# Factors affecting germination of *Deschampsia antarctica* and *Colobanthus quitensis* seeds from an altitudinal gradient on Livingston Island (South Shetland Islands, Antarctica)

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**Abstract**

The temperature, photoperiod, stratification and seed age affected on seed germination of the two Antarctic native vascular plants *Deschampsia antarctica* and *Colobanthus quitensis* from different altitudes (10 to 147 m) on Livingston Island, South Shetland Islands. Germination tests were carried out in the laboratory over a two year period. The seeds of *D. antarctica* germinated first and had a higher germination success (mean 28,7±4,9%) that those of *C. quitensis* (mean 6,86±1,8%), but germination of seeds from the current year was nil or very low (5%) in *D. antarctica*, while higher in *C. quitensis* (maximum 53%).

Some seeds of both species germinated in darkness at low temperatures (4-5ºC), but a higher germination took place with a photoperiod dark/light. The optima temperature, after a cold pre-treatment, seems to be with daily fluctuations between 4-5º dark /15-19ºC light. Seeds subjected to constant temperatures of 3-4ºC and photoperiod of 8h dark/16h light over 3 months did not germinate (with the exception of a few *Colobanthus* seeds).

The seeds of *D. antarctica* from the lowest site had a higher germination (maximum 100%) than those from higher altitudes (maximum 75%). *Colobanthus* seeds from higher altitude germinated in darkness at the beginning of the experiment, but the germination was nil when after were subjected to treatments with a dark/light photoperiod carried out with short term stratification. Seeds of *Colobanthus* from lower altitudes that were stored for a long time at low temperature showed enhanced germination.

Most of the seeds collected from the 2-4 cm layer of soil were poorly developed or showed signs of fungal degradation and did not germinate.

**Keywords**: Altitudinal variation; Antarctica; vascular plants; age and storage seeds; germination; temperature and photoperiod treatments.

**Running title** : Factors affecting germination of Antarctic plants.

## Introduction

*Deschampsia antarctica* Desv. (Poaceae) and *Colobanthus quitensis* (Kunth) Bartl (Caryophyllaceae) are the only native vascular plants in Antarctica and are widespread in the maritime Antarctic (Holtom & Greene 1967; Corner 1971; Greene & Holtom 1971). An increase of temperature over the past c.50 years in the maritime Antarctic has caused a rapid expansion of these plants (Fowbert & Lewis Smith 1994; Lewis Smith 1994; Grobe et al. 1997; Day et al. 1999; Robinson et al. 2003; Convey et al. 2011; Lewis Smith 2003), in response to a warming trend in summer temperatures. Colonization and dispersal in *Colobanthus* occurs apparently only as a result of seed production, while *D. antarctica* is also able to reproduce and disperse by the production of ramets (Fowbert & Lewis Smith 1994; Vera 2011). Viable seeds of both species can remain dormant for lengthy periods when they are stored experimentally at 3º C (McGraw & Day 1997; Ruhland & Day 2001). However the long-term viability of seeds in the soil seed bank in natural conditions, where the seeds are subjected to a wide range of temperature and soil moisture, and the degree of microbial degradation, is unknown.

It is postulated that the sexual reproductive capacity of each species will be influenced by altitude (and thus temperature), in terms of seed production, germination, viability, establishment and distribution. In fact, in this area of study, the production of ripe seeds, seedling establishment and distribution of these species was greater at lower than at higher altitudes (Vera 2011).

Previous studies show different germination responses with the altitude (Miller & Cummins 1987; Lavorel 1987; Holm 1994; Vera 1997; Lohengrin & Arroyo 2000). The percentage of seed germination may also be influenced by seed size (Harper 1977; Tripathi & Khan 1990; Lusk 1995; Vera 1997).

Temperature acts to regulate germination, by determining the capacity and rate of germination, by terminating dormancy, and by inducing secondary dormancy. Differences in the temperature requirements for germination can determine the distribution of plants by limiting the species to areas that have suitable temperatures for germination. Germination behaviour may also differ according to provenance of the seed (Bewley & Black 1994).

Previous studies have documented the influence of temperature on germination in Antarctic vascular plants (Corte 1961; Holtom & Greene 1967; Corner 1971; Edwards 1974), but the experiments were shorter, with fewer temperature treatments and seeds were not collected along an altitudinal gradient.

The present study analyses the effects of temperature, photoperiod, stratification, seed size and age of seed on germination of *D. antarctica* and *C. quitensis* seeds from different altitudes and sites over two years. Different germination responses to varying temperature, and the potential effects of climate change on the spread of these species are assessed. This investigation forms part of a comprehensive study on Livingston Island that aims to provide a better knowledge on processes of colonization and reproduction of both vascular Antarctic plants and how the effects of climate change could influence populations of these species (Vera 2011; Vera 2017, Vera et al.2013).

## Material and methods

### ***Study area***

Samples of *D. antarctica* and *C. quitensis* were collected during January and February 2002 near the Spanish Antarctic Base Juan Carlos I in South Bay, Livingston Island, South Shetland Islands (62º 39' 46'' S, 60º 23' 20'' W). The highest point in this zone, Mount Reina Sofía reaches 276 m altitude. A preliminary study was undertaken with samples of both species collected on January of 2000 in the same area.

The study area is formed by raised beaches, morainic deposits, ridges and erosive platforms (López-Martínez et al. 1992). The area investigated includes turbidic deposits, formed fundamentally of shales, sandstones and conglomerates, assigned to the Miers Bluff formation (Arche et al. 1992; Pallás et al. 1992). Some of these sites are also covered by pyroclastic material.

The mean summer (December-February) air temperature from 1988-2002 was 2.1ºC, the precipitation 109 mm and the average relative air humidity 80% (Bañon 2004). Soil surface temperatures along the altitudinal gradient where the seeds could germinate, were recorded at one hour intervals from 27 January 2002 to 21 February 2002, using two thermometers (Optic StowAway temp, Onset) placed on the bare ground surface at 20 and 147 m of altitude. A variation about 1ºC mean temperature was obtained along the altitudinal range. The average temperature during this period was 3.96ºC at 20 m and 2.99ºC at 147 m, although considerable fluctuations occurred (at 20 m, between -0.81ºC and 21,47ºC; at 147m, between -2.03ºC and 20.78ºC (Fig. 1).

