WEED RESEARCH An International Journal of Weed Biology, Ecology and Vegetation Management

DOI: 10.1111/wre.12085

Assessment of daily heat pulse regimes on the germination of six *Amaranthus* species

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Received 6 November 2013
Revised version accepted 24 February 2014
Subject Editor: José Gonzalez-Andujar, CSIC, Spain

Summary

In Mediterranean environments, thermal requirements for seed germination of *Amaranthus* species are met in spring. Nonetheless, seedlings of these species start emerging in the field from late winter, although the theoretical heat sum thresholds required for germination are not likely to be reached under field conditions. We hypothesised that soil thermal fluctuations can reduce the heat requirements of these species. Cardinal temperatures and thermal times of six *Amaranthus* species (*A. albus*, *A. cruentus*, *A. deflexus*, *A. graecizans*, *A. retroflexus* and *A. viridis*) were determined on non-dormant seeds at constant temperatures ranging from 10 to 40°C. Subsequently, germination response to heat pulses was studied by imposing a thermal fluctuation regime of 32/8°C with two different thermoperiods of 3/21 and 6/18 h. In the two thermoperiods, exposure to 32°C was imposed for a different number of days: from 1 to 12 and from 1 to 6 heat pulses cycles in the 3/21 and 6/18 h thermoperiods respectively. Cumulative germination, germination rate and mean germination time were evaluated. Heat sum requirements and final germination percentage were affected by thermoperiod and number of thermal cycles. *Amaranthus* spp. germination was higher and faster when seeds were submitted to 6 h compared with 3 + 3 h of heat pulses. Our data showed that heat sum requirements for germination may change depending on the way in which varying temperatures are imposed on germinating seeds.

Keywords: amaranth, pigweed, cardinal temperature, cumulative heat pulse, germination rate, mean germination time, thermoperiod.

CRISTAUDO A, GRESTA F, CATARA S & MINGO A (2014). Assessment of daily heat pulse regimes on the germination of six *Amaranthus* species. *Weed Research*.

Introduction

Amaranthus species are a group of important summer annual weed species widely adapted to several environmental conditions, causing crop damage in many agro-ecosystems throughout the world's temperate, Mediterranean, tropical and subtropical regions (Costea & Tardif, 2003; Costea *et al.*, 2004). These species are characterised by a highly competitive strategy, depending also on a series of adaptive traits that allow their seeds to trigger germination and maximise seedling establishment under different environmental conditions (Guo & Al-Khatib, 2003). Due to their short life cycle, early time of emergence, high growth rate and capability to produce two or more generations per growing season (Royer & Dickinson, 1999),

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these species easily outcompete most crops and some native species (Viegi, 2001).

The stimulating effect of relatively high temperatures on seed germination is well known for several species of the *Amaranthus* genus; many studies have demonstrated that the seeds of these species need to be exposed to temperatures ranging from 30 to 40°C to achieve full germination (Steinmaus *et al.*, 2000; Faccini & Vitta, 2005; Cristaudo *et al.*, 2007). However, in spite of such high temperature requirements, early germination of *Amaranthus* species is frequently observed in natural conditions when temperatures are far below the optimum, within the concept of thermal time (Trudgill *et al.*, 2005). This approach is based on the estimation of base temperature (T_b), that is, the threshold below which germination rate is zero (Steinmaus *et al.*, 2000), and on the calculation of the heat sum (d°C) required for a given percentile of seed population to germinate (Larsen & Bibby, 2005). Several models aiming to predict the time-course of germination in field crops or in weed dynamics models are based on the combined evaluation of T_b and thermal time or heat sum (Gardarin *et al.*, 2010, 2011; Sakanoue, 2010). They are frequently adopted to quantify heat unit accumulation on daily basis (Cesaraccio *et al.*, 2001) or to compare germination patterns among different species, climates and locations (Wagenvoort & Bierhuizen, 1977; Moot *et al.*, 2000). These approaches are based on the assumption that thermal time for germination is constant for a given subpopulation of seeds (Trudgill *et al.*, 2005), so that germination is predicted to occur in the field only when the fixed sum of day degrees is reached, independently of how such heat sum was achieved. It is commonly observed, however, that different patterns of temperature regime may result in marked differences in germination responses, even if the total heat sum provided to germinating seeds is the same. Fluctuating temperature regimes may strongly affect germination patterns, and thermal model suitability under field variable temperatures has been the object of both experimental and modelling researches (Leon *et al.*, 2004; Hardegree *et al.*, 2010). In our view, more attention should be devoted to verify the extent to which thermal time do really behave as a constant, or, on the contrary, if it changes depending on the particular regime of fluctuating temperatures experienced by germinating seeds. This research was aimed to test the hypothesis that different time and number of exposures to heat pulse would determine a different cumulative effect of the same heat sum on germination process. An experimental protocol was implemented on six *Amaranthus* species with the following objectives: (i) to estimate the cardinal temperatures (base and optimal) for the six species; (ii) to quantify the germination responses at constant temperatures under different daily heat pulse treatments for a variable number of cycles; (iii) to compare thermal time requirements for 50% germination among species and treatments and to verify whether these parameters are constant or vary depending on thermal fluctuation.

Materials and methods

Seed collection

Mature seeds of six summer annual *Amaranthus* species (*A. albus* L., *A. cruentus* L., *A. deflexus* L., *A. graecizans* L., *A. retroflexus* L. and *A. viridis* L.) were collected randomly in October 2003, from more than 100 plants per species. The seeds were collected at altitudes ranging between 50 and 400 m a.s.l., in a zone with a thermo-Mediterranean climate (Catania plane, Sicily, Southern Italy) from spontaneous populations growing in irrigated crops (citrus groves, fruit orchards and horticultural crops). Seed material was stored for 270 days in cloth bags at room temperature (20 ± 2°C), so that the experiment could start when a considerable release from dormancy was presumably attained (Cristaudo *et al.*, 2007).

Germination trials

Two kinds of germination trials were conducted: in a first experiment, base and optimal temperatures were estimated by putting seeds to germinate in seven different germination chambers (Angelantoni MCT 200, Italy), in the dark, in which constant temperatures were set to 10, 15, 20, 25, 30, 35 and 40°C. In a second experiment, two thermal fluctuation regimes of 32/8°C were imposed on seeds (details provided below). In both experiments, four replicates (or pseudo-replicates, as they were placed in the same germinator) of 25 seeds each were adopted for each treatment. Petri dishes were distributed randomly within each chamber, and to ensure no systematic effects due to position within the chamber, Petri dishes were rerandomised daily.

