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Loss of soluble carbohydrates and changes in freezing point of Antarctic bryophytes after leaching and repeated freeze-thaw cycles

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Abstract: Healthy samples of *Grimmia antarctici* (turf and cushion ecodemes), *Ceratodon purpureus*, *Bryum pseudotriquetrum* and *Cephaloziella exiliflora* were collected in late summer in Wilkes Land together with senescing and dead *G. antarctici* material. Plant material was subjected to leaching in water and up to 16 freeze-thaw cycles. Gas chromatography revealed that following 16 days immersion, loss of carbohydrates (mainly glucose and fructose) was relatively low (*c.* 10–29% of the total sugar pool) for healthy material, with the loss of 69% from the dead *G. antarctici* material. Freeze-thaw cycles greatly increased rates of sucrose leakage and led to a 2–3 times rise in total sugar loss in all samples except the dead brown tissue which was not significantly different from the leached control treatment. After 16 freeze-thaw cycles *Bryum pseudotriquetrum* had lost 65% of total sugar pool. Losses for other species were below 28%. Differential thermal analyses showed freezing points of tissue varied from -8.3 to -3.5°C with dead material having the highest freezing temperatures. There was no significant correlation within species of freezing temperature changes with progressive sugar loss. The results are discussed in relation to nutrient cycling, soil microbial activity and the viability of bryophyte species in the Antarctic environment.

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Key words: Antarctica, freeze-thaw, bryophytes, carbon flow, sugars, sugar leaching

Introduction

In polar regions cryptogams are considered to play a major role in nutrient cycling (Stoner *et al.* 1982, Smith 1985, Longton1988). Studies of maritime Antarctic fellfields have shown large increases in soil sugar and microbial populations which have been attributed to the leaching of soluble carbohydrates and polyols from vegetation during periods of thaw (Wynn-Williams 1980, Tearle 1987). Since freezing may increase rates of leakage from cells it has been suggested that cold shock may significantly increase leaching in the field (Wynn-Williams 1980, Tearle 1987). However, Hurst *et al.* (1985) found repeated freeze-thaw made no significant difference to nutrient leaching from mature and senescent leaf material of two phanerogams in the Subantarctic. Similarly, Greenfield (1989) found that in dried samples of Antarctic terrestrial plants and microbes freeze-thaw cycling made little difference to the release of water soluble extracts. However, these studies acknowledged a need for the study of live tissues.

In this paper the responses of live bryophytes from the Windmill Islands, Wilkes Land, (66°S, 110°E) to freeze-thawing and leaching are examined. The loss of water soluble carbohydrates from live samples was studied to determine if leachates from these communities were likely to make a significant contribution to the availability of carbohydrates in the soils. Although these cryptogamic communities in Wilkes Land represent the most extensive vegetation communities on continental Antarctica they contain relatively little litter which is thought to be a potential source of carbohydrates in the subantarctic ecosystems (Walton 1985, Hurst *et al.* 1985). Carbohydrate and polyol leaching from the standing vegetation constitute a major nutrient input to the ecosystem (in areas away from penguin colonies) and thus may be expected to affect the soil microfloral activity.

The dominant bryophytes in the Wilkes Land communities are turfs of *Grimmia antarctici* Card. and *Ceratodon purpureus* (Hedw.) Brid. with smaller patches of *Bryum pseudotriquetrum* (Hedw.) Gaertn., Meyer et Scherb., and *Cephaloziella exiliflora* (Tayl.) Steph. (Selkirk & Seppelt 1987, Smith 1988). Cushion ecodemes of *G. antarctici* may occur (Kappen *et al.* 1989) together with heavily convoluted patches of turf. The convoluted turf form results from freeze-thaw activity and the moss sometimes appears to be senescent and is frequently encrusted by lichens (Smith 1988). Sugars and polyols are thought to play a role in the cryoprotection of plant tissue (Levitt 1980, Kaurin *et al.* 1981) so the effects of leaching and freeze-thaw cycles on both the loss of sugars and changes in the relative freezing temperatures of tissue were also examined in relation to plant vigour. The responses of browning, apparently senescent specimens of *G. antarctici* and dead brown stem material have been compared with those of healthy samples.

