Seed Science Research (2014) **24**, 321–330
doi:10.1017/S0960258514000221

Thermal time model of *Solanum sarrachoides* germination

J.P. del Monte[1]*, P.L. Aguado[1] and A.M. Tarquis[2,3]

[1]Dpto. de Producción Vegetal: Botánica and [2]Dpto. De Matemática Applicada. E.T.S. de Ingenieros Agrónomos (UPM), Ciudad Universitaria s.n. 28040 Madrid, Spain; [3]C.E.I.G.R.A.M. (Centro de Estudios e Investigación de Gestión de Riesgos Agrícolas y Medioambientales) (UPM), Ciudad Universitaria s.n. 28040 Madrid, Spain

(Received 7 March 2014; accepted after revision 4 July 2014; first published online 16 September 2014)

Abstract

A population-based modelling approach was used to predict the occurrence of germination in *Solanum sarrachoides* (SOLSA) for different treatments. Seeds collected in Toledo (Spain) were exposed to constant temperatures, to temperatures alternating between 10 and 30°C and to gibberellins (GAs; 0, 50, 100, 150 and 1000 ppm) during a 24-h imbibition period. The following parameters were measured: base temperature (*Tb*), mean thermal time (θ_T(50)) and the standard deviation of thermal time ($\sigma_{\theta T}$). The SOLSA seeds only germinated at constant temperatures when the highest GA concentration was applied. The thermal model suggests that the induction and loss of physiological dormancy following seed dispersal is achieved when temperatures vary and when a mean thermal time of 66 growing degree-days (d°C) and a *Tb* value of 16°C are achieved when no GA treatment was added. The concentration of GA applied under conditions of alternating temperatures has an additive effect, reducing θ_T (50) up to threefold, from basal level (66 d°C) to 19.40 d°C, when the 1000 ppm GA treatment was applied. In this last case, the germination was accelerated by reducing *Tb* to 14°C. A 5–10°C change in temperature and a range of average temperatures of 20–27.5°C promoted the germination of SOLSA seeds to the greatest extent in the absence of GA. However, these conditions are not frequently encountered in the irrigated areas of the studied region; this finding could explain the limited ability of SOLSA to expand its range within this area.

Keywords: base temperature, dormancy, gibberellins, modelling, *Solanum sarrachoides*, thermal time

*Correspondence
Email: jp.monte@upm.es

Introduction

Solanum sarrachoides Sendtner (SOLSA) is an annual species that is part of the so-called '*Solanum nigrum* complex' (*Solanum* section *Solanum*), which is composed of many morphogenetically different taxa (Edmonds and Chweya, 1997). This species is morphologically similar to the species *Solanum physalifolium* Rusby var. *nitidibacatum* (Bitter) Edmonds (SOLPH); for this reason, both species have frequently been considered to represent the same species (*Solanum sarrachoides* Stend.) (Edmonds and Chweya, 1997). Both species are native to South America and currently distributed worldwide (Edmonds and Chweya, 1997). Both species are also present in Spain as non-native weeds in fields of irrigated crops (Sobrino and Del Monte, 1994). However, the distribution and habitat of these species differ completely. For example, SOLPH is widely distributed in the Northern Plateau (and continues to expand its range), whereas SOLSA is confined to a small area in central Spain (Toledo) (Sobrino and Del Monte, 1994), near to the location where it was initially discovered by Laorga (1983). This suggests that the germination process of SOLSA may be restricted and its range therefore limited.

The germination process is very important in the life cycle of a plant, affecting the survival, development and population dynamics of a species. These processes are controlled by both genetics and the environment (Gutterman, 2000; Meyer and Pendelton, 2000; Del Monte and Dorado, 2011). A plant species can adapt to local habitats by adopting genotypic changes; in addition, phenotypic variability can produce temporal variations in the germination process by maintaining a seed bank in the soil that facilitates dispersal over time. However, dormancy is a common attribute of many weed seed populations, which can only be measured by the absence of germination. Dormancy ['internal condition of the seed that impedes its germination under otherwise adequate hydric, thermal and gaseous conditions' (Benech-Arnold *et al.*, 2000)] should not just be associated with the absence of germination; rather, it is a

characteristic of the seed that determines the conditions required for germination. When dormancy is considered in this way, any environmental cue that alters the conditions required for germination is, by definition, altering dormancy (Finch-Savage and Leubner-Metzger, 2006).

The loss of dormancy following seed dispersion, and subsequent germination, can be promoted by: (1) environmental factors such as the ambient temperature, water potential of the soil, the amount of light, etc. (Bewley and Black, 1994; Pérez-Flores *et al.*, 2003; Finch-Savage and Leubner-Metzger, 2006) [Each of these factors may act individually or in combination; in addition, these factors tend to vary by season in the area where the seeds are distributed. Seeds often incorporate the effects of these factors over time (Del Monte and Dorado, 2011).]; (2) endogenous growth regulators, i.e. promoters such as gibberellic acid (GA) or inhibitors such as abscisic acid (ABA) (Karssen *et al.*, 1987; Groot and Karssen, 1992; Ni and Bradford, 1993).

Temperature is the environmental factor that has the greatest effect on dormancy and seed germination. The temperature affects germinability by regulating dormancy or the germination rate of seeds that do not exhibit dormancy (Totterdell and Roberts, 1980; Roberts and June, 1983; Murdoch *et al.*, 1989; Bouwmeester and Karssen, 1992). Numerous studies have sought to determine the effect of different temperature regimens (T), i.e. constant temperatures (Covell *et al.*, 1986; Dahal *et al.*, 1990; Bradford *et al.*, 1993) or alternating temperatures (Murdoch *et al.*, 1989; Ellis and Barrett, 1994), on seed germination.

The loss of dormancy in seeds is associated with an increase in GA biosynthesis and a degradation of ABA (Karssen *et al.*, 1987; Groot and Karssen, 1992; Ni and Bradford, 1993). The ABA–GA balance is modified by environmental factors. For example, a regimen of alternating temperatures appears to promote germination by enabling the embryo to overcome physical limitations. It is also possible that the regulation of dormancy status results from the response of this balance through hormone signalling networks that influence sensitivity to ABA and GA (Huarte and Benech-Arnold, 2010, Footitt *et al.*, 2011).

