



Light intensity triggers different germination responses to fire-related cues in temperate grassland species

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Abstract Fire is a widespread disturbance that affects plant individuals and populations. In fire-prone environments, such as Mediterranean-type ecosystems, many species are stimulated to germinate by fire-related cues, for example heat and smoke. However, little is known about the effect of fire on seed germination of species from herbaceous communities like temperate grasslands. In this study we assessed the germination response to direct (heat shock and smoke) and indirect (light intensity) fire-related cues of five herbaceous and one shrubby species that occur in natural grasslands of eastern Uruguay. All species are native and belong to the Poaceae (2 species) and Asteraceae (4 species) families. Seeds were subjected to smoke and heat shock treatments (50°C, 100°C) under two light intensities (high-light and low-light). We found that direct fire-related cues did not stimulate germination in any of the species studied. Most of the species showed a reduced percentage of germination or a delayed mean germination time, relative to the control treatment, when exposed to heat shock at 100°C or smoke. However, the seeds survived the exposure to high temperatures, indicating that they

can tolerate a fire event. Light was found to be a key germination cue. Treated seeds responded to fire-related cues mainly when we simulated the high-light environment after fire. Our results contribute to the growing body of evidence that many species of South American grasslands do not rely on recruitment by seeds after a fire event, but rather on the ability to resprout by means of subterranean or protected basal buds.

Keywords heat shock · smoke · high light conditions · seed germination · prescribed burning · Uruguay

Introduction

Fire is a widespread disturbance that affects plant individuals and populations. After a burning event, plants may persist by vegetative regeneration (‘resprouters’), by seed recruitment (‘seeders’), or by combining both strategies (Pausas et al. 2004; Paula and Pausas 2008; Le Stradic et al. 2015; Zupo et al. 2016). Many grassland taxa are hemicryptophytes or geophytes (Raunkiaer 1934) and bear their regenerative buds at or below the soil surface. On the other hand, seeders rely on regeneration from seeds for post-fire recovery (Pausas et al. 2004; Paula and Pausas 2008). Typically, seeders allocate most of their resources to aboveground growth and establish rapidly after fires (Verdú 2000).

To achieve successful establishment, seeds must survive during fire, and germination may be stimulated by some fire-related cues, such as heat shock and smoke (Keeley and Fortheringham 2000; Dayamba et al. 2008;

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Fidelis et al. 2016). Heat shock can break dormancy by physiologically affecting the embryo or by physically affecting the pericarp (Baskin and Baskin 2014), and plant-derived smoke or aqueous smoke extracts promote germination in many plant families (Van Staden et al. 2000; Dayamba et al. 2008). Another important effect of burning is the removal of aboveground biomass and litter, resulting in an increase in light intensity at ground level (Hulbert 1988; Keeley and Forthringham 2000). Seeds of some plant species require light for germination, especially those with small seeds (Milberg et al. 2000; Zhang et al. 2017), and the reduction of above-ground plant biomass after a fire may trigger the germination of these species. Also, canopy consumption changes light quality (low-red/far-red ratio) and daily soil temperature fluctuations, promoting germination in some species (Santana et al. 2013). However, no general trends arise from studies that tested the interaction of light with heat shock and smoke (Long et al. 2011; Koo et al. 2015; Tavşanoğlu et al. 2017).

The response of seeds to fire-related cues has been extensively investigated in fire-prone environments, such as Mediterranean shrublands (Bond and Keeley 2005; Keeley et al. 2012) and savannas (Dayamba et al. 2008; Fichino et al. 2016; Ramos et al. 2016), but to a lesser extent in herbaceous communities like temperate grasslands (Ortega and Lateralra 2003; Overbeck et al. 2006; Le Stradic et al. 2015). The Río de la Plata grasslands is one of the largest areas of natural temperate sub-humid grasslands of the world, covering more than 700,000 km² across eastern Argentina, Uruguay and southern Brazil (Soriano et al. 1991). Although the history of fire in the Río de la Plata grasslands is largely unknown, some studies from Argentina and Brazil have addressed its current effect. In southern Brazil, fire and grazing prevent the development of forests, which would be expected according to the humid climatic conditions of the region (Overbeck and Pfadenhauer 2007). In the Flooding Pampa, winter and spring burns of grasslands dominated by the tussock grass *Paspalum quadrifarium* Lam. (Panicoideae) favour the establishment of alien species (Lateralra 1997; Lateralra et al. 2003; Ortega and Lateralra 2003). Additionally, other studies have analysed the effect of heat and smoke on seed germination of species that thrive in these grasslands (e.g. Overbeck et al. 2006; Fidelis et al. 2010, Fidelis et al. 2016; Kin et al. 2016). In broad

terms, fire-related cues did not stimulate seed germination of the species tested, suggesting a different pattern to that observed in some fire-prone environments like Mediterranean systems. To our knowledge, no studies have been conducted on the effect of fire on the germination of Uruguayan grassland species.

