

Simulating the germination response to diurnally alternating temperatures under climate change scenarios: comparative studies on *Carex diandra* seeds

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- **Background and Aims** Environmental temperature regulates plant regeneration via seed in several superimposed ways, and this complex regulation will be disrupted by climate change. The role of diurnally alternating temperatures (ΔT) in terminating dormancy will be a major factor in this disruption, as its effects on seed germination are immediate.
- **Methods** The effect of ΔT on seed germination was modelled using two populations of the wetland sedge *Carex diandra*, one from a montane site and one from a subalpine site. A cardinal-temperature model was fitted to germination results obtained from a thermal gradient plate, and the model was used to simulate changes in germination under two possible future climate scenarios (RCP2-6 and RCP8-5, for representative concentration pathways) as defined by the Intergovernmental Panel on Climate Change.
- **Key Results** Scenario RCP2-6 projected moderate increases in average temperatures and ΔT , whereas RCP8-5 projected greater warming and higher ΔT . Increasing ΔT decreased the base temperature for seed germination and the thermal time required for germination. The effect of higher ΔT together with the higher temperatures increased germination under both climate scenarios.
- **Conclusions** *Carex diandra* germination is highly responsive to potential changes in ΔT , and thus this study highlights the role of ΔT in seed responses to climate change. Comprehensive cardinal-temperature models, encompassing the different effects of temperature on seed germination, are needed to understand how climate change will affect plant regeneration.

Key words: Cardinal-temperature models, climate change, germination thermal threshold models, physiological dormancy, plant–climate interactions, plant regeneration, representative concentration pathways, seed ecology.

INTRODUCTION

Climate change (IPCC, 2013) will disrupt the many interactions between biology and climate, from enzymatic reactions to ecological patterns. Climate thoroughly controls key processes such as plant regeneration, as is exemplified by the thermal regulation of seed germination (Probert, 2000; Fenner and Thompson, 2005). Temperature drives local adaptation (Wagmann *et al.*, 2012; Fernández-Pascual *et al.*, 2013a) and phenotypic plasticity (Fenner, 1991; Hoyle *et al.*, 2008; Figueroa *et al.*, 2010; Chiang *et al.*, 2011; Fernández-Pascual and Jiménez-Alfaro, 2014) in germination traits, as well as the physiological processes of dormancy loss (Pritchard *et al.*, 1996; Batlla and Benech-Arnold, 2003, 2004, 2005; Finch-Savage and Leubner-Metzger, 2006; Steadman and Pritchard, 2004) and germination elicitation (García-Huidobro *et al.*, 1982; Pritchard and Manger, 1990; Hardegrave, 2006). In seasonal climates, germination traits interplay with annual temperature cycles to ensure that seed emergence and seedling establishment occur in the most favourable season (Vleeshouwers *et al.*, 1995; Donohue *et al.*, 2010). Given the significance of germination in the life history of a plant, it is not surprising that its timing is a central scenario for natural selection (Donohue *et al.*, 2005a, b, c). However, the complex

thermal control of germination timing is highly responsive to climate change (Walck *et al.*, 2011). New environmental temperatures may not match the temperatures that alleviate dormancy (Orrù *et al.*, 2012) and elicit germination (Cochrane *et al.*, 2011). This mismatch could alter recruitment from the soil seed bank (Ooi *et al.*, 2009; Ooi, 2012; Hoyle *et al.*, 2013) and shift germination timing (Mondoni *et al.*, 2012), compromising plant regeneration and community composition.

Nonetheless, many aspects of the potential seed response to climate change remain unstudied. Together with a steady increase in average temperatures, climate change projections point to more frequent hot and fewer cold daily extremes (IPCC, 2013). Depending on the relative change in daily maxima and minima, diurnal thermal oscillations could actually rise, fall or remain unchanged, affecting the physiological processes of species (Vasseur *et al.*, 2014). It has been known for a long time that diurnally alternating temperatures (hereafter ΔT) promote germination in many plant species (Morinaga, 1926; Probert, 2000; Fenner and Thompson, 2005). This germination response to ΔT is especially common in wetland and disturbed habitats, where it detects favourable establishment environments with regard to burial depth, soil perturbation

