

## Germination parameterization and development of an after-ripening thermal-time model for primary dormancy release of *Lithospermum arvense* seeds

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- **Background and Aims** Models based on thermal-time approaches have been a useful tool for characterizing and predicting seed germination and dormancy release in relation to time and temperature. The aims of the present work were to evaluate the relative accuracy of different thermal-time approaches for the description of germination in *Lithospermum arvense* and to develop an after-ripening thermal-time model for predicting seed dormancy release.
- **Methods** Seeds were dry-stored at constant temperatures of 5, 15 or 24 °C for up to 210 d. After different storage periods, batches of 50 seeds were incubated at eight constant temperature regimes of 5, 8, 10, 13, 15, 17, 20 or 25 °C. Experimentally obtained cumulative-germination curves were analysed using a non-linear regression procedure to obtain optimal population thermal parameters for *L. arvense*. Changes in these parameters were described as a function of after-ripening thermal-time and storage temperature.
- **Key Results** The most accurate approach for simulating the thermal-germination response of *L. arvense* was achieved by assuming a normal distribution of both base and maximum germination temperatures. The results contradict the widely accepted assumption of a single  $T_b$  value for the entire seed population. The after-ripening process was characterized by a progressive increase in the mean maximum germination temperature and a reduction in the thermal-time requirements for germination at sub-optimal temperatures.
- **Conclusions** The after-ripening thermal-time model developed here gave an acceptable description of the observed field emergence patterns, thus indicating its usefulness as a predictive tool to enhance weed management tactics.

**Key words:** *Lithospermum arvense*, winter annual weed, thermal-time model, cardinal temperatures, primary dormancy, after-ripening thermal-time, storage temperature, field germination, seedling emergence.

### INTRODUCTION

*Lithospermum arvense* L. [= *Buglossoides arvensis* (L.) I.M.Johnst.] is a dicotyledonous facultative winter annual plant, native to Eurasia. In Argentina, particularly in the semi-arid area of the Buenos Aires Province, *L. arvense* has become an increasingly important weed in winter crops, mainly due to the adoption of low-impact tillage systems during the last few years (Chantre *et al.*, 2009).

At seed maturity, *L. arvense* seeds (nutlets) show physiological dormancy, requiring an after-ripening period for dormancy release (Baskin and Baskin, 1998). According to Baskin and Baskin (1976, 1986, 1998), after-ripening is the process by which winter annual species lose primary dormancy after exposure of dry seeds to warm temperatures. Temperature is the major environmental factor governing changes in the degree of dormancy in temperate environments (Benech-Arnold *et al.*, 2000); in general, the higher the storage temperature, the greater the loss of dormancy (Thompson, 1973; Bell, 1993; Murdoch and Ellis, 2000). Allen *et al.* (1995) and Bauer *et al.* (1998) found that after-ripening increased both the percentage and the germination rate of *Bromus tectorum* seeds. Favier (1995) proposed a germination

rate model for dormancy loss of *Hordeum vulgare* seeds where the rate of change of mean germination time was described as function of after-ripening temperature. Using a thermal-time approach, Steadman *et al.* (2003a, b) found that the rate of dormancy loss in *Lolium rigidum* seeds was a positive linear function of after-ripening temperature.

Many thermal-germination models have been constructed on the basis of seed-to-seed response variation in order to understand the behaviour of the seed population. Most thermal-germination models use cardinal temperatures (base, optimal and maximal) and sub-optimal ( $\theta_1$ ) and supra-optimal ( $\theta_2$ ) thermal-times to quantify within-population variability (García-Huidobro *et al.*, 1982; Covell *et al.*, 1986; Ellis *et al.*, 1986, 1987; Ellis and Butcher, 1988; Murdoch *et al.*, 1989; Bradford, 1996; Hardegree *et al.*, 1999). The most accepted cardinal thermal-germination approach assumes a constant value of base temperature ( $T_b$ ) for the entire seed population, and a normal or log-normal distribution of  $\theta_1$  in the sub-optimal temperature range (Covell *et al.*, 1986; Ellis *et al.*, 1986; Benech-Arnold *et al.*, 1990; Steinmaus *et al.*, 2000; Alvarado and Bradford, 2002). In the supra-optimal thermal range, a common value of  $\theta_2$  and a normal or log-normal distribution of the maximal germination temperature ( $T_c$ ) are usually accepted (Covell *et al.*, 1986; Ellis *et al.*,

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1986; Ellis and Butcher, 1988). However, Hardegree (2006) obtained a better model fit using four rangeland grass species by assuming a common value of  $T_c$  and a log-normal distribution of  $\theta_2$ . Working with *Stellaria media* seeds, Grundy *et al.* (2000) proposed the idea of threshold temperatures for germination as a complement to the cardinal temperatures concept. According to this approach, each seed has lower and upper temperature thresholds for germination that are normally distributed in the population and define the temperature range within which each seed can germinate. Batlla and Benech-Arnold (2003) associated the concept of threshold temperatures to the dormancy status of a population of *Polygonum aviculare* seeds and successfully developed a thermal-time model for dormancy release.

The period of primary dormancy in seeds of winter annual species occurs during the summer season, and it coincides with the average length of the unfavourable season for seedling establishment (Schütz *et al.*, 2002). As these seeds are released from dormancy, the permissive thermal-germination range widens and allows them to germinate at progressively higher temperatures (Karssen, 1982), such those prevailing during autumn after seed dispersal (Baskin and Baskin, 1986, 1998). Temperature is the main factor regulating seed dormancy status. As a result, the use of thermal-time models based on the accumulation of biological time can be very helpful to account for changes in the distribution of the population thermal parameters in relation to the dormancy-breaking conditions. The objectives of the present work were: (1) to evaluate the relative accuracy of different thermal-time models for the description of *L. arvense* germination under a range of constant temperatures, and (2) to develop an after-ripening thermal-time model for primary-dormancy release, based on the changes of the seed population thermal-time parameters.

## MATERIALS AND METHODS

### After-ripening treatments

Seeds of *Lithospermum arvense* L. [= *Buglossoides arvensis* (L.) I.M.Johnst.] were collected in a wheat field located in Bahía Blanca (38°44'S, 62°16'W), Argentina, at the time of their natural dispersal (December 2005). After harvest, seeds were cleaned and placed in paper bags before dry storage in growth chambers at constant temperatures of 5, 15 and 24 °C for up to 210 d. Seed germinability was assessed on freshly matured seeds and after 45, 100, 150 and 210d of dry storage. The water content of seeds at harvest was 8–10 % (d. wt basis).

### Germination test

An aluminum temperature gradient bar designed according to Chatterton and Kadish (1969) was used for germination trials. Batches of 50 seeds were incubated at eight constant temperature regimes of 5, 8, 10, 13, 15, 17, 20 or 25 °C over a 30-d period. A 12-h photoperiod was applied by means of fluorescent lamps providing a photosynthetic photon flux density of 80  $\mu\text{mol m}^{-2} \text{s}^{-1}$ . Germination was monitored daily, visible protrusion of the radicle being the criterion for

germination. Germination percentages were calculated for the viable fraction of the seed population. To test seed viability, ungerminated seeds were sliced longitudinally and incubated in 0.1 % (wt/vol.) tetrazolium chloride (2,3,5-triphenyltetrazolium chloride) solution for 24 h in the dark at 30 °C (International Seed Testing Association, 1999). Seeds with pink- or red-stained embryos were considered viable.