The aim of the present study was to assess the germination response to direct (heat shock and smoke) and indirect (light intensity) fire-related cues of five herbaceous and one shrubby species that occur in natural grasslands of eastern Uruguay. The species belong to the Poaceae (2 species) and Asteraceae (4 species) families. All the species are native perennials (Fig. 1d–i) and five of them thrive well in burned areas – the grasses *Erianthus angustifolius* Ness (Panicoideae) and *Aristida laevis* (Nees) Kunth (Aristidoideae), the composite forb *Pterocaulon balansae* Chodat and *Stenachaenium megapotamicum* Baker, and the shrub *Acanthostyles buniifolius* (Hook. ex Arn.) R.M. King & H. Rob. Accordingly, we expected a positive to neutral effect of the fire-related cues on the germination of these species. The composite forb *Solidago chilensis* Meyen is observed mostly thriving in unburnt areas, so we expected an opposite response in comparison to the other species.

Material and methods

Study system

The Río de la Plata grasslands host about 5000 species (8% endemic) Asteraceae, Poaceae and Fabaceae being the plant families with the largest number of species (Andrade et al. 2018). The entire region is considered a continuous plain, but hills or tablelands, with a maximum elevation of 500 m above the plain, occur in some areas (Soriano et al. 1991). In Uruguay, 60% of the territory is occupied by natural grasslands (Baeza et al. 2019), and grazing by livestock is the main productive activity. The geomorphological region of Sierras del Este encompasses an area of 25,000 km² (14.3% of the country's surface), and consists of a set of elevations oriented in the south-west to north-east direction, being the region with the highest energy relief of the country (Fig. 1a). Three grassland communities are recognized in this region: densely vegetated grasslands (associated with medium and deep soils), sparsely vegetated grasslands (associated with

