Alp Botany (2015) 125:59–68
DOI 10.1007/s00035-015-0155-1

OPINION PAPER

Seeds at risk: How will a changing alpine climate affect regeneration from seeds in alpine areas?

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Received: 19 February 2015 / Accepted: 31 August 2015 / Published online: 10 September 2015

Abstract Alpine areas are both regional water reservoirs and zones of high species endemism. Increasing temperatures and earlier snowmelt have already caused upward migration of species, changes in flowering phenology and increasing frost damage in plants. Thus, significant loss of diversity in alpine areas is imminent. Plant migration and distribution shifts occur mainly via seeds, which also provide the genetic diversity required for adaptation. The ability of plants to shift their distribution in response to climate change will depend on seed dispersal, germination and seedling success under new environmental conditions. Despite the critical importance of seeds and seedlings for species adaptation, migration and persistence, the majority of studies concerning climate change in alpine areas have mostly focused on the response of adult plants to warming. Temperature during seed development, as well as the temperature to which seeds are exposed post-dispersal, has been found to have strong effects on seed longevity, germination and seedling survival. Therefore, global change (particularly, warming) is expected to greatly impact regeneration of seeds in alpine areas. Despite evidence that climate change is advancing flowering phenology in several mountain ranges around the world, under natural and artificial warming, the cascade effects that early flowering can have on seeds and seedlings have been poorly studied. Indeed, while a literature search on Web of Science using the search terms "germination", "alpine plants" and "climate change" revealed 50 studies, of which only 7 directly examined the effect of warming on germination and establishment of seeds of alpine plant species. Here, we discuss the findings of these studies. We identify critical questions regarding seeds and seedlings of alpine species that require urgent research and recommend experimental approaches. Answering these questions will assist in predicting the impacts of global warming and in conservation and management of plants in alpine areas.

Keywords Seeds · Seedlings · Climate change · Alpine areas · Germination phenology · Early snowmelt

Electronic supplementary material The online version of this article (doi:10.1007/s00035-015-0155-1) contains supplementary material, which is available to authorized users.

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Introduction

Until relatively recently, alpine vegetation was considered to be dominated by highly clonal, vegetatively spreading species with only a minority of species reproducing frequently via seed (Billings and Mooney 1968). Sexual reproduction was considered by early alpine ecologists to be disadvantageous in harsh alpine environments, because establishment of seedlings might be detrimentally affected by a range of extreme conditions. However, recent studies on alpine seedling survival and establishment contradict this idea (Venn and Morgan 2009). Many species typical of alpine communities (alpine specialists as well as species that extend below treeline) have per capita recruitment rates and seedling survival rates similar to those of perennial plants from other environments (Forbis 2003; Forbis and Doak 2004). The exception is the alpine tundra, where seedling survival is very low (Arctic Council 2013; Cramer et al. 2014). Furthermore, a sizeable persistent soil seed bank has been found in alpine zones [e.g. 899 seeds/m^2 in the Andes of Central Chile (Cavieres and Arroyo 2001) and 150 ± 27–1330 ± 294 seed/m^2 in alpine areas of Australia (Venn and Morgan 2010)] (see also Kalin Arroyo et al. 1999; Molau and Larsson 2000; Ma et al. 2010; Venn and Morgan 2010; Hoyle et al. 2013). Finally, the high gene flow found in some alpine areas suggests that sexual reproduction is frequent and seedling recruitment common (Pluess and Stöcklin 2004).

Seeds are a means by which many plants persist in the landscape (i.e. as seeds in the soil seedbank) or migrate across it. Upslope migrations of alpine species have been already found for most of the European alpine areas, with the exceptions of boreal–temperate and Mediterranean mountain regions where, instead, species contractions have been seen (Gottfried et al. 2012; Pauli et al. 2012). Shifts in species' distribution range are related to seed dispersal, germination and establishment in these new environments. Therefore, insights into how climate change affects seed production and dispersal, germination and seedling establishment are urgently needed to predict plant species movement, plant persistence and adaptation to novel alpine environments under future scenarios.