### Determination of sub-optimal and supra-optimal temperature range for germination

Germination time-course curves obtained after different storage periods were used to estimate the time taken for cumulative germination to reach subpopulation percentiles of 10, 20, 30, 40, 50, 60, 70, 80 and 90 %. These estimates were obtained by linear interpolation between daily germination percentages at each incubation temperature. Germination rates at each percentile (reciprocals of time estimates of each fraction) were then plotted against incubation temperature to estimate the optimum temperature for germination (maximal germination rate) by means of a linear regression model. A linear relationship between germination rates and incubation temperature was assumed (Garcia-Huidobro *et al.*, 1982; Covell *et al.*, 1986). For the sub-optimal range,

$$r_1 (= 1/t_g) = (T - T_b)/\theta_1 \quad (1)$$

while for the supra-optimal range,

$$r_2 (= 1/t_g) = (T_c - T)/\theta_2 \quad (2)$$

where  $r$  is the germination rate (reciprocal of germination time of fraction  $g$ ),  $T$  is the incubation temperature,  $T_b$  is the base temperature for germination,  $T_c$  is maximum or 'ceiling' temperature, and  $\theta_1$  and  $\theta_2$  are the sub- and supra-optimal thermal-time, respectively.

Optimum temperature ( $T_o$ ) values were calculated for each subpopulation as the intercept of the sub- and supra-optimal temperature-response functions (Hardegree, 2006). Linear regression equations for each percentile were recalculated and constrained to pass through the average  $T_o$  value in order to identify the sub- and supra-optimal population thermal range for germination.

### Parameterization of thermal-germination models

Experimentally obtained cumulative-germination curves were used to perform a non-linear regression procedure to assess the relative accuracy of different thermal-germination models in predicting germination response under constant incubation temperatures. Seed germination follows a binomial cumulative distribution function within the population due to seed-to-seed response variation. Thus, simulation of observed data was based on the cumulative distribution function of the normal distribution according to the 'central limit theorem' (Sokal and Rohlf, 1981).

*Model 1.* In this model a normal distribution of  $\theta_1$  was assumed while  $T_b$  was considered constant for the entire population (Covell *et al.*, 1986; Ellis *et al.*, 1986, 1987). In the

supra-optimal temperature range,  $T_c$  was considered to be normally distributed in the population while commonality of  $\theta_2$  was assumed (Ellis *et al.*, 1986). For the sub-optimal temperature range  $\theta_1 \sim N(\theta_{50}, \sigma_\theta^2)$ , therefore the proportion of germinating seeds was described by the following equation:

$$p(\theta_{1(g)}) = \Phi[(\theta_{1(g)} - \theta_{50})/\sigma_\theta] \quad (3)$$

where  $p$  is the proportion of germinating seeds at a given  $\theta_1$  of a  $g$  fraction of the population,  $\Phi$  is the normal probability integral, and  $\theta_{50}$  and  $\sigma_\theta$  are the mean and standard deviation of the normal distribution, respectively.

For the supra-optimal temperature range, germination prediction based on  $T_c \sim N(T_{c50}, \sigma_{T_c}^2)$  followed the equation:

$$p(T_{c(g)}) = 1 - [\Phi(T_{c(g)} - T_{c50})/\sigma_{T_c}] \quad (4)$$

where  $p$  is the proportion of germinating seeds at a given  $T_c$  of a  $g$  fraction of the population,  $\Phi$  is the normal probability integral, and  $T_{c50}$  and  $\sigma_{T_c}$  are the mean and standard deviation of the normal distribution, respectively.

*Model 2.* A normal distribution of both  $T_b$  and  $T_c$  was assumed while  $\theta_1$  and  $\theta_2$  remained constant for all subpopulations. The equation for the sub-optimal temperature range is

$$p(T_{b(g)}) = \Phi[(T_{b(g)} - T_{b50})/\sigma_{T_b}] \quad (5)$$

where  $T_{b50}$  and  $\sigma_{T_b}$  are the mean and standard deviation of the normal distribution, respectively. For the supra-optimal range eqn (4) was applied.

*Model 3.* The germination-threshold approach proposed by Grundy *et al.* (2000) was used to estimate seed maximum germination percentages in relation to the after-ripening treatments. According to this approach each seed of the population is capable of germinating in a temperature range within a lower and an upper temperature threshold, and both are considered to be normally distributed in the population. The proportion of germinating seeds at a given temperature was calculated as:

$$p(T) = \{\Phi[(T - m_L)/S_L] - \{1 - \Phi[(T - m_U)/S_U]\}\} \quad (6)$$

where  $p(T)$  is the proportion of seeds germinating at temperature  $T$ . Means and standard deviations of the lower and upper threshold distributions were  $m_L$ ,  $S_L$  and  $m_U$ ,  $S_U$ , respectively.

Experimentally obtained germination curves were simulated within both temperature-threshold limits (germinable fraction of the population) assuming a normal distribution of both  $\theta_1$  and  $\theta_2$ . Base and maximum germination temperatures were estimated from eqns (1) and (2).

Optimal thermal parameters for germination models were obtained by a non-linear least-squares curve-fitting method using an optimization program (Premium Solver Platform 7.0; Frontline systems, Inc). Maximum fit between simulated and experimentally obtained data was achieved by an iterative technique using a quasi-Newton algorithm.

#### Goodness-of-fit and statistical significance of thermal-germination models

Assessment of goodness-of-fit was performed by means of the coefficient of determination ( $R^2$ ). The fraction variance accounted for by the simulation model  $R^2$  was calculated as:

$$R^2 = 1 - [\sum(y_{\text{obs}} - y_{\text{sim}})^2 / \sum(y_{\text{obs}} - \bar{y}_{\text{obs}})^2] \quad (7)$$

where  $y_{\text{obs}}$  are observed values,  $y_{\text{sim}}$  are simulated values. An  $R^2$  value of 1 indicates a perfect fit of the model to the observed data.

The relative accuracy of the models tested for the prediction of *L. arvense* germination response was evaluated in terms of the statistical significance of the comparison between  $R^2$  coefficients. The following  $F$ -test was used (Vohnout, 2003):

$$F = [(n - p - 1)(R^2 - *R^2)]/q(1 - R^2) \quad (8)$$

where  $R^2$  is the coefficient of the model that best fits the data,  $*R^2$  is the coefficient of the model to compare,  $n$  is the total number of cases in the model,  $p$  is the number of variables related to  $R^2$  and  $q$  is the number of variables related to  $*R^2$ . The statistical significance was obtained from the  $F$ -distribution with  $q$  and  $n - p - 1$  degrees of freedom.

#### Development of dormancy model

The process of model development is summarized below.