or water level (Thompson and Grime, 1983; Liu *et al.*, 2013). Physiologically, ΔT may act through a decrease in the ratio of abscisic acid to gibberellins, which reduces the embryo's water potential and promotes radicle growth (Huarte and Benech-Arnold, 2005, 2010; Huarte, 2013). The effect of thermal oscillations is almost immediate, and on the first day after transfer from constant to ΔT regimes imbibed seeds undergo a large transcriptome change involving metabolism, plant hormones and the circadian clock (Foley *et al.*, 2010). ΔT is thus an 'immediate' signal for dormancy alleviation which marks that emergence conditions are immediately favourable, contrasting with slower dormancy loss associated with seasonal change, for example overwintering (Finch-Savage and Leubner-Metzger, 2006). This immediacy means that if climate change modifies ΔT , plant regeneration will be strongly affected as small changes in amplitude will translate into quick changes in emergence.

Recent research has begun to explore seed responses to climate change using cardinal-temperature models (Orrù *et al.*, 2012; Porceddu *et al.*, 2013). In this approach, germination experiments are employed to fit germination models based on two thresholds, namely (1) the base temperature (T_b) below which the germination rate is zero and (2) the thermal time (θ) or degrees-day above the T_b required to reach successive percentage germination increases in a seed population (Hardegre, 2006; Porceddu *et al.*, 2013). Subsequently, the models are tested under average temperature projections to simulate potential emergence patterns in the future (Orrù *et al.*, 2012). In terms of cardinal-temperature models, increasing ΔT decreases the base temperature and the thermal time for germination, i.e. germination is faster (Qiu *et al.*, 2006), similar to other dormancy-breaking factors such as chilling (Steadman and Pritchard, 2004; Pritchard *et al.*, 1996, 1999). It is therefore clear that in those species with a germination response to ΔT , an accurate simulation will require the incorporation of the dormancy-breaking effect of ΔT into the models. To our knowledge this step in model design and testing has not yet been taken.

Here we describe for the first time a simulation of seed germination under projected diurnal thermal oscillations according to climate change scenarios. To do so we chose as a study species the wetland sedge *Carex diandra* (Cyperaceae). *C. diandra* is a widely distributed northern hemisphere sedge that occupies transitional mire soils (Habitats Directive 7140 Transition mires and quaking bogs; Jiménez-Alfaro *et al.*, 2014) in the margins of lakes and ponds (Tutin *et al.*, 1980; Castroviejo *et al.*, 2007), habitats where the germination response to ΔT seems to be a pervasive adaptation. We used seeds from two contrasting altitudes to establish comparative cardinal-temperature models describing the fresh germination response to ΔT . We then used the models to simulate germination changes under the ΔT projected in representative concentration pathway (RCP) scenarios (Vuuren *et al.*, 2011). RCPs define different pathways of total radiative forcing produced by accumulation of all sources of human greenhouse gas emissions. RCPs do not represent specific scenarios of environmental/economic projections; instead each of them could result from different combinations of economic, technological, demographic,

policy and institutional futures (http://sedac.ipcc-data.org/ddc/ar5_scenario_process/RCPs.html). We chose RCP2-6 (lowest radiative forcing, i.e. lowest climatic alteration) and RCP8-5 (highest radiative forcing, i.e. highest climatic alteration). We address two specific hypotheses in freshly dispersed seeds with conditional dormancy: (1) increasing ΔT will lower the base temperature and shorten the thermal time for germination; and (2) simulated germination timing will change under the ΔT projections of the RCPs.

MATERIALS AND METHODS

Study system

Carex diandra Schrank produces abundant seeds that disperse in summer and show conditional physiological dormancy, being able to germinate at warm temperatures immediately after dispersal and acquiring the capacity to germinate at cooler temperatures as they lose dormancy (Fernández-Pascual *et al.*, 2013b). In late July 2012, ripe *C. diandra* seeds were collected from two ponds of the Cantabrian Mountains, the montane site 'La Mina' (43°16'N, 4°59'W, 1110 m asl) and the subalpine site 'La Recoleta' (43°1'N, 6°7'W, 1780 m asl) (Molina *et al.*, 2009). The day after collection, seeds were cleaned and kept in controlled conditions (15 °C, 15 % relative humidity) until further use.

To calculate present and future values of environmental average temperatures and ΔT at the population sites, present model-derived monthly maximum and minimum temperatures at the population sites were downloaded from WorldClim (Hijmans *et al.*, 2005), and projections of the same variables for the year 2070 under the RCP2-6 and RCP8-5 scenarios were downloaded from the HadGEM2-ES model (Jones *et al.*, 2011).

