ECOLOGICAL SIGNIFICANCE OF POLYOL CONCENTRATIONS IN SUBARCTIC LICHENS

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A thesis submitted to the faculty of Graduate Studies and Research in partial fulfillment of the requiremen[illegible] for the degree of Master of Science

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GENERAL ABSTRACT

It has been suggested that the high polyol concentrations of lichens function to buffer against stress, at the cost of lowered production. We investigated this hypothesis. A quantitative study of loss of polyols through leakage from rewetting dry lichens was done for 21 subarctic lichen species. The quantity of polyol lost was proportional to the polyol concentration present. In some species the total rainfall influenced the quantity of polyol lost. We conclude that leakage of polyol must be a major part of the lichen carbon budget, because daily growth rates are of the same order of magnitude as leakage. Since loss is proportional to polyol concentration, this supports the hypothesis that polyol concentration affects production.

If polyols buffer against stress, then higher polyol concentrations should be found in lichen species in less favorable environments. This prediction was not confirmed. Instead lichens with higher saturation water contents and in more favorable environments had higher polyol concentrations. We suggest that polyol concentration may be functioning to affect osmotic potential or metabolic rates, thus allowing more carbon uptake. High polyol concentrations may be one lichen strategy to increase production which is effective in lichens with high saturation water contents.

RÉSUMÉ GENERAL

Il a été suggéré que les fortes concentrations en polyols particulières aux lichens ont une fonction tampon dans des situations de stress. Les faibles taux de croissances des lichens seraient aussi une conséquence de cette spécialisation. Cette hypothèse a été examiné dans cette présente thèse. Une étude quantitative des pertes en polyols par écoulement suite à la ré-hydratation des lichens secs chez 21 espèces de lichens sub-arctiques fût entreprise. La quantité de polyols perdue est proportionelle à la quantité présente avant ré-hydratation. Chez quelques espèces les précipitations totales influencent la quantité de polyols perdue. Les taux de croissance journaliers étant du même ordre de grandeur que l'ampleur des pertes par écoulement, nous concluons que l'écoulement de polyols doit être une composante du budget carbonique chez les lichens. Comme ces pertes sont proportionelles à la concentration en polyols, ceci supporte l'hypothèse selon laquelle la concentration en polyols affecte la croissance.

Si les polyols exercent un effet tampon contre le stress, alors de fortes concentrations de polyols devraient être associées aux espèces de lichens particulières aux habitats moins favorables. Cette prédiction ne fût pas confirmée par les présent résultats. Au contraire, les espèces ayant des contenus en eau plus élevés au point de saturation et vivant dans des habitats plus favorables ont de plus fortes concentrations en polyols. Nous suggérons que la concentration en polyols à possiblement pour fonction d'agir sur le potentiel osmotique ou les taux metaboliques, permettant ainsi une plus grande fixation de carbone. De fortes concentrations en polyols constitueraient possiblement une stratégie favorable chez les espèces à forte teneur en eau au point de saturation pour augmenter leurs taux de croissance.

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Chapter 1

PREFACE

As permitted by the regulations of the Faculty of Graduate Studies and Research, this thesis is composed of two articles to be submitted for publication with the co-authorship of my advisor Martin J. Lechowicz. As required, a general introduction and a summary have been added to combine the papers into a unified thesis. In all cases, I am the first author and am primarily responsible for the content and presentation of the papers.

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The research was supported by the National Science and Engineering Reseach Council and the Canadian Wildlife Service. I thank the Canadian Wildlife Service and the McGill Faculty of Graduate Studies for scholarship support. The McGill University Computing Center and the Faculty of Graduate Studies and Research provided computing time.

I would like to thank my advisor Martin Lechowicz for his continued support and aid through both deed and example.

GENERAL INTRODUCTION

In 1976 Farrar published his physiological buffering hypothesis (Farrar,1976; Farrar and Smith,1976). He had observed that the lichen's response to dessication and recovery from dessication was high rates of turnover through respiration and depletion in the polyol pool, while the proteins and other insoluable material were relatively unaffected. He suggested that the lichen's response to stress, particularly recovery from dessication, is respiratory loss. Having such high polyol concentrations restricts such loss to the polyol pool. The polyol pool is therefore a buffer for the proteins and structural material against environmental stress. Lichens are often dessicated. The need to maintain a high polyol content could therefore account for the low rates of synthesis of macromolecules, high allocation to polyol (Farrar,1978), and the low net annual production of lichens.

Though Farrar presented this idea simply as a hypothesis, the idea of physiological buffering is widely referred to in subsequent ecophysiological literature (Mathes & Feige,1983; Bewley,1979; Lawrey,1984; Hill,1981), because of its explanatory value. It suggests a mechanism for recovery from dessication and an identifiable allocation of carbon to recovery. It justifies the high polyol concentrations and low production rates of lichens. Moreover it has predictive value. It suggests that distribution and production of lichens should be correlated with polyol concentrations. Though many authors have suggested that physiological buffering may explain a given phenomenon (Lechowicz.1981;

Hill,1981) none have directly tested the physiological buffering hypothesis.

The physiological evidence for this hypothesis is ambiguous. Recovery from stress may require energy, and this energy may result from respiration of polyols, but this does not imply that high levels of polyols are necessary for recovery. In mossses it has been shown that resaturation respiration is at least partly uncoupled from ATP production (Bewley & Krochko,1983). Thus the loss of polyol through resaturation respiration as well as that from leaching may be wasteful, but Farrar (1976) argues that it is the high loss of polyols that requires maintenance of high polyol concentrations in order to ensure that adequate polyols are present.

One can examine the effect of phenomena that cause polyol depletion in the lichen. Polyols are lost during recovery from dessication, and during a short activity period there may not be sufficient time to accumulate more polyols (Farrar,1976). The physiological buffering hypothesis thus predicts that many short activity periods will impair metabolism. Metabolic rates are lessened by short activity periods (Farrar,1976; Link & Nash,1984), and this is correlated with lower polyol concentrations. But other explanations may be proposed for the deleterious effects of short wettings such as nutrient loss through leaching or incomplete repair of membranes or proteins.

Therefore in this thesis I intend more direct tests of the physiological buffering hypothesis. The relationship between polyol concentration, the more easily measured variable, and allocation to polyols, the more difficult variable affecting production, can be partly established by looking at leaching of polyols upon rewetting. The leakage of polyols is expected to be one reason for the high turnover of polyols (Farrar, 1976) and it is expected to be a function of the polyol concentration (Simon, 1974).

The physiological buffer hypothesis is an ecological hypothesis because it suggests that the polyol allocation functions to resist stress. The most appropriate test is an ecological one: is allocation to polyols a function of the distribution of the lichen species? Confirmation of this hypothesis would not simply support the hypothesis but also make predictions about the relation of production to distribution.

LITERATURE CITED

Bewley, J.D. 1979. Physiological aspects of dessication tolerance. Annual Review of Plant Physiology 30:195-238.

Bewley, J.D. and J.E. Krochko. 1983. Dessication tolerance. Encyclopedia of Plant Physiology 12B:325-378

Farrar, J.F. 1978. Ecological physiology of the lichen Hypogymnia physodes. IV. Carbon allocationa at low temperatures. New Phytologist 81:65-69

Farrar, J.F. 1976. Ecological physiology of the lichen Hypogymnia physodes II. Effects of wetting and drying and the concept of physiological buffering. New Phytologist 77: 105-113.

Farrar, J.F. and D.C. Smith. 1976. Ecological physiology of the lichen Hypogymnia physodes III. The importance of the rewetting phase. New Phytologist 77:115-125.

Hill, D.J. 1981. The growth of lichens with special reference to the modelling of circular thalli. Lichenologist 13:265-287.

Lawrey, J.D. 1984. Biology of lichenized fungi. Praeger Publishers, New York.

Lechowicz, M.J. 1981. The effects of climatic pattern on lichen productivity: Cetraria cucullata (Bell.) Ach. in the Arctic tundra of northern Alaska. Oecologia 50: 210-216.

Link, S.O. and T.H. Nash III. 1984. Ecophysiological studies of the lichen Parmelia praesignis Nyl. Population variation and the effect of storage conditions. New Phytologist 96:249-256.

Mathes, U. and G.B. Feige. 1983. Ecophysiology of lichen symbioses. Encyclopedia of Plant Physiology 12C: 423-467.

Simon, E.W. 1974. Phospholipids and plant membrane permeability. New Phytologist 73:377-420.

Chapter 1

A quantitative description of the leaching of polyols in 21 species of subarctic lichens

By Susan A. Dudley and Martin J. Lechowicz

Abstract. Lichens have been observed to lose polyols through leakage upon rewetting, but no quantitative analysis of these losses exists. We observed the leaching of 23 species of subarctic lichens by synthetic rain at various intensities. Average polyols leached for each species was strongly correlated with the average polyol concentration of the species. Species had different responses to rainfall. Of those species that did have a significant response to rainfall the majority had increased leaching with increased total rainfall, rather than responding to rainfall intensity. The biomass lost through through leaching is of the same order of magnitude as literature values for daily relative growth rates for these lichens. This strongly suggests that loss of polyols through leaching is a major portion of the carbon balance of subarctic lichens.

INTRODUCTION

Lichens have low production rates because of their low photosynthetic rates and their poikilohydric water economy (Farrar,1976b; Kallio and Karenlampi,1975). Using equations predicting net photosynthesis as a function of lichen water content, light levels and tissue temperature (Lange et al,1977), yearly carbon uptake has been estimated quite well for desert lichens. Desert lichens are primarily wetted by dew and water vapor (Kappen et al.,1979); however wetting by liquid water causes resaturation respiration and leakage of solutes after rewetting (Farrar,1976b) A transient period of major leakage occurs in dessication tolerant plants that seems to be associated with temporary dessication induced membrane damage (Dhindsa & Bewley,1977; Bewley,1979). Therefore rain-wetted lichens, unlike the desert lichens Lange studied, have a time dependant loss of carbon after wetting, which complicates estimates of the carbon balance. Lechowicz (1981) had good agreement between measured growth rates and simulated productivity using a model that included the initial loss of carbon dioxide through resaturation respiration. But the high polyol content of lichens (Lewis & Smith,1967a) together with the high rates of leakage expected (Dhindsa & Matowe,1981) imply that leakage of polyols upon rewetting may also be an important part of the carbon balance.

Earlier studies of leakage of polyols have either been qualitative (Cooper & Carroll,1978) or have been done with the lichens immersed in solution (Farrar & Smith,1976) and so the results are not useful for predicting quantities of polyols leached in nature. The period of leakage from the cells is over within a few minutes of rewetting (Bewley,1979), but the period of leaching of polyols from the thallus should be known to relate climatic data to predictions of leaching. The intensity of rainfall is expected to affect the rates of leakage because a rapid inrush of water may displace membranes (Bewley,1979), and because low intensities may allow the lichen to take up polyols from the free space once membrane repair is completed (Farrar & Smith,1976). The amount of loss is expected to be influenced only by the concentration gradient and to have a low Q_{10} (Simon,1974). The purpose of this study is two-fold: to test if leakage of polyols is a significant part of the lichen carbon balance, and to attempt to predict leakage as a function of the polyol concentration, the rainfall intensity and the duration of rain.

METHODS AND MATERIALS

Collections of 21 lichen species were made near Schefferville in subarctic Quebec in September,1983, a subarctic area unusually rich in species (Waterway et al.,1984). These foliose and fructicose species are from alpine tundra, subalpine heath, open lichen woodland and closed spruce-moss forests, the major habitat types in the area where lichens are found (Waterway et al.,1984), and have distributions from arctic-to-temperate to arctic.

The lichens were collected during a rain to avoid damaging the brittle and fragile tips. The wet lichens were collected in paper bags, dried at room temperature, and shipped by air to Montreal. They were stored air-dry at room temperature and analyzed within a month.