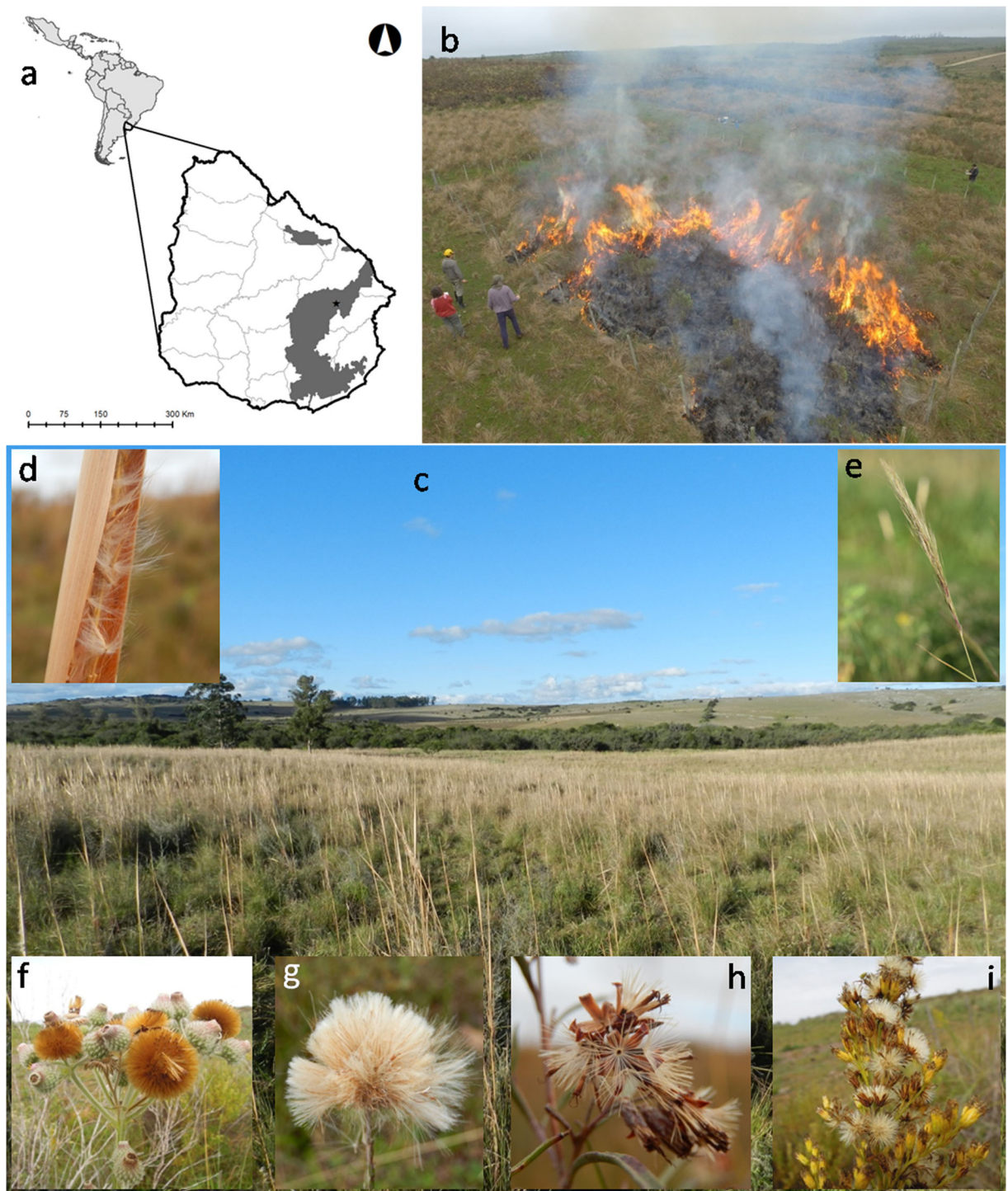


Fig. 1 **a** – Geographic location of the geomorphological region Sierras del Este (Uruguay). The grey area denotes the limit of the region, and the star shows the location of seed collection in Paisaje Protegido Quebrada de los Cuervos. About 65% of the Sierras del Este region is covered by natural grasslands. **b** – Prescribed fire in the study area. **c** – Landscape view of a tall, dense grassland

dominated by *Erianthus angustifolius*. **d–i** – Inflorescences and/or infructescences of the six species under study, growing in the study area: *Erianthus angustifolius* (**d**), *Aristida laevis* (**e**), *Stenachaenium megapotamicum* (**f**), *Pterocaulon balansae* (**g**), *Acanthostyles buniifolius* (**h**) and *Solidago chilensis* (**i**).

shallow soils), and tall and densely vegetated grasslands (associated with humid or temporarily inundated soils – Lezama et al. 2019). The latter are dominated by the tall tussock grass *Erianthus angustifolius* (Fig. 1c). The tussock growth-form consists of bunch like tufts sometimes more than a metre high, in which the hard, old, withered leaves are intermingled with the fresh, young, green leaves (Walter 1979). The tussocks are of little grazing value, and for this reason prescribed burning is used to reduce their cover (Royo Pallarés et al. 2005, Fig. 1b). The large amount of dry standing dead matter and litter that accumulates over many growing seasons provides the necessary combustible fuel for fire ignition and spread. Prescribed burning not only enhances the forage quality of *E. angustifolius* plants, but may also create open spaces prone to be colonized by other species that propagate vegetatively or by seeds (López-Mársico, pers. observ.).

Seed collection

Mature achenes and caryopses (hereafter referred to as ‘seeds’) were harvested manually from plants growing in grasslands that had not been burned for at least five years, in the protected area *Paisaje Protegido Quebrada de los Cuervos, Departamento de Treinta y Tres* (32°55' S, 54°26' W; Fig. 1a). The mean annual precipitation in the region is 1,318 mm and the annual temperature averages 17°C (INIA-GRAS 2018). Seeds were harvested from 8 to 10 randomly selected individuals widely distributed over the area, and stored in paper bags at room temperature until the start of the experiment.

Seed processing and treatments

About 30% of the collected seeds were discarded because they exhibited conspicuous signs of damage, easily detected by visual inspection (Table 1). An additional 1,200 seeds of each species were set apart for length and width measurements ($n = 100$), 1,000-seed fresh mass estimation, and an initial viability assessment (4 replicates of 25 seeds each; Table 1). Seed length and width were measured under a binocular stereo microscope, fresh seed mass was estimated using a precision balance (with 0.01-g accuracy) and viability was assessed with the tetrazolium test. Seeds were dissected longitudinally, immersed in a 1% 2,3,5-triphenyl-tetrazolium chloride

Table 1 Main characteristics of six native species of Uruguayan grasslands and time of seed collection.

Species	Seed collection date	Seed dispersal period	Plant family	Life form	Bud position	Seed length ± SD [mm]	Seed width ± SD [mm]	1,000-seed weight [g]	Damaged seeds [%] ± SD (1)	Initial viability (%) ± SD (2)
<i>Erianthus angustifolius</i>	April 2015	February–June	Poaceae	Tussock grass	Hemicryptophyte	2.31 ± 0.12	0.69 ± 0.07	1.16	17.8 ± 9.4	81.0 ± 11.3
<i>Aristida laevis</i>	January 2015	December–March	Poaceae	Caespitose grass	Hemicryptophyte	9.57 ± 0.66	0.40 ± 0.04	1.36	21.6 ± 4.0	95.0 ± 8.2
<i>Stenachaenium megapotaemicum</i>	April 2015	March–May	Asteraceae	Forb	Geophyte (rhizome)	3.60 ± 0.24	0.87 ± 0.07	0.55	29.2 ± 6.4	69.5 ± 14.2
<i>Pterocaulon balansae</i>	April 2015	February–May	Asteraceae	Forb	Hemicryptophyte	0.79 ± 0.05	0.20 ± 0	0.03	21.2 ± 5.8	24.5 ± 5.0
<i>Solidago chilensis</i>	April 2015	February–May	Asteraceae	Forb	Geophyte (rhizome)	1.47 ± 0.25	0.30 ± 0.07	0.10	52.6 ± 6.1	49.5 ± 12.6
<i>Acanthostyles bunifolius</i>	April 2015	March–May	Asteraceae	Shrub	Nanophanerophyte (xylopodium)	2.30 ± 0.18	0.37 ± 0.05	0.24	24.6 ± 6.5	75.5 ± 13.8

