

# Fire and legume germination in a tropical savanna: ecological and historical factors

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- **Background and Aims** In many flammable ecosystems, physically dormant seeds show dormancy-break patterns tied to fire, but the link between heat shock and germination in the tropical savannas of Africa and South America remains controversial. Seed heat tolerance is important, preventing seed mortality during fire passage, and is usually predicted by seed traits. This study investigated the role of fire frequency (ecological effects) and seed traits through phylogenetic comparison (historical effects), in determining post-fire germination and seed mortality in legume species of the Cerrado, a tropical savanna–forest mosaic.
- **Methods** Seeds of 46 legume species were collected from three vegetation types (grassy savannas, woody savannas and forests) with different fire frequencies. Heat shock experiments (100 °C for 1 min; 100 °C for 3 min; 200 °C for 1 min) were then performed, followed by germination and seed viability tests. Principal component analysis, generalized linear mixed models and phylogenetic comparisons were used in data analyses.
- **Key Results** Heat shocks had little effect on germination, but seed mortality was variable across treatments and species. Seed mortality was lowest under the 100 °C 1 min treatment, and significantly higher under 100 °C 3 min and 200 °C 1 min; larger seed mass decreased seed mortality, especially at 200 °C. Tree species in Detarioideae had the largest seeds and were unaffected by heat. Small-seeded species (mostly shrubs from grassy savannas) were relatively sensitive to the hottest treatment. Nevertheless, the presence of physical dormancy helped to avoid seed mortality in small-seeded species under the hottest treatment.
- **Conclusions** Physical dormancy-break is not tied to fire in the Cerrado mosaic. Heat tolerance appears in both forest and savanna species and is predicted by seed traits (seed mass and physical dormancy), which might have helped forest lineages to colonize the savannas. The results show seed fire responses are better explained by historical than ecological factors in the Cerrado, contrasting with different fire-prone ecosystems throughout the world.

**Keywords:** Cerrado, Fabaceae (Leguminosae), fire ecology, heat shock, physical dormancy, seed traits, tropical savanna.

## INTRODUCTION

Regeneration from seed is often strongly tied to fire in fire-prone ecosystems (Keeley *et al.*, 2011) because fire-related cues such as heat shock and smoke can break dormancy and thus enhance germination (and fitness) in post-fire environments (Auld and O'Connell, 1991; Herranz *et al.*, 1998; Williams *et al.*, 2003; Moreira *et al.*, 2010). Heat shocks may rupture the water-impermeable seed coat of seeds having physical dormancy (PY) (Morrison *et al.*, 1998), which occurs in many lineages and ecosystems (Baskin *et al.*, 2000; Willis *et al.*, 2014; Rubio de Casas *et al.*, 2017).

In some cases, dormancy is not alleviated by heat; rather, seeds tolerate heat shocks, avoiding seed mortality and ensuring seed supply for post-fire regeneration (Pausas *et al.*, 2004; Paula and Pausas, 2008; Jaureguiberry and Díaz, 2015; Fichino *et al.*, 2016). Some seed traits (e.g. seed mass, seed shape, dormancy class) are predictors of seed responses to fire temperatures

(Ribeiro *et al.*, 2015; Gómez-González *et al.*, 2016; Ramos *et al.*, 2016; Saracino *et al.*, 2017). For instance, larger seeds tend to show higher heat tolerance (Gashaw and Michelsen, 2002; Ribeiro *et al.*, 2015), while smaller ones are more easily stimulated to germinate following fires (Hanley *et al.*, 2003).

Most studies of fire and seed germination have been conducted in Mediterranean ecosystems, which are subject to high-intensity crown fires (Keeley *et al.*, 2011, 2012). In grassland and savanna ecosystems, low-intensity surface fires are frequent disturbances, shaping vegetation structure and plant traits (Bond and Keeley, 2005; Miranda *et al.*, 2009; Dantas *et al.*, 2013). Fire also stimulates seed germination in Australian tropical savannas (Williams *et al.*, 2003, 2005; Scott *et al.*, 2010), but there is debate about its effects in Africa (e.g. Mbalo and Witkowski, 1997; Gashaw and Michelsen, 2002; Dayamba *et al.*, 2008) and South America (e.g. Ribeiro *et al.*, 2013; Fichino *et al.*, 2016; Daibes *et al.*, 2017).

In Brazil, *in situ* assembly of the Cerrado's megadiversity of woody species occurred through the migration and subsequent radiation of forest woody lineages over the last 10 Myr (Simon *et al.*, 2009). Cerrado fires have helped to shape vegetation structure, resulting in a dynamic savanna–forest mosaic (Hoffmann *et al.*, 2012). Thus, different vegetation types, such as grassy and woody savannas and fire-free seasonal forests, are related to different fire frequencies (Coutinho, 1982; Oliveira-Filho and Ratter, 2002; Dantas *et al.*, 2015). Therefore, fire has been suggested as an environmental filter and a selective pressure on Cerrado plant traits (Simon and Pennington, 2012), including the presence of underground organs (capable of post-fire resprouting; Pausas *et al.*, 2018) and thick corky barks (Dantas and Pausas, 2013). Regarding regeneration from seed, current literature suggests that savanna fires do not affect emergence from soil seed banks (de Andrade and Miranda, 2014), and heat shocks also seem to have little effect on germination of Cerrado species (Ribeiro *et al.*, 2013; Fichino *et al.*, 2016). However, while seeds from fire-prone grasslands and savannas are expected to be heat-tolerant (Le Stradic *et al.*, 2015; Fichino *et al.*, 2016), seeds from fire-free forest trees can be heat-sensitive, showing higher mortality (Ribeiro and Borghetti, 2014; Ribeiro *et al.*, 2015). Fire could therefore be an environmental filter preventing the establishment of forest species in savannas (Hoffmann, 2000; Dantas *et al.*, 2013).

