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RESEARCH PAPER

Geographic variation in the flood-induced fluctuating temperature requirement for germination in *Setaria parviflora* seeds

F. P. O. Mollard & P. Insausti

IFEVA-CONICET, Facultad de Agronomía, Universidad de Buenos Aires, Buenos Aires, Argentina

Keywords

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Correspondence

F. P. O. Mollard, IFEVA-CONICET, Facultad de Agronomía, Universidad de Buenos Aires, Av. San Martín 4453, C1417DSE, Buenos Aires, Argentina.
E-mail: fmollard@ifeva.edu.ar

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ABSTRACT

Our aim was to search for specific seed germinative strategies related to flooding escape in *Setaria parviflora*, a common species across the Americas. For this purpose, we investigated induction after floods, in relation to fluctuating temperature requirements for germination in seeds from mountain, floodplain and successional grasslands. A laboratory experiment was conducted in which seeds were imbibed or immersed in water at 5 °C. Seeds were also buried in flood-prone and upland grasslands and exhumed during the flooding season. Additionally, seeds were buried in flooded or drained grassland mesocosms. Germination of exhumed seeds was assayed at 25 °C or at 20 °C/30 °C in the dark or in the presence of red light pulses. After submergence or soil flooding, a high fraction (>32%) of seeds from the floodplain required fluctuating temperatures to germinate. In contrast, seeds from the mountains showed maximum differences in germination between fluctuating and constant temperature treatment only after imbibition (35%) or in non-flooded soil conditions (40%). The fluctuating temperature requirement was not clearly related to the foregoing conditions in the successional grassland seeds. Maximum germination could also be attained with red light pulses to seeds from mountain and successional grasslands. Results show that the fluctuating temperature requirement might help floodplain seeds to germinate after floods, indicating a unique feature of the dormancy of *S. parviflora* seeds from floodplains, which suggests an adaptive advantage aimed at postponing emergence during inundation periods. In contrast, the fluctuating temperature required for germination among seeds from mountain and successional grasslands show its importance for gap detection.

INTRODUCTION

Seedlings of species that have a broad ecological distribution are subject to different mortality factors along the environmental range where they are found (Vandvik & Vange 2003). Therefore, natural selection may benefit or hinder the development of different features of young individuals in diverse habitats. Some of these features are associated with the dynamics of seed dormancy and its effect on the timing of seed germination (Masuda & Washitani 1992; Meyer *et al.* 1997; Vandvik & Vange 2003; Donohue *et al.* 2005). An appropriate germination response to environmental signals is the first requirement for a plant to successfully establish in a location (Donohue *et al.* 2005). Thus, study of the relationship between the dynamics of seed dormancy of different populations in contrasting habitats may identify survival strategies of individuals. With respect to flood-prone and wetland habitats, there are insufficiently data available to indicate which features associated with seed dormancy could have adaptive value (Davy *et al.* 1990).

Seeds of grasslands species are capable of germinating in a wide range of habitats and conditions (Vandvik & Vange 2003) and many environmental requirements are known to lift or break their dormancy (Simpson 1990; Baskin & Baskin 1998). On the other hand, seedlings of wetland species generally emerge under a more narrow range of conditions (Moore & Keddy 1988; Bakker *et al.* 2007). In fact, seeds of wetland species more often require fluctuating temperatures for germination, compared to species from other habitats, including grasslands (Thompson & Grime 1983). In seeds of wetland species, sensitivity to fluctuating temperatures is part of a mechanism that would allow them to detect a decrease in water depth, *i.e.*, temperature fluctuation increases as water depth decreases (Thompson & Grime 1983; Pons & Schroder 1986; Ekstam & Forseby 1999). This enables wetland seeds to germinate and emerge after flooding recedes, a period in which high survival is expected due to the lack of oxygen deprivation for seedlings. Furthermore, changes in the sensitivity of seeds to factors that can promote seed germination are associated with changes in dormancy levels (Benech-Arnold *et al.* 2000; Finch-Savage & Leubner-Metzger 2006). So, the different soil conditions experienced by seeds modify their dormancy level and change their germinative response to environmental signals (Batlla *et al.* 2007; Mollard *et al.* 2007). For instance, seeds of the grass *Setaria parviflora* (Poir.) Kerguélen from a flood-prone grassland experience, after submergence, secondary dormancy and require fluctuating temperatures for germination (Mollard *et al.* 2007). The background presented so far suggests that the induction of a requirement for temperature fluctuations for germination is of critical importance in setting germination timing of wetland species and might be subject to high selection pressure.

