Cold resistance in Antarctic angiosperms

León A. Bravo a,*, Nancy Ulloa b, Gustavo E. Zuñiga c, Angélica Casanova b, Luis J. Corcuera a and Miren Alberdi b

aDepartamento de Botánica, Facultad de Ciencias Naturales y Oceanográficas, Universidad de Concepción, Casilla 160-C, Concepción, Chile
bInstituto de Botánica, Facultad de Ciencias, Universidad Austral de Chile, Valdivia, Chile
cDepartamento de Biología, Facultad de Química y Biología, Universidad de Santiago, Santiago, Chile
*Corresponding author, e-mail: lebravo@udec.cl

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Deschampsia antarctica Desv. (Poaceae) and Colobanthus quitensis (Kunth) Bartl. (Caryophyllaceae) are the only two vascular plants that have colonized the Maritime Antarctic. The primary purpose of the present work was to determine cold resistance mechanisms in these two Antarctic plants. This was achieved by comparing thermal properties of leaves and the lethal freezing temperature to 50% of the tissue (LT50). The grass D. antarctica was able to tolerate freezing to a lower temperature than C. quitensis. The main freezing resistance mechanism for C. quitensis is supercooling. Thus, the grass is mainly a freezing-tolerant species, while C. quitensis avoids freezing. D. antarctica cold acclimated by reducing degrees of polymerization were mostly between 3 and 10. C. quitensis showed little cold-acclimation capacity. Because day length is highly variable in the Antarctic, the effect of day length on freezing tolerance, growth, various soluble carbohydrates, starch, and proline contents in leaves of D. antarctica growing in the laboratory under cold-acclimation conditions was studied. During the cold-acclimation treatment, the LT50 was lowered more effectively under long day (21.3 h light/dark) and medium day (16/8) light periods than under a short day period (8/16). The longer the day length treatment, the faster the growth rate for both acclimated and non-acclimated plants. Similarly, the longer the day treatment during cold acclimation, the higher the sucrose content (up to 7-fold with respect to non-acclimated control values). Oligo- and polyfructans accumulated significantly during cold acclimation only with the medium day length treatment. Oligofructans accounted for more than 80% of total fructans. The degrees of polymerization were mostly between 3 and 10. C. quitensis under cold acclimation accumulated a similar amount of sucrose than D. antarctica, but no fructans were detected. The suggestion that survival of Antarctic plants in the Antarctic could be at least partially explained by accumulation of these substances is discussed.

Introduction

Freezing-resistant plants may either avoid ice formation in the tissues (avoidance) or tolerate the various strains exerted by extracellular ice formation (tolerance) (Levitt 1980, Alberdi and Corcuera 1991). Supercooling is a frequent avoidance mechanism against freezing injury in plants from regions where frost occurs during periods of high metabolic and developmental activity (Levitt 1980, Sakai and Larcher 1987). Freezing tolerance is usually observed in tropical environments at high altitude, where sub-zero temperatures may occur any night of the year or in zones with a seasonal climate (Alberdi et al. 1985, Larcher 1995). In high tropical Andean habitats, ground-level plants showed freezing tolerance, while arborescent forms showed supercooling as the main mechanism of cold resistance (Goldstein et al. 1985, Squeo et al. 1991). They suggested that a combination of freezing tolerance and avoidance by insulation is less expensive and a more secure mechanism than supercooling alone. The maximum freezing tolerance of plants is usually induced in response to low, non-freezing temperatures (below approximately 10°C). This phenomenon is known as cold acclimation or cold hardening (Levitt 1980, Alberdi and Corcuera 1991). Freezing tolerance is a result of several cryoprotective mechanisms operating concurrently (Sakai and Larcher 1987). Because compatible solutes accumulate during cold acclimation, it is thought that this accumulation is a cryoprotective mechanism in some plants (Alberdi and Corcuera 1991, Livingston 1996). Soluble carbohydrates and free proline may be involved in freezing point depression of

Abbreviations – DP, degree of polymerization; LD, long day; LT50, lethal temperature to 50% of the tissue; MD, medium day; SD, short day; SPS, sucrose phosphate synthase.
cell sap, prevention of plasmolysis during cell dehydration caused by freezing, and protein and lipid stabilisation (Strauss and Hauser 1986, Livingston et al. 1989, Santarius 1992). Proline accumulates in a variety of plants subjected to cold and its content has been correlated with frost tolerance (Alberdi et al. 1993, Dörfling et al. 1997, Bravo et al. 1998, Wanner and Juntila 1999). Fructans (polyfructosylsucrose) have been described as storage sugars in vegetative tissues of several plant groups (Nelson and Spollen 1987). Their physiological role is not completely understood, although, there is evidence that fructan’s role is not merely storage (for review see Vijn and Smeekens 1999). Several reports correlated cold acclimation with an increase in fructan contents (Santoiani et al. 1993, Puebla et al. 1997, Koroleva et al. 1998) and also with depolymerization of polyfructans (fructo-polysaccharides) into oligofructans (fructo-oligosaccharides) (Pontis 1989, Suzuki 1989, Livingston 1991). It is difficult, however, to establish a direct correlation between stress and fructan accumulation (Vijn and Smeekens 1999). High irradiance and low temperatures favor accumulation of fructans (Nelson and Spollen 1987). Long days (LDs) have been reported to favor accumulation of fructans (Hendry 1987), but this accumulation was probably caused by increased total irradiance in LDs (Solhaug 1991). Fructan synthesis under cold and water stress was compared in two Bromus species adapted to different climatic conditions (Puebla et al. 1997). It was found that species adapted to a cold desert climate exhibited constitutive fructan synthesis, whereas species adapted to a warmer climate produced fructans only under cold stress. There is no evidence of direct involvement of fructans in cryoprotection. However, they have a well-established osmotic activity, which could indicate a possible function as volume regulators of vacuoles (Pontis 1989). Further research is necessary to clarify if fructans are involved in cold resistance.