Seeds were placed in 9–cm-diameter Petri dishes with a double layer of filter paper moistened with 6 ml of distilled water. To determine the influence of heat pulse on germination of *Amaranthus* seeds, before treatments, all seeds of the second experiment were properly imbibed for 24 h at 8°C, in distilled water. Two germination chambers were used in darkness. To guarantee a quick temperature change in the alternating temperature treatments, Petri dishes were manually shifted between germination chambers fixed at 8 and 32°C. All treatments were carried out simultaneously.

Black Petri dishes were used to exclude any effect of light. The germination papers were wetted periodically to obtain non-limiting water conditions.

Germinated seeds were counted and removed daily under a green safelight. Seeds were considered germinated when they exhibited radicle length of ≥2 mm. The incubation period was 15 days; this time was sufficient for all the species and treatments to reach the asymptotes of germination curves. At the end of the experiments, non-germinated seeds were maintained at optimal temperature (32°C) to perform an *a posteriori* validation test for seed viability. Non-viable seeds represented less than 0.5% and were then considered negligible and not analysed.

Cardinal temperatures and thermal time at constant temperature

Cardinal temperatures were estimated by analysing germination responses previously conducted at constant temperatures. The number of days necessary to achieve a given germination percentile was determined for each species and treatment, through nonlinear regression; their reciprocal values were taken as corresponding germination rates (Bierhuizen & Wagenvoort, 1974). Germination percentiles were scaled to the maximum germination percentage expressed among all the tested temperatures, to separate any residual dormancy effect before determining germination rates.

A Weibull model was interpolated to daily germination data:

$$Gp = Gp_{\max}(1 - e^{-r(t-t_0)}) \quad (1)$$

where Gp is the germination percentage referred to the number of sown seeds, as a function of time in days (t); $Gp_{\max}$ is the maximum germination percentage; t_0 is the lag time to start germination; r is a parameter associated with the maximal curve slope. This model gets negative values when $t < t_0$, so the model fitting was limited to the time series following lag time, and Gp was set to assume 0 value for $t < t_0$. The model was fitted to the average values of the four pseudo-replicates. Model fitting was evaluated as the R^2 coefficient of the linear regression of predicted vs. real values. Once the best fitting model parameters had been identified, the number of days necessary to achieve any given percentile was obtained by the model inverse formula. Ten percentiles ranging from 10 to 100% were examined, in 10% increments. For each percentile, germination rates were then plotted against temperature, and a log-normal type model was fitted to these data (Hardegree, 2006, modified) to determine the optimal temperatures:

$$R = R_{\max} e^{-\frac{\ln(2)}{\ln(a)^2}\ln(\frac{(T-To)(a^2-1)}{ab}+1)^2} \quad (2)$$

where R is germination rate as a function of temperature (T); T_o is a model parameter which closely identifies the x co-ordinate of the curve peak, so providing a good estimate of the optimal temperature; $R_{\max}$ is the maximal germination rate achieved at such temperature; a and b are shape parameters associated with curve wideness and asymmetry. This model was chosen because it gives a good estimate of the optimal temperature and it was found to provide the desired fitting values on the studied data set.

Once the optimal temperatures were determined, linear regressions of germination rates to temperature were obtained in the suboptimal range (Garcia-Huidobro *et al.*, 1982a). Intersections with the x-axis were taken as the base temperatures for each germination percentile, and the mean of these values was assumed as the final estimate of base temperature for the species.

New linear regressions, forced to intercept the x-axis at the estimated value of species base temperature, were then interpolated to suboptimal data for each percentile; the reciprocals of their angular values were taken as estimates of thermal times required to achieve the given germination percentile (Garcia-Huidobro *et al.*, 1982a). Finally, the ten percentiles were plotted against the corresponding thermal times and fitted by a new Weibull-type model:

$$G = 1 - e^{-\alpha(\theta-\theta_0)} \quad (3)$$

where G is the germination percentile as a function of thermal time (θ), θ_0 is the lag thermal time below which G was set to assume 0 value, and α is the parameter associated with germination rate/maximal curve slope. Heat sum corresponding to 50% germination (θ_{50}) was determined by the inverse formula. The Weibull model had been previously adopted by different authors (Brown & Mayer, 1988; Larsen & Bibby, 2005), as this model reflects the most typical germination pattern, with a lag time to start germination and a basic thermal sum requirement below which germination rate is zero.

Heat pulses

For each of the six species, 18 seed samples, each consisting of four replicates of 25 seeds, were subjected to temperature lower than the base temperature with heat pulses at near-optimal temperature. As absolute lowest base and optimal temperatures were found to be, respectively, around 8 and 32°C, the regime of alternating temperature was set at 32/8°C, with two different

thermoperiods of 3/21 and 6/18 h. In the two thermoperiods, exposure to 32°C was imposed for a different number of days (cycles), till reaching the equal dose of heat pulses of 36 h: from 1 to 12 (12 seed samples) and from 1 to 6 (six seed samples), heat pulses cycles were imposed in the 3/21 and 6/18 h thermoperiods respectively (Fig. 1). After the established number of heating cycles was reached, Petri dishes were let at constant 8°C till the end of the experiment (15 days) to allow for residual effect of heat pulse on germination. Two more samples were placed at constant temperature of 8 and 32°C, respectively, as controls (Fig. 1).

Germination trials under fluctuating temperature were analysed as follows: first, the number of cycles was scaled to heat sum through the following function (Bierhuizen & Wagenvoort, 1974; Garcia-Huidobro *et al.*, 1982b):

$$\theta = (T - T_b)t \quad (4)$$

where θ is the heat sum or thermal time in degree days, T is the temperature imposed during heating pulses (32°C), T_b is the species base temperature, and t is the total time (days) spent at 32°C. The resulting heat sums are reported in Table 1. Germination percentages were then plotted against heat sums and fitted to the Weibull model previously described (3), taking into account that G was now representing the percentage of the final germination estimated for each heat sum (θ). All model fittings were performed with the SPSS nonlinear regression facility and with Excel tools expressly implemented for that purpose.