Population-based models have been used to successfully predict the occurrence of seed germination. Two approaches, an empirical model and a mechanistic model, have been used to model seed germination. Empirical models incorporating various levels of empiricism can effectively match individual-level data on germination over time; however, these models may need more empirical variables (Brown and Mayer, 1988). The empirical approach may be useful for a specific purpose; however, it is difficult to interpret the biological significance of derived model parameters (Bradford, 1990; Forcella *et al.*, 2000). Mechanistic threshold models for seed germination have achieved some success (Forcella, 1993; Benech-Arnold and Sánchez, 1995; Allen *et al.*, 2000; Roman *et al.*, 2000; Bradford, 2002; Rowse and Finch-Savage, 2003). Population-based modelling approaches investigating the occurrence of germination as a function of temperature and/or water potential have been well developed in recent decades, using concepts of thermal time (θ_T) (Garcia-Huidobro *et al.*, 1982) and hydrothermal time (θ_{HT}) (Bradford, 1995; Finch-Savage *et al.*, 1998; Allen *et al.*, 2000, Dorado *et al.*, 2009).

Thermal time (degree-day or degree-hour) is a well-established developmental mathematical parameter that is used to model the effect of temperature on the development of seed germination (García-Huidobro *et al.*, 1982; Ellis *et al.*, 1986; Dahal *et al.*, 1990; Bradford, 1995; Kebreab and Murdoch, 1999a, b, 2000; Alvarado and Bradford, 2002). According to the model, the thermal time $\theta_T(g)$ for the percentile g is:

$$\theta_T(g) = (T - T_b)t_g \quad (1)$$

where T is the actual temperature (incubation mean temperature), T_b is the base or minimum temperature required for germination and t_g is the time to germination for a given percentile g. Because the germination rate (GR) is defined as the inverse of the time to radicle emergence of a specific percentile of the population, equation (1) can be rewritten:

$$GR_g = \frac{(T - T_b)}{\theta_T(g)} = \frac{1}{t_g}. \quad (2)$$

GR is linearly related to temperature within a range of sub-optimal temperatures ranging from the base temperature (T_b) to the optimal temperature (T_o), i.e. the temperature at which maximum germination rate occurs for non-dormant seeds. The linear relationship between GR_g and T [i.e. the slope of the linear regression line, which is equal to the reciprocal of thermal time ($\theta_T(g)$)] varies among different subpopulations. Equation (2) was also described previously by Covell *et al.* (1986), indicating that it was also valid for a range of temperatures above the optimal temperature.

If the variation in $\theta_T(g)$ within a seed population follows a log-normal distribution (Covell *et al.*, 1986; Ellis *et al.*, 1986), then the change in germination time with thermal time can be described using repeated probit analysis:

$$probit(g) = \left(\frac{1}{\sigma_{\theta_T}}\right)^* \log((T - T_b)t_g) - \frac{\log(\theta_T(50))}{\sigma_{\theta_T}} \quad (3)$$

where *probit(g)* is the probit transformation of the cumulative germination percentile g, $\theta_T(50)$ is the thermal time to 50% germination (or median thermal time to germination) and σ_{θ_T} is the standard deviation of θ_T for individual seeds in the population. Once T_b is estimated, the thermal time to germination *t(g)* can be normalized on a thermal time scale by multiplying this

https://doi.org/10.1017/S0960258514000221 Published online by Cambridge University Press

Table 1. Typical temperatures encountered at La Puebla de Montalban, Toledo, Spain during different seasons: T_{max}, average maximum temperature; T_m, average medium temperature; and T_{min}, average minimum temperature. Historical data obtained from SIAR (2013)

Seasons	T_{max} (°C)	T_m (°C)	T_{min} (°C)
Winter	13.2	7.4	1.4
Spring	25.2	16.7	9.4
Summer	32.6	23.6	14.1
Autumn	16.3	11.3	4.3

parameter by $(T - T_b)$. Thermal time models have been used successfully to predict the occurrence of seed germination under non-water-limiting conditions, thus explaining approximately 80% of the variation in the cumulative percentile (Garcia-Huidobro *et al.*, 1982; Covell *et al.*, 1986).

Under current environmental conditions, the habitat of SOLSA appears to be restricted to a small area in the centre of the Iberian Peninsula where its potential to act as an invasive weed is currently limited. In this paper, we propose a thermal model of SOLSA germination (using different temperature regimes and GA concentrations as a way to overcome dormancy) to model, understand and to justify its behaviour in this environment and under a warmer climate in the near future, if increased average temperatures in the Mediterranean area occur as a result of global warming (UNEP and WMO, 2007), which could increase the distribution area of this weed.

Materials and methods

Plant material

SOLSA seeds were collected in 2009 from corn fields in La Puebla de Montalban (Toledo, Spain) where the plant grew as a weed. SOLSA normally germinates in late spring and early summer among irrigated crops. The soil water content did not limit germination, as the zone is irrigated. The typical temperature regimen in this area is shown in Table 1 (SIAR, 2013). The climate of the area is classified as continental Mediterranean and characterized by hot and dry summers.

Weed populations were mostly concentrated along the edge of the crops and consisted of 11–15 individual plants. The mature berries of all observed specimens were collected and pooled; the berries were crushed and the seeds were washed for 1–2 min in a sieve under running water to eliminate all traces of the berries; the seeds were then cleaned, initially dried on filter paper to eliminate excess water and then air dried and stored at room temperature for 2 months. Experiments lasted for 16 weeks thereafter. A previous experiment with 1-year-old SOLSA seeds showed no variation in the germination levels. Immediately prior to the germination experiments, the seeds were treated with 1% sodium hypochlorite for 3 min (to prevent infections during the assay) (Chen and Chang, 1972), washed with distilled water to eliminate any residue and dried at room temperature.

Experimental design

Germination tests were carried out in a growth chamber with a constant 12 h light/12 h dark photoperiod and two different types of temperature regimens: a constant temperature regimen ($\Delta T = 0$) and alternating temperatures ($\Delta T \geq 5$°C) (see Table 2). The factors used to build the model included the average incubation temperature (T_m) with constant and alternating temperatures (when using alternating temperatures the thermal regime was 12/12 h with the high temperature coinciding with the period of light, and obviously $T_m \pm \frac{1}{2}$ temperatures), the temperature difference (ΔT) and the concentrations of GA in which the seeds were incubated. Fifteen thermal treatments with different values of T_m and ΔT and five treatments with GA doses ranging from 0 to 1000 ppm (G0 to G4) were established, for a total of 75 distinct treatments with three replicates per treatment. Each treatment is defined by a specific combination of a thermal treatment and a GA concentration, as shown in Table 2 (e.g. T10/0G0 = average temperature of 10°C, temperature range of 0°C (constant temperature) and GA concentration of 0 ppm).

