Germination responses to water potential in *Bromus sterilis* L. under different temperatures and light regimes

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ABSTRACT


Barren brome (*Bromus sterilis* L.) is a troublesome weed of winter cereals in western and central Europe and its control requires an exact estimation of emergence time. The study focused on the germination response of populations from the Czech Republic to water availability at different temperatures and under different light regimes. Seeds were able to germinate even at very low water potential ($\Psi$) close to the wilting point, but decreasing temperatures below 25°C and exposure to light decreased the germination percentage (GP) and prolonged the time to reach 50% germination ($T_{50}$). At higher temperatures of 15, 20, and 25°C, seeds germinated up to a $\Psi$ value of –1.5 MPa; however, the GP differed between light (0–3%) and darkness (50–75%). At the highest temperature of 25°C and germination in water, $T_{50}$ was less than 1 day, but a decrease in $\Psi$ to –1.5 MPa prolonged the $T_{50}$ to 5 days; however, this occurred without any significant effect of light regime. With decreasing temperature and $\Psi$, seeds were more sensitive to the light regime and the disproportion between $T_{50}$ in light and darkness increased. At a $\Psi$ of less than –1.0 MPa, seeds needed twice as long for germination in light than in darkness when germinating at 20°C or 15°C. The results may be of value for the development of predictive models and for identifying times when weed control may be the most effective.

Keywords: weedy grass; seed ecology; water stress; hydrothermal time

Barren brome (*Bromus sterilis* L. (syn. *Anisantha sterilis* (L.) Nevski) is one of the most troublesome annual weedy grasses in western and central Europe, occurring mainly in cereals in simple crop rotations with reduced soil tillage (Andersson et al. 2002). Its control is difficult and can be performed only by a limited number of post-emergence herbicides (Jursík et al. 2016). For successful weed control, proper control timing is the most important consideration, because weed emergence is a crucial phase for further crop-weed competition resulting in yield loss, as well as for future weed population dynamics (Forcella et al. 2000). Water and temperature are primary ecological regulators influencing seed germination (Bradford 2002). Germination and emergence are possible when seeds are non-dormant and environmental (hydric, thermal and gaseous) conditions are adequate. Water influences enzyme activation, breakdown, translocation, and the use of reserve storage material (Copeland and McDonald 2001), while temperature affects the beginning and speed of germination. Seed germination is triggered by the amount of water the seed can imbibe, which is influenced by the water potentials of both the soil and the seed (Bradford 2002, Bewley et al.

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In general, air-dry seeds can have water potential values of $-50$ to $-350$ MPa (Bewley et al. 2013). The amount of water available for plant uptake is limited by available water capacity and its accessibility by wilting point. The wilting point is estimated to be the water content at a soil matric potential of $\Psi = -1.5$ MPa (Kirkham 2014). Common weedy plants can usually grow up to a $\Psi$ value of $-0.5$ MPa (Brant et al. 2005). The speed of germination is dependent on the temperature, but if water availability is suboptimal, it acts as a modifier of the germination speed. Similarly, light can stimulate germination under some conditions or inhibit it under others (Hulbert 1955). The germination of *B. sterilis* seeds can be inhibited by white and red light (660 nm), but this response can be reversed by exposure to far-red light (Hilton 1984).

The known concept of thermal time can be extended to take into account water availability via the use of hydrothermal time (Dahal and Bradford 1994). Hydrothermal time models have considerable potential for characterizing and quantifying the effects of thermal and hydric environments on seed germination and seedling emergence (Bradford 2002). Models that estimate germination and weed seedling emergence are valuable management decision tools that can be used to optimise weed control schedules (Forcella 1998, Dastheib et al. 2003).

The main aim of this article was to investigate the germination characteristics of *B. sterilis* at different levels of water availability over a range of temperatures and light conditions, and to obtain exact data for further use in decision support systems in plant protection. It was hypothesized that germination percentage and germination speed under water stress would be negatively influenced and delayed by suboptimal temperatures and light conditions and that germination would be fully inhibited at a certain point. Germination and emergence models based on hydrothermal conditions have already been published for different *Bromus* species (Meyer and Allen 2009, García et al. 2013) but not yet for *B. sterilis*.

