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# An after-ripening thermal-time model for *Lithospermum arvense* seeds based on changes in population hydrotime parameters

G R CHANTRE, M R SABBATINI & G A ORIOLI
*Departamento de Agronomía y CERZOS, Universidad Nacional del Sur, CONICET, Bahía Blanca, Argentina*

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## Summary

*Lithospermum arvense* seeds show primary physiological dormancy. Changes in population hydrotime parameters during after-ripening were used to model primary dormancy loss. *Lithospermum arvense* seeds were dry-stored at constant temperatures of 5, 15, 24 and 30°C for 180 days. After different storage periods, seeds were incubated at 10°C at a range of water potentials (0 to −1.2 MPa). Experimentally obtained cumulative–germination curves were analysed by repeated probit regressions to obtain seed population hydrotime parameters. The population mean base water potential ($\Psi_b(50)$) showed a progressive decrease as after-ripening time progressed and the dormancy release rate was positively related to storage temperature. The hydrotime constant ($\theta_H$) and the SD of the base water potential ($\sigma_{\Psi b}$) were unaffected by after-ripening time or storage temperature. To account for the effect of after-ripening time and temperature on dormancy release, an after-ripening thermal-time model was developed. The model consisted of the description of $\Psi_b(50)$ changes as a function of an after-ripening thermal-time index ($\theta_{AT}$). An exponential decay function accurately described ($R^2 = 0.92$) the decrease pattern of $\Psi_b(50)$ as function of $\theta_{AT}$. Model evaluation under fluctuating soil water regimes showed a good correlation between observed and predicted data ($r = 0.94$ and 0.96). This indicated that the after-ripening process could be adequately described as a thermal-time response, further suggesting the potential applicability of the model to predict *L. arvense* emergence in the field.

**Keywords:** modelling, dormancy loss, germination, field gromwell, water potential, fluctuating soil water regimes, winter annual weed, weed management.

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## Introduction

Dormancy is defined as an internal condition of the seed that impedes its germination under otherwise adequate hydric, thermal and gaseous conditions (Benech-Arnold *et al*., 2000). It provides a mechanism to avoid germination under unfavourable environmental conditions for seedling survival (Schütz *et al*., 2002), extending seed longevity in the soil (Baskin & Baskin, 1998). Dormancy is the most important attribute of weed seedbank dynamics (Forcella *et al*., 2000; Grundy, 2003) and this complicates the task of predicting both the extent and the timing of seedling emergence under field conditions (Benech-Arnold & Sánchez, 1995). Batlla and Benech-Arnold (2007) highlighted the importance of developing models that account for changes in the dormancy status of weed seedbanks in order to achieve more effective weed control tactics in agricultural systems.

*Lithospermum arvense* L. (field gromwell, corn gromwell) is a facultative winter annual weed of

*Correspondence*: Guillermo R Chantre, Departamento de Agronomía, Universidad Nacional del Sur, CONICET Av. Colón 80, Bahía Blanca (8000), Argentina. Tel: (+54) 291 4595102; Fax: (+54) 291 4595127; E-mail: gchantre@criba.edu.ar

 

increasing importance in wheat crops of the semiarid area of the Buenos Aires Province in Argentina (Chantre *et al*., 2009b). *Lithospermum arvense* seeds show primary physiological dormancy, requiring an after-ripening period for dormancy release (Baskin & Baskin, 1998). Temperature is the governing environmental factor that regulates seed dormancy in temperate environments (Benech-Arnold *et al*., 2000). Recently, Chantre *et al*. (2009a) developed an after-ripening thermal-time model to characterise *L. arvense* primary dormancy release based on changes in seed population thermal parameters, the mean maximum germination temperature and the thermal-time required for seed germination at suboptimal temperatures. The developed thermal-time model allowed the prediction of the dormancy status of the seed population under a fluctuating thermal field environment. As a result, *L. arvense* germination 'time window' as well as the proportion of the seed population germinating within this time-period were satisfactorily predicted by the model.

The dormancy status can also be quantified by monitoring changes in the base water potential ($\Psi_b$) of a seed population, based on the hydrotime concept proposed by Gummerson (1986). According to the hydrotime approach, germination rates for the different fractions of a seed population ($1/t_g$) decrease linearly with a common slope ($1/\theta_H$) as the incubation water potential ($\Psi$) is reduced, establishing different thresholds or base water potential values ($\Psi_b(g)$). The hydrotime model (Gummerson, 1986; Bradford, 1990) describes the above-mentioned relation as:

$$GR_g = 1/t_g = (\psi - \psi_b(g))/\theta_H \quad (1)$$

or

$$\theta_H = (\psi - \psi_b(g))t_g \quad (2)$$

where $GR_g$ is the germination rate ($1/t_g$) of a given $g$ fraction of the seed population, $\Psi$ is the water potential of the incubation medium, $\Psi_b(g)$ is the base water potential (i.e., the threshold under which it is too dry to allow germination) for a specific population fraction $g$, $\theta_H$ is the hydrotime constant and $t_g$ is the germination time. A normal distribution of $\Psi_b(g)$ was generally assumed in the seed population based on large experimental evidence (Gummerson, 1986; Bradford, 1990; Dahal & Bradford, 1990).

