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Short communication

Germination requirements of two sheep-preferred grasses (*Hordeum comosum* and *Koeleria vurilochensis* var. *patagonica*) from semiarid Patagonian steppes

P.E. Gundel[a,c,*], J.G.N. Irisarri[b], N.S. Sorzoli[a], C.E. Mosso[a], G. García-Martínez[d], R. Golluscio[b]

[a] *IFEVA-Facultad de Agronomía (UBA)/CONICET, Cátedra de Ecología, Av. San Martín 4453 (C1417DSE), Buenos Aires, Argentina*
[b] *IFEVA-Facultad de Agronomía (UBA)/CONICET, Cátedra de Forrajicultura, Av. San Martín 4453 (C1417DSE), Buenos Aires, Argentina*
[c] *MTT Agrifood Research Finland, Plant Protection, 31600 Jokioinen, Finland*
[d] *INTA, EEA Esquel, AER Gobernador Costam, Estrada 850, (CP 9223) Chubut, Argentina*

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ABSTRACT

Grazing by domestic exotic herbivores on native vegetation in Patagonian steppes has led to the deterioration of forage resources, where grasses are replaced by shrubs, and preferred grasses by non-preferred ones. Therefore, attempts at breeding and regeneration should start by identifying species with potential forage capability. Two perennial high-preferred species are the focus of this paper: *Hordeum comosum* and *Koeleria vurilochensis*. For the first time, we studied their morphological traits, dormancy level, and their temperature and water germination requirements, through thermal- and hydrotime models. *H. comosum* seeds were solid, large and heavy, while *K. vurilochensis* seeds were soft, small and light. Neither light-quality nor alternating temperature affected the dormancy level in seeds of both species. Seeds of *H. comosum* and *K. vurilochensis* required, respectively, 768.74 °Ch and 2217.89 °Ch to germinate; alternatively, base temperature was higher for *H. comosum* than for *K. vurilochensis*. The hydrotime required by *H. comosum* was 26 MPah and 110 Mpah for *K. vurilochensis* but, base water potential was −0.99 MPa and −1.45 MPa, respectively. For all the conditions, *H. comosum* displayed a higher germination rate compared to *K. vurilochensis* seeds. Deeper knowledge on the morphological and germination traits of seeds is a key step toward breeding and restoration of forage potential species.

1. Introduction

Patagonia (Argentina) covers an extensive area of South America between 39° and 55° South latitude, and from the southern end of the Andes Mountains to the Atlantic Ocean (Soriano et al., 1983; León et al., 1998). Its arid and semiarid steppes are mainly devoted to sheep production for lamb and wool (Soriano et al., 1983). These production activities are based on continuous and extensive grazing directly on natural vegetation composed by shrubs, and non-preferred and preferred grasses. Since the introduction of exotic herbivores (mainly sheep) in the 19th century, vegetation has experienced changes in the relative cover of different functional types (Soriano et al., 1983; Perelman et al., 1997; Bertiller and Bisigato, 1998). Replacements of grasses by shrubs, and preferred grasses by non-preferred ones are some of the most important recorded changes (Aguiar and Sala, 1998; Bertiller and Bisigato, 1998).

Mainly controlled by temperature and water (Allen et al., 2000; Bradford, 2002), seed germination is a critical step in the process of seedling recruitment of populations (Arnott, 1969; Baskin and Baskin, 1998; Benech-Arnold and Sánchez, 2004). Water availability for germination may depend not only on rainfall but also on soil characteristics (Bertiller et al., 1995; Defossé et al., 1997; Rotundo et al., 2006; Cipriotti et al., 2008). In Patagonia, small rainfall (or snow) events occur in late-autumn, winter and early spring; however, germinated seeds will face different constraints depending on whether they germinate in autumn or in spring, periods in which adequate soil humidity overlaps with relatively high temperatures (Soriano, 1960; Bertiller, 1992; Defossé et al., 1997; Cipriotti et al., 2008). Although early germination means a significant reduction in the seed bank for most grasses, some seeds may persist until next autumn, increasing their probability of successful establishment (Bertiller, 1992). Higher likelihood of survival has been observed for seeds that germinated in autumn, suggesting that winter cold temperatures are not as threatening as summer high temperatures are for seeds germinated in spring (Bertiller, 1992; Defossé et al., 1997). Seed germination in a safe-site and favorable time will depend not only on functional traits governing their response to temperature and water availability but

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* Corresponding author. IFEVA-CONICET, Cátedra de Ecología, Facultad de Agronomía, Universidad de Buenos Aires. Av. San Martín 4453, Buenos Aires (C1417DSE), Argentina. Tel.: +54 11 45248070x8125; fax: +54 11 45148730.
E-mail address: gundel@agro.uba.ar (P.E. Gundel).

