Annals of Botany **112**: 1887–1897, 2013
doi:10.1093/aob/mct238, available online at www.aob.oxfordjournals.org

ANNALS OF BOTANY
Founded 1887

Thermal niche for *in situ* seed germination by Mediterranean mountain streams: model prediction and validation for *Rhamnus persicifolia* seeds

Marco Porceddu[1], Efisio Mattana[1,2,*], Hugh W. Pritchard[2] and Gianluigi Bacchetta[1]

[1]*Centro Conservazione Biodiversità (CCB), Dipartimento di Scienze della Vita e dell'Ambiente, Università degli Studi di Cagliari, Viale Sant'Ignazio da Laconi, 11–13, Cagliari, 09123, Italy and* [2]*Seed Conservation Department, Wellcome Trust Millennium Building, Royal Botanic Gardens, Kew, Wakehurst Place, Ardingly, West Sussex RH17 6TN, UK*
** For correspondence. E-mail e.mattana@kew.org*

Received: 14 June 2013 Returned for revision: 29 July 2013 Accepted: 23 August 2013 Published electronically: 7 November 2013

• *Background and Aims* Mediterranean mountain species face exacting ecological conditions of rainy, cold winters and arid, hot summers, which affect seed germination phenology. In this study, a soil heat sum model was used to predict field emergence of *Rhamnus persicifolia*, an endemic tree species living at the edge of mountain streams of central eastern Sardinia.
• *Methods* Seeds were incubated in the light at a range of temperatures (10–25 and 25/10 °C) after different periods (up to 3 months) of cold stratification at 5 °C. Base temperatures (T_b), and thermal times for 50 % germination (θ_{50}) were calculated. Seeds were also buried in the soil in two natural populations (Rio Correboi and Rio Olai), both underneath and outside the tree canopy, and exhumed at regular intervals. Soil temperatures were recorded using data loggers and soil heat sum (°Cd) was calculated on the basis of the estimated T_b and soil temperatures.
• *Key Results* Cold stratification released physiological dormancy (PD), increasing final germination and widening the range of germination temperatures, indicative of a Type 2 non-deep PD. T_b was reduced from 10·5 °C for non-stratified seeds to 2·7 °C for seeds cold stratified for 3 months. The best thermal time model was obtained by fitting probit germination against log °Cd. θ_{50} was 2·6 log °Cd for untreated seeds and 2·17–2·19 log °Cd for stratified seeds. When θ_{50} values were integrated with soil heat sum estimates, field emergence was predicted from March to April and confirmed through field observations.
• *Conclusions* T_b and θ_{50} values facilitated model development of the thermal niche for *in situ* germination of *R. persicifolia*. These experimental approaches may be applied to model the natural regeneration patterns of other species growing on Mediterranean mountain waterways and of physiologically dormant species, with overwintering cold stratification requirement and spring germination.

Key words: Base temperature, climate change, cold stratification, physiological dormancy, Rhamnaceae, *Rhamnus persicifolia*, seed germination model, soil heat sum, thermal time.

INTRODUCTION

Seed dormancy prevents germination in a specified period of time, under any combination of environmental factors that otherwise favour germination (Baskin and Baskin, 2004). Thus, dormancy is an adaptive trait that optimizes the distribution of germination over time in a population of seeds (Copete *et al.*, 2011). In seasonal climates, temperature is usually the main environmental factor governing seed germination in moist soil (Fenner and Thompson, 2005). Seeds of many temperate plant species are dormant at the time of dispersal, and specific temperature requirements must be met before dormancy is lost and germination is possible (Baskin and Baskin, 1998). Depending on the species and timing of dispersal, seeds may experience a warm period before autumn and winter begin, or be subjected to cold stratification during winter immediately after autumn shedding (Baskin and Baskin, 1989; Noronha *et al.*, 1997). The requirement for chilling, widespread amongst temperate species, represents a natural mechanism which ensures that germination occurs in the spring (Probert, 2000). During exposure to low temperatures, the range of temperatures over which seeds will germinate, as well as germination percentages, increases (Baskin and Baskin, 1988).

The Mediterranean climate is characterized by its seasonality in temperature and precipitation, which leads to a hot drought in summer and a cool, wet, winter (Joffre *et al.*, 1999). This peculiarity has important implications for plant germination physiology, since dry summer conditions limit water availability and thus germination and growth, while cool winter temperatures can limit germination during the season with high water availability (Rundel, 1996).

The canopies of woody plants modify the microclimate beneath and around them through interception of precipitation and by shading, which influence maximum soil temperature and the amount of soil moisture available to plants (Breshears *et al.*, 1998). As the course of action and relative importance of factors regulating germination in the laboratory may be quite different from those occurring under field conditions (Thompson, 1973), linkage between field, garden and laboratory studies is crucial (Brändel and Schütz, 2005).

As reproduction niche and reproductive success are related to temperature, all aspects of the plant reproductive cycle are

potentially sensitive to climate change (Bykova *et al.*, 2012). The Intergovernmental Panel on Climate Change (IPCC) has predicted temperature increases of approx. 2–4 °C by 2090–2099. Furthermore, in the Mediterranean region, a declining trend of precipitation was observed from 1900 to 2005 (IPCC, 2007). In response to climate change, plants can adapt to the new environmental conditions or, when possible, migrate to track their climatic niches (Meineri *et al.*, 2013).

In non-dormant seeds, the germination response to accumulated temperature has been modelled by a thermal time (θ) approach (Garcia-Huidobro *et al.*, 1982; Covell *et al.*, 1986; Ellis *et al.*, 1986, 1987; Pritchard and Manger, 1990; Hardegree, 2006). In this model, seeds accumulate units of thermal time (°Cd) to germinate for a percentile g of the population. When seeds are subjected to temperatures (T) above a base temperature for germination (T_b), the germination rate increases linearly with temperature to an optimum temperature (T_o), above which germination rate starts to decrease (Garcia-Huidobro *et al.*, 1982). Thus, in this sub-optimal range (T_o–T_b), germination occurs in the time t_g, when the thermal time accumulated has reached the critical value (θ_g) for a percentile g of the population, and can be described as $\theta_g = (T - T_b)t_g$.

Intraspecific variation in T_b among seed populations may be due to different environmental conditions during seed development (Daws *et al.*, 2004). However, T_b has also been found to change with dormancy status. In particular, Pritchard *et al.* (1999) found that T_b decreased by 1 °C every 6 d of pre-chilling at 6 °C in *Aesculus hippocastanum* seeds. Thus seed dormancy release in this species could be described simply in terms of T_b reduction, gradually allowing germination to occur at progressively lower temperatures (Pritchard *et al.*, 1999). In addition, subsequent seed germination may be predicted in relation to thermal time accumulation (heat sum, °Cd) above a gradually reducing T_b (Steadman and Pritchard, 2004). This approach has been used to predict seed germination in the field (i.e. Hardegree and Van Vactor, 2000; Steadman *et al.*, 2003; Chantre *et al.*, 2009) and, more recently, to assess the impact of different simulated climate change scenarios on seed dormancy release and germination timing in *Vitis vinifera* subsp. *sylvestris* (Orrù *et al.*, 2012).

Sardinian massifs represent a southern European refugium for some temperate tree species *sensu* Tzedakis *et al.* (2002). In this region, vegetation among mountain waterways is mainly constituted by *Alnus glutinosa* woods, where the rare Sardinian endemic *Rhamnus persicifolia* may also be found. Seeds of the Rhamnaceae have an investing embryo (Martin, 1946) and can be non-dormant or, following the dormancy classification system (Baskin and Baskin, 1998, 2004), show physiological (PD), physical dormancy (PY) or combined (physical and physiological; PY + PD) dormancy. Physical dormancy is the most represented class in this family (61 % of the investigated species), followed by PY + PD (22 %), PD (12 %) and non-dormancy (ND) (6 %; Walck *et al.*, 2012). Mattana *et al.* (2009) reported that germination of *R. persicifolia* seeds could be achieved, without any scarification, at warm temperatures (≥20 °C), excluding the presence of PY. Whilst there was no obligate requirement for alternating temperature or light, pre-chilling had a positive effect on the germination rate, reducing T_{50} by >50 % and indicating the presence of PD in seeds of this species. However, the effect of pre-chilling on seed germination over a wide range of temperatures, and the identification of the type of PD according to the seed dormancy classification system (Baskin and Baskin, 2004), remain to be investigated.

The aims of this work were to (1) investigate the thermal requirements for seed dormancy release and germination of the rare *R. persicifolia* and (2) develop a thermal-time model, based on a soil heat sum approach, in order to characterize the thermal niche for seed germination and predict the seed germination phenology in the field.

