Reports from the Kevo Subarctic Research Station (Rep. Kevo Subarctic Res. Stat.)

Rep. Kevo Subarctic Res.
Stat. 7, 1—8. 1970.

Distribution of chlorophyll in the lichen Cladonia alpestris

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Received Febr. 10, Printed December 20, 1970

Abstract

KÄRENLAMPI, LAURI. (Botany Dept., Univ., Turku 2, Finland) Distribution of chlorophyll in the lichen Cladonia alpestris. REP KEVO SUBARCTIC RES STAT 7. 1—8. Illus. 1970. — The chlorophyll content and growth activity are greatest in the top parts of the thallus. A good logarithmic regression was found between the proportion of chlorophyll and the dry weight of the thallus, and a corresponding logarithmic regression was found between the rate of CO_2 exchange (per gram dry wt.) and the dry weight. Good linear regressions were found between the net photosynthesis and the dry weight, and between the net photosynthesis and the chlorophyll content. Within the size classes studied there seems to be linear regression between the rate of CO_2 exchange (per gm. dry wt.) and the proportion of chlorophyll. The ratio of chlorophyll a to chlorophyll b was found to be less than 2 in the top and more than 2 in the lower parts. There is a clearly defined active zone in the top of *Cladonia alpestris.* Therefore the suggestion is made that methods are to be developed for dividing the lichen biomass into different parts when making measurements on an areal basis. A further suggestion is that even the simple sampling procedure of taking 1 cm from the top of the thallus might give more easily comparable material e.g. for chemical analyses.

1. Introduction

Since 1968 the author has been carrying out work for the IBP at the Kevo Subarctic Research Station of Turku University. The aim has been to measure the primary productivity of the ecosystems of the area. Among the primary producers, the reindeer lichens are very abundant in the area and constitute an important part of the winter food of the domesticated reindeer. Therefore the role of the reindeer lichens, especially *Cladonia alpestris,* has been recognized as very important and has received considerable attention.

The amount of chlorophyll is thought to give a measure of the size of the photosynthetic system. What role does lichen chlorophyll play in the total photosynthetic system of the Lapponian ecosystems? How does the amount of chlorophyll correlate with the productivity? These questions supplied the starting-points of this study. But no final answers can be given in this paper. It rather gives some advice on how to sample reindeer lichens when measuring biomass and chlorophyll on an areal basis. On the other hand, the comparisons between chlorophyll content and activity may help to further the understanding of the relationship between chlorophyll and productivity.

In lichen biomass studies it is always difficult to distinguish between the active and inactive parts of the thallus. It is known how the apices branch and the upper internodes increase in length, while the lower internodes slowly become inactive and decay. It can also be assumed that the chlorophyll content is correlated with growth activity. One of the aims of this study is to investigate this relation.

If the active thallus top is more or less clearly differentiated, we should try to develop some method for dividing the biomas into different parts. This might give more valid and easily comparable results when measuring the biomass. Thus, in addition to the »total lichen biomass» figure, some »upper biomass» could be measured by taking a portion of known length from the top of the thallus. It should be possible to use the internode numbers in dividing the thallus biomass.

The phycobiont of *Cladonia alpestris* is a green alga of the genus *Trebouxia.* It is a close relative of the genus *Chlorella,* which has frequently been used as material in chlorophyll studies. In this study the chlorophyll content is not determined on the basis of the algal weight or volume, but on the basis of the total lichen weight.

2. Material

The lichen specimens were collected at the end of August 1969. They were growing in dry pine forest in the vicinity of the Kevo Station. The specimens were dried in the laboratory for two days. The temperature and moisture of the laboratory air were regulated. When the lichens had reached equilibrium with the air moisture, each specimen was weighed separately and subsamples were taken to be oven-dried (80°C). The specimens were divided into weight classes according to the estimated oven-dry weight; the following classes being used in the chlorophyll determinations: 0—10, 10—20, 20—30, 30—40, 50—70 and 100—130 mg. The smallest specimens represent 6 to 10 mm long, young, regenerating individuals, and the tallest ones about 30—34 mm long individuals. The smallest ones are probably in the period of generation and the tallest ones in an early part of the period of renovation (periods according to Andreev 1954, abstracted by Ahti 1959 and 1961).

In collecting the specimens, an attempt was made to cut them all in the same way at the point where they emerged from the dense layer of litter and moss. Since the plants always extended below this point, part of the individual remained uncollected, this part being largest in the case of the older lichens. However, this sampling method was found satisfying.

