Effect of soil moisture during stratification on dormancy release in seeds of five common weed species

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Summary

Although the effects of cold stratification on the release of physiological dormancy in seeds have been studied extensively, knowledge of the role of soil moisture content on seed dormancy release during cold stratification is limited. Our study determined seed dormancy characteristics and the effect of soil moisture content on seed dormancy breakage during cold stratification in the five common weed species Amaranthus retroflexus, Chenopodium album, Chenopodium hybridum, Plantago lanceolata and Setaria glauca. Seeds of all five species were dormant at the time of harvest and their germination response to light and temperature varied. Soil moisture content had a significant effect on seed dormancy release of all species except P. lanceolata. Germination percentage of A. retroflexus, C. album, C. hybridum increased and then decreased as soil moisture content increased, regardless of germination test temperature. The optimal soil moisture content and seed moisture content for dormancy breakage of A. retroflexus, C. album, C. hybridum were 8%, 12%, 8% and 22.0%, 37.7%, 25.7% respectively. Dry storage (after-ripening) significantly increased germination of S. glauca. Moreover, increasing soil moisture content first slowed and then increased dormancy breakage in S. glauca. These results suggest that data on soil moisture content should be incorporated into models that predict weed seed dormancy breakage and timing of seedling emergence as well as those for weed management.

Keywords: buried weed seeds, cold stratification, germination temperature, seed dormancy, seed moisture content.


Introduction

Seed dormancy is the failure of viable seeds to germinate in a specified period under conditions optimal for germination of non-dormant seeds (Baskin & Baskin, 2004). Cold-wet stratification is one of the most important ways in which non-deep physiological dormancy (see Baskin & Baskin, 2004) is broken in seeds of summer annuals and many perennials in the temperate zone (Baskin & Baskin, 1988; Probert, 2000). During exposure to low temperatures, percentages and rates of germination in these species increase, and often the temperature range at which seeds can germinate increases with an increase in stratification time (Baskin & Baskin, 2014). Hence, a cold stratification requirement represents a mechanism to ensure that germination occurs in spring or early summer (Probert, 2000), when habitat conditions are favourable for the survival
of seedlings of temperate zone species (Van Assche & Vanlerberghe, 1989).

Both temperature and seed moisture content can alter the rate of dormancy alleviation during cold-wet stratification (Batlla & Benech-Arnold, 2006; Baskin & Baskin, 2014). Generally, the effective temperatures for cold stratification are between 1 and 10°C with speed of dormancy breakage being highest at 2–5°C (Stokes, 1965). The optimum seed moisture content for dormancy breakage during cold stratification has been determined for a relatively few species, including the trees Alnus glutinosa (L.) Gaertn., 30% (O’Reilly & Atrip, 2007); Betula pubescens Ehr., 35% (O’Reilly & Atrip, 2007); Picea glauca (Moench) Voss., ≥25% (Downie et al., 1998); and Picea sitchensis (Bong.) Carr., 25% and 30% (Gosling & Rigg, 1990). However, aside from these few tree species, little is known about the relationship between soil moisture content, seed moisture content and dormancy breakage during cold stratification in winter.

Under field conditions, soil moisture content during winter may vary in time and space depending on rainfall amount, microtopography (Bo et al., 2014), soil depth (Qiu et al., 2001), tillage (Kováč et al., 2014) and irrigation management (Kang et al., 2002). Moreover, climate change also may alter soil moisture conditions, thus affecting seed dormancy release and consequently seedling recruitment and community composition (Walck et al., 2011). Thus, understanding the role of soil moisture content in controlling seed dormancy release is of fundamental importance in predicting species distribution, invasion and plant conservation, as well as seasonal pattern of weed emergence and consequently weed management during agricultural practice. However, with the exception of Polygonum aviculare L. (Batlla & Benech-Arnold, 2006; Batlla et al., 2007), little is known about the effect of soil moisture content on dormancy breakage of weed seeds during cold stratification. In P. aviculare, fluctuations in soil moisture content affected seed dormancy level (Batlla & Benech-Arnold, 2006) and seed germination responses to light (Batlla et al., 2007); however, these authors did not quantitatively determine the relationship between soil moisture content and dormancy release.