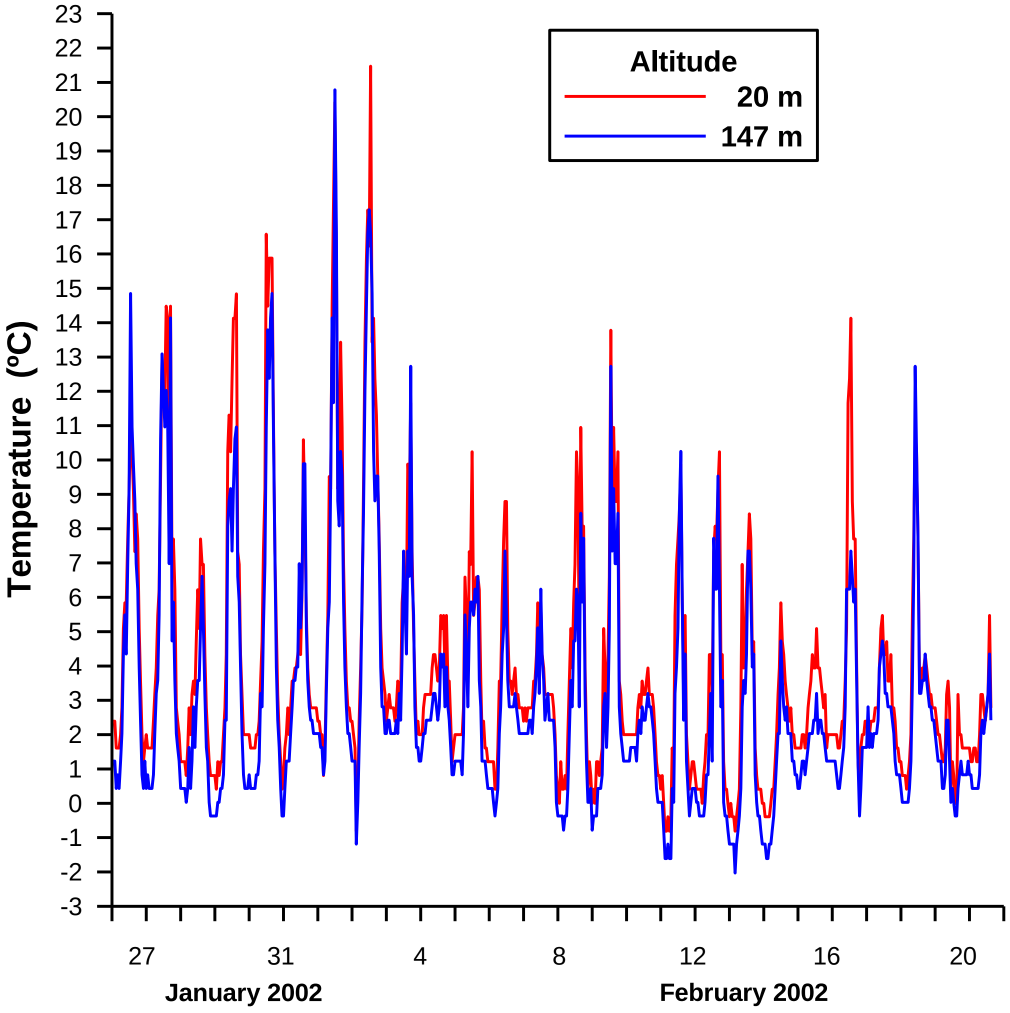

**Figure 1.** Variation of bare ground surface temperature at 20 and 147 m of altitude from 27 January to 21 February 2002.

To compare the temperature between different orientations of a ridge at 20 m and also the variation between soil cover with vegetation and bare ground, temperature data were recorded hourly using a Squirrel data logger (Grant Instruments, UK) from 10 until to 17 February 2002. Temperature sensors were inserted at 1 cm depth in the plants. There were wide fluctuations with the different situations, although the oscillations were mitigated in the *Colobanthus* cushion. The mean temperature within the tufts of *D. antarctica* growing on the flat ridge top was 4.73ºC, in *D. antarctica* occurring on the slopes facing NE was 5.89º C and in SW from the ridge was 3.44º C. The mean temperature within the compact cushion structure of *C. quitensis* growing on the flat ridge top was 4.34ºC. The mean temperature on bare ground was 3.10ºC.

The study sites were selected in 2002 from close to sea level up to the highest site where the two vascular plants occur together (147 m) in the examined area. The descriptions of sites are: at 10 m: Flat raised beach; at 20 m, three sites were studied: Top flat ridge, NE side of the ridge and SW side of the ridge; at 97 m: Top flat of an erosion platform and 147 m: Top flat morainic deposit.

### *Samples collected in January 2000 (preliminary study)*

Samples (seven at 10 m and four at 120 m) of *Deschampsia antarctica* with attached soil (about 5 cm deep) were collected on 6$^{th}$ of January of 2000. The samples were kept frozen (about -14ºC) until October-2000, when experiments were conducted in the laboratory in the University of Oviedo, Spain.

The seeds were then thawed and stored at 4-5ºC from 26-October-2000 until January 2001. For the first 40 days they were stored in dry conditions and later were stored moist (moist seeds subjected to cold stratification for a period could increasing their germination (Holtom & Greene 1967; Körner 1999; Ruhland &. Day 2001).

Ripe seeds (fully developed) were sampled from panicles that had developed in the previous growing season (1998-1999); Also, ripe seeds were recovered from the upper soil layer between 0 and 2cm depth (largely organic soil stratum with leaves and mineral constituents) , and from the deeper soil horizon (mineral soil stratum at 2-4cm below the surface). Seeds (105 and 31 from 10 m and 120 altitude respectively; the ripe seed production varied with altitude, Vera 2017) were placed on two moistened filter papers in Petri dishes (7 dishes from 10 m altitude, and 4 dishes from 120 m altitude) on 10 January 2001 and kept moist in a growth chamber at 20°C in a 8h dark/16h light regime until 12 February 2001. As no germination had occurred after 30 days the seeds were transferred to a 5°C 8h dark/19°C 16h light regime for 5 months. Seeds were watered regularly with deionised water and examined for germination weekly.

### *Samples collected in January-February 2002*

Six randomly chosen samples from individuals with similar size of *D. antarctica* and *C. quitensis* with attached soil **(**up to 10 cm deep in those more developed) were collected on 25-26 of January and on 21-22 of February 2002 at the different sites. The samples were shipped frozen (-20ºC) to Spain. From mid-April until July 2002 they were kept at 4ºC-5 º in a dry state.

Dry ripe (filled) seeds of both species from each site were selected (the number selected varied depending on ripe seed production in each site and month, Vera 2017; *D. antarctica:* January between 47 and 104; February: between 52 and 191, divided between 2 to 6 replicas depending on the amount of seeds; *C. quitensis*: January between 24 and 87; February: between 35 and 140, divided between 2 to 4 replicas depending on the amount of seeds) and stored moist under darkness at 4º-5ºC for two and a half months from 26 July until 9 October 2002. A percentage of seeds had germinated after of this period in darkness. In October the selected ripe seeds for each species from the different altitudes, microhabitats and experimental categories were sown in Petri dishes, on two layers of filter paper saturated with deionised water. Two replicates for each case and treatment were used, except sites with low production in *C. quitensis* (sites: 97 m and 147 m).The experimental categories were: (1) current year's seeds obtained from flowers developed during the growing season 2001-02 until end of February (in some sites the production was nil, see Table 3 and 4); (2) previous seasons' seeds from the surface to 2 cm of depth (organic stratum: within the cushion of *C. quitensis* or leaves and mineral soil in *D. antarctica*; (3) older seeds obtained from deeper soil, between 2 and 4 cm depth (mineral soil stratum). The relationship between depth of soil at which the seeds are buried and the time when they were released is based on Mcgraw & Vavrek (1989), Legg et al. (1992), and Bruggink (1993).