MATERIALS AND METHODS

Study species

Rhamnus persicifolia is a small dioecious tree or shrub. It is closely related to *R. cathartica*, but with elliptic–lanceolate leaves and reddish ripe drupes. It is endemic to Central–Eastern Sardinia (Italy), occurring at 600–1500 m a.s.l. on both limestone and siliceous substrata. This species grows in scattered groups or as single trees, in riparian woods or hygrophilous scrubs along mountainous waterways (Mattana *et al.*, 2009). *Rhamnus persicifolia* is included in the Italian Red Book as vulnerable (Conti *et al.*, 1992, 1997), because of its narrow distribution and population decline, induced by human activities and by climate change (Arrigoni, 1977). To date, only six populations are known; half of these are threatened by low plant numbers or an unbalanced sex ratio (Bacchetta *et al.*, 2011).

Seed lot details

Fruits of *R. persicifolia* were collected directly from 15 plants on 16 September 2011 along the Rio Correboi (RC; Villagrande Strisaili, Ogliastra) and from five plants on 30 September 2011 along the Rio Olai (RO; Orgosolo, Nuoro) streams in Central–Eastern Sardinia (see Table 1). The low number of sampled plants was due to the few female individuals found in these two populations (see Bacchetta *et al.*, 2011). Seeds were immediately separated from the pulp by rubbing the fruits through sieves under running water. The cleaned seeds were then spread out and left to dry at room temperature, until the experiments started, as specified below.

Germination tests under controlled conditions

For the RC provenance collection, three replicates of 20 seeds were sown on the surface of 1 % agar water in 90 mm diameter plastic Petri dishes and incubated in the light (12 h light/12 h darkness) for 1–4 months under a range of constant temperatures (10, 15, 20 and 25 °C) and under an alternating temperature regime (25/10 °C). In the alternating temperature regime, the 12 h light period coincided with the elevated temperature period. At the same time, three different cold stratification periods were started (5 °C in 1 % agar water in 90 mm diameter plastic Petri dishes for 1, 2 and 3 months: C1, C2 and C3 treatments, respectively) and, at the end of each pre-treatment, seeds were incubated, as detailed above.

Due to the low availability of seeds collected in the RO (see Table 1), these seeds were only stratified for 3 months at 5 °C

TABLE 1. *Locations, habitat characteristics and dates of experimental trials carried out in each site (Rio Correboi, RC; Rio Olai, RO) of the two natural populations of* R. persicifolia

Population	Soil substrate type	Experimental sites	Habitat	Altitude (m a.s.l.)	Aspect	Date of field sowing	Dates of exhumation and days after sowing
Rio Correboi (Villagrande Strisaili, Ogliastra), RC	Metamorphytes	RC1 IN	Riparian wood with *Alnus glutinosa* – Mantle shrubs with *Rubus ulmifolius*.	1209	0	30/09/2011	26/04/2012 (209 d), 25/06/2012 (269 d)
		RC1 OUT	Open grassland of *Carici-Genistetea lobelioidis*.		NE		
		RC2 IN	Riparian wood with *A. glutinosa* – Mantle shrubs with *R. ulmifolius*.	1267	0	30/09/2011	09/12/2011 (70 d), 29/03/2012 (181 d), 26/04/2012 (209 d), 25/06/2012 (269 d)
		RC2 OUT	Open grassland of *Carici-Genistetea lobelioidis*.		NE		
		RC3 IN	Shady rocky outcrop with *Ribes multiflorum*. subsp. *sandalioticum* and *Rubus ulmifolius*.	1347	NE	30/09/2011	26/04/2012 (209 d), 25/06/2012 (269 d)
		RC3 OUT	Open grassland of *Carici-Genistetea lobelioidis*.		NE		
Rio Olai (Orgosolo, Nuoro), RO	Metamorphytes	RO IN	Riparian wood with *A. glutinosa* – Mantle shrubs with *R. ulmifolius*.	970	0	05/10/2011	09/12/2011 (65 d), 29/03/2012 (176 d), 26/04/2012 (204 d), 25/06/2012 (264 d)
		RO OUT	Open grassland of *Carici-Genistetea lobelioidis*.		0		

For each experimental site, IN and OUT differentiate between underneath and outside the canopy, respectively.

and then incubated at 25 °C (12 h light/12 h darkness). These conditions were chosen on the basis of earlier findings (Mattana *et al.*, 2009).

Germination was defined as visible radicle emergence (>1 mm). Germinated seeds were scored three times a week. At the end of the germination tests, when no additional germination had occurred for 2 weeks, a cut test was carried out to determine the firmness of the remaining seeds and the number of empty seeds. Firm seeds were considered to be viable. This methodology was chosen on the basis of previous findings on seeds of this species, which highlighted a very high seed viability, with 100 % of non-empty seeds staining uniformly red in 1 % solution of 2,3,5-triphenyl-tetrazolium chloride (Mattana *et al.*, 2009).

Field experiments

Within 15–20 d of collection, seeds were placed in fine-mesh polyester envelopes (three replicates of 25 seeds) and buried in soil at a depth of 2–3 cm. Envelopes were buried both underneath (IN) and outside (OUT) the canopy, with a distance between them of approx. 6 m, at each experimental site of the two original populations, for a total of six experimental sites for RC, in order to cover the whole altitudinal range of this population, and two for RO (Table 1). Envelopes buried in experimental sites RC2 and RO were exhumed at intervals of about 3 months from September 2011 to June 2012 (with an intermediate exhumation also in April 2012; Table 1). Alternatively, those buried in experimental sites RC1 and RC3 were exhumed only in April and June 2012. Retrieved envelopes were analysed in the laboratory, where they were washed under running water and opened. The number of germinated seeds was recorded, and a cut test was carried out to check the viability of any remaining non-germinated seeds, as described above.

Soil temperatures at the level of the envelopes were recorded IN and OUT of the canopy at 90 min intervals, using data loggers (TidbiT® v2 Temp logger, Onset Computer Corporation, Cape Cod, MA, USA).

Data analysis

The final germination percentage was calculated as the mean of the three replicates ± standard deviation (s.d.), on the basis of the total number of filled seeds. Generalized linear models (GLMs) were used to compare: (1) final germination percentages and T_b achieved under controlled conditions for seed collected in RC, followed by a *post hoc* pairwise comparisons *t*-test (with Bonferroni adjustment); and (2) the field germination percentages at each experimental site (RC1, RC2, RC3 and RO) on different exhumation dates (December 2011, March 2012, April 2012 and June 2012), both IN and OUT of the canopy (see Table 1). Generalized linear models, with a logit link function and quasi-binomial error structure, were used when analysing germination percentages, whereas a GLM with a log link function and quasi-poisson error structure was used for analysing T_b values. Quasi-binomial and quasi-poisson error structures and *F*-tests with an empirical scale parameter instead of χ^2 on the subsequent analysis of variance (ANOVA) were used in order to overcome residual overdispersion (Crawley, 2007).

Thermal time analyses were carried out for RC seeds germinating at constant temperatures for untreated seeds (0, control) and after each cold pre-treatment (C1, C2 and C3). Estimates of time

(t_g, d) taken for cumulative germination to reach different percentiles (g) for successive increments of 10 % germination were interpolated from the germination progress curves (Covell *et al.*, 1986). The germination rate ($1/t_g$) was regressed, using a linear model, as a function of temperature according to the following equation (Garcia-Huidobro *et al.*, 1982):

$$1/t_g(d^{-1}) = (T_g - T_b)/\theta \quad (1)$$

An average ($\pm$ s.d.) of the x-intercept among percentiles was calculated for the sub-optimal temperature range (10–20 °C) to establish the T_b for each treatment (Ellis *et al.*, 1986; Pritchard and Manger, 1990). Linear regression equations were then recalculated for each percentile, but constrained to pass through T_b (Hardegree, 2006). A comparison of regressions was then made between this model and one in which the T_b were allowed to vary for all the percentiles, and the best estimate was considered to be that which resulted in the smallest residual variance (Covell *et al.*, 1986). Thermal time (θ, °Cd) estimates for each treatment were then calculated separately as the inverse of the sub-optimal regression equations [Covell *et al.*, 1986; see eqn (1)].

The T_b values were fitted as a function of the length of the stratification period using a linear model. Generally, θ did not accumulate during pre-treatments because the stratification temperature (5 °C) was lower than the T_b. However, in seeds stratified at 5 °C for 120 d (C3), T_b reduced during stratification to below the stratification temperature itself. Using the relationship between rate of decline of T_b and temperature, and assuming that the rate of reduction of T_b continued unchanged, according to Steadman and Pritchard (2004), θ accumulated during the C3 stratification phase (θ_s) was calculated.