Freezing tolerance seems to be improved by short days (SDs) and low temperature in some woody plants (Levitt 1980). In pine seedlings, even at warm temperature, SDs induced higher frost resistance. In other plants, SDs alone are ineffective; a combination of SDs and low temperature is necessary to result in hardening in these plants (Sakai and Larcher 1987, Larcher 1995). In winter cereals, the day length seems to be only of secondary importance, if at all (Sakai and Larcher 1987). Low temperatures and SDs appeared to increase tolerance via an increase in carbohydrate content, particularly sucrose in woody plants (Aronson et al. 1976). Carbohydrate accumulation is required for hardening in cereals (Levitt 1980, Alberdi et al. 1993, Maldonado et al. 1997).

Deschampsia antarctica Desv. (Poaceae) and Colobanthus quitensis (Kunth) Bartl. (Cariophyllaceae) are the only two angiosperms that have colonized the Antarctic islands (Edwards and Lewis-Smith 1988, Casaretto et al. 1994). The native Antarctic vegetation must have one or various mechanisms that allow the maintenance of metabolism at low temperature during the Antarctic summer (growing season) and survival during winter. Previous studies have shown that D. antarctica does not have unusual contents in polar lipids or degree of unsaturation of fatty acids compared with other Poaceae (Zühiga et al. 1994). High amounts of sucrose and fructans are found mainly towards the end of summer under field conditions (Zühiga et al. 1996). This is consistent with the findings that D. antarctica and C. quitensis have relatively high net photosynthetic rates on cool days (Xiong et al. 1999). At 0°C, these plants maintain about 30% of the photosynthetic rate found at the optimum temperature (Edwards and Lewis-Smith 1988). The purpose of the present work was to determine freezing tolerance and the supercooling capacities of these two Antarctic plants. In addition, we hypothesized that day length during cold acclimation influences the accumulation of non-structural carbohydrates (soluble sugars and fructans) and proline, and therefore, freezing resistance in both plants.

Materials and methods

Plant material and growth conditions

Deschampsia antarctica Desv. (Poaceae) was collected on the Coppermine Peninsula on Robert Island, Maritime Antarctic (62°22′S, 59°43′W) and Colobanthus quitensis (Kunth) Bartl. (Cariophyllaceae) was collected on King George Island, Maritime Antarctic (62°14′S, 58°48′W). A description of the environmental conditions of the habitat has been published elsewhere (Zühiga et al. 1996). Plants of both species were transported in plastic bags to the laboratory. Plants were reproduced vegetatively in plastic pots, using a soil:peat mixture (2:1) and maintained at 13–15°C in a growth chamber (Forma Scientific, Inc., Marietta, OH, USA) with a photon flux density of 180 μmol m−2 s−1 at the top of canopy and 16/8 h light/dark period. The light source consisted of cool-white fluorescent tubes F40CW (General Electric, Charlotte, NC, USA). Plants were fertilized with Phostrogen® (Solaris, Buckinghamshire, UK) using 0.12 g 1−1 once every 2 weeks. The cold-acclimation treatment consisted of transferring plants described above to other growth chambers set at 4°C with the same light intensity and 3 different day lengths for D. antarctica (8/16 h = SD, 16/8 h = medium day [MD], and 21/3 h light/dark = LD). Radiation integrals were 5.2, 10.4, and 13.6 mol m−2 day−1 for the SD, MD, and LD treatments, respectively. Because of lack of sufficient material, only MD treatment was used for C. quitensis. Samples were taken at different times of cold acclimation from the MD photoperiod for determination of freezing resistance mechanisms. Unless otherwise indicated, values are means of two separate experiments consisting of a sample of pooled leaves from 4 to 5 pots. The pooled leaves from each sample were divided into 3 replicates. A 3-way ANOVA (P = 0.05; acclimation time, day length and temperature) demonstrated that the variability among these subsamples was equal or greater than the variability between the experiments for the measurements mentioned below. For this reason, n = 6 was considered for the following analyses: freezing tolerance, non-structural soluble sugars, starch and free proline contents. These analyses were performed in non-acclimated (control) and cold-acclimated plants under the 3 day-length treatments. Plant material was collected 2 h after the beginning of the photoperiod to avoid diurnal variations in compounds.

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Water content and mean relative growth rate (MRGR)

Four genets containing 4 rams each with the same number of leaves (two developed and one emergent leaves) were taken from *D. antarctica* plants growing at 13–15°C and considered as a sample unit for mass measurements. Four of these samples were harvested at the beginning of the treatments to determine dry weight (W0) using an analytical balance (Sartorius-Werke GmbH, Göttingen, Germany). Four other samples were subjected to cold acclimation at each day length described above and were harvested after 21 days of treatment. Total dry weight (shoots and roots) was determined (W21) and mean relative growth rates were calculated as follows: MRGR = (ln W21 − ln W0)/Δt.

