

Lobe Growth in Lichen Thalli

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Abstract

Using pairs of photographs taken one month to 2 years apart, measurements were made of thallus growth, lobe width and distance between lobe divisions. Thallus growth was directly correlated with lobe width in thalli of *Parmelia saxatilis*. Lobe growth was related to lobe width in *Xanthoria parietina* and *Diploicia canescens*. In *P. saxatilis*, lobe width increased with thallus size. Distance between lobe divisions was independent of growth rate. These rates are discussed in terms of developmental and growth processes involving both symbionts in lichen thalli.

Keywords: growth, lichen, symbiosis, *Parmelia saxatilis*

1. Introduction

Most measurements of growth rate of flat lichen thalli have been of the increase in linear size of the whole thallus and have been found to be affected by environmental factors (Armstrong, 1988; Hale, 1974; Hill, 1991; Lawrey and Hale, 1977), thallus size (Hill, 1981) and related to thallus morphology (Rogers, 1990). In lobed thalli although the growth rate of individual lobes has been measured (Fisher and Proctor, 1978; Hale, 1970; Lawrey and Hale, 1977), it has not been directly related to other measurable lobe features or the growth of the thallus as a whole. Hooker (1980) observed competition between lobes in the placodioid lichen *Xanthoria elegans* (Link) Th.Fr. and found that, as

the lobes could not overlap, some lobes became "engulfed" when neighbouring lobes divided. The frequency of "engulfed" lobes at the thallus perimeter varied with thallus diameter. Hill (1984) observed that lobes in *Diploicia canescens* (Dickson) Massal. thalli were similar but the angle of divergence when lobes divided differed in this species, that lobe division appeared to maintain circularity and that the distance between previous successive lobe divisions was constant with thallus diameter.

Growth analysis of flat circular thalli, based on the physiological functions of photosynthesis, respiration, transport and growth in area and weight, has been used to investigate the relationship between growth rate and thallus size (Aplin and Hill, 1979; Childress and Keller, 1980; Keller and Childress, 1981; Hill, 1981). The formulation of an equation to describe the relationship involved the use of five coefficients which were amalgamated into two. One included net photosynthetic production (a with dimensions time^{-1}) and the other the lateral transport of photosynthate (s with dimension distance). In practice, the predicted relationship between growth rate and thallus size matched observed growth as closely as, or more closely than, models published previously or since, none of which have been based on physiological function (Innes, 1985). In the revised form (Hill, 1981) this relationship is given by:

$$dr/dt = asr/(r + 2s) \quad (1)$$

where r is radius and t time. Using measurements of growth of a number of thalli of different sizes in different lichens in the field, the values for the two constants were found to be $a = 0.3-2y^{-1}$ and $s = 0.1-0.05$ cm depending on the species. From the small value for s , significant amounts of photosynthetic products would be expected to move no further than within a lobe. This indicates, as has been observed by Gilbert (1971) and Armstrong (1979), that growth rate was unaffected by removal of the central portion of the thallus. As a corollary of this, lobes grow as independent physiological units as predicted in theory (Aplin and Hill, 1979; Hill, 1984) and found experimentally by Armstrong (1984) despite the fact that they appear to function together in forming circular thalli. In this paper the relationships between the lobe growth, lobe width and lobe division are investigated in the context of the growth, physiology and development of the thallus. The overall aim of this research is to provide more information on the pattern of growth of lichen thalli and how this may relate to the co-development of the two symbionts. The term growth in a lobe is used to refer to increase in its length.

2. Materials and Methods

This investigation is based on photographs of thalli taken one or two years apart at the following sites:

- (a) *Parmelia saxatilis* (L.) Ach. on gravestones, Killin Churchyard, Perthshire, Scotland August to August the next year.
- (b) *Xanthoria parietina* (L.) Th.Fr. on walls, Vatches Farm, Barton, Cambridgeshire, England April to April two years later.
- (c) *Diploicia canescens* on walls, Prince of Wales Road, Exeter, England, December to January one month later (taken by Dr. M.C.F. Proctor).