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also on morphological traits, such as size/hardness and dormancy mechanisms (Arnott, 1969; Baskin and Baskin, 1998; Allen et al., 2000; Bradford, 2002; Benech-Arnold and Sánchez, 2004). Therefore, perspectives for breeding and regeneration of wild native grass species for forage use should start by examining morphological and functional aspects of target species seeds (Soriano, 1960; Bertiller, 1992; Cipriotti et al., 2008).

In this work, we focused on two sheep-preferred species: *Hordeum comosum* and *Koeleria vurilochensis* var. *patagonica* (hereafter: *H. comosum* and *K. vurilochensis*). While the former is common in plant communities of the Occidental and Sub-Andean Districts, where annual precipitation ranges from 300 to 600 mm, the latter is majorly found in the Sub-Andean District of West Patagonia and the ecotone with the Occidental district (Bertiller, 1992; Bertiller and Bisigato, 1998; León et al., 1998) where annual precipitation ranges from 600 to 800 mm (Jobbágy et al., 1995). Although information on the ecology of both species is sparse (but see Soriano, 1960, and Bertiller, 1992), the negative relationships between their relative cover and grazing pressure indicate that both are highly preferred by sheep (León and Aguiar, 1985; Perelman et al., 1997). We are presenting a morphological characterization of seeds from both species, and a basic experimental study to determine general level of dormancy, and seed germination requirements of temperature and water. To address this last goal, actual data of germination response to temperature and water were described by means of the thermal-time and hydrotime models, respectively. These population-based models have been found to be more than merely another way of describing germination time course data, since their parameters have proven to have physiological basis and ecological significance (Allen et al., 2000; Bradford, 2002).

2. Materials and methods

Seeds of both species were collected in January 2009 from three sites in North-Western Patagonia [Bariloche (−41° 7′ S Lat, −71° 17′ W Long), Montoso (−42° 26′ S Lat, −71°5′ W Long), and Tecka (−43° 29′ S Lat, −70° 49′ W Long)] and another one from a southern position [Río Mayo (−45° 40′ S Lat, −70° 15′ W Long)] in which only *H. comosum* was found. All sites have a modest stocking rate and are subjected to controlled grazing systems (Golluscio et al., 1998). The material was brought to the laboratory for threshing, and the amount of seeds was evaluated. Due to insufficient seeds from some sites, seeds were pooled by species. The seeds were stored in dry condition at 10 °C until they were used for the experiments three months later. A set of evaluations and experiments was carried out to obtain information on morphological and functional traits.

Morphological traits (length, width and height) were measured on 50 seeds per species using a caliper. 1000-seed weight (g) was estimated on three groups of 20 seeds per species. Seeds were subjected to a set of dormancy release treatments. Thirty seeds were sown in Petri dishes on a filter paper with 5 mL distilled water, and incubated for 4 h at 20 °C. After this pre-treatment, 3 dishes were randomly assigned to one of the 4 treatments: incubation in the dark at constant 25 °C, red light pulse and incubation in the dark at 25 °C, far-red light pulse and incubation in the dark at 25 °C, and incubation in the dark at alternating temperature (20–30 °C, 12 h, day/night). Light quality treatments were 1 h of red pulse (R, 660 nm, 30 μmol m^{-2} s^{-1}) or far-red (FR, 730 nm, 17 μmol m^{-2} s^{-1}). Light and dark treatments were placed inside the same incubator, while alternating temperature treatment was carried out in another incubator. One week afterward, seed germination was recorded and expressed as percentage of the seeds sown per dish. Standard one-way ANOVA was performed using R software (R Development Core Team, 2011) for testing the effect of treatment within species; assumptions were achieved without data transformation.