The hydrotime model provides parameters (i.e., $\Psi_b(50)$, $\theta_H$ and $\sigma_{\Psi b}$) that allow for the prediction of both the extent and timing of germination, for a certain seed population, at any given $\Psi$ possible. The $\theta_H$ value is a measure of the inherent speed of germination of the population; $\Psi_b(50)$ is the mean base water potential, or median $\Psi_b(g)$ value, an indicator of the average stress tolerance; and $\sigma_{\Psi b}$ is the SD of $\Psi_b(g)$ values, an estimator of the uniformity in germination timing among seeds (Bradford, 2002).

As suggested by Bradford (1995), progressive loss of dormancy in a seed population may be related to a progressive decrease in $\Psi_b(50)$ values. Seed dormancy release due to after-ripening in the winter annual weeds *Bromus tectorum* L. (Christensen *et al*., 1996; Bauer *et al*., 1998; Bair *et al*., 2006) and *Elymus elymoides* (Raf.) Swezey (Meyer *et al*., 2000) were adequately described through a reduction in $\Psi_b(50)$ values. Batlla and Benech-Arnold (2004), working with the summer annual *Polygonum aviculare* L., satisfactorily applied the hydrotime approach to describe changes in $\Psi_b(50)$ as a function of a stratification thermal-time index.

Population-based threshold models that account for the distribution of threshold sensitivities among seeds in the population, as well as for shifts in the median sensitivity of a population to environmental factors, can be very helpful for quantifying a wide array of germination and dormancy ecophysiological responses (Bradford, 2005). However, threshold models have seldom been applied under variable field conditions for describing the effect of temperature and water potential on seed germination (Finch-Savage, 2004) and seed dormancy (Grundy, 2003).

In semiarid regions, soil water availability might be a key environmental factor regulating weed seedbank dynamics (Chantre *et al*., 2009b). Cyclic fluctuations in the soil water content for seeds buried near the soil surface have been regarded to affect the dormancy status and the pattern of weed emergence in the field (Bouwmeester, 1990). Therefore, considering the characteristic erratic distribution of the rain patterns and the predominance of sandy soils in the semiarid region under study, the dormancy status of buried weed seeds might be affected by large fluctuations in the seed-zone soil water content. Thus, the objectives of the present work were to: (i) develop an after-ripening thermal-time model for *L. arvense* based on changes in the hydrotime parameters of the seed population, (ii) test the developed model under fluctuating soil water regimes in the field.

## Materials and methods

### *After-ripening treatments*

*Lithospermum arvense* seeds were collected in a wheat field located in Bahía Blanca (latitude 38°44′S, longitude 62°16′W), Argentina, at the time of their natural dispersal on December 20, 2006. After harvest, seeds were cleaned and placed in paper bags before dry storage in growth chambers at constant temperatures

 

of 5, 15, 24, and 30°C for 180 days. Seed germinability was assessed on freshly matured seeds and after 60, 87, 118 and 180 days of dry storage. The water content of seeds at harvest was 9–10% (dry weight basis).

### *Germination test*

Seed germination was evaluated by placing 50 seeds evenly in a 9 cm diameter Petri dish containing two layers of Whatman No. 1 filter paper moistened with 5 ml of distilled water or different polyethylene glycol solutions, establishing water potentials of −0.2, −0.4, −0.8 and −1.2 MPa. Solutions of polyethylene glycol (PEG 6000; Merck KGaA, Darmstadt, Germany) were prepared according to Michel (1983) and water potential values of the solutions were confirmed by measurement with a vapour pressure osmometer (VAPRO 5520; Wescor Inc., Logan, UT, USA). In order to keep the water potential of the medium constant, PEG solutions were renewed after the first 24 h of incubation and subsequently at weekly intervals (Ni & Bradford, 1992). Petri dishes were sealed with parafilm to prevent water evaporation and wrapped in aluminium foil to provide darkness. *Lithospermum arvense* seeds were incubated at an optimal constant temperature of 10°C (Chantre *et al*., 2009a) in the dark, as no light requirement was detected for germination in this species (Chantre *et al*., 2009b). A randomised complete-block design with three replicates was used, except for model evaluation with independent field data ($n = 6$). Each replication was arranged on a different shelf in the incubator and considered as a block. Germination counting was performed under white fluorescent light at regular intervals over a 21 day incubation period, with the criterion for germination being visible protrusion of the radicle. 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% 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.

### *Calculation of hydrotime parameters*

Germination time–course curves obtained under the different water potentials (0, −0.2, −0.4, −0.8 and −1.2 MPa) for seeds after-ripened at each combination of storage time and temperature were analysed by repeated probit regressions to calculate seed population hydrotime parameters ($\Psi_b(50)$, $\theta_H$ and $\sigma_{\Psi b}$). The applied hydrotime approach was previously described by Bradford (1990, 1995) and Dahal and Bradford (1990).

### *Dormancy model development*

In order to quantify the effect of storage time and temperature on *L. arvense* seed population dormancy status, Chantre *et al*. (2009a) developed an after-ripening thermal-time model to account for changes in population thermal parameters. Derived equations related the rate of change of these parameters to the accumulation of after-ripening thermal-time units (°Cd) above a 'base' temperature for dormancy loss to occur, according to the following equation:

$$\theta_{AT} = (T_s - T_l)t_{ar} \quad (3)$$

where $\theta_{AT}$ is the thermal-time requirement for after-ripening, $T_s$ is the storage or after-ripening temperature, $T_l$ is the base temperature (at or below which after-ripening does not occur), and $t_{ar}$ is the time required for after-ripening. The after-ripening base temperature for *L. arvense* seeds was estimated to be −6°C (Chantre *et al*., 2009a).

