

A field perspective on effects of fire and temperature fluctuation on Cerrado legume seeds

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Abstract

Information from a field perspective on temperature thresholds related to physical dormancy (PY) alleviation and seed resistance to high temperatures of fire is crucial to disentangle fire- and non-fire-related germination cues. We investigated seed germination and survival of four leguminous species from a frequently burned open Neotropical savanna in Central Brazil. Three field experiments were conducted according to seed location in/on the soil: (1) fire effects on exposed seeds; (2) fire effects on buried seeds; and (3) effects of temperature fluctuations on exposed seeds in gaps and shaded microsites in vegetation. After field treatments, seeds were tested for germination in the laboratory, together with the control (non-treated seeds). Fire effects on exposed seeds decreased viability in all species. However, germination of buried *Mimosa leioccephala* seeds was enhanced by fire in an increased fuel load treatment, in which we doubled the amount of above-ground biomass. Germination of two species (*M. leioccephala* and *Harpalyce brasiliiana*) was enhanced with temperature fluctuation in gaps, but this condition also decreased seed viability. Our main conclusions are: (1) most seeds died when exposed directly to fire; (2) PY could be alleviated during hotter fires when seeds were buried in the soil; and (3) daily temperature fluctuations in gaps also broke PY of seeds on the soil surface, so many seeds could be recruited or die before being incorporated into the soil seed banks. Thus seed dormancy-break and germination of legumes from Cerrado open savannas seem to be driven by both fire and temperature fluctuations.

Keywords: *campo sujo*, experimental fire, Fabaceae, fire-prone, Neotropical savanna, physical dormancy

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Introduction

Recruitment from seeds following fire is a key factor for the maintenance of populations of many species with physically dormant seeds throughout the world (e.g. Auld and O'Connell, 1991; Moreira and Pausas, 2012; Ooi *et al.*, 2014). Physical dormancy (PY), or seed coat impermeability to water (Rolston, 1978; Baskin and Baskin, 2004; Finch-Savage and Leubner-Metzger, 2006), is common in many ecosystems and in many plant families, but it is especially relevant in tropical Fabaceae (Baskin *et al.*, 2000; Jayasuriya *et al.*, 2013; Baskin and Baskin, 2014). Although PY may have emerged as a response to distinct selective pressures rather than fire, such as drought and seasonality (Bradshaw *et al.*, 2011; Santana *et al.*, 2013; Willis *et al.*, 2014; Jaganathan, 2015; Jayasuriya *et al.*, 2015), its alleviation under natural conditions has been suggested to be fire dependent in many fire-prone habitats (Auld and O'Connell, 1991; Auld and Denham, 2006; Keeley *et al.*, 2011; Moreira and Pausas, 2012; Ooi *et al.*, 2014).

Nevertheless, daily temperature fluctuations may also play a role in PY alleviation in fire-prone ecosystems (Auld and Bradstock, 1996; Santana *et al.*, 2010, 2013). Relatively high temperature fluctuations occur in post-fire vegetation due to increased solar irradiance (Auld and Bradstock, 1996; Fidelis and Blanco, 2014), but temperature fluctuations also occur in undisturbed vegetation gaps (Santana *et al.*, 2013). On the other hand, there are many cases in which the temperature required to break PY is higher than those reached in vegetation gaps, and thus fire passage would be necessary to break dormancy (Moreira and Pausas, 2012). PY has been reported to be broken at different temperature thresholds, and these data have been used to classify species into functional groups according to their fire dependency: fire-obligate or fire-facultative, depending on the temperatures required to break PY (Ooi *et al.*, 2014).

Determination of the temperature thresholds related to PY alleviation is crucial to disentangling

the role of fire and temperature fluctuations as germination cues in fire-prone environments (Moreira and Pausas, 2012; Ooi *et al.*, 2014), and it is also important to clarify seed tolerance to fire-related temperatures (Jaureguiberry and Díaz, 2015; Ribeiro *et al.*, 2015). Moreover, it is necessary to determine such temperature thresholds from a field perspective (e.g. Santana *et al.*, 2013). Therefore, if PY break is fire dependent, one should expect dormancy to be alleviated only at temperatures higher than those found in daily temperature fluctuations at the soil surface (Moreira and Pausas, 2012; Ooi *et al.*, 2014).