Gibberellin treatments and temperature regimes

Gibberellic acid 3 (GA_3, the most commonly used commercial form of GA) has been shown to disrupt

Table 2. Thermal treatments and gibberellic acid concentrations used in the experiment

Treatment	T_m	ΔT	Treatment	[GA] ppm
T10/0	10	0	G0	0
T12.5/5	12.5	5	G1	50
T15/0	15	0	G2	100
T15/10	15	10	G3	150
T17.5/5	17.5	5	G4	1000
T17.5/15	17.5	15		
T20/0	20	0		
T20/10	20	10		
T20/20	20	20		
T22.5/5	22.5	5		
T22.5/15	22.5	15		
T25/0	25	0		
T25/10	25	10		
T27.5/5	27.5	5		
T30/0	30	0		

https://doi.org/10.1017/S0960258514000221 Published online by Cambridge University Press

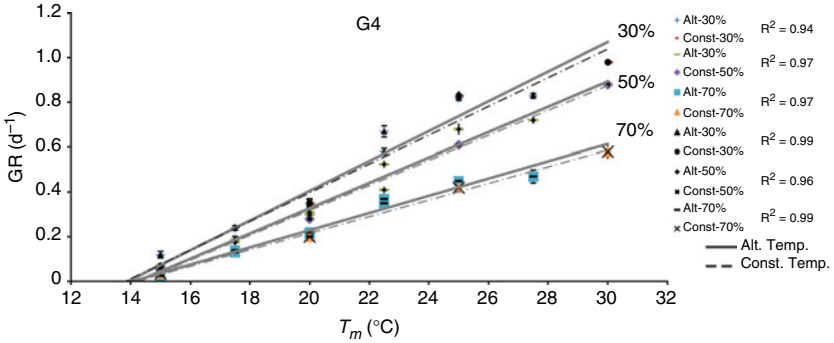

Figure 1. (colour online) Relationship between germination rate (GR) (30, 50 and 70%) and T_m with $\Delta T = 0$°C (dashed line) or $\Delta T \geq 5$°C (solid line) in the G4 treatment. T_b is the point of intersection of the regression lines and the *x*-axis. The percentiles shown in the figures were of the treatments: T15/0 (all), T20/0 (all), T25/0 (all), T30/0 (all), T15/10 (all), T17.5/5 (all), T17.5/15 (all), T20/10 (all), T20/20 (all), T22.5/5 (all), T22.5/15 (all), T25/10 (all) and T27.5/5 (all).

dormancy in, and promote the germination of, the seeds of several genera of plants. The GA used in this experiment consisted mostly (90%) of GA_3 (Fluka, Sigma-Aldrich, St. Louis, Missouri, USA), a form of gibberellin frequently used in seed germination assays. This commercial formulation of GA_3 contains no surfactants or other related compounds. Two types of experiments have been conducted to examine the association between GA and seed germination: in a first type of experiment, the germination solution contained a constant concentration of the plant growth regulator (Groot and Karssen, 1992; Ni and Bradford, 1993); in a second type of experiment, the plant growth solution was applied only at certain times (mainly at the imbibition phase) and replaced with a water solution (Andreoli and Khan, 1999; Jusaitis *et al.*, 2004, Machado de Mello *et al.*, 2009). We used the latter approach in this experiment.

Seeds were imbibed for 24 h (Machado de Mello *et al.*, 2009) on paper soaked in 5 ml of one of the different GA solutions in Petri dishes (9-cm diameter) at each temperature regimen [this time period (24 h) was included to calculate the accumulated thermal time, because in some of the treatments the seed germination started during this period]. Subsequently, the seeds were rinsed with distilled water and immediately placed in Petri dishes (9-cm diameter) only with water up to the end of the assay.

Five different concentrations of GA_3 (Table 2) were prepared by dissolving GA in distilled water. The solutions had a final pH of 7 (± 0.1), they were buffered and the pH adjusted with phosphate buffer (pH 7.0). Initially, only GA concentrations lower than 1000 ppm were used. However, because no significant differences between these concentrations and G0 were observed, we increased the GA concentration to 1000 ppm, as the aim of the study was not to determine the minimum dose needed to reach germination but rather to determine the role of GA in the germination process.

Germination tests

Three replicates of at least 90 seeds were placed on blotter paper in 9-cm glass Petri dishes and moistened with distilled water (3 ml). All of the replicates were randomized within each temperature and incubated in a growth chamber. Three identical growth chambers were used, and the temperatures were monitored every 20 min using temperature sensors linked to HOBO_U12 data loggers (Onset Computer Corporation, Pocasset, Massachusetts, USA). Data were considered acceptable if the recorded temperature remained within the prescribed limit of ± 0.5°C. A 12 h light/12 h dark photoperiod was used for all of the temperature regimens. All the experiments were set up at 10.00 hours, the first hour of the light cycle. The occurrence of germination was recorded daily for at least 21 d. The emergence of a radicle of length 1.0 mm was counted as a germinated seed. All Petri dishes were moistened with distilled water as needed to replace evaporation losses. Mean germination rate (GR_{50}, d^{-1}) is the inverse of mean time (t_{50}) value, and t_{50} is the time taken for seeds to reach 50% germination.

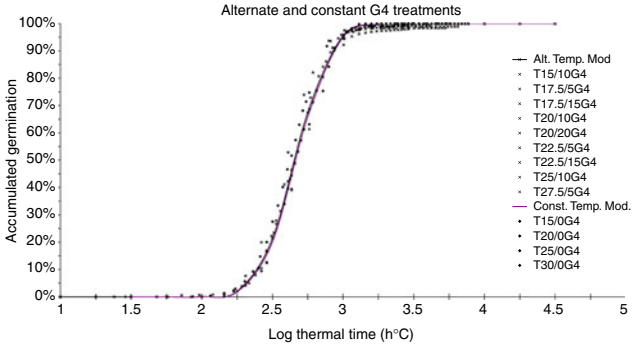

Figure 2. (colour online) Relationship between accumulated germination (%) and log $\theta_T(g)$ in the G4 treatments.

https://doi.org/10.1017/S0960258514000221 Published online by Cambridge University Press