- (1) Determine seed population thermal parameters that maximize the fit between the experimentally obtained germination time-course curves for seeds stored dry at 5, 15 and 24 °C during different after-ripening time periods and the predicted germination curves for each germination model.
- (2) Assess the relative accuracy of each germination model in the prediction of the germination response by means of the determination of  $R^2$  coefficients and the statistical significance of their comparison.
- (3) Select the germination model that best fits the observed data.
- (4) Characterize changes in the seed population thermal parameters over time as a function of storage temperature, and derive thermal-time equations relating rate of change of these parameters to the after-ripening thermal-time.
- (5) Using equations developed in (4), predict changes in the population thermal parameters of seeds after-ripened in the laboratory and in the field.
- (6) Use predicted values of the population thermal parameters to simulate germination time-course curves and compare with experimentally obtained emergence data.

#### Model evaluation

In order to evaluate model performance with independent data, results from an experiment carried out under field conditions were used. Seeds of *L. arvense* were collected in a wheat field at Bahía Blanca, Argentina, at the time of their natural dispersal (January 2005). After harvest, seeds were

cleaned and placed in paper bags before dry storage in growth chambers at constant temperatures of 20 and 40 °C. The water content of seeds at harvest was 6–8 % (d. wt basis). *Lithospermum arvense* seeds were divided into three different seed lots that were subjected to different after-ripening treatments. Seed lot 1 was stored at 20 °C for 33 d; seed lot 2 was stored at 20 °C for 20 d plus 30 d at 40 °C; and seed lot 3 was exposed to a constant temperature regime of 40 °C for 70 d. Subsequently, *L. arvense* seeds were buried at 1 cm depth in an experimental field of the CERZOS, Universidad Nacional del Sur and CONICET, located in Bahía Blanca, Argentina (38°39'54''s 62°13'58''W). Seeds belonging to each seed lot were buried in plots of 0.8 × 0.8 m following a completely randomized factorial design with three replicates (80 seeds per plot). Seed burial date varied according to the duration of each pre-burial after-ripening treatment; thus seeds were buried in the field on 24 February 2005 (seed lot 1), 11 March 2005 (seed lot 2) and 30 March 2005 (seed lot 3).

Seedling emergence was recorded every 2 weeks from early autumn until early spring in order to include the complete emergence season of this species. Seedlings were counted as emerged when cotyledons were visible, and seedlings were removed immediately after counting. Plots were irrigated periodically using a localized drip irrigation system in order to avoid effects on emergence caused by erratic precipitation in the region under study. Field temperature was recorded every 2 h at 1 cm depth using temperature data loggers (Thermochron Ibuttons, Model DS1921G-F50, Maxim Integrated Products, Inc).

## RESULTS

### *Germination time-course curves and median germination rates*

Maximum germination values of 50–60 % were observed for recently harvested seeds of *L. arvense* incubated at low temperatures (5–10 °C) while germination was almost negligible at incubation temperatures higher than 13 °C (Fig. 1A). As after-ripening time progressed, *L. arvense* seeds were able to germinate at higher incubation temperatures (compare rows in Fig. 1). The percentage of germinating seeds at a given time of the after-ripening process was clearly influenced by the storage temperature. Germination percentages were higher and seeds were able to germinate at higher temperatures as the storage temperature increased (compare columns in Fig. 1).

Median germination rate (reciprocal of time for 50 % subpopulation germination) also increased with after-ripening time and temperature (Fig. 2). Lowest germination rates were observed at an after-ripening temperature of 5 °C (Fig. 2A) while a significant increase was detected for seeds after-ripened at higher temperatures (Fig. 2B, C). As shown in Figs 1 and 2, maximum germination percentages and the highest median germination rate were obtained for seeds after-ripened for 210 d at 24 °C. Final germination percentages for these seeds were 85 % at 5 °C, 100 % between 8–15 °C, 77 % at 17 °C, 20 % at 20 °C and 0 % at 25 °C (Fig. 1G), while the fastest germination was observed at incubation temperatures of 10, 13 and 15 °C (Fig. 2C).

From these results, it is clear that *L. arvense* after-ripening patterns were characterized by a widening in the thermal-

germination range associated with a progressive increase in the maximum germination temperature, as expected for a winter annual species (Foley, 1994; Allen *et al.*, 1995; Baskin and Baskin, 1998).

### *Goodness-of-fit and statistical analysis of thermal-germination models*

The most accurate approach for the simulation of *L. arvense* germination response was achieved by assuming a normal distribution of both  $T_b$  and  $T_c$  in the seed population (Model 2; Table 1). An  $R^2$  value of 0.956 was obtained after fitting the model to the observed data considering the complete (global) germination thermal range. Highly significant differences were observed between best-fit values ( $R^2$  coefficients of Model 2) and the rest of the coefficients obtained under different models assumptions (Table 1). Residuals and normal probability plots indicated no evidence of lack of homocedasticity or normality in the selected model (data not shown).

From fitting *L. arvense* subpopulation germination rate to a linear temperature function a normal distribution of both  $T_b$  and  $T_c$  was inferred in the seed population, as suggested by the selected thermal-germination approach (Table 1). An example of this population attribute is shown in Fig. 3. Germination rates for the different subpopulations obtained from the sub-optimal thermal range of seeds after-ripened at 15 °C for 210 d could be described as a series of positive linear functions with different  $x$ -intercepts or  $T_{b(g)}$  values. Similarly, a series of negative linear functions with different  $T_{c(g)}$  values gave an acceptable description of the behaviour of germination rates in the supra-optimal thermal range. The slopes of the regression functions of different population percentiles were relatively similar within each thermal range; thus indicating a small variation in the germination thermal-time estimates among subpopulations (Fig. 3).

*Lithospermum arvense* germination rates for the different fractions of the seed population were also fitted to quadratic and Gaussian functions, as a plateau of optimum temperatures was observed at 10 and 20 % percentiles of the population (Fig. 3). In spite of this fact, the most accurate description of the observed germination rates was achieved by the linear regression model (data not shown).

### *Changes in seed population thermal parameters in relation to after-ripening time and storage temperature*

A noticeable increment in the mean maximum germination temperature ( $T_{c(50)}$ ) was observed as after-ripening time and storage temperature increased (Table 2; Fig. 4A). The estimated temperature increment between recently harvested seeds and those after-ripened at 24 °C for 210 d was 8 °C. Conversely, the mean base temperature for germination ( $T_{b(50)}$ ) showed no consistent variation when regressed as a linear function of the after-ripening time ( $P = 0.68$ ,  $R^2 = 0.02$ ) and storage temperature ( $P = 0.42$ ,  $R^2 = 0.07$ ). In the sub-optimal temperature range, lower germination thermal-time ( $\theta_1$ ) values were predicted as storage temperature increased ( $P < 0.01$ ,  $R^2 = 0.57$ ), irrespective of the after-ripening time. Average  $\theta_1$  values were reduced by 17.6, 34.6

