

SHORT RESEARCH PAPER

Germination sensitivities to water potential among co-existing C₃ and C₄ grasses of cool semi-arid prairie grasslands

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ABSTRACT

An untested theory states that C₄ grass seeds could germinate under lower water potentials (Ψ) than C₃ grass seeds. We used hydrotimology to study seed water relations of C₄ and C₃ Canadian prairie grasses to address Ψ divergent sensitivities and germination strategies along a risk-spreading continuum of responses to limited water. C₄ grasses were *Bouteloua gracilis*, *Calamovilfa longifolia* and *Schizachyrium scoparium*; C₃ grasses were *Bromus carinatus*, *Elymus trachycaulus*, *Festuca hallii* and *Koeleria macrantha*. Hydrotimology parameters were obtained after incubation of non-dormant seeds under different Ψ PEG 6000 solutions. A *t*-test between C₃ and C₄ grasses did not find statistical differences in population mean base Ψ ($\Psi_b(50)$). We found idiosyncratic responses of C₄ grasses along the risk-spreading continuum. *B. gracilis* showed a risk-taker strategy of a species able to quickly germinate in a dry soil due to its low $\Psi_b(50)$ and hydrotimology (θ_H). The high $\Psi_b(50)$ of *S. scoparium* indicates it follows the risk-averse strategy so it can only germinate in wet soils. *C. longifolia* showed an intermediate strategy: the lowest $\Psi_b(50)$ yet the highest θ_H . *K. macrantha*, a C₃ grass which thrives in dry habitats, had the highest $\Psi_b(50)$, suggesting a risk-averse strategy for a C₃ species. Other C₃ species showed intermediate germination patterns in response to Ψ relative to C₄ species. Our results indicate that grasses display germination sensitivities to Ψ across the risk-spreading continuum of responses. Thus seed water relations may be poor predictors to explain differential recruitment and distribution of C₃ and C₄ grasses in the Canadian prairies.

INTRODUCTION

Grasses with a C₄ photosynthetic pathway often co-dominate with C₃ species in cool semi-arid grasslands such as the Canadian prairies (Coupland 1950), including the mixed grass prairie, the fescue prairies and their transition zones. However, temperatures in some of these grasslands are marginally favourable for C₄ grasses as average daily air temperatures within any month are much below 21 °C (Government of Canada 2014), a threshold considered to predict a decline in physiological advantages for C₄ plants and their ability to compete against C₃ species (Ehleringer *et al.* 1997; Collatz *et al.* 1998; Sage & Kubien 2007). The marginal distribution of C₄ grasses in Canadian mixed grass prairies is further illustrated by the regional climate dictating that these prairies belong to a C₃ exclusive grassland type according to C₃/C₄ distribution models (Collatz *et al.* 1998). The unexpected abundance of C₄ grasses in Canadian cool prairies may be partially explained by advantages during recruitment over C₃ species, as this process is a key factor determining geographic ranges in grasslands (Minnick & Coffin 1999).

Differences in temperature requirements for seed germination between C₄ and C₃ grasses are well documented, with base and optimal temperatures for seed germination higher for C₄ than C₃ grasses (Jordan & Haferkamp 1989). Germination is

especially sensitive to low water availability (Evans & Etherington 1990; Ludewig *et al.* 2014). Moreover, differences in water requirements for germination between C₃ and C₄ grass species could be anticipated because the warm season is also the driest in most ecosystems (Baskin & Baskin 2001). While the ability of C₄ grass seeds to germinate at lower water potentials than those of C₃ grasses was previously studied, results are far from conclusive. After a compilation of data from different sources, Baskin & Baskin (2001) proposed that C₄ grass seeds are able to germinate under greater water stress (lower water potentials) than those of C₃ grasses. While extensive in the number of species (*n* = 25) studied, the Baskin & Baskin (2001) review included data obtained through different experimental methods and were deficient in statistical analyses of results. Qi & Redmann (1993), who independently tested the same hypothesis on a smaller pool of cultivated and native pasture species (*n* = 6), did not find differences in germination responses to water stress between C₄ and C₃ grasses. Besides the hypothesis of seed water relations, differences between C₃ and C₄ grasses do not take into account that recruitment is naturally episodic for species in arid and semi-arid lands due to high annual variations in amount and timing of precipitation (Lauenroth *et al.* 1994). Species may depict different risk-spreading strategies for germination under restricted soil water (Daws *et al.* 2008). Some species are risk-takers, able to rapidly progress towards

