

# **Environmental factors affecting flower and seed production in *Deschampsia antarctica* and *Colobanthus quitensis* on Livingston Island (South Shetland Islands, Antarctica)**

**MARÍA LUISA VERA**

Departamento de Biología de Organismos y Sistemas. Universidad de Oviedo. 33071 Oviedo. Spain. [mlvera@uniovi.es](mailto:mlvera@uniovi.es)

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## **Abstract**

The variation in floral and seed development, as well as the seed bank, along an altitudinal gradient (10 to 147 m) and on different orientations (NE and SW), of the two native vascular species was studied on Hurd Peninsula at Livingston Island, Antarctica. The phenological cycles have been described quantifying the flower and seed production. Flower density in both species increased during the austral summer from early January until the end of February, except for *C. quitensis* at the highest altitude. The more advanced flower and seed maturation at lower sites suggests an important effect of temperature on reproduction through seed production. The production of ripe seeds from the current and previous seasons' seed decreased with altitude. Seeds of both species at lower sites can reach full maturation in one growing season. A higher production of ripe seeds was found on NE orientated slopes (warmer than the other orientations) and in less compacted soils.

A better development of the reproductive structures at the lower altitudes, with more probability to complete the full maturation of seeds in the current year than at the higher sites (altitudinal gradient about 1° C) shows that the Antarctic vascular plants are highly sensitive to temperature. An increase in reproduction through seed production over the last decades (mean summer temperature rise of more than 1 °C over the last three decades) appears closely linked to the rapid expansion of both species (distribution and abundance) in the Antarctic Peninsula area.

**Key words:** Antarctic vascular plants reproduction, altitude and orientation effect, climate change, seed bank.

## Introduction

*Deschampsia antarctica* E. Desv. (Poaceae) and *Colobanthus quitensis* (Kunth) Bartl. (Caryophyllaceae) are the only native vascular plants living in Antarctica, but they also occur outside the Antarctic biome (Holtom & Greene 1967; Greene 1970; Corner 1971; Greene & Holtom 1971; Convey 1996; Lewis Smith 2003). Both species are widespread in the Maritime Antarctic region. In Antarctica both species occur at the southern limit of their geographical range, and changes in their physiology, phenology and microdistribution can be used as both indicators and monitors of biological responses to global climate change. An increase of more than 1°C mean summer air temperature has been measured in the Maritime Antarctic region since 1950 (Lewis Smith 1994; Fowbert & Lewis Smith 1994; Turner et al. 2005; [www.antarctica.ac.uk/met/gjma/main.html](http://www.antarctica.ac.uk/met/gjma/main.html)), and the consequent increase in length of the growing season has caused a rapid expansion of these plants during the last decades (Fowbert & Lewis Smith 1994; Lewis Smith 1994; Grobe et al. 1997; Day et al. 1999; Walther et al. 2002; Robinson et al. 2003; Gerighausen et al. 2003, Convey et al. 2011, Vera 2011).

To understand the potential effects of climate change on the spread of the species, quantitative analysis of seasonal reproductive performance, seedling development and demographic structure of populations under different environmental conditions is critical, as is comparative analysis of new and well established populations.

Temperature is a critical factor in the reproductive success when a species occurs at the edge of its ecological range (Wookey et al. 1993; Havström et al. 1993; Körner 1999). To determine the influence of temperature and other environmental factors on sexual reproduction of species it is necessary to know how these physical parameters affect the different phases of the reproductive cycle. Field manipulation experiments have shown that the development of reproductive structures in Antarctic vascular plants improved in response to warming (Edwards 1974; Day et al. 1999). However, few floral characters were measured under experimental conditions. The responses of the Antarctic flowering plants to changes in temperature or other environmental conditions have been scarcely explored in the field. Previous data on floral and seed development in both species on islands of the maritime Antarctic were based mainly on studies of herbarium specimens or field observations, with few analyses of reproductive characteristics (Holtom & Greene 1967; Greene & Holtom 1971; Corner 1971; Edwards 1974; Convey 1996). Most of the observations have been carried out in different years, and few reflect responses to orientation and elevation, key parameters that can determine reproductive and colonization capacity. Therefore, a precise estimation of the size of reproductive structures and efficacy of seed production along an altitudinal gradient in a single growing season (current year), as well as a comparison with previous years, is important since the number of ripe seeds developed in both species growing in the severe Antarctic environment can vary widely between years. A comparison between the present results and those of studies on reproductive performance of *D. antarctica* and *C. quitensis* carried out more than three decades earlier (Holtom & Greene 1967; Greene & Holtom 1971; Edwards 1974), may allow an estimate of the effect of climatic change on the reproductive process.

Several studies have suggested the importance of seed size for successful germination and seedling establishment. Larger seeds contribute to a higher germination percentage in some species (Harper 1977; Gross 1984; Tripathi & Khan 1990; Lusk 1995; Vera 1997). Edwards (1974) indicated a minimum seed size for successful germination in *D. antarctica* (1.4 mm) and *C. quitensis* (0.5 mm). However, few seed measurements in populations from different sites have been recorded and most of them were from studies made more than three decades ago (Holtom & Greene 1967; Greene & Holtom 1971; Corner 1971; Edwards 1974).

The soil seed bank density relative to annual seed production in both species is another key feature (Thompson 1992), because the persistence of viable seed in the soil may affect the persistence of these species in places where they already exist, or their ability to colonise new sites if the seeds can be dispersed. Seed bank densities have been estimated from germinated seedlings (Mcgraw & Day 1997; Ruhland & Day 2001). However, the density of the seed bank could be underestimated when factors such as seed dormancy, stratification history, seed storage time are not known and germination tests are run for only a short period of time or optimal conditions during germination experiments have not been attained. A viable seed bank of these flowering plants can persist when vegetation/soil samples were stored experimentally at 3°C (Ruhland & Day 2001). However, soils in Antarctica can attain much higher temperatures under ambient conditions (Bañón et al. 2013, Vera 2017) and the viability of seeds may decrease with time. There is usually a relationship between the depth of soil at which the seeds are buried and the time when they were released (Mcgraw & Vavrek 1989; Legg et al. 1992; Bruggink 1993). However, no previous studies have recorded the variation in the seed bank at different soil depths in Antarctica.

The aim of this paper was the quantification of the development of reproductive structures (maturation stages, floral and seed development, flower and seed production) of *D. antarctica* and *C. quitensis* along an altitudinal gradient and at different orientations. The influence of these variables on the success of seed maturation and on seed bank structure was also analysed, with special emphasis on seed size, maturation degree and seed germination capabilities. The results were compared with historical data. This paper forms part of a comprehensive study that aims to establish a consistent baseline for a long term monitoring program studying the effects of climate change driving modifications to Antarctic flowering plant populations (Vera 2011, 2017, Vera et al. 2013).

## Material and Methods

### *Study area*

Sampling was carried out near the Spanish Antarctic Base Juan Carlos I in South Bay, Hurd Peninsula, Livingston Island, South Shetland Islands (62° 39' 46'' S, 60° 23' 20'' W), in February 2000 and during January and February 2002. A diverse array of sites with elevations from near sea level up to 276 m, Mount Reina Sofía, the highest point in this zone. Glacial retreat has been reported in recent years from near the Spanish Base, exposing new sites for colonization (Calvet *et al.* 1992). The investigated sites occur on raised beaches, moraine deposits, ridges and erosive platforms, as described by López-Martínez *et al.* (1992) and Vera (2011).

Preliminary studies were carried out with seven samples of *D. antarctica* collected at 10 m, and only four samples at 120 m, collected on 6<sup>th</sup> of January of 2000.

The sites studied in 2002 were selected from close to sea level up to the highest site where the two vascular plants occurred together (147 m). Characteristics of the sample sites are given in Table 1. The top sites (at 20, 97 and 147 m) are flat, windy promontories where snow is frequently blown away. At the lowest sites (10 m) and slopes of the ridge (20 m) sites there is an extended period with late lying snow. At the Spanish Base, in December-February (austral summer), between 1988-2002 the average relative air humidity was 80%, mean air temperature was 2.1° C, and precipitation in this period was 109 mm (Bañón 2004).

**Table 1.** Site descriptions.

Sites	Altitude (a.s.l.) (m)	Characteristics
1	10	Sides N-NE of microhabitat 2.
2	10	Pebbles. Flat raised beach.
3	20	Flat top of a ridge.
4	20	NE slope of the ridge. Unstable morainic deposit.
5	20	SW slope of the ridge. Unstable morainic deposit.
6	97	Flat top of an erosion platform, with stones.
7	147	Flat top of a morainic deposit.