The heavy grazing of the area made it difficult to find suitable material, since the growth of the plants was often interrupted and there were few tall individuals. The taller plants were often found in shaded localities (e.g. under trees or between shrubs), where their development probably differs from that of the specimens in the open lichen patches. Of course it would have been possible to include specimens from the boulder fields, where very old undisturbed individuals are found, but the comparability of such material is dubious.

3. Methods

The specimens were transported to Turku, where the analyses were performed in the Botany Department. The specimens analysed last were kept in air-dry condition for about three weeks (water about 8—10 per cent dry weight). The delay was not observed to occasion any significant decrease in absorption.

The lichens were pressed like herbarium specimens, and divided into parts, being cut through horizontally 2, 4, 7, 12, 17, 22 millimetres from the top of the thallus. The method of division is shown in Fig 1. From each weight class 7—24 specimens were taken for division and chlorophyll determinations. The corresponding parts were put together, weighed and homogenized, and the chlorophyll content of two to four subsamples was measured. The figures were averaged and the value per mm thallus length was calculated. For the extraction of chlorophyll the method of Hill and Woolhouse (1966: 211) was used. It comprises crushing in 80 % pyridine, stirring after adding methanol and centrifuging after adding 80 % acetone.

The distribution of biomass in the thallus was used as an indication of growth activity. The more or less conical top of *Cladonia alpestris* increases in length and thickness while the biomass of the more basal parts remains constant or even decreases. In the distribution curve (Fig. 2 A), the biomass increases sharply from the top to the mode, and this part of the curve (plus about 2 mm downwards) was taken as roughly representing the actively growing zone. The morphological development of the thallus will be discussed in more detail in a later study.

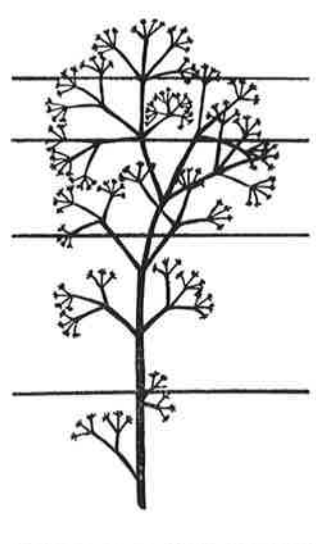

Fig. 1. Scheme of the thallus of *Cladonia alpestris* according to des Abbayes (1939). The lines superimposed on the figure show the parts into which the thalli were divided in this study.

The difference in activity between small and taller specimens was measured with CO_2 experiments. The URAS apparatus at Kevo was used. Measurements were made of the rate of photosynthesis at 10°C, a light intensity of 10 000 lux and about optimum moisture (water 150 per cent dry weight), and of respiration in the dark at the same temperature and moisture. The experimental conditions were not kept exactly as mentioned, but the maximum differences from the values given above were 1°C and 1000 lux. The CO_2 exchange under the above conditions was then predicted using a multiple regression model. Three to five experiments were made to obtain one figure. Only the linear form of the independent variables was used, because the predicted value was so close to those of the observations. This procedure was thought to give more precise results, than experiments in which it was attempted to maintain the given conditions exactly. In computing the data the electronic desk-top computer *Programma 101* was used.

4. Results

4.1. Distribution of chlorophyll (a + b) in the thallus

Fig. 2 shows the results of the chlorophyll determinations and weighings of the parts of the thallus. The curves are slightly smoothed. Smoothing was accomplished using the logarithmic regression model ln y = A + B ln x. The independent variable (x) was the average weight of the individual and the dependent variable (y) the weight of the given part or its chlorophyll content. All the regression curves have rising logarithmic form. In most cases the correlation coefficient was more than 0.95 and only twice did it lie between 0.85—0.90. Using these regression models the dry weight and the chlorophyll content of each part was predicted for the following values of x : 10, 30, 50, 75 and 100 mg. These predicted values were used in Fig. 2 (A: weight, B: amonut of chlorophyll). The chlorophyll percentage values given in Fig. 2 C are computed from the values in A and B of the same diagram.

The curves of Fig. 2 B show the distribution of chlorophyll by distance from the top of the thallus, the content being calculated as mg per mm. The mode does not change with the weight of the specimens.

