

Short Communication

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From germination to seedling development: the effects of smoke on a native and an invasive grass species of the Cerrado

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Abstract

The Brazilian Cerrado, a Neotropical savanna, is a fire-prone ecosystem where the ground layer biomass consists mainly of graminoids. However, as for other savannas, the effects of fire cues (such as smoke) on Cerrado grasses do not present a clear pattern, either for germination or seedling development. Smoke can stimulate different stages of the plant life cycle, which can alter the community and invasion processes. So far, most research on the subject focuses on germination, not addressing post-germinative phases, a sensitive stage of plant development. Here, we investigated the effect of smoke on a native (*Echinolaena inflexa*) and an invasive (*Urochloa decumbens*) grass species common in the Cerrado. We analysed germinative parameters and seedling mass and length after exposing the seeds to dry smoke for 5, 10, 15 or 20 min. Seedling development was assessed by measuring shoot and root systems after cultivating germinated seeds for 3, 7 or 15 d. Smoke did not affect germination percentages. However, fumigation reduced the mean germination time of both species and the germination onset of *E. inflexa*. *U. decumbens* had higher length values in all periods of cultivation, whereas mass values only surpassed that of *E. inflexa* at 15 d. Smoke exposure reduced the aboveground length of 7-d seedlings of *U. decumbens*, and mass of 15-d plants of both species. Also, smoke enhanced the root investment of the native and invasive species in different cultivation periods. Therefore, studying post-germinative parameters on seedling development may bring further insights into the smoke effects.

Introduction

The plant communities in fire-prone ecosystems are largely affected by fire, making the understanding of its effects imperative for the study of the communities (Bond and Keeley, 2005). In the Brazilian Cerrado, a Neotropical savanna, adaptations to fire date from 9.8 to 0.4 Mya (million years ago) (Simon et al., 2009). The vegetation in this biome presents numerous fire adaptations, such as high resprouting capacity, thermal insulation by a thick bark and synchronous flowering after burns (Neves and Damasceno-Junior, 2011; Dantas and Pausas, 2013; Pausas et al., 2018; Pilon et al., 2018). Despite the possible negative impacts of fire, burned areas represent new establishment opportunities for the surviving organisms and seedling recruitment due to the nutrient input and alleviated interplant competition (Miranda and Klink, 1996a,b; Lamont and Downes, 2011; Musso et al., 2015). Although vegetative reproduction is prevalent in the Cerrado, seed reproduction is also important for the persistence of species in fire-prone ecosystems (Pilon et al., 2021). In this context, fire-related cues – such as heat and smoke – may have a high adaptive value for pyrophytic species. Accordingly, fire-related cues have been reported to stimulate germination and seedling growth traits (Lange and Boucher, 1990; Baxter et al., 1994; Clarke and French, 2005; Sparg et al., 2005; Light et al., 2009; Moreira et al., 2010; Ghebrehiwot et al., 2012; Mojzes et al., 2015; Tavşanoğlu et al., 2015; Zirondi et al., 2019), which are both common strategies for population maintenance (Labouriau et al., 1963; Salazar et al., 2011; Andrade and Miranda, 2014).

In the Cerrado, grasses correspond to the majority of the biomass in the ground layer (Castro and Kauffman, 1998), and most of the species present a perennial life cycle, with seed dispersal through the dry season (Sarmiento, 1992; Munhoz and Felfili, 2007), when wildfires are frequent (Pivello, 2011). As in other savannas, Cerrado fires are sustained by the ground layer and have a low residence time of high temperatures (Miranda et al., 1993). Several studies suggest that germination responses are idiosyncratic among grassland species worldwide and report that grass seeds can tolerate heat shocks up to 100°C without detrimental effects (Clarke and French, 2005; Overbeck et al., 2006; Dayamba et al., 2008; Ramos et al., 2016; Paredes et al., 2018; Ramos et al., 2019; Dairel and Fidelis, 2020a; Gorgone-Barbosa et al., 2020). Likewise, smoke stimuli on germination do not present a

clear pattern for grasses (Dayamba et al., 2008; Fernandes et al., 2021). On the other hand, the effect of smoke on grass seedling development is still scarce and indicates that measurable parameters might be noticeable only in post-germinative phases (Taylor and van Staden, 1996; Blank and Young, 1998; Daws et al., 2007; Ghebrehiwot et al., 2012). In addition, early seedling development is a critical phase in the plant life cycle, and previous studies have shown noticeable effects of smoke during this stage (Sparg et al., 2005).

Different aspects of smoke may result in a broader ecological significance than heat-shock. First, smoke may influence areas that have not been burned but are adjacent to its occurrence. Curtis (1998) and Lamont and Downes (2011) inferred that an increased blooming in an unburned area resulted from smoke drift from burned adjacent areas (200–1000 m apart). However, few studies address the effects of dry smoke, which surrogates field conditions in the dry season (Sparg et al., 2005; Dayamba et al., 2008). Second, smoke-stimulated germination is registered in a large number of clades around the world and may be an ancestral feature in plant phylogeny (Keeley and Pausas, 2018). Third, the smoke compound credited for triggering the processes (butenolide, karrikinolide-1) acts in small concentrations and can be originated by any cellulose combustion (Flematti et al., 2004; Light et al., 2009). Also, the butenolide penetrates the soil, possibly influencing the soil seed bank, which is isolated from high temperatures (Stevens et al., 2007; Ghebrehiwot et al., 2012). In this context, the smoke could alter the plant community by affecting native and exotic plants present in the areas.

African grasses represent a threat to Cerrado's biodiversity leading to changes in the local species composition (Pivello et al., 1999; Zenni and Ziller, 2011). For the ground layer vegetation, the threat increases if a positive interaction between smoke and the invasive species is presented. Among the alien grasses, *Urochloa decumbens* (Stapf) R. D. Webster – a perennial C4 grass – is the most widespread due to pasture formation in the region (Loch, 1977; Zenni and Ziller, 2011). *U. decumbens* is a strong competitor in the soil bank by forming a transient soil seed bank larger than native species, also by excluding other invaders (Correia and Martins, 2015; Dairel and Fidelis, 2020b). Although transient, the seed bank is continuously replenished by several flowering episodes throughout the year (Florencio et al., 2009; Dantas-Junior et al., 2018; Xavier et al., 2019). The *U. decumbens* invasiveness may also be associated with its high vegetative reproduction rates (Loch, 1977). Among the native Cerrado grasses, *Echinolaena inflexa* (Poir.) Chase – a perennial C3 species – is dominant in the ground layer, with broad distribution through the biome (Klink and Joly, 1989; Pivello et al., 1999). This species has a set of morphological plasticity traits influenced by environmental conditions, which allow colonization of burned areas through seed dispersal and vegetative resprouts (Miranda and Klink, 1996a,b). Also, *E. inflexa* presents dominance in the soil seed bank in the months after burns (Andrade and Miranda, 2014). Both *E. inflexa* and *U. decumbens* present a similar seed morphology and seedling emergence, high vegetative reproduction rates, stoloniferous growth habit, and are widely distributed in Brazil. Even though *U. decumbens* was not able to alter the distribution of *E. inflexa* in degraded Cerrado areas (Pivello et al., 1999), due to their similarities, these grasses could compete for similar niches in cases of co-occurrence, therefore comparing both species is a coherent approach for studying their interaction.

