

NON-STRUCTURAL CARBOHYDRATES IN *DESCHAMPSIA ANTARCTICA* DESV. FROM SOUTH SHETLAND ISLANDS, MARITIME ANTARCTIC

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Deschampsia antarctica Desv. (Angiosperm: family Gramineae) plants were collected from Robert Island, South Shetland Islands, Maritime Antarctic, during February, 1992 and January, 1993, and were extracted with 80% ethanol. Total soluble sugars were analyzed in leaves and roots by colorimetric and HPLC techniques. Compared with other gramineae, the levels of sucrose and fructans were higher. These substances reached their maximum levels by the end of summer. The levels of sucrose and fructans found in February, 1992 were twice the level found in January, 1993. We suggest that the unusually high accumulation of sucrose and fructans may be one of the protective mechanisms against low temperature that has allowed *D. antarctica* to grow in the Maritime Antarctic.

Key words: Gramineae, chilling, cold stress, fructans, freezing tolerance, low temperature stress, sucrose.

INTRODUCTION

Deschampsia antarctica (Desv.) is the only gramineous plant that has colonized the Maritime Antarctic.^(1,2) The Maritime Antarctic has a cold, moist maritime climate with mean monthly temperatures near 0 °C in the Austral summer.⁽³⁾ During this period, when irradiation is sufficient to allow photosynthesis, temperatures often fall below 0 °C. Thus, plants and other organisms are regularly exposed to diurnal cycles of freezing and thawing in the Antarctic. The mechanisms that allow *D. antarctica* to tolerate the adverse Antarctic climatic conditions are unknown. Based on photosynthetic and respiratory studies in growth chambers, it has been suggested that *D. antarctica* has not developed special metabolic adaptations for survival, i.e. not different from those found in plants from other cold regions.⁽⁴⁾ Nonetheless, the native Antarctic vegetation must have one or various mechanisms that allow the maintenance

of metabolism during the Antarctic summer and survival during the winter. A low concentration of phosphatidylglycerol (PG) in leaves of field collected *D. antarctica* has been found.⁽⁵⁾ A low unsaturation index of this fraction appears to be associated with chilling sensitivity in plants,⁽⁶⁾ although elevated levels of high melting point PG did not induce chilling sensitivity in an *Arabidopsis* mutant.⁽⁷⁾ Low temperature enhances the accumulation of lipids, especially unsaturated fatty acids⁽⁸⁾ in some plants.⁽⁹⁾ A high unsaturation ratio of fatty acids permits plants to maintain membrane fluidity, thereby facilitating metabolic reactions.^(10,11)

The accumulation of low-molecular weight solutes and certain sugars is frequently observed in plants subjected to low temperature in the field or laboratory.^(12–14) These compounds appear to be related to chilling tolerance and avoidance.^(15,16) In some gramineae, fructan accumulation is thought to be involved in increased resistance to low tem-

perature.⁽¹⁷⁾ Accumulated fructans probably enhance growth at low temperature rather than induce freezing tolerance.⁽¹⁸⁾ Since *D. antarctica* is the only grass naturally growing under the harsh Antarctic conditions, it is hypothesized that high accumulation of sugars takes place during the summer as a protective mechanism against freezing. In this article we report the content of non-structural carbohydrates (glucose, sucrose, fructose, and fructans) in leaves and roots of *D. antarctica* growing under summer field conditions.

MATERIALS AND METHODS

Sampling and collection site.

The most common growth form of *D. antarctica* on the Coppermine Peninsula on Robert Island, Maritime Antarctic (62°22'S, 59°43'W) is of small tussocks less than 10 cm high, with numerous aerial ramets, being recently described as a colonizing species in this area.⁽¹⁹⁾ Plants were randomly collected from tussocks in this island in summer (February 1992 and January 1993). Daily leaf and air temperatures were registered with a data logger in January, 1993. Leaf temperature (20 leaves) was monitored with a thermocouple inserted in the aerial part of the tussock. Air temperature was measured with thermocouples (two) placed 30 cm above the soil. Two thermocouples were inserted 5 cm below the ground near the tussocks for measurements of soil temperature.

Sugar extraction.

Five samples of leaves (100 mg of fresh weight, which are about 50 leaves) collected from Robert Island were extracted with 80% ethanol (2 ml) for 24 h. Leaf extracts were then filtered (0.45 µm, Millipore filter). Extracts from fresh samples were immediately analyzed using the anthrone reagent. Extracts from liquid N₂-frozen samples were later analyzed by HPLC. The recovery of sugars after the first extraction was 85%.

Sugar analyses.