(1) Collected seeds exhibiting conspicuous signs of damage easily detected by visual inspection, and discarded from the experiment. Five replicates of 100 seeds per species were analysed under a stereomicroscope. (2) According to the tetrazolium test. Four replicates of 25 seeds per species were analyzed. Non-viable seeds were incorporated into the experiment because they appeared undamaged externally

solution and left in the dark at room temperature (15–20°C) for 24 h. Embryos and cotyledons were then examined carefully for staining (Table 1).

The remaining seeds were used for germination experiments. Four treatments were performed in order to assess the effects of fire-related cues on seed germination: 1 – control (C; no heat shock and no smoke), 2 – smoke (S), 3 – heat shock of 50°C (H50) and 4 – heat shock of 100°C (H100). The selected temperatures are within the range of temperatures used by Overbeck et al. (2006), which are supposed to cover the thermal conditions potentially encountered by seeds during fire in grasslands similar to the eastern grasslands of Uruguay. Heat treatments were applied to the seeds for a period of 2 minutes using a preheated oven. For the smoke treatment, seeds were placed into a 60×40×30 cm plastic chamber and fumigated with smoke derived from burning 50 g of dry biomass of *E. angustifolius* in a bee smoker. Once the chamber was saturated with smoke, it was left sealed for 5 minutes.

Control and treated seeds were placed on two layers of moist filter paper in sterilized Petri dishes (4 replicates of 25 seeds per species and treatment). The long awns of the lemma of *A. laevis* were cut off before placing the seeds into the dishes. The Petri dishes were sealed, labelled and placed in a germination chamber with a 12/12 h light/dark and 25°/20°C regime, simulating the environmental conditions of eastern Uruguay during spring (INIA-GRAS 2018). The four treatments were repeated 40 days later, using a lower light intensity during the incubation period. High-light and low-light intensities were achieved by turning on 12 or 3 white light tubes (cool daylight, 36 W each) of the germination chamber, respectively. The high-light incubation aimed to reproduce the light intensity after a burning event. The low-light incubation reduced incident light by 73% (from 300 to 80 lx with 12 and 3 light tubes, respectively) and simulated the interception of light by the canopy of *E. angustifolius* (83%; from 538 ± 192 to 89 ± 30 lx above and below the canopy, respectively, $n = 10$; unpublished data). Incident light was measured using a digital lux meter (Tondaj LX1010B). Field data was registered in spring, at around midday.

Seed germination (protrusion of the radicle through the seed coat) was recorded daily during the first week, and later every two or three days. Emerging seedlings

and seeds covered with fungal mycelium were removed from the Petri dishes. The experiments lasted a maximum of 30 days.

Data analyses

Percentage of germination (%G), mean germination time (MGT) and the standard deviation of germination time (SD_{MGT}) were calculated for each species (Ranal and Santana 2006):

$$\%G = \frac{\text{Seeds germinated at the end of the experiment}}{\text{Total number of seeds}} \times 100,$$

$$MGT = \frac{\sum_{i=1}^k n_i t_i}{\sum_{i=1}^k n_i} \text{ and}$$

$$SD_{MGT} = \sqrt{\frac{\sum_{i=1}^k n_i (t_i - MGT)^2}{\left(\sum_{i=1}^k n_i - 1\right)}},$$

where n_i is number of seeds germinated on day i , t_i is the number of days from the start of the experiment to the i_{th} day, and k is the last day of germination.

As most germination variables did not meet the assumption of normality, even when transformed, non-parametric Kruskal–Wallis tests were performed for each species separately in order to investigate for significant differences among treatments. The interaction between light and direct fire-related cues (heat shock, smoke) was analysed using the adjusted rank transform test (Sawilowsky 1990). All tests were performed using InfoStat (Di Rienzo et al. 2016).

Results

Direct fire-related cues (heat shock and smoke) did not stimulate germination in any of the species studied. When significant differences were found, the species had a reduced percentage of germination or delayed their mean germination time, relative to the control treatment. Otherwise, a neutral effect was observed. Heat shock at 100°C and smoke (applied separately) were the only treatments that influenced the germination of the species. Light was found to be a key germination cue (Figs 2 and 3).

