Germination response of common annual and perennial forbs to heat shock and smoke treatments in the Chaco Serrano, central Argentina

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Running title: Germination response of forbs to fire cues

Abstract

Fire is a key ecological factor affecting plant dynamics. In the last decades, fire occurrence in the Chaco region has increased noticeably, challenging the adaptive capacity of plants to regenerate after a fire. Broad-leaved forb species have been much less studied than woody and graminoids, although they are an important component of fire dynamics. Here we analyzed the germination response to heat shock of 70ºC and 110ºC, smoke and their combination in10 broad-leaved herbaceous species frequently occurring in the Chaco Serrano of Córdoba province, central Argentina, including five annual (*Bidens subalternans*, *Conyza bonariensis, Schkuhria pinnata, Tagetes minuta* and *Zinnia peruviana*) and five perennial species (*Borreria eryngioides, Sida rhombifolia*, *Solidago chilensis, Taraxacum officinale* and *Verbena litoralis*). We also compared the response of annual vs perennial species. Six species had highest germination when treated with heat and smoke combined, whereas two had lowest germination under this treatment, indicating synergistic and antagonistic interaction of these factors, respectively. Most of the species tolerated heat shock (i.e. germination was similar to that in control treatment), whereas others had higher germination in response to heat shock, especially under the moderate 70ºC treatment. Germination was higher than control (i.e. no heat and no smoke) after smoke treatment in four species. Perennial species showed higher average germination than annuals in both heat treatments and in the control. Annual species had higher average germination for all treatments involving smoke. The high variability observed at the species level, and the limited number of species studied calls for precaution in interpreting and extrapolating results. Nevertheless, our study shows a general positive response of both perennial and annual species to fire cues, suggesting an advantage of these species for colonizing post-fire environments, and being favoured under scenarios of increasingly frequent low-to-medium intensity fires.

Key words: broad-leaved herbs, germination cues, life history strategies, post-fire regeneration, seed mass

Introduction

Germination is one of the most significant stages in a plant´s life cycle on which the development of the following generation depends (Harper 1977). Seeds always aim to maximize their establishment and survival (Reigosa *et al.* 2004), and therefore, physiological processes and traits related to germination are key aspects of a plant´s response to different disturbances.

Fire is a widely distributed ecological factor in worldwide ecosystems. It is one of the main factors affecting vegetation dynamics (Bond & Keeley 2005), causing direct removal of plant biomass and affecting species survival and growth, as well as the establishment of new individuals from seeds, especially in fire-prone regions. As a consequence, several species have developed different recruitment strategies to optimize post-fire survival. For example, in regions with Mediterranean climates, where fire has been a very important evolutionary factor, seeds of many species can tolerate or be stimulated by high temperatures (e.g. 120C°) or other fire-related factors, such as ashes and smoke (Hanley & Lamont 2000; Keeley & Fotheringham 2000; Hanley *et al.* 2001; Luna *et al.* 2007; Ghebrehiwot *et al.* 2011; Zuloaga-Aguilar *et al.* 2011).

Heat shock influences germination by removing inhibitory waxy substances covering the seed and/or scarifying seeds that have hard and impermeable covers, thereby allowing water and oxygen exchange (Keeley & Bond 1997; Keeley & Fotheringham 2000; Hanley *et al.* 2001). Furthermore, smoke can promote germination by increasing the permeability of solutes (ions and gases) in the subdermal cuticle of the seed (a semipermeable membrane), which would be induced mainly by the presence of nitrogen oxides (Keeley & Fotheringham 1997). The mechanism underlying this compound, however, is still unknown (Brown & Staden 1997; Keeley & Fotheringham 1998).

Seed germination response to both heat and smoke depends on the duration and intensity of treatments applied to seeds (Keeley *et al.* 1985; Keeley 1987; Auld & O'Connell 1991; Keith 1997; Plummer *et al.* 2001). Germination response can also vary depending on whether these factors are applied individually or combined (Keith 1997; Gilmour *et al.* 2000; Morris 2000; Kenny 2000; Tieu *et al.* 2001; Thomas *et al.* 2003).

While in the Chaco region there are historical records of fires produced by indigenous communities as a hunting and/or defence technique (Morello & Saravia Toledo 1959; Morello 1983) as well as of natural fires induced by lightening, there is no experimental evidence of recurring fire having been a strong natural selection pressure over evolutionary time, indicating that fire history in the region has presumably not been as long or intense as that of the Mediterranean systems.