Germination experiment

The germination experiment began in November 2012. For each population, 25 seeds were sown onto 169 Petri dishes (1 % agar). Each population was placed on a bidirectional thermal gradient plate (Model GRD1, Grant Instruments, Cambridge, UK). While one gradient remained constant, the other changed direction every 12 h, providing 169 thermoperiod combinations with averages from 7.5 to 37.5 °C and ΔT from 0 to 30 °C (Fig. 1). The thermoperiods were coupled with a 12/12-h light–dark photoperiod, half of the dishes having a warmer day regime and half a warmer night regime. Actual temperatures in the gradient were recorded with five temperature probes (one in the centre and one in each corner of the thermal gradient plate), placed into the agar Petri dishes and set to record every 10 min for the duration of the experiment. From these records we calculated the average day and night temperatures of each Petri dish, and used these averages in further calculations. Germination (radicle emergence) was scored daily, and the dishes were replenished periodically to prevent the agar from drying. The experiment lasted 5 weeks by which time no further germination was observed. A random sample ($n = 300$ per population) of non-germinated seeds were cut open to determine the occurrence of empty and fungus-infected seeds. The

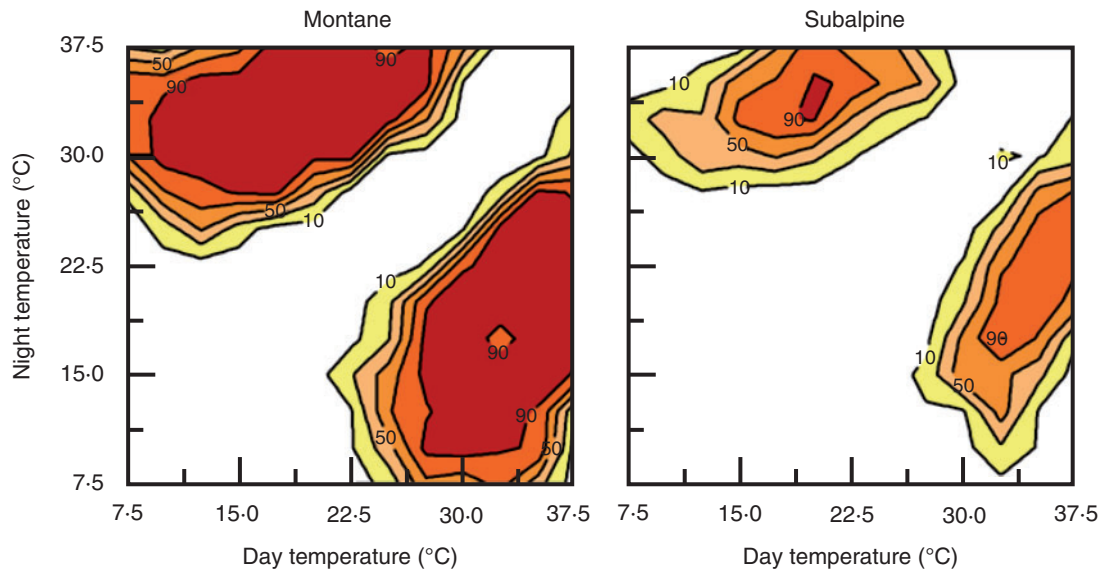


FIG. 1. Germination of *C. diandra* on the thermal gradient plate. Contour plots with points of equal percentage germination connected by germination isopleths. The shading represents 20 % germination increases, from lower (lighter filling) to higher (darker filling). Seeds were collected from a montane site (left panel) or subalpine site (right panel). The transverse axes of symmetry from the bottom-left corner to the top-right corner are constant thermal regimes. Points above and below the axes represent diurnally alternating temperature regimes, with greatest amplitude at the top-left (warm night, cold day) and bottom-right (warm day, cold night) corners.

percentage on non-viable seeds was low (4 %) and therefore the germination percentages were calculated over the total number of seeds sown (25).