Amaranthus retroflexus L., Chenopodium album L., Chenopodium hybridum L., Plantago lanceolata L. and Setaria glauca (L.) P. Beauv. are five widespread weeds in agricultural and disturbed habitats throughout the northern temperate regions of the world. This group of weeds represents four eudicotyledons and one monocotyledon, four annuals and one perennial and two C₄ and three C₃ species. On the semi-arid Loess Plateau of China, these five species are typically found in cropland, abandoned land and along roadsides, but rarely in grasslands (Hu XW, pers. comm.). Seeds of these five species have non-deep physiological dormancy at the time of dispersal (Baskin & Baskin, 2004, 2014; Hu et al., 2017). A range of environmental factors, such as temperature, light and soil chemistry, have been reported to play a role in regulating seed dormancy release and germination in these species (Baskin & Baskin, 2014). For example, cold stratification could release seed dormancy of A. retroflexus (Kępczyński & Sznigir, 2013), C. album (Baskin & Baskin, 2014), C. hybridum (Hu et al., 2017) and Setaria glauca (Amini et al., 2015), depending on stratification duration (Kępczyński & Sznigir, 2013; Baskin & Baskin, 2014; Amini et al., 2015) and seed sources (Amini et al., 2015). However, no studies have reported the effects of soil moisture content during cold stratification on the dormancy status of these species.

Thus, in our study, we asked (i) to what extent is seed dormancy release influenced by soil moisture during cold stratification, and (ii) are there differences among the five species in seed dormancy release in response to soil moisture content? To answer these questions, we determined the soil moisture content requirement for seed dormancy breakage of five common weeds during cold stratification. Further, using the results from our study, we addressed the possibility of managing seed dormancy by manipulation of field soil moisture content, that is the ecological and practical significance of regulating seed dormancy release via control of soil moisture content.

Materials and methods

Species and seed collection

Five weed species, Amaranthus retroflexus, Chenopodium album, Chenopodium hybridum, Plantago lanceolata and Setaria glauca, which extensively occur in crop land on the Loess Plateau, were used in this study. Except for P. lanceolata, the other study species are annuals that grow from spring to early autumn, and seeds mature in the autumn. Plantago lanceolata is a perennial, and its seeds mature from late summer to autumn, depending on climatic conditions (e.g. mature early in dry conditions). These five species were selected because they are the most common weed species and co-exist in the same agricultural system, while they vary in seed dormancy and life form as well as seed germination requirements based on preliminary research findings.

Seeds of A. retroflexus, C. album, C. hybridum, P. lanceolata and S. glauca were collected in autumn 2013 from a field on the Yuzhong Campus of Lanzhou
University (35°57′N, 104°10′E, 1700 m a.s.l.), Gansu Province, China. Mean annual temperature is 6.7°C, and mean annual rainfall is 400 mm, with most of it falling from July to September (Hu et al., 2014). Infructescences with ripe seeds were collected from several hundred individual plants of each species and taken to the laboratory, where the seeds were separated from other plant material. Seeds were dried at room temperature (RH 20–35%, 18–25°C) for 1 week and then stored dry at 4°C until used in experiments, which commenced within 4 weeks after seed collection. The initial seed moisture content of A. retroflexus, C. album, C. hybridum, P. lanceolata and S. glauca was 6.9%, 6.5%, 6.7%, 10.3% and 5.3%, respectively. Three replicates of 200 seeds each were used to measure seed moisture content after oven-drying at 101°C for 16 h.

Effect of light and temperature on germination of fresh seeds

To determine whether the seeds were dormant at maturity, freshly collected seeds after drying (hereafter fresh seeds) were tested in both light and darkness at 20°C, 25°C, 10/20°C, 15/25°C, 20/30°C and 25/35°C (12 h/12 h) with a 12 h/12 h daily photoperiod (hereafter light) or in continuous darkness. The light source was white fluorescent tubes, and the photon irradiance was 60 μmol m⁻² s⁻¹ (400–700 nm). For continuous darkness, Petri dishes were covered with two layers of aluminium foil. For each treatment, three replicates of 50 seeds each were placed in 11 cm diameter Petri dishes on two sheets of filter paper (Shuangquan, Hangzhou, China) moistened with 8 mL of distilled water. All Petri dishes were randomly placed within the incubator. Seeds incubated in light were examined for germination daily for at least 14 days until no further germination occurred or for three consecutive days, as described above. No seeds of the five tested species germinated during cold stratification. Seed moisture content was determined according to Eqn (1):

\[ SMC_i = \frac{W_i - W_f + SMC_t \times W_i}{W_i}, \]

where SMCᵢ and SMCᵣ are seed moisture content before and after/during cold stratification, respectively, and \( W_i \) and \( W_f \) are seed mass before and after during cold stratification, respectively, thus assuming that the seed weight gain during stratification was due to imbibition. SMCᵢ was determined by drying a sample seed at 101°C in a drying oven for 16 h.

