A quantitative analysis of dormancy loss dynamics in *Polygonum aviculare* L. seeds: Development of a thermal time model based on changes in seed population thermal parameters

Diego Batlla* and Roberto Luis Benech-Arnold

IFEVA/Cátedra de Cerealicultura, CONICET/Facultad de Agronomía, Universidad de Buenos Aires, Av. San Martín 4453, C1417DSE-Buenos Aires, Argentina

Abstract

A model for simulating *Polygonum aviculare* L. seed dormancy loss in relation to stratification temperature was developed. The model employs the lower limit temperature for germination (T_l) as an index of seed population dormancy status. While population mean for T_l ($T_{l(50)}$) and T_l distribution within the population (σ_{Tl}) are allowed to vary as seeds are released from dormancy, other thermal parameters characterizing the germination thermal responses (base, optimal and maximal temperatures, and thermal time required for germination) and the higher limit temperature for germination (T_h) are held constant. In order to relate changes in $T_{l(50)}$ and σ_{Tl} to variable time and temperature, a stratification thermal time index (S_{tt}) was developed, which consists of the accumulation of thermal time units under a threshold temperature for dormancy loss to occur. Therefore, $T_{l(50)}$ and σ_{Tl} varied in relation to the accumulation of S_{tt} according to time and temperature. To derive model equations, changes in seed population thermal parameters were estimated for buried seeds stored at 1.6, 7 and 12°C for 110 d. Seeds were exhumed at regular intervals, and were incubated at 15°C and at a gradually increasing temperature regime in the range 6–25°C. The germination time-course curves obtained were reproduced using a mathematical model. Thermal parameters that best fit simulated and experimentally obtained germination time-course curves were determined. Model performance was evaluated against data of two unrelated experiments, showing acceptable prediction of timing and percentage of germination of seeds exhumed from field and controlled temperature conditions.

Keywords: dormancy, germination, *Polygonum aviculare*, stratification, thermal time

*Correspondence
Fax: +54–11–4524–8039
Email: batlla@mail.agro.uba.ar

Introduction

Dormancy is a common attribute of many weed seed populations, and this hampers the task of predicting emergence from weed seed banks under field conditions (Benech-Arnold and Sánchez, 1995). Consequently, accurate predictions of timing and proportion of weed emergence depend largely on an understanding of the dynamics of dormancy in relation to the environment (Murdoch, 1998). Although there is abundant information related to dormancy in weed species, few attempts have been made to model seed dormancy changes as affected by the environment (Forcella *et al.*, 2000).

In adapted weed species, dormancy is alleviated during the season preceding the period with favourable conditions for seedling development and plant growth, while dormancy induction takes place in the period preceding the season with environmental conditions unsuitable for plant survival (Karssen, 1982; Benech-Arnold *et al.*, 2000). Vegis (1964) suggested that dormancy alleviation could be associated with a widening of the thermal range that permits seed germination. Conversely, dormancy induction would be associated with a narrowing of the thermal range. Germination under field conditions is, therefore, restricted to the period when field temperature and the temperature range for germination overlap (Vleeshouwers *et al.*, 1995; Vleeshouwers and Bouwmeester, 2001).

Of the many environmental factors that regulate seed behaviour under field conditions, temperature has been identified as one of the main factors governing changes in dormancy status of seed populations (Bouwmeester and Karssen, 1992). For the case of the summer annual *Polygonum aviculare* L., Kruk and Benech-Arnold (1998) demonstrated that low winter temperatures alleviate dormancy, producing a widening of the thermal range permissive for germination as a consequence of a progressive decrease of the lower limit temperature

for germination of the population (T_l). In contrast, high summer temperatures reinforce dormancy, which results in a narrowing of the thermal range permissive for germination through an increase of T_l. In this work, the authors clearly distinguished between thermal parameters associated with dormancy status of the seed population (limit temperatures establishing the permissive germination range) and thermal parameters that characterize the germination response of the seed population (base temperature (T_b), optimal temperature (T_o), maximal temperature (T_m) and mean thermal time required for germination ($\theta_{(50)}$). Changes in seed population dormancy status were associated with changes in T_l, while other seed population germination thermal parameters (i.e. T_b, T_o, T_m and $\theta_{(50)}$) were assumed to remain almost constant irrespective of changes in seed dormancy status.

Totterdell and Roberts (1979) hypothesized that temperature-dependent changes in dormancy of summer annuals result from two simultaneous subprocesses: (1) relief of primary dormancy as a result of exposure to temperatures less than 15°C; (2) induction of secondary dormancy with a rate that rises with higher prevailing temperatures. Based on these hypotheses, Bouwmeester and Karssen (1992) developed a simulation model of seasonal changes in dormancy for *Polygonum persicaria* L. that considers dormancy status as a function of cold and heat unit sums. This model allowed the estimation of the width of the thermal range permissive for germination for seeds that had been buried for different periods of time and, thus, exposed to a variable thermal environment. The model successfully predicted the timing of emergence of this weed species in the field. However, it was not an aim of the model to estimate which proportion of the seed bank population would have emerged within the emergence 'time window'.

The possibility of predicting seedling emergence is essential for improving weed management decisions (Murdoch, 1998). For this purpose, not only would it be necessary to predict changes in mean population thermal parameters that characterize seed dormancy status, but also to account for changes in their distribution within the population. Several reports on germination behaviour of different species indicate that limit temperatures demarcating the germination range vary among individuals belonging to the same seed population (Washitani, 1987; Kebreab and Murdoch, 2000). The importance of accounting for this variation to predict germination percentages of a seed population can be exemplified by considering two seed populations with the same mean T_l ($T_{l(50)}$) (i.e. 15°C), but different standard deviations of T_l (σ_{Tl}) (i.e. batch (A) $\sigma_{Tl} = 4$; batch (B) $\sigma_{Tl} = 1$). Assuming a normal distribution of T_l within the population (Washitani, 1987; Kebreab and Murdoch, 2000) and

that all seeds with a T_l lower than germination test temperature would germinate, incubating seeds at 14°C will result in 40% of germination in batch (A), while only 15% of the seed population will germinate in batch (B). If dormancy status of a seed population could be characterized by population mean limit temperatures for germination, dispersion of these parameters could be correlated with variation in the degree of dormancy observed among seeds belonging to the same population. However, no research has been conducted to investigate how dormancy changes can affect the distribution of these parameters within the population. Such a population approach has been used to characterize seed responses to other factors affecting seed germination and dormancy, e.g. water availability, hormones and light (Bradford, 1996, 2002).

In this work, the effects of stratification temperature on the dynamics of dormancy release of *P. aviculare* seeds were studied. Changes in dormancy status were assessed by changes in seed thermal parameters (lower and higher limit temperature permissive for germination) and their distribution within the population, using a modified version of the screening methodology proposed by Washitani (1987). Based upon these results, a simple thermal time model was developed to predict changes in seed mean thermal parameters and their distribution within the population in relation to temperatures that alleviate dormancy.

