The effect of fire on seed germination of campo rupestre species in the South American Cerrado

Alessandra Furtado Fernandes [1], Yumi Oki [2], Geraldo Wilson Fernandes [2], Bruno Moreira [1,3*]

[1]Centre for Functional Ecology, Department of Life Sciences, University of Coimbra, Calçada Martim de Freitas, 3000-456, Coimbra, Portugal

[2]Evolutionary Ecology and Biodiversity/DGEE, ICB/Universidade Federal de Minas Gerais, Belo Horizonte, 30161-970, Brazil

[3]CIDE-CSIC, Campus IVIA, Ctra. Náquera Km. 4.5, 46113, Montcada, Valencia, Spain

Abstract

Fire is an important disturbance in terrestrial ecosystems and plays a key role in the germination process and seedling establishment of many species. In grassland ecosystems, seeds normally tolerate heat-shocks associated with low intensity fires but are negatively affected by higher heat doses. Additionally, smoke-stimulated germination is not commonly recognized as important in neotropical grasslands. This may result from a negative relationship between the ability to resprout after fire and fire-stimulated germination ability, as many species in these grasslands regenerate well after fire by resprouting. To ascertain the role of heat and smoke in the post-fire germination response of campo rupestre vegetation, we exposed seeds of nine species (that regenerate after fire by resprouting) to various treatments related to fire (heat, smoke, and heat plus smoke) and analysed their germination behaviour. Our results suggest that these species have high seed germination in the absence of specific dormancy breaking conditions and/or that stimulated germination triggered by fire-related cues is not common. Additionally, while seeds from some species can withstand temperatures of 80 °C for 5 min without affecting germination, most species are negatively affected by higher heat doses. However, our results suggest an important role of smoke enhancing germination rate (speed) of campo rupestre species. In view of the characteristics of campo rupestre environmental conditions and vegetation, we provide a novel outlook of enhanced germination rate by smoke as an important mechanism for seed regeneration after fire of campo rupestre's species that resprout, particularly for seeds resulting from post-fire seed dispersal.

ArticleNote

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Introduction

Fire is an essential factor in the formation and maintenance of several biomes worldwide (Bond and Keeley **2005**). Fire is particularly important for the maintenance of open vegetation formations in South American Cerrado savannas have occurred naturally in the region for more than 4 million years (Simon et al. **2009**).

The Brazilian campo rupestre (or rupestrian grasslands) is a Neotropical ecosystem and part of the Cerrado biome. In addition to fire, edapho-climatic factors, such as poor and shallow soils, hamper tree growth and help maintain its open physiognomy (Fernandes **2016**). It includes a high diversity of communities, under different environmental conditions, such as rock outcrops dominated by drought-tolerant species, but also grasslands and shrublands dominated by resprouter species (Conceição et al. **2016**). Water availability is expected to be more important on rock outcrops, with an increasing importance of fire on grasslands and shrublands. Fires in the campo rupestre are typically fast and of low intensity (Figueira et al. **2016**). Soil temperature increases during fire are generally small, and the maxima observed are likely to have little direct effect on buried seeds, or underground organs of plants. However, temperatures and residence times near the soil surface, that can affect recently dispersed seeds may be much higher (Miranda et al. **1993**).

Understanding the fire ecology of this region is important not only to assist with ecological restoration of degraded areas but also in the implementation of fire management programs (Fernandes **2016**). Previous studies show that after fire, most species in campo rupestre regenerate well by resprouting (Hoffmann **1998**; Barbosa et al. **2014**; Le Stradic et al. **2018a**). Additionally, several species produce flowers from resprouting individuals within the 1st years after fire (Ribeiro et al. **2007**; Conceição and Orr **2012**; Le Stradic et al. **2015a**). Generally, the post-fire environment is favourable for plant growth and reproduction because the higher availability of soil nutrients and light and lower competition (Lamont and He **2017**; Pyke **2017**). Thus, even though the small number of species exhibiting fire-dependent flowering (i.e., flowering only after a fire), post-fire flowering species are relatively common across fire prone areas (Lamont and Downes **2011**; Pyke **2017**).

