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Losses of Polyol through Leaching in Subarctic Lichens

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ABSTRACT

Upon rewetting, lichens lose polyols through leaching. We quantified leaching losses for 21 species under simulated rainfall. Polyol concentrations in these lichens range from 1.0 to 8.8%, with a mean of 2.8%. Leaching losses range up to about 7.5 mg (polyol)/g (lichen dry weight) in a typical rain event. The rate of polyol leaching declines exponentially, becoming negligible within 1 hour of continuous rain. The response of polyol leaching rate to rainfall intensity and amount varies between species—six species showed no response, one had increased leaching with increased rainfall intensity, four had increased leaching with increased amount of rainfall, and one had decreased leaching with increased total amount of rainfall. Polyol leaching rates are positively correlated with polyol concentration for 20 species. Literature values of average daily growth rates for subarctic lichens are of the same order of magnitude as leaching rates, suggesting that polyol leaching is an important part of the carbon budget of lichens.

The cells of desiccation-tolerant plants lose solutes such as inorganic ions, amino acids, and soluble carbohydrates upon rewetting (1). The desiccated cell membranes are leaky, exposing cell contents to the surrounding solution. Though repair of membranes starts within a few minutes of rewetting, ending the leakage of solutes, nonetheless from 20 to 50% of solute present in the cell can be lost to the surrounding solution (4). In nature, lichens may be rewetted several hundred times a year (5) and have concentrations of soluble sugar alcohols (polyols) between 1 and 10% (11); thus, relatively large biomass losses would be possible if rates of polyol leaching are substantial under field conditions.

It has not been demonstrated that any loss of polyol from lichens occurs in the field. Lichens wetted by rainfall may take up leaked solutes before they are leached out of the thallus (5). For example, Crittenden (3) found that *Stereocaulon paschale* wetted by immersion lost a large amount of potassium, but when wetted by natural rainfall it lost little or no potassium. A qualitative study of leaching under simulated rain, however, did find measurable polyol loss (2).

Furthermore, there are large discrepancies between observed and estimated growth for several models of lichen production that predict growth from climatic variables (6). These discrepancies suggest that an additional source of carbon loss exists; leaching losses may well account for discrepancies in production models. Polyols are the principal transport and storage compounds in lichen metabolism (9) and polyol losses would therefore have deleterious effects on net production. Consequently, we wish to predict how much polyol is lost through leakage for a variety of subarctic lichens under field conditions.

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MATERIALS AND METHODS

To evaluate the importance of polyol leaching in subarctic lichens under natural conditions, we designed a series of laboratory experiments where simulated rain fell on air-dry lichen thalli from which all leachate was collected. Using this protocol, we determined the function describing polyol leaching rates with time for *Cladina stellaris* which is the most abundant lichen in subarctic Quebec. To determine the magnitude of the effect of polyol concentration on polyol leaching rate, we correlated the leaching rate and polyol concentration for 21 sympatric species. We also determined the relation between rain intensity and leaching rate for 11 of these subarctic species.

Collection. Collections of 21 lichen species (Table I) were made near Schefferville in subarctic Quebec (55°N, 67°W) in September 1983. These foliose and fructicose species are from alpine tundra, subalpine heath, open lichen woodland, and closed spruce-moss forests, which are the major habitat types where lichens are found (14). They represent diverse growth forms. The lichens were collected during a rain to avoid damaging thalli, dried at room temperature, shipped by air to Montreal, stored air-dry and frozen, and analyzed within a month.

Sampling. Lichens show a gradient in physiological activity from the growing tips to the senescing bases (10). To control for such within-thallus variation and to sample equivalent portions across all species, we used only the portion of intact thalli with living algae as judged by microscopic examination of algal distribution and external color gradients. Each replicate was composed of several thalli to give a relatively high dry weight of tissue in each leaching trial.

