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Photoinhibition of germination in grass seed – Implications for prairie revegetation

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ABSTRACT

Germination photoinhibition is not a recognized cause of revegetation failure; yet prolonged sunlight exposure can inhibit germination of several grass species. This research addressed susceptibility to photoinhibition of selected native grass species used to restore Canadian prairies, and reclamation treatments to alter environmental conditions in order to release seeds from photoinhibition. Under laboratory conditions effects of photoinhibition were tested on the ability of seeds to germinate at low water potential and effects of daily alternating temperatures and nitrates to break photoinhibition. Whether surficial mulch can release seeds from photoinhibition was assessed in a field experiment. Germination photoinhibition was evident in *Festuca hallii* and *Koeleria macrantha* seeds even under very low irradiances. The prolonged exposure to light decreased germination rates and ability of seeds to germinate at low water potentials. Daily fluctuating temperatures released a fraction of *Bromus carinatus* and *Elymus trachycaulus* seeds from photoinhibition yet did not improve *F. hallii* or *K. macrantha* germinability. Nitrates failed to break seed photoinhibition in all species tested. In the field experiment, mulched *F. hallii* seeds (covered with an erosion control blanket) showed a tenfold increase in germination percentages relative to seeds exposed to direct sunlight, indicating the facilitative effects of mulching on attenuation of the light environment. We conclude that germination photoinhibition as a cause of emergence failures in land reclamation where seed is broadcast or shallow seeded should be recognized and germination photoinhibition included in the decision making process to select revegetation seeding techniques.

1. Introduction

Broadcast seeding is more commonly used than drill seeding for prairie revegetation. Rationale for that cultural decision is based on a balance of economic, aesthetic and logistic (Rowe, 2010), rather than biological factors. Broadcast seeding has well recognized limitations generally attributed to insufficient seed imbibition due to poor seed-soil contact and high desiccation rates on the soil surface; these limitations are compensated for by doubling or tripling seeding rates (Doerr and Redente, 1983; Rowe, 2010). Germination inhibition from exposure to long photoperiods of sunlight of surface lying or lightly covered seeds (Pons, 2000) could be an abiotic cause of unexpected revegetation failures.

Prolonged sunlight exposures can inhibit germination of grass species of several different genera (Hilton, 1984; Ellis et al., 1986; Hou and Simpson, 1991; Andersson et al., 2002; Goggin et al., 2008; Barrero et al., 2012). Photoinhibition was observed in row crop grasses, associated grass weeds (Hilton, 1984; Hou and Simpson, 1991; Goggin et al., 2008; Barrero et al., 2012) and grassland species (Probert et al., 1985; Dobarro et al., 2010). The consequences of photoinhibition are easily recognizable when negatively photoblastic seeds fail to germinate after broadcasting or shallow planting. Thus, photoinhibition and ways to overcome it are of great concern as many grassland reclamation attempts are susceptible to emergence failure.

Seed light responses are tightly controlled by environmental cues such as temperature regime and the soil chemical environment (Hilhorst and Karssen, 2000; Pons, 2000). In positively

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photoblastic seeds of many grassland species, germination is promoted by a combination of fluctuating temperatures and light, signals related to vegetation gaps (Thompson and Grime, 1983; Probert and Smith, 1986). In several species, light stimulation of germination require exposure of seeds to cycles of alternating temperatures (Probert and Smith, 1986; Casal and Sánchez, 1998). Soil nitrates in high concentrations can also break seed dormancy indicating vegetation gaps and a safe site for germination (Pons, 1989; Hilhorst and Karssen, 2000). The interaction between nitrates and light in germination induction has been shown, with positively photoblastic seeds from different habitats requiring a simultaneous combination of both signals to break dormancy (Pons, 1989; Hilhorst and Karssen, 2000; Mollard and Insausti, 2009a). Therefore since seed light responses can be modulated by alternating temperatures and nitrates, these factors may alleviate seeds from photoinhibition.

The inhibitory effects of prolonged photoperiods on germination can be increased under conditions of low water availability as partially hydrated seeds are more sensitive to photoinhibitory effects than fully hydrated ones (Hsiao and Simpson, 1971; Hilton, 1984; Fellner and Sawhney, 2002). Photoinhibition responses can appear in partially hydrated seeds that do not have negative photoblastic behavior in otherwise fully hydrated conditions (Niedzwiedz-Siegen and Lewak, 1992). In this way, seeds covered by light amounts of soil or surface applied mulch may still show photoinhibition if kept under suboptimal hydration in the seedbed.

Broadcast seeding under photoinhibitory conditions may fail to give expected emergence rates according to seeding calculations derived from laboratory germination tests that do not test photoinhibition. Thus revegetation may benefit from practices focused on attenuating irradiations. Under field conditions, photoinhibition may be released with use of surface applied mulch to attenuate light. The aim of this research was to determine to what extent grass seeds of native species used to reclaim grasslands in Canadian prairies are subjected to photoinhibition and to explore different conditions to alleviate it. We addressed the following questions: Are grass seeds commonly used for reclamation in the prairies susceptible to photoinhibition? Can photoinhibitory conditions change ability of seeds to germinate at low water potentials? Can grass seed photoinhibition be relieved by nitrates or daily fluctuating temperature regimes? Can grass seeds be released from photoinhibition through the use of mulch?