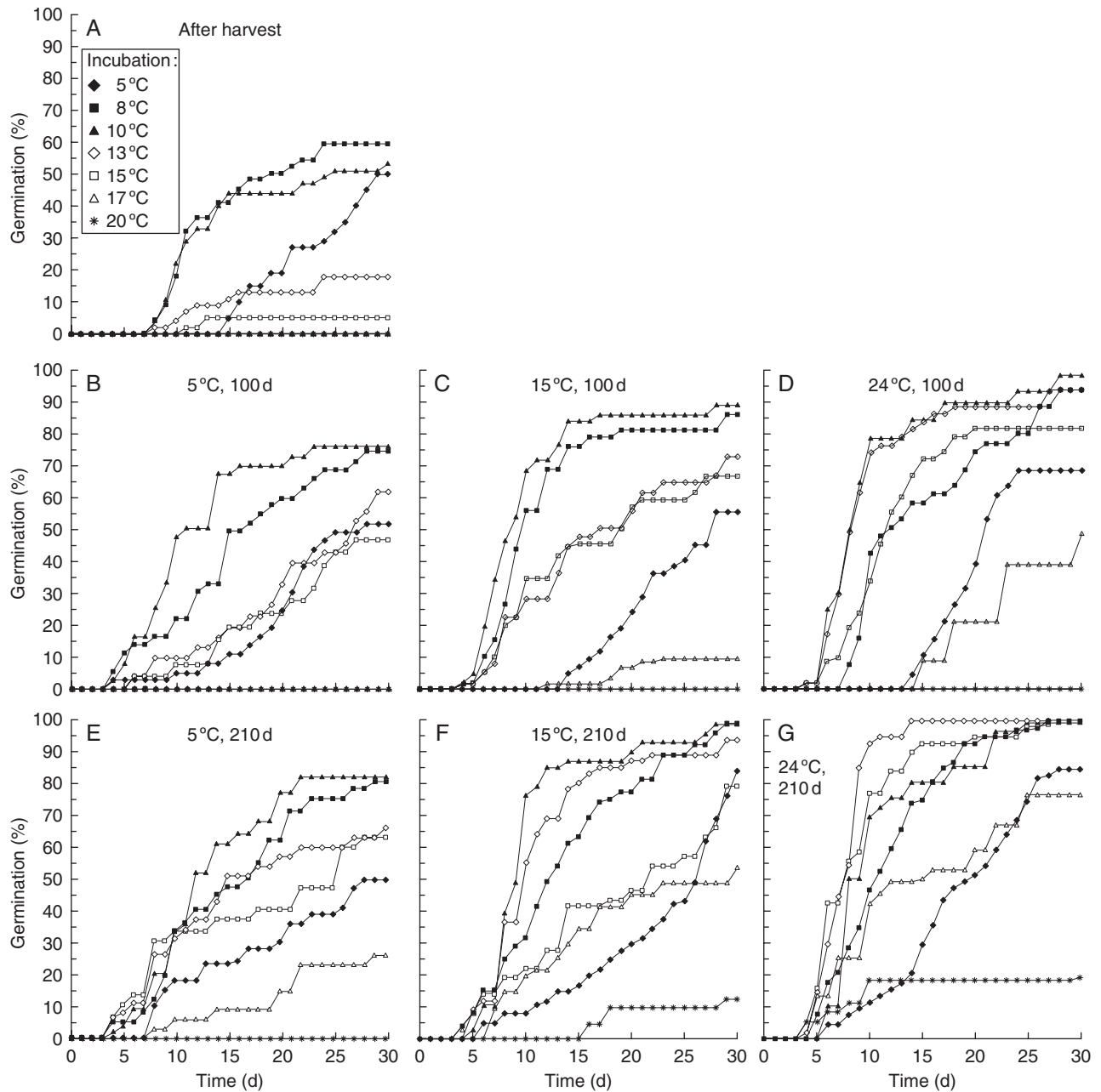


FIG. 1. Observed germination time-course curves for *L. arvense* seeds after-ripened at 5, 15 or 24 °C for (A) 0, (B–D) 100 or (E–G) 210 d and subsequently incubated at 5, 8, 10, 13, 15, 17 or 20 °C for 30 d as indicated. No germination was registered at 25 °C for any of the after-ripening treatments.

and 40.4 °Cd when seeds were after-ripened at 5, 15 and 24 °C, respectively, compared to recently harvested seeds (Table 2). Observed variations in  $\sigma_{T_b}$  and  $\sigma_{T_r}$  values, as well as changes in thermal-time estimates for the supra-optimal thermal range ( $\theta_2$ ) were not related to the after-ripening time ( $P \geq 0.34$ ).

*Development of dormancy model*

The process of seed dormancy release in *L. arvense* was characterized by progressive changes in seed population parameters ( $T_{c(50)}$  and  $\theta_1$ ) as a function of after-ripening time

and storage temperature. In addition, estimated  $T_{c(50)}$  values (Fig. 4A) were higher as after-ripening time and storage temperature increased.

In order to quantify the effect of temperature on seed population dormancy status, changes in  $T_{c(50)}$  were predicted as a function of the accumulation of after-ripening thermal-time units (°Cd) above a ‘base’ temperature for dormancy loss to occur, according to the following equation:

