

Understanding erratic seedling emergence in perennial grasses using physiological models and field experimentation

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Abstract Year-to-year variability in field seedling emergence in arid rangelands poses questions about the viability of perennial grasses threatened by overgrazing. There are practical difficulties in estimating long-term seedling emergence for perennial species with erratic behavior (high temporal variability). We present a novel approach combining seed germination studies, modeling, and field experiments to estimate long-term germination probability using *Poa ligularis* as a perennial grass model. Additional objectives were to evaluate: (i) germination requirements of different *P. ligularis* populations, (ii) field emergence behavior at two extreme locations within the distributional range in

the southern region, and (iii) the influence of microsite or local soil on field emergence. Germination parameters were obtained from 8 populations collected across a 12° latitude transect. Germination parameters were combined with a soil water model to estimate long-term germination (used as a proxy of field emergence) in the southernmost location. Germination requirements differed across populations and correlated with environmental conditions of collection sites, demonstrating ecological significance. Field experiments showed very low emergence. Simulations for the southern location indicated erratic emergence. Fifty percent of the years had nil simulated field emergence and the magnitude of the events, when occurred, was small. The magnitude of these events depended on the population, demonstrating an additional genetic constraint. Erratic field emergence arises from the mismatch between environmental conditions and germination requirements. Knowing favorable climatic conditions triggering seed germination (and thus emergence) events are key for opportunistic management of species with this erratic behavior.

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Introduction

Seedling emergence is key demographic process in arid rangelands for the regeneration of palatable

perennial grasses threatened by overgrazing and frequent drought (ÓConnor 1991). Several perennial grasses from different dry rangelands present very low and high year-to-year variability in seedling emergence (e.g., Lauenroth et al. 1994; Vega and Montaña 2004; Vilá and Lloret 2000; Morgan 2001). Assuming that all germinating seeds produce an emerged seedling, erratic field emergence can be a consequence of species having inherently low germination (i.e., dormancy) and/or being associated with low frequency of suitable environmental conditions for germination to occur. Albeit the many germination requirement type of studies, long-term year-to-year variability in field seedling emergence for these dominant species has not been systematically studied. In general, long-term data are missing precluding estimations of frequency and magnitude of germination or emergence occurrence. Practical difficulties to conduct long-term studies may be responsible of this lack. This knowledge is, however, crucial to assess population viability of threatened species under human use intensification, habitat loss, and climate change (Silvertown and Lovett-Doust 1993; Westoby and Burgman 2006). Here, we present a novel approach that combines physiological, modeling, and field studies to estimate long-term probability and magnitude of germination in a palatable grass species threatened by overgrazing.

Long-term probability of field emergence events and its magnitude can be estimated with empirical or physiological models (Bradford 2002). Empirical models are based on correlations between observed emergence and soil moisture and/or temperature. This approach is, in general, limited because numerous years of field data are required to construct these relationships. Physiological models of seed germination (taken as a proxy for field emergence) constitute a more rational approach since they focus on knowledge of seed functional parameters associated with temperature and water availability (García-Huidobro et al. 1982; Bradford 1995). These models have been frequently applied to characterize seed germination requirements in weeds (Forcella et al. 2000; Grundy 2003), cultivated species (Finch-Savage and Phelps 1993; Finch-Savage et al. 1998; Still and Bradford 1998; Cheng and Bradford 1999), and dry rangelands species (Hardegree and Van Vactor 2000; Hardegree et al. 2003, 2010). However, less work has been conducted on using these parameters to model long-

term germination behavior using field environmental information on temperature and soil moisture.

There is evidence that the seed physiological parameters that determine germination and seedling emergence are under genetic control and that selection operates upon them in contrasting environments (Bradford 1995; Allen et al. 2000). Therefore, it is expected that species with a large distribution range present intra-population variability. Although theory indicates that selection for germination parameters in perennial species is scarce (Rees 1994; Venable and Brown 1988), the empirical evidence indicates the contrary (Allen et al. 2000; Smith et al. 2000). Studying populations distributed along the species geographic range allows an evaluation of intra-specific variability in germination requirements. Evaluating their performance in common gardens located in central and peripheral locations is necessary to separate genetic and environmental influences on the episodic behavior.

To test these ideas, we used the perennial grass *Poa ligularis* as study system which is native to three large phytogeographical Provinces of temperate South America rangelands: Pampa, Monte and Patagonia (approximately 10⁶ km² in area) (Fig. 1). This species is particularly important as forage for both wildlife and domestic large herbivores (Graff et al. 2013; Leva et al. 2013). The population in the Patagonian location (close to the southern distributional edge) exhibited abundant seed production all years but very poor field emergence. In a series of unpublished greenhouse and field experiments in different years, with different seed cohorts and experimental treatments, we observed good germination in greenhouse but nil or lack of emergence under field conditions (a description of the experiments are reported in Rotundo and Aguiar 2004 and 2005 only for *Bromus pictus* since *P. ligularis* showed this field emergence syndrome). On the contrary, in a northern rangeland site (Monte phytogeographical province), located close to the center of the distributional range, field emergence germination is less erratic (Bisigato 2000). These features make this system ideal to study the controls of erratic seedling emergence in arid environments.

The general objective of this paper was to understand the processes associated with erratic field emergence of a perennial grass dominant to temperate grasslands of South America. We had four specific objectives. First, to quantify intra-specific variability