Materials and methods

Stratification treatments

Seeds of *P. aviculare* were collected in a wheat field at Balcarce (latitude 37°45′S, longitude 58°15′W), Argentina, at the time of their natural dispersal (March 2000). Seeds were winnowed, in order to eliminate light seeds, and stored in glass jars at ambient temperature (*c.* 20°C) for 40 d until the experiment commenced. In May 2000, groups of approximately 300 seeds were placed inside nylon mesh bags and buried at 5 cm depth in 12 cm diameter black plastic pots filled with soil, previously oven-dried at 70°C for 3 d. Pots were irrigated to saturation, sealed at top with black nylon and stored at different temperatures (1.6, 7 and 12°C). Pots were allowed to drain for 48 h, following which the black nylon that sealed the pots was removed, and the pots were weighed to determine the weight corresponding to field capacity for each pot. At regular intervals during the storage period, pots were re-weighed, and water was added until they reached their original weight to maintain initial field capacity status. Soil temperature in the pots was recorded hourly during

the storage period using temperature sensors (LI-COR model 1015, Lincoln, NE, USA) connected to a DataLogger (LI-COR model 1000, Lincoln, NE, USA). At the beginning of the experiment, three replicates of 35 seeds that had not been buried were exposed to an initial germination test, to quantify the initial dormancy status of the population. At intervals of 15 d throughout the storage period, three nylon mesh bags containing seeds (three replications) were exhumed from pots placed at each storage temperature and tested for germination. The whole experiment lasted 110 d. Afterwards, one pot from each temperature was transferred to a chamber at 22°C to determine whether non-dormant seeds could be re-induced into secondary dormancy at higher storage temperatures. After 12 d of storage at 22°C, seeds were exhumed and exposed to the germination test.

Germination test

Seeds were exhumed from the mesh bags under fluorescent white light and were rinsed with distilled water to remove adhered soil particles. Replicates of 35 seeds were then placed in 9-cm diameter Petri dishes containing 5 ml of distilled water on two discs of Whatman No. 3 filter paper. Three replications were exposed to a gradually increasing temperature regime, ranging from 6 to 25°C. Since germination rate is generally greater at higher temperatures in the physiological range, the duration of exposure to each temperature differed, depending on the prevailing incubation temperature (Washitani, 1987): 6 d at 6°C, 5 d at 10°C, 4 d at 15°C, 3 d at 20°C, and 2 d at 25°C. Another three replications were incubated at 15°C in a controlled temperature chamber for 15 d. After 24 h of incubation in either regime, seeds were exposed to a 15 min red-light pulse, to bypass the light requirement for germination (Baskin and Baskin, 1988). The number of germinated seeds was recorded immediately before each temperature change in the increasing temperature regime, and at regular intervals during the incubation period at the constant 15°C regime. Seeds were exposed to white fluorescent light during germination counting, but otherwise were maintained in the dark inside temperature chambers. The criterion for seed germination was visible radicle protrusion, and germinated seeds were removed after they were counted.

Quantification of seed population thermal parameters

Thermal parameters for seeds exhumed during the storage period were quantified using a mathematical germination model described in detail by Washitani (1987). The model predicts germination dynamics of a seed population as a function of time and temperature. Thus, germination time-course curves obtained for seeds stored at each temperature (1.6, 7 and 12°C) using the previously described germination test (germination curves from seeds incubated at the increasing temperature regime and at the constant 15°C regime), were reproduced by simulation for each exhumation. The model allows the estimation of two kinds of population thermal parameters in relation to observed data: (1) those describing dormancy status of the seed population, and (2) those describing the relationship between germination rate and temperature of individual seeds. Briefly, model assumptions are as follows (Washitani, 1987):

(1) A given seed can germinate only within the temperature range between two limits, i.e. a lower limit temperature (T_l) and a higher limit temperature (T_h), and both limit temperatures vary among seeds within a seed population following a normal distribution function. Therefore, the fraction (F_l) of seeds with lower limit temperature below a given temperature (T) can be given by the following distribution function:

$$F_l(T) = \int_{-\infty}^{(T-\mu_{Tl})/\sigma_{Tl}} \left(1/\sqrt{2\pi}\right) \cdot \exp\left(-\chi^2/2\right) dx \quad (1)$$

where μ_{Tl} and σ_{Tl} are the mean and standard deviation of T_l. Similarly, the fraction (F_h) with a higher limit temperature higher than T, can be given by the following distribution function:

$$F_h(T) = \int_{(T-\mu_{Th})/\sigma_{Th}}^{\infty} \left(1/\sqrt{2\pi}\right) \cdot \exp\left(-\chi^2/2\right) dx \quad (2)$$

where μ_{Th} and σ_{Th} are the mean and standard deviation of T_h.

(2) The temperature dependency of the mean germination rate of individual seeds is assumed to be approximated by two linear equations with four parameters, i.e. base temperature (T_b), optimal temperature (T_o), maximal temperature (T_m), and thermal time required for germination (θ):

for the suboptimal range,

$$r = 1/\theta \cdot (T - T_b), \quad (3)$$

while for the supraoptimal range,

$$r = 1/\theta \cdot (T_o - T_b) \cdot [(T_m - T)/(T_m - T_o)] \quad (4)$$

Cardinal temperatures $(T_b, T_o$ and $T_m)$ are assumed not to vary among individual seeds of the same population.

(3) The distribution of θ within a seed population can be described by the following distribution function:

$$F_t(\theta) = 1 - [3D^{-3}(\theta - m + D)^3 + 1]^{-\frac{1}{2}}, \quad (5)$$

where m is the median of the distribution, i.e. the required thermal time for the seeds that germinate at the cumulative percentage of 50%, and D is the differential thermal time between 0 and 50% germination.

(4) The distribution of T_l, T_h and θ are assumed independent of each other.

Therefore, the cumulative percentage of germination (G) after time (t) from the start of imbibition for seeds incubated at a constant temperature (T) regime can be calculated combining the following equations:

when $T < T_b$ or $t(T - T_b) < m - D$ or $T > T_m$,

$$G(T, t) = 0, \quad (6)$$

when $T_b < T < T_o$ and $t(T - T_b) > m - D$,

$$G(T,t) = F_l(T) \cdot F_h(T) \cdot F_t[t(T - T_b)], \quad (7)$$

when $T_o < T < T_m$ and $t(T - T_b) > m - D$,

$$G(T,t) = F_l(T) \cdot F_h(T) \cdot F_t[t(T_m - T) \cdot (T_o - T_b)/ \\ (T_m - T_o)] \quad (8)$$

For seeds incubated at the increasing temperature regime, the cumulative germination percentage (G_i) at the time of temperature change from the ith to the $i+1$th temperature can be approximated by the following expressions:

$$G_0 = 0, \quad (9)$$

$$G_i = G_{i-1} + [F_l(T_i) \cdot F_t(h_i) - G_{i-1}] \cdot F_h(T_i), \quad (10)$$

where h_i is the accumulated thermal time at the time of temperature change from the ith to $i+1$th temperature.

T_l and T_b should be clearly distinguished from each other in the suboptimal range. T_l is a parameter for characterizing the dormancy status of the population, and indicates the lower limit of the thermal range permissive for germination. T_b is the theoretical temperature above which thermal time is accumulated after temperature enters that permissive thermal range. While T_l is distributed within the seed population, T_b is assumed to be constant among individual seeds. Similarly, in the supraoptimal range, T_h (a parameter characterizing dormancy) should be distinguished from T_m. On the other hand, m should also be clearly distinguished from D. While m is the thermal time accumulated between seed imbibition and 50% germination, D is the thermal time accumulated between the germination of the first seed (just after 0% germination) and 50% germination.