2.1. Thermal-time model

Seeds were sown in Petri dishes (30 seeds each) on a filter paper with 5 mL of distilled water. Three dishes were assigned to one of the three incubation constant temperatures: 6, 15 and 25 °C. Selection of treatments was based on the fact that a linear relationship is predicted for the germination rate and temperature within the range of suboptimal temperatures (Bradford, 2002). Germination (radicle protrusion) was periodically recorded. Dynamics of germination in relation to incubation time was analyzed running the thermal-time model by means of repeated probit analysis. The thermal-time model has proven to produce good fits for the cumulative germination in relation to incubation temperature (Bradford, 2002). The foundations of the thermal-time model start with formula:

$$\theta_T(g) = [T - T_b]tg$$

where $\theta_T(g)$ is thermal-time (°Ch) for germinating a "g" seed fraction in the population, T is incubation temperature (°C), T_b is constant base temperature of the population, and tg is the time to germinate a "g" fraction of seeds. θ_T is assumed to vary normally, while T_b is assumed to remain constant, which means the threshold to get germination (Bradford, 2002).

2.2. Hydrotime model

Seeds were sown in Petri dishes (30 seeds each) on a filter paper moistened with 5 mL of distilled water or different solutions depending on the treatment. Three dishes were assigned to one of the 4 water potentials: 0, −0.5, −1.0 and −1.25 MPa. Selection of treatments was based on the fact that the lower the medium water potential, the lower the germination rate for a given seed fraction (Bradford, 2002). The water potentials were obtained with concentrations of polyethylene glycol (PEG-6000) solutions following Steuter et al. (1981). After 24 h, solutions were changed for fresh ones in every dish. Germination was recorded periodically and germination dynamics in relation to water availability was analyzed running the hydrotime model by means of probit analysis.

At a particular water potential (Ψ) in the germination medium, the hydrotime model has proven to have good fits for the cumulative germination in relation to imbibition time; the most important attribute is the derivation of physiologically-based parameters with ecological significance (Allen et al., 2000). The foundations of the hydrotime model start with formula:

$$\theta_H = [\Psi - \Psi b(g)]tg$$

where θ_H is constant hydrotime (MPah), Ψ is water potential (MPa) in the medium, $\Psi b(g)$ is base water potential for a "g" seed fraction in the population, and tg is the time to germinate the same "g". θ_H is assumed to remain constant among seeds, while $\Psi b(g)$ is assumed to vary normally. $\Psi b(g)$ means the threshold to get germination in a "g" (Bradford, 2002). Linear regression analyses between actual and predicted data for both species were used to evaluate the accuracy of the thermal-time and hydrotime models.

3. Results

While *H. comosum* seeds were solid, *K. vurilochensis* seeds presented a liquid/soft endosperm. In addition, *H. comosum* seeds were larger than *K. vurilochensis* seeds, although they had similar height. In comparison with *K. vurilochensis*, 1000-seed weight of

Table 1
Morphological and functional seed traits for the two Patagonian grasses. Morphological variables and germination levels (dormancy) are shown as mean values plus coefficients of variation [CV = (SD/mean)*100]. $\theta_T(50)$ is thermal-time for germinating 50% of seeds and $\sigma_{\theta T}$ is its standard deviation, while T_b is the constant base temperature. θ_H is the constant hydrotime while $\Psi_b(50)$ is the base water potential for 50% of seeds and $\sigma_{\Psi b}$ is its standard deviation.

Seed trait	Grass species	
	Hordeum comosum	*Koeleria vurilochensis var. vurilochensis*
Morphological		
Length (mm)	5.21 (7.56)	2.88 (12.55)
Width (mm)	1.17 (9.56)	0.1 (<1.00)
Height (mm)	0.58 (18.03)	0.72 (18.71)
1000-seed weight (g)	2.86 (11.61)	0.34 (4.62)
Functional		
Dormancy level (%)		
Red light pulse	94.58 (7.99)	78.16 (15.56)
Far-red light pulse	98.04 (3.96)	81.24 (11.87)
Full darkness	90.22 (4.23)	72.65 (15.74)
Alternating temperature	90.22 (11.53)	79.09 (12.69)
Thermal-time parameters		
$\theta_T(50)$ (°Ch)	768.74	2217.89
T_b (°C)	−1.1	−4.8
$\sigma_{\theta T}$	1.85	1.26
Hydrotime parameters		
θ_H (MPah)	26	110
$\Psi_b(50)$ (MPa)	−0.99	−1.45
$\sigma_{\Psi b}$	0.31	0.38

H. comosum was 8.41 fold higher (Table 1). Overall dormancy was low for both species, as neither *H. comosum* ($F_3 = 0.81$, $P = 0.52$) nor *K. vurilochensis* ($F_3 = 0.34$, $P = 0.79$) were affected by any of the treatments (Table 1). *K. vurilochensis* showed a lower dormancy level and a higher viability in the general response (see CV in Table 1).