$$\Theta_{AT} = (T_s - T_1)t_{ar} \tag{9}$$

where  $\Theta_{AT}$  is the thermal-time requirement for after-ripening,

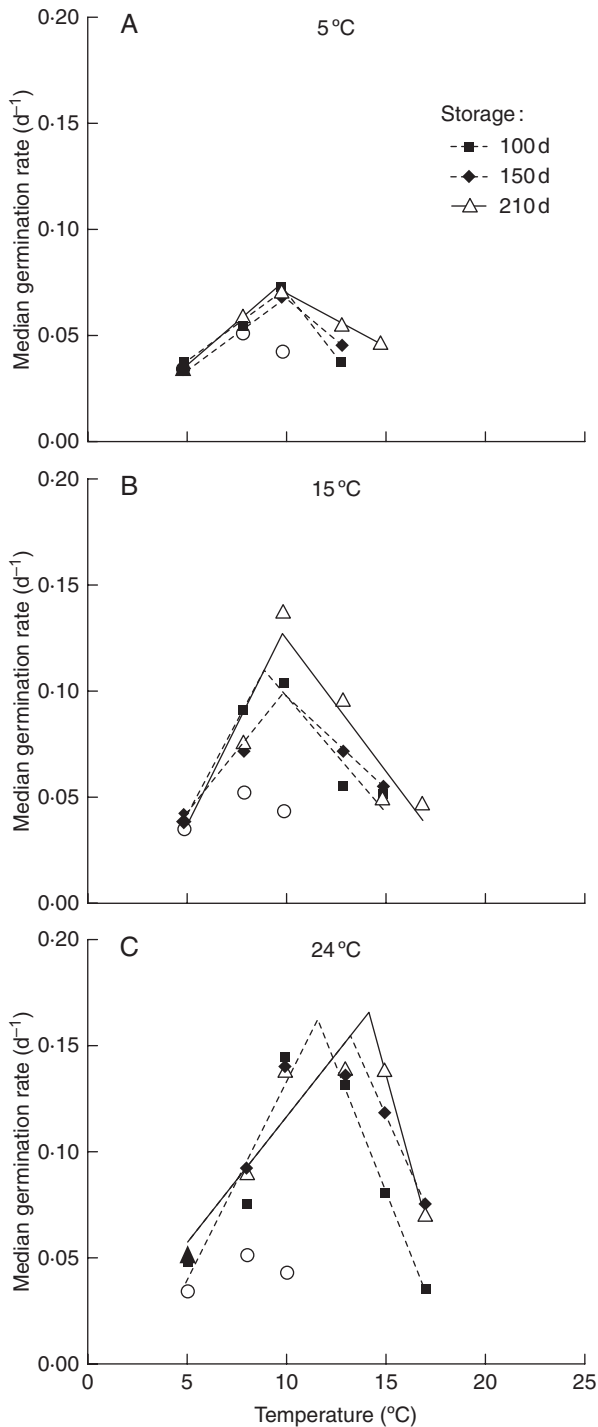


FIG. 2. *Lithospermum arvense* median germination rate (reciprocal of time for 50% subpopulation germination) regressed as a function of incubation temperature for seeds after-ripened at (A) 5 °C, (B) 15 °C, or (C) 24 °C for 100, 150 or 210 d of dry storage.  $R^2$  values for seeds after-ripened for 100, 150 and 210 d, respectively, were: (A) 0.98, 0.98 and 0.99; (B) 0.94, 0.98 and 0.90; (C) 0.94, 0.87 and 0.91. Median germination rate for recently harvested seeds is shown (circles) although regression analysis was not performed due to lack of sufficient data.

$T_s$  is the storage or after-ripening temperature,  $T_1$  is the lowest or base temperature (at or below which after-ripening does not occur), and  $t_{ar}$  is the time required for after-ripening.

As observed in Fig. 4B, changes in  $T_{c(50)}$  values were accurately described by a quadratic function of the after-ripening thermal-time. Optimal  $T_1$  was obtained by an iterative procedure until the best fit of the polynomial regression between  $T_{c(50)}$  and  $\Theta_{AT}$  was attained. Maximum data fit was achieved by considering a  $T_1$  value of  $-6$  °C according to the following equation:

$$T_{c(50)} = -2.66e^{-07} \Theta_{AT}^2 + 0.003 \Theta_{AT} + 11.14 \quad (10)$$

Changes in  $\theta_1$  values were also observed during the process of seed dormancy release (Fig. 5A). Lower values of  $\theta_1$  were recorded for seeds after-ripened at 15 and 24 °C compared to seeds stored at 5 °C after 100, 150 and 210 d of storage. To account for the effect of  $\Theta_{AT}$  on  $\theta_1$ , a nonlinear regression analysis was performed. The presence of an outlier value was detected, and thus it was excluded from the definitive data analysis (data not shown). The results presented in Fig. 5B indicate that the reduction in  $\theta_1$  values was a positive function of the accumulation of after-ripening thermal-time and storage temperature increase. Both variables were combined into a single expression of the form:  $\ln[(\Theta_{AT}/100) + 1]T_s$ . An accurate description of the changes in  $\theta_1$  values was achieved by means of the following quadratic function:

$$\theta_1 = 0.0059[\ln[(\Theta_{AT}/100) + 1]T_s]^2 - 1.0095[\ln[(\Theta_{AT}/100) + 1]T_s] + 115.3 \quad (11)$$

#### Model performance

Model performance was evaluated using emergence data from an independent experiment. The model was tested under field conditions in order to assess its relative accuracy in the prediction of *L. arvense* emergence patterns in the region under study. *Lithospermum arvense* seed lots were exposed to different after-ripening temperatures in the laboratory and the field (see above), and the model was used to estimate the changes in  $T_{c(50)}$  and  $\theta_1$  values using eqns (10) and (11) according to  $\Theta_{AT}$  accumulation (eqn 9) and after-ripening temperature. The total accumulation of after-ripening thermal-time units (°Cd) for each seed lot was the sum of the thermal units accumulated by after-ripening in the laboratory and the field. Accumulation of  $\Theta_{AT}$  began when the daily mean soil temperature recorded in the experimental field at a seeding depth of 1 cm was above the previously determined  $T_1$  value ( $-6$  °C). Predicted values of  $T_{c(50)}$  and  $\theta_1$  for the different seed lots were used to simulate germination dynamics in the field by assuming a normal distribution of both  $T_b$  and  $T_c$  in the population. Equations (4) and (5) were combined and a seed viability term was added:

$$p(T_{b(g)}, T_{c(g)}) = (1 - G_u) \{ \Phi[(T_{b(g)} - T_{b50})/\sigma_{Tb}] - \{1 - [\Phi(T_{c(g)} - T_{c50})/\sigma_{Tc}]\} \} \quad (12)$$

where  $G_u$  is the proportion of non-viable seeds at the time of seeding (Scott *et al.*, 1984);  $T_{b(50)}$ ,  $\sigma_{Tb}$ ,  $\sigma_{Tc}$  and  $\theta_2$  were considered constant for the simulation process. The following

TABLE 1. Comparison between thermal-germination model outputs obtained after data fitting. Residual sum of squares (SS) and total SS were calculated for the sub-optimal and supra-optimal thermal ranges as well as for the complete germination thermal range (global). Models goodness-of-fit assessment were based on the  $R^2$  coefficient (eqn 7). The relative accuracy of each thermal-germination model was assessed by means of an F-test (eqn 8) using  $R^2$  coefficients of Model 2 as best-fit values ( $R^2$  of eqn 8). Statistical significance was obtained from the F-distribution with  $F_{0.01} \sim (q, n - p - 1)$

Germination model	Thermal range	Residual SS $\Sigma(y_{\text{obs}} - y_{\text{sim}})^2$	Total SS $\Sigma(y_{\text{obs}} - \bar{y}_{\text{obs}})^2$	$R^2$	Statistical significance
Model 1	Suboptimal	71749.9	1045581.5	0.931	**
	Supraoptimal	49642.4	1246363.2	0.960	—
	Global	121392.3	2568344.4	0.953	**
Model 2	Suboptimal	61423.7	959252.9	0.936	—
	Supraoptimal	49642.4	1246363.2	0.960	—
	Global	111066.1	2523758.4	0.956	—
Model 3	Suboptimal	69983.2	1023260.7	0.932	**
	Supraoptimal	65413.1	1127764.0	0.942	**
	Global	135396.3	2494402.2	0.946	**

average values were used:  $T_{b(50)} = 1.8^\circ\text{C}$ ,  $\sigma_{T_b} = 3.8^\circ\text{C}$ ,  $\sigma_{T_c} = 3.0^\circ\text{C}$ , and  $\theta_2 = 44.0^\circ\text{Cd}$ . A  $G_u$  value of 0.3 was used in the model and the estimation of this coefficient was obtained by performing a tetrazolium chloride viability test (see germination test, above) on samples of 50 seeds obtained from each seed lot prior to seeding (seed viability =  $70 \pm 6\%$ ,  $n = 3$ ).