To run the model for simulation of the germination curves obtained with the germination test, values for T_b and T_o were given *a priori* to the program. Such values were those reported for *P. aviculare* by Kruk and

Benech-Arnold (1998) ($T_b = 0°C$ and $T_o = 16°C$). The program relating the equations of the germination model previously described was developed in the Squeak Smalltalk (Anonymous, 2000) computing environment, and is a modified version of that previously developed in DOS language by Kruk and Benech-Arnold (1998). This version has an optimization module, which optimizes values for each seed population thermal parameter to maximize the fit of simulated curves with experimentally obtained ones. Optimization is performed by a dynamic hill-climbing global optimization algorithm (Russell and Norvig, 1995). The criterion used for thermal parameter optimization was minimum root mean square error (RMSE). The value of RMSE used for optimization was the average of the RMSE resulting from the fit of both germination regime curves of the germination test.

Generation of the dormancy loss model

Our objective was to establish functional relationships between dormancy loss in the seed population and stratification temperature. As pointed out earlier, dormancy loss in *P. aviculare* and other summer annuals can be quantified by changes in T_l, so this thermal parameter was used as an indicator of the dormancy status of the seed population. However, dormancy status varies among seeds belonging to the same population (Ransom, 1935; Probert, 1992; Bradford, 1996). Thus, in order to predict the fraction of the seed population that will emerge at a certain time, functional relationships between changes in the distribution (standard deviation) of T_l within the population as a result of dormancy changes and stratification temperature must be established. The process of model development is summarized in the following paragraphs:

(1) Determine seed population thermal parameters that maximize the fit between experimentally obtained germination test curves for seeds buried at 1.6, 7 and 12°C and simulated germination curves constructed with the mathematical germination model for each exhumation during the storage period.

(2) Characterize changes in $T_{l(50)}$ and σ_{Tl} of the seed population over time as a function of seed stratification temperature, and derive thermal time equations relating rate of change in $T_{l(50)}$ and σ_{Tl} to temperature.

(3) Using the developed equations relating changes in $T_{l(50)}$ and σ_{Tl} of the seed population to thermal time, predict changes in both parameters of seeds buried under measured temperature in field and environmentally controlled situations.

(4) Use predicted values of $T_{l(50)}$ and σ_{Tl} to simulate germination curves using the equations described

in the mathematical germination model, and compare results with experimentally obtained independent data curves.

Evaluation of the model

In order to evaluate model performance with independent data, results from two unrelated experiments carried out under field and controlled conditions were used.

Field experiment

Seeds of *P. aviculare* were collected in a wheat field at Balcarce, Argentina, at the time of their natural dispersal (March 1999). Seeds were winnowed to eliminate light seeds and stored in glass jars at ambient temperature (*c.* 20°C) for 45 d until the experiment was set up. In May 1999, groups of approximately 300 seeds were placed inside nylon mesh bags and buried 5 cm deep in the experimental field of the Facultad de Agronomía, Universidad de Buenos Aires, Buenos Aires (latitude 34°25′S, longitude 58°25′W), Argentina. Around the time of previously reported emergence season for this species (late winter–early spring) (Kruk and Benech-Arnold, 1998) during two consecutive years, seeds were exhumed and exposed to a similar germination test system as the one described previously. Seeds were incubated at 15°C and at a gradually increasing temperature regime (7 d at 4°C, 6 d at 8°C, 5 d at 12°C, 4 d at 16, 20 and 24°C, 3 d at 28°C, 2 d at 32°C and 1 d at 36°C). Soil temperature in the plots was recorded hourly at 5 cm depth using temperature sensors (LI-COR model 1015) connected to a DataLogger (LI-COR model 1000). Plots were hand-weeded throughout the experimental period.

Experiment under controlled conditions

Seeds of *P. aviculare* contained in nylon mesh bags were buried in pots and exposed to storage temperatures (1.6, 7 and 12°C) as in the treatments described previously. Seeds were exhumed at regular intervals and exposed to a 60 min red-light pulse. Afterwards, dishes containing seeds were wrapped in aluminium foil, in order to avoid light reaching the seeds, and were incubated at constant 15°C for 15 d. Germination was recorded at the end of the incubation period. Handling and manipulation of seeds during exhumation and conditioning of seeds for incubation were carried out in total darkness.

Results
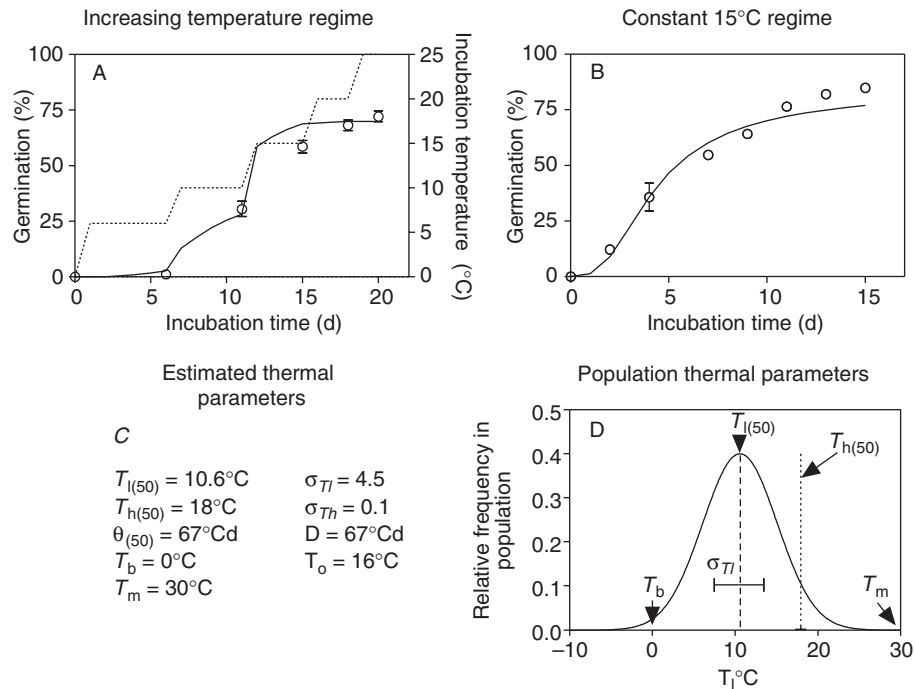
Seed population thermal parameters

Seed population thermal parameters that maximize the fit of simulated germination curves with curves obtained from experimental data were estimated for each exhumation date at each storage temperature. An example of simulated and experimental curves fit for the increasing temperature regime and the constant 15°C regime, and optimum thermal parameters obtained for seeds exhumed after 110 d of storage at 7°C, is shown in Fig. 1.

To estimate seed population thermal parameters, it was assumed that T_b and T_o did not vary during the stratification period, so fixed values of 0 and 16°C, respectively, were used for parameter optimization. These assumptions were based on previous reports for *P. aviculare* and other annual weed species, which suggested no variation of these parameters during different burial periods in the soil (Vleeshouwers, 1997; Bauer *et al.*, 1998; Kruk and Benech-Arnold, 1998, 2000). Values for other parameters were allowed to vary over a reasonable range, based on previously reported data for *P. aviculare* or related summer annual species found in the literature (Washitani, 1987; Bouwmeester, 1990; Kruk and Benech-Arnold, 1998). Results of a preliminary quantification of changes in seed population thermal parameters at the three tested storage temperatures showed little variation in T_h (18°C) and T_m (30°C) during the stratification period. This was consistent with the fact that, generally, very few seeds (0–9%) germinated above 15°C when seeds were exposed to the increasing temperature regime (data not shown). Almost full germination of remaining non-germinating seeds during the germination test with further incubation for extended periods at 6°C, showed that non-germinating seeds were viable but dormant (data not shown). Therefore, final estimation of optimum seed population thermal parameters was made using constant values of T_b (0°C), T_o (16°C), T_h (18°C) and T_m (30°C) (Tables 1a, b, c and 2).