In adult plants, floods were shown to exert selective pressure that favoured the appearance of locally adapted populations of different species (Keeley 1979; Davy *et al.* 1990; Huber *et al.* 2009; Mollard *et al.* 2010). Take this finding into account – and due to the abovementioned differences in germination behaviour among seeds harvested from wetland and grassland species – a species such as *S parviflora* that grows both in floodplains and in non-flooded grasslands might have locally adapted populations with different seed dormancy and germination responses to environmental cues.

In spite of the expected differences between populations of *S. parviflora* from different habitats, the patterns of dormancy response to submergence are broadly similar when seeds from a flood-prone habitat are compared with those of a parapatric population in an upland grassland (Mollard *et al.* 2007). The lack of substantial germinative differences between populations may be due to the proximity of the flooded and non-flooded areas and, consequently, there could be a high degree of seed dispersal and cross-pollination that reduces the effects of divergent selection. *S. parviflora* can also be found in distant allopatric populations situated in landscapes where flooding does not occur over a large part of the total area, *i.e.*, mountain grasslands and agroecosystems. Therefore, these populations of *S. parviflora* provide suitable tools for probing geographic variation in seed germinative responses to floods.

Setaria parviflora (Poir.) Kerguélen is a perennial grass species that grows across the Americas in different ecosystems subjected to frequent disturbances (Dekker 2000; Pensiero *et al.* 2005). *S. parviflora* is a preferentially allogamous species with the ability to produce viable seeds through self-fertilisation (Pensiero *et al.* 2005); its ripe fruits are dispersed at the end of summer and form large soil seed banks (D'Angela *et al.* 1988). Germination on floodplains takes place in late spring or summer, after the flooding period during winter or early spring. The aim of this study is to determine the existence of geographic variations in the germinative responses of *S. parviflora* seeds to floods and, consequently, the strategies used by particular seed populations of this widespread species to escape flooding. The hypothesis is that the dormancy of *S. parviflora* seeds harvested from allopatric populations is expressed differently after seeds are exposed to floods and that the differences can be related to their habitat: *i.e.*, floods can induce fluctuating temperature requirements for germination in seeds of *S. parviflora* from floodplain grasslands but not in those from mountain or successional, non-flooded grasslands.

MATERIAL AND METHODS

Sites description

In March 2004 and March 2006, *S. parviflora* ripe fruits (hereinafter referred to as seeds) were harvested from grasslands located in three different regions of Argentina (Fig. 1; Table 1):

1 Mountain grassland (Sierras Pampeanas region): a subhumid temperate, non-flooded natural grassland, dominated by tussock grasses that grow in a shallow, thick-textured soil over a granitic substrate.

Table 1. Characteristics of the habitats of grasslands types where *Setaria parviflora* seeds were harvested.

	elevation (m a.s.l.)	soil	mean temperature (°C)		precipitation (mm)
			January	July	
mountain grassland	1030	litic ustortents	22.2	10.1	825
floodplain grassland	27	typic natraquoll	21.9	8.1	1024
successional grassland	88	typic argiudoll	23.3	9.2	1021

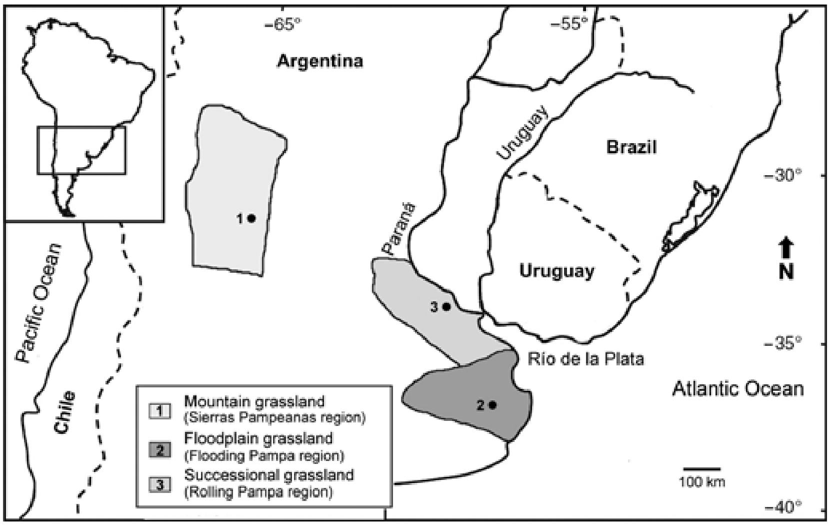

Fig. 1. Populations from which *Setaria parviflora* seeds were harvested.

2 Floodplain grassland (Flooding Pampa region): a humid temperate natural grassland within the River Salado Basin that experiences temporary floods due to its clayey soils with poor drainage and flat relief.

3 Successional grassland (Rolling Pampa region): a humid to sub-humid temperate, non-flooded grassland associated with soils having good drainage. This grassland belongs to a late post-agricultural secondary succession. In the entire region, the original vegetation was replaced for crop production so there are no longer any natural grasslands.