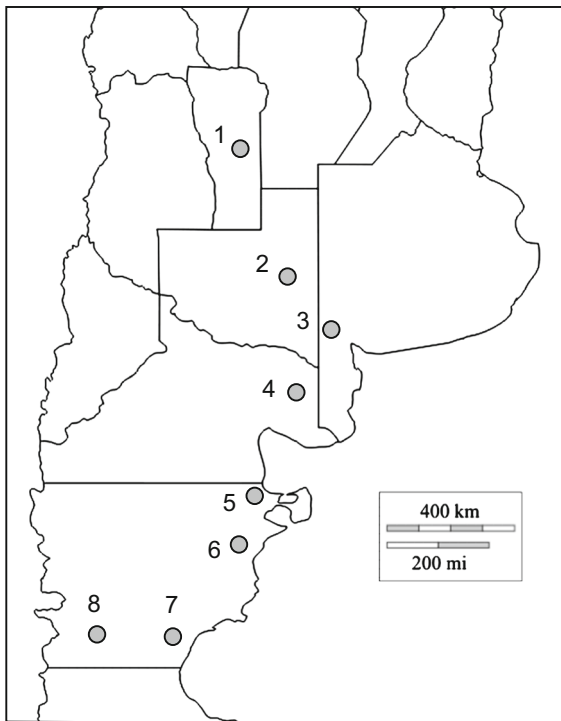


Fig. 1 Geographical distribution of seed collection sites. 1 San Luis, 2 La Pampa, 3 Algarrobal, 4 Conesa, 5 Pirámides, 6 Km1458, 7 Castillo and 8 Río Mayo. Field experiments were conducted at locations 4 and 8

in germination requirements using thermal- and hydro-time models for eight populations collected from different sites from its biogeographic range (spanning 12° latitudes). Second, we assessed field emergence of these *P. ligularis* populations in two common gardens established at one central and the southern border locations of the distribution range. In order to increase robustness of inferences, we repeated the experiment two consecutive years. Third, we evaluated microsite and soil type effects on emergence since micro-environmental conditions are critical for field emergence in arid ecosystems (Aguiar and Sala 1994; Aguiar et al. 1992). Common garden experiments aimed to evaluate specific adaptation for seedling emergence to regional environment neglects these microsite effects (e.g., Hierro et al. 2009; Keller and Kollmann 1999; Meyer and Allen 1999). Fourth, we estimated the probability and magnitude of germination events (used as a surrogate of field emergence) in the southern location using a modeling approach and taking into account intra-specific variability on germination parameters.

Materials and methods

Seed collection and collection sites

Seeds were collected in January (2001 and 2002) directly from plants previous to seed dispersal. Seeds were maintained at 20 °C in the lab until they were prepared for planting. No dormancy-breaking treatment was applied to the seeds previous to planting. Seed viability was checked (>95 %) with tetrazolium before the initiation of experiments.

Collection areas were approximately 25 ha located at eight sites spanning approximately 12 latitude degrees from northern (Villa Mercedes, San Luis province, 33°40′27″S) to southern (Río Mayo, Chubut province, 45°41′0″S) temperate grasslands of South America (Fig. 1). Meteorological data were obtained from stations located near (<20 km) the collection sites. Plant taxonomy was confirmed by expert analysis of floral characters (Giussiani, L. pers. com.).

Hydro and thermal requirements for germination

Hydro-time model

Seeds of the eight populations were incubated in plastic Petri dishes at 10 and 20 °C under 6 different water potentials (Ψ): 0, −0.4, −0.8, −1.2, −1.6, and −2 MPa. The different water potentials were obtained using Polyethyleneglycol 6000 (PEG 6000, Biopack, Argentina) solutions at different concentrations. Nominal values of Ψ used for the preparation of PEG solutions were calculated according to Michel (1983). Actual Ψ values were measured using a vapor pressure osmometer (Wescor 5520, Wescor Inc., Logan, UT, USA). Filter paper embedded in PEG solution were measured simulating conditions in Petri dishes for germination. Measured values were used for deriving the germination models.

Thirty seeds per Petri dish (replication) were sown with three replicates per treatment. Each Petri dish had two filter papers. PEG solutions were replaced each seventh day during the duration of the experiment. Replacing PEG solutions avoids increases in Ψ that may result as a consequence of water molecules being absorbed into filter paper fibers (Bradford 1995).

Germination was recorded daily until day 35. Germination time courses were analyzed using the

hydro-time model (Gummerson 1986; Bradford 1995), as follows:

$$\theta_H = (\Psi - \Psi_{b(g)}) \cdot t_g,$$

where θ_H is the hydro-time requirement (MPa day), Ψ is the water potential of the medium (MPa), $\Psi_{b(g)}$ is the water potential of the medium that impedes the germination of fraction g , and t_g is the time in days required for the germination of fraction g . Germination time courses in different Ψ can be described with the Probit equation (Bradford 1995):

$$\text{Probit}(g) = [\Psi - (\theta_H/t_g) - \Psi_{b(50)}] / \sigma_{\Psi_b},$$

where probit (g) is the accumulated germination in probit scale, $\Psi_{b(50)}$ is the water potential of the medium that impede the germination of the 50 % of the population, and σ_{Ψ_b} is the standard deviation of $\Psi_{b(g)}$ within the seed population. Parameters of the model were obtained by regressing probit (g) with $\Psi - (\theta_H/t_g)$ for all the treatments. A value of θ_H is obtained iteratively to accommodate all the treatments in a best fit linear model. This analysis was carried out for each replication and for each incubation temperature.

Differences among populations in the hydro-time parameters were evaluated by means of an analysis of variance (ANOVA, SAS 1988). Post hoc comparisons were carried out using a Tukey test (SAS 1988).

Thermal-time model

Seeds of the eight populations (Fig. 1) were incubated in plastic Petri dishes at 5, 10, 15, 20, and 25 °C in 0 MPa (distilled water). Seed germination responses to temperature were analyzed with the thermal-time model (Garcia-Huidobro et al. 1982), which is expressed as follows:

$$\theta_{T(g)} = (T - T_b) \cdot t_g,$$

where $\theta_{T(g)}$ is the thermal time required for the germination of fraction g , T is the medium temperature, T_b is the base temperature below which germination does not occur, and t_g is the time required for the germination of the fraction g . Data were analyzed by the Probit procedure (Bradford 2002), regressing germination data (Probit scale) against $\theta_{T(g)}$, according to the equation:

$$\text{Probit}(g) = [((T - T_b) \cdot t_g) - \theta_{T(50)}] / \sigma_{\theta_T},$$

where probit (g) is the accumulated germination in probit scale, $\theta_{T(50)}$ is the requirement of thermal time for the germination of 50 % of the population, and σ_{θ_T} is the standard deviation of the thermal time requirement within the population.