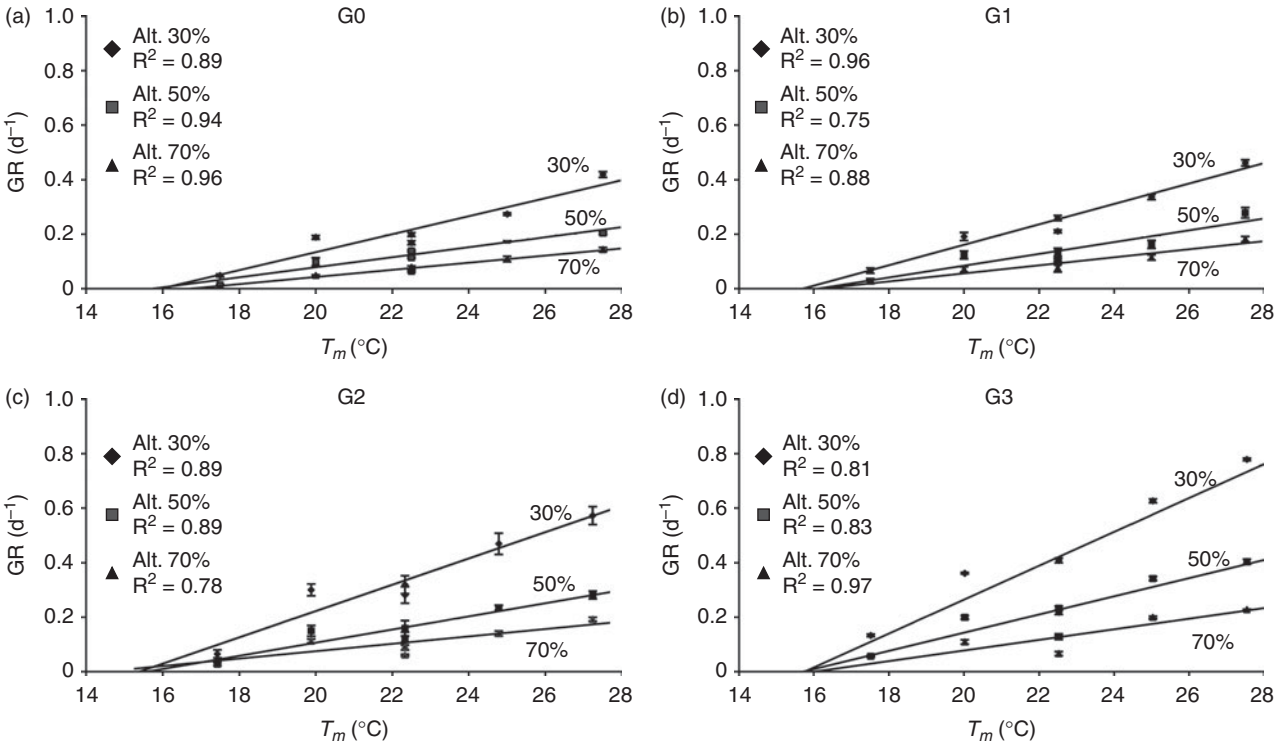

Figure 3. Relationship between germination rate (GR) (30, 50 and 70%) and T_m with $\Delta T \geq 5$°C for different concentrations of GA: (a) G0, (b) G1, (c) G2 and (d) G3. T_b is the point of intersection of the regression line with the *x*-axis. The percentiles shown in the figures were of the treatments: T17.5/15 (30 and 50%), T20/10 (all), T22.5/5 (all), T22.5/15(all), T25/10 (all) and T27.5/5 (all).

Data analysis for the thermal time model

The final germination percentile was calculated on the total incubated population per treatment. Germination rates (GR_g) for $g = 30$, 50 and 70% within a given GA treatment were calculated using equation (2) ($1/t_g$) when the percentiles of germination in each treatment reached the mentioned values of g. The T_b parameter was obtained by combining the data from a range of mean incubation temperatures (T_m) and germination rates (g) within a given GA treatment (see Figs 1 and 3). A linear regression analysis was used to obtain the intercept; the average intercept of all cases represents the estimated value of T_b. Although this method is more time consuming than other commonly used methods (Ellis *et al.*, 1986), we were able to verify that T_b had a constant value for each population.

The mean thermal time to 50% germination for the log thermal time distribution at alternating temperatures ($\theta_T(50)$) was calculated using the T_b value obtained previously for a g value of 50%:

$$\theta_T(50) = \frac{(T_m - T_b)}{GR_{50}}. \quad (4)$$

T_m values (i.e. temperatures above T_b) were used to estimate the thermal time; more specifically, T_m was multiplied by a factor that expressed the proportion of hours per day during which this temperature occurred. If T_m was lower than T_b, the thermal time was null (but never negative). Data from different temperature regimens can be normalized on a thermal time basis (Covell *et al.*, 1986) and will follow the linear relationship described in equation (3). The estimated thermal time $\theta_T(50)$ values were used in equation (3) to calculate the standard deviation of the log thermal time distribution at alternating temperatures ($\sigma_{\theta t}$). Using the probit model, the median germination time was estimated using values corresponding to probit 0.5 (Bradford, 1990; Tompsett and Pritchard, 1998). An analysis of variance (Statgraphic Plus 5.1, Statistical Graphics Corp., Virginia, USA) was performed to probe relationships between T_m, ΔT and GA. Statistical significance was defined as $P < 0.05$. Tranformation of the data was applied as necessary to achieve normality.

Results and discussion

The final germination percentiles achieved in each treatment and the results of the multiple range tests are presented in Table 3. In treatments with $\Delta T = 0$°C and GA <1000 ppm, no germination (i.e. percentile germination of 0%) occurred after 3 weeks of incubation. This suggests that the natural dormancy of SOLSA cannot be overcome by increasing T_m and

https://doi.org/10.1017/S0960258514000221 Published online by Cambridge University Press

Table 3. SOLSA germination percentage ± standard deviation and results of the multiple range test for the different treatments ($n = 90$; $P < 0.05$)