The presence of fire also seems to be related to the recent high diversification rates in some legume lineages (Simon *et al.*, 2009). The Cerrado is a global biodiversity hotspot (Myers *et al.*, 2000), and Leguminosae is one of its richest taxonomic groups, comprising ~10 % of the >12 000 species (The Brazilian Flora Group, 2015). Legumes have also undergone several evolutionary transitions from water-impermeable to permeable-coated seeds, showing considerable interspecific variation in seed dormancy (Rubio de Casas *et al.*, 2017). In addition, the availability of well-resolved phylogenies for the family (The Legume Phylogeny Working Group, 2017) make Leguminosae an ideal model for studying whether fire has shaped the variability in seed dormancy and heat tolerance.

Despite all recent efforts to understand how fire affects germination in savanna and forest species, studies addressing the effect of fire on seed germination unfortunately suffer from several caveats, including (1) small numbers of species, (2) a lack of phylogenetically explicit analyses, (3) concentration on a single vegetation type and (4) no evaluation of seed traits that could drive the response patterns. Therefore, we still lack a general mechanistic understanding on how fire drives vegetation dynamics through the regeneration niche. Here, we aim to investigate how fire affects seed germination and seed mortality of 46 legume species occurring along a gradient of fire frequency in the Cerrado, by integrating experimental essays, phylogenetic information and functional traits (seed morphological and physiological traits) that could provide a mechanistic understanding of the observed patterns. Our sample includes a high number of species from different growth-forms in a savanna–forest mosaic.

Specifically, we addressed the role of (1) vegetation type (grassy savanna, woody savanna and forest) and (2) seed traits (seed mass, seed shape and dormancy) in predicting seed germination and mortality due to fire. Vegetation type is a proxy for

fire frequency (present-day ecological factors), while seed traits and phylogenetic affiliation provide information on historical contingencies. We expected a higher degree of dormancy break and higher heat tolerance (lower mortality) in savanna vegetation types (grassy and woody savannas) compared to forest species. Alternatively, seed traits could be the key drivers of seed mortality, depending primarily on the species' phylogenetic affiliation irrespective of vegetation type. For instance, lineages with larger and rounder seeds could better protect the embryos from heat shocks (Ribeiro *et al.*, 2015; Gómez-González *et al.*, 2016). Thus, we used phylogenetically controlled analyses to disentangle the ecological (i.e. fire frequency) vs. historical (i.e. seed traits) factors driving seed germination and mortality.

## MATERIAL AND METHODS

### Seed collection

Seeds of 46 legume species were collected, mostly in 2014 and 2015, across different vegetation types (grassy savannas, woody savannas and forests) in central and south-eastern Brazil (see Supplementary Data Table S1 for details). Grassy savannas (locally termed *campo sujo*) form an almost treeless ecosystem, rich in herbaceous species and small, thin-branched shrubs (Coutinho, 1982; Ribeiro and Walter, 2008). This grassy savanna is subject to low-intensity grass-fuelled fires that consume most of the fine fuel load (Rissi *et al.*, 2017) and occur at intervals of 3–4 years (Miranda *et al.*, 2009). Woody savannas (cerrado *sensu stricto*) are open communities dominated by short trees with an herbaceous layer in the understorey (Oliveira-Filho and Ratter, 2002); they are also subject to grass-fuelled fires, but with longer fire intervals than the grassy savannas (but shorter than 10 years; see Dantas *et al.*, 2015). Tree species in these woody savannas show different traits that enable them to avoid fire damage, such as thick corky bark, unlike forest species (Hoffmann *et al.*, 2003; Dantas *et al.*, 2013). Forests are closed-canopy ecosystems and include forest-like and semi-deciduous seasonal forest trees (Supplementary Data Table S1), which rarely burn (Dantas *et al.*, 2015). All sites show a similar climate, with a marked dry season from May to September and a high precipitation level (up to 2000 mm year<sup>-1</sup>) concentrated during the wet months (Eiten, 1972).

Because shrubs occur exclusively in the grassy savannas, we classified our study species according to vegetation type associated with growth-forms: (1) savanna shrubs, (2) savanna trees and (3) forest trees. Some *Mimosa* species are subshrubs, showing herbaceous aerial parts and woody underground organs (suffrutescent geoxyles, see Simon *et al.*, 2009; Pausas *et al.*, 2018), and these were included as shrubs in the analyses (Supplementary Data Table S1). Generalist *Senna* species, described as shrubs and/or trees but collected in the *campo sujo*, were also included as savanna shrubs (Table S1).

For each species, seeds from three to 11 individual plants were pooled and kept under low temperatures (~5 °C) until their use in the experiments. Most seeds were kept stored for a period between 4 and 6 months, and only a few were stored for 1–3 years before the trials (Supplementary Data Table S1).

Initial germination, total viability and dormancy class were assessed for the controls for each species (see Tables S2–S4).

#### Heat shocks and germination procedures

Experimental heat shocks were set up under laboratory conditions, based on our previous recordings of fire temperatures in the field (see Daibes et al., 2017, 2018). Seeds were subjected to the following four treatments: (1) a laboratory control (untreated seeds); (2) 100 °C for 1 min; (3) 100 °C for 3 min; and (4) 200 °C for 1 min. Heat shocks were applied in an electric oven (muffle furnace) with five replicates per treatment. Replicates were separately exposed to their respective temperature/time to avoid pseudoreplication (Morrison and Morris, 2000). Each replicate consisted of 20 seeds depending on seed availability (Supplementary Data Table S4). To avoid misinterpretation with soil temperature fluctuations, which reach up to 60 °C (Zupo et al., 2016; Daibes et al., 2017), lower temperatures were not tested. Therefore, we applied 100 °C as a typical fire-related heat dose (e.g. Ribeiro et al., 2013; Fichino et al., 2016), while 200 °C was considered the average lethal temperature for Cerrado seeds when directly exposed to fires (Rizzini, 1976; Daibes et al., 2018). The duration of heat shocks was selected based on the average time of heat pulses during Cerrado fires, which spread rapidly (Miranda et al., 1993; Rissi et al., 2017). After heat shock treatment, seeds were tested for germination under optimal laboratory conditions.