Germination parameters and weather conditions of collection sites

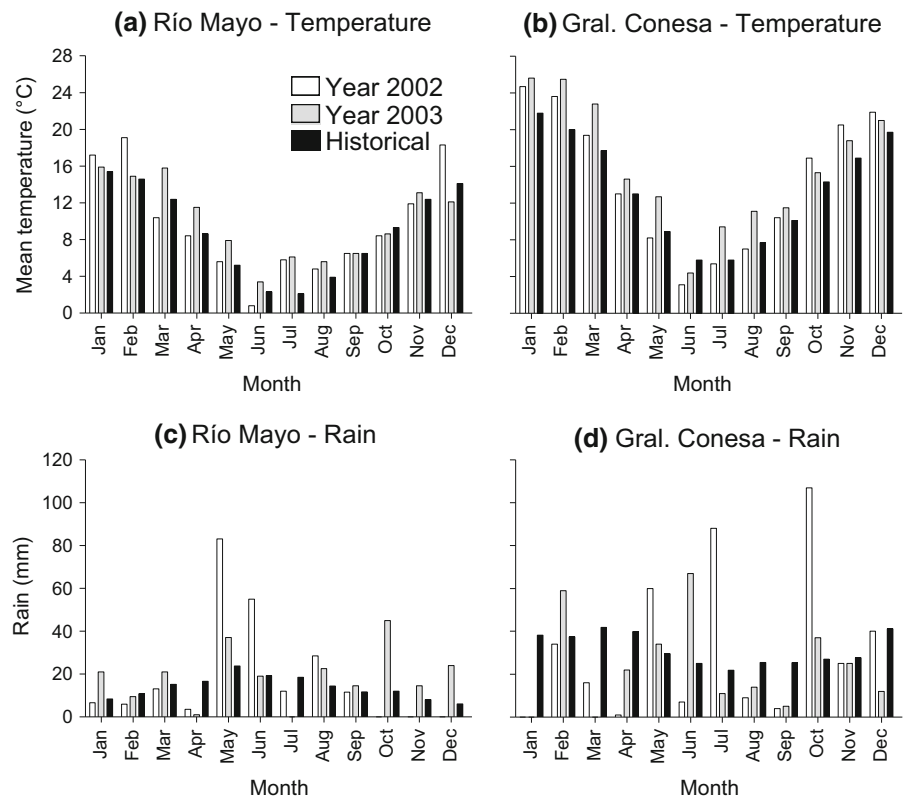
Correlation analyses were performed between seed physiological parameters and mean monthly precipitation and temperature of each collection site. Months were selected according to the most frequent seed germination period in the field. The underlying hypothesis was that populations from drier sites will have lower $\Psi_{b(50)}$ than populations from wetter sites. On the other hand, populations from warmer sites will have a higher T_b compared to populations from colder sites.

Field experimental sites

Experiments were conducted in Río Mayo (45°42'S, 70°33'W, close to the southern edge of distribution range) and in Conesa (39°58'S, 64°43'W, center of distribution range) (Fig. 1, sites 8 and 4, respectively). Río Mayo is located in the south portion of the distribution range of *P. ligularis* and corresponds to a grass-shrub steppe of the Occidental District of Patagonia. Dominant shrubs are *Mulinum spinosum*, *Adesmia volkmanni*, and *Senecio filaginoides*. Dominant grasses are *Pappostipa speciosa*, *P. humilis*, *Poa ligularis*, and *B. pictus* (León et al. 1998). Mean annual precipitation is 156 mm, most of which fall between May and November (winter and early spring) (Fig. 2c). Soil water potential falls from 0 MPa in September to −9 MPa in January. Daily mean temperature ranges between 14 °C in January and 2 °C in July (Fig. 2a). Strong dry winds (15 km h^{−1} annual mean) blow from the west. Soil is sandy with high percentages of stones of variable diameter.

The Conesa site is located in the central portion of the north–south distribution range of *P. ligularis* and it corresponds to a tall shrub steppe of the Monte Phytogeographic province (León et al. 1998). Dominant woody species are *Geophroea decorticans*, *Chuquiraga erinacea*, and *Condalia microphylla*. Dominant grasses are *P. speciosa*, *P. tenuis*, *Aristida*

Fig. 2 Climatic data of Río Mayo (close to the southern edge of species distribution) and Conesa (center of species distribution). **a, c** Show average temperature and monthly precipitation of Río Mayo. **b, d** Show the average temperature and monthly precipitation of Conesa. Data correspond to year 2002 (*open bars*), year 2003 (*gray bars*) and the historical average (*closed bars*)



mendocina, and *Poa ligularis*. Mean annual precipitation is 380 mm (Fig. 2d). Compared to the Río Mayo in Conesa rain is less seasonally distributed site (Fig. 2b). Mean temperature is 21.8 °C in January and 5.8 °C in June (Fig. 2d). Soil is sandy with high percentage of silt on surface.

Field experiments

Year 2002

Poa ligularis field emergence was evaluated in a completely randomized factorial experiment carried out to assess the effect of site (Río Mayo vs. Conesa), population origin (see Fig. 1), and microsite type (bare soil or grass patch). Seeds collected the previous growing season were sown at 1 cm depth in plastic nylon net containers (7-cm long, 3-cm wide, and 5-cm height) filled with local top-soil on May 2002. Containers were located on bare soil patches >20 cm diameter (bare soil microsite) or near (<2 cm) an adult grass (grass microsite). There were five replicates ($12 \text{ seeds} \times \text{rep}^{-1} = 1 \text{ container}$) for each combination of experimental factor level. Seedling emergence was

recorded in November 2002. After the experiment, we remove containers. In the lab, we confirmed that field emergence was equivalent to germination rate.

Year 2003

We repeated the 2002 experiment with the addition of a new (fourth) factor for assessing the influence of local soil differences between the two sites. This new factor (soil type) had two levels, local soil or sand. Plastic nylon net containers were filled either with local top-soil or sand, sowed with 50 seeds each and placed at ground level. Seeds were sown on May 2003. Seedling emergence was recorded in November 2003.

For both experiments, we used analysis of variance (SAS 1988) and proportion data were transformed by the root square arcsine. Tukey test was utilized to assess specific treatment differences.