**Table 1**. Treatments of temperature applied to samples of *D. antarctica* and *C. quitensis* collected in January and February 2002 (A to E: seeds placed in chambers in October 2002, after of 5'5 months of cold storage: 3 months in dry conditions and later 2'5 months in moist conditions; F and G, only for *C. quitensis,* seeds placed in the chambers in November 2003, after of 19 months of cold storage in dry conditions). The duration which the seeds were subjected to different temperatures and photoperiods is shown in parentheses. For a better interpretation of the Figures 3 and 4, significant periods for germination (months since the beginning of the germination test) have been marked with bold letter.

| A | B | C | D | E | F | G |
|---|---|---|---|---|---|---|
| 3ºC dark/ 3ºC light (9/10/02 to 13/1/03) | 4ºC dark/ 4ºC light (9/10/02 to 13/1/03) | 3ºC dark / 3ºC light (9/10/02 to 18/11/02) | 4ºC dark/ 4ºC light (9/10/02 to 18/11/02) | 4ºC dark/19ºC light (9/10/02 to 8/1/04) | | |
| 4ºC dark/8ºC light (13/1/03 to 7/2/03) | 4ºC dark/8ºC light (13/1/03 to 7/2/03) | 4ºC dark/11ºC light (18/11/02 to 23/9/03) **(1-11.5$^{th}$ months test)** | 4ºC dark/11ºC light (18/11/02 to 23/9/03) **(1-11.5$^{th}$ months test)** | | | |
| 4ºC dark/9ºC light (7/2/03 to 30/5/03) **(4-7.5$^{th}$ months test)** | 4ºC dark/9ºC light (7/2/03 to 30/5/03) **(4-7.5$^{th}$ months test)** | | | | | |
| 4ºC dark/11ºC light (30/5/03 to 23/9/03) | 4ºC dark/11ºC light (30/5/03 to 23/9/03) | | | | | |
| 4ºC dark/15ºC light (23/9/03 to 8/1/04) | 4ºC dark/15ºC light (23/9/03 to 8/1/04) | 4ºC dark/15ºC light (23/9/03 to 8/1/04) | 4ºC dark/15ºC light (23/9/03 to 8/1/04) | | 4ºC dark/15ºC light (4/11/03 to 8/1/04) | 4ºC dark/19ºC light (4/11/03 to 8/1/04) |
| 4ºC dark/15ºC dark (8/1/04 to 23/1/04) **(15$^{th}$ month test)** | 4ºC dark/15ºC dark (8/1/04 to 23/1/04) **(15$^{th}$ month test)** | 4ºC dark/15ºC dark (8/1/04 to 23/1/04) **(15$^{th}$ month test)** | 4ºC dark/15ºC dark (8/1/04 to 23/1/04) **(15$^{th}$ month test)** | 4ºC dark/19ºC dark (8/1/04 to 23/1/04) **(15$^{th}$ month test)** | 4ºC dark/15ºC dark (8/1/04 to 23/1/04) | 4ºC dark/19ºC dark (8/1/04 to 23/1/04) |
| 4ºC dark/15ºC light (23/1/04 to 12/2/04) | 4ºC dark/15ºC light (23/1/04 to 12/2/04) | 4ºC dark/15ºC light (23/1/04 to 12/2/04) | 4ºC dark/15ºC light (23/1/04 to 12/2/04) | 4ºC dark/19ºC light (23/1/04 to 12/2/04) | 4ºC dark/15ºC light (23/1/04 to 12/2/04) | 4C dark/19ºC light (23/1/04 to 12/2/04) |
| 4ºC dark/15ºC dark (12/2/04 to 2/3/04) | 4ºC dark/15ºC dark (12/2/04 to 2/3/04) | 4ºC dark/15ºC dark (12/2/04 to 2/3/04) | 4ºC dark/15ºC dark (12/2/04 to 2/3/04) | 4ºC dark/19ºC dark (12/2/04 to 2/3/04) | 4ºC dark/15ºC dark (12/2/04 to 2/3/04) | 4ºC dark/19ºC dark (12/2/04 to 2/3/04) |
| 4ºC dark/15ºC light (2/3/04 to 19/4/04) | 4ºC dark/15ºC light (2/3/04 to 19/4/04) | 4ºC dark/15ºC light (2/3/04 to 19/4/04) | 4ºC dark/15ºC light (2/3/04 to 19/4/04) | 4ºC dark/19ºC light (2/3/04 to 19/4/04) | 4ºC dark/15ºC light (2/3/04 to 19/4/04) | 4ºC dark/19ºC light (2/3/04 to 19/4/04) |
| 4ºC dark/15ºC dark (19/4/04 to 11/5/04) **(19$^{th}$ month test)** | 4ºC dark/15ºC dark (19/4/04 to 11/5/04) **(19$^{th}$ month test)** | 4ºC dark/15ºC dark (19/4/04 to 11/5/04) **(19$^{th}$ month test)** | 4ºC dark/15ºC dark (19/4/04 to 11/5/04) **(19$^{th}$ month test)** | 4ºC dark/19ºC dark (19/4/04 to 11/5/04) **(19$^{th}$ month test)** | 4ºC dark/15ºC dark (19/4/04 to 11/5/04) **(5.5-6$^{th}$ months test)** | 4ºC dark/19ºC dark (19/4/04 to 11/5/04) **(5.5-6$^{th}$ months test)** |
| 4ºC dark/15ºC light (11/5/04 to 13/6/04) | 4ºC dark/15ºC light (11/5/04 to 13/6/04) | 4ºC dark/15ºC light (11/5/04 to 13/6/04) | 4ºC dark/15ºC light (11/5/04 to 13/6/04) | 4ºC dark/19ºC light (11/5/04 to 13/6/04) | 4ºC dark/15ºC light (11/5/04 to 13/6/04) | 4ºC dark/19ºC light (11/5/04 to 13/6/04) |
| 4ºC dark/15ºC dark (13/6/04 to 5/7/04) | 4ºC dark/15ºC dark (13/6/04 to 5/7/04) | 4ºC dark/15ºC dark (13/6/04 to 5/7/04) | 4ºC dark/15ºC dark (13/6/04 to 5/7/04) | 4ºC dark/19ºC dark (13/6/04 to 5/7/04) | 4ºC dark/15ºC dark (13/6/04 to 5/7/04) | 4ºC dark/19ºC dark (13/6/04 to 5/7/04) |
| 4ºC dark/15ºC light (5/7/04 to 27/7/04) | 4ºC dark/15ºC light (5/7/04 to 27/7/04) | 4ºC dark/15ºC light (5/7/04 to 27/7/04) | 4ºC dark/15ºC light (5/7/04 to 27/7/04) | 4ºC dark/19ºC light (5/7/04 to 27/7/04) | 4ºC dark/15ºC light (5/7/04 to 27/7/04) | 4ºC dark/19ºC light (5/7/04 to 27/7/04) |

| | | | | | | |
|---|---|---|---|---|---|---|
| 4ºC in darkness (27/7/04 to 7/9/04) | 4ºC in darkness (27/7/04 to 7/9/04) | 4ºC in darkness (27/7/04 to 7/9/04) | 4ºC in darkness (27/7/04 to 7/9/04) | 4ºC in darkness (27/7/04 to 7/9/04) | 4ºC in darkness (27/7/04 to 7/9/04) **(8.5-10th months test)** | 4ºC in darkness (27/7/04 to 7/9/04) **(8.5-10th months test)** |
| 4ºC dark/15ºC light (7/9/04 to 9/12 /04) | 4ºC dark/15ºC light (7/9/04 to 9/12 /04) | 4ºC dark/15ºC light (7/9/04 to 9/12 /04) | 4ºC dark/15ºC light (7/9/04 to 9/12 /04) | 4ºC dark/19ºC light (7/9/04 to 9/12 /04) | 4ºC dark/15ºC light (7/9/04 to 9/12 /04) | 4ºC dark/19ºC light (7/9/04 to 9/12 /04) |

The length of seeds was determined with a micrometer under a stereoscopic microscope in dry conditions. The mean size (referred to the length) of filled (ripe) seeds in dry conditions from the different sites did not vary greatly (*D.antarctica*, populations mean varied between 1.39 and 1.49 mm, range seed length:1.10-1.90 mm; *C.quitensis*, populations mean varied between 0.61 and 0.68 mm, range seed length: 0.50-0.85 mm).