2. Methods

2.1. Species used in experiments

Seed photoinhibition was studied in native grass species commonly used for reclamation in western Canada. Most experiments were conducted with *Festuca hallii* (Vasey) Piper or *Koeleria macrantha* Schultes seeds to test for photoinhibition in materials that may show contrasting light sensitivity. *Festuca hallii* has notoriously poor emergence when broadcast seeded but establishes adequate plant stands when seeds are hay transferred (Desserud and Naeth, 2013a, 2013b). *Koeleria macrantha* var. 'ARC Mountain View' is a popular and reliable choice for revegetation with a potentially low sensitivity to photoinhibition due to its relatively good broadcast seeding performance. Research to address photoinhibition in different species was conducted with seeds of *Bromus ciliatus* L., *Bromus carinatus* Hook. & Arn., *Deschampsia caespitosa* (L.) P. Beauv. or *Elymus trachycaulus* (Link) Gould ex Shinners ssp. *trachycaulus* (Syn. = *Agropyron trachycaulum* (Link) Malt.). Seeds (caryopses, 1-seeded florets or seeds) were purchased from seed distributors in Alberta, Saskatchewan or Manitoba. Seeds were stored dry at −20 °C immediately after being obtained to avoid after-ripening as this process can change seed light responses (Mollard and Insausti, 2009b) and experiments were conducted throughout 2012 and 2013.

2.2. First laboratory experiment: photoinhibition at different irradiances

Seeds of *F. hallii* and *K. macrantha* were put into a growth chamber (Conviron, PGR15, Controlled Environments Ltd., Winnipeg Manitoba) at continuous temperatures of 20 °C under white light provided by 32 fluorescent tubes (Sylvania Pentron 4100 K FB39/841/HO/ECO) and twelve 40 W incandescent lamps (Frosted Globe, Globe Electric Company, Montreal, Canada). A light period of 14 h per day was used as representative of the photoperiod during the early growing season in the Canadian prairies (Ripley, 1973). The photosynthetic photon flux density (PPFD) was 678 ± 43 μmol m^{-2} s^{-1}. Light had a red to far red ratio (R/FR) of 1.3 ± 0.1, which is close to that of sunlight (R/FR = 1.1). Germination boxes were transparent polystyrene boxes (11 × 11 × 3.5 cm) containing one layer of absorbent cotton and white tissue paper saturated with distilled water. Positions of germination boxes were rotated within the chamber after each monitoring.

The following treatments were replicated four times, with 30 seeds for each species per replication.

- Continuous light treatment with germination boxes subjected to the above described growth chamber light conditions.
- Light pulses of 1 h per day during the first five days (germination boxes were wrapped during dark periods).
- Darkness.
- 21% full light (147 μmol m^{-2} s^{-1}, R/FR light of 1.14).
- 14% full light (98 μmol m^{-2} s^{-1}, R/FR light of 1.10).
- 5.5% full light (38 μmol m^{-2} s^{-1}, R/FR light of 1.06).

Germination boxes were wrapped with two layers of reflective surface black polyethylene (IFlex Ayr-foil reflective insulation, Soprema North America, USA) in the pulse and darkness treatments. In the 21, 14 and 5.5% full light treatments, germination boxes were wrapped with one, two or three layers of white 92 bright, 75 gm^{-2} printer paper (Aspen 30, Boise Paper Holding, Boise, USA), respectively. Daily light pulses and darkness treatments were used as controls.

Photoinhibition was indicated by a significant reduction in seed germination rate at each monitoring period, or final germination in any of the long photoperiod treatments, relative to the controls. The daily light pulses treatment was used to provide light cues to break dormancy (Mollard and Insausti, 2009a, 2009b, 2011) and to fulfill requirements of short light photoperiods for germination (Casal and Sánchez, 1998). Germination was assessed under very low intensity safe green light (wavelength = 527 ± 0.6 nm) during the shortest time possible and at irradiances below detection limits of the quantum meters. Tests were carried out on seeds of *F. hallii* and *K. macrantha* to justify the use of safe light as recent research indicates seeds may be sensitive to green light (Goggin and Steadman, 2012). There were no differences in germination after ten days between treatments subjected to 5 min of green light and those in total continuous darkness.

The chamber temperature below the continuous light treatment germination boxes was 20.5 ± 1 °C (mean ± SD); measured with a HOBO temperature logger (Onset Computer Corporation, Cape Cod, Massachusetts, USA). Germination was monitored every second day for 20 days, the period which ensured that germination rates were closely related to current seed conditions. Already counted germinated seeds were removed at each monitoring. Those

germinated seeds with a radicle protrusion of ≥2 mm were considered as germinated the day before the monitoring date.

2.3. Second laboratory experiment: effects of low water potentials on photoinhibition

Seeds of *Festuca halli* and *K. macrantha* were subjected to one of 0, −0.3, −0.5 or −0.8 MPa water potentials in the substrate. Water potential values in the germination media were generated with solutions containing different concentrations of polyethylene glycol 6000 (PEG) following equations published by Michel and Kaufmann (1973). The actual water potential was measured using a Decagon WP4-T dewpoint potentiometer (Decagon Devices, Inc. Pullman, WA). Two layers of Whatman no. 1 filter paper were used per germination box and soaked with one of the PEG solutions. Water potential treatments were combined with shading (21% full light, identical to the second treatment in the first laboratory experiment) or continuous darkness. Seeds incubated on solutions containing PEG were transferred to fresh solutions on the second, fifth and tenth day after seeding to best maintain PEG concentrations. Germination was monitored following the same protocols from the first laboratory experiment. Germination boxes were rotated within the chamber after each monitoring.