All sites have contrasting soil water regimes due to their topographic and soil characteristics (Table 1).

Seed treatments

Seeds harvested in 2004 for the laboratory experiment were stored under dry and dark conditions (20 °C) for 3 months before use. Dry conditions during storage ensure the attenuation of primary dormancy through the after-ripening process (Baskin & Baskin 1998). For the field and mesocosm experiments, the seeds were harvested in March 2006 and stored dry under dark conditions at −10 °C until June 2006, when they were used in experiments. Dry conditions and freezing temperatures during storage ensured the maintenance of primary dormancy of seeds. In field and mesocosm experiments, conditions that favour the attenuation of primary dormancy through the after-ripening process occurred in the soil while seeds were buried.

S. parviflora seeds were subjected to laboratory, field and mesocosm pretreatments. After all pretreatments, seed germination was tested under laboratory controlled conditions.

Laboratory pretreatments

Seeds from each population (n = 3) of *S. parviflora* were placed in groups of 200 (n = 4) in plastic boxes of 15 × 10 × 6 cm, lined with cotton wool covered in white absorbent paper soaked in distilled water, or were immersed below 5 cm of distilled water in the same type of box. The boxes were wrapped in opaque black polyethylene to keep the light out and were kept in an incubator at 5 °C for 2 months to simulate temperature conditions in the grassland soil during winter.

Field pretreatments

S. parviflora seeds were buried in the soil of two grasslands sites (used as common gardens): (i) a non-flooded upland grassland with well-drained soils (soil type: thapto-argic Hapludoll); and (ii) a flood-prone lowland grassland with poorly-drained soil and flat relief (soil type: typic Natraquoll). In June 2006, 200 seeds (n = 6) per population were placed in polyester open-weave bags (10 × 8 cm, 500 μm opening) and were buried at a depth of 3 cm at each site (n = 3) inside fenced exclosures to keep out large herbivores. Six holes of 10 cm in diameter were dug, disturbing the vegetation as little as possible. One bag per population was buried inside each hole and then attached to a landmark and the holes were covered with soil. The seeds were exhumed at night in the dark during the flooding period in the lowlands (24 August 2006). Immediately after exhumation, the bags were covered with black polyethylene and taken to the laboratory for germination experiments.

The soil volumetric water content of each experimental site was measured with ECHO Probe EC-10 sensors (Decagon Devices, Pullman, WA, USA) installed in Campbell 21-X data-loggers (Campbell Scientific, Logan, UT, USA). The occurrence of floods was recorded using the same sensors situated vertically in contact with the soil to detect the presence of water. The data-logger from the upland site stopped working on 6 August 2006 so, during the following period, two measures of volumetric soil water content were made with a Theta Probe ML2X (Delta–T Devices Ltd., Cambridge, UK).

Mesocosm pretreatments

Experiments with flooding under field conditions are difficult to carry out due to annual variability of the extent and duration of floods and the challenge of maintaining simultaneous controls (non-flooded situation). Therefore, we used grassland mesocosms to analyse the impact of soil flooding on the dormancy level of *S. parviflora* seeds and their requirement for environmental signals to germinate. In June 2006 groups (n = 3) of 200 seeds from each of the three different populations were placed in polyester open-weave bags (10 × 8 cm, 500 μm opening) and buried 3-cm deep in the soil of eight previously marked sites in the flood-prone lowland grassland. In July, eight mesocosms (20 × 20 × 25 cm) with intact vegetation containing the seed bags were dug up, placed in containers and taken to the experimental garden. For 20 days the mesocosms received a flooding treatment (n = 4), applied following a randomised design, and a control (n = 4). The treatment consisted of flooding the mesocosms and maintaining a water level of 2 cm above the soil surface for 20 days. The control mesocosms were watered daily and allowed to drain freely. At the end of the experimental period, the seeds bags were exhumed at night in the dark and taken to the laboratory for germination studies.

Germination treatments

Seeds of *S. parviflora* were placed on filter paper saturated with distilled water in transparent crystal polystyrene boxes (6 × 7 × 1 cm). The seeds harvested in 2004 received the following light treatments: (i) R: red light pulses for 20 min per day (R/FR = 4.09, irradiance = 18 $\mu mol \cdot m^{-2} \cdot s^{-1}$, FR = far-red light), provided by two Philips fluorescent TL 40W/15 tubes covered in red acetate; and (ii) darkness (control). The light treatments were combined with a treatment of fluctuating temperatures 20/30 °C (9/15 h day^{-1}), the R light treatment was performed during the high temperature phase. The control had a constant temperature of 25 °C. Germination boxes (n = 4 per treatment) had 25 seeds each. The seeds harvested in 2006 (field and mesocosm experiments) were subjected to the same treatments, with exception of the combination of fluctuating temperature and R light pulses. After each irradiation period, the boxes were covered with opaque black polyethylene. Seeds were rapidly distributed within the boxes under green light of very low intensity to avoid reaction of the seed photoreceptors with light. Dim green light was supplied from a 20 W white fluorescent tube wrapped in green acetate. Germination was recorded after 10 days of incubation under the different treatments. The 10-day period before the final germination count ensured that the response of seeds and the germination percentages were closely related to current dormancy level and not to other unexpected processes that could affect imbibed seeds over longer periods.