**MATERIAL AND METHODS**

Seed material. The influence of water availability on the germination of *B. sterilis* seeds at different temperatures and under different light regimes was tested in laboratory conditions between 2011 and 2013 on seeds collected each year in mid-July at two localities (Dřemčice and Opolany) in Central Bohemia with the regular occurrence of this weed. Seeds were air-dried and stored for two months in dry conditions in darkness at a room temperature of approximately $20^\circ$C until use; damaged seeds were excluded. The experiments were conducted with after-ripened, non-dormant seeds.

**Germination test.** The germination of seeds was tested in a water potential gradient under the influence of different temperatures and light conditions. The effect of water potential on germination was simulated by means of polyethylene-glycol solutions (PEG 6000 Carbo wax 6000, Union Carbide Corporation) following the methodology proposed by Burlyn and Kaufmann (1973). The concentrations of PEG solutions for individual values of water potential were calculated according to the algorithm suggested by Michel and Kaufmann (1973). Solutions with water potentials ($\Psi$) of $0$, $-0.25$, $-0.5$, $-0.75$, $-1$, $-1.25$, $-1.5$ MPa were prepared and used for germination tests at 15, 20 and 25°C. Weaker negative water potentials ($\Psi = -0.075$, $-0.1$ MPa) were included for testing at the lowest temperature of $10^\circ$C. Germination was tested under controlled light and temperature conditions in Sanyo MLR-350H growth chambers (the temperature distribution stated by the manufacturer is $\pm 1.0^\circ$C when lamp off and $\pm 2.5^\circ$C when lamp on). Two different light regimes were used: (i) 12 h light/12 h darkness (L) and (ii) permanent darkness (D). The light in the growth chamber had a photo flux density of approximately $160 \mu$E/m$^2$/s and a $670$–$730$ nm R/FR ratio. Seeds were germinated in 7 cm diameter Petri dishes inserted upside-down in 12 cm diameter Petri dishes. Cellulose filter paper strips measuring $50 \times 200$ mm (Filpap filter paper of weight 80 g/m$^2$) were passed through the smaller Petri dishes to deliver the PEG solution for germination. The absorbent cellulose filter paper was moistened from the larger Petri dish containing 20 mL of distilled water or polyethylene-glycol 6000 (PEG) solution delivered by pipette (Handy Step®, S, Brand). Subsequently, 25 seeds were placed in each Petri dish, with four replicates for each treatment. All treatments with PEG 6000 solutions were wrapped in parafilm ‘M’ (Bemis®) to minimize evaporation and changes in the water potential of the solutions during the test. The permanent darkness condition was as-
measured by wrapping the Petri dishes in one layer of aluminium foil (18 µm) before the test. The number of germinated seeds was inspected and counted each day for 2 weeks. The counting of dark-germinated seeds took place under inactinic green light, which, in general, should have no effect on seed germination (Guillemin et al. 2013). A seed was considered as germinated when the length of the radicle exceeded 1 mm. Germinated seeds were removed from the Petri dish. Each remaining non-germinating seed was tested for viability by means of the tetrazolium chloride test after the germination test was terminated. Non-germinating seeds were split lengthways and placed in a Petri dish containing 10 mL of a freshly prepared 0.2% solution of 2, 3, 5-triphenyl tetrazolium chloride and incubated for half an hour at room temperature (Patil and Dadlani 2009). Seeds coloured pink or red were considered as viable but dormant.

Statistical analysis. The outputs of the germination experiments for each treatment were the seed germination percentage (GP, %) and the time taken for 50% of all seeds to achieve germination (T\textsubscript{50}, days). The cumulative germination (y, %) at a certain water potential was calculated each day from the beginning of the experiment up to the 14\textsuperscript{th} day after the seeds were put in the germinator. Using a non-linear regression log-logistic four parameters model, T\textsubscript{50} values were calculated as the time to achieve the germination of 50% of seeds. For the calculation of estimated germination, the equation devised by Nielsen et al. (2004) was used:

\[ y = c + \frac{d - c}{1 + e^{(b(\log x - \log T_{50}))}} \]

Where: \( y \) – estimated cumulative germination (%); \( c, d \) – coefficients corresponding to lower and upper limits; \( T_{50} \) – time (in days) taken for 50% of seeds to germinate and is an inflection point between the lower and upper limits; \( b \) – slope parameter; \( x \) – independent time variable (in days). Coefficients \( c \) and \( d \) conformed to the values 0 and the maximum percentage of germinated seeds in a respective test (% of germination).