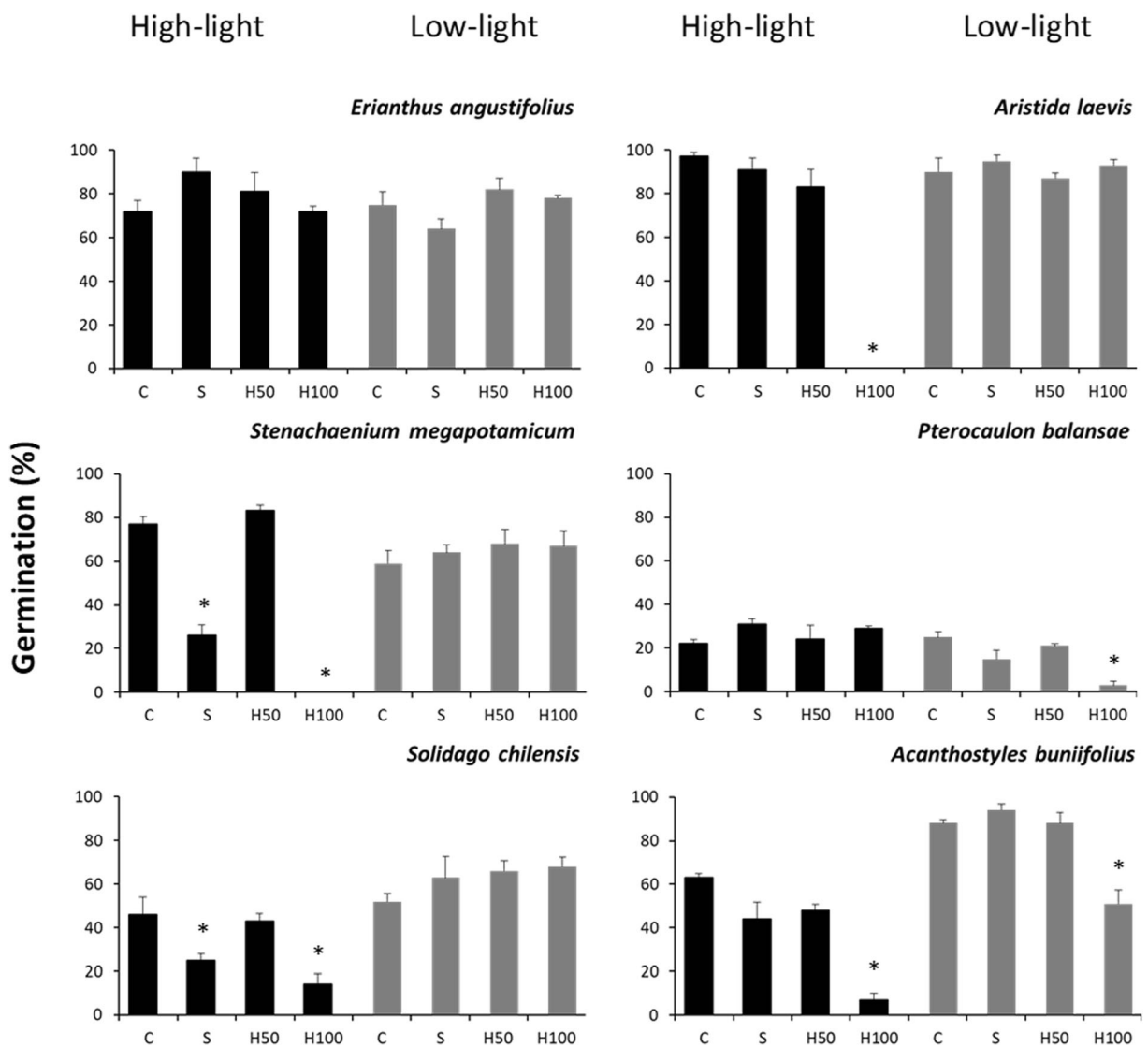


Fig. 2 Percentage of seed germination (mean \pm SE) of six native species from Uruguayan grasslands subjected to smoke, heat shock and control treatments under high-light and low-light conditions: C (control, no heat shock and no smoke), S (smoke, 5 min), H50 (heat shock at 50°C, 2 min) and H100 (heat shock at

100°C, 2 min). Statistical significance ($P < 0.05$) between experimental and control treatments is indicated by asterisks above the bars. Four replicates of 25 seeds per species and treatment were employed. Germination tests were performed at 25°/20°C under a 12-h photoperiod and lasted 30 days.

High-light experiment

Grass species were only affected by heat whereas most composites were affected both by heat and smoke (Figs 2 and 3). When exposed to 100°C, seeds of *A. laevis* and *S. megapotamicum* did not germinate whereas those of *S. chilensis* and *A. buniifolius* experienced a germination decrease from 46% to 14% ($H = 10.1$, $P = 0.02$) and from 63% to 7% ($H = 11.5$, $P < 0.01$) relative to the control, respectively (Fig. 2). In addition, heat

shock at 100°C increased the MGT of *E. angustifolius* by 49% ($H = 9.8$; $P = 0.02$; Fig. 3), and nearly tripled the MGT of *A. buniifolius* ($H = 11.9$; $P < 0.01$; Fig. 3) and the SD_{MGT} of *S. chilensis* ($H = 11.4$; $P < 0.01$), respectively. Smoke reduced the %G of *S. megapotamicum* from 77% to 26% ($H = 13.2$; $P < 0.01$) and that of *S. chilensis* from 46% to 25% ($H = 10.1$, $P = 0.02$; Fig. 2), and delayed the MGT of *A. buniifolius* and the two latter species ($P < 0.01$, Fig. 3).