Several studies show that resprouting, which is a widespread trait both in fire-prone and non-fire-prone ecosystems (Wells 1969; Bellingham *et al.* 1994; Lloret *et al.* 1999; Pausas & Verdú 2005), is the main post-fire regeneration strategy of dominant Chaco species, with some species showing a high resprouting capacity following low to medium intensity fires (e.g. Gurvich *et al.* 2005; Bravo *et al.* 2014; Torres *et al.* 2014). Furthermore, studies on germination response of some species conducted in the Chaco region have generally been focused on response to variations in environmental temperature ranging between 5° and 35°C (Funes & Venier 2006; Funes *et al.* 2009; Venier *et al.* 2012). Nevertheless, Jaureguiberry and Díaz (2015) reported that dominant trees, shrubs and herbs of Chaco forest can tolerate (or even be stimulated) by heat treatments of low and medium intensity (70-100°C). However, at high intensity treatments (120-180°C), practically all species exhibited low or no germination.

Yet, fire recurrence has increased notably in the last decades, mainly due to land use changes and population expansion (Grau *et al.* 2005; Boletta *et al.* 2006; Bravo *et al.* 2010).

This phenomenon is especially noticeable in the Chaco Serrano (central Argentina), where fires of different characteristics, ranging from low to medium-high intensity fires depending on environmental conditions and the vegetation physiognomy, occur every year (Bravo *et al.* 2001; Kunst & Bravo 2003; PPMF 2007; Kunst 2011; Argañaraz *et al.* 2015a), challenging the adaptive capacity of plants to regenerate after a fire.

Herbaceous plants play a key role in fire dynamics, since they provide the fine fuel needed for fire ignition and propagation (Kunst & Bravo 2003). They also have an important ecological role in the post-fire environment, for example in preventing erosion processes by stabilizing the soil (especially in areas with high slope), and in hastening functional restoration by preventing non-native species invasion and in some cases facilitating the establishment of woody species (Keeley *et al.* 1981; Spittler 1995; Maestre *et al.* 2001; Floyd *et al.* 2006; Giorgis *et al.* 2013). However, little is known about the effects of fire on their seeds, with most studies focusing on woody species (Keeley 1987; Hanley & Lamont 2000; Enright & Kintrup 2001; Tieu *et al.* 2001). Particularly in the Chaco Serrano, broad-leaved forbs are an important component of the ecosystem, whereas other herbaceous species such as graminoids are relatively less frequent, especially in areas with mature forest (Giorgis *et al.* 2011).

While there is considerable evidence showing that herbaceous perennial and annual species differ in several ecological aspects (Pitelka 1977; Garnier 1992; Garnier & Laurent 1994; Fenner *et al.* 1999; Corbin & D'antonio 2004; Roumet *et al.* 2006), there is a surprisingly low number of studies on germination response to heat and smoke in those species (e.g. Keeley *et al.* 1981; Keeley *et al.* 1985; Paula & Pausas 2008). Some studies have reported annual species having a high tolerance (or even stimulation) to the mentioned factors, and in some cases they have reported a higher tolerance or stimulation than perennial herbs (Keeley 1991; Keeley & Bond 1997; Paula & Pausas 2008); however none of them compares these strategies explicitly. Still, based on those studies, we can assume that, at least within a given temperature

range that does not pose physical limitations to seeds (Bond *et al.* 1999; Hanley *et al.* 2003), annual species in fire-prone ecosystems such as the Chaco region might exhibit a greater tolerance to factors such as heat and smoke than perennial herbs, since they depend solely on the reproductive success of their seeds for survival (Pausas & Keeley 2014).

The aim of this work was to evaluate the effects of heat shock and smoke experimental treatments on the germination response of annual and perennial herbaceous species that are frequent in the Chaco Serrano of Córdoba province, central Argentina. The experimental treatments will simulate low (70°C) and medium-high (110°C) fire intensities. Considering previous studies on germination response of herbaceous species in other regions of the world and those reported for the Chaco region (Torres *et al.* 2014; Jaureguiberry & Díaz 2015), as well as the above mentioned fire history in the study area, we expect a decrease in the germination capacity of most of the species under the most intense heat treatment and tolerance under the less intense heat treatment. Furthermore, the high variability observed under different smoke treatments in species from other regions, along with the lack of previous experiments involving Chaco species, does not allow us to make a more specific prediction for this treatment. We also compare the germination response between annual and perennial species. Based on the mentioned records, we expect that annual species will exhibit higher tolerance to heat and/or smoke treatments than perennial species.