Photographic methods

Accurate and standardised duplicate photographs without parallax errors were taken of *P. saxatilis* using a camera (Exacta Varex IIa with Zeiss Tessar 50 mm f2.8 lens) supported in a metal frame, surrounding the camera body and fixed at the tripod screw socket and the two strap eyelets. To this frame were bolted four legs of adjustable length at the distal ends of which was bolted another larger flat rectangular frame whose size was just smaller than the field of view of the camera. The legs were adjusted to bring the larger frame exactly into the focal plane of the lens at its closest focal setting. When the larger frame was placed against the substratum (gravestone) around the thalli to be photographed, the camera was held still in an exactly re-locateable position with preset focus. The frame was re-located in the field by reference to a print of the earlier photograph. Life size prints were made from negatives (Ilford FP3 film) without intervening enlarger adjustment which could have affected magnification between successive prints.

Measurement of thalli

Measurements to an accuracy of 0.01 cm were made directly from the prints using vernier scale calipers and a hand lens. Means of the same two diameters (largest and smallest) of thalli were taken before and after the growth period; this ensured that imperfections in circularity did not bias the data. The widths of lobes which were selected randomly (but excluding any that appeared atypical e.g. those damaged) were measured by positioning the calipers at right angles to the mid-line of the lobe immediately after the previous lobe division. Some judgement was required in a few cases but the possible errors were small compared with the range in width measurement. A maximum of

6 lobes were measured in 85% of thalli (but fewer in the other 15% which had fewer than 6 lobes) before and after the growth period. Mean lobe width and after the growth period was not statistically significant ("t" test, $p \geq 0.05$). Therefore overall mean including before and after measurements were used. In lobe branching studies, lobe growth and width were measured to the nearest 0.5 mm and distance between lobe branches to 0.1 mm.

Measurements of *X. parietina* thalli were made from photographs taken using the tripod described by Hill (unpublished). For *D. canescens*, measurements were made from photographs taken without a special camera tripod. For both these species, the growth of individual lobes was measured rather than the whole thallus.

Handling of data

Some of the investigations required calculations of the ratio of thallus growth rate to lobe width in *P. saxatilis* which incidentally caused increased variation in the data. To overcome this difficulty, the 74 sets of measurements were reduced to 15 by block means as follows. Data for mean thallus diameter (geometric mean before and after growth period), growth rate and lobe width, were ranked according to mean thallus diameter and then grouped into blocks (the sequence of the size of the blocks being 1.5, 1.5, 1.5, 1.5, 3, 3, 3, 5, 5, 5, 10, 20 mm of mean diameter). The number of individual thalli in each block ranged from 3 to 10 (median 6). The mean was then calculated for each of the three parameters within each block. As there were few data for thalli above 60 mm diameter, these thalli were treated as individuals.

3. Results

Correlation of growth rate with lobe width

Growth rate ranged from close to zero to 0.5 cm per year in *P. saxatilis* (Fig. 1). Since the thallus diameters, and not individual lobes, were measured, some lobes may have grown faster than this maximum. The growth rate (increase in diameter) in *P. saxatilis* thalli was in direct linear proportion to the mean lobe width of each thallus.

Using both faster and slower growing thalli of *X. parietina* over a two-year period, it was found that lobe growth rate was closely related to lobe width (Fig. 2), although the relationship is apparently not linear.