Here, we investigate the effects of smoke on germination and early seedling development of *E. inflexa* and *U. decumbens*. By evaluating the changes in germinative parameters, seedling mass and seedling length, we aim to provide an overview of responses of these species to different dry smoke exposure periods. Given the evolutionary history of *E. inflexa* with fire in the Cerrado, we initially hypothesized that exposure to smoke would result in overall beneficial effects for the native grass, whereas the invasive would present negative or no responses. In addition, considering that smoke is known to affect the germination of a large number of plants (Keeley and Pausas, 2018) and invasive grasses may alter the ecosystem's plant community, our study can be valuable for management purposes and provide further insights into the response of *E. inflexa* to smoke and in the invasion success of *U. decumbens* in the Cerrado.

Materials and methods

Studied species and seed sorting

The species used in this study were *E. inflexa* and *U. decumbens*. Seeds of *E. inflexa* were collected in the Reserva Ecológica of IBGE (35 km South of Brasília, DF, 15°55'S, 47°52'W) at the end of the rainy season in 2017 (March–April). Seeds of *U. decumbens* were bought for presenting low quality and quantity in field conditions. The diaspores of both species were manually sorted and tested, selecting only those containing a full caryopsis (Brasil, 2009; Aires et al., 2014). The seeds were stored in ambient conditions (~25°C, 50% R.U.) inside paper bags in the laboratory cabinets until the experiment.

Fumigation

The fumigation was carried out in January 2018, with dry smoke derived from the burn of leaf litter collected in an open savanna (cerrado *sensu stricto*) area at the Reserva Ecológica of IBGE. Before burning, the litter was dried in an oven at 60°C for 48 h and homogenized to ensure a similar smoke composition in all replicates (Light et al., 2009). The smoke passed through a chimney, ca 2 m distant from the heat source, avoiding any temperature effect on seeds. Smoke and air temperatures (T_s and T_a) were attested by two thermocouples (type k: chromel/alumel, 30 swg): the first placed over the fine-mesh (1 mm) metallic support where the seeds were positioned during fumigation, and the second 2 m apart from the heat source in the upwind direction ($T_s = 1011 \times T_a$; $r^2 = 0.9852$; $P = 0.0001$). The seeds were arranged on the mesh without overlapping, to ensure a homogeneous exposure to the smoke. The seeds were smoked for 5, 10, 15 or 20 min and a control group (without exposure), waiting for complete smoke dissipation between replicates. For each species and treatment, we used five replicates with 70 seeds each, randomly selected from the previously sorted seeds. To avoid pseudoreplication, each replicate was fumigated separately (Morrison and Morris, 2000).

The number of seeds per replicate (70) was established by a 48-h viability test under dark conditions, applying a 1% solution of 2,3,5-triphenyl tetrazolium chloride (Lakon, 1949), carried out 5 months before the fumigation. The tetrazolium solution was applied to seeds of each species, with no optimization for either. The viability was assessed by cutting all seeds under a dissecting microscope to determine the presence of a coloured embryo.

Germination

After the smoke exposure, the seeds were sown in Petri dishes with filter paper and moistened with distilled water. No chemical or physical treatment was applied to the seeds to prevent contamination by pathogens or fungi (Paredes et al., 2018). The dishes were placed in a greenhouse under white light (12 h/12 h), ambient temperature and humidity (~27°C; 54%) and kept continually moistened by distilled water. The number of germinated seeds was counted daily for 30 d. The seeds were considered germinated when presenting aerial parts and the geotropic curvature of the radicle (Kumar et al., 2012). We also registered the initial time of germination (T₀) and calculated the mean germination time (MGT). The formula used for the MGT was $(\Sigma(n \times d))/N$, where n is the number of germinated seeds per day, d is days passed since the beginning of the experiment and N is the total number of germinated seeds (Kochankov et al., 1998). After the experiment period, the non-germinated seeds were tested for viability as described previously.

Seedling parameters and Root:Shoot ratio

Concurrent with germination, we evaluated changes in the mass and length of seedlings after cultivation for 3, 7 or 15 d. All seedlings were cultivated under the same light, temperature and humidity conditions as previously described. For each smoke treatment, the germinated seeds were transferred from the Petri dishes to trays with moistened filter paper. The seedlings were identified by replicate and germination date. After 3 and 7 d of cultivation, five seedlings of each replicate were harvested for mass and length measurements. Also, five seedlings of each replicate were transferred to pots (5 cm diameter × 9 cm depth) in which they were cultivated for 15 d. The pots contained a commercial substrate made with *Sphagnum* peat moss, coconut fibre, rice husk, Pinus bark, vermiculite, NPK and micronutrients, and pH 6.0–6.5 (Pires et al., 2018). To avoid damage to the root system and biomass loss, the seedlings cultivated for 3 and 7 d were carefully removed with tweezers from the filter paper. Seedlings grown for 15 d were carefully rinsed under running water to remove the substrate. The seedlings were cut directly below the cotyledon, at the beginning of the root system, and the aerial and root parts were measured in length, dried for 48 h in an oven (60°C), and then weighted for attaining the dry mass (0.00001 g precision). For the control group, the data collection followed the same procedures described above. With the length and dry mass data, we conducted the Root:Shoot (R:S) analysis to assess the investment in root development.

Data analysis

We applied a generalized linear mixed model (GLMM) with a binomial error distribution and Logit link function to analyse the differences in the percentages of germination and viability of non-germinated seeds between species for each treatment. In the GLMM analysis, we used the replicates as random effects. For the T₀ analysis, we used a generalized linear model (GLM) with a Quasi-Poisson error distribution and Log link function, since it consisted of underdispersed count data. The effects of the treatments on the MGT were evaluated using a GLM with a Gamma error structure and an Identity link function, with species and smoke treatment as predictor variables and the MGT as

response variable. To analyse the effects of smoke in the development of seedlings for each cultivation period (3, 7 or 15 d), we used the same method of MGT. After adjustments all models were attained, we tested the interaction between our predictors (species and treatment). Whenever significant, the interactions were compared pairwise utilizing Tukey ($P < 0.05$), otherwise the effects of treatments were considered similar for both species.