Modeling of seed germination at the Río Mayo location

For this modeling exercise, seed germination is taken as a surrogate for field emergence. Since we do not

have long-term microsite measurements of soil temperature and water potential we used meteorological data to estimate them. To simulate long-term field emergence of the eight populations in the site of Río Mayo, we first estimated daily soil water potential in the upper soil layer using DINAQUA (Paruelo and Sala 1995). The model DINAQUA was developed to simulate water balance at this particular study site. Model inputs are vegetation parameters for grasses and shrubs (biomass, phenology, root vertical distribution, and transpiration coefficients) and soil parameters (field capacity and wilting point). Daily precipitation, mean temperature, and solar radiation for the Río Mayo site for the period 1989 to 2003 (except 1995) were utilized. Output of the model was daily soil water content for the first 10 cm of soil where most of the seeds are located (Rotundo and Aguiar 2004). Water availability content was converted to soil Ψ (MPa) with the equations proposed in Paruelo et al. (1988).

We simulated the field germination of the 8 populations of *P. ligularis* in the Río Mayo site using daily values of soil Ψ_a (MPa) and T° , and the physiological parameters of the hydro- and thermal-time models. No intention was made of constructing a joint hydro-thermal-time model. Our rationale to incorporate the effects of soil Ψ (MPa) and T° on seed germination was to accumulate hydro-time (with parameters estimated at 10 °C) in the days when temperature is above T_b . Because air temperature could be above or below 10 °C and thus increase or decrease the accumulation of hydro-time, we corrected the accumulation of hydro-time according to the daily average temperature. We assumed a linear relationship to obtain a multiplier (between 0 and 1) that corrects the accumulated hydro-time each day. When the temperature is T_b , the multiplier is 0. Based on each population's response to temperature, we set the temperature factor equal to 1 at 15 °C. This temperature was selected since all the populations had maximum germination rate (estimated as days to 50 % germination) when incubated at 15 °C (0 MPa). On average, days to 50 % germination was 16 days at 10 °C, 10 days at 15 °C, and 14 days at 20 °C ($P < 0.05$, data not shown). Simulations with the historical climatic series of Río Mayo and the physiological parameters of the 8 populations were carried out. Model results were validated against 2002 and 2003 field experiments.

Results

Hydro and thermal requirements for germination

Hydro-time model

The regression coefficient of germination in probit scale [$\Psi - (\theta_H/t_g)$] was greater than 76 % for all the populations at both temperatures evaluated (R^2 in Tables 1 and 2). Overall, the root-mean-square-deviation (RMSD) of observed vs. modeled germination was 2.8 for 10 °C and 5.7 for 20 °C (Fig. S1). The higher RMSD at 20 °C compared to 10 °C is probably due to populations like Km1458 and Pirámides at 20 °C that had small difference in germination between -0.62 and 0 MPa. Also, all populations but Castillo, Km1458 and La Pampa expressed some degree of dormancy when incubated at 20 °C. This effect is observed as an increase in $\Psi_{b(50)}$ (less negative) when incubated at 20 °C compared to 10 °C (Tables 1 and 2).

The parameters of the model differed significantly among the populations (Tables 1 and 2). The θ_H at 10 °C was highest for Castillo population. The $\Psi_{b(50)}$ at 10 °C varied from -1.17 (Castillo) to -0.35 MPa (La Pampa). The σ_{Ψ_b} at 10 °C differed statistically among the populations (Table 1). The θ_H and the $\Psi_{b(50)}$ at 20 °C varied significantly among the populations while σ_{Ψ_b} did not vary significantly among the populations (Table 2).

Thermal-time model

The regression coefficient of germination in probit scale against $\theta_{T(g)}$ was greater than 75 % for all the populations (Table 3). The RMSD of observed vs. modeled germination was 3.7 (Fig. S2).

The T_b and $\theta_{T(50)}$ were significantly different among populations (Table 3). The two northern populations (La Pampa and San Luis) had the highest T_b . The $\theta_{T(50)}$ of San Luis population was the lowest, and was significantly different from those of the rest of the populations (Table 2). The σ_{θ_T} did not vary significantly among the evaluated populations.

Germination parameters and weather conditions of the collection sites

There were significant associations between average monthly precipitation and temperature at collection

Table 1 Parameters of the hydro-time model determined for the different populations of *P. ligularis* at 10 °C

R^2	Population	θ_H		$\Psi_{b(50)}$		σ_{Ψ_b}	
0.90	Río Mayo	9.9	<i>0.5 a</i>	−0.84	<i>0.04 b</i>	0.38	<i>0.01 b</i>
0.86	Castillo	16	<i>1.4 b</i>	−1.17	<i>0.04 a</i>	0.35	<i>0.02 b</i>
0.93	Km1458	9.8	<i>0.7 a</i>	−1	<i>0.02 a</i>	0.26	<i>0.02 ab</i>
0.91	Pirámides	8.6	<i>1.5 a</i>	−0.62	<i>0.08 cd</i>	0.3	<i>0.03 ab</i>
0.92	Conesa	5.6	<i>0.4 a</i>	−0.48	<i>0.02 de</i>	0.23	<i>0.03 a</i>
0.93	Algarrobal	6.8	<i>1 a</i>	−0.59	<i>0.02 d</i>	0.28	<i>0.02 ab</i>
0.93	La Pampa	7.3	<i>1 a</i>	−0.35	<i>0.04</i>	0.27	<i>0.03 ab</i>
0.88	San Luis	7.9	<i>0.1 a</i>	−0.69	<i>0.02 c</i>	0.3	<i>0.05 ab</i>
		$F_{7,23} = 11.74$		$F_{7,23} = 41.81$		$F_{7,23} = 2.88$	
		$P < 0.0001$		$P < 0.0001$		$P = 0.0377$	

The values in italics correspond to the mean standard error. Different letters show statistically significant differences (Tukey $P < 0.05$). θ_H is the hydro-time constant (MPa day), $\Psi_{b(50)}$ is the water potential of the medium that prevents the germination of 50 % of the population and σ_{Ψ_b} is the standard deviation of $\Psi_{b(g)}$ within the seed population