Seeds were allowed to germinate in Petri dishes, upon a double layer of filter paper moistened with distilled water. They were incubated in germination chambers at a constant temperature of 27 °C (12 h light), which is an average temperature of the soil surface in Central Brazil (Daibes et al., 2017) and is considered optimal for germination of Cerrado species (see also Fichino et al., 2016). Germination was scored at 2- to 3-d intervals for 1 month, and seeds exhibiting radicle protrusion were considered germinated. Germinated seeds were discarded after scoring. By the end of the trials, ungerminated seeds were tested for seed viability. Hard seeds (remaining dormant) were mechanically scarified with sandpaper and set to germinate for an additional week; those that germinated within that period were scored as viable. Imbibed but ungerminated seeds were carefully cut with a razor blade and subjected to tetrazolium tests (1 %, pH 7). Seeds with embryos that stained red were counted as viable (Hilhorst, 2011), while non-stained or damaged seeds were scored as dead.

#### Seed trait measurements

Different seed traits were measured, including seed mass, water content, seed shape and proportion of seeds with water-permeable coat. Morphological traits (seed mass and seed shape) were measured individually on nearly 20 seeds per collected individual (see Supplementary Data Table S4). Seed mass was measured for dried seeds after oven-drying at 80 °C for 48 h. Fresh mass was previously weighed, and water content (on a fresh mass basis) was obtained as the adjusted difference between fresh and dry seed mass. Seed shape was based on

three dimensions: length, breadth and width (in millimetres). We measured each dimension, using a digital caliper, and divided each value by the largest dimension of the seed – usually the length – then calculated the variance among them (Pérez-Harguindeguy et al., 2013). Thus, seed shape varies between zero (a sphere) and one; the closer to one, the more flattened and/or elongated is the seed (see Thompson et al., 1993).

Our study species have never shown any other type of seed dormancy except PY. Therefore, the proportion of permeable (non-dormant) seeds was obtained as the adjusted proportion of germinated seeds in relation to the total initial seed viability of each species, based on germination of controls (untreated seeds; ~100 seeds per species) as described above. Total viability was considered as the sum of non-dormant + dormant seeds (ungerminated but viable by the end of tests). Based on the proportions of non-dormant seeds, we categorized seed dormancy into three classes (see Baskin and Baskin, 2014; Dayrell et al., 2017): physically dormant seeds (PY), with less than 30 % adjusted germination in relation to viable seeds; intermediate dormancy (IN), with 30–70 % adjusted germination; and non-dormant (ND), exhibiting more than 70 % adjusted germination (Supplementary Data Table S4).

#### Data analyses

All analyses were performed in R software version 3.2.5 (R Core Team, 2016). First, we performed generalized linear mixed models (GLMMs), separately for each species, to evaluate germination percentage and total viability as functions of the heat shock treatments (see Supplementary Data Tables S2 and S3). We used a binomial distribution and considered replicates as random effects (Zuur et al., 2009). A principal component analysis (PCA) was conducted to evaluate whether seed traits could be used to group species according to their vegetation type. Data were log-transformed, centred and rescaled before the analysis. PCA was run using the *stats* package and plotted with the *ggbiplot* function, available from *github*.

Next, we calculated the magnitude of the effects of the heat shock treatments in relation to the controls. Such an effect size was obtained by subtracting the number of germinated seeds in each treatment from the number of seeds germinated in the control, and was then calculated as a proportion of total viability:  $(N_{germ}^{(treat)} - N_{germ}^{(control)})/N_{viab}^{(control)}$ . This index can be positive or negative (i.e. with more or less germination in the treatment than in the control, respectively). Such an effect was compared as a function of the interaction between vegetation type and heat shock treatments, using linear mixed effect models (LMMs) and considering the species as random effects. All mixed-effects models were performed using the *lme4* package (Bates et al., 2015).

Seed mortality was first evaluated as the difference between seed viability in the controls and the heat shock treatment:  $(N_{viab}^{(control)} - N_{viab}^{(treat)})/N_{viab}^{(control)}$ . Then, we assessed whether heat shocks increased seed mortality, using a GLMM with binomial distribution. Seed mortality was also evaluated as a function of the interaction between vegetation type and heat shock treatments. In all analyses, vegetation type categories were considered as a gradient of increasing fire frequency: forest, woody savanna and grassy savanna. Thus, forest (fire-free)

and the 100 °C 1 min treatment (lowest heat dose) served as the baseline (intercept) for the statistical analyses. Species were considered as random effects.