Almost no seeds germinated in the initial germination test, indicating the intense dormancy of the population shortly after dispersal. This prevented the quantification of initial seed population thermal parameters (Table 1a, b, c). On the other hand, seeds buried at 12°C did not germinate until 38 d of storage, thus impeding the calculation of seed population thermal parameters for shorter storage periods in this treatment (Table 1c). RMSE was calculated as an estimator of the goodness of curve fitting for the estimated thermal parameters. Low values of RMSE were obtained for seeds stored at 1.6 and 7°C (Table 1a, b); however, seeds buried at 12°C showed relatively high values of RMSE (Table 1c). This was due to the fact that seeds stored at 12°C showed higher germination percentages at the constant 15°C incubation regime for most exhumation dates than would be expected using the model equations from germination percentages registered in the increasing temperature regime.

Figure 1. Quantification of population thermal parameters for *P. aviculare* seeds stored for 110 d at 7°C using the germination test system and the germination model equations. (A) Predicted (solid line) and observed (symbols) germination time-course curves for *P. aviculare* seeds incubated at the increasing temperature regime. Dotted line (right axis) represents the course of temperature change during seed incubation. Vertical bars indicate SE. (B) Predicted (solid line) and observed (symbols) germination time-course curves for *P. aviculare* seeds incubated at the constant 15°C regime. Vertical bars indicate SE. (C) Estimated thermal parameter values obtained by simulation of the germination time-course curves for seeds incubated at the increasing temperature regime (A) and at the constant 15°C regime (B). Simulation was performed using equations corresponding to the germination model, and optimum thermal parameters were obtained by minimizing root mean square error between simulated and experimentally obtained data in both incubation regimes. (D) T_l plotted as a normal frequency distribution. The solid curve is the normal distribution of T_l values among individual seeds, which is characterized by $T_{l(50)}$ and its corresponding standard deviation (σ_{Tl}). Values of T_b, T_m and $T_{h(50)}$ are indicated by arrows.

Effect of stratification temperature on changes in seed population thermal parameters

A progressive decrease in $T_{l(50)}$ was observed at all seed burial temperatures during the storage period (Fig. 2A). The rate of change was dependent on the temperature at which the seeds were stratified. Lower temperatures produced a greater rate of $T_{l(50)}$ decrease in comparison to higher temperatures of storage. The rate at which $T_{l(50)}$ decreased, as a function of days of storage at any temperature, was accurately described by linear regression functions.

Changes in the values of σ_{Tl} showed different patterns, depending on stratification temperature (Fig. 3A). Seeds stored at 1.6°C showed a low σ_{Tl} (close dispersion of T_l around mean for the population) after 12 d of burial; however, 12 d later a marked increase in this parameter was observed.

From there onward, exhumations showed a progressive decrease in σ_{Tl}, accompanying that observed in $T_{l(50)}$. A similar trend was observed for seeds stored at 7°C, although estimated σ_{Tl} values were always lower than those observed for seeds stored at 1.6°C. Finally, seeds stored at 12°C showed low σ_{Tl} values during the entire burial period. $T_{h(50)}$ was assumed constant (18°C) during burial at any temperature, and T_h generally showed low σ_{Th} values (Table 1a, b, c). However, seeds stored at 1.6°C displayed relatively high values in σ_{Th} after 38 d of burial (Table 1a). $\theta_{(50)}$ showed little variation between exhumations for seeds buried at the three tested temperatures (Table 1a, b, c). Nevertheless, seeds stored at 1.6°C mostly showed lower values for this parameter than seeds stored at the other two temperatures. In addition, an increase in the value of $\theta_{(50)}$ was detected in seeds stored at 12°C when

Table 1. Estimated population thermal parameters for *P. aviculare* seeds stored at (a) 1.6°C; (b) 7°C and (c) 12°C. Parameters were obtained by simulation of the germination time-course curves for seeds incubated at the increasing temperature regime and at the constant 15°C regime for each exhumation day. Simulation was performed using the equations described in the germination model (equations 1–10). A dash means that estimation of population thermal parameters was not possible, due to zero germination in the germination test

(a) 1.6°C

Days of storage	$T_{1(50)}$ (°C)	σ_{Tl}	$T_{h(50)}$ (°C)	σ_{Th}	$\theta_{(50)}$ (°Cd)	D (°Cd)	RMSE
0	–	–	–	–	–	–	–
12	15.5	6.2	18	0.9	57	55	2.4
24	13.9	13.9	18	0.1	44	44	2.8
38	13	13	18	5	49	49	4.2
53	12	11.7	18	2	47	47	2.1
67	11.5	11.5	18	3	40	40	3.2
81	9	9	18	5	37	37	4.7
97	8	8	18	9	57	57	7.8
110	7	7	18	9	56	56	5.6

(b) 7°C

Days of storage	$T_{1(50)}$ (°C)	σ_{Tl}	$T_{h(50)}$ (°C)	σ_{Th}	$\theta_{(50)}$ (°Cd)	D (°Cd)	RMSE
0	–	–	–	–	–	–	–
12	18	1.8	18	0.2	77	20	0.3
24	17	2	18	0.19	75	75	8.3
38	17.2	7.3	18	0.1	74	66	2.4
53	15	5	18	0.1	60	60	11.2
67	14	4.5	18	0.1	64	64	10
81	8.9	7	18	2.6	45	45	1.8
97	10.7	6	18	0.1	56	56	3.3
110	10.6	4.5	18	0.1	67	67	5

(c) 12°C

Days of storage	$T_{1(50)}$ (°C)	σ_{Tl}	$T_{h(50)}$ (°C)	σ_{Th}	$\theta_{(50)}$ (°Cd)	D (°Cd)	RMSE
0	–	–	–	–	–	–	–
12	–	–	–	–	–	–	–
24	–	–	–	–	–	–	–
38	17.5	2	18	0.1	55	15	4.3
53	17	1.1	18	0.1	60	30	2
67	16	3.5	18	0.1	55	20	17.8
81	15.5	3	18	0.1	55	40	11.7
97	14	2.5	18	0.1	85	85	19
110	14	1.5	18	0.1	80	80	24.2

Table 2. Estimated population thermal parameters for *P. aviculare* seeds stored at 1.6, 7 and 12°C for 110 d, followed by storage at 22°C for 12 d. Parameters were obtained by simulation of the germination time-course curves for seeds incubated at the increasing temperature regime and at the constant 15°C regime. Simulation was performed using the equations described in the germination model (equations 1–10). A dash means that estimation of population thermal parameters was not possible, due to the low germination percentage (less than 7%) obtained in the germination test

