

# A quantitative analysis of temperature-dependent seasonal dormancy cycling in buried *Arabidopsis thaliana* seeds can predict seedling emergence in a global warming scenario

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**Highlight**

A model that simulates seasonal dormancy changes in buried *Arabidopsis thaliana* seeds can predict germination in a realistic global warming scenario.

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## Abstract

Understanding how the environment regulates seed bank dormancy changes is essential for forecasting seedling emergence in actual and future climatic scenarios, and to interpret studies of dormancy mechanisms at the physiological and molecular level. Here we used a population threshold modelling approach to analyse dormancy changes through variations in the thermal range permissive for germination in buried seeds of *Arabidopsis thaliana* Cvi a winter annual ecotype. Results showed that changes in dormancy level were mainly associated with variations in the higher limit of the thermal range permissive for germination. Changes in this limit were positively related to soil temperature during dormancy release and induction and could be predicted using thermal time. From this we developed a temperature driven simulation to predict the fraction of the seed bank able to germinate in a realistic global warming scenario that approximated seedling emergence timing. Simulations predicted, in accordance with seedling emergence observed in the field, an increase in the fraction of the seed bank able to emerge as a result of global warming. In addition, our results suggest that buried seeds perceive changes in the variability of the mean daily soil temperature as the signal to change between dormancy release and induction according to the seasons.

Keywords: *Arabidopsis thaliana*, dormancy, global warming, population threshold models, seed bank, seedling emergence, simulation, soil temperature.

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## Introduction

Seedling emergence at the right time and in the right place is essential for ensuring species persistence in a given ecological scenario (Donohue et al., 2010). Thus, sophisticated mechanisms have evolved to ensure seeds germinate in the right context. The most common of these mechanisms is seed dormancy, a trait through which seeds are able to sense the environment around them and adjust their response for timing germination completion to occur under favorable conditions (Finch-Savage and Leubner-Metzger, 2006; Willis et al., 2014; Arana et al., 2016).

Dormancy can be defined as an internal impedance to the seeds progress towards germination completion under moisture, thermal and gaseous conditions that, otherwise, would result in germination (Egley 1986; Benech-Arnold et al. 2000). It is a common feature in seeds forming persistent seed-banks. Usually seed-banks show cyclic seasonal changes in their dormancy level which determine their seedling emergence season. The dynamics of these cyclic changes in relation to seasonal changes throughout the year depends on the species' life cycle. In winter annuals the dormancy level usually decreases during summer and increases during winter (Baskin and Baskin, 1998). This regulation ensures that the seasonal emergence window occurs during autumn when seeds have a relatively low dormancy level. The resulting plants overwinter in the rosette stage and flower, and set seeds, in the following spring and early summer. In contrast, summer annual species, generally show the reverse dormancy pattern, in which dormancy is relieved during winter, establishing their emergence window in spring. For many species in the absence of germination at these allotted times for both summer and winter annuals the seeds dormancy is subsequently reinforced to restart the seasonal dormancy cycle (Finch-Savage and Footitt, 2017).

These seasonal changes in seed-bank dormancy level are mainly driven by soil temperature (Finch-Savage *et al.*, 2007; Bewley *et al.*, 2013). For example, in winter annuals high summer temperatures determine release from primary dormancy, while low winter temperatures promote the induction into secondary dormancy. These changes in dormancy level are related to changes in the range of temperatures permissive for seed germination, which widens as dormancy release takes place and narrows as seed dormancy level increases (Vegis, 1964; Vlesshouwers et al., 1995; Probert, 2000). This range can be characterized through lower and higher limit temperatures that define the range so that dormancy changes can be quantified through changes in these parameters (Batlla and Benech-Arnold, 2015). In winter annuals, for example, seasonal changes in the permissive thermal range (i.e. widening and narrowing) are usually determined by gradual changes in the higher temperature limit for germination (Baskin and Baskin, 1998), thus, changes in this single temperature limit can be monitored to quantify changes in the seed population dormancy level (Chantre et al., 2009).

Normally, emergence in the field takes place when seeds display a relatively low -or minimum- dormancy level, which maximizes the probability of an overlap between prevailing environmental conditions and the environmental range within which germination can proceed. We can characterise this as the window of maximum opportunity, or germination window during which seeds become sensitive to signals (e.g. light) that remove the final layer of dormancy, so allowing germination to proceed. For example, in winter annual species seeds present a maximum permissive thermal range for seed germination at the end of the summer, so germination takes place when descending autumn temperatures overlap the range (Probert, 2000). Thus quantifying changes in the permissive thermal range for germination of seeds buried in the field and relating these changes to prevailing soil temperature allows the prediction of the germination window to indicate emergence timing from seedbanks. This approach has been demonstrated for summer annual species such as *Polygonum aviculare* (Batlla and Benech-Arnold, 2003; Fernandez et al., 2019, 2021; Malavert et al., 2020), but not for winter annuals.

Understanding, and eventually predicting, seedling emergence is essential for forecasting how plant populations will behave in a new ecological scenario or respond to changing climatic conditions, for example those resulting from anthropomorphic climate change. Extensive field studies have described and contrasted the timing of seedling emergence in response to the environment in both obligate winter and summer annual *Arabidopsis thaliana* ecotypes (Finch-Savage and Footitt, 2017; Footitt et al., 2021). In the present work we analysed changes in the dormancy level of seeds of an obligate winter ecotype (Cape Verde Islands (Cvi)) of *A. thaliana* buried for two years in the soil using a population threshold modelling approach. This enabled quantification of seasonal changes in the thermal range permissive for seed germination. From this we developed a temperature driven simulation able to predict the fraction of the seed bank able to germinate in a realistic global warming scenario that approximated actual seedling emergence timing.

## Material and Methods

### Seed production

Seeds of the *Arabidopsis thaliana* ecotype Cape Verde Islands (Cvi; N8580) were produced (February to May 2007) in a temperature controlled glasshouse compartment at Wellesbourne, UK (52°11'N, 1°35'W). Heating and venting were set to provide a temperature of 16-18 °C during the 16 h day and 10-15 °C at night. Supplementary lighting (400 W high pressure sodium lamps; Osram Ltd, UK) was supplied when light intensity fell below 300 w m<sup>2</sup> during the 16 h day. Nondormant seeds were sown into compost (Levingtons F2/sand /vermiculite at a ratio of 6/1/1) in multi-cell trays held in capillary matting lined seed trays. Four cohorts each of 96 plants were produced with two cohorts placed on each side of the compartment with at least 1 m distance between each tray. Temperature and light conditions differed subtly across the compartment so that small differences in the behaviour of the

seeds produced was expected. Mature seeds were harvested separately from each tray by hand threshing and equilibrated at 15% RH/ 5°C for 7 days to produce an equilibrium moisture content of 5-7 % on a dry weight basis. Seeds of each cohort were stored separately at -80°C in sealed tubes. Prior experiments have shown such storage conditions effectively halt changes in dormancy characteristics over the time frame of the experiments conducted.

### Burial experiments

Seeds of each cohort were dispersed in soda lime Ballotini balls (Potters Ballotini, UK) in nylon mesh bags and buried in the field at Wellesbourne, UK, on 11 October 2007 at a depth of 5 cm. Seeds of each cohort were buried in adjacent plots in the field trial area. At monthly intervals for 2 years, a bag of seeds for each cohort was recovered and seeds for the physiological analysis shown here were separated from Ballotini balls in the light by first washing bags in cold water to remove excess soil then separating Ballotini balls from seeds by sedimentation in cold water. SM200 soil moisture sensors (Delta-T Devices, UK) and Thermistore temperature probes (Betatherm, USA) linked to a data logger (Delta-T Devices, UK) recorded soil moisture and temperature at seed depth in dummy bags. Full details of seed burial and recovery are given in the supplemental information accompanying Footitt et al., (2011).

### Seed germination assays

To evaluate changes in thermo-dormancy during burial, recovered seeds were treated as follows. Seeds were surface-sterilized in a 0.125% sodium hypochlorite solution [household bleach (5% sodium hypochlorite) diluted to 2.5%] for 5 min and then washed three times in water. Using Ultipette BARKY CP-100 tips (Barky Instruments International, UK), 50 seeds were plated out in three replicate plates (124 × 88 × 22 mm) (Stewart Plastics, UK). Each plate contained two sheets of Whatman 3MM chromatography paper and 8 mL of water. Plates were placed in sealable freezer bags and incubated at 5, 10, 15, 20, and 25 °C under continuous light. Germination was scored at 2- to 3-d intervals for 28 d. Germination was recorded as the emergence of the radicle through the endosperm and seed coat. Seeds that did not become decayed in the test were considered viable but deeply dormant. In addition, the initial level of seed dormancy (primary dormancy) in seed before burial was evaluated on water as above for buried seeds.