The question regarding fire-mediated PY break has been mainly addressed in Australia (e.g. Auld and O'Connell, 1991; Knox and Clarke, 2006; Scott *et al.*, 2010; Ooi *et al.*, 2014) and Mediterranean vegetation (e.g. Herranz *et al.*, 1998; Reyes and Trabaud, 2009; Moreira *et al.*, 2010; Santana *et al.*, 2013). In the Cerrado, few data are derived from field experiments (but see Hoffmann, 2000; Dodonov *et al.*, 2014) and most data obtained from laboratory studies suggest that fire-related heat shock does not break PY (Ribeiro *et al.*, 2015; Fichino *et al.*, 2016; Zupo *et al.*, 2016). Therefore, we still lack a comprehensive idea of the overall effects of fire on dormancy alleviation and seed germination in grassy biomes, which have been typically neglected (Parr *et al.*, 2014). Such a field-related perspective should help us to understand germination responses to fire in the Neotropical savannas, such as the open Cerrado formations (*campo sujo*; Coutinho, 1982). The Cerrado is a Neotropical complex of open and closed savannas (Furley, 1999; Oliveira-Filho and Ratter, 2002), where fire has been present for at least four million years (Simon *et al.*, 2009).

Our purpose was to evaluate different scenarios for seed germination and survival from a field perspective, aiming to disentangle the role of fire-related and non-fire-related germination cues in a Neotropical savanna. Thus we carried out three field experiments with legume shrub seeds from Cerrado open savannas, according to where seeds could be located in/on the soil. Specifically, we tested the effects of (1) fire on exposed seeds (in the upper layer of the soil), (2) fire on buried seeds and (3) temperature fluctuations under non-fire-related conditions on dormancy break and maintenance of seed viability.

Additionally, we addressed the following hypotheses. (1) As recurrent Cerrado fires consume up to 90% of the grassy fuel load (Coutinho, 1982; Kauffman *et al.*, 1994; Miranda *et al.*, 2009), we expected fire to kill most of the seeds located on the soil surface, as reported for other fire-prone ecosystems (Carrington, 2010; Dayamba *et al.*, 2010). (2) Below-ground soil heating during fires could be increased by a higher amount of fuel load (Choczynska and Johnson, 2009; Gagnon *et al.*, 2012, 2015), which in this case is the amount of live and dead grassy

biomass, so we expected fires to have a positive effect on seed germination of buried seeds at increased fuel load conditions. (3) Natural temperature fluctuations, especially in vegetation gaps, could alleviate PY in the Cerrado, as reported for Australian legumes (Santana *et al.*, 2010) and Mediterranean Basin obligate seeders (Santana *et al.*, 2013).

Materials and methods

Study site and seed collection

Three separate field experiments (Table 1) were carried out at the *Reserva Natural Serra do Tombador*, in Central Brazil (47°45'–51°W and 13°35'–38°S, 8900 ha, 560–1118 m above sea level). The area has been subjected to natural and anthropogenic fires, with a mean return interval of 3 years (Daldegan *et al.*, 2014). The climate has a marked dry season from May to September (total precipitation during this period <60 mm) and a wet season from October to April, when most of the annual precipitation (1300–1500 mm/year) occurs. Annually, mean temperatures range from 22 to 25°C and September is usually the hottest month. Minimum temperatures may be <15°C and maximum temperatures up to 36°C (Fundação Grupo Boticário, 2011). Vegetation is predominantly *campo sujo*, which is an open savanna dominated by a species-rich herbaceous layer with scattered shrubs and small trees (Coutinho, 1982).

We collected seeds from four leguminous shrub species commonly found in the area: *Mimosa leioccephala* Benth., *Harpylyce brasiliiana* Benth., *Senna corifolia* (Benth.) H.S. Irwin and Barneby, and *Bauhinia dumosa* Benth. (Table 1). Seeds from all species were collected from several individuals ($n > 10$) and stored at 5°C for up to 1 year until the beginning of the experiments. All species had physically dormant seeds (Table 1), except *B. dumosa*, which has permeable seed coats (Zupo *et al.*, 2016).

Fire effects on exposed seeds

We evaluated fire effects on survival of exposed seeds by carrying out experimental fires with seeds placed in the upper soil layer. The experiment was performed in July 2014 (mid-dry season), a period in which the study species are shedding seeds (authors' personal observation) and when most anthropogenic fires occur in the Cerrado (Ramos-Neto and Pivello, 2000; Miranda *et al.*, 2009). We avoided patches invaded by *Urochloa brizantha*, an invasive African grass species, because it can influence fire parameters (Gorgone-Barbosa *et al.*, 2015).

Seeds were placed in experimental plots subjected to fires (Fig. 1). We independently burned four distinct

Table 1. Experiment set-up, species, date of collection, date of experiment, presence of dormancy and number of seeds per dish used in the tests at field conditions in an open savanna in Central Brazil

Experiment	Species	Seed collection date	Date of experiment	Dormancy	Seeds per dish (<i>n</i>)
Fire effects on exposed seeds	<i>Mimosa leiocephala</i>	October 2013	July 2014 (mid-dry season)	PY	10
	<i>Harpalyce brasiliiana</i>	July 2013		PY	10
	<i>Senna corifolia</i>	October 2013	October 2015 (late-dry season)	PY	9
	<i>Bauhinia dumosa</i>	October 2013		ND	8
Fire effects on buried seeds	<i>Mimosa leiocephala</i>	October 2014	October 2015 (late-dry season)	PY	10
	<i>Bauhinia dumosa</i>	October 2015		ND	10
Temperature fluctuation effects	<i>Mimosa leiocephala</i>	October 2013	July–October 2014 (dry season)	PY	10
	<i>Harpalyce brasiliiana</i>	July 2013		PY	10
	<i>Senna corifolia</i>	October 2013		PY	9
	<i>Bauhinia dumosa</i>	October 2013		ND	8