The soil temperature was measured every hour on the surface of bare ground at 20m and at 147 m altitude from 27 January 2002 to 21 February 2002, using temperature loggers (Optic StowAway temperature-Onset). Temperature of the compact cushion structure of *C. quitensis* and the caespitose *D. antarctica* growing at 20 m (three orientations) were measured. Temperature sensors were inserted at 1 cm depth in the plants from 10 to 17 February 2002, and data recorded hourly using a Squirrel data logger (Grant Instruments, UK). The depth of soil was recorded by inserting a fine steel rod into the soil and measuring the maximum depth. The granulometry: gravels, sand and small particles (20.0-2.0; 2.0-0.25; < 0.25 mm respectively) were ascertained by passing samples through a set of soil sieves.

The pH of three samples of soil from beneath each species and on nearby bare soil was measured for each altitude, using a glass electrode inserted into a suspension of a soil sample saturated with distilled water. Soil organic matter (determined by loss on ignition) was only measured at 20 and 147 m from soil samples beneath *D. antarctica* where roots were present.

Six discrete individuals of about 6 cm diameter of both species were collected at each site. Based on the annual growth rates published for *C. quitensis* (Edwards 1975; Lewis Smith 1994; Vera 2011) this size represents well developed individuals of similar age. For *D. antarctica* an increase in diameter is obtained by tillering and it is not possible to determine age this way, and individuals with a similar size (6 cm diameter) were analysed only for comparative purposes.

Samples of the plants were collected with the soil underneath (up to 10 cm deep in those more developed) with a knife, then carefully introduced into paper bags, on 25-26 of January and on 21-22 of February 2002. The samples were stored at 4°C in the laboratory at the Spanish base and subsequently shipped frozen (-20°C) to Spain at the end February. From mid-April 2002 onwards they were thawed and kept at 4°C in dry conditions for later analysis in the laboratory in Spain.

### ***Flower production and maturation stages***

Due to their high density, for *C. quitensis*, numbers of flowers were estimated in subsamples 3x3 cm<sup>2</sup> cut from the middle of the cushions. For *D. antarctica* all inflorescences were counted. The numbers of *C. quitensis* flowers and *D. antarctica* inflorescences at each maturation stage, as defined by Holtom and Greene (1967) but excluding stage (0), were counted (see legends in Figs. 2 and 4). The number of inflorescences of *D. antarctica* developing during the current season (greenish colour) and those from the previous season (straw-coloured) were recorded in each sample collected in January and February 2002. The number of flowers of *D. antarctica* per m<sup>2</sup> was calculated considering

the number of inflorescences per m<sup>2</sup> multiplied by average number of spikelets per inflorescences x 2 flowers (mean number of flowers formed per spikelet). The number of flowers detached and lying on the soil mixed with leaves was also estimated. For comparative purposes, the data on density of reproductive structures were referred to 1 m<sup>2</sup>, assuming 100 % cover for both *D. antarctica* and *C. quitensis*. As complementary measurements of inflorescence development, the panicle length, the stalk (culm) length, 2 uppermost spikelets and the lowermost spikelets for each of 5 more mature new inflorescences of *D. antarctica* were measured. The length of 20 sepals and 20 capsules from the most mature flowers of *C. quitensis* were measured.

### ***Seed development and production***

The size of three seeds in each of the 20 most mature capsules of *C. quitensis*, and those seeds from 5 spikelets (two upper, two middle and the lowest) in each one of five most mature inflorescences of *D. antarctica* was measured. Exceptionally, some individuals of *D. antarctica* had fewer than five inflorescences. Seed length was determined with a micrometer under a stereoscopic microscope in dry conditions. For *D. antarctica* the style was excluded from the seed length. In order to compare the present with historical data the results of seed size were recalculated for populations from the flat top ridge at 20 m as: (1) five longest seeds per specimen (Holtom & Greene 1967); (2) two largest seeds from each of five inflorescences or five most mature capsules (Greene & Holtom 1971).

The length of filled seeds (considered ripe) of both species was also measured, as well as for seeds of *D. antarctica* collected in January 2000.

An estimate of seed production for *C. quitensis* was obtained by multiplying the number of capsules per m<sup>2</sup> by the mean number of seeds in development from 20 capsules from the most advanced maturation stage. Flower production in *D. antarctica* was utilised as an estimation of potential seed production. The maturation seed capacity was estimated as percentage ripe seeds in relation to the number of current season flowers for *D. antarctica* or total number of seeds for *C. quitensis* in February.

### ***Seed bank***

The seed bank produced in previous years was estimated from samples collected in February 2002 in three different strata: (1) Organic stratum (surface to 2 cm depth, or 1.5 cm at the highest altitude), consisting of the cushion of *C. quitensis* or leaves and mineral constituents for *D. antarctica*; (2) Mineral soil stratum (2 to 4 cm depth) interspersed with roots and some organic constituents; (3) Deep mineral soil stratum (4 to 6 cm) in sites with better developed soils. Ripe seeds, identified as pale brown coloration and fully developed, with a minimum seed size for successful germination in *D. antarctica* (1.4 mm) and *C. quitensis* (0.5 mm), based on data from Edwards (1974) and Vera (unpublished data), were counted in samples of 36 cm<sup>2</sup> (*D. antarctica*) or 9 cm<sup>2</sup> (*C. quitensis*) in all strata. All other seeds: incompletely filled or poorly developed seeds were also counted. These data are given as number of seeds per m<sup>2</sup>.

As a measure of the dynamics and characteristics of the seed bank, numbers of old flowers in the soil were counted and compared to the number of new flowers produced in *D. antarctica* and old seeds with respect to new seeds in *C. quitensis*. The percentage of ripe seeds in relation to numbers of old flowers (*D. antarctica*) or total seeds (*C. quitensis*) was also calculated.

### Statistical analysis

To determine the differences between altitudes and sites for different flower characters and production, seed size and production, and granulometric composition of soils, a one way Analysis of Variance (ANOVA) was used.

To examine the relationships between the size of different floral characters, number of flowers formed, seed production and size, and the characteristics of the soil, Pearson's correlation was utilised.

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

## Results

### Temperature and soil conditions

Site temperature data are shown in Table 2. There were considerable variation in temperatures resulting from changes in altitudes, orientation and between surface of bare soil and within of plants. Higher temperatures were measured within plants than in bare soil. The altitudinal temperature gradient ranged between 0.76 to 0.85 °C per 100 m. This gradient is intermediate between the expected adiabatic gradient for wet and dry, but can be used as a guide to estimate the mean temperatures at intermediate sampling sites. The differences in soil surface temperatures between northeast and southwest facing slopes and horizontally orientated surfaces are consistent with heating by solar radiation. NE facing slopes are warmer than those facing SW or having flat-topped surfaces. There were strong temperature fluctuations at all situations.

The soils are poorly developed, ranging from none (bare rock) to 10 cm depth (Table 3). Soils differ in the proportion of different types of soil particles with altitude ( $P < 0.001$ ), although no differences were found in soils underneath either vascular species. The lower sites with better developed soils had a lower percentage of gravel than those from higher altitudes with a more youthful appearance. Soil characteristics varied between microhabitats at the 20 m site, with soil from the flat top of the ridge being more compact and stable than that from the sides of the ridge.

**Table 2.** Mean temperatures (minimum and maximum) on bare ground and on plants in sites 3, 4, 5 and 7, during two periods.

	20 m			147 m
	Top ridge	NE side of ridge	SW side of ridge	Top
Bare ground (27 January to 21 February 2002)	3.96°C (-0.81°, 21.47°)	-	-	2.99° C (-2.03°, 20.78°)
<i>D. antarctica</i> plant (10 to 17 February 2002)	4.73°C (-2.70°, 25.13°)	5.89° C (-1.50°, 30.55°)	3.44° C (-0.80°, 15.65°)	-
<i>C. quitensis</i> cushion (10 to 17 February 2002)	4.34 °C (0.05°, 16.05°)	-	-	-
Bare ground (10 to 17 February 2002)	3.10°C (-0.81°, 14.13°)	-	-	2.02°C (-2.03, 12.73°)

Soil organic matter was low, corresponding to recently developed soils, ranging from 3.6 to 7.2 % under *D. antarctica*. Soil pH in all bare ground sites ranged from 5.4 to 7.1, while the soil pH is lower under plants (Table 3), due to the input of humic acids.