The simulated field germination dynamics and the observed emergence patterns are shown in Fig. 6. The simulation process was started when field temperatures were proximate to entering the permissive range for germination of *L. arvense* seeds. Dormancy model outputs for seed lot 1 ( $\Theta_{AT} = 1787.3^\circ\text{Cd}$ ; Fig. 6A), seed lot 2 ( $\Theta_{AT} = 2426.7^\circ\text{Cd}$ ; Fig. 6B) and seed lot 3 ( $\Theta_{AT} = 3220^\circ\text{Cd}$ ; Fig. 6C) were  $T_{c(50)} = 15.54$ ,  $16.65$  and  $17.69^\circ\text{C}$ , respectively; estimated values for  $\theta_1$  were  $76.44$ ,  $72.3$  and  $70.53^\circ\text{Cd}$ , respectively.

A fairly acceptable description of the observed emergence patterns was achieved by the simulation model for the different seed lots (Fig. 6). However, the model clearly over-estimated germination percentages at the beginning of the field germination ‘time window’ (April 2005) for seed lot 1 (Fig. 6A) and

seed lot 2 (Fig. 6B), and also for the predicted maximum germination plateau in the former. Higher simulated germination values were obtained at the beginning of the evaluated time period, as seeds accumulated after-ripening thermal-time and were progressively released from dormancy. A similar trend was observed for emergence data, as evidenced by a faster rate of seedling emergence in seed lot 3 (Fig. 6C).

Field emergence data was also simulated by assuming a constant  $T_b$  value and a normal distribution of  $\theta_1$  in order to determine whether the predictability of the model could be improved by Model 1 assumptions. A significantly better fit to the field data ( $F$ -value = 4.93,  $P < 0.05$ ) was obtained by assuming a normal distribution of  $T_b$  ( $R^2 = 0.73$ ) compared with a constant value of  $T_b$  ( $R^2 = 0.66$ ).

## DISCUSSION

Based on the results observed, we found that the most accurate thermal-time approach for the simulation of germination response of *L. arvense* to constant temperatures was achieved by assuming a normal distribution of both base and maximum germination temperatures ( $T_b$  and  $T_c$ ) in the seed population ( $R^2 = 0.956$ ; Table 1). According to this approach, each fraction of the seed population will germinate within a specific temperature range delimited by a unique set of cardinal temperatures. Thus, the probability of germination for each subpopulation will result from the combination of the cumulative normal distributions of both  $T_b$  and  $T_c$  (eqn 12).

Our results contradict the widely accepted assumption of a single  $T_b$  value for the entire seed population. Although many authors have assumed  $T_b$  as a unique value, it should be noted that most of these germination models were developed for crop cultivars (Garcia-Huidobro *et al.*, 1982; Covell *et al.*, 1986; Gummerson, 1986; Dahal *et al.*, 1990) in which  $T_b$  has been reported as a constant attribute (Bradford, 1995). Conversely, in non-crop species previous reports have also indicated intra-population variation and lack of stability in  $T_b$  values (Pritchard *et al.*, 1999; Forcella *et al.*, 2000; Wang *et al.*, 2004; Hardegee, 2006). This situation could be associated with a distinct genetic variability within the seed population, as proposed for *Setaria viridis* (Wang *et al.*, 1995) and *Eurotia lanata* (Wang *et al.*, 2004). An alternative

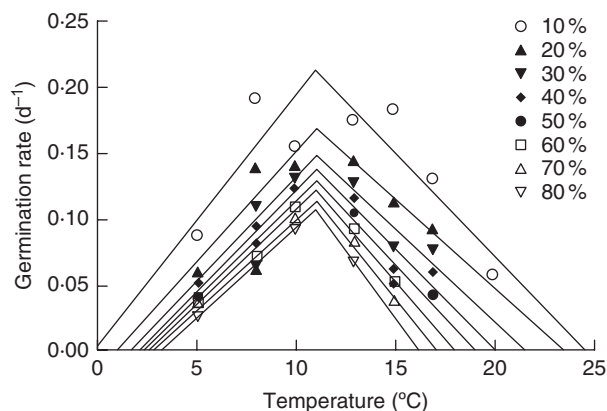


FIG. 3. Germination rates for *L. arvense* subpopulations regressed as linear functions of incubation temperature. Germination percentiles of 10% ( $R^2 = 0.74$ ), 20% ( $R^2 = 0.88$ ), 30% ( $R^2 = 0.91$ ), 40% ( $R^2 = 0.91$ ), 50% ( $R^2 = 0.93$ ), 60% ( $R^2 = 0.98$ ), 70% ( $R^2 = 0.95$ ) and 80% ( $R^2 = 0.99$ ) obtained from seeds after-ripened at  $15^\circ\text{C}$  for 210 d are shown.

TABLE 2. Estimated population thermal parameters for *L. arvense* seeds after-ripened at 5, 15 or 24 °C. Seeds were incubated at 5, 8, 10, 13, 15, 17, 20 or 25 °C after 0, 45, 100, 150 or 210 d of dry storage. Parameters were obtained by simulation of the germination time-course curves assuming a normal distribution of both  $T_b$  (eqn 5) and  $T_c$  (eqn 4) in the seed population.  $R^2$  values for each after-ripening treatment as well as the coefficients of variation (CV) of the population thermal parameters are included

Storage temperature (°C)	Days of storage	$T_{b(50)}$ (°C)	$\sigma_{Tb}$	$\theta_1$ (°Cd)	$R^2$	$T_{c(50)}$ (°C)	$\sigma_{Tc}$	$\theta_2$ (°Cd)	$R^2$
—	0	2.11	4.64	114.67	0.90	11.64	3.07	39.70	0.99
5	45	1.51	7.33	82.70	0.88	12.44	4.07	30.79	0.95
	100	1.79	4.01	104.42	0.94	15.15	3.44	61.65	0.93
	150	2.11	6.30	103.05	0.93	15.54	3.26	44.89	0.94
	210	1.70	4.78	98.23	0.97	16.18	3.60	44.27	0.92
15	45	1.87	4.96	95.34	0.94	12.45	2.56	36.37	0.99
	100	2.11	2.50	72.10	0.89	16.75	3.33	54.93	0.92
	150	1.79	2.71	82.36	0.90	18.42	3.49	54.01	0.91
	210	2.11	2.42	70.53	0.96	18.56	2.97	54.00	0.94
24	45	1.51	2.62	79.51	0.92	13.98	2.18	47.34	0.99
	100	1.95	2.65	70.97	0.94	18.07	1.55	39.36	0.98
	150	1.51	1.94	74.81	0.89	19.62	2.62	33.56	0.98
	210	1.51	2.65	71.76	0.94	19.52	2.24	31.39	0.97
CV		13.81	44.24	17.54		17.28	23.50	22.56	