To calculate germination percentages, dead seeds were subtracted from the total number of seeds per box. For each replicate, seeds were checked to determine if they were dead or alive by gauging the hardness of the seeds using histology tweezers after the final germination count. Additionally, at the same time as germination treatments were set, a group of 25 seeds subjected to the same pretreatments was used for tetrazolium chloride tests to study potential seed viability [1% solution of 2,3,5-triphenyltetrazolium chloride (w/v) for 24 h in the dark at 25 °C]. The germination percentages were transformed using the arc sine formula $\sqrt{x}$ and were analysed with analysis of variance. For the laboratory controlled experiment, differences in response to germination treatments among seed populations were tested by studying triple and double interactions between populations; temperature and light treatments were evaluated using a three-way analysis of variance. For field and mesocosms experiments, the interaction between population and germination treatments was analysed with two-way analysis of variance. Multiple comparisons were performed with Tukey tests ($P < 0.05$; Sokal & Rohlf 1969).

RESULTS

Laboratory experiment

After the submergence period in water, *S. parviflora* seeds of the three populations showed differences in germination percentage in response to temperature treatments in controlled unsubmerged conditions (population × temperature: $P < 0.001$; seeds submerged; Fig. 2a, c, e). After water immersion, a high percentage of the mountain grassland seeds germinated and there were no significant differences between treatments (Fig. 2a). In contrast, seeds from the floodplain grassland had a significantly lower germination percentage at constant temperature than at fluctuating temperatures (Fig. 2c). Seeds from the successional grassland showed a high germination after immersion in all the treatments, except at a constant temperature in darkness (Fig. 2e). The different populations of *S. parviflora*, which had been incubated on water-saturated filter paper, had different germination percentages when exposed to temperature treatments (population × temperature: $P < 0.026$; seeds imbibed; Fig. 2b,d,f). Seeds from the mountain grassland showed high germination percentages at fluctuating temperatures, while germination was significantly lower at constant temperature (Fig. 2b). In turn, seeds from the floodplain grassland had high germination in all treatments (Fig. 2d). The percentage germination of seeds from the successional grassland was high in all treatments, except at constant temperature in darkness (Fig. 2f). Viability of the seeds after the laboratory experiment remained high, between 80% and 92%, for all populations and pretreatments.

Field burial experiment

During the time seeds were buried, soil in the flood-prone grassland was flooded for nearly 22 days during the winter, whereas no flooding occurred in the upland (Fig. 3). The germination of seed populations exhumed from flooded grassland varied greatly in response to treatment (population × treatment: $P < 0.001$; Fig. 4a,c,e). Fluctuating temperatures had a favourable effect on all seed populations when compared to controls at constant temperature in darkness (Fig. 4a,c,e). However, seeds from the mountain and

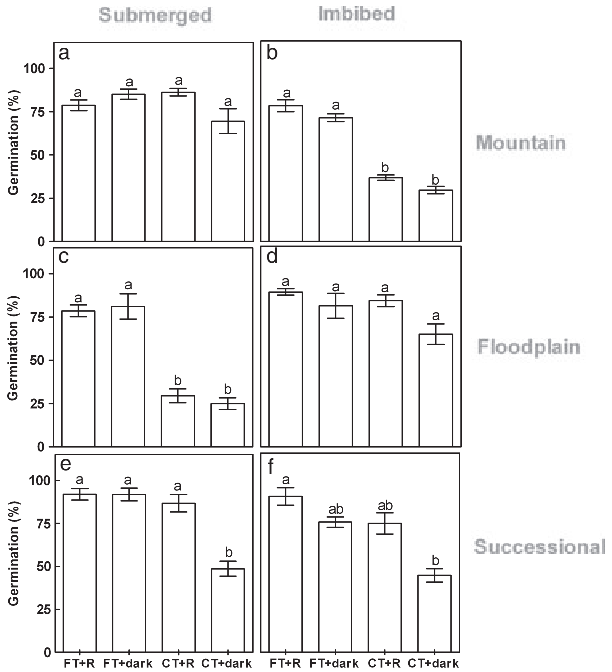

Fig. 2. Germination (%) under temperature and light treatments of *Setaria parviflora* seeds previously submerged in water (a, c, e) or imbibed (b, d, f) for 60 days at 5 °C. Seeds harvested in mountain (a, b), floodplain (c, d) or successional (e, f) grasslands. FT, fluctuating temperature (20–30 °C), CT, continuous temperature (25 °C), R, daily light pulses with high R/FR (Red/Far-Red) light, dark, darkness. Values are means ± SE (n = 4). Different letters indicate significant differences ($P < 0.05$).