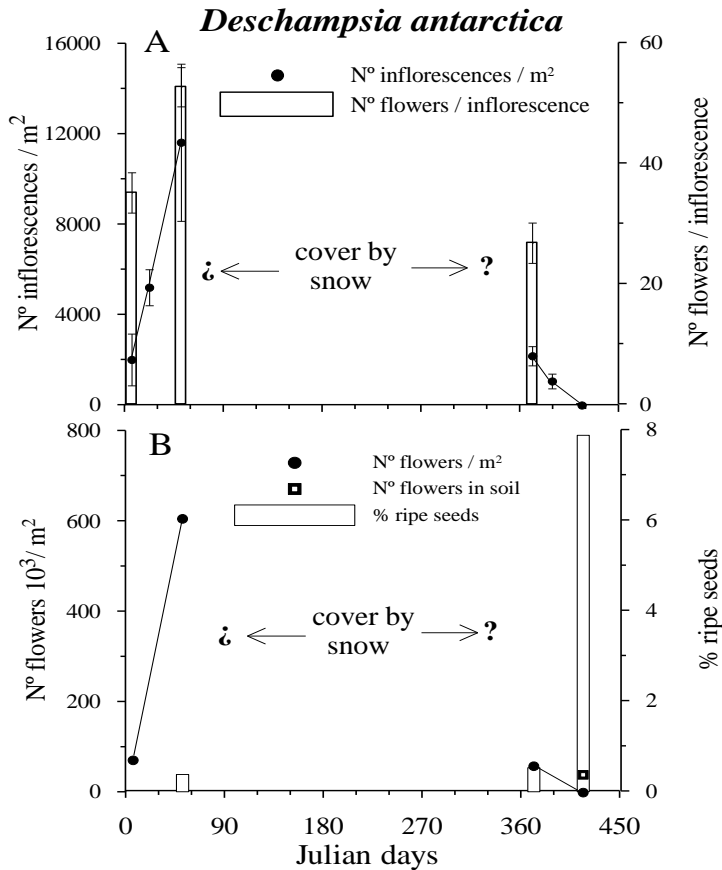
**Table 3.** Soil depth, pH and granulometry on bare ground and under *D. antarctica* and *C. quitensis*, at each site (see table 1).

Sites	Soil depth (cm)	Soil pH			% gravel (20.0-2.0 mm)		% sand (2.0-0.25 mm)		% particle <0.25 mm	
		Bare ground	Under <i>Desch.</i>	Under <i>Colob.</i>	Under <i>Desch.</i>	Under <i>Colob.</i>	Under <i>Desch.</i>	Under <i>Colob.</i>	Under <i>Desch.</i>	Under <i>Colob.</i>
1	0 - 2	-	-	-	-	-	-	-	-	-
2	2 - 5	-	-	-	-	-	-	-	-	-
3	4-10	6.4 -6.7	4.8-5.8	5.3-5.6	9.2	12.2	69.3	70.4	21.5	17.4
4	2 - 6, 5	6.4-6.7	-	-	19.3	14.3	57.4	63.0	23.3	22.7
5	2- 6	6.4-6.8	-	-	21.5	25.3	47.9	47.7	30.6	27.0
6	2 - 5	5.4-6.7	4.6-5.2	5.8-6.0	48.9	29.2	35.0	39.2	16.1	31.6
7	1- 3, 5	6.8-7.1	5.2-5.8	5.3-5.5	28.6	38.5	45.6	42.3	25.8	19.2

### ***Characteristics and production of reproductive structures in D. antarctica***

The *D. antarctica* populations sampled in the year 2000 did not show much interannual difference from those collected in 2002 (Tables 4-6), and thus could be used to reconstruct a phenological cycle of the species in the sample area at 10-20 m (Fig. 1). At the beginning of January, when the snow melted, the grass inflorescences were poorly developed (most inflorescences not visible, inside of sheaths) and the upper spikelet of the new inflorescence was smaller than in more advanced summer inflorescences. During January and February the number of inflorescences and flowers per inflorescence increased (Fig. 1 A), with a greater number of flowers per surface unit at the end of February (Fig. 1 B). The proportion of flowers in the plant with ripe seeds was lower (0.38 %) at the end of February (Table 6, Fig. 1 B). Some new flowers (current year) were found detached on the soil in February (Table 7). Many winter surviving inflorescences remained on the plants in January, but several flowers had already detached from the inflorescences and only 0.53 % of the old flowers remaining on the plants contained ripe seeds (Table 6, Fig. 1 B). At the end of February *D. antarctica* plants retained no old inflorescences at 20 m and only a few old inflorescence remained at the highest altitude. The proportion of flowers detached on the soil was lower with respect to those developing on the plants (Tables 4 and 7, Fig. 1 B). The proportion of ripe seeds in old flowers in or on the soil (from different years) was higher than ripe seeds developed in one growing season (Tables 6 and 7, Fig. 1 B). Variations on this cycle at other sites are shown in the Tables 4, 6 and 7.

No significant differences were detected in the inflorescence and flower numbers in response to altitude. Site orientation had some effect the density of flowers and inflorescences, with the lowest density at the SW oriented site (Table 4).



**Figure 1.** Phenologic cycle of *Deschampsia antarctica* at sea level reconstructed (see methods) with data from January 2000 at 10 m altitude and January and February 2002 at 20 m altitude on the flat top sites (time is expressed in Julian days).

A: Inflorescence density (N°/m<sup>2</sup>) and flowers per inflorescences.

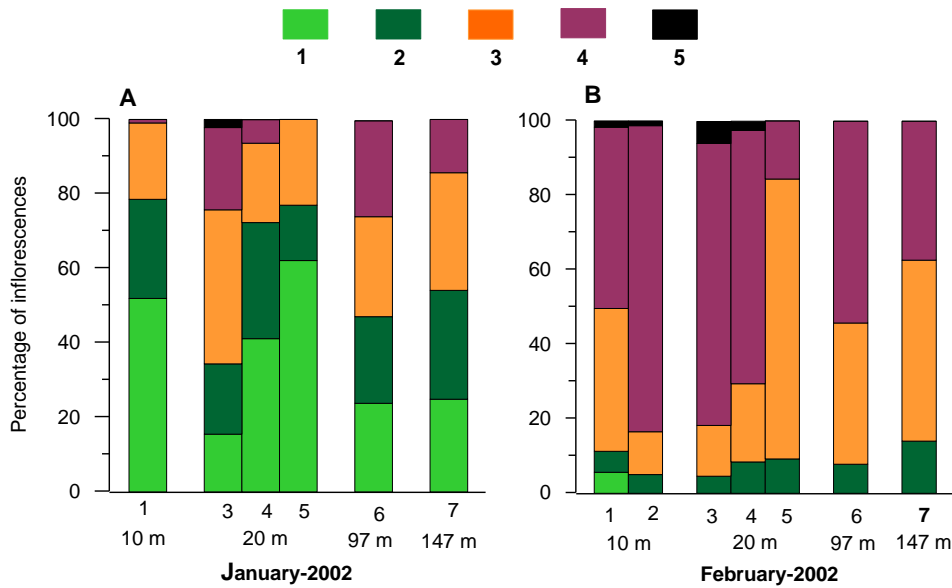
B: Flower density (N°/m<sup>2</sup>), detached flowers on soil in February, and percentage of ripe seeds related to new flowers, except the last bar where the percentage is related to old flowers in surface soil.

**Table 4.** *D. antarctica* average inflorescence number (SE) in the previous (2000-2001) and current (new) (2001-2002) season, flowers per old and new inflorescence and total new and flowers in the plant sampled in January 2000, 2002 and February 2002. Asterisks, significance of differences between altitudes in top sites; crosses, significance of differences between microhabitats at 20 m: \*\*\*, +++  $P < 0.001$ ; \*\*, ++  $P < 0.01$ ; \*, +  $P < 0.05$ ; n.s., non significant (only for 2001-2002 data). Data of 1998-1999 and 1999-2000 seasons correspond to samples collected in early January 2000.

	Sites						
	10 m stony soil	10 m top	20 m top	20 m NE	20 m SW	97 m top	147 m top
<b>Inflorescences of the previous season / m<sup>2</sup></b>							
January 2002 n.s.; n.s.	-	1907 (650)	1064 (326)	1556 (321)	1177 (265)	685 (574)	994 (330)
<b>New inflorescences / m<sup>2</sup></b>							
January 2002 n.s.; +++	-	2716 (438)	5214 (796)	1210 (424)	274 (126)	4494 (586)	3691 (814)
February 2002 n.s.; ++	4916 (610)	8600 (1846)	11634 (3478)	3878 (7200)	2039 (5000)	10529 (941)	7103 (1486)
<b>Flowers per old inflorescence</b>							
1998-1999 (10 and 120 m.)		26.80 (3.35)	-	-	-		17.78 (2.60)
<b>Flowers per new inflorescence</b>							
January 2000 (10 and 120 m)	-	35.14 (10.68)	-	-	-		28.90 (1.65)
February 2002 n.s.; +++	-	-	52.7 (3.25)	38.7 (4.35)	29.6 (1.58)	60.1 (6.89)	50.6 (4.3)
<b>New flowers / m<sup>2</sup>.</b>							
January 2000 (10 and 120 m)	-	71453	-	-	-		9775
February 2002 n.s.; +	-	-	605958 (188267)	168293 (69251)	60188 (24090)	649000 (120150)	379071 (93425)
<b>Old flowers/m<sup>2</sup></b>							
Previous season's 1999-2000 (10 and 120 m)		58395	-	-	-		23034



*Deschampsia antarctica* Inflorescence Maturation Stages



**Figure 2.** Percentage of inflorescences in each maturation stage of *D. antarctica* in January and February 2002 at different altitudes and microhabitats (see Table 1). The maturation stages were: inflorescences sheaths swollen but unopened (1); sheaths open with tops of inflorescences visible (2); inflorescences <1/2 emerged (3); inflorescences >1/2 emerged, most branches erect to slightly spreading (4); inflorescences >1/2 emerged, most branches moderately to fully spreading (5).