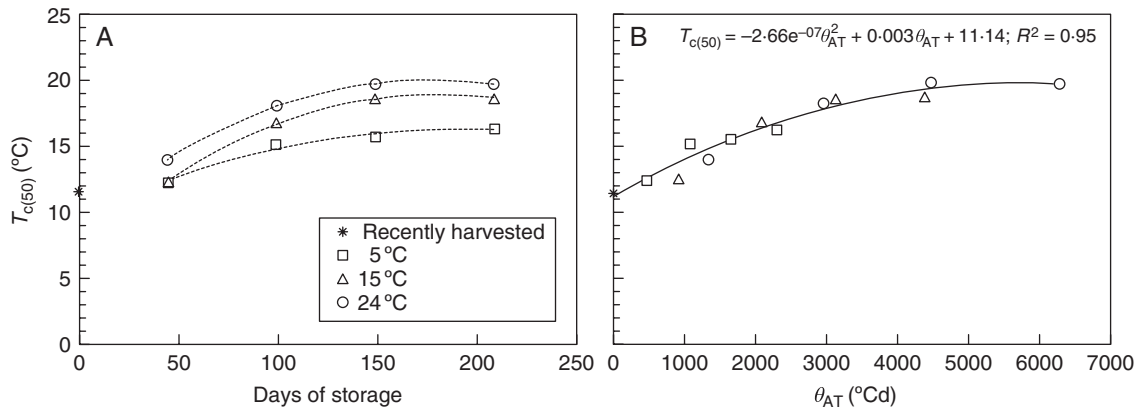


FIG. 4. Estimated values of the mean maximum-germination temperature ( $T_{c(50)}$ ) for recently harvested seeds of *L. arvense* and for seeds after-ripened at 5, 15 or 24 °C as indicated, plotted against (A) days of storage, and (B) against after-ripening thermal-time ( $\Theta_{AT}$ ). The dotted lines in (A) are fitted quadratic equations for each storage temperature, with  $R^2$  values of 0.96, 0.99 and 0.99, respectively. The quadratic function fitted in (B) was obtained by a repeated regression analysis in which the base after-ripening temperature ( $T_i$ ; eqn 9) was varied until the best fit was obtained.

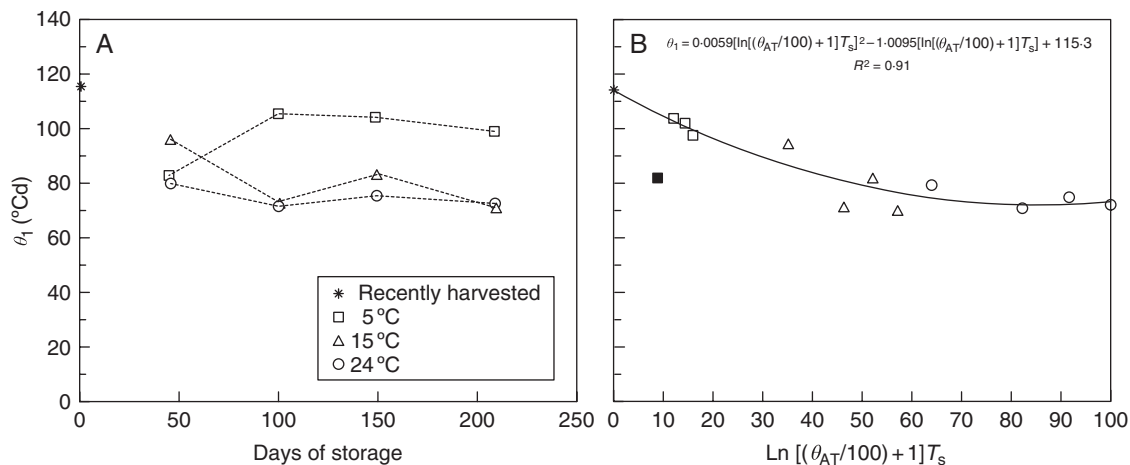


FIG. 5. Estimated values of the sub-optimal germination thermal-time ( $\theta_1$ ) for recently harvested seeds of *L. arvense* and for seeds after-ripened at 5, 15 or 24 °C as indicated, plotted against (A) days of storage, and (B) against  $\ln[(\Theta_{AT}/100) + 1]T_s$ , where  $\Theta_{AT}$  is the after-ripening thermal-time and  $T_s$  is the after-ripening or storage temperature. In (B) changes in  $\theta_1$  values were adequately described by fitting a quadratic function (eqn 11) after excluding an outlier value (solid square) from the definitive data analysis.



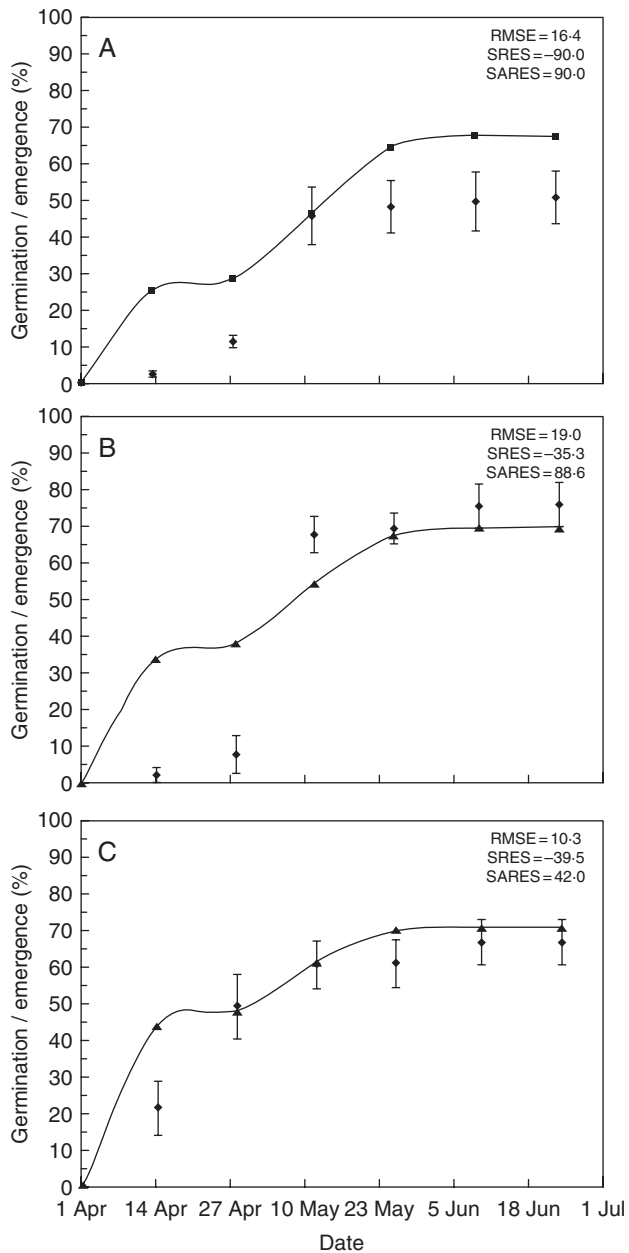


FIG. 6. Predicted field germination percentages and observed emergence data for *L. arvense* seed lots exposed to different after-ripening treatments in the laboratory and in the field. (A) Seed lot 1 was after-ripened at 20 °C for 33 d and buried in the field on 24 February 2005, (B) seed lot 2 was after-ripened at 20 °C for 20 d plus 30 d at 40 °C and buried on 11 March 2005, and (C) seed lot 3 was exposed to a constant temperature regime of 40 °C for 70 d and buried on 30 March 2005. The simulation process was started in April 2005 when field temperatures were proximate to entering the permissive range for germination of *L. arvense* seeds. Dormancy model outputs for seed lot 1 ( $\Theta_{AT} = 1787.3$  °Cd), seed lot 2 ( $\Theta_{AT} = 2426.7$  °Cd) and seed lot 3 ( $\Theta_{AT} = 3220$  °Cd), respectively, were  $T_{c(50)} = 15.54$ , 16.65 and 17.69 °C; estimated values of  $\theta_1$  were 76.44, 72.3 and 70.53 °Cd, respectively. The lines represent field germination values predicted from simulation modelling, assuming a seed viability of 70%; symbols represent observed emergence data, and vertical bars indicate s.e. ( $n = 3$ ). RMSE, root-mean-square error; SRES, sum of residuals; SARES, sum of absolute residuals.