### Quantification of seasonal changes in the thermal range permissive for seed germination during burial

Changes in the thermal range permissive for seed germination during burial were quantified through a mathematical model based on the conceptual model presented in Batlla and Benech-Arnold (2015). The model assumes that the seed population thermal range permissive for germination is limited by two thresholds temperatures ( $T_h$  and  $T_l$ , the higher and lower limit temperature for seed germination, respectively). Both limit temperatures are normally distributed within the seed population and can be characterized by their mean values ( $T_h(50)$  and  $T_l(50)$ ) and their corresponding

standard deviations ( $\sigma_{T_l}$  and  $\sigma_{T_h}$ ). According to this, the fraction of germinating seeds (GF) at a given temperature ( $T$ ) can be calculated as:

$$GF(T) = (\Phi((T - T_l(50))/\sigma_{T_l}) - (1 - \Phi((T - T_h(50))/\sigma_{T_h}))) \quad (1)$$

where  $\Phi$  is the normal probability integral. The model assumes that for the fraction of seeds for which  $T$  is within the limits  $T_l$  and  $T_h$ , germination rate depends on the accumulation of thermal time above or below a threshold value (i.e. base or maximum temperature, respectively). Thermal time is considered to be normally distributed within the seed population with a mean ( $\theta(50)$ ) and a standard deviation ( $\sigma_\theta$ ); while the cardinal temperatures for seed germination, base temperature ( $T_b$ ), optimum temperature ( $T_o$ ) and maximum temperature ( $T_m$ ) are considered constant for the entire seed population (Garcia-Huidobro et al., 1982). Thermal time in the sub-optimal thermal range ( $\theta_{sub}$ ; between  $T_b$  and  $T_o$ ) and the supra-optimal thermal range ( $\theta_{sup}$ , between  $T_o$  and  $T_m$ ) can be calculated for different fractions of the population ( $g$ ) with equations 2 and 3, respectively:

$$\theta_{sub}(g) = (T - T_b) t_g \quad (2)$$

$$\theta_{sup}(g) = (T_m - T) t_g \quad (3)$$

Where  $t_g$  is the time required for completing the germination of the fraction  $g$  of the population. The model assumes that cardinal temperatures for seed germination ( $T_b$ ,  $T_o$  and  $T_m$ ) remains invariable during seed dormancy release and induction (Kruk and Benech-Arnold, 1998; Batlla et al., 2009; Arana et al., 2016). Based on preliminary model adjustments and bibliographic data cardinal temperatures values were considered 0°C for  $T_b$ , 15°C for  $T_o$  and 35°C for  $T_m$  (Baskin and Baskin, 1972; Salaita et al., 2005; Soltani et al., 2017). Parameters other than cardinal temperatures for seed germination were derived by minimizing the root-mean-square error (RMSE) between simulated and germination time-courses at 5, 10, 15, 20 and 25 °C for each burial time through a non-linear optimization procedure using Solver tool of Microsoft Excel (2003–2010). Parameters were only derived for those cases in which germination was > 10% at least at one of the tested incubation temperatures.

Permissive thermal range limit temperature ( $T_l$  and  $T_h$ ) values obtained for seeds during seed burial were used to develop functions able to predict changes in both limits in relation to soil temperature. According to the model framework (Batlla and Benech-Arnold, 2015) the percentage of seeds in the seed bank that can complete germination and could result in emerged seedlings is predicted when soil temperature is within the limits of that fraction. Once temperature is within the permissive thermal range, seed germination rate depends on the accumulation of thermal time ( $\theta$ ) according to the prevailing temperature. Its important to note that  $T_l$  and  $T_h$  are different from  $T_b$  and  $T_m$ . While  $T_l$  and  $T_h$  are the lower and higher, respectively, limit temperatures of the thermal range permissive for seed germination and are related to the seed population dormancy level.  $T_b$  and  $T_m$  are the threshold values above or below which, respectively, thermal time for seed germination is

accumulated once soil temperature is within the range of temperatures permissive for seed germination (Batlla and Benech-Arnold et al., 2015).

Collection of seedling emergence data in a global warming scenario used to validate the simulation model

Seedling emergence was determined in a global warming scenario using a thermogradient tunnel. The data used here was part of a large screen reported in Footitt et al., (2021). Here, the *A. thaliana* Cvi data is used to validate the model. Full details of the experiment set and operation are in the supplemental information of Footitt et al (2021). Brief details follow. The polyethylene tunnel (32 m long × 9 m wide) structure enabled the fraction of the seed population that resulted in seedling emergence to be monitored under natural day lengths with a high percentage (76%) of natural irradiance levels. The ambient air temperature was constantly monitored outside of the tunnel. Reacting to this, an electronic climate control system operated fans that generated opposing warmed and ambient air flows to establish and maintain a temperature gradient from ambient at one end of the tunnel to approximately ambient +4 °C at the other end (Wurr et al., 1996). Air and soil temperatures were monitored continuously along the tunnel. Realistic seasonal and diurnal temperature fluctuations were therefore maintained within the tunnel, but with varying degrees of simulated climate warming depending on the position that seeds were buried along the tunnel.

A projected median emissions scenario for the local experimental area used in this work (West Midlands, UK) indicates an increase in the summer mean temperature of 3.7 °C by 2080 (UK Climate Change Projections, 2014; <http://ukclimateprojections.metoffice.gov.uk/>). We therefore adjusted the tunnel to a gradient from ambient to approximately + 4 °C this gave a soil temperature gradient of 2.5°C.

*A. thaliana* Cvi seeds were produced from two replicate plants in a growth cabinet at 15°C under a 16 h light/8h dark cycle at a light intensity of 100  $\mu\text{moles m}^{-2} \text{sec}^{-1}$  and 80% RH (RH was gradually reduced to 45%). Seed harvesting, processing, and storage was as described above. Seedling emergence was performed in 9 cm square pots 2 cm deep with a 125  $\mu\text{m}$  nylon mesh bottom.

Each pot contained 50 mL of sterile soil, on top was dispersed 10 mg of seed which was covered with a further 75 mL of sterile soil. Seeds from the two replicate plants were sown in individual pots and placed in 1.2 metre square and 12 cm deep gravel trays (1.2 x 1.2 m x 12 cm deep; Garland Products Ltd, Kingswinford, UK) filled with sand to the lip of the seed containing pots.

Trays were placed at three positions along the thermogradient tunnel being 1.45 m, 14.5 m and 28.5 m from the ambient end of the tunnel, these positions were termed Ambient (cool), Middle, and Warm, respectively. Trays were watered regularly using mist spray irrigation to prevent soil drying. The experiment was set up to simulate seed dispersal in the spring on 15-16 May 2013 (winter annual behaviour). Soil in each pot was disturbed by stirring every 2 weeks allowing exposure to light, and emerged seedlings recorded and removed. Thermistore temperature probes (Betatherm) linked to a data logger (Delta-T Devices, UK) recorded soil temperature in each tray.



## Results

### Germination behaviour in the first year of burial

All cohorts (4) had a high dormancy level at burial (11 October 2007) with maximum germination ranging from 21 – 30% at 10°C, above and below this temperature germination decreased (Fig. S1). Once buried, seeds remained dormant at all times as they were not exposed to light and therefore did not germinate in the soil. When exhumed, the seeds were exposed to light, but only germinated when in the low dormancy phase. In this phase they were sensitive to light which removed the final layer of dormancy to enable germination. Exhumed seeds of the four cohorts presented a clear seasonal pattern of change in their dormancy level (Figs. 1-4), however differences between cohorts were observed.

For most cohorts no germination or very low germination values (<5%) were seen, on exposure to light, in seed exhumed during the first 7 months after burial (data not shown). The exception was cohort 4, which showed a fraction of the seed population (< 20%) able to germinate at low temperatures (5°C) following exhumations from February to the beginning of April (Fig. 4). In the first week of May all cohorts showed a slight increase in germination capacity at low temperatures (5°C) (Figs. 1-4). For seeds exhumed in June, a fraction of the seed population acquire the capacity to germinate at 10 °C, while seeds exhumed in July were able to germinate in a wider thermal range (most seeds germinated at temperatures below 20°C, and just a small fraction germinated at 25°C). This capacity to germinate on exposure to light in a wide thermal range was maintained for seeds exhumed in August in most cohorts. The exception was cohort 1, in which a significant decrease in the fraction of germinated seeds at incubation temperatures below 15°C was seen. Across all 4 cohorts, seeds exhumed in September and October showed a gradual decrease in the temperature range under which they were able to germinate (germination below 10°C only), while seeds exhumed in November showed almost no germination at any incubation temperature (data not shown). Although there were differences between cohorts in the first year their behaviour was broadly similar, whereas in the second year differences between cohorts became more pronounced.

### Germination behaviour in the second year of burial

In the second year of burial, seeds of all cohorts were exhumed each month as in year one. Where exhumed seeds did not germinate > 10% when exposed to light on water between 5 - 25°C no data is shown; for example in exhumed seeds of cohorts 1 and 2 from November to May. During this period cohort 3 showed very low germination at 5 and 10°C for seeds exhumed in March (Fig. 3), while cohort 4 showed germination values close to 20% at 5 and 10 °C during February, March, and April (Fig. 4). This explains why there are more data points for cohort 4 in the second year of the experiment. In all cohorts an increase in the capacity of seeds to germinate in the thermal range tested was seen in the summer of the second year.

However, the responses seen were not as synchronised between cohorts as in the first year. Cohort 1 showed low germination values (<20%) at 5 and 10 °C when exhumed in June and July (Fig. 1). Cohort 2 showed maximum germination capacity in June (most seeds were able to germinate below 15°C and 40 % of the seeds to germinate at 20°C), while a decrease in seed germination capacity was observed in seeds exhumed in July (Fig. 2). Cohort 3 achieved maximum germination capacity in August, when almost the entire seed population germinated below 20°C and a small fraction at 25°C (Fig. 3). Finally, cohort 4 showed almost half of the seed population able to germinate at low temperatures (< 10°C) in May (Fig. 4). In July seeds acquired the capacity to germinate at 15°C, and a fraction (20%) was able to germinate at 20°C. Seed exhumed in October showed a reduction in the temperature range under which they could germinate, showing similar germination behaviour to those exhumed in May. Except for cohort 4, no germination was observed in the exhumations of October of the second year of burial.