In the analysis of seedling development, we used the average of the parameters (length and mass of the root and shoot systems) of five cultivated seedlings as input for the models, to overcome the lack of independence between seedlings pooled in the same Petri dishes. Whenever necessary, we removed outliers from the models based on Cook's distance method. In order to assure all models' adjustments, the distribution and variance of residuals was visually assessed and analysed by the Shapiro–Wilk and Levene tests. All GLM and GLMM analyses were tested for over- and under-dispersion of residuals using a goodness-of-fit ratio (residual deviance/degrees of freedom; Dunn and Smyth, 2018) and the testDispersion() function from the DHARMA package (Hartig, 2022; see also Brooks et al., 2019). The analyses were carried out using the R software (version 4.0.0, R Core Team, 2021), the models were constructed with the stats (R Core Team, 2021) and lme4 (Bates et al., 2015) packages, and the graphics were built using the ggplot2 package (Wickham, 2016). Our model analyses were overall based on Zuur et al. (2009).

Results

Germination

The germination of *U. decumbens* was greater than the germination of *E. inflexa* ($P < 0.0001$), and we did not find any effect of smoke on the germination of either species. On average, the germination of the exotic grass was $65 \pm 14\%$, and for *E. inflexa* was $32 \pm 10\%$ (Fig. 1A). From the initial tetrazolium solution tests, the seeds of *U. decumbens* displayed $78 \pm 8\%$ viability, 5.2-fold greater than that of *E. inflexa* ($15 \pm 10\%$; $t = -11.172$; $P < 0.0001$). On the other hand, the percentage of non-germinated viable seeds was 3.3-fold greater in *E. inflexa* ($P < 0.0001$). After 20 min of smoke exposure, *E. inflexa* presented a significantly greater percentage of fertile non-germinated seeds ($22 \pm 5\%$), whereas in *U. decumbens* it was reduced to $4 \pm 2\%$ ($P < 0.05$; Fig. 1B).

Compared to the control, there was a reduction in MGT of both species after 5 min of fumigation ($P < 0.05$; Fig. 1C). However, *E. inflexa* generally presented a higher MGT ($P < 0.0001$). Except for 10 min, there was a reduction in T₀ for fumigated *E. inflexa* seeds compared to the control group, reaching 4 ± 0 d after the 5 or 20 min of smoke exposure ($P < 0.05$; Fig. 1D). On the other hand, T₀ of *U. decumbens* was synchronic, just 1 d after the fumigation of seeds.

Seedling parameters and R:S ratio

After 3 d of development, the length of the shoot and root systems of seedlings of *U. decumbens* were 2.3-fold and 1.9-fold greater than seedlings of *E. inflexa*. However, *E. inflexa*'s seedlings presented greater aboveground mass (1.2-fold; $P < 0.0001$) than *U. decumbens*'s, with no difference in the root system mass. The smoke treatment did not affect any of these parameters (Table 1). After 7 d, the same pattern was maintained when comparing the species ($P < 0.0001$). However, seeds of *U. decumbens*

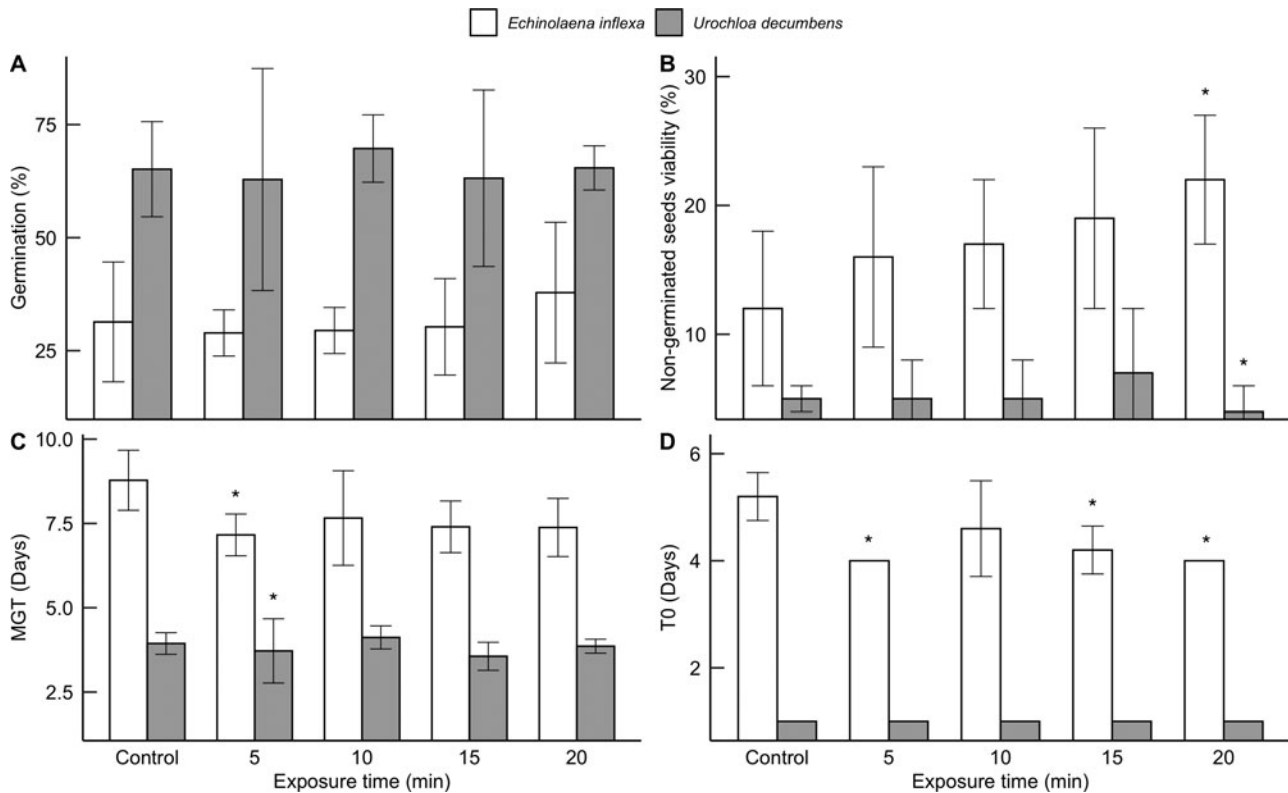


Figure 1. Germinative parameters measured for a native (*Echinolaena inflexa*) and an invasive (*Urochloa decumbens*) grass species common in the Cerrado, after different periods of smoke exposure. (A) Germination; (B) percentage of viable non-germinated seed remaining after the experiment; (C) mean germination time; and (D) time necessary to the first germination. Asterisks denote statistical significance between treatments within species. Data are presented as mean \pm standard deviation.

fumigated for 15 min increased the aboveground length from 1.9 ± 0.6 cm in the control to 2.6 ± 0.7 cm ($P < 0.01$; Table 1). After 15 d, *U. decumbens* had higher mass and length values than *E. inflexa* for both shoot and root systems ($P < 0.0001$; Tables 1 and 2). Also, smoke treatment affected the shoot system in both species, reducing the mass in all treatments, reaching a maximum reduction (1.3-fold) after 20 min of treatment ($P < 0.0001$; Table 2). The fumigation altered the aboveground length as well, resulting in a reduction after 5 min in both species ($P < 0.001$; Table 1). There was no significant alteration in the root system of the species.