The present work will allow improving our understanding of plant dynamics in fire-prone regions and the post-fire colonization strategy of forb species in burned areas of the Chaco Serrano and similar ecosystems. For example, if the studied species show low tolerance to heat and/or smoke, then it is probable that post-fire colonization of spaces occur mainly by seed dispersal from nearby areas, or by basal resprouting in the case of perennial herbs whose resprout tissues have survived fire.

Materials and Methods

144 ***Study area***

145 The study area is located within the Chaco Serrano District (Cabrera 1976). Vegetation
146 is composed mostly of secondary forest communities and substitute shrublands, alternated with
147 grasslands (Cabido *et al.* 2010; Giorgis *et al.* 2011). The families Fabaceae, Asteraceae and
148 Poaceae are the most numerous in the flora of the study area, and Bromeliaceae, Cactaceae and
149 Solanaceae are also well represented. The most representative species include *Lithraea*
150 *molleoides* (Vell.) Engl., *Acacia caven* (Molina) Molina var. *caven*, *Zanthoxylum coco* Gillies ex
151 Hook. f. & Arn. and *Jarava pseudoichu* (Caro) F. Rojas (Zuloaga *et al.* 1994; Zuloaga &
152 Morrone 1996; Zuloaga & Morrone 1999; Giorgis *et al.* 2011).

153 Fires season spans from June through December (i.e. early winter through early
154 summer), with highest occurrence of fires in August-September (PPMF 2007; Argañaraz *et al.*
155 2015a). Fire frequency varies considerably within the study region, mainly depending on
156 climatic variables, human population density and vegetation type (Argañaraz *et al.* 2015b).
157 Overall, effective burnt area in the study region for the period 1999-2011 was approximately
158 25%, with some areas burnt every 3-4 years (especially grassland areas), others every 5-6 years,
159 and others burnt every 10 or more years (Argañaraz *et al.* 2015a).

160

161 ***Species selection and seed collection***

162 The study focused on forb species common in the Chaco Serrano forest. Sampling sites
163 were located between 500 and 1300 m.a.s.l. For species selection, on the basis of Giorgis *et al.*
164 (2011), who reported a total of 383 forb species for the Chaco Serrano, and considering the
165 logistic restrictions and limitations in seed availability in the field during the proposed sampling
166 period (February-April 2015), we collected seeds from 10 forb species (Table 1): five annual
167 and five perennial species. The selected species are highly frequent in the study area (relative
168 frequency ranging between 6.86% and 69.11%; Annex 1 of Giorgis *et al.* 2011), seven of them
169 being in the top 8% most frequent. Annual species included: *Bidens subalternans* DC., *Conyza*
170 *bonariensis* (L.) Cronquist*, Schkuhria pinnata* (Lam.) Kuntze ex Thell.*, Tagetes minuta* L. and

Zinnia peruviana (L.) L. (all five are Asteraceae). Perennial species included: *Borreria eryngioides* Cham et. Schltdl. (Rubiaceae), *Sida rhombifolia* L. (Malvaceae), *Solidago chilensis* Meyen (Asteraceae), *Taraxacum officinale* G. Weber ex F.H. Wigg. (Asteraceae) and *Verbena litoralis* Kunth (Verbenaceae) (following Zuloaga *et al.* 1994; Zuloaga & Morrone 1996; Zuloaga & Morrone 1999; and updates, http://www.darwin.edu.ar/Proyectos/FloraArgentina/fa.htm).

The germination period of the selected species is concentrated between the end of winter and spring; the vegetative phase (including sprouting of perennial species) occurs during the spring and summer; and the flowering and fructification occurs in summer and early fall (Atlas de malezas INTA; http://rian.inta.gov.ar/atlasmalezas/atlasmalezasportal/DetalleMaleza.aspx?pagante=CXF&idmaleza=23585).

Seeds were collected from areas of mature forest, shrubland areas and areas with low and open vegetation. A total of 12 sites were visited for seed collection, located in several points along the Sierras Chicas range, Córdoba province, approximately between 30º58'00''S and 31º29'00''S. For each species, seeds of at least 20 healthy-looking (i.e. no evidence of physical damage), sexually mature, randomly selected individuals located at different sites of the sampling area were collected. Seeds were stored in paper bags in a refrigerator for three weeks to 3 months (depending on the species and the collection sites) until the experiments to prevent predation by insects and fungi. Before the experiment samples were mixed to prevent bias from seed collection location. Most of the seed mass data reported in Table 1 were obtained from previous works conducted in the study area and are part of TRY global database (Kattge *et al.* 2011). Seed mass of *B. eryngioides*, *S. rhombifolia* and *V. litoralis* was calculated in the present work, following the method described by Pérez-Harguindeguy *et al.* (2013).