#### Changes in limit temperatures permissive for seed germination and germination rate related parameters

These germination data (Figs 1-4) were quantified using the mathematical model described above (equations 1 to 3) that was based on the conceptual model presented in Batlla and Benech-Arnold (2015). In general the model accurately described changes in seed germination behavior in the range 5-25°C during burial (Table S1; colour lines in Figs. 1-4). The temperature limits determined through the adjustment of the model showed that variations in germination behaviour under the thermal range tested were associated with changes in the higher temperature limit permissive for germination ( $T_h$ , max 24, min 4.3 °C across cohorts, Table S1; Fig 5). In contrast, the lower temperature limit ( $T_l$ ) varied little during burial ( $P > 0.05$ ), with mean values of  $4.5 \pm 1.1^\circ\text{C}$ ,  $3.9 \pm 1.3^\circ\text{C}$ ,  $3.7 \pm 0.9^\circ\text{C}$  and  $3.9 \pm 0.9^\circ\text{C}$  for cohorts 1, 2, 3 and 4, respectively. The increase in germination capacity at higher temperatures during dormancy release (Figs 1-4) was mirrored by an increase in  $T_h$  (50) from June to July (Fig. 5) for all cohorts when maximum values of  $T_h$  (50) were seen (around 23-24 °C). High  $T_h$  (50) values were maintained in August for most cohorts (except cohort 1, in which  $T_h$  (50) decreased). Values subsequently diminished to reach low values again in October. The increase and decrease in  $T_h$  (50) during the first year of burial was consistent with increasing soil temperature during late spring and summer and its decrease during autumn, respectively (Fig. 5).

During the second year of burial the seasonal pattern of change in  $T_h$  (50) was similar to that seen in year one (Table S1; Fig. 5). However, in accordance with the observed germination performance (Figs. 1-4), behaviour of the cohorts differed from the first year. Cohort 2 showed its maximum  $T_h$  (50) in June (15.4 °C), while cohort 1 and cohort 4 showed their maximums in July; with the value of  $T_h$  (50) for cohort 4 (19.4 °C) more than double to that of cohort 1 (8.73°C). Finally, cohort 3 showed its maximum  $T_h$  (50) in August (19.4°C). Maximum  $T_h$  (50) values seen in the second year of burial were lower than in year one.

The standard deviation values of both limits ( $\sigma_{T_h}$  and  $\sigma_{T_l}$ ) showed no significant differences between cohorts ( $P>0.05$ ), and no trend was seen during dormancy release or induction (Table S1). Standard deviation of  $T_l$  ( $\sigma_{T_l}$ ) had a mean value of  $0.93\pm 0.9^\circ\text{C}$ , and minimum and maximums of 0.1 and  $3.38^\circ\text{C}$  respectively, while standard deviation of  $T_h$  ( $\sigma_{T_h}$ ) had a mean value of  $2.45\pm 1.6^\circ\text{C}$  and minimum and maximums of  $0.24^\circ\text{C}$  and  $8.73^\circ\text{C}$ , respectively.

Thermal time for seed germination in the sub-optimal thermal range ( $\theta_{sub}$ ), that is germination between  $T_b$  and  $T_o$ , showed little variation between cohorts ( $P>0.05$ ), with a mean value of  $70 \pm 16.4^\circ\text{Ch}$  and minimum and maximum values of 20.6 and  $108^\circ\text{Ch}$ , respectively (Table S1). During burial no clear pattern of change in this parameter was seen during dormancy release ( $P>0.05$ ), although an increasing trend was seen during dormancy induction ( $P<0.05$ ). Thermal time for seed germination in the supra-optimal thermal range, that is germination between  $T_o$  and  $T_m$ , was only determined when seeds germinated  $>10\%$  above  $15^\circ\text{C}$ . Values for  $\theta_{sup}$  were higher than those for  $\theta_{sub}$  (mean  $177\pm 72.8^\circ\text{Ch}$ ), particularly during the second year of burial in which the higher  $\theta_{sup}$  values were registered. Deviation values of  $\theta_{sub}$  were not significantly different between cohorts ( $P>0.05$ ) with a mean of  $9.7\pm 6.5^\circ\text{Ch}$  and minimum and maximum values of 1 and  $25.3^\circ\text{Ch}$ , respectively. During burial an increasing and decreasing trend of  $\sigma_{\theta_{sub}}$  was observed during dormancy release and induction respectively ( $P<0.05$ ). Standard deviation of  $\theta_{sup}$  ( $\sigma_{\theta_{sup}}$ ) was calculated for just few exhumations showing values always higher than those determined for  $\sigma_{\theta_{sub}}$ .

#### Development of the simulation procedure

Parameters identified in quantifying the germination data above were used to develop dormancy induction and release equations as the basis of a simulation procedure for the fraction of the seed bank able to germinate in variable field environments. In order to do this it was necessary to establish functional relationships between changes in  $T_h$  during dormancy release (when  $T_h$  (50) values increase) and induction (when  $T_h$  (50) values decrease) with time and soil temperature. Therefore  $T_h$  (50) values during both processes were regressed against the following thermal time equation:

$$TT_d = (T_s - T_{bd}) \text{ days} \quad (4)$$

Where  $TT_d$  is dormancy release or induction thermal time ( $^\circ\text{Cd}$ ),  $T_s$  is soil temperature ( $^\circ\text{C}$ ) and  $T_{bd}$  is the temperature ( $^\circ\text{C}$ ) above which seed dormancy changes take place. Through an iterative procedure we identified the values of  $T_{bd}$  that establish the maximum  $R^2$  of a linear relationship between  $T_h$  (50) values and Eq 4 during dormancy release and induction (Figs. 6). These values were  $9.2$  and  $0^\circ\text{C}$  for dormancy release and induction respectively, and the corresponding linear models were the following:

$$T_h(50) = T_{hd}(50) + 0.01419 * TT_{dr} \quad \text{for dormancy release (5)}$$

$$T_h(50) = T_{hnd}(50) * -0.01272 * TT_{di} \quad \text{for dormancy induction (6)}$$

Where  $TT_{dr}$  and  $TT_{di}$  are thermal time for dormancy release and induction, respectively,  $T_{hd}(50)$  is the value of  $T_h(50)$  in dormant seeds (that before dormancy release) and  $T_{hnd}(50)$  is the  $T_h(50)$  in non-dormant seeds (that before the beginning of dormancy induction). The dormancy release model (eq. 5) was developed using  $T_h(50)$  values of the 4 cohorts over the two years of burial. The dormancy induction model (eq. 6) used data of the same cohorts but only of the first year of burial, because data of the second year was more erratic and scarce (Table S1; Fig. 6).

No significant relationship between  $\sigma_{Th}$  and  $TT_{dr}$  and  $TT_{di}$  was observed ( $P > 0.05$ ) (Fig. 6). In relation to thermal time for seed germination,  $\theta(50)_{sub}$  had no significant relationship with  $TT_{dr}$  accumulation ( $P > 0.05$ ), while a significant positive linear relationship was observed with  $TT_{di}$  ( $P < 0.05$ ) (i.e.  $\theta(50)_{sub}$  increases during dormancy induction) (Fig. S2). On the contrary, standard deviation of  $\theta_{sub}$  ( $\sigma_{\theta_{sub}}$ ) showed a positive linear relationship to  $TT_{dr}$  accumulation ( $P < 0.05$ ) and no significant ( $P > 0.05$ ) relationship to  $TT_{di}$  (Fig. S2).

#### Application of the simulation procedure developed

To validate the ability of the simulation procedure developed above to predict the fraction of the seed bank able to germinate over the season and therefore the pattern of seedling emergence in the field we contrasted simulated final germination using equations 1 to 6 with emergence data obtained from an independent burial experiment. In this experiment *A. thaliana* Cvi seedling emergence was monitored along a thermal gradient from ambient (cool) temperature to ambient + 4°C (warm) in a global warming scenario. Seed were buried at three positions along the gradient to produce contrasting temperature regimes.

To predict the fraction of the seed population able to germinate at a given time we simulated changes in the higher limit temperature ( $T_h$ , Figs. 5, 6) in relation to burial time and soil temperature (Fig. 7) during dormancy release and induction using equations 5 and 6 (Fig. 8). For this simulation we assumed fixed values of  $\sigma_{Th}$  of 1.8°C during dormancy release and 2.8°C during dormancy induction; (values are the mean of all data used to develop the model during dormancy release (increasing  $T_h(50)$ ) and induction (decreasing  $T_h(50)$ ), respectively; Table S1). We also assumed  $T_l$  to remain constant during burial with a  $T_l(50)$  of 3.75 °C (the mean of the 4 cohorts x all exhumation dates used to develop the dormancy release and induction model; Table S1). To run the simulation we assumed that at burial seeds were fully dormant and that “full” dormant seeds have the same mean values and standard deviations for  $T_h$  and  $T_l$  (i.e. the thermal range permissive for seed

germination is null). So we used a  $\sigma_{T_h}$  of 1.8°C (similar to  $\sigma_{T_h}$  in order to equalise both ranges) and an initial  $T_h(50)$  of 3.75°C. This value was included as the initial  $T_{hd}(50)$  in equation 5.

To establish the date on which the simulation model changed from the dormancy release mode (equation 5) to the dormancy induction mode (equation 6) we analyzed the moment when  $T_h(50)$  began to decrease for each cohort in relation to soil temperature (Figure. 5). We observed that this moment takes place when the mean temperature of a 30 days period (burial time was divided in windows of 30 days) began to decrease in most cohorts (Fig. 7).

Therefore, to simulate dormancy release we used Eq. 5 assuming an initial value of  $T_h(50)$  of 3.75 °C for recently dispersed seeds (i.e. dormant seeds) and a fixed value of  $\sigma_{T_h}$  of 1.8 °C to determine the increase in  $T_h$  for different seed fractions in relation to the different soil temperature environments along the thermal gradient (Fig. 8). Once the 30 day period mean temperature began to decrease (i.e. the difference between one period and the next become positive) we started to simulate the decrease in  $T_h$  according to soil temperature with Eq. 6. For this we used a fixed value of  $\sigma_{T_h}$  of 2.8 °C, in which  $T_{hnd}(50)$  was the value of  $T_h(50)$  before changing from Eq. 5 to Eq. 6 (i.e. from the dormancy release model to the dormancy induction model). Once  $T_h(50)$  had decreased back to a value of 3.75 °C we assumed seed were “fully” dormant and the range of temperatures permissive for seed germination was null (seed cannot germinate at any temperature because  $T_h(50)$  is equal to  $T_l(50)$ ). This situation of “full” dormancy remains until mean daily soil temperature is greater than 9.2 °C (the base temperature for accumulation of  $TT_{dr}$ ), and  $T_h(50)$  increases again according to the accumulation of  $TT_{dr}$  using equation 5. Seed fractions are considered able to germinate when soil temperature is below the  $T_h$  value for that fraction. Then seed germination is predicted to occur once sufficient mean thermal time for seed germination (i.e.  $\theta(50)_{sub}$  if ambient temperature is below the optimum  $T_{op}-15^\circ\text{C}$ , or  $\theta(50)_{sup}$ , if above the optimum) has accumulated in the prevailing soil temperature using equations 2 for  $\theta(50)_{sub}$  or 3 for  $\theta(50)_{sup}$ .