PY, physical dormancy; ND, non-dormant.

plots (15 × 20 m, excluded from fire for 2 years), which were located at least 20 m from each other. Seeds were placed in aluminium dishes (7 cm diameter × 0.5 cm deep) filled with local soil and positioned on the soil surface. Seeds were carefully placed within the aluminium dishes, so that they would have at least part of their seed coat exposed directly to fire. Three aluminium dishes were placed under the native vegetation (grass tussocks) in each plot (3 dishes × 4 plots = 12 samples). We conducted head fires (wind direction), and fires were set in the morning for safety reasons (Whelan, 1995); all plots were burned in the same week. Fire temperatures were recorded every 2 s with type K thermocouple sensors attached to data loggers (Novus LogBox). We calculated fire residence times at temperatures ≥60°C, which is a standardized temperature threshold lethal for plant tissue (Daniell *et al.*, 1969; Dayamba *et al.*, 2010). Additionally, we calculated residence time of fire temperatures ≥100°C, a threshold easily reached by fire under exposed conditions. The number of seeds per dish varied between species due to constraints in seed availability (see Table 1). Control seeds were transported to the field and kept stored in thermal bags at room temperature, thus not heated. After fires, both control and treatment seeds were tested for germination in the laboratory (see germination procedures below).

Fire effects on buried seeds

To test the effects of fire on the survival and germination of buried seeds, we used seeds of two species with different traits in relation to seed coat permeability: one with impermeable-coated seeds (PY, *M. leiocephala*) and the other with permeable seeds (non-dormant, *B. dumosa*, see Table 1). Since the amount of fuel (live and dead grassy species) can influence both fire intensity and temperatures reached during fires (Gagnon *et al.*, 2015; M.N. Rissi *et al.*,

unpublished data), we manipulated the fuel load to assess the effects of doubling the amount of above-ground biomass on soil heating and thus on seed germination and survival of buried seeds. Therefore, the following treatments were carried out in the field: (1) no manipulation of fuel load (~700 g m⁻²) and (2) increased fuel load by adding 700 g m⁻² of biomass collected from neighbouring plots to double the fuel load. For this treatment, four 30 × 30 m plots were individually burned, and we established three subplots (2 × 2 m) for each biomass manipulation treatment within each plot (3 subplots × 4 plots = 12 subplots/treatment). In each subplot, we placed an aluminium dish with 10 seeds/species buried 1 cm below ground (Fig. 1; Table 1). Fires were set in October 2015 (late-dry season), using experimental procedures similar to those described above, and fire temperatures were recorded (type K thermocouple sensors attached to data loggers) every 2 s at 1 cm below ground in each subplot/treatment. However, measurements in most sensors at the increased fuel load treatment failed and therefore they are not presented in detail. Control seeds were transported to the field, but kept stored at room temperature, and after fires both control and treatment seeds were tested for germination in the laboratory (see below).

Temperature fluctuation effects

We used seeds of the four species at two different field treatments to test the effects of temperature fluctuations on seed germination: (1) shaded: seeds placed under native grass tussocks; and (2) gap: seeds placed in bare soil gaps. The soil-filled aluminium dishes (0.5 mm deep) with seed samples were placed at soil level in shade or in a gap in the field for *ca* 75 days from July to October (dry season). Soil temperatures were recorded every 30 min by sensors coupled to data loggers. The sensors were placed at the soil surface in each