Data analysis

The effects of light, incubation temperature and their interaction and of soil moisture content, incubation temperature and their interaction on seed germination of each species were tested by fitting generalised linear
models (GLM). Seed germination was a probability ranging from 0 to 1; hence, we applied a binomial estimation of the model using a logit link function. Tukey’s test was used for multiple comparisons when significant differences were found. Regression analysis was used to determine the relationship between soil moisture content and seed moisture content after cold stratification. All data were processed with GenStat, version 18.0 (VSN International Ltd., Hemel Hempstead, UK).

To determine the critical and optimal seed moisture content for dormancy breakage during cold stratification, the observed data were fitted to nine mathematical equations (linear, logarithmic, inverse, quadratic, cubic, power, S-curve, logistic and exponential) with curve estimation function of SPSS (IBM SPSS statistics 22, IBM, USA). To identify the best model for estimating the critical and optimal seed moisture content for dormancy breakage, the root-mean-squared errors (RMSEs; Eqn 2) and the corrected Akaike information criterion (AICc; Eqn 3) were used.

\[
\text{RMSE} = \sqrt{\frac{\text{RSS}}{n}} = \sqrt{\frac{\sum_{i=1}^{n} (Y_{\text{obs}} - Y_{\text{pred}})^2}{n}}
\]

(2)

\[
\text{AICc} = n \ln \left( \frac{\text{RSS}}{n} + 2k \right) + \frac{2k(k+1)}{n-k-1}
\]

(3)

where RSS is the residual sum of squares, \(Y_{\text{obs}}\) and \(Y_{\text{pred}}\) observed and predicted values, respectively, \(n\) the number of observations and \(k\) the number of model parameters. The model that yields the most accurate estimate is the one with the lowest RMSE and AICc values. In the case of contradictory results between RMSE and AICc, the model with the best goodness of fit was selected.

The quadratic model was the best fit for the relationship between seed moisture content and germination at each temperature for \(A.\ retroflexus\), and the cubic model was the best fit for \(C.\ album\), \(C.\ hybridum\) and \(S.\ glauca\) (Tables S1–S4). There was no significant correlation between seed moisture content and germination regardless of germination test temperature in \(P.\ lanceolata\). The goodness of fit between predicted and observed data was assessed by means of the coefficient of determination (\(R^2\)). The fraction variance accounted for by the simulation model \(R^2\) was calculated using Eqn (4):

\[
R^2 = 1 - \frac{\sum_{i=1}^{n} (Y_{\text{obs}} - Y_{\text{pred}})^2}{\sum_{i=1}^{n} (Y_{\text{obs}} - \bar{Y}_{\text{obs}})^2},
\]

(4)

where \(Y_{\text{obs}}\) is observed values, \(Y_{\text{pred}}\) predicted values and \(\bar{Y}_{\text{obs}}\) the mean of observed values. An \(R^2\) value of 1 indicates a perfect fit of the model to the observed data.

The optimal seed moisture content for dormancy breakage was calculated from the vertex of the regression curve (seed moisture content vs. germination percentage). The critical seed moisture content for dormancy breakage was determined from the regression curve in which seed germination percentage was equal to that of the fresh seeds.

### Results

**Effect of light and temperature on germination of fresh seeds**

Germination of fresh seeds varied with species, temperature and light condition (Table 1, Fig. 1). Light and its interaction with temperature, but not the main effect of temperature, had a significant effect on seed germination of \(A.\ retroflexus\). Light significantly promoted seed germination of \(A.\ retroflexus\) at 25, 20/30 and 25/35°C but not at the other temperatures. Under alternating temperatures, germination increased with an increase in temperature regardless of light condition. The highest germination (71%) was for seeds incubated in light at 25/35°C.

Light, temperature and their interaction had significant effects on germination of \(C.\ album\). Seeds of \(C.\ album\) germinated equally well in the dark and in the light at 10/20, 25/35 and 20/30°C; however, at 20, 25 and 15/25°C, germination was significantly higher in the light than in the dark. The highest germination (39%) was for seeds incubated in light at 20°C.