### Simulation validation

The simulation gave an acceptable description of the seasonal pattern of seedling emergence timing along the thermal gradient (Fig. 9 panels a-c). During the first year the model predicted that the germination window of seeds in the soil seed bank peaked before and partially overlapped with actual seedling emergence at all thermal environments. Moreover, the predicted fraction of the seed bank able to germinate during the first year of burial was close to 10% of the buried seeds, similar to the actual seedling emergence recorded (Fig. 9 a, b and c). During the second year, the predicted germination window began earlier than the actual seedling emergence and the germination fraction was overestimated, particularly in the warm environment. In relation to the effect of differences in soil thermal regimes along the gradient, the simulation predicted minor changes in the timing of the germination window.

However, it simulated a higher proportion of the seed bank able to germinate in the warmer environment. This is denoted by an increase in the maximum values of  $T_h$  simulated with increasing temperatures (compare maximum  $T_h$  values in panels a, b and c in Fig. 8), and the higher emergence fraction predicted in the second year of burial (Fig. 9). This prediction is in accordance with the increase in seedling emergence recorded in the field along the thermal gradient (Fig. 9).

## Discussion

We analysed changes in seed germination responses to incubation temperature (5–25°C) of four *A. thaliana* Cvi cohorts over two years of burial using a population-based threshold modelling approach. This allowed a comprehensive understanding of how the timing of seedling emergence from the soil seedbank is determined. This timing resulted from temperature-driven seasonal changes in the seed populations' thermal range permissive for germination. Overlap of this range with changing ambient soil temperature establishes the timing and fraction of the seed-bank able to emerge in this obligate winter annual ecotype (Fig. 8). From this understanding we derived population threshold parameters and developed a thermal-time based simulation of the seasonal changes in the thermal range permissive for germination in relation to soil temperature during dormancy release and induction. This simulation procedure was then validated by predicting seed germination/emergence patterns in a field-based global warming scenario (Fig. 9 panels a-c).

Germination results showed that in *A. thaliana*, as in many other winter annuals, changes in seed dormancy level are related to variations in the seeds capacity to germinate at higher temperatures (Figs. 1-4). Indeed, exhumed seeds incubated at 25°C in the light did not exceed 25 % germination at any point during the two years of burial, illustrating germination suppression at higher temperatures. Similar results have previously been reported for this species (Baskin and Baskin 1972, 1983; Ali-Rachedi et al., 2004; Burghardt et al., 2016). Here, changes in the seeds capacity to germinate at higher temperatures during dormancy release and induction were accounted for by variations in the higher temperature limit to the thermal range permissive for germination ( $T_h$ ) (Table S1; Fig. 5). In contrast, the lower limit temperature ( $T_l$ ) showed only minor changes during burial.

The thermal range permissive for seed germination (i.e. dormancy level) widened as soil temperature increased during spring and summer. It stayed at its maximum for approximately 1 month, before decreasing as temperature decreased during autumn and winter (Fig. 5). Even though in the present work seeds were buried outside their normal dispersal time, an almost identical pattern of changes in *A. thaliana* seed dormancy in relation to the seasonal dynamic of soil temperature was observed in burial experiments by Derkx and Karssen (1994), Baskin and Baskin (1972; 1983), Footitt et al., (2014) and Buijs et al. (2020). This behaviour suggests that, although in

*A. thaliana*, and other winter annuals prolonged exposure to low temperatures induces dormancy (Finch-Savage et al., 2007; Penfield and Springthorpe, 2012, Footitt et al., 2017), in the soil seed bank, dormancy induction also responds to another kind of temperature signal. This signal may be related to the decrease of temperature from mid-summer onwards, i.e. a relative change (pattern) rather than absolute temperature. Both processes, dormancy release (increase in  $T_h$ ) and induction (decrease in  $T_h$ ), were adequately described by the accumulation of degree days over a base temperature (i.e. dormancy thermal time) (Fig. 6). However, the threshold values for accumulating dormancy degree days differed between processes (9.2 °C for dormancy release and 0°C for dormancy induction). A similar thermal time model with a  $T_b$  of 0°C was developed for dormancy induction in *A. thaliana* (Col accession) by Footitt et al., (2017).

This raises questions about the nature of the environmental signal that makes buried seeds switch from a dormancy release to a dormancy induction mode. Our results suggest this signal is related to the change in temperature that takes place in the transition from the summer to the autumn. In the present simulation, acceptable predictions of seasonal emergence patterns in the global warming scenario were obtained when this change was associated with the moment the mean temperature recorded in a 30 days period from the date of burial began to decrease in late summer (August) (Fig. 7). Further analysis of the soil thermal environment shows that this transition coincides with the time of year when the variation in the mean daily temperature from one day to the next was minimal (i.e. similar mean daily temperatures between days) (see red line in Fig. 7). This suggests that buried seeds are capable of detecting changes (decrease or increase) in temperature alternation (variability) and that this could be the signal for switching from the dormancy release to the dormancy induction mode. Recent results reported by Buijs et al., (2020) for the *A. thaliana* ler ecotype showed that large fluctuation in soil temperature over a period of days (i.e. a dip in temperature followed by a period with higher temperatures) affected the expression of genes associated with dormancy release and induction during dormancy cycling of buried seeds. A similar signal (an abrupt decrease in the daily mean temperature followed by a sudden increase) was related to the beginning of *Echinochloa colona* L. seedling emergence in the field (Picapietra et al., 2020). In accordance with previous mentioned results abrupt changes in daily mean soil temperature were registered in the present work previous to dormancy release (between April and May) and dormancy induction (between August and September) during the first year of burial (Fig. 5). These observations support the idea that changes in the fluctuations of the mean daily soil temperature could be the signal perceived by buried seeds to change from the dormancy release mode to the induction mode and *vice versa*.

In the second year of burial, results were dissimilar between cohorts, unlike in the first year (Figs. 1-4), and seasonal changes in  $T_h$  (50) were not so clear (Fig. 5). This may be the result of the small differences in production environment, which is known

to influence dormancy behaviour. However, it could also be the result of differences in soil micro-climate across the field trial area such as the occurrence of very low soil moisture contents during this period. There is evidence that water stress can provoke and/or enhance dormancy induction in seeds of many species, including *A. thaliana* (Auge *et al.*, 2015; Footitt *et al.*, 2017). This enhancement of dormancy induction by water stress may explain the fact that during the second year of burial, seeds presented a higher dormancy level (i.e. lower  $T_h$  (50) values) than in the first year. Although, in earlier versions of the model we tried to include soil water content as a variable modulating the effect of soil temperature on dormancy changes (i.e. a threshold soil water content affecting the accumulation of dormancy thermal time), we did not obtain a better fit in comparison to that obtained with just temperature as the driver of dormancy changes. This is consistent with temperature being the dominant environmental signal driving changes in the depth of dormancy, probably, in the present case, masking the effect of soil water content.

The model gave acceptable predictions of the seasonal pattern of seedling emergence along the thermal gradient in the global warming scenario (Fig. 9 panels a-c). Particularly during the first year of burial, it adequately predicted the timing and the fraction of the seed bank able to germinate (10%). These results highlight the importance of using a population-based threshold approach that takes into account the variability inherent in the seed population when predicting germination or emergence proportions (Bradford, 2005, 2018). The model clearly shows that soil temperature overlaps with the permissive thermal range of only a fraction of the seed-bank population when temperature is falling during late summer to early autumn (Fig. 8), as recently observed for *A. thaliana* Cvi by Footitt *et al.* (2020; 2021). Seeds in this fraction of the population will then germinate and emerge, but only if exposed to a signal such as light (during soil disturbance) that removes the final layer of dormancy. Meanwhile, the majority of the seeds remain dormant in the seed-bank even if exposed to light until the next emergence season. This behaviour is consistent with what generally happens in species forming persistence seed-banks, where the fraction of the seed-bank that emerges each year is low (Roberts, 1963; Forcella *et al.*, 1997). However, the model showed some discrepancies with the temporal pattern of seedling emergence observed along the thermal gradient and overestimated seedling emergence in the second year of burial (Fig. 9 panels a-c). This overestimation of the emergence proportion in the second year may result from the inability of the model to quantify the depth of dormancy in seeds after the winter of the second year (as dormancy is quantified through germination, we cannot measure the dormancy level of non-germinating seeds).

Results published by Footitt *et al.*, (2011 and 2017), using gibberellins to break dormancy, indicate that in the winter (i.e. cold temperatures) seeds enter into a very deep dormant state. However as explained above, this cannot be evaluated by the model which assumes a  $T_h$  (50) value of 3.75°C for “full” dormant seeds. Letting  $T_h$  (50) reach a lower value after the winter (i.e. 2°C) gives a better simulation of the



fraction of the seed bank able to emerge in the second year of burial (see the comparison of  $T_h$  (50) 3.75 (Fig. 9 panels a-c) vs 2°C (Fig. 9 panels d-f), in correspondence with the above proposed hypothesis. Alternatively, the inability of the model to correctly predict the temporal extension of the emergence during the first year of burial is probably related to an overestimation of the rate of induction into secondary dormancy. As observed for *A. thaliana* by Auge et al., (2015) and *P. aviculare* by Malavert et al., (2017) and Fernandez et al., (2019) the rate of induction into secondary dormancy is negatively related to seed dormancy level (less dormant seeds present a lower rate of induction into secondary dormancy). During the first year of burial, maximum  $T_h$  (50) (that before induction into secondary dormancy) was lower than that experimentally observed, thus the rate of induction into secondary dormancy should have been lower than that simulated with equation 6. Indeed decreasing by 23.6 % the rate of dormancy induction (from 0.01272 to 0.00972 in equation 6) resulted in a better simulation of the temporal pattern of seedling emergence during the first year of burial under the three thermal regimes used, supporting the above proposed hypothesis (Fig. 9 panels g-i).