When the R:S ratio was calculated with length values (length-R:S), after 3 d of development, the length-R:S of *E. inflexa* was 1.4-fold greater than *U. decumbens* ($P < 0.001$), with no significant differences between treatments. After 7 d, the length-R:S did not present significant differences comparing species or treatments. For 15 d of development, the *E. inflexa*'s length-R:S (0.76 ± 0.54) remained higher than that of *U. decumbens* (0.54 ± 0.18 ; $P < 0.0001$), and was increased in both species after 5 min of exposure to smoke ($P < 0.05$; Table 1). When calculated with the mass data (mass-R:S), the R:S after 3 d increased after 10 min of exposure to smoke in both species (*U. decumbens*: 1.2-fold; *E. inflexa*: 1.8-fold; $P < 0.05$), but there was no significant difference between species. After 7 d, the mass-R:S of *U. decumbens* was 1.3-fold higher ($P < 0.01$), with no significant differences between treatments (Table 2). For 15 d of development, the mass-R:S of *U. decumbens* remained higher than *E. inflexa* ($P < 0.0001$), however none showed treatment effects (Table 2).

Discussion

In summary, *U. decumbens* showed higher values for all studied germinative parameters than *E. inflexa*. The invasive species showed higher and faster germination rates, a smaller number of dormant seeds, and did not show smoke effects in the germination percentages. Accordingly, the low occurrence of such responses in grasses has been previously shown (Pérez-Fernández and Rodríguez-Echeverría, 2003; Clarke and French, 2005; Daws et al., 2007; Dayamba et al., 2008) including for both species addressed here (Le Stradic et al., 2015; Gorgone-Barbosa et al., 2020; Fernandes et al., 2021). For the Cerrado, smoke-enhanced germination is only reported for *Aristida* spp. (Le Stradic et al., 2015; Ramos et al., 2019). In addition, *Andropogon gayanus*, another African invasive species in Cerrado, showed slower germination rates after being treated with aerosol smoke (Dayamba et al., 2008). In contrast, the MGT of *U. decumbens* was not affected by smoke in our experiment. Such an outcome may be a consequence of the capacity of species for overcoming environmental stresses (Pereira et al., 2012; Dantas-Junior et al., 2018; Xavier et al., 2019). Despite not presenting tangible responses to smoke, the germination rate of *U. decumbens* seeds also depicted the invasive potential of the species, as all replicates germinated 3 d earlier than the first *E. inflexa* seed.

Albeit lower than *U. decumbens*' germination, *E. inflexa*'s germination was more responsive to smoke, as the fumigation anticipated the onset of germination of the native species. Although higher than some values reported for the species in previous

Table 1. Effects of different smoke exposure periods on the length of shoot and root systems, and the Root:Shoot ratio of *Echinolaena inflexa* and *Urochloa decumbens*

Treatment (min)	<i>Echinolaena inflexa</i>			<i>Urochloa decumbens</i>		
	Shoot system (cm)	Root system (cm)	Root:Shoot	Shoot system (cm)	Root system (cm)	Root:Shoot
3 d						
Control	0.8 ± 0.3 Ba	1.1 ± 0.5 Ba	1.60 ± 1.04 Ba	1.9 ± 0.5 Aa	2.7 ± 1.0 Aa	1.40 ± 0.38 Aa
5	0.7 ± 0.3 Ba	1.5 ± 0.5 Ba	2.42 ± 1.24 Ba	2.0 ± 0.7 Aa	3.0 ± 1.0 Aa	1.48 ± 0.40 Aa
10	0.7 ± 0.3 Ba	1.5 ± 0.6 Ba	2.37 ± 1.08 Ba	1.7 ± 0.4 Aa	2.7 ± 0.9 Aa	1.61 ± 0.39 Aa
15	0.8 ± 0.3 Ba	1.4 ± 0.7 Ba	1.98 ± 0.97 Ba	2.0 ± 0.6 Aa	2.6 ± 0.9 Aa	1.33 ± 0.45 Aa
20	1.0 ± 0.3 Ba	1.6 ± 0.5 Ba	1.89 ± 0.94 Ba	1.7 ± 0.4 Aa	2.5 ± 0.8 Aa	1.46 ± 0.34 Aa
7 d						
Control	1.2 ± 0.4 Ba	1.5 ± 0.7 Ba	1.39 ± 0.74 Aa	1.9 ± 0.6 Aa	2.7 ± 0.8 Aa	1.49 ± 0.68 Aa
5	1.2 ± 0.3 Ba	2.0 ± 0.9 Ba	1.82 ± 0.98 Aa	2.2 ± 0.6 Aa	2.9 ± 1.2 Aa	1.39 ± 0.68 Aa
10	1.3 ± 0.4 Ba	1.4 ± 0.7 Ba	1.14 ± 0.59 Aa	2.5 ± 0.6 Aa	3.2 ± 1.0 Aa	1.32 ± 0.43 Aa
15	1.3 ± 0.5 Ba	1.9 ± 1.0 Ba	1.54 ± 0.73 Aa	2.6 ± 0.7 Ab	3.6 ± 1.4 Aa	1.40 ± 0.49 Aa
20	1.1 ± 0.3 Ba	1.9 ± 0.7 Ba	1.72 ± 0.49 Aa	2.4 ± 0.6 Aa	3.3 ± 1.1 Aa	1.42 ± 0.57 Aa
15 d						
Control	4.3 ± 1.0 Ba	2.8 ± 1.1 Ba	0.64 ± 0.21 Ba	15.4 ± 3.8 Aa	7.5 ± 1.7 Aa	0.51 ± 0.15 Aa
5	3.3 ± 1.2 Ba	2.6 ± 1.6 Ba	1.02 ± 0.98 Bb	12.7 ± 3.2 Aa	7.3 ± 1.3 Aa	0.61 ± 0.19 Ab
10	4.1 ± 0.8 Ba	2.9 ± 1.6 Ba	0.74 ± 0.49 Ba	14.5 ± 3.2 Aa	7.1 ± 1.9 Aa	0.50 ± 0.17 Aa
15	3.9 ± 1.0 Ba	2.8 ± 1.7 Ba	0.71 ± 0.39 Ba	14.8 ± 3.4 Aa	6.9 ± 1.7 Aa	0.49 ± 0.16 Aa
20	3.8 ± 0.5 Ba	2.7 ± 1.0 Ba	0.72 ± 0.22 Ba	12.2 ± 3.4 Aa	6.6 ± 1.3 Aa	0.59 ± 0.22 Aa

Upper case letters represent statistical differences between species when analysing the same treatment, whereas lower case letters represent statistical differences between treatments within the same species.

studies (e.g. Le Stradic et al., 2015; Musso et al., 2015), the germination values of *E. inflexa* showed no difference after fumigation. Such behaviour may be due to dormancy of *E. inflexa*'s seeds (Aires et al., 2014; Le Stradic et al., 2015; Ramos et al., 2016) and an inability of the smoke to alleviate it. Nevertheless, the anticipation in T0 may represent an opportunity for earlier establishment and competitive advantage. It is also noteworthy that the MGT of *E. inflexa* corresponds to the lowest values measured among other native grasses (Aires et al., 2014). Therefore, *E. inflexa* should have an advantage in colonizing recently burned areas in the Cerrado or competing with other native species for establishment opportunities. However, all *U. decumbens* replicates presented faster initial germination and overall lower MGT, which suggests it would be a better competitor than *E. inflexa*.

