistaceae (*Halimium ocyroides*, *C. ladanifer* and *C. salvifolius*), the direct effect of fire was mainly responsible for seed germination (approx. 70 % of the total seed bank). However, these authors suggested that there were other environmental factors involved in seed

TABLE 3. Parameters estimated by the GLMM models for seedlings emerged and seeds remaining ungerminated in soil

Species	Fixed effects	Estimate	s.e.	d.f.	t-value	P-value
Seedlings emerged						
<i>Ulex</i>	Intercept	-1.747	0.214	44	-8.163	< 0.001
	Brushing	1.418	0.210	40	6.748	< 0.001
	Gap	1.377	0.210	40	6.547	< 0.001
	Fire	-0.340	0.143	44	-2.375	0.022
<i>Cistus</i>	Intercept	-4.285	0.395	44	-10.824	< 0.001
	Brushing	0.516	0.456	40	1.133	0.264
	Gap	1.858	0.414	40	4.489	< 0.001
	Fire	0.406	0.207	44	1.963	0.056
<i>Rosmarinus</i>	Intercept	-6.301	1.819	42	-3.464	0.001
	Brushing	3.745	1.804	40	2.076	0.044
	Gap	5.013	1.789	40	2.801	0.007
	Fire	2.518	1.841	42	1.367	0.178
	Brushing × fire	-1.672	1.880	42	-0.889	0.379
Seeds remaining ungerminated						
<i>Ulex</i>	Intercept	0.743	0.191	42	3.891	0.004
	Brushing	-2.270	0.293	40	-7.725	< 0.001
	Gap	-1.866	0.279	40	-6.692	< 0.001
	Fire	-0.010	0.237	42	-0.043	0.965
	Brushing × fire	0.363	0.363	42	1.001	0.322
	Gap × fire	0.734	0.338	42	2.166	0.036
	Fire	0.696	0.147	45	4.718	< 0.001
<i>Cistus</i>	Intercept	-0.446	0.206	40	-2.166	0.036
	Gap	-0.613	0.205	40	-2.983	0.004
	Fire	0.445	0.205	44	2.168	0.035
<i>Rosmarinus</i>	Intercept	-1.343	0.214	40	-6.271	< 0.001
	Gap	-3.359	0.381	40	-8.821	< 0.001
	Fire	-1.033	0.204	44	-5.040	< 0.001
	Fire	-1.033	0.204	44	-5.040	< 0.001

P-values < 0.05 are highlighted in bold.

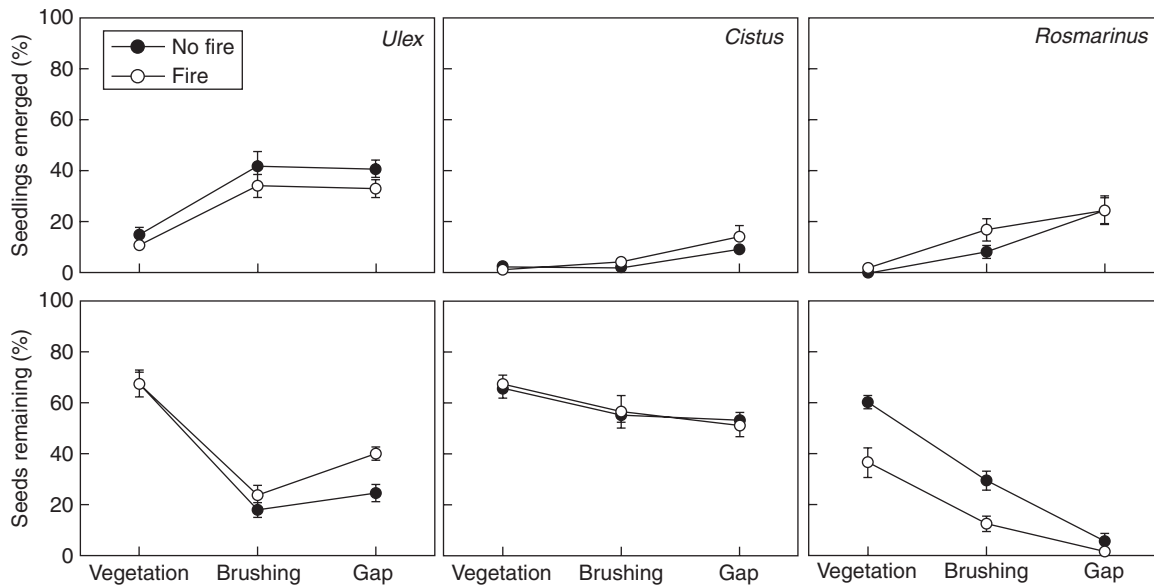


FIG. 2. Fate of the seeds placed inside the containers in the different microhabitats and for the three species. Bars represent the standard error ($n = 15$).

softening (mainly temperature fluctuation) since seed bank depletion was always >90 %. Therefore, despite daily temperature fluctuations possibly playing a decisive role in seed bank dynamics, further studies are required to determine their specific level of influence, in terms of fire temperature intensity, as well as possible complex interactions with other direct fire cues (e.g. smoke). Indeed, this modulated response

may differ according to species since, for example, *U. parviflorus* and *R. officinalis* viability was sensitive to these low intensity temperatures, unlike *C. albidus*.

