

## Base Temperatures for Germination of Selected Weed Species in Iran

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### Abstract

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Weed emergence models require the estimation of base temperature for germination ( $T_b$ ) that was estimated for *Abutilon theophrasti*, *Echinochloa crus-galli*, *Amaranthus retroflexus*, *Sorghum halepense*, *Amaranthus albus*, and *Amaranthus hybridus* in Iran, to calibrate an existing model called AlertInf. Two statistical procedures were adopted: Model 1 – linear regression of germination rate and Model 2 – probit analysis. Model 1 provided lower  $T_b$  values. *Abutilon theophrasti* and *A. hybridus* presented lower  $T_b$  values (about 4 and 8°C), while the remaining species had values above 10°C. Since the estimated values of  $T_b$  were in agreement with those adopted for the Alertinf model in Italy, the first step was achieved to adapt AlertInf to Iranian conditions.

**Keywords:** *Abutilon theophrasti*; *Amaranthus retroflexus*; *Amaranthus albus*; *Amaranthus hybridus*; *Echinochloa crus-galli*; emergence model; population variability; *Sorghum halepense*

The efficacy of weed management operations is strongly dependent on their correct timing according to the dynamics of seedling emergence (MASIN *et al.* 2011). This awareness induced increasing interest in the development of models that can simulate seedling emergence and the potential benefits, but also challenges, of their adoption were recognised and thoroughly reviewed (FORCELLA *et al.* 2000; GRUNDY 2003). Several models have been created for seedling emergence of various weed species in the main crops such as maize (DORADO *et al.* 2009a; MASIN *et al.* 2012), soybean (MASIN *et al.* 2014; WERLE *et al.* 2014) or winter cereals (ROYO-ESNAL *et al.* 2010, 2015; GARCÍA *et al.* 2013; IZQUIERDO *et al.* 2013). These models are often based on the hydrothermal time concept (BRADFORD 2002) and require the estimation of biological parameters, base temperature,

and base water potential for germination ( $T_b$  and  $Y_b$  hereinafter), to simulate seedling emergence according to weather trends. Adopting inaccurate values of  $T_b$  and  $Y_b$  could notably influence the precision of model prediction, so several procedures have been proposed to estimate these parameters, such as linear or nonlinear regression with resampling methods (MASIN *et al.* 2010; ONOFRI *et al.* 2014), population-based threshold models (DORADO *et al.* 2009b), probit analysis (ZAMBRANO-NAVEA *et al.* 2013) or survival analysis (ONOFRI *et al.* 2010).

Inter-population variability of weed species could limit the transferability of emergence models across different agricultural areas and thus be a constraint to the widespread adoption of these tools and other Integrated weed management (IWM) strategies (GRUNDY 2003; LODDO *et al.* 2014). Local weed

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populations have been reported to have developed different germination behaviour as an adaptation to local environmental and agronomic conditions (LODDO *et al.* 2014), and inter-population differences were detected between  $T_b$  values of weed species such as *Datura stramonium* L. and *Conyza canadensis* L. (LODDO *et al.* 2013; TOZZI *et al.* 2014). Therefore investigating the behaviour of local populations could be an important step when trying to calibrate an existing emergence model to geographical areas other than that of creation.

According to this approach, the objective of this study was to estimate  $T_b$  for several weed species collected in the area of Gorgan, Golestan province, northern Iran, as the first step to calibrate an existing model for weed emergence called AlertInf (MASIN *et al.* 2012) for this area. The following weed species were selected for the study according to their distribution and agronomic importance in Gorgan: *Abutilon theophrasti* Medik., *Echinochloa crus-galli* (L.) Beauv., *Amaranthus retroflexus* L., *Sorghum halepense* (L.) Pers., *Amaranthus albus* L., and *Amaranthus hybridus* L.

Lastly, two statistical procedures, i.e. (1) logistic model of germination time course plus linear regression of germination rate against incubation temperature with resampling bootstrap methods (MASIN *et al.* 2010), (2) probit analysis (DAHALL & BRADFORD 1990; DAHAL *et al.* 1990; ZAMBRANO-NAVEA *et al.* 2013) were adopted independently, starting with the same germination data, to calculate  $T_b$  of all studied species in order to assess potential differences between the results obtained by the two procedures.