2.4. Third laboratory experiment: effects of nitrates on photoinhibition

Four replications, with 30 seeds each, were evaluated under continuous light with germination boxes subjected to the same conditions as in the first laboratory experiment, light pulses of 1 h per day during the first five days after experiment setup (germination boxes were unwrapped during light periods) and continuous darkness. Light treatments were combined with a potassium nitrate solution of 20 mM KNO_3 and a control with distilled water. The potassium nitrate solution is expected to be on the optimal range to stimulate germination (Pons, 1989; Mollard and Insausti, 2009a). Two layers of Whatman #1 filter paper were used per germination box and soaked with either distilled water or KNO_3 solution. Growth chamber and monitoring protocols were the same as in the first laboratory experiment.

2.5. Fourth laboratory experiment: effects of fluctuating temperatures on photoinhibition

Seeds of *F. hallii*, *K. macrantha*, *B. carinatus* and *E. trachycaulus* were put to germinate in two growth chambers (Conviron, E15, Controlled Environments Ltd., Winnipeg, Manitoba) at a continuous temperature of 20 °C or daily fluctuating temperatures of 15/25 °C (10/14 h day^{-1}), under white light provided by twelve fluorescent tubes (Philips Silhouette F3975/835HO). Photoperiod was of 14 h per day at a PPFD of 390 ± 62 μmol m^{-2} s^{-1} and R/FR light of 2.3 ± 0.1. Germination was monitored in seeds subjected to the above described light conditions as well as in darkness and in seeds subjected to daily light pulses. Light pulses were of 1 h per day during the first five days after experiment setup (germination boxes were unwrapped during light periods). The light pulse treatment (similar to the second treatment in the first laboratory experiment) was performed during the high temperature phase in the daily fluctuating temperature regime. Four germination boxes per treatment had 30 seeds each. Other methods and monitoring protocols followed those described for the first laboratory experiment.

2.6. Field experiment: effects of mulching on photoinhibition

An experiment was initiated May 16, 2013 to study *F. hallii* photoinhibition and effect of mulch in semi-controlled field conditions, at the Ellerslie Research Station of the University of Alberta, Edmonton, Canada (53°25′08″ N, 113°32′43″ W). Germination boxes were of transparent crystal polystyrene (11 × 11 × 3.5 cm) containing one layer of absorbent cotton and white tissue paper saturated with distilled water. Four replications containing 30 seeds each were used per treatment. Treatments were sunlight, one layer of erosion control blanket covering the germination boxes, two layers of erosion control blanket covering the germination boxes, continuous darkness, and continuous darkness under two layers of erosion control blanket.

The erosion control blanket was of agricultural straw/coconut fiber SC150BN (North American Green, North Evansville, IN). Each layer of erosion control blanket gives a mulch rate equal to 0.35 kg m^{-2} (North American Green, 2013), values that generate typically used straw mulch rates for grassland restoration (Kiehl et al., 2006; Kiehl and Wagner, 2006). Germination boxes were wrapped with two layers of IFlex reflective insulation foil (Ayr-foil, Soprema North America, USA) to generate continuous darkness treatments. Germination boxes in the unwrapped treatments were re-watered to keep the substrate saturated. The wrapped treatments did not require watering. An extra set of four germination boxes was used in each of the continuous darkness treatments to study germination at two monitoring dates: seven and fifteen days after experiment setup. Three additional germination boxes per treatment were drilled to place an S-TMB-M002 temperature sensor (Onset Computer Corporation, Cape Cod, Massachusetts, USA) under the germination substrate. Temperature was logged hourly by HOBO Micro Stations (Onset Computer Corporation, Cape Cod, Massachusetts, USA).

2.7. Light quantity and quality measurements

PPFD was measured with an Apogee MQ-200 (Apogee Instruments, Logan, Utah, USA) punctual, cosine corrected PAR sensor. The ratio of red (R) (660 nm) to far red radiation (FR) (730 nm) was measured with a Skye SKR110 sensor (Skye Instruments Ltd., Llandridod Wells, UK). Transmitted PPFD was measured at noon during a clear day under one and two layers of erosion control blankets with a Li-Cor LI-191 Line Quantum sensor (Li-Cor Biosciences, Lincoln, Nebraska, USA).

2.8. Statistical analyses and germination modeling

Seed germination was analyzed using the germination rate index (GRI, Steinmaus et al., 2000) during a 20 (experiments 1 and 3) or 15 day period (experiment 4). The 15 or 20 day periods before the final germination count ensured that germination rates were closely related to the current dormancy level and not to other unexpected processes (i.e. warm stratification) that could affect imbibed seeds over longer periods. The germination rate index (GRI) was calculated with the following equation:

$$\mathrm{GRI} = \frac{\mathrm{Gtotal}}{p}\sum_{i=0}^{20}\frac{g_i}{t_i} \quad (1)$$

where G_{total} = the total number of germinated seeds at the end of the germination test (day 20), p = the total number of seeds per germination box, and g_i = the number of seeds germinated between time t_{i-1} and t_i (in days) (Steinmaus et al., 2000). As it was not possible to transform GRI indices to meet assumptions of normality and homogeneity of variance, data were analyzed with

