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NOTES AND COMMENTS

Seed longevity and fire: post-germination responses of *Rumex acetosella* L. in northwest Patagonian grasslands (Argentina)

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Abstract

The cosmopolitan herb *Rumex acetosella* forms persistent soil seed banks and increases in cover after fire. We investigated how the interaction between seed age and fire affects seedling growth by exposing different-aged seeds to heat, smoke, charcoal, and ash treatments. We measured growth of germinated seedlings that were transplanted and allowed to grow for 65 days in a greenhouse. Seedlings from seeds >8 years old did not reach an appropriate radicle length for transplantation. Seedling growth decreased with increasing temperature of the heat treatment. As seed age increased, growth decreased with smoke and charcoal, and increased with ash treatment. Height was negatively correlated with seed age. Our results suggest that fire and seed age could affect demographic responses of *R. acetosella* seedling populations. Post-fire recruitment could be partially favored by the positive effect of nutrient input from ash on seedling growth. High fire intensities, however, would be detrimental to seedling vigor.

Keywords: exotic herb, plant invasions, post-fire establishment, seedling growth, soil seed bank.

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Introduction

Seedling establishment is the last stage of the recruitment process (Harper 1977) and plays an important role during the introduction of non-native species in natural plant communities (Ferreras & Galetto 2010). Exotic species often establish during post-fire recovery. Post-fire plant communities may be more easily invasible because of reduced competition, increases in pre-existing resources and/or the creation of new resources (Bond & van Wilgen 1996; Davis *et al*. 2000).

Post-fire regeneration of many species comes from seeds stored in the soil profile (Whelan 1995; Bond & van Wilgen 1996). Seed age and fire-related abiotic factors (e.g. heat and smoke) can influence both germination and subsequent seedling development (Hanley *et al*. 2001; Sparg *et al*. 2005; Valleriani & Tielbörger 2006). The interaction between seed age and fire effects on seedling growth is particularly relevant when considering the post-fire recruitment of invasive non-native species that form persistent seed banks. To our knowledge, however, there are no studies that consider seed age and fire effects on such species.

One of the most abundant non-native species in the seed bank of northwestern Patagonian grasslands (Argentina) is *Rumex acetosella* L. (sheep sorrel, Polygonaceae) (Ghermandi 1997). It is a cosmopolitan perennial herb which forms persistent seed banks (Thompson *et al*. 1997). This exotic herb increases in cover after disturbances, particularly fire (Ferrandis *et al*. 1999; Ghermandi *et al*. 2004). A recent germination study examining the interaction between fire-related abiotic factors and seed age indicated that *R. acetosella* seeds <1 year old were particularly responsive to fire, and that a short exposure to smoke stimulated their germination (Franzese & Ghermandi 2011). However, no studies have evaluated its post-germination responses from different-aged seed cohorts exposed to diverse fire-related abiotic factors. We carried out laboratory experiments to investigate how the interaction between seed age and a short exposure of

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seeds to fire factors (i.e. heat, smoke, charcoal and ash) affects seedling growth in *R. acetosella*.

Materials and methods

Study area

Seeds were collected in a semi-arid grassland in northwest Patagonia, Argentina (41°03′S, 71°01′W). Mean annual precipitation is 580 mm (60% in fall and winter), and mean annual temperature is 8.6°C. The grassland is dominated by the perennial tussock grasses *Pappostipa speciosa* (Trin. et Rupr.) Romash and *Festuca pallescens* (St. Yves) Parodi. The dominant shrubs are *Fabiana imbricata* Ruiz et Pavón, *Mulinum spinosum* (Cav.) Pers. and *Senecio bracteolatus* Hook et Arnott. Vegetation cover is approximately 55% and the gaps (intertussock areas) are colonized by herbaceous species like the native forbs *Triptilion achilleae* Ruiz et Pavón, *Plagyobothrys verrucosus* (Phil.) Johnst., and *Microsteris gracilis* (Hook.), and the exotic forbs *Erophila verna* (L.) Chevall., *Holosteum umbellatum* L., and *R. acetosella*.