Germination percentages were transformed to probits using tabular data from Finney (1971). Linear regression was used to express probit (g) as a function of thermal time (θ_g) and the form of cumulative germination response of seeds described by the equation (Covell *et al.*, 1986):

$$\text{probit}(g) = K + \theta_g/\sigma \quad (2)$$

where K is an intercept constant when θ_g is zero, θ_g may be normal or log-normal distributed (and the best model evaluated on the basis of the r^2 values; Hardegree, 2006), and σ is the standard deviation of the response to θ_g (i.e. the reciprocal of the slope), and represents the sensitivity of the population to θ_g (Covell *et al.*, 1986). Thus the flatter the slope of the fitted line, the greater the variation in response to thermal time between individual seeds (Daws *et al.*, 2004).

A heat sum approach was used to predict seed germination in the field, according to Orrù *et al.* (2012). These authors used environmental temperatures of the original populations above T_b to assess the temperature accumulation until the achievement of θ_{50} (Orrù *et al.*, 2012). In this study, soil heat sum was calculated, starting from the date of sowing, according to the following equation, modified from Daws and Jensen (2011):

$$\text{Soil heat sum (°Cd)} = \{\sum[(T_S - T_b) \times t]\}/18 \quad (3)$$

where T_S is the temperature at each logging interval recorded by data loggers, T_b is the base temperature for germination calculated day by day, according to the length of stratification experienced in the field, t is the length of the logging interval expressed in hours and 18 is the number of logging records per day. All statistical analyses were carried out using R v. 2·14·0 (R Development Core Team, 2011).

Pluviometric data for RC (monthly rainfall averages from 1922 to 2009 from the nearby climatic station of Fonni, Nuoro) and RO (monthly rainfall averages from 1936 to 2009 from the nearby climatic station of Montes, Orgosolo, Nuoro) were acquired from Regione Autonoma della Sardegna (http://www.regione.sardegna.it/j/v/25?s=131338&v=2&c=5650&t=1). The presence/absence of the tree canopy of riparian wood with *A. glutinosa* was observed at each field excursion realized during this study.

RESULTS

Seed germination under controlled conditions

The fitted GLM highlighted a statistically significant effect ($P <$ 0·001) on germination of temperature (T) and treatment (S) factors and of their interaction ($T \times S$; Fig. 1) for seeds collected in RC (see Table 1). Untreated seeds (0) germinated at percentages ranging from approx. 50 % to approx. 87 % at all the tested temperatures, except at 10 °C where germination was <15 % (Fig. 1). The applied cold stratification treatments increased seed germination percentages and widened the range of germination temperatures (Fig. 1). In particular, the effect of cold stratification was positive and statistically significant ($P <$ 0·001) at 10 °C, with germination increasing with the length of stratification from 12 $\pm$ 8 % (0) to 92 $\pm$ 8 % (C3), and at 15 °C, with percentages increasing from 61 $\pm$ 5 % (0) to 87 $\pm$ 3 % (C3). Untreated and cold-stratified seeds reached high germination when incubated under the alternating temperature regime (25/10 °C), with percentages >80 % for 0, C1 and C2

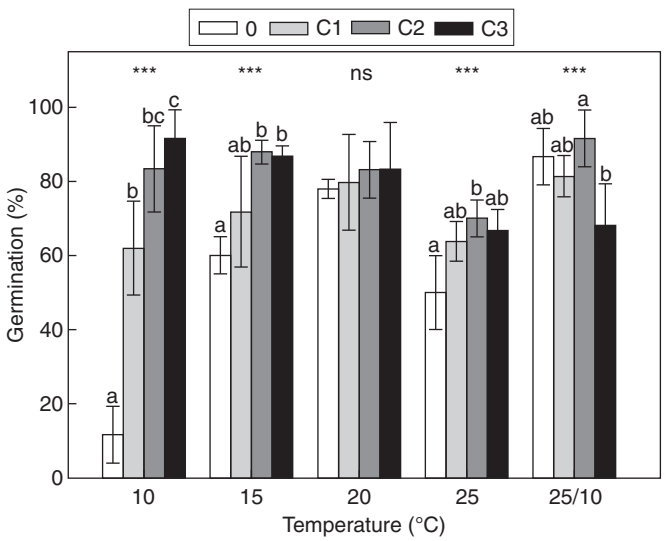

FIG. 1. Effects of temperatures and cold treatments (0, control; C1, C2 and C3 cold stratification at 5 °C for 1, 2 and 3 months, respectively) on final germination for *Rhamnus persicifolia* seeds collected in Rio Correboi. Data are the mean of three replicates (1 $\pm$ s.d.). Temperatures, treatments and their interaction are statistically significant ($P <$ 0·001 by GLM). *Post hoc* pairwise t-test comparisons (with Bonferroni adjustment) were carried out for each germination temperature, and bars with different letters indicate significant ($P <$ 0·05) variation.

treatments, without statistically significant differences ($P > 0{\cdot}05$); whereas after C3, germination significantly ($P < 0{\cdot}05$) decreased to 68 ± 11 % (Fig. 1).

Final germination for seeds collected in RO incubated at 25 °C after 3 months at 5 °C was 60 ± 7 %.

Thermal requirement for germination

Goodness of fit (r^2) for the linear regressions of $1/t$ against temperature for RC collections showed that the best sub-optimal model included data only up to 20 °C (i.e. excluding 25 °C; Fig. 2A). Based on germination rate responses for each 10th percentile from 10 to 80 % germination, it was possible to estimate the mean base temperature for germination (T_b) in the sub-optimal temperature range for each treatment (Fig. 2A). Average T_b values were 10·5 ± 0·6, 8·5 ± 0·9, 6·1 ± 1·4 and 2·7 ± 0·8 °C, for 0, C1, C2 and C3 treatments, respectively. For the different treatments, linear regressions were re-calculated for each percentile, constraining them to pass through the mean T_b. This model led to no differences in residual sum of squares and showed higher values of r^2 for all of the linear regression equations ($r^2 > 0{\cdot}75$ for 0, $r^2 > 0{\cdot}93$ for C1, $r^2 > 0{\cdot}81$ for C2