Several capsules of *Colobanthus quitensis* (from the current and previous seasons) collected at 10 and 20 m altitude were also stored in darkness in dry conditions at 4ºC for 19 months from April 2002 until November 2003. Seeds from these capsules were sown in Petri dishes in November 2003 as described above.

The Petri dishes were kept in different chambers with a determined temperature, and according to the type of treatment, the temperature were changed in the chambers throughout the germination test period (Table 1). The seeds were exposed to a photoperiod of 8 h darkness/16h light (range 60-115 µmol$^{-2}$ s$^{-1}$ ), except some periods which were in darkness (to stimulate the germination, when the germination was very low or nil) . The different treatments of temperature and darkness periods are shown in the Table 1. Not all seeds from different sites were subjected at each treatment, since the production of ripe seeds was scarce in some site (see tables 3 and 4). A record of successful seed germination was made every week. The length of germinated seeds was determined as above, but in moist conditions (seed size usually increased 0.05 and 0.1 mm when soaked in water).

### *Statistical analyses*

Analysis of variance (ANOVA) was used to examine the differences in germination between seeds subjected to different temperature treatments, seed size and sites.

Pearson's correlation was applied to examine the relationships between the size (length) germinated seeds and the treatments, and between the percentage of germination of seeds collected from different altitudes and the different treatments.

The statistical analyses were only carried out for the study using the comprehensive samples collected in 2002.

## Results

### *Germination in darkness*

A small percentage of moist seeds of both species germinated in darkness at low temperature: 4º-5ºC between 26 July and 9-October 2002 (Table 2). However, no current year's seeds of *D. antarctica* remaining in the panicles germinated, and only a few of the current season's seeds of *C. quitensis* from the 10 m altitude site germinated. All germinated seeds of *D. antarctica* and most of those of *C. quitensis* were derived from the previous growing seasons. No significant differences were found between sites, although seeds of *Colobanthus* from higher altitudes tended to germinate better.

Rarely, seeds of both species placed in growth chamber germinated in the short periods in darkness. However, *D. antarctica* seeds, generally enhancing germination when these were placed in darkness for some weeks during the treatments (15th month since test the beginning of the germination test) and subsequently they were again exposed to light. In the case of *C. quitensis*, this only occurred in the seeds placed in growth chambers in November 2003 (treatments F and G), being remarkable its germination to the few days at the end of the period in darkness in the 10th month germination test.

**Table 2.** Mean (±SE) germination percentage of current (Curr.) and previous (Prv. ) seasons' *Deschampsia antarctica* and C*olobanthus quitensis* seeds after two months in darkness at low temperature (4-5ºC), collected in January (Janu.) and February (Febr.) 2002. The mean germinated seed size and ranges are shown at the sites where seeds were in good condition (only for *D.antarctica*).

| | Sites | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 10 m | | Top ridge-20 m | | NE 20 m | | SW 20 m | 97 m | | 147 m | |
| | Janu. | Febr. | Janu. | Febr. | Janu. | Febr. | Febr. | Janu. | Febr. | Janu. | Febr.: |
| *D.antarctica* | Prv. 0% | Prv.6 % (±3)<br>1.45 mm<br>(1.4-1.5) | Prv.8 % (±1) | Curr.0 %<br>Prv.4 % (±2)<br>1.67 mm<br>(1.65-1.7) | Curr. 0%<br>Prv. 5 % (±5) | Curr. 0 %<br>Prv.10 % (±3)<br>1.48 mm<br>(1.4-1.6) | Curr. -<br>Prv.: 18 % (±4)<br>1.49 mm<br>(1.4-1.7) | Prv.1 % (±1) | Curr.0 %<br>Prv.11 % (±8)<br>1.45 mm<br>(1.3-1.6) | Prv.0<br>% | Curr.-<br>Prv.7 % (±6)<br>1.45 mm<br>(1.3-1.6) |
| *C. quitensis* | Prv. 0% | Curr. 1 %<br>Prv.2 % (±2) | Prv. 0% | Curr. 0 %<br>Prv.3%<br>(±0.2) | Curr. 0%<br>Prv. 0 % | Curr. 0 %<br>Prv. 2 % (±2) | Curr. -<br>Prv. 2% (±2) | Prv. 0 % | Curr.:0 %<br>Prv.9% (±9) | Prv.0<br>% | Curr. 0 %<br>Prv.6 % (±6) |

### *Effect of temperature, site and longevity on germination*

The percentages of *Deschampsia antarctica* seeds, collected in January 2000 at 10 and 120 m, that germinated are shown in Fig. 2. The results were similar for both altitudes, although germination was slightly higher in seeds obtained from the lowest altitude. A contrasting thermoperiod: 5º/19ºC applied one month after sowing the seeds improved the germination percentage. Only seeds from the previous growing seasons and released into the soil germinated. Filled seeds from the previous season and remaining in the panicle did not germinate.

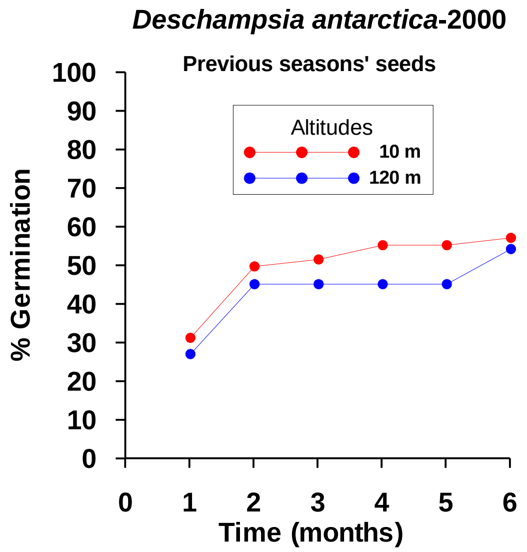


**Figure 2.** Cumulative germination percentage during six months after sowing of *Deschampisa antarctica* seeds (collected in January 2000) from previous seasons' production at different altitudes. Seeds were incubated at 20ºC with a photoperiod 8 h darkness/16 h light from 10/1/2001 until 12/2/2001, and at 5ºC dark/19ºC light from 12/2/2001 until 12/7/2001.

Seeds of *D. antarctica* collected in 2002 had a higher germination percentage than those of *C. quitensis* (Tables 3 and 4, Figures 3 and 4). The responses in germination to different temperature treatments varied according to the origin and age of the seeds of *D. antarctica* . The previous seasons' seeds collected in February at lower sites and subjected to treatments C and D seem to have a better germination (reaching in the most cases the 100%) and were the first to begin germination (Table 3 and Fig.3). Unfortunately, these treatments were not applied to seed collected in February from higher altitudes. The seeds from higher altitudes germinated better with lower temperature treatments (A and B), and lower germination with the treatment with higher temperature treatments (E). The *Deschampsia* seeds subjected to a constant temperature of 3º or 4 º C (the 3 first months of treatment A or B) did not germinate (Fig.3). The germination of most seeds with treatments A and B was stimulated when the temperature was increased from 4º/11º C to 4º/15ºC and after the seeds were subjected to a dark vernalisation period and a later dark/light regime (Table 1 and Fig. 3). This treatment had little or no effect in the other experiments. Some seeds of *D. antarctica* collected from 20 m on a NE facing ridge germinated at 4ºC dark/15ºC light almost two years after sowing following placement at 4ºC in darkness for five weeks (Fig. 3).