Experimental Design. To estimate the time course of leaching, four replicates of *Cladina stellaris* were leached at approximately 3 mm/h. Polyol leaching rates per 15 min interval were measured over 1 h. In a rain intensity experiment, 9 replicates of each of 11 species were leached at rainfall intensities between 1 and 6 mm/h. Actual rainfall intensities could not be exactly controlled so the total amount of rainfall was also measured as a covariate in this experiment. Finally, the average polyol leaching rates and average polyol concentrations of 21 species were correlated to determine the effect of polyol concentration. The data from the rainfall intensity experiment were averaged by species and 3 replicates of 9 other species were leached at 3 mm/h.

Leaching. Simulated rain was generated by a Bete-Fog synthetic rain nozzle with tap water filtered through activated carbon. The nozzle was oriented to project the water upwards; the rain then fell with only the acceleration of gravity. Air-dry lichen replicates were placed in plastic mesh baskets that were, in turn, placed in large plastic containers, and all rainfall passing through the lichens was collected. After the leaching period was completed the lichens and leachate were weighed to 0.01 g, for calculation of rainfall volume and intensity. The lichens were dried at 100°C and weighed to 0.01 g. A subsample of leachate was weighed, concentrated by evaporation under an airstream to a known weight, and frozen until analysis for polyols. The lichens were dried, ground into a powder, and stored at room temperature

Table I. *Study Species Collected at a Single Site in Subarctic Québec*

Species Name	Acronym	Number of Replicates
Alectoria ochroleuca (Hoffm.) Massal.	ALOCH	9
Alectoria sarmentosa (Ach.) Brodo & D. Hawksw.	ALSAR	9
Bryoria lanestris (Ach.) Brodo & D. Hawksw.	BRLAN	9
Bryoria nitidula (Th. Fr.) Brodo & D. Hawksw.	BRNIT	9
Cetraria delisei (Bory ex Schaerer) Nyl.	CEDEL	3
Cetraria islandica (L.) Ach.	CEISL	3
Cetraria nigricans Nyl.	CENIG	3
Cetraria nivalis (L.) Ach.	CENIV	3
Cladina mitis (Sandst.) Hale & Culb.	CLMIT	3
Cladina rangiferina (L.) Harm.	CLRAN	3
Cladina stellaris (Opiz) Brodo	CLSTE	4
Cladonia amaurocraea (Floerke) Schaerer	CLAMA	3
Cladonia bellidiflora (Ach.) Schaer.	CLBEL	9
Cladonia gracilis (L.) Willd.	CLGRA	3
Cladonia subfurcata (Nyl.) Arn.	CLSUB	9
Cladonia sulphurina (Michx.) Fr.	CLSUL	3
Nephroma arcticum (L.) Torss.	NEART	9
Peltigera aphthosa (L.) Willd.	PEAPT	9
Peltigera scabrosa Th. Fr.	PESCA	9
Stereocaulon paschale (L.) Fr.	STPAS	9
Umbilicaria proboscidea (L.) Schrad.	UMPRO	9

until analyzed. Subsamples of the lichen powder were analyzed for polyols.

Polyol Analysis. We used a periodate reduction assay for polyols modified from Lewis and Smith (11) to include a simpler and more effective method of purification and a sample blank to correct for color in the sample. Two replicates of each tissue sample were analyzed.

Samples of approximately 0.10 g of lichen powder were dried at 100°C, cooled in a desiccator, weighed to 0.0001 g, and extracted three times with 10 ml boiling 80% ethanol for 10 min. The three extracts were pooled.

The ethanol was evaporated off under an air stream at 40°C and distilled H_2O added to maintain a volume of 20 ml. The solution was saturated with $(NH_4)_2SO_4$ to precipitate protein and to coagulate fine crystals, and left overnight. The following day the solution was filtered, washing with saturated $(NH_4)_2SO_4$ solution, and then made up with distilled H_2O to 50 ml in a volumetric flask. The $(NH_4)_2SO_4$ did not affect the spectrophotometric assay.

The spectrophotometric assay estimated polyol concentration (mg mannitol/L) by the reduction in periodate, a reaction specific for polyols. A sample blank, as well as a reagent blank, was included to correct for color in the sample solution.