Laboratory experiment

Seeds were subjected to the following treatments: control (no heating); heat shock at 70°C for one hour, and heat shock at 110°C for five minutes. Treatments consisted of placing seeds in glass dishes and heating them in a convection oven. The heat shock treatments were applied simultaneously to all seeds, although it is acknowledged that separate applications would have produced a more appropriate replication of these treatments (Morrison & Morris 2000).

Heat treatments were selected to cover a range of conditions that seeds might endure in the soil during a fire or in open sites, considering, on the one hand, the temperatures recorded at the soil level in other studies conducted both in the Chaco region (e.g. Bravo *et al.* 2010; Ledesma *et al.* 2011) and in other semiarid regions (Bóo *et al.* 1996; Bradstock *et al.* 1992); and, on the other hand, the effect of soil insulation even at very low depth (Beadle 1940; Valette *et al.* 1994; Bradstock & Auld 1995). Moreover, the selected temperatures are within the range reported as stimulating for several species in similar studies conducted in other regions (Keeley 1987; Hanley *et al.* 2001; Luna *et al.* 2007; Ne'eman *et al.* 2009).

In addition, two treatments combining both heat shock conditions plus a commercial smoke extract (Montazeri *et al.* 2013) diluted in distilled water at a concentration of 1:30 v/v (Baxter *et al.* 1994; Brown & Staden 1997; Doherty & Cohn 2000) were performed (hereafter referred to as 70ºC+S and 110ºC + S). The diluted smoke was applied to each pertinent replicate (see below) at the start of incubation. Finally, another treatment including only the diluted smoke extract was performed. Hence, each species was subjected to a total of six treatments.

Seeds were incubated in Petri dishes lined with filter paper moistened with distilled water. Fifty seeds were placed in each dish, with three dishes (replicates) per treatment per species being used. Before incubation, each dish was treated with a broad-spectrum fungicide (Carbendazim and Benzimidazol) to avoid fungal proliferation. Petri dishes were placed in a

germination chamber at 25°C and a 12-hour photoperiod for 40 days (in some species, the experiment was ended after 36 days of incubation because no germination had been recorded for one week). This temperature mimics the average maximum spring temperature in the region (Capitanelli 1979), which is the most common germination season for most of the studied species, and has been used in previous studies as an optimum germination temperature (Cony & Trione 1996; Funes *et al.* 2009; D'agostino *et al.* 2012; Venier *et al.* 2012). The Petri dishes were periodically checked for seed germination, with the germination criterion being seeds with radicle emergence. Germination data were used to calculate germination percentage of each treatment for each species. No viability tests were performed.

Data analyses

Statistical analyses were performed using InfoStat software version 2017 (Di Rienzo *et al.* 2017, Grupo InfoStat, Facultad de Ciencias Agropecuarias, Universidad Nacional de Córdoba, Argentina). Differences in germination percentages among species and among treatments within species were determined using a Generalized Linear Model (Bates *et al.* 2014; Di Rienzo *et al.* 2017), which included the experimental treatments (i.e. smoke and heat), species and their interactions as fixed factors, assuming a binomial error distribution. The obtained means were then compared using the DGC *a posteriori* test (Di Rienzo, Guzmán, Casanoves pair-wise comparison procedure; Di Rienzo *et al.* 2002). Model selection was based on the Akaike criterion (1972). Model fit was performed via InfoStat, which uses the *lme4* library (Bates *et al.* 2014) in R (R Development Core Team 2008).

Results

General patterns

Nine of the ten species showed a significant increase in germination with relation to control (i.e. no heat and no smoke) under at least one of the treatments; being *T. officinale* the only species that did not follow this pattern (Fig. 1). The model showed a high influence of

'species' in explaining the patterns observed under the experimental treatments ($p<0.0001$; deviance=2377.89; Table 2); and there was a significant but relatively weak interaction between heat shock, smoke and species ($p<0.0001$; deviance = 165.36; Table 2). The strength and nature of the interaction varied greatly depending on the species (Fig. 1; see following sections).