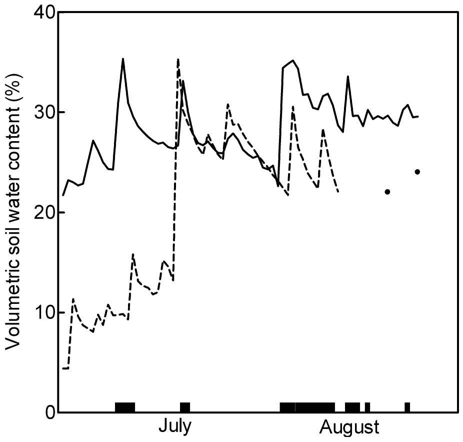

Fig. 3. Soil volumetric water content (%) time courses in the flood-prone lowland (continuous line) or in the nearby non-flooded upland (dotted line) grasslands where seeds were buried. Points indicate the measurements of soil volumetric water content in the upland grassland after the datalogger stopped working. The horizontal line above the *X*-axis shows flooding periods in the flood-prone lowland grassland.

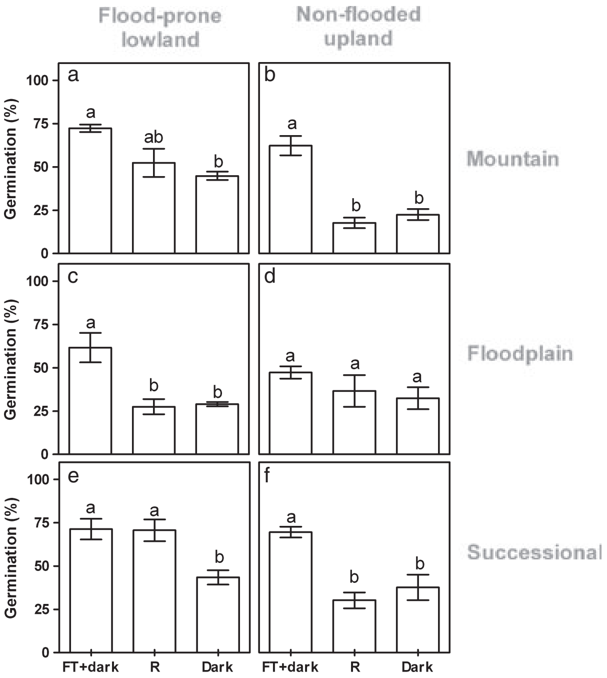

Fig. 4. Germination (%) of *Setaria parviflora* seeds exhumed in late winter during the flooding period. Seeds were buried in a flood-prone lowland (a, c, e) or in a nearby non-flooded upland grassland (b, d, f). Seeds were harvested in mountain (a, b), floodplain (c, d) or successional (e, f) grasslands. FT, fluctuating temperature (20–30 °C) (other treatments were at continuous 25 °C), R, daily light pulses with high R/FR, dark, darkness. Values are means ± SE (n = 4). Different letters indicate significant differences ($P < 0.05$).

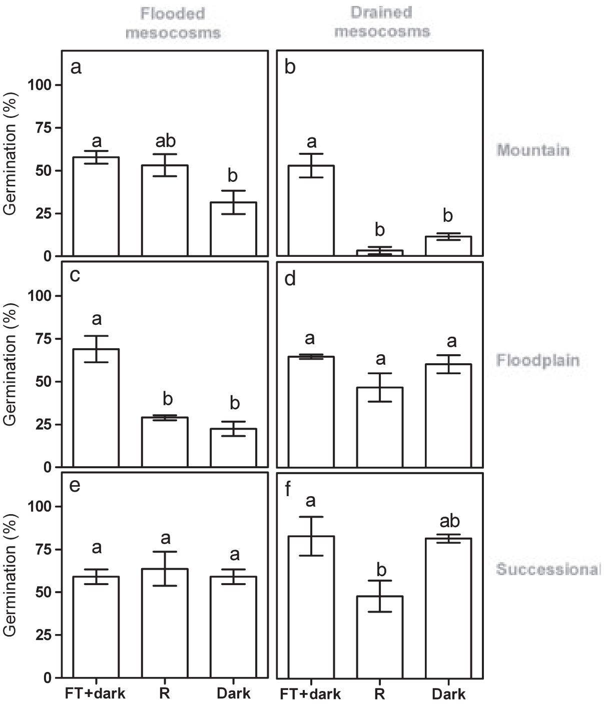

Fig. 5. Germination (%) of *Setaria parviflora* seeds exhumed from mesocosms. Seeds were buried in flooded (a, c, e) or drained mesocosms (b, d, f). Seeds harvested in mountain (a, b), lowland (c, d) or successional (e, f) grasslands. FT, fluctuating temperature (20–30 °C) (other treatments were at continuous 25 °C), R, light with high R/FR, dark, darkness. Values are means ± SE (n = 4). Different letters indicate significant differences ($P < 0.05$).