Materials and methods

Collection and treatment of samples

Healthy material of *Grimmia antarctici* (cushion and turf ecodemes), *Ceratodon purpureus*, *Bryum pseudotriquetrum*, and *Cephaloziella exiliflora* together with samples of senescent and dead brown material of *G. antarctici* were collected in late February 1991 from the Site of Special Scientific Interest 16 on Bailey Peninsula near Casey Station. Samples were collected in the early morning and stored in sealed plastic bags. Plant material was blotted dry then microscopically examined to ensure freedom from lichen contamination. Any senescent moss samples with lichen contamination were discarded. Approximately 150 mg of plant material were placed into pyrex vials and 4 ml of ultra-pure water (Millipore Waters Milli-Q, conductivity 0.05 mS cm^{-1}) were added to each. Replicates were used to determine the dry weight and organic content of samples.

Leaching of water soluble carbohydrates was examined in laboratory experiments at Casey over a 16 day period at a temperature of 4°C. The filtrates from five replicates of each sample were collected and the samples dry weighed after immersion for 1, 2, 4, 8 and 16 days. Five replicates of each sample were freeze-thawed in 12:12 h cycles of -15°C to +4°C in a variable temperature freezer and growth cabinet at a gradient of freezing of *c.* 5°C.h^{-1} . These conditions approximated typical diurnal freeze-thaw cycles measured in the moss beds in the late summer. After 1, 2, 4, 8 and 16 cycles these samples were then treated as described for the leaching treatments. The total ethanol soluble low molecular weight sugar content of the plant material was determined for five replicate samples of each treatment allowing the percentage loss of leachates to be calculated. A further five replicates of all treatments were retained for the freezing point experiment. Statistically significant differences in sugar content of extracts and freezing points were determined according to Student's *t*-test.

Plant material was weighed fresh. Replicate samples were dried to constant weight at 80°C to determine water content. Organic matter was determined by measuring ignition loss of dried samples at 450°C.

Soluble carbohydrate measurements

Plant material was weighed, ground in a mortar and pestle with a small quantity of acid washed sand and extracted in 70% ethanol. The ethanol suspension was then sonicated (Branson 450 sonifier probe) at 50 watts for 2x30 s and centrifuged (1000G for 10 min) to remove suspended material. Triplicate 0.5ml aliquots were evaporated to dryness under compressed air. Leaching filtrates were directly evaporated under a stream of air and reduced to dryness over silica gel. All extracts were silylated with a 2:1:10 mixture of hexamethyldisilazane (HMDS) and trimethylchlorosilane (TMCS) and pyridine for 30 min at 37°C as described by Sweeley *et al.* (1963). An 0.4 ml aliquot of this mixture was chromatographed directly on a Varian 3400 gas chromatograph fitted with a flame ionization detector using a 12 m (i.d. 0.53 mm) SGE 12QC5/BP1 fused silica column with He carrier gas at 1ml min^{-1}. Injector temperature was set at 250°C and detector at 300°C. Ramp conditions were 5°C min^{-1} isothermal from 100°C (2 min hold) to 290°C (5 min hold). The identification and quantification of the peaks was achieved by comparison and co-chromatography to known standards and related to the calibration curves for the individual carbohydrates. Threitol (which was first shown to be absent) was used as an internal standard.