Dynamics of seed germination of *H. comosum* and *K. vurilochensis* in response to temperature was well described by the thermal-time models (R^2: 0,94, $P < 0{,}001$; and R^2: 0,97, $P < 0{,}001$, respectively). Accordingly, the thermal-time model provided cumulative seed germination curves that described actual data reasonably well (Fig. 1). The seed germination requirement expressed in thermal units was 768.74 °Ch and 2217.89 °Ch for *H. comosum* and for *K. vurilochensis*, respectively. On the contrary, the T_b was higher for *H. comosum* than for *K. vurilochensis* (−1.1 vs −4.8 °C). *H. comosum* seeds displayed a higher germination rate compared to *K. vurilochensis* seeds.

The fitted hydrotime models showed a very good description of seed germination dynamics in response to water availability for *H. comosum* (R^2: 0,99, $P < 0{,}001$) and *K. vurilochensis* (R^2: 0,98, $P < 0{,}001$). Both species differed in their water requirement to germinate. The θ_H was 26 MPah for *H. comosum* and 110 MPah for *K. vurilochensis*. The $\Psi_b(50)$ was −0.99 MPa for *H. comosum* and −1.45 MPa for *K. vurilochensis* (Table 1). Modeled curves of cumulative seed germination derived from the hydrotime model showed a general good fit to the actual data for both species under each water availability treatment. *K. vurilochensis* germination was more inhibited by water shortage than *H. comosum* germination (Fig. 2).

4. Discussion

Seed size and hardness are important characteristics to manage harvesting, conditioning and storage of seeds (Arnott, 1969; Leishman and Westoby, 1994; Benech-Arnold and Sánchez, 2004). In this regard, *H. comosum* seeds show better characteristics since their dimensions are not different from other commercial forage species (e.g., *Lolium perenne*; Arnott, 1969); therefore, their management could be easy. Alternatively, *K. vurilochensis* seeds are extremely light and very small, and they show a liquid/soft endosperm, a trait that has already been described for the genus (Terrell, 1971). Such attributes would make harvest and management difficult. Three months after harvesting, seeds did not show any specific response to factors related to release from or induction to dormancy. Compared to germination in the dark, neither alternating temperature/pulse of red light nor far-red light promoted or reduced final germination, respectively. Grass seeds can initially have primary dormancy and inducible secondary dormancy; however, they can have slight general level of dormancy (Soriano, 1960; Baskin and Baskin, 1998). Our results suggest that dormancy would not be an obstacle in either species, which is in accordance with results previously found for *H. comosum* (Soriano, 1960; Benech-Arnold and Sánchez, 2004). Under realistic ecological scenarios, seed size is associated with the existent trade-off between seed longevity and the probability of being eaten (Arnott, 1969; Leishman and Westoby, 1994; Baskin and Baskin, 1998); therefore, *H. comosum* may be the fittest in places without predation, while *K. vurilochensis* may be favored under high predation rates. This speculative reasoning is possible keeping every other variable equal, but more research is needed.

Both species presented contrasting patterns in the response to temperature and water. At all temperatures, *H. comosum* seeds displayed faster germination than *K. vurilochensis* seeds. *K. vurilochensis* showed a significant lower T_b, but a higher $\theta_T(50)$. Therefore, in spite of the fact that the lower T_b allows seeds to accumulate thermal units earlier, the thermal requirements for germination completion were even higher. Comparatively, *H. comosum* seeds presented a higher T_b, but a lower thermal requirement, which meant a fast and synchronized germination