Storage temperature (°C)	$T_{1(50)}$ (°C)	σ_{Tl}	$T_{h(50)}$ (°C)	σ_{Th}	$\theta_{(50)}$ (°Cd)	D (°Cd)	RMSE
1.6	12	8	18	2	60	20	11.2
7	14	4.8	18	1.2	67	23	2.8
12	–	–	–	–	–	–	–
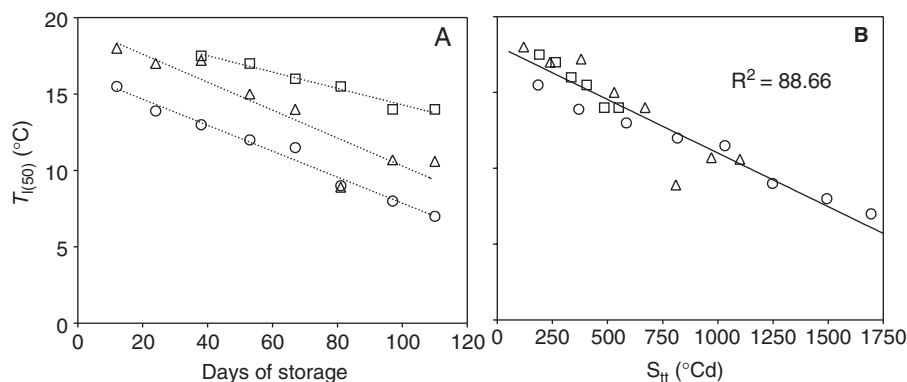
Figure 2. Estimated values of the mean lower limit temperature ($T_{l(50)}$) for *P. aviculare* seeds stored at 1.6°C (○), 7°C (△) and 12°C (□), plotted against days of storage (A), and against stratification thermal time (S_{tt}) (B). The dotted lines in (A) were fitted linear equations for each storage temperature, with R^2 values of 0.98, 0.84 and 0.96, respectively. The fitted line in (B) is the result of repeated regression analysis to obtain the threshold 'ceiling' temperature (T_c) with the best fit according to equation (11). The value of parameters corresponding to equation (12) are slope ($T_{l(50)}$ decrease rate) = −0.007 ± 0.0005°C per °Cd and y-axis intercept (initial $T_{l(50)}$ of the seed population) = 18.07 ± 0.4°C.

exhumed 97 and 110 d after burial (Table 1c). The thermal parameter *D* is an estimator of the distribution of θ within the seed population. For seeds buried at 1.6 and 7°C, high values of *D* were estimated during almost the whole storage period, suggesting that θ was widely distributed within the seed population (Table 1a, b). However, seeds stored at 12°C showed a progressive increase of *D* with days of burial (Table 1c). These results suggest that the θ distribution within the seed population could be modified during initial phases of the process of seed dormancy loss. Washitani (1985) found a wider distribution of θ within a *Geranium carolinianum* L. seed population, as a consequence of dormancy loss due to storage at 4°C under moist conditions.

For seeds that had been released previously from dormancy at 1.6 and 7°C, storage at 22°C for 12 d resulted in an increase in $T_{l(50)}$ values, suggesting an induction of secondary dormancy due to seed exposure to high temperatures (Table 2). Induction of secondary dormancy in *P. aviculare* seeds by temperatures of *c.* 20°C has been documented previously by other authors (Courtney, 1968; Kruk and Benech-Arnold, 1998). Slight changes in $θ_{(50)}$ were observed in relation to the values estimated during the low-temperature burial period; however, the lower *D* values obtained suggest that high storage temperatures produce a narrowing of the distribution of θ within the population. Finally, storage at 22°C of seeds that had previously been buried at 12°C showed very low germination percentages (less than 7%) at both incubation regimes of the germination test; this high dormancy status of the seeds prevented calculation of seed population thermal parameters (Table 2).

Model development

A simple dormancy loss model was developed to predict progressive changes in seed population dormancy parameters ($T_{l(50)}$ and $σ_{Tl}$) as a function of stratification temperature. The results showed that the lower the temperature, the higher the rate of dormancy loss, verified as a progressive decrease of $T_{l(50)}$ (Fig. 2A). This suggests that for *P. aviculare*, as in other summer annual weed species, the rate of dormancy loss during burial is negatively correlated with the prevailing temperature. To account for the effect of temperature on seed population dormancy status, changes in $T_{l(50)}$ were predicted as a function of the accumulation of stratification thermal time units (S_{tt}) under a 'ceiling' threshold temperature for dormancy release to occur, according to the following equation:

$$S_{tt} = Days \cdot (T_c - T_s) \quad (11)$$

where T_c is the dormancy release 'ceiling' temperature (the temperature at, or over, which dormancy release does not occur) and T_s is the daily mean storage temperature. Optimal T_c was obtained using different values of this parameter until best fit of the linear regression between $T_{l(50)}$ and S_{tt} was attained. Figure 2B shows a high correlation coefficient between $T_{l(50)}$ and S_{tt} for a T_c of 17°C, according to the following function:

$$T_{l(50)} = −0.007 \cdot S_{tt} + 18.07 \quad (12)$$

where 18.07 (the initial $T_{l(50)}$ of the population) was obtained by extrapolation of the linear function.

$σ_{Tl}$ also varied during dormancy loss (Fig 3A). However, in contrast to the negative linear

relationship observed between $T_{l(50)}$ and days of storage at the three burial temperatures, σ_{Tl} showed particular trends depending on the temperature experienced by the seeds. For quantification of changes in σ_{Tl} in relation to S_{tt}, a new equation was developed (Fig. 3B). This equation relates changes in σ_{Tl} to the $\ln(S_{tt}/100)/T_s$, using the following function:

$$\sigma_{Tl} = -11.28 \cdot (\ln(S_{tt}/100)/T_s)^2 + 23.91 \cdot (\ln(S_{tt}/100)/T_s) \quad (13)$$

The relationship found between σ_{Tl} and $\ln(S_{tt}/100)/T_s$ implies that changes in σ_{Tl} depend not only on the accumulation of S_{tt}, but also on the daily mean temperature at which a certain value of S_{tt} was accumulated.

Based on results obtained, and previous results reported by other authors, and for practical modelling reasons, the present model assumes that $\theta_{(50)}$ and $T_{h(50)}$ did not change during seed burial.

Model performance

Model performance was evaluated using data from a field experiment and an independent experiment carried out under controlled conditions, as described above. To contrast model output against the data obtained under field conditions for each exhumation date, changes in $T_{l(50)}$ and σ_{Tl} were simulated using equations (11–13) of the dormancy loss model, in relation to daily mean soil temperature recorded at 5 cm deep in the experimental field plots. Accumulation of S_{tt} began when the recorded daily mean soil temperature was below T_c (17°C). To calculate $T_{l(50)}$ and σ_{Tl} for seeds exhumed during the second year (2000), S_{tt} was reset to 0 at the beginning of the year. This assumption was based on the fact that seeds, exhumed during summer of the first year