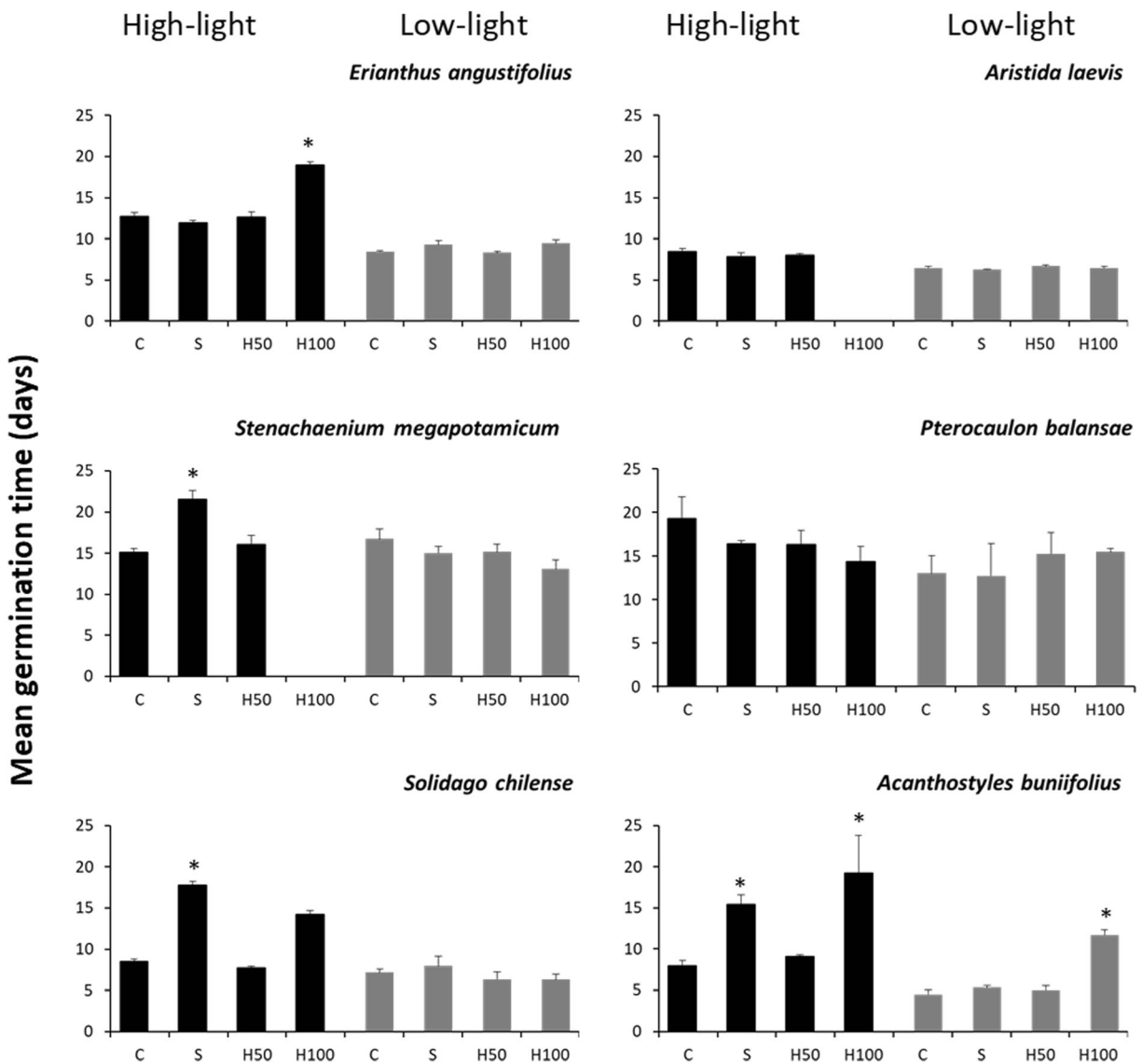


Fig. 3 Mean germination time (mean \pm SE) of six native species from Uruguayan grasslands subjected to smoke, heat shock and control, under high-light and low-light conditions: C (control, no heat shock and no smoke), S (smoke, 5 min), H50 (heat shock at 50°C, 2 min) and H100 (heat shock at 100°C, 2 min). Statistical

significance ($P < 0.05$) between experimental and control treatments is indicated by asterisks above the bars. Four replicates of 25 seeds per species and treatment were employed. Germination tests were performed at 25°/20°C under a 12-h photoperiod and lasted 30 days.