Total soluble sugars were determined using 100 µl of ethanolic extract. The extracts were mixed with 3 ml of anthrone reagent and left at 100 °C for 10 min.⁽²⁰⁾ Absorbance was read at 625 nm. Total soluble sugars were expressed as glucose equivalents. For fructan determinations ($n=5$), 100 µl of

ethanolic extract were mixed with 1 ml of absolute ethanol. The mixture was maintained at 4 °C for 24 h and then centrifuged at 9000 g. The pellet was resuspended with 2 ml of anthrone and the absorbance was read at 517 nm. Total fructans were expressed as inulin equivalents.

Carbohydrates were separated by HPLC with a Partisil 10 carbohydrate column (Whatman) at room temperature. The mobile phase consisted of a mixture of acetonitrile:water (75:25 v/v), with a flow rate of 1 ml min⁻¹. A Knauer differential refractometer was used for detection and quantification of carbohydrates, using pure fructose, glucose and sucrose as standards. Sugar concentrations were expressed on a dry weight basis.

RESULTS AND DISCUSSION

Field microclimatic conditions.

Thermal conditions were recorded over a period of 20 days during the 1993 expedition. Fig. 1 shows air and leaf temperatures during this time, which had a 2 h dark period daily. Even when maxima for air temperature were always below 8 °C, unusually high episodic temperatures were reached by the leaves when exposed to the sun. Although detailed daily temperature data are not available for the 1992 expedition, individual temperatures for several days at 15:00 h are shown in Fig. 1 (Legend). The variations in temperatures of *D. antarctica* tussocks and surrounding soil and air of January 24, 1993 are shown in Fig. 2. Leaf temperature was higher than air and soil temperatures. As expected, soil temperature was less variable than leaf temperature.

*Non-structural carbohydrates in leaves and roots of *D. antarctica*.*

The contents of soluble sugars, total fructan and sucrose in leaves and roots from field-collected plants were analyzed by colorimetric and HPLC procedures (Table 1). Sucrose contents were higher in young leaves than in roots, and in young leaves than in complete tops, which included leaves of various ages and stems ($P \leq 0.05$, Duncan's test).⁽²¹⁾ The level of total soluble sugars was about 8-fold higher than those found in leaves of cold-acclimated barley and oat.^(22,23) In addition, the amounts of total fructans were significantly higher in young leaves than in shoots and roots ($P \leq 0.05$) and higher