A reagent blank of 1 ml distilled H_2O, 1 ml 1 M (pH 4.50) acetate buffer, and 1 ml (0.500 g/L) sodium metaperiodate solution was mixed in a quartz cuvette, and used to set the spectrophotometer at 0.800 *A* at 260 nm. The sample solution of 1 ml purified extract, 1 ml buffer, and 1 ml periodate solution was read at exactly 3.00 min after the periodate solution was added. A sample blank of 1 ml solution, 1 ml distilled H_2O, and 1 ml buffer was read against a distilled H_2O blank for each sample. The final absorbance reading for a sample was 0.800 minus the sample solution absorbance plus the sample blank absorbance.

Final results for leachate concentrations are expressed as (g polyol leached/g lichen dry weight) × 100. Tissue polyol concentrations are expressed as (g polyols/g lichen dry weight) × 100 and are the sum of polyols present in the tissue after leaching and polyols leached from the sample. Original polyol concentrations before wetting would also include polyols respired during resaturation respiration in the experiment, but this respiratory loss could not be measured.

Analyses. Statistical analyses were done on the McGill computer system using the General Linear Model procedure of Statistical Analysis System, version 82.4 (12).

RESULTS

The quantity of polyol lost from *Cladina stellaris* decreased exponentially over the 60 min measured (Fig. 1). The majority of loss occurred in the first 15 min. By 45 min the rate of polyol loss was negligible. The best fit regression for the leaching rate of *Cladina stellaris* expressed as the percentage polyol loss over time since wetting was:

$$\log(\text{leaching rate}) = -0.030\,(\text{min}) - 1.004$$

with $p = 0.0001$, $r^2 = 0.80$, and $n = 16$ (Fig. 1).

We found no statistically significant effect of either rainfall intensity or polyol concentration on polyol leaching rate in *C. stellaris*.

The response to rainfall was also analyzed separately for each of 11 other lichen species. Two multiple regressions were calculated for each species: polyol leaching rate as a function of polyol concentration and either of two rainfall variables—total rainfall (mm) or rain intensity (mm/h). The total amount of rain varied from 1 to 3 mm, while rain intensity varied between 1 and 6 mm/h; the two variables were not correlated. Response to rainfall is strongly species specific. Six species show no significant response at all to the amount or intensity of rainfall. Only one species, *Cladina rangiferina*, showed a small but statistically significant increase in leaching rate with increased rain intensity (slope = 0.07). Five species had a significant correlation of polyol leaching rate with the total amount of rainfall. *Alectoria sarmentosa*, *Peltigera apthosa*, *Peltigera scabrosa*, and *Umbilicaria proboscidea* had varying degrees of positive correlation of leaching rate with the total amount of rainfall (slopes = 0.10, 0.36, 0.40, and 0.10, respectively), and one species, *Stereocaulon paschale*, actually showed lower leaching of polyols with increased total rainfall (slope = −0.40).

The greater a species polyol concentrations, the greater will be the rate of polyol leaching in a rain episode, (Fig. 2). Polyol loss

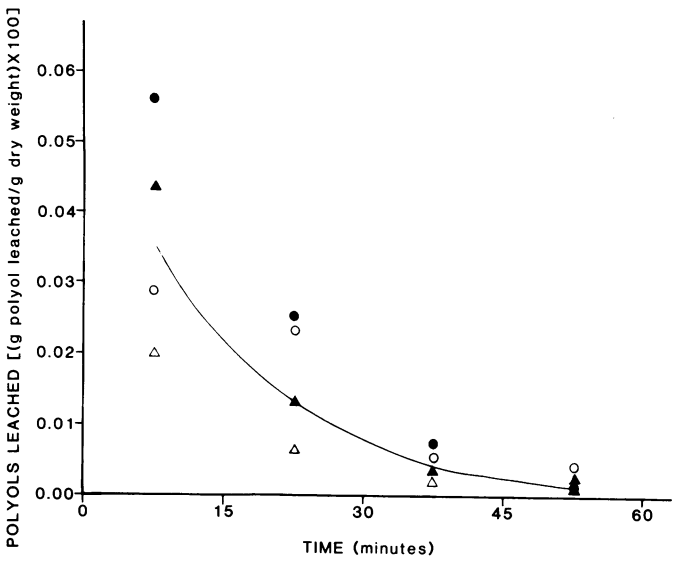

FIG. 1. The time course of polyol leaching rate (polyol leached per 15 min interval) for four replicates of *C. stellaris*. The different symbols denote the individual replicates. The transformed regression of log (leaching rate) on time has been plotted.