germination even at low soil water potentials. In contrast, other species are risk-averse as they avoid desiccating conditions and rely on the onset of prolonged wet periods for recruitment (Daws *et al.* 2008). Further studies including different recruitment strategies are needed to draw a comprehensive framework for seed germination sensitivity to water potential designed for C₃ and C₄ species.

Population base hydrotime threshold models have been used to study seed germination sensitivity to water stress in multiple plant species, including vegetables (Bradford 1990), weeds, invasive plants (Batlla & Benech-Arnold 2004; Allen *et al.* 2007), forest species (Daws *et al.* 2008) and range grasses (Gundel *et al.* 2012). We used hydrotime modelling as a tool to study seed water relations (hydrotime parameters) of native C₃ and C₄ grasses in an attempt to address divergent sensitivities between groups and their germination strategies along the risk-spreading continuum of responses to limited water. Outcomes will assist in elucidating the processes that control ecological sorting of grass species in Canadian prairies.

MATERIAL AND METHODS

Plant material

We studied germination and seed water relations of grass species native to Canadian mixed-prairie grasslands. This is an appropriate system to study the hypothesis of functional divergences between C₃ and C₄ grasses in seed water relations because the climate of the mixed-prairie is dry sub-humid to semi-arid, with individual years ranging from humid to arid (Coupland 1992). In the mixed-prairie grasslands dry years as well as moist years tend to be grouped (Coupland 1992), providing different opportunities for seedling establishment. The climate in Canadian mixed prairie grasslands is continental, characterised by long cold winters and short summers (Coupland 1992). Mean July and January daily temperatures recorded for a representative weather station for the region at Swift Current, Saskatchewan (50°16' N, 107°44' W) are 18.5 °C and -10.6 °C, respectively (Government of Canada 2014). Mean annual precipitation in the area is 357 mm, more than two-thirds occurring during the growing season as rainfall. The average length of the frost-free period is 122 days.

Seeds of tested species were purchased from licensed seed companies and included registered varieties, wild harvested seeds and seeds from cultivated stands derived from plants collected in Alberta, Saskatchewan and Manitoba. The C₃ grasses were *Bromus carinatus* Hook. & Arn., *Elymus trachycaulus* (Link) Gould ex Shinners subsp. *trachycaulus* (Syn = *Agropyron trachycaulum* (Link) Malt.), *Festuca hallii* (Vasey) Piper and *Koeleria macrantha* (Ledeb.) Schultes 'ARC Mountain View'. The C₄ grasses were *Bouteloua gracilis* (Willd. ex Kunth) Lag. ex Griffiths 'Bad River', *Calamovilfa longifolia* (Hook.) Scribn. and *Schizachyrium scoparium* (Michx.) Nash (Syn = *Andropogon scoparius* Michx.). *Festuca hallii*, *C. longifolia*, and *S. scoparium* were harvested from cultivated wild-harvested sources in the above-mentioned provinces. *Bromus carinatus* seeds were wild-harvested and *E. trachycaulus* seeds belong to a commonly used genotype native from the area whose provenance was not disclosed by the seed company. Seeds harvested during 2011 were purchased in 2012 and stored dry at -20 °C immediately after purchase. We assumed that seed underwent dry after-

ripening during seed company storage. Species seed weights were obtained from an unpublished literature review of one of the authors (M. A. Naeth, unpublished data).