Seed germination and seedling establishment clearly play an important role in alpine community composition and dynamics, yet the ecology and physiology of the transition from seed to seedling, which includes crucial stages involved in becoming an established adult plant (Fig. 1, stages 1–7), is poorly researched. Most studies concerning the effect of climate change on alpine floras have focused on how adult plants (Fig. 1, stages 8–12) function and will respond to warming.

Alpine areas have been identified as one of the ecosystems most at risk under climate change (Arctic Council 2013; Theurillat and Guisan 2001; Cramer et al. 2014), with average temperatures already increasing across mountain areas around the world (Beniston et al. 1997; Diaz and Bradley 1997; Vuille et al. 2003; Pickering et al. 2004; Gobiet et al. 2014). The net result of this warming is expected to be reduced snowfall and earlier snowmelt (Beniston 2012; Pederson et al. 2013; Sánchez-Bayo and Green 2013). Thus, the motivation to prepare for the effects of climate change is high.

Under snow, both plants and seeds in the soil seed bank remain at very stable temperatures (close to 0 °C) even when air temperatures are well below freezing (Björk and Molau 2007; Mondoni et al. 2012; Briceño et al. 2014). The length of the alpine growing season is controlled by snow duration. Early snowmelt due to climate warming is both advancing and increasing the length of the alpine growing season; however, it is also exposing plants that rely on snow insolation to more frequent and severe freezing events (Inouye 2008; Wipf et al. 2009; Gerdol et al. 2013; Wheeler et al. 2014). Thus, an increase in temperature is not the only factor to consider when assessing the impacts of a changing climate in alpine areas. The question of how subzero temperatures and the associated lack of snow will affect the production and survival of seeds, seed germination strategies and seedling establishment in alpine areas is becoming increasingly relevant.

Warming and advanced snowmelt could potentially increase plant fitness by prolonging the growth period and hence increasing resource allocation to seed production (Galen and Stanton 1993). However, there is increasing evidence that early snowmelt also increases the risk of frost damage in adult life stages. We already know that naturally occurring warm spells and early snowmelt increase the mortality of flower buds due to frost damage (Molau 1993; Inouye 2008; Lambert et al. 2010). Further, experimental snow reduction and advancing snowmelt have been linked to reductions in biomass and fruit production, again resulting from frost damage (Gerdol et al. 2013). Thus, paradoxically, warming and the associated early snowmelt will expose seeds in the soil, seedlings that germinate under snow and adult perennial plants to freezing events, potentially offsetting the positive effects of a longer growing season.

Here, we highlight the potential impacts of climate change, particularly warming and early snowmelt, on seed germination and seedling establishment for plant species typical of alpine environments. We outline the questions that need urgent attention and suggest empirical approaches, already utilized in other areas, to answer these questions. We advocate for an increased focus on seed and seedling ecology to fill critical knowledge gaps and position us to conserve and manage alpine communities effectively in the face of warmer and less snowy alpine areas.

Climate change effects on seed recruitment in alpine areas

Plant regeneration via seeds has been globally recognized as critical for species persistence and migration under future climate scenarios (Walck et al. 2011). Temperature is one of the most influential climatic variables for seed germination, since it synchronizes germination and emergence with environmental conditions optimal for subsequent seedling establishment. Temperature influences the number of seeds that germinate and the rate at which they germinate (Probert 2000). Global climate change is altering the environmental