## MATERIAL AND METHODS

**Germination tests.** Seeds were collected in August to September 2014 from spontaneous populations in the area of Gorgan, Golestan province, northern Iran. The area (36°51'N, 54°16'E, 13.3 m a.s.l.) has a cold semi-arid climate reported in the Bsk class according to the Köppen-Geiger climate classification updated by PEEL *et al.* (2007) with cool rainy winters and hot dry summers, mitigated by the proximity to the Caspian sea, with about 6.1 h/day and 2223 h/year of bright sunshine. Annual average temperature is 18.2°C, January is the coldest month (average monthly minimum and maximum temperatures of 3.4 and 12.4°C, respectively) and August the hottest one (average monthly minimum and maximum temperatures of

22.9 and 32.7°C, respectively). Annual precipitation is about 523.5 mm, with a rainy period from October to April (average monthly precipitation 50–60 mm) and a dry period from May to September (average monthly precipitation 20–30 mm). One single seed batch corresponding to a single local population was collected per weed species, apart from *A. retroflexus* for which two separate seed batches corresponding to two distinct populations were included. Only ripen seeds were collected from several plants in order to obtain a representative sample of intra-population variability. Seeds were cleaned and left to dry at room temperature for several days and then stored in paper bags at room temperature (20°C) for 3–4 months until the start of the germination test. The experiments started in January 2015 in the Laboratory of Seed Science and Technology of the Agronomy Department, Gorgan University of Agricultural Science and Natural Resources, Iran. Since low germination percentages were obtained for all species in preliminary germination tests with untreated seeds (data not shown), specific seed pre-treatments were performed to break dormancy and promote germination. Seed dormancy was removed in *A. theophrasti* seeds by soaking them in boiling water for 10 second. Seed dormancy of *A. hybridus* was broken by soaking seeds for 24 h in a 200 ppm gibberellic acid (GA3) solution. Seed dormancy of the other species was interrupted by dipping them in a concentrated sulphuric acid (98%) solution for a time ranging from 1 min to 30 min; seeds were then washed with distilled water. Maximum germination was achieved from soaking periods of 2.5, 5, 20, and 30 min for *A. albus*, *A. retroflexus*, *E. crus-galli*, and *S. halepense*, respectively. Seeds were put on moistened filter paper in Petri dishes to imbibe in light and then incubated in the dark in a germination chamber. Monitoring of germination was also conducted in light. This procedure ensured the photostimulation necessary to promote seed germination since the studied species, as many other weeds, are very sensitive even to short light exposures as that occur during soil tillage (BATLLA & BENECH-ARNOLD 2014). Seeds were incubated at a series of constant temperatures (6, 8, 10, 12, 14, 16, 18, 20, 24, 28, and 32°C), chosen according to previous experiments conducted on different species (STEINMAUS *et al.* 2000; STECKEL *et al.* 2004; MASIN *et al.* 2010). These temperatures are within the range of suboptimal temperatures for germination of the studied species. Three replicates of 50 seeds were included for each combination of

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species  $\times$  incubation temperature. Germinated seeds were counted and removed twice daily. Seeds were considered germinated when the radicle length reached 2 mm. Tests were considered concluded when no further germination occurred for 5 days.

**Data analyses and estimation of base temperature for germination.** Determination of  $T_b$  was performed adopting two different procedures:

**Model 1: Logistic model plus linear regression with bootstrap.** This approach, originally developed by ROCHÉ *et al.* (1997), has already been adopted to estimate  $T_b$  for seed germination or rhizome sprouting (MASIN *et al.* 2010; LODDO *et al.* 2012). A logistic function (Eq. 1) in the BIOASSAY97 program (ONOFRI 2005) was used to analyse the germination dynamics of each replicate:

$$CG = 100 / (1 + \exp(a \times (\ln(t + 0.0000001 - \ln(b)))))(1)$$

where: CG – cumulative germination percentage;  $t$  – time (h);  $a$  – slope of the curve;  $b$  – inflection point

The time necessary for the germination of half of the total germinated seeds ( $t_{50}$ ) was calculated for each replicate. The  $t_{50}$  in the above equation corresponds to the inflection point ( $b$ ). The germination rate was estimated as  $1/t_{50}$  and a linear regression was performed with germination rates of the three replicates against incubation temperature for each species.  $T_b$  was estimated as the intercept of the regression line with the temperature axis ( $x$  axis). A bootstrap method using an artificial resampling procedure was adopted to calculate statistical 95% confidence intervals for the estimated values of  $T_b$  that are reported hereinafter as  $T_b \pm$  half of the 95% statistical confidence intervals.