On the contrary, post-fire seedling recruitment from soil seed banks is not so common in campo rupestre. Post-fire germination may be triggered by different mechanisms, with heat and smoke-derived compounds being the main fire-related germination cues (van Staden et al. **2000**). Heat can trigger germination by accelerating after-ripening in species with a water-permeable seed coat, or by rupturing the seed coat layer, allowing water uptake, in species with water-impermeable seeds (Baskin and Baskin, **2014**). Smoke-stimulated germination is mainly attributed to the presence of butenolide molecules (karrikins), a family of structurally related plant growth regulators (Flematti et al. **2004**; Nelson et al. **2009**) but there are probably many different compounds responsible for stimulating germination (Keeley and Pausas **2018**).

Post-fire seedling recruitment from soil seed banks may be limited by the fact that campo rupestre plant communities present soil seed banks with low seed density and species richness (Medina and Fernandes **2007**, Luz et al. **2018**), most species produce non-dormant seeds (Le Stradic et al. **2015a**; Silveira et al. **2016**), only show moderate resistance to heat shock (Ribeiro et al. **2013**) and stimulated germination percentage by fire-related cues is not common (Le Stradic et al. **2015a**; Oliveira et al. **2016**). Additionally, most species of the campo rupestre, particularly those with small seeds, typically present a positive photoblastic behaviour (Abreu and Garcia **2005**; Oliveira and Garcia **2011**; Mota and Garcia **2013**). This could result in a portion of the seed pool (if buried) remaining dormant until a fire occurs, if dark inhibition is overcome by fire-related cues (e.g., Keeley **1987**). Yet, to the best of our knowledge, there is no study on dark inhibition being overcome by fire-related cues for campo rupestre's species.

Recent studies suggest that Cerrado seeds show complex responses to fire cues. Zirondi et al. (**2019**) showed that species with impermeable seed coats generally lack dormancy broken by heat shock, but the combination of heat and smoke increases germination in several species, differing from other-fire prone ecosystems (e.g., Mediterranean vegetation). These authors suggest that these complex responses to fire cues may be because plants in the Cerrado are subject to higher fire frequencies and heterogeneity (Miranda et al. **1993**), comparing to other fire-prone ecosystems such as Mediterranean type environments (Zirondi et al. **2019**). Another important, yet unexplored, role of fire-related cues on campo rupestre species is regarding enhanced germination rate after post-fire seed dispersion from post-fire resprouting species. Le Stradic et al. (**2015a**, **b**) showed that species resprouting and flowering after fire had high and rapid germination, suggesting an adaptive advantage for regeneration after fire. Considering the environmental conditions of campo rupestre, where plants have to establish in shallow, nutrient-poor and water-limited soils, that makes plant recruitment from seeds very difficult (Nunes et al. **2016**), and the characteristics presented by plants (i.e., frequent post-fire resprouting and quick flowering and fruit production, high germination percentage without any dormancy break treatment and/or germination percentage not stimulated by fire-related cues, low seedbank density and only moderate resistance to heat shock), enhanced germination rate after post-fire seed dispersion might allow plants to take full advantage of the favourable

conditions for plant growth and reproduction of the post-fire environment. The active compounds of smoke affecting seed germination have been shown to remain for a long time in the soil (Preston and Baldwin **1999**) and could have an important role on seeds dispersed from species showing quick post-fire resprouting, flowering and fruit production. This effect is expected to be particularly important in species with seeds with water permeable seed coats because it would not require the action of heat shock to overcome physical dormancy (Chamorro and Moreno **2019**, Zarondi et al. **2019**).

We hypothesise that, in campo rupestre, fire stimulated seedling recruitment from soil seed banks is negligible. However, enhanced germination rate by smoke-derived compounds (henceforth "smoke" will be used as a shorthand), acting on seeds produced as a result of post-fire resprouting, may still be important for post-fire seedling recruitment. Thus, we expect that species in campo rupestre (1) will have a high germination percentage without any dormancy break treatment (control), (2) do not have germination percentage stimulated by fire-related cues (Le Stradic et al. **2015a**; Nunes et al. **2016**), (3) are negatively affected by high temperatures (Ribeiro et al. **2013**), but (4) have germination rate enhanced by smoke. We also expect (5) a positive photoblastic behaviour in germination, which is not overcome by fire-related cues, in accordance with fast recruitment after dispersal and not from the soil seed bank.