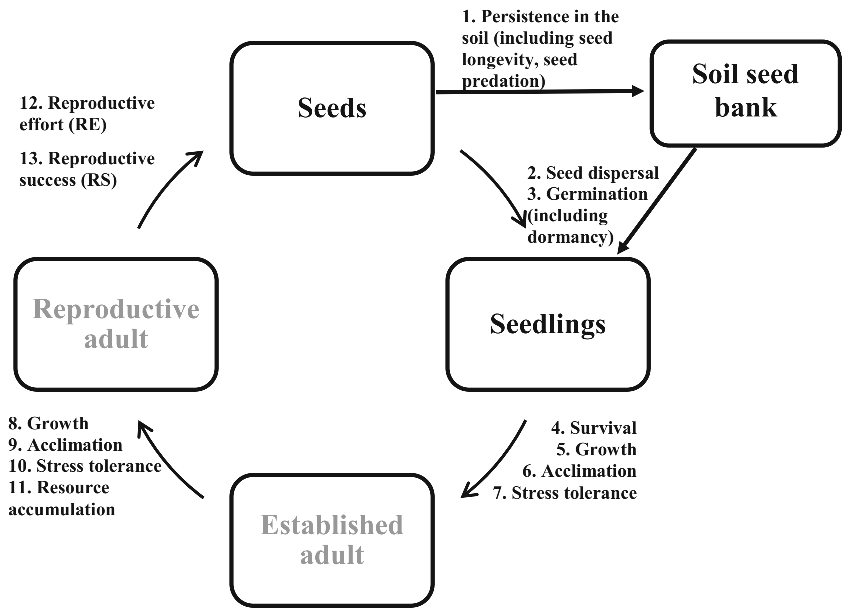

Fig. 1 To date, research into the potential effects of climate change upon alpine flora has focused on how temperature increases will affect adult plant growth, reproductive effort and success (*7–12*). The effects of climate change on the transition from seed to seedling (*1–7*) has been neglected, even though the success of plant establishment depends critically on seed dispersal (*2*), seed germination (*3*) and seedlings' success (*4–7*). The germination strategy of seeds from alpine plants includes mechanisms that regulate seasonal emergence patterns, dormancy and persistence in the soil (*1*) as well as the transition from seedling to established adult (*4–7*). The effect of temperature, both warming and changes in exposure to extreme high and low temperatures, on germination strategies has received scarce attention despite the fact that germination and seedling survival are highly temperature sensitive and an important component of the plant life cycle

cues that seeds receive, potentially resulting in compromised seedling emergence and changes to seedling performance. However, to our knowledge, only seven studies have specifically asked questions about the impacts of warming and early snowmelt on seeds or seedlings of alpine species (Graae et al. 2008, 2009; Milbau et al. 2009; Shevtsova et al. 2009; Klady et al. 2011; Mondoni et al. 2012; Hoyle et al. 2013). The effect of climate change on seeds and seedlings of alpine species has been largely neglected. For instance, we have learnt a great deal about how warming and snow reduction impact several vegetative traits in adult plants (see Henry and Molau 1997; Arft et al. 1999; Wipf and Rixen 2010). Some of these studies consider seed size and seed production. However, the extent to which warming affects the germination or establishment of seeds produced under warmer conditions has not received adequate attention (but see Klady et al. 2011). Warming and early snowmelt are likely to affect seeds and seedlings at every stage of the life cycle, from seed development, dispersal and persistence in the seed bank, to seedling germination and establishment.

Effects on seed production and dispersal

One of the widespread effects of warming in alpine areas is advanced flowering phenology (Dunne et al. 2003; Molau et al. 2005; Inouye 2008; Forrest et al. 2010; Hoffmann et al. 2010; Lambert et al. 2010). This advance in flowering phenology is often seen as a prolongation of the growing season leading to a longer period for resource accumulation and allocation to reproduction, which could be reflected in increased seed size and/or seed production as shown by some studies (Alatalo and Totland 1997; Totland 1997; Wagner and Reichegger 1997; Welker et al. 1997; Arft et al. 1999; Nyléhn and Totland 1999; Totland 1999; Sandvik and Totland 2000). A study in the High Arctic tundra, using open top chambers to increase temperature, reported that 12 years of heating increased reproduction (flowering, seed production and seed germination) in several species, with shrubs and graminoids showing greater response than forbs (Klady et al. 2011). To the best of our knowledge, the latter study is the only one to date addressing how in situ warming directly influences the germination of seeds produced under warmer climate. A reduction in seed production has also been found when the warming treatment was applied only in winter (Liu et al. 2012) or when increases of temperature advanced flowering and exposed reproductive organs to freezing damage (Molau 1996; Gerdol et al. 2013). Therefore, an advance in flowering phenology might not necessarily translate into increased seed production (see Scheepens and Stöcklin 2013), particularly if warming is coupled with early snowmelt, exposing reproductive organs to freezing damage.

In addition, there is increasing evidence that conditions during seed development (pre-dispersal) also influence seed and seedling performance. Small changes in climatic conditions can result in large changes in seed responses: for instance, climatic changes during development can alter seed longevity (Ooi et al. 2009; Kochanek et al. 2010, 2011; Mondoni et al. 2014), dormancy status (Steadman et al. 2004; Hoyle et al. 2008) and seed mass (Hovenden et al. 2008). This means that as the plant's maternal environment changes, seed characteristics will do so as well. Such 'carry-over effects' on seeds and seedlings of alpine plant species have not yet received much attention.