Seed viability of *U. decumbens* was also significantly higher (5.2-fold) than that of *E. inflexa*, determined by the initial tetrazolium solution test. Nevertheless, the germination percentage of the invasive species was only twice the value of the native. Also, the number of non-germinated viable seeds, assessed by viability tests after the experiment period, was 3.3-fold larger for *E. inflexa*. These results suggest that the viability of the fumigated *E. inflexa* seeds was higher than previously calculated by the initial tetrazolium solution test, 5 months prior. It is worth mentioning that Aires et al. (2014) also reported an increase in the viability of *E. inflexa* seeds after 1-year of storage, which supports this inference. In addition, despite the low initial viability, the germination results were consistent with the varied values reported by previous studies on *E. inflexa* (Musso et al., 2015 (8–19%);

Paredes et al., 2018 (52%); Fontenele et al., 2020 (20%)). We did not reassess seed viability closer to the fumigation, leading to germination values larger than predicted by the tetrazolium results for the native species. Nevertheless, the germination results show that the comparison between the species was fairer than the initial viability suggested.

Although *U. decumbens* showed higher length values of root and shoot systems in all periods of cultivation, the mass values only surpassed *E. inflexa*'s after 15 d. A possible explanation is cellular elongation in the early days of *U. decumbens*' seedlings development (Kutschera, 2000). Meanwhile, considering length-R:S, *E. inflexa* presented a higher root investment, whereas *U. decumbens* had a longer aerial part. Moreover, smoke effects were recorded in the endmost periods of cultivation, with 5 min of exposure increasing the root investment. Furthermore, analysing the root and shoot system length could indicate eventual detrimental effects of smoke, since these post-germinative parameters have shown sensitivity in response to stressors, such as herbicides and allelopathic substances (Sparg et al., 2005; Navas and Pereira, 2016; Muniz et al., 2019). Nonetheless, when calculated with mass, *U. decumbens* presented a higher root investment than *E. inflexa*. Also, 10 min of smoke exposure led to higher values in the early stages of development in both species.

In addition, both length-R:S and mass-R:S results indicate cellular elongation in seedlings of *U. decumbens*, as an effort to develop the aerial part and photosynthetic surface and later allocating the resources to the root system. The development of the

Table 2. Effects of different smoke exposure periods on mass of aerial and root systems, and the Root:Shoot ratio of *Echinolaena inflexa* and *Urochloa decumbens*

Treatment (min)	<i>Echinolaena inflexa</i>			<i>Urochloa decumbens</i>		
	Shoot system (mg)	Root system (mg)	Root:Shoot	Shoot system (mg)	Root system (mg)	Root:Shoot
3 d						
Control	4.96 ± 0.76 Ba	0.24 ± 0.24 Aa	0.04893 ± 0.05045 Aa	4.16 ± 0.49 Aa	0.29 ± 0.15 Aa	0.06985 ± 0.03567 Aa
5	4.77 ± 0.76 Ba	0.36 ± 0.23 Aa	0.07546 ± 0.04955 Aa	3.78 ± 0.89 Aa	0.38 ± 0.20 Aa	0.14459 ± 0.25347 Aa
10	4.72 ± 0.78 Ba	0.42 ± 0.27 Aa	0.08982 ± 0.05312 Ab	3.99 ± 0.46 Aa	0.33 ± 0.17 Aa	0.08283 ± 0.04356 Ab
15	4.70 ± 0.80 Ba	0.31 ± 0.20 Aa	0.06736 ± 0.04285 Aa	4.06 ± 0.35 Aa	0.37 ± 0.16 Aa	0.09345 ± 0.04116 Aa
20	4.86 ± 0.57 Ba	0.35 ± 0.16 Aa	0.07271 ± 0.03582 Aa	4.07 ± 0.65 Aa	0.25 ± 0.18 Aa	0.06270 ± 0.04903 Aa
7 d						
Control	4.48 ± 0.94 Ba	0.29 ± 0.18 Aa	0.06737 ± 0.04421 Ba	3.83 ± 0.37 Aa	0.39 ± 0.16 Aa	0.10200 ± 0.04396 Aa
5	4.47 ± 1.06 Ba	0.42 ± 0.27 Aa	0.09654 ± 0.05833 Ba	3.72 ± 0.46 Aa	0.46 ± 0.19 Aa	0.12668 ± 0.05588 Aa
10	4.67 ± 0.85 Ba	0.43 ± 0.32 Aa	0.09336 ± 0.06541 Ba	3.92 ± 0.41 Aa	0.40 ± 0.16 Aa	0.10394 ± 0.04593 Aa
15	4.67 ± 0.61 Ba	0.40 ± 0.29 Aa	0.08573 ± 0.06475 Ba	3.64 ± 0.45 Aa	0.43 ± 0.25 Aa	0.12227 ± 0.08017 Aa
20	4.71 ± 0.75 Ba	0.53 ± 0.26 Aa	0.11079 ± 0.04916 Ba	3.75 ± 0.52 Aa	0.53 ± 0.27 Aa	0.14023 ± 0.06680 Aa
15 d						
Control	6.56 ± 1.29 Ba	0.58 ± 0.20 Ba	0.08935 ± 0.03343 Ba	9.86 ± 2.25 Aa	1.58 ± 0.57 Aa	0.16051 ± 0.04511 Aa
5	4.68 ± 0.86 Bb	0.54 ± 0.30 Ba	0.11293 ± 0.06322 Ba	8.15 ± 1.79 Ab	1.58 ± 0.54 Aa	0.19650 ± 0.07253 Aa
10	5.34 ± 0.82 Bb	0.43 ± 0.15 Ba	0.08080 ± 0.02944 Ba	12.46 ± 15.90 Ab	1.56 ± 0.46 Aa	0.16265 ± 0.04713 Aa
15	5.44 ± 1.27 Bb	1.00 ± 1.99 Ba	0.17319 ± 0.32674 Ba	8.94 ± 2.22 Ab	1.71 ± 0.76 Aa	0.18714 ± 0.05284 Aa
20	4.94 ± 0.61 Bb	0.54 ± 0.36 Ba	0.10903 ± 0.06651 Ba	7.57 ± 1.88 Ab	1.61 ± 0.66 Aa	0.21559 ± 0.08901 Aa

Upper case letters represent statistical differences between species when analysing the same treatment, whereas lower case letters represent statistical differences between treatments within the same species.

root system in the seedlings is essential for the recruitment, since it increases seedling fixation to the soil and favours water and nutrient accessibility (Ries and Svejcar, 1991; Leskovar and Stoffella, 1995; Lynch, 1995). Also, investment in the root system enables the storage of soluble carbohydrates, which are important for protection of tissues to stresses, sustenance during periods of photosynthesis limitation (e.g. drought, post-fire), and play a role in the osmotic adjustment (Souza et al., 2010; Moraes et al., 2016). Therefore, well-developed underground organs are essential for Cerrado grasses survival and resprout (Pilon et al., 2021). A heavier root system, thus denser, suggests a larger carbon allocation and cellular development by the native species in the early days. On the other hand, photosynthetic areas represent independence of the seed's reserves and carbon fixation by the seedling (Wright and Westoby, 2000), which would later provide more resources for the root system development. In this context, the reduction in aboveground mass of 15-d seedlings of both species in all fumigation periods suggests detrimental effects of smoke on the early development of these grasses.