Cardinal-temperature model

Cardinal-temperature models (García-Huidobro *et al.*, 1982; Pritchard and Manger, 1990; Hardegrave, 2006; Qiu *et al.*, 2006; Orrù *et al.*, 2012; Porceddu *et al.*, 2013) were used to describe the germination thermal thresholds under different ΔT regimes. For each dish, the time (t_g , days) taken for cumulative germination to reach successive 10 % increases (g) was calculated by linear interpolation on the germination curves. Data from dishes were grouped according to their regime of ΔT ($\pm 2^\circ\text{C}$), and for each regime the rate of germination ($1/t_g$) was plotted against the average germination temperature. Available treatments did not allow analysis of the supra-optimal temperature range, which would not be relevant ecologically for the environment of the Cantabrian Mountains (see Discussion). The suboptimal germination temperature range was identified by visual inspection of the germination rate plots, and a linear regression was fitted to it according to:

$$1/t_g (\text{d}^{-1}) = (T - T_b)/\theta_g.$$

The average x -intercept among g percentiles was the T_b for each ΔT , and T_b was allowed to vary among g to consider variation among individual seeds.

Subsequently the thermal time required to reach successive g (θ_g , $^\circ\text{Cd}$) was estimated as the inverse of the slope of the sub-optimal linear regression equations. Values of g were transformed into probits, and new linear regressions were fitted between probit (g) and θ_g expressed as both linear θ_g and $\log \theta_g$.

Linear θ_g gave higher R^2 values, and therefore linear equations were used to describe the germination of seeds as a function of thermal time under each ΔT regime:

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

The reciprocal of the slope (σ) represents the sensitivity of the population to thermal time; flatter slopes indicate higher variability among individual seeds in their response. K is the intercept when θ is zero.

Statistical analysis

To determine how the thermal thresholds varied depending on the ΔT regime, the seed population and the phase when temperatures were higher (day or night), Generalized Linear Models (gamma regression) were fitted to the values of T_b and θ_{50} . Then, considering only the dishes with a warmer day regime (the ecologically meaningful situation) and separately for each population, linear or logarithmic equations were used to estimate T_b and θ_{50} as a function of ΔT values at present and in the RCP2-6 and RCP8-5 scenarios. Finally, the thermal thresholds and the accumulation of degrees-day for germination were simulated under (1) present average monthly temperatures and ΔT , (2) RCP average monthly temperatures but present ΔT , and (3) RCP average monthly temperatures and ΔT .

RESULTS

Germination response to temperature

Germination of *C. diandra* did not occur under constant thermoperiods; a ΔT of at least 2°C was necessary for some

TABLE 1. Sources of variation in the thermal thresholds, base temperature for germination (T_b) and thermal time for 50 % germination (θ_{50}): results of a main effects Generalized Linear Model (gamma regression) that included diurnal thermal amplitude (ΔT), population of origin and warmer phase (i.e. whether the warmer phase of the photoperiod was coupled to the light or the dark phase) as fixed predictors

Effect	T_b		θ_{50}	
	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
ΔT	36.626	<0.001	19.01	<0.001
Population	20.404	<0.001	1.943	0.172
Warmer phase	0.092	0.763	8.739	0.005

germination to take place (Fig. 1). The highest germination percentages occurred with an average (day + night) temperature of around 25 °C and a ΔT of 10–14 °C. The montane population had higher germination (a total of 1798 germinated seeds) than the subalpine (687 germinated seeds). These differences were due to dormancy and not seed quality, as the subalpine seeds were able to produce >95 % germination in some treatments (e.g. 35/20 °C). Germination was possible both under warmer day and warmer night regimes, and although slightly more seeds germinated when the warm phase coincided with light, the differences were minimal (1258 seeds vs. 1227 seeds, averaging both populations).

Cardinal-temperature model and effect of ΔT

T_b was significantly different among the two populations, being estimated at 16.8 ± 0.5 °C for the montane seeds and 20.3 ± 0.6 °C for the subalpine seeds whereas the phase of higher temperatures did not affect T_b significantly (Table 1). Conversely, θ_{50} was not significantly different among populations but was significantly higher in warmer day regimes (258.8 ± 27.5 °Cd), compared with warmer night regimes (162.1 ± 19.0 °Cd) (Table 1).

We were able to establish sub-optimal cardinal-temperature models for ΔT regimes from 2 to 24 °C. ΔT significantly influenced both T_b and θ_{50} (Table 1). In both cases the effect followed a logarithmic function (Fig. 2), with a sharp decrease as ΔT increased from 0 to 10–14 °C and stabilization with higher values. T_b followed a linear decrease between 0 and 14 °C; as this matched the range of ΔT found in the temperatures of the models, we used a linear equation to estimate the monthly T_b under the monthly ΔT projected at present and under the two RCPs. For θ_{50} , we estimated an annual average using a logarithmic equation and the annual average ΔT under each scenario.