**Model 2: Probit analysis.** Germination time course data were analysed by repeated probit regression using

the thermal time approach previously described by DAHAL and BRADFORD (1990) and DAHAL *et al.* (1990). Seed germination response over time was linearised by transforming cumulative germination percentage to probits and plotting on a logarithmic time scale. Germination data lower than 1% or exceeding 99% (which carry little weight in probit analysis and skew germination response curves) were omitted according to GIANNETTI and COHN (2007). This procedure can be formulated by the following Eq. (2):

$$\text{Probit}(g) = \{\log[(T - T_b)t_g] - \log \theta_T\} / \sigma_{\theta T} \quad (2)$$

where:  $T$  – temperature;  $T_b$  – base temperature;  $t_g$  – time required for the fraction or percentage;  $g$  – seeds to germinate;  $\theta T$  – mean thermal time or  $\theta T(50)$ ;  $\sigma_{\theta T}$  – inverse of the slope or the standard deviation of log thermal time for germination

Although  $T_b$  is initially unknown, probit analyses can be conducted with  $T_b$  set to different values; the best estimate of  $T_b$  of Eq. (2) is provided by the model that results in the lowest residual variance. Statistical 95% confidence intervals for the estimated values of  $T_b$  were calculated. Values of  $T_b$  are reported hereinafter as  $T_b \pm$  half of the 95% statistical confidence intervals.

## RESULTS

Germination percentage notably increased with a rise in incubation temperatures for all the studied species apart from *A. theophrasti* that presented no significant differences in germination percentages obtained in the range of incubation temperatures from 10°C to 24°C (Table 1), while germination rate increased for all studied species (Figures 1 and 2).

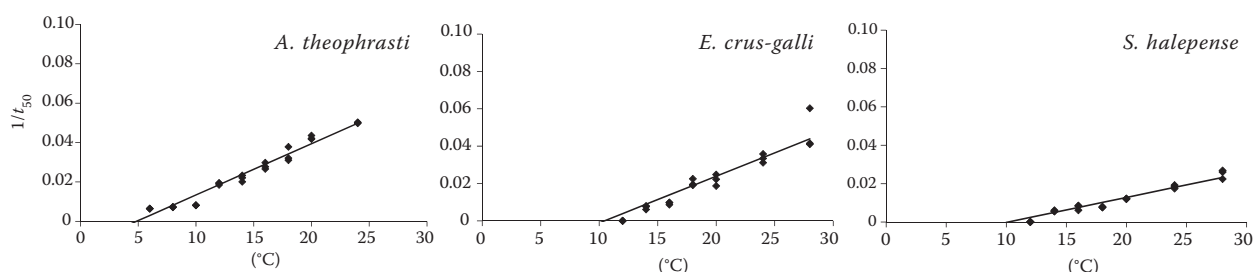


Figure 1. Estimation of base temperatures for germination ( $T_b$ ) for *Abutilon theophrasti*, *Echinochloa crus-galli*, and *Sorghum halepense* according to Model 1 (logistic model of germination time course plus linear regression of germination rate against incubation temperature). The points are the calculated germination rates ( $1/t_{50}$ ) of the three replicates at different incubation temperatures and the solid line represents the linear regression line. The  $T_b$  for germination was estimated as the intercept of the regression line with the incubation temperature axis