explanation could be related to the effect that a heterogeneous maternal environment during seed maturation might have on the seed population response to temperature (Baskin and Baskin, 1998).

From an ecological perspective, the different physiological responses to temperature observed among subpopulations of *L. arvense* might be considered as an adaptive strategy, where the different fractions of the population accumulate thermal-time at distinct rates, spreading germination over time and thus increasing the probability of seedling establishment.

Some of the estimations of the standard deviations of the normal distribution of  $T_b$  predicted extremely low values of this population thermal parameter (Table 2). This might be related to the way in which the mathematical model applied calculates  $T_b$  values for each percentile of the population. In fact, even very small changes in the germination rates of the different subpopulations at low incubation temperatures are likely to have a significant influence on estimates of  $T_b$ , as observed in the present case.

In the present work, an after-ripening thermal-time model for dormancy release of *L. arvense* seeds was developed. Model development consisted of the quantification of the observed changes in the optimal thermal-germination parameters previously obtained by assuming a normal distribution of both cardinal temperatures ( $T_b$  and  $T_c$ ) within the population, and the derivation of equations relating the rate of change of each parameter ( $T_{c(50)}$  and  $\theta_1$ ) to the after-ripening thermal-time accumulation and storage temperature. Changes in  $T_{c(50)}$  values were adequately described by a quadratic function of  $\Theta_{AT}$  (Fig. 4B; eqn 10), indicating that the increment in the mean maximum-germination temperature was positively related to the accumulation of  $\Theta_{AT}$ . Thus, it could be inferred that the  $T_{c(50)}$  rate of increase is a positive function of storage temperature that regulates the rate of after-ripening thermal-time accumulation. These results concur with previous reports that found that the rate of dormancy release was positively related to the after-ripening temperature in winter annual species (Baskin and Baskin, 1976; Foley, 1994; Allen *et al.*, 1995; Bauer *et al.*, 1998; Steadman *et al.*, 2003a, b).

Observed changes in  $\theta_1$  with after-ripening time (Fig. 5A) were described as a function of  $\Theta_{AT}$  and  $T_s$  (Fig 5B; eqn 11). The relationship found between  $\theta_1$  and  $\ln[(\Theta_{AT}/100) + 1]T_s$  clearly indicates that changes in  $\theta_1$  depend not only on the accumulation of  $\Theta_{AT}$ , but also on the temperature experienced by the seeds during the after-ripening process. Thus, it might be inferred that  $\theta_1$  changes during *L. arvense* seed dormancy release depend on the amount of accumulated thermal-time units (°Cd) and the rate of  $\Theta_{AT}$  accumulation, which depends on  $T_s$ , as previously mentioned. A similar equation was developed by Batlla and Benech-Arnold (2003) for *Polygonum aviculare* seeds to describe changes in the standard deviation of the lower-limit germination temperature as a function of stratification temperature and thermal-time accumulation.

An after-ripening base temperature value of  $-6$  °C gave an accurate description of *L. arvense* seed dormancy loss (Figs 4B and 5B). Available evidence is scarce relating to the effects of

sub-zero temperatures during seed dormancy release. Sivakumar *et al.* (2006) observed a significant reduction in the levels of primary physiological dormancy of *Strychnos nux-vomica* seeds after exposure to sub-zero temperatures during dry storage. Experimental evidence of the existence of seed metabolic activity at sub-zero incubation temperatures was provided by Wang *et al.* (2004), who found that *E. lanata* seeds were capable of accumulating thermal-time and thus germinating when imbibed at temperatures close to  $-1^{\circ}\text{C}$ . The potential occurrence of after-ripening at such low temperatures should make researchers aware of the thermal conditions in which seeds should be stored in order not to alter their dormancy level.

The molecular mechanisms of after-ripening are still not known (Finch-Savage and Leubner-Metzger, 2006); however, the physical properties of the membrane lipids are apparently involved in the perception of temperature by seeds (Hallett and Bewley, 2002). Steadman *et al.* (2003b) suggested that the higher dormancy-release rates observed at increasing after-ripening temperatures in *Lolium rigidum* seeds are related to increased membrane fluidity and inherent enzyme temperature–activity relationships promoted by warmer temperatures. An analogous mechanism may act to mediate the role of temperature in the regulation of *L. arvense* after-ripening process.

The after-ripening thermal-time model developed in this study was capable of predicting the ‘time window’ for *L. arvense* field germination and the fraction of the seed population that will germinate within this time period, as evidenced by previous seed-bank studies performed by Chantre *et al.* (2009). Simulated field-germination curves and observed emergence patterns clearly indicate that as *L. arvense* seeds are progressively released from dormancy, as  $\Theta_{\text{AT}}$  is accumulated, the proportion of germinating seeds and emerged seedlings will increase at the beginning of the field germination ‘time window’ in early autumn (Fig. 6). This is mainly due to a widening in the germination-permissive thermal range associated with an increment in the mean maximum-germination temperature, as expected for a winter annual species (Baskin and Baskin, 1998), and a reduction in the thermal-time requirement for germination at sub-optimal temperatures.

The model performance for predicting *L. arvense* emergence patterns was quite acceptable considering that seedling emergence involves additional post-germination seedling growth compared with seed germination in the laboratory. In addition, possible fluctuations in the seed water content due to sporadic rainfall events during the after-ripening period in the field could have altered the rate of dormancy release, as suggested by previous reports in other species (Leopold *et al.*, 1988; Foley, 1994; Steadman *et al.*, 2003b; Bair *et al.*, 2006).

From a modelling point of view, we have found strong evidence to support the development of a germination model and an after-ripening thermal-time model for *L. arvense* based on the concept of a normal distribution of  $T_b$  in the seed population. Such evidence was provided by a better fit of the germination response of *L. arvense* to the applied model (Table 1) and a more accurate prediction of the field emergence data in comparison to the assumption of a constant  $T_b$ . However, it should be noted that some of the variation observed in  $T_b$

might be associated with the method of data analysis, and also with the unavoidable difficulty in correctly estimating germination rates at low incubation temperatures. Thus, further investigations should be performed in order to increase the predictive capability of the developed model to account for variations in the above mentioned factors.

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