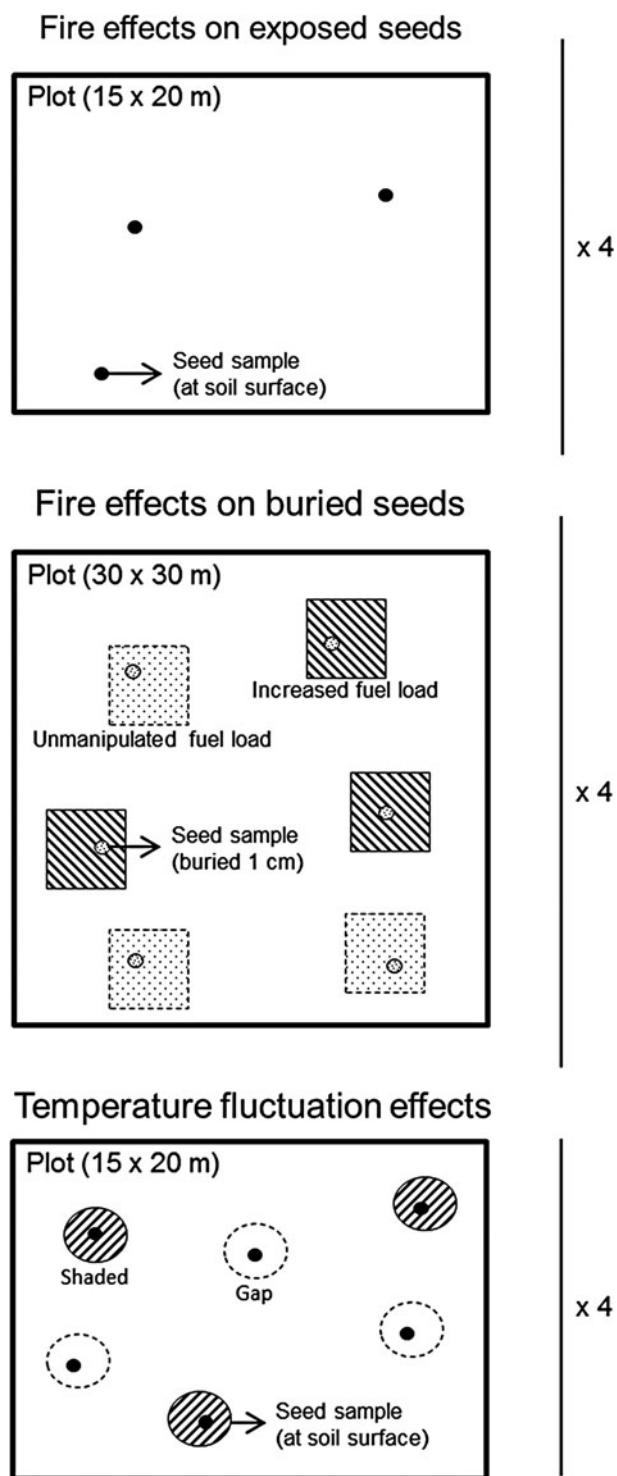


Figure 1. Experimental design of the different experiments tested under field conditions in an open savanna in Central Brazil: (1) fire effects on exposed seeds; (2) fire effects on buried seeds at different fuel load treatments: unmanipulated and increased amount of above-ground biomass; and (3) temperature fluctuations in shaded and gap conditions. Black circles show aluminium dishes containing seed samples at soil surface, and grey dotted circles show aluminium dishes containing seed samples at 1 cm below ground. Number of seeds per species/sample is shown in Table 1.

treatment, near the aluminium dishes: in the shaded treatment, sensors were placed under tussocks of native grasses; and in the gap treatment they were exposed to sunlight. The treatments were applied in four unburned plots and followed the same experimental design as described above (3 dishes \times 4 plots = 12 samples/treatment; Fig. 1; Table 1). After 75 days, treatment and control (stored at room temperature) seeds were tested for germination, as described below.

Germination tests after field treatments

Upon the completion of the field treatments, seeds were packed in thermal bags and immediately transported to the laboratory. Treatment and control seeds were then placed in Petri dishes (60 mm diameter) with a double layer of filter paper saturated with distilled water and set to germinate in germination chambers at 27°C (12 h of light); the average temperature was recorded at soil level in the study site and in accordance with other studies performed with Cerrado seeds (Fichino *et al.*, 2016). We used a constant temperature to avoid possible additional effects of alternating temperature in seed germination. Seeds were considered germinated when exhibiting radicle protrusion (Bewley *et al.*, 2013) and germination was recorded three times a week for four consecutive weeks.

At the end of the germination tests, non-germinated seeds were scarified, allowed to imbibe and then cut and tested for the viability using tetrazolium solution (1%, buffer pH 7). Seeds with embryos that stained red were considered to be viable, and those with non-stained embryo or those that were visibly burned or showed flaccid tissues were considered to be dead (Lakon, 1949).

Data analyses

All our experiments followed a similar experimental design (Fig. 1), with the seed samples nested within the experimental plots (3 dishes \times 4 plots = 12 samples/species/treatment). Therefore, we performed generalized linear mixed models (GLMMs) with a binomial distribution (see Santana *et al.*, 2013) to separately evaluate: seed germination (binary data considering that a seed could germinate or not) and seed viability (a seed could be viable or not at the end of the trials). As the dishes were nested within the plots, they were considered as the random effects in the analyses. The field treatments (fire treatments or shaded/gap temperature fluctuations, as well as their respective controls) were treated as fixed effects. The controls were considered as the baseline for the intercept in the models. Models were fitted for each species separately in

each experiment. *Post hoc* Tukey's tests were performed to evaluate multiple comparisons among treatments. All analyses were performed using the packages lme4 (Bates *et al.*, 2015) and multcomp (Hothorn *et al.*, 2008) in R software 3.2.5 (R Core Team, 2016) and significance was determined with $\alpha = 0.05$.