Treatment	Final germination percentage (%)	Treatment	Final germination percentage (%)	Treatment	Final germination percentage (%)
T10/0G0	0 d	T17.5/15G0	64 ± 1.32 b	T22.5/15G0	68 ± 3.32 b
T10/0G1	0 d	T17.5/15G1	65 ± 1.75 b	T22.5/15G1	74 ± 1.76 b
T10/0G2	0 d	T17.5/15G2	64 ± 0.99 b	T22.5/15G2	78 ± 1.45 b
T10/0G3	0 d	T17.5/15G3	66 ± 1.06 b	T22.5/15G3	77 ± 2.04 b
T10/0G4	0 d	T17.5/15G4	97 ± 1.12 a	T22.5/15G4	99 ± 0.67 a
T12.5/5G0	0 d	T20/0G0	0 d	T25/0G0	0 d
T12.5/5G1	0 d	T20/0G1	0 d	T25/0G1	0 d
T12.5/5G2	0 d	T20/0G2	0 d	T25/0G2	0 d
T12.5/5G3	0 d	T20/0G3	0 d	T25/0G3	0 d
T12.5/5G4	0 d	T20/0G4	96 ± 3.54 a	T25/0G4	96 ± 4.37
T15/0G0	0 d	T20/10G0	81 ± 5.07 a	T25/10G0	83 ± 7.30 a
T15/0G1	0 d	T20/10G1	81 ± 2.91 a	T25/10G1	84 ± 2.03 a
T15/0G2	0 d	T20/10G2	92 ± 2.33 a	T25/10G2	92 ± 2.65 a
T15/0G3	0 d	T20/10G3	98 ± 2.49 a	T25/10G3	91 ± 3.63 a
T15/0G4	98 ± 2.13 a	T20/10G4	99 ± 0.58 a	T25/10G4	98 ± 1.03 a
T15/10G0	0 d	T20/20G0	27 ± 0.60 cd	T27.5/5G0	88 ± 1.93 a
T15/10G1	0 d	T20/20G1	28 ± 3.18 cd	T27.5/5G1	92 ± 4.16 a
T15/10G2	0 d	T20/20G2	35 ± 1.76 c	T27.5/5G2	89 ± 4.91 a
T15/10G3	0 d	T20/20G3	37 ± 2.32 c	T27.5/5G3	91 ± 3.72 a
T15/10G4	97 ± 1.13 a	T20/20G4	95 ± 3.61 a	T27.5/5G4	100 ± 0.0 a
T17.5/5G0	35 ± 5.06 c	T22.5/5G0	82 ± 3.52 a	T30/0G0	0 d
T17.5/5G1	35 ± 3.71 c	T22.5/5G1	84 ± 8.18 a	T30/0G1	0 d
T17.5/5G2	31 ± 3.38 c	T22.5/5G2	88 ± 2.65 a	T30/0G2	0 d
T17.5/5G3	37 ± 4.41 c	T22.5/5G3	91 ± 2.04 a	T30/0G3	0 d
T17.5/5G4	98 ± 2.33 a	T22.5/5G4	98 ± 0.92 a	T30/0G4	96 ± 4.37

GAs below 150 ppm. These results are not consistent with those obtained by Dahal *et al.* (1990) in different tomato cultivars (Solanaceae), where the authors observed a linear relationship between germination and incubation temperatures ranging from the base temperature to the optimal temperature.

To overcome this presumed dormancy of SOLSA seeds, a treatment of 1000 ppm GA (G4) was applied to new batches of seeds incubated in temperature regimens with $\Delta T = 0$°C and fixed T_m values (previously used). High concentrations of GA have been similarly used for this purpose by Riley (1987), who used concentrations of up to 2000 ppm, and Rosner *et al.* (2002) and Machado de Mello *et al.* (2009) who used concentrations of up to 1000 ppm. Once applied, the G4 treatment was able to disrupt seed dormancy in thermal treatments with T_m values greater than 15°C. In all cases, full germination (>90%) was achieved and no significant differences among the treatments were observed (Table 3). As can be deduced, the disruption of dormancy requires a high concentration of GA (G4) and a minimum value of T_m. This relationship between T_m and germination has been observed previously in other crops, such as melon (Welbaum and Bradford, 1991), weeds such as *Solanum* (Del Monte and Tarquis, 1997) and nine annual weed species (Steinmaus *et al.*, 2000).

The relation between germination rate GR_g and temperature was plotted for different values of g (30, 50 and 70%) in Fig. 1 (dashed line) and a linear relationship was observed. The intercept on the x-axis (14 ± 0.27°C) of previous linear relationships for the different values of g represented the estimated T_b value of the population under the experimental conditions (Ellis *et al.*, 1986; Kebreab and Murdoch, 1999a,b; Steinmanus *et al.*, 2000). Similar linear relationships and similar T_b values (14 ± 0.23°C) were obtained for

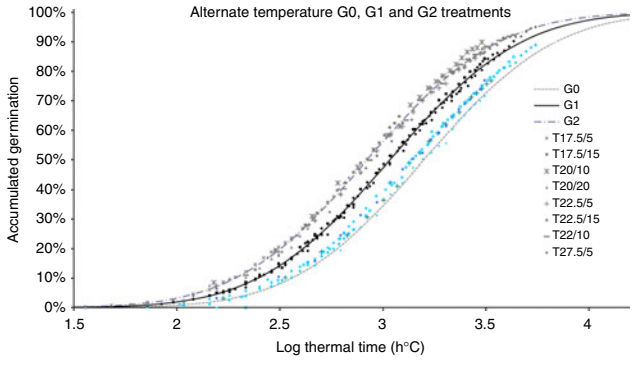

Figure 4. (colour online) Relationship between accumulated germination (%) and log $\theta_T(g)$ for G0, G1 and G2 treatments with $\Delta T \geq 5$°C.

https://doi.org/10.1017/S0960258514000221 Published online by Cambridge University Press

Table 4. Parameters of the model for SOLSA germination at different alternating temperatures and for different GA treatments. Log $\theta_T(50)$ = log thermal time to 50% germination in d°C; $\sigma_{\theta T}$ = standard deviation of the log thermal time distribution within the seed population; $\theta_T(50)$ = thermal time to 50% germination in d°C; T_b = base temperature of the alternating regimen

GA (ppm)	Log $\theta_T(50)$ (log d°C)	$\sigma_{\theta T}$ (log d°C)	$\theta_T(50)$ (d°C)	T_b (°C)
0	1.78 ± 0.02	0.50	66.00	16 ± 0.6
50	1.65 ± 0.03	0.50	45.02	16 ± 0.4
100	1.54 ± 0.02	0.50	35.00	16 ± 0.4
150	1.44 ± 0.03	0.50	27.30	16 ± 0.3
1000*	1.28 ± 0.01	0.20	19.40	14 ± 0.3

* Alternating and non-alternating regimens are included.

G4 treatments with ΔT values greater than 5°C (Fig. 1, solid line). The T_b value obtained in treatments with G4 explains the absence of germination for T_m values less than 15°C.