In *D. canescens* in which narrow and wide lobes are found in the same thallus, the relationship between lobe width and monthly increase in lobe

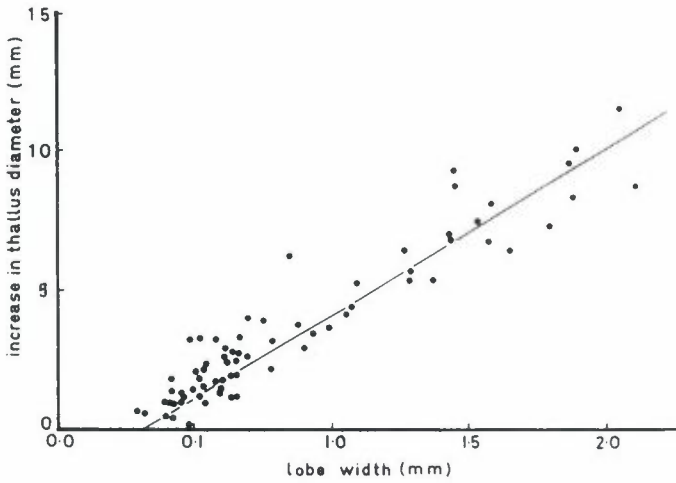


Figure 1. Relationship between growth rate and lobe width in *Parmelia saxatilis*. Rate of growth measured as increase in thallus diameter during 1 year.

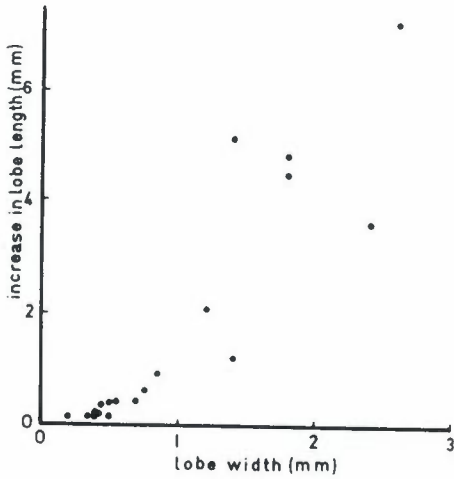


Figure 2. Relationship between growth rate and lobe width in *Xanthoria parietina*. Rate of growth measured as increase in lobe length during 2 years.

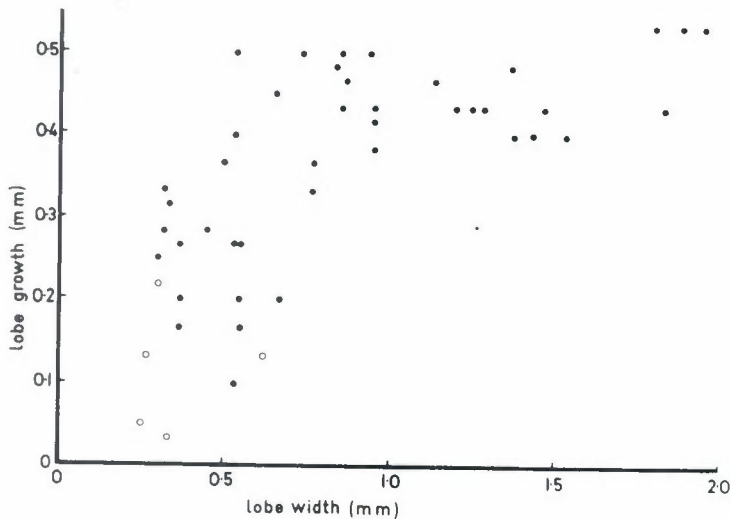


Figure 3. Relationship between growth rate and lobe width in *Diploicia canescens*. Rate of growth measured as increase in lobe length during one month (31 days December to January).

length was asymptotic (Fig. 3). In lobes less than 1 mm wide, growth was apparently linearly related to width although some of the smaller lobes were being "engulfed". In those wider than 1 mm, growth was independent of width. This, unlike the other two foliose species, has a placodioid thallus construction with a relatively uniform mean lobe width in different thalli.

Although all three species show positive correlation between lobe width and growth rate, there appeared to be differences in the type of correlation. A further feature in common was that it appeared that lobes of width less than approximately 0.3 (0.2–0.4) mm width would not be capable of growth.