Study species

Rumex acetosella is native to Europe and is widely distributed in the temperate environments of the world, commonly in open and/or disturbed habitats (Correa 1984). This herb has been identified as an invasive species in some countries such as New Zealand (Fan 1996), USA (Frey *et al*. 2008), and Argentina (Speziale & Ezcurra 2011). *Rumex acetosella* has an extensive root system, flowers in late spring, and produces abundant seeds in early summer that are mostly dispersed by barochory at the end of the season. After disturbance this species can form dense monospecific patches. In the study area, *R. acetosella* dominates the grassland gaps.

Experimental design

To study the effect of fire and seed age on *R. acetosella* post-germination responses, we used seedlings from seeds exposed to treatments of heat, smoke, charcoal, ash, and control. Seeds were collected in 13 different years within a 19-year period (1989 to 2008), and stored in paper bags at room temperature until they were required for experimentation. In October 2008, at the time of experimentation, seed ages were: 19, 15, 14, 13, 12, 8, 7, 5, 4, 3, 2, 1, and <1 year old (fresh seeds). For the heat treatments, seeds were placed in an oven for 5 min at 60, 90 or 120°C, and for the smoke treatment, seeds were exposed to an environment saturated with cooled smoke for 10 min. For the charcoal and ash treatments, seeds were placed in Petri dishes and watered with 4 mL of 10 g/L solution of pulverized charcoal or ash, respectively. The smoke, charcoal and ash were prepared from the combustion of biomass belonging to the dominant grassland species. Seeds in the control treatment were not fire treated. After treatment, all seeds were placed in Petri dishes in a germination chamber for 2 months, simulating an autumn photoperiod (see detailed methodology in Franzese & Ghermandi 2011). Ten emerged seedlings were selected by seed age and fire treatment (10 replicates × 7 treatments × 13 seed ages = 910 seedlings) and transplanted into individual trays (8 × 5 cm) that contained grassland soil. Seedlings were chosen at random among those that reached an appropriate radicle length (i.e. ≥1 cm). There were 18 days ± 1.2 (mean ± SE) between germination and transplanting. Before transplanting, height and root length of all seedlings were measured. Seedlings were watered regularly and grown for 65 days in a greenhouse, with an even light supply across all trays. At the end of the experiment, height and root length of all seedlings were measured.

Data analysis

We analyzed the growth of seedlings (total seedling length = height plus root length) during the growth period in the greenhouse. Data were standardized subtracting the value measured after 65 days (final measure) from the value measured before transplanting the seedlings (initial measure). Then, the variability from different seed germination days was eliminated from the variable. We performed a multiple regression to model the influence of seed age (quantitative), fire treatment (qualitative with six levels), and their interaction on the difference in total length (post-transplant value minus pretransplant value) using a general linear model with variance heterogeneity (gls function, nlme package, R software; Pinheiro *et al*. 2012). We log10 transformed the response variable to achieve normality, and this assumption was checked by the inspection of residual graphs and the use of a Kolmogorov–Smirnov test. The residual variance was different among fire treatments; therefore, we fitted a heteroscedastic model using the VarIdent function in the nlme package (Pinheiro *et al*. 2012). After performing the analysis of variance, the significance of each fire factor was assessed with a likelihood-ratio test, by comparing the goodness of fit of models with and without the effect of each evaluated fire factor. We did not analyze growth data from seeds >8 years old and those from the 120°C treatment because there were no recently germinated seeds with the appropriate radicle length for transplantation. For the control treatment, we evaluated the relationship between height and seed age (seed age range: <1–8 years old), and between root length and seed age with Spearman correlations. The significance level was $\alpha = 0.05$ and marginal significances were reported.

Results

The effect of fire treatments on the studied variable depended on the age of seeds (Table 1), except for the highest temperature heat treatment (120°C for 5 min), which had a strong negative impact on the development of all seedlings. The 90°C for 5 min diminished growth at greater seed age, and the 60°C for 5 min did not show any effects on this variable (Fig. 1). These results showed that under similar exposure times, the impact of heat on seedling total length decreases when the temperature diminishes. We observed a negative effect from smoke and charcoal treatments on total seedling length when seed aged (Fig. 1). Ash solution was the only treatment that had a positive effect on seedling length at increasing seed age (Fig. 1). Seed age showed a negative correlation with height (Spearman's $r = -0.20$, $P = 0.06$), but it was not correlated with root length ($P > 0.05$).