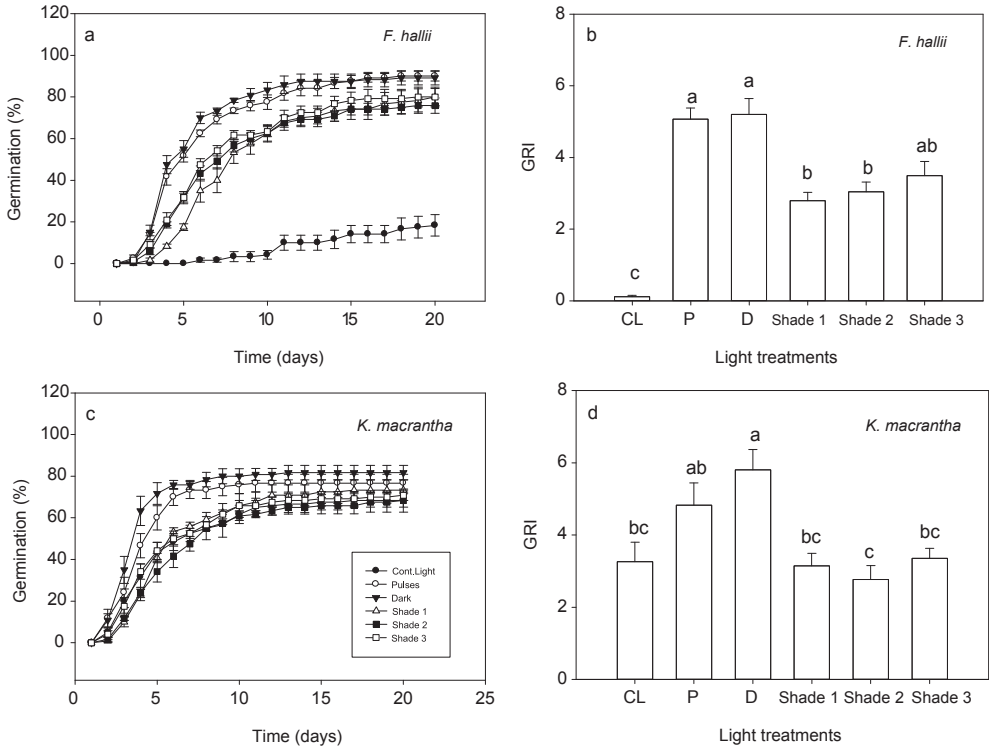

Fig. 1. Cumulative germination time courses (a, c) and germination rate indices (GRI) (b, d) of *Festuca hallii* (a, b) and *Koeleria macrantha* (c, d) seeds subjected to light treatments. CL = continuous light, P = daily light pulses, D = continuous darkness. Shaded 1, 2 and 3 = light was filtered through one, two or three layers of white paper. Data are means ± SE ($n = 4$). GRI bars not sharing the same letter are significantly different ($p < 0.05$).

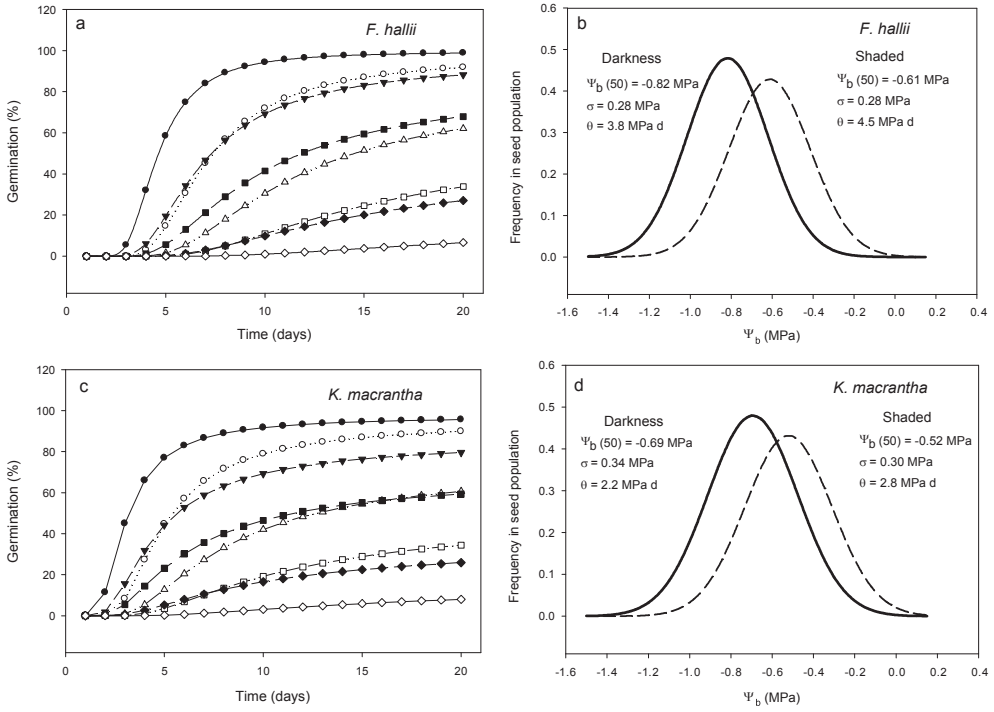

Fig. 2. Modeled cumulative germination time courses (a, c) and frequency distributions of base water potential (b, d) of *Festuca hallii* (a, b) and *Koeleria macrantha* (c, d) seeds subjected to shaded continuous light or darkness under low water potentials. Data points in (a, c) represent modeled germination at −0 MPa (circles), −0.3 MPa (triangles), −0.5 MPa (squares), −0.8 MPa (diamonds). Solid and open data points represent treatments in darkness or shaded continuous light, respectively. Calculated hydrotime parameters are shown in (b, d) panels.