successional grasslands also had high germination percentages at constant temperature after irradiation with red light pulses (Fig. 4a, e). In fact, in both populations, there were not statistical differences in germination between seeds subjected to fluctuating temperatures or daily red light pulses ($P > 0.05$; Fig. 4a, e). This was not the case among seeds from the floodplain grassland because an important fraction of the population was only able to germinate at fluctuating temperatures (Fig. 4c). The seed populations exhumed from the upland grassland also showed differences in germination in response to treatment (population × treatment: $P < 0.008$; Fig. 4b, d, f). Seeds from both the mountain and successional grassland germinated more in response to fluctuating than constant temperatures (Fig. 4b, f). On the other hand, seeds from the floodplain grassland did not show significant differences in germination percentages between treatments (Fig. 4d). After exhumation from both flood-prone and upland grasslands, seeds from the mountain grassland maintained high viability (>90%) while viabilities of seeds from the floodplain and successional grasslands were between 72% and 80%.

Mesocosm experiment

Seed populations of *Setaria parviflora* exhumed from flooded and drained mesocosms showed differences in germination responses to treatments (population × treatment: $P < 0.001$ in both flooded and drained mesocosms; Fig. 5). Seeds from the mountain grassland exhumed from flooded mesocosms showed a significant effect of fluctuating temperatures on germination (Fig. 5a). However, similar high germination percentages could also be reached using red light pulses at constant temperature ($P > 0.05$; Fig. 5a). In contrast, an important fraction of seeds from the floodplain grassland exhumed from flooded mesocosms were only able to germinate at fluctuating temperatures (Fig. 5c). In effect, seeds from the floodplain grassland had approximately 40% higher germination at fluctuating than at constant temperatures either in darkness or under daily red light pulses (Fig. 5c). Seeds from the successional grassland exhumed from flooded mesocosms showed no differences in germination between treatments (Fig. 5e). Seeds from the mountain grassland exhumed from drained mesocosms only reached high germination percentages when they were incubated at fluctuating temperatures (Fig. 5b). In contrast, there were no differences in germination percentages among seeds from the floodplain and successional grassland that were subjected to fluctuating and constant temperatures in darkness (Fig. 5d, f). After exhumation, from both flooded and drained mesocosms, seeds from the mountain grassland were 96% viable while viability of seeds from the floodplain and successional grasslands was between 56% and 68%.

DISCUSSION

Natural selection can produce geographic variations in germination behaviour among populations of the same species that live at considerable distances from one another (Meyer *et al.* 1997; Donohue *et al.* 2005) and even among populations from adjacent areas in contrasting habitats (Masuda & Washitani 1992; Pezzani & Montaña 2006). In a previous work we found that despite small quantitative differences, the patterns of germination after flooding were broadly similar between seeds of *S. parviflora* harvested from a non-flooded and a nearby flood-prone habitat within a floodplain (Mollard *et al.* 2007). In the present study, we demonstrate that there are conspicuous population differences in germination requirements that are related to flooding escape between seeds of *S. parviflora* from allopatric, well separated and different habitats. Hence, our results highlight some unique features of dormancy in *S. parviflora* seeds from floodplain grasslands that suggest an adaptive advantage aimed at postponing emergence during inundation. This behaviour was not present in *S. parviflora* seeds from non-flooded mountain and successional grasslands.

Seeds of many plant species from wetland and flood-prone habitats need a drawdown period for germination and successful seedling establishment (Lenssen *et al.* 1998; Nishihiro *et al.* 2004). For such plants, seed behaviours that ensure germination when sites are not flooded are considered highly adaptive (Nishihiro *et al.* 2004). Our results show that a considerable fraction of *S. parviflora* seeds from a floodplain grassland, after submergence or flooding, was highly dependent on fluctuating temperatures for germination. Sensitivity to fluctuating temperatures allows seeds from wetlands to detect a decrease in water depth (Thompson & Grime 1983; Pons & Schroder 1986; Ekstam & Forseby 1999). Therefore, the existence of this requirement in relation to floods could be an adaptation to avoid germination until the risk of seedling damage due to the flood conditions has passed.