Table 2 Parameters of the hydro-time model determined for the different populations of *P. ligularis* at 20 °C

R^2	Population	θ_H		$\Psi_{b(50)}$		σ_{Ψ_b}	
0.83	Río Mayo	5.5	<i>0.3 abc</i>	−0.51	<i>0.02 abc</i>	0.49	<i>0.03 a</i>
0.79	Castillo	6.4	<i>1.2 abc</i>	−0.94	<i>0.12 a</i>	0.38	<i>0.04 a</i>
0.76	Km1458	5.2	<i>0.2 abc</i>	−0.85	<i>0.02 ab</i>	0.31	<i>0.01 a</i>
0.91	Pirámides	7.8	<i>0.2 bc</i>	−0.25	<i>0.08 cd</i>	0.59	<i>0.06 a</i>
0.86	Conesa	6	<i>1.5 abc</i>	−0.51	<i>0.16 abc</i>	0.53	<i>0.09 a</i>
0.83	Algarrobal	4.4	<i>0.2ab</i>	−0.18	<i>0.09 d</i>	0.6	<i>0.13 a</i>
0.80	La Pampa	8.3	<i>0.6 c</i>	−0.62	<i>0.04 abc</i>	0.42	<i>0.04</i>
0.82	San Luis	3.2	<i>0.3 a</i>	−0.22	<i>0.05 cd</i>	0.67	<i>0.17 a</i>
		$F_{7,23} = 5.35$		$F_{7,23} = 11.14$		$F_{7,23} = 1.97$	
		$P = 0.0026$		$P < 0.0001$		$P = 0.1246$	

The values in italics correspond to the standard error. Different letters show statistically significant differences (Tukey $P < 0.05$). θ_H is the hydro-time constant (MPa day), $\Psi_{b(50)}$ is the water potential of the medium that prevents the germination of 50 % of the population and σ_{Ψ_b} is the standard deviation of $\Psi_{b(g)}$ within the seed population

sites, and the hydro and thermal germination parameters measured for the different populations collected in those sites (Fig. 3). $\Psi_{b(50)}$ measured at 10 °C for each population was positively correlated with average precipitation of the site of origin during both May and September. These are the months during which fall and spring field emergence, respectively, usually takes place (Fig. 3a). On the other hand, T_b measured for each population positively correlated with average temperature of the site of origin of the population during September (Fig. 3b).

Seedling emergence in field experiments

Year 2002

There were no significant differences between grass and bare soil microsites in seedling emergence. Across populations, field emergence varied between 0 and 14 %, and was greater in Río Mayo than in Conesa (Fig. 4) (Site main effect $F_{7,128} = 7.82$, $P < 0.001$). The effect of site, however, depended on the population (Site \times Population $F_{7,128} = 2.53$, $P = 0.018$).

Table 3 Parameters of the thermal-time model determined for the different populations of *P. ligularis*

R^2	Population	T_b		θ_T (50)		σ_{θ_T}	
0.92	Río Mayo	2.2	0.2 b	147	15 a	57	2.7 a
0.96	Castillo	2.3	0.2 b	130	10 ab	39	3.5 a
0.88	Km1458	2.5	0.3 b	110	11 ab	36	6.6 a
0.86	Pirámides	2.5	0.6 b	138	13 a	54	1.4 a
0.84	Conesa	2.8	0.1 b	148	9 a	48	7.3 a
0.92	Algarrobal	2.5	0.3 b	128	6 ab	39	3.0 a
0.75	La Pampa	4.9	0.3 a	139	14 a	52	5.1 a
0.80	San Luis	4.9	0.8 a	78	15 b	71	22 a
		$F_{7,23} = 8.79$		$F_{7,23} = 4.1$		$F_{1,23} = 2.88$	
		$P = 0.0002$		$P = 0.0104$		$P = 0.0507$	

The values in italics correspond to the standard error. Different letters show statistically significant differences (Tukey $P < 0.05$). T_b is the base temperature below which germination does not occur, $\theta_{T(50)}$ is the requirement of thermal time for the germination of 50 % of the population and σ_{θ_T} is the standard deviation of the thermal time requirement within the population

Northern populations (Algarrobal, La Pampa and San Luis) showed no germination at any sites. Pirámides emerged 6 % in Río Mayo compared to 1 % in Conesa. The percentage of emergence of Castillo was 14 % in Río Mayo and 4.2 % in Conesa. The emergence of Km1458 was 11.7 % in Río Mayo and 4.2 % in Conesa. Finally, Conesa and Río Mayo populations emerged very little at both sites (<2.5 %), displaying no significant differences between the sites.

Year 2003

The effect of population and site depended on type of microsite and soil type (Fig. 5). There were two triple interactions [Site \times Population \times Microsite ($F_{7,243} = 3.8$; $P < 0.001$); Site \times Population \times Soil Type ($F_{7,243} = 6.40$; $P < 0.001$)]. The emergence on local soil was, on average, greater in Río Mayo (5.3 %) than in Conesa (0.9 %) (Fig. 5). The difference between sites diminished when seeds were sown in sand (e.g., Fig. 5a vs. e and f, c vs. g and h).

The four northern populations (Conesa, Algarrobal, La Pampa and San Luis) had emergence less than 1 % at both experimental sites. As in the 2002 experiment, population emergence varied with site. In treatments with local soil, averaged across microsites, Castillo emerged 11.2 % in Río Mayo and 2.6 % in Conesa, and Km1458 emerged 5.8 % in Río Mayo and 0 % in Conesa. On the other hand, Río Mayo and Pirámides populations had low values of emergence (<2.8 %) with no significant differences between sites.

When comparing the emergence in local soil at Río Mayo with the emergence in sand at Conesa, the detrimental effect of Conesa environment was smaller. The reduction due to Conesa seeded in sand for Castillo population was 28 % (compared to a 77 % reduction due to Conesa in local soil) and for Km1458 was of 75 % (compared to a 100 % reduction in Conesa local soil). On the contrary, emergence of Río Mayo population was higher in the Río Mayo site with local soil compared to the emergence in Conesa with sand.