In this study, changes in $\Psi_b(50)$ were also quantified as a function of storage time and temperature by means of $\theta_{AT}$. The process of model development is summarised as:

(1) Determine seed population hydrotime parameters for seeds dry-stored at 5, 15, 24 and 30°C during different after-ripening time-periods.
(2) Characterise changes in $\Psi_b(50)$ over time as a function of storage temperature, and derive equations relating the rate of change of this parameter to the after-ripening thermal-time.
(3) Use the equation developed in (2) to predict changes in $\Psi_b(50)$ of seeds after-ripened in the laboratory and the field.
(4) Use predicted values of $\Psi_b(50)$ to simulate germination time–course curves and compare with experimentally obtained data.

### *Model evaluation*

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 on January 1, 2008. After harvest, seeds were cleaned and stored in a growth chamber at 25°C during 17 days before burial in the field. The water content of seeds at harvest was 6–9% (dry weight basis). The experiment consisted of seeds buried at 2 cm exposed to two different fluctuating soil water regimes (rain-fed and rain-fed plus irrigation), and retrieved from the field at different after-ripening time-periods (30, 70 and 95 days of burial). Each treatment (combination of a

 

given after-ripening time-period and soil water regime) was applied to a mesh bag containing 350 seeds. The experiment was performed using a completely randomised factorial design with six replicates. *Lithospermum arvense* seeds were buried in the experimental field of the CERZOS (38°39′54″S 62°13′58″W), Universidad Nacional del Sur and CONICET (Bahía Blanca, Argentina) on January 18, 2008 and retrieved from the field on February 18, March 28 and April 22, 2008. After retrieval, *L. arvense* seeds were rinsed with distilled water to remove the soil prior to incubation at different water potentials (0, −0.2, −0.4, −0.8 and −1.2 MPa). Seed subsamples obtained from each replicate were used for gravimetric seed water content determination. Seeds were dried at 103°C for 17 h and then reweighed, expressing seed water content percentages on dry weight basis.

The mesh bags of 10 × 10 cm were made of permeable nylon to emulate natural soil conditions (water and air diffusion). The soil of the experimental site was loamy sand with an organic matter content of 1.3%. A localised irrigation system was used for artificial irrigation. Irrigation was performed every third week and the amount of water was adjusted to achieve a soil water condition close to field capacity in the first 10 cm of the soil profile. Field temperature and soil volumetric water content values ($m^3\ m^{-3}$) were registered hourly at 2 cm depth using temperature data loggers (Thermochron Ibuttons, Model DS1921G-F50; Maxim Integrated Products Inc., Sunnyvale, California, USA) and capacitance sensors (ECHO Dielectric Aquameter, Model EC-20; Decagon Devices Inc., Pullman, Washington, USA) connected to a datalogger, respectively. A soil-specific calibration for capacitance sensors was performed according to Starr and Paltineanu (2002). A soil water release curve was developed according to Richards (1949), to obtain soil water potential values between 0 and −1.5 MPa. Soil water contents corresponding to water potentials of −100 and −1000 MPa were experimentally determined using an air dried and an oven dried soil, respectively.

### *Goodness of fit assessment*

Model evaluation was based on the coefficient of determination ($R^2$) and the root mean square error (RMSE). $R^2$ indicates the fraction variance accounted for by the simulation model:

$$R^2 = 1 - \left[\sum (y_{obs} - y_{sim})^2 / \sum (y_{obs} - \bar{Y}_{obs})^2\right] \quad (4)$$

RMSE is an estimator of the difference between observed and predicted germination values. A small value of RMSE indicates a close agreement between observed and simulated data (McMaster *et al.*, 1992).

$$\text{RMSE} = \left[\sum (y_{obs} - y_{sim})^{2/n}\right]^{1/2} \quad (5)$$

where $n$ is the divisor of the expression $\sum (y_{obs} - y_{sim})^2$ where $y_{obs}$ are the observed values and $y_{sim}$ the simulated values. $n$ is the number of cases in the model.

## Results

### *Germination time–course curves*

After harvest, a high percentage of *L. arvense* seeds were able to germinate at 0 MPa (88 ± 6%); however, seed germination was drastically reduced to 31% (±24) and 13% (±11), when seeds were incubated at −0.2 and −0.4 MPa, respectively. No germination was registered at −0.8 and −1.2 MPa during the 21 day incubation period. As after-ripening time increased, *L. arvense* seeds were capable of germinating at progressively more negative water potentials, as evidenced by the higher germination percentages observed in the evaluated osmotic range (compare columns in Fig. 1). Germination percentages at a given after-ripening time-period also increased when seeds were after-ripened at higher storage temperatures (compare rows in Fig. 1).