Seed base water potential for germination can vary according to the dormancy status of each seed lot (Bradford 1996). As our aim was to obtain a quantitative mathematical description of potential seed germinability under low water potentials, we excluded seed lots with noticeable dormancy. Species were included in the study if any seed lot had unnoticeable or low seed dormancy as determined by >70% germination after 30 days at 20 °C in distilled water and darkness and by a lack of significant germination percentage differences between seeds incubated at 20 °C and those incubated at fluctuating temperatures 15/25 °C for 12/12 h·day⁻¹. We followed this procedure because diurnally fluctuating temperatures are known to be the main environmental signal breaking seed dormancy in grassland species (Thompson & Grime 1983). The seven species included in this study were selected from an initial set of 13 grass species. Species not included according to the above-mentioned selection process were *Bromus ciliatus* L., *Deschampsia caespitosa* (L.) P. Beauv., *Hesperostipa comata* Trin. & Rupr., *Pascopyrum smithii* (Rydb.) A. Löve, *Nassella viridula* (Trin.) Barkworth and *Sporobolus cryptandrus* (Torr.) A. Gray.

Laboratory experiments

Seed experiments were conducted in a growth chamber (Model DS33SD; Powers Scientific Inc., Pipersville, USA) at 20 °C, the temperature associated with highest germination across water stress treatments for both C₄ and C₃ grass species (Qi & Redmann 1993). Experiments were conducted with ambient dim white light provided by two fluorescent tubes (Sli Lighting Eco-Line 4100K F17T8/84). Photoperiod was 12 h day⁻¹ at an average irradiance rate of 14 ± 2 µmol·m⁻²·s⁻¹. Pure seed units were selected by diaphanoscopes. Then, four replications, each with 30 seeds (caryopsis, one seeded florets or seeds), at each of 0, -0.3, -0.5, -0.8, -1 and -1.5 MPa water potential treatments were evaluated for each species. Water potentials in the germination media were generated with solutions containing different concentrations of polyethylene glycol (PEG) 6000 following equations published in Michel & Kaufmann (1973). Actual water potential was measured using a Decagon WP4-T dewpoint potentiometer (Decagon Devices, Inc., Pullman, USA). Transparent polystyrene boxes (11 × 11 × 3.5 cm) lined with two layers of Whatman no. 1 filter paper were used; filter paper was evenly soaked with one of the PEG solutions. Germination boxes were covered with plastic film to prevent evaporation. Seeds incubated with solutions containing PEG were transferred to fresh solutions in days 2, 5 and 10 after seeding to correct for increases in PEG concentration. Position of germination boxes was rotated within each growth chamber after each germination monitoring. Germination was recorded as the initiation of embryo growth, verified as the protrusion of the radicle or coleoptile emergence (in *C. longifolia*) through seed coats. Germination was monitored every day and ended after 20 days. This period ensured that germination rates were closely related to current seed conditions. Counted germinated seeds were removed from germination boxes. Seed mortality at the end of the experiments was determined by applying gentle pressure with tweezers or by diaphanoscopes (e.g. small seeds) and dead seeds were excluded from the data.

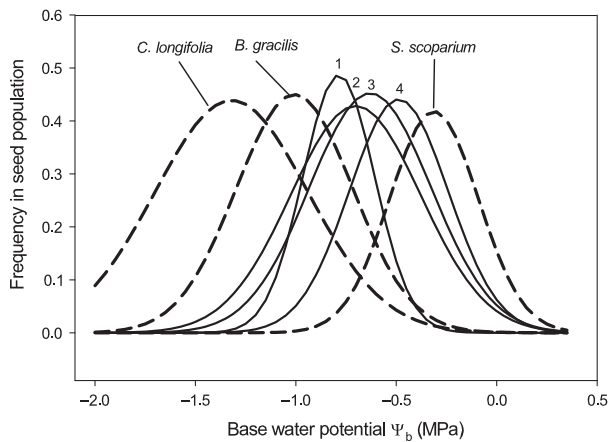


Fig. 1. Frequency distributions of base water potential (Ψ_b) in seed populations of C₃ and C₄ grasses. Distribution curves are of C₃ grasses (1) *Festuca hallii*, (2) *Elymus trachycaulus*, (3) *Bromus carinatus*, (4) *Koeleria macrantha*. Distribution curves of C₄ grasses (*Bouteloua gracilis*, *Calamovilfa longifolia* and *Schizachyrium scoparium*) are labelled in the figure.