**Table 3**. Mean (±SE) germination percentage of current (except in sites with no or low production) and previous seasons' seeds *Deschampsia antarctica*, collected in January and February 2002, after 26 months from sowing for different treatments (see table 1). Not all seeds were subjected at each treatment (-). All previous seasons' seeds were obtained from 0-2 cm depth (see methods). The mean germinated seed size and ranges (mm) are shown. Asterisks indicate level of significance of differences between treatments: *** $P$<0.001; ** $P$<0.01; * $P$<0.05; n.s., non significant.

| | Treatments | | | | |
|---|---|---|---|---|---|
| Sites | A | B | C | D | E |
| 10 m. January<br>Previous seasons' seeds. n.s. | 0 | 20 % (±20)<br>1.7 ( 1.7-1.7) | 0 | 0 | 0 |
| 10 m. February<br>Previous seasons'seeds * | 80 % (±10)<br>1.7 (1.6-1.9 ) | 60 % (±5)<br>1.65 (1.5-1.85) | 100% (±0)<br>1.56 (1.4-1.8) | 100 % (±0)<br>1.59 (1.4-1.75) | 85 % (±1.2)<br>1.51 (1.15-1.8) |
| Top ridge 20 m. January<br>Previous seasons'seeds | 0 | 0 | - | - | 0 |
| Top ridge 20 m. February<br>Current season's seeds | 0 | 0 | 0 | 0 | 0 |

| | | | | | |
|---|---|---|---|---|---|
| Top ridge 20 m. February<br>Previous seasons'seeds * | 0 | 0 | 40 % (±10)<br>1.67 (1.65-1.7) | 25 % (±5)<br>- | 34 %(±15.5)<br>1.48 (1.4-1.6) |
| NE 20 m. January<br>Previous seasons' seeds n.s. | 0 | 0 | - | - | 13 % (±6.6)<br>1.5 (1.5-1.5) |
| NE 20 m. February<br>Current season's seeds | 0 | 0 | - | - | 5 %<br>1.8 (1.8-1.8) |
| NE 20 m. February<br>Previous seasons' seeds n.s. | 81 % (±6.2)<br>1.50 (1.4-1.7) | 81 % (±6.2)<br>1.48 (1.3-1.6) | 100 % (±0)<br>1.47 (1.4-1.55) | 87 % (±10.6)<br>1.48 (1.35-1.65) | 78 % (±15.7)<br>1.53 (1.3-1.8) |
| SW 20 m. February<br>Previous seasons' seeds n.s. | 44 % (±6)<br>1.60 (1.5-1.65) | 78 % (±7)<br>1.52 (1.3-1.6) | - | - | 68 % (±5.1)<br>1.51 (1.4-1.6) |
| 97 m. January<br>Previous seasons' seeds n.s. | 12 % (±12)<br>1.5 (1.5-1.5) | 0 | 0 | 0 | 0 |
| 97 m. February<br>Previous seasons' seeds * | 75 % (±12)<br>1.47 (1.3-1.6) | 50 % (±5)<br>1.41 (1.3-1.5) | - | - | 20 % (±12.2)<br>1.39 (1.3-1.5) |
| 147 m. January<br>Previous seasons' seeds | 0 | 0 | 0 | 0 | 0 |
| 147 m. February<br>Previous seasons' seeds * | 62 % (±6)<br>1.54 (1.4-1.6) | 75 % (±7)<br>1.56 (1.45-1.65) | - | - | 21 % (±8.2)<br>1.40 (1.3-1.5) |

## *Deschampsia antarctica* - 2002

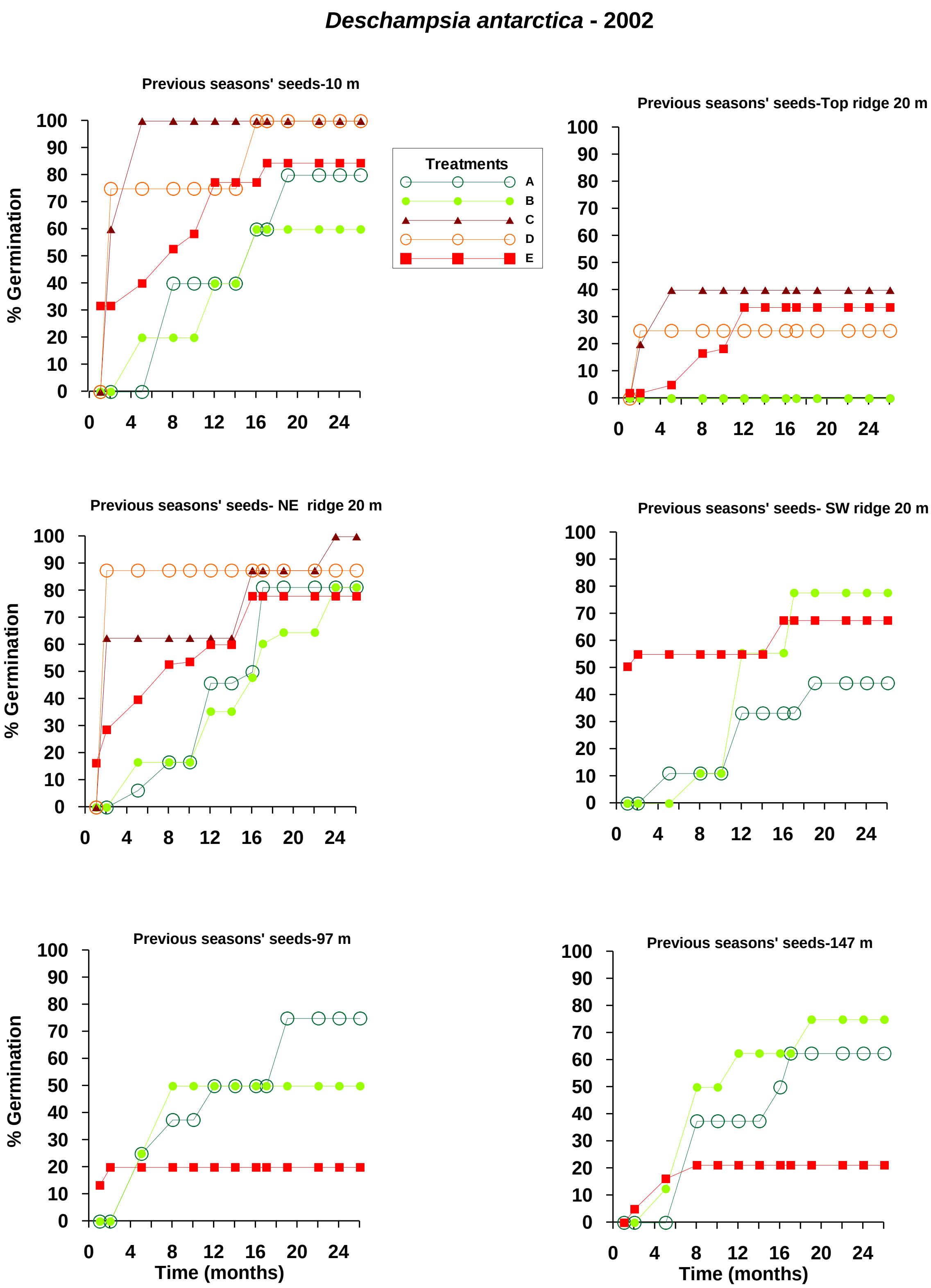


**Figure 3.** Cumulative germination percentage over 25 months from sowing of *Deschampsia antarctica* seeds collected in February 2002 at different sites for different light and temperature treatments (see Table 1). In those sites with low seed production not all treatments could be applied (Table 3). In the cases, where the germination was nil or low, are not showed in the graphic (Table 3).