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

A progressive decrease in $\Psi_b(50)$ values was observed as storage time increased ($r^2 = 0.57$, $P < 0.001$), irrespective of storage temperature. $\Psi_b(50)$ values of −0.36 and −1.04 MPa were estimated for recently harvested seeds and after storage for 180 days at 30°C, respectively (Table 1). $\Psi_b(50)$ values were also reduced as storage temperature increased ($r^2 = 0.40$, $P < 0.01$). The rate of decrease of $\Psi_b(50)$ over storage time or dormancy release rate (expressed as MPa per day) was positively related to storage temperature (Fig. 2). After 180 days of storage, $\Psi_b(50)$ values were reduced to −0.84, −0.96, −1.00 and −1.04 MPa for seeds after-ripened at 5, 15, 24 and 30°C, respectively (Table 1). Conversely, $\sigma_{\Psi b}$ values showed no consistent variation when regressed as a linear function of after-ripening time ($r^2 = 0.01$, $P = 0.73$) or storage temperature ($r^2 = 0.06$, $P = 0.32$). The hydrotime constant was the less variable parameter (CV = 12.3%; Table 1), being unaffected by after-ripening time or storage temperature ($P > 0.05$).

### *Model development*

The dormancy release process was characterised by a progressive reduction in $\Psi_b(50)$ values as a function of

 

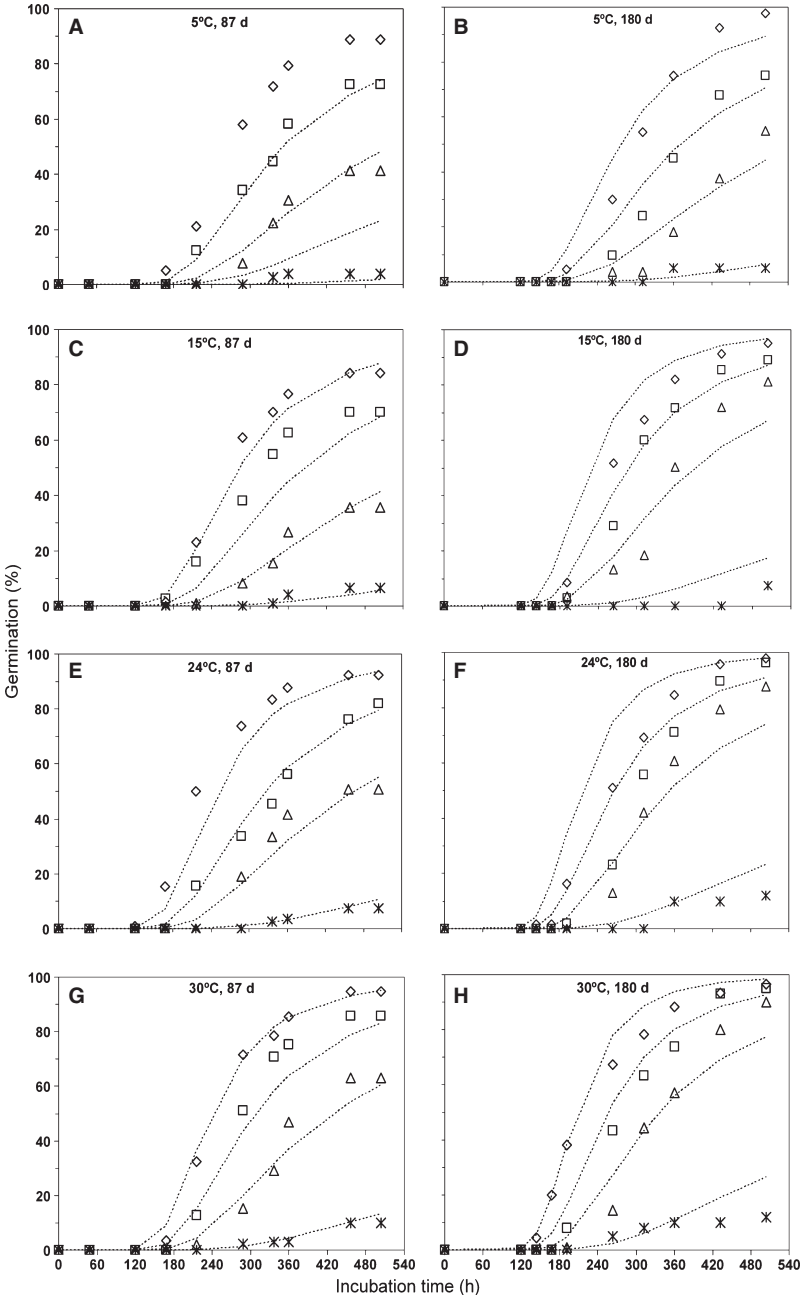


**Fig. 1** Observed and predicted germination time–course curves for *L. arvense* seeds after-ripened at 5, 15, 24 and 30°C for 87 (A, C, E, G) and 180 (B, D, F, H) days, and subsequently incubated at 0 (◇), −0.2 (□), −0.4 (△) and −0.8 (✱) MPa at 10°C for 21 days. No germination was observed at −1.2 MPa for any of the after-ripening treatments. The dotted lines represent predicted values from simulation modelling, while symbols represent observed germination data.