Seed dispersal will be critical for alpine plant migration under future climate. The effects of warming on seed dispersal patterns are difficult to predict and will be highly reliant on the effect of warming on vectors (e.g. animals, wind). How the dispersal of seeds of alpine species will be affected by climatic change is, to our knowledge, unknown. However, mathematical models evaluating shifts in species distribution in alpine areas consider the seed dispersal mechanism as an important factor. Indeed, models considering seed dispersal and species niche show a less optimistic future scenario than models that only consider species niche by itself (Parolo and Rossi 2008; Dullinger et al. 2012). Therefore, the effects on seed dispersal due to climatic change in alpine areas merit pressing attention.

Soil seed bank-mediated effects

Seeds of many alpine plant species do not germinate immediately after dispersal and may remain viable in the transient soil seed bank until the following growing season or enter the permanent soil seed bank where they can remain viable for years (Molau and Larsson 2000; Baskin and Baskin 2001). The duration of seed persistence in the soil varies among species and populations, and depends on the physical and physiological characteristics of seeds and how they are affected by the biotic and abiotic environment [see Long et al. (2015) for a review]. There is only one study that directly addresses the question of how soil warming will affect germination from the soil seed bank in alpine areas. In the Australian Alps, soil warming leads to reduced overall germination from the soil seed bank (Hoyle et al. 2013). However, germination response to soil temperature was species specific, such that the total number of species that germinated actually increased with soil warming. A total of 39 species germinated from warmed soil seed bank compared to 27 from ambient soil conditions. Interestingly, Poaceae seeds made up the bulk of the germinable soil seed bank (63.5 % of total germinants), and their total percentage of germination and the rate at which they germinated were significantly reduced by soil warming throughout the entire experiment when compared with germination from soil at cooler temperatures (Hoyle et al. 2013). Warming, as well as freeze/thaw cycles due to early snowmelt, could have direct effects on seed longevity in alpine soil, for example by altering the metabolic state or dormancy status of seeds (see below) and could also have indirect effects on persistence as a result of altered predator/pathogen distributions. Additionally, non-climatic factors such as seed predation and soil properties can constrain regeneration of seeds in alpine areas (Brown and Vellend 2014), but this too has not been widely studied to date. An integrated understanding of the eco-physiological mechanisms of seed persistence, including seed predation, is essential if we are to improve our ability to predict how long seeds can survive in soils, both now and under future climatic conditions. Seed persistence allows a species, population or genotype to survive long after the death of parent plants, thus distributing genetic diversity through time. Therefore, the persistent soil seed bank can act as a reservoir of seeds that can buffer future losses of diversity.

Germination phenology (post-dispersal effects)

One of the most significant research gaps concerning the germination of seeds of alpine species lies in germination phenology: when and under what conditions do seeds germinate today and in future? Climate change has the potential to alter where and when seeds germinate. However, much about the mechanisms that regulate seasonal emergence patterns of alpine seedlings, otherwise known as germination strategies (mechanisms including environmental germination requirements and seed dormancy), remains unknown. How will warm spells and cold snaps in winter and summer influence germination? The answers to this question are still obscure. For example, an increase in winter temperature did not have an effect on subsequent germination of the dwarf shrub *Empetrum nigrum* (Ericaceae) from alpine tundra, but germination increased with the increased incubation temperature to which seeds were exposed in controlled conditions (Graae et al. 2008). Similarly, summer warming (+2.5C ° increase of incubation temperature) accelerated germination time in 19 out of 23 subarctic species, whereas colder winter soil temperatures (stratifying seeds to freezing temperatures and simulating thin snow cover) delayed germination in 10 species. The combination of colder winter and summer warming accelerated germination in most of the species (Milbau et al. 2009). The authors concluded that a future warming climate could improve regeneration by seeds/seedlings by advancing and prolonging the growing season. Mondoni et al. (2012) exposed seeds to soil temperatures that mimicked current and projected alpine seasonal cycles and found that warming shifted germination of many alpine species from spring to autumn. The atypical autumn germinants could then be subject to frequent and severe freezing events that they might not be able to cope with, such that increased germination would be followed by increased mortality.