In Fig. 2 C the chlorophyll content is calculated as mg per gm dry weight. The modes of the distribution curves tend to move towards the thallus top. The distribution of the chlorophyll remains approximately the same for specimens of different weight. The decrease in the thallus top values of the heavier specimens shown by this material, was not thought to be significant. The curves of Fig. 2 A show the biomass distri-

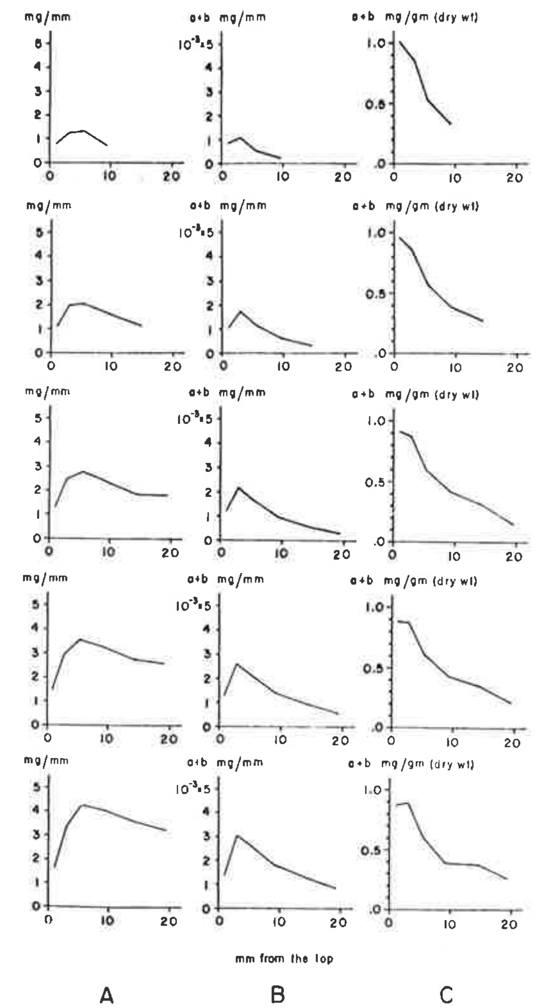

Fig. 2. Distribution of biomass (A), chlorophyll as mg/mm (B) and chlorophyll as mg/gm (C) in *Cladonia alpestris.* The uppermost diagrams refer to thalli weighing 10 mg, the others to specimens weighing 30, 50, 75 and 100 mg respectively.

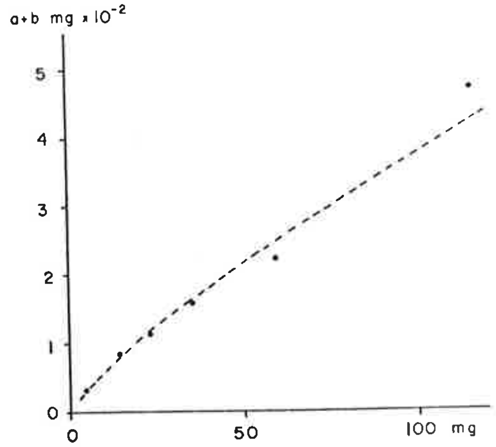

Fig. 3. The chlorophyll content plotted against the dry weight of the individual thallus, and the logarithmic regression curve.

bution and here, the position of the mode remains constant, though it tends to occur more to the right (nearer the thallus base) than in the chlorophyll distribution curves. Reference to Fig. 1 and 2 shows that the results of the chlorophyll determinations and weighings can be summarized as follows: the amount of chlorophyll per gm dry weight is highest in the two top parts of the thallus; the biomass per mm thallus length is highest in the third part, and the amount of chlorophyll per mm thallus length is highest in the second.

4.2. Chlorophyll in relation to the growth activity of the thallus

The steepness of the rise of the biomass curve from the thallus top to the mode is used as a measure of growth activity in this study. It is seen that the strongest growth is concentrated in the zone of the two uppermost parts and that the chlorophyll content is also highest in the these two parts. Thus, in spite of the limitations of this paper regarding information on the growth rates, there seems to be sufficient evidence to justify the conclusion that the zone of the highest chlorophyll content per gm dry weight coincides with the zone of the greatest growth activity of the thallus, and that they are clearly differentiated from the remainder of the thallus.