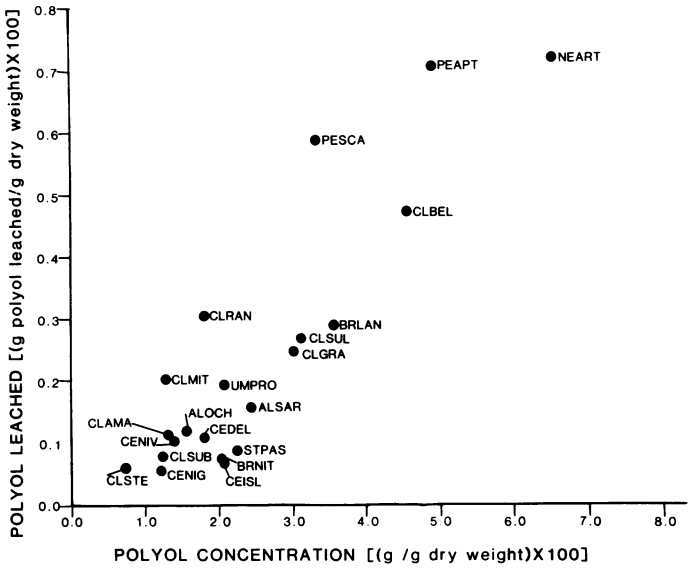

FIG. 2. The average leaching of polyols (polyols leached per rewetting episode) *versus* the average concentration of polyols for 21 species of lichens. The acronyms are defined in Table I.

can be predicted from polyol concentration using the equation below where all units are (g [polyol]/g [dry biomass] expressed as a percentage loss) as in Figure 2:

$$\text{(polyol loss)} = 0.14\ \text{(polyol concentration)} - 0.088$$

with $p = 0.0001$, $r^2 = 0.71$, and $n = 20$. This relationship applies between about 1.0 and 9.0% polyol concentration.

DISCUSSION

Only about 10% of the polyol pool and up to 0.7% of the lichen biomass will be lost by a lichen in a natural wetting event, a small loss compared to some rates reported for vascular plants. For example, up to 6% of dry weight equivalent was leached from young bean leaves over 24 h (13). Although in both comparative and absolute measures the polyol leaching rate is low in lichens under natural rain regimes, leaching losses may nonetheless be a substantial part of the lichen carbon balance under field conditions.

Compared to higher plants, lichens grow slowly enough that even these low leaching rates can influence production. A comparison of daily lichen growth rates with polyol leaching rates for subarctic reindeer lichens demonstrates that leaching losses are likely to be an important part of the lichen carbon balance. In summer, subarctic lichens have been observed to grow from 0.0 to 0.8% of their biomass per day, the rate depending on rainfall and species (7, 8). But an activity period is usually initiated by rainfall, implying that from 0.01 to 0.7% of the biomass, depending on the thallus polyol concentration, will be lost through leaching of polyols in each activity period. We conclude that a large proportion of the gross daily production must be allocated to replenishing the lost polyol pool, since leaching rates are of the same order of magnitude as growth rates.

Polyol leaching is thus likely to be a significant component of the lichen carbon balance under natural precipitation regimes. Though unremarkable as a physiological phenomenon, polyol leaching is important as an ecological one. Any loss of carbon is disadvantageous to production limited lichens, and leaching appears to be a significant loss. Since polyol concentration is the most important predictor of polyol leaching rate, leaching rates can be readily predicted for incorporation into a carbon budget model. Integrating predictions of polyol leaching rate into comprehensive carbon budgets of several lichen species can give an assessment of the importance of leaching, and also a measure of the cost for the lichens of maintaining high polyol concentrations.

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