Dry weights of roots and shoots were measured separately in order to calculate the shoot/root ratios. Fresh weight (FW) was measured immediately after harvesting and dry weight (DW) was determined after drying the tissue for 10 min at 105°C and then at 80°C until constant mass was obtained. Water content (%) was calculated as follows: WC = 100 × (FW − DW)/FW.

Thermal analysis

One expanded leaf with the apex removed was attached to a thermocouple (Copper-constantan thermocouples, Gauge 30; Cole Palmer Instruments Co., Vernon Hills, IL, USA), and immediately enclosed in small, tightly closed cryotubes to avoid changes in tissue water content. Temperature was continuously monitored and recorded (1 measurement per s) with a ACjr data acquisition board connected to a multi-channel temperature terminal panel (Strawberry Tree Inc. Sunnyvale, CA, USA). The tubes were placed in a cryostat and the temperature was lowered from 0°C to −17°C at a rate of approximately 2°C h⁻¹. The temperature at the initiation of the freezing exotherm corresponds to the ice nucleation temperature, while the highest point of the exotherm represents the freezing temperature of the water in the apoplast (including symplastic water driven outwards by the water potential difference caused by apoplastic ice formation) (Larcher 1995).

Freezing tolerance

Freezing tolerance was determined by measuring the lethal temperature of 50% of the leaf tissues (LT₅₀, also called index of freezing injury) by ion leakage (Flint et al. 1967). Sixteen leaf segments (1 cm length) collected from 4 different pots were placed at the bottom of a vial using silver iodide as a nucleating agent. The vials were stoppered and pots were placed at the bottom of a vial using silver deionized by passing them through a Dowex 50-1X8 column. Then, the degree of polymerization (DP) of fructo-oligosaccharides was determined by TLC using butanol:acetic acid:water (2:1:1 v/v) as mobile phase. A solution composed of 5.6 ml H₃PO₄ + 94.4 ml of a butanol water mixture (99:20 v/v) + 3 g urea dissolved in 5 ml ethanol was used to stain the ketose containing spots (Vieira and Figueiredo-Ribeiro 1993). They were identified by comparison with oligosaccharide standards of the inulin series prepared from dormant tubers (subjected to 4°C for 3 months) of *Helianthus tuberosus* according to Pollock (1982a). Isokestose and nystose (kindly provided by Dr...
The ice nucleating temperature of temperatures during a cold-acclimation period (Table 1). Dichloroethane and the A520 was compared with a standard Ninhydrin–proline complexes were extracted in 1,2- 2.5 ml of extract was used for the acid ninhydrin reaction. After filtration through Whatman No. 1 filter paper, B Bates et al. (1973). Leaves (0.2 g fresh weight) were ground Free proline was determined colorimetrically according to Proline quantified from the residues of the ethanolic extractions Hokkaido, Japan) and raffinose (Sigma Chemical Co., St ulty of Dairy Science, Rakuno Gakuen University, Ebetsu, Norio Shiomi from the Department of Food Science, Fac- 

Table 1. Relationship between cold injury, ice nucleation and freezing temperature in cold-acclimated Antarctic plants (4°C, day length 16/8 h light/dark, photon flux density 180 μmol m⁻² s⁻¹). Values are means of 3 replicates for the thermal analyses and 4 replicates ± SD for the LT₅₀. Values without a common letter are significantly different (P≤0.05). Lower case letters compare nucleation and freezing temperatures (columns and rows). Upper case letter compare only within the LT₅₀ column. LT₅₀ = lethal temperature for 50% of leaf tissue; AS = avoidance by supercooling; FT = freezing tolerance. FT/AS, presents both mechanisms.

<table>
<thead>
<tr>
<th>Plant species</th>
<th>Acclimation time (days)</th>
<th>Nucleation temperature (°C)</th>
<th>Freezing temperature (°C)</th>
<th>LT₅₀ (°C)</th>
<th>Freezing resistance mechanism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deschampsia antarctica</td>
<td>0</td>
<td>-5.3 ± 0.3a</td>
<td>-3.7 ± 0.2d</td>
<td>-12.0 ± 2.0A</td>
<td>FT/AS</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>-5.8 ± 0.2ac</td>
<td>-4.0 ± 0.2d</td>
<td>-21.5 ± 0.4B</td>
<td>FT/AS</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>-7.5 ± 0.3b</td>
<td>-6.5 ± 0.3be</td>
<td>-24.0 ± 0.2C</td>
<td>FT/AS</td>
</tr>
<tr>
<td></td>
<td>21</td>
<td>-10.4 ± 0.3c</td>
<td>-9.2 ± 0.5cf</td>
<td>-26.6 ± 0.6D</td>
<td>FT/AS</td>
</tr>
<tr>
<td>Colobanthus quitensis</td>
<td>0</td>
<td>-6.6 ± 0.7bc</td>
<td>-1.6 ± 0.3g</td>
<td>-4.8 ± 0.2E</td>
<td>AS</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>-6.2 ± 0.2ac</td>
<td>-1.6 ± 0.2g</td>
<td>-5.7 ± 0.4E</td>
<td>AS</td>
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<tr>
<td></td>
<td>14</td>
<td>-8.2 ± 0.2bf</td>
<td>-3.0 ± 0.5d</td>
<td>-4.3 ± 0.2E</td>
<td>AS</td>
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<tr>
<td></td>
<td>21</td>
<td>-9.4 ± 0.5c</td>
<td>-3.8 ± 0.3d</td>
<td>-5.8 ± 0.2E</td>
<td>AS</td>
</tr>
</tbody>
</table>

Norio Shiomi from the Department of Food Science, Faculty of Dairy Science, Rakuno Gakuen University, Ebetsu, Hokkaido, Japan) and raffinose (Sigma Chemical Co., St Louis, MO, USA) were also used as standards. Starch was quantified from the residues of the ethanolic extractions after acid hydrolysis with perchloric acid, using the phenolsulfuric method (Dubois et al. 1956).