Kruskal–Wallis tests by ranks followed by pair-wise comparisons between treatments ($p < 0.05$) (Conover, 1999). Germination percentages on the second monitoring date of the field experiment were analyzed with parametric one-way ANOVA after arc sine $\sqrt{x}$ transformation of data. Statistical analyses for data obtained in the first monitoring date were carried out through non-parametric Kruskal–Wallis tests followed by pair-wise comparisons due to failures in transforming germination percentages to meet ANOVA assumptions of normality and homogeneity of variance. All statistical analyses were conducted using STATISTICA version 10 (StatSoft Inc. Tulsa, OK). All results are presented as untransformed means of four replicates ± standard error.

The hydrotime model was used to describe the effect of a prolonged photoperiod on the ability of seeds to germinate under low water potentials. The hydrotime model relates the time to germination to the difference between actual seed water potential (Ψ) and minimum or base water potential $\Psi_b(g)$ allowing germination of fraction g through the following equation (Gummerson, 1986; Bradford, 1990; Allen et al., 2007):

$$\theta_H = [\Psi - \Psi_b(g)]t_g \quad (2)$$

where θ_H is accumulated hydrotime (expressed in units of MPa d) required from imbibition to germination; and t_g is time for the g fraction to germinate (in days). The model assumes θ_H is constant for a seed lot so variation in time to germination among fractions depends on the difference between Ψ and $\Psi_b(g)$ (Bradford, 1990). The model assumes variation in Ψ_b within a seed lot is normally distributed to use probits for analysis (Bradford, 1990); being $\sigma\psi_b$ the standard deviation of the population. Values of $\Psi_b(50)$, θ_H and $\sigma\psi_b$ were determined using repeated probit regression analysis to determine the best fit (least residual variance) to the data as previously described by Bradford (1990).

3. Results

3.1. First laboratory experiment: photoinhibition at different irradiances

Effects of a long photoperiod of continuous white light (14 h photoperiod) on germination time courses and germination rate indices (GRI) of *F. hallii* and *K. macrantha* seeds are presented in Fig. 1. Photoinhibition treatments were compared to controls (continuous darkness, daily light pulses) and shade treatments aimed to ameliorate photoinhibition. Germination percentages and rates of *F. hallii* seeds were reduced relative to controls by mild irradiances (compared to sunlight) obtained in the growth chamber ($p < 0.05$; Fig. 1a, b). *Festuca hallii* germination rates were increased by shade treatments ($p < 0.05$; Fig. 1b). *Koeleria macrantha* seeds showed less photoinhibition than *F. hallii*, affecting germination rates ($p < 0.05$; Fig 1c, d) but not germination percentages ($p = 0.19$; Fig. 1c). Despite the low inhibitory effect of long white light irradiances on *K. macrantha* seeds, germination rate was not improved by shade treatments, as the same fraction of seeds was still inhibited relative to seeds incubated in darkness ($p < 0.05$; Fig 1d).

3.2. Second laboratory experiment: effects of low water potentials on photoinhibition

Effects of long photoperiods of low light intensities (98 µmol m^{-2} s^{-1}) and their interaction with low water potentials are presented in the hydrotime modeled germination time courses in Fig. 2. The light treatment yielded similar results in both *F. hallii* and *K. macrantha* germination behavior. For seeds incubated under the same water potential, continuous light increased lag phases and decreased final germination percentages relative to seeds incubated in darkness (Fig 2a, c). Continuous light changed the

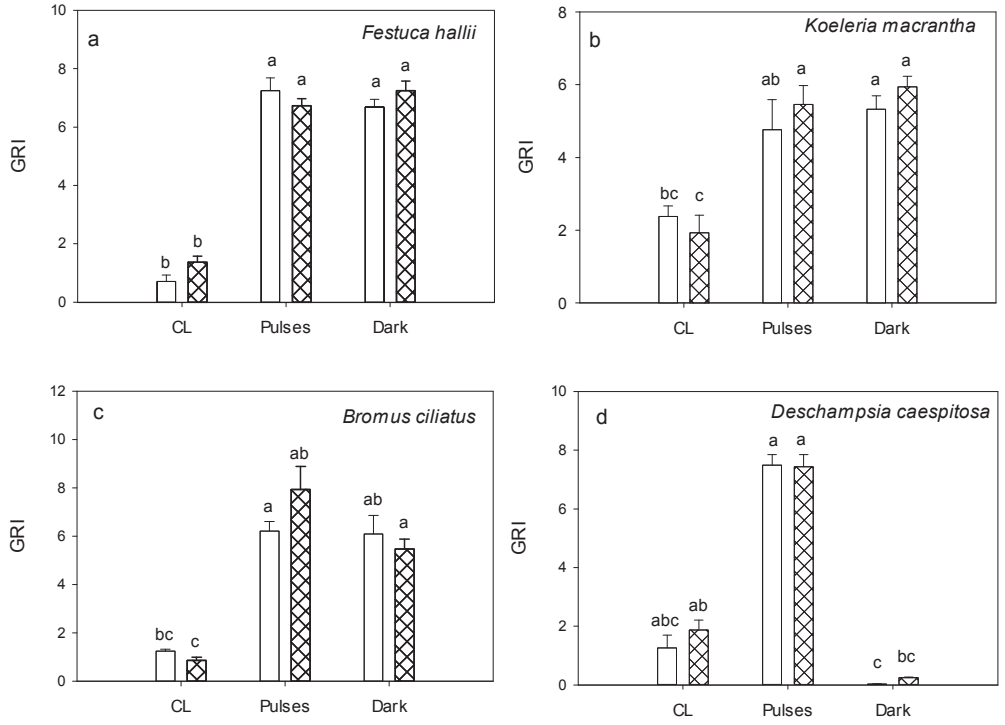

Fig. 3. Effects of light treatments and nitrate on germination rate indices (GRI) of *Festuca hallii* (a), *Koeleria macrantha* (b), *Bromus ciliatus* (c), *Deschampsia caespitosa* (d) seeds. Open bars = seed incubated in distilled water; gridded bars = seed incubated in 20 mM KNO_3. CL = continuous light treatment. Data are means ± SE ($n = 4$). GRI bars not sharing the same letter are significantly different ($p < 0.05$).