Material and methods

Study area and species selection

Seed collection was carried out in Serra do Cipó, Brazil (between the parallels 19 and 20° S and 43 and 44° W in the southern portion of the Espinhaço mountain range) in areas with altitudes varying from 900 to 1100 m a.s.l., with dominant vegetation of campo rupestre. The regional climate is classified as tropical altitudinal, with rainy summers and dry winters (Cwb, Köppen). The annual rainfall is ca. 1370 mm and annual average temperature ranges from 20° to 22 °C, with maximum reaching 34° to 36 °C in the dry season and minimum ranging from 0° to 4 °C. Despite the seasonal climate, campo rupestre vegetation produces flowers and fruits all year long without a clear seasonal pattern (Le Stradic et al. **2018b**). Most fires in campo rupestre occur between the end of the dry season and the beginning of the wet season (Alvarado et al. **2017**).

Species selected belong to the most common and abundant families in the studied plant community (Asteraceae, Poaceae, Velloziaceae and Xyridaceae): *Lychnophora ericoides* Mart. (Asteraceae), *Echinolaena inflexa* (Poir.) Chase, *Homolepis longispicula* (Döll) Chase (Poaceae), *Xyris pilosa* Kunth (Xyridaceae), *Vellozia caruncularis* Mart. ex Seub, *Vellozia epidendroides* Mart. ex Schult. & Schult.f., *Vellozia resinosa* Mart. ex Schult. & Schult.f., *Vellozia variabilis* Mart. ex Schult. & Schult.f., and *Vellozia nanuzae L.B.Sm. & Ayensu* (Velloziaceae). All species are perennial hemicryptophytes (except for Lychnophora ericoides and Vellozia variabilis which are nanophanerophyte) which can resprout from underground buds and organs which remain viable after fire (Fidelis et al. **2010**; Le Stradic et al. **2015b**, **2018b**). Although these are not fire-dependent flowering species (Lamont and Downes **2011**; Pyke **2017**), they typically resprout and flower within 1 year after fire (Table **1**).

Table 1 Growth form (NA: Nanophanerophyte, He: Hemicryptophyte) and fire-related traits (post-fire resprouting and flowering) for the studied species: *Lychnophora ericoides*, *Echinolaena inflexa Homolepis longispicula*, *Xyris pilosa*, *Vellozia caruncularis*, *Vellozia epidendroides*, *Vellozia resinosa*, *Vellozia variabilis* and *Vellozia nanuzae*

Family	Species	Life form	Fire-related traits		
			Resprout and flowering		References
Asteraceae	*Lychnophora ericoides*	Na	Resprouts and flowers within 12 months after fire		Ribeiro-Silva et al. (**2017**)
Poaceae	*Echinolaena inflexa*	He	Resprouts and flowers within 12 months after fire		Moraes et al. (**2016**)
	Homolepis longispicula	He	Resprouts and flowers within 12 months after fire		Le stradic et al. (**2015a**)
Xyridaceae	*Xyris pilosa*	He	Resprouts after fire		Le Stradic et al. (**2015a**)
Velloziaceae	*Vellozia caruncularis*	HeHeHeNaHe	Example of resprout and flower soon after fire in *Vellozia sincorana*		Conceição and Orr (**2012**)

	V. epidendroides			
	V. resinosa			
	V. variabilis			
	V. nanuzae			

Seeds of each species were collected from at least 50 different randomly selected individuals presenting fruits and at least 10 m apart. Seed collection was performed considering the fructification period of each species (from June to August 2015). Seeds were collected in paper bags, taken to the laboratory for processing (cleaning) and stored at 20 °C in dark conditions until the beginning of the experiments in September 2015.