Altitude has an effect on inflorescence stalk and spikelet length, both being shorter at higher altitudes (Table 5). Significant variations in these inflorescence characters and panicle length were also found between microhabitats located at the 20 m site, both being shorter on the slopes. The number of spikelets per inflorescence was positively correlated with the panicle length ( $r=0.345$ ,  $P<0.001$ ,  $n=114$ ) and with inflorescence stalk length ( $r=0.321$ ,  $P<0.001$ ,  $n=112$ ).

Altitude does not seem to have any significant effect on inflorescence maturation stages of *D. antarctica* (sites 2,3,6,7 flat and deeper soil sites with early snow cover disappearance) in the early austral summer, but maturation could be delayed by a prolonged snow cover (sheltered and slopes sites 1, 4 and 5) (Fig. 2 A). This delayed maturation could be compensated for at low altitudes when the snow cover disappears as the summer progresses (site 1) or on warmer NE orientated slopes (site 4) (Fig. 2 B). In contrast, during February maturation was slower at higher altitudes (sites 6 and 7) and on the colder SW orientated slopes (site 5). Another indicator of the effect of altitude on maturation of the plants was the presence of the current year's ripe seed at 20 m in February, but not at higher sites (Fig.3). At this time, most seeds in new inflorescences were green, with the largest seeds measuring 1.60 mm, although they did not reach the size of previous seasons (Table 6). Significant differences in seed length were found between altitudes and orientations at the end of February in 2002 (Table 6). The longest seeds developed during the current year were observed at lower altitudes. In the microhabitats located at 20 m, the seeds were smaller on the NE and SW orientated slopes than on the flat top.

**Table 5.** *D. antarctica* average (SE) size of several new flower characters (except for 1998-1999: old flowers). Asterisks, significance of differences between altitudes in top sites; crosses, significance of differences between microhabitats at 20 m: \*\*\*, +++  $P < 0.001$ ; \*\*, ++  $P < 0.01$ ; \*, +  $P < 0.05$ ; n.s., non significant (only for 2001-2002 season). Data of 1998-1999 and 1999-2000 seasons correspond to samples collected in early January 2000.

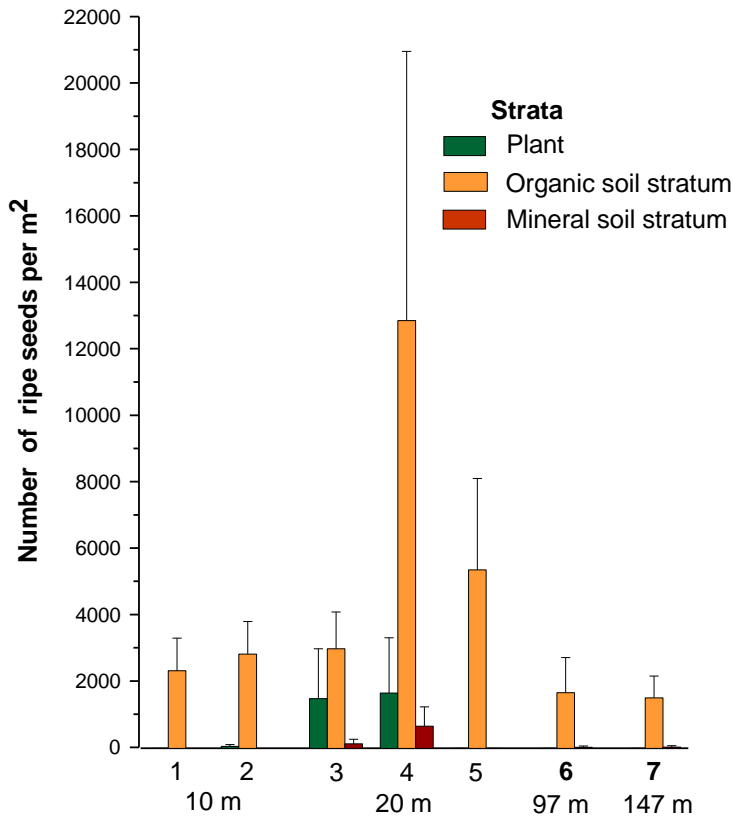
	Sites						
	10 m stony soil	10 m top	20 m top	20 m NE	20 m SW	97 m top	147 m top
<b>Panicle length (mm)</b>							
February 2002 n.s.; ++	-	-	25.33 (0.91)	21.41 (1.29)	17.7 (1.21)	26.60 (1.41)	22.88 (0.85)
<b>Inflorescence stalk length (mm)</b>							
February 2002 ***, +++	-	-	28.76 (0.79)	14.9 (1.14)	9.1 (0.43)	19.13 (1.40)	12.40 (0.76)
<b>Upper spikelet length (mm) of the inflorescence.</b>							
1998-1999 (10 and 120 m)		5.03 (0.19)				4.69 (0.13)	
January 2000 (10 and 120 m)		4.97 (0.11)				4.57 (0.35)	
February 2002 ***, +	-	-	5.07 (0.05)	4.88 (0.05)	4.93 (0.07)	4.72 (0.08)	4.81 (0.06)
<b>Lower spikelet length (mm) of the inflorescence</b>							
February 2002 ***, +++	-	-	4.77 (0.06)	4.28 (0.07)	4.55 (0.14)	4.55 (0.09)	4.31 (0.07)

**Table 6.** Percentage of ripe seeds per new (most remaining in the plant) collected in February 2002 and per old flowers, January 2000, in the *D. antarctica* plant, and average length (SE and [intervals]) of more mature seed from the current and previous seasons collected. Asterisks, significance of differences between altitudes in top sites; crosses, significance of differences between microhabitats at 20 m: \*\*\*, +++  $P < 0.001$ ; \*\*, ++  $P < 0.01$ ; \*, +  $P < 0.05$ ; n.s., non significant (only for 2001-2002 data).

	Sites						
	10 m stony soil	10 m top	20 m top	20 m NE	20 m SW	97 m top	147 m top
<b>% new ripe seeds / total new flowers</b>							
February 2002	-	-	0.38	1.22	0	0.01	0
<b>% incompletely filled seeds/total new flowers</b>							
February 2002		-	1.64	0.08	0	0	0
<b>% old ripe seeds / total old flowers</b>							
January 2000 (10 and 120 m)		0.53				0	
<b>Seed length of the five more new mature inflorescences (mm)</b>							
February 2002 ***, +++	-	1.17 (0.07) [1.00-1.30]	1.15 (0.02) [0.40-1.60]	0.90 (0.03) [0.30-1.55]	0.55 (0.03) [0.20-1.20]	0.59 (0.02) [0.20-0.90]	0.48 (0.01) [0.20-0.90]
<b>Length of previous seasons' ripe seeds (mm)</b>							
January 2000 (10 and 120 m)		1.49 (0.01) [1.35-1.80]				1.52(0.06) [1.35-1.70]	
February 2002 *, +++	1.30 (0.01) [1.10-1.50]	1.48 (0.02) [1.10-1.80]	1.53 (0.01) [1.20-1.80]	1.40 (0.01) [1.10-1.75]	1.39 (0.01) [1.10-1.60]	1.43 (0.01) [1.10-1.70]	1.48 (0.01) [1.20-1.90]

The number of ripe and incompletely filled seeds estimated in relation to flowers developed in the current year (Table 6) was very low at the end of February (about 1 or 2 %) at 20 m. This percentage decreases for SW orientated sites or at higher altitudes. The current year ripe seed production was higher in the lower sites and on NE facing slopes (Fig. 3), but no significant differences were found with regard to altitude and orientation. These seeds had a green or green-clear brown coloration.

### *Deschampsia antarctica*



**Figure 3.** Production of ripe seeds of *D. antarctica* along the altitudinal gradient and microhabitats (see Table 1) in plants (from current year) with green or green-clear brown coloration and different soil strata (see methods for strata description) most with coloration clear brown in February 2002 .