The simulation was relatively accurate at predicting seedling emergence timing under a global warming scenario. Simulations predicted an increase in the seed bank fraction able to emerge as a consequence of global warming (Fig. 9), in coincidence with seedling emergence behaviour observed in the field and recent results reported for *A. thaliana* Cvi by Footitt et al. (2021). This is not surprising, as dormancy release in *A. thaliana* Cvi has been shown to be positively associated with soil temperature (Footitt et al., 2011). This prediction (i.e. increase of seedling emergence as a consequence of global warming) would probably apply to many other winter annuals species in which dormancy release is positively associated with temperature (Walck et al., 2011); summer annuals, in which dormancy release is promoted by low temperatures, will probably show the reverse pattern, as proposed by Fernández-Pascual et al., (2019) and suggested by results reported by Footitt et al. (2018). On the other hand, coincident with seedling emergence observed in the field, the simulation did not predict major changes in the temporal pattern of seedling emergence along the thermal gradient. These results contradict other studies in which a warmer soil environment provoked a delay in the timing of seedling emergence, as observed previously in *A. thaliana* Cvi (Footitt et al., 2021). However, the fact that in the present simulation the time seeds go into secondary dormancy is tied to the decrease in the mean temperature in a 30 day window, could have probably limited the possibility of simulating changes in the temporal pattern of seedling emergence.

A greater understanding of the seasonal pattern of change in dormancy status in natural seed-banks is essential for modelling weed/crop competition dynamics and natural population's responses to future climate change scenarios. In this study we presented an analytical framework to understand how temperature dependent changes in seed dormancy establish the timing and fractions of the seed bank

emerging in winter annual ecotypes of *A. thaliana*. This same approach could be used to analyse similar effects in other winter annual species. Results presented here also suggest that changes in the fluctuations of soil temperature (i.e. decrease or increase of the variability in the mean daily temperature across days) could be the environmental signal that allows buried seeds to perceive seasonal changes and adjust their dormancy level. Research is needed to confirm the proposed environmental signal used by seeds to perceive seasonal changes as this is fundamental to understanding how dormancy cycling is controlled in natural field soil seed-banks.

### Supplementary data

**Fig S1.** Germination for cohorts of *Arabidopsis thaliana* ecotype Cvi before burial.

**Fig S2.** Changes in mean thermal time for seed germination and its standard deviation during dormancy release and induction.

**Table S1.** Estimated population thermal parameters for different cohorts of *Arabidopsis thaliana* ecotype Cvi seeds buried in the field

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## **Author contributions**

**DB: conceptualization, project administration and writing-original draft preparation; DB, RLB-A, WEF-S and SF: funding acquisition and resources, DB, WEF-S and SF: supervision and validation; DB, CM and RBFF: methodology and visualization; CM, SF and RBFF formal analysis, WEF-S and SF investigation; DB, WEF-S, SF and RLB-A Writing – Review & Editing.**

## **Data availability**

**All data supporting the findings of this study are available within the paper and within its supplementary data published online.**

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## FIGURE LEGENDS

**Figure 1.** Observed (symbols) and predicted (dotted colour lines) cumulative germination (%) at 5 °C (○), 10 °C (□), 15 °C (△), 20 °C (▽) and 25 °C (◇) for *Arabidopsis thaliana* Cvi seeds (Cohort 1) buried in the field during 2008 (a-f) and 2009 (g-h). Exhumation dates are indicated above each figure panel. Error bars indicate SEM; n= 3

**Figure 2.** Observed (symbols) and predicted (dotted colour lines) cumulative germination (%) at 5 °C (○), 10 °C (□), 15 °C (△), 20 °C (▽) and 25 °C (◇) for *Arabidopsis thaliana* Cvi seeds (Cohort 2) buried in the field during 2008 (a-f) and 2009 (g-h). Exhumation dates are indicated above each figure panel. Error bars indicate SEM; n= 3.

**Figure 3.** Observed (symbols) and predicted (dotted colour lines) cumulative germination (%) at 5 °C (○), 10 °C (□), 15 °C (△), 20 °C (▽) and 25 °C (◇) for *Arabidopsis thaliana* Cvi seeds (Cohort 3) buried in the field during 2008 (a-f) and 2009 (g-h). Exhumation dates are indicated above each figure panel. Error bars indicate SEM; n= 3.

**Figure 4.** Observed (symbols) and predicted (dotted colour lines) cumulative germination (%) at 5 °C (○), 10 °C (□), 15 °C (△), 20 °C (▽) and 25 °C (◇) for *Arabidopsis thaliana* Cvi seeds (Cohort 4) buried in the field during 2008 (a-i) and 2009 (j-p). Exhumation dates are indicated above each figure panel. Error bars indicate SEM; n= 3.

**Figure 5.** Changes in the mean higher limit temperature of the thermal range permissive for seed germination ( $T_{h(50)}$ ) for *Arabidopsis thaliana* Cvi seeds buried in the field. Soil temperature (°C, red line; left y-axis) and soil water content (% , blue dotted line; right y-axis) during seed burial (from 2007 to 2009). Lines and symbols (n= 3) represent changes in  $T_{h(50)}$  for cohorts 1, 2, 3 and 4. The horizontal black dotted line represents the constant value (3.75 °C) assumed for  $T_{l(50)}$ . Coloured blocks indicate the seasons: green, autumn; blue, winter; yellow, spring; red, summer.

**Figure 6.** Estimated values of the mean higher limit temperature ( $T_{h(50)}$ , upper panels) and their standard deviation ( $\sigma_{Th}$ , lower panels) during dormancy release (a, c) and induction (b, d) for *Arabidopsis thaliana* Cvi seeds buried in the field over two years (2008 open symbols and 2009 black symbols) plotted against thermal time required for dormancy release ( $TT_{dr}$ ) and thermal time required for dormancy induction ( $TT_{di}$ ), respectively. Thermal parameters were estimated from germination data of cohorts 1 (□, ■), 2 (○, ●), 3 (△, ▲) and 4 (◇, ◆). Thermal time (°Cd) was



accumulated over a base temperature of 9.2 °C for dormancy release (a, c) and 0 °C for dormancy induction (b, d). Equations and  $R^2$  are indicated inside each figure panel. When no clear patterns were observed, mean values were estimated.

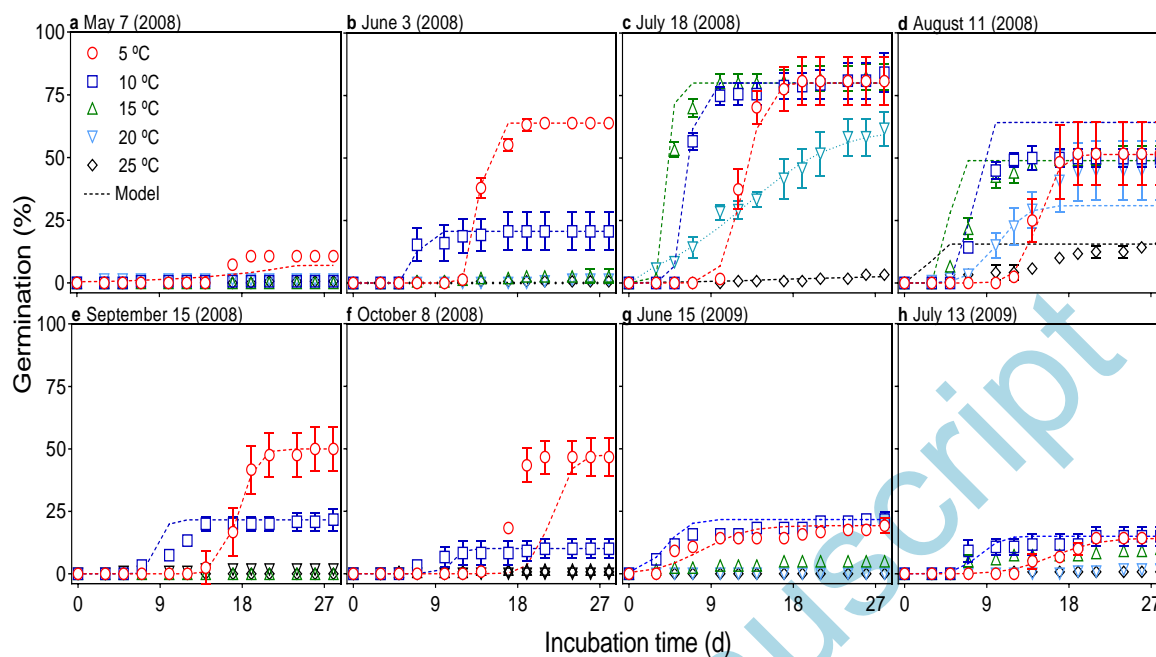
**Figure 7.** Seasonal changes in soil temperature at seed depth (5-cm) from 2007 to 2009. Mean daily temperature (blue line), mean temperature in a 30-day time window date (black points; time from burial date was divided in periods of 30 days), and difference in mean daily soil temperatures between consecutive days (red line); zero line indicates no difference in mean daily temperature between days. Black arrows indicate the time cohorts 1 to 4 (C1 to C4) beginning to enter secondary dormancy (i.e. change from dormancy release to dormancy induction). Brown bars indicate the period in which the difference in mean daily temperature between consecutive days is minimum (i.e. days show similar mean daily temperatures), which coincides with the time seeds of different cohorts switch from the dormancy release mode to the dormancy. Coloured blocks indicate the seasons: green, autumn; blue, winter; yellow, spring; red, summer.