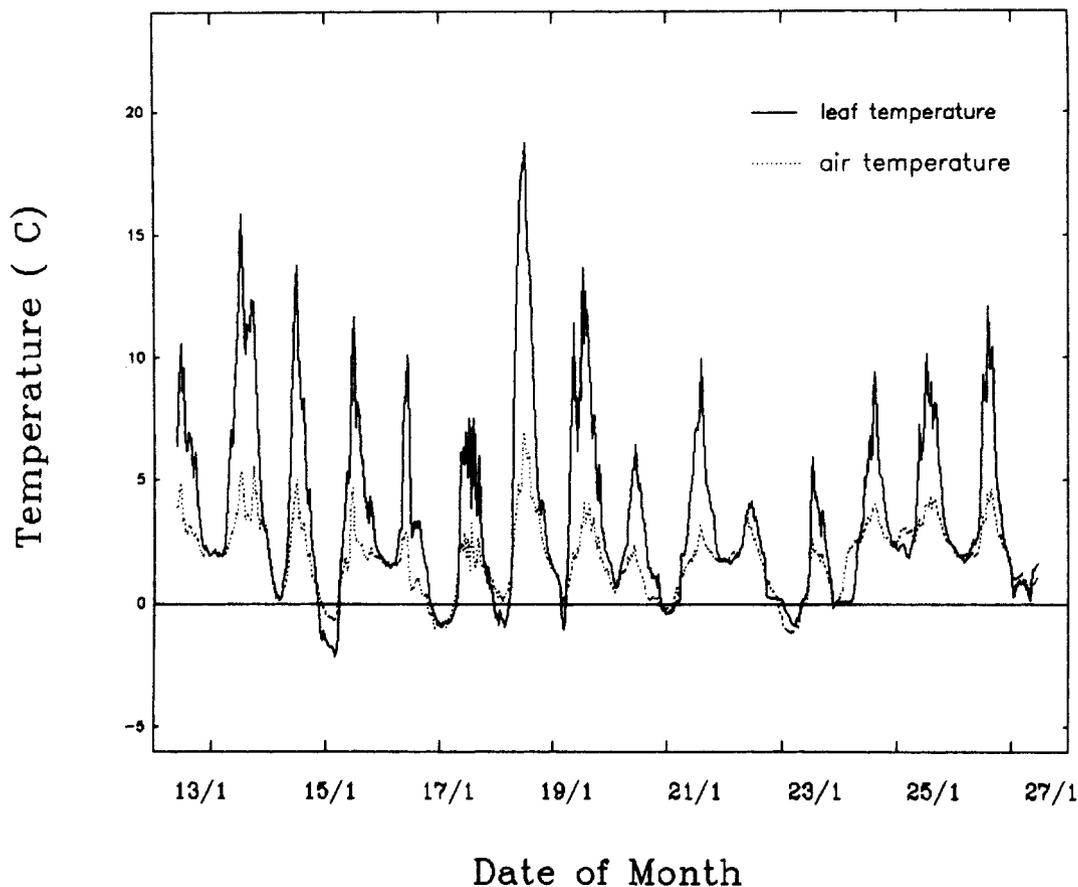


Fig. 1. Air and leaf temperature changes in tussocks of *Deschampsia antarctica* recorded between 13 and 27 January 1993 on Robert Island. Air temperatures at 15 h during the 1992 expedition were as follows: 2.6, 1.6, 1.2, and 0.8°C for February 17, 19, 21, and 23, respectively.

than those described for *Deschampsia flexuosa* (76 mg g⁻¹ dry weight), *D. caespitosa* (49 mg g⁻¹ dry weight) and *Hordeum bulbosum* (132 mg g⁻¹ dry weight), and similar to those in *Poa arctica* R. Br. (200 mg g⁻¹ dry weight) acclimated to 10/5 °C with a 12 h photoperiod.⁽²⁴⁾

Variation of sucrose and fructan contents during the summer. The amounts of sucrose and total fructans were determined in samples collected in February of 1992 and January of 1993. The content of both types of sugars increased and reached maximum levels by the end of the month (Fig. 3; $P < 0.05$). The contents of sucrose and total fructans were higher in February 92 than in January 93. As a result of restricted access to the Antarctic, it was not possible to perform these measurements in the same

year. Nonetheless, mean monthly temperature was lower in February, 1992 (1.2 °C) than in January, 1993 (2.8 °C).

Glucose, fructose, sucrose and fructan contents during a daily cycle. Glucose, fructose and sucrose contents were determined by HPLC (Fig. 4). The highest levels of glucose and fructose were found in the morning. The highest level of sucrose was found at noon and the lowest at night. The levels of fructans increased steadily during the day (Fig. 4).

Several studies have suggested that cold hardiness is a result of several cryoprotective mechanisms operating simultaneously or sequentially.^(3,15) One of these includes accumulation of carbohydrates.⁽¹²⁾ Proposed cryoprotective mechanisms involving carbohydrates are freezing point depression of cell