Proline

Free proline was determined colorimetrically according to Bates et al. (1973). Leaves (0.2 g fresh weight) were ground in liquid nitrogen and extracted with 5 ml 3% sulphosalicylic acid. After filtration through Whatman No. 1 filter paper, 2.5 ml of extract was used for the acid ninhydrin reaction. Ninhydrin–proline complexes were extracted in 1,2- dichloroethane and the A₅₂₀ was compared with a standard curve obtained with pure proline (Merck KGaA).

Statistics

The statistical differences in ice nucleation, freezing temperature and freezing tolerance (LT₅₀), of cold-acclimated and non-acclimated plants were calculated using one-way ANOVA (level of significance was P ≤ 0.05). A Tukey test was then used to identify those values with significant differences. To analyze the combined effects of cold acclimation and day length on the different parameters studied, a two-way ANOVA was also performed in each case (SigmaStat 2.0 software (SPSS® Inc., Chicago, IL, USA) was used for both analyses. For comparisons among plant species, a one-way ANOVA was used.

Results

Cold resistance mechanisms

D. antarctica leaves decreased its ice nucleation and freezing temperatures during a cold-acclimation period (Table 1). The ice nucleating temperature of D. antarctica leaves was -5.3 in control plants and -10.4 in cold-acclimated plants and the LT₅₀ reached down to -26.6°C. Thus, D. antarc- tica is able to avoid freezing by supercooling and after ice nucleation occurs, it is able to tolerate freezing. In contrast, C. quitensis maintained its freezing tolerance (LT₅₀) nearly constant. A small but significant decrease in its ice nucleation temperature was observed during cold acclimation. Because LT₅₀ values of C. quitensis were always higher than ice nucleation temperature, it may be concluded that this species cannot tolerate freezing. Thus, the main mechanism for surviving low temperature in C. quitensis is supercooling. Thermal analysis experiments were executed without adding a nucleating agent. When AgI was added, no low temperature exotherm was observed in these plants down to -20°C.

Growth rates

The combinations of cold and day length affected MRGR (interaction P ≤ 0.05). The highest MRGR was found in D. antarctica plants growing at the LD treatment (Table 2) in both non-acclimated and cold-acclimated plants. MRGR decreased significantly in plants growing at the SD and MD
treatments. At the MD, MRGR were not significantly influenced by cold. MRGR of \textit{C. quitensis} were similar to those of \textit{D. antarctica} at the corresponding day length at both the cold-acclimated and non-acclimated state. With the exception of plants growing at the SD treatment no statistically significant differences were found in the shoot/root ratios of cold-acclimated and non-acclimated plants of \textit{D. antarctica}. In \textit{C. quitensis}, the shoot/root ratios of plants growing at the MD treatment were significantly lower than in \textit{D. antarctica} at the same treatment. These ratios were also not influenced by the low temperature.

Water content

Water contents of cold-acclimated \textit{D. antarctica} plants were frequently lower (\(P \leq 0.05\)) than those of control plants (Fig. 1). At the end of the cold treatment, lower water contents (\(P \leq 0.05\)) and thus higher dry weights with respect to control plants were obtained at all investigated day lengths. The lowest water contents were obtained in cold-acclimated plants under LD conditions (\(P \leq 0.05\)). In \textit{C. quitensis} water content decreased from 90 down to 77\% (\(P \leq 0.05\)) at 21 days of cold acclimation (values not shown).

Day length and cold hardening

Plants of \textit{D. antarctica} significantly decreased (\(P \leq 0.05\)) their LT\(_{50}\) over a period of 21 days of cold acclimation. This decrease in LT\(_{50}\) was lower at SD conditions (about 9°C) than at MD or LD treatments (about 13°C). Thus, low temperature exposure can improve significantly the freezing tolerance of this species, being enhanced by exposure to longer light periods (Fig. 2). The LT\(_{50}\) profiles showed a rapid decrease during the first week of acclimation, from −11.8 to around −23°C (\(P \leq 0.05\)) at the MD and LD treatments. At the SD treatment the LT\(_{50}\) profiles decreased down to approximately −18°C at a slower rate than in treatments with longer light periods. Experiments to cold acclimate plants of \textit{C. quitensis} were conducted only at the MD treatment. In contrast to \textit{D. antarctica}, the LT\(_{50}\) of leaves of \textit{C. quitensis} did not change significantly after 3 weeks of cold acclimation (Table 1).

Non-structural carbohydrates

Concomitantly with the exposure of \textit{D. antarctica} to low temperature, there was an increase in freezing tolerance and in sucrose (Fig. 2). Sucrose content showed a rapid increase during the first week of cold acclimation at LD and MD treatments, decreasing afterwards. Significant differences (\(P \leq 0.05\)) between sucrose contents of cold-acclimated and non-acclimated plants were found at the MD and LD light periods. In the SD treatment the small differences between non-acclimated and acclimated plants were statistically significant only at the end of cold treatment. In \textit{C. quitensis} grown at the MD treatment, sucrose content was 20 mg g\(^{-1}\) dry weight in non-acclimated plants and 130 mg g\(^{-1}\) dry weight in cold-acclimated plants (values not included in Fig. 2). Thus, \textit{C. quitensis} and \textit{D. antarctica} accumulated a similar amount of sucrose when cold acclimated at the MD treatment.