Figure 2 shows the relationship between the percent germination and log $\theta_T(g)$ of SOLSA seeds treated with G4 for all thermal regimes. The similarity of the two curves confirms that both types of treatments (constant and alternating temperatures) have common $\theta_T(50)$ and $\sigma_{\theta T}$ values (19.40 d°C and 0.2, respectively). Regardless of the ΔT, full germination was achieved in all treatments, albeit at different times. More specifically, the time to full germination was increased as T_m approached T_b (14°C). In treatments with alternating temperatures ($\Delta T \geq 5$°C) and GA values of less than 1000 ppm, the germination pattern was different from that observed in the previous cases. Alternating temperatures favoured the disruption of SOLSA seed dormancy (Table 3). These results are consistent with those obtained by Totterdell and Roberts (1980) and Murdoch *et al.* (1989) in different plant species. Ellis and Barrett (1994) studied the germination of lentils (*Lens culinaris* Medic.) under an alternating temperature regimen and proposed a model whereby temperature changes decrease the thermal time $\theta_T(g)$ while maintaining T_b constant.

As with G4 treatments, in treatments with GA doses ≤ 150 ppm, a linear relationship between GR_g and T_m was observed for different values of g. In Fig. 3 this linear relationship is shown for treatments which reached values of 30, 50 and 70 percentiles. However, the estimated T_b was 16 ± 0.54°C in these treatments, and germination did not occur in treatments with $T_m < 16$°C. These results suggest that T_b was constant when GA dose was ≤ 150 ppm and that T_b up to 14°C when GA was 1000 ppm. The occurrence of a constant T_b in *Lens culinaris* Medic. was described by Ellis and Barrett (1994). Other authors (Covell *et al.*, 1986; Del Monte and Tarquis, 1997; Grundy *et al.*, 2000; Kebreab and Murdoch, 2000; Alvarado and Bradford, 2002) have stated that T_b varies with alternating temperatures.

If we ignore the results obtained with G4, the highest percent germination was achieved in treatments with a ΔT value between 5 and 10°C and a T_m value between 20 and 27.5°C (Table 3); these conditions were thus considered optimal for germination. A clear reduction in the final percentage germination was observed only when ΔT was ≥15°C and when T_m approached T_b, such as happens on treatments T17.5/5 and T17.5/15 which have the same T_m but ΔT was different (5°C and 15°C, respectively). In addition, this reduction increased as ΔT increased. This inhibition of germination with increasing values of ΔT was consistent with the observations of Kebreab and Murdoch (1999a). The optimal conditions described in this experiment suggest that with these conditions the seeds of SOLSA reach full germination without requiring the addition of GA. This could be due to *de novo* synthesis of GA or by reducing the balance of ABA/GA. This aspect has not been evaluated in this paper, but in both cases germination was promoted (Table 3).

The *de novo* synthesis of GA appears to be an important requirement for overcoming dormancy (Jacobsen and Olszewski, 1993; Huarte and Benech-Arnold, 2010), as an increase in GA resulted in the disruption of dormancy and induction of seed germination (Finch-Savage and Leubner-Metzger, 2006). According to Kucera *et al.* (2005), the production of GA may result in an increased growth potential of the embryo during germination by relieving the mechanical constraints associated with the layered lining of the seed, and acting to weaken the tissues surrounding the radicle. The germination of Solanaceae seeds may be inhibited by the micropylar endosperm cap. Therefore, an increase in the biosynthesis of GA and an increased degradation of ABA in Solanaceae could result in a mitigation of the overall effect produced by these tissues. The members of Solanaceae appear to be highly sensitive to GA (Finch-Savage and Leubner-Metzger, 2006), as the addition of GA appeared to facilitate the germination of seeds.

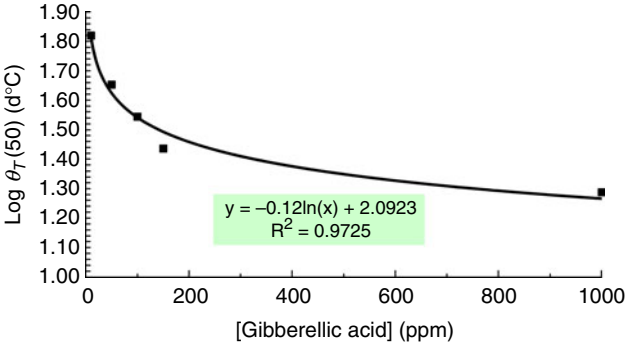

Figure 5. (colour online) Relationship between log $\theta_T(50)$°C and GA concentration.

https://doi.org/10.1017/S0960258514000221 Published online by Cambridge University Press

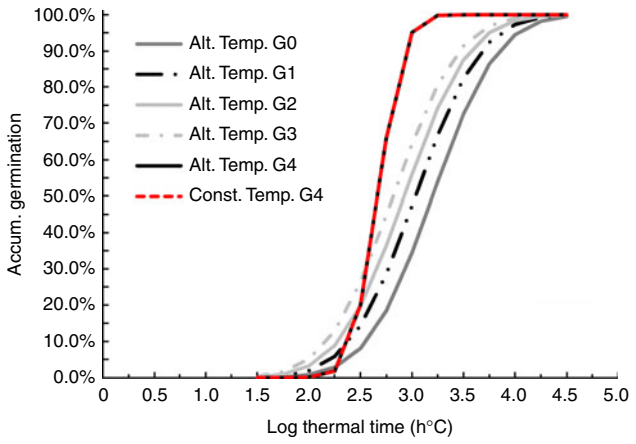

Figure 6. (colour online) Relationship between g and log $\theta_T(g)$.

Nevertheless, according to Huarte *et al.* (2014), alternating temperatures reduce ABA/GA ratios through a reduction in ABA accumulation during incubation but without altering GA synthesis as compared to that observed under constant temperatures; and alternating temperatures did not increase sensitivity to GA.

The germination rate of SOLSA appears to be influenced by the concentration of GA. Figure 4 shows the relationship between g and log $\theta_T(g)$ for treatments with $\Delta T \geq 5$°C and varying GA concentrations (G0, G1 and G2). Under these conditions, $\theta_T(g)$ was inversely related to the concentration of GA, whereas $\sigma_{\theta T}$ was not affected by increasing values of GA (Table 4). According to these results, we can observe a logarithmic relationship between GA concentrations and the log $\theta_T(50)$ characterized by an R^2 value of 0.97 (Fig. 5). Figure 6 illustrates the relationship between accumulated germination and log $\theta_T(g)$ at different concentrations of GA; the thermal time decreased for each percentile increase in the concentration of GA. As was discussed above, for alternating and constant temperatures, thermal time values were markedly lower in the G4 treatment than in the other GA treatments.