Table 1 Analysis of variance results for seedling growth response to seed age, fire treatment and their interaction

Source of variation	d.f.	Difference in total length† F statistic	P-value
Seed age	1	3.39	0.06
Fire treatment	5	16.08	<0.001
Seed age × fire treatment	5	3.45	0.004
Error	447		

† Variable log-transformed.

Discussion

Abiotic factors related to fire produced heterogeneous effects on seedlings of *R. acetosella* originated from different-aged seeds. Heat consistently decreased total length of seedlings and its impact increased with increasing temperature. Reduced seedling growth could reflect internal seed damage produced by high temperatures (Hanley & Fenner 1998). In several Mediterranean species, the exposure of seeds to temperatures higher than 80°C decreased growth and survival (Hanley & Fenner 1998). Similarly, *R. acetosella* seedlings from heated seeds may have low survival and limited recruitment in field conditions. Particularly, fires of high intensity would prevent seedling emergence or establishment.

Varied effects of smoke on weed species growth have been reported, which would differ from those effects observed in germination (Daws *et al.* 2007). Smoke had a neutral effect on germination of *R. acetosella* from aged

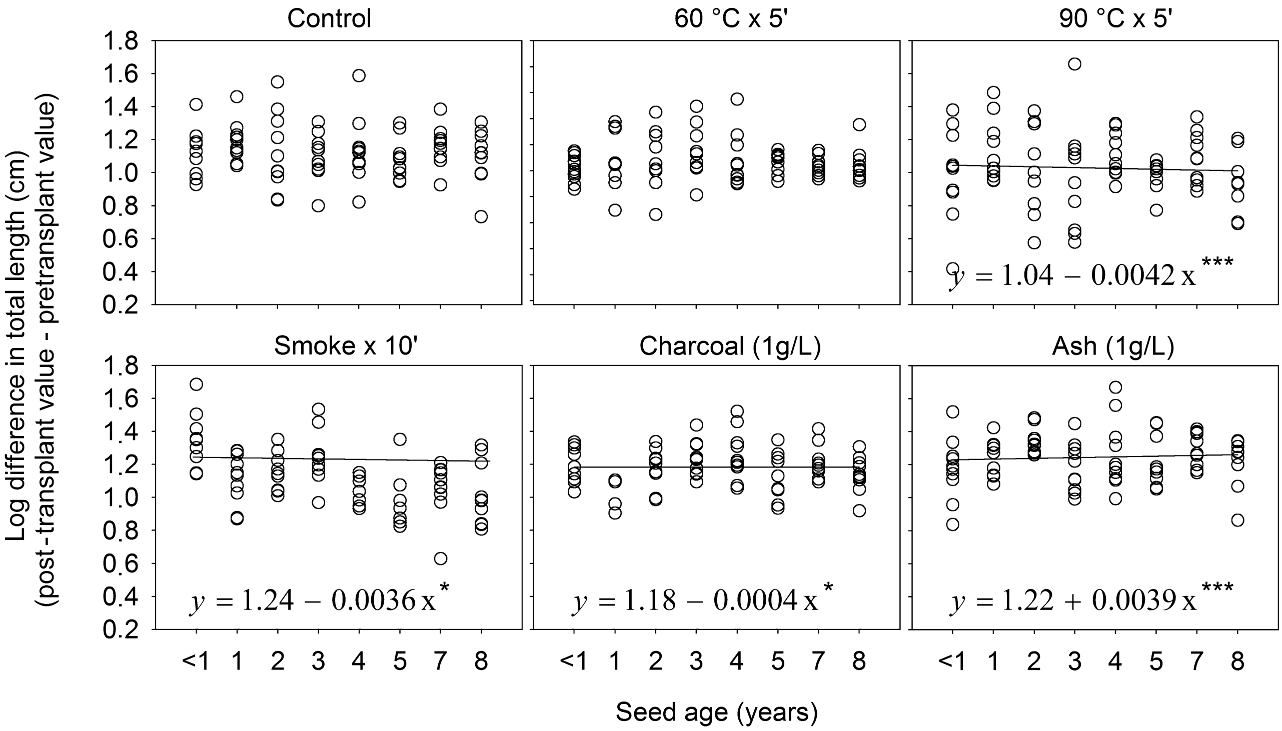

Fig. 1 Relationships between log difference in total length (cm) and seed age (years) for each fire treatment and control. Ten replicates were used by seed age and treatment, except for seedlings from seed ages (years): <1 (control: $n = 9$), 1 (charcoal: 4), 2 (smoke, 60°C, and charcoal: 9), 3 (60°C: 9), 4 (smoke: 8), 5 (90°C: 8, ash: 9, and smoke: 7), 8 (90°C: $n = 9$). The figure shows the equations of those fire treatments with significant effects. Statistical comparisons by likelihood-ratio test. * = $P < 0.05$, *** = $P < 0.001$.