Other aspects of lobe width in P. saxatilis

Using block mean data, it was found that both lobe width and thallus growth rate were positively correlated with thallus diameter (Fig. 4). The relationships were apparently not linear but were similar. Since growth rate and lobe width were linearly related, change in lobe width with thallus diameter could account for the widely reported association between growth rate and thallus size (first reported by Armstrong (1973) and reviewed by Hill (1981)). If this were correct, there would be no correlation between the ratio of growth rate to lobe width and thallus diameter. This is tested in Fig. 5 which shows that in very small thalli growth rate was slower for other reasons.

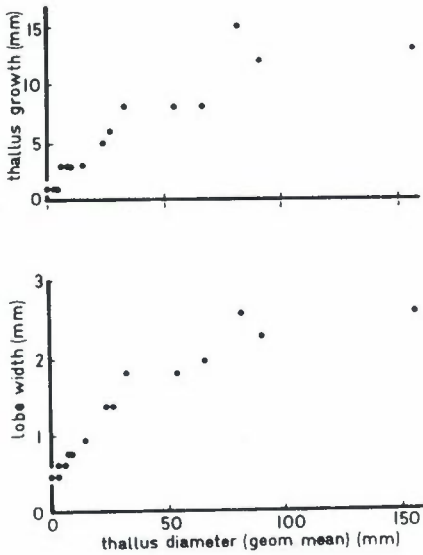


Figure 4. Thallus growth rate in *Parmelia saxatilis* and lobe width plotted as a function of thallus size. Block mean data and thallus size geometric mean of diameter before and after growth interval (1 year).

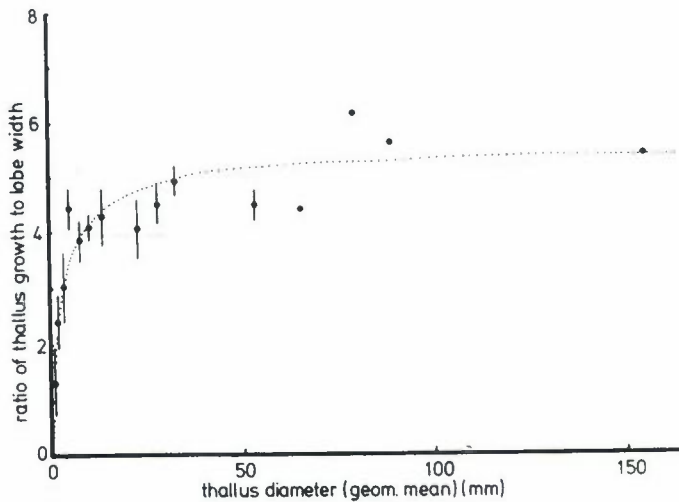


Figure 5. Thallus growth in *Parmelia saxatilis* as ratio to lobe width (x-axis) plotted as function of thallus size (y-axis) (see Fig. 4). Vertical lines indicate calculated standard deviations. The dotted line is $dy/dx = 5.5x/(x+0.396)$ ($a = 2.81y^{-1}$, $s = 0.198$ cm).

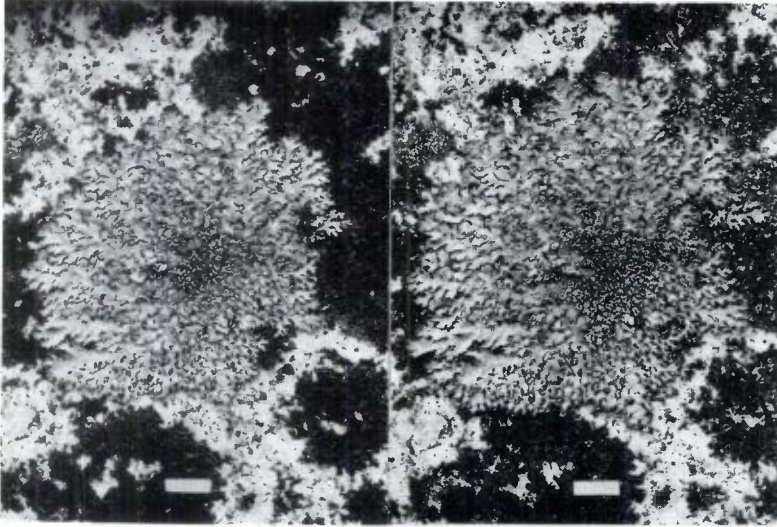


Plate 1. Thallus of *Parmelia saxatilis* photographed a year apart showing slower growing narrow lobes and faster growing wide lobes in the same thallus. Scale is 1 cm.