The estimated values of \( T_{50} \) were plotted and their dependence on \( \Psi \) for temperatures of 10, 15, 20 and 25°C were calculated by means of an exponential regression model. Statistical evaluation of differences in germination was carried out by ANOVA with the Tukey’s post-hoc test (\( \alpha = 0.05 \)) in the Statistica (StatSoft CR s.r.o., Prague, Czech Republic, version 13.2) programme and the relationship between water potential and \( T_{50} \) was evaluated using simple non-linear regression in MS Excel 2013 (Microsoft, Redmond, USA).

RESULTS

Germination percentage across the water potential gradient. As expected, GP decreased with lower values of osmotic potential but was also influenced by both light regime and temperature. Large differences in GP were found between the tested light regimes at all tested temperatures (Figure 1a–f), with significantly (\( P < 0.05 \)) higher GP in darkness when the water potential was lower than –0.5 MPa (Table 1). Temperature, however, was also an important factor affecting GP, besides the light regime. At higher temperatures close to the germination optimum (20°C and 25°C), the differences in GP between D and L were relatively small over the range of water potentials between 0 and –0.75 MPa, but at suboptimal temperatures of 15°C and 10°C, larger differences in GP between D and L were already visible at –0.25 MPa and –0.075 MPa, respectively (Figure 1e–h). At temperatures of 15, 20 and 25°C, seeds germinated up to a water potential as low as –1.5 MPa (permanent wilting point); however, the GP values were almost zero (0–3%) in L, but surprisingly high (50–75%) in D. The germination of seeds at 10°C in L declined very sharply with dwindling water availability. The control and the following three lowest PEG concentrations up to –0.25 MPa showed 70% GP. The majority of the remaining seeds were secondary dormant (20–28%) and only a few were non-viable. With a decrease in water potential, the GP also decreased and the seeds stopped germinating at –1.0 MPa in L and at –1.25 MPa in D at the lowest temperature of 10°C (Figure 1g,h).

Dormancy in seeds increased with decreasing water potential, as did the share of nonviable seeds. The highest percentage of secondary dormant seeds was observed at the lowest values of water potential: 63–82% in L and 5–20% in D at the higher temperatures of 20°C and 25°C (Figure 1a–d). However, at the lowest temperature of 10°C, a very high proportion of dormant seeds (i.e., ≥ 50%) was already found at \( \Psi = –0.75 \) MPa in L and \( \Psi = –1.25 \) MPa in D. The percentage of nonviable seeds was higher at lower \( \Psi \), mostly between 20–35% in L and less than 20% in D.
Comparing the localities, the GP was slightly higher ($P < 0.05$) at Dřemčice than at Opolany but only at the higher temperatures of 15, 20, and 25°C (Figure 1). Dependencies of GP on water potential, temperature and light regime were similar for both localities.

**Time taken for 50% germination ($T_{50}$) across the water potential gradient.** It is obvious that a decrease in water availability and temperature caused an increase in $T_{50}$ values. As shown in Table 1, the GP of *B. sterilis* seeds exceeded 50% in most cases at temperatures of 15, 20 and 25°C; however, many experimental treatments at $\Psi = -1.25$ and $-1.5$ MPa did not attain 50% GP in L within the 14-day duration of the germination experiment. GP was also low or zero for 10°C and respective $\Psi$ values were lower than $-0.75$ MPa in L and $-1.25$ MPa in D; thus, it was not possible to determine the $T_{50}$ values and calculate regressions for these combinations of water potentials and temperatures. The data presented in Figure 2 show an evident negative correlation between