Germination experiments

Smoke and heat treatments

For each species we used 5 replicates of 25 seeds per treatment (4 replicates in the case of *L. ericoides* and *E. inflexa*, due to insufficient number of seeds). Since many of campo rupestre species present a large proportion of unviable seeds (Dayrell et al. **2016**), seeds were screened before applying any treatment in order to discard any that did not contain an embryo or exhibited symptoms of decay and exclude them from use in the experiment. Two heat, one smoke, and one combined treatment of heat and smoke were then performed. Additionally, a control treatment (seeds not subjected to heat or smoke) was used. All five treatments were duplicated in light and dark conditions. We performed a trial with a small set of seeds, before the start of the experiment, in order to test the effect of smoke concentration in the germination of the species that would be used. For this we used several dilutions (1:10, 1:100, 1:200) and, in all cases, germination in response to the diluted solutions was equal or lower to germination in response to the concentrated solution. For this reason, we used only the concentrated smoke solution in the experiment.

For the heat treatments, we choose temperatures of 80° or 100 °C and an exposure time of 5 min which been used in previous works on Cerrado (Ribeiro et al. **2013**; Le Stradic et al. **2015a**) based in data obtained during prescribed fires (Miranda et al. **1993**). This aims to simulate the range of temperatures and exposure time in the top layer of soil or during more intense fires that are expected from changes in fire regimes associated to fire exclusion (resulting in the accumulation of biomass, increasing the intensity and severity of fires). These temperatures are also within the range of temperatures and residence times used in other studies on grassland species worldwide, allowing comparison (Clarke and French 2005; Dayamba et al. **2008**, **2010**; Gonzales and Ghermandi **2012**; Tavşanoğlu et al. **2015**; Cox et al. **2017**). For each heat treatment, seeds were placed in aluminium dishes (one for each species), which were arranged randomly in the centre of a tray to avoid the extremes and thus minimize the variation in temperature, and the tray was quickly introduced into an electric oven (pre-heated at the desired temperature). This operation was repeated five times for each treatment and species (five independent replicates, Morrison and Morris **2000**). After these treatments, seeds were incubated in water for 24 h (for appropriate comparison with smoke treatments, see below). For the smoke treatments we produced a smoke solution using dry leaves and thin stems of *Actinocephalus robustus* (Eriocaulaceae), which were ground in an electric grinder. Biomass of this species was used because it was available from another, unrelated, experiment for which it had been collected (small amounts from several different individual to minimize damages on the plants). The active compounds in smoke that stimulate germination result from a ubiquitous plant material and are broadly found in different plant species (Jäger et al. **1996**). Five batches (replicates) of 5 g plant biomass were separately heated in an oven for 30 min at 195 °C (± 5 °C), next we added 50 ml of distilled water, mixed it, and after 10 min filtered it to obtain the aqueous smoke solution, as described in Jäger et al. (**1996**). Seeds were then incubated in the respective smoke solutions (independent replicates) for 24 h. Additionally, a combined treatment of heat and smoke was performed. For this, seeds were exposed to 100 °C for 5 min (see heat treatments above) and soaked in smoke solution (see smoke treatments above) for 24 h before sowing in petri dishes. The control group consisted of seeds not subjected to high temperatures nor to liquid smoke; seeds were only incubated in water for 24 h before sowing in petri dishes.

Germination conditions and monitoring

Seeds were sown in Petri dishes containing agar (0.9%) as substrate and were incubated in a germination chamber type Biochemical Oxygen Demand (BOD). The germination chamber was maintained at a constant

temperature of 25 °C with a photoperiod of 12 h (light and dark). These are optimum conditions for the germination of campo rupestre plant species (Le Stradic et al. 2015a; Nunes et al. 2016). To test germination in dark conditions, the respective Petri dishes were wrapped in double sheets of aluminium foil. The germination of the seeds incubated in the light was monitored for 30 consecutive days (Baskin et al. 2006), being verified every 24 h. The germination of the seeds incubated in the dark was checked after 30 (in which seeds were briefly exposed to light) and again after 60 days, since in the first observation germination was null. Germination was determined as the visible emergence of the radicle. Germinated seeds or contaminated seeds that did not germinate were scored and removed from the Petri dishes.