after-ripening time and storage temperature. In order to quantify the effect of temperature on seed population dormancy status, changes in $\Psi_b(50)$ were predicted as a function of the accumulation of after-ripening thermal-time units (°Cd), according to Eqn (3). An after-ripening base temperature of −6°C was used (Chantre *et al.*, 2009a). As observed in Fig. 3, changes in $\Psi_b(50)$ were adequately described by the following exponential decay function:

$$\psi_b(50) = 0.7198e(-4.583e^{-04}\theta_{AT}) - 1.083 \quad (6)$$

This equation could be written in terms of the biological meaning of its parameters, as:

$$\psi_b(50) = [\psi_{bi}(50) - \psi_{bf}(50)]e(-K\theta_{AT}) + \psi_{bf}(50) \quad (7)$$

where $\Psi_{bi}(50)$ and $\Psi_{bf}(50)$ are predicted $\Psi_b(50)$ values for recently harvested and fully after-ripened seeds, respectively. Thus, $\Psi_{bi}(50)$ represents the initial value of $\Psi_b(50)$ where $\theta_{AT}$ equals zero, and $\Psi_{bf}(50)$ is the *plateau* of the exponential decay function. $K$ is the exponential rate at which $\Psi_b(50)$ is reduced from $\Psi_{bi}(50)$ to $\Psi_{bf}(50)$ as after-ripening thermal-time is accumulated.

### *Model evaluation*

Experimentally obtained germination data used for the determination of *L. arvense* population hydrotime

 

**Table 1** Estimated population hydrotime parameters for *L. arvense* seeds after-ripened at 5, 15, 24 and 30°C

| Storage temperature (°C) | Days of storage | $\psi_b(50)$ (MPa) | $\sigma_{\psi b}$ (MPa) | $\theta_H$ (MPa h) | $R^2$ |
|---|---|---|---|---|---|
| | 0 | −0.36 | 0.17 | 199 | 0.94 |
| 5 | 60 | −0.48 | 0.29 | 238 | 0.92 |
| | 87 | −0.75 | 0.29 | 190 | 0.97 |
| | 118 | −0.67 | 0.38 | 154 | 0.94 |
| | 180 | −0.84 | 0.20 | 238 | 0.97 |
| 15 | 60 | −0.72 | 0.29 | 242 | 0.93 |
| | 87 | −0.74 | 0.31 | 194 | 0.97 |
| | 118 | −0.81 | 0.30 | 190 | 0.95 |
| | 180 | −0.96 | 0.26 | 242 | 0.95 |
| 24 | 60 | −0.82 | 0.28 | 247 | 0.97 |
| | 87 | −0.81 | 0.29 | 197 | 0.97 |
| | 118 | −1.01 | 0.37 | 235 | 0.95 |
| | 180 | −1.00 | 0.23 | 245 | 0.93 |
| 30 | 60 | −0.63 | 0.28 | 214 | 0.94 |
| | 87 | −0.98 | 0.27 | 235 | 0.98 |
| | 118 | −1.00 | 0.37 | 238 | 0.96 |
| | 180 | −1.04 | 0.28 | 233 | 0.97 |
| Mean (CV) | | −0.80 (24.1) | 0.29 (19.3) | 219 (12.3) | |

Seeds were incubated at different water potentials (0, −0.2, −0.4, −0.8 and −1.2 MPa) at an optimal temperature of 10°C after 0, 60, 87, 118 and 180 days of storage. Parameters were obtained by repeated probit regressions until the best fit to the observed germination data was attained. $R^2$ values for each after-ripening treatment as well as the coefficients of variation (CV) for each parameter are included.

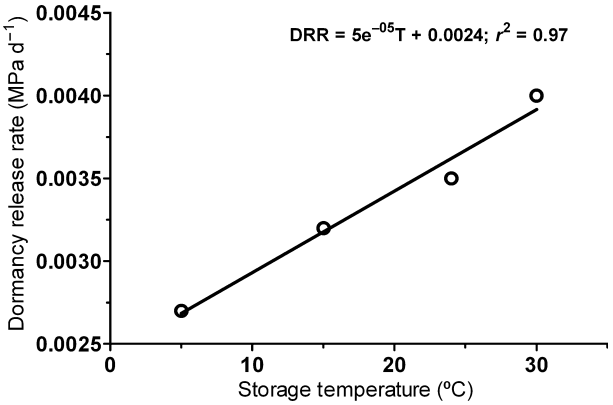


**Fig. 2** Linear relation between dormancy release rate (rate of $\Psi_b(50)$ decrease over storage time, expressed as MPa per day) and storage temperature for *L. arvense* seeds.

parameters were compared with predicted germination values, in order to assess the goodness of fit of the developed model. Based on the assumption of a normally distributed $\Psi_b$ in the seed population (Gummerson, 1986; Bradford, 1990; Dahal & Bradford, 1990), the proportion of germinating seeds was described by the following equation:

$$p(\psi_b(g)) = \phi\left[(\psi_b(g) - \psi_b(50))/\sigma_{\psi b}\right] \qquad (8)$$