Lechowicz (1983) demonstrated that lichens show a gradient in physiological activity from the growing tips to the senescing bases. To control for such within-thallus differences in activity and to sample equivalent portions across all species, we used all of intact thalli with living (green) algae. Microscopic examination of algal distribution and external color gradients were used to decide what part of the thalli would be used in experiments. In these experiments each replicate is composed of several thalli, to give a high dry weight of tissue in each leaching trial.

Synthetic rain was generated by a Bete-Fog synthetic rain nozzle oriented to project the water vertically; the rain therefore falls with only the acceleration of gravity. The water used was tap water filtered through activated carbon.

At any point in the sample grid the intensity of the rain shadow varied across the area and between trials. The different rain intensities in these experiments were generated by placing the lichen sample in places of different expected intensities and measuring the final intensities received. Air-dry lichen replicates were placed in plastic mesh baskets that were in turn placed in large plastic containers that allowed all rainfall passing through the lichens to be collected. After leaching, the lichens were dried at 100C°, weighed to 0.01 g, and analyzed for polyol concentration. The leachate was collected, weighed for measurement of rain intensity, and sampled for analysis of polyols in the leachate. The subsample was weighed, concentrated by evaporation to a known weight, and frozen until analysis.

We used a periodate reduction assay for polyols modified from Lewis and Smith (1967b). The modifications were a simpler and more effective method of purification and a sample blank to correct for color in the sample. Each lichen replicate was dried at 100C°, and ground into powder. Two replicate analyses of this powder were done. Small samples of approximately 0.10 g of lichen powder were dried at 100C°, cooled in a dessicator, weighed to 0.0001g, and extracted three times with 10 mls. boiling 80% ethanol. The three extracts were pooled. The ethanol was evaporated off under an air stream at about 40C°. The solution was saturated with ammonium sulphate and left overnight. The following day the solution was filtered and made up to 50 mls in a volumetric flask.

The polyol concentration is estimated by the reduction in periodate, a reaction specific for polyols. Three minutes after mixing the sample (1 ml sample solution, 1 ml 1M pH 4.50 acetate buffer, and 1 ml sodium metaperiodate solution(0.500 g/l)) is read at 260 hm on an ultraviolet spectrophotometer. Concentration in mannitol equivalents is estimated from absorbance using a mannitol standard curve. The reagent blank is 1 ml distilled H_2O, 1 ml buffer and 1 ml sodium metaperiodate. A sample blank was also read for each sample- 1 ml sample solution, 1 ml buffer and 1 ml water. The leachate solutions were treated as the purified sample solutions were.

Final results for leachate concentrations are expressed as (g polyol leached/g lichen dry weight) x100. Tissue polyol concentrations are expressed as (g polyols/g lichen dry weight) x 100 and are the sum of polyols present in the tissue after leaching and polyols leached for the sample. Original polyol concentrations before wetting would also include polyols respired during resaturation respiration in the experiment, but this was not measured.

A time course of polyol loss was done for <u>Cladina stellaris</u>. Four samples were leached at 3 mm / hour rainfall for one hour. The leachate was collected at 15 minute intervals and polyol concentration analyzed.

In the prediction of leakage experiment it was intended to analyze polyols leached as function of polyols present and rain intensity. We attempted to vary intensity while holding total rainfall constant by subjecting lichen to rainfall of 6mm / hour for 15 minutes, 3 mm / hour for 30 minutes, or 1.5mm / hour for 60 minutes. Because of the uncontrolled variance in intensity, however, total rainfall also varied and was measured. For 11 species we had sufficient material for nine replicates, three at each time. For the other species three replicates were leached at 30 minutes.

For the time course of leaching a linear regression was calculated of leaching through time, using the General Linear Model procedure of Statistical Analysis Systems version 82.4 (SAS Institute, 1982) and choosing the model with good residuals and the highest correlation coefficient.

Preliminary scatter plots for the prediction of polyol leached suggested that a differential response to rainfall existed between species. We therefore regressed polyol leached on polyol present using average values for each species. The response to rainfall was done separately for each species where nine replicates had been done. Multiple regressions were calculated of polyol leached as a function of polyol present and one of the three rainfall variables -- total rainfall, rain intensity, or time leached.

RESULTS

The best fit model for leaching as a function of time calculated from the four replicates of Cladina stellaris is log (leachate) = -1.004 + -0.030(time) ($p=0.0001, r2=0.80$)(Figure 1). Neither intensity nor polyol concentration had a significant effect on the polyols leached for these four curves. There appears to be a negative exponential loss of polyols over time, with the majority lost in the first 15 minutes, and over 90% lost within 30 minutes.

Polyols lost through leakage(Figure 2) for 21 species are directly proportional to polyols present; polyols leached= -0.088+0.14(polyol concentration) $p=0.0001, r2=0.71$. Using average values per species gives a better fit model than using all replicates, probably because the variance between replicates for each species is high.

The rainfall responses vary between species (Table 2). Five species show no response at all to the rainfall variables. One species showed a small increase in leaching with increased rain intensity. Bryoria lanestris lost less polyols during the longer rains. Five species had a significant correlation of polyols leached with total rainfall: four had varying degrees of positive correlation, and Stereocaulon paschale showed lower leaching of polyols with increased rainfall. Rainfalls varied from 1 to 3 mm, while intensity varied between 1 and 6 mm/hour. There was no correlation between intensity and rainfall.

DISCUSSION

The exponentially declining loss of polyols was similar to that reported for other dessication tolerant plant tissues (Bewley,1979) since the polyols are from cell contents temporarily exposed because of leaky membranes (Simon,1974) and repair of membranes starts within the first few minutes (Bewley, 1979) after rewetting. The exponential decline of loss is also the pattern shown by leakage of polyol for immersed lichens (Farrar and Smith,1976), of amino acids for immersed mosses (Dhindsa & Bewley,1977) and of the mineral nutrients potassium and nitrogen from lichens immersed in a solution (Lang et al.1976), and under natural rainfall (Crittenden,1983). Millbank (1982) saw the same amount of nitrogen leached from lichens whether rained upon 15 or 60 minutes after dampening, suggesting that the major loss is occuring within the first 15 minutes after wetting.

The result that polyols are leached as a function of the polyol concentration of the lichen is consistent with the theories of leaky membranes, since amount of leakage is expected to be a function of the concentration gradient(Simon,1974). The rate of leaching observed here is low; Dhindsa & Matowe (1981) found that 20% to 50% of rubidium leaked out of a dessication tolerant moss immersed in water. This difference is likely attributable to the method of wetting; rainfall perhaps allows uptake of the leached material before it is washed out (Farrar & Smith,1976). Crittenden (1983) notes that considerable potassium is leached from immersed <u>Stereocaulon paschale</u>, while the same lichen under natural rainfall shows little or no loss of potassium.

Sources of the polyol leached may be both the cytoplasm and the extracellular space, since in lichen with green algae polyols are transferred between algae and fungi. One of the studies species, Peltigera scabrosa, has only blue-green algae, which transfer glucose to the fungus, but it does have a high rate of leakage, suggesting that much leakage is from the cytoplasm. The quantity of polyol leached may well vary depending on lichen pretreatment; in mosses more leakage is seen from rapidly dried samples than slowly dried ones (Dhindsa & Matowe, 1981). The rapidity of drying may well influence the amount of extracellular polyol, since transfer in some lichens only occurs at saturation (MacFarlane & Kershaw, 1982). That polyol loss continues through the rainfall is unlikely, since the diffusive transfer of polyols appears quite efficient; fungal ablility to take up polyol is high (Collins & Farrar, 1978) and Hill (1976) found it difficult to wash out the glucose transferred in his study species.

It was hypothesized that all species would show a similar and positive response of polyols leached to rain intensity. Instead species varied in their responses, with almost half of them showing no response to rain, and the majority of the other species having increased leaching with increased total rainfall. One source of variation may be the species specific rate of rehydration under similar rain. Crittenden (1983) also sees more leaching of nitrogen with higher total rainfalls rather than rain intensities. The negative correlation of leaching to total rainfall for Stereocaulon paschale needs more investigation, since it may be a chance relationship. Any more detailed conclusions on which species show responses to rainfall should be avoided because sample sizes for each species are small and the experiment was not designed to show an effect of total rainfall. It is interesting, however, that the two Peltigera species exhibit the strongest response to rainfall. They were found in low stress environments (Dudley & Lechowicz, in prep). In mosses, differences in repair rates of membrane damage between a dessication-tolerant and -sensitive species have been observed (Dhindsa & Matowe, 1981). If accurate predictions of leaching of polyols for a given species are needed a more detailed study should be done but generally it appears that the polyol concentration of the lichen is the most important variable to predict polyols leached.

Kallio and Karenlampi(1975) and Karenlampi(1971) have measured daily relative growth rates for some of the reindeer lichens studied here. In summer their growth rate is 0.03 to 0.8 percent increase in dry weight per day, within an order of magnitude of the leaching expected by one rainfall for these species (Fig. 2). Therefore loss of biomass through leaching can be considered a significant proportion of the carbon budget.

These results also have interesting implications for the physiological buffering hypothesis of Farrar (1976a). These results show that a high concentration of polyols results in a higher loss of biomass through leaching, and within a species a higher polyol concentration also results in higher resaturation respiration (Smith and Molesworth, 1973).

Therefore the losses from the polyol pool upon rewetting are proportional to the polyol concentration. Lichens will replenish their polyol concentration from either photosynthesis (Farrar, 1976a) or stored compounds (Cowan et al., 1979). Therefore a high polyol concentration implies a continued high allocation to polyols, as Farrar (1976a) implies.

These results demonstrate that leaching of polyols is occuring during natural rainfall. The rates of leaching are high enough to be of considerable importance to the production rates. Species respond differently to rainfall and this may have physiological or ecological interest, but leaching can be simply predicted as a function of the polyol concentration.

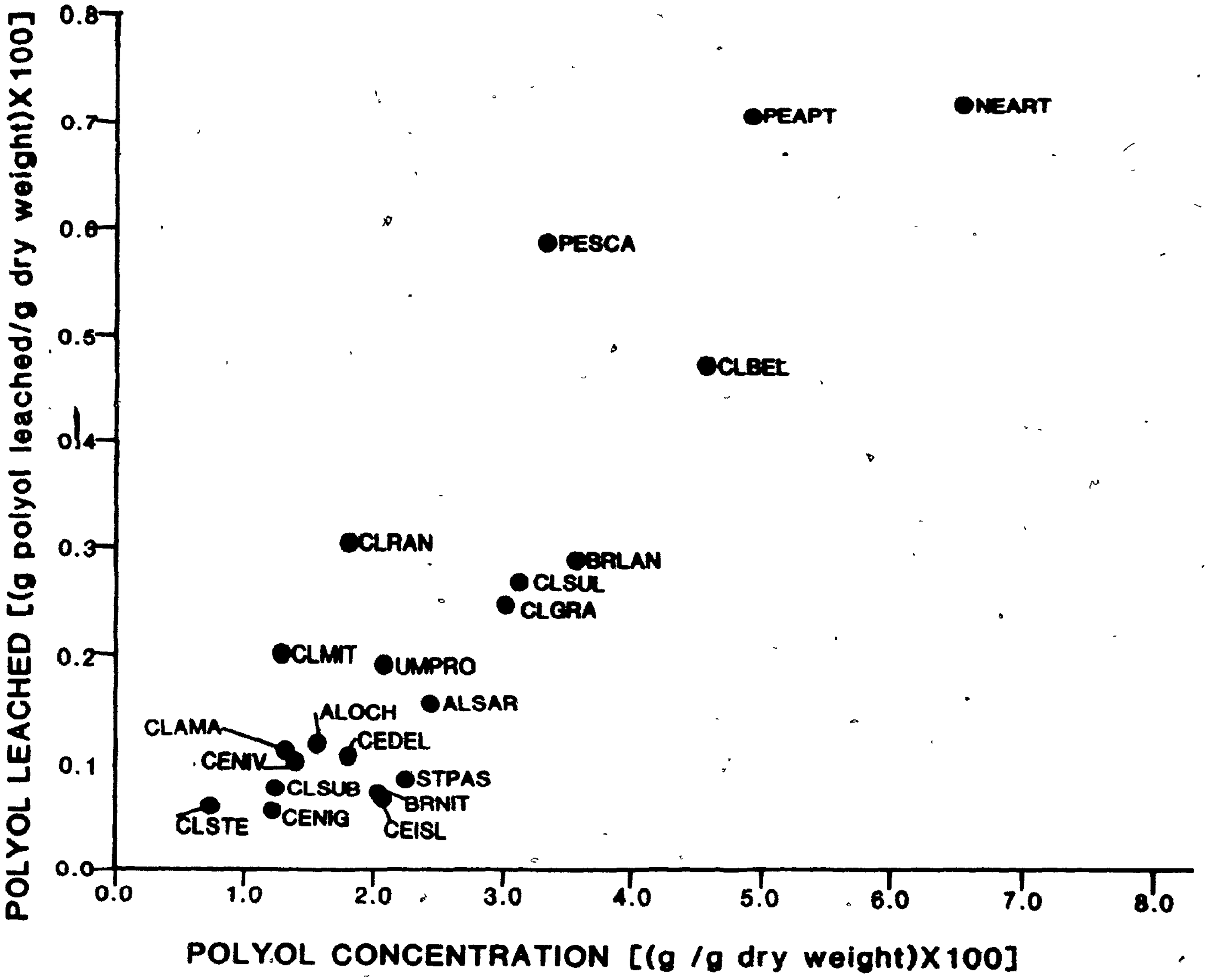
Table 1.The study species and the acronyms used to identify them in subsequent tables and figures.