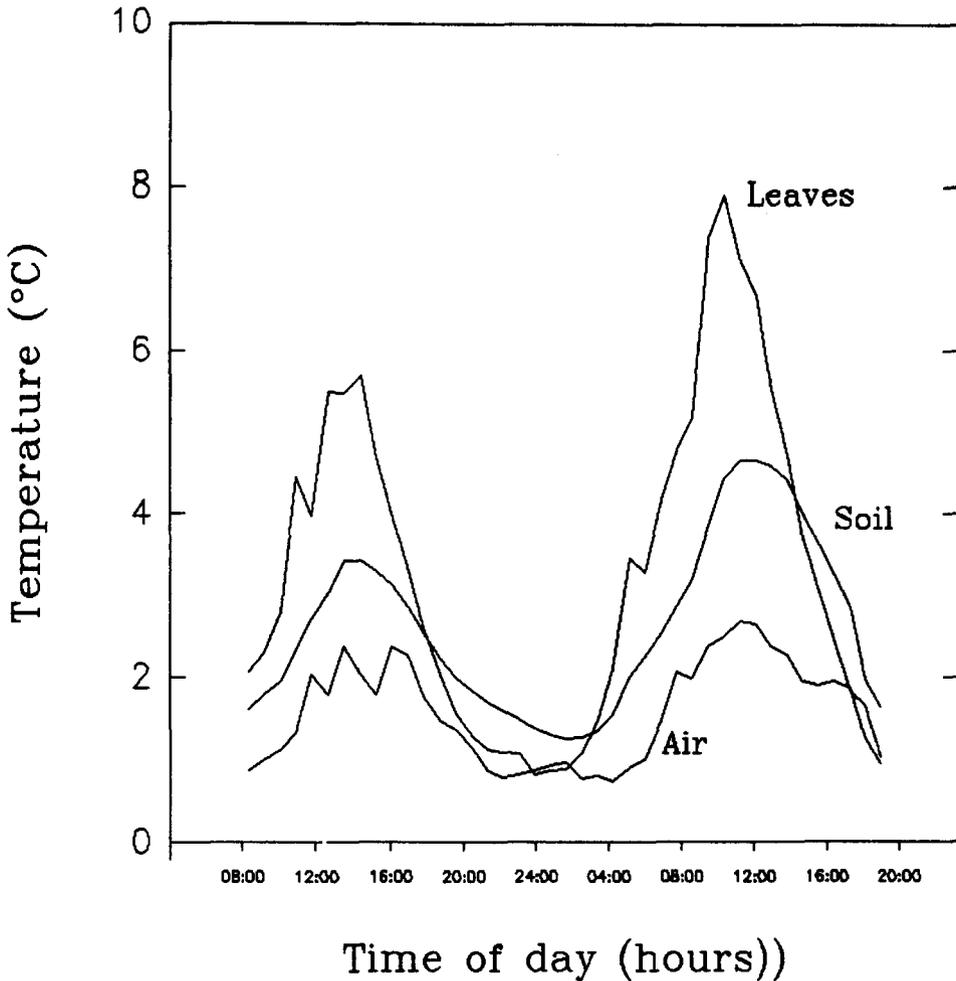


Fig. 2. Diurnal variations of air, leaves, and soil temperatures in tussocks of *Deschampsia antarctica* recorded on January 24, 1993, on Robert Island.

sap,⁽¹⁵⁾ prevention of plasmolysis as water is removed during freezing,⁽¹⁵⁾ and protein stabilization.⁽¹⁷⁾ The accumulation of sucrose is stimulated by low temperatures in some plants.⁽⁹⁾ In some cereal crops, such as barley and oat, the accumulation of sugars is induced during the acclimation of plants to low temperature.⁽²²⁾ The levels of sugars found in these cereals are, however, lower than those found in *D. antarctica*. Moreover, the amount of sucrose in *D. antarctica* growing in a growth chamber at 13 ± 1.5 °C (optimum growth temperature)⁽⁴⁾ is very similar to that of barley under similar conditions (10 mg g^{-1} dry weight).^(23,25)

Although the accumulation of sugars during cold-hardening has been described for different species, the role of soluble sugars as cryoprotective substances is still unclear. Positive correlations have been shown between fructan content and cold tolerance in several grasses.^(26,27) In some gramineae there is a marked depolymerization of highly polymerized fructans into low-molecular weight sugars during the winter period, providing the main substrate for sucrose synthesis.⁽²⁸⁾ Although the accumulation of fructans may contribute to the osmoregulation of cells,^(17,29,30) it is not clear if these compounds have a role as cryoprotectants.⁽²⁶⁾ As

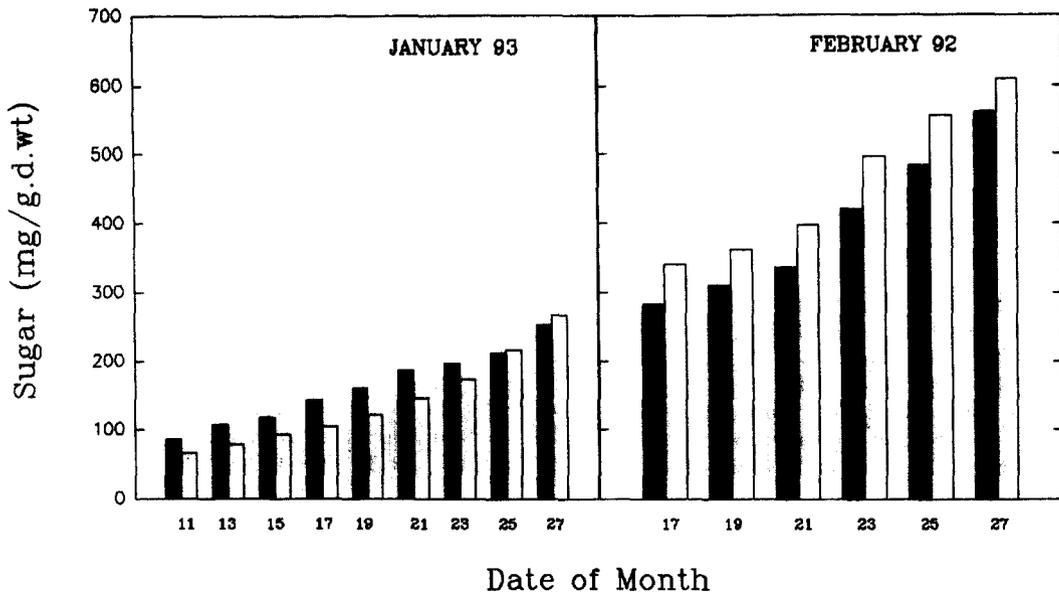


Fig. 3. Variation in the content of sucrose (■) and total fructans (□) during January 1993 and February 1992. Each value is the average of five samples \pm 1 s.e.