Statistical analysis

A three-way ANOVA with species, thermoperiods (3/21–6/18 h) and cumulative heat pulse (from 6 to 36 h at 32°C) as main factors was applied to the fluctuating temperature experiment. To avoid any problem related to an unbalanced data matrix, the analysis was restricted to the six thermal doses that were common either to 3/21 or to 6/18 h cycle; intermediate odd points of the 3/21 h cycle were excluded. SPSS 13.0 for Windows package was used to perform the analysis.

Results were evaluated in terms of final germination percentage (FGP) and mean germination time (MGT). Final germination percentage data were previously arcsine-square-root transformed to achieve normality; back-transformed data were used for table and graphic representation. Mean germination time was calculated for each species and thermal cycle by the following formula (Labouriau, 1983):

$$\mathrm{MGT} = \frac{\sum_{i=1}^{k} n_i t_i}{\sum_{i=1}^{k} n_i} \quad (5)$$

where n_i is the number of seeds germinated on day-number t_i, and k is the duration in days of the test.

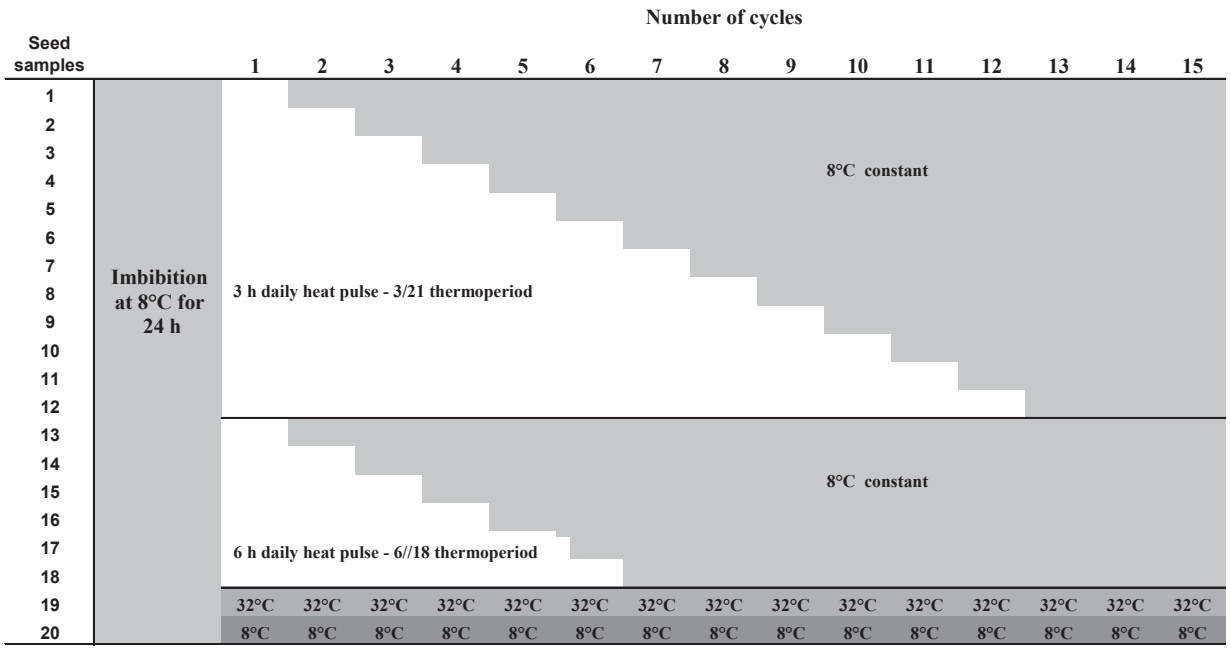

Fig. 1 Experimental design. Eighteen seed samples were incubated at 8°C (grey) and then submitted at 32/8°C (white) with thermoperiods of 21/3 and 18/6 h. At the end of the heat pulse period, seeds were kept at 8°C for up to 15 days. Two seed samples were kept at constant 8 and 32°C, respectively, as controls.

Table 1 Total heat sums (d°C) supplied to each of the six *Amaranthus* species submitted to fluctuating temperature regimes (32/8°C), either with a 3/21 h (from 1 to 12 cycles) or with a 6/18 h (from 1 to 6 cycles) thermoperiod, calculated in relation to the base temperature (T_b) of each species

	Number of heat pulses											
Pulse duration: 3 h	1	2	3	4	5	6	7	8	9	10	11	12
Pulse duration: 6 h	–	1	–	2	–	3	–	4	–	5	–	6
	Heat sum (d°C)											
A. albus	1.6	3.2	4.8	6.5	8.1	9.7	11.3	12.9	14.5	16.2	17.8	19.4
A. cruentus	2.9	5.8	8.7	11.6	14.5	17.5	20.4	23.3	26.2	29.1	32.0	34.9
A. deflexus	1.6	3.1	4.7	6.2	7.8	9.3	10.9	12.4	14.0	15.5	17.1	18.6
A. graecizans	1.3	2.5	3.8	5.1	6.3	7.6	8.9	10.1	11.4	12.7	13.9	15.2
A. retroflexus	2.5	5.0	7.6	10.1	12.6	15.1	17.6	20.2	22.7	25.2	27.7	30.2
A. viridis	1.5	3.0	4.5	6.0	7.5	9.0	10.5	12.0	13.5	15.0	16.5	18.0

Results

Germination at constant temperatures

Germination responses at constant temperatures are represented in Fig. 2. Some residual dormancy effects were observed in some species, whose intensity varied depending on the incubation temperature. *A. albus* attained maximum germination at 35°C, reaching around 90% cumulative germination after 9 days. Maximum germination percentages of this species decreased both at higher and lower temperatures, and no germination was observed under 15°C.