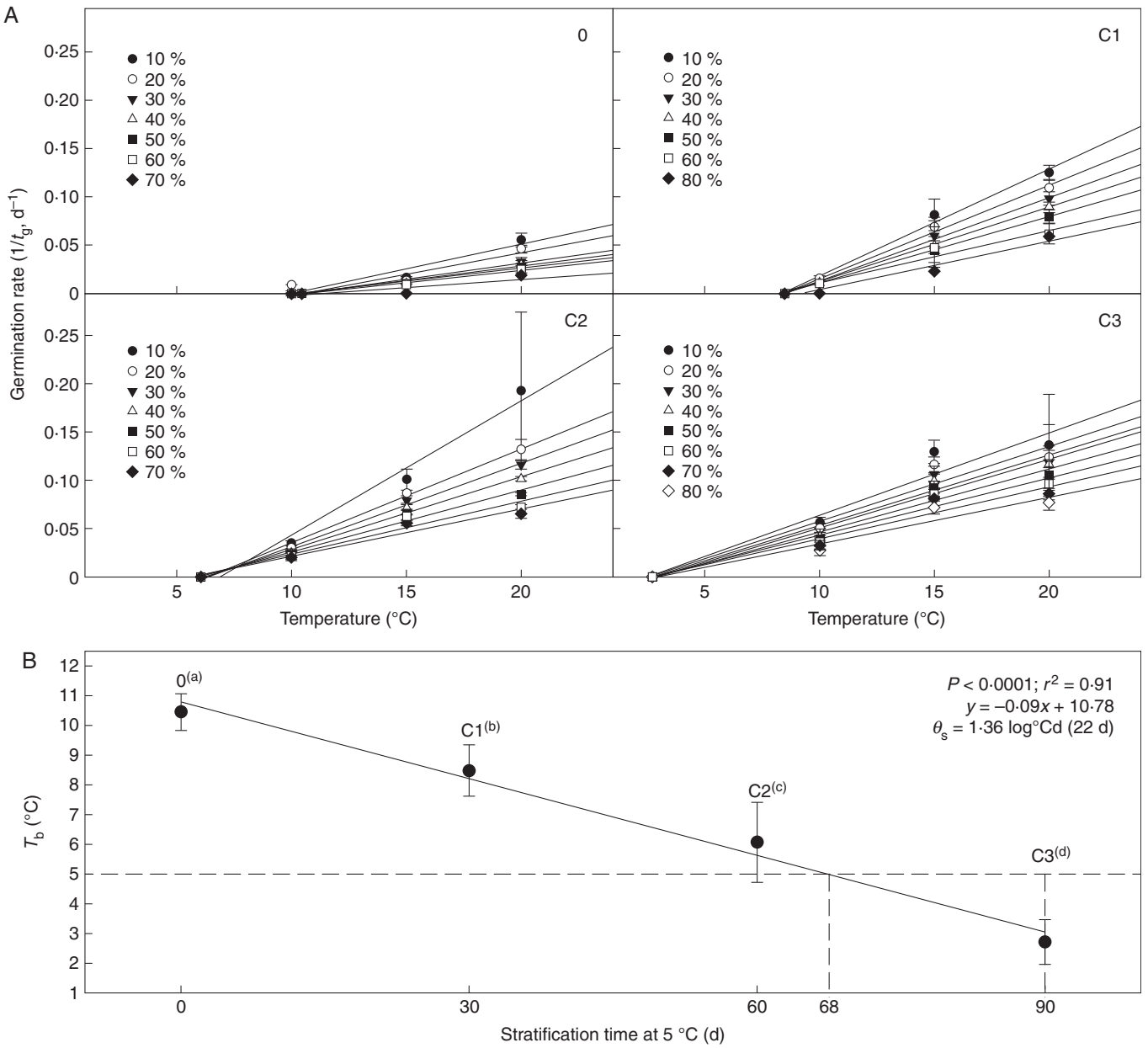

FIG. 2. (A) Base temperatures (T_b), calculated for different germination percentiles of *Rhamnus persicifolia* seeds, after each pre-treatment (0, control; C1, C2 and C3 cold stratifications at 5 °C for 1, 2 and 3 months, respectively) and incubation at the sub-optimal temperatures (10–20 °C). Within each pre-treatment, the linear regressions for the different percentiles were constrained to the common value of T_b. Linear regressions of percentiles with $P > 0{\cdot}05$ were not included. (B) Relationship between T_b and stratification time at 5 °C. Data are the mean ± s.d. of T_b of each percentile. Statistical differences among pre-treatments were analysed by GLM followed by *post hoc* pairwise *t*-test comparisons (with Bonferroni adjustment). Mean T_b values with different letters are significantly different at $P < 0{\cdot}05$.

and $r^2 > 0{\cdot}81$ for C3) than the model where T_b varied for each percentile ($r^2 > 0{\cdot}73$ for 0, $r^2 > 0{\cdot}87$ for C1, $r^2 > 0{\cdot}73$ for C2 and $r^2 > 0{\cdot}54$ for C3). The T_b values were statistically different ($P < 0{\cdot}001$) by GLM, and the *post hoc* pairwise *t*-test comparison (with Bonferroni adjustment) highlighted significant differences among all treatments (Fig. 2B). The relationship between T_b and the length of the stratification period at 5 °C is shown in Fig. 2B. The linear regression highlighted that this negative relationship was statistically significant ($r^2 = 0{\cdot}91$, $P < 0{\cdot}0001$; Fig. 2B), with T_b decreasing by 0·09 °C d^{-1} of stratification or by 1 °C for every 11 d of chilling. After 68 d of stratification, T_b decreased below 5 °C, and seeds accumulated 1·36 log °Cd (θ_s) in the next 22 d until the end of the C3 treatment at 90 d.

Figure 3 shows the relationship between log thermal time (θ) and germination expressed in probits, calculated according to eqn (2). The relationship between log θ and probit germination had better residual sums of square (0·1091, 0·0961, 0·0228 and 0·1366 for 0, C1, C2 and C3, respectively) and r^2 (0·95, 0·97, 0·99 and 0·96 for 0, C1, C2 and C3, respectively) than when expressed on a linear scale (data not shown). Thermal time for 50 % of germination (θ_{50}) was greater for the control (2·59 log °Cd) compared with the cold-treated seeds (from 2·17 to 2·19 log °Cd; Fig. 3). Seeds of 0 and C2 had a greater σ value (0·26 and 0·25 log °Cd, respectively) compared with C1 and C3 (0·18 and 0·12 log °Cd, respectively; Fig. 3).

Seed germination in the field

In December 2011, the great majority of seeds (>85 %) were dormant (Table 2), although a few seeds (<3 %) had started to germinate in RO. In March 2012, seeds also started germinating in RC, while the majority of the remaining seeds were still dormant, and the level of dead seeds was always <7 % (Table 2). In RO, the majority of the seeds germinated, reaching values of approx. 70 % both IN and OUT, and the remaining seeds were mainly dead (Table 2). By April 2012, germination in RC1 was approx. 60 %, with approx. 25 % of seeds remaining dormant and 15 % dead, for both IN and OUT. In RC2 IN and OUT, approx. 75 and 35 % of the seeds, respectively, had germinated; approx. 14 and 45 % of seeds were dormant and approx. 11 and 20 % of seeds were dead. For RC3 OUT, germination reached approx. 43 %, with approx. 10 and 47 % being dormant or dead, respectively. No germination data were available for RC3 IN due to predation by animals (Table 2).

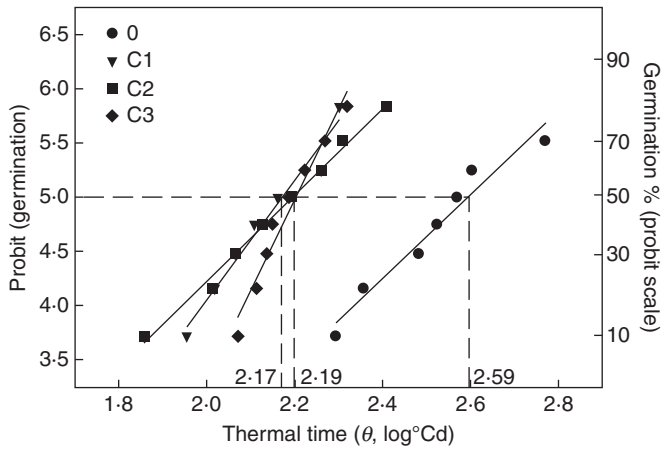

FIG. 3. Probit germination after each pre-treatment (0, control; C1, C2 and C3 cold stratification at 5 °C for 1, 2 and 3 months, respectively) as a function of log-thermal time requirement. Thermal times were calculated from germination time-courses from estimated T_b of 10·5, 8·5, 6·1 and 2·7 °C for 0, C1, C2 and C3, respectively. Thermal times to reach θ_{50} are also shown (dashed lines).

At the last exhumation, in June 2012, the percentage of dead seeds was high for all the experimental sites in both populations, ranging from 24 ± 9 % for RC1 OUT to 91 ± 4 % for RC2 OUT, and all the remaining seeds germinated (Table 2). The bag in RC1 IN could not be retrieved, as it was probably washed away, while seeds in that of RC3 IN were predated by animals (Table 2).

Generalized linear models highlighted a statistically significant ($P < 0{\cdot}001$) effect for all the factors (date, D; position, P; site S) as well as for their interactions, except for the two-way interaction D × P and the three-way interaction D × P × S for which $P > 0{\cdot}05$ (Table 3).

Soil heat sum and thermal niche for in situ *seed germination*

The establishment of the tree canopy of *A. glutinosa* woods was very similar in the two streams (RC and RO), starting at the end of April and disappearing in mid-October (Fig. 4). In detail, the annual trend of soil temperatures could be divided into six periods, according to the presence/absence of the canopy and to the seasons, for RC1, RC2 and RO experimental sites: (I) from the sowing at the end of September/early October to the disappearance of the tree canopy in mid-October; (II) from the disappearance of the canopy in mid-October to the start of the stratification period, when mean daily temperatures fell to 5 °C in December; (III) the main stratification period, from December to March, when mean daily temperatures are close to 5 °C; (IV) from the end of the stratification period in March to the appearance of the canopy in April; (V) from the appearance of the canopy in April to the start of the summer droughts in June/July; and (VI) the summer drought period when rainfall drastically reduces (Fig. 4, Table 2). The absence of a riparian wood in RC3 (see Table 1) led to only four environmental periods: (I) from sowing to the start of the stratification period in December; (II) the stratification period until March; (III) from the end of the stratification period to the start of the summer droughts in June/July; and (IV) the summer drought period.