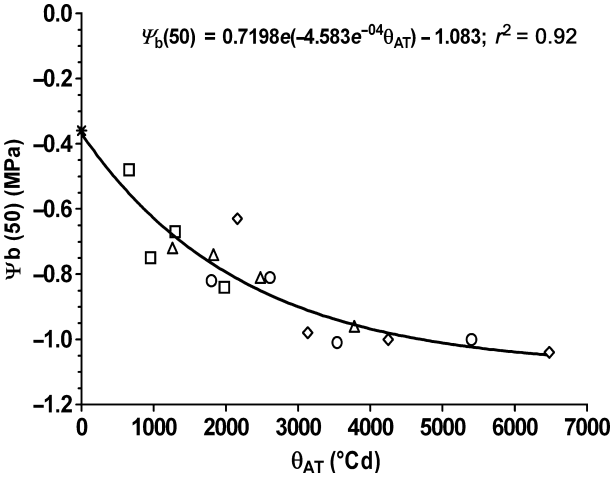


**Fig. 3** Estimated values of the mean base water potential ($\Psi_b(50)$) for recently harvested seeds of *L. arvense* (✱) and for seeds after-ripened at 5 (□), 15 (△), 24 (○) and 30°C (◇), plotted against after-ripening thermal-time ($\theta_{AT}$). The solid line corresponds to an exponential decay function.

where $p$ is the proportion of germinating seeds at a given $\Psi_b$ of a $g$ fraction of the population, $\Phi$ is the normal probability integral, $\Psi_b(50)$ and $\sigma_{\Psi b}$ are the mean and SD of the normal distribution. The equation variable $\Psi_b(g)$ could also be described as $[\Psi - (\theta_H/t_g)]$, where $\Psi$ is the water potential of the incubation medium, $\theta_H$ is the hydrotime constant and $t_g$ is the germination time of a given $g$ fraction of the seed population.

Simulation of germination data for the different after-ripening treatments was based on the estimation of changes in $\Psi_b(50)$ values according to Eqns (3) and (6). $\sigma_{\Psi b}$ and $\theta_H$ were considered constant for the simulation process; thus the following average values were used: $\sigma_{\Psi b}$ = 0.29 MPa and $\theta_H$ = 219 MPa h (Table 1).

Observed germination data were adequately described by the developed model ($R^2$ = 0.90; RMSE = 10), suggesting that most of the germination behaviour of *L. arvense* seeds could by satisfactorily explained by a progressive reduction in $\Psi_b(50)$ values as a function of $\theta_{AT}$, according to Eqn (6). However, the model underestimated germination time–course curves at some after-ripening treatments, where $\theta_H$ values were lower than the average value used for simulation (e.g., Fig. 1A and C; Table 1). On the contrary, the model overestimated germination percentages in some cases where observed $\theta_H$ values were higher than the mean, mainly at 0 and −0.8 MPa (e.g., Fig. 1D and F).

Despite the contrasting variations in the soil water content (Fig. 4A) and soil water potentials values (Fig. 4B) between regimes, and the subsequent differences in seed water content at exhumation (rain-fed = 10.3 ± 1.2%; irrigated = 19.1 ± 5%), model predictions were fairly accurate under both rain-fed (RMSE =

 