Temperature, but not light, had a significant effect on germination of \(P.\ lanceolata\). Germination increased and then decreased with increasing

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Effect of light, incubation temperature and their interaction on germination of fresh seeds of three weed species using generalised linear model (GLM) (n = 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parameters</td>
<td>Estimate</td>
</tr>
<tr>
<td><strong>Amaranthus retroflexus</strong></td>
<td></td>
</tr>
<tr>
<td>Light (L)</td>
<td>5.204</td>
</tr>
<tr>
<td>Temperature (T)</td>
<td>−0.068</td>
</tr>
<tr>
<td>L × T</td>
<td>0.79</td>
</tr>
<tr>
<td><strong>Chenopodium album</strong></td>
<td></td>
</tr>
<tr>
<td>Light (L)</td>
<td>1.717</td>
</tr>
<tr>
<td>Temperature (T)</td>
<td>−0.57</td>
</tr>
<tr>
<td>L × T</td>
<td>0.2919</td>
</tr>
<tr>
<td><strong>Plantago lanceolata</strong></td>
<td></td>
</tr>
<tr>
<td>Light (L)</td>
<td>−0.084</td>
</tr>
<tr>
<td>Temperature (T)</td>
<td>0.459</td>
</tr>
<tr>
<td>L × T</td>
<td>−0.2127</td>
</tr>
</tbody>
</table>

SE, standard error of estimate value; \(t\), \(t\) ratio from regression analysis.
temperature in both the light and dark. Light promoted germination, but a significant effect was observed only at 25 and 25/35°C. The highest germination (90%) was for seeds incubated in light 15/25°C. No fresh seeds of *C. hybridum* or *S. glauca* germinated at any combination of temperature and light.

**Relationship between soil moisture content and seed moisture content**

There was a significant linear correlation between seed moisture content and soil moisture content for each species (*P* < 0.001, Fig. 2). Seed moisture content increased as soil moisture content increased, while the slope of regression varied with species. The fastest increase in seed moisture content in response to increasing soil moisture content was for *P. lanceolata* and the slowest for *S. glauca*.

**Effect of soil moisture content during cold stratification and incubation temperature on seed dormancy**

Effect of soil moisture content during cold stratification, incubation temperature and their interaction on seed germination varied with species. Soil moisture content, temperature and their interaction had a significant effect on germination of *A. retroflexus* and *C. hybridum*, whereas temperature had no effect on *C. album* and soil moisture content had no effect on *P. lanceolata*. The interaction of soil moisture content and temperature had no effect on seed germination of *P. lanceolata* or *S. glauca* (Table 2).

Germination percentages of *A. retroflexus* seeds increased and then decreased at all tested temperatures, except 10/20°C, as soil moisture content increased (Fig. 3). The effect of soil moisture content on seed germination varied with incubation temperature. For example, the highest germination at 15/25, 20/30 and 25/35°C was attained when seeds were stratified in soil with 8%, 16% and 8% moisture content respectively. Germination percentage increased with increasing germination temperature regardless of soil moisture content during stratification.

Germination percentages of *C. album* seeds increased and then decreased at all test temperatures as soil moisture content increased during stratification (Fig. 3). The effect of soil moisture content on seed germination varied with incubation temperature. For example, the highest germination percentage at 10/20 and 15/25°C was attained when seeds were stratified in soil with 16% and 12% moisture content respectively. Seeds germinated to the highest percentage at 15/25°C and to the lowest percentage at 25/35°C regardless of soil moisture content during stratification.

Germination percentages of *C. hybridum* seeds increased and then decreased as the soil moisture content increased during stratification (Fig. 3). The effect of soil moisture content on seed germination varied with incubation temperature. For example, seeds germinated to the highest percentage at 20/30 and 25/35°C when stratified in soil with 8% and 12% moisture content respectively. Germination percentage increased with increasing germination temperature regardless of soil moisture content during stratification.

Germination percentages of *S. glauca* seeds decreased and then increased as the soil moisture content increased.
content increased (Fig. 3). No difference in germination percentage was observed when soil moisture content was higher than 8%. Germination percentages of *P. lanceolata* showed little response to stratification soil moisture content or incubation temperature, except a decrease in germination at 25/35°C when seeds were stratified in soil with 16% moisture content.