Tissue freezing points

Tissue freezing temperatures were determined by differential thermal analysis (DTA) (George *et al.* 1974, Sakai 1978). The tip of a water saturated gametophyte was surface blotted dry and then placed in contact with a thermistor probe (Fenwal 3K), wrapped in aluminium foil and placed in a vacuum flask within a deep freeze chamber at a constant -30°C. A second matched reference probe, connected in series with the first probe, was also wrapped in foil and placed in the container. With this procedure a cooling rate of 15°C h^{-1} was achieved. Temperature difference between the probes was measured via a potentiometer circuit box and recorded on a chart recorder. This procedure was repeated for all replicates of each treatment.

Results

Soluble carbohydrates

Sucrose was the major carbohydrate present in all the bryophytes examined together with lesser amounts of fructose and glucose. Trehalose was also relatively common in the liverwort *C. exiliflora*. *Bryum pseudotriquetrum* contained small quantities of an unknown sugar, which eluted after sucrose and trehalose suggesting that it may be either a disaccharide or an oligosaccharide. The soluble carbohydrate composition of each sample is given in Table I.

Healthy tissue demonstrated the lowest leakage of carbohydrates with 10–29 % loss of total soluble carbohydrates from samples leached for up to 16 days (Table II). Over 80% of the sugars lost in the leachate from healthy tissues were accounted for by the monosaccaharides fructose and glucose.

Freeze-thaw cycles significantly increased (p>0.05) sugar leakage in all cases, except for the dead brown stem material (Table II). In healthy tissue, freeze-thawing resulted in a two to three-fold increase in sugar loss, largely accounted for by a significant increase in the proportion of sucrose in the extracts. As in the control experiment, the greatest sugar loss from the healthy tissue was observed in *B. pseudotriquetrum*. Freeze-thawing also doubled the sugar leakage from senescent

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Table I. Major ethanol soluble sugars (mg g^{-1} dry wt) in the bryophyte samples (n=5 ± s.d.).

Sample	fructose	glucose	sucrose	trehalose	unknown	total
Grimmia antarctici						
healthy turf	6.26 ± 0.61	5.80 ± 0.52	53.28 ± 2.31			65.34 ± 4.18
healthy cushion	4.10 ± 0.51	3.81 ± 0.34	35.15± 2.99			43.05 ± 3.44
senescent	2.98 ± 0.91	3.10 ± 0.80	19.70 ± 3.23			25.70 ± 4.21
brown stems	0.68 ± 0.12	0.71 ± 0.09	10.50 ± 0.91			11.89 ± 1.11
Bryum pseudotriquetrum						
healthy turf	7.11 ± 1.23	7.14 ± 1.11	38.75 ± 4.01		2.11 ± 0.91*	55.00 ± 5.79
Ceratodon purpureus						
healthy turf	1.61 ± 0.51	1.01 ± 0.32	19.2 ± 2.60			21.80 ± 4.01
Cephaloziella exiliflora						
healthy	0.23 ± 0.04	0.19 ± 0.06	4.32 ± 0.71	1.1 ± 0.34		5.84± 0.92

*Quantification of unknown sugar calculated on calibration curve for sucrose.

Table II. Percentage loss of total soluble low molecular weight carbohydrates from bryophyte samples after leaching and freeze-thaw cycles (n=5 ± s.d.).