Amaranthus cruentus reached almost full germination in about 2–3 days under a wide set of temperatures ranging from 20 to 35°C, with lower and slower

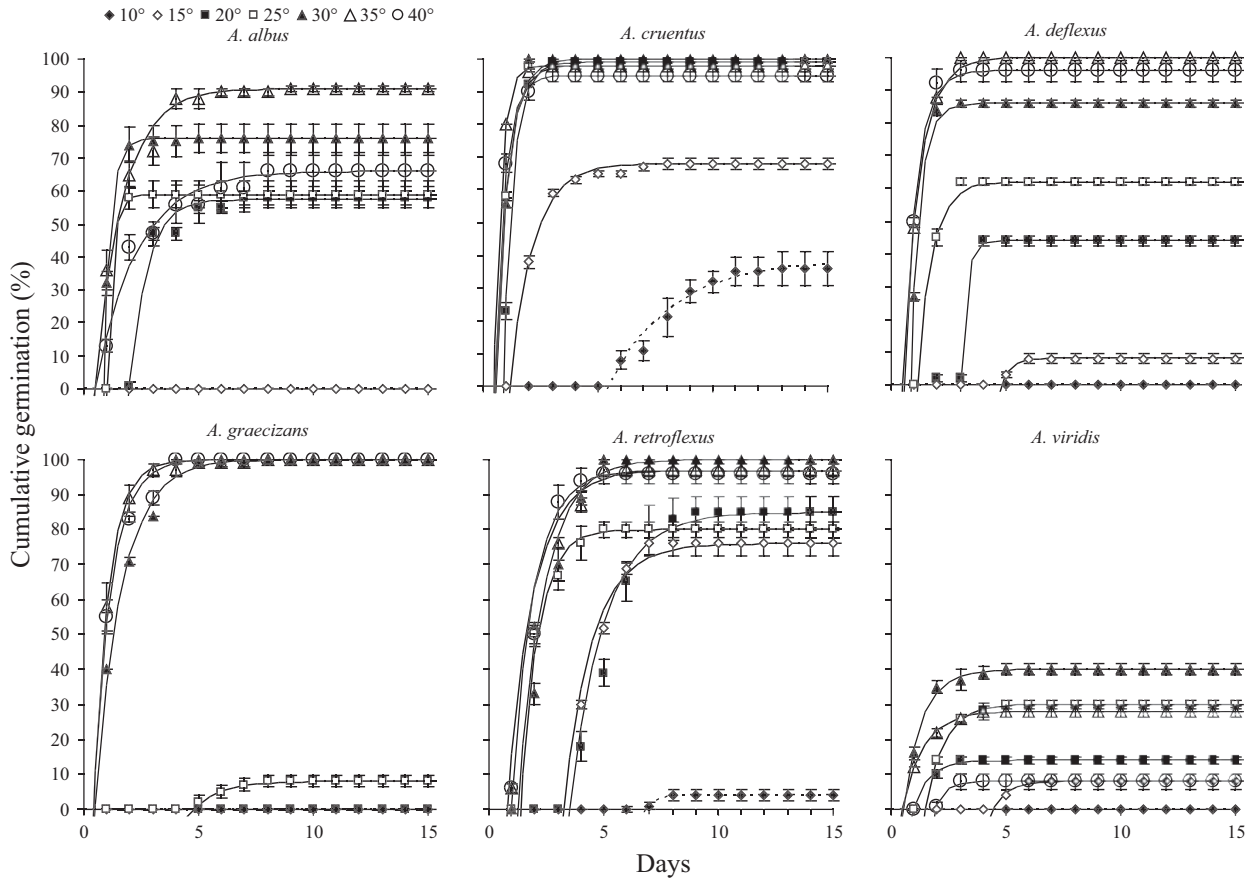

Fig. 2 Cumulative germination during time of six *Amaranthus* species subjected to seven different constant temperatures regimes ranging from 10 to 40°C, expressed as percentage of the viable seeds. Lines represent Weibull models (Eqn 1 in *Materials and methods*) interpolated to data points. Each point represents the average of four replicate dishes; vertical bars represent standard errors.

germination at 10 and 15°C. *Amaranthus deflexus* only attained full germination at 35–40°C, taking about 3 days to reach maximum cumulative values, with lower germination percentages at the other tested temperatures and no germination at 10°C. *Amaranthus graecizans* obtained full germination in about 4 days in the 30–40°C range, with very low germination at 25°C and no germination under 20°C. *Amaranthus retroflexus* took about 5 days at 30–40°C to reach full germination, with decreasing values at lower temperatures and very low percentages at 10°C. *Amaranthus viridis* only attained 40% FGP at 30°C in about 5 days, with lower values at all the other temperature tested and no germination at 10°C. Previous studies (Cristaudo *et al.*, 2007) showed that *A. viridis* exhibited a very low germination under constant temperature and required both alternating temperature and light to germinate. A promoting effect of alternating temperature on seed germination was also observed in *A. tuberculatus* (Leon *et al.*, 2007) and *A. blitoides* (Cristaudo *et al.*, 2007).

Cardinal temperatures

A graphic representation of the model used to estimate base and optimal temperatures is reported in Fig. 3; the values assumed by T_b and T_o are reported in Table 2. In the cases of *A. graecizans* and *A. cruentus,* the estimates of cardinal temperatures were quite consistent among the different percentiles; similarly, a relatively high stability of T_o estimates was observed on *A. deflexus*, although the variability was greater for T_b, with the lowest percentiles showing sensibly lower T_b values compared with the average estimated value.