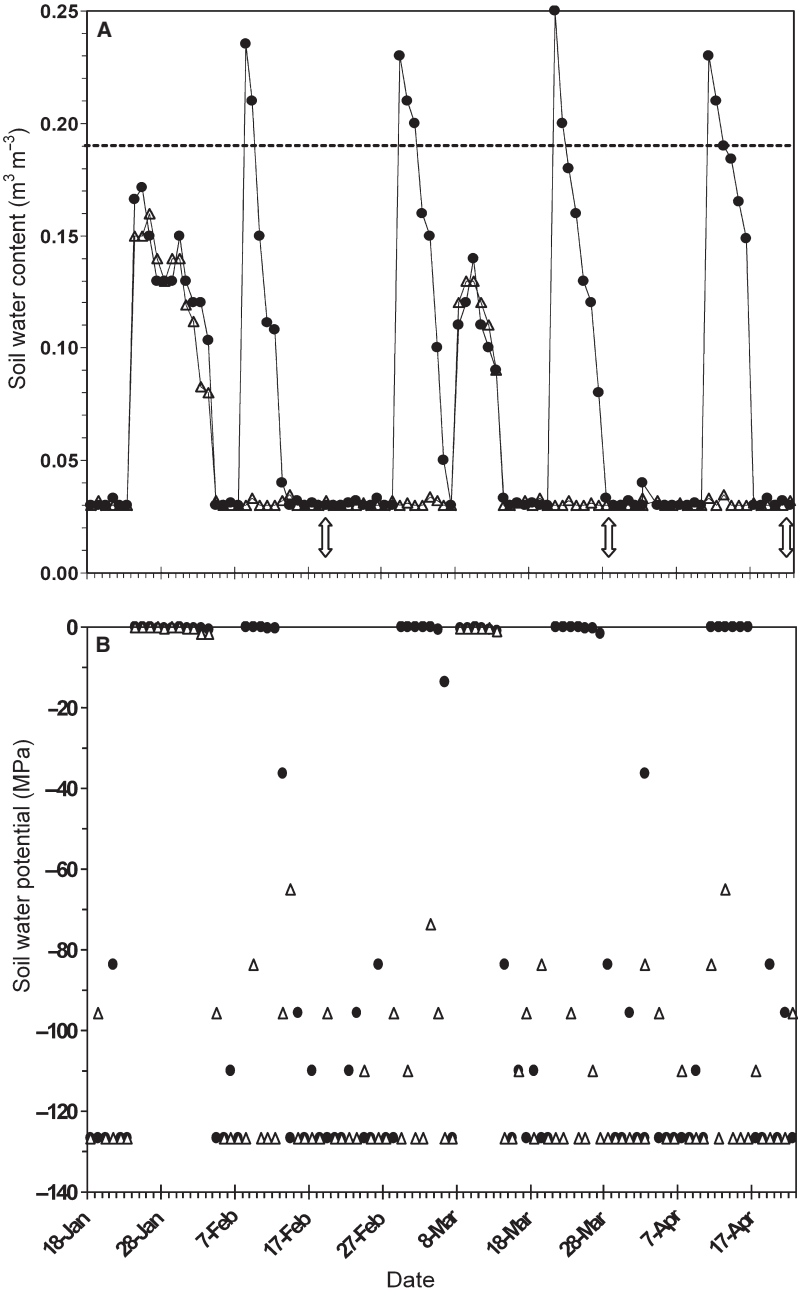


**Fig. 4** Soil volumetric water content (A) ($m^3m^{-3}$) and soil water potential (B) (MPa) values measured at 2 cm depth in the field for *L. arvense* seeds after-ripened under a (△) rain-fed or (●) rain-fed plus irrigation water regime. Seeds were buried on January 18, 2008 and retrieved from the field after 30 (February 18), 70 (March 28) and 95 days (April 22) of burial (exhumation dates are indicated by vertical arrows). The dotted horizontal line in (A) indicates the soil water content value corresponding to field capacity.

10.8) and irrigation (RMSE = 13.5). A good correlation between observed and predicted germination data was obtained (Pearson's correlation coefficient, $r_{\text{rain-fed}}$ = 0.96; $r_{\text{irrigated}}$ = 0.94).

However, the model underestimated germination time–course curves at 0, −0.2 and −0.4 MPa for seeds after-ripened under a rain-fed regime for 70 days (Fig. 5A) and also at 0 and −0.2 MPa for seeds after-ripened for 95 days under the same soil water regime (Fig. 5C). Overestimation was observed for *L. arvense* seeds incubated at −0.2 and −0.4 MPa after 95 days of burial under the irrigated regime (Fig. 5D).

## Discussion

In this study, $\Psi_b(50)$ was used as an index of mean seed population dormancy status. Model development consisted of the estimation of the hydrotime parameters, assuming a normal distribution of base water potentials in the seed population (Gummerson, 1986; Bradford, 1990; Dahal & Bradford, 1990) and the quantification of changes in $\Psi_b(50)$ as a function of after-ripening time and temperature.

The evaluated dormancy release index showed a progressive decrease as after-ripening time progressed

 

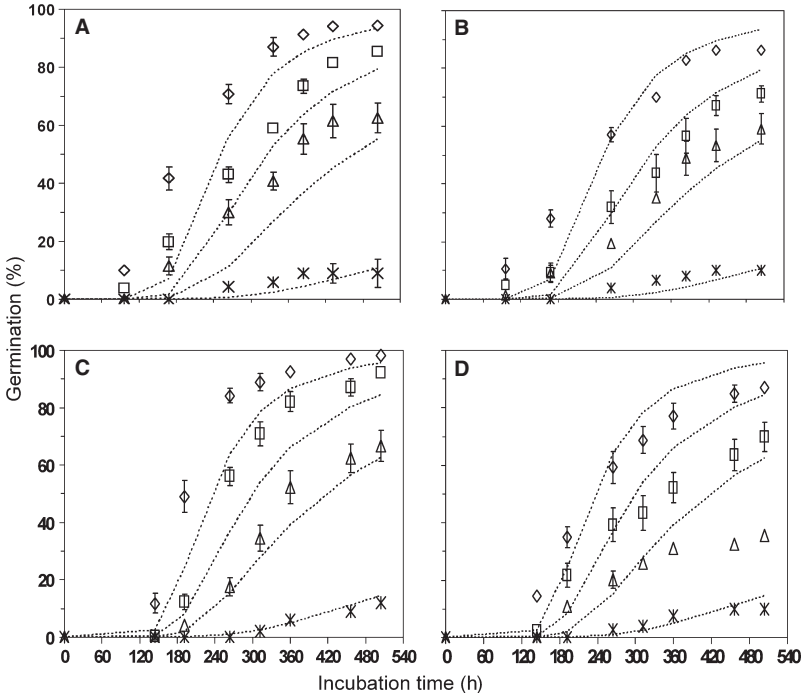


**Fig. 5** Observed and predicted germination time–course curves for *L. arvense* seeds after-ripened in the field for 70 and 95 days under a rain-fed (A and C) or rain-fed plus irrigation (B and D) water regime, and subsequently incubated at 0 (◇), −0.2 (□), −0.4 (△) and −0.8 (✱) MPa at 10°C for 21 days. No germination was observed at −1.2 MPa for any of the after-ripening treatments. The dotted lines represent predicted values from simulation modelling, while symbols represent observed germination percentages. Vertical bars indicate SE if larger than the symbols ($n = 6$).

($P < 0.001$). The rate of decrease of $\Psi_b(50)$ over storage time or dormancy release rate was appropriately described as a positive linear function of storage temperature (Fig. 2). These results concur with previous reports indicating a positive relation between storage temperature and dormancy release rate for *L. arvense* (Chantre *et al*., 2009a) and other winter annuals species (Bauer *et al*., 1998; Steadman *et al*., 2003).