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**Figure 1.** Germinating, dormant and non-viable seeds of *Bromus sterilis* at different water potentials ($\Psi$) and temperatures and under different light regimes (O – Opolany; D – Dřemčice)
The seeds germinated very quickly at higher temperatures in water and in the lowest concentrations of PEG. The T\textsubscript{50} value was very small, only 1–2 days, when seeds were germinating in water and at the higher temperatures of 15, 20 and 25°C, regardless of light regime. The differences in T\textsubscript{50} values (the distances between curves constructed for a certain Ψ gradient) between the L and D regimes strongly increased with a decrease in temperature. While there was almost

![Figure 2](image_url)

Figure 2. Relationship between time taken for 50% of all seeds to achieve germination (T\textsubscript{50}) and water potential (Ψ) at different temperatures and under different light regimes (exponential model, T\textsubscript{50} = a × e\textsuperscript{b×Ψ}). L – 12 h light/12 h darkness; D – permanent darkness
DISCUSSION

Both the conditions during seed maturation and the post-dispersal environment influence germination behaviour (Finch-Savage and Leubner-Metzger 2006). As pointed out by Chaturvedi et al. (2014), temperature acts to control germination in seeds and regulates it by driving enzymatic activity and other metabolic processes. This study revealed that temperature, when close to the optimum value for germination, diminished the influence of the other studied factors such as light regime and water potential. A similar finding was obtained by Haliniarz et al. (2013): under water stress (−0.9 MPa), Bromus secalinus germinated from only 75% of seeds at the lower temperatures of 18°C and 14°C, but from 90% at optimum temperatures of 22–25°C. Low temperature and the presence of light were also found to have a negative influence on seed germination in both Bromus sterilis and Bromus tectorum by Andersson et al. (2002).

Water availability and temperature influence the germination capacity of many species, a fact which is related to the different strategies they adopt in order to accommodate the heterogeneity of habitats and climatic seasonality (Allen and Meyer 2002, Shaban 2013). Our study showed that Bromus sterilis is probably more tolerant to drought when germinating close to its temperature optimum (20°C) than Bromus diandrus, which was studied by Del Monte and Dorado (2011). In our study, Bromus sterilis germinated in darkness even at the very low water potential value of −1.5 MPa (wilting point), which is usually considered as a limit for plant growth. For some other grasses, such as Lolium multiflorum and Lolium perenne, the limiting Ψ value for germination at 20°C was −0.5 MPa (Brant et al. 2005).

Each seed in the population has its own specific thermal time requirements in order to become responsive to light (Steadman 2004), so the seed sample germinates gradually at a specific rate. The rate of seed imbition decreases with decreasing soil water potential, causing a water deficit and, consequently, decreased germination (Asgarpour et al. 2015). In our study, Bromus sterilis seeds germinated within 1 day at the optimal temperature and without water stress, but the effect of insufficient imbition at lower Ψ on T50 was clearly visible at the lowest temperature in L, which prolonged the T50 by about 5–6 days. It has been shown many times that Bromus sterilis seeds germinate better in darkness than in light (Hilton 1984, Ellis et al. 1986), but our study only confirmed this finding at lower temperature and lower Ψ. At the highest tested temperature, T50 was not sensitive to light regime, only to Ψ. Based on the tetrizolium test, a high percentage of nonviable seeds after the germination test was also found in unfavourable conditions. According to Afzali et al. (2006), reductions in seed germination under stress conditions can result from the occurrence of metabolic disorders which can lead to the mortality of seeds during germination.

On the basis of the calculation of T50, it is possible to determine the expected germination time for seeds deposited on the soil surface or in upper layers of soil in field conditions at a certain soil temperature and with a certain level of soil moisture. The germination ability of Bromus sterilis in conditions of very low water availability, i.e. close to the wilting point, brings this species a competitive advantage over crop and other weed species. Light and low temperatures are factors inhibiting the germination and allowing seeds to remain dormant on the soil surface if the water conditions are not favourable for further growth, while seeds in the soil profile are able to germinate even at lower water potentials close to the wilting point. The data obtained in this research can be utilized by decision support systems in order to increase the effectiveness of Bromus sterilis control based on exact estimations of its germination time and emergence with regard to the factors studied. Such knowledge can be applied when preparing seedbeds for winter crops attempting to destroy emerging weeds by delayed soil tillage or by applying herbicides (either between crops or selective
post-emergence) at the proper estimated time (e.g.

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