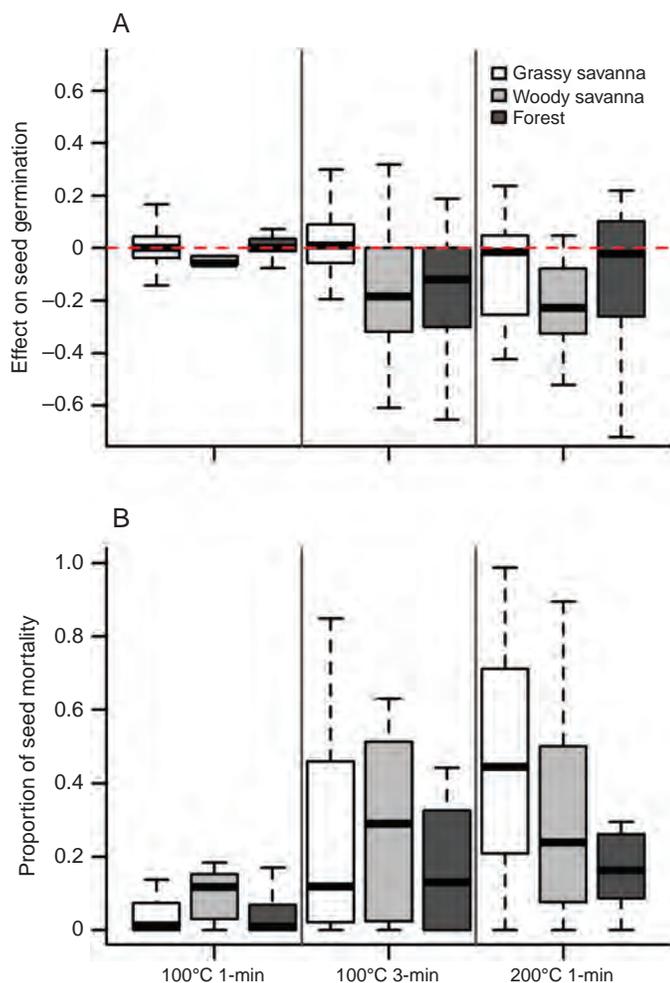


FIG. 1. Relationship between (A) seed germination (effect size in relation to controls) and (B) seed mortality (dead seeds in relation to controls) under different heat shock treatments for the different vegetation types [grassy savanna (shrubs), woody savanna (trees), forest (trees)]. See Table 1 for statistical tests.

TABLE 1. Coefficients of the generalized linear mixed models for seed germination and seed mortality in relation to the heat shock treatments (100 °C 1 min, 100 °C 3 min, 200 °C 1 min) and vegetation type (forest trees, woody savanna trees, grassy savanna shrubs) – the 100 °C 1 min treatment (lowest heat dose) and forest vegetation type (fire-free) were considered as the baseline (intercept) in the analyses

	Germination			Mortality		
	Estimate	s.e.	P	Estimate	s.e.	P
Intercept (forest and 100 °C 1 min)	0.012	0.065	—	−4.051	0.481	—
Treatment (100 °C 3 min)	−0.166	0.069	n.s.	<b>1.971</b>	<b>0.206</b>	<b>&lt;0.001</b>
Treatment (200 °C 1 min)	−0.105	0.069	n.s.	<b>2.170</b>	<b>0.204</b>	<b>&lt;0.001</b>
Woody savanna	−0.076	0.101	n.s.	1.168	0.732	n.s.
Grassy savanna	−0.037	0.081	n.s.	0.987	0.588	n.s.
Treatment 100 °C 3 min × woody savanna	0.041	0.110	n.s.	−0.075	0.295	n.s.
Treatment 100 °C 3 min × grassy savanna	0.190	0.087	n.s.	−0.431	0.242	n.s.
Treat 200 °C 1 min × woody savanna	−0.106	0.107	n.s.	−0.478	0.288	n.s.
Treat 200 °C 1 min × grassy savanna	−0.012	0.086	n.s.	<b>0.688</b>	<b>0.239</b>	<b>0.004</b>

s.e. = standard error; n.s. = non-significant.

Because seeds rarely died in the 100 °C 1 min treatment (see Results), we analysed seed mortality in relation to seed traits only for the treatments where mortality was statistically different from the baseline. Given the correlation between some seed traits (see Results), we investigated seed mortality in relation to seed mass (seed size), seed shape (three-dimensional perspective) and proportion of permeable seeds. Hence, we conducted GLMMs with a binomial distribution, considering species as random effects. Whenever relevant, we also conducted this analysis separately for (1) all the study species, and (2) the different growth-forms: trees (from forest and woody savanna) and shrubs (from grassy savanna). Categories of seed dormancy were also evaluated in a separate analysis, considering only small-seeded species (seed mass <0.05 g, to avoid bias of larger seed mass) and grouped IN + ND species in comparison to PY.

Finally, the relationship between seed mortality and seed traits was also investigated through a phylogenetic comparative analysis, conducted with the function *compar.gee* in the package *APE* (Paradis et al., 2004). To do so, we used a previously reconstructed phylogenetic tree for legumes, based on *matk839c* (Simon et al., 2009), which was kindly provided by Dr M. F. Simon (Embrapa/Cenargen, Brazil). We pruned this phylogeny, leaving our species or genera and adding missing species with Mesquite software version 3.2 (Maddison and Maddison, 2017). When possible, polytomies were resolved according to updated published phylogenies on *Mimosa*, *Senna* and *Chamaecrista* (Marazzi et al., 2006; Conceição et al., 2009; Simon et al., 2011). Branch lengths were calibrated based on the initial divergence times dated in the original tree, and an average estimation was made for the added species using the Phylocom software and the *bladj* function (Webb et al., 2008).

## RESULTS

Overall, seed germination was unaffected by heat shock treatments, irrespective of growth-form and vegetation type (Fig. 1A; Table 1). Species-based analyses indicated that heat shocks had a positive effect on the seed germination of only six shrubs – four *Mimosa* and two *Senna* – out of the 46 study species (Supplementary Data Tables S1 and S2). Effect sizes in relation to controls were never greater than 30 % for all species (Fig. 1A). Regarding seed viability, nearly all species tolerated

the 100 °C 1 min treatment, while approximately one-third and one-half of species had decreased viability under the 100 °C 3 min and 200 °C 1 min treatments, respectively (Table S3). Therefore, seed mortality was significantly higher under 100 °C 3 min and 200 °C 1 min (Fig. 1B; both  $P < 0.001$ , see Table 1). The interaction between treatment and vegetation type was significant, and mortality was highest for grassy savannas under the hottest treatment (200 °C 1 min;  $P = 0.004$ ; Table 1).

Considering seed traits, the two first principal components of the PCA explained 56 % of the variation in the data. Seed mass and length were correlated, providing a major contribution to PC1, whereas PC2 was primarily linked to seed permeability and water content (Fig. 2). Grassy savanna shrubs were more clustered (due to their smaller seeds) than savanna and forest trees, which were scattered through the multi-dimensional space (Fig. 2). Both savanna and forest species have shown variable proportions of permeable seeds (Supplementary Data Fig. S1); therefore, dormancy class ranged from PY to ND across study species (Table S4).