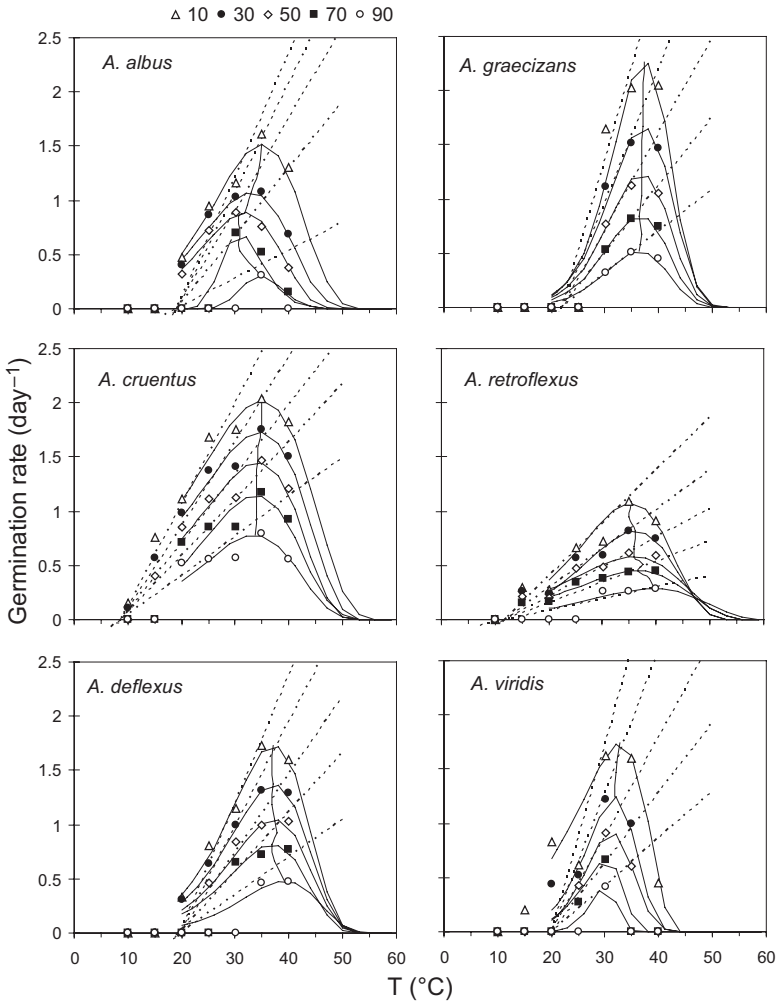

Fig. 3 Germination rates of six *Amaranthus* species in response to temperature. Five percentiles are reported in the figure, namely the 10th (open triangle), 30th (filled circle), 50th (open rhombus), 70th (filled square) and the 90th (open circle). To each percentile, a curve-peak model (see Eqn 2 in *Materials and methods*) was interpolated, in order to determine the optimal temperature. Dotted lines represent the linear regressions forced to intercept the *x*-axis at base temperature. A full near-vertical line connecting curve peaks separates the suboptimal from the supra-optimal range.

Table 2 Estimates of base (T_b) and optimal (T_o) temperatures for germination of the six studied *Amaranthus* species

Cardinal temperatures	T_b		T_o	
	Mean	SE	Mean	SE
A. albus	19.1	2.2	32.8	0.6
A. cruentus	8.7	0.6	34.3	0.2
A. deflexus	19.6	1.6	37.1	0.3
A. graecizans	21.9	0.1	36.7	0.2
A. retroflexus	11.8	1.8	36.1	0.8
A. viridis	20.0	1.5	31.5	0.3

In contrast, *A. albus* and *A. retroflexus* showed a high variability both for T_b and T_o estimates. *Amaranthus viridis* estimates were strongly affected by the limited germination achieved at constant temperatures, as only 40% germination was achieved.

Values for T_o, obtained by averaging the curve peaks of the interpolated models, were included in a relatively narrow interval, ranging approximately from 32 to 37°C according to the species. T_b values, in contrast, were quite differentiated among species: *A. cruentus* and *A. retroflexus* showed the lowest values of T_b, while the values estimated for the other four species were considerably higher, with the maximum value recorded on *A. graecizans*.

Germination under fluctuating temperatures

ANOVA revealed that both thermoperiods (3/21 and 6/18 h) and cumulative heat pulse (from 6 to 36 h at 32°C) had marked effects on FGP and on MGT of all species (Table 3). Both variables differed significantly among species, between thermoperiod type and among cumulative heat pulse, as well as among treatment interactions.

The average differences between the two thermoperiod types as a function of cumulative heat pulse are shown in Fig. 4. Final germination percentage tended to increase with total time of incubation at 32°C and was generally higher on seeds treated with 6/18 h relative to those treated with 3/18 h thermoperiod (Fig. 4A). Mean germination time was generally lower under 6/18 h compared with the 3/21 h thermoperiod (Fig. 4B); it tended to decrease with the increase in heat pulse, reaching relatively stable minimum values after about 12 h at 32°C (corresponding to 4 and 2 thermal cycles, respectively, for the 3/21 and 6/18 h thermoperiods). Seeds subjected to 3/21 h tended to germinate later and to a relatively lower extent than those treated with 6/18 h thermoperiod. Regarding control treatments, no species germinated under constant 8°C temperature. At constant 32°C, the lowest MGT was reached, and full germination was

Table 3 Main parameters of 3-way analysis of variance (ANOVA) with species, thermoperiod type (3/21–6/18 h) and cumulative heat pulse (6–36 h at 32°C) as main factors. Mean germination time (MGT) and final germination percentage (FGP) were the variable tested. FGP values were previously arcsine-square-root transformed in order to achieve normality

	Model	Main factor			2-way			3-way			
	Corrected model	Intercept	Species	Thermop. type	Cum. h. p.	Species × Thermop.	Species × Cum. h. p.	Thermop. × Cum. h. p.	Species × Thermop. × Cum. h. p.	Error	Total
MGT											
d.f.	62	1	5	1	5	5	22	5	19	61	124
F-values	199.2	65164.7	367.3	6600.9	208.02	83.2	18.3	69.3	9.7		
P<	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001		
FGP											
d.f.	71	1	5	1	5	5	25	5	25	72	144
F-values	28.5	5102.9	70.0	168.1	226.2	19.6	5.9	15.6	2.0		
P<	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.013		

Thermop., Thermoperiod; Cum. h. p., Cumulative heat pulse.

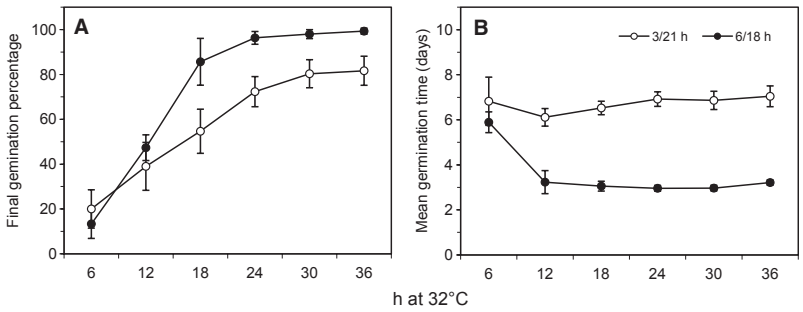

Fig. 4 Final germination percentage (A) and mean germination time (B) as a function of total incubation time at 32°C, under either a 3/21 or a 6/18 h thermal regime. Points and bars represent averages of the six *Amaranthus* species ±SE, as resulting by ANOVA analysis – factor interaction between 'cycle type' (3/21–6/18 h) and 'cumulative pulse time' (6–36 h). Open circles: 3/21 h; filled circles: 6/18 h. See ANOVA (Table 3) for significance of factor effects and interactions.

attained by *A. cruentus*, *A. deflexus* and *A. graecizans*; germination was almost complete for *A. retroflexus* and *A. albus*, while it resulted in quite low germination in *A. viridis* (data not shown).