A proposed model for the relationship between SOLSA germination and temperature regimens (alternating or constant) shows that dormancy can only be overcome with an exogenous treatment of 1000 ppm GA, a thermal time of 19.40 d°C and when $T_m > T_b$ (14°C). Similar values were obtained for an alternating temperature regimen and the same exogenous GA treatment. For other alternating temperature treatments, dormancy was overcome when $T_m > T_b$ (16°C) and when a minimum thermal time of 66.00 d°C was achieved in the absence of exogenous GA. The thermal time was reduced to 27.30 d°C with the addition of 150 ppm GA. These results indicate that a direct relationship exists between germination, T_m, $\Delta T \geq 0$°C, and decrease in the ratio ABA/GA (by means of an increase in GA biosynthesis or a degradation of ABA). These results also suggest that a ΔT value between 5 and 10°C and a T_m value between 20 and 27.5°C were optimal for germination.

Values of T_m near T_b and ΔT values greater than 15°C are commonly encountered in the study area (Table 1). These values appear to negatively affect SOLSA germination and establishment, which enables other species to compete with SOLSA for resources. In the study to assess the impact of global warning in Spain (Castro *et al.*, 2005) an increase in the average temperature, every 30 years, throughout the 21st century is foreseen, between 1.1 and 1.2°C in winter and between 1.8 and 2°C in summer. If this increase in the average temperature occurs, SOLSA could readily germinate, the time when it germinates will also be longer and its range will extend. In these circumstances, the presence of SOLSA as a weed in crops is likely to be more frequent in the Iberian Peninsula. However, the temperature difference will still occur, which may act as partial restriction on their ability to germinate.

Financial support

This research received no specific grant from any funding agency, commercial or not-for-profit sectors.

Conflicts of interest

None.

References

Allen, P.S., Meyer, S.E. and Khan, M.A. (2000) Hydrothermal time as a tool in comparative germination study. pp. 401–410 *in* Black, M.; Bradford, K.J.; Vazquez-Ramos, J. (Eds) *Seed biology: Advances and applications*. Oxon, CABI Publishing.

Alvarado, V. and Bradford, K.J. (2002) A hydrothermal time model explains the cardinal temperatures for seed germination. *Plant Cell & Environment* **25**, 1061–1069.

Andreoli, C. and Khan, A. (1999) Matriconditioning integrated with gibberellic acid to hasten seed germination and improve stand establishment of pepper and tomato. *Brasilia* **34**, 1953–1958.

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

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

Bewley, J.D. and Black, M. (1994) *Seed physiology and germination* (2nd edition). New York, Plenum.

Bouwmeester, H.J. and Karssen, C. (1992) The dual role of temperature in the regulation of the seasonal changes

https://doi.org/10.1017/S0960258514000221 Published online by Cambridge University Press

in dormancy and germination of seeds of *Polygonum persicaria* L. *Oecologia* **90**, 88–94.

Bradford, K.J. (1990) A water relation analysis of seed germination rates. *Plant Physiology* **94**, 840–849.

Bradford, K.J. (1995) Water relations in seed germination. pp. 351–396 *in* Kigel, J.; Galili, G. (Eds) *Seed development and germination*. New York, Marcel Dekker.

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

Bradford, K.J., Tarquis, A.M. and Durán, J.M. (1993) A population-based threshold model describing the relationship between germination rates and seed deterioration. *Journal of Experimental Botany* **44**, 1225–1234.

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

Castro, M., Martín-Vide, J. and Alonso, S. (2005) *Impactos del cambio climático en España. 1. El clima de España, pasado, presente y escenarios de clima para el Siglo XXI*, Ed.MAGRAMA- Universidad de Castilla La Mancha, Madrid.

Chen, S.S.C. and Chang, J.L.L. (1972) Does gibberellic acid stimulate seed germination via amylase synthesis? *Plant Physiology* **49**, 441–442.

Covell, S., Ellis, R.H., Roberts, E.H. and Summerfield, R.J. (1986) The influence of temperature on seed germination rate in grain legumes I: A comparison of chickpea, lentil, soybean and cowpea at constant temperatures. *Journal of Experimental Botany* **37**, 705–715.

Dahal, P., Bradford, K.J. and Jones, R.A. (1990) Effects of priming and endosperm integrity on seed germination rates of tomato genotypes I: Germination at suboptimal temperature. *Journal of Experimental Botany* **41**, 1431–1439.

Del Monte, J.P. and Dorado, J. (2011) Effects of light conditions and after-ripening time on seed dormancy loss of *Bromus diandrus* Roth. *Weed Research* **51**, 551–590.

Del Monte, J.P. and Tarquis, A.M. (1997) The role of temperature in the seed germination of two species of the *Solanum nigrum* complex. *Journal of Experimental Botany* **48**, 2087–2093.

Dorado, J., Fernández-Quintanilla, C. and Grundy, A.C. (2009) Germination patterns in naturally chilled and non-chilled seeds of fierce thornapple (*Datura ferox*) and velveleaf (*Abutilon theophrasti*). *Weed Science* **57**, 155–162.

Edmonds, J. and Chweya, J.A. (1997) *Black nightshades.* Solanum nigrum *L.* and *related species*. Promoting the conservation and use of underutilized and neglected crops, 15. Rome, IPGRI.

Ellis, R.H. and Barrett, S. (1994) Alternating temperatures and rate of seed germination in lentil. *Annals of Botany* **74**, 519–524.

Ellis, R.H., Covell, S., Roberts, E.H. and Summerfield, R.J. (1986) The influence of temperature on seed germination rate in grain legumes II: Intraspecific variation in chickpea *Cicer arietinum* L. at constant temperatures. *Journal of Experimental Botany* **37**, 1503–1515.

Finch-Savage, W.E. and Leubner-Metzger, G. (2006) Seed dormancy and the control of germination. *New Phytologist* **171**, 501–523.

Finch-Savage, W.E., Steckel, J.R.A. and Phelps, K. (1998) Germination and post-germination growth to carrot seedling emergence: Predictive threshold models and sources of variation between sowing occasions. *New Phytologist* **139**, 505–516.

Footitt, S., Doureterlo-Soler, I., Clay, H. and Finch-Savage, W.E. (2011) Dormancy cycling in *Arabidopsis* seeds is controlled by seasonally distinct hormone-signaling pathways. *Proceedings of the National Academy of Sciences, USA* **108**, 20219–20224.

Forcella, F. (1993) Seedling emergence model for velvetleaf. *Agronomy Journal* **85**, 929–933.

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

Garcia-Huidobro, J., Monteith, J.L. and Squier, G.R. (1982) Time, temperature and germination of pearl millet *Pennisetum typhoides*, Sand H. *Journal of Experimental Botany* **33**, 288–296.