There were significant differences ($p<0.0001$) in germination of the previous seasons' seeds of *D. antarctica* from different sites. The highest germination occurred at the lowest site (10 m) and the 20 m NE facing ridge site. The percentage of germination six months after sowing did not differ greatly with that obtained for seeds collected in 2000. Only seeds from 10 m and the 20 m NE facing ridge from the 2002 year reached a higher germination with treatments C and D in the first six months of the germination trial.

The germination response of *Colobanthus quitensis* seeds to treatment temperatures A, B, C, D and E varied slightly at the end of experiment (Table 4), with the lowest germination in treatment E.

The different experimental temperature regimes influenced on germination of *C. quitensis*. There was no germination (with one exception) in the treatments A,B,C and D (similar to the results obtained for *D. antarctica*), when the temperatures were low (3º-4ºC) during all photoperiod (dark/light) regimes. The germination response of seeds to a higher temperature varied between treatments. Seeds cultivated at lower temperatures for longer times (A and B) tended to have a better response to a higher temperature (4º/11º) than those at low temperature for a short time (C and D) (Table 1, Fig. 4). However, the current year's seeds collected at 10 m and subjected to treatments C and D showed a slight increase in germination when placed at 4º/15ºC.

The previous seasons' seeds of *C. quitensis* from the lowest site stored in dry conditions at 4ºC during for 19 months (treatments F and G) had a higher germination after twelve months from sowing (Table 4. Fig. 4) than those subjected to other treatments. Most seeds subjected to treatments F and G germinated in the 10th-11th months of the germination test (4ºC dark/15ºC light and 4ºC dark/18ºC light respectively) after that they were stored for five weeks at 4ºC in darkness and moist conditions (Table 1, Fig. 4).

There were significant differences ($p=0.001$) in responses of *Colobanthus* seeds from different sites. The current season's seeds from near level sea had the best germination (except treatments F and G). Seeds from higher altitudes showed no germination, but these seeds were not stored long term at low temperatures (treatments F and G). The seeds collected from 20 m also showed no or low germination (Table 4).

Seeds age had an effect on germination. The *Colobanthus* current season's seeds from sites with higher temperature at 10 m and from the 20 m NE ridge had better germination that the previous seasons' seeds collected in January and February, except if they were subjected to long term cold treatment (treatments F and G). The response of *Deschampsia* seeds collected in 2002 was different, as there was no or very low germination of current season's filled seeds at the lower sites (current season's filled seeds were not found at the higher altitudes) (Table 3). The previous seasons' seeds of *D. antarctica* collected in February 2002 usually had a higher germination than those collected in January (seeds from the ridge top at 20 m showed no germination with treatments A and B, because they were infected by fungi). Only some of the previous seasons' seeds from plants collected in January 2002 and collected from the soil germinated (Table 3), while the percentage germination of seeds from those plants collected in January 2000 was higher, as indicated above. The seeds with dark coloration did not germinate. Few seeds from the 2000 year collected from 2-4cm depth in the soil germinated (about 10 % of total germination ), while there was no germination of seed collected in 2002 at the same site.

**Table 4.** Mean (±SE) germination percentage of current (except in sites with no or low production) and previous seasons' *Colobanthus quitensis* seeds, collected in January and February 2002, after 26 months from sowing for the treatments: A to E, and after 12 months for the treatments: F and G (see table 1). Not all seeds were subjected at each treatment (-). All previous seasons' seeds were obtained from the upper 2 cm of *Colobanthus* cushion. The mean germinated seed size and ranges (mm) are shown. Asterisks indicate level of significance of differences between treatments: *** *P*<0.001; ** *P*<0.01; * *P*<0.05; n.s., non significant.

| | Treatments | | | | | | |
|---|---|---|---|---|---|---|---|
| Sites | A | B | C | D | E | F | G |
| 10 m. January * Previous seasons' seeds | 4 % (±4)<br>0.7 (0.7-0.7) | 11 % (±1)<br>0.7 (0.7-0.7) | 0 | 0 | 0 | - | - |
| 10 m. February * Current season's seeds | 32 % (±4)<br>0.74 (0.65-0.8) | 25 % (±5)<br>0.7 (0.65-0.8) | 43 % (±5)<br>0.78 (0.75-0.8) | 40 % (±5)<br>0.7 (0.7-0.7) | 4 % (±2) | 40 % (±10)<br>0.72(0.6-0.8) | 53 % (±13.3)<br>0.75 (0.7-0.8) |
| 10 m. February * * Previous seasons' seeds | 12% (±12)<br>0.7 (0.7-0.7) | 0 | 17 % (±17)<br>0.6 (0.6-0.6) | 0 | 0 | 64 % (±22)<br>0.78(0.6-0.9) | 54 % (±4.1)<br>0.73(0.65-.8) |
| Top ridge 20 m. January Previous seasons' seeds | 0 | 0 | - | - | 0 | - | - |
| Top ridge 20 m. February Current season's seeds | 0 | 0 | 0 | 0 | 0 | - | - |
| Top ridge 20 m. February Previous seasons' seeds | 0 | 0 | 0 | 0 | 0 | - | - |
| NE 20 m. January Previous seasons' seeds | 0 | 0 | 0 | 0 | 0 | - | - |
| NE 20 m. February ** Current season's seeds | 13 % (±3)<br>0.7 (0.7-0.7) | 12 % (±4)<br>0.72 (0.7-0.75) | 0 | 0 | 4 % (±2.8)<br>0.73(0.7-0.8) | 0 | 0 |
| NE 20 m. February n.s. Previous seasons' seeds | 0 | 0 | 0 | 0 | 0 | 0 | 17 % (±17)<br>0.6 (0.6-0-6) |
| SW 20 m. February n.s. Previous seasons' seeds | 0 | 0 | - | - | 17 % (±17) | - | - |
| 97 m. January Previous seasons' seeds | - | - | - | - | 0 | - | - |
| 97 m. February Previous seasons' seeds | 0 | 0 | - | - | - | - | - |
| 147 m. January. Previous seasons' seeds | 0 | 0 | - | - | 0 | - | - |
| 147 m. February Current season's seeds | 0 | 0 | - | - | - | - | - |
| 147 m. February Previous seasons' seeds | 0 | 0 | - | - | 0 | - | - |

