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Research article

Assessing the importance of cold-stratification for seed germination in alpine plant species of the High-Andes of central Chile

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A B S T R A C T

Snow cover duration, which depends on winter precipitation and temperature, is decreasing along several alpine areas around the world as a consequence of the global warming and further increases are expected in the future. Thus, alpine species that requires cold stratification to initiate seed germination may be threatened in the future. Here we report the results of an exploratory experimental survey to assess the importance of cold stratification for the seed germination in alpine plant species from the High-Andes of central Chile. In addition, we assessed how this requirement varies with elevation at the community level. Seeds of a total of fifty plant species were collected along an altitudinal range from 2200 to 3600 m a.s.l. Eighteen species corresponded to the Low-Andean vegetation belt, while thirteen and nineteen species corresponded to the Mid- and High-Andean vegetation belts, respectively. For each species, seeds that experienced cold-stratification at 4 °C for 3 months and control seeds (stored at room temperature and dry conditions for 3 months) were placed on moist paper in 4 Petri dishes containing 50 seeds each, and arranged in a growth chamber set at 20 °C/10 °C and a photoperiod of 14/10 h. While 36% of the studied species were able to germinate without cold-stratification, after this pretreatment 74% of these species germinated, suggesting that in general cold-stratification promotes seed germination of these alpine plant species. This positive effect was particularly evident on the species from the lower elevations. At highest elevation both the number of species that showed seed germination and the percentage and velocity of seed germination were low. Nonetheless, non-germinated seeds remained viable, suggesting a deeper dormancy. Our results suggest that cold stratification could be an important requirement for successful seed germination in species from lower elevations, while species from higher elevations could require other factors than cold-stratification to break seed dormancy. Nonetheless, as we did not use temperatures as those experienced in the field, further studies are needed to gain insights into the importance of the duration of cold stratification and the underlying mechanisms involved in the seed germination in the field of high-Andean plant species.

1. Introduction

The germination of seeds, that is the transition from seed to seedling, is a high-risk period in the life cycle of many plant species (Baskin and Baskin, 2014); hence, the mechanisms regulating the timing of this transition are expected to be under strong selective pressure (Angevine and Chabot, 1979; Willis et al., 2014). If seeds germination timing is an adaptive process, then natural selection should favor seed germination requirements that reduce the probability of facing environmental conditions that are not appropriate for seedlings establishment (Vleeshouwers et al., 1995; Willis et al., 2014; Baskin and Baskin, 2014). Thus, seed germination requirements should vary depending on the environmental risks associated to seedlings mortality (Meyer and Monsen, 1991; Meyer et al., 1989, 1990, 1995).

Alpine environments are characterized by low temperatures, strong winds, unstable substrates, and short growing seasons (Körner, 2003). The short growing season of alpine habitats is a major barrier for plants recruitment as it constrains seedlings growth and the period favorable for their establishment (Chambers et al., 1990; Forbis, 2003) because seedlings have to attain a critical biomass by the end of growing season to withstand the harsh and long-lasting winter conditions (Schütz, 2002).

It has been suggested that seeds from plant populations that normally experience long snow cover periods and adverse winter conditions, as occur in alpine habitats, would require an amount of time experiencing the moderately low and constant temperatures that occur

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under the snow cover to allow or promote their germination (Meyer and Monsen, 1991; Meyer et al., 1989; Meyer et al., 1990; Schütz and Rave, 1999). In the seed germination literature the process of seeds experiencing low and constant temperatures to simulate natural winter conditions as if they were under snow is called cold stratification (Bewley et al., 2013; Baskin and Baskin, 2014). The requirement of a cold stratification period would allow seeds to sense the presence of snow, thereby timing the germination to a period appropriate for seedling survival and establishment. This way, cold stratification requirements would prevent precocious germination under autumn conditions where appropriate soil moisture and temperatures to complete seed germination and seedling establishment are not likely to persist for more than a few days (Meyer and Monsen, 1991). In addition, cold stratification requirements allow germination to occur only in spring after the snow melt, preventing young seedlings from being damaged by freezing temperatures (Billings and Mooney, 1968). Thus, it may be expected that a cold stratification period would be a common requirement for seed germination in alpine plant species. In an early review, Amen (1966) concluded that cold stratification is not a common requirement for the seed germination of several alpine plant species from different mountains in the USA (see also Sayers and Ward, 1966; Marchand and Roach, 1980; Kaye 1997). In contrast, Söyrinki (1938, cited in Körner, 2003) experimenting with 91 alpine species from the Alps, found that storage at winter temperatures (cold stratification) increased seed germination in the great majority of species. More recently, Sommerville et al. (2013) and Hoyle et al. (2015) showed that in 19 and 54 Australian alpine plant species, respectively, a cold stratification period significantly increased seed germination only in half of the species tested. Shimono and Kudo (2005) reported that cold stratification increased the seed germination over a range of temperatures in most of the 27 plant species studied in the alpine zone of Japan. Baskin and Baskin (2014) showed that seeds of most tundra species are dormant, and only those with physiological dormancy require cold stratification to release dormancy. Therefore, how important is cold stratification for seed germination in alpine plant species remains elusive. Further, although studies conducted in species with broad altitudinal distribution (e.g., Cavieres and Arroyo, 2000; García-Fernández et al., 2015) and in species living in habitats with contrasting snow cover duration (Meyer et al., 1989, 1995), have found a positive relationship between the duration of snow cover and duration of cold stratification for maximum seed germination (but see Schütz and Milberg, 1997; Schütz,2002), few studies have analyzed seed germination patterns in high elevation species at the community level, and as far as we are aware, none has evaluated the altitudinal variation of cold stratification requirements for seed germination at this scale.