Species name	Acronym	Number of replicates
Alectoria ochroleuca (Hoffm.) Massal.	ALOCH	9
Alectoria sarmentosa (Ach.)	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

Table 2. The slope of leaching on each of the rainfall variables for each species. **-p=0.01,*-p=0.05, no entry-p is not significant. All probabilities are of the slope of the variable in a multiple regression with polyol concentration as the second independent variable and polyols leached as the dependent variable.

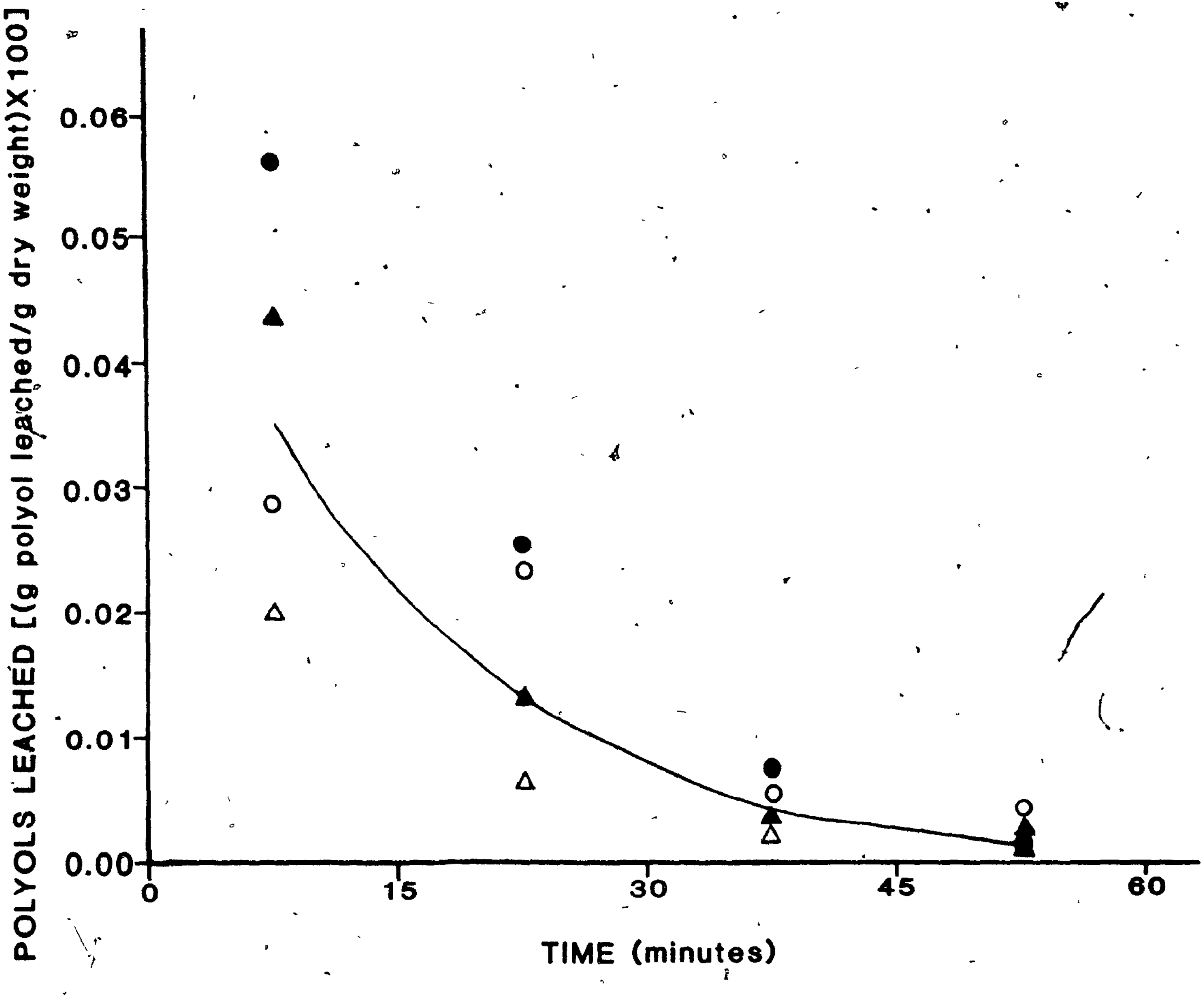
Species Acronym	Total Rainfall	Rain Intensity	Duration of Rainfall
ALOCH			
ALSAR	0.10**		
BRLAN			-0.006*
BRNIT			
CLBEL			
CLRAN		0.07*	
CLSUB			
NEART			
PEAPT	0.36*		
PESCA	0.40*		
STPAS	-0.40*		
UMPRO	0.10**		

Figure 1. The time course of leaching of four replicates of <u>Cladina stellaris</u>. The different symbols denote the individual replicates.



POLYOL LEACHED [(g polyol leached/g dry weight)X100]
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
POLYOL CONCENTRATION [(g /g dry weight)X100]
0.0
1.0
2.0
3.0
4.0
5.0
6.0
7.0
8.0
NEART
PEAPT
PESCA
CLBEL
CLRAN
BRLAN
CLSUL
CLGRA
CLMIT
UMPRO
ALSAR
ALOCH
CLAMA
CEDEL
CENIV
STPAS
CLSUB
BRNIT
CENIG
CEISL
CLSTE

Figure 2. Leaching of polyols versus the concentration of polvols in the thallus. The points are average values for each species.


POLYOLS LEACHED [(g polyol leached/g dry weight)X100]
0.06
0.05
0.04
0.03
0.02
0.01
0.00
0
15
30
45
60
TIME (minutes)

REFERENCES

Bewley, J.D. (1979). Physiological aspects of dessication tolerance. Annual Review of Plant Physiology 30:195-238.

Collins, C.R. & Farrar, J.F. (1978). Structural resistances to mass tranfer in the lichen Xanthoria parietina. New Phytologist 81: 71-83.

Cooper, G. & Carroll, G.C. 1978. Ribitol as a major component of water soluable leachates from Lobaria oregana. Bryologist 81: 568-572.

Cowan, P.A., Green, T.G.A. & Wilson, A.T. (1979). Lichen metabolism I The use of tritium labelled water in studies of anhydrobiotic metabolism in Ramalina celastri and Peltigera polydactyla. New Phytologist 82:489-503.

Crittenden, P.D. (1983). The role of lichens in the nitrogen economy of subarctic woodlands; nitrogen loss from the nitrogen fixing lichen Stereocaulon paschale during rainfall. In Nitrogen as an ecological factor (Ed. by J.A. Lee, S.McNeil, &I.H.Rorison), pp 43-68. Blackwell Scientific Publication, Oxford & London.

Dhindsa, R.S. & Bewley, J.D. (1977). Water stress and protein synthesis. V. Protein synthesis, protein stability and membrane permeability in a drought-sensitive and a drought-tolerant moss. Plant Physiology 59: 295-300.

Dhindsa, R.S. & Matowe, W. (1981). Drought tolerance in two mosses: correlated with enzymatic defense against lipid peroxidation. Journal of Experimental Botany 32: 79-91.

Farrar, J.F. (1976a). Ecological physiology of the lichen Hypogymnia physodes II. Effects of wetting and drying and the concept of physiological buffering. New Phytologist

77: 105-113.

Farrar, J.F. (1976b). The lichen as an ecosystem: observation and experiment. In Lichenology: Progress and Problems. (Ed. by D.H Brown, D.L. Hawksworth, & R.H. Bailey), pp. 385-406. Academic Press, London & New York.

Farrar, J.F. & D.C. Smith. (1976). Ecological physiology of the lichen Hypogymnia physodes III. The importance of the rewetting phase. New Phytologist 77: 115-125.

Hill, D.J. (1976). The physiology of lichen symbiosis. In Lichenology: progress and problems (Ed. by D.H. Brown, D.L. Hawksworth & R.H. Bailey). pp 457-496. Academic Press, London & New York.

Kallio, P. & Karenlampi, L. (1975). Photosynthesis in mosses and lichens. In Photosynthesis and Productivity in Different Environments (Ed. by J.P. Cooper), pp 393-423. Cambridge University Press, Cambridge.

Kappen, L., Lange, O.L., Schulze, E.-D., Evanari, M., & Buschbom, U. (1979). Ecophysiological investigation on lichens of the Negev Desert. VI. Annual course of the photosynthetic production of Ramalina maciformis. Flora 168: 85-108.

Karenlampi, L. (1971) Studies on the relative growth rates of some fructicose lichens. Reports of the Kevo Subarctic Research Station 7: 33-39.

Lang, R.E., Reiners, W.A. & Heier, R.K. (1976). Potential alteration of precipitation chemistry by epiphytic lichens. Oecologia 25: 229-241.

Lange, O.L., Geiger, I.L., & Schulze, E.-D. (1977). Ecophysiological investigations on lichens of the Negev

Desert. V. A model to simulate net photosynthesis and respiration of Ramalina maciformis. Oecologia 28: 247-259.

Lechowicz, M.J. (1983). Age dependence of photosynthesis in the caribou lichen Cladina stellaris. Plant Physiology 71: 893-895.

Lewis, D.H. & Smith,D.C. (1967a). Sugar alcohols (polyols) in fungi and green plants. I. Distribution, physiology and metabolism. New Phytologist 66:143-184.

Lewis, D.H. and D.C. Smith. (1967b). Sugar alcohols (polyols) in fungi and green plants. II. Methods of detection and quantitative estimation in plant extracts. New Phytologist 66: 185-204

MacFarlane,J.D. & K.A. Kershaw. (1982). Physiological-environmental interactions in lichens XIV. The environmental control of glucose movement from alga to fungus in Peltigera polydactyla, P. rufescens, and Collema furfuraceum. New Phytologist 91:93-101.

Millbank, J.W. (1982). The assessment of nitrogen fixation and throughput by lichens III. Losses of nitrogenous compounds by Peltigera membranacea, P. polydactyla, and Lobaria pulmonaria in simulated rainfall episodes. New Phytologist 97:229-234

SAS Institute Inc. (1982). SAS User's Guide: Statistics, 1982 Edition. Cary, NC: SAS Institute Inc.

Simon, E.W. (1974). Phospholipids and plant membrane permeability. New Phytologist 73, 377-420.

Smith, D.C. & Molesworth, S. (1973). Lichen physiology. XIII. Effects of rewetting dry lichens. New Phytologist

72:525-533.