By combining eqn (3) and the equation in Fig. 2B, where T_b was calculated day by day, for RC seeds, according to the length of stratification experienced in the field, it was possible to calculate the soil heat sum reached by the seeds at the different exhumation times for each experimental site of both populations (Table 2). The values calculated for RC2 and RO (for which there was a complete temporal sequence) were compared with those estimated using the thermal time (θ) model, expressed as probit germination and log °Cd (for germination values from 10 to 80 %; see Fig. 3). The linear regression highlighted a statistically significant relationship between calculated and estimated data ($n = 5$; $P = 0{\cdot}0018$; $r^2 = 0{\cdot}97$; $y = 1{\cdot}0992x - 0{\cdot}1739$).

In RC2 (Fig. 4A), the length of the effective stratification periods was 92 d for IN and 98 d for OUT (with 41 and 47 d of snow cover, respectively), leading to T_b values at the end of the stratification period of 2·9 and 2·5 °C for IN and OUT, respectively. Before (periods I and II) and during stratification (period III), mean soil temperatures were similar or lower than T_b (10·2 °C), preventing the soil heat sum accumulation for germination. However, after

TABLE 2. *Evaluation categories of the exhumed seeds (%), recorded soil temperatures (°C), calculated soil heat sum (log °Cd) and field germination percentages (mean ± s.d.) for each experimental site (Rio Correboi, RC; Rio Olai, RO) underneath (IN) and outside (OUT) the canopy at the different exhumation dates*

		Evaluation categories of the exhumed seeds (%, mean ± 1 s.d.)											Recorded mean soil temperature (°C)		Calculated soil heat sum (log °Cd)		Predicted soil heat sum (log °Cd)	
		IN					OUT											
Date of exhumation	Experimental site	G	V	D	NT	P	G	V	D	NT	P	Period	IN	OUT	IN	OUT	IN	OUT
09/12/2011	RC2	0 ± 0	95 ± 2	5 ± 2	–	–	0 ± 0	98 ± 2	1 ± 2	–	–	II	4·9	3·0	1·54	0·95	–	–
	RO	1 ± 2	83 ± 8	16 ± 11	–	–	3 ± 2	87 ± 6	11 ± 4	–	–	II	7·5	6·6	1·68	1·88	–	–
29/03/2012	RC2	32 ± 18	61 ± 19	7 ± 2	–	–	2 ± 3	95 ± 5	5 ± 5	–	–	IV	6·3	3·5	1·96	1·43	2·15	–
	RO	73 ± 12	6 ± 7	21 ± 16	–	–	75 ± 3	8 ± 7	17 ± 7	–	–	IV	8·2	11·0	2·16	2·43	2·29	2·28
26/04/2012	RC1	57 ± 24	28 ± 13	15 ± 15	–	–	61 ± 12	25 ± 13	13 ± 2	–	–	IV	8·9	10·4	2·44	2·37	2·22	2·23
	RC2	74 ± 5	14 ± 5	11 ± 2	–	–	35 ± 12	45 ± 8	20 ± 7	–	–	IV	9·5	9·7	2·34	2·28	2·28	2·16
	RC3	–	–	–	–	100	43 ± 25	10 ± 9	47 ± 33	–	–	III	6·7	16·2	1·94	2·69	–	2·18
	RO	55 ± 11	7 ± 4	38 ± 11	–	–	73 ± 3	14 ± 11	13 ± 8	–	–	IV	11·7	13·7	2·49	2·68	2·21	2·27
25/06/2012	RC1	–	–	–	100	–	76 ± 9	0 ± 0	24 ± 9	–	–	V	17·4	27·5	3·01	3·15	–	2·29
	RC2	71 ± 21	0 ± 0	29 ± 21	–	–	9 ± 4	0 ± 0	91 ± 4	–	–	V	19·3	27·0	3·02	3·11	2·26	–
	RC3	–	–	–	–	100	4 ± 4	0 ± 0	96 ± 4	–	–	III	16·6	27·6	2·90	3·22	–	–
	RO	45 ± 24	0 ± 0	55 ± 24	–	–	57 ± 11	0 ± 0	43 ± 11	–	–	V	17·1	27·1	3·02	3·19	2·18	2·22

The soil heat sum values, predicted on the basis of the thermal time (θ) model (expressed as probit germination and log °Cd; see Fig. 3), are also reported for the different germination percentages for values from 10 to 80 % (see Fig. 3).

G, germinated seeds; V, viable dormant seeds; D, dead seeds; P, predated seeds; NT, envelopes not retrieved.

Periods, identified according to the presence/absence of the canopy and to the seasons for all the experimental sites for RC1, RC2 and RO, correspond to: (I) from sowing to the disappearance of the tree canopy; (II) from the disappearance of the canopy to the start of the stratification period; (III) the stratification period; (IV) from the end of the stratification period to the appearance of the canopy; (V) from the appearance of the canopy to the start of the summer droughts; and (VI) the summer drought period. For RC3 they correspond to: (I) from sowing to the start of the stratification period; (II) the stratification period; (III) from the end of the stratification period to the start of the summer droughts; and (IV) the summer drought period.

TABLE 3. *GLM results for the effect on seed germination in the field of the following factors: 'Date' (D: December 2011, March 2012, April 2012 and June 2012), 'Position' (P: IN and OUT) and 'Experimental site' (S: RC1, RC2, RC3 and RO)*

	d.f.	Deviance	Residual d.f.	Residual deviance	*F*	*P*
Null			62	3105·85		
Date (D)	3	1371·24	59	1734·61	59·3520	<0·001
Position (P)	1	98·21	58	1636·40	12·7530	<0·001
Site (S)	3	456·67	55	1179·73	19·7661	<0·001
D × P	3	34·36	52	1145·37	1·4872	>0·05
D × S	5	408·83	47	736·54	10·6173	<0·001
P × S	2	385·86	45	350·68	25·0519	<0·001
D × P × S	3	10·34	42	340·34	0·4474	>0·05