As shown by a lower T0 and MGT, dry smoke exposure hastens the germination process of *E. inflexa*, a native grass of Cerrado. On the one hand, this result is not enough to overcome the invasive *U. decumbens*, which shares several functional traits. On the other hand, when considering post-germinative parameters, *E. inflexa* presents a greater seedling development in its early days, contributing to the native's success in an eventual competition. Since Keeley and Pausas (2018) argued that responses to smoke might be an exaptation present in several groups of plants, such effects could

likely comprise different life stages and appear erratically in related species, as evidenced here. Therefore, analysing post-germinative parameters and seedling development may bring elucidating answers on the smoke effects.

Due to the expansion of *U. decumbens*' invasion in the Cerrado (Macedo, 2005), the interaction between the studied species has become more frequent, leading to a possible scenario of competitive exclusion. In this context, the invasion success should be lower due to the shared functional traits of exotic species with natives (MacArthur and Levins, 1967; Symstad, 2000; Diez et al., 2008). Nevertheless, the values of T0, MGT and R:S suggest that the niche similarity between native and invasive grasses may not ensure resistance to the *U. decumbens* invasion in the Cerrado (see also Damasceno et al., 2018). However, Klink (1996) reports that seedlings of *E. inflexa* showed a continual survival rate in the field, while the seedlings of the invasive *A. gayanus* were largely threatened by predation. In this context, high seedbank occupancy (Dairel and Fidelis, 2020b), greater and faster germination rates, and enhanced root investment of *U. decumbens* may not be fully reflected in the seedling recruitment. Such considerations reinforce that different traits should also be taken in consideration for management purposes (Assis et al., 2021) and for assessing the competition between native and alien grasses.

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References

- Aires SS, Sato MN and Miranda HS (2014) Seed characterization and direct sowing of native grass species as a management tool. *Grass and Forage Science* **69**, 470–478.
- Andrade LAZ and Miranda HS (2014) The dynamics of the soil seed bank after a fire event in a woody savanna in central Brazil. *Plant Ecology* **215**, 1199–1209.
- Assis GB, Pilon NA, Siqueira MF and Durigan G (2021) Effectiveness and costs of invasive species control using different techniques to restore Cerrado grasslands. *Restoration Ecology* **29**, e13219.
- Bates D, Maechler M, Bolker B and Walker S (2015) Fitting linear mixed-effects models using lme4. *Journal of Statistical Software* **67**, 1–48.
- Baxter BJM, Van Staden J, Granger JE and Brown NAC (1994) Plant-derived smoke and smoke extracts stimulate seed germination of the fire-climax grass *Themeda triandra*. *Environmental and Experimental Botany* **34**, 217–223.
- Blank RR and Young JA (1998) Heated substrate and smoke: influence on seed emergence and plant growth. *Journal of Range Management* **51**, 577–583.
- Bond WJ and Keeley JE (2005) Fire as a global ‘herbivore’: the ecology and evolution of flammable ecosystems. *Trends in Ecology & Evolution* **20**, 387–394.
- Brasil (2009) *Regras para análise de sementes*. Brasília, Brazil, Ministério da Agricultura.
- Brooks ME, Kristensen K, Darrigo MR, Rubim P, Uriarte M, Bruna E and Bolker BM (2019) Statistical modeling of patterns in annual reproductive rates. *Ecology* **100**, e02706.
- Castro EA and Kauffman JB (1998) Ecosystem structure in the Brazilian Cerrado: a vegetation gradient of aboveground biomass, root mass and consumption by fire. *Journal of Tropical Ecology* **14**, 263–283.
- Clarke S and French K (2005) Germination response to heat and smoke of 22 Poaceae species from grassy woodlands. *Australian Journal of Botany* **53**, 445–454.
- Correia GGDS and Martins SV (2015) Banco de sementes do solo de floresta restaurada, Reserva Natural Vale, ES. *Floresta e Ambiente* **22**, 79–87.
- Curtis NP (1998) A post-fire ecological study of *Xanthorrhoea australis* following prescribed burning in the Warby Range State Park, North-eastern Victoria, Australia. *Australian Journal of Botany* **46**, 253–272.
- Dairel M and Fidelis A (2020a) How does fire affect germination of grasses in the Cerrado? *Seed Science Research* **30**, 275–283.
- Dairel M and Fidelis A (2020b) The presence of invasive grasses affects the soil seed bank composition and dynamics of both invaded and non-invaded areas of open savannas. *Journal of Environmental Management* **276**, 111291.
- Damasceno G, Souza L, Pivello VR, Gorgone-Barbosa E, Giroldo PZ and Fidelis A (2018) Impact of invasive grasses on Cerrado under natural regeneration. *Biological Invasions* **20**, 3621–3629.
- Dantas VDL and Pausas JG (2013) The lanky and the corky: fire-escape strategies in savanna woody species. *Journal of Ecology* **101**, 1265–1272.