Low-light experiment

Heat shock at 100°C was the only treatment that affected germination, and only in two species: *A. buniifolius* and *P. balansae*. The seeds of *A. buniifolius* reduced their %G from 88% to 51% ($H = 9.7$, $P = 0.02$; Fig. 2), but their MGT nearly tripled ($H = 9.3$, $P = 0.03$; Fig. 3). The %G of *P. balansae* decreased from 25% to 3% ($H = 9.9$, $P = 0.02$; Fig. 2). The SD_{MGT} was not affected by the treatments.

Interaction between light and fire-related cues

For most species, significant interactions between light and heat were found (Table 2). All germination variables measured on *A. laevis*, *S. megapotamicum* and *S. chilensis*, and MGT of *E. angustifolius* were affected by heat shock at 100°C only under high-light conditions. Conversely, heat shock at 100°C reduced the %G of *P. balansae* only under low light.

Table 2 Effect of the interaction between light and direct fire-related cues (heat shock and smoke) on the germination of six native species of Uruguayan grasslands. Values are the results of factorial ANOVAs on adjusted ranked data. Only interactioneffects are presented because the adjusted rank transform test (ART) removes the main effects. %G – percentage of germination; MGT – mean germination time; SD_{MGT} – standard deviation of mean germination time.

Species		Light × Temperature			Light × Smoke		
		<i>d.f.</i>	<i>F</i>	<i>P</i>	<i>d.f.</i>	<i>F</i>	<i>P</i>
<i>Erianthus angustifolius</i>	%G	2	0.13	0.88	1	7.72	0.0167
	MGT	2	51.66	< 0.0001	1	3.77	0.0761
	SD _{MTG}	2	1.70	0.2105	1	2.48	0.1415
<i>Aristida laevis</i>	%G	2	124.8	< 0.0001	1	0.60	0.4539
	MGT	2	101	< 0.0001	1	0.45	0.513
	SD _{MTG}	2	4.80	0.0214	1	0.65	0.4344
<i>Stenachaenium megapotamicum</i>	%G	2	37.63	< 0.0001	1	36.79	< 0.0001
	MGT	2	7.15	0.005	1	23.58	0.0004
	SD _{MTG}	2	7.01	0.0056	1	0.01	0.9266
<i>Pterocaulon balansae</i>	%G	2	13.79	0.0002	1	9.22	0.0103
	MGT	2	0.43	0.6545	1	0.08	0.7812
	SD _{MTG}	2	0.40	0.6736	1	0.01	0.9248
<i>Solidago chilensis</i>	%G	2	10.76	0.0008	1	4.35	0.0590
	MGT	2	40.27	< 0.0001	1	36.57	< 0.0001
	SD _{MTG}	2	14.92	0.0002	1	0.04	0.8517
<i>Acanthostyles buniifolius</i>	%G	2	1.89	0.1799	1	9.22	0.0103
	MGT	2	1.67	0.2161	1	29.88	0.0001
	SD _{MTG}	2	1.89	0.1792	1	0.23	0.6362

Significant differences ($p < 0.05$) are in bold

The effect of the interaction between light and smoke was significant only for %G and MGT (Table 2). When seeds were exposed to smoke, the %G of *E. angustifolius* and *P. balansae* increased at high-light but decreased under the low-light treatment, relative to the control conditions. *Stenachaenium megapotamicum*, *A. buniifolius* and, marginally, *S. chilensis* reduced the %G and increased the MGT only under the high-light treatment.

Discussion

Contrary to our expectation, we found that species that thrive well in burned areas of eastern Uruguay did not exhibit an improvement on their germination performance when their seeds were exposed to high temperatures or smoke. Conversely, most of them had a reduced percentage of germination in the different experimental treatments. The only species that was not affected on the germination percentage was *E. angustifolius*, the dominant tussock grass of the community. On the

other hand, the prediction that the forb *S. chilensis* (which does not prosper in burned areas) would be negatively affected by fire cues was confirmed. The lack of species stimulated to germinate by fire-related cues was also reported for grassland species from southern Brazil and central Argentina (Overbeck et al. 2006; Fidelis et al. 2010; Fidelis et al. 2016; Kin et al. 2016) for herbaceous species from rocky grasslands of south-eastern Brazil (Le Stradic et al. 2015) and also from Brazilian savannas (Fichino et al. 2016; Ramos et al. 2016). These results contrast with those reported for Mediterranean-type ecosystems, where both heat and smoke promote germination in a wide range of species (e.g. Dixon et al. 1995; Keeley and Fotheringham 1998; Moreira et al. 2010; Keeley et al. 2012). In addition, the treatments delayed the mean germination time of four species and increased the germination dispersal over time in one of them. In certain environments, like deserts, extended germination over time is considered a beneficial trait (Venable and Lawlor 1980).

However, in fire-prone ecosystems, fast germinating and growing species are at an advantage because fire creates an environment free of competitors and promptly releases plenty plant-bound nutrients, which are ready to be colonized (Le Stradic et al. 2015; Carthey et al. 2018).