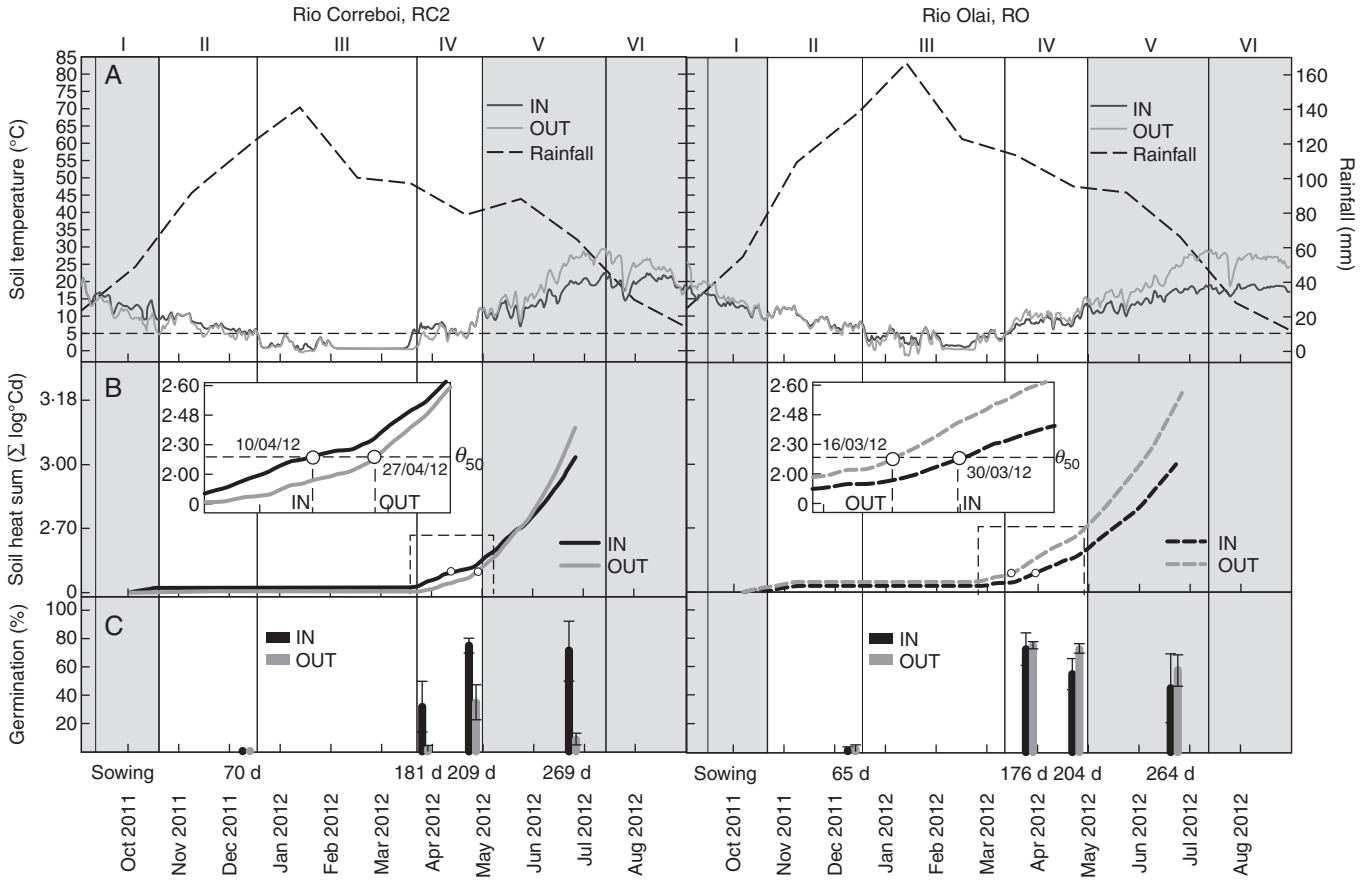

FIG. 4. Soil temperatures, soil heat sum and field germination for Rio Correboi (RC2) and Rio Olai (RO). (A) Annual trends of mean daily temperatures recorded in the soil both underneath (IN) and outside (OUT) the tree canopy and mean monthly rainfall (data from the nearby weather stations of Fonni and Montes for RC2 and RO, respectively); (B) soil heat sum (expressed in log °Cd); and (C) field germination (three replicates of 25 seeds each) IN and OUT at each time of exhumation, expressed in days after sowing. The inset plots in (B) show the detail of the achievement of the θ_{50} threshold value (2·19 log °Cd). The background grey squares correspond to the presence of the tree canopy. The details of periods I, II, III, IV, V and VI are as given for Table 2

stratification (period IV), the lower T_b values and the increasing soil temperatures allowed the threshold of 2·19 log °Cd (which corresponds to the value to achieve 50 % of germination in the laboratory, θ_{50}) to be reached 194 (IN) and 211 (OUT) d from sowing (Fig. 4B). This estimated time was confirmed by the germination recorded in the field (Table 2, Fig. 4C).

In RC1, the length of the stratification period was 90 d for IN and 104 d for OUT environmental conditions, leading to T_b values at the end of the stratification period of 2·9 and 1·9 °C for IN and OUT, respectively. After stratification, the threshold for θ_{50} was reached 186 and 200 d after sowing for IN and OUT, respectively, consistent with the field values presented in Table 2. In RC3, the effective stratification period was 116 d for IN and 93 d for OUT, leading to T_b values at the end of the stratification period of 2·5 and 2·9 °C for IN and OUT, respectively. Therefore, the threshold for θ_{50} was reached in period III, 171 (OUT) and 219 (IN) d after sowing. Although few field germination data were available for this experimental site, the

highest germination (43·0 ± 25·2 % for OUT) was recorded in April (Table 2).

In RO, the length of the stratification period was 75 d for both IN and OUT (with 15 and 20 d of snow cover, respectively), leading to T_b values at the end of the stratification period of 4·4 °C for each site (Fig. 4). Before (periods I and II) and during stratification (period III), mean soil temperatures were similar or lower than T_b (10·2 °C), leading to a slow accumulation of heat sum (1·73 log °Cd for IN and 1·97 log °Cd for OUT) by the end of period III (Fig. 4B). After stratification (period IV), the lower T_b values and the increasing soil temperatures enabled θ_{50} for RC seeds to be reached 164 (OUT) and 178 (IN) d after sowing (Fig. 4B). Although these times were estimated using data from seeds belonging to a different population (RC), the estimated dates were confirmed by the high germination percentages recorded in the field from March to April (Fig. 4C).

DISCUSSION

Type of dormancy

Final germination of *R. persicifolia* seeds was significantly improved by cold stratification (5 °C) at intermediate and low temperatures, confirming the presence of PD and supporting earlier observations (Mattana *et al.*, 2009). Physical dormancy is also known in seeds of *R. cathartica*, *R. caroliniana*, *R. frangula* and *R. purshiana* (Baskin and Baskin, 1998), *R. alaternus* and *R. cathartica* (Dupont *et al.*, 1997; García-Fayos *et al.*, 2001), and *R. alnifolia* and *R. lanceolata* (Sharma and Graves, 2005). As just 1 month of cold stratification is sufficient to break *R. persicifolia* seed dormancy, the seeds appear to have non-deep PD (Baskin and Baskin, 2004). Further, as the temperature range at which the *R. persicifolia* seeds could germinate widened from higher to lower, the seeds have Type 2 non-deep PD (Baskin and Baskin, 2004).

Thermal requirements for germination

The optimal temperature for germination of non-dormant seeds of *R. persicifolia* is presumed to be around 20 °C, as the best fit of the germination rate data in the sub-optimal temperature range excluded 25 °C, which fell in the supra-optimal temperature range. The T_b in seeds of *R. persicifolia* varied from approx. 10 °C for non-treated seeds to approx. 3 °C for seeds cold stratified for 3 months. To our knowledge, this is the first report of T_b for a member of the Rhamnaceae. Constraining the linear regressions of each percentile for germination through the mean T_b improved the residual sum of squares and r^2 values; therefore, T_b can be used to describe the whole population response in *R. persicifolia* seeds, as previously reported for other species (e.g. Covell *et al.*, 1986; Ellis *et al.*, 1987; Pritchard and Manger, 1990; Orrù *et al.*, 2012).

Treatments for dormancy release clearly modified T_b in *R. persicifolia* seeds, and the widening of the range of temperatures for germination can be used as a surrogate for the efficient removal of dormancy. Chilling at 5 °C reduced T_b in *R. persicifolia* seeds by approx. 0·09 °C d^{-1} of chilling, such that T_b reached the chilling temperature after 68 d of stratification. A similar trend has been observed in *A. hippocastanum* seeds, with T_b reducing by 0·17 °C d^{-1} of chilling at 6 °C (Pritchard *et al.*, 1999). In both these species, the sequential removal of dormancy lowers T_b until the stratification temperature becomes permissive for germination growth *per se* (Pritchard *et al.*, 1999). However, the process is nearly twice as rapid in *A. hippocastanum* seeds, with T_b reducing by 1 °C for every 5·9 d of chilling compared with 11·1 d of chilling in *R. persicifolia*. Consequently, it is clear that the quantitative impacts of a shortened cold season as a result of climate change will be highly species-specific with respect to the efficiency of dormancy loss and the timing of germination.

The best model was obtained by fitting germination expressed in probit and log-normal (log °Cd) rather than normal distributed thermal times (°Cd), as previously reported for other herbaceous (Covell *et al.*, 1986; Ellis and Butcher, 1988) and tree species (Pritchard and Manger, 1990). Seeds of *R. persicifolia* vary in their thermal time estimates to reach θ_{50}, depending on treatment. Chilling increased the rate of accumulation of thermal units (°Cd) at any temperature in the sub-optimal range, leading to a reduction in θ_{50} values from 2·59 log °Cd (385 °Cd) for untreated seeds to about 2·18 log °Cd (150 °Cd) for cold-stratified seeds. Batlla and Benech-Arnold (2003) also detected a cold-induced decrease in θ_{50}, from 80 °Cd to 56 °Cd, for seeds of *Polygonum aviculare* stratified at 12 and 1·6 °C, respectively. Similarly, the thermal history of *V. vinifiera* subsp. *sylvestris* seed lots varying with maternal environment is known to affect θ_{50} (33·6 to 68·6 °Cd) for non-dormant, cold-stratified seeds (Orrù *et al.*, 2012).

Soil heat sum and thermal niche for in situ *seed germination*

Maximum germination of Mediterranean species is typically in the range 5–15 °C and is limited to autumn and winter, and usually decreases markedly above 20 °C (Thanos *et al.*, 1995; Luna *et al.*, 2012). *Rhamnus persicifolia* showed a typical germination phenology of temperate and alpine plants, where spring germination prevails due to temperatures being too low to stimulate emergence following autumn dispersal or due to a requirement for cold stratification over winter (Baskin and Baskin, 1998; Walck *et al.*, 2011; Mondoni *et al.*, 2012). However, under harsh Mediterranean climatic conditions, the topsoil in the mountains remains moistened for only a few weeks after snow-melt, such that adaptation for fast germination in the early spring is an advantage (Giménez-Benavides *et al.*, 2005; Mattana *et al.*, 2010). The dormancy breaking and thermal time requirements identified in this study, together with the recorded annual trends in soil temperature, allowed a model for the thermal niche of seed germination to be constructed and spring emergence to be predicted for *R. persicifolia* seeds. Soil temperatures of around 5 °C (i.e. the stratification temperature tested in the controlled conditions) from December to February for RO (approx. 75 d) and from December to March (approx. 95 d) for RC facilitate both a fall in T_b to approx. 3 °C and efficient germination of the seeds in March and April when the mean soil temperatures are approx. 10 °C.