seeds (Franzese & Ghermandi 2011), but produced a negative effect on growth of seedlings originated from them. Smoke could contain inhibitor compounds (Daws *et al*. 2007), which would be leached from the seeds by rainfall in natural conditions, but are kept at a high concentration in germination studies using Petri dishes (Sparg *et al*. 2005).

The presence of charcoal and ash in the post-fire environment modifies the availability and spatial distribution of nutrients, which may influence the growth and distribution of seedlings after fire (Kutiel & Naveh 1987; Ne'eman *et al*. 1992; Gundale & DeLuca 2007). For example, the short-term increase in availability of N and P immediately after fire may be essential for the establishment of Mediterranean legume species whose seeds have low contents of these macronutrients (Hanley & Fenner 1997). In our study, the ash solution increased seedling growth as the seeds increased in age. Seeds are likely to have a lower availability of reserves as they age, so the increase in nutrients from ash could have favored old seeds more than the young. On the other hand, internal reserves of small seeds are minor compared with the reserves of large seeds (Hanley & Fenner 1997). *Rumex acetosella* seeds are among the smallest of the community (mean seed weight: 0.5 mg vs. seed weight range of the species in the community: 0.012 to 165 mg); and among the smallest within the perennial species (mean seed weight: 9.8 mg) (Gonzalez & Ghermandi 2004). A greater efficiency in the capture and use of resources by small-seeded species (Burke & Grime 1996) suggests that immediately after fire, the establishment of *R. acetosella* could be disproportionately benefited by ash derived nutrients compared to other, larger seeds. Unexpectedly, the charcoal solution diminished the growth of seedlings at greater seed age. Gundale and DeLuca (2007) found that charcoal generated in the laboratory at a low temperature diminished growth of young plants of *Koeleria macrantha*, a perennial grass species. Nevertheless, under similar experimental conditions, they found that natural charcoal collected from a wildfire showed a positive effect on this species growth. The authors suggested that laboratory-generated charcoal may not adequately represent natural charcoal since the latter may have been generated under different conditions and later exposed to several environmental factors, which could influence its chemical and physical properties. Further studies that consider these aspects are required to understand the effect of charcoal on the studied species.

Seedling vigor can be considered an indirect indicator of seed deterioration (Dell'Aquilla 1987, Newton *et al*. 2006). The poor development of seedlings from seeds >8 years old, and the decrease in the height of seedlings that came from the oldest studied seeds may indicate seed deterioration due to the aging process. Aging occurs gradually and negatively affects the integrity of the protein synthesis system (Dell'Aquila 1987), which in turn affects the biochemical processes necessary for germination (Dell'Aquila 1994) and seedling development (Newton *et al*. 2006). Although old seeds of *R. acetosella* can show relatively high percentages of germination (e.g. 19-year-old seeds: 38% ± 9.7, mean ± SE; Franzese & Ghermandi 2011), seedlings which developed from these seeds lacked vigor, which would prevent their establishment under field conditions. This suggests that the relationship between seed longevity and seed germination does not necessarily reflect the potential establishment of a species, and that seedling growth is an important factor in evaluating the potential for seedling establishment.

Our results suggest that fire affects demographic responses of *R. acetosella* seedling populations, which are mediated in part by seed age. The diverse effects of fire-related abiotic factors on early growth of seedlings are likely to influence their competitive abilities during post-fire establishment (Blank & Young 1998; Sparg *et al*. 2005), even though differences in size between neighboring plants could be established for only a short period (Hanley *et al*. 2001). Abundant post-fire recruitment of *R. acetosella* could be partially caused by the nutrient input from ash that positively impacts the early seedling growth. Fire intensity would, however, also be an important factor in determining the potential establishment from the soil seed bank.

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