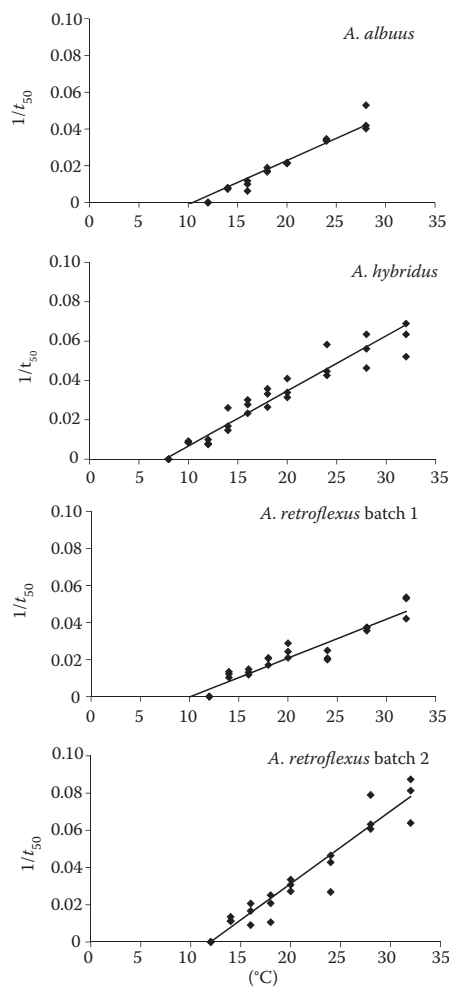


Figure 2. Estimation of base temperatures for germination ( $T_b$ ) for *Amaranthus* species (*A. retroflexus*, *A. albus*, and *A. hybridus*) according to Model 1 (logistic model of germination time course plus linear regression of germination rate against incubation temperature). The points are the calculated germination rates ( $1/t_{50}$ ) of the three replicates at different incubation temperatures and the solid line represents the linear regression line. The  $T_b$  for germination was estimated as the intercept of the regression line with the incubation temperature axis

Differences were observed between the values of  $T_b$  estimated by the two models for each species, by Model 1 providing generally lower values than Model 2, except for *A. theophrasti* with  $T_b$  values of  $4.9 \pm 0.16$  and  $3.5 \pm 1.15^\circ\text{C}$  for Model 1 and Model 2 (Table 2). The differences can be considered statistically significant for *A. hybridus*, *A. retroflexus* batch 1, *A. albus*, *E. crus-galli*, and *S. halepense* because in these cases the confidence intervals of the two values did not overlap (Table 2).  $T_b$  for germination varied between the species, with the lowest values

Table 1. Germination percentages obtained for the various species at the different incubating temperatures

Species	Incubating temperature (°C)										
	6	8	10	12	14	16	18	20	24	28	32
<i>Abutilon theophrasti</i>	55.5 ± 4.99	62.5 ± 2.22	67.5 ± 3.20	68.5 ± 1.26	69.0 ± 1.29	64.0 ± 5.48	71.0 ± 5.26	63.5 ± 2.75	70.0 ± 2.16	–	–
<i>Amaranthus albus</i>	0	0	0	0.7 ± 0.67	12.7 ± 2.67	16.7 ± 1.76	42.0 ± 1.15	74.0 ± 6.43	82.0 ± 4.16	88.0 ± 1.15	–
<i>Amaranthus hybridus</i>	0	0	6.7 ± 2.91	18.7 ± 5.70	58.0 ± 5.03	66.0 ± 8.00	78.7 ± 0.67	82.0 ± 3.46	64.7 ± 2.67	91.3 ± 1.33	97.3 ± 1.33
<i>Amaranthus retroflexus</i> batch 1	0	0	0	0	14.7 ± 4.06	34.0 ± 0.58	35.3 ± 3.53	48.0 ± 4.16	68.0 ± 1.15	74.0 ± 3.46	79.3 ± 1.76
<i>Amaranthus retroflexus</i> batch 2	0	0	0	0	12.7 ± 3.53	28.7 ± 5.33	47.3 ± 5.46	35.3 ± 3.18	55.3 ± 3.53	71.3 ± 3.71	70.0 ± 5.29
<i>Echinocloa crus-galli</i>	0	0	0	2.0 ± 1.15	13.3 ± 2.67	24.0 ± 2.31	60.0 ± 4.62	86.0 ± 5.29	87.3 ± 3.71	90.7 ± 1.76	–
<i>Sorghum halepense</i>	0	0	0	0	13.3 ± 5.33	21.3 ± 1.76	53.5 ± 1.76	54.0 ± 6.11	74.7 ± 2.91	76.0 ± 7.21	–