Waterway, M.J., Lechowicz, M.J., & Moore, T.R. (1984). Vegetation of the Schefferville region, Nouveau Quebec. McGill Subarctic Research Papers, in press.

Chapter 2

Correlations between polyol levels, morphology and habitat in lichens: a test of the 'physiological buffering' hypothesis.

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Abstract. The physiological buffer hypothesis proposes that lichens allocate carbon to polyols as a method for tolerating stress. The amount of allocation to polyols should strongly affect the rate of production of lichen species. We test the hypothesis that lichens living in less favorable habitats have higher polyol concentrations. Favorableness can be considered an inverse function of the probability of short wettings, since short wettings cause loss of polyols.

For 23 species of lichens no positive correlation between any measure of short wetting periods and polyol concentration was found. Instead higher polyol concentrations were found in lichen species with higher saturation water contents and growing in more favorable environments.

The physiological buffer hypothesis is not supported by these results. Of the other functions that have been proposed for polyols only that of lowering the osmotic potential would account for differing allocation to polyols. The lower osmotic potential caused by higher polyols does not necessarily account for the observed correlation between polyols concentration and saturation water content. But the combination of high saturation water contents and high polyols may allow the lichen to take up water vapor and become metabolically active, allowing more carbon uptake than lichens remaining dormant. Therefore rather than greater polyol allocation lowering production, high polyol concentrations may be one lichen strategy for increasing production.

INTRODUCTION

A great deal is known about patterns of photosynthesis and respiration in lichens (Lawrey,1984), but as with higher plants growth is not simply a function of carbon uptake but also of patterns of carbon allocation (Mooney and Chiarello,1983). Lichens production is known to be somewhat uncoupled from photosynthesis(Hill,1981; Bewley and Krocko,1983); Farrar (1976a) suggests that the high allocation of photosynthate to polyol (sugar alcohol) compared to structural compounds or non-soluble storage compounds explains this lack of relation. The impact of polyol allocation to production can only be predicted if the function of polyols is better understood.

It is known that polyols are important metabolically, being the primary respiratory substrate of lichens as well as the form in which photosynthate is transferred between the alga and the fungus (Farrar,1973). The concentrations found in the lichen are quite high, from 1 to 10% of the dry biomass, depending on species (Lewis and Smith,1967a).

Farrar (1976b) suggested that the high polyol concentrations of lichens are a means for tolerating the stresses imposed by dessication and rewetting. He observed that under a severe environmental regime all loss of carbon was from polyols; no loss was from proteins or other insoluble material. He suggested that the polyol pool is a 'physiological buffer' against stress. The function of the polyol pool is suggested not to be a physico- chemical one, but simply to be a store of respiratory substrate for recovery from stress, to be used for resaturation respiration and repair after dessication.

Polyols are metabolized for 'resaturation respiration' which occurs immediately upon rewetting and declines gradually over time (Smith and Molesworth,1973). Presumably this provides energy for recovery and repair, though in mosses, another poikilohydric plant, resaturation is at least partly uncoupled from ATP production (Bewley and Krochko,1983). The polyol concentration must suffice to provide energy for subsequent wetting periods since the lichen may dry before it replenishes its polyol pool. Therefore according to the physiological buffering hypothesis polyol concentration should be higher if the lichen is more likely to be subjected to brief wetting periods.

The physiological buffer hypothesis implies that higher production will be found in less stressful environments. It proposes that lichens trade productivity for stress resistance. Higher polyol concentrations imply greater allocation of photosynthate to polyols (Dudley and Lechowicz,in prep) and the physiological buffer hypothesis implies that at least some of the photosynthate allocated to polyols will be lost to the environment. Therefore lichens living in less favorable environments should have lower productivity because of high physiological buffering, and that this productivity should be less coupled with photosynthetic rates than for lichens living in more favorable habitats. The 'physiological buffering' hypothesis predicts that species living in less favorable habitats should have higher polyol concentrations. If this prediction is born out it would support both the physiological buffer hypothesis itself as well as the hypothesis that productivity and environment are correlated in lichens.

METHODS AND MATERIALS

Site

We did the study near Schefferville in subarctic Quebec, an area unusually rich in plant species. Both arctic and temperate species can be found there, and the region is characterised by Picea mariana – Cladina stellaris woodlands (Waterway et al,1984). We chose a site that contained a variety of microhabitats and a large number of species within an area of less than 0.25 square kilometers. The general habitat types found in this site -- alpine tundra,, subalpine heath, open lichen woodland and closed spruce forests -- give the range of moisture regimes of the Schefferville area where lichens are found (Waterway et al.,1984). It is reasonable to assume that the entire site experiences identical weather, therefore differences in the probability of stress will be caused by overhead canopy interception of rain causing differences in the number of short wetting events, and by differences in the dryness of the environment.

Experimental design and sampling

Each observation was based on a microsite: a fairly dense population of the study species in an area of 1 to 3 square meters. After preliminary survey of all potential microsites, 24 microsites with 23 different species were chosen (Table 1). All field observations for a species were made at its microsite and lichens for lab work were collected at or very near the microsite. We collected lichens twice: the first collection in

August 1982 for studies of evaporative resistance and the second collection in September 1983 for studies of polyols and saturation water content. Both times we collected during a rain to avoid damaging fragile tips. The wet lichens were collected in paper bags, dried at room temperature and shipped to McGill. The 1982 collection was frozen at -20C until used, and the 1983 collection was kept at room temperature and analyzed within a month.

Lechowicz (1983) demonstrated that lichens show a gradient in physiological activity from the growing tips to the senescing bases. To control for such within-thallus differences in activity and to sample equivalent portions across all species we used all of an intact thallus with living (green) algae. Microscopic examination of algal distribution and external color gradients were used to decide what part of the thallus would be used in experiments.

Rain

Because all the lichens studied occur in such a small area no climatic differences in number of short wetting events are expected. Since canopy interception of rainfall may, however, differ between microsites, we compared rainfall at the different microsites. Five bottle and funnel rain gauges were placed around the circumference of each microsite, or with the corticolous lichens among the branches of the tree. Measurements were taken only if the foliage was dry at the onset of rain. Six rainfalls were recorded; the rain collected was weighed to 0.01 g and then converted to millimetres rainfall per storm. We averaged the rainfall of the 5 rain gauges at each microsite and took the geometric mean of the six experiments as the rain value for each microsite. No true overhead canopy storage occured: even in low rainfalls all microsites received some rain but overhead canopy reduced the rainfall.

Drying

The probability of short activity periods increases with the water evaporation rate of the lichen. Evaporation in lichens in a physical process, not a physiological one. The evaporation rate is affected by both the environment of the lichen and the lichen morphology (Larson and Kershaw, 1976) but unlike plants with stomates the lichen cannot control water loss. The evaporation rate of the lichen is described by the following equation from Larson and Kershaw(1976)

$$\text{evaporation rate} = \frac{\rho c'}{\lambda \gamma} \; \frac{(Es(To)-Ea)}{r(a)+r(i)} \qquad \text{Eq. 1}$$

The boundary layer resistance of the lichen to water loss, r(a), decreases with increased wind. The internal resistance of the lichen to diffusion, r(i), is a function of the adsorption properties of the thallus and is a power function of the thallus water content (Hoffman and Gates, 1970; Lechowicz, 1976). Constants in the equation are ρ, the density of air; c', the heat capacity of the air; λ, the latent heat of vaporization; and γ, the psychometric constant. Es(t0) is the vapor pressure at the lichen thallus, assumed to be the saturation vapor pressure at the lichen surface temperature. Ea is the vapor pressure of the atmosphere. Evaporation rate here is expressed as g(H20)/g (dry wt)/s rather than per cm2 even though evaporation is a surface property, because a lichen can have a high evaporation rate per unit surface area yet lose water fairly slowly; it is the drying rate of the whole lichen that interests us.

Index of favorableness

Index of favorableness measures stress level at each microsite. It integrates all the environmental effects on drying rate. Equation 1 shows that evaporation rate of the lichen increases with windspeed and radiation and decreases with environmental vapor pressure. These three variables often vary over the lichen thallus (Kershaw and Field, 1975). To estimate the overall effect on drying rate of these variables we measured the evaporation rate of a 'standard sponge'. Lichens hold water in capillary spaces, by matric adhesion and osmotically. Sponges have only matric and intercapillary water storage. Nevertheless since most water in lichens is held matrically and in intercapillary spaces the manner of sponge drying approximates that of lichen drying. The sponges should therefore respond to the same environmental variables as lichens do while having uniform internal resistance, allowing a comparison of external influences on drying rates. Thus they can be placed in roughly the same environment as the lichens, will respond similarly to the environment, can be assumed to have equal evaporative resistances, and give simultaneous measurements for all 24 microsites.

At the start of 1982 field season 10 sponge sites were placed randomly into each microsite. The lichens present and any other vegetation were removed in a 5 x 5 cm area. We did eight experiments. At the start of each experiment we wetted the sponges with an average 3 ml per sponge (equivalent to an absolute water content of 240%), watered the microsite to simulate a brief wetting, placed out the sponges, and recorded the time out at each microsite. Every half hour we collected at random one sponge from each of the 24 microsites and weighed it to 0.001 g. Results are expressed as absolute water content(AWC) (g H_2O/g sponge dry wt) at time(minutes) since placing out. We regressed AWC against time for the first four sponges collected in seven experiments, giving rates of water loss for the first three hours of the drying course. The slope, which is the negative of 100 times the evaporation rate, is taken as the index of favorableness: the higher the index the moister the environment.

Evaporative resistance

The morphology of the lichen modifies its wetting regime. The ratio of biomass to evaporating surface, the strength of the adhesion of water to the thallus, and the boundary layer resistance constitute the evaporative resistance and affect the speed at which the lichen comes into equilibrium with its environment. We measured total evaporative resistance (ra+ri) at 1m/s windspeed over the course of drying using the method of Larson and Kershaw (1976). Lichen evaporation rate, thallus and air temperature, and atmospheric vapor pressure are monitored in a wind tunnel as the lichen drys. The air speed was 1.0 m/s. For each species we did three replicates, each replicate comprising several individuals. At each measuring interval both internal resistance and relative water content(RWC)(absolute water content/saturation water contentx100) were calculated. Absolute water content (AWC) is the g H_2O present/g lichen dry wtx100. Saturation water content (SWC) is g H_2O at saturation/g lichen dry wt x100. We fit a power curve model, log resistance =a + b log(RWC), for each species. Though these parameters describe the evaporative resistance of lichens, to compare species we need one number which integrates both measures. The drying time of the lichens in the same environmental conditions will differ depending on the resistances. Therefore the time to dry is a logical measure of evaporative reistance, and one that is implicitly defined in the drying equation. We calculate the time taken to dry to a given RWC under identical conditions using the drying equation. Redefine the constant terms as k. Define the vapor pressure gradient (Es(To)-Ea) equal to v. Then evaporation=kv/resistance. This equation is actually a differential equation since resistance=a(RWC)b and evaporation rate is the change in water content over time and is equal to dAWC/dtx100.

$$\frac{d(AWC)}{dt(100)} = \frac{kv}{10^{a}(RWC)^{b}} = \frac{d(RWC) \times SWC}{dt \times 100^{2}} \qquad \text{Eq. 2}$$

$$d(RWC) \times RWC^{b} = \frac{kvdt \times 100^{2}}{10\ SWC} \qquad \text{Eq. 3}$$

Integrating both sides

$$\frac{(RWC)^{b+1}}{b+1} = \frac{kvt \times 100^{2}}{10^{a}SWC} + C \qquad \text{Eq. 4}$$

At t=0, RWC=100. Therefore

$$t(\text{minutes}) = \frac{10\ (RWC^{b+1} - 100^{b+1})SWC}{kv \times 100^{2} \times 60} \qquad \text{Eq. 5}$$

We used equation 5 to calculate time from 100% to 5% RWC, assuming all lichens at 20C° in an atmosphere at 20C° and 50% humidity, and ignoring differences that evaporational cooling and absorbance of radiation may cause in temperature.