Germination response to the combination of heat shocks and smoke extract

Six species had the highest germination percentages under one of the treatments combining heat shock and smoke (Fig. 1). Four of them (*S. pinnata, S. chilensis, V. litoralis* and *Z. peruviana*) exhibited significantly higher germination percentage in the combined treatment of 70°C + S (Fig. 1). For *B. subalternans*, the treatment combining 110°C +S showed a significantly higher germination, and for *B. eryngioides*, germination percentage was equally high for both combined treatments (Fig. 1).

Furthermore, *S. rhombifolia* and *T. minuta* also had the highest germination percentages in the treatment combining 70°C + S, but the values did not differ significantly from the germination response obtained in other treatments within the species (70°C for one hour in *S. rhombifolia* and smoke extract in *T. minuta*) (Fig. 1). Finally, *C. bonariensis* and *T. officinale* were the only species that showed decreased germination in response to combined treatments of heat and smoke extract with respect to control (Fig. 1).

Germination response to heat shock (no smoke)

Germination percentages obtained in the control treatments were highly variable. Two species showed a germination percentage below 5% (*S. pinnata* and *S. rhombifolia*), whereas others, such as *T. minuta* and *T. officinale*, exceeded 60% (Appendix S1; Fig. 1).

Six species did not show significant differences between control and heat shock treatments; four species showed a significantly higher germination than the control under the 70°C treatment: *B. eryngioides, C. bonariensis, S. rhombifolia* and *S. chilensis*. Finally, only *S. chilensis* had a significantly higher germination percentage under 110°C, but without significant differences from those under 70°C (Fig. 1).

Germination response to smoke treatment (no heat)

Germination response to the application of a commercial smoke extract was very variable among species. Four species exhibited a significantly higher germination percentage than control and both heat treatments: *B. subalternans, B. eryngioides, T. minuta* and *Z. peruviana (*Fig. 1). Furthermore, five species did not show significant differences from control, and only *T. officinale* showed a lower germination percentage than control under the smoke treatment (Fig. 1).

Germination response of annual vs. perennial forb species

In general, annual species showed a higher mean germination than perennials under the three treatments involving smoke (Fig. 1), and also a higher impact of these treatments (i.e. greater difference with respect to control treatment) than perennial species (Fig. 2; Appendix S2). Germination response was on average higher in perennial species under the control treatment and the two heat shock treatments (Fig. 2; Appendix S2), although differences with respect to control were not as high as those mentioned for annuals above.

Discussion

Germination response to the combination of heat and smoke extract

The six species that had their highest germination in one of the combined heat and smoke treatments showed a synergistic effect of these factors on germination (i.e. a positive interaction between heat and smoke). Accordingly, previous studies conducted with species

304 from other regions also reported highest germination under this type of treatments (Keith 1997;
305 Morris 2000; Enright & Kintrup 2001; Tieu *et al.* 2001; Thomas *et al.* 2003). On the other hand,
306 the antagonistic patterns such as the one observed in *C. bonariensis* and *T. officinale* (Fig. 1) has
307 also been reported for other species (Tierney 2006), although the mechanism underlying this
308 pattern is not clear. A possible explanation is that heat might produce chemical changes in the
309 compounds present in smoke, which in turn can produce changes in seed incubation conditions,
310 such as pH, which may affect germination (Keeley & Fotheringham 1998). In the case of *T.*
311 *officinale* the two combined heat and smoke treatments showed similar germination levels to
312 that with smoke individually, indicating that smoke, regardless of heat application, is the
313 inhibiting factor for this species.
314
315 ***Germination response to heat shock (no smoke)***
316 All the studied species were capable of tolerating the110ºC treatment (i.e. germination
317 similar to the control treatment); *S. chilensis* even showed a significantly higher germination
318 under this treatment (Fig. 1). Bond *et al.* (1999) suggest that high heat tolerance of small-seeded
319 species (less than 2 mg *sensu* Bond *et al.* 1999), such as most in the present work (with the
320 exception of *Z. peruviana*; Table 1) and of broad-leaved forbs in general, may be because these
321 seeds germinate from shallow soil areas, since seedlings cannot emerge from higher depths.
322 Therefore, small seeds are more exposed than large seeds (more than 10 mg s*ensu* Bond *et al.*
323 1999) to factors such as heat generated by fire or the high insolation in certain regions. This
324 situation might act as a selective pressure for developing higher tolerance to heat shock in
325 small-seeded species (Hanley & Lamont 2000; Hanley *et al.* 2003), at least within a given
326 temperature range.
327
328 Furthermore, the fact that germination was stimulated mostly by the less intense heat
329 shock treatment (70ºC) suggests that such stimulation is not necessarily an indicator of adaptive
330 response to fire, since similar temperatures can occur due to sun exposure in open sites

331 generated by overgrazing, erosion, or burned soils after a fire (Auld & Bradstock 1996; Keeley
332 & Fotheringham 2000). Moreover, our results show that heat-stimulated species also geminated
333 under the control treatment, indicating that they are not strictly tied to post-fire environments.
334 Therefore, both species that tolerated heat shocks and those that were stimulated have great
335 resistance to heat but do not depend on it to germinate (Ooi *et al*. 2014).