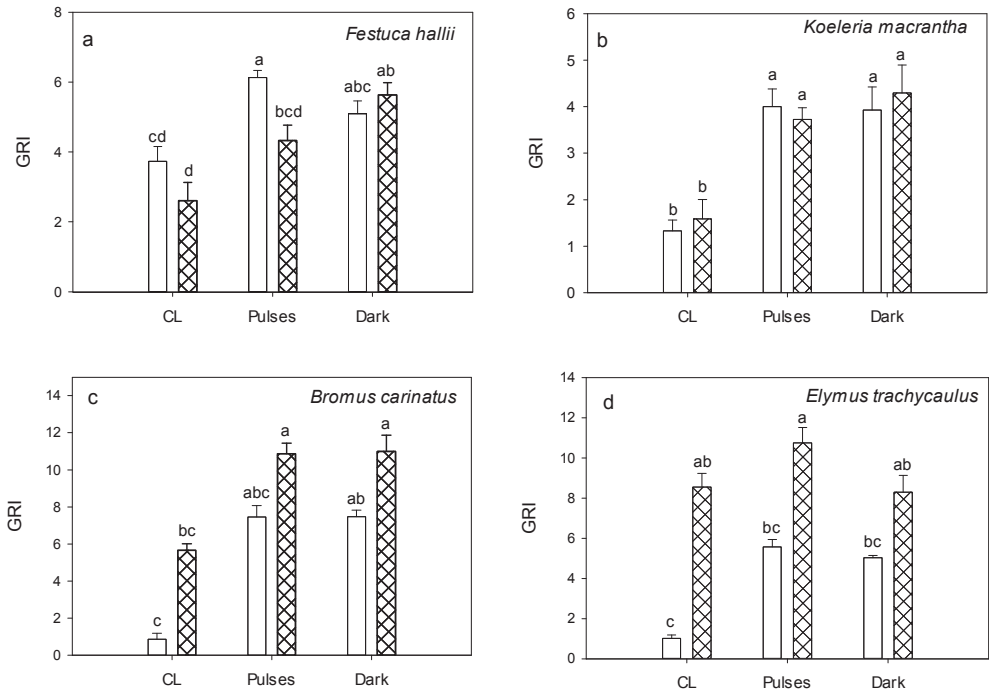

Fig. 4. Effects of light treatments and daily alternating temperature on germination rate indices (GRI) of *Festuca hallii* (a), *Koeleria macrantha* (b), *Bromus carinatus* (c), *Elymus trachycaulus* (d) seeds. Open bars = seed incubated under continuous temperature; gridded bars = seed incubated under daily fluctuating temperatures. CL = continuous light, P = daily light pulses, D = continuous darkness. Data are means ± SE ($n = 4$). Bars not sharing the same letter are significantly different ($p < 0.05$).

distribution of the seeds' germination tolerance to low water availability by increasing $\Psi_b(g)$ (less negative), then reducing the fraction of seeds able to germinate at a given water potential (Fig. 2b,d).

3.3. Third laboratory experiment: effects of nitrates on photoinhibition

Nitrates did not relieve *F. hallii*, *K. macrantha* or *B. ciliatus* seeds from photoinhibition as GRI indices under continuous light were not significantly different when seeds were incubated on KNO_3 solutions or distilled water ($p > 0.05$; Fig. 3a, b, c). Potential effects of nitrates to relieve seeds from photoinhibition were tested in *D. caespitosa* in a seed lot known to have requirements of light signals to germinate (Fig. 3d). Long photoperiods generated a disadvantageous light environment for *D. caespitosa* seeds as they failed to improve germination rates relative to either light pulses or continuous darkness (Fig. 3d). In this species, nitrates were unable to improve germination relative to seeds incubated in water ($p > 0.05$; Fig. 3d).

3.4. Fourth laboratory experiment: effects of fluctuating temperatures on photoinhibition

Alleviation of photoinhibition by daily alternating temperatures was species-specific (Fig. 4). Daily alternating temperatures relieved neither *F. hallii* nor *K. macrantha* seeds from white light photoinhibition as seen by a lack of significant differences in germination rates between seeds incubated under alternating temperature relative to those under continuous temperature ($p > 0.05$; Fig. 4a,b). Only final germination percentages of *B. carinatus* and *E. trachycaulus* seeds showed a significant light × temperature regime interaction ($p < 0.01$, data not shown). Kruskal–Wallis tests did not show sufficient power to find differences between GRI indices of *B. carinatus* seeds subjected to alternating or continuous temperatures (Fig. 4c). However, final germination percentages showed statistically significant differences and indicated that a fraction of seeds acquired the ability to germinate under a long photoperiod of white light when incubated under alternating temperatures ($p < 0.05$ [Tukey tests]; data not shown). Seeds of *E. trachycaulus* were relieved from photoinhibition by alternating temperature regimes and were able to recover germination rates representative of dark conditions ($p < 0.05$; Fig. 4d).