Fluctuating temperature, in general, appeared to reduce the heat sum requirements for germination. The comparison between the fluctuating temperature regime and the predicted thermal time model was worked out for each of the six species by modelling germination responses as a function of heat sums. The Weibull function (Eqn 3) was interpolated to germination values as a function of thermal times (Fig. 5), while obtaining the parameters that maximise model fitting (Table 4). Thermal time to achieve 50% germination (θ_{50}) was always lower under fluctuating regimes compared with the values obtained with the predicted thermal time models, based on germination responses at constant temperature in the 10–40°C range. A similar pattern was observed for the lag thermal time (θ_0), with the only exception of *A. graecizans*. In general, θ_{50} also differed sensibly between the 6/18 and the 3/21 h thermoperiods: *A. albus, A. deflexus and A. graecizans* showed θ_{50} values decreasing in the order: 3/21 h > 6/18 h (Table 4). In contrast, relatively lower heat sum requirements were found under the 3/21 compared with the 6/18 h thermoperiod for *A. cruentus* and *A. retroflexus*. This last species was the most heat sum requiring species when analysed at constant temperature in the 10–40°C range, but fluctuating temperature drastically reduced its heat sum requirement, reaching the lowest θ_{50} values found among the six species. *Amaranthus viridis* did not show substantial differences in terms of θ_{50} between the two fluctuating temperature regimes.

Discussion

Predicting weed emergence in field crops is an essential target for weed management. It can help farmers to programme and schedule weed control treatments, avoiding damage to the crop. The question is: Does *Amaranthus* seeds only germinate when a fixed amount of day degrees is reached or may small consecutive daily heat pulses reduce these thermal requirements? In this latter case, *Amaranthus* seeds would germinate in the field relatively earlier compared with the former hypothesis.

The first step to address this question was to determine the cardinal temperatures for the studied seed populations. This was a necessary step to transform the time of exposure at 32°C, to compare species and treatments on a common thermal scale. Some of the parameters resulting from the present work are quite consistent with previous determinations available in literature. The base temperature values found by Benvenuti and Macchia (1993), Masin *et al.* (2010) and Guillemin *et al.* (2013) for *A. retroflexus* are in agreement with our results. They found for *A. retroflexus*, a threshold germination temperature (T_b) of 11.6, 12.1–12.3 and 8.9°C respectively.

The following step was to assess whether either thermal time requirements or dormancy patterns may be affected by the regime of fluctuating temperature experienced by seeds during germination. In our experiment, both heat sum requirements and FGP were affected by thermoperiod and by the number of heat pulse cycles, with a variable intensity depending on the studied species. Two or three cycles of heat pulses of six daily hours at 32°C generally satisfied the germination requirements of all species, but in at least three species, a longer period of exposure was necessary to achieve the same germination values when 3/21-h thermoperiod was applied.

The role of alternating temperatures on seed germination was the object of several previous studies (Totterdell & Roberts, 1980; Probert *et al.*, 1986). Referring to the *Amaranthus* genus, the need for alternating temperatures to break dormancy had already

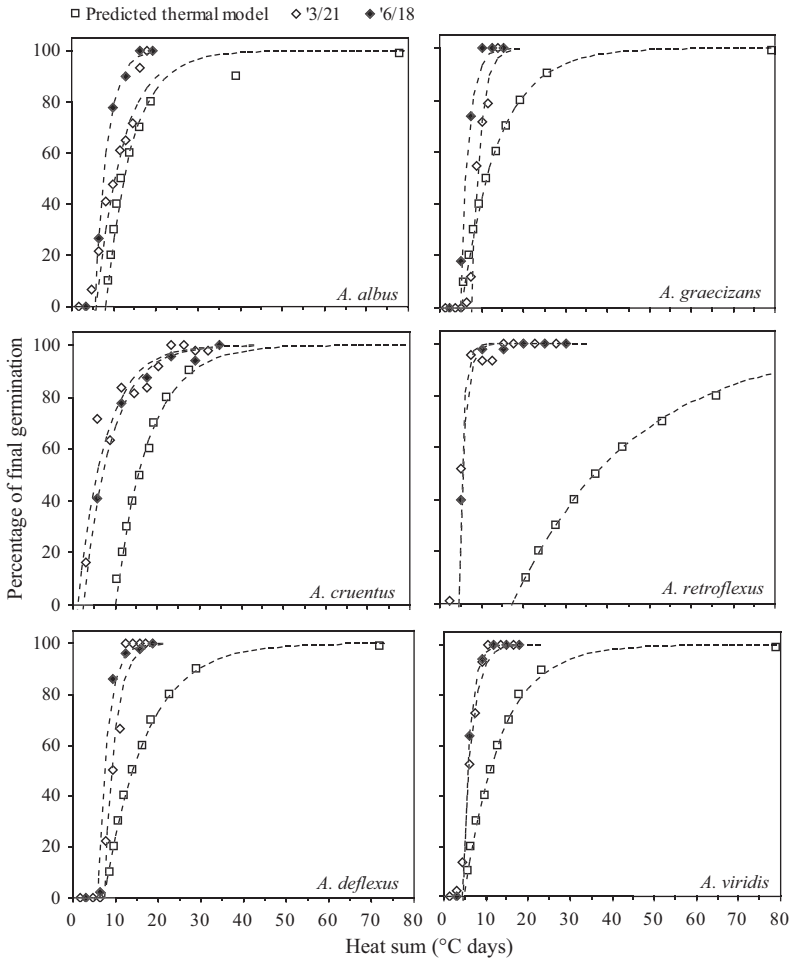

Fig. 5 Germination response of six *Amaranthus* species to heat sum accumulation, either at constant (squares) or at alternate temperature (rhombus: 3/21 h; circles: 6/18 h). Dotted lines represent Weibull-type models (Eqn 3) interpolated to data points. The predicted thermal time models were obtained by plotting ten germination percentiles, studied at constant temperature in the 10–40°C range, against the corresponding estimated thermal times. The responses at fluctuating temperature were modelled by plotting cumulative germination percentages against the heat sums supplied. See Table 4 for model parameters and fitting.

been found for *A. tuberculatus* (Leon *et al.*, 2007), *A. blitoides* and *A. viridis* (Cristaudo *et al.*, 2007). In addition to what had already been documented, in the present experiment, we found that the effect of heat pulse treatments on seed germination not only depends on total heat sum but it may also be affected by the thermoperiod (3/21 and 6/18 h).