To account for the effect of after-ripening time and storage temperature on the dormancy release process, an after-ripening thermal-time model was developed. An exponential decay function accurately described the decrease pattern of $\Psi_b(50)$ values as a function of $\theta_{AT}$ (Eqn 6). As inferred from Fig. 3, the rate of decrease of $\Psi_b(50)$ values was faster during the first 3000°Cd of storage, becoming slower as *L. arvense* seeds fulfilled their after-ripening requirement for dormancy loss. Batlla and Benech-Arnold (2004) reported a negative exponential decrease pattern of $\Psi_b(50)$ as a function of stratification thermal-time accumulation for *P. aviculare* seeds. Similarly, Bair *et al*. (2006) observed a negative exponential decrease pattern of $\Psi_b(50)$ as *B. tectorum* seeds accumulated after-ripening thermal-time at a range of osmotic potentials between −300 and −80 MPa.

*Lithospermum arvense* germination time–course curves for the different after-ripening treatments in the laboratory were adequately described by the developed model ($R^2 = 0.90$; RMSE = 10); thus, indicating that accounting for changes in $\Psi_b(50)$ alone, while considering $\sigma_{\Psi b}$ and $\theta_H$ constant, was a suitable model approach. These results coincide with previous reports in other species (Bauer *et al*., 1998; Batlla & Benech-Arnold, 2004; Gianinetti & Cohn, 2007). Despite this fact, the model underestimated (Fig. 1A and C) or overestimated (Fig. 1D and F) germination data at certain water potentials, when observed $\theta_H$ values were lower or higher than the average $\theta_H$ value used for simulation, respectively. As previously mentioned, $\theta_H$ is a measure of the speed of germination of the population. Thus, lower $\theta_H$ values indicate a reduction of the required time for seed germination or a faster germination rate, for a given $\Psi - \Psi_b(g)$ difference (Eqns 1 and 2). In an analogous way, higher $\theta_H$ values suggest a reduction in the speed of germination. As mentioned before, $\theta_H$ was unaffected by after-ripening time or storage temperature ($P > 0.05$). Thus, observed variations in $\theta_H$ may be related to the time required for imbibition and metabolic activation processes during germination, which may be relatively unaffected by seed dormancy status, as suggested by Alvarado and Bradford (2005).

Fluctuations in the seed water content during after-ripening have been suggested to alter the dormancy release rate in many species (Steadman *et al*., 2003; Gallagher *et al*., 2004). Hydration–dehydration cycles have been associated with an alteration of the dormancy status and the field emergence pattern of buried weed seeds (Bouwmeester, 1990; Batlla & Benech-Arnold, 2006).

In this study, *L. arvense* seeds were exposed to widely fluctuating soil water potential cycles in the field, as a result of the interaction between water regimes and soil water loss dynamics. Despite the variations in the frequency and the magnitude of the fluctuations in the

 

soil water content (Fig. 4A) and water potentials values (Fig. 4B) between regimes, model predictions were fairly accurate in both cases ($RMSE_{rain\text{-}fed}$ = 10.8; $RMSE_{irrigated}$ = 13.5). A possible explanation for the capability of the thermal-time model to describe *L. arvense* seed dormancy loss under the evaluated range of fluctuating soil water regimes might be found in the conceptual model proposed by Bair *et al.* (2006). According to Bair *et al.* (2006), *B. tectorum* seeds, stored at constant water potentials between −150 and −40 MPa, experience after-ripening as a linear function of temperature alone, whereas storage water potential does not affect the after-ripening rate. In contrast, prolonged seed exposure to constant water potentials above −40 MPa would hinder the after-ripening process, promoting seed deterioration or accumulation of progress towards germination. As observed in Fig. 4B, soil water potential values for *L. arvense* seeds ranged from −127 to −0.02 MPa under both regimes. However, such water potential values remained below −40 MPa during 82% and 54% of the after-ripening time-period for the rain-fed and the irrigated regimes, respectively. Despite the greater time of seed exposure under the latter regime to high water potentials (close to field capacity = −0.033 MPa), the after-ripening process was satisfactorily described as a thermal-time response (Fig. 5). This could be explained by the fact that seed exposure to high $\Psi$ values took place during intermittent short-time intervals, because of the applied irrigation pattern and the occurrence of a sandy soil texture favouring rapid soil water loss by evaporation and infiltration. No evidence of seed deterioration or accumulation of progress towards germination was observed during the course of the after-ripening process.

These results agree with previous seedbank studies, which suggested that the rate of primary dormancy release of *L. arvense* was unaffected by soil water content fluctuations in the same study area (Chantre *et al.*, 2009b). Previously, Bauer *et al.* (1998) had successfully applied an after-ripening thermal-time model to predict *B. tectorum* seed dormancy loss in the field, when water potentials were above −150 MPa.

Based on the observed results, we conclude that the after-ripening thermal-time model developed in this work would be a valuable tool to enhance the prediction of both the extent and timing of *L. arvense* germination under field conditions. However, it should be considered that under drier soil water conditions, such as those that prevail during very hot and dry summers in semiarid regions, the after-ripening model alone may not be an adequate predictor of the seed dormancy status. Thus, further work should be performed, in order to account for the effect of a wider range of soil water potentials on *L. arvense* seed dormancy loss.

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