336 Patterns of high to medium germination observed under control treatment (i.e. no heat
337 and no smoke) in *T. officinale*, *T. minuta, C. bonariensis* and *S. chilensis* agrees with previous
338 findings (Fenner 1980; Washitani 1984; Correia *et al.* 1999; Ferreira *et al.* 2001). The low
339 germination of some of the species has also been reported elsewhere. One possible cause for this
340 is the presence of some type of seed dormancy. For example, *S. rhombifolia* has been reported
341 to have physical dormancy due to a hard seed coat (Chauhan & Johnson 2008), whereas *S.*
342 *pinnata* has been reported to have physiological dormancy (Fenner 1980). Likewise, *Z.*
343 *peruviana* has been reported as having moderate seed dormancy (Rodríguez-Arévalo *et al.*
344 2016).Therefore, in these species the performed treatments were able to break seed dormancy, at
345 least partially. The lack of germination of the remaining seeds could be due to seeds being non-
346 viable (which was not tested in this study) or to specific germination requirements that were not
347 met in the present study.

348

349 ***Germination response to smoke extract (no heat)***

350 The germination response under the smoke extract treatment was variable; five species
351 did not show significant differences in germination from control, whereas *B. eryngioides, B.*
352 *subalternans, T. minuta* and *Z. peruviana* increased germination with respect to control and to
353 both heat treatments. These results are in agreement with other studies in which smoke was
354 found to be effective in inducing and/or increasing germination of both annual and perennial
355 herbs (Dixon *et al.* 1995; Jäger *et al.* 1996; Keeley & Bond 1997; Lloyd *et al.* 2000; Enright &
356 Kintrup 2001; Ghebrehiwot *et al.* 2011).

357

The decreased germination observed in *T. officinale* under this treatment might be attributed to the potentially inhibitory capacity of smoke extract (Brown & Staden 1997; Jäger *et al.* 1996; Gilmour *et al.* 2000), which occurs at different concentrations depending on the species (Keeley & Fotheringham 1998). This could explain the variability observed in our study under the same extract concentration.

Germination response of annual vs perennial forbs

While on average perennial species exhibited a higher germination percentages than annual species under control and 70ºC and 110ºC treatments (Fig. 2), annual species had higher average germination percentages under smoke, 70ºC + S, and 110ºC + S than perennial species, showing and opposite relationship to that observed in control treatments (Fig. 2). Thus, the effects of these treatments were much higher for annual species than for perennial ones. The results obtained under smoke, 70ºC + S and 110ºC + S agree with previous findings showing that in some ecosystems, annual forbs are more tolerant or more stimulated by heat or smoke (Keeley & Bond 1997; Paula & Pausas 2008), and support our hypothesis postulating that annual species are more tolerant to heat and smoke than perennial species. However, when considering all treatments, the hypothesis and prediction formulated are only partially supported.

The high variability observed on species' germination and the high influence of species reported by the model makes it difficult to reach firm conclusions regarding differences between life strategies, at least for the group of species selected for this study. Nevertheless, despite the limited number of species used, which demands precaution in the interpretation of the results and in the extrapolation of the results, we believe that our study provides novel and valuable information to better understand the post-fire dynamics of forb species with different life strategies.

Ecological implications

Our results suggest that the studied species are capable of tolerating temperatures associated with low and medium intensity fires, and that smoke (both acting alone or in combination with heat shock) is an effective promoter of germination for most of the species, therefore favouring their performance in post-fire environments, allowing them to colonize the free spaces generated by fire (Keeley & Fotheringham 2000; Hanley & Lamont 2000; Hanley *et al.* 2001). Additionally, under increasing fire frequency scenarios herbaceous species could be favoured with respect to woody species, since these latter require longer times between fires to regenerate and be able to colonize post-fire spaces, either through resprouting or recruitment of new individuals from seeds (Silvertown *et al.* 1993; Moles *et al.* 2004). Under such scenarios, it could be expected that the herbaceous species studied here, and others common in the study region, will become more frequent and produce changes in vegetation structure (e.g. species diversity and/or composition, physiognomy) (Verzino *et al.* 2005; Casillo *et al.* 2012; Giorgis *et al.* 2013), a process that has already been reported for other fire-prone semi-arid regions (Pausas & Keeley 2009; Keeley & Brennan 2012).