Saturation water content

The saturation water content of the lichens is the weight of water that is held by the lichen after complete wetting. It affects the evaporative resistance since it is the amount of water with which the lichen starts the drying course, and it varies with the morphology of the lichen. Lichens placed on a plastic mesh platform were subjected to synthetic rain using a Bete Fog synthetic rain nozzle for 15 to 60 minutes. Lichen and platform were then weighed. Weight of the wet lichen was calculated as weight of lichen and platform minus dry weight of platform and minus an estimate of water droplets adhering to the platform.

Polyols

We used a periodate reduction assay for polyols modified from Lewis and Smith (1967b). The modifications were a simpler and more effective purification technique and the addition of a sample blank. For each species several individuals were dried at 100 C, combined, and ground to give an average sample for the species. We did two replicate analyses of this powder for each species. Small samples of approximately 0.10 g of ground lichen tissue were dried at 100 C, weighed to 0.0001 g and extracted 3 times with boiling 80% ethanol. The three extracts were pooled. The ethanol was evaporated off. The solution was saturated with ammonium sulphate and left overnight. The following day the solution was filtered and made up to 50 mls. The polyol concentration is estimated by the reduction in periodate a reaction that is specific for polyols. The sample (1 ml sample solution, 1 ml sodium metaperiodate solution (0.500g/l), 1 ml 1M acetate buffer pH 4.5) is read 3 minutes after mixing at 260nm on an ultraviolet spectrophotometer. The reagent blank is 1 ml H_2O, 1 ml periodate, and 1 ml buffer. A sample blank (1 ml H_2O, 1 ml buffer, 1 ml solution) was also read for each solution. Concentration of the sample solution was estimated from a mannitol standard curve. No attempt was made to determine what polyols were present. Final results are expressed in (g polyol/g dry wt)x100. The average of the two replicates is the species polyol concentration.

Data analysis

General Linear Models and Correlation procedures from the Statistical Analysis System Version 82.4 (SAS Institute,1982) were performed on the data. Normality is assumed because all variables are the means of several observations.

RESULTS

The range of polyol concentration (Figure 1) is similar to that reported in the literature and polyol concentrations show an eight-fold variation between species, sufficient to test the hypothesis. Foliose lichens have the highest polyol concentrations, ranging from 4 to 9%. Cladina stellaris, the abundant reindeer lichen of the open lichen woodlands, had the lowest polyol concentration at 1% and Alectoria ochroleuca, the most abundant lichen in the alpine tundra, also had a low polyol content at 1.5%. Except for the foliose lichen there is no marked similarity of polyol concentrations within genera.

There were few sites with any reduction of rainfall by overhead canopy (Figure 2). It was assumed, therefore, that the index of favorableness could be used to indicate differences in probability of short wettings.

The time courses of sponge water contents vary more strikingly within the major habitats than between them. (Figure 3, Table 2). Differences in drying rates are associated with the growth form of the lichen as well as the environment. For the forest lichens the highest indices of favorableness were for the lichens growing in moss(CLRAN,PEAPT,CLTUR), the lichens on trees and logs had the lowest indices of favorableness (BRLAN,ALSAR,CLSUL) while mat lichens had values between these extremes (CLSUB,CLSTE). Ridge and heath lichens, solitary lichens growing on earth (BRNIT,CLCOC,CLDEL) or rock (UMPRO) tended to have lower indices of favorableness while lichens growing in mixed or pure mats (STPAS,CLAMA, CENIV,ALOCH) had high indices of favorableness.

Lichens can modify the lengths of their activity periods by their evaporative resistance. The resistance - RWC (Fig. 4) curves differ in both of the estimated parameters for the resistance-RWC relationship which are mean resistance (a) and rate of change in resistance as RWC decreases (b) (Table 2). Foliose lichens have higher slopes than fructicose lichens: complexly branched lichens have some of the lowest resistances and simple fructicose lichens have some of the highest resistances. Under identical conditions drying times are estimated to vary from nine minutes for Bryoria nitidula to 101 minutes for Peltigera aphthosa (Fig. 8), reflecting different rates of approach to equilibrium with the environment.

Saturation water content does not determine length of wetting period. It was originally measured as a subsidiary variable in the evaporative resistance equations but we are including it as a primary independant variable because it was found to be correlated with polyol concentration (Table 3). The saturation water contents we obtained are higher than those commonly reported in the literature (Lange and Mathes,1981) because of the method we used. The usual method is to immerse the lichens for several minutes and then either shake or blot them to remove surface water. This method may both supersaturate the internal thallus and remove the surface water. Differences in the amount of the surface water has been shown to affect drying times of lichens (Snelgar and Green, 1981).

Index of favorableness is the major measure of the probalitity of short wettings: the physiological buffer hypothesis predicts that index of favorableness will be negatively correlated with polyol concentration. This result is not seen. Table 3 shows the correlation matrix. The largest observed correlation is between polyols and saturation water content (Fig 5). This correlation was unexpected and isn't easily explicable -- most of the water is held outside the cytoplasm (Harris, 1976) while the majority of polyols are inside the cells. Having a high saturation water content does not always result in a long drying time; the correlation between these variables though positive, is not significant (p=0.06).

Index of favorableness and evaporative resistance interact to determine the probability of short wetting periods. It had been previously observed that lichens living in xeric habitats will be less resistant to water loss (Larson and Kershaw, 1976), but our results do not conform to that generalization (Fig. 6). Our index of favorableness results are somewhat anomalous; the index of favorableness for Cladina stellaris, the abundant mat lichen of the mesic lichen woodlands, is lower than that of Alectoria ochroleuca, the abundant mat lichen of the more xeric alpine tundra. This is perhaps explained because in pure species mats there is a direct causal relationship between evaporative resistance and index of favorableness; the lower the evaporative resistance of the lichens, the higher the evaporation rate of the lichens, and since the sponges are surrounded by the evaporating lichen species, the moister the environment of the sponges. Temperature and moisture profiles are quite different within the mat than in the ambient environment(Kershaw and Field,1975). Cladina rangiferina has a low evaporative resistance but lives in a moist environment. This species has a wide range of distributions and is often found in more xeric environments. The other major outliers to the suggested positive relationship between evaporative resistance and index of favorableness are the cup lichens Cladonia coccifera and Cladonia sulphurina, the foliose lichen Umbilicaria proboscidea, and the arboreal fruticose Bryoria lanestris, which have high evaporative resistances and low indices of favorableness. The cup lichens are abundant in early succession after fire in the Schefferville region. The functional significance of a correlation between evaporative resistance and index of favorableness is evasion of heat stress (Mathes and Feige,1983); perhaps these outliers are unusually heat resistant when moist.

Index of favorableness and evaporative resistance together are the determinants of short activity periods for the lichens in our sites, but no significant correlation of polyols as a function of both these variables was found. The only significant multiple regression shows that polyol concentration of a species is higher if the saturation water content of the species is high and if the index of favorableness of the microsite is high (Fig. 7)

polyols=1.68+0.0039(SWC)+1.922(index of favorableness)

(p[0.0001,r2=0.46.) This result is contrary to the predictions of the physiological buffer hypothesis.

DISCUSSION

We find that polyols are most strongly correlated with saturation water content, a morphological variable important for lichen water relations. This is contrary to the predictions of the physiological buffer hypothesis which suggests that lichens allocate polyols solely in response to the environment. The physiological buffer hypothesis suggested that the index of favorableness should be negatively correlated with polyol concentration. But allocation to polyol is clearly not in response to greater probability of stress since, controlling for saturation water content, high polyol concentrations are found with high index of favorableness. The physiological evidence for the physiological buffering hypothesis is ambiguous (Bewley and Krochko,1983), so that this type of ecological test is both necessary and decisive. Our results do not deny any role of polyols in providing energy for recovery from dessication, but do refute the hypothesis that high allocation of photosynthate to polyols is due to 'buffering' against stress.

To predict the effect of polyol allocation on production a hypothesis for the function of polyol concentration is needed. A number of functions have been proposed for polyols -- storage, protection of macromolecules during dessication and freezing, enhancing photosynthesis by promoting production, and lowering osmotic potential(Lewis and Smith,1967a; Farrar,1976b). The first two roles are unlikely. Storage is not a logical function, since an insoluable compound such as starch would be less vulnerable to leakage. All the lichens we studied are equally subject to dessication and freezing; the hypothesis of protection of macromolecules would predict the same pattern of allocation as the physiological buffering hypothesis (Hill,1981) There is literature support for the hypothesis that polyols affect metabolic rates. For example polyol concentration was correlated with photosynthetic rate over the course of a severe wetting and drying regime (Farrar,1976a). Feeding a lichen polyols speeds photosynthetic recovery (Groulx and Lechowicz,unpubl) in subsequent recovery from dessication. The possible implications of the lowered osmotic potential caused by higher polyol concentrations are quite interesting. The possesion of a high osmotic potential will not prevent the lichen from drying when the environment is dry, but it may well extend its activity period. The environmental conditions in which lichen is active and the time the lichen takes to dry may determine whether it is positive or negative carbon balance.

Neither the enhancement of photosynthesis nor the lowering of osmotic potential hypotheses explain the results we obtained. Though increasing polyol concentration in a given lichen will decrease its osmotic potential and so should increase its saturation water content, this mechanism can not be assumed to be the cause of the the correlation in lichen species between saturation water content and polyol concentration. It is believed that most differences in saturation water contents between species are because of differences in extracellular water, not cytoplasmic water, though no studies on the distribution of water in the thallus have been done for lichens (Harris,1976).

It may be that higher polyol concentrations increase carbon uptake; either the lower osmotic potential could extend the time the lichen can photosynthesize or the stimulation of photosynthesis could increase the rate at which the lichen takes up carbon. These gains must then be balanced against the high losses of polyol through leakage upon resaturation (Dudley and Lechowicz,in prep) which are proportional to polyol concentration. We would deduce from our results that in lichens living in more favorable environments and with higher saturation water contents the gains are greater than the losses. The lowered osmotic potential may allow the lichens to avoid some of the losses, since wetting by water vapor could occur and it does not cause resaturation respiration or leaching.

Why saturation water content could affect carbon uptake is uncertain: saturation water content has not often been viewed as an important variable for lichen productivity. Lichens with high SWC have been observed to have lower depression of net photosynthesis at high thallus water content (Turk,1983), perhaps because they have a looser thallus (Reid,1960) that allows more diffusion of CO2. If water is lost from walls and intercellular spaces before it is lost from cells as Dilks and Procter (1983) imply occurs in mosses, then high SWC allows metabolic activity at lower RWC. Water potential of the environment and water content of the lichen are directly related (Brock,1975) but this relation varies between lichens, depending on how tightly water is bound. Saturation water content may be correlated with the matric potential of the extracellular spaces because it is the weight of water that can be held against gravity. The relation between the saturation water content and the distribution of water between the cells and the extracellular spaces is not known, but our results imply that it is the key relation.

No evidence has been found to support the hypothesis the polyol pool is present as a mechanism for stress tolerance. The evidence suggests that polyols serve to lower osmotic potential, which could determine under what conditions the lichen is active. The implication is that rather than polyol allocation lowering production, it is one of the strategies by which a lichen can increase its opportunities for production; the loss through increased leaching and resaturation respiration can presumably be balanced by the gain in carbon uptake under some environmental conditions. Therefore it cannot be assumed that lichens with higher polyols will have lower rates of production. But it is perhaps significant that the two most abundant lichens in subarctic Quebec, <u>Cladina stellaris</u> and <u>Alectoria ochroleuca</u>, have very low polyol concentrations, while the lichens with the highest polyol concentrations are more rare species found in isolated spots within the forests. Perhaps lichens which are able to minimize polyol allocation are the most successful species.

Table 1. The study species and the acronyms used in subsequent figures and tables.

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

<u>Umbilicaria</u> <u>proboscidea</u> (L.) Schrad. UMPRO

Table 2. Estimated parameters for the regressions log(resistance)=a+b(log(RWC)) and AWC=a+b(time).