#### *D. antarctica* seed bank

The number of ripe seeds in good condition varied significantly among the different soil strata ( $P=0.001$ ) (Fig. 3). Higher numbers of ripe seeds were counted at the surface, the majority corresponding to the pale brown coloration of seeds developed during the previous years (about 90 %), and only a 5 % corresponding to the green colour seeds released from the current year's inflorescences. Ripe seeds with dark colour (about 5 %) were not considered (they are degraded by fungi and a germination test showed that they are not viable). All seeds from the 147 m altitude site had a pale brown coloration. Fewer numbers of ripe seeds were found in the deeper soil stratum (nil between 4 to 6 cm depth) (Fig. 3). Most of the ripe seeds were enclosed between the lemma and palea of the flowers.

The number of ripe seeds per m<sup>2</sup> of *D. antarctica* collected in January 2000 estimated at the soil surface (10 m: 3492 and 120 m: 1172) show no great differences compared to the results for *D. antarctica* collected in 2002. There was no significant difference between the number of ripe and incompletely filled seeds (data not shown) between sites, although higher numbers tended to be found at lower altitudes, mainly on NE orientated slopes. The granulometric composition did not seem to influence seed densities in soils, although a higher number of ripe seeds was found in less compacted soils with a higher proportion of gravel.

Significant variation in ripe seed size from previous seasons was found between the different microhabitats (Table 6), but not between populations from different altitudes. Seed size from the previous year showed a significant positive correlation with seed size from the current year in the same individuals ( $r=0.222$ ,  $P=0.009$ ,  $n=136$ ). Plants which had better developed seeds in the growing season 2001-2002, also produced larger seeds in the previous years.

Numbers of old flowers (previous years) found at different soil depths are shown in Table 7. A higher proportion of old flowers with respect to new flowers was found at the highest altitude and the slopes orientated NE and SE (Table 7). The proportion of old flowers with ripe seeds varied between altitudes and microhabitats. A higher percentage of swollen seeds was found at 20 m, mainly on NE oriented slopes. The percentage of ripe seeds in soil (0-2 cm) in relation to new flowers is lower, although also varying between sites.

**Table 7.** *D. antarctica* old flower / m<sup>2</sup> (estimation of seeds) per soil stratum, January 2000 and February 2002, at each site. The new flowers detached from plant at soil in February 2002 are shown between brackets. The relation between new (most in the plant) and old flowers, as well as the percentage of old ripe seeds formed in relation to the total number of flowers developed during the previous years (old flowers) and the current year (new flowers) found in the different strata of soil are also shown.

	Sites					
	10 m	20 m top	20 m NE	20 m SW	97 m top	147 m top
<b>Old flowers / m<sup>2</sup> in the soil</b>						
January 2000 (10 and 120 m)						
Organic soil stratum 0-2 cm	23425					18123
Mineral soil stratum 2-4 cm	608					69
February 2002						
Organic soil stratum 0-2 cm	-	27864 + 2357 (new)	62876 + 480 (new)	29991	28753 +72 (new)	43665
Mineral soil stratum 2-4 cm	-	11539	5614	1693	11232	3357
<b>New flowers /old flowers soil 0-6 cm</b>						
February 2002						
	-	14.28	2.38	1.89	14.86	8.06
<b>% ripe seeds/ total old flowers</b>						
January 2000 (10 and 120 m)						
Organic soil stratum 0-2 cm	14.90					6.46
Mineral soil stratum 2-4 cm	8.27					0.64
February 2002						
Organic soil stratum		7.89	20.96	18.85	7.02	3.48
Mineral soil stratum 2-4 cm		1.20	11.86	0	1.13	1.22
<b>% ripe seeds (soil 0-2 cm)/total new flowers</b>						
February 2002						
	-	0.36	7.83	9.39	0.31	0.40

### ***Characteristics and production of reproductive structures in C. quitensis***

The number of flowers (=capsules) increased substantially from January until February 2002 (Table 8) in most of the populations, since the plants produced new flowers in this period; one exception occurring in populations from the highest altitude. These differences arise on the NE oriented slopes. Flower production scarcely varied between altitudes. However, at the 20 m site, there were significantly higher numbers of flowers on the flat top than on the NE and SW oriented slopes. The number of flowers of *C. quitensis* per m<sup>2</sup> was slightly higher on plants growing in soils having a higher percentage of sand ( $r=0.356$ ,  $P=0.02$ ,  $n=29$ ).

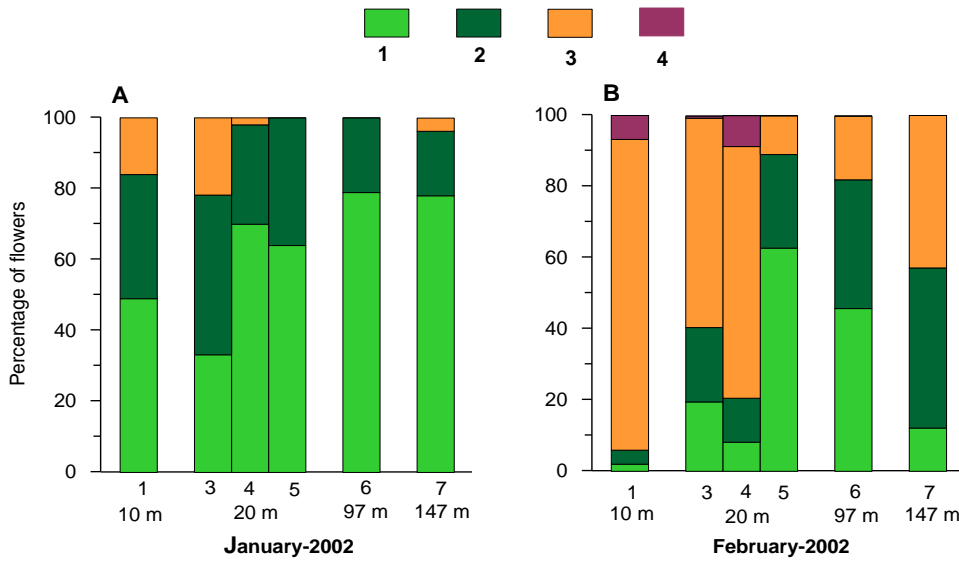
Table 8. *C. quitensis* average (SE) of current year capsule production (end of January and February 2002). Current year seed production per m<sup>2</sup>, percentage of new ripe (full-size) seeds in relation to the total number of new (current year) seeds and mean (SE) and [intervals] of current and previous seasons seed size (February 2002) of *C. quitensis* in each site. Asterisks at each parameter indicate the significance between the top sites at different altitudes, and the crosses between the microhabitats at 20 m: \*\*\*, +++  $P < 0.001$ ; \*\*, ++  $P < 0.01$ ; \*, +  $P < 0.05$ ; n.s., non significant.

	Sites					
	10 m top	20 m top	20 m NE	20 m SW	97 m top	147 m top
<b>Capsules per m<sup>2</sup></b>						
January 2002 n.s.; ++	47036 (3613)	82221 (10464)	35925 (5105)	27222 (10512)	73703 (12106)	88518 (13546)
February 2002 *, +++	81678 (16613)	145998 (17436)	122443 (15780)	66110 (17676)	142220 (26664)	85276 (6457)
<b>Number of seeds per capsule</b>						
February 2002 ***, +++	20.53 (1.36)	13.34 (0.70)	18.13 (0.90)	11.62 (1.08)	13.40 (0.71)	10.98 (1.01)
<b>Number of current year seeds per m<sup>2</sup></b>						
February 2002 n.s.; n.s.	1767194 (499656)	1914662 (353953)	2215495 (882166)	914430 (149280)	1597787 (679447)	1204380 (282256)
<b>% new ripe seeds/total current year seeds</b>						
February 2002	1.29	0.88	0.57	0	0	0.35
<b>Seed length of the current year twenty more mature capsules (mm)</b>						
January 2002 (range)	0.2-0.5	0.2-0.5	0.1-0.4	0.1-0.2	0.1-0.2	0.1-0.7
February 2002 ***, +++	0.55 (0.004) [0.40-0.70]	0.59 (0.003) [0.40-0.80]	0.57 (0.003) [0.30-0.75]	0.30 (0.007) [0.15-0.65]	0.39 (0.004) [0.20-0.70]	0.43 (0.008) [0.20-0.70]
<b>Length of previous season's ripe seeds (mm)</b>						
February 2002 ***, +++	0.68 (0.01) [0.50-0.80]	0.68 (0.007) [0.50-0.85]	0.65 (0.005) [0.50-0.85]	0.61 (0.01) [0.50-0.70]	0.66 (0.01) [0.55-0.80]	0.64 (0.005) [0.50-0.80]

The maturation of flowers can be recognized by changes in some morphological characteristics, such as the sepals (Table 9). Their full size was effectively attained at the end of January, with little evident increase during February. However, capsule length increased strongly during February. Capsule size varied significantly with altitude and microhabitat, being smallest at the higher altitudes and on SW oriented slopes. Capsule length was positively correlated with the number of seeds ( $r=0.445$ ,  $P < 0.001$ ,  $n=290$ ) and with seed size ( $r=0.740$ ,  $p < 0.001$ ,  $n=329$ ). Seeds in capsules smaller than 0.9 mm did not usually develop to full-size.