Simulated germination under climate change

The average annual temperature at present according to models was 6.7 °C with a ΔT of 9.5 °C. The RCP2.6 projected an average of 8.8 °C with a ΔT of 9.9 °C. The RCP8.5 projected an average of 11.0 °C with a ΔT of 10.3 °C. As a consequence of the increasing ΔT of the RCPs, T_b (averaging the two

populations) was projected to decrease from 18.6 °C at present to 18.2 °C in RCP2.6 and to 17.8 °C in RCP8.5 (Fig. 3). Present T_b and environmental temperatures would allow some accumulation of degree-days for fresh germination at the montane site, but prevent it at the subalpine site (Fig. 4). Under RCP2.6, the accumulation of degree-days would increase notably in the montane population, but would still not occur in the subalpine population (Fig. 4). Under RCP8.5, accumulation would be high in both populations (Fig. 4). When the lowering of T_b as a consequence of the new ΔT is incorporated into the simulation, the accumulation of degree-days increases in both sites and RCPs, the effect being more marked in the subalpine site where accumulation appears under RCP2.6 and doubles under RCP8.5 (Fig. 4).

The value of θ_{50} would also vary with the ΔT of each climatic scenario, although the change would be minimal (Fig. 4). At the montane site it was projected to decrease by 4 % from the present value in RCP2.6 and by 8 % in RCP8.5. At the subalpine site the decrease would be comparatively smaller, 3 % in RCP2.6 and in 5 % RCP8.5. Nonetheless, the combined effects of a higher accumulation of degree-days and a lower θ_{50} would change seed germination patterns in both sites (Fig. 4). In the montane site, 50 % fresh germination would occur in the summer season of dispersal under either scenario. The subalpine population would be unaffected under RCP2.6, but under RCP8.5 significant fresh germination would occur at dispersal, 50 % if the ΔT effect is considered.

DISCUSSION

Germination response to ΔT

Germination in *C. diandra* has an absolute requirement for ΔT . Such a strong germination response to ΔT is a common trait of sedges (Schütz, 1997, 2000; Schütz and Rave, 1999; Budelsky and Galatowsky, 1999) and wetland species in general (Thompson and Grime, 1983; Maas, 1989; Baskin and Baskin, 1998; Patzelt et al., 2001; Carta et al., 2013). In the case of *C. diandra*, it could allow seeds to detect a fall of the water table in the pond margins it colonizes. ΔT marks the beginning of the favourable season for dormancy-breaking and germination, at least in related fen soils of the Cantabrian Mountains where amplitude is minimal in winter, increases in spring and peaks in summer (unpubl. result). Nevertheless, there are differences among sites and ΔT is less successful in alleviating dormancy in the subalpine population, following a pattern of deeper dormancy at higher altitudes that is common to many species (Cavieres and Arroyo, 2000; Fernández-Pascual et al., 2013a; Zhou and Bao, 2014). Conditional dormancy in *C. diandra* can also be alleviated in the long term by cold-overwintering (Fernández-Pascual et al., 2013b), and this signal of slow seasonal change (Finch-Savage and Leubner-Metzger, 2006) is probably more significant in the subalpine site, where winter is harsher and premature emergence riskier.

These dormancy differences operate through T_b , mirroring the positive latitudinal correlation that has been found in the T_b of *Aesculus hippocastanum* L. populations (Daws et al., 2004). On the other hand, θ_{50} does not vary among populations, contrasting with the negative correlation between altitude and θ_{50} that has been found in *Vitis vinifera* L. (Orrù et al., 2012). T_b ,