The importance of thermoperiod had already been explored by several authors (Totterdell & Roberts, 1980; Murdoch *et al.*, 1989), although the results of these studies did not present sufficient homogeneity to draw general conclusions. Van Assche and Van Nerum (1997), in a study conducted on *Rumex obtusifolius*, concluded that the 'activation' of seeds was influenced by the rate of temperature change, rather than by the total heat sum, as only fast heating treatments were able to trigger germination of this species. Moreover, repeated rapid heating cycles appeared to enhance germination response, independently of the total heat sum provided. In the present experiment, seed heating during heat pulses was extremely fast, as Petri dishes were directly moved from 8 to 32°C incubators and vice versa. The germination of seeds treated with two cycles of heat pulses of 3/21 h thermoperiod was often lower compared with seeds treated with a single heat pulse of 6/18 h thermoperiod. Thus, for the six *Amaranthus* species analysed, the duration of heat pulse (thermoperiod) rather than their total number (6 h instead of 3 + 3 h) is the determining factor for seed germination.

What is the ecological meaning of such behaviour? Germination sensitivity to thermal changes had already been envisaged as a mechanism aimed to prevent germination when the seed is deeply buried in the soil (Thompson & Grime, 1983). In fact, diurnal temperature fluctuations are greatest near the soil surface and

Table 4 Model parameters and curve-fitting values of the Weibull-type models (Eqn 3) interpolated to per cent germination values as a function of thermal time. The predicted thermal time models were obtained by interpolating the model to the ten studied germination percentiles as a function of the corresponding thermal times, resulting from the whole set of germination trials conducted at constant 10, 15, 20, 25, 30, 35 and 40°C respectively. See model Eqn (3) in *Materials and methods* for any explanation about the meanings of each single parameter, and Fig. 5 for a graphic representation of data points and models

Species	α day^{-1} * °C^{-1}	θ_0 days * °C	θ_{50} days * °C	Curve fitting	
				R^2	Fit St. Err.
Amaranthus albus					
Predicted TT	0.16 ± 0.01	7.96 ± 0.26	12.35 ± 0.24	0.982	0.038
3/21	0.16 ± 0.02	4.74 ± 0.44	9.15 ± 0.42	0.941	0.072
6/18	0.35 ± 0.03	5.59 ± 0.12	7.55 ± 0.10	0.996	0.022
Amaranthus cruentus					
Predicted TT	0.12 ± 0.004	9.92 ± 0.15	15.60 ± 0.12	0.996	0.020
3/21	0.16 ± 0.03	1.14 ± 0.81	5.53 ± 0.53	0.901	0.077
6/18	0.15 ± 0.01	2.19 ± 0.53	7.62 ± 0.28	0.988	0.026
Amaranthus deflexus					
Predicted TT	0.11 ± 0.002	7.49 ± 0.11	13.91 ± 0.08	0.998	0.012
3/21	0.40 ± 0.07	7.25 ± 0.29	8.97 ± 0.23	0.942	0.069
6/18	0.61 ± 0.03	6.18 ± 0.03	7.31 ± 0.06	0.999	0.012
Amaranthus graecizans					
Predicted TT	0.11 ± 0.00	4.97 ± 0.04	11.33 ± 0.04	1.000	0.004
3/21	0.34 ± 0.05	6.54 ± 0.25	8.58 ± 0.31	0.936	0.092
6/18	0.52 ± 0.07	4.70 ± 0.12	6.04 ± 0.14	0.990	0.038
Amaranthus retroflexus					
Predicted TT	0.03 ± 0.001	17.23 ± 0.31	37.61 ± 0.42	0.999	0.011
3/21	0.81 ± 0.19	4.13 ± 0.23	4.98 ± 0.08	0.961	0.027
6/18	0.66 ± 0.09	4.27 ± 0.11	5.32 ± 0.04	0.999	0.009
Amaranthus viridis					
Predicted TT	0.11 ± 0.004	4.69 ± 0.17	10.82 ± 0.14	0.995	0.021
3/21	0.31 ± 0.04	3.25 ± 0.24	5.48 ± 0.30	0.944	0.080
6/18	0.62 ± 0.03	4.33 ± 0.09	5.46 ± 0.04	0.999	0.005

diminish rapidly with depth (Thompson *et al.*, 1977). In our view, this aptitude could additionally be related to the detection of the best suited climatic conditions for germination during spring. Late winter and early spring germination may confer a great advantage in terms of competitive resource pre-emption, as well as the opportunity to bring on a higher number of life-cycles during one growth season. Cold-sensitive species, however, would risk being seriously damaged if they emerged too soon, as late freezing events could partly destroy seedling populations. Thus, the ability to finely trigger seed germination as a function of seasonal stage could be a crucial adaptive trait to allow these species to colonise their habitats. A threshold of the heat pulse duration would act as the signal to discriminate premature vs. good conditions for a successful early germination.

Acknowledgements

We are very grateful to Dr Andrea Onofri for his precious support in statistical analysis. We also thank two anonymous reviewers who significantly contributed to improve this manuscript.

References

Benvenuti S & Macchia M (1993) Calculation of threshold temperature for the development of various weeds. *Agricoltura Mediterranea*, **123**, 252–256.

Bierhuizen JF & Wagenvoort WA (1974) Some aspects of seed germination in vegetables. 1. The determination and application of heat sums and minimum temperature for germination. *Scientia Horticulturae*, **2**, 213–219.

Brown RF & Mayer DG (1988) Representing cumulative germination. 2. The use of the Weibull function and other empirically derived curves. *Annals of Botany*, **61**, 127–138.

Cesaraccio C, Spano D, Duce P & Snyder RL (2001) An improved model for determining degree-day values from daily temperature data. *International Journal of Biometeorology*, **45**, 161–169.