Values are presented as mean of 3 replicates  $\pm$  standard errors

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Table 2. Base temperatures for germination ( $T_b$ ) estimated for the different weed species and according two different statistical procedures (Model 1 – logistic model of germination time course plus linear regression of germination rate against incubation temperature; Model 2 – probit analysis)

Species	Base temperature ( $T_b$ )	
	Model 1	Model 2
<i>Abutilon theophrasti</i>	$4.9 \pm 0.16$	$3.5 \pm 1.15$
<i>Amaranthus albus</i>	$10.4 \pm 0.42$	$13.1 \pm 0.46$
<i>Amaranthus hybridus</i>	$7.6 \pm 1.73$	$9.6 \pm 0.25$
<i>Amaranthus retroflexus</i> batch 1	$10.0 \pm 1.30$	$12.3 \pm 0.40$
<i>Amaranthus retroflexus</i> batch 2	$12.0 \pm 1.27$	$12.9 \pm 0.37$
<i>Echinochloa crus-galli</i>	$10.4 \pm 0.49$	$13.3 \pm 0.27$
<i>Sorghum halepense</i>	$10.1 \pm 0.33$	$12.4 \pm 0.47$

Values are presented as  $T_b \pm$  half of the 95% statistical confidence intervals

estimated for *A. theophrasti* ( $4.9 \pm 0.16$  and  $3.5 \pm 1.15^\circ\text{C}$  for Model 1 and Model 2) and *A. hybridus* ( $7.6 \pm 1.73$  and  $9.6 \pm 0.25^\circ\text{C}$  for Model 1 and Model 2, respectively), while the remaining *Amaranthus* species, *E. crus-galli* and *S. halepense* had values of  $T_b$  in the range of  $10\text{--}13^\circ\text{C}$  (Table 2).

## DISCUSSION

The increase of germination percentage and germination rate with the rise of temperature demonstrated that seeds were incubated in the range of suboptimal temperatures for the various species. This dynamics also suggested the presence of an intraspecific variability regarding the range of temperatures permissive for germination, i.e. only a limited fraction of seeds was able to germinate at the lowest incubation temperatures. For example, germination percentage for *A. hybridus* ranged from  $6.7 \pm 2.91\%$  at  $10^\circ\text{C}$  to  $97.3 \pm 1.33\%$  at  $32^\circ\text{C}$  (Table 1). The differences observed between the values of  $T_b$  estimated by the two models were probably related to their specific statistical approaches, since this situation was constant for almost all the studied species. It should be underlined that Model 1 takes into account only seeds germinated at different temperatures to calculate germination rate ( $1/t_{50}$ ). Consequently the fraction of ungerminated seeds is not considered for the estimation of  $T_b$ , which could therefore be assumed as representative of only more germinable (less dormant) seeds. This limitation could be rel-

evant if the total germination percentage is low or if different percentages are obtained for the different incubation temperatures, as observed in this study, since different percentiles of the total population would be compared, which is inherently misleading and can underestimate the differences in germination rates ( $1/t_{50}$ ) (BEWLEY *et al.* 2013). The probit analysis adopted for Model 2 instead combines the whole dataset (seeds germinated at all incubation temperatures and also ungerminated seeds), so the estimated values of  $T_b$  could be more representative of the whole population. The probit analysis adopted for Model 2 could be considered more adequate to estimate  $T_b$  when the aim is to characterise the whole population from an ecological point of view or compare several different populations of the same weed species. However, the procedure used for Model 1 has been successfully adopted to calculate parameters for weed emergence models because in those situations it could be appropriate to focus on the subpopulation of less dormant seeds that are more likely to germinate and produce seedlings during the cropping season.