**Correlation of seed moisture content during stratification and germination**

The strength of the correlation between seed moisture content and germination varied with species and incubation temperature (Fig. 4, Tables S1–S5). A quadratic regression provided the best fit for the relationship between seed moisture content and seed germination at all test temperatures for *A. retroflexus*, *C. album*, *C. hybridum*, except for *A. retroflexus* at 10/20°C and *C. hybridum* at 10/20°C and 15/25°C (Fig. 4, Tables S1–S4). In *S. glauca*, there was a significant cubic regression between seed moisture content and germination at each incubation temperature. However, there was no significant relationship between seed

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Estimate</th>
<th>SE</th>
<th>T</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Amaranthus retroflexus</em></td>
<td>Soil moisture content</td>
<td>4.9</td>
<td>1.28</td>
<td>3.82</td>
</tr>
<tr>
<td></td>
<td>Temperature (T)</td>
<td>1.3233</td>
<td>0.0563</td>
<td>23.49</td>
</tr>
<tr>
<td></td>
<td>Soil moisture content × T</td>
<td>1.561</td>
<td>0.485</td>
<td>3.22</td>
</tr>
<tr>
<td><em>Chenopodium album</em></td>
<td>Soil moisture content</td>
<td>21.37</td>
<td>1.09</td>
<td>19.62</td>
</tr>
<tr>
<td></td>
<td>Temperature (T)</td>
<td>-0.0272</td>
<td>0.042</td>
<td>-0.65</td>
</tr>
<tr>
<td></td>
<td>Soil moisture content × T</td>
<td>-3.317</td>
<td>0.374</td>
<td>-8.87</td>
</tr>
<tr>
<td><em>Chenopodium hybridum</em></td>
<td>Soil moisture content</td>
<td>-12.05</td>
<td>1.67</td>
<td>-7.21</td>
</tr>
<tr>
<td></td>
<td>Temperature (T)</td>
<td>0.2443</td>
<td>0.0561</td>
<td>4.36</td>
</tr>
<tr>
<td></td>
<td>Soil moisture content × T</td>
<td>3.544</td>
<td>0.522</td>
<td>6.79</td>
</tr>
<tr>
<td><em>Plantago lanceolata</em></td>
<td>Soil moisture content</td>
<td>-1.12</td>
<td>1.85</td>
<td>-0.61</td>
</tr>
<tr>
<td></td>
<td>Temperature (T)</td>
<td>-0.4226</td>
<td>0.0753</td>
<td>-5.61</td>
</tr>
<tr>
<td></td>
<td>Soil moisture content × T</td>
<td>-0.515</td>
<td>0.588</td>
<td>-0.88</td>
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<tr>
<td><em>Setaria glauca</em></td>
<td>Soil moisture content</td>
<td>38.22</td>
<td>2.09</td>
<td>18.26</td>
</tr>
<tr>
<td></td>
<td>Temperature (T)</td>
<td>0.3342</td>
<td>0.058</td>
<td>5.76</td>
</tr>
<tr>
<td></td>
<td>Soil moisture content × T</td>
<td>-0.475</td>
<td>0.773</td>
<td>-0.61</td>
</tr>
</tbody>
</table>

SE = standard error of estimate value; $t = t$ ratio from regression analysis.
moisture content and germination for *P. lanceolata* regardless of incubation temperature.

The optimal seed moisture content and the critical seed moisture content for dormancy release varied with incubation temperature and species (Table 3). For example, the optimal seed moisture content of *A. retroflexus* that resulted in the highest germination at 15/25, 20/30 and 25/35 °C was 27.5%, 27.3% and 22%, respectively, whereas the optimal seed moisture content for *C. album* for highest germination at 15/25 °C and 20/30 °C was 37.7% and 39.7% respectively. The optimal seed moisture content for dormancy breakage of *A. retroflexus*, *C. album* and *C. hybridum* was 22.0%, 37.7% and 25.7%, respectively, and the critical seed moisture content for dormancy breakage was 4.7%, 16.0% and 9.5% respectively.