**Figure 8.** Simulated values of  $T_h$  for *Arabidopsis thaliana* Cvi seed percentiles (50, 75 and 95) exposed to different soil thermal environments in a global warming scenario. Red line indicate soil temperature at 2-cm in (a) cool, (b) middle, and (c) warm scenarios. Values were simulated according to soil temperature using Eqs. 4, 5 and 6 and a fixed standard deviation of  $T_h$  of 1.8 °C during dormancy release and 2.8 °C during dormancy induction. Dormancy release mode (eq. 5) was switched to the dormancy induction mode (eq. 6) when mean temperature in a 30-day time window (time from burial date was divided in periods of 30 days) began to decrease. The horizontal black dotted line represents the  $T_{l(50)}$  (3.75 °C). Germination of different percentiles of the seed population is predicted once soil temperature (red line) is lower than the  $T_h$  of that percentile (see predictions in Fig. 9. Panels a, b and c). Coloured blocks indicate the seasons: green, autumn; blue, winter; yellow, spring; red, summer.

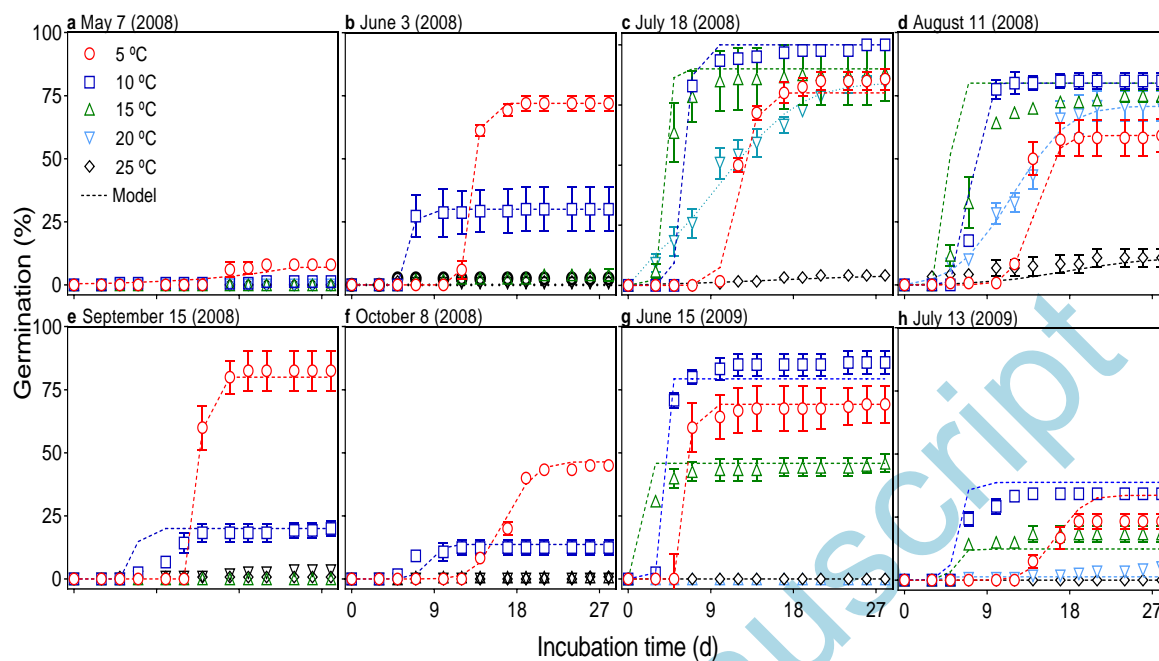
**Figure 9.** Simulation validation using *Arabidopsis thaliana* Cvi seedling emergence recorded in a global warming scenario. Observed emergence (green) vs simulated germination (red) for different soil thermal environments (cool, middle and warm; left, middle and right panels, respectively) along a thermal gradient. Three different simulations were performed: (1) a-c germination was simulated using equations 1, 2 and 3 according to soil temperature and the values simulated in Fig. 8. Simulations were performed using an initial value of  $T_{h(50)} = 3.75$  °C after the winter (i.e. “full” dormant seeds). Standard deviation of  $T_h$  was considered constant, with values of 1.8 °C during dormancy release and 2.8 °C during dormancy induction; (2) d-f simulations were performed for the 2nd year as in (1), but using an initial value of  $T_{h(50)} = 2$  °C after the winter; (3) g-i simulations were performed for the 1st year as in (1), but using a slower dormancy induction rate. The rate of dormancy induction was

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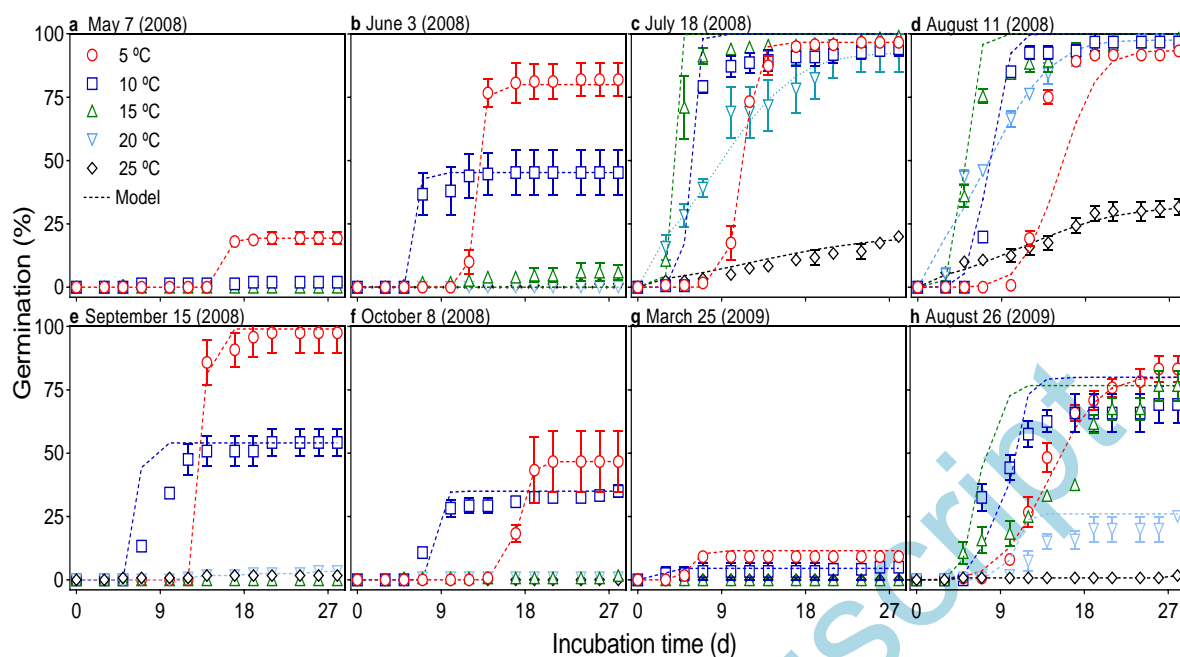
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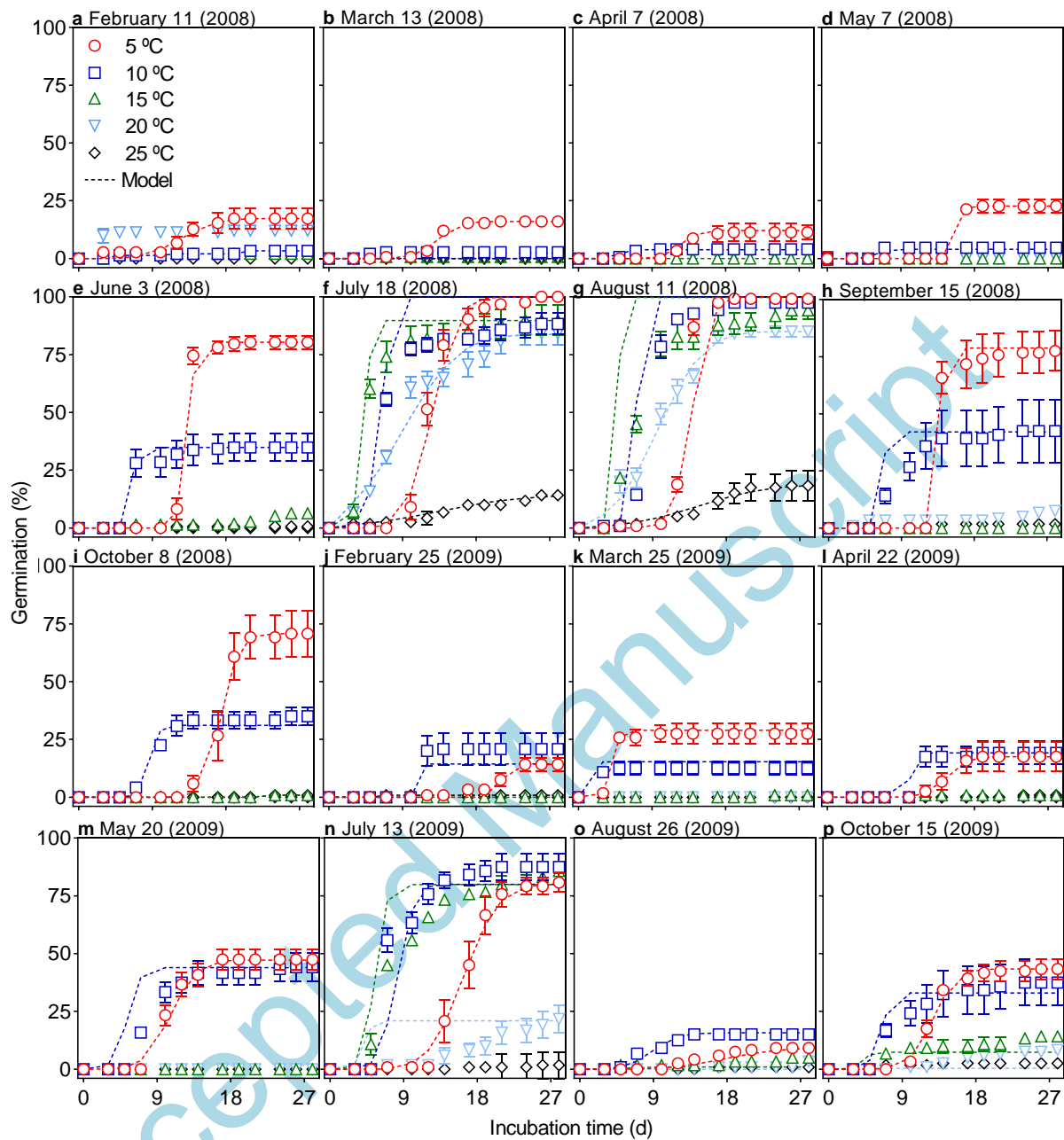
**Figure 1.** Observed (symbols) and predicted (dotted colour lines) cumulative germination (%) at 5 °C (○), 10 °C (□), 15 °C (△), 20 °C (▽) and 25 °C (◇) for *Arabidopsis thaliana* Cvi seeds (Cohort 1) buried in the field during 2008 (a-f) and 2009 (g-h). Exhumation dates are indicated above each figure panel. Error bars indicate SEM; n= 3.