Plant distribution and competitiveness are highly dependent on environmental envelopes or niches (Walck *et al.*, 2011; Bykova *et al.*, 2012). For *R. persicifolia* habitat, up to six temperature periods were identified throughout the year, which contribute to a better understanding of the field germination period in this and other species growing along Mediterranean mountain waterways; especially as there have, hitherto, been no historical series of monthly averages of temperatures and rainfall at altitudes higher

than approx. 1100 m a.s.l. in Sardinia. In each investigated site, seed germination of *R. persicifolia* was obtained after cold stratification, when the canopy was absent. Tree canopy seems therefore to have no influence on seed germination *sensu stricto*, but closure of the canopy could influence survival of newly established seedlings due to microclimate amelioration (moister and cooler) during the dry and hot Mediterranean summers (Valiente-Banuet *et al.*, 1991; Greenlee and Callaway, 1996; Gómez-Aparicio *et al.*, 2005). This was confirmed by the high germination percentages reached under controlled conditions by untreated and cold-stratified seeds (>80 %) when incubated under the alternating temperature regime (25/10 °C). The ecological significance of germination stimulation by alternating temperature can be interpreted as a season-sensing system for temperate plants because the diurnal fluctuation of the soil surface temperature is large in the spring before dense vegetation covers the ground of deciduous forest or grassland (Shimono and Kudo, 2003).

The ecology of germination identified in this study for *R. persicifolia* explains the present distribution of this species which is mainly limited to small 'temperate' refuge areas along mountain waterways (Mattana *et al.*, 2009), where the general lack of rainfall during summer is overcome by the moisture of the soil. These findings confirm the identification of *R. persicifolia* as a species with a relic distribution, as previously reported by Arrigoni (1977) and Bacchetta *et al.* (2011).

The quantification of thermal time for germination has been used in different studies to characterize changes in seed dormancy and subsequent germination in the field (i.e. Forcella *et al.*, 2000; Hardegree and Van Vactor, 2000; Steadman *et al.*, 2003; Chantre *et al.*, 2009). Recently, Orrù *et al.* (2012) used an environmental heat sum approach (using mean monthly temperatures) to predict germination timing under two simulated IPCC scenarios (+1·8 °C for B1 and + 3·4 °C for A2; IPCC, 2007) for *V. vinifera* subsp. *sylvestris* seeds. The B1 scenario of +1·8 °C would still adequately overcome dormancy for all the investigated populations, whereas under the A2 scenario with +3·4 °C the higher winter temperature would not allow seed dormancy loss in the lowest investigated population (Orrù *et al.*, 2012). The same altitude-related pattern of seed dormancy release and germination in response to global warming can be assumed for *R. persicifolia* seeds. An increase of +1·8 °C (B1) would not reduce the stratification period at 5 °C for the high RC population (approx. 90 d, leading to a T_b of approx. 3 °C), whereas it could affect that of the low RO population (approx. 21 d; T_b of approx. 9 °C). However, an increase of +3·4 °C (A2) would reduce the cumulative stratification time at 5 °C to only 50 (T_b of approx. 6·5 °C) and 17 d (T_b of approx. 9 °C) for RC and RO, respectively. According to the B1 scenario, these changes in T_b and the increased soil temperatures would affect the germination time, by anticipating field germination to February–March and March–April, for RO and RC, respectively. An increase of 3·4 °C (A2) could lead to germination in the field in autumn (November) in both sites. This phenological shift to germination in autumn is possible as seeds of this species may also germinate at temperatures ≥15 °C without any cold stratification. Therefore, warmer temperatures and a consequent reduction of the stratification period would not be detrimental *per se* for seed germination. However, seedling survival over winter might then become the limiting event for the natural regeneration of the species. Moreover, projections for Mediterranean mountains predict lower precipitations mainly during spring (Nogués-Bravo *et al.*, 2008), and the seedling growing season could also be shortened by a reduction in soil moisture and water availability.

Conclusions

In conclusion, Type 2 non-deep PD was identified for *R. persicifolia* seeds, the thermal niche requirements for dormancy release and germination were quantified, and predictions for germination were validated through field observations of emergence. Overall, the results confirm the value of using a soil heat sum approach to predict the effects of subtle changes in field temperature on germination performance. The soil heat sum model developed for seed germination in this species may have applicability to predictions of *in situ* regeneration of other species growing by Mediterranean mountain waterways and of PY species of temperate and alpine regions, where spring germination prevails due to a requirement for cold stratification over winter.

ACKNOWLEDGEMENTS

We thank Eva Cañadas Sánchez (CCB) for helpful advice with the R package, and Rosangela Picciau (CCB) for helping with field work. The Royal Botanic Gardens, Kew, receives grant in-aid from Defra, UK. This work was supported by Ente Foreste della Sardegna. We gratefully acknowledge the Sardinia Regional Government for the financial support of the PhD scholarship of M.P. (P.O.R. Sardegna F.S.E. Operational Programme of the Autonomous Region of Sardinia, European Social Fund 2007–2013 – Axis IV Human Resources, Objective l.3, Line of Activity l.3·1.).

LITERATURE CITED

Arrigoni PV. 1977. Le piante endemiche della Sardegna: 2–4. *Bollettino della Società Sarda di Scienze Naturali* **16**: 269–280.

Bacchetta G, Fenu G, Mattana E, *et al.* 2011. Genetic variability of the narrow endemic *Rhamnus persicifolia* Moris (Rhamnaceae) and its implications for conservation. *Biochemical Systematics and Ecology* **39**: 477–484.

Baskin CC, Baskin JM. 1988. Germination ecophysiology of herbaceous plant species in a temperate region. *American Journal of Botany* **72**: 286–305.

Baskin CC, Baskin JM. 1998. *Seeds: ecology, biogeography, and evolution of dormancy and germination.* San Diego: Academic Press.

Baskin JM, Baskin CC. 1989. Physiology of dormancy and germination in relation to seed bank ecology. In: Leck MA, Parker VT, Simpson RL, eds. *Ecology of soil seed banks*. San Diego: Academic Press, 53–66.

Baskin JM, Baskin CC. 2004. A classification system for seed dormancy. *Seed Science Research* **14**: 1–16.

Batlla D, Benech-Arnold RL. 2003. A quantitative analysis of dormancy loss dynamics in *Polygonum aviculare* L. seeds: development of a thermal time model based on changes in seed population thermal parameters. *Seed Science Research* **13**: 55–68.

Brändel M, Schütz W. 2005. Temperature effects on dormancy levels and germination in temperate forest sedges (*Carex*). *Plant Ecology* **176**: 245–261.

Breshears DD, Nyhan JW, Heil CE, Wilcox BP. 1998. Effects of woody plants on microclimate in a semiarid woodland: soil temperature and evaporation in canopy and intercanopy patches. *International Journal of Plant Sciences* **159**: 1010–1017.

Bykova O, Chuine I, Morin X, Higgins SI. 2012. Temperature dependence of the reproduction niche and its relevance for plant species distributions. *Journal of Biogeography* **39**: 2191–2200.

Chantre GR, Batlla D, Sabbatini MR, Orioli G. 2009. Germination parameterization and development of an after-ripening thermal-time model for

primary dormancy release of *Lithospermum arvense* seeds. *Annals of Botany* **103**: 1291–1301.

Conti F, Manzi A, Pedrotti F. 1992. *Libro rosso delle piante d'Italia. Ministero dell'Ambiente, WWF Italia*. Poligrafica Editrice, Roma: Società Botanica Italiana.

Conti F, Manzi A, Pedrotti F. 1997. *Liste rosse regionali delle piante d'Italia. WWF Italia*. TIPAR Poligrafica Editrice, Camerino: Società Botanica Italiana.

Copete E, Herranz JM, Ferrandis P, Baskin CC, Baskin JM. 2011. Physiology, morphology and phenology of seed dormancy break and germination in the endemic Iberian species *Narcissus hispanicus* (Amaryllidaceae). *Annals of Botany* **107**: 1003–1016.