These few studies clearly illustrate that germination will be affected by changes in temperature; however, the direction and extent of the effect are likely to be species and season dependent. In addition, a warm spell in winter will have a different effect than a warm spell in summer, and thus both conditions need evaluation.

Effects on seedling survival

The consequences of changes in germination phenology under a future climate will depend on how seedlings tolerate the novel conditions to which they are subsequently exposed.

Of the seven studies on the effect of warming on regeneration by seeds, only two included the effects on seedlings and neither of these was specifically alpine. One examined summer warming using heat pulses (known as the free air temperature increased method, or FATI) on soil-sown seeds of Arctic herbaceous species and demonstrated delayed and decreased seedling emergence with warming (Graae et al. 2009). Another study on tundra species used the same warming methodology, but zoom into the life phases where the effects of warming actually take place. The authors broke down the whole-season warming effect into full factorial combination of early-, mid-, and late-season warming periods. Whole-season heating reduced germination and establishment in four out of ten species. Early-germinating species were susceptible to warming; the critical phases were early summer for germination and midsummer for seedling survival. Graminoids (*Deschampsia flexuosa, Festuca ovina* and the rush *Luzula multiflora*), which emerged later, were less susceptible, although some negative effects during late summer were observed (Shevtsova et al. 2009).

These results confirm that the effect of summer warming depends on species-specific time of germination. Thus, germination phenology seems to be a strong determinant of the fate of seedlings, which was also found for alpine plant species (Shimono and Kudo 2003). Furthermore, seedling survival in alpine areas is also influenced by microsite conditions such as plant interactions, presence of litter and soil conditions (Venn and Morgan 2009; Graae et al. 2011); hence, we cannot evaluate seedling survival ignoring these factors.

What are we missing?

The few studies mentioned above suggest that climate change will interfere with seed production, seed germination and seedling survival. However, with so little research on the topic, we cannot yet draw general conclusions. Here, we highlight what we think are key questions to evaluate how seeds and seedlings from alpine areas will be affected by warming and snow reduction and suggest potential methods, most of which are readily available, to answer these questions (see Online Resource Table 1).

Seed production and pre-dispersal and dispersal effects

Previous experiments have looked at how warming influences seed production (Welker et al. 1997; Arft et al. 1999) and the resulting germination of these seeds (Klady et al. 2011); however, studies on seedlings are still absent. Pressing answers are needed for some questions such as: Is warming increasing or decreasing seed size? Is warming advancing or delaying germination? Perhaps the fastest way to approach this gap is to compare seed size and germination of seeds produced under in situ warming experiments (such as OTC's or similar passive warming treatments), to seeds produced under natural conditions, as well as comparing the carry-over effects on seedling performance.

The effects of climate change on seed dispersal are complex to evaluate, but mathematical models under current and future climate have already been applied to other environments and can be of use for alpine areas (Engler and Guisan 2009; Mokany et al. 2014).

Persistent soil seed bank

Will warming and increasing freeze/thaw cycles increase or decrease and accelerate or decrease germination of the alpine soil seed bank? Hoyle et al. (2013), using soil samples collected at different elevations, showed that warming will interfere with seed germination from the soil seed bank. Similar experiments can be done considering the effect of a decrease, or both an increase and decrease in soil temperatures, on germination of seeds from the soil seed bank. Soil collection for this kind of experiment needs to be done before the dispersal of the current season seeds, to ensure exclusion of transient soil seed bank.

Germination of the soil seed bank usually increases after disturbance (Venn and Morgan 2010; Hoyle et al. 2013), making it likely that the predicted increases in soil freeze/thaw cycles, due to lack of snow insulation, could increase germination from the soil seed bank. Questions concerning how freezing interferes in seed germination, persistence and longevity in the soil need answers to evaluate whether this seed reservoir will persist in future climates. In situ burial and retrieval experiments (see Cavieres and Arroyo (2001) for seed burial methodology), in contrasting micro-sites (wet versus dry, high versus low elevation, early snowmelt versus late snowmelt), may reveal how environmental conditions affect seed longevity and persistence (see Long et al. 2008, 2015). For instance, seed burial experiments done in zones of early and late snowmelt can give us insight into how future changes in snow regimes will affect seed persistence and longevity in the soil (Shimono and Kudo 2003, 2005).