The combinations of cold and day length affected mainly total carbohydrates, fructans, and glucose (interaction \(P \leq 0.05\)). Total carbohydrates and fructan contents were significantly higher in cold-acclimated \textit{D. antarctica} plants at the MD light period than in the SD and LD treatments (\(P \leq 0.05\); Table 3). After 21 days of cold acclimation, plants showed 2.6–9.6-fold increases in total carbohydrates and 0.6–16-fold increases in fructan contents, with respect to the controls. The main increase in total fructans (16-fold) at the MD treatment corresponds to the fructo-oligosaccharide fraction (Fig. 3). At this light period, the polyfructan fraction increased fivefold over the whole period of acclimation (\(P \leq 0.05\)). The increment in the fructo-oligosaccharide fraction represents nearly 90\% of the total fructan increase at this day length. Low molecular mass forms are the main component of the fructo-oligosaccharide fraction, with a DP of 3 in both cold-acclimated and control plants (Fig. 4).
Higher DP forms were proportionally less abundant and only present under cold-acclimation conditions at the MD and LD treatment (Fig. 4). In *C. quitensis* no fructans were found (Table 3). Two compounds were detected in the TLC experiments in *C. quitensis* (TLC not shown). Although the identity of these compounds is not firmly established, it is likely that they correspond to sucrose and raffinose because they are ketose containing compounds (as indicated by the urea reagent), they give a negative reaction with the Fehling reagent and have the same R<sub>f</sub> as sucrose and raffinose.

Glucose and fructose contents were higher in cold-acclimated *D. antarctica* plants than in controls at the end of treatments in all day lengths (Table 3). However, the levels of these carbohydrates were significantly higher at the SD treatments in all day lengths (Table 3). However, the levels of these carbohydrates were significantly higher at the SD treatments in all day lengths (Table 3). However, the levels of these carbohydrates were significantly higher at the SD treatments in all day lengths (Table 3). However, the levels of these carbohydrates were significantly higher at the SD treatments in all day lengths (Table 3). However, the levels of these carbohydrates were significantly higher at the SD treatments in all day lengths (Table 3). However, the levels of these carbohydrates were significantly higher at the SD treatments in all day lengths (Table 3). However, the levels of these carbohydrates were significantly higher at the SD treatments in all day lengths (Table 3). However, the levels of these carbohydrates were significantly higher at the SD treatments in all day lengths (Table 3). However, the levels of these carbohydrates were significantly higher at the SD treatments in all day lengths (Table 3). However, the levels of these carbohydrates were significantly higher at the SD treatments in all day lengths (Table 3). However, the levels of these carbohydrates were significantly higher at the SD treatments in all day lengths (Table 3). However, the levels of these carbohydrates were significantly higher at the SD treatments in all day lengths (Table 3). However, the levels of these carbohydrates were significantly higher at the SD treatments in all day lengths (Table 3). However, the levels of these carbohydrates were significantly higher at the SD treatments in all day lengths (Table 3). However, the levels of these carbohydrates were significantly higher at the SD treatments in all day lengths (Table 3). However, the levels of these carbohydrates were significantly higher at the SD treatments in all day lengths (Table 3). However, the levels of these carbohydrates were significantly higher at the SD treatments in all day lengths (Table 3). However, the levels of these carbohydrates were significantly higher at the SD treatments in all day lengths (Table 3). However, the levels of these carbohydrates were significantly higher at the SD treatments in all day lengths (Table 3). However, the levels of these carbohydrates were significantly higher at the SD treatments in all day lengths (Table 3).

**Free proline**

The combinations of cold and day length affected proline content in *D. antarctica* (interaction *P* ≤ 0.05). Free proline contents of *D. antarctica* increased up to eightfold (*P* ≤ 0.05) under cold-acclimation conditions with respect to the controls at the SD and MD treatments (Table 3). At the LD treatment the highest proline increase with respect to the controls was at the LD treatment, where a small increase was found in the last weeks of cold acclimation (6.8 and 7.6 mg g<sup>-1</sup> dry weight).

**Table 3.** Changes in contents of carbohydrates (mg g<sup>-1</sup> DW) and proline in plants of *D. antarctica* and *C. quitensis* subjected to cold acclimation (4°C, photon flux density of 180 μmol m<sup>-2</sup> s<sup>-1</sup>) at different day length treatments. 0 = non-acclimated plants at beginning of the experiment; SD = short day (8/16 h light/dark); MD = medium day (16/8 h light/dark); LD = long day (21/3 h light/dark). Values are means ± SD of two separate experiments with 3 replicates each. Total fructan contents were estimated by addition of the oligo- and polyfructan fractions. In these fractions, fructose and fructose equivalents from sucrose were subtracted. The effect of cold acclimation and day length treatments on the various parameters was determined by a two-way ANOVA and Tukey test in *D. antarctica*. Differences between species were determined by a one-way ANOVA and Tukey test. Different letters indicate statistically significant differences (*P* ≤ 0.05).