Sample	No. days leaching/freeze-thaw cycles				
	1	2	4	8	16
Grimmia antarctici (turf)					
Control	11.13 ± 0.90	12.60 ± 1.54	13.21 ± 1.61	14.68 ± 1.95	14.83 ± 1.70
Freeze-thaw	19.60 ± 2.35	23.81 ± 2.89	28.30 ± 2.40	28.37 ± 3.60	27.88 ± 3.25
Grimmia antarctici (cushion)					
Control	1 0.80 ± 1.95	14.18 ± 1.92	15.00 ± 1.02	15.47 ± 2.67	16.43 ± 1.44
Freeze-thaw	16.59 ± 1.35	18.40 ± 2.34	31.55 ± 3.23	30.20 ± 2.03	31.20 ± 3.56
Grimmia antarctici (senescent)					
Control	18.72 ± 2.40	18.62 ± 3.64	22,92 ± 2.20	29.66 ± 4.52	27.92 ± 3.80
Freeze-thaw	34.81 ± 4.41	41.27 ± 7.51	45.10 ± 6.28	48.53 ± 7.26	48.18 ± 7.30
Grimmia antarctici (brown stems)					
Control	64.27 ± 12.65	58.43 ± 18.86	68.21 ± 15.85	66.33 ±8.45	68.83 ±7.05
Freeze-thaw	69.37 ± 8.33	74.33 ± 10.79	78.07 ± 10.61	69.20 ± 10.51	76.30 ± 12.06
Bryum pseudotriquetrum					
Control	20.37 ± 2.10	23.04 ± 3.83	26.23 ± 3.50	29.10 ± 3.05	28.97 ± 2.42
Freeze-thaw	24.05 ± 2.80	52.00 ± 6.91	53.78 ± 5.93	64.37 ± 8.39	65.83 ± 10.68
Ceratodon purpureus					
Control	9.03 ± 1.46	11.65 ± 1.77	11.67 ± 2.02	13.76 ± 1.87	12.73 ± 2.20
Freeze-thaw	16.53 ± 4.36	19.70 ± 3.82	27.37 ± 5.92	30.20 ± 6.85	29.17 ± 4.95
Cephaloziella exiliflora					
Control	9.77 ± 1.01	8.83 ± 1.26	9.34 ± 1.17	10.73 ± 2.15	10.43 ± 2.46
Freeze-thaw	11.00 ± 2.60	17.77 ± 2.07	29.98 ± 2.47	25.01 ± 3.20	29.22 ± 5.65

G. antarctici samples (Table II).

Tissue freezing points were all above -9°C but showed some variation between samples. Within *G. antarctici* material, significantly higher (p>0.05) freezing temperatures were observed in the dead brown stems and freeze-thawed senescent samples with relatively low sugar content (Table III). However, regression analyses revealed no significant correlation between progressive sugar loss and freezing temperatures within species or treatments. All samples produced one exotherm with no sign of a secondary exotherm with continued temperature decrease.Water and organic matter content of the samples generally reflected plant vigour, healthy material containing higher moisture contents than dead or senescent specimens. Organic matter content was also highest in the healthy tissue with the dead and senescent material recording the lowest values (Table IV).

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Table III. Freezing point (°C) of tissue samples after leaching and freeze-thaw cycles (n=5 ± s.d.).

Sample	No. days leaching/freeze-thaw cycles				
	1	2	4	8	16
Grimmia antarctici (turf)					
Control	-7.3 ± 0.4	-7.9 ± 0.9	-7.7 ± 0.6	-7.5 ± 0.5	-7.6 ± 1.2
Freeze-thaw	-6.7 ± 0.5	-7.8 ± 1.7	-7.3 ± 0.9	-6.1 ± 1.7	-6.8 ± 2.1
Grimmia antarctici (cushion)					
Control	-6.8 ± 0.5	-8.3 ± 0.5	-7.0 ± 0.9	-6.6 ± 1.9	-7.0 ± 0.5
Freeze-thaw	-6.5 ± 0.6	-7.0 ± 1.4	-6.4 ± 0.2	-5.9 ± 1.2	-5.5 ± 0.3
Grimmia antarctici (senescent)					
Control	-4.6 ± 1.3	-5.7 ± 2.1	-5.0 ± 0.3	-5.1 ± 0.2	-4.9 ± 1.2
Freeze-thaw	-4.4 ± 0.5	-4.9 ± 0.9	-4.6 ± 0.9	-3.9 ± 0 .4	-3.9 ± 0.4
Grimmia antarctici (brown stems)					
Control	-4.7 ± 0.5	-4.3 ± 0.8	-4.1 ± 0.8	-4.3 ± 05	-5.0 ± 1.0
Freeze-thaw	-4.3 ± 0.3	-4.3 ± 0.8	-3.5 ± 0.6	-4.0 ± 0.8	-4.3 ± 0.6
Bryum pseudotriquetrum					
Control	-6.0 ± 0.6	-6.2 ± 0.4	-6.1 ± 0.3	-5.8 ± 0.3	-7.4 ± 1.6
Freeze-thaw	-5.5 ± 0.3	-5.8 ± 1.5	-5.2 ± 0.3	-4.5 ± 0.1	-4.9 ± 1.6
Ceratodon purpureus					
Control	-6.3 ± 1.3	-6.1 ± 0.3	-5.0 ± 1.7	-5.8 ± 0.9	-4.6 ± 0.8
Freeze-thaw	-6.4 ± 0.4	-4.2 ± 0.3	-4.0 ± 0.2	-3.8 ± 0.9	-5.2 ± 1.9
Cephaloziella exiliflora					
Control	-6.7 ± 0.5	-6.3 ± 1.1	-7.0 ± 0.8	-6.2 ± 0.1	-5.4 ± 1.4
Freeze-thaw	-6.0 ± 0.1	-6.0 ± 0.6	-6.4 ± 0.2	-5.3 ± 0.4	-5.3 ± 2.0