Model description and data analysis

We used the hydrotime model as a population base threshold model to describe the germination response of seeds to different water potentials, which is based on the linear relationship between germination rate and water potential (Bradford 1990; Allen *et al.* 2007). The hydrotime model relates time to germination to the difference between the actual seed water potential (Ψ) and the minimum or base water potential $\Psi_b(g)$, allowing germination of fraction 'g' through the following equation (Gummerson 1986):

$$\theta_H = [\Psi - \Psi_{b(g)}]t_g \quad (1)$$

where θ_H is the accumulated hydrotime (in MPa day⁻¹) required from imbibition to germination; and t_g is time for the 'g' fraction to germinate (in days). The model assumes that θ_H is constant for a seed lot, thus variation in time to germination among fractions depends on the difference between Ψ and $\Psi_b(g)$ (Bradford 1990). The model also assumes that variation in Ψ_b within a seed lot is normally distributed to use probits for the analysis (Bradford 1990); σ_{Ψ_b} being the standard deviation of the population. The values of the parameters $\Psi_b(50)$

(population mean base Ψ), θ_H and σ_{Ψ_b} were determined using repeated probit regression analysis to determine the best fit (least residual variance) to the data, as previously described by Bradford (1990). Differences among species in 95% confidence intervals for $\Psi_b(50)$ and σ_{Ψ_b} were determined through regression analyses of cumulative germination (probits) versus estimated base water potentials. In this probit analysis, σ_{Ψ_b} is the reciprocal of the slope of the regression line (Bradford 1990). Regression and *t*-test analyses between C₃ and C₄ species in $\Psi_b(50)$ were conducted using STATISTICA version 10 (Stat-Soft Inc. Tulsa, OK, USA).

RESULTS

Germination rates and final germination percentages were progressively inhibited when seeds were subjected to more negative water potentials (Figure S1). None, or a small percentage, of *B. carinatus*, *E. trachycaulus*, *F. hallii*, *K. macrantha* and *S. scoparium* seeds germinated at -1.0 MPa; however, even with an increased lag period, approximately 40% of *B. gracilis* and *C. longifolia* seeds germinated at that water potential (Figure S1). At -1.5 MPa, observed germination was prevented or negligible in all species but *C. longifolia* (Figure S1).

The hydrotime model showed that there were significant differences in base water potentials among species as determined by their confidence intervals (Fig. 1, Table 1). *C. longifolia* and *B. gracilis*, species with the C₄ pathway, had lower $\Psi_b(50)$ values in the distribution (Fig. 1, Table 1); *S. scoparium*, another C₄ species, had a $\Psi_b(50)$ higher than all the C₃ species (Fig. 1, Table 1). Other hydrotime parameters varied across the studied species and respective C₄ or C₃ groups. The hydrotime model and regression analyses showed that there were significant differences among species in the distribution of population base water potentials along seed subpopulations, as determined by σ_{Ψ_b} ($P < 0.001$; Fig. 1, Table 1). The σ_{Ψ_b} indicated that differences in germination uniformity within seed subpopulations were higher in some species, such as *C. longifolia*, and smaller in others, such as *F. hallii* (Fig. 1, Table 1). The θ_H , accumulated hydrotime required from imbibition to germination, indicated that germination speed also varied among species; with *C. longifolia* seeds the most slow to germinate (Table 1). The fraction of total variation accounted for by the hydrotime model was high for all species and allowed calculation of hydrotime parameters and the modelling of germination time courses (Figure S1).