Species	Evaporative resistance				Drying Index			
Acronym	a	b	r2	p	a	b	r2	p
ALOCH	-0.22	-1.60	0.82	0.0001	228	-0.73	0.48	0.0001
ALSAR	-0.99	-1.18	0.82	0.0001	228	-0.85	0.70	0.0001
BRLAN	-1.62	-0.80	0.58	0.0001	215	-1.37	0.61	0.0001
BRNIT	-1.33	-1.20	0.85	0.0001	226	-1.32	0.80	0.0001
CEDEL	-0.47	-1.50	0.80	0.0001	228	-1.03	0.61	0.0001
CEISL	-0.64	-1.25	0.81	0.0001	221	-0.80	0.47	0.0001
CENIG	-1.31	-0.98	0.71	0.0001	231	-1.21	0.76	0.0001
CENIV	-0.93	-1.17	0.92	0.0001	244	-0.77	0.49	0.0001
CLAMA	-1.35	-0.97	0.88	0.0001	231	-0.57	0.31	0.0004
CLBEL	-0.12	-1.52	0.94	0.0001	224	-0.49	0.26	0.0015
CLCOC	-0.93	-0.92	0.92	0.0001	232	-1.30	0.64	0.0001
CLGRA	-0.99	-1.12	0.86	0.0001	235	-0.53	0.29	0.0007
CLMIT	-0.74	-1.52	0.95	0.0001	237	-0.81	0.42	0.0001
CLRAN	-0.79	-1.38	0.86	0.0001	234	-0.02	0.00	0.8798
CLSTE	0.05	-1.59	0.87	0.0001	237	-0.89	0.54	0.0001
CLSUA	-0.85	-1.26	0.88	0.0001	246	-0.63	0.28	0.0008
CLSUB	-1.40	-0.97	0.93	0.0001	243	-0.80	0.41	0.0001
CLSUL	-0.35	-1.42	0.93	0.0001	231	-1.04	0.60	0.0001
CLTUR	-0.55	-1.39	0.95	0.0001	237	-0.43	0.16	0.0144
NEART	-1.31	-1.03	0.94	0.0001	237	-0.61	0.31	0.0004
PEAPT	-1.01	-0.76	0.88	0.0001	244	0.16	0.01	0.4999
PESCA	-1.35	-0.85	0.85	0.0001	233	-0.44	0.28	0.0009

STPAS	-0.58	-1.19	0.88	0.0001	240	-0.76	0.54	0.0001
UMPRO	-0.77	-1.39	0.83	0.0001	221	-1.34	0.78	0.0001

Table 3. Pearson correlation matrix for all variables. *-p=0.05, **p=.01.

	Polyols	Rain	Drying Index	Evaporative Resistance	Saturation Water Content
Rain	-0.18				
Drying Index	0.33	-0.23			
Evaporative Resistance	0.37	-0.11	0.35		
Saturation Water Content	0.59**	-0.26	-0.04	0.39	

Figure 1. A frequency histogram of the polyol concentrations of the study species.

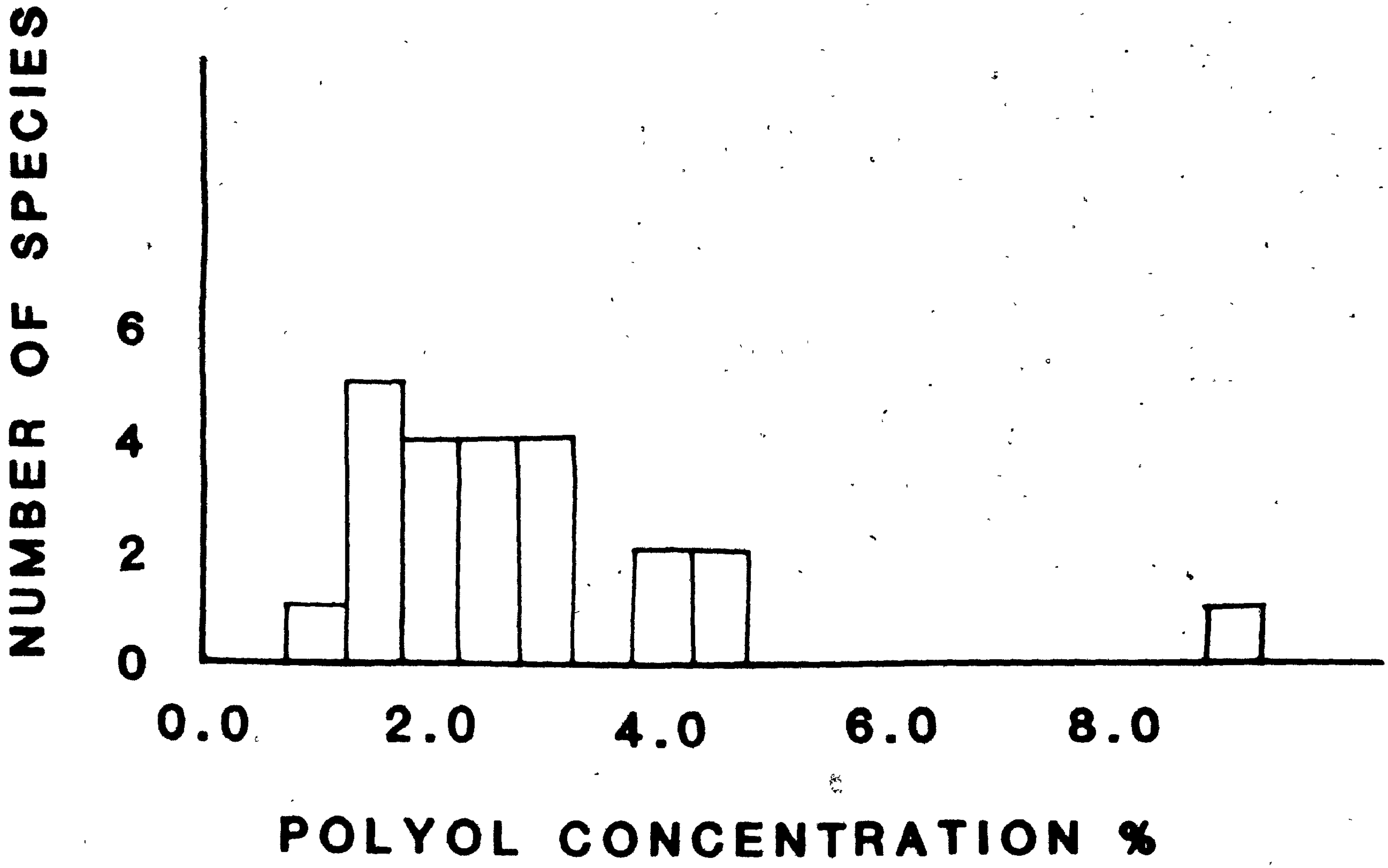

NUMBER OF SPECIES
6
4
2
0
0.0
2.0
4.0
6.0
8.0
POLYOL CONCENTRATION %

Figure 2. A frequency histogram of the logarithmic means of the rainfall of six storms for the twenty-four microsites.

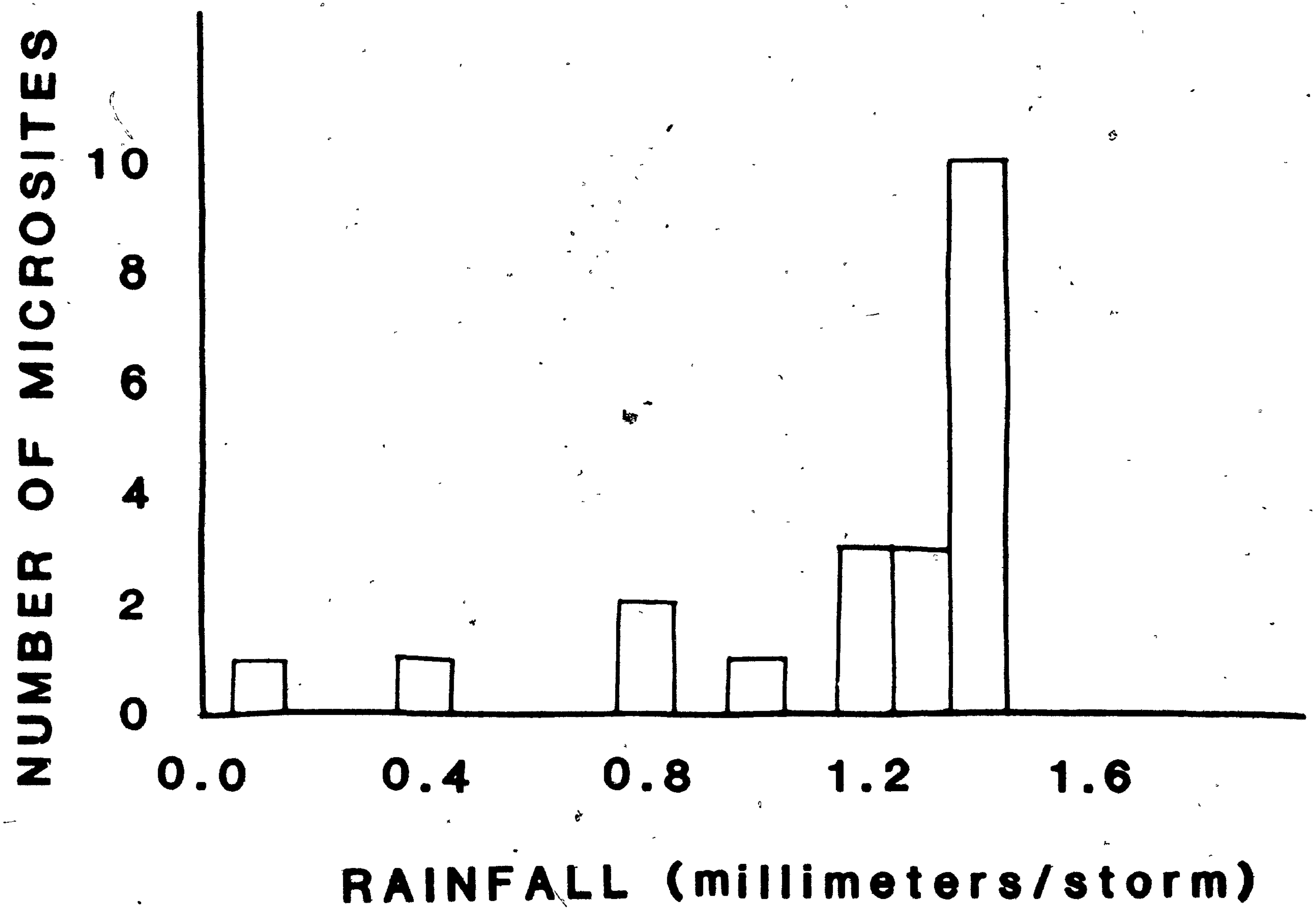
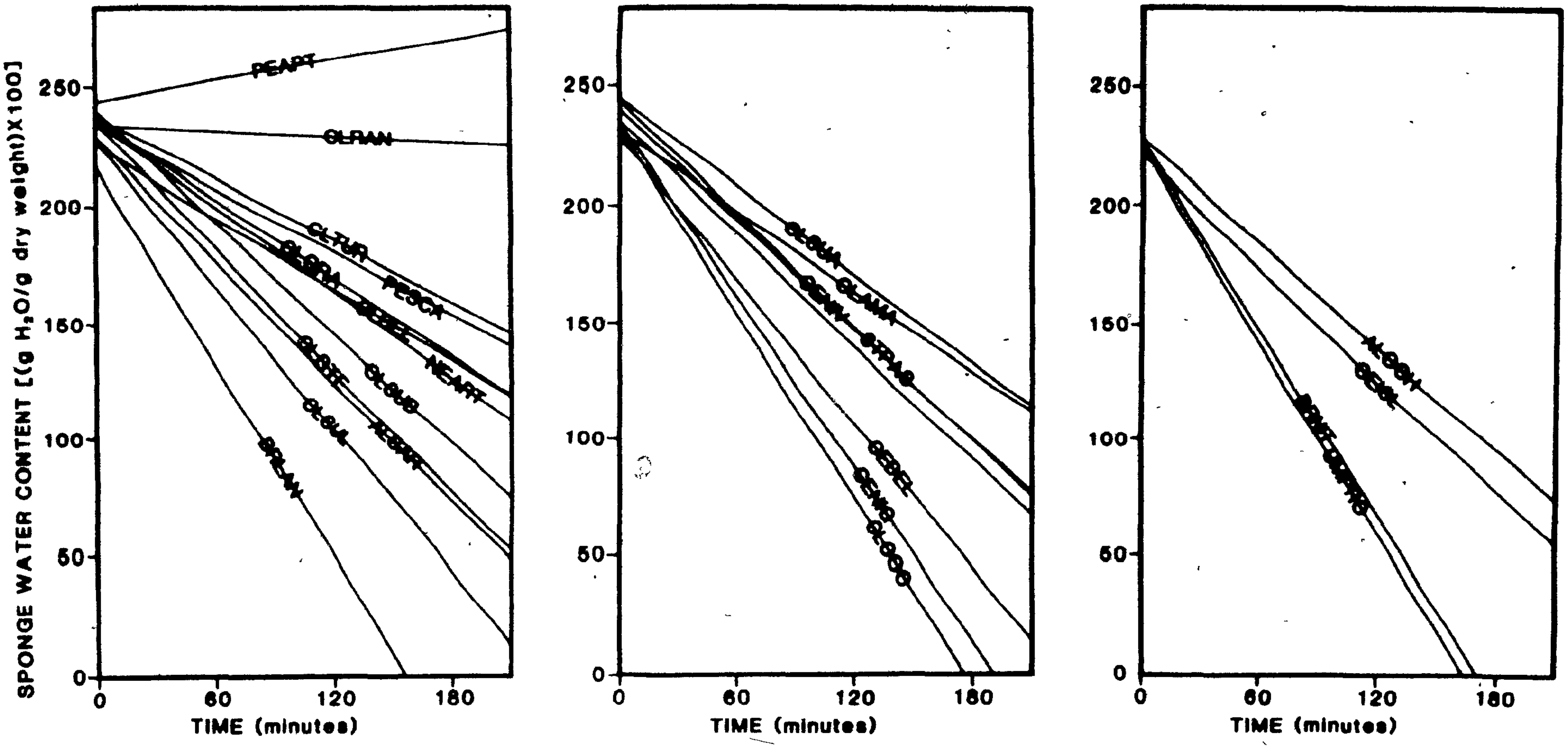