No significant effect was detected regarding seed mortality and seed traits in the 100 °C 3 min treatment (Fig. 3A; Table 2). Under 200 °C 1 min, seed mortality was negatively related to seed mass, with larger-seeded species showing lower seed mortality when considering all species together ( $P < 0.001$ ; Fig. 3B; Table 2). Grassy savanna shrubs had smaller seed mass (ranging from 0.003 to 0.063 g) in comparison to savanna and forest trees (which varied from 0.01 to 3.45 g; Fig. 3C; Supplementary Data Table S4). Considering tree species separately, phylogenetically controlled analysis also detected a significant effect of seed mass on seed mortality under 200 °C (Table 2). Species in Detarioideae (e.g. genera *Hymenaea* and *Copaifera*) had the largest seeds (Fig. S2a), which were unaffected by heating (Fig. 4; Table S3, Fig. S2b). Caesalpinioideae (including the

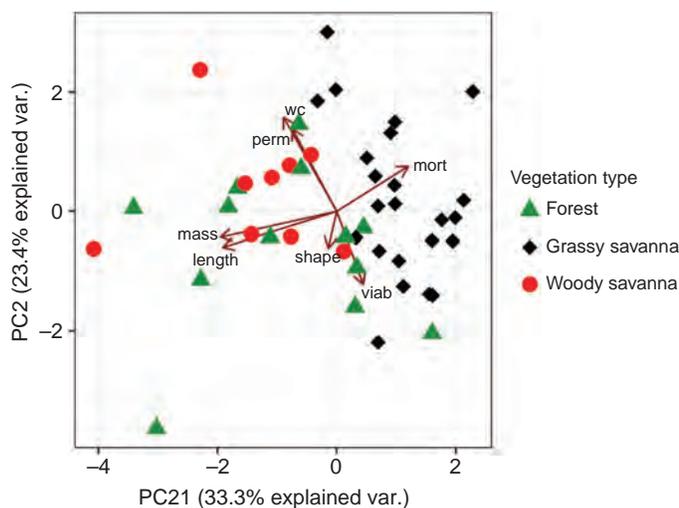


FIG. 2. Principal component analysis of seed traits in 46 legume species from different vegetation types (grassy savannas, woody savannas and forest) of the Cerrado. PC1 had major contributions from seed mass (−0.60) and seed length (−0.59), whereas PC2 was mainly related to seed coat permeability (0.50) and seed water content (0.58). perm = proportion of permeable seeds; wc = water content; viab = seed viability in control treatments; mort = seed mortality under 200 °C 1 min.

Mimosoid clade) had the smallest seeds and the highest seed mortality under 200 °C (Figs 3B and 4).

Seed shape varied from spherical to elongate-flattened in shrubs (0.05–0.2; see Supplementary Data Table S4) as well as in savanna and forest trees (0.04–0.28). The relationship between seed shape and seed mortality was not significant, regardless of the treatment (Table 2). Evaluating shrub species separately, there was a significant effect of permeability on seed mortality in the hottest treatment (Table 2). Regardless of the vegetation type (excluding large-seeded species), small-seeded species with higher numbers of non-dormant seeds

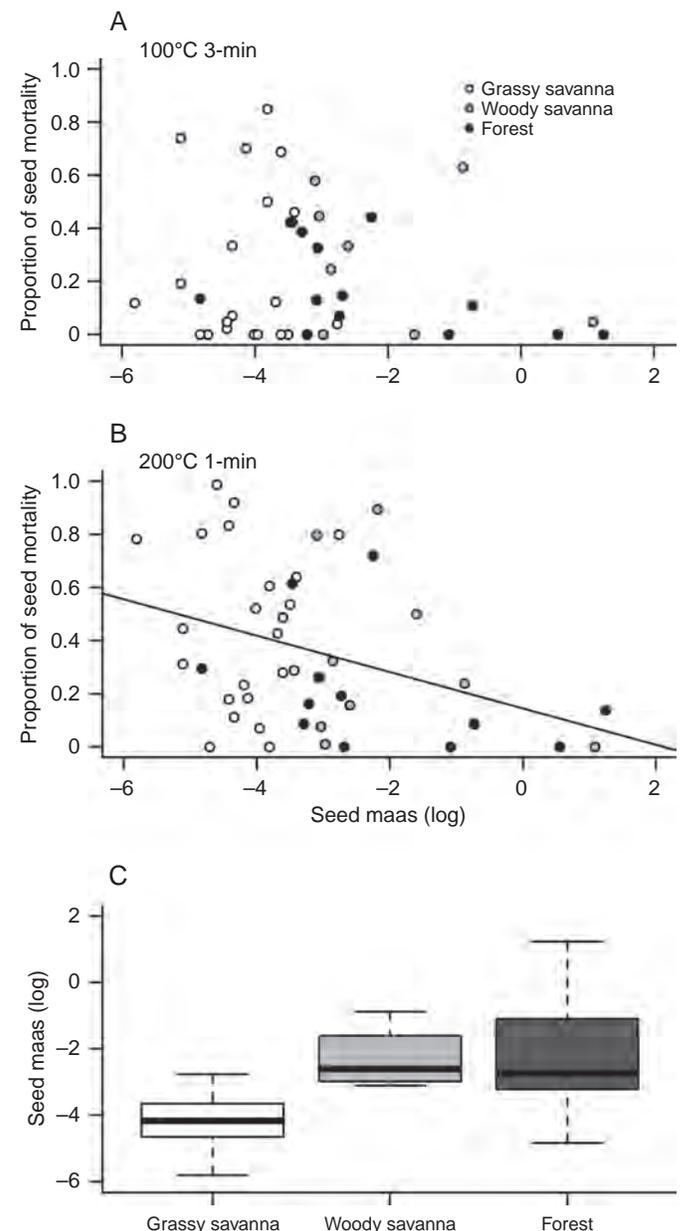


FIG. 3. (A) Seed mortality (dead seeds relative to controls) in relation to seed mass (log) in the 100 °C 3 min treatment (not significant; Table 2). (B) Seed mortality in relation to seed mass in the 200 °C 1 min treatment. (C) Seed mass (log) for the different vegetation types (significantly smaller in shrubs than savanna and forest trees; Supplementary Data Table S4).