Table 1. Estimated population hydrotime parameters for germination of C₃ and C₄ grass species native to the Canadian mixed grass prairie.

species	carboxylation pathway	seed weight (mg)	$\Psi_b(50)$ (MPa)	$\Psi_b(50)$ CI (MPa)	σ_{Ψ_b} (MPa)	θ_H (MPa day ⁻¹)	r^2
<i>B. gracilis</i>	C4	0.63	-1.00	-1.03/-0.97	0.41	2.6	0.90
<i>B. carinatus</i>	C3	4.80	-0.63	-0.65/-0.60	0.44	2.4	0.91
<i>C. longifolia</i>	C4	1.90	-1.32	-1.37/-1.27	0.55	6.3	0.84
<i>E. trachycaulus</i>	C3	3.55	-0.70	-0.73/-0.66	0.46	3.1	0.85
<i>F. hallii</i>	C3	2.55	-0.78	-0.80/-0.76	0.26	2.7	0.94
<i>K. macrantha</i>	C3	0.27	-0.48	-0.50/-0.47	0.36	2.8	0.98
<i>S. scoparium</i>	C4	2.00	-0.31	-0.33/-0.31	0.31	1.4	0.95

$\Psi_b(50)$ is the mean base water potential in MPa, $\Psi_b(50)$ CI are the 95% confidence intervals for base water potential, σ_{Ψ_b} is the SD of the base water potential, θ_H is the hydrotime constant (MPa day⁻¹).

Coefficients of determination indicate the fraction of total variation explained by the model.

A *t*-test analysis for independent samples gave non-significant differences between C₃ and C₄ species in $\Psi_b(50)$ ($t = 0.75$, $P = 0.53$). The $\Psi_b(50)$ mean ($\pm$ SE) for C₃ species ($n = 4$) was -0.65 ± 0.06 MPa; that of C₄ species ($n = 3$) was -0.88 ± 0.30 MPa.

DISCUSSION

According to a compilation of mean Ψ that reduced germination to 50% in grasses, C₃ species would have a higher (less negative) Ψ threshold for germination relative to C₄ species (Baskin & Baskin 2001). Our data indicate that the global tendency shown by Baskin & Baskin (2001) was not displayed by Canadian prairies grasses, as C₄ species $\Psi_b(50)$ were not significantly lower (more negative) than those of C₃ grasses. The ecological relevance of this finding is that germination sensitivity to Ψ is not tightly associated to functional groups, and thus seed water relations may be poor predictors to explain recruitment and distribution of C₃ and C₄ grasses in the Canadian prairies.

Phenology differences between C₄ and C₃ species which prompted the hypothesis that C₄ grasses could germinate on drier soils during the warm season led to the proposition of Qi & Redmann (1993) and Baskin & Baskin (2001) about C₄ and C₃ functional divergences in germination sensitivities to water potentials. However, differences in Ψ thresholds for germination may arise as a result of local adaptation to different habitats (Allen *et al.* 2000; Finch-Savage & Leubner-Metzger 2006). Adaptation to a site that is subjected to frequent and intense water deficits, such as the Canadian prairies, may drive a continuum of responses between two contrasting germination strategies that reduce risks of desiccation: the risk-taker strategy of fast emerging species able to accumulate hydrotime (θ_H) and rapidly germinate at low Ψ and the risk-averse strategy of species that slowly germinate at high Ψ thus emerging during prolonged wet periods (Daws *et al.* 2008). Our C₄ grasses, which were anticipated to have a low Ψ threshold for germination, showed contrasting strategies along the continuum. *Bouteloua gracilis* clearly showed the risk-taker strategy of a species able to quickly germinate in a relatively dry soil due to its low $\Psi_b(50)$ and reduced θ_H . The *B. gracilis* risk-taker strategy that would allow it to rapidly emerge and establish may support its pioneer nature in sandy-loam soils and its vulnerability to be outcompeted by taller colonisers (Coupland 1992). In contrast, the high $\Psi_b(50)$ of *S. scoparium* indicates that this species follows the risk-averse strategy, so it can progress towards germination only in wet soils. The risk-averse strategy of *S. scoparium* seems to support its mesic habitat preference. Thus, although *S. scoparium* can occur in relatively moist soils in the mixed grass prairies of western Canada (Coupland 1950;