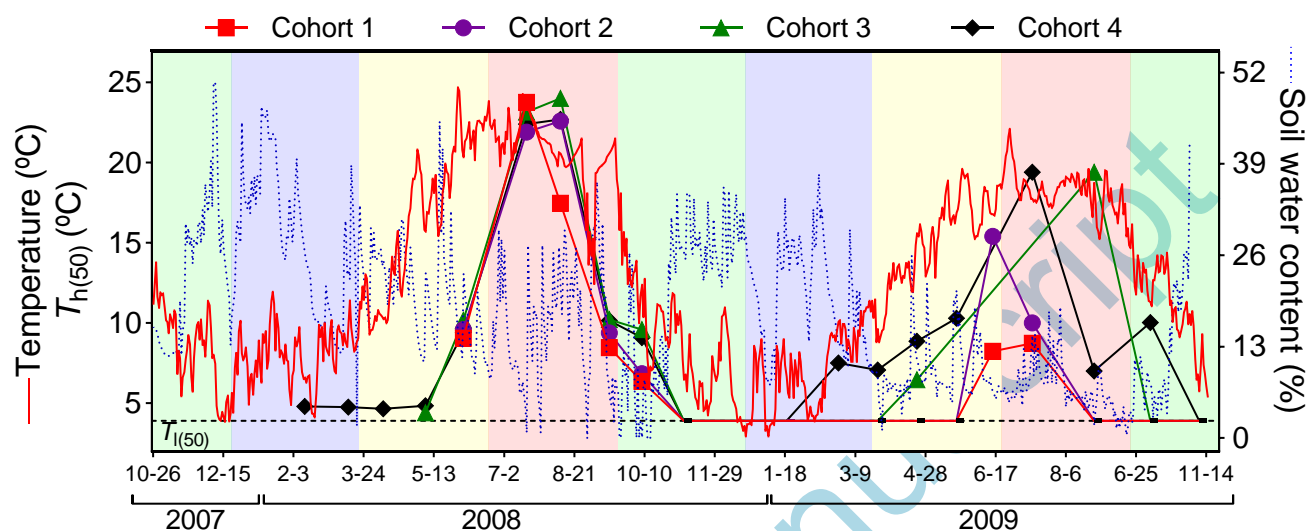
**Figure 2.** Observed (symbols) and predicted (dotted colour lines) cumulative germination (%) at 5 °C (○), 10 °C (□), 15 °C (△), 20 °C (▽) and 25 °C (◇) for *Arabidopsis thaliana* Cvi seeds (Cohort 2) buried in the field during 2008 (a-f) and 2009 (g-h). Exhumation dates are indicated above each figure panel. Error bars indicate SEM; n= 3.



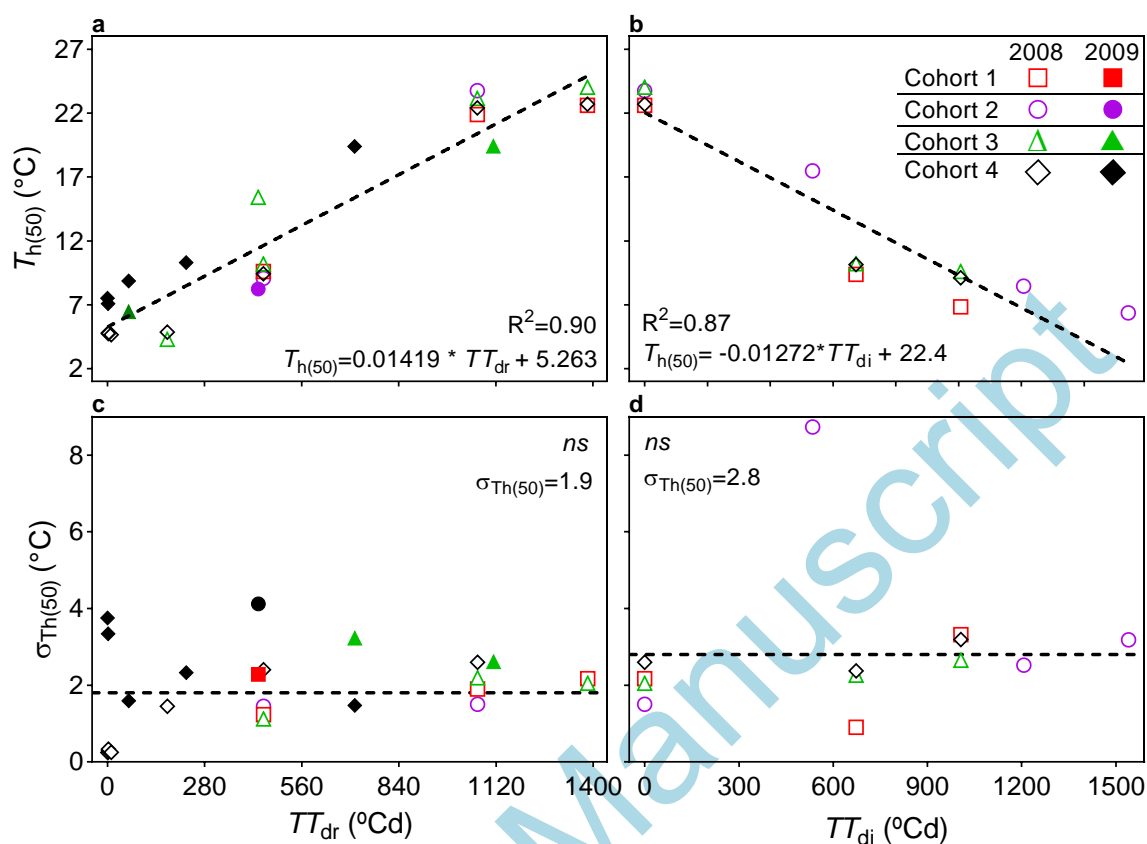
**Figure 3.** Observed (symbols) and predicted (dotted colour lines) cumulative germination (%) at 5 °C ( $\circ$ ), 10 °C ( $\square$ ), 15 °C ( $\Delta$ ), 20 °C ( $\nabla$ ) and 25 °C ( $\diamond$ ) for *Arabidopsis thaliana* Cvi seeds (Cohort 3) buried in the field during 2008 (a-f) and 2009 (g-h). Exhumation dates are indicated above each figure panel. Error bars indicate SEM; n= 3.



**Figure 4.** Observed (symbols) and predicted (dotted colour lines) cumulative germination (%) at 5 °C (○), 10 °C (□), 15 °C (△), 20 °C (▽) and 25 °C (◇) for *Arabidopsis thaliana* Cvi seeds (Cohort 4) buried in the field during 2008 (a-i) and 2009 (j-p). Exhumation dates are indicated above each figure panel. Error bars indicate SEM; n=3.

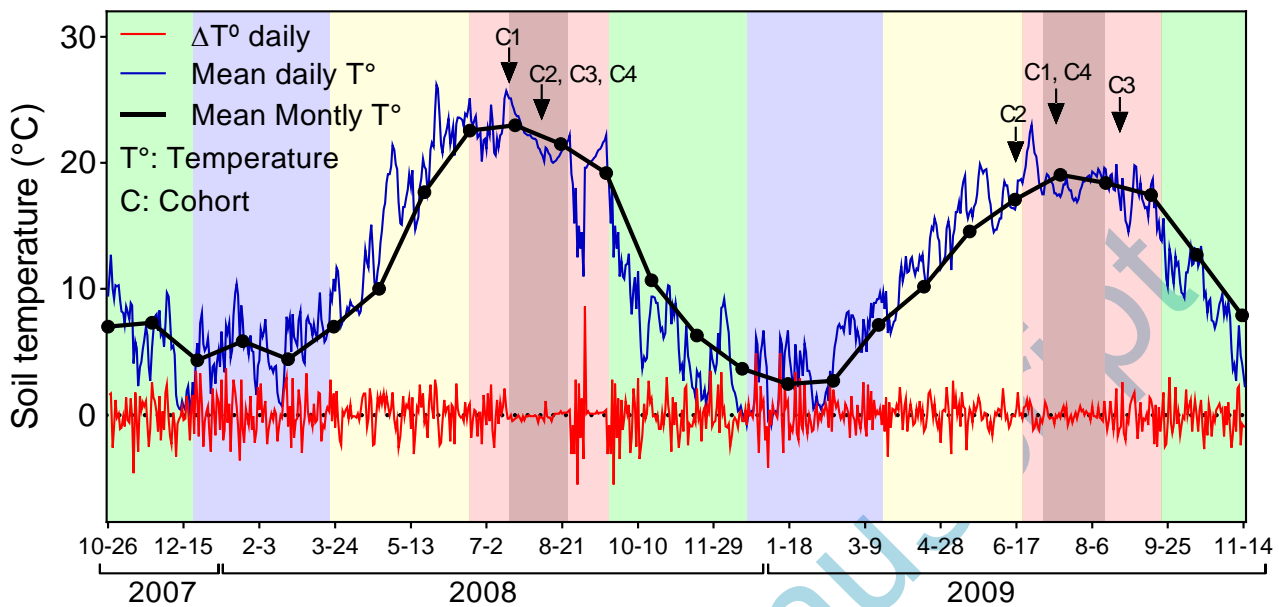


**Figure 5.** Changes in the mean higher limit temperature of the thermal range permissive for seed germination ( $T_{h(50)}$ ) for *Arabidopsis thaliana* Cvi seeds buried in the field. Soil temperature ( $^{\circ}\text{C}$ , red line; left y-axis) and soil water content (%; blue dotted line; right y-axis) during seed burial (from 2007 to 2009). Lines and symbols ( $n=3$ ) represent changes in  $T_{h(50)}$  for cohorts 1, 2, 3 and 4. The horizontal black dotted line represents the constant value (3.75  $^{\circ}\text{C}$ ) assumed for  $T_{l(50)}$ . Coloured blocks indicate the seasons: green, autumn; blue, winter; yellow, spring; red, summer.