TABLE 2. Coefficients of the generalized linear mixed models (GLMMs) and phylogenetic comparisons of how seed mass, seed shape and the proportion of permeable seeds (*perm*) explain seed mortality in the heat shock treatments that killed a significant proportion of the seeds (100 °C 3 min and 200 °C 1 min). Forest and savanna trees were grouped in this analysis because this growth-form showed less effect from heat shocks in comparison to shrubs (Table 1)

		GLMMs			Phylogenetic comparison		
		Estimate	s.e.	<i>P</i>	Estimate	s.e.	<i>P</i>
<b>All species</b>							
100 °C 3 min	Intercept	-4.141	1.393	—	-0.369	0.867	—
	mass	-0.427	0.289	n.s.	0.255	0.172	n.s.
	shape	0.006	0.071	n.s.	0.001	0.042	n.s.
	<i>perm</i>	0.009	0.012	n.s.	-0.009	0.007	n.s.
<b>All species</b>							
200 °C 1 min	Intercept	-4.229	1.007	—	-1.526	0.926	—
	mass	-0.731	0.211	<b>&lt;0.001</b>	-0.188	0.190	n.s.
	shape	0.011	0.054	n.s.	0.009	0.037	n.s.
	<i>perm</i>	0.017	0.009	n.s.	0.006	0.006	n.s.
<b>Trees (forest and woody savanna)</b>							
200 °C 1 min	Intercept	-4.055	1.157	—	-3.899	1.041	—
	mass	-0.500	0.351	n.s.	-0.696	0.269	<b>0.032</b>
	shape	0.049	0.076	n.s.	0.004	0.055	n.s.
	<i>perm</i>	0.009	0.011	n.s.	0.006	0.008	n.s.
<b>Shrubs (grassy savanna)</b>							
200 °C 1 min	Intercept	-2.917	2.284	—	-2.722	1.673	—
	mass	-0.114	0.495	n.s.	-0.041	0.323	n.s.
	shape	0.117	0.115	n.s.	0.190	0.080	n.s.
	<i>perm</i>	0.034	0.012	<b>0.006</b>	0.024	0.010	n.s.

s.e. = standard error; n.s. = non-significant.

(intermediate and non-dormant categories: IN and ND) had higher seed mortality under 200 °C ( $P = 0.004$ ; Fig. 5) in comparison to PY seeds. Such dormancy patterns were not significant under 100 °C 3 min treatment (Table 2; Fig. 5).

## DISCUSSION

Our study is the first to show an overall lack of germination responses to fire across vegetation types, growth-forms and phylogenetic affinities in a large group of species (Leguminosae) in the Cerrado mosaic. Although fire is known to have a minor effect on germination of Cerrado seeds (e.g. Ribeiro *et al.*, 2013; Fichino *et al.*, 2016), previous research has focused on physically dormant species, which represent the clearest examples of fire-mediated dormancy break at a global scale (Auld and O'Connell, 1991; Herranz *et al.*, 1998; Williams *et al.*, 2003; Moreira and Pausas, 2012; Ooi *et al.*, 2014). Contrary to the findings of studies on Mediterranean-type ecosystems and Australian savannas, heat shocks (100 °C 3 min and/or 200 °C 1 min treatments) enhanced germination in only a few species (six out of 46), with effect sizes limited to 30 %. These results suggest a minimal role of fire temperature in enhancing recruitment, which strongly contrasts with other fire-prone vegetation, where PY break seems to be closely tied to fire-related temperatures (>80 °C, see Moreira and Pausas, 2012; Ooi *et al.*, 2014).

Savannas have assembled relatively recently in the Earth's history (around 8 Mya), following the expansion of  $C_4$  grasses and resulting from fire-climate feedback (Beerling and Osborne, 2006). Woody lineages of Cerrado species have originated from surrounding fire-free forests (Simon *et al.*, 2009), which emerged in the Late Palaeocene (~60 Mya, Wing *et al.*, 2009) under a climate of non-fire conditions in the Cenozoic.

Fire-climate relationships affect the savannas in contrasting ways across continents (Lehmann *et al.*, 2014), which may also apply to fire-mediated germination. For example, Australian savannas may form persistent seed banks that are highly stimulated by fire (Williams *et al.*, 2005; Scott *et al.*, 2010), because they evolved from lineages in arid environments that originated in the fire-prone Cretaceous (Crisp *et al.*, 2011), which is not the case for Brazilian savannas. In contrast, Cerrado species acquired other mechanisms that enabled them to persist under the high frequency of savanna fires, such as strong basal resprouting and thick corky barks (Simon *et al.*, 2009; Dantas and Pausas, 2013; Pausas *et al.*, 2018). Therefore, contemporary effects of fire on seed germination need to be examined from an historical and phylogenetic perspective.