Previous (Mollard *et al.* 2007) and present results tell us more about the ability of *S. parviflora* to colonise temporarily flooded communities. It is well known that in temperate regions seeds may loss their primary dormancy and enter secondary dormant/non-dormant cycles depending on soil temperature and other environmental factors during residence in the soil bank (Baskin & Baskin 1998; Finch-Savage & Leubner-Metzger 2006). In the huge Flooding Pampa region, inundation can induce both entrance into secondary dormancy of previously non-dormant *S. parviflora* seeds (Mollard *et al.* 2007) as well as release from primary dormancy of seeds of other dominant species of the floodplain (Insausti *et al.* 1995; Cornaglia *et al.* 2009). Our results show that the attenuation of primary dormancy can also act in flooded *S. parviflora* seeds from the floodplain to a level where a fluctuating temperature requirement is essential for germination in an important fraction of the population. Taken together, these results suggest that, in unpredictable floodplain soils, both primary dormancy attenuation and secondary dormancy induction in inundated conditions drive *S. parviflora* seeds to a physiological state in which specific signals associated with a drawdown period are required for germination. Populations of *S. parviflora* seeds from non-flooded habitat, mountain and successional grasslands had a lower requirement than seeds from the floodplain grassland to attain maximum germination after submergence or flooding. The absence or loss of specific requirements for germination during a season with frequent soil inundation suggests that there are no physiological obstacles that would prevent germination during such periods. Therefore, our results indicate that *S. parviflora* seeds from mountain and successional grasslands would retain the ability to germinate during flooding, a behaviour that could lead to the death of emerging seedlings. Fluctuating temperatures also increased germination percentages of seeds from mountain and successional grassland, but this effect was not related to previous immersion or flooded conditions. Moreover, similar germination percentages could also be reached using red light pulses, a mechanism for gap detection that proved as good as fluctuating temperature. In this regard, the redundancy of particular light and temperature signals has been shown to be crucial for germination in grassland seeds (Insausti *et al.* 1995; Insausti & Grimoldi 2006). Similarly, the effect of fluctuating temperatures on germination of seeds from mountain and successional grassland is related to the importance of such temperatures for a gap detection mechanism.

Oxygen plays a key role in the germination metabolism of *Setaria* spp. (Dekker 2000) and also interacts with some dormancy release factors such as light (Corbineau & Côme 1995). In effect, in some species, the acquisition and maintenance of seed sensitivity to light depends on oxygen availability (Corbineau & Côme 1995). Besides, considering that oxygen depletion is one of the first and most consistent outcomes of soil flooding, different responses to light between seeds subjected to this treatment and those in drained conditions were expected (Insausti *et al.* 1995; Baskin *et al.* 2000). Our results show that all seed populations submerged or exhumed after flooding conditions had more similar responses to light than seeds subjected to non-flood conditions. Since the presence of nitrates partly regulated the *S. parviflora* seed light response (Mollard & Insausti 2009), the interaction of light with both the soil gaseous environment and soil chemistry deserves further population comparative studies.

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REFERENCES

Bakker C., van Bodegom P.M., Nelissen H.J.M., Aerts R., Ernst W.H.O. (2007) Preference of wet dune species for waterlogged conditions can be explained by adaptations and specific recruitment requirements. *Aquatic Botany*, **86**, 37–45.

Baskin C.C., Baskin J.M. (1998) *Seeds: ecology, biogeography and evolution of dormancy and germination*. Academic Press, San Diego, CA, USA.

Baskin C.C., Baskin J.M., Chester E.W. (2000) Effect of flooding on the annual dormancy cycle and on germination of seeds of the summer annual *Schoenoplectus purshianus* (Cyperaceae). *Aquatic Botany*, **67**, 109–116.

Batlla D., Nicoletta M., Benech-Arnold R.L. (2007) Sensitivity of *Polygonum aviculare* seeds to light as affected by soil moisture conditions. *Annals of Botany*, **99**, 915–924.

Benech-Arnold R.L., Sánchez R.A., Forcella F., Kruk B., Ghersa C.M. (2000) Environmental control of dormancy in weed soil seed banks. *Field Crops Research*, **67**, 105–122.

Corbineau F., Côme D. (1995) Control of seed germination and dormancy by the gaseous environment. In: Kigel J., Galili G. (Eds), *Seed development and germination*. Marcel Dekker, New York, NY, USA, pp 397–424.

Cornaglia P.S., Schrauf G.E., Deregibus V.A. (2009) Flooding and grazing promote germination and seedling establishment in the perennial grass *Paspalum dilatatum*. *Austral Ecology*, **34**, 343–350.

D'Angela E., Facelli J.M., Jacobo E. (1988) The role of the permanent soil seed bank in early stages of a post-agricultural succession in the Inland Pampa, Argentina. *Vegetatio*, **74**, 39–45.