On the other hand, the prevalence of the annual and/or perennial strategies will depend on factors such as relative abundance in the ecosystem, dispersal ability, relative growth rate, and seed size and production (Garnier 1992; Leishman *et al.* 1995; Moles *et al.* 2004). This could be highly relevant for invasion processes, especially by annual exotic species, many of which are known to outcompete native perennial species under disturbance scenarios (Seabloom *et al.* 2003; Corbin & D'Antonio 2004).

Conclusions

Germination of all of the studied species was stimulated by at least one of the experimental treatments performed. Eight species responded positively to the combined heat and smoke treatments, four of which showed a synergistic effect on germination. This positive

412 response was mostly under the 70°C temperature, suggesting that the studied species might have
413 an advantage in colonization of environments after low-to-medium intensity fires. Unlike
414 expected, germination response did not decrease under the most intense heat treatment (i.e.
415 110ºC).

416 The prediction of germination response in annual vs. perennial species was partially
417 supported, since although on average germination was higher in annual species in all treatments
418 involving smoke, perennial species had higher average germination under the control treatment
419 (i.e. no heat and no smoke) and the two heat treatments alone.

420

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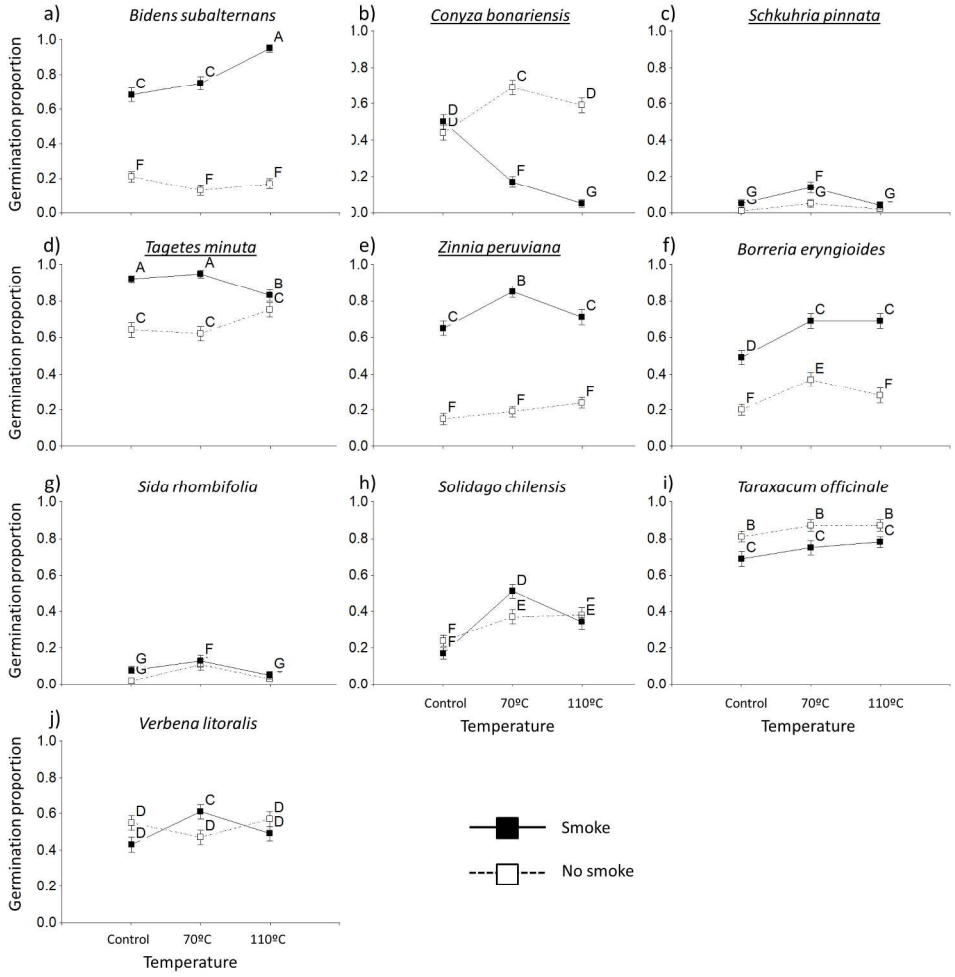
Table 1. List of studied species, indicating botanical family, life history strategy and seed mass. Nomenclature follows Zuloaga et al. (1994), Zuloaga and Morrone (1996, 1999) and updates (following http://www.darwin.edu.ar/Proyectos/FloraArgentina/fa.htm). References on seed mass: [a]Kattge, J. et al. (2011); [b]this study.