A lack of fire-stimulated germination has also been shown for fire-prone Brazilian subtropical grasslands, the Argentinean Chaco and the Chilean matorral (Overbeck *et al.*, 2006; Jaureguiberry and Díaz, 2015; Fidelis *et al.*, 2016; Gómez-González *et al.*, 2017). Irrespective of germination, seed traits were important predictors of seed mortality under the hottest treatment. Growth-form is also a crucial factor, globally recognized as strongly related to seed size (Moles *et al.*, 2005, 2007). This helps to explain how small-seeded shrubs could show higher seed mortality under 200 °C, despite facing frequent fires in the grassy savannas. Trees, however, usually have relatively larger seeds (Moles *et al.*, 2005; Rubio de Casas *et al.*, 2017), which may provide protection to the embryo (Ribeiro *et al.*, 2015). Moreover, this trait is phylogenetically conserved (Moles *et al.*, 2005; Table 2), thus clarifying why lineages of forest species have heat-tolerant seeds. Large-seeded forest species might have played a role in the colonization of savannas, radiating lineages of vicariant congeneric pairs with

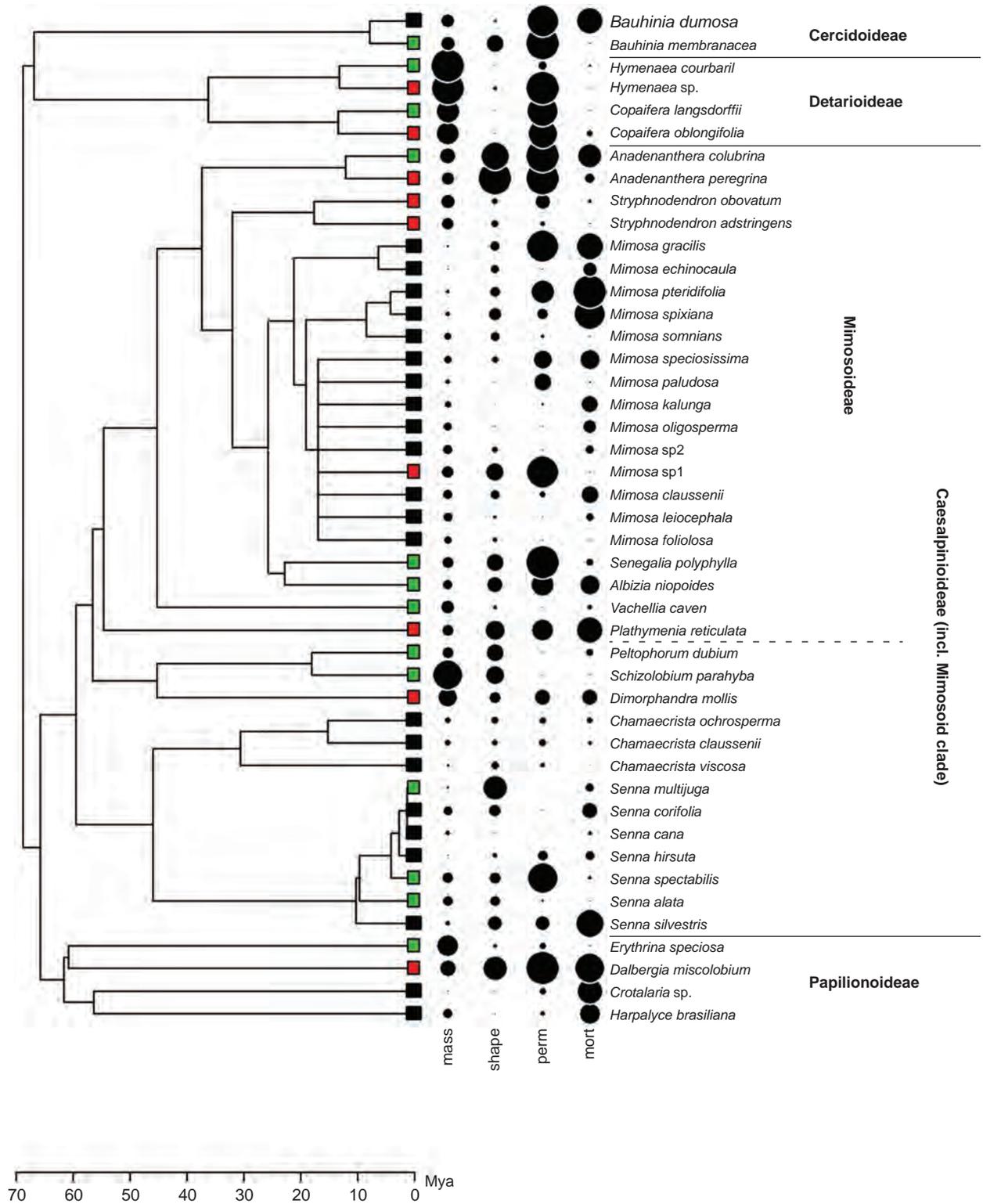


FIG. 4. Time-calibrated phylogenetic tree and seed traits (seed mass, seed shape, proportion of permeable seeds, and mortality at 200 °C 1 min) for legume species of the Cerrado. *Chamaecrista desvauxii* was excluded from the figure because it was not tested under 200 °C. Subfamilies are classified according to The Legume Phylogeny Working Group (2017). Square colours indicate vegetation type + growth-form: forest trees (green), woody savanna trees (red), and grassy savanna shrubs (black). Circle size is proportional to trait value.

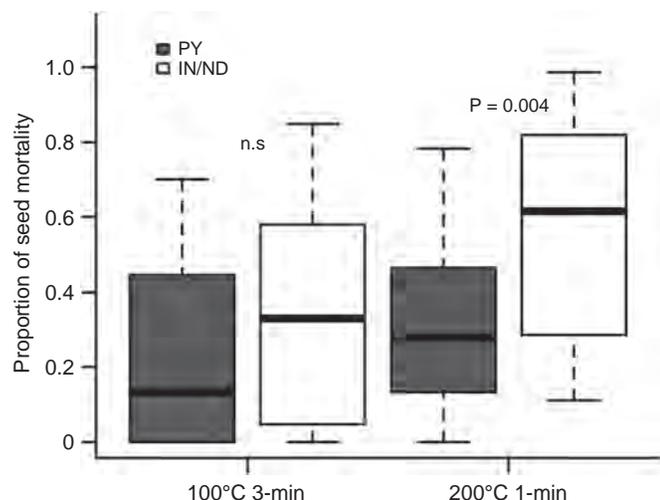


FIG. 5. Seed mortality analysis considering only small-seeded species (<0.05 g), regardless of vegetation type, under 100 °C 3 min and 200 °C 1 min, in relation to dormancy level (PY = physical dormancy; IN/ND = species with intermediate and/or non-dormant seeds) in legumes of the Cerrado.

different establishment strategies in contrasting habitats (see Hoffmann, 2000).