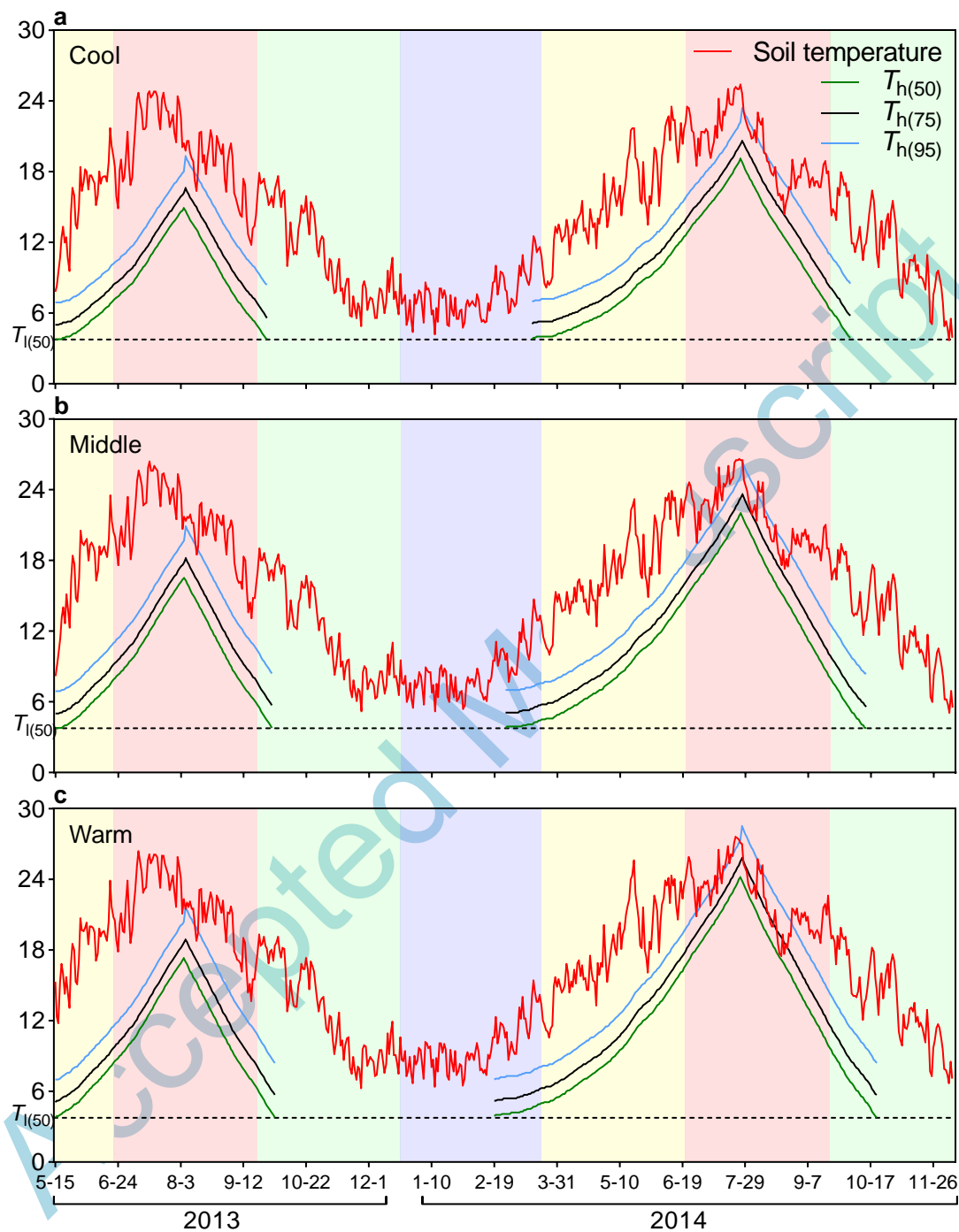


**Figure 6.** Estimated values of the mean higher limit temperature ( $T_{h(50)}$ , upper panels) and their standard deviation ( $\sigma_{Th}$ , lower panels) during dormancy release (a, c) and induction (b, d) for *Arabidopsis thaliana* Cvi seeds buried in the field over two years (2008 open symbols and 2009 black symbols) plotted against thermal time required for dormancy release ( $TT_{dr}$ ) and thermal time required for dormancy induction ( $TT_{di}$ ), respectively. Thermal parameters were estimated from germination data of cohorts 1 (□, ■), 2 (○, ●), 3 (△, ▲) and 4 (◇, ◆). Thermal time (°Cd) was accumulated over a base temperature of 9.2 °C for dormancy release (a, c) and 0 °C for dormancy induction (b, d). Equations and  $R^2$  are indicated inside each figure panel. When no clear patterns were observed, mean values were estimated.





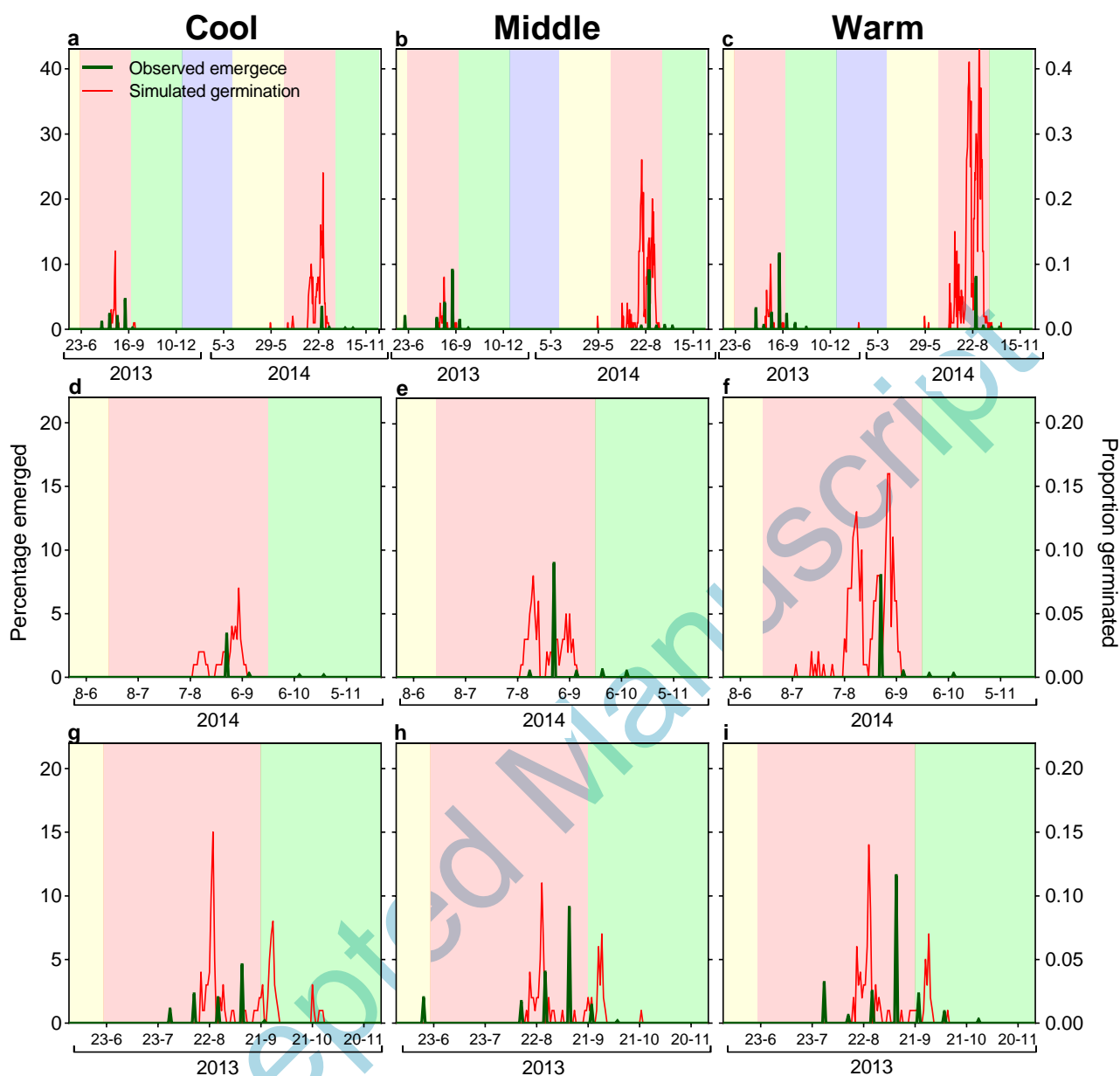
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