While most thalli had either predominantly narrower or wider lobes, a few of the larger thalli were seen to be composed of sectors of each (Plate 1). On the assumption that such thalli represent single individuals or ramets, questionable despite the fact that this species almost exclusively reproduces vegetatively, they would indicate that lobe width is phenotypically rather than genotypically determined.

Linear regression analysis of the correlation between distance between divisions and lobe width (Fig. 6) indicated that the gradient was highly significant (from zero, $p \leq 0.001$) but the intercept was not significant from zero. This indicates that distance between lobe divisions is related to lobe size rather than growth rate. On the other hand the number of divisions during the year was not significantly related to the amount of growth (Fig. 7) but that the intercept, using a linear regression, was highly significant from zero ($p \leq 0.001$).

Some unrestricted lobes in very small thalli growth much more slowly than others. Previously (Hill, 1984) it was shown that in very small thalli, lobe division was not sufficient to provide a contiguous series of lobes found the perimeter. In larger thalli lobe division gave rise to too many lobes and as growth

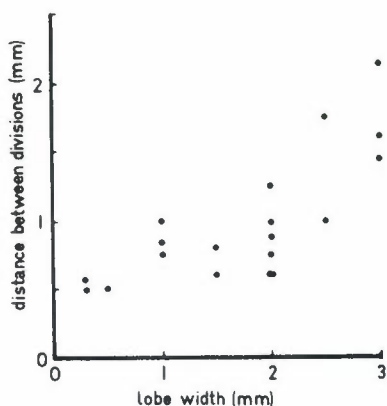


Figure 6. Relationship between distance between lobe divisions and lobe width in *Parmelia saxatilis*.

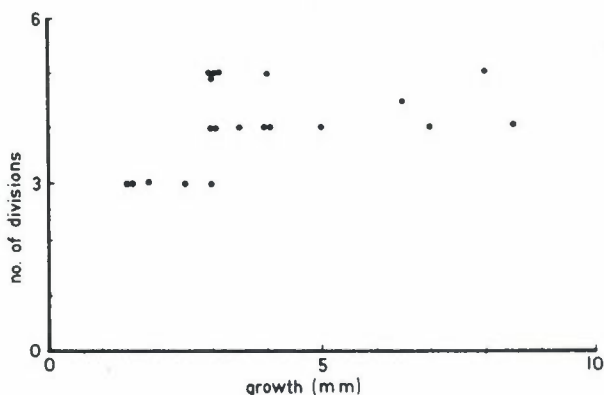


Figure 7. Number of lobe divisions occurring during growth over 1 year in *Parmelia saxatilis*.

proceeded some lobes became stunted or engulfed or overlapped. Figure 8 shows silhouettes of small thalli made from photographs taken one year apart and it can be seen that in each, a number of unrestricted lobes grew either much slower than others or apparently grow not at all. This is also apparent in photographs in Lawrey and Hale's (1977) paper as is the polar i.e. directional growth in very young thalli.

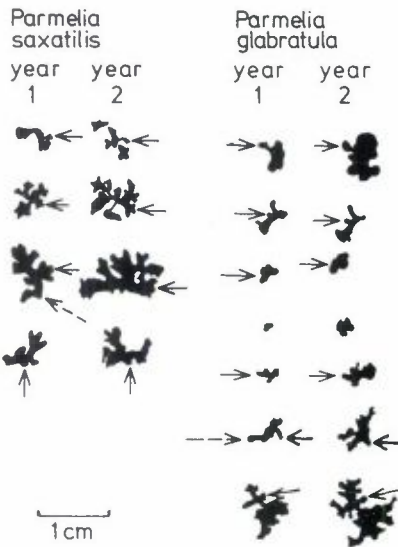


Figure 8. Silhouettes of actively growing young thalli of *Parmelia saxatilis*. Arrows point to lobes that have grown little or not at all. Scale is 1 cm. Note some lobes (dashed arrow) present in the first year but missing in the second.