Table IV. Water contents and organic matter of bryophyte samples (n=5 ± s.d.).

Sample	Moisture content (% fresh wt)	Organic matter (% dry wt)
Grimmia antarctici		
healthy turf	75.2 ± 2.1	91.2 ± 4.1
healthy cushion	69.1 ± 1.2	88.9 ± 2.3
senescent	39.1 ± 3.2	76.7 ± 3.8
Bryum pseudotriquetrum		
healthy turf	66.9 ± 5.9	85.7 ± 4.2
Ceratodon purpureus		
healthy turf	73.1 ± 3.2	88.0 ± 3.5
Cephaloziella exiliflora		
healthy	77.8 ± 1.9	88.1 ± 3.2

Discussion

Earlier studies of senescent and dried Antarctic and subantarctic plants have shown freeze-thaw to have no significant effect on nutrient loss (Hurst *et al.* 1985, Greenfield 1989). However, these studies suggest that since the action of freeze-thaw is in part a membrane rupturing process, it might be expected to affect young actively growing material more than dead or senescent material. The present study supports this speculation. There is a definite increase in sugar leakage from live bryophytes in response to freeze-thaw cycles, varying with the species and vigour of the plants. In their study of senescent leaves and litter of two subantarctic phanerogams, Hurst *et al.* (1985) found leaves immersed in water lost up to 80% of their total available soluble carbohydrates in the first 8h. This situation is similar to that observed for the dead brown *G. antarctici* material in which the majority of cell leakage occurred in the initial 24h and the samples showed no significant change in sugar leakage with exposure to freeze-thaw cycling. In contrast, all live material demonstrated much slower initial rates of release with significant increases still being observed following up to eight days of freeze-thaw cycling. These differences may reflect the nature of the cell membranes. In dried material it is suggested that cell leakage occurs until the membrane becomes fully hydrated (Simon 1974), whereas in green moist tissue initial leakage may be slower, due to the relatively hydrated membrane, but increases as freeze-thaw activity decreases membrane integrity (Burke *et al.* 1976, Levitt 1980, p. 254). The mechanisms of leakage may also explain the differences in the types of sugars lost. The monosaccharides glucose and fructose leached in preference to the disaccharide sucrose in control extractions, with significant loss of sucrose apparent only in the freeze-thaw treatments. This may be due to the rupturing of membranes increasing the leakage of the larger sucrose molecule. Possible losses of sucrose resulting from hydrolytic activity by microorganisms in the samples appeared to be negligible as the combined sucrose content of plant tissues and leachates remained relatively constant in all treatments for each species.