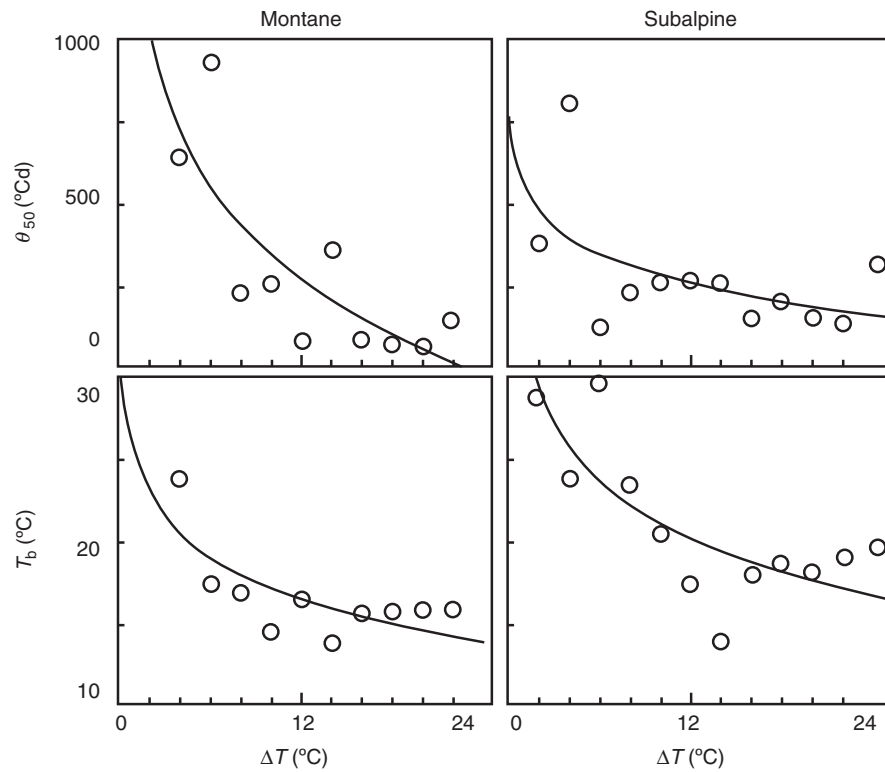


FIG. 2. Effect of diurnal thermal amplitude (ΔT) on the base temperature for germination (T_b) and the thermal time for 50 % germination (θ_{50}) for seeds from montane and subalpine sites. The symbols represent the average values of T_b and θ_{50} at each ΔT regime; the lines are the logarithmic fits to the data.

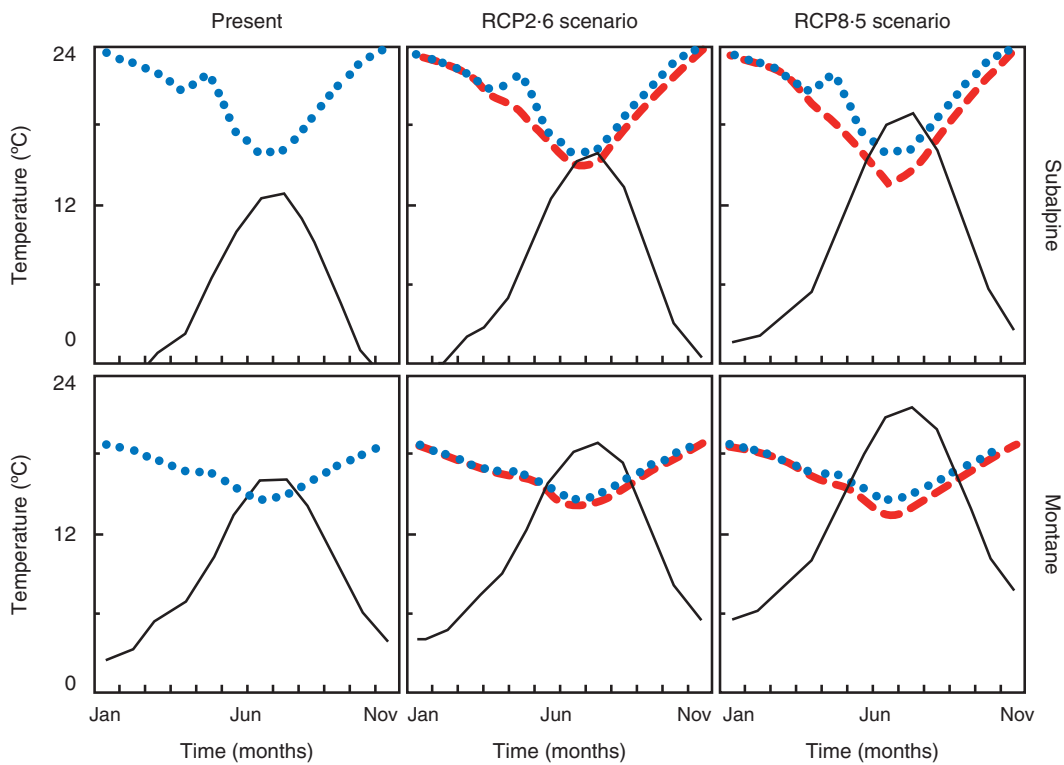


FIG. 3. Simulated environmental temperature and base temperature (T_b) for germination at present and under the RCP2.6 and RCP8.5 scenarios. Each panel shows the results for one population under a given scenario. The solid line is the environmental monthly average temperature, the dotted line represents T_b projected on the basis of the present diurnal thermal amplitude (ΔT) and the broken line is for T_b projected in relation to the ΔT of the scenario.