Post-treatment seed viability

We used tetrazolium chloride to test post-treatment seed viability. Seeds were incubated in a 1% solution of 2,3,5-triphenyl tetrazolium chloride for 24 h at 30 °C in dark conditions (International Seed Testing Association 1999). After this period of immersion in tetrazolium, seeds produced almost no or very variable staining (the same happened following additional 24 h of immersion after cutting seeds). Thus, we could only obtain trustworthy post-treatment seed viability data with the cut test, which has been shown to provide a reliable estimate of seed viability (Ooi et al. 2004). Not all species patently presented firm, moist and white endosperms. Thus, when possible this was the criteria used, if not seeds were scored as viable if firm endosperm was present while soft and mouldy seeds were scored as inviable. Empty/embryo less seeds, without any signs of heat-damage and that were detected in the initial screening (pre-treatment), were not considered for the analysis (i.e., were subtracted from the initial number of seeds).

Statistical analysis

The effects of heat and smoke on seed germination were evaluated by changes in the number of seeds germinated and germination rate (t_{50}). For each species, the final germination of each treatment was compared with its respective control using GLM (generalized linear models) and assuming a binomial error distribution. The time to 50% germination (t50) was calculated as t50 = ti + [(N/2−ni) (ti−tj)]/ni−nj, where, N is the final number of seeds germinated and ni, nj the cumulative number of seeds germinated by adjacent counts at times ti and tj, respectively when ni < N/2 < nj (Coolbear et al. 1984, Farooq et al. 2007). The effects of heat and smoke on germination rate were evaluated by comparing the time to 50% germination of total seed population (t_{50}) for each treatment with the respective control, using a GLM with a gamma distribution. For species with low germination (*Homolepis longispicula* and *Lychnophora ericoides*), time to another percentile (t_4) was used (Soltani et al. 2016). In both cases, changes in the number of seeds germinated and germination rate (t_{50}), multiple post hoc pairwise comparisons were fitted using the function glht() from R package multcomp (Hothorn et al. 2008).

Results

Germination percentage

Except for *E. inflexa* in which the germination was extremely low regardless of the treatment, germination of all species occurred in all treatments light/dark photoperiod (Fig. 1). On the contrary, germination was null or very low in the dark condition for all species studied, independent of the treatment (data not shown).

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Fig. 1 Germination percentage (mean ± s.e.) for the studied species under the following treatments: Control, Smoke, 80 °C (H80), 100 °C (H100), and 100 °C plus smoke (HS). The significance of the pairwise comparison of each treatment with its corresponding control is also included (ns, not significant; *$P < 0·05$; **$P < 0·01$; ***$P < 0·001$). See text for details of treatments

The germination of *V. nanuzae* and *V. epidendroides* increased in the heat treatments. In *V. nanuzae*, maximum germination was obtained in the heat treatment of 80 °C and, for *V. epidendroides*, both heat treatments resulted in higher germination compared to the control treatment (Fig. 1, Table S1). *Vellozia variabilis, V. resinosa* were not affected by the heat treatment of 80 °C but the heat treatment of 100 °C negatively affected their germination, probably as result of seed mortality as suggest by the decrease in post-treatment seed viability (Table S2). The remaining species (*L. ericoides, H. longispicula*, and *X. pilosa* and *V.*

caruncularis) were the least resistant to the heat and germination was negatively affected by both treatments (Fig. **1**, Table S1), probably as result of seed mortality as suggest by the decrease in post-treatment seed viability (Table S2). Smoke significantly enhanced germination percentage in *V. epidendroides* but the magnitude of stimulation was low (*ca.* 13%). For all the remaining species, the effect of smoke was not significant. In most cases, germination in the combined treatment of heat and smoke was similar to the germination of the corresponding heat treatment alone (100 °C). However, for *X. pilosa* the combined treatment of heat and smoke resulted in maximum germination, with smoke apparently overcoming the negative effect of the high temperatures on seed germination (Fig. **1**, Table S1). There was a decrease in seed viability for *X. pilosa* in the heat treatment of 100 °C heat treatment (Table S2).