Despite the relative importance of seed mass in predicting seed tolerance to fire (see Ribeiro *et al.*, 2015), a considerable number of small-seeded species (from both savanna and forest) were still able to tolerate the heat shock treatments. In such cases, PY represented an important trait that decreases seed mortality under the hottest treatment (200 °C; Fig. 5). Seeds exposed in the soil surface would face severe fire temperatures (see Daibes *et al.*, 2017, 2018); thus, PY can be important for avoiding seed mortality. In an evolutionary context, water-impermeable seeds evolved independently in at least 15 families of flowering plants, and there is fossil evidence (*Rhus rooseae*) of PY appearing around 43 Mya (Baskin *et al.*, 2000; Willis *et al.*, 2014). Some authors consider that multiple environmental factors shaped PY in fire-prone ecosystems (Santana *et al.*, 2010, 2013), and therefore heat tolerance could be argued as an exaptation (Jaganathan, 2015).

Other evidence indicates that hard-seededness could be even more ancient, originating during the fire-prone Cretaceous (Lamont *et al.*, 2019), following the origins of Fabales (~100 Mya; see Bello *et al.*, 2012; Li *et al.*, 2015). Either way, water-impermeable seed coats offer a physical barrier protecting the embryo against environmental hazards, mostly by providing desiccation tolerance (Rolston, 1978; Tweddle *et al.*, 2003). PY seeds have very low water contents, and this trait seems to be an important proxy in predicting heat tolerance in seasonal vegetation (Tangney *et al.*, 2019). PY is today found mainly in seasonal environments (Rubio de Casas *et al.*, 2017), irrespective of fire. Hence, if acquired during the Cretaceous, fire-related PY-breaking mechanisms would have been lost during the radiation of Neotropical legumes in the Tertiary (see Lavin *et al.*, 2005). What we know so far is that different types of seed dormancy may drive propagule persistence (Dalling *et al.*, 2011; Long *et al.*, 2015), independent of their evolutionary origins.

Other seed traits, such as seed shape, had no effect on seed mortality in our study species, contrasting with the findings that

rounded seeds should be more heat-tolerant in Mediterranean ecosystems (Gómez-González *et al.*, 2016). Some biometrical components of seed shape, such as seed length, are correlated with seed mass and have therefore been suggested to explain heat-tolerance in African savanna plants (Gashaw and Michelsen, 2002). Although seed mortality was significant under the 100 °C 3 min treatment, 65 % of the study species remained unaffected. Therefore, most seeds would be able to survive fires when incorporated into soil seed banks, where fire temperatures at 1 cm below ground would reach <60 °C during fire passage (Miranda *et al.*, 1993). Below-ground temperatures could increase during hotter fires, but still would not reach temperatures high enough to affect viability of seeds buried in soil seed banks (Daibes *et al.*, 2017). Moreover, because smaller seeds incorporate easily into the soil (Saatkamp *et al.*, 2014), they would be similarly likely as larger seeds of surviving milder fires.

Nonetheless, the Cerrado forms mostly transient seed banks, where propagules recruit (or die) seasonally, showing a low density of buried propagules (de Andrade and Miranda, 2014). Moreover, some tropical legumes have been shown to have PY-break mediated by a combination of moisture and heating (van Klinken *et al.*, 2006). In the Cerrado, several species disperse seeds in synchrony with the onset of the rainy season, probably as a strategy for seedling recruitment in less stressful conditions (Salazar *et al.*, 2011; Ramos *et al.*, 2017; Escobar *et al.*, 2018). Therefore, our evidence suggests that direct fire heat shocks may not have been selected as the main dormancy-breaking factor in Cerrado, and a combination of environmental cues, such as soil moisture and temperature fluctuation, could potentially be identified as factors contributing to PY alleviation under field conditions (Daibes *et al.*, 2017).

## CONCLUSIONS

We have found little evidence to support fire-mediated PY dormancy break in the Cerrado mosaic. Forest lineages emerged before the settlement of the Cerrado savannas, and larger seeds, by offering some protection to the embryo, probably helped tree species to colonize and persist in the fire-prone environment. The presence of PY is also an important seed trait driving heat tolerance of small-seeded species under severe conditions. Overall, historical factors (seed traits and phylogenetic affiliation) better explained germination responses to fire in the Cerrado than ecological factors such as fire regime. This is a very different pattern from other fire-prone ecosystems around the world. A global analysis would be desired to fully understand the relative role of fire, climate and historical factors in shaping seed responses to fire.

## SUPPLEMENTARY DATA

Supplementary data are available online at <https://academic.oup.com/aob> and consist of the following. Table S1: study species, vegetation type, growth-form, collection site, date of collection and date of the experimental set-up for 46 legumes of the Cerrado. Table S2: seed germination after fire-related heat shocks. Table S3: seed viability after fire-related heat shocks. Table S4: number of sampled individuals, number of measured

seeds in morphological traits, seed mass and shape values, number of seeds per replicate in germination tests and seed dormancy class for 46 legumes of the Cerrado. **Fig. S1**: proportion of non-dormant seeds between forest and savannas in the Cerrado. **Fig. S2**: relationship between seed mass and seed mortality of 19 tree species of the Cerrado as a function of their legume phylogenetic groups: Detarioideae, Mimosoideae and Caesalpinioideae.

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