of burial, showed similar germination response (no germination) to that observed in the germination test for recently dispersed seeds prior to burial. Using predicted values of $T_{l(50)}$ and σ_{Tl} for each exhumation date, germination dynamics in the increasing temperature regime and the constant 15°C regime were predicted by replacing these parameters in the equations describing the Washitani germination model [equations (1–10)]. To simulate germination under both incubation regimes, $\theta_{(50)}$, $T_{h(50)}$ and σ_{Th} were assumed constant during burial in the soil, with values of 60°Cd, 18°C and 0.1, respectively. θ was assumed to be widely distributed within the seed population, so a D value of 60°C was used. Population cardinal temperatures characterizing the response of seed germination to temperature were: $T_b = 0°C$, $T_o = 16°C$ and $T_m = 30°C$. Therefore, if the prevailing germination test temperature enters the permissive range for germination of a certain fraction of the seed population, which depends on predicted values of $T_{l(50)}$ and σ_{Tl}, accumulation of θ for that fraction above T_b was assumed to start simulating germination dynamics at each incubation regime. For seeds exhumed on July 1999, the dormancy model output was $T_{l(50)} = 16.24°C$ and $\sigma_{Tl} = 1.66$ (Fig. 4, upper panels). Although the model predicted low germination percentages at both incubation regimes, simulated data overestimated observed germination values. However, the model gave a good prediction of the temperature at which the seed population began germination (15°C) in the increasing temperature regime. At the end of the 2000 autumn season (May), low values of S_{tt} had accumulated, resulting in a $T_{l(50)}$ of 17.7°C and a low value of σ_{Tl} (0.22) (Fig. 4, middle panels). Simulated values matched those registered in the increasing temperature regime, where no germination was observed. In the constant incubation
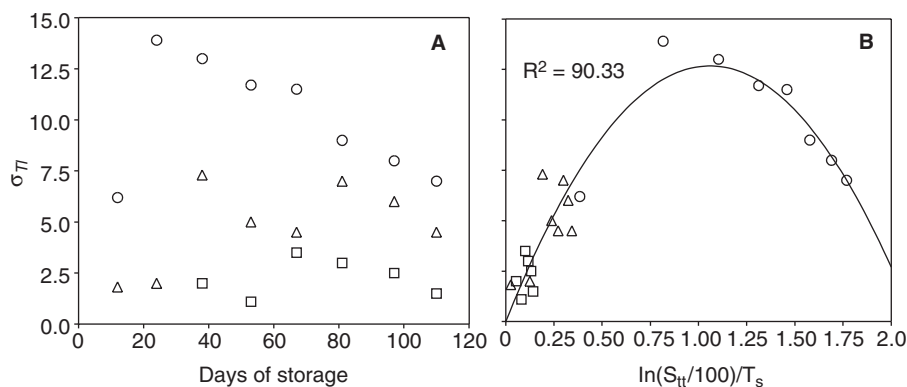
Figure 3. Estimated values of standard deviation of the lower limit temperature (σ_{Tl}) for *P. aviculare* seeds stored at 1.6°C (○), 7°C (△) and 12°C (□), plotted against days of storage (A), and against the $\ln(S_{tt}/100)/T_s$, where S_{tt} is stratification thermal time and T_s is the daily mean storage temperature (B). The line (B) was fitted according to equation (13). Coefficients of the quadratic function (13) are A = 0 (fixed constant value), B = 23.91 ± 1.3 and C = −11.28 ± 0.9.
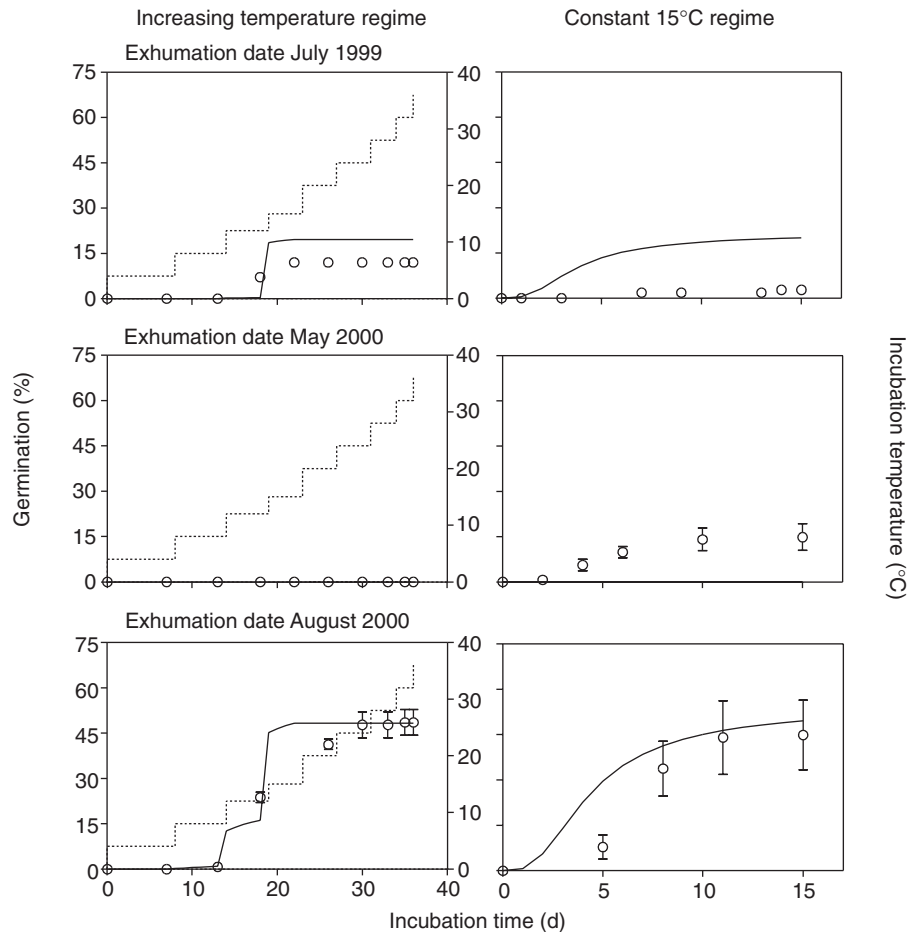
Figure 4. Predicted and observed germination time-course curves for seeds of *P. aviculare* exhumed from the field during the 1999 and 2000 emergence season, and incubated at the increasing temperature regime (left panels) and at the 15°C constant regime (right panels). Symbols represent observed germination percentages for seeds exhumed from the field, while solid lines represent values predicted from simulation modelling for both panels. The dotted line in the left panels (right axis) represents the course of temperature change during incubation of seeds at the increasing temperature regime. Vertical bars indicate SE.

regime, low germination values were registered, while simulation predicted no germination. Finally, for seeds exhumed at the end of winter (August 2000), the model estimated values of $T_{l(50)} = 14.6$°C and $\sigma_{Tl} = 3.12$ (Fig. 4, lower panels). In this case simulated data provided a good description of the observed seed population germination dynamics during both incubation regimes.

To contrast the model output against data obtained for the independent controlled conditions experiment, changes in $T_{l(50)}$ and σ_{Tl} were simulated using equations (11–13) of the dormancy loss model in relation to daily mean soil temperature recorded in the pots at 5 cm depth. Once these parameters were estimated, final germination percentages of seeds incubated at a 15°C constant regime for 15 d were predicted, using the previously described germination model equations. The same values of fixed thermal parameters as those described for simulated field experimental data were used. In this experiment, where seeds had been stored at three different constant temperatures for a period of 125 d, the model also gave a good description of the dynamics of changes in germination percentages in response to burial temperatures (Fig. 5).