NUMBER OF MICROSITES
10
8
6
4
2
0
0.0
0.4
0.8
1.2
1.6
RAINFALL (millimeters/storm)

Figure 3. The estimated regression lines of absolute water content as a function of time for the 24 microsites.

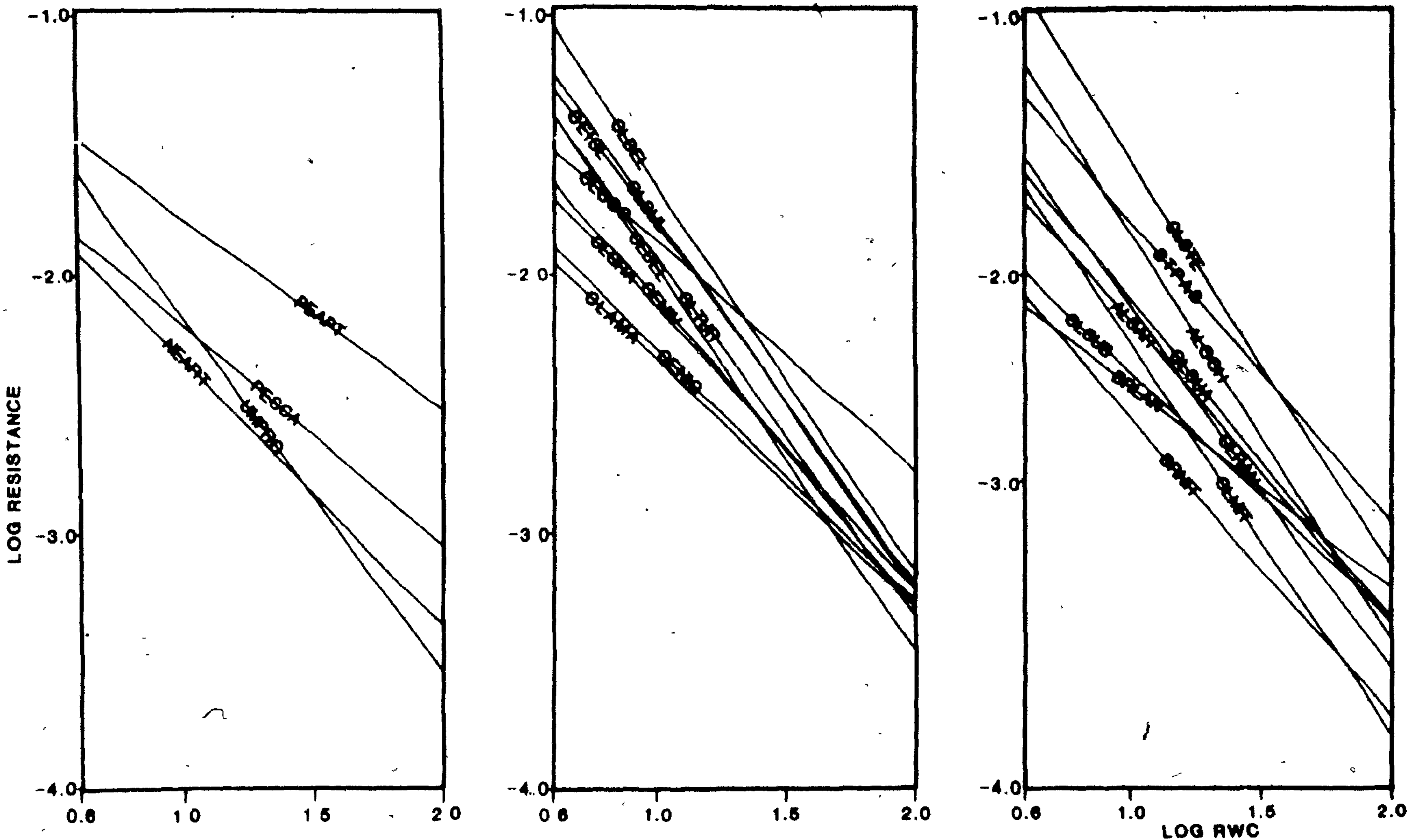


a) forest

b) heath

c) ridge

Figure 4. The estimated regression lines of resistance to water loss as a function of relative water content for the 24 lichen species.



a)foliose

b)simple fruticose

c)complex fruticose

Figure 5. Scatter plot of polyol concentration of each species versus its saturation water content.

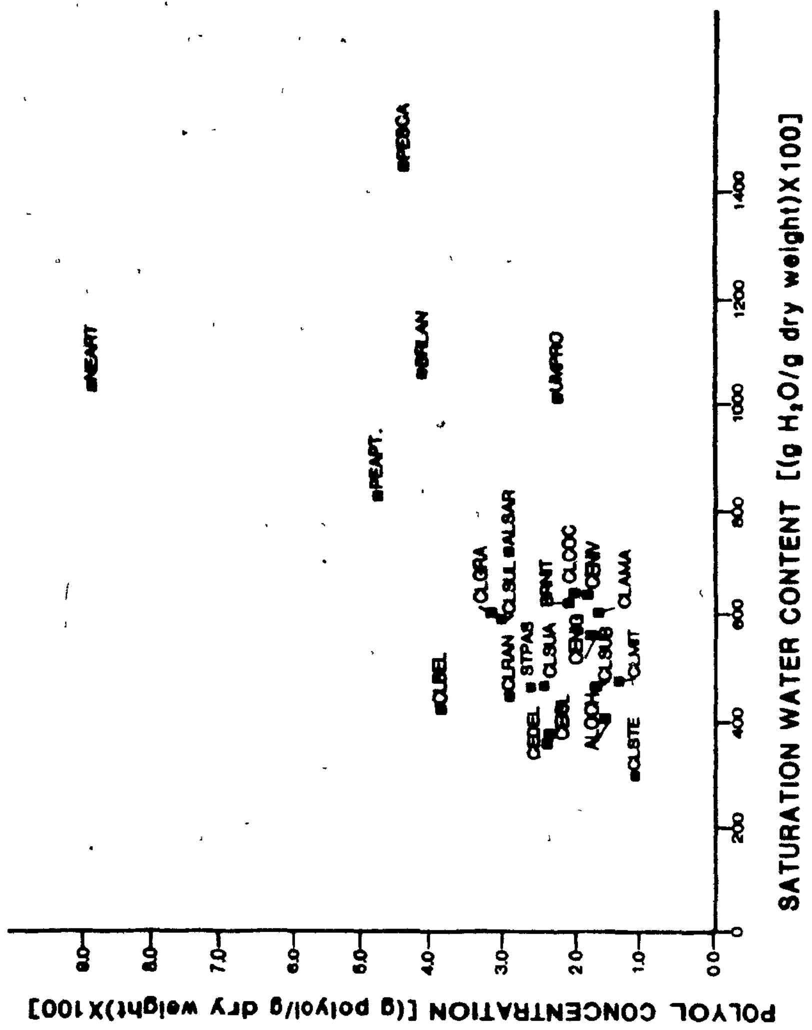

POLYOL CONCENTRATION [(g polyol/g dry weight)X100]
SATURATION WATER CONTENT [(g H₂O/g dry weight)X100]
0.0
1.0
2.0
3.0
4.0
5.0
6.0
7.0
8.0
9.0
0
200
400
600
800
1000
1200
1400
NEAPT
PESCA
BRLAN
UMPRO
PEAPT
CLBEL
CLGRA
CLSUL
ALSAR
CLRAN
STPAS
BRNIT
CLOOC
CEMIN
CLAMA
CLSUA
CEMIG
CLSUB
CLMIT
CEDEL
CEBIL
ALOCH
CLSTE

Figure 6. Scatter plot of evaporative resistance (minutes to dry from 100%RWC to 5%RWC) versus index of favorableness. Closed squares are non pure mat-forming lichens, open squares are pure mat-forming lichens.