4. Discussion

The relationship between lobe width and growth rate may be universal and such a relationship is apparent between species, for example, in Rogers (1990) in his triangulation of "morphology index" and "relative growth rate". It is, however, necessary to consider how this relationship comes to be.

Considering growth in a lobe as independent of the rest of the thallus, Hill (1981), Proctor (1978) and Armstrong (1987) have assumed that growth depends directly on the photosynthetic production of the photobiont. However it is impossible to link photopynthesis and growth unless it is known if lateral transport of photosynthetic products occurs in the lobe. Transport is extremely difficult to investigate experimentally within a lobe owing to the small distances involved and the poikilohydric nature of the symbionts causing leakiness (Farrar and Smith, 1976) and consequently has not been attempted. Interestingly, Armstrong (1987) has obtained some indirect evidence based on the effect of removal of central thallus of non-lobe forming *Rhizocarpon geographicum* (L.) D.C. thalli on carbohydrate content of the areolae and hypothallus. Only removal to 1–2 mm of the perimeter reduced growth rate of the marginal hypothallus.

Thus it might be supposed that the wider the lobe the greater the photosynthetic area and hence the faster the growth rate. However, it can be shown

that even if the rate of increase in lobe length is directly dependent on the distance through which transport occurs, lobe growth rate would be independent of lobe width. Since the rate of growth of the lobe (length) is not independent of lobe width (Fig. 1), this supposition appears to be inappropriate and an alternative explanation should be sought. In the following discussion we do not assume that growth rate determines lobe width or lobe width determines growth rate but the two appear to be correlated.

Lobe growth appears to be initiated at the apex but extends some way back from the apex (Fisher and Proctor, 1978; Hale, 1970; Hill, 1981). On a microscopic level, analysis of the way in which fungal hyphae push the photobiont cells apart after division (Honegger, 1987) indicates how the plate of photobiont cells, which is of uniform thickness in thallus lobes (Hill, 1985), expands. Transfer of products from the photobiont cell to the contacting hypha followed by growth of that hypha without any substantial transport could largely account for the observed growth pattern.

In addition, some other process must also occur to cause directional growth and the cessation of growth in older regions. Hill (1989) proposed that in younger regions where photobiont cells followed cyclical cell division (phase 1 cells) were under different regulation than in the more mature i.e. older regions where cells no longer divided but continued to grow in size. These older (phase 2) cells may not easily re-enter the cell division cycle. This would impose directionality on growth and could account for polarity in very young thalli.

The relationship between growth rate, and thallus size, with the effect of lobe width removed (Fig. 5) indicates that photosynthate transport may apply to a small element of growth and could well determine this directionality. It was found that, by using residuals of regressions of logarithmic plots (using the integrated form of equation (1) (Aplin and Hill, 1979), the ratio of growth to lobe width, rather than direct measurement of growth rate, conformed closest to the analysis of growth in the introduction. This model can be used to find the extent of an assumed transport of photosynthetic products and the distance coefficient s would then be about 1 mm.

Lobes can become wider as they grow and when of sufficient width divide to form two new lobes. The ratio of width to length (i.e. distance between divisions) is variable between species (Hill, 1984). Indeed since lobe length appears to be relatively constant, this with angle of divergence of lobe divisions, may be a useful taxonomic character. Some lobes were also observed to become narrower as they grow in length. The limit to this can be seen in Figs. 1-3 in which lobe growth ceases at a lobe width of about 0.3 mm. The significance

of the dimension of this is not clear at present but may be a general feature of the structure, function and development of lichen thalli.

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