Modeling of seed germination at the Río Mayo location

For Río Mayo, seedling emergence and seed germination are equivalent since non-emerged germinated seeds were not observed at this site (after careful examination of net container recover from the field. pers. obs.). Therefore, we assumed that modeling and field observed data are equivalent. Model validation showed that it explained 84 % of the variation in field observed emergence (Fig. 6, RMSD 1.9 %, $P < 0.0001$). The model, however, tended to overestimate to some degree seedling emergence.

The simulation with historical weather data for the Río Mayo site showed that germination probability is low. Populations from southern sites had no germination in 50 % of the 14 simulated years (Fig. 7a). The germination of Río Mayo population was >10 % only in 12 % of the years (Fig. 7a). Castillo and Km1458

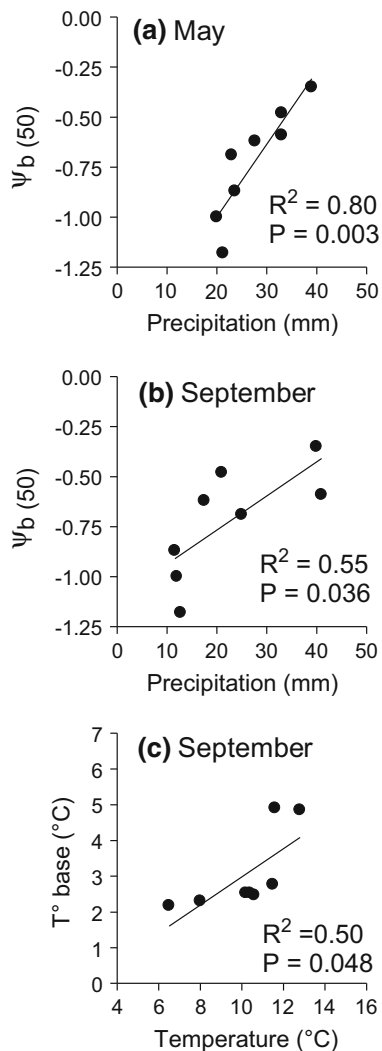


Fig. 3 Correlation between parameters of hydro- and thermal-time models against historical precipitation and temperature from each collection site. **a** Correlation between $\Psi_{b(50)}$ at 10 $^\circ\text{C}$ and May average precipitation. **b** Correlation between $\Psi_{b(50)}$ at 10 $^\circ\text{C}$ and September average precipitation. **c** Correlation between T_b at 0 MPa and September mean temperature. Each point corresponds to one of the eight evaluated populations

had germination >10 % in 40 % of the years. Pirámides had an intermediate behavior, showing germination >10 % in 28 % of the years.

Populations from northern sites had nil values of simulated germination in more than 50 % of the years (Fig. 7b). In particular, San Luis and Conesa populations had no germination in 80 and 70 % of the years, respectively (Fig. 7b). Both populations had simulated germination >10 % only in 8 % of the years (Fig. 7b). Algarrobal population had no germination in 58 % of

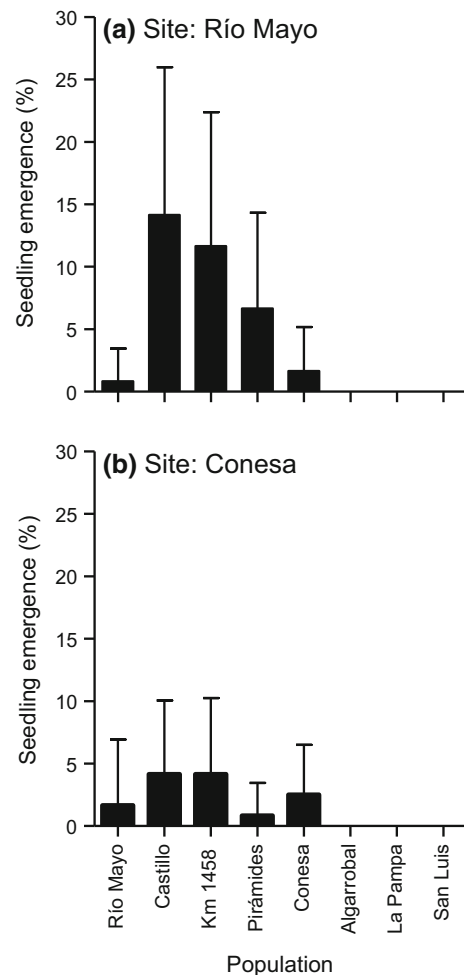


Fig. 4 Seedling field emergence of *P. ligularis* populations from eight different origins arranged from south to north sown in two common gardens, Río Mayo (**a**) close to the southern edge of distribution) and Conesa (**b**) center of species distribution) in 2002. Bars are mean \pm SE

the years and germination >10 % in 20 % of the years. Finally, La Pampa population had no germination in 65 % of the years and germination >10 % in 30 % of the years (Fig. 7b).

Discussion

Perennial grasses dominant in dry rangelands that are obligated seed reproducers frequently present local erratic or episodic recruitment. This fact, together with