Discussion

Existing models for simulating dormancy changes under field conditions predict the occurrence of the 'time window' for weed emergence with reasonable accuracy. However, these models cannot predict which percentage of the population will germinate in
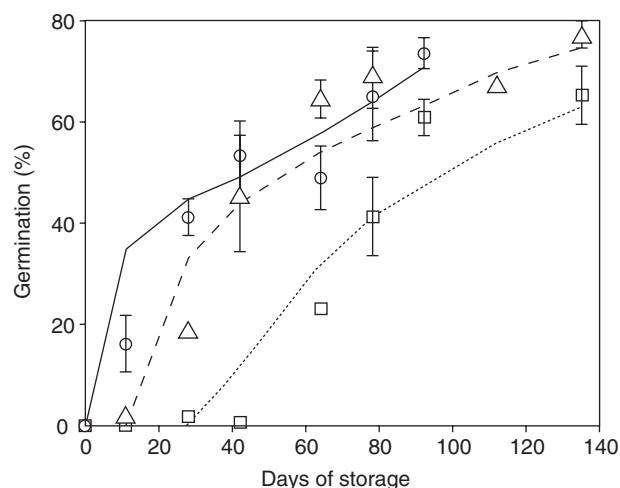
Figure 5. Predicted and observed germination percentages for *P. aviculare* seeds exhumed after different storage durations at constant temperatures. Symbols represent observed germination percentages for seeds stored at 1.6°C (○), 7°C (△) and 12°C (□), while lines represent values predicted from simulation modelling for storage temperatures of 1.6°C (solid line), 7°C (dashed line) and 12°C (dotted line). Vertical bars indicate SE.

that 'time window' (Murdoch, 1998). In the present work, a simulation model for *P. aviculare* dormancy loss was developed. This model can predict the timing of weed emergence, and which percentage of the weed seed population will emerge at that time. The model is based on the assumption that dormancy loss, due to the effect of low temperatures in summer annual weed species, can be related to changes in a single variable, T_l, while other thermal parameters associated with the germination response of the seed population, such as T_b, T_o, T_m, $\theta_{(50)}$ and T_h, remain constant, or change very little during burial (Kruk and Benech-Arnold, 1998). Although changes in thermal parameters other than T_l cannot be totally ruled out, the ability of the model to account for changes in seed germination behaviour as dormancy loss proceeds, suggests that to consider changes in a single variable (T_l) is an appropriate modelling framework. On the other hand, the model presents a novel population approach that permits the prediction of changes in the distribution within the seed population of the thermal parameters that characterize seed dormancy status (T_l and T_h). Quantification of this variation within the seed population allows consideration of both germinable and non-germinable seeds as part of the same continuous dormancy distribution (Bradford, 1996, 2002).

Estimation of seed population thermal parameters for establishing functional relationships between

dormancy status and temperature was achieved by optimizing parameters values that maximized the fit of simulated germination curves with experimentally obtained ones (Fig. 1). For this purpose, a screening germination test system and a mathematical model describing germination response of seeds to temperature were used, based on a methodology developed by Washitani (1987) and Kruk and Benech-Arnold (1998). The screening system permitted a population approach for quantification of changes in seed population thermal behaviour in relation to dormancy loss. Moreover, the system allowed a separate treatment of temperature effects on dormancy and germination in the seed population. However, it is important to note that seed exposure to low temperatures (i.e. 6°C), or to temperature shifts in the increasing temperature regime, may cause some dormancy alleviation in the seed population. This would probably determine changes in the germination behaviour of seeds at subsequent temperatures in the test, thus leading to an erroneous estimation of seed population thermal parameters. To minimize these effects in the present work, seeds were also incubated at a temperature that would cause minor or no dormancy alleviation of seed populations during incubation (constant 15°C) (Ransom, 1935). The good fit observed, between simulated and experimentally obtained time-course curves for both germination test regimes (the gradually increasing temperature and constant 15°C) using a single set of seed population thermal parameters, indicates that these effects were not meaningful in this case. However, in species that exhibit marked changes in dormancy in response to short periods of exposure to low temperature, or/and extreme sensitivity to low-amplitude temperature shifts, the use of a gradually increasing temperature regime may not be satisfactory for determining seed population thermal parameters.

The modelling process related changes in $T_{l(50)}$ at different burial temperatures to the accumulation of stratification thermal time (S_{tt}) units under a 'ceiling' threshold temperature for dormancy release to occur [Fig. 2B; equations (11,12)]. The utilization of this index was possible because of the linear relationship found between days of storage and changes in $T_{l(50)}$ at the three storage temperatures (Fig. 2A). Pritchard *et al.* (1996) found linear relationships between changes in germination percentage and time at four storage temperatures, and used a similar thermal time approach to quantify low-temperature effects on *Aesculus hippocastanum* L. dormancy loss. A model based on thermal time was also used successfully by Bauer *et al.* (1998) to predict changes in *Bromus tectorum* L. dormancy status as a function of the mean base water potential of the population. The 'ceiling' temperature for dormancy loss that maximized the

linear regression fit between $T_{l(50)}$ and S_{tt} for *P. aviculare* was 17°C. Similar border temperatures for net dormancy loss have been reported for a number of cultivated and weed species (Roberts and Smith, 1977; Totterdell and Roberts, 1979; Bouwmeester, 1990; Pritchard *et al*., 1996). In the case of *Polygonum* spp., Ransom (1935) observed that seed stratification can take place at 12°C, but this process did not occur when seeds were stored at 18, 20 or 30°C. These results suggest that accumulation of thermal time units, under a 'ceiling' temperature for that process to occur, can be used as a simple tool for quantifying the effects of temperature on seed dormancy loss.

As discussed earlier, distribution of T_l can be correlated with variations in the dormancy states among seeds of the same population. Results obtained in this work showed that the standard deviation of T_l changed considerably as seeds were released from dormancy (Fig. 3A). Considerable variation of dormancy states among seeds of *P. aviculare* belonging to the same population was reported previously by Ransom (1935) and Hammerton (1964). However, the pattern of change observed in σ_{Tl} was different, depending on the temperature at which the seeds were buried. These results suggest that changes in σ_{Tl} are related to S_{tt} accumulation, and that the temperature at which S_{tt} accumulated has an important effect on σ_{Tl}. In the present model, an index based on S_{tt}, which is affected by temperature, was used in order to predict changes in σ_{Tl} at any seed burial temperature [Fig. 3B; equation (13)]. The relationship between these two parameters can be described by a quadratic function. Under field situations this implies that changes in σ_{Tl} would depend on the prevailing winter temperature at which dormancy release occurs, and the duration of this 'dormancy release season', which would be determined by the time that the daily mean soil temperature is below 17°C. For example, under the Argentinean pampas conditions, where a short, mild winter is the usual situation, low temperatures (3–6°C) will yield high values of σ_{Tl} (wide distribution of T_l within the population), associated with low values of $T_{l(50)}$. In contrast, if dormancy loss takes place with relatively high winter temperatures (8–12°C), lower values of σ_{Tl} (narrow distribution of T_l within the population) associated with a high value of $T_{l(50)}$ would be expected. On the other hand, in regions with longer and colder winter seasons, the accumulation of many S_{tt} units would be permitted, and the seed population would reach spring with low values of both σ_{Tl} and $T_{l(50)}$, resulting in a high percentage of the seed bank population germinating in the field. Results obtained from simulation suggest that inaccurate prediction of changes in this parameter could lead to important over- or underestimation of the proportion of the seed population that will germinate at a determinate time.

The model performed good simulations of the percentage germination of seeds buried under variable field conditions (Fig. 4) and in controlled conditions under a constant temperature regime (Fig. 5). Although several assumptions were made, whether for theoretical or practical reasons, the ability of the model to predict germination percentages successfully under both situations suggests the validity of these assumptions. However, some discrepancies between observed and simulated data were observed when low germination percentages were registered in seeds exhumed from the field experiment (Fig. 4, upper and middle panels).