Germination rate

Vellozia caruncularis, *V. resinosa* and *X. pilosa* had significantly faster germination in the smoke treatments than in the control (Table **2**). *V. nanuzae* had significantly faster germination in both smoke and heat (80 °C) treatments (Table **2**). For all the remaining species, despite lower t_{50} values in smoke than in control, these differences were not statistically significant (Table **2**). The effect of heat was not as consistent as for smoke. *X. pilosa* was negatively affected by heat, particularly with the heat treatment of 100 °C. Germination rate in *V. nanuzae* was positively affected by the heat treatment of 80 °C while in *V. epidendroides* it was negatively affected by the combined treatment of heat and smoke.

Table 2 Germination rate (t_{50} in days, mean ± s.e) for the studied species under the following treatments: Control, Smoke, 80 °C (H80), 100 °C (H100), Smoke and 100 °C (HS)

Species	Control	Smoke	H80	H100	HS
E. inflexa	NA	NA	NA	NA	NA
H. longispicula (t_4)	6.0 ± 0.0	5.0 ± 0.6 ns	NA	NA	NA
L. ericoides (t_4)	10.5 ± 1.2	9.0 ± 0.0 ns	NA	NA	NA
X. pilosa	15.8 ± 0.9	8.3 ± 0.8***	16.3 ± 2.0 ns	21.8 ± 2.2 ns	10.8 ± 0.9*
V. caruncularis	7.5 ± 0.6	5.6 ± 0.7**	NA	NA	NA
V. epidendroides	14.1 ± 1.2	11.4 ± 1.0 ns	10.7 ± 0.4 ns	15.5 ± 1.1 ns	19.0 ± 5.2*
V. resinosa	8.7 ± 0.4	6.0 ± 0.8***	8.0 ± 0.6 ns	NA	NA
V. nanuzae	11.3 ± 0.7	8.4 ± 1.9**	7.0 ± 0.8***	13.0 ± 0.9 ns	10.2 ± 0.4 ns
V. variabilis	7.2 ± 1.2	5.9 ± 0.1 ns	6.8 ± 0.4 ns	NA	NA

The effects of heat and smoke on germination rate were evaluated by comparing the time to 50% germination (t_{50}) for each treatment with the respective control, by one-way analysis of variance (ANOVA).

The significance of the pairwise comparison between each treatment and control is also included (ns, not significant; $*P < 0·05$; $**P < 0·01$; $***P < 0·001$). For *Homolepis longispicula* and *Lychnophora ericoides*, due to low germination rate refers to t_4 values (in days, mean ± s.e). NAs represent treatments for which germination rate was not possible to calculate due to low or null germination values. See text for details of treatments

Discussion

Our results support previous studies showing that resprouting species in campo rupestre do not require dormancy breaking conditions to promote high germination percentages and/or that seed germination triggered by fire-related cues is not common (Le Stradic et al. **2015a**; Fichino et al. **2016**; Nunes et al. **2016**; Ramos et al. **2017**). Additionally, while seeds from some species can withstand moderate heat doses (sensu Paula and Pausas **2008**; temperature below 100 °C and/or low exposure times) without affecting germination, most species are negatively affected by higher heat (Ribeiro et al. **2013**; Le Stradic et al. **2015a**; Fichino et al. **2016**; Ramos et al. **2017**). The germination observed in the combined smoke x heat treatment suggests that for some species (e.g., *X. pilosa*), seeds do not immediately die when exposed to high heat. Instead, it is likely that high heat weakens seeds and they subsequently become more susceptible to fungi or other type of deterioration. Smoke, possibly because of increased germination rate, may be important to avoid seed mortality.

The positive photoblastic behaviour in germination and the fact that light requirement is not overcome by fire-related cues is also in accordance with a reduced importance of germination from the soil seed bank and consistent with recruitment in open habitats in which seeds may be subject to high light intensities. Indeed, the seed bank of campo rupestre is characterized by low species richness and low density of seeds relative to adjacent communities (Medina and Fernandes **2007**; Luz et al. **2018**).