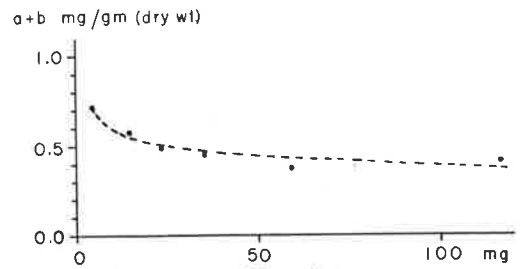

Fig. 4. The average chlorophyll content per gm dry weight plotted against the dry weight of the thallus, and the logarithmic regression.

4.3. Chlorophyll content in relation to the weight of the individual thallus

Fig. 3 shows chlorophyll content plotted against the weight of the individual. The relationship is appoximated by the logarithmic regression (broken line in the figure), ln y = —0.0545 + 0.798 ln x, r = 0.996, (x = dry weight in mg, y = chlorophyll content in 10^{-6} gm). The values of the heaviest specimens deviate most from expectation. This is thought to be the effect of the slightly differing environment of the taller individuals, because they could not be found in open lichen patches.

Fig. 4 shows the relationship between the average chlorophyll content per mg dry weight and the dry weight of the individual. The relationship is approximated by the logarithmic regression (broken line in the figure), ln y = 2.246 — 0.200 ln x, r = —0.947, (x = dry weight in mg, y = chlorophyll content in 10^{-4} gm/gm dry weight). The proportion of chlorophyll is smaller in the heavier individuals because they have a proportionally larger basal part, which has a low chlorophyll content (see Fig. 2).

4.4. The relation of chlorophyll to activity in individuals of different weight

Fig. 5 shows the net photosynthesis (10°C, 10 000 lux) of individual thalli plotted against their dry weights. The relationship is very clearly linear: regression y = —0.000253 + 0.000676 x, r = 0.998 (x = dry weight in mg, y = uptake of CO_2 in mg/h).

Fig. 6 shows the net photosynthesis (10°C, 10 000 lux) of individual thalli plotted against their chlorophyll contents. The rela-

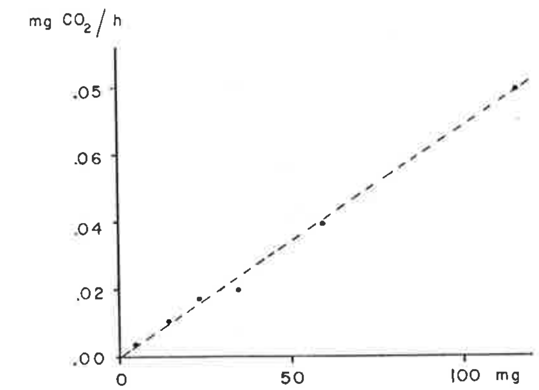

Fig. 5. The net photosynthesis at 10°C and 10,000 lux plotted against the dry weight of the individual thallus, and the linear regression.

tionship is approximated by the linear regression y = —0.00327 + 1.748 x, r = 0.995 (x = chlorophyll content of individual thallus in mg, y = uptake of CO_2 in mg/h).

So it seems to be possible to estimate the size of the lichen photosynthetic system, or photosynthetic capacity, indirectly from the weight alone. But it is dubious, whether this relationship obtains in older, very heavy individuals. Another complication is that variation may be caused by differences in the sites. On the whole, the size of the photosynthetic system can probably be more pre-

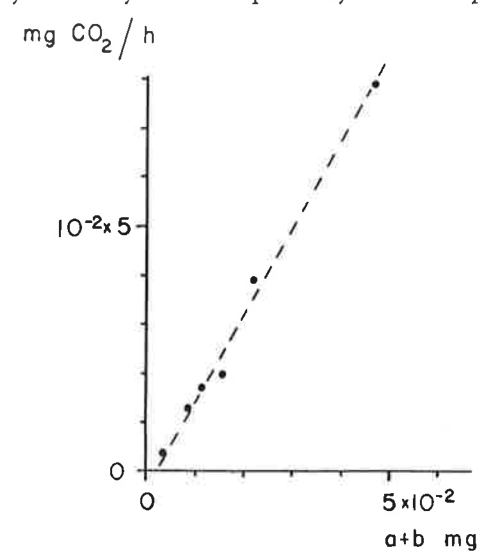

Fig. 6. The net photosynthesis at 10°C and 10,000 lux plotted against the chlorophyll content of the individual thallus, and the linear regression.