Plants of *C. quitensis* from lower altitudes had more developed flowers at the end of January than those from higher altitudes (Fig. 4 A). Plants from altitudes below 147 m developed new flowers in February, which leads to an increase in the proportion of less advanced flower maturation stages at the end of February (Fig. 4 B). However, only plants from lower altitudes had open capsules in this time. The development of flowers is also influenced by microhabitat characteristics. Most plants growing on slopes at 20 m, both NE and SW orientation, presented a more delayed flower maturation in January. During February, the retarded development of flowers was compensated with a very fast maturation on the NE slopes, having several open capsules with ripe seeds, and surpassing the development stages of individuals growing on the flat ridge tops.

**Colobanthus quitensis Flower Maturation Stages**



**Figure 4.** Percentage of flowers in each maturation stage of *C. quitensis* in January and February 2002 at different altitudes and microhabitats (see Table 1). The maturation stages were: flower buds visible, with sepals closed (1); sepals open, capsule closed and <1/2 the length of the sepals (2); sepals open, capsule closed and >1/2 the length of the sepals (3); capsules open, seeds visible (4).

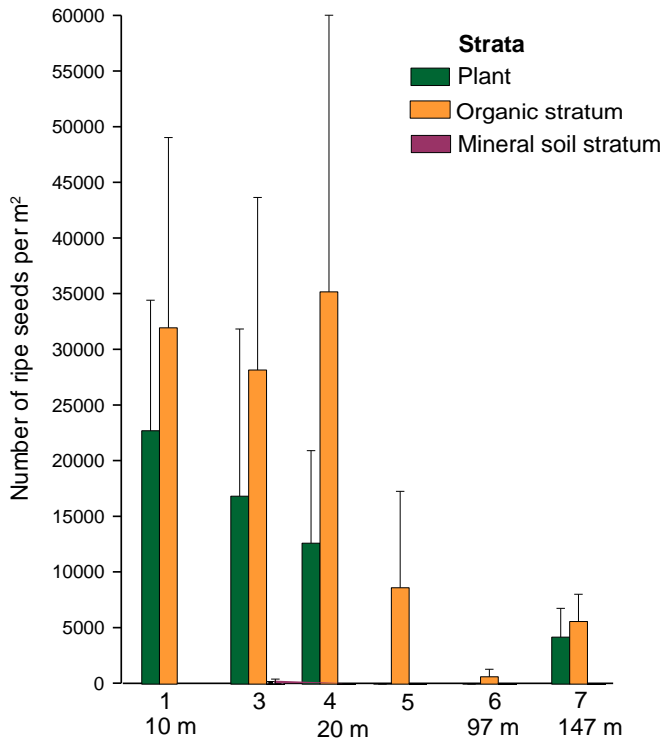
**Table 9.** *C. quitensis* average (SE) size of sepals and capsules (end of January and February 2002). Asterisks at each parameter indicate the significance between the top sites at different altitudes, and the crosses between the microhabitats at 20 m: \*\*\*, +++  $P < 0.001$ ; \*\*, ++  $P < 0.01$ ; \*, +  $P < 0.05$ ; n.s., non significant.

		Sites					
		10 m top	20 m top	20 m NE	20 m SW	97 m top	147 m top
<b>Sepal length (mm)</b>							
January 2002	*, +	2.60 (0.05)	2.48 (0.03)	2.45 (0.04)	2.24 (0.12)	2.45 (0.03)	2.41 (0.08)
February 2002	***, +++	2.58 (0.03)	2.60 (0.02)	2.67 (0.02)	2.46 (0.02)	2.44 (0.02)	2.57 (0.02)
<b>Capsule length (mm)</b>							
January 2002	***, +++	1.24 (0.10)	1.31 (0.05)	1.02 (0.05)	0.76 (0.10)	0.97 (0.04)	1.00 (0.09)
February 2002	***, +++	2.12 (0.06)	1.92 (0.04)	2.10 (0.04)	1.03 (0.03)	1.37 (0.03)	1.31 (0.04)

The highest numbers of seeds per capsule were found in plants growing near to the sea level (10 m), while the lowest were found at the highest altitude (147 m) (Table 8). Individuals on the NE side of the ridge at 20 m, receiving more insolation than at other situations (flat top or SW side), had higher number of seeds per capsule. Length of the current year’s seeds, as well as the length of ripe seeds formed in the previous growing seasons, varied along the altitudinal gradient and between microhabitats (Table 8). A significant increase in size was detected between the seeds collected in January and February. However, most of the current year seeds at the end of February did not reach fully maturation. The percentage of the current season’s filled and clear brown seeds (considered ripe) in relation to the total seed number at the end of February was very low or nil (around 1 % in the lower flat sites) (Table 8). Altitude had no effect on the production of ripe current year seeds, although higher numbers were found at the lower altitudes (Fig. 5). Similarly there were no significant differences between sites at 20 m, although no full-sized seeds were found at the SW side.

Neither altitude nor site orientation had a significant effect on the potential seed production per m<sup>2</sup> of the current season, although this tended to be lower at higher altitudes and on the SW orientated slopes (Table 8).

### *Colobanthus quitensis*



**Figure 5.** Production of clear brown ripe seeds of *C. quitensis* along the altitudinal gradient and microhabitats (see Table 1) in each studied stratum: plant (from current year), organic stratum and mineral soil stratum (see methods for strata description) in February 2002.

### *C. quitensis* seed bank

In the organic stratum, numbers of ripe old seed per m<sup>2</sup> tended to decrease with altitude ( $P=0.1$ ), but no differences were detected between microhabitats (Fig. 5). Those plants with a higher proportion of ripe seeds from the growing seasons 2001-2002 had more ripe seeds from previous years in the organic stratum ( $r=0.395$ ,  $P=0.02$ ,  $n=26$ ). The proportion of seeds (old) in the seed bank related to newly produced seed varied between microhabitats and altitude (Table 10).

Only a relatively small percentage of the total numbers of old seeds (the percentage decreased in deeper layer) had attained maturation, although the percentage was higher at lower altitudes (Table 10). This percentage varied mainly between microhabitats, being highest on NE oriented slopes. The proportion of ripe seeds was lower ( $P = 0.01$ ) in the mineral soil stratum than in the organic stratum. In the mineral soil stratum, the number of ripe seeds was very low or nil (Fig. 5). Only about 50 % of filled seeds found at the 10 and 20 m sites had clear brown coloration, but almost 100% at the 97 and 147 m sites. The greater proportion of well developed seeds was found in capsules or released into the organic stratum (*C. quitensis* cushion) formed during previous years.

**Table 10.** *C. quitensis* seed per m<sup>2</sup> (including, poorly developed seeds) in February 2002 on the organic stratum (cushion portion developed in the previous seasons on 0-2 cm) and mineral soil stratum (under the cushion on 2 cm of depth) at each site. The relation between new seeds (current year) and old seeds (previous years) and the percentage of old ripe seeds per total number of old seeds are also shown.

	20 m top	20 m NE	20 m SW	97 m top	147 m top
<b>Total old seeds/ m<sup>2</sup></b>					
Organic stratum 0-2 cm	101333	490708	213089	1124907	416769
Mineral soil stratum 2-4 cm	22222	39600	11955	23842	6221
<b>New seeds/ old seeds</b>	1.84	4.17	4.06	1.39	2.84
<b>% old ripe seeds/ total old seeds</b>					
Organic stratum 0-2 cm	6.07	21.23	5.45	0.06	1.34
Mineral soil stratum 2-4 cm	0.02	1.01	0	0	0

A higher number of ripe seeds tended to be found in soils with a higher proportion of sand ( $r=0.30$ ,  $P=0.05$ ,  $n=31$ ), while more compacted soils, with a higher proportion of small particles ( $<0.25$  mm), are less favourable for seed maturation because they retain more moisture and are thus colder ( $r=-0.33$ ,  $P=0.03$ ,  $n=31$ ).

The size of the previous season's fully mature seeds decreased significantly with altitude (Table 8). The seed size also varied between microhabitats, being smaller on slopes (NE and SW) than on the flat ridge tops.