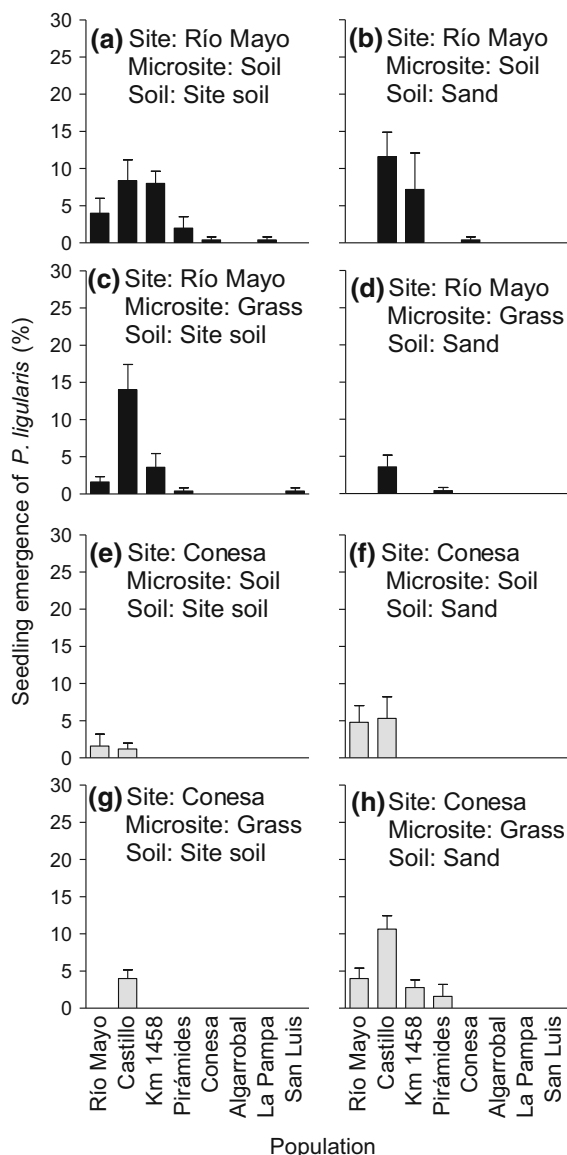


Fig. 5 Seedling field emergence of *P. ligularis* populations from eight different origins arranged from south to north sown in two common gardens, Río Mayo (close to the southern edge of distribution) and Conesa (center of species distribution) in 2003. Microsite soil refers to sowings made in bare soil patches >20 cm in diameter; microsite grass refers to sowings made close to a tussock individual (<2 cm far). Soil refers to substrate of seeding bed: soils from the site or sand. Bars are mean \pm SE

drought and grazing, may be a critical constraint for their long-term population viability (ÓConnor 1991). *Poa ligularis* is an excellent study system to evaluate questions regarding these concerns because of its ample distribution, forage value, and intra-specific genecological variability with latitude (Leva et al.

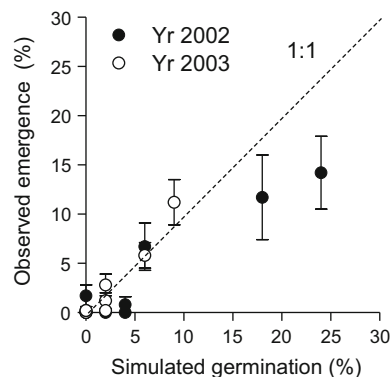


Fig. 6 Validation of the model for simulation of *P. ligularis* emergence in field. Each point corresponds to a different population in 1 year. Values are mean \pm standard deviation

2013). Our results indicate that populations from different locations in the distribution range presented differences in germination parameters. Even though, the possibility that these differences arise from initial differences in dormancy cannot be ruled out; however, since seeds from different locations were harvested at the same time, stored during the same period until experimental utilization, and all of them displayed final germination percentages close to 100 % during the germination studies, it is unlikely that differences in the germination parameters value can be ascribed to reasons other than genetic differences between population in germination responses to temperature and water availability. Parameters correlated with environmental conditions from collection sites. However, common garden field experiments located in central and peripheral locations indicated that such populations had nil or low emergence. Because *P. ligularis* seedlings are small sized, chances of survival through dry summers are very small (Aguiar et al. 1992, Leva et al. 2013). This suggests that populations of *P. ligularis* are threatened up to some level by germination requirements under scenarios of climate change. Also soil seed bank has short persistence (50 % decreases in seed viability after 2 years) (unpublished data and Rotundo and Aguilar 2005). Simulations to study long-term dynamics of germination for Río Mayo (location, close to the southern distributional edge) are a first approach to infer long-term germination dynamics. Although DINAQUA provides soil water information for the first 10 cm and seeds are mostly in the first 5 cm, we submit that germination simulations provide information to explore future

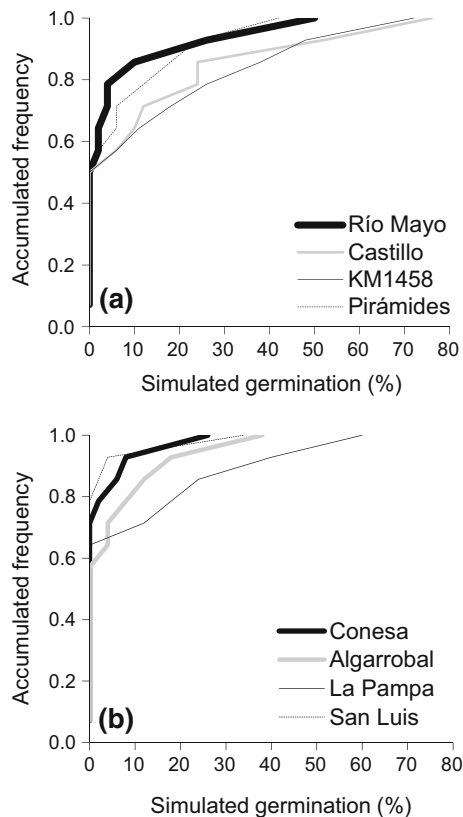


Fig. 7 Modeled cumulative frequency of germination of eight populations of *P. ligularis* in the site of Río Mayo. **a** Results for the four populations from the sites at low latitude. **b** Results for the four populations from the sites at high latitude

dynamics. In particular, we are interested in comparing populations rather than obtaining absolute estimations. Our results showed that there were differences in the frequency of germination episodes, but all populations showed erratic germination (more than half of the 14 years without germination). In other words, *P. ligularis* life-span should be larger to 7 years in order to produce some seedling that may confront other constraint to achieve establishment. Interaction with other species and climate vagaries can reduce life-span.

Germination parameters varied widely among populations. The populations with the highest emergence in common gardens had the lowest $\Psi_{b(50)}$ (i.e., more negative) at 10 °C. Because $\Psi_{b(50)}$ is an indicator of the ability of seeds to tolerate lack of water (Bradford 2002), it is surprising that still the population with the lowest $\Psi_{b(50)}$ (Castillo = −1.17 MPa) had a $\Psi_{b(50)}$ higher (i.e., less negative)

than those of perennial grasses from humid grasslands (Larsen et al. 2004). These authors reported values of $\Psi_{b(50)}$ measured at 10 °C of between −1.14 up to −1.95 MPa. Allen et al. (2000) reported values for perennial grasses from arid ecosystems within the range of −0.73 and −1.49 MPa. Larsen et al. (2004) studied commercial cultivars whereas Allen et al. (2000) and ourselves worked with wild populations. Since the physiological parameters are under genetic control, it is probable that commercial cultivars have undergone a process of selection for characters that accelerate germination as occurred with grain crops (Evans 1993).