Costea M & Tardif FJ (2003) The biology of Canadian weeds. 126. *Amaranthus albus* L., *A. blitoides* S. Watson and *A. blitum* L. *Canadian Journal of Plant Science*, **83**, 1039–1066.

Costea M, Weaver S & Tardif FJ (2004) The biology of Canadian weeds. 130. *Amaranthus retroflexus* L., *A. powellii* S. Watson and *A. hybridus* L. *Canadian Journal of Plant Science*, **84**, 631–668.

Cristaudo A, Gresta F, Luciani F & Restuccia A (2007) Effects of after-harvest period and environmental factors

on dormancy breaking of *Amaranthus* species. *Weed Research*, **47**, 327–334.

Faccini D & Vitta JI (2005) Germination characteristics of *Amaranthus quitensis* as affected by seed production date and duration of burial. *Weed Research*, **45**, 371–378.

Garcia-Huidobro J, Monteith JL & Squire GR (1982a) Time, Temperature and Germination of Pearl Millet (*Pennisetum typhoides* S. & H.). I. Constant temperature. *Journal of Experimental Botany*, **33**, 288–296.

Garcia-Huidobro J, Monteith JL & Squire GR (1982b) Time, temperature and germination of pearl millet (*Pennisetum typhoides* S. & H.). II. Alternating temperature. *Journal of Experimental Botany*, **33**, 297–302.

Gardarin A, Guillemin JP, Munier-Jolain NM & Colbach N (2010) Estimation of key parameters for weed population dynamics models: base temperature and base water potential for germination. *European Journal of Agronomy*, **32**, 162–168.

Gardarin A, Dürr C & Colbach N (2011) Prediction of germination rates of weed species: relationships between germination speed parameters and species traits. *Ecological Modelling*, **222**, 626–636.

Guillemin JP, Gardarin A, Granger S, Reibel C, Munier-Jolain N & Colbach N (2013) Assessing potential germination period of weeds with base temperatures and base water potentials. *Weed Research*, **53**, 76–87.

Guo P & Al-Khatib K (2003) Temperature effects on germination and growth of redroot pigweed (*Amaranthus retroflexus*), Palmer amaranth (*A. palmeri*), and Common waterhemp (*A. rudis*). *Weed Science*, **51**, 869–875.

Hardegree SP (2006) Predicting germination response to temperature. I. Cardinal-temperature models and subpopulation-specific regression. *Annals of Botany*, **97**, 1115–1125.

Hardegree SP, Moffet CA, Roundy BA *et al.* (2010) A comparison of cumulative-germination response of cheatgrass (*Bromus tectorum* L.) and five perennial bunchgrass species to simulated field-temperature regimes. *Environmental and Experimental Botany*, **69**, 320–327.

Labouriau LG (1983) *A germinação das sementes*. Série de Biologia. Monografia 24. Secretaria Geral da Organização dos Estados Americanos - OEA. Washington, USA.

Larsen SU & Bibby BM (2005) Differences in thermal time requirement for germination of three turfgrass species. *Crop Science*, **45**, 2030–2037.

Leon RG, Knapp AD & Owen MDK (2004) Effect of temperature on the germination of common waterhemp (*Amaranthus tuberculatus*), giant foxtail (*Setaria faberi*), and velvetleaf (*Abutilon theophrasti*). *Weed Science* **52**, 67–73.

Leon RG, Bassham DC & Owen MDK (2007) Thermal and hormonal regulation of the dormancy–germination transition in *Amaranthus tuberculatus* seeds. *Weed Research*, **47**, 335–344.

Masin R, Loddo D, Benvenuti S, Zuin MC, Macchia M & Zanin G (2010) Temperature and water potential as parameters for modeling weed emergence in Central-Northern Italy. *Weed Science*, **58**, 216–222.

Moot DJ, Scott WR, Roy AM & Nicholls AC (2000) Base temperature and thermal time requirements for germination and emergence of temperate pasture species. *New Zealand Journal of Agricultural Research*, **43**, 15–25.

Murdoch AJ, Roberts JH & Goedert GO (1989) A model for germination response to alternating temperatures. *Annals of Botany*, **63**, 97–111.

Probert RJ, Smith RD & Birch P (1986) Germination responses to light and alternating temperatures in European populations of *Dactylis glomerata* L. V. The principle components of the alternating temperature requirement. *New Phytologist*, **102**, 133–142.

Royer F & Dickinson R (1999) *Weeds of Canada and the Northern United States*. University of Alberta Press, Edmonton, AB, Canada.

Sakanoue S (2010) Thermal time approach to predicting seedling emergence dates of red clover, white clover and lucerne in farm fields. *Grass and Forage Science*, **65**, 212–219.

Steinmaus SJ, Prather TS & Holt JS (2000) Estimation of base temperatures for nine weed species. *Journal of Experimental Botany*, **51**, 275–286.

Thompson K & Grime JP (1983) A comparative study of germination responses to diurnally-fluctuating temperatures. *Journal of Applied Ecology*, **20**, 141–156.

Thompson K, Grime JP & Mason G (1977) Seed germination in response to diurnal fluctuations of temperature. *Nature*, **267**, 147–149.

Totterdell S & Roberts EH (1980) Characteristics of alternating temperatures which stimulate loss of dormancy in seeds of *Rumex obtusifolius* L. and *R. crispus* L. *Plant Cell and Environment*, **3**, 3–12.

Trudgill DL, Honek A, Li D & Van Straalen NM (2005) Thermal time – concepts and utility. *Annals of Applied Biology*, **146**, 1–14.

Van Assche JA & Van Nerum DM (1997) The influence of the rate of temperature change on the activation of dormant seeds of *Rumex obtusifolius* L. *Functional Ecology*, **11**, 729–734.

Viegi L (2001) Investigation on some reproductive features of invasive alien plants in Italy. In: *Plant Invasions: Species Ecology and Ecosystem Management* (eds G Brundu, J Brock, I Camarda, L Child & M Wade). 255–262. Backhuys Publishers, Leiden, The Netherlands.

Wagenvoort WA & Bierhuizen JF (1977) Some aspects of seed germination in vegetables. II. The effect of temperature fluctuation, depth of sowing, seed size and cultivar, on heat sum and minimum temperature for germination. *Scientia Horticulturae*, **6**, 259–270.