3.5. Field experiment: effects of mulching on photoinhibition

The field experiment tested *F. hallii* germination inhibition at higher irradiances than those that can be reached in growth chambers and the effect of mulch to relieve seeds from photoinhibition. The erosion control blanket changed the quantity of transmitted light reaching the seeds; transmitted light indicated that one and two layers of erosion control blanket filter out 96.0 ± 0.8% and 99.7 ± 0.1% of PAR light, respectively (data not shown). *F. hallii* seeds subjected to unshaded sunlight conditions showed no germination on the first monitoring date (Fig. 5a) and a poor final germination relative to seeds kept in darkness ($p < 0.05$; Fig. 5b). Treatments aimed to amend irradiances by surficial placement of erosion control blankets improved germination percentages relative to seeds under direct sunlight ($p < 0.05$, Fig. 5a, b). Two layers of erosion control blankets on top of germination boxes improved germination percentages relative to seed kept in darkness without any blanket cover ($p < 0.05$, Fig. 5b). Supra-optimal temperatures may explain the inhibition shown in seeds that were in darkness but not covered by an erosion control blanket relative to seeds that were covered by two erosion control blankets. The combination of darkness and erosion control blankets created the lowest daily average temperatures (Fig. 5c) and the lowest maximum temperatures of all the treatments (Fig. 5d).

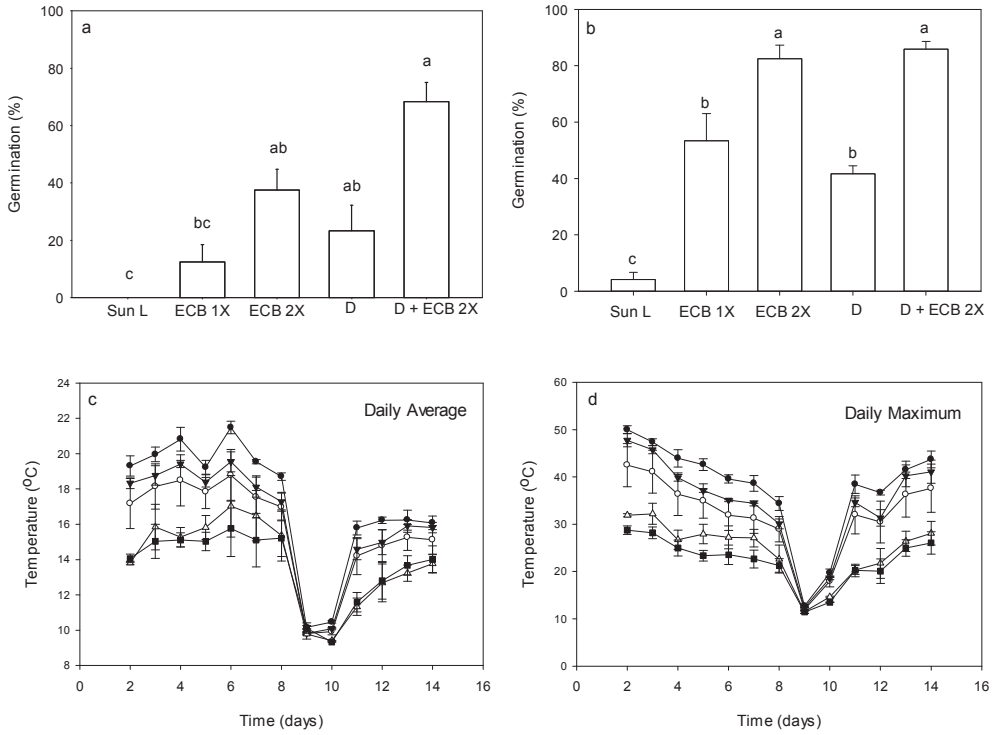

Fig. 5. Field germination of *Festuca hallii* seed. Panels represent germination during the first (a) and second (b) monitoring dates, daily average temperature (c) and daily maximum temperature (b) in germination boxes. Sun L = seeds subjected to direct sunlight, D = seeds in darkness, ECB 1X = germination boxes covered by one layer of erosion control blanket, ECB 2X = germination boxes covered by two layers of erosion control blanket. Data are means ± SE with $n = 4$ in (a, b) and $n = 3$ in (c, d). Bars not sharing the same letter are significantly different ($p < 0.05$).

4. Discussion

Seeds whose germination is inhibited by high photon flux densities have been found in several grass species (Hilton, 1984; Ellis et al., 1986; Hou and Simpson, 1991; Barrero et al., 2012). However, detrimental effects of prolonged sunlight exposure on broadcast seeding revegetation practices have not been addressed. Results of this research show that photoinhibition can limit revegetation efforts in grasslands, as evidenced by the inhibitory effects of long photoperiods detected here in some of the most popular species used for revegetation in the Canadian prairies.

Difficulties in establishing *F. hallii* stands through broadcast seeding in semi-arid grasslands have long plagued revegetation practitioners (Desserud and Naeth, 2013a, 2013b). *Festuca hallii* seedling emergence is improved with native hay transfer (Desserud and Naeth, 2011) suggesting that, aside from the benefits of spreading a seed-bearing material (Desserud and Naeth, 2011), seedbed condition improvement through surface mulching may be facilitating *F. hallii* emergence (Desserud and Naeth, 2013a). Our results indicate that a long photoperiod representative of the light environment during the early growing season for the Canadian prairies can inhibit *F. hallii* seed germination, and that inhibition can be relieved by shade or mulch amendments.