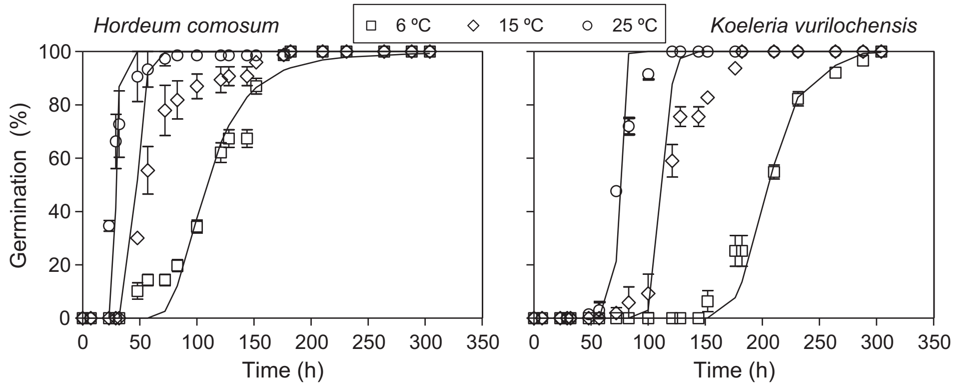

Fig. 1. Cumulative seed germination for the two Patagonian grasses under different temperatures. Symbols are actual data (mean ± s.e.) and lines are modeled data using parameters from the thermal-time model.

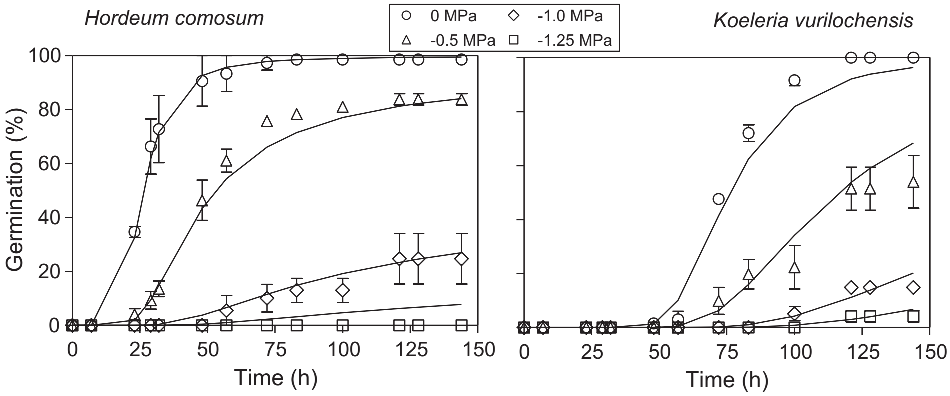

Fig. 2. Cumulative seed germination for the two Patagonian grasses under different water potentials. Symbols are actual data (mean ± s.e.) and lines are the modeled data using parameters from the hydrotime model.

(although $\sigma_{\theta T}$ were similar between species). Interestingly, we found that germination rate in *H. comosum* responds linearly to temperature up to 25 °C, marking a slight difference with the previous findings which suggested that the range goes up to 18 °C (Soriano, 1960). The lower the temperatures in the original ecological habitat, the lower the T_b will be (Allen et al., 2000). Accordingly, both species showed a relatively low T_b, but the value for *K. vurilochensis* was strikingly lower. Similarly, *K. vurilochensis* presented a significant lower $\Psi_b(50)$ but a higher θ_H, and *H. comosum* presented a higher $\Psi_b(50)$ but a lower θ_H. As a result of a lower θ_H and a relatively lower $\sigma_{\Psi b}$, *H. comosum* seeds had a faster and synchronized germination than *K. vurilochensis*. The total germination was lower for *K. vurilochensis* when water availability was reduced. The parameters profile for *K. vurilochensis* suggests that its populations could be more associated to sandy soils (see Allen et al., 2000). Although both species occur in similar habitats, subtle variations in soil features, associated with micro-site differences (Golluscio et al., 2009), should be taken into account for any effort at restoration.

The information presented here is valuable in studies for breeding native preferred grass species from Northwest Patagonia. Ever since the introduction of exotic domestic herbivores, the energy flow from primary producers to exotic herbivores has shown symptoms of deterioration (Soriano et al., 1983; Aguiar and Sala, 1998; Bertiller and Bisigato, 1998). Although this problem has been considered for more than 50 years, few efforts have been devoted to native preferred species restoration. Furthermore, in spite of some exceptions (e.g., *Bromus catharticus*, *Paspalum dilatatum*), there are almost no native domesticated species from temperate South America (Cornaglia et al., 2005). As the studied species are among those which disappear under high sheep-grazing pressure (León and Aguiar, 1985; Perelman et al., 1997), our results provide valuable information for future breeding programs devoted to delay the rate of deterioration in Patagonia. The reintroduction of these grass species may lead to improve the primary productivity and the precipitation use efficiency, key biophysical variables in this system (Verón and Paruelo, 2010).

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