Moss 1983), it is more abundant in the more humid mixed and tall grass prairies of eastern Canada (Best *et al.* 1971). That *C. longifolia* showed an intermediate strategy, with the lowest $\Psi_b(50)$ and the highest θ_H , indicates its seeds are able to progress towards germination at suboptimal water availability but with a slow germination rate characteristic of risk-averse species.

Our pool of native C₃ species encompasses a broad spectrum of geographic, habitat and soil ranges. *Koeleria macrantha*, the species with preference for dry environments and broad spectrum soil textures, had the highest $\Psi_b(50)$, suggesting a risk-averse strategy for a C₃ species. The other C₃ grasses, which thrive in more structured soils (*e.g.* *E. trachycaulus*) or moister and protected habitats (*e.g.* *F. hallii*), showed similar and intermediate germination patterns in response to Ψ relative to C₄ species. The small differences in $\Psi_b(50)$ and θ_H among C₃ grasses are not surprising as C₃ species are able to germinate early in the spring due to their low base and optimum temperature for germination (Jordan & Haferkamp 1989). They then avoid emergence during the growing season, which is the period of higher variation in soil water content in the Canadian prairies (James *et al.* 2003).

Our results indicate that grasses display germination sensitivities to water potential across the continuum of responses expected according to the risk-spreading theory depicted for sites with restricted soil water for recruitment (Daws *et al.* 2008). In semi-arid grasslands, where recruitment is an episodic phenomenon (Lauenroth *et al.* 1994), a further refinement of the risk-spreading theory to include a higher number of species and functional groups may be conducive to understanding germination niches and ecological sorting of plant species.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Figure S1. Observed and modelled cumulative germination time courses of C₃ and C₄ grasses, showing the effect of low water potentials on cumulative germination.

REFERENCES

- Allen P.S., Meyer S.E., Khan M.A. (2000) Hydrothermal time as a tool in comparative germination studies. In: Black M., Bradford K.J., Vazquez-Ramos J. (Eds), *Seed biology: Advances and applications*. CAB International, Wallingford, UK, pp 401–410.
- Allen P.S., Benech-Arnold R.L., Batlla D., Bradford K.J. (2007) Modeling of Seed Dormancy. In: Bradford K.J., Nonogaki H. (Eds), *Seed development, dormancy and germination*. Annual plant reviews, 27. Blackwell, Sheffield, UK, 72–112.
- Baskin C.C., Baskin J.M. (2001) *Seeds. Ecology, Biogeography, and Evolution of dormancy and germination*. Academic Press, San Diego, CA, USA, pp 666.
- Batlla D., Benech-Arnold R.L. (2004) Seed dormancy loss assessed by changes in *Polygonum aviculare* L. population hydrotime parameters. Development of a predictive model. *Seed Science Research*, **14**, 277–286.
- Best K.F., Looman J., Campbell J.B. (1971) *Prairie grasses identified and described by vegetative characters*. Department of Agriculture, Ottawa, ON, Canada, pp 239.
- Bradford K.J. (1990) A water relations analysis of seed germination rates. *Plant Physiology*, **94**, 840–849.
- Bradford K.J. (1996) Population-based models describing seed dormancy behavior: Implications for experimental design and interpretation. In: Lang G.A. (Ed.), *Plant dormancy: physiology, biochemistry and*