Davy A.J., Noble S.M., Oliver R.P. (1990) Genetic variation and adaptation to flooding in plants. *Aquatic Botany*, **38**, 91–108.

Dekker J. (2000) Emergent weedy foxtail (*Setaria* spp.) seed germinability behaviour. In: Black M., Bradford K.J., Vásquez-Ramos J. (Eds), *Seed biology: advances and applications*. CAB International, Wallingford, UK, pp 411–423.

Donohue K., Dorn L., Griffith C., Kim E., Aguilera A., Polisetty C.R., Schmitt J. (2005) The evolutionary ecology of seed germination of *Arabidopsis thaliana*: variable natural selection on germination timing. *Evolution*, **59**, 758–770.

Ekstam B., Forseby Å. (1999) Germination response of *Phragmites australis* and *Typha latifolia* to diurnal fluctuations in temperature. *Seed Science Research*, **9**, 157–163.

Finch-Savage W.E., Leubner-Metzger G. (2006) Seed dormancy and the control of germination. *New Phytologist*, **171**, 501–523.

Huber H., Jacobs E., Visser E.J.W. (2009) Variation in flooding-induced morphological traits in natural populations of white clover (*Trifolium repens*) and their effects on plant performance during soil flooding. *Annals of Botany*, **103**, 377–386.

Insausti P., Grimoldi A.A. (2006) Gap disturbance triggers the recolonization of the clonal plant *Ambrosia tenuifolia* in a flooding grassland of Argentina. *Austral Ecology*, **31**, 828–836.

Insausti P., Soriano A., Sanchez R.A. (1995) Effects of flood-influenced factors on seed germination of *Ambrosia tenuifolia*. *Oecologia*, **103**, 127–132.

Keeley J.E. (1979) Population differentiation along a flood frequency gradient – physiological adaptations to flooding in *Nyssa sylvatica*. *Ecological Monographs*, **49**, 89–108.

Lenssen J.P.M., Ten Dolle G.E., Blom C.W.P.M. (1998) The effect of flooding on the recruitment of reed marsh and tall forb plant species. *Plant Ecology*, **139**, 13–23.

Masuda M., Washitani I. (1992) Differentiation of spring emerging and autumn emerging ecotypes in *Galium spurium* L. var. *echinospermon*. *Oecologia*, **89**, 42–46.

Meyer S.E., Allen P.S., Beckstead J. (1997) Seed germination regulation in *Bromus tectorum* (Poaceae) and its ecological significance. *Oikos*, **78**, 475–485.

Mollard F., Insausti P. (2009) Breaking *Setaria parviflora* seed dormancy by nitrates and light is part of a mechanism that detects a drawdown period after flooding. *Aquatic Botany*, **91**, 57–60.

Mollard F., Insausti P., Sánchez R.A. (2007) Flooding induces secondary dormancy in *Setaria parviflora* seeds. *Seed Science Research*, **17**, 55–62.

Mollard F.P.O., Striker G.G. Polschuk E.L. Insausti P. (2010) Subtle topographical differences along a floodplain promote different plant strategies among *Paspalum dilatatum* subspecies and populations. *Austral Ecology*, **35**, 189–196.

Moore D.R.J., Keddy P.A. (1988) Effects of a water depth gradient on the germination of lakeshore plants. *Canadian Journal of Botany*, **66**, 548–552.

Nishihiro J., Araki S., Fujiwara N., Washitani I. (2004) Germination characteristics of lakeshore plants under an artificially stabilized water regime. *Aquatic Botany*, **79**, 333–343.

Pensiero J.F., Gutiérrez H.F., Exner E. (2005) Sistema de polinización y su efecto sobre la producción y el peso de semillas en nueve especies sudamericanas del género *Setaria*. *Interciencia*, **30**, 495–500.

Pezzani F., Montaña C. (2006) Inter- and intraspecific variation in the germination response to light quality and scarification in grasses growing in two-phase mosaics of the Chihuahuan Desert. *Annals of Botany*, **97**, 1063–1071.

Pons T.L., Schroder H.F.J.M. (1986) Significance of temperature fluctuation and oxygen concentration for germination of the rice field weeds *Fimbristylis littoralis* and *Scirpus juncoides*. *Oecologia*, **68**, 315–319.

Simpson G.M. (1990) *Seed dormancy in grasses*. Cambridge University Press, New York, NY, USA.

Sokal R.R., Rohlf F.J. (1969) *Biometry*. Freeman, San Francisco, CA, USA.

Thompson K., Grime J.P. (1983) A comparative study of germination responses to diurnally fluctuating temperatures. *Journal of Applied Ecology*, **20**, 141–156.

Vandvik V., Vange V. (2003) Germination ecology of the clonal herb *Knautia arvensis*: regeneration strategy and geographic variation. *Journal of Vegetation Science*, **14**, 591–600.