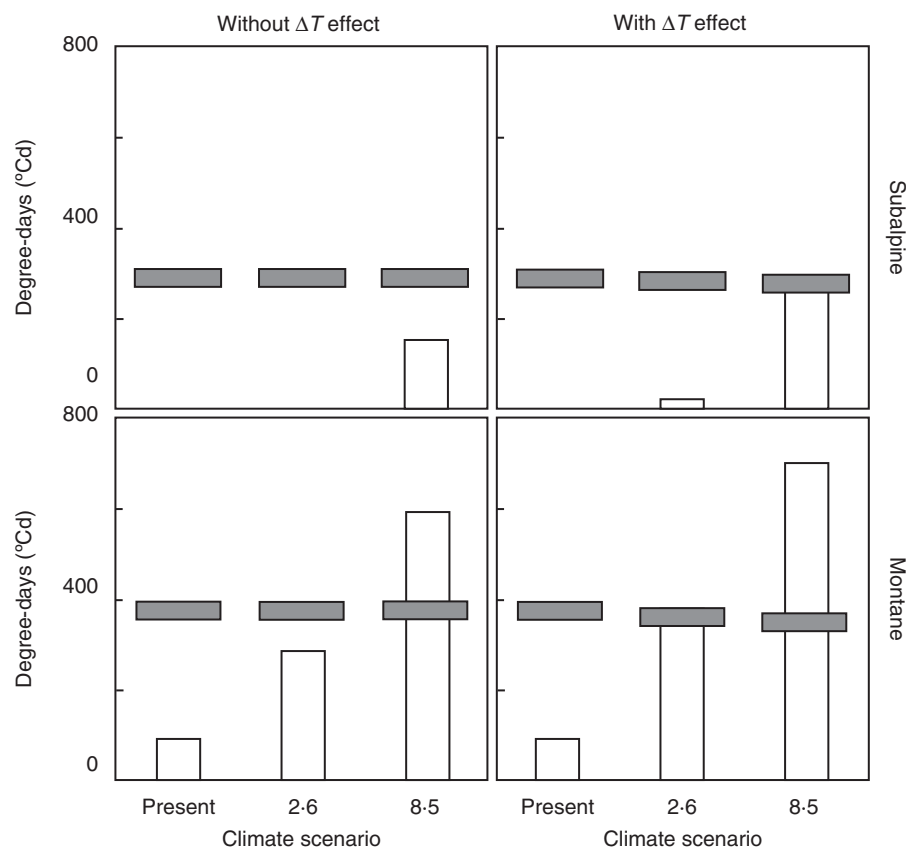


FIG. 4. Annual accumulated environmental heat sum for germination (EHR) and thermal time required for 50 % germination (h50) at present and under the RCP2-6 (2-6) and RCP8-5 (8-5) scenarios for montane and subalpine populations. The vertical columns are EHR and the grey horizontal bars are h50. The results of the simulation are shown without (left panels) and with (right panels) taking into account the effect of the diurnal thermal oscillations (ΔT).

as well as the total number of germinated seeds, does not vary depending on whether the light phase coincides with the warm or the cool phase of the thermoperiod. This agrees with previous work that shows that the amplitude of the thermal oscillation is the main parameter that explains the germination response to ΔT (Roberts and Totterdell, 1981; Benech-Arnold *et al.*, 1990a, b; Batlla *et al.*, 2003). Conversely, θ_{50} is significantly lower when the night is warmer than the day, i.e. seeds germinate faster in that case. It is difficult to interpret this abnormal and ecologically unrealistic situation, but it could be related to the role of the phytochrome in the response to ΔT (Probert, 2000).