Covell S, Ellis RH, Roberts EH, Summerfield RJ. 1986. The influence of temperature on seed germination rate in grain legumes. I. A comparison of chickpea, lentil, soyabean and cowpea at constant temperatures. *Journal of Experimental Botany* **37**: 705–715.

Crawley MJ. 2007. *The R book*. Chichester, UK: John Wiley & Sons Ltd.

Daws MI, Jensen M. 2011. Effects of developmental heat sum on fruit traits of clonal lines of *Quercus petraea* grown under controlled conditions. *Plant Growth Regulation* **64**: 203–206.

Daws MI, Lydall E, Chmielarz P, *et al*. 2004. Developmental heat sum influences recalcitrant seed traits in *Aesculus hippocastanum* across Europe. *New Phytologist* **162**: 157–166.

Dupont É, Dulière JF, Malaisse F. 1997 *Aspects de l'ornithochorie et de la germination des semences des arbustes en fruticée calcicole de Calestienne*. University of Gembloux.

Ellis RH, Butcher PD. 1988. The effects of priming and 'natural' differences in quality amongst onion seed lots on the responses of the rate of germination to temperature and the identification of the characteristics under genotypic control. *Journal of Experimental Botany* **39**: 935–50.

Ellis RH, Covell S, Roberts EH, Summerfield RJ. 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.

Ellis RH, Simon G, Covell S. 1987. The influence of temperature on seed germination rate in grain legumes. III. A comparison of five faba bean genotypes at constant temperatures using a new screening method. *Journal of Experimental Botany* **38**: 1033–1043.

Fenner M, Thompson K. 2005. *The ecology of seeds*. Cambridge: Cambridge University Press.

Finney DJ. 1971. *Probit analysis*, 3rd edn. Cambridge: Cambridge University Press.

Forcella F, Benech Arnold RL, Sanchez R, Ghersa CM. 2000. Modeling seedling emergence. *Field Crops Research* **67**: 123–139.

García-Fayos P, Gulias J, Martinez J, Marzo A, *et al*. 2001. *Bases ecológicas para la recolleción, almacenamiento y germinación de semillas de especies de uso forestal de la Comunidad Valenciana.* Banc de Llavors forestals, Valencia, Spain.

Garcia-Huidobro J, Monteith JL, Squire GR. 1982. Time, temperature and germination of pearl millet (*Pennisetum typhoides* S. & H.). *Journal of Experimental Botany* **33**: 288–296.

Giménez-Benavides L, Escudero A, Pérez-García F. 2005. Seed germination of high mountain Mediterranean species: altitudinal, interpopulation and interannual variability. *Ecological Research* **20**: 433–444.

Gómez-Aparicio L, Gómez JM, Zamora R, Boettinger JL. 2005. Canopy vs. soil effects of shrubs facilitating tree seedlings in Mediterranean montane ecosystems. *Journal of Vegetation Science* **16**: 191–198.

Greenlee JT, Callaway RM. 1996. Abiotic stress and the relative importance of interference and facilitation in montane bunchgrass communities in Western Montana. *American Naturalist* **148**: 386–396.

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

Hardegree SP, Van Vactor SS. 2000. Germination and emergence of primed grass seeds under field and simulated-field temperature regimes. *Annals of Botany* **85**: 379–390.

IPCC. 2007. Climate change 2007: synthesis report. In: Core Writing Team (Pachauri RK, Reiginger A, eds) *Contribution of Working Groups I, II, III to the 4th Assessment Report of the Intergovernmental Panel on Climate Change*. Geneva: IPCC.

Joffre R, Rambal S, Damesin C. 1999. Functional attributes in Mediterranean-type ecosystems. In: Pugnaire FI, Valladares F, eds. *Handbook of functional plant ecology*. New York: Marcel Dekker, 347–380.

Luna B, Pérez B, Torres I, Moreno J. 2012. Effects of incubation temperature on seed germination of mediterranean plants with different geographical distribution ranges. *Folia Geobotanica* **47**: 17–27.

Martin AC. 1946. The comparative internal morphology of seeds. *American Midland Naturalist* **36**: 513–660.

Mattana E, Daws MI, Bacchetta G. 2009. Effects of temperature, light and pre-chilling on germination of *Rhamnus persicifolia*, an endemic tree species of Sardinia (Italy). *Seed Science and Technology* **37**: 758–764.

Mattana E, Daws MI, Bacchetta G. 2010. Comparative germination ecology of the endemic *Centranthus amazonum* (Valerianaceae) and its widespread congener *Centranthus ruber*. *Plant Species Biology* **25**: 165–172.

Meineri E, Spindelböck J, Vandvik V. 2013. Seedling emergence responds to both seed source and recruitment site climates: a climate change experiment combining transplant and gradient approaches. *Plant Ecology* **214**: 607–619.

Mondoni A, Rossi G, Orsenigo S, Probert RJ. 2012. Climate warming could shift the timing of seed germination in alpine plants. *Annals of Botany* **110**: 155–164.

Nogués-Bravo D, Araújo MB, Lasanta T, López Moreno JI. 2008. Climate change in Mediterranean mountains during the 21st century. *Ambio* **37**: 280–285.

Noronha A, Andersson L, Milberg P. 1997. Rate of change in dormancy level and light requirement in weed seeds during stratification. *Annals of Botany* **80**: 795–801.

Orrù M, Mattana E, Pritchard HW, Bacchetta G. 2012. Thermal thresholds as predictors of seed dormancy release and germination timing: altitude-related risks from climate warming for the wild grapevine *Vitis vinifera* subsp. *sylvestris*. *Annals of Botany* **110**: 1651–1660.

Pritchard HW, Manger KR. 1990. Quantal response of fruit and seed germination rate in *Quercus robur* L. and *Castanea sativa* Mill., to constant temperatures and photon dose. *Journal of Experimental Botany* **41**: 1549–1557.

Pritchard HW, Steadman KJ, Nash JV, Jones C. 1999. Kinetics of dormancy release and the high temperature germination response in *Aesculus hippocastanum* seeds. *Journal of Experimental Botany* **50**: 1507–1514.

Probert RJ. 2000. The role of temperature in germination ecophysiology. In: Fenner M, ed. *Seeds – the ecology of regeneration in plant communities*. Wallingford, UK: CAB International, 261–292.

R Development Core Team. 2011. *R: a language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org.

Rundel PW. 1996. Monocotyledoneous geophytes in the California flora. *Madroño* **43**: 355–368.

Sharma J, Graves WR. 2005. Propagation of *Rhamnus alnifolia* and *Rhamnus lanceolata* by seeds and cuttings. *Journal of Environmental Horticulture* **23**: 86–90.

Shimono Y, Kudo G. 2003. Intraspecific variations in seedling emergence and survival of *Potentilla matsumurae* (Rosaceae) between alpine fellfield and snowbed habitats. *Annals of Botany* **91**: 21–29.

Steadman KJ, Pritchard HW. 2004. Germination of *Aesculus hippocastanum* seeds following cold-induced dormancy loss can be described in relation to a temperature-dependent reduction in base temperature (T_b) and thermal time. *New Phytologist* **161**: 415–425.

Steadman KJ, Bignell GP, Ellery AJ. 2003. Field assessment of thermal after-ripening time for dormancy release prediction in *Lolium rigidum* seeds. *Weed Research* **43**: 458–465.

Thanos CA, Kadis CC, Skarou F. 1995. Ecophysiology of germination in the aromatic plants thyme, savory and oregano (Labiatae). *Seed Science Research* **5**: 161–170.

Thompson PA. 1973. Seed germination in relation to ecological and geographical distribution. In: Heywood VA, ed. *Taxonomy and ecology*. London: Academic Press, 93–119.

Tzedakis PC, Lawson IT, Frogley MR, Hewitt GM, Preece RC. 2002. Buffered tree population changes in a quaternary refugium: evolutionary implications. *Science* **297**: 2044–2047.

Valiente-Banuet A, Ezcurra E. 1991. Shade as a cause of the association between the cactus *Neobuxbaumia tetetzo* and the nurse plant *Mimosa luisana* in the Tehuacan Valley, Mexico. *Journal of Ecology* **79**: 961–971.

Walck JL, Hidayati SN, Dixon KW, Thompson KEN, Poschlod P. 2011. Climate change and plant regeneration from seed. *Global Change Biology* **17**: 2145–2161.

Walck JL, Shea Cofer M, Gehan Jayasuriya KMG, Fernando MTR, Hidayati SN. 2012. A temperate rhamnaceous species with a non-enclosing stone and without physical dormancy. *Seed Science Research* **22**: 269–278.