**Discussion**

*Seed dormancy and germination requirements*

As a portion of the fresh viable seeds of *A. retroflexus*, *C. album*, *C. hybridum*, *P. lanceolata* and *S. glauca* did not germinate at any test condition, we concluded that they were dormant. These results confirm those of previous research that seeds of the five study species have non-deep physiological dormancy (see Baskin & Baskin, 2014). Although these five species occupy a similar habitat, their germination requirements are different. For example, high temperature favoured seed germination of *A. retroflexus*, but it significantly reduced seed germination of *P. lanceolata* and *C. album*. These results are in agreement with those of Guo and Al-Khatib (2003) who showed that rise temperatures caused higher germination rates in seeds of *A. retroflexus*. Moreover, light had no significant effect on germination percentage of *P. lanceolata*, but significantly increased germination percentages of *A. retroflexus* and *C. album*. A possible reason for the different response to light is that *P. lanceolata* seeds are larger than those of *A. retroflexus* and *C. album*. This is consistent with previous research that the incidence of light-dependence declines with increasing seed size (Fenner & Thompson, 2005). The differences in germination requirements of *P. lanceolata*, *A. retroflexus* and *C. album* may partly contribute to their differentiation in seedling emergence time as well as other life history stages and thus favour their coexistence (Hu et al., 2015). In a three-year field experiment, seeds of *C. album* usually germinated earlier in the spring than those of *A. retroflexus* (Baskin & Baskin, 1977; Agir & Dawson, 1984), which reflects the differences in their temperature requirements for germination.
Relationship between soil moisture content and seed moisture content

There is a positive significant linear relationship between soil moisture content and seed moisture content, suggesting that the variation in seed moisture content is mainly attributed to the changes of soil moisture content. However, due to variation in seed traits such as seed size and seed chemical composition, the relationship between seed moisture content and soil moisture content varies with species. For example, seeds of *P. lanceolata* have a much higher seed moisture content than the other four species at each given soil moisture content. A possible reason for this is that seeds of *P. lanceolata* have a mucilage layer that favours seed imbibition during stratification. As water uptake of seeds is driven by the water potential gradients between seeds and soil, the relationship between soil and seeds may be influenced by the soil types, with texture being the main factor determining soil water retention curves (water potential vs. soil moisture content). Thus, using soil moisture content to explore its role and possible ecological significance in regulating seed dormancy is limited to the cultivated Loess soils in this study, and further study involving soil water potential determination is needed to determine whether these results are consistent across soil types.

Effect of seed moisture content on seed dormancy breakage

It is clear from our results that cold stratification had a significant effect on seed dormancy breakage. This is consistent with earlier reports of common weeds in which seed dormancy is broken by cold stratification, for example *A. retroflexus* (Kępczyński & Szniģir, 2013), *C. album* (Baskin & Baskin, 1987; Matsuo & Kubota, 1988), *C. suecicum* (Scholz) Murr. (Milberg & Andersson, 1998) and *C. rubrum* L. (Galinato & Van Der Valk, 1986).

A major contribution of our study is that soil moisture content (and thus seed moisture content) plays a key role in the breaking of seed dormancy during cold stratification in four of the five tested species. These results agree with those of previous research on trees that seed moisture content must reach a critical level for dormancy breakage to occur during cold stratification (e.g. Jensen, 1997; O’Reilly & Atrip, 2007). In our study, germination of *A. retroflexus*, *C. album* and *C. hybridum* seeds increased and then decreased as soil moisture content increased, suggesting that a certain seed moisture content is necessary for dormancy breakage during cold stratification. However, high seed moisture content inhibited dormancy breakage during
cold stratification. A possible explanation for this inhibition is that some biochemical reaction involving dormancy breaking occurs only when seed moisture content reaches a critical value (Obroucheva & Antipova, 2000), whereas too much water may restrict oxygen exchange, thereby inhibiting seed dormancy breakage. Excess water may also cause decay of the seeds due to fungal or microbial infection. Seeds of Prunus spinosa L. adjusted to 25% moisture content during cold stratification germinated to a higher percentage than those fully imbibed (30%) (Afroze & O’ 2016).

In our study, the optimal soil moisture content for dormancy breakage of A. retroflexus, C. album and C. hybridum seeds was 8%, 12% and 8% respectively. Furthermore, the average monthly soil moisture content at a depth of 0–10 cm in the study area ranges from 6.5% to 14.6% from October to December, which is the time when dormancy release mainly occurs (Li et al., 2014). Thus, in non-irrigated soils of the semi-arid Loess Plateau, it is reasonable to conclude that dormancy release of buried seeds is not restricted by low soil moisture content. On the other hand, dormancy breakage is likely to be restricted by high soil moisture content, especially when seeds are buried at deeper depths, which generally have a relatively high soil moisture content (Bo et al., 2014). Moreover, cool season irrigation traditionally has been used to improve perennial crops in the study area thereby increasing the chance of subjecting seeds to too much soil moisture content for dormancy breakage to occur. However, Boddy et al. (2013) suggested that winter flooding may help control Echinochloa oryzicola Vasinger. in rice fields in California (USA) by decreasing seed dormancy and time to seedling emergence in spring, provided wintertime soil saturation can be maintained for an extended period of time.