<table>
<thead>
<tr>
<th>Day length (D/ N)</th>
<th>Acclimation time (days)</th>
<th>Total carbohydrates</th>
<th>Total fructans</th>
<th>Glucose</th>
<th>Fructose</th>
<th>Starch</th>
<th>Proline (μmol g&lt;sup&gt;-1&lt;/sup&gt; DW)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>D. antarctica</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SD</td>
<td>0</td>
<td>55 ± 2a</td>
<td>20 ± 1a</td>
<td>5 ± 1a</td>
<td>4 ± 1a</td>
<td>5.3 ± 0.5a</td>
<td>4 ± 1a</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>102 ± 4b</td>
<td>17 ± 1a</td>
<td>20 ± 1b</td>
<td>6 ± 1ab</td>
<td>5.3 ± 0.4a</td>
<td>20 ± 1b</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>130 ± 5c</td>
<td>23 ± 2ab</td>
<td>20 ± 2b</td>
<td>10 ± 1b</td>
<td>5.1 ± 0.5a</td>
<td>21 ± 1b</td>
</tr>
<tr>
<td></td>
<td>21</td>
<td>202 ± 8d</td>
<td>31 ± 1b</td>
<td>47 ± 1c</td>
<td>10 ± 4b</td>
<td>5.0 ± 0.1a</td>
<td>26 ± 2b</td>
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<td>MD</td>
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<td>345 ± 12e</td>
<td>89 ± 4c</td>
<td>37 ± 2d</td>
<td>11 ± 1b</td>
<td>3.3 ± 0.3b</td>
<td>16 ± 1b</td>
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<tr>
<td>LD</td>
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<td>233 ± 12h</td>
<td>32 ± 3b</td>
<td>5 ± 1af</td>
<td>9 ± 2b</td>
<td>5.5 ± 0.5ac</td>
<td>209 ± 23c</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>229 ± 1h</td>
<td>51 ± 1g</td>
<td>7 ± 2af</td>
<td>9 ± 1b</td>
<td>6.8 ± 0.4cd</td>
<td>244 ± 14d</td>
</tr>
<tr>
<td></td>
<td>21</td>
<td>199 ± 10d</td>
<td>54 ± 1g</td>
<td>10 ± 3ef</td>
<td>9 ± 1b</td>
<td>7.6 ± 0.5de</td>
<td>279 ± 5e</td>
</tr>
<tr>
<td><em>C. quitensis</em></td>
<td></td>
<td>33 ± 3i</td>
<td>0</td>
<td>6 ± 1af</td>
<td>2 ± 1c</td>
<td>10.3 ± 0.1c</td>
<td>40 ± 11b</td>
</tr>
<tr>
<td>MD</td>
<td>21</td>
<td>146 ± 3c</td>
<td>0</td>
<td>6 ± 1af</td>
<td>7 ± 1b</td>
<td>8.9 ± 0.3ce</td>
<td>30 ± 1b</td>
</tr>
</tbody>
</table>

![Fig. 2. Freezing tolerance and sucrose contents of *Deschampsia antarctica* upon cold acclimation under 3 day length treatments: SD = short day (8/16 h light/dark); MD = medium day (16/8 h light/dark); LD = long day (21/3 h light/dark). Lethal temperature of 50% of leaf tissue (LT<sub>50</sub>) was evaluated by ion leakage. Sucrose was determined by HPLC in ethanolic extracts. Values are means ± SD of three separate experiments with three replicates each; vertical bars are not shown when they do not exceed the size of symbols.](image-url)
control (up to eightyfold) was found at the end of cold treatment. At this time, proline contents were around 10-fold higher than the proline contents of cold-acclimated plants at the SD and MD treatments. In *C. quitensis* no statistically significant differences between proline contents of cold-acclimated and non-acclimated plants were found (Table 3).

**Discussion**

The two Antarctic vascular plants showed different strategies to resist cold temperature. The grass *D. antarctica* is mainly a freezing-tolerant species, while *C. quitensis* avoids freezing by supercooling. *D. antarctica* was able to cold acclimate, reducing its LT50 and ice nucleation temperature. *C. quitensis* showed little capacity to cold acclimate. The sensitivity of a variety of plants to frost is affected by epiphytic bacteria with ice nucleating activity (Lindow et al. 1982). Ice nucleating bacteria reduce protection of the tissues against frost damage by decreasing supercooling. It is unknown; however, whether these bacteria are present in Antarctic plants. *D. antarctica* is found in more exposed areas than *C. quitensis* in the Antarctic. The population of *D. antarctica* is expanding in the Maritime Antarctic (Casaretto et al. 1994). For *C. quitensis*, the status of its population dynamics is not known. Distribution of these plants in the Antarctic may increase as global warming occurs. The predicted warming for the Antarctic Peninsula is about 3°C over the next 30 years (Mitchell et al. 1990). Both plants have a photosynthetic optimum near 15°C (Xiong et al. 1999), and are able to maintain about 30% of that optimum at 0°C (Edwards and Lewis-Smith 1988). A small change in temperature may increase its photosynthetic rate significantly. For example, at 5°C this percentage may increase up to 70 and 60% of maximum photosynthetic rate in *D. antarctica* and *C. quitensis*, respectively. Field experiments near Palmer Station in the Antarctic show that warming by 2.3°C significantly increases growth and sexual reproduction of these plants (Grobe et al. 1997, Day et al. 1999).