Seeds can withstand very low temperatures if they are dehydrated, which is the principle method of preservation for orthodox seeds ex situ. However, imbibed seeds (for example, following snowmelt) may be unable to tolerate subzero temperatures if ice crystals form in the seeds. Cold tolerance thresholds of seeds from alpine species can be examined ex situ. The vulnerability of seeds to freezing damage depends on a variety of factors such as seed moisture and oil content (Bonner 1990). Exposing seeds of different moisture contents to a range of freezing temperatures and exploring seed viability after treatments could determine the minimum temperature and maximum moisture content at which seeds of different species remain viable (see Marcante et al. (2012) for seed freezing methodology).

As seed persistence in the soil seed bank is also influenced by seed predation and soil pathogens, the effects of climatic change on these factors are also important to evaluate. Soil and seed reciprocal transplant experiments along the elevation gradient can give us insight into whether different soil property changes in predation and climatic variables with elevation have a significant effect on germination of seeds from the soil seed bank (Brown and Vellend 2014).

Impacts of climate change on soil seed bank dynamics will not only facilitate accurate predictions of species distributions and risk of extinction, but also highlight species best suited to alpine restoration, ensuring successful establishment and survival of these species.

Thermal niche of germination

Changes in temperature associated with future climate may uncouple the temperatures that seeds experience from the temperatures at which germination is able to occur (germination niche). It has been hypothesized that species with a broad distribution range (e.g. species distributed all along the elevation gradient in alpine areas) germinate over a broad range of temperatures (Fig. 2, species 1), while species with a restricted distribution range might germinate over a narrow range of temperatures (Fig. 2, species 2, 3) (Luna and Moreno 2010; Cochrane et al. 2011; Luna et al. 2012). Will temperature requirements for germination of narrowly distributed alpine species be met under future climate conditions? Exposing seeds to a gradient of temperatures enables investigation of the relationships between temperature and seed germination (Cochrane et al. 2011). This approach is particularly useful for identifying species that have narrow thermal ranges for germination and thus may be most at risk under climate change. For instance, the breadth of germination niche for alpine species inhabiting the entire alpine elevation gradient could be compared with species inhabiting more restricted microhabitats, such as warmer low elevation areas. Interestingly, most of the studies on germination of seeds of alpine species focus on finding the optimal conditions for seed germination. However, in the context of climate change, the edges and shape of the germination niche (low and high temperatures for germination) will be equally important for potential species' niche expansion. By determining the range of temperatures within which alpine species have, for example, >90 % germination success (thermal optimums for germination) and the thermal edges for germination, we can forecast the potential distribution expansion of species (independent of other (e.g. dispersal) limitations, see above) will change under future climate.

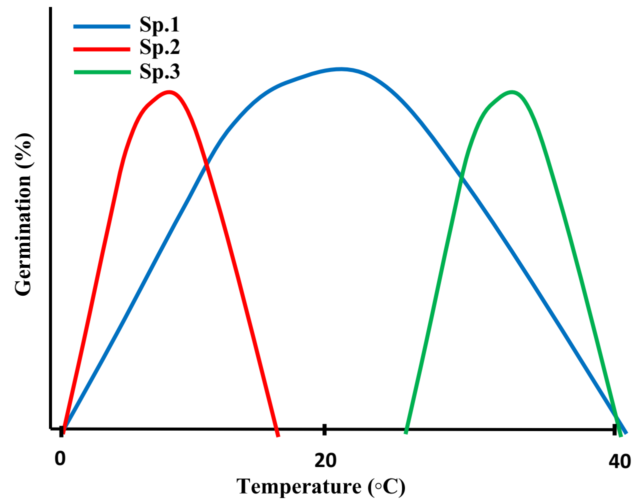

Fig. 2 Hypothetical germination range of three alpine species. Species 1 represents alpine species with a wide distribution range along the elevational gradient, with seeds which have a broad temperature range for germination. Species 2 and 3 represent species with a more restricted distribution and, therefore, seeds of these species germinate over narrow temperature ranges. Perhaps, suitable temperature conditions for germination will be less common under future scenarios, and thus seed recruitment, particularly for species 2 and 3, will be negatively affected by climate change. Identifying species with a narrow germination niche might help us to target potential endangered alpine species (color figure online)