Koeleria macrantha is a highly reliable species for broadcast seeding during revegetation and is one of the most widespread grass species used by reclamation practitioners in the Canadian prairies. The cultivar 'ARC Mountain View' was developed for revegetation of disturbed sites (Woosaree et al., 2004), and is expected to have been subjected to selection and domestication processes during breeding and propagation in nurseries. Thus selection may have reduced any photoinhibitory response that wild ancestors of *K. macrantha* might have possessed. However, germination inhibition of long white light photoperiods was evident even under very low irradiances indicating that a fraction of seeds exhibited a residual photoinhibition.

The ability of seeds to germinate under low water potentials may be critical in soils with low water holding capacity or under water limited regimes commonly found in the prairies. The net effects on germination of the interaction between light and low water potential are complex to predict as they depend on quality and quantity of light and on response to light of individual species (Casal and Sánchez, 1998). White light can increase embryo growth potential, the main requisite for germination, in water stressed, positively photoblastic seeds (Sanchez and Mella, 2004), or decrease even further the already reduced germination of negatively photoblastic seeds subjected to low water potentials (Hsiao and Simpson, 1971; Niedzwiedz-Siegen and Lewak, 1992). Our results show that a long photoperiod of low white light moved $\Psi_b(g)$ frequency distribution of *F. hallii* and *K. macrantha* seeds to higher (less negative) values relative to seeds under continuous darkness. These results indicate that grass seeds with a barely noticeable response to long photoperiods (*K. macrantha*) may express photoinhibition in dry soils even when covered by light amounts of surface applied mulch. The ability to germinate at low water potential is affected by low irradiances such as those that can be generated through mulch amendments.

Daily alternating temperatures and nitrates, especially when combined with light, are efficient treatments to promote grassland species seed germination (Thompson and Grime, 1983; Pons, 1989; Mollard and Insausti, 2009a, 2009b). Daily alternating temperatures relieved *B. carinatus* and *E. trachycaulus* from photoinhibition yet did not promote either *F. hallii* or *K. macrantha* seed germination. Thus seeds of some grass species can benefit from a daily alternating temperature regime and escape from photoinhibition. Although implications should be confined to light fluences as low as those tested in our experiments (390 ± 62 μmol m^{-2} s^{-1}), results

indicate the relevance of mulch treatments aimed to reduce transmitted light while maintaining fluctuating temperature regimes in the seedbed. Relief of photoinhibition by exposure to KNO_3 solutions has been previously documented yet results seem to be ascribed to only a few species (Ellis et al., 1986; Bell et al., 1999). None of the species tested in our research showed the ability to escape from photoinhibition when seeds were incubated in nitrate solutions. Our results and those in the literature suggest that nitrate fertilization may not be successful as a technique to overcome seed photoinhibition.

A quick and synchronized germination can determine success of a revegetation plan aimed to achieve a high and rapid cover on an erodible soil or on sites where competition with fast growing undesirable species is a concern. From our field experiment we attributed the poor germination rates of *F. hallii* seeds lying on the soil surface to photoinhibition. The experiment demonstrates that photoinhibition takes place even under optimal hydrologic conditions as water availability was controlled throughout the study. The field experiment also suggests that initial seedling densities during the first two weeks may not be recovered by doubling or tripling rates over those of drill seeding, an expensive practice that is in use to overcome broadcast seeding limitations (Doerr and Redente, 1983; Rowe, 2010), as *F. hallii* seeds covered by layers of erosion control blanket showed a tenfold or higher increase in germination relative to seeds exposed to direct sunlight. The field experiment stresses the facilitative effects of mulching for attenuation of the light environment for a photoinhibition sensitive species such as *F. hallii*. Basically, an erosion control blanket cover relieved seeds from photoinhibition. As erosion control blanket application rates were similar to those commonly used in straw or hay amendments for grassland restoration (Kiehl et al., 2006; Kiehl and Wagner, 2006), a desirable seed germination pattern and extent of broadcast seeded seeds can be recovered with surface mulching.

The results from this research have great potential for revegetation with common species that are notoriously difficult to establish even with seed lots with high germination and viability. The results are sufficiently positive to recommend testing of other common and desirable species for revegetation. Further field testing can provide more detailed reclamation practices, such as amounts and kinds of mulches to increase germination of many photoinhibited species. The challenge is then to determine how we can facilitate germination of photoinhibited species seeded with those that require high light fluence for germination.

5. Conclusions

Germination photoinhibition has yet to be recognized as a cause of emergence failures in revegetation scenarios. Germination drops and delays such as those that are shown by our experiments jeopardize traditional practitioner seeding strategies, such as synchronization of seeding with the window of opportunity for seedling establishment. Our data show that seed sensitivity to photoinhibition should be included in revegetation plans for practitioners whose cultural practices include surface seeding (e.g. broadcast seeding, hydroseeding). By including germination photoinhibition in the decision making process of selecting broadcast or drill seeding, practitioners may make more robust choices and generate more realistic expectations about the outcome of their practices. Techniques aimed to alleviate photoinhibition as straw or hay mulching may represent a more economic choice than buying expensive native seeds as the above mentioned materials are highly accessible to revegetation practitioners in the Canadian prairies.

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