Snow cover duration, which depends on winter precipitation and temperature, is decreasing on several alpine areas around the world as a consequence of the recent climate change (Beniston, 1997, 2012), and further decreases are expected to occur in the future due to global warming (op. cit.). Thus, alpine species that require to experience a time of low and constant temperatures as occur under snow (a cold stratification) to initiate their seed germination may be threatened in the future, whilst species with no such requirements may be indirectly favored, generating important changes in the composition and dynamics of alpine plant communities (Briceño et al., 2015; Walder and Erschbamer, 2015). In this study we evaluated the importance of a cold stratification period for seed germination in 50 high elevation plant species from the Andes of central Chile, and whether the requirement of cold stratification varies with elevation at the community level. We expected that a cold stratification period is an important factor for seed germination in these species, and that this importance increases with the altitude (i.e., higher duration of snow cover) at which a community develops.

2. Materials and methods

2.1. Seeds sources

Seeds of the studied alpine plant species were collected in the central Chilean Andes, in the surroundings of La Parva (33°21′S, 70°19′W) and Valle Nevado (33°19′S, 70°15′W) Ski resorts, distanced at 50 and 80 km east of Santiago, respectively. This area is characterized by an alpine climate with strong influence of the Mediterranean-type climate that prevails in the lowlands (di Castri and Hajek, 1976). Precipitation mainly occurs as snow during winter, with summer months usually receiving very few or no rain (Santibáñez and Uribe, 1990). Snow cover remains for 3–5 months depending on the altitude and slope aspect (Rozzi et al., 1989).

The alpine vegetation of the central Chile Andes can be found from immediately above the *Kageneckia angustifolia*'s tree line at 2200 m up to 3600 m a.s.l. (Cavieres et al., 2000). Based on the altitudinal distribution of plant life-forms, this vegetation has been divided in three altitudinal vegetation belts (Cavieres et al., 2000). The Low-Andean belt span from 2200 to 2500 m a.s.l., and is dominated by prostrate shrubs species such as *Chuquiraga oppositifolia* D. Don (Asteraceae), and *Anarthrophyllum cumingii* Hook. et Arn. J.F. Phil. (Fabaceae), with several annual species growing in open spaces between shrubs (Cavieres et al., 2000). The Mid-Andean belt extends from 2600 to 3200 m a.s.l., and is characterized by the dominance of cushion plants (e.g., *Laretia acaulis* Gillies & Hook. (Apiaceae), *Anarthrophyllum gayanum* (A. Gray) B.D. Jacks (Fabaceae)), and perennials herbs such as *Phacelia secunda* J.F. Gmel (Boraginaceae), *Nassauvia aculeata* (Less.) Poepp. et Endl. (Asteraceae), and *Melosperma andicola* Benth. (Plantaginaceae) (Cavieres et al., 2000). The High-Andean belt can be found from 3200 to 3600 m elevation, and is characterized by a low plant cover, where the dominant species are small rosette-forming herbs such as *Pozoa coriacea* Lag. (Apiaceae), *Chaetanthera* sp. (Asteraceae) and *Montiopsis potentilloides* (Barneoud) Ford (Montiaceae) (Cavieres et al., 2000).

The studied species were selected on basis of their seeds availability, to represent different growth-forms and families, and for to be important component of the plant communities in the study area (Table 1). Seeds of fifty plant species were collected along an altitudinal range from 2200 to 3600 m a.s.l.; eighteen species corresponded to the Low-Andean belt, while thirteen and nineteen species corresponded to the Mid- and High-Andean belts, respectively.

All seeds were collected during the natural period of seed dispersal (January-March), stored in paper bags, and transported to the laboratory at the University of Concepción. To assure high intra-specific representation for each species seeds were collected from at least 50 different individuals sparsely distributed within the corresponding vegetation belt. Depending on the species, some fruits (e.g., caryopses, achenes) were treated as seeds. In the laboratory, seeds were immediately cleaned, removing non-seed material by hand and blow away. Then visually-healthy seeds were selected, discarding those aborted and/or predated. The selected seeds of each species were allocated to treatments of cold stratification and control as follow.

2.2. Cold stratification

A widely used standard protocol for the stratification of seeds was used (Baskin and Baskin 2014). For each species, 200 seeds were placed in plastic boxes (20 × 15 × 6 cm) between two layers of paper previously wet. For this, we added distilled water until the papers were saturated and the excess of water was drain out from the plastic boxes. The boxes were completely wrapped with aluminum foil to avoid the passage of light, and were arranged into a growth chamber (Pitec, Bioref-19L) at 4 °C in darkness during three months. We monthly checked that papers remained moist during the cold stratification treatment, and we added distilled water when it was necessary. This procedure was done in dark conditions to avoid light effects on seed