Results

Fire effects on exposed seeds

Maximum fire temperatures were $225 \pm 152^\circ\text{C}$, with residence time of temperatures $\geq 60^\circ\text{C}$ equal to 80 ± 26 s and $\geq 100^\circ\text{C}$ always less than 1 min. For *M. leiocephala*, germination decreased from $26 \pm 9\%$ (control) to $9 \pm 7\%$ [fire treatment, $P = 0.002$; Table 2 (a); Fig. 2A]. Germination of *H. brasiliensis* and *S. corifolia* was nearly null in both the control and the fire exposure treatments [Table 2 (a); Fig. 2A]. However, for *B. dumosa*, a species with permeable seeds, fire exposure reduced germination from 88 ± 4 to $11 \pm 22\%$ [($P < 0.001$); Table 2

(a); Fig. 2A]. Initial seed viability ranged from 63% (*H. brasiliensis*) to 90% (*B. dumosa*), and seed viability of all four species decreased after fire exposure to less than 30% [all ($P < 0.001$); Table 2 (a); Fig. 2B].

Fire effects on buried seeds

Below-ground temperatures reached mean maxima of $54 \pm 14^\circ\text{C}$, and thus they did not exceed the threshold of 60°C for residence time calculations in the unmanipulated treatment. Sensors failed to measure below-ground temperatures in the increased fuel load condition, however; recordings of fire temperatures in the same area point out that mean maxima temperatures reach around 80°C at 1 cm depth in sites under high biomass accumulation (see Discussion).

Regarding seed germination, there was no difference in germination percentage of *M. leiocephala* seeds between the control ($5 \pm 4\%$) and the unmanipulated treatment [$3 \pm 5\%$, Table 2 (b); Fig. 3A]. Moreover, the increased fuel load treatment had a positive effect on

Table 2. Parameters estimated by the GLMM models for seed germination and viability of four legume shrub species exposed to: fire effects on exposed seeds (a); fire effects on buried seeds (b); and temperature fluctuations (c), in an open savanna in Central Brazil

Experiment	Species	Treatment	Seed germination			Seed viability		
			Estimate	SEM	<i>P</i> -value	Estimate	SEM	<i>P</i> -value
Fire effects on exposed seeds (a)	<i>Mimosa leiocephala</i>	Intercept	-1.093	0.264	<0.001	2.034	0.736	0.006
		Fire	-1.362	0.448	0.002	-3.862	1.051	<0.001
	<i>Harpalyce brasiliensis</i>	Intercept	-3.136	0.457	<0.001	0.632	0.696	0.364
		Fire	-1.644	1.103	0.136	-4.051	1.202	<0.001
	<i>Senna corifolia</i>	Intercept	-2.632	0.522	<0.001	1.344	0.436	0.002
		Fire	-2.020	1.133	0.075	-2.357	0.621	<0.001
	<i>Bauhinia dumosa</i>	Intercept	2.331	0.873	0.008	2.530	0.874	0.004
		Fire	-6.2931	1.615	<0.001	-6.468	1.604	<0.001
Fire effects on buried seeds (b)	<i>Mimosa leiocephala</i>	Intercept	-3.700	0.729	<0.001	1.418	0.339	<0.001
		Unmanipulated	-0.565	1.151	0.578	-0.031	0.476	0.950
		Increased	2.225	0.869	0.011	-0.339	0.469	0.470
	<i>Bauhinia dumosa</i>	Intercept	6.058	1.496	<0.001	6.058	1.496	<0.001
		Unmanipulated	-2.250	1.511	0.137	-2.250	1.511	0.137
		Increased	-2.434	1.510	0.107	-2.434	1.510	0.107
Temperature fluctuation effects (c)	<i>Mimosa leiocephala</i>	Intercept	-1.098	0.272	<0.001	1.874	0.398	<0.001
		Shaded	-0.641	0.406	0.114	-0.381	0.549	0.488
		Gap	1.435	0.376	<0.001	-1.231	0.535	0.021
	<i>Harpalyce brasiliensis</i>	Intercept	-3.216	0.489	<0.001	0.551	0.293	0.060
		Shaded	0.947	0.579	0.102	-0.121	0.413	0.769
		Gap	1.754	0.546	0.001	-0.900	0.414	0.030
	<i>Senna corifolia</i>	Intercept	-2.543	0.393	<0.001	1.192	0.306	<0.001
		Shaded	-1.318	0.815	0.106	0.065	0.423	0.879
		Gap	0.156	0.539	0.772	-1.402	0.415	<0.001
	<i>Bauhinia dumosa</i>	Intercept	1.989	0.296	<0.001	2.190	0.334	<0.001
		Shaded	-0.682	0.378	0.071	-0.425	0.440	0.335
		Gap	-1.788	0.358	<0.001	-1.780	0.403	<0.001

Significant *P*-values (≤ 0.05) are highlighted in bold.