- Dantas-Junior AB, Musso C and Miranda HS (2018) Seed longevity and seedling emergence rate of *Urochloa decumbens* as influenced by sowing depth in a Cerrado soil. *Grass and Forage Science* **73**, 811–814.
- Daws MI, Davies J, Pritchard HW, Brown NA and Van Staden J (2007) Butenolide from plant-derived smoke enhances germination and seedling growth of arable weed species. *Plant Growth Regulation* **51**, 73–82.
- Dayamba SD, Tigabu M, Sawadogo L and Oden PC (2008) Seed germination of herbaceous and woody species of the Sudanian savanna-woodland in response to heat shock and smoke. *Forest Ecology and Management* **256**, 462–470.
- Diez JM, Sullivan JJ, Hulme PE, Edwards G and Duncan RP (2008) Darwin’s naturalization conundrum: dissecting taxonomic patterns of species invasions. *Ecology Letters* **11**, 674–681.
- Dunn PK and Smyth GK (2018) Models for proportions: binomial GLMs, pp. 333–369 in Dunn PK and Smyth GK (Eds) *Generalized linear models with examples in R*. Heidelberg, Springer Texts in Statistics.
- Fernandes AF, Oki Y, Fernandes GW and Moreira B (2021) The effect of fire on seed germination of campo rupestre species in the South American Cerrado. *Plant Ecology* **222**, 45–55.
- Flematti GR, Ghisalberti EL, Dixon KW and Trengove RD (2004) A compound from smoke that promotes seed germination. *Science* **305**, 977–977.
- Florencio C, Arruda R and de Figueiredo RA (2009) Phenology of herbaceous species in Cerrado: comparisons between intact, burnt, and reforested areas. *Naturalia* **32**, 1–12.
- Fontenele HG, Figueirôa RN, Pereira CM, Nascimento VTD, Musso C and Miranda HS (2020) Protected from fire, but not from harm: seedling emergence of savanna grasses is constrained by burial depth. *Plant Ecology & Diversity* **13**, 189–198.
- Ghebrehiwot HM, Kulkarni MG, Kirkman KP and Van Staden J (2012) Smoke and heat: influence on seedling emergence from the germinable soil seed bank of mesic grassland in South Africa. *Plant Growth Regulation* **66**, 119–127.
- Gorgone-Barbosa E, Daibes LF, Novaes RB, Pivello VR and Fidelis A (2020) Fire cues and germination of invasive and native grasses in the Cerrado. *Acta Botanica Brasílica* **34**, 185–191.
- Hartig F (2022) DHARMA: Residual Diagnostics for Hierarchical (Multi-Level/Mixed) Regression Models. R Package (Version 0.4.5). Available at: <https://CRAN.R-project.org/package=DHARMA>
- Keeley JE and Pausas JG (2018) Evolution of ‘smoke’ induced seed germination in pyroendemic plants. *South African Journal of Botany* **115**, 251–255.
- Klink CA (1996) Germination and seedling establishment of two native and one invading African grass species in the Brazilian Cerrado. *Journal of Tropical Ecology* **12**, 139–147.
- Klink CA and Joly CA (1989) Identification and distribution of C3 and C4 grasses in open and shaded habitats in São Paulo State, Brazil. *Biotropica* **21**, 30–34.
- Kochankov VG, Grzesik M, Chojnowski M and Nowak J (1998) Effect of temperature, growth regulators and other chemicals on *Echinacea purpurea* (L.) Moench seed germination and seedling survival. *Seed Science and Technology* **26**, 547–554.
- Kumar B, Verma SK, Ram G and Singh HP (2012) Temperature relations for seed germination potential and seedling vigor in Palmarosa (*Cymbopogon martinii*). *Journal of Crop Improvement* **26**, 791–801.
- Kutschera U (2000) Cell expansion in plant development. *Brazilian Journal of Plant Physiology* **12**, 65–95.
- Labouriau LG, Valio IM, Salgado-Labouriau ML and Handro W (1963) Nota sobre a germinação de sementes de plantas de cerrados em condições naturais. *Revista Brasileira de Biologia* **23**, 227–237.
- Lakon G (1949) The topographical tetrazolium method for determining the germinating capacity of seeds. *Plant Physiology* **24**, 389–394.
- Lamont BB and Downes KS (2011) Fire-stimulated flowering among resprouters and geophytes in Australia and South Africa. *Plant Ecology* **212**, 2111–2125.
- Lange JH and Boucher C (1990) Autecological studies on *Audouinia capitata* (Bruniaceae). I. Plant-derived smoke as a seed germination cue. *South African Journal of Botany* **56**, 700–703.
- Leskovar DI and Stoffella PJ (1995) Vegetable seedling root systems: morphology, development, and importance. *HortScience* **30**, 1153–1159.
- Le Stradic S, Silveira FA, Buisson E, Cazelles K, Carvalho V and Fernandes GW (2015) Diversity of germination strategies and seed dormancy in herbaceous species of campo rupestre grasslands. *Austral Ecology* **40**, 537–546.
- Light ME, Daws MI and Van Staden J (2009) Smoke-derived butenolide: towards understanding its biological effects. *South African Journal of Botany* **75**, 1–7.
- Loch DS (1977) *Brachiaria decumbens* (signal grass)—a review with particular reference to Australia. *Tropical Grasslands* **11**, 141–157.
- Lynch J (1995) Root architecture and plant productivity. *Plant Physiology* **109**, 7–13.
- MacArthur R and Levins R (1967) The limiting similarity, convergence, and divergence of coexisting species. *The American Naturalist* **101**, 377–385.