The correlation between physiological parameters for seed germination and environmental conditions of collection sites suggests that the parameters have ecological and evolutionary significance as previously pointed out by Bradford (1995, 2002) and Allen et al. (2000). Supporting this notion, genecological regional analysis of *P. ligularis* indicated that local adaptation was important (Leva et al. 2013). The $\Psi_{b(50)}$ was lower (i.e., more negative) in populations from sites with low mean precipitation during autumn and spring (seasons with cool temperatures). This would indicate that those sites with low precipitation selected phenotypes able to germinate under shortage or uncertainty of water availability. On the other hand, the T_b was higher in those sites with higher average temperature. Again, *P. ligularis* data suggest that in those sites with low temperature, phenotypes were selected to tolerate low temperatures. However, this does not preclude a mismatch between water availability (winter and early spring) and temperature not enough for triggering germination, determining erratic germination. The variation in hydro- and thermal-time parameters could be either genotypic or phenotypic (Bradford 1995). It is well documented that the germination ability is heritable (Bradford 1995). Nevertheless, part of the observed variation can also result from differences in environmental conditions during seed development that could alter the seed physiological attributes (Wulff 1995). Experiments with *Bromus tectorum*, in which germination characteristics of populations cultivated in common gardens were compared with populations harvested in situ showed that, although maternal effect existed, it was relatively low (Meyer and Allen 1999). Smith et al. (2000) found a correlation between germination characteristics and site in seeds cultivated in one common garden. These authors

suggest this correlation indicates that the maternal component of the variation in germination behavior was small, and that most of the variation would be explained by a genetic component.

Our field experiments showed very low seedling emergence for most populations at both experimental locations. Emergence never surpassed 14 % and commonly was between ca. 5 and 0 %. Because grass seedling survival during the first year is, in general, low (<7 %, Rotundo and Aguiar 2005), recruitment chances are very low and it would be restricted to infrequent good germination years.

From a biogeographical perspective, previous studies reported that erratic field emergence tends to occur more frequently at the edges of species distributional range (Brown et al. 1996; Hengeveld 1990; Jonas and Geber 1999; Nantel and Gagnon 1999). In our field experiments, emergence was always low but higher in Río Mayo (location closer to the southern distributional edge) than in Conesa (location at the center of distributional range). A detailed analysis of precipitation at both sites revealed that during experimental years 2002 and 2003 precipitation in Conesa was close or lower than the historical average (+2.8 % in 2002 and –25 % in 2003), while in Río Mayo it was 30 and 39 % higher than historical average. In 1999, 2000, and 2001, when *P. ligularis* emergence in Río Mayo was nil (data not published), precipitation was equal or smaller than historical average (–37 % in 1999, +5 % in 2000 and +2 % in 2001). This indicates that in Conesa, unlike Río Mayo, emergence occurred even in years with precipitation near or below the historical average. Supporting this idea, previous works on population dynamics of *P. ligularis* in the Monte Austral did show evidence of frequent emergence across years (Bisigato 2000). This circumstantial evidence of emergence led us to propose that in the long-term emergence and probably germination may be less erratic at Conesa compared to Río Mayo. Unfortunately, there is no soil water model calibrated to the Conesa site, precluding the utilization of seed germination parameters to directly test this hypothesis.

Low emergence at Conesa, located in the center of the distribution range, may be explained by two particular issues. Soil physical characteristics, as shown by the 2003 field experiment, affected seedling emergence and may be an alternative explanation for

the lower emergence in Conesa compared to Río Mayo. Results suggested that sand increased seedling emergence compared to local soil that has high silt content in the top-soil. Crust formation may, in some cases, prevent seedling emergence, even though, seed germination occurred (pers. obs.). The lower seedling emergence may be also related to physiological constraints (i.e., dormancy) and local controls (i.e., soil type). The hydro-time model showed that most populations expressed dormancy at 20 °C. Dormancy was evidenced as a displacement of $\Psi_{b(50)}$ toward less negative values when measured at 20 °C. Mean temperature during spring in Conesa double that of Río Mayo (ca. 16 vs. 8 °C).

Low germination rate can be compensated by high number of seeds produced each year. A recent study performed at the Río Mayo site (Graff et al. 2013) reports that *P. ligularis* female plants present secondary anti-herbivore compounds. Authors associated this trait with the experimentally confirmed less competitive ability of female compared to male plants. In turn, this results in establishment and growth of female plants predominantly in open space, far from competing non-palatable species (males grow predominantly close to refuge plants). This spatial pattern determines that female plants are more frequently found and browsed by sheep than male plants despite the secondary compounds. Accordingly, sex ratio is laden toward male when passing from moderately grazed (1.5:1, female:male) to heavily grazed fields (1:2). Overall, *P. ligularis* under grazing intensification may be constrained by both seed production and emergence rate.

Erratic or episodic seedling emergence (i.e., demographic inertia sensu Westoby et al. 1989) is a clear example of non-linearity in ecological processes. Westoby et al. (1989) included this idea in their often cited state and transition conceptual framework for arid ecosystem management. According to these authors, the circumstances (e.g., environmental) that allow rangeland recovery (e.g., rangelands with low *P. ligularis* density transitioned to a state with high *P. ligularis* density, López et al. 2013) represent management opportunities. Nevertheless, success in this opportunistic management depends on two aspects: knowledge of favorable climatic conditions and capacity to predict them. Climate uncertainty and episodic events are intrinsic driving forces of arid and semi-arid systems (Noy-Meir 1973; Call and Roundy

1991; Wiegand and Jeltsch 2000). Our study presents a research protocol to deal with them and remarks the potential importance of using a biogeographical analysis for estimating future scenarios in which intensification of land use concur with directional climate change (Westoby and Burgman 2006).

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