Germination phenology

One of the major gaps in the ecology of germination for seeds of alpine species is seed germination phenology: when and under what conditions do seeds germinate in situ under current conditions and how will this change as a result of warming? Ex situ experiments that expose seeds to soil temperatures that mimic current and projected alpine seasonal cycles could help to disentangle germination strategies of alpine species. This approach should identify when seedling emergence currently occurs in the field and whether or not the germination strategy involves dormancy (Fig. 3a). Mimicking future conditions, such as warmer, colder and/or more variable soil temperatures may indicate the potential for shifts (advance or delay) in germination phenology (Fig. 3b). Understanding shifts in germination phenology is crucial; germination at the wrong time may be lethal for alpine seedlings.

Alternatively, the effect of climate change on germination strategies could also be inferred from in situ seed sowing experiments using a reciprocal design along an elevation gradient (Giménez-Benavides et al. 2007), or contrasting early and late snowmelt sites (Shimono and Kudo 2003). Assessing patterns of germination and embryo development before and during burial would establish the impact of contrasting environmental conditions on subsequent germination.

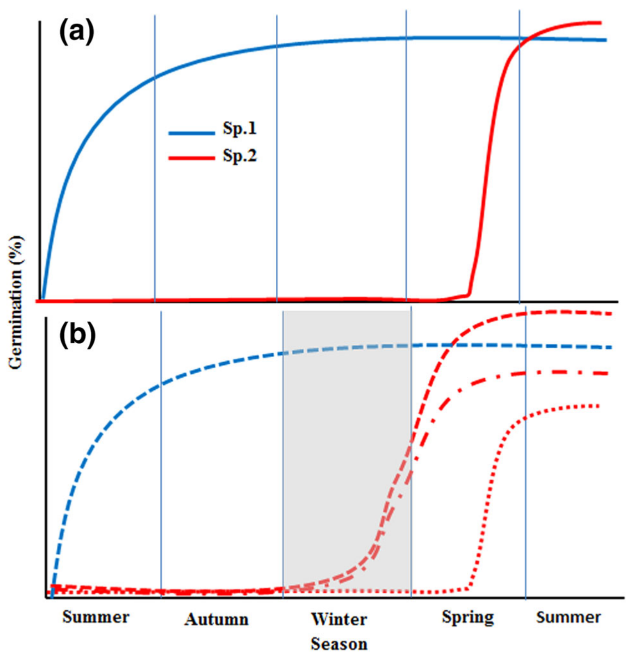

Fig. 3 The germination phenology of two hypothetical species (*red* and *blue*) determined by ex situ mimicking of soil temperatures for each alpine season and periodically scoring germination under **a** current conditions (*solid lines*) and **b** simulated future climate (e.g. warmer winter and early snowmelt, depicted by *dashed lines*). Under current conditions, species 1 germinates immediately after seeds are dispersed in summer, whereas dormancy prevents immediate germination of species 2 until the following year in late spring. Under warmer conditions, the germination phenology of species 1 may not be affected; however, the germination phenology of species 2 may be reduced and/or advanced to late winter. A reduction in germination may occur if warming is associated with direct reductions in longevity and/or increased seed mortality as a result of greater frequency of soil freezing events (particularly after seeds are imbibed) or if conditions do not effectively alleviate dormancy. Persistence of species 2 under a scenario of advanced germination (late winter) will depend on seedlings' cold tolerance (color figure online)

Seedling survival and establishment

What are the environmental tolerance limits of seedlings and how will they cope with novel environments? Are germination phenology and seedling thermotolerance linked? For instance, will seedlings that emerge in summer (Fig. 2, species 1) differ in the conditions they tolerate, compared to seedlings that emerge in spring (Fig. 2, species 2)? Furthermore, will climate change expose seedlings to conditions they are not able to tolerate? These questions need to be urgently addressed, particularly if warming will advance germination phenology and expose seedlings to conditions outside of their range of tolerance.