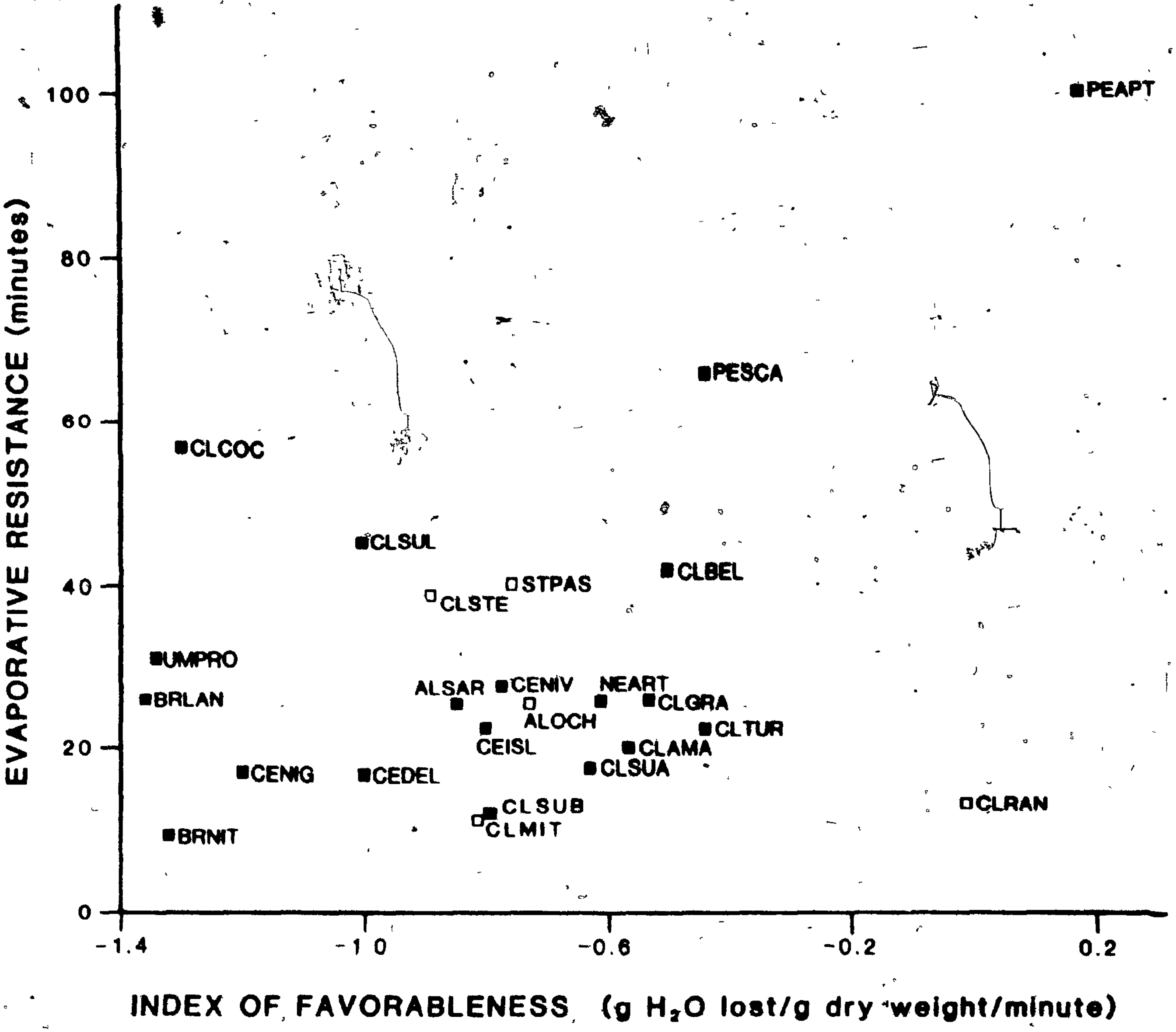
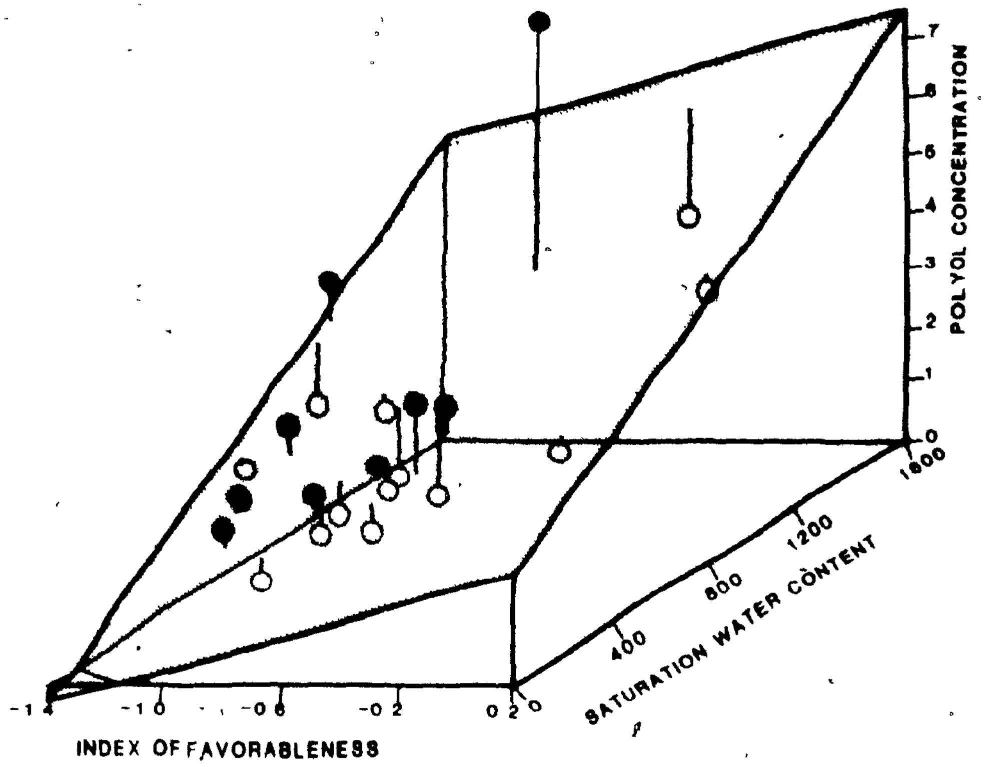

EVAPORATIVE RESISTANCE (minutes)
100
80
60
40
20
0
-1.4
-1.0
-0.6
-0.2
0.2
INDEX OF FAVORABLENESS (g H_2O lost/g dry weight/minute)
PEAPT
PESCA
CLCOC
CLSUL
CLBEL
STPAS
CLSTE
UMPRO
BRLAN
ALSAR
CENIV
NEART
CLGRA
ALOCH
CLTUR
CEISL
CLAMA
CLSUA
CENIG
CEDEL
CLSUB
CLMIT
CLRAN
BRNIT

Figure 7. Polyol concentration as function of saturation water content and index of favorableness. The shaded plane is the fitted regression plane. Closed circles are species above the regression plane, open circles are species below the regression plane, and lines represent magnitude of the residuals.


POLYOL CONCENTRATION
0
1
2
3
4
5
6
7
SATURATION WATER CONTENT
400
800
1200
1600
INDEX OF FAVORABLENESS
-1 4
-1 0
-0 6
-0 2
0 2

LITERATURE CITED

Bewley, J.D. and J.E. Krochko. 1982. Dessication tolerance. Encyclopedia of Plant Physiology 12B:325-378

Brock, T.D. 1975. The effect of water potential on photosynthesis in whole lichens and in their liberated algal components. Planta 124:13-23.

Dilks, T.J.K. and M.C.F. Procter. 1979. Photosynthesis, respiration, and water content in bryophytes. New Phytologist 82:97-114.

Farrar, J.F. 1973. Lichen physiology: progress and pitfalls. Pages 238-282 n *in* B.W. Ferry, M.S. Baddely and D.L. Hawksworth, editors. Air Pollution and Lichens. University of Toronto Press, Toronto.

------------. 1976a. Ecological physiology of the lichen *Hypogymnia physodes*. II. Effects of wetting and drying cycles and the concept of 'physiological buffering'. New Phytologist 77:105-113.

-----. 1976b. The lichen as an ecosystem: observation and experiment. Pages 385-406 *in* D.H Brown, D.L. Hawksworth and R.H. Bailey, editors. Lichenology: progress and problems. Academic Press, New York.

Harris, G.P. 1976. Water content and productivity of lichens. Pages 452-468 *in* O.L. Lange, L. Kappen and E.-D. Schulze, editors. Water and plant life: problems and modern approaches. Springer-Verlag, Berlin.

Hill, D.J. 1981. The growth of lichens with special reference to the modelling of circular thalli. Lichenologist 13:265-287.

Hoffman, G.R., and D.M. Gates. 1970. An energy budget approach to the study of water loss in cryptogams. Bulletin of the Torrey Botanical Club 107:361-366.

Kershaw, K.A. and G.F. Field. 1975. XV. The temperature and humidity profiles in a *Cladina alpestris* mat. Canadian Journal of Botany. 53:2614-2640.

Lange, O.L. and U. Mathes. 1981. Moisture dependent CO_2 exchange of lichens. Photosynthetica 15:555-574.

Larson, D.W. 1982. Environmental stress and *Umbilicaria* lichens: the effect of high temperature pretreatments. Oecologia 55: 102-107.

-------, and K.A. Kershaw. 1976. Studies on lichen-dominated ecosystems. XVII. Morphological control of evaporation in lichens. Canadian Journal of Botany 54:2061-2073.

Lawrey, J.D. 1984. Biology of lichenized fungi. Praeger Publishers, New York.

Lechowicz, M.J. 1976. Environmental response structure of *Cladonia* lichens from contrasting climates. Ph.D. Thesis, University of Wisconsin, Madison.

--------. 1983. Age dependence of photosynthesis in the caribou lichen *Cladina stellaris*. Plant Physiology 71:893-895.

Lewis, D.H. and D.C. Smith. 1967a. Sugar alcohols (polyols) in fungi and green plants. I. Distribution, physiology and metabolism. New Phytologist 66:143-184.

Lewis, D.H. and D.C. Smith. 1967b. Sugar alcohols (polyols) in fungi and green plants. II. Methods of detection and quantitative estimation in plant extracts. New Phytologist 66: 185-204

Mathes, U. and G.B. Feige. 1983. Ecophysiology of lichen symbioses. Encyclopedia of Plant Physiology 12C: 423-467.

Mooney, H.A. and N.R. Chiariello. 1984. The study of plant function: the plant as a balanced ecosystem. in R. Dirzo and J Sarukhan, eds, Perspectives on plant population ecology. Sinauer Associates, Sunderland, MA.

Reid, A. 1960. Thallusbau und Assimilationshaushalt von Laub- und Krustenflechten. Biologisches Zentralblatt 79:129-151.

SAS Institute Inc. (1982). *SAS User's Guide: Statistics, 1982 Edition*. Cary, NC: SAS Institute Inc.

Smith, D.C. and S. Molesworth. 1973. Lichen physiology. XIII. Effects of rewetting dry lichens. New Phytologist 72:525-533.

Snelgar, W.P. and T.G.A. Green. 1981. Ecologically linked variation in morphology, acetylene reduction and water relations in *Pseudocyphellaria dissimilis* New Phytologist 87:403-411.

Turk, R. 1983. Laboruntersuchungen uber den CO2-gaswechsel von flechten aus den mittleren Ostalpen.II. Die Abhangigkeit des CO2- Gaswechsels epigaischer, subalpiner Flechten und von *Pseudevernia furfuracea* vom Wassergehalt der Thalli. Phyton (Austria) 23:1-18

Waterway, M.J., Lechowicz, M.J., & Moore, T.R. (1984). Vegetation of the Schefferville region, Nouveau Quebec. *McGill Subarctic Research Papers, in press*.

CONCLUSION

This work has significantly advanced the understanding of the ecological significance of polyol concentrations in lichens. Preliminary work had suggested that polyol concentrations could be important to lichen ecology. Our results demonstrate that polyol concentrations are indeed important, but not for the reasons previously suggested.

The leaching results present proof that leaching of polyols are a major drain on the carbon balance under natural conditions, and provide a method for predicting these losses. The magnitude of polyol loss is much higher than had been previously suspected. It also provides evidence that allocation to polyols must be in proportion to the polyol present. We argue as others have in the past, that polyol concentration must be important to lichens, because the cost, in biomass, is so high.

We have provided evidence against a major theory in the lichen ecophysiological literature, the physiological buffering hypothesis. The major prediction it makes, that polyol concentration should be higher in lichens in less favorable environments, was falsified. Instead a relation between polyol concentration and saturation water content was found. This is a new and unexpected result, and suggests that saturation water content deserves more consideration as a variable in lichen production.

We suggest new theories for the function of polyol concentration, ones that make quite different predictions for the relation between polyol concentration and production. Instead of high polyol allocation lowering production, as the physiological buffering hypothesis suggested, we suggest that higher polyol concentration, by altering the rates of gas exchange and the dependency of gas exchange on environmental moisture levels, may allow higher carbon gain under some sets of environmental and morphological conditions.

Both these sets of results provide a means for unifying gas exchange studies and allocation studies: we suggest that polyol concentration affects relation between gas exchange and environment, and the leaching of polyol may well be the cost of having high carbon uptake at low water levels.