A main effect of ΔT in *C. diandra* is that T_b decreases as ΔT increases, thus facilitating the accumulation of degree-days for germination (i.e. under a given average temperature, more degrees above T_b can be accumulated each day). The relationship between T_b and ΔT is linear up to 10–14 °C, whereas it becomes asymptotic with larger ΔT values, as was found in certified seeds of *Dactylis glomerata* L. (Qiu *et al.*, 2006). ΔT above the optimal amplitude did have a negative effect in wild European populations of *D. glomerata* (Probert *et al.*, 1986). However, supraoptimal values do not seem realistic in *C. diandra* field conditions and therefore lack ecological significance as temperatures above 25 °C will not be reached in the Cantabrian Mountains, even under the more extreme climatic scenarios (Álvarez García *et al.*, 2009). The optimal ΔT in

C. diandra and *D. glomerata* contrasts with what was found in grassland species living in disturbed soils at very high altitudes in the Tibetan Plateau, where the optimum ΔT was 20 °C and 10 °C had no effect (Liu *et al.*, 2013). Such high amplitudes can be expected in the extreme Tibetan conditions, and therefore the optimal ΔT might be closely related to the thermal range of the specific habitat.

Implications for climate change

Simulation of *C. diandra* germination under RCP scenarios points to an increase in emergence parallel to the intensity of the RCP scenario and to the associated increase in average temperatures. However, the full magnitude of the change is only appreciated when the response to ΔT is incorporated into the simulation, as this increases the daily accumulation of degree-days at the same time that it decreases the cumulative degree-days needed for the population to germinate. The effect of incorporating ΔT is particularly relevant at the subalpine site, where the accumulation of degree-days doubles under the RCP8-5 scenario. Total germination increases are higher at the montane site, where a high percentage of the seeds are not expected to overwinter and join the soil seed bank even under the moderate RCP2-6 scenario. However, as germination at dispersal at the montane site seems to be possible at present, it

is to be expected that seedlings might survive winters there. Conversely, the harsher winters at the subalpine site make premature germination in autumn or during early winter a more deleterious trait, and accordingly dormancy there is deeper. Therefore, the change could have more negative consequences at the subalpine site in the RCP8.5 scenario, as germination in the same summer of dispersal would change from negligible to affecting half the seed population. Although our findings are based on a simulation of how one species might respond across two sites, they do highlight the probable importance of ΔT on regeneration under climate change of the many plant species that show a response to ΔT . The latest climate change analysis at the *C. diandra* study sites reports a higher increase of daily maxima in comparison with daily minima (Álvarez García *et al.*, 2009), so ΔT could already be modifying germination patterns.

Note that our simulation is a simplification of nature that concentrates on the effect of ΔT on fresh germination, whereas in the field temperature will influence germination in other ways. A new climate will modify germination traits through phenotypic plasticity (Fenner, 1991; Daws *et al.*, 2004; Hoyle *et al.*, 2008; Chiang *et al.*, 2011; Fernández-Pascual *et al.*, 2013a; Fernández-Pascual and Jiménez-Alfaro, 2014). For example, present differences in T_b between the montane and subalpine sites are larger (16.8 vs. 20.3 °C) than the differences in T_b projected among scenarios (18.6–17.8 °C). Therefore, if all the present variation among sites is attributable to phenotypic plasticity, this plasticity would accommodate the effect produced by climate change (Cochrane *et al.*, 2014). However, our experimental design does not at this stage allow for differentiation between phenotypic plasticity and genetically based local adaptation (Fernández-Pascual *et al.*, 2013a).

Furthermore, our simulation is only valid for the germination of fresh seeds, i.e. seed germination between dispersal in summer and the beginning of the winter season in November. In the field, temperatures during overwintering would alleviate dormancy (Fernández-Pascual *et al.*, 2013b), probably slowly lowering T_b (Pritchard *et al.*, 1996, 1999; Batlla and Benech-Arnold, 2003; Steadman and Pritchard, 2004; Porceddu *et al.*, 2013) and reducing relative sensitivity to ΔT (Benech-Arnold *et al.*, 1990a, 2000; Batlla *et al.*, 2003). Such anticipated changes require consideration. Finally, *in situ* soil temperatures can be expected to vary from those projected by air models, especially in fen soils where the buffer effect of the spring water is strongly felt (unpubl. res.). Overall, further complexity is needed in the development of cardinal-temperature models before they can encompass all the thermal determinants of seed germination and accurately predict plant regeneration under climate change.

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