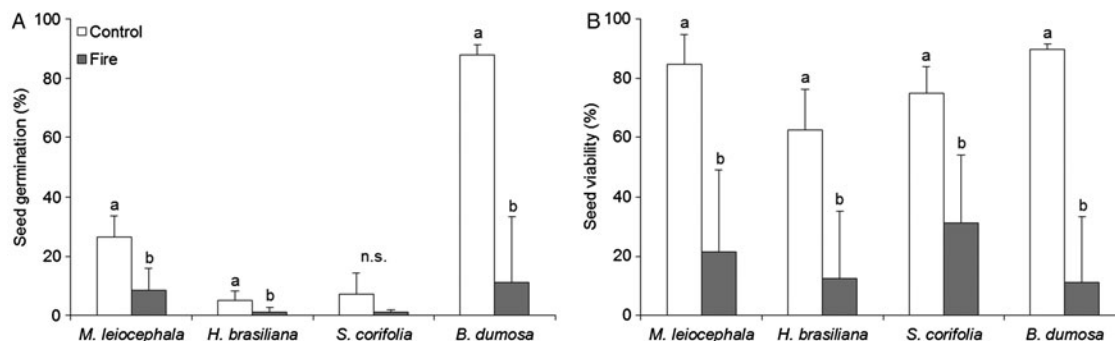


Figure 2. Fire effects on exposed seeds: (A) seed germination and (B) seed viability (%; mean \pm SD) of four leguminous shrub species from an open savanna subjected to experimental fires in Central Brazil. Control refers to untreated seeds, set to germinate in the laboratory. Different letters (a, b) denote significant differences ($P \leq 0.05$) among treatments; statistical comparisons were performed separately for each species; n.s., not significant.

seed germination of *M. leiocephala*, resulting in a 6-fold increase in germination [$P = 0.011$; Table 2 (b); Fig. 3A]. There was no difference in seed viability among treatments for this species [Table 2 (b)], which was over 70% for all treatments (Fig. 3B). For *B. dumosa* seeds, both seed germination and viability percentages were not affected by the treatments [Table 2 (b)], remaining $\geq 88\%$ (Fig. 3A and B).

Temperature fluctuation effects

Soil temperature under the shaded condition ranged from 20 to 38°C (Fig. 4) and had little or no effect on seed germination for any species [Table 2 (c); Fig. 5A]. On the other hand, soil temperature in gaps ranged from 18 to 53°C and remained $\geq 50^\circ\text{C}$ for at least 2 h each day (Fig. 4).

Temperature fluctuations in the gap treatment had a positive effect in alleviating PY in *M. leiocephala* ($P < 0.001$) and *H. brasiliiana* [$P = 0.001$; Table 2 (c)]. For *M. leiocephala*, the gap condition enhanced germination percentage ($58 \pm 17\%$) in relation to both

shaded treatments ($16 \pm 4\%$) and the control ($26 \pm 9\%$; Fig. 5A). For *H. brasiliiana*, the gap condition increased germination to $20 \pm 5\%$ compared with the control ($5 \pm 3\%$, Fig. 5A). However, none of the treatments alleviated dormancy in *S. corifolia* seeds [Table 2 (c); Fig. 5A]. Seeds of *B. dumosa* were negatively affected by temperature fluctuation in the gaps [$P < 0.001$; Table 2 (c); Fig. 5A].

Seed viability of all species in the shaded treatment did not differ from their respective controls [Table 2 (c); Fig. 5B]. Nonetheless, the gap treatment had a negative effect on seed viability from 20 to 30% across species [Table 2 (c); Fig. 5B].

Discussion

There is an ongoing debate on the relative importance of fire-related temperatures *vs* daily temperature fluctuations in alleviating PY in fire-prone ecosystems (Moreira *et al.*, 2010; Santana *et al.*, 2010, 2013; Ooi *et al.*, 2014), and our study helps to disentangle these germination cues from a field perspective in a