There were significant interaction effects between soil moisture contents during cold stratification and incubation temperature. For example, at 4% soil moisture content cold stratified seeds of C. hybridum germinated to their highest percentage at 10/20°C (corresponding to spring), whereas >4% soil moisture content decreased seed germination at 10/20°C but increased it at the other test temperatures (the other seasons). These results suggest that soil moisture content during cold stratification may play a role in fine-tuning seed germination to temperature and thus timing of the germination season.

In contrast to A. retroflexus, C. album and C. hybridum, seed dormancy of S. glauca was broken in seeds buried in dry soil, suggesting that dry after-ripening occurs in this species. This result is in agreement with previous results in which 4 months of dry storage broke seed dormancy of S. glauca seeds (Steel et al., 1983). However, cool and moist conditions obviously accelerated the breaking of seed dormancy in this species (Steel et al., 1983; Fig. 2). Bewley et al. (2013) suggest that after-ripening generally occurs in seeds below a certain water content (20–40%), and it may be prevented by a higher seed water content. In our study, after-ripening of S. glauca seeds seems to be

### Table 3

<table>
<thead>
<tr>
<th>Species</th>
<th>Temperature (°C)</th>
<th>Equation</th>
<th>Prob. &gt; F</th>
<th>Goodness of fit (R²)</th>
<th>Optimal seed moisture content (%)</th>
<th>Critical seed moisture content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amaranthus retroflexus</td>
<td>10/20</td>
<td>$y = -0.004x^2 + 0.185x - 1.254$</td>
<td>0.294</td>
<td>0.13</td>
<td>23.1</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>15/25</td>
<td>$y = -0.103x^2 + 7.879x - 37.724$</td>
<td>0.000</td>
<td>0.92</td>
<td>27.5</td>
<td>6.6</td>
</tr>
<tr>
<td></td>
<td>20/30</td>
<td>$y = -0.156x^2 + 8.529x - 36.213$</td>
<td>0.000</td>
<td>0.85</td>
<td>27.3</td>
<td>8.0</td>
</tr>
<tr>
<td></td>
<td>25/35</td>
<td>$y = -0.076x^2 + 3.345x + 57.245$</td>
<td>0.000</td>
<td>0.71</td>
<td>22.0</td>
<td>4.7</td>
</tr>
<tr>
<td>Chenopodium album</td>
<td>10/20</td>
<td>$y = -0.0072x^2 + 0.5342x - 9.2793x + 53.873$</td>
<td>0.000</td>
<td>0.94</td>
<td>38.2</td>
<td>17.6</td>
</tr>
<tr>
<td></td>
<td>15/25</td>
<td>$y = -0.0055x^2 + 0.3756x - 4.861x + 32.697$</td>
<td>0.000</td>
<td>0.95</td>
<td>37.7</td>
<td>16.0</td>
</tr>
<tr>
<td></td>
<td>20/30</td>
<td>$y = -0.0076x^2 + 0.6087x - 12.406x + 90.764$</td>
<td>0.000</td>
<td>0.94</td>
<td>39.7</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>25/35</td>
<td>$y = -0.005x^2 + 0.387x - 7.842x + 59.511$</td>
<td>0.001</td>
<td>0.67</td>
<td>37.8</td>
<td>–</td>
</tr>
<tr>
<td>Chenopodium hybridum</td>
<td>10/20</td>
<td>$y = 0.0029x^2 - 0.2688x + 7.2519x - 47.559$</td>
<td>0.001</td>
<td>0.69</td>
<td>19.9</td>
<td>9.7</td>
</tr>
<tr>
<td></td>
<td>15/25</td>
<td>$y = 0.003x^2 - 0.2953x + 8.418x - 56.105$</td>
<td>0.009</td>
<td>0.50</td>
<td>20.9</td>
<td>9.6</td>
</tr>
<tr>
<td></td>
<td>20/30</td>
<td>$y = 0.0035x^2 - 0.3582x + 10.607x - 72.803$</td>
<td>0.000</td>
<td>0.75</td>
<td>29.7</td>
<td>10.5</td>
</tr>
<tr>
<td></td>
<td>25/35</td>
<td>$y = 0.0025x^2 - 0.2837x + 9.639x - 68.213$</td>
<td>0.000</td>
<td>0.79</td>
<td>25.7</td>
<td>9.5</td>
</tr>
<tr>
<td>Setaria glauca</td>
<td>10/20</td>
<td>$y = -0.029x^2 + 1.632x - 21.652x + 80.345$</td>
<td>0.000</td>
<td>0.79</td>
<td>28.9</td>
<td>6.7</td>
</tr>
<tr>
<td></td>
<td>15/25</td>
<td>$y = -0.03x^2 + 1.644x - 22.31x + 96.723$</td>
<td>0.000</td>
<td>0.73</td>
<td>27.5</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>20/30</td>
<td>$y = -0.035x^2 + 1.976x - 29.145x + 144.171$</td>
<td>0.001</td>
<td>0.67</td>
<td>27.6</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>25/35</td>
<td>$y = -0.033x^2 + 1.835x - 25.988x + 120.368$</td>
<td>0.001</td>
<td>0.67</td>
<td>27.5</td>
<td>–</td>
</tr>
</tbody>
</table>