The near absence of species stimulated to germinate by fire-related cues in South American grasslands may lie in the predominance of resprouters in these communities, which do not rely on regeneration from seeds (Overbeck and Pfadenhauer 2007). Resprouting confers tolerance to fire and can be considered a functional trait (i.e. phenotypic trait that influences fitness, Reich et al. 2003). Resprouting ability is determined by the development, protection and resourcing of a viable bud bank (Clarke et al. 2013). The six species under study can be considered to be resprouters, as they possess subterranean bud-bearing organs (rhizomes, xylopodia) or, in the case of the grasses, meristems which are protected by closely packed leaf sheaths at the soil surface (Gibson 2009). It has been hypothesized that because resprouters are already able to persist in fire-prone ecosystems, they have been subjected to lower selective pressure to acquire traits allowing or enhancing post-fire recruitment (Paula and Pausas 2008). A pertinent question that emerges from the South American data is whether the fire regime (historical and/or current) is a selective force that promotes resprouting. Since resprouting is a widespread trait in angiosperms (Bond and Keeley 2005), and basal meristems and below-ground nutrient reserves are also associated with grazing and/or aridity tolerance (Coughenour 1985), only phylogenetic analyses can evaluate the adaptive value of resprouting in response to fire (Bond and Keeley 2005).

Light was found to be a key germination cue for the species under study. Although most of the control seeds were insensitive to light intensity, treated seeds responded to fire-related cues mainly under high-light conditions. In other words, heat and smoke reduced the germination percentage of four out of six species when we simulated the full light conditions of a post-fire environment, but had a neutral effect in most of them when we simulated the light intensity under the canopy of *E. angustifolius*. This would mean that seeds that lie beneath the canopy of the tussock grasses in adjacent unburned patches would keep their germination parameters unchanged even when the area of influence of smoke and heat extends beyond the actual burned area, affecting at least the closest neighbours. The effect of the

interaction of light and other fire-related cues remains elusive. In smoke-responsive species, smoke can promote or inhibit germination. From the thousands of smoke compounds, a group of closely related small organic compounds (i.e. karrikins) have been isolated and proved to stimulate germination (Flematti et al. 2015) whereas other related compounds show an antagonistic effect against the most active karrikin, KAR₁ (Burger et al. 2018). On the other hand, plants respond to high temperatures or heat stress by turning on heat shock genes. The products of heat shock genes are heat shock proteins or HSPs, which prevent irreversible protein aggregation (Waters 2013). Some studies indicate that both karrikins and HSPs may interact with light. Merritt et al. (2006) found that exposure to smoke replaces the light requirement for the germination of five Australian composites. Likewise, Koo et al. (2015) found that administering a heat shock treatment to tobacco seeds releases their dependence on light for germination. On the contrary, Long et al. (2011) reported that the effect of light on the germination of eight Brassicaceae weed species was independent of the effect of smoke. However, the exact ecophysiological mechanisms underlying the interactions are still unknown, and more studies are needed to predict their consequences.

Even when the seeds of most species were negatively affected by the fire-related cues, all survived the different treatments. The survival of seeds exposed to high temperatures constitutes a physiological resistance to heat (Overbeck et al. 2006) and should be considered as an important trait in flammable ecosystems (Fidelis et al. 2016). The most striking result was observed in the germination of *A. laevis* and *S. megapotamicum* in the heat shock treatment at 100°C. Under the high-light incubation, seeds did not germinate, but their germination did not differ from the control under low-light conditions. Our results are consistent with those reported by Overbeck et al. (2006) for seeds of *A. laevis*, which did not germinate at temperatures greater or equal to 110°C, under a 16/8 h light/dark photoperiod, reinforcing the importance of our findings about the effects of the interaction of light with the fire-related cues on the germination response of these species.

Conclusions

To our knowledge, this is the first study that analyses the effect of fire-related cues on the germination of

Uruguayan grassland species. We found that heat and smoke do not enhance germination in any species (regardless of whether or not they thrive in burned areas), but instead germination is reduced in most of them. Our results contribute to the growing body of evidence that many species from South American grasslands do not rely on recruitment by seeds after a fire event, but rather on the ability to resprout by means of subterranean or protected basal buds. Resprouters might still benefit from rapid post-fire germination if it allows greater total recruitment (Carthey et al. 2018), but for this to happen, species must generate a fire-resistant seed bank. As most species tested in our study tolerate temperatures of 100°C, it is likely that they can survive a fire event. Another important finding was that fire-related treatments were more deleterious when we simulated the high-light environment after fire, emphasizing the strong interaction between direct and indirect effects of fire. Finally, our research may also provide useful information for conservation purposes. Natural grasslands in Uruguay have been largely devoted to livestock grazing since European settlement about 400 years ago, but during the last two decades a large proportion (close to 10%) switched to agriculture (mainly soybean) and afforestation (MGAP-DIEA 2011). In this scenario of accelerated change, a better understanding of the germination requirements of native species may improve restoration plans of highly degraded landscapes (Jiménez-Alfaro et al. 2016).

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