## Discussion

The responses of the two Antarctic flowering plants growing on Hurd Peninsula (Livingston Island) have been analysed in comparison with previous published data in the area. The phenological and sexual reproductive traits and their responses can thus be related to trends in climate warming.

### *Phenology and maturation stages*

Based on field observations and studies of herbarium material Holtom & Greene (1967) and Greene & Holtom (1971) defined the beginning of production of *D. antarctica* inflorescences as being in December- January of the current summer. Later, Edwards (1974) suggested initiation of flowering took place at the end of the previous summer, since small inflorescences were observed in November, or even October (with a similar stage of development to those recorded the previous March, late in the previous summer) and visible inflorescences could be found at the end December. On Hurd Peninsula (Livingston Island), the presence of inflorescences in January showing good condition and green colour (the most inside of sheaths at beginning of January, or with more advanced stage of development at the end of January), suggests that their development took place after winter. Extrapolating from the lineal increase in inflorescence numbers, initiation of the process may occur in December, with subsequent development and maturation over the summer. Inter annual variations in inflorescence initiation time can occur (Greene & Holtom 1971), due to variability in seasonal temperatures and depth and duration of snow cover. New inflorescences of *D. antarctica* develop during January and February on Hurd Peninsula, and some of them reach an advanced stage of development by the end of February. It is likely that these inflorescences, overwintering, and continue their development through the following growing season. The significant increase in inflorescence numbers in February has not been clearly noted in previous papers, and only Edwards (1974) indicated the presence of new inflorescences in March under field



conditions, without quantifying this increase. The parallel increase in the number of flowers per inflorescence during the reproductive seasons has previously only been mentioned by Edwards (1974).

The reproductive cycle of *C. quitensis*, from initiation of flowers to seed production, takes usually more than one year (Greene & Holtom 1971; Edwards 1974; Lewis Smith 2003; Vera 2013). Initiation floral development occurs in November-December, depending on ambient conditions during the year (Greene & Holtom 1971; Edwards 1974). It is probable that the first flowers from the plant populations studied on Hurd Peninsula (Livingston I.), appeared in December of the austral summer 2001-2002, because the flowers had not reached an advanced stage of development in January. At lower altitude sites, mainly NE in orientation, some plants had their capsules open with ripe seeds in a single growing season. As for *D. antarctica*, new flowers of *C. quitensis* were formed in February, except in the highest altitude populations. Based on the frequency distribution of capsule size of plants in March, a second set of flowers was noted by Edwards (1974), whose results and those of this paper confirm an increase in flower number (and also for *D. antarctica* inflorescences) under different temperature and photoperiod experiments (Greene 1970).

The maturation stage varied with altitude and orientation. Maturation is delayed with altitude and in those microhabitats having a higher duration of snow for both *D. antarctica* and *C. quitensis*. However, there is a compensatory effect on warmer slopes with a NE orientation, where development of reproductive structures is accelerated. These results suggest a strong influence of temperature on the maturation of reproductive structures, and support the findings of increased development of reproductive structures in *D. antarctica* and *C. quitensis* under experimental warming conditions (Day et al. 1999).

The responses of phenological traits of both species to temperature and altitude reflect changing climatic patterns, as has been recognized in plants and animals in other regions (Root *et al.* 2003; Walther *et al.* 2002; Peñuelas & Filella 2001). A revision of the phenological traits of these two species, using published data from the 1960's and from other high latitude islands, together with current observations, is given in Table 11.

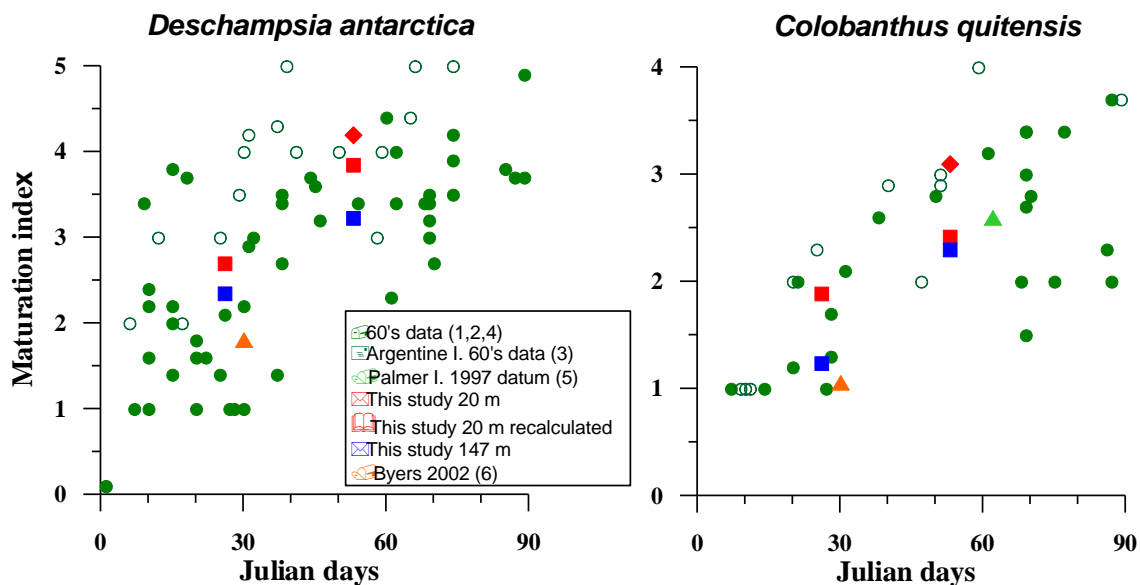
**Table 11.** Source of data shown in figures 6 and 7

Refs.	Authors	Sites	Latitude
1	Holtom & Greene (1967)	South Orkney Is. South Shetlands Is.	60°S 61°-63°37'S
2	Greene & Holtom (1971)	South Orkney Is. South Shetlands Is.	60°S 61°-63°37'S
3	Corner (1971)	Argentine Is.	65°S
4	Edwards (1974)	South Orkney Is.	60° S
5	Day et al. (1999)	Anvers Is.	64° 47' S
6	Vera et al. (2013)	Byers, Livingston Is.	62°34' -40'

Variation in size of different characters of *D. antarctica* inflorescences along the altitudinal gradient also suggests an effect of temperature on their formation. Inflorescence stalks from populations studied in the 1960's (Corner 1971, Edwards 1974) were shorter than in the present study. Smaller capsule size in *C. quitensis* at higher altitudes in the present study and during the 1960's (Edwards 1974) suggests an effect of climate warming on flowers development in the Antarctic Peninsula area. Other evidence for change with climate warming may be the new inflorescences in *D. antarctica* and flowers in *C. quitensis* that have formed February, except in *C. quitensis* populations

at the highest altitude. This phenomenon seems to have been observed one month later, in March, four decades ago (Edwards 1974).

However, no great differences have been found when considering changes in the maturation index (Fig. 6). If data from the Argentine I. is not included, the population at the flat ridge top at 20 m represents one of the highest maturation indices for February in the recalculated index. The results obtained for flower maturation stages of *C. quitensis* are coincident with the results obtained by Day et al. (1999), who studied a population on Palmer Island, where all flowers were considered. Further possible evidence that temperatures may affect maturation of reproductive structures comes from the response of populations from higher altitudes in the present study being comparable to data obtained from on Byers Peninsula (west of Livingston I.) with lower temperatures (Vera et al. 2013) or from lower altitude populations in the 1960's. Although data from the present study was recalculated to enable comparisons with previous studies, such comparisons are always difficult because of insufficient data recorded in previous studies and the methodologies used are not always well defined.



**Figure 6.** Maturity index of *D. antarctica* inflorescences and *C. quitensis* flowers (January-March) obtained from different sources (Table 11) under ambient conditions and this study on Hurd Peninsula (20 and 147 m). The recalculated results are also shown without considering new flowers produced in February.