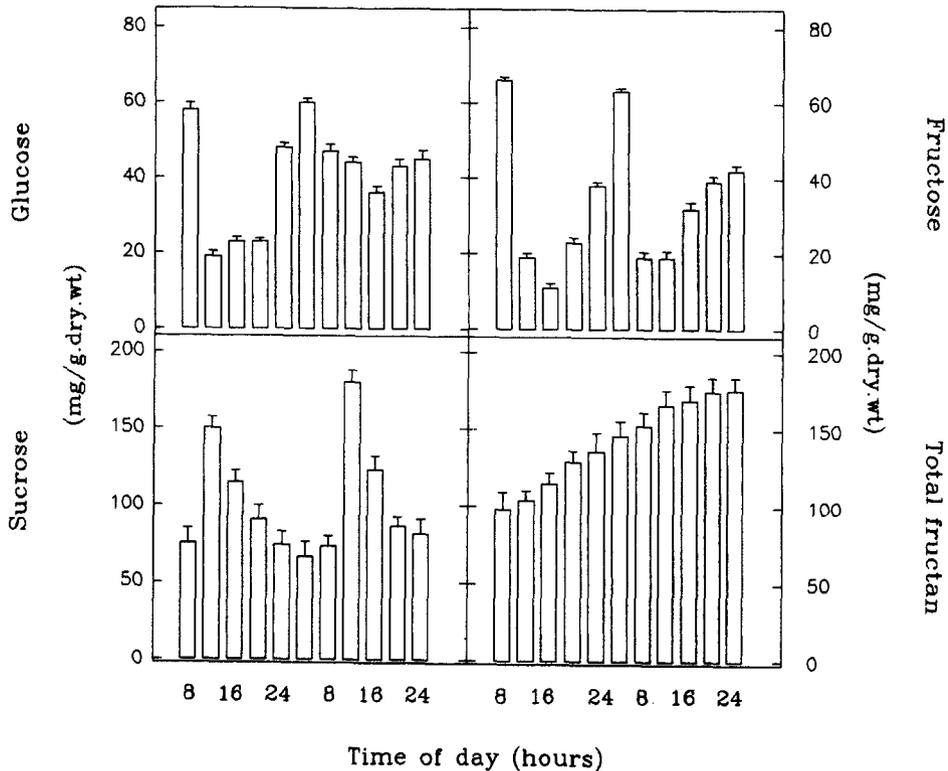


Fig. 4. Daily changes in the contents of non-structural carbohydrates in shoots of *Deschampsia antarctica*. Fructans were analyzed colorimetrically and other sugars by HPLC, as described in the methods section. Each value is the average of five samples \pm 1 s.e.

Table 1. Contents of non-structural carbohydrates in *Deschampsia antarctica*¹

Organ	TSS ²	Fructan ³ (mg/g dry wt)	Sucrose ⁴
Young leaves	708 ± 38a	112 ± 10a	358 ± 21a
Shoot	393 ± 23b	79 ± 8b	206 ± 26b
Roots	168 ± 11c	70 ± 7b	118 ± 16c

¹Plants were collected from Robert Island during February 1992. Water content was 88% in young leaves, 84% in shoots and 78% in roots.

²Total Soluble Sugars. Colorimetric determination using anthrone reagent (glucose equivalents).

³Colorimetric determination using anthrone reagent (inulin equivalents).

⁴Determined by HPLC as described in Experimental.

Each value is the average of five samples ± 1 s.e. Column values with different letters indicate statistically significant differences at $P \leq 0.05$ (Duncan's test).

suggested by Pollock *et al.*⁽¹⁸⁾ for other plants, it is likely that the accumulation of fructans and sucrose would enhance survival of *D. antarctica* because they are readily accessible energy reserves during growth periods with negative carbon balance. The high level of both sugars found at the end of the summer supports this hypothesis. In addition, the effects of the daily fluctuations of freezing and thawing during summer could be reduced by the high sugar content acting as a cryoprotectant. The data of the present study support the hypothesis that the high accumulation of sugars, which takes place during the summer growing period, is a cryoprotective mechanism enabling *D. antarctica* to survive and grow in the Maritime Antarctica. It would be of interest to identify the different fructan types accumulated by *D. antarctica* to evaluate their cryoprotective activity.⁽³¹⁾

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