- Macedo MCM (2005) Pastagens no ecossistema cerrados: evolução das pesquisas para o desenvolvimento sustentável, pp. 56–84 in *42 Reunião Anual Da Sociedade Brasileira De Zootecnia*. Goiânia, Brasil.
- Miranda MI and Klink CA (1996a) Colonização de campo sujo de cerrado com diferentes regimes de queima pela gramínea *Echinolaena inflexa* (Poaceae), pp. 46–52 in Miranda HS; Saito CH and Dias BFS (Eds) *Impactos de queimadas em áreas de cerrado e de restinga*. Brasília, Universidade de Brasília.
- Miranda MI and Klink CA (1996b) Influência do fogo na alocação de biomassa de *Echinolaena inflexa* em duas áreas de campo sujo de cerrado, pp. 37–45 in Miranda HS; Saito CH and Dias BFS (Eds) *Impactos de queimadas em áreas de cerrado e de restinga*. Brasília, Universidade de Brasília.
- Miranda AC, Miranda HS, Dias IFO and Dias BFS (1993) Soil and air temperatures during prescribed cerrado fires in central Brazil. *Journal of Tropical Ecology* **9**, 313–320.
- Mojzes A, Csontos P and Kalapos T (2015) Is the positive response of seed germination to plant-derived smoke associated with plant traits? *Acta Oecologica* **65**, 24–31.
- Moraes MG, Carvalho MAM, Franco AC, Pollock CJ and Figueiredo-Ribeiro RDCL (2016) Fire and drought: soluble carbohydrate storage and survival mechanisms in herbaceous plants from the Cerrado. *BioScience* **66**, 107–117.
- Moreira B, Tormo J, Estrelles E and Pausas JG (2010) Disentangling the role of heat and smoke as germination cues in Mediterranean Basin flora. *Annals of Botany* **105**, 627–635.
- Morrison DA and Morris EC (2000) Pseudoreplication in experimental designs for the manipulation of seed germination treatments. *Austral Ecology* **25**, 292–296.
- Munhoz CBR and Felfili JM (2007) Reproductive phenology of an herbaceous-subshrub layer of a Savannah (Campo Sujo) in the Cerrado Biosphere Reserve I, Brazil. *Brazilian Journal of Biology* **67**, 299–307.
- Muniz DR, Garcia JS, Braga TC, Fátima A and Modolo LV (2019) Pre-emergence application of (thio)urea analogues compromises the development of the weed species *Bidens pilosa*, *Urochloa brizantha*, and *Urochloa decumbens*. *Journal of Advanced Research* **17**, 95–102.
- Musso C, Miranda HS, Aires SS, Bastos AC, Soares AM and Loureiro S (2015) Simulated post-fire temperature affects germination of native and invasive grasses in Cerrado (Brazilian savanna). *Plant Ecology & Diversity* **8**, 219–227.
- Navas R and Pereira MRR (2016) Efeito alelopático de *Raphanus sativus* em *Urochloa decumbens* e *Lactuca sativa*. *Revista Agro@mbiente On-line* **10**, 228–234.
- Neves DRM and Damasceno-Junior GA (2011) Post-fire phenology in a campo sujo vegetation in the Urucum plateau, Mato Grosso do Sul, Brazil. *Brazilian Journal of Biology* **71**, 881–888.
- Overbeck GE, Müller SC, Pillar VD and Pfenhauer J (2006) No heat-stimulated germination found in herbaceous species from burned subtropical grassland. *Plant Ecology* **184**, 237–243.
- Paredes MV, Cunha ALD, Musso C, Aires SS, Sato MN and Miranda HS (2018) Germination responses of native and invasive Cerrado grasses to simulated fire temperatures. *Plant Ecology & Diversity* **11**, 193–203.
- Pausas JG, Lamont BB, Paula S, Appezzato-da-Glória B and Fidelis A (2018) Unearthing belowground bud banks in fire-prone ecosystems. *New Phytologist* **217**, 1435–1448.
- Pereira MRR, Martins CC, Souza GSF and Martins D (2012) Influence of saline and water stress on germination of *Urochloa decumbens* and *Urochloa ruziziensis*. *Bioscience Journal* **28**, 537–545.
- Pérez-Fernández MA and Rodríguez-Echeverría S (2003) Effect of smoke, charred wood, and nitrogenous compounds on seed germination of ten species from woodland in central-western Spain. *Journal of Chemical Ecology* **29**, 237–251.
- Pilon NA, Hoffmann WA, Abreu RC and Durigan G (2018) Quantifying the short-term flowering after fire in some plant communities of a Cerrado grassland. *Plant Ecology & Diversity* **11**, 259–266.
- Pilon NA, Cava MG, Hoffmann WA, Abreu RC, Fidelis A and Durigan G (2021) The diversity of post-fire regeneration strategies in the Cerrado ground layer. *Journal of Ecology* **109**, 154–166.
- Pires HRA, Franco AC, Piedade MTF, Scudeller VV, Kruijt B and Ferreira CS (2018) Flood tolerance in two tree species that inhabit both the Amazonian floodplain and the dry Cerrado savanna of Brazil. *AoB Plants* **10**, pty065.
- Pivello VR (2011) The use of fire in the Cerrado and Amazonian rainforests of Brazil: past and present. *Fire Ecology* **7**, 24–39.
- Pivello VR, Carvalho VMC, Lopes PF, Peccinini AA and Rosso S (1999) Abundance and distribution of native and alien grasses in a “Cerrado” (Brazilian Savanna) biological reserve. *Biotropica* **31**, 71–82.
- R Core Team (2021) *R: a language and environment for statistical computing*. Vienna, Austria, R Foundation for Statistical Computing. Available at: <https://www.R-project.org/>
- Ramos DM, Liaffa AB, Diniz P, Munhoz CB, Ooi MK, Borghetti F and Valls JF (2016) Seed tolerance to heating is better predicted by seed dormancy than by habitat type in Neotropical savanna grasses. *International Journal of Wildland Fire* **25**, 1273–1280.
- Ramos DM, Valls JF, Borghetti F and Ooi MK (2019) Fire cues trigger germination and stimulate seedling growth of grass species from Brazilian savannas. *American Journal of Botany* **106**, 1190–1201.
- Ries RE and Svejcar TJ (1991) The grass seedling: when is it established? *Journal of Range Management* **44**, 574–576.
- Salazar A, Goldstein G, Franco AC and Miralles-Wilhelm F (2011) Timing of seed dispersal and dormancy, rather than persistent soil seed-banks, control seedling recruitment of woody plants in Neotropical savannas. *Seed Science Research* **21**, 103.
- Sarmiento G (1992) Adaptive strategies of perennial grasses in south American savannas. *Journal of Vegetation Science* **3**, 325–336.
- Simon MF, Grether R, Queiroz LP, Skema C, Pennington RT and Hughes CE (2009) Recent assembly of the Cerrado, a neotropical plant diversity hotspot, by in situ evolution of adaptations to fire. *Proceedings of the National Academy of Sciences of the United States of America* **106**, 20359–20364.
- Souza A, Sandrin CZ, Caliό MFA, Meirelles ST, Pivello VR and Figueiredo-Ribeiro RCL (2010) Seasonal variation of soluble carbohydrates and starch in *Echinolaena inflexa*, a native grass species from the Brazilian savanna, and in the invasive grass *Melinis minutiflora*. *Brazilian Journal of Biology* **70**, 395–404.
- Sparg SG, Kulkarni MG, Light ME and Van Staden J (2005) Improving seedling vigour of indigenous medicinal plants with smoke. *Bioresource Technology* **96**, 1323–1330.
- Stevens JC, Merritt DJ, Flematti GR, Ghisalberti EL and Dixon KW (2007) Seed germination of agricultural weeds is promoted by the butenolide 3-methyl-2 H-furo [2, 3-c] pyran-2-one under laboratory and field conditions. *Plant and Soil* **298**, 113–124.
- Symstad AJ (2000) A test of the effects of functional group richness and composition on grassland invasibility. *Ecology* **81**, 99–109.
- Taşanođlu Ç, Çataş ŞS and Özüdođru B (2015) Fire-related germination and early seedling growth in 21 herbaceous species in Central Anatolian steppe. *Journal of Arid Environments* **122**, 109–116.
- Taylor JLS and van Staden J (1996) Root initiation in *Vigna radiata* (L.) Wilczek hypocotyl cuttings is stimulated by smoke-derived extracts. *Plant Growth Regulation* **18**, 165–168.
- Wickham H (2016) *ggplot2: Elegant Graphics for Data Analysis*. New York, Springer-Verlag.
- Wright IJ and Westoby M (2000) Cross-species relationships between seedling relative growth rate, nitrogen productivity and root vs leaf function in 28 Australian woody species. *Functional Ecology* **14**, 97–107.
- Xavier RO, Leite MB and Matos DMS (2019) Phenological and reproductive traits and their response to environmental variation differ among native and invasive grasses in a Neotropical savanna. *Biological Invasions* **21**, 2761–2779.
- Zenni RD and Ziller SR (2011) An overview of invasive plants in Brazil. *Brazilian Journal of Botany* **34**, 431–446.
- Zirondi HL, Silveira FA and Fidelis A (2019) Fire effects on seed germination: heat shock and smoke on permeable vs impermeable seed coats. *Flora* **253**, 98–106.
- Zuur AF, Ieno EN, Walker NJ, Saveliev AA and Smith GM (2009) *Mixed effects models and extensions in ecology with R*, Vol. 574. New York, Springer.