In the absence of freeze-thaw cycling the amounts of sugar leached from the healthy bryophytes are low compared to some rates reported for vascular plants. Tukey (1970) reports losses of up to 6% dry weight equivalent leached from young bean leaves over 24h. However, carbohydrate loss from the

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bryophytes, though small, is similar to that reported for subarctic lichens by Dudley & Lechowicz (1987) who found about 10% of the polyol pool was leached in a 1h wetting event. Only in the freeze-thaw treatments did sugar leakage from healthy green material greatly exceed these levels, with species showing considerable variability in response. *Bryum pseudotriquetrum* appeared to be the most susceptible species, losing up to 65.8% of total sugars after 16 cycles. *Grimmia antarctici* also demonstrated significantly more sugar leakage after freeze-thaw cycles with maximum losses amounting to 27.9% of the sugar pool.

Other workers have noted the release of carbohydrates from live cryptogam tissue which has been desiccated and rewetted (Farrar 1976, Gupta 1977). Desiccation may be responsible for the relatively high loss of nutrients from senescing *G. antarctici* which had lower moisture content than the green turf and cushion material. Presumably membrane leakage has occurred upon initial hydration. Since mosses, unlike vascular plants, have effectively no protective cuticle this is not unexpected.

Sugars, including sucrose, have been shown to confer freezing protection (Lineberger & Steponkus 1980, Kaurin *et al.* 1981). However, although the bryophyte samples we studied demonstrated significant differences between the rates of sugar loss, few general trends in tissue freezing points were apparent. All freezing points were relatively high (> -9°C) suggesting that the plants do not avoid freezing but merely tolerate the freeze-thaw process. No decrease in freezing temperature could be confidently discerned with loss of sugars, although the senescent and brown *G. antarctici* material did have significantly higher freezing temperatures than healthy tissue. The modes of cryoprotection are complex. Actual freezing point depression may involve higher concentrations of sugars than encountered in this study (Santarius & Giersch 1983). In higher plants it has been demonstrated that significant changes in the cryoprotection of thylakoid membranes may involve relatively minor differences in sugar concentrations, depending upon salt concentrations at the membrane (Santarius & Giersch 1983). Therefore, the differences observed in sugar loss from the bryophytes we studied could have a significant impact on plant survival. Increased rates of leakage from senescent mosses are likely to be detrimental to frost sensitive membranes.

The findings of this study experimentally support previous speculation that freeze-thaw activity may increase availability of metabolites in the soil and melt water during periods of thaw as found in maritime Antarctic peat (Wynn-Williams 1980, Tearle 1987). The presence of more extensive peat and litter in maritime areas suggests that the large nutrient fluxes recorded are due to a combination of leaching from dead matter and slower release from live material after freeze-thaw. In the Wilkes Land sites that we studied peat and litter accumulation is relatively low. The area of vegetation cover is much more limited than in the maritime Antarctic and levels of soluble sugars and polyols in the vegetation are lower than those reported for cryptogams there by Tearle (1987). In soils from lichen dominated regions near Casey Station, Bölter (1990a) found an active microbial population with the potential for significant activity during short periods of high temperature and moisture. However, he notes that the numbers of microbes and periods of activity appear to be limited by comparison with results from the maritime Antarctic or subantarctic islands (Bölter 1990b).

The limitations of the present study must be acknowledged. There is likely to be considerable variability in the response of bryophytes to leaching, given the differences in microhabitat microclimate and plant development between the samples. There may also exist a continuum of plant responses to leaching over environmental gradients in the field. Thus estimates of nutrient turnover are extremely difficult. However, within the terrestrial ecosystem in Wilkes Land there appears to be the potential for significant nutrient cycling over short periods when environmental conditions are favourable. Freeze-thaw activity and the presence of free water may result in nutrient loss from the standing vegetation and stimulation of soil microbes. For plants in more extreme habitats growth may be inhibited not only by lack of moisture but also by an increased leakage of sugars on the relatively few occasions free water may be available.

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