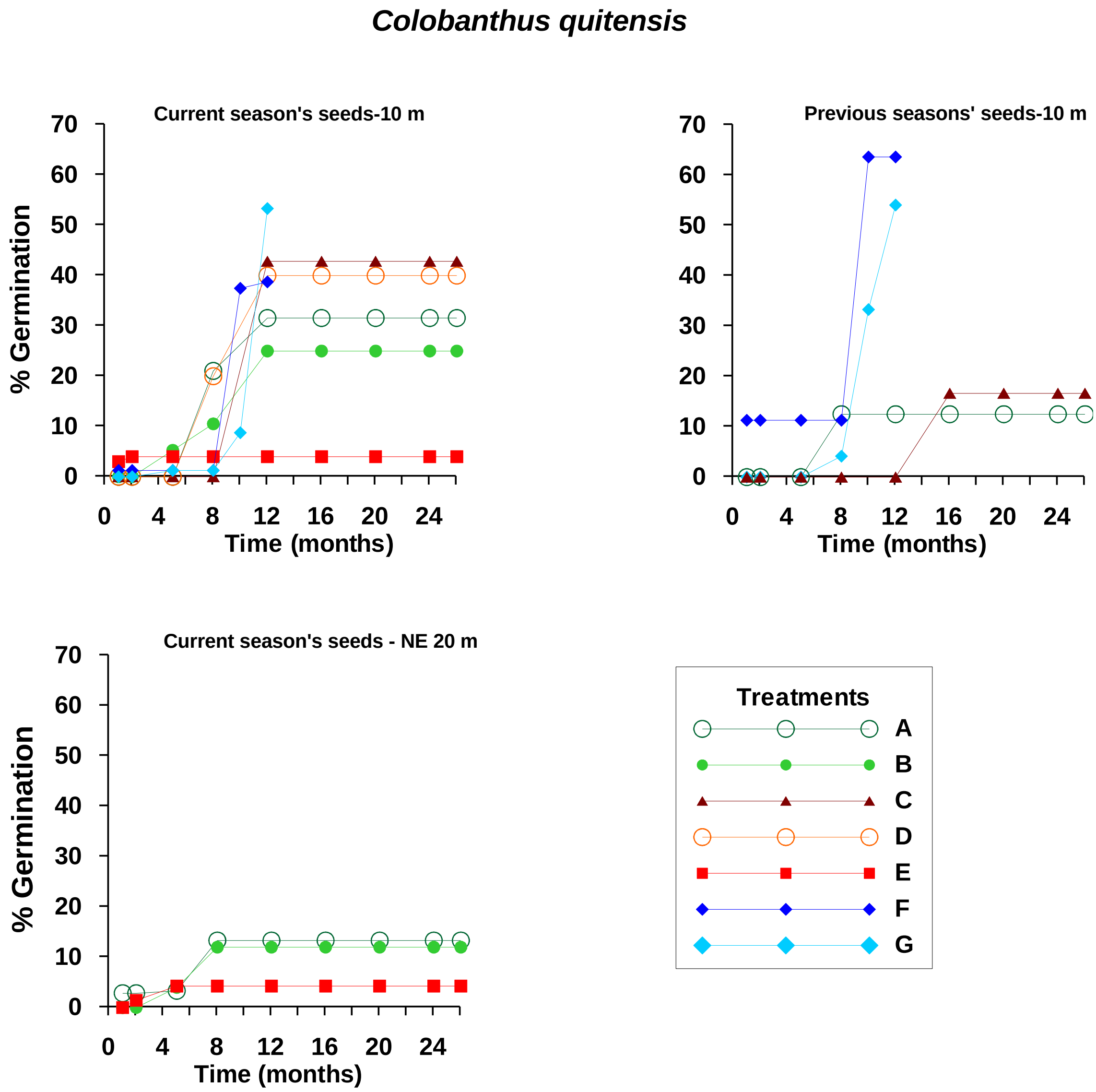


**Figure 4.** Cumulative germination percentage over 25 months from sowing of *Colobanthus quitensis* seeds collected in February 2002 at different sites for different light and temperature treatments (12 months for F and G treatments) (see Table 1). In those sites with low seed production not all treatments could be applied (Table 4). In the cases, where the germination was nil or low, are not showed in the graphic (Table 4).

### *Effect of seed size on germination*

Most of the germinated seeds (89%) of *D. antarctica* collected in 2002 had a length ≥ 1. 3 mm in moist conditions, while all germinated seeds from 2000 had a size ≥ 1. 4 mm.

A negative correlation was shown between the germinated seed size and the temperatures of different treatments (r=-0.178, p=0.007, n=191). The mean germinated seed size was usually larger in the colder treatments in each site.

The mean size of germinated *Deschampsia* seed varied significantly with the site (p=0.01). Germinated seed size decreased with altitude. However, the germination of moist seed stored in darkness (July to October 2002) at low temperatures did not vary significantly with seed size.

There was no significant difference in relation to germinated size seed of *C. quitensis* with the type of treatment or site.

## Discussion

### *Effect of light*

The percentage of germination of both species stored at 4-5ºC in darkness (before the beginning of treatments) is similar to results obtained in previous studies (Corner 1971). *Colobanthus* seeds from higher altitudes only germinated when they were stored at low temperatures in darkness at the beginning of this study, but there was no germination after they were subjected to dark/light treatments carried out with short or no period of low temperatures (seeds from high sites were not subjected to long period of cold stratification). The great number of germinated *Colobanthus* seeds with emergent radicles found inside the cushions could be explained because the seeds germinate with little light or in darkness. However, both species only germinated rarely when they were placed in darkness for some weeks during the treatments.

Light appeared to have a positive effect on germination. Although some seeds of both species germinated in darkness, a larger proportion germinated with a dark/light treatment. However, a long period with light could induce a secondary dormancy in moist seeds, and this dormancy could be broken when the seeds were subsequently held in darkness, enhancing germination when the seeds were again exposed to light. Light may inhibit the germination of some types of grass seeds that have imbibed water (Simpson 1990).

### *Effect of cold storage, longevity and altitude*

The different germination responses to cold pre-treatments (stratification) and temperature treatments in *Colobanthus* seeds suggests the importance of a long period of cold stratification for germination as implied in previous studies (Holtom & Greene 1967; Ruhland & Day 2001). In the present study, seeds with similar or shorter cold pre-treatments germinated better than in other investigations. The current season's seeds from the lowest site subjected to short cold stratification (6 months) had a higher percentage of germination (treatments A,B,C and D) than seeds with a similar period of cold storage: 4 months (6% germination) placed at constant temperature (21º) ( Ruhland & Day 2001). Also, germination was higher in current and previous seasons' seeds after 19 months of cold storage compared to the results obtained with a longer stratification: >4 years (38 % germination) (Holtom and Greene 1967; Ruhland & Day 2001). A possible explanation may be that the longer experimental duration of the present study, or, concurring with Holtom and Greene (1967), an alternation of low temperatures in darkness and a higher temperatures in the light could improve germination if the seeds are subjected to cold stratification over several months. Other previous studies show that seeds from arctic and high elevations, where environmental conditions are also severe, require a long period of cold stratification to germinate (Basking & Basking 1998; Lohengrin & Arroyo 2000). *Colobanthus quitensis* germination is higher with seeds which have overwintered and have remained moist (Holtom & Greene 1967; Edwards 1974 ). In the present germination experiments, few current season's seeds germinated before February 2003. Chilling represents a natural dormancy-breaking mechanism in many species when followed by favourable temperatures (Baker 1989; Probert 1992; Vera 1997; Basking & Basking 1998; Körner 1999; Lohengrin & Arroyo 2000). However, in Antarctic *C. quitensis* the current year's seeds seem to require a longer period of cold stratification in order to germinate than seeds collected in other more temperate regions, where the response seems to be different. Most of the current year's seeds from Navarino Island (Chile), latitude 54º 55' S, germinated (at 4ºCdark/15ºClight) in the first month of a germination trial after they were stored for only seven months under dry conditions (with or without cold pre-treatment), while seeds in cold storage under moist conditions had a low germination (Vera unpublished data).