Day length is a highly variable characteristic in the Maritime Antarctic, ranging from 21 h at the beginning of summer to 3 h in winter. For this reason, studying the effect
of the day length on cold-acclimation responses in Antarctic plants is especially pertinent. Because the availability of \textit{C. quitensis} was limited, studies of the effect of day length were performed only with \textit{D. antarctica}. The main effects of cold were observed with MD and LD treatments. This was expected because plants are usually covered with snow during most of fall, winter and spring in this area, when the shortest days occur. During cold acclimation, the most effective day length for total carbohydrates and fructans accumulation was MD, which occurs in the field by the end of summer. LD treatment was more effective for sucrose and proline accumulation than MD and SD treatments. This is consistent with the photosynthetic requirement for cold acclimation and solute accumulation in other plants. For example, it is known that illumination favors proline accumulation in barley and wheat subjected to water and cold stress respectively by converting glutamate into proline (Stewart 1978, Hanson and Tully 1979). A continuous source of sugars (sucrose) from photosynthesis is needed to provide precursors of glutamate (\(\alpha\)-ketoglutarate) by dark respiration (Charest and Ton Phan 1990). The accumulation of soluble carbohydrates in leaves of some plants exposed over a long term to low growth temperatures is associated with increased activities of photosynthetic enzymes (Guy et al. 1992). Hurry et al. (1995) suggested that the increases in activity of these enzymes represent an acclimation to low growth temperature. Because \textit{Juniperus chinensis} accumulated sugars and proline at low temperature (\(1^\circ\text{C}\)) independently of the photoperiod (Bigras et al. 1989), this may not be a general behavior of plants. \textit{C. quitensis} accumulated a large amount of sucrose, but little proline during cold acclimation under MD treatment. Studies separating the effect of photoperiod and irradiance integral may be necessary for better understanding of metabolic acclimation responses of these Antarctic plants. There are small but significant differences in the freezing tolerance of \textit{Lolium perenne} observed at equivalent radiation integrals, generated by varying irradiance rather than photoperiod, suggesting that factors other than total intercepted light may have some effect upon hardening (Pollock et al. 1988).

Leaf fructan contents in cool-season grasses grown at low temperature (10\(^{\circ}\text{C}\), day/night) range from zero in the genera \textit{Stipa} and \textit{Phragmites}, up to approximately 400 mg \text{g}^{-1} dry weight in accessions of \textit{Bromus inermis}, \textit{B. carinatus} and \textit{Phalaris aquatica} (Chatterton et al. 1989). Species with circumpolar habitats, such as \textit{Poa alpina} and \textit{Deschampsia caespitosa}, have moderate fructan contents ( >10 and <50 mg \text{g}^{-1} dry weight). In \textit{D. antarctica}, the highest fructan accumulation with respect to the other day length treatments was found after 21 days of cold treatment at the MD. This value was similar or slightly lower than the higher values found in cool season grasses by Chatterton et al. (1989) and higher than fructan contents (112 mg \text{g}^{-1} dry weight) of leaves of \textit{D. antarctica} growing in the Antarctic during summer (naturally LD conditions) (Zúñiga et al. 1996). Based on the assumption that LD favored fructan accumulation in some plants (Hendry 1987), we had expected to find higher fructan contents at the LD treatment and in the Antarctic, with respect to MD. This was not the case. The MD treatment was the most effective in fructan accumulation at low temperature. Sucrose but not glucose and fructose were significantly higher under LD conditions than under other day length treatments during exposure to cold. Probably, a depolymerization of fructans through the activity of a fructan exo-hydrolase occurs at this photoperiod (Morvan et al. 1997). This enzyme catalyzes sequential removal of terminal fructose residues from the fructan chain (Lea and Leegood 1993). The released fructose could be converted by fructose kinase, sucrose phosphate synthase (SPS) and sucrose phosphatase to sucrose. That is consistent with the augmented SPS activity in \textit{D. antarctica} during the light period (Zúñiga et al. 1998). It is generally accepted that fructans accumulate under conditions that favor photosynthesis but not growth. \textit{Poa pratensis} accumulates more fructans under SD than in LD without increases in the relative growth rates (Solhaug 1991). According to Solhaug (1991), the rapid increases in growth of this species in the LD plants compared with the SD plants is associated with allocation of more assimilates to leaf blade growth in LD, and to storage as fructans in SD. Our growth analysis showed that the longer the photoperiod, the higher the growth rate of \textit{D. antarctica} in both control and cold-acclimation conditions. The same irradiance was used for the SD, MD and LD treatments. For this reason, plants under MD and LD conditions received a higher total photosynthetic active radiation than the SD plants during the period of the experiments. MD- and LD-treated plants had more time for photosynthesis and more assimilates to divert for growth than SD-treated plants (Solhaug 1991). The higher concentration in total carbohydrates achieved during cold acclimation in the MD- and LD-treated plants in relation to SD-treated plants is also consistent with this explanation. When the MRGR of both MD- and LD-treated plants are compared, it was found that plants with the largest irradiation integral grew at a higher rate than those with the smaller irradiation integral (\(P \leq 0.05\)). Total carbohydrates and fructans were lower in LD treated plants with respect to MD-treated plants (\(P \leq 0.05\)). Concomitantly, there was more growth in LD-treated plants than in MD-treated plants. This behavior is also observed in grasses from a range of maritime environments from 52°N northwards exposed to cool long days, without increasing the supply of PAR (Hay 1990). Hay (1990) suggests that this response is not a specific adaptation to the cool long days of the high latitude summer. Instead, the related short day repression of growth, which facilitates the cold hardening of these grasses in autumn is likely to be more important. Grasses and cereals from lower latitudes are equipped to grow well during the growing season at high latitudes. However, they are subject to winter kill because they do not recognize the correct photoperiodic cue in autumn (Hay 1990). Thus, plants can have a different critical photoperiod for stimulation of growth. Probably, a 21-h day length is above the critical photoperiod for growth stimulation of \textit{D. antarctica}. The shoot/root ratio was practically unaffected by day length extension in \textit{D. antarctica}. Similar results have also been reported for other grasses (Ryle 1966, Hay 1990).