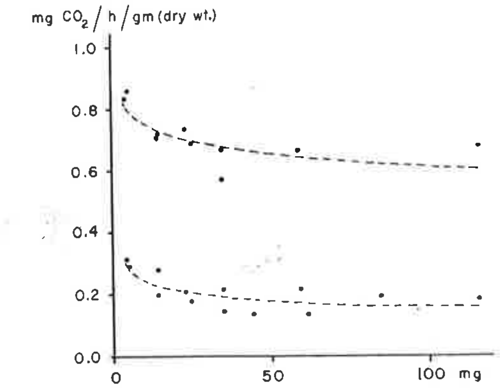

Fig. 7. The rate of net photosynthesis (dots, 10°C, 10,000 lux) and the rate of respiration in the dark (circles, 10°C), in mg CO_2/h/gm dry weight, plotted against the dry weight of the thallus. The broken lines show the logarithmic regressions.

cisely estimated from measurements of the amount of chlorophyll.

Fig. 7 (dots) shows the rate of net photosynthesis, in mg/h/gm dry weight, plotted against the dry weight of individual. The relationship is approximated by the logarithmic regression model ln y = —0.0713 — 0.0911 ln x, r = —0.736, (x = dry weight in mg, y = photosynthesis in mg CO_2/h/gm dry weight). The curve corresponds very well to the curve in Fig. 4 representing the relationship between the amount of chlorophyll and the weight of the individual. This suggests that the rate of photosynthesis and the clorophyll content per unit weight may be in linear correlation within the measured range. This is shown in Fig. 8, regression y = 0.436 + 0.520 x, r = 0.739, p = 5—10 %, (x = chlorophyll content in mg per gm, y = mg CO_2/h/ gm dry weight).

Figs. 7 and 8 also show respiration in the dark at 10°C (small circles in figures). The curve in Fig. 7 is the logarithmic approximation ln y = —0.961—0.194 ln x, r = —0.700, (x = dry weight in mg, y = respiration in dark in mg CO_2/h/gm dry weight). In small individuals the respiration in the dark is about 0.3 whereas in heavier ones it is about 0.2. The high value of the smallest individuals creates a slight positive correlation between the CO_2 released in the dark and the average chlorophyll content per mg. This is shown in Fig. 8, y = 0.080 + 0.285 x, r = 0.778, p = 5—10 per cent, (x = chlorophyll

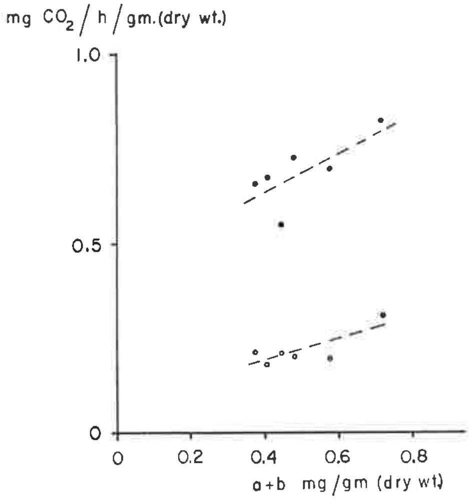

Fig. 8. The rate of net photosynthesis (dots, 10°C, 10,000 lux) and the rate of respiration in the dark (circles, 10°C), in mg CO_2/h/gm dry weight, plotted against the average chlorophyll content per gm dry weight. The broken lines give the linear regressions.

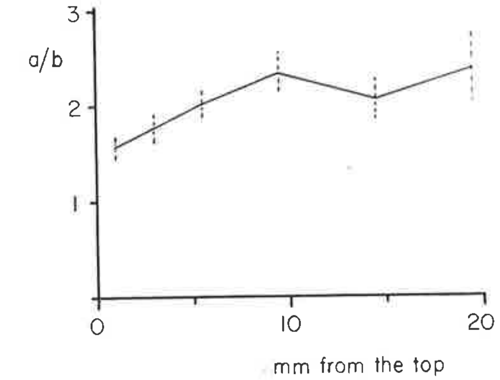

Fig. 9. The ratio of chlorophyll a to chlorophyll b in the top and middle parts of the thallus of *Cladonia alpestris*. The broken lines show the mean error of the mean.

content in mg per gm dry weight, y = respiration in dark in mg CO_2/h/gm dry weight). The reason for the rate of higher respiration in younger individuals cannot be sought here. It may be attributable to a larger proportion af algae, or to the active growth of the fungus or to both.