On the other hand, model building resulted in several insights into characteristics of the dynamics of dormancy loss in *P. aviculare* seed populations. Our results suggest that $T_{h(50)}$ (18°C) is relatively constant during stratification. Kruk and Benech-Arnold (1998) obtained similar results for this species. Bouwmeester and Karssen (1992) also assumed constant values of T_h to simulate dormancy cycling of *P. persicaria* under field conditions. $\theta_{(50)}$ displayed some minor variations between exhumations at the three tested storage temperatures (Table 1a, b, c); however, in the model $\theta_{(50)}$ was assumed to be constant during seed burial. Similar assumptions were used successfully to model dormancy changes in other weeds (Bauer *et al*., 1998; Kruk and Benech-Arnold, 1998).

In the present work, evidence of dormancy induction by temperatures around 20°C was presented (Table 2). However, in order to complete the model for simulating dormancy cycling observed under field conditions, functional relationships between secondary dormancy induction and temperature for *P. aviculare* seeds have to be derived. Although Totterdell and Roberts (1979) hypothesized that the rate of induction into secondary dormancy rises with higher prevailing temperatures over 0°C, the gradual decrease of $T_{l(50)}$ observed when seeds were stored for 110 d at 12°C (Fig. 2A) suggests no induction of secondary dormancy at this storage temperature in *P. aviculare* seeds. Similar results were obtained by Pritchard *et al*. (1996) with *A. hippocastanum* seeds, suggesting that a threshold temperature for dormancy induction may exist in certain species.

The development of quantitative models to predict dormancy changes and germination of weed seed populations can be an important method to increase understanding of the system, and may be used to improve weed control strategies. In the present work, a novel population approach for assessing and modelling dormancy loss in weed seeds in relation to temperature was developed. However, dormancy and emergence from seed banks under field situations can be influenced by many environmental factors, such as light, temperature and

soil water content fluctuation, nitrate content, etc. The construction of quantitative population models that include the effects of these environmental factors on seed dormancy and germination would lead to a better understanding and prediction of weed emergence patterns under field situations.

Acknowledgements

References

Anonymous (2000) Welcome to squeak! Available at http://www.squeak.org. Accessed 24 November, 2000.

Baskin, C.C. and Baskin, J.M. (1988) Germination ecophysiology of herbaceous plant species in a temperate region. *American Journal of Botany* **75**, 286–305.

Bauer, M.C., Meyer, S.E. and Allen, P.S. (1998) A simulation model to predict seed dormancy loss in the field for *Bromus tectorum* L. *Journal of Experimental Botany* **49**, 1235–1244.

Benech-Arnold, R.L. and Sánchez, R.A. (1995) Modelling weed seed germination. pp. 545–566 *in* Kigel, J.; Galili, G. (Eds) *Seed development and germination.* New York, Marcel Dekker.

Benech-Arnold, R.L., Sánchez, R.A., Forcella, F., Kruk, B.C. and Ghersa, C.M. (2000) Environmental control of dormancy in weed seed banks in soil. *Field Crops Research* **67**, 105–122.

Bouwmeester, H.J. (1990) The effect of environmental conditions on the seasonal dormancy pattern and germination of weed seeds. Ph.D. thesis, Wageningen Agricultural University.

Bouwmeester, H.J. and Karssen, C.M. (1992) The dual role of temperature in the regulation of the seasonal changes in dormancy and germination of seeds of *Polygonum persicaria* L. *Oecologia* **90**, 88–94.

Bradford, K.J. (1996) Population-based models describing seed dormancy behaviour: Implications for experimental design and interpretation. pp. 313–339 *in* Lang, G.A. (Ed.) *Plant dormancy: Physiology, biochemistry, and molecular biology.* Wallingford, UK, CAB International.

Bradford, K.J. (2002) Applications of hydrothermal time to quantifying and modeling seed germination and dormancy. *Weed Science* **50**, 248–260.

Courtney, A.D. (1968) Seed dormancy and field emergence in *Polygonum aviculare. Journal of Applied Ecology* **5**, 675–684.

Forcella, F., Benech-Arnold, R.L., Sánchez, R.A. and Ghersa, C.M. (2000) Modelling seedling emergence. *Field Crops Research* **67**, 123–139.

Hammerton, J.L. (1964) Variations in the after-ripening requirement of *Polygonum aviculare* L. seeds. *Proceedings of the British Weed Control Conference* **7**, 628–634.

Karssen, C.M. (1982) Seasonal patterns of dormancy in weed seeds. pp. 243–270 *in* Khan, A.A. (Ed.) *The physiology and biochemistry of seed development, dormancy and germination.* Amsterdam, Elsevier.

Kebreab, E. and Murdoch, A.J. (2000) The effect of water stress on the temperature range for germination of *Orobanche aegyptiaca* seeds. *Seed Science Research* **10**, 127–133.

Kruk, B.C. and Benech-Arnold, R.L. (1998) Functional and quantitative analysis of seed thermal responses in prostrate knotweed (*Polygonum aviculare*) and common purslane (*Portulaca oleracea*). *Weed Science* **46**, 83–90.

Kruk, B.C. and Benech-Arnold, R.L. (2000) Evaluation of dormancy and germination responses to temperature in *Carduus acanthoides* and *Anagallis arvensis* using a screening system, and relationship with field-observed emergence patterns. *Seed Science Research* **10**, 77–88.

Murdoch, A.J. (1998) Dormancy cycles of weed seeds in soil. *Aspects of Applied Biology* **51**, 119–126.

Pritchard, H.W., Tompsett, P.B. and Manger, K.R. (1996) Development of a thermal time model for the quantification of dormancy loss in *Aesculus hippocastanum* seeds. *Seed Science Research* **6**, 127–135.

Probert, R.J. (1992) The role of temperature in germination ecophysiology. pp. 285–325 *in* Fenner, M. (Ed.) *Seeds. The ecology of regeneration in plant communities*. Wallingford, UK, CAB International.

Ransom, E.R. (1935) The inter-relations of catalase, respiration, after-ripening, and germination in some dormant seeds of the Polygonaceae. *American Journal of Botany* **22**, 815–825.

Roberts, E.H. and Smith, R.D. (1977) Dormancy and the pentose phosphate pathway. pp. 385–411 *in* Khan, A.A. (Ed.) *The physiology and biochemistry of seed dormancy and germination*. Amsterdam, North Holland Publishing Company.

Russell, S.J. and Norvig, P. (1995) *Artificial intelligence: A modern approach*. Englewood Cliffs, New Jersey, Prentice Hall.

Totterdell, S. and Roberts, E.H. (1979) Effects of low temperatures on the loss of innate dormancy and the development of induced dormancy in seeds of *Rumex obtusifolius* L. and *Rumex crispus* L. *Plant, Cell and Environment* **2**, 131–137.

Vegis, A. (1964) Dormancy in higher plants. *Annual Review of Plant Physiology* **15**, 185–224.

Vleeshouwers, L.M. (1997) Modelling weed emergence patterns. Ph.D. thesis, Wageningen Agricultural University.

Vleeshouwers, L.M. and Bouwmeester, H.J. (2001) A simulation model for seasonal changes in dormancy and germination of weed seeds. *Seed Science Research* **11**, 77–92.

Vleeshouwers, L.M., Bouwmeester, H.J. and Karssen, C.M. (1995) Redefining seed dormancy: An attempt to integrate physiology and ecology. *Journal of Ecology* **83**, 1031–1037.

Washitani, I. (1985) Germination-rate dependency on temperature of *Geranium carolinianum* seeds. *Journal of Experimental Botany* **36**, 330–337.

Washitani, I. (1987) A convenient screening test system and a model for thermal germination responses of wild plant seeds: behavior of model and real seed in the system. *Plant, Cell and Environment* **10**, 587–598.