Altogether, campo ruprestre's plant physiognomy is similar to other grasslands worldwide, in which most species resprout after fire, typically seeds tolerate heat-shocks associated with low intensity fires but are negatively affected by higher heat doses, and fire-stimulated seed germination by heat and smoke is uncommon (Clarke and French 2005; Dayamba et al. **2008**, **2010**; Gonzalez and Ghermandi **2012**; Tavsanoglu et al. **2015**; Cox et al. **2017**; Ramos et al. **2017**). On the contrary, post-fire seedling recruitment from soil seed banks is not so common. This post-fire regeneration strategy may be hampered by the fact that plants apparently lack most of the characteristics presented by plants in other ecosystems where fire-stimulated seedling emergence from soil seed banks is important; i.e., in Mediterranean climate ecosystems such as California (Keeley and Fotheringham **1998**, **2000**), South Africa (Brown **1993**), southern Australia (Dixon et al. **1995**), and the Mediterranean Basin (Moreira et al. **2010**; Moreira and Pausas **2018**). Species that recruit from soil stored seed banks have a set of important traits that maximize and enhance the probability of successful seedling recruitment after fire. These include seed dormancy that allows seed persistence in the soil between fires, seed resistance to high temperatures (in many cases temperatures above 100 °C) that allows survival of seeds present in the soil seed bank to the passage of fire and dormancy break and germination stimulated by fire-related cues such as heat and smoke (Keeley and Fotheringham **1998**; Keeley et al. 2012). This may potentially be due to negative relationship between the ability to resprout after fire and fire-stimulated germination ability (Paula and Pausas **2008**), as most species regenerate well after fire by resprouting. However, our study supports that smoke may have an important role in the regeneration of campo rupestre's plants, via enhancing germination rate. The ecological significance of this mechanism (enhanced germination rate by smoke) is expected to be particularly relevant for species that resprout and flower after fire, acting on seeds resulting from post-fire seed dispersal. Many of campo rupestre's species resprout vigorously and flower rapidly, which creates an abundance of seeds soon after fire (Oliveira et al. **2016**), overcoming the limitations of a poor seedbank. Early emergence allows seedlings to take advantage of reduced competition and increased resource availability and have long-term effects on plant fitness (de Luis et al. **2008**). In our study, most species showed earlier germination of only a few days. Future studies should assess the occurrence and ecological implications (effects on plant fitness) of earlier emergence under natural conditions in campo rupestre. In this region, most fires usually occur at the end of the dry season; the high germination percentage and the enhanced germination rate by smoke exhibited by campo rupestre species may allow recruitment of seedlings soon after fire, when there is an increased availability of resources, competition is low and still during the wet season, before the less favourable conditions of the dry season (Alvarado et al. **2017**). The potential effect of smoke on these seeds dispersed as a result of post-fire resprouting and flowering is not surprising as the active compounds of smoke affecting seed germination have been shown to remain in the soil for a long time after fire (over 7 years according to Preston and Baldwin **1999**) although possibly declining through time and with soil depth due to its water-soluble nature (Ghebrehiwot et al. **2011**). Thus, enhanced germination rate by smoke may be important for post-fire seedling recruitment and thus for post-fire persistence and community dynamics of campo rupestre flora. It provides an alternative mechanism, to fire-stimulated seedling emergence from seed banks, for seedling establishment in post-fire environments that may be equally effective, to facilitate plant recruitment success.

Similarly, a previous study, performed with plant species from Central Anatolian steppe has shown seedling vigour enhanced by smoke, despite the lack of smoke stimulated germination, and suggested the use of enhanced seedling vigour by fire-related cues, rather than germination percentages, as a useful tool to understand plant regeneration in these fire-prone grasslands (Tavsanoglu et al. **2015**). Considering this lack of stimulated germination (percentages) by fire-related cues among plant species from grasslands, it would be interesting to know how other characteristics, such as germination rate (this study) and seedling vigour (Tavsanoglu et al. **2015**), affect plant regeneration in fire-prone grasslands worldwide.

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ArticleNote

Electronic supplementary material

Below is the link to the electronic supplementary material. Supplementary file1 (DOCX 20 kb)

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