It will be interesting to see what new light is thrown on the results of this section when the productivity and growth rates of small and taller thalli are investigated, and compared with their chlorophyll contents and CO_2 exchange capacities.

4.5. *Ratio of chlorophyll a to chlorophyll b*

Fig. 9 shows the a/b ratio in the different parts of the thallus. The ratio is less than 2 in the top and rises above 2 in the middle and lower parts. The mean error of the mean is high and becomes higher in the basal parts.

Fig. 10 shows the relationship between the amounts of chlorophylls a and b, and the parabolic approximations. It may also be seen that the proportion of b increases when the total chlorophyll content increases, as happens in the top of the thallus. The coefficients of the parabolic regressions are as follows: A:$y = -0.2807 + 0.4617\,x + 0.2835\,x^2$, B: $y = 0.9819 - 0.1514\,x + 0.1226\,x^2$, and C: $y = 0.06145 + 0.3230\,x + 0.03801\,x^2$.

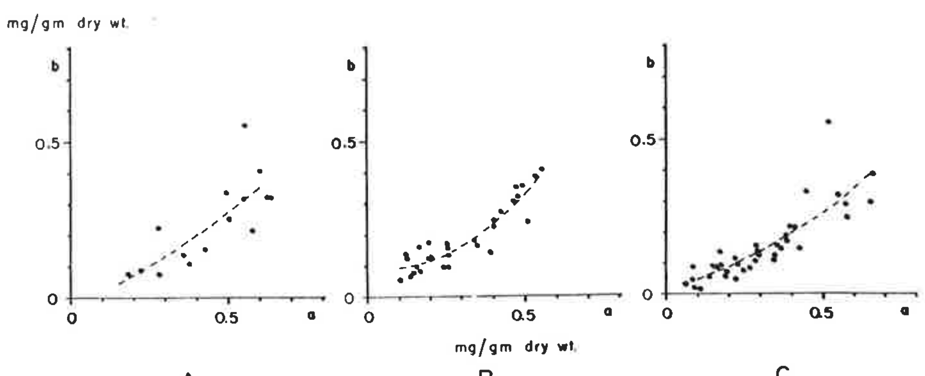

Fig. 10. Relationship between chlorophyll a and chlorophyll b. A: Thalli weighing 0—20 mg, B: thalli weighing 20—40 mg and C: thalli weighing 50—70 and 100—130 mg. The broken lines show the parabolic regressions.

5. Discussion

HILL & WOOLHOUSE (1966) presented following results concerning the chlorophyll content of *Xanthoria parietina:* specimens on trees 4.1 mg per gm dry weight, on roofs 3.5 mg and on rocks by the sea 1.7 mg. In this study the highest values in the top of young thalli were about 1 mg per gm dry weight. The difference might be expected because the thallus of *Xanthoria* is thicker having a well-developed algal layer, whereas the algal layer is sparse and scattered in *Cladonia*. The results of WILHELMSEN (1959) show that the chlorophyll content varies significantly from season to season, and it is higher in winter. Therefore the comparisons are, to some extent, dubious.

The good correlation between chlorophyll content and growth seems to suggest that no significant translocations of energy need be assumed. The assimilates are produced mainly where the growth takes place. This makes productivity studies easier to perform.

It must be remembered that this study has at least two limitations. Firstly, its material does not include very old thalli, and, secondly, no investigations were made of the extent to which the chlorophyll content and the ratio of chlorophyll a to chlorophyll b vary from season to season.

As regards the regression approximations of the results, it must be emphasized that they are only to be used within the range of the present observations. Outside they would give invalid results.

Some of the results, e.g. the type of correlation between the rate of CO_2 exchange and the chlorophyll content (presented in Fig. 8), may raise questions of a physiological nature. But it must be noted that the goals and methods of this study are mainly ecological in character, and do not allow physiological considerations. All the results are intended to further the assessment of the biomass and productivity of lichens and of their role in the Lappish ecosystems. Some features of the CO_2 exchange experiments prevent an exact physiological interpretation of their results. E.g. in the photosynthesis experiments some parts of the thallus are shaded by others, and these conditions change with the weight of the studied individuals. It should also be remembered in connection with Fig. 8, that the chlorophyll values are the averages of thallus parts that occur in different proportions and whose chlorophyll content varies. Nevertheless, it is hoped that the results may prove significant and useful from the ecological standpoint.