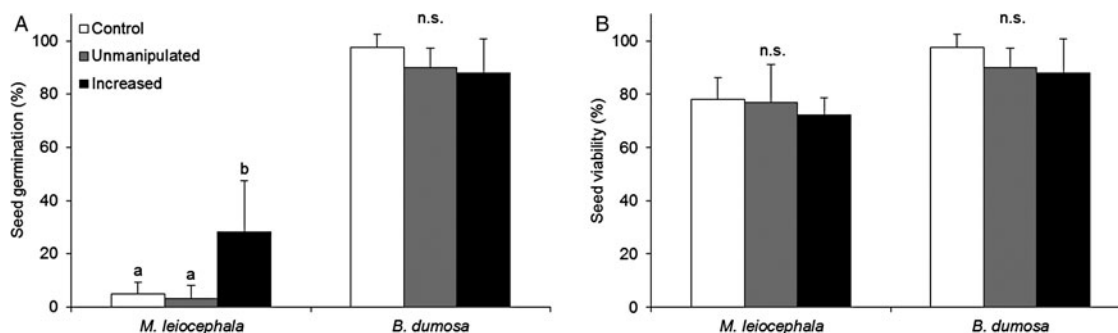


Figure 3. Fire effects on buried seeds: (A) seed germination and (B) seed viability (%; mean \pm SD) of buried seeds (1 cm below ground) of two legume shrub species from an open savanna, subjected to experimental fires in Central Brazil. Control: untreated seeds, set to germinate in the laboratory; Unmanipulated: treatment with no manipulation of fuel load; Increased: above-ground biomass was manipulated to double its average amount. Different letters (a, b) denote significant differences ($P \leq 0.05$) among treatments; statistical comparisons were performed separately for each species; n.s., not significant.

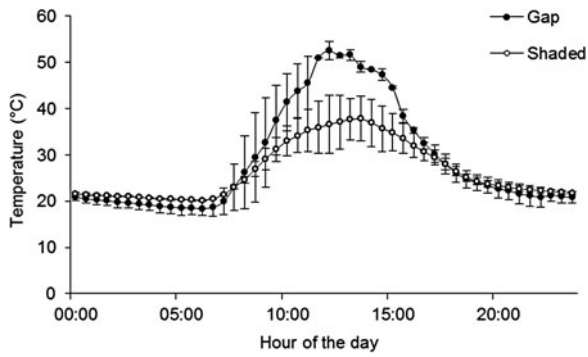


Figure 4. Daily temperature fluctuations (mean \pm SD) at the soil surface under vegetation gaps (filled circles) and under shaded conditions (open circles) in an open savanna in Central Brazil for 75 days in the dry season (July to October 2014).

Neotropical savanna. Theoretically, PY should be broken at different temperature thresholds, so dormancy alleviation could be classified as being fire related or not (Moreira and Pausas, 2012; Ooi *et al.*, 2014). However, we argue that Cerrado leguminous seeds have their PY (or at least part of it) alleviated by two different mechanisms: (1) during hotter fires, if seeds are stored in the soil, or (2) when exposed to daily temperature fluctuations.

Although not tested under the buried conditions in our field experiments, some seeds of *H. brasiliensis* have part of their PY alleviated after heat shocks applied in the laboratory (H.L. Zironi *et al.*, unpublished data). Therefore, seeds of both *M. leioccephala* and *H. brasiliensis* should be classified as facultative regarding their functional responses to fire (see Ooi *et al.*, 2014). PY of *S. corifolia* seeds, on the other hand, was neither alleviated from laboratory heat shocks (data not shown) nor from the enhanced field temperature fluctuations, which could suggest that these legume seeds have other habitat-specific dormancy-breaking mechanisms

under natural conditions, perhaps related to wetter conditions (van Klinken and Goulier, 2013).

Fire effects on exposed seeds caused a high mortality, as reported for most seeds throughout the world when placed in the upper layer of the soil (Auld and O'Connell, 1991; Bradstock and Auld, 1995; Carrington, 2010; Dayamba *et al.*, 2010). However, seeds buried in the soil would be insulated and could have PY alleviated, as reported for Australian leguminous seeds (Auld and O'Connell, 1991; Bradstock and Auld, 1995). Conversely, below-ground temperatures required for PY break seem to be only reached under more intense fires (Knox and Clarke, 2006). Despite that, Cerrado fires are mostly fast and below-ground soil heating is usually negligible ($\sim 50^\circ\text{C}$; Miranda *et al.*, 1993), with minor consequences to the seed bank (Andrade and Miranda, 2014).

Nevertheless, hotter fires may occur in the Cerrado when the return intervals take 4 years or more, due to a substantial accumulation of fuel load (Coutinho, 1982), which would also enhance below-ground fire temperatures, as reported for other high-diversity flammable communities (e.g. Gagnon *et al.*, 2015). In our study area, previous measurements of fire temperatures 1 cm below ground in plots with 4 years of fuel load accumulation, detected temperatures reaching $\geq 60^\circ\text{C}$ for about 3 min, occasionally recording temperatures $\geq 100^\circ\text{C}$ (mean maxima = 80°C ; maximum observed = 154°C ; T. Zupo *et al.*, unpublished data). On the other hand, anthropogenic fires have been suggested to change fire regimes in Cerrado open savannas, by shifting season of occurrence from wet to the dry season and shortening the frequency of return (Ramos-Neto and Pivello, 2000; Miranda *et al.*, 2009).

Fire-stimulated germination traits should not have been favoured in fire-prone systems that undergo surface fires (Bond and Midgley, 2003; Bradshaw *et al.*, 2011), such as the open savannas where fire episodes are frequent and less severe (Bond and Keeley, 2005).