- molecular biology*. CAB International, Wallingford, UK, pp 313–339.
- Collatz G.J., Berry J.A., Clark J.S. (1998) Effects of climate and atmospheric CO₂ partial pressure on the global distribution of C₄ grasses: present, past, and future. *Oecologia*, **114**, 441–454.
- Coupland R.T. (1950) Ecology of mixed prairie in Canada. *Ecological Monographs*, **20**, 271–315.
- Coupland R.T. (1992) Mixed-prairie. In: Coupland R.T. (Ed.), *Ecosystems of the World, 8 A. Natural grasslands introduction and western hemisphere*. Elsevier, Amsterdam, The Netherlands, pp 151–182.
- Daws M.I., Crabtree L.M., Dalling J.W., Mullins C.E., Burslem D.F.R.P. (2008) Germination responses to water potential in neotropical pioneers suggest large-seeded species take more risks. *Annals of Botany*, **102**, 945–951.
- Ehleringer J.R., Cerling T.E., Helliker B.R. (1997) C₄ photosynthesis, atmospheric CO₂, and climate. *Oecologia*, **112**, 285–299.
- Evans C.E., Etherington J.R. (1990) The effect of soil water potential on seed germination of some British plants. *New Phytologist*, **115**, 539–548.
- Finch-Savage W.E., Leubner-Metzger G. (2006) Seed dormancy and the control of germination. *New Phytologist*, **171**, 501–523.
- Government of Canada (2014) Canadian Climate Normals. http://climate.weather.gc.ca/climate_normals/index_e.html (27 November 2014)
- Gummerson R.J. (1986) The effect of constant temperatures and osmotic potentials on the germination of sugar beet. *Journal of Experimental Botany*, **37**, 729–741.
- Gundel P.E., Irisarri J.G.N., Sorzoli N.S., Mosso C.E., García-Martínez G., Golluscio R. (2012) Germination requirements of two sheep-preferred grasses (*Hordeum comosum* and *Koeleria vuilochensis* var. *patagonica*) from semiarid Patagonian steppes. *Journal of Arid Environments*, **78**, 183–186.
- James S.E., Partel M., Wilson S.D., Peltzer D.A. (2003) Temporal heterogeneity of soil moisture in grassland and forest. *Journal of Ecology*, **91**, 234–239.
- Jordan G.L., Haferkamp M.R. (1989) Temperature responses and calculated heat units for germination of several range grasses and shrubs. *Journal of Range Management*, **42**, 41–45.
- Lauenroth W.K., Sala O.E., Coffin D.P., Kirchner T.B. (1994) The importance of soil water in the recruitment of *Bouteloua gracilis* in the shortgrass steppe. *Ecological Applications*, **4**, 741–749.
- Ludewig K., Zelle B., Eckstein R.L., Mosner E., Otte A., Donath T.W. (2014) Differential effects of reduced water potential on the germination of floodplain grassland species indicative of wet and dry habitats. *Seed Science Research*, **24**, 49–61.
- Michel B.E., Kaufmann M.R. (1973) The osmotic potential of polyethylene glycol 6000. *Plant Physiology*, **51**, 914–916.
- Minnick T.J., Coffin D.P. (1999) Geographic patterns of simulated establishment of two *Bouteloua* species: implications for distributions of dominants and ecotones. *Journal of Vegetation Science*, **10**, 343–356.
- Moss E.H. (1983) *Flora of Alberta*, 2nd edition. University of Toronto Press, Toronto, ON, Canada, pp 687.
- Qi M.Q., Redmann R.E. (1993) Seed germination and seedling survival of C₃ and C₄ grasses under water stress. *Journal of Arid Environments*, **24**, 277–285.
- Sage R.F., Kubien D.S. (2007) The temperature response of C₃ and C₄ photosynthesis. *Plant, Cell & Environment*, **30**, 1086–1106.
- Thompson K., Grime J.P. (1983) A comparative study of germination responses to diurnally-fluctuating temperatures. *Journal of Applied Ecology*, **20**, 141–156.