– indicates no data.
inhibited by a small (0–4%) increase in soil moisture content, as seeds subjected to 4% soil moisture content (seed moisture content 15.3%) did not germinate regardless of germination test temperature. Although the effects of after-ripening and cold stratification in controlling seed dormancy were not clarified in detail in our study, there obviously is a transition from after-ripening to cold stratification effects on dormancy breakage as soil moisture content increases. Moreover, unlike *A. retroflexus*, *C. album* and *C. hybridum*, high soil moisture content did not significantly inhibit seed dormancy release in *S. glauca*.

The response of seed dormancy release to soil moisture content may have important ecological consequences, as soil moisture content could be changed by many environmental factors as well as by human activities. For example, seeds subjected to different burial depths may have a different moisture content (e.g. soil surface vs. deep soil). In addition, tillage (changing seed position) and irrigation (Kang et al., 2002) may change the moisture condition of seeds and consequently dormancy breaking and germination behaviour. From a practical viewpoint, no-tillage may maintain seeds on the soil surface and thus decrease seed dormancy release efficiency during winter due to the relatively low soil moisture content on the soil surface. Also, in some areas, winter irrigation may expose seeds to high soil moisture content, which could limit seed dormancy release, as discussed above. Thus, the effect of soil moisture content on dormancy status of seeds in the seedbank should be taken into account if weed emergence models are to predict seedling emergence with precision (Batlla & Benech-Arnold, 2006).

Moreover, in a scenario of global climate change, both temperature and rainfall changes potentially may affect seed dormancy release dynamics and thus arrest, delay or enhance regeneration from seeds (Walck et al., 2011). For example, increasing temperature coupled with decreasing rainfall in autumn on the Loess Plateau (Wu et al., 2008) may result in a drier winter, which would reduce seed dormancy release and consequently seedling emergence the next spring. Thus, the breaking of seed dormancy during winter in relation to soil moisture content is of fundamental importance for weed management, as well as understanding ecological processes such as plant species regeneration, migration and co-existence under climate change.

In summary, our study clearly shows that soil moisture content plays a key role in regulating seed dormancy release, and this may have important ecological and agricultural implication in weed management. However, it is also worth noting that there are at least two limitations to this study. First, we use one rather than several durations of stratification, and thus, the possible interaction of effects between stratification duration and soil moisture content in regulating seed dormancy is not considered. The second limitation is that the effect of soil moisture on seed moisture content was examined for only one soil type, and thus, it may be difficult to extrapolate the findings to these species growing on other soil types. Obviously, then, further investigations involving soil water potential measurement are needed to determine the effect of soil moisture content on dormancy breakage in weeds.

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**References**


### Supporting Information

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

- **Table S1** Parameters of regression analyses of seed moisture content and germination percentage at different incubation temperatures of *Amaranthus retroflexus*
- **Table S2** Parameters of regression analyses of seed moisture content and germination percentage at different incubation temperatures of *Chenopodium album*
- **Table S3** Parameters of regression analyses of seed moisture content and germination percentage at different incubation temperatures of *Chenopodium hybridum*
- **Table S4** Parameters of regression analyses of seed moisture content and germination percentage at different incubation temperatures of *Setaria glauca*
- **Table S5** Parameters of regression analyses of seed moisture content and germination percentage at different incubation temperatures of *Plantago lanceolata*