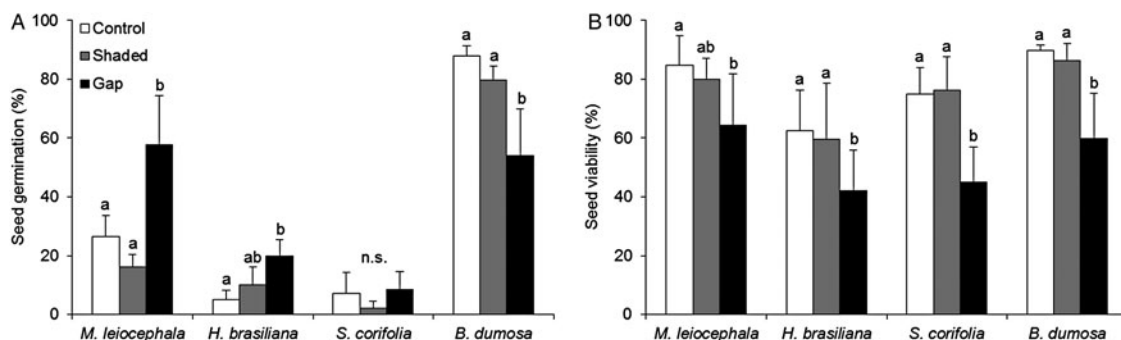


Figure 5. Temperature fluctuation effects (A) on seed germination and (B) on seed viability (%), mean \pm SD) of four legume shrub species from an open savanna, subjected to daily temperature fluctuation in Central Brazil for 75 days. Control: untreated seeds, set to germinate in the laboratory; Shaded: temperature fluctuations under the shade of grasses; Gap: temperature fluctuations in the gaps of bare soil. Different letters (a, b) denote significant differences ($P \leq 0.05$) among treatments; statistical comparisons were performed separately for each species.

Although there has been no evidence of fire-stimulated germination from laboratory experiments (Le Stradic *et al.*, 2015; Ribeiro *et al.*, 2015; Fichino *et al.*, 2016; Zupo *et al.*, 2016), we argue that fire-related conditions can potentially break PY, as was observed in at least 30% of *M. leiocephala* buried seeds under increased fuel load conditions. Therefore, fire could play a role in seed recruitment for a proportion of seeds from the Cerrado soil seed bank.

Fire also has been recognized as having an indirect effect on PY alleviation by removing vegetation and consequently increasing daily temperatures at upper soil layers (~0.5 cm), therefore reaching temperature thresholds required for gap-dependent seeds to break PY (Ooi *et al.*, 2014), which do not seem to occur in vegetated sites (Auld and Bradstock, 1996; Santana *et al.*, 2010). However, gaps in unburned sites seem to have the same effect in seedling recruitment, suggesting that biomass removal, which increases the daily temperature fluctuations, plays a role in regeneration from seeds irrespective of fire (Santana *et al.*, 2013).

Previous laboratory simulations of temperature fluctuations had already recognized a decrease in germination in the permeable-coated *B. dumosa* seeds, but such experiments had no effect on PY alleviation of *M. leiocephala* (Zupo *et al.*, 2016). Instead, our results show that temperature fluctuation in gaps enhanced germination for *M. leiocephala* and *H. brasiliiana* seeds, even though PY was not fully alleviated. Thus, from a field perspective, vegetation gaps provide an important micro-environment with higher temperature fluctuations that could lead to the alleviation of PY in legume seeds, enabling their germination in both post-fire environments (where temperature fluctuations will be higher due to the removal of the above-ground vegetation), as well as in fire-free intervals if seeds reach such gaps.

PY release has not yet been fully explained for Cerrado open savannas. Our four study species are dominant in their plant community, and distinct traits related to dormancy relief and germination strategies could explain a diversity of regeneration niches and species co-existence (see Grubb, 1977). Therefore, other species-specific processes may be related to releasing PY in these species. Further studies are warranted to understand if water availability could play any role coupled with daily heating temperatures (Moreno-Casasola *et al.*, 1994; van Klinken *et al.*, 2006), and if seeds could become sensitive to dormancy-breaking conditions as they age in soil seed banks (van Assche *et al.*, 2003; Jayasuriya *et al.*, 2009; Gama-Arachchige *et al.*, 2012; Liyanage and Ooi, 2017).

This was the first attempt to test fire- and non-fire-related cues of seed germination within an experimental field approach for the Cerrado open savannas. We draw three main conclusions from our experiments: (1) most seeds die when exposed directly to fire; (2) PY could be alleviated during hotter fires when seeds

were buried, and thus stored in the soil seed bank; and (3) daily temperature fluctuations in gaps also break PY in seeds of some species on the soil surface, and thus many seeds may germinate or die instead of being incorporated into the soil seed banks.

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