The temperature range that alpine seedlings are able to tolerate (thermotolerance) could be assessed by exposing whole seedlings, or seedling leaves, to temperatures ranging from below freezing to above average leaf temperatures and measuring seedling damage (Buchner and Neuner 2003; Sierra-Almeida and Cavieres 2012; Briceño et al. 2014). Additionally, thermotolerance needs to be considered in the context of the germination strategy: species that germinate immediately following dispersal may differ in thermal tolerance from those that postpone germination.

Finally, transplanting seedlings in the field along an elevational gradient or a snowmelt gradient will improve our understanding of how warming and early snowmelt will interfere with seedling survival. Survival, growth and thermotolerance could be monitored to measure the acclimation capacity of these seedlings. Reciprocal transplants will reveal whether seedlings from early snowmelt sites have a greater capacity to tolerate freezing events than those from late snowmelt sites (Briceño et al. 2014). Transplanting seedlings in the field is also a realistic way to consider nonclimatic factors (such as species interactions, soil properties and soil microbiota) as important determinants of seedling survival.

Suggested action plan

Alpine areas have been described as a mosaic of microenvironments (Scherrer and Körner 2010). Factors such as topography, elevation, aspect and snow duration modify plant temperatures. Therefore, alpine areas provide an ideal space in which to look into the future climate effects on plants at the whole ecosystem scale. The air temperature gradient with elevation and patches of different snow duration can be seen as "natural experimental treatments", where inter- and intraspecific differences to climatic conditions can be tested. In addition, the alpine environment, as a mosaic of microenvironments, offers excellent opportunities to test the contribution of pre-dispersal factors (such as parental effects or carry-over effects) on seeds and seedling performance. We recommend using the unique alpine landscape to approach the questions mentioned above and listed in Online Resource Table 1 from different perspectives: interspecific and intraspecific differences in seed production and dispersal, seed longevity, germination strategies under current and predicted thermal conditions and seedlings' tolerance to extreme temperatures as well as the contribution of parental effects on these traits.

Conclusions

We strongly encourage greater investigation into the potential effects of climate change on seed recruitment in alpine areas. The threat of climate change to alpine plant biodiversity is real, and by filling the gaps outlined here we will be in a better position to manage, maintain and restore alpine areas effectively. An increased understanding of the basic mechanisms of plant recruitment by seeds in alpine areas is needed to bridge the current gaps in the literature and aid prediction about the effects of climate change on species persistence and distribution. Basic information regarding when and under what conditions seeds germinate in the field, and how seedlings cope with frost and heat, is a crucial prerequisite prior to tackling the more complex questions regarding how these processes will be affected by climate change. Furthermore, an appreciation of the within-species variation in germination and seedling success, and how seed provenance and/or parental effects contribute to this variation, will enable identification of both vulnerable species and those more likely to be resilient to climate change. For example, some species may be able to shift germination phenology without compromising recruitment, while others with narrow germination windows may not be exposed to the physical conditions that meet germination requirements under a future climate.

In addition, future climate regimes are predicted to include more frequent disturbance due to fire in some alpine areas and human impact leading to greater reliance on restoration [e.g. through assisted migration and targeted restoration (Havens et al. 2015)] to maintain ecosystem functioning. Such restoration requires an understanding of which species are short lived in the soil seed bank, when and from where to collect seeds (e.g. high or low elevations, snowbeds, fellfields, etc.), how to germinate the seeds and where and at which stage (seedlings, juvenile or adult) they can be planted in the field. Great effort is currently focused on the establishment of ex situ seed banks (Walker et al. 1993; Cochrane et al. 2007), including alpine seed banks, for both long-term conservation and to support restoration. Information on optimal storage conditions, germination requirements and how seed origin (both long-term population and short-term microenvironment) affects seed production, quality, germination and seedling survival are essential if we are to rely on the use of seed collections for conservation and restoration of alpine areas in the future. While there is plenty of excellent work on how alpine adult plants will be affected by climate change, we must not forget that to become an established adult plant, successful germination and seedling survival are needed.

Acknowledgments The authors would like to thank the Nicotra Lab for their constructive comments on this manuscript as well as Daniel Harris-Pascal for his assistance in revision of this article. V. F. Briceño is supported by the CONICYT-PAI (82140011) fellowship.

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