### ***Production of reproductive structures and seed size***

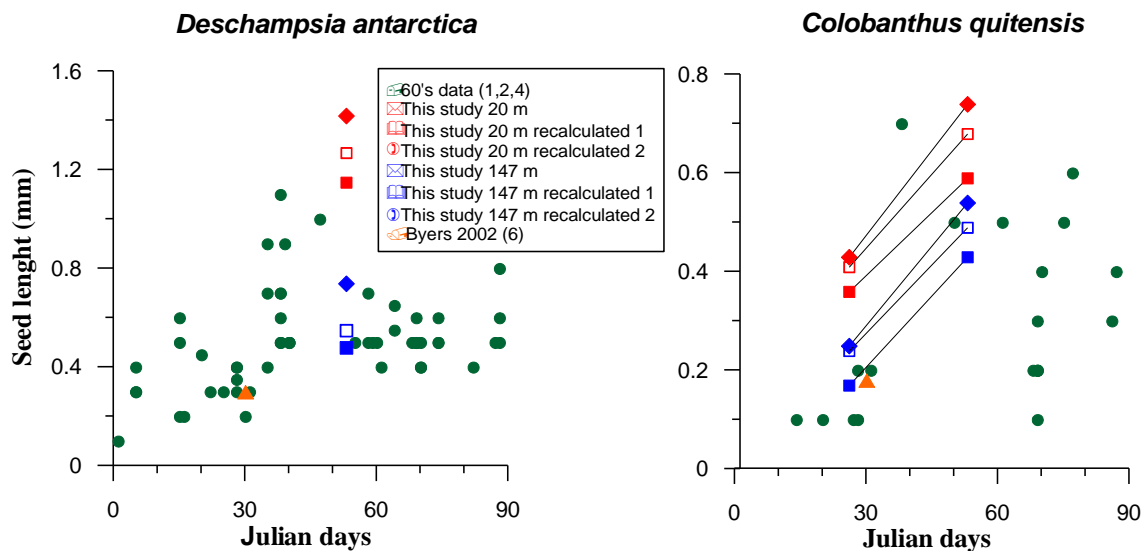
Seed size and number of ripe seeds produced by *D. antarctica* and *C. quitensis* may be more indicative of response to climate warming in the Antarctic Peninsula area than inflorescences and flowers maturation indices. Seed size and numbers of ripe seeds are not always well correlated. The fact that both species have produced mature seeds, even in one season, suggests a improvement in success of sexual reproduction, when historically the proportion of years in which mature seed is produced is very low (Corner 1971, Greene & Holtom 1971, Edwards 1974). Neither mature seeds in both species, nor flowers of *C. quitensis* with open capsules, were observed at the end of the growing season in the South Shetlands during the 1960's or in previous years (Greene & Holtom 1971). In the present study, neither the plants of *C. quitensis* from higher altitudes had open current year capsules at the end of February, however some apparently ripe filled seeds with pale browns coloration were observed inside, although it is likely that these current

seeds were not fully developed at this time (this could not be confirmed with germination test, Vera 2017). Anyway, this would need to be confirmed at the end of the growing season.

The significant increase in the current year seed length with respect to observations from the 1960's for plants at lower altitudes, and seeds in the higher altitude populations studied being of similar length to lower altitude populations studied in the 1960's and to those areas like on Byers Peninsula (West Livingston I.) with lower temperature than Hurd Peninsula (Vera et al., 2013), may reflect direct responses to climate warming ( Fig. 7). Differences in the maturation reproductive structures between Byers and Hurd Peninsulas (Fig. 6) are also indicated by Vera et al. (2013).

The mean number of seeds per capsule and the size of ripe seeds in *C. quitensis* on Livingston I. are affected by temperature, since these parameters decreased along of the altitudinal gradient (Table 8) and cover the range estimated by other authors for several Antarctic Islands. These results agree with the patterns arising from a comparative analysis of previous population studies in the Antarctic Islands (Corte 1961; Edwards 1974; Convey 1996; Day et al. 1999; Day et al. 2001), in which the mean number of seeds per capsule decreased with altitude and latitude. This also agrees with results from experimental warming, in which capsules produced more seeds (Day et al. 1999). Seed development in *C. quitensis* was influenced negatively by soil moisture; dry soils with a higher proportion of sand particulates are usually warmer than moist soils.

A lower flower production in *D. antarctica* populations at NE and SW oriented sites could explain the greater percentage of ripe seeds in the soil. In moist soils, the flowers tended to produce fewer ripe seeds. Corner (1971) noted the presence of sterile *D. antarctica* plants on moist habitats.



**Figure 7.** Current year seed length of *D. antarctica* and *C. quitensis* obtained from different sources (Table 11) under ambient conditions and this study on Hurd Peninsula (20 and 147 m). For results recalculated, see methods.

### ***Seed bank***

The percentage of ripe seeds from the previous years' seed production was higher than the current year's seeds in the seed bank, but these data could be influenced by degradation or germination of seeds. Probably most of the seeds continue their development until the end of the growing season or even into the next season, and some complete their development to full-size, as has been detected in *D. antarctica* seeds during winter (Edwards 1974). For *D. antarctica*, the percentage of ripe seeds in relation to the total flowers produced in the previous years (old) in the soil could be overestimated because a higher proportion of flowers without swollen seed may become detached and lost, particularly in windy sites and there is a higher ratio of new flowers to old flowers. The percentage of ripe seeds found in the organic stratum 0-2 cm (dominated by the previous year's seed) in relation to the total current year's flowers gives a more precise measure of maturation efficiency, although it is necessary to assume a similar number of flowers produced each year. This percentage is an underestimate, because a portion of the ripe seeds is lost by germination, dispersion and degradation (Edwards 1974; Leck *et al.* 1989; Vera 2017). Due to the cushion habit of *C. quitensis*, dispersion of flowers and seeds is difficult. In this study the number of old seeds remaining in the cushion with respect to new seeds was lower on the NE and SW slopes than on the flat ridge top. Gravity, water flow may lead to greater down slope dispersal of seeds away from the parent plants than on the flatter ridge tops. The seedlings of *C. quitensis* were more abundant on steep unstable soil, below the parent cushions, (Edwards 1974).

The seed number of both species in the seed bank tended to decrease with altitude. Most seeds found at the highest altitude are clear, in contrast to a black coloration when they are attacked by fungi. Colder temperatures at the higher elevations results usually in better preservation of the seeds (Miller & Cummins 1987; McGraw & Vavrek 1989). Furthermore, better preservation of old *D. antarctica* flowers was also observed at the higher sites and the number of old flowers also increased with altitude in relation to flower production. Well drained soils, found mostly on sloping sites, seems also to contribute to a better conservation of the seeds of both species.

The number of ripe seeds in the organic stratum of the soil tended to be higher on the NE slopes. This may be one of the reasons for the higher presence of these species at the warmer sites of north facing slopes (Edwards 1972; Longton 1985).

The number of seeds per m<sup>2</sup> for *C. quitensis* at low altitudes on Livingston Island is generally higher than that estimated for the southernmost locality of the species, near Palmer Station (64° 47'S), when related to the cover percentage per unit area of this species (McGraw & Day 1997; Ruhland & Day 2001). Seed bank size from the highest altitudes on Hurd Peninsula (area of this study in Livingston Island) is similar to that observed at lower altitudes on Byers Peninsula, also in Livingston I., but with colder temperatures (Vera *et al.* 2013) and at Palmer Station.

The production of ripe seeds in both species at the highest altitudes as well the observation of current year's seedlings (235 per m<sup>2</sup> for *C. quitensis* and 7 per m<sup>2</sup> for *D. antarctica*, - Vera 2011) and the presence of smaller plants at this high site (Vera 2011), suggests that these species are not at their limit of sexual reproductive capacity. Edwards (1972) and Lewis Smith (2003) considered that vegetative propagation (often by birds) may be the most frequent means of dispersal and establishment of *D. antarctica*. In contrast, the establishment of grasses from seed is the main form of colonization in the Arctic (Grulke & Bliss 1988 in Convey 1996). The presence of populations of the two species on the windy top sites at higher altitudes could be related with the presence of nesting skuas in snow free areas in the early austral summer (Vera 2011). The birds may transport and disperse plant fragments (mainly ramets of *D. antarctica*) and seeds.

A decrease in numbers of ripe seeds with depth in the soil may indicate that a higher proportion of seeds from the deeper layers have germinated and higher numbers of seeds destroyed than in the surface layers, since seeds in depth are older than those from the surface level (McGrav & Vavrek 1989; Legg et al. 1992; Bruggink 1993).

Responses of *D. antarctica* and *C. quitensis* to climate warming in the Antarctic Peninsula area have been demonstrated in this study. There is a better development of reproductive structures of both species at lower altitudes than at the higher sites (with a mean adiabatic temperature variation about 1°C). All sites studied have a similar snow-free period, including the possibility to complete formation of seeds in the current year. Also, a small variation in the temperature affected to recruitment of seedlings on Livingston Island (Vera 2011). This suggests that the Antarctic vascular plants are highly sensitive to temperature shifts. There has been a rapid increase in the abundance and distribution of these species in the last decades (Lewis Smith 1994; Fowbert & Lewis Smith 1994; Lewis Smith 2003) in response to an increase of summer air temperature (more than 1°C in the Maritime Antarctic since 1950) and a longer growing season, although, a recent study (Steig et al. 2009) indicates a lesser warming trend in the Antarctic Peninsula region. The results and methodologies presented in this paper suggest the efficacy of future monitoring of climatic change through phenological responses and range changes of the vascular plants.

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