Groot, S.P.C. and Karssen, C.M. (1992) Dormancy and germination of abscisic acid-deficient tomato seed. Studies with the *sitiens* mutant. *Plant Physiology* **99**, 952–958.

Grundy, A.C., Phelps, K., Reader, R.J. and Burston, S. (2000) Modelling the germination of *Stellaria media* using the concept of hydrothermal time. *New Phytologist* **148**, 433–444.

Gutterman, Y. (2000) Genotypic and phenotypic germination survival strategies of ecotypes and annual plant species in the Negev Desert of Israel. pp. 389–399 *in* Black, M.; Bradford, K.J.; Vazquez-Ramos, J. (Eds) *Seed biology: Advances and applications*. Oxon, CABI Publishing.

Huarte, H.R. and Benech-Arnold, R.L. (2010) Hormonal nature of seed responses to fluctuating temperatures in *Cynara cardunculus* (L.). *Seed Science Research* **20**, 39–45.

Huarte, H.R., Luna, V., Pagano, E.A., Zavala, J.A. and Benech-Arnold, R.L. (2014) Fluctuating temperatures terminate dormancy in *Cynara cardunculus* seeds by turning off ABA synthesis and reducing ABA signalling, but not stimulating GA synthesis or signalling. *Seed Science Research* **24**, 79–89.

Jacobsen, S.E. and Olszewski, N.E. (1993) Mutations at the SPINDLY locus of *Arabidopsis* alter gibberellin signal transduction. *Plant Cell* **58**, 887–896.

Jusaitis, M., Polomka, L. and Sorensen, B. (2004) Habitat specificity, seed germination and experimental translocation of endangered herb *Brachycome muelleri. Asteraceae*. *Biological Conservation* **116**, 251–266.

Karssen, C.M., Groot, S.P.C. and Koornneef, M. (1987) Hormone mutants and seed dormancy in *Arabidopsis* and tomato. pp. 119–133 *in* Thomas, H.; Grierson, D. (Eds) *Developmental mutants in higher plants*. Cambridge, UK, Cambridge University Press.

Kebreab, E. and Murdoch, A.J. (1999a) A model of the effects of a wide range of constant and alternating temperatures on seed germination of four *Orobanche* species. *Annals of Botany* **84**, 549–557.

Kebreab, E. and Murdoch, A.J. (1999b) Modelling the effects of water stress and temperature on germination rate of *Orobanche aegyptiaca* seeds. *Journal Experimental Botany* **50**, 655–664.

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

Kucera, B., Cohn, M.A. and Leubner-Metzger, G. (2005) Plant hormone interactions during seed dormancy

release and germination. *Seed Science Research* **15**, 281–307.

Laorga, S. (1983) Datos floristicos sobre la comarca de la Sagra Toledo, España III. *Lazaroa* **5**, 321–323.

Machado de Mello, A., Streck, N.A., Blankenship, E.E. and Paparozzi, E.T. (2009) Gibberellic acid promotes seed germination in *Penstemon digitalis* cv. Husker Red. *HortScience* **44**, 870–873.

Meyer, S.E. and Pendleton, R.L. (2000) Genetic regulation of seed dormancy in *Purshia tridentate Rosaceae*. *Annals of Botany* **85**, 521–529.

Murdoch, A.J., Roberts, E.H. and Goedert, C.O. (1989) Model for germination responses to alternating temperatures. *Annals of Botany* **63**, 97–111.

Ni, B.R. and Bradford, K.J. (1993) Germination and dormancy of abscisic acid- and gibberellin-deficient mutant tomato *Lycopersicon esculentum* seeds. *Plant Physiology* **101**, 607–617.

Pérez-Flores, L., Carrari, F., Osuna-Fernandez, R., Verónica Rodriguez, M., Enciso, S., Stanelloni, R., Sanchez, R.A., Bottini, R., Iusem, N.D. and Benech-Arnold, R.L. (2003) Expression analysis of a GA 20-oxidase in embryos from two sorghum lines with contrasting dormancy: possible participation of this gene in the hormonal control of germination. *Journal of Experimental Botany* **54**, 2071–2079.

Riley, J.M. (1987) Gibberellic acid for fruit set and seed germination. *California Rare Fruit Growers Journal* **19**, 10–12.

Roberts, H.A. and June, E. (1983) Field emergence and temperature requirements for germination in *Solanum sarrachoides* Sendt. *Weed Research* **23**, 247–252.

Roman, E.S., Murphy, S.D. and Swanton, C.J. (2000) Simulation of *Chenopodium album* seedling emergence. *Weed Science* **48**, 217–224.

Rosner, L.S., Harrington, J.T., Dreesen, D.R. and Murray, L. (2002) Effect of gibberellic acid and standard seed treatments on mountain snowberry germination. *Native Plants Journal* **3**, 155–162.

Rowse, H.R. and Finch-Savage, W.E. (2003) Hydrothermal threshold models can describe the germination response of carrot *Daucus carota* and onion *Allium cepa* seed populations across both sub- and supra-optimal temperature. *New Phytologist* **158**, 101–108.

SIAR (2013) Servicio Integral de Asesoramiento al Regante de Castilla La Mancha. Available at: http://www.crea.uclm.es/siar/datmeteo/ (accessed 3 January 2014)

Sobrino, E. and Del Monte, J.P. (1994) Two alien species new to the Spanish flora, and their characterization within the *Solanum nigrum* complex Solanaceae. *Flora Mediterranea* **4**, 101–109.

Steinmaus, S.J., Prather, T.S. and Holt, J.S. (2000) Estimation of base temperatures for nine weed species. *Journal of Experimental Botany* **51**, 275–286.

Tompsett, P.B. and Pritchard, H.W. (1998) The effect of chilling and moisture status on the germination, desiccation, tolerance and longevity of *Aesculus hippocastanum* L. seed. *Annals of Botany* **82**, 249–261.

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

UNEP (United Nations Environment Programme) and WMO (World Meteorological Organization) (2007) *Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change, 2007*. Solomon, S.; Qin, D.; Manning, M.; Chen, Z.; Marquis, M.; Averyt, K.B.; Tignor, M.; Miller, H.M. (Eds). Cambridge, UK, Cambridge University Press.

Welbaum, G.E. and Bradford, K.J. (1991) Water relations of seed development and germination in muskmelon *Cucumis mello* L. VII: Influence of after-ripening and ageing on germination responses to temperature and water potential. *Journal of Experimental Botany* **42**, 1137–1145.