The clear delimitation and the coincidence of the zones of maximum chlorophyll content and growth activity in the *Cladonia alpestris* thallus allow the following suggestions. In measurements of the lichen biomass carried out on an areal basis the biomass of *Cladonia alpestris,* and probably of other lichens, too, should be divided into parts. It would then be much easier to compare the figures of different areas and sites. And the biomass figures would reveal the amount of the productive upper biomass, or the amount of reindeer forage, and the age of the lichen stand, amongst other important data. How this dividing should be done is not easy to decide. The points at which the divisions should be made can probably be determined by reference to the internode numbers and lengths. It would also be desirable to ascertain, whether the same methods can be applied to other reindeer pasture lichens, too.

To start with, a simple method might be to cut off the upper 1 cm of the lichen thalli (tried by ANDREEV 1954). Of course, 1 cm pieces taken from the tops of lichens of different regions (e.g. continental contra maritime) and different ages would not be exactly similar, but the degree of similarity might be sufficient for some purposes. This might give better estimates than other sampling methods of the amount and character of the reindeer forage, when material is collected for chemical analyses, e.g. investigations of radioactive fallout.

Acknowledgements My sincerest thanks are due to Prof. PAAVO KALLIO, the Head of the Kevo Subarctic Research Station and the leader of the Kevo IBP/PT project, for allowing me the use of all the facilities of the station. Financial support received from the Finnish State Council for Natural Sciences is gratefully acknowledged. I am very indebted to Mrs. ANNA A. DAMSTRÖM, M. A., for correcting the English language.

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Rep. Kevo Subarctic Res. Stat. 7, 9—15. 1970.

Morphological analysis of the growth and productivity of the lichen Cladonia alpestris

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Received April 14, 1970 Printed December 20, 1970

Abstract

KÄRENLAMPI, LAURI. (Botany Dept., Univ., Turku 2, Finland) Morphological analysis of the growth and productivity of the lichen Cladonia alpestris. REP KEVO SUBARCTIC RES STAT 7. 9—15. Illus 1970. — Measurements were made of the lengths and diameters of *Cladonia alpestris* thalli and the lengths of their internodes. The weight distribution in the thallus was studied by dividing thalli and weighing the different parts. The relationships between the observed values were studied and conclusions were drawn concerning growth and productivity. There are definite relationships between the length, diameter and weight of the thallus. In the Kevo area the internode seems to continue growing up to the age of eight years. The relative length growth rate of the internode is highest when it is young. A diagram was prepared which illustrates both the weight distribution and the internode lengths. This is thought to show the pattern of growth. There is a definite growth zone at the top of the older thalli. The relative length, diameter and weight growth rates were examined by means of growth analysis. The RGR values of the young individuals were found to be much higher than those of the older ones.

1. Introduction

The productivity of lichens is one of the main interests of the Kevo IBP group. Several methods for estimating their productivity are to be developed and tried. One possibility is to base productivity calculations on the age of the thallus. The age of reindeer lichens can be estimated by counting the number of nodes (see e.g. ANDREEV 1954).

This paper gives the results of a morphological analysis of the *Cladonia alpestris* thallus. The observations include measurements of the lengths and diameters of the thalli, measurements of the internode lengths and data on the pattern of weight (biomass) distribution. This paper is one of a series of publications on the productivity of reindeer lichens. The synthesis, which will describe possible methods for making practical productivity measurements, will be presented later. Here only the actual analytic results are given.

2. Material

The lichen specimens were collected at the beginning of September in 1969. They were growing in dry pine forest in the vicinity of the Kevo Station. The material does not include any very old individuals, because the area is heavily grazed by reindeer. The lichens are mostly in the period of generation and some in the early part of the period of renovation (periods according to ANDREEV 1954).

According to the dry weight, the material was divided into classes, the range of each class being 10 mg between 0 and 100 mg and 25 mg between 100 and 175 mg. The measurements of the tallest individuals are not based on strictly comparable material because the taller specimens were often found in differing microhabitats (outside the heavily grazed lichen patches). The same difficulty was experienced in an earlier study (KÄRENLAMPI 1970).

The specimens were collected by cutting the taller ones at the point where they emerged from the dense layer of litter and moss, and the smaller ones at the point where they had started their growth.