Grasses from cold regions are usually characterized by accumulation of fructans of a high degree of polymerization (DP) (Solhaug 1991, Solhaug and Aarøes 1994). Fructans
with different molecular mass occur in temperate grasses with different levels of cold hardiness (Suzuki 1989). A typical change during cold acclimation of hardy grasses is a decrease in high-DP fructan (DP ≥ 35) and an increase in medium DP fructan (11–34), with little change in low DP fructans (3–10). In D. antarctica fructo-oligosaccharides with DP higher than 4 were found only in cold-acclimated plants at the MD and LD treatments. An increase in low DP fructans (4–7) with respect to non-acclimated was also found in whole-crown tissues of cold-acclimated oats (2°C) (Livingston and Henson 1998). Winter cereals hydrolyze fructans during frost events, thus permitting fructose transport to the apoplast, where it can act as cryoprotector by diminution of the adhesive energy between frost crystals and the interfacial liquid of cell surfaces (Olien 1992). This reduction in the DP of fructans seems to be important in cold-resistant cereals because substances such as glucose, sucrose or raffinose are more efficient cryoprotectants than higher fructan polymers (Santarius and Bauer 1983, Livingston et al. 1993). In the apoplastic solution of cold-treated (−3°C) barley and winter oat an increase of 3–4-fold of fructans was correlated with a higher frost tolerance (Livingston 1996, Livingston and Henson 1998). In oats, lower DP fructans (DP3 and DP4) increased more than higher DP fructans (DP7 and DP > 7) in the apoplastic fluid (Livingston and Henson 1998). These authors suggest that the accumulation of these sugars in specific regions could lower the freezing point enough to increase the survival of the whole plant to freezing.

Members of the order Cariophyllales, which includes C. quitensis, have not been shown to accumulate fructans. Among the Angiosperms, fructans are found in the most evolved orders, such as Poales, Liliales, Asterales, Campanulales, Dipsacales, Polemoniales and Ericales (Hendry 1993), but not in Cariophyllales, which is more primitive (Frohne and Jensen 1992). While it can be argued that the accumulation of fructans may be some form of low temperature protection, Hendry (1993) questioned this assumption based on the origin and evolution of the majority of fructan-rich angiosperm families, which inhabit regions with seasonal rainfall and more prolonged water shortages on most continents.

The accumulation of sucrose and fructans in cold-acclimated D. antarctica and sucrose in C. quitensis reflects an efficient carbon metabolism under these conditions. These plants maintain a positive carbon balance at 0°C (Edwards and Lewis-Smith 1988). D. antarctica and C. quitensis show no photoinhibition on clear and cold days in the Antarctic and in the laboratory at low temperature and high intensity (M. A. Casanova (1997) M.Sc. Thesis, Univ. Austral de Chile, Valdivia, Chile; Xiong et al. 1999). This high photochemical efficiency of the PSII requires the existence of important sinks for the trapped energy. A strong correlation between CO₂ assimilation rate and SPS, which is the main enzyme of sucrose biosynthesis in leaves, indicates that sucrose synthesis may be a strong sink at low temperature (Hurry et al. 1994, 1995, Savitch et al. 1997). SPS increases its activity and levels under cold conditions in rye (Hurry et al. 1995) and ivy (Bauer et al. 1996). D. antarctica increases its SPS activity during cold acclimation at a LD photoperiod (Zúñiga et al. 1998). For other plants, it has been suggested that a low demand in sugar, a decrease of sucrose cleavage caused by low temperature exposure, and low utilization of sucrose as substrate for fructan biosynthesis during the first week of cold acclimation could also cause an increase in sucrose (Pollock 1984). Low temperature may also decrease the rate of sucrose phloem transport (Strand et al. 1999).

In cold climates, fructan accumulation appears to be strongly related to elevated sucrose levels (Housley and Pollock 1985). In cold-acclimated wheat, fructan synthesis began after 14 days, when the sucrose level was constant (Van den Ende and Van Laere 1996). Our results in D. antarctica showed that sucrose accumulated during cold acclimation and reached the highest level prior to maximum fructan accumulation at both LD and MD treatments. The significance of carbohydrate accumulation during cold acclimation remains to be fully understood. Its role in freezing point depression is marginal with respect to the changes in Lₜ₀ in cold-acclimated plants (Pollock et al. 1988). Nonetheless, non-coligative properties of carbohydrates may be important in cryoprotection (Crowe et al. 1993). It has been suggested that carbohydrates are also easily accessible reserves during periods of negative carbon balance (Pollock 1982b); these reserves could also be of significance prior to the onset of growth after winter (Pollock and Jones 1979). Environmental conditions that cause periods of negative carbon balance are common in the Antarctic. If the above propositions are correct, the high content of these substances found in Antarctic plants under field conditions (Zúñiga et al. 1994) and under cold acclimation in the laboratory, may favor the survival of these plants in the Antarctic.

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References


Deschampsia antarctica

Colobanthus quitensis


Lolium tumulentum


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