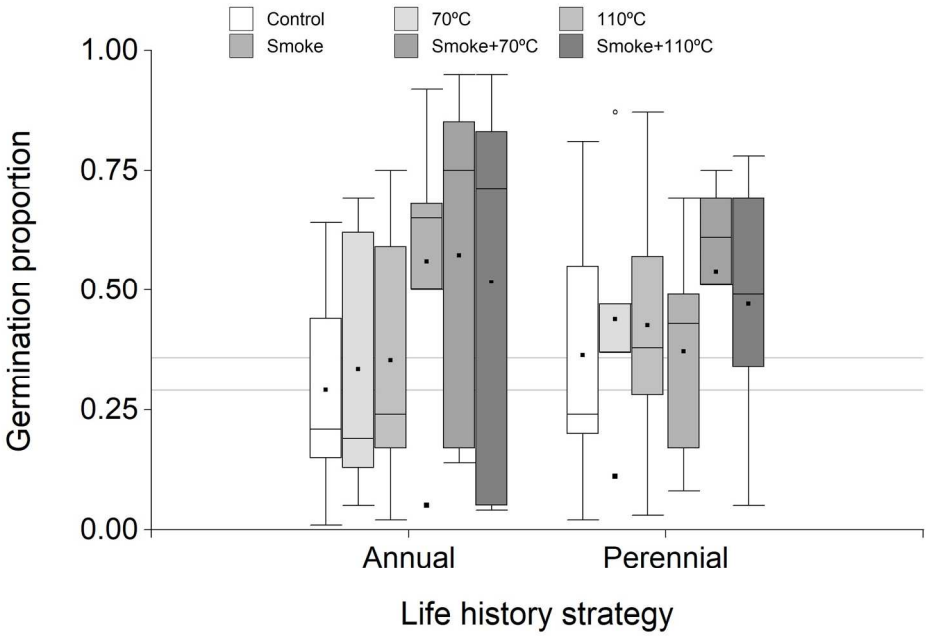
Species	Family	Life history strategy	Seed mass (mg)
Bidens subalternans DC.	Asteraceae	Annual forb	1.6[a]
Conyza bonariensis (L.) Cronquist.	Asteraceae	Annual forb	1.1 [a]
Schkuhria pinnata (Lam.) Kuntze ex Thell.	Asteraceae	Annual forb	1.0 [a]
Tagetes minuta L.	Asteraceae	Annual forb	0.78 [a]
Zinnia peruviana (L.) L.	Asteraceae	Annual forb	4.86 [a]
Borreria eryngioides Cham et. Schltdl.	Rubiaceae	Perennial forb or subshrub	0.28[b]
Sida rhombifolia L.	Malvaceae	Perennial forb or subshrub	1.99[b]
Solidago chilensis Meyen	Asteraceae	Perennial forb	0.13 [a]
Taraxacum officinale G. Weber ex F.H. Wigg.	Asteraceae	Perennial forb	0.55 [a]
Verbena litoralis Kunth	Verbenaceae	Perennial forb	0.23[b]

Table 2. Sequential hypothesis testing for fixed effects (i.e. smoke, temperature, species and their interactions) of the generalised linear model used in the present study. "P" values (last column) lower than 0.05 indicate a significant effect of the corresponding factor.

	Df	Deviance	Resid. Df	Resid. Dev	Pr(>Chi)
NULL			179	3896.76	
Smoke	1	169.83	178	3726.94	<0.0001
Temperature	2	35.81	176	3691.12	<0.0001
Species	9	2377.89	167	1313.23	<0.0001
Smoke:Temperature	2	8.00	165	1305.23	0.0183
Smoke:Species	9	757.72	156	547.51	<0.0001
Temperature:Species	18	104.94	138	442.58	<0.0001
Smoke:Temperature:Species	18	165.36	120	277.219	<0.0001



394x393mm (150 x 150 DPI)



450x300mm (150 x 150 DPI)