The response of Antarctic previous seasons' seeds of *C. quitensis* was very different. Previous seasons' seeds collected in January and February had no or low germination if they were not subjected to a long-term stratification. This suggests that these seeds could have a strong dormancy, and a long period of stratification is necessary to break dormancy. However, those seeds having experienced long term stratification treatments under dry conditions (F and G) showed little germination until they were stored moist for five more weeks in darkness at 4-5ºC after the 8th month of the germination trial. Other seeds subjected to other temperature treatments and stored under these conditions (after 21 months of the germination test: treatments A-E) did not show this response, although many seeds were in good condition. Previous studies show that germination is much faster in seeds stored at low temperatures in a moist rather than dry state (Körner 1999), but the different results of the present study and the results from Navarino Island, Chile, seeds show that cold storage under moist conditions may not always be better than storage under dry conditions. This variation may be due to a number of factors, for example origin of the seeds, their age, storage time and temperature treatments.

Seeds of *C. quitensis* collected from higher sites did not germinate. Unfortunately, *Colobanthus* seeds from higher altitudes were not stratified for long periods (F and G). Given longer stratification the seeds may germinate, because several seeds were apparently filled and many seedlings were observed in the higher altitude sites (Vera 2011).

The percentage of germination of *D. antarctica* was very variable. There was no or little germination of the current season's seeds under all experimental conditions. Few seeds may have been entirely mature in February 2002, although they were apparently filled. No filled current season's seeds were found at the higher altitudes. Germination of the previous seasons seeds collected from between the leaves and mixed with soil in January 2000 and 2002 was different. Germination of those from January 2000 was higher than those seeds collected in January 2002. The seeds were subjected to different treatments, with longer chilling of the seeds collected in 2000. Inter-annual variations in maturation of seeds could also explain in part this difference (especially regarding summer weather conditions, length of growing/snow-free season and inter-site microclimate variation). The low germination of the previous seasons' seeds collected from the vegetation cover mixed with soil in January 2002, compared to February 2002, could be due to the spikelets from previous growing season and released to the vegetation cover not yet being fully ripe in January 2002 and thus few seeds germinated. By February 2002 some of the previous growing season's seeds may have reached maturity and having fallen to the soil, increased the percentage of germination. This different response between the two months suggests that most of the previous seasons' seeds germinated from samples collected in February 2002 correspond to seeds from spikelets formed in the previous growing season, 2000-2001. However, full sized previous year seeds (only present at lowest altitudes) remaining in the panicle did not germinate, perhaps because they were not completely ripe.

All seeds which germinated were obtained from current year reproductive structures (rarely in *D. antarctica*), or from the previous seasons' flowers released onto the surface of the soil (surface to 2 cm depth) and mixed with leaves of *D. antarctica* or from cushions of *C. quitensis* (up to 2 cm depth in cushions). Most seeds below a depth of 2 cm of the surface were poorly developed or in degraded by fungal decay, frequently with dark coloration, and did not germinate. Only some *D. antarctica* seed with a clear brown coloration found in the 2-4 cm soil depth horizon germinated. This suggests what the seeds do not have great longevity under natural conditions, although they could remain viable for several years, as this study has shown, since some seeds germinated almost 2 years after sowing, even with temperatures of 15ºC. These seeds were also collected prior to the germination experiments. Seed longevity greater than 4 years has been shown for seeds stored at 3ºC (Ruhland & Day 2001), but no long term experiments have been conducted at higher temperatures. In the Maritime Antarctic, the surface of soil can reach 15ºC in the growing season (Edwards 1972; Vera, this study). At these temperatures seeds can be readily infected by fungi. At the end of the germination experiments some seeds were observed to be contaminated with fungi and at a depth below 2 cm in the soil; most seeds were dark and degraded by fungi, especially those from low altitudes (Vera 2017). Also, many *Colobanthus* filled seeds are attacked by fungi below 1cm of the surface of the cushions. Most of the old *Deschampsia* spiketets had seeds in a poor state of conservation and none germinated.

### *Effect of temperature*

Seed germination was influenced by temperature in both species, confirming results of previous studies (Corte 1961; Holtom & Greene 1967; Corner 1971; Edwards 1974). The optimal temperature, after pre-treatment at low temperature, varied with daily fluctuations between 4-5ºC dark/15-19ºC light, although there was a tendency for higher germination in this experiment using 4º/15ºC. Various experiments with arctic and alpine species confirmed the high temperature requirements for germination in cold climate plants, ensuring germination and growth under warm conditions (McGraw & Vavrek, 1989; Körner 1999; Lohengrin & Arroyo 2000). A constant temperature is less effective (Vera, this study; Holtom & Greene 1967). High continuous temperatures can introduce secondary dormancy in grass seeds (Simpson 1990). However, a high germination of *D. antarctica* has been achieved experimentally with continuous 20ºC (Corner 1971), but not as successfully as in the present experiments with seeds from low sites. Seeds of *D. antarctica* from higher altitudes had a better germination in the treatments with initial low temperatures (A and B, with respect to E). A stratification period may be important for breaking natural dormancy in theses seeds. Variations in the daily temperature of the soil surface in the study sites allows seeds of either species can germinate successfully , when these overwinter in darkness and low temperatures.

### *Effect of seed size*

The effect of seed size on germination success of *D. antarctica* varied in relation to the characteristics of the site from where the seed was collected. The mean germinated seed size was usually higher at the lowest altitude (10 m) , although the seed size from different altitudes was similar. Larger seeds contain a higher amount of nutritive substances for seedling development, survival and growth (Harper 1977; Gross 1984; Tripathi & Khan 1990; Reyes & Casal 1994; Vera 1997). The larger seeds from the different sites had a better germination with the colder treatments. Possibly improved germination at suboptimal temperatures may be due to higher storage reserves of these seeds (Gross 1984; Tripathi & Khan 1990; Arista *et al*. 1992; Vera 1997).

## Conclusions

Seeds of *D. antarctica* and C. *quitensis* require warm conditions with daily temperature fluctuations for germination. Similar conditions were recorded on the bare ground surface ensuring germination.

Seeds, mainly *C. quitensis,* improved germination when they are subjected to cold pre-treatment in darkness a long period. In the Antarctica with dark and cold winters may facilitate seed germination, when these are exposed later to periods with daily dark/light and temperature fluctuations.

Although the germination percentage of *D. antarctica* seeds seems to be higher, a greater number of seedlings of *C. quitensis* were found in the studied area. This could be due to *C. quitensis* produces more ripe seeds than *D. antarctica* (Vera 2011, 2017) and their current year´s ripe seeds overwintering at low temperatures has a higher germination than *D. antarctica.* However, the mortality of *C. quitensis* seedlings is elevated (Edwards 1974; Fowbert & Lewis Smith 1994; Vera 2011).

The reproductive capacity by seeds is lower at the high altitudes in the study area (Vera, 2011). A lower number of ripe seeds were found at higher altitudes (Vera 2017), and this study shows that seeds from high altitudes had a lower germination.

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