The range of  $T_b$  values estimated for *A. theophrasti* ( $3.5 \pm 1.15^\circ\text{C}$  to  $4.9 \pm 0.16^\circ\text{C}$ ) was in complete agreement with the results of previous studies on an Iranian population (SADEGHLOO *et al.* 2013), but also on European populations (MASIN *et al.* 2010; LODDO *et al.* 2013), while DORADO *et al.* (2009b) reported a value of around  $7^\circ\text{C}$  as  $T_b$  for a Spanish population. Results obtained for *A. albus* ( $T_b$  from  $10.4 \pm 0.42^\circ\text{C}$  to  $13.1 \pm 0.46^\circ\text{C}$ ) confirmed previous findings by STECKEL *et al.* (2004), who observed almost no germination for this species at  $10^\circ\text{C}$ , while higher values of  $T_b$ , ranging from  $14^\circ\text{C}$  to  $17^\circ\text{C}$ , were estimated by STEINMAUS *et al.* (2000). The values of  $T_b$  estimated for batches 1 of *A. retroflexus* ( $10.0 \pm 1.30^\circ\text{C}$  to  $12.3 \pm 0.40^\circ\text{C}$ ) were slightly lower than those for batches 2 ( $12.0 \pm 1.27^\circ\text{C}$  to  $12.9 \pm 0.37^\circ\text{C}$ ), but no significant differences could be detected. Moreover, all these values were in complete agreement with previous findings obtained on two Italian populations of *A. retroflexus* (MASIN *et al.* 2010) while a significantly lower  $T_b$  value of about  $9^\circ\text{C}$  was reported by GUILLEMIN *et al.* (2013) for a French population. Regarding *A. hybridus*, no proper value of  $T_b$  has ever been reported but a previous study observed little germination at  $10^\circ\text{C}$  and none at  $5^\circ\text{C}$  (STECKEL *et al.* 2004), supporting the range of  $T_b$  estimated in the present experiment ( $7.6 \pm 1.73^\circ\text{C}$  to  $9.6 \pm 0.25^\circ\text{C}$ ). *Amaranthus hybridus* had the lowest  $T_b$  values among

the *Amaranthus* species included in this study. The two grass species, *E. crus-galli* and *S. halepense*, had a similar range of  $T_b$  values (from 10°C to 13°C). These results were confirmed for *S. halepense* by previous studies on Italian and US populations that reported values of  $T_b$  around 12°C (HOLT & ORCUTT 1996; MASIN *et al.* 2010). Regarding *E. crus-galli*, values of  $T_b$  in agreement with the findings of the present study were estimated by STEINMAUS *et al.* (2004) and MASIN *et al.* (2010) for Californian and Italian populations, while significantly lower  $T_b$  of about 6 and 5°C were reported for a French and Iranian population, respectively (GUILLEMIN *et al.* 2013; SADEGHLOO *et al.* 2013).

The differences observed between the values of  $T_b$  estimated in the present work and the results of previous studies could be related to several factors. The adaptation process of the studied weed populations to local environmental conditions (i.e. France vs Iran) could have led to interpopulation variability. For example, lower  $T_b$  for germination has been reported for populations of *D. stramonium* and *C. canadensis* growing in colder climates (LODDO *et al.* 2013; TOZZI *et al.* 2014). The lower values of  $T_b$  estimated for French populations of *A. retroflexus* and *E. crus-galli* (GUILLEMIN *et al.* 2013) could be related to a similar phenomenon. However, differences in the values of  $T_b$  reported in different studies could be partially related to the specific statistical procedures adopted. As observed in the present study, distinct statistical procedures could estimate significantly different values of  $T_b$  even starting with the same germination data because different parts of the same population of seeds are taken into account. This issue should not be overlooked when findings of studies based on different statistical procedures are compared.

Since the values of  $T_b$  estimated in this study for some species were in complete agreement with the values adopted by MASIN *et al.* (2012) for the Alertinf model in Italy, the first positive step was achieved to adapt AlertInf to local Gorgan area conditions. This result was not presumable *a priori* since values of  $T_b$  for local populations had previously been studied only for *A. theophrasti* and *E. crus-galli* (SADEGHLOO *et al.* 2013), while for the remaining species the available  $T_b$  values were determined in other geographical areas such as the US or Europe. However, further experiments are necessary to estimate other parameters required for the Alertinf model, such as base water potential for germination ( $Y_b$ ). Moreover,

field experiments should be conducted in the Gorgan area to evaluate the weed emergence patterns and calibrate the AlertInf equation parameters.

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