The important role of microhabitat modification (and the subsequent daily temperature fluctuation in summer) supports the hypothesis of climate being a decisive selective pressure in MB obligate seeders (Pausas *et al.*, 2006; Baeza and Roy,

2008). Breakage of seed dormancy by summer temperatures may enhance plant fitness via different mechanisms. For example, it may avoid germination and seedling establishment failures before and during a dry summer period. This response is also critical in the species' ability to detect vegetation gaps, where not only is competition with other species poor, but the inhibiting low red/far-red ratio noted under canopies was absent (Gorsky *et al.*, 1998; Rees, 1997). Nonetheless, and despite this possible relevance, there are very few studies available which have investigated the role of seasonal climatic patterns on the seed germination and seedling emergence of MB species. After an experimental fire performed in October (autumn), De Luis *et al.* (2008) found that Cistaceae (*Helianthemum marifolium* and *C. albidus*) and Fabaceae species (*Ononis fruticosa* and *U. parviflorus*) displayed a bimodal pattern of germination, with the first peak just after burning and a second one in the following autumn (after summer). Baeza and Roy (2008) noted a similar pattern in *U. parviflorus* after an autumn brushing. Similarly, in fire-prone ecosystems from Australia, Ooi *et al.* (2012) also found that Leguminosae species with physical dormancy germinate readily after being exposed to summer temperature fluctuation. The scarcity of studies that determine the role of seasonal climatic patterns in seed bank dynamics of fire-prone areas is surprising, especially when considering that physical and other dormancy types in arid systems and other less fire-prone systems depend on season-long high temperature fluctuations being broken (Baskin and Baskin, 1998; Ooi *et al.*, 2009).

The seeding capacity of MB species evolved during the Quaternary period and concomitantly with the Mediterranean climate and frequent fires (Pausas *et al.*, 2006; Saura-Mas and Lloret, 2007). In this sense, a question that arises in this study is whether the post-fire seeding trait is an adaptation to fire or, if instead, it is an exaptation from the species' ability to respond to seasonal temperature fluctuations (for a clear explanation of these concepts, see Bradshaw *et al.*, 2011; Keeley *et al.*, 2011). Studies that have worked with a wide range of MB species have denied that germination is exclusively linked to fire heat (Buhk and Hesen, 2006; Luna *et al.*, 2007); i.e. post-fire recruitment was probably the result of seeds being tolerant to fire rather than being stimulated directly by it. Most of the species studied, including the three herein, were able to germinate in response to moderate pulses of heat (approx. 80 °C for a few minutes), but the germination of only a few species was stimulated by fire peak temperatures (100–120 °C). Even these high temperatures were deleterious (in terms of seed viability) in most species (Baeza *et al.*, 2002b; Buhk and Hesen, 2006; Luna *et al.*, 2007; Reyes and Trabaud, 2009). Additionally, several germination experiments applying other fire products, such as smoke and charred wood, obtained poor results in MB flora (with only some response in the *Lamiaceae* species; Keeley and Baer-Keeley, 1999; Reyes and Trabaud, 2009; Moreira *et al.*, 2010). This limited evidence for fire-cued germination in MB species supports the results obtained in this study, thus suggesting that climatic factors are a decisive selective pressure on the post-fire seeding trait (but see Lloret *et al.*, 2005; Moreira *et al.*, 2012). These results contrast with those obtained in studies into other Mediterranean-type ecosystems, such as the

Californian chaparral, where direct fire cues are essential for the germination of main seeder species (Tyler, 1995; Keeley and Fotheringham, 2000). In this case, it is postulated that the rise of seeding capacity pre-dates the appearance of the Mediterranean climate, during the Tertiary period and under other fire-prone climatic conditions (Pausas *et al.*, 2006).

In summary, the response of MB seeder species to seasonal temperature fluctuations suggests that seeding capacity may have evolved in response to a wide range of disturbances, and not exclusively to fire (Ackerly, 2004). The seeder trait is associated with species that act as opportunistic colonizers (Verdú, 2000); thus, it may also have evolved in response to the long history of disturbance in the MB by either human use or herbivory.

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