Symbiosis, 22 (1997) 293–302
Balaban, Philadelphia/Rehovot

Factors Associated with Degeneration of the Thallus Centre in Foliose Lichens

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Received January 30, 1996; Accepted November 11, 1996

Abstract

Degeneration of the older parts of foliose lichen thalli often lead to the formation of a space or 'window' in the centre of the colonies. The percentage of thalli of different size which exhibited 'windows' was studied in twenty saxicolous lichen populations in south Gwynedd, Wales. The proportion of thalli with 'windows' increased with thallus size. The size class at which 50% and 100% of thalli exhibited 'windows' varied between populations. Differences between populations were not correlated with distance from the sea, aspect, slope or porosity of the substrate or the total number of lichen species present. However, a higher percentage of smaller thalli had 'windows' on rock surfaces with a greater lichen cover. There were no significant differences in the levels of Ca, Mg, Cu or Zn in large (>4 cm) and small (<2 cm) *Parmelia conspersa* (Ehrh. ex Ach.)Ach. thalli or in the centres and marginal lobes of these thalli. The concentration of ribitol, arabitol and mannitol was significantly reduced in the centre of large thalli compared with the margin of large thalli and the centre of small thalli. However, carbohydrate levels were similar in the centre of large thalli and the margin of small thalli. The data suggest that loss of the thallus centre is a degenerative process related to thallus size. In the field, the formation of 'windows' may be related to the intensity of competition on a substrate. Central degeneration was not associated with a deficiency or an accumulation of Ca, Mg, Cu and Zn in the thallus centre. However, degeneration may be associated with a reduction in carbohydrates in the centre compared with the marginal lobes.

Keywords: Lichen, thallus degeneration, 'windows', inorganic ions, carbohydrate

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0334-5114/97/$05.50

1. Introduction

The older parts of foliose lichen thalli often degenerate leading to the formation of a space or 'window' in the centre (Lawrey, 1984).

Larger thalli appear to especially vulnerable suggesting that 'window' formation is an age-related degenerative process. However, little is known about the frequency of 'window' formation in different lichen species in the field or whether this process is influenced by environmental factors. Hence, the first objective was to test the following hypotheses: 1) that the formation of 'windows' was related to thallus size and 2) that 'window' formation was related to the physical and/or competitive environment of the substrate.

In addition, the factors which are associated with central degeneration have not been established. First, photosynthesis may be reduced in older tissue (Nash et al., 1980, Lechowicz, 1983). This could lead to a deficiency in the soluble carbohydrate pool in the centre since it is unlikely that carbohydrates are transported from margin to centre (Armstrong, 1979, 1991). Second, degeneration could be related to a deficiency or accumulation of inorganic ions in the centre compared with the margin. Calcium is required in relatively large amounts by calcicoles to maintain the integrity of cell membranes (Bates, 1982). A reduction in the level of calcium in the centre of larger thalli could result in the leaching of ions (Fletcher, 1976) and fragmentation. By contrast, lichens accumulate metal ions relative to the substratum (Brown and Slingsby, 1972; Puckett et al., 1973; Nieboer et al., 1972; Nash, 1989). Although lichens can concentrate metal ions without damage (Nash, 1989), excessive accumulation in the centre of older thalli could be associated with degeneration. Hence, the second objective was to test the hypothesis that degeneration was associated with a reduction in carbohydrates or changes in inorganic ions in the centre of larger thalli.

2. Materials and Methods

Site

The study was carried out at a site of Ordovician slate in south Gwynedd, Wales (Nat. Grid Ref. SN 6196) described previously (Armstrong, 1974).

Frequency of thalli with 'windows'

Twenty foliose lichen populations located on separate rock surfaces were studied: 1) 10 populations of *Parmelia glabratula* ssp. *fuliginosa* (Fr. ex Duby) Laund., 2) 5 populations of *Parmelia saxatilis* (L.)Ach., 3) 3 populations of *Physcia orbicularis* (Neck.) Poetsch and 4) 2 populations of *Parmelia conspersa* (Ehrh. ex

Ach.) Ach. For each population, the greatest diameter of all thalli was measured in 25, 10 × 10 cm quadrats located at random. Whether a thallus had an entire centre or possessed 'windows', was recorded. Several aspects of the rock surface environment were measured using previously described methods (Armstrong, 1974): 1) distance of the rock surface from the sea, 2) aspect, 3) slope, 4) rock porosity, 5) the number of lichen species present and 6) the degree of lichen cover of the substrate (Armstrong, 1981). For each population, the relationship between percent thalli with 'windows' and mid-point of thallus size class was fitted by a first, second or third order polynomial (Statcalc software, MacMillan Education Ltd.). A higher order function was accepted when a significant reduction in the sums of squares was achieved relative to a linear fit (Snedecor and Cochran, 1980). The size class at which 50% and 100% of thalli within each population had 'windows' was then determined from the fitted curve. Third, whether the size classes at which 50% and 100% of thalli had 'windows' varied between species was tested using a 1-way analysis of variance (ANOVA) (Statview II software, Abacus Concepts). Since no significant differences were found, all populations were pooled to test the correlation (Pearson's 'r') between size class at which 50% and 100% of thalli had 'windows' and the environmental factors. Since aspect is a circular variable, it was divided into three categories for analysis, viz., 90–179°, 180–269° and 270–360° and differences between these three groups tested by a 1-way ANOVA (Statview II).

Analysis of inorganic ions

On 24, Sep 1994, four replicate samples of the marginal lobes and centres of large (>4 cm diameter) and small (<2 cm diameter) *P. conspersa* thalli were collected from a large population on a south facing rock surface. All small thalli sampled had entire centres. However, the majority of thalli >4 cm in diameter had 'windows', so large thalli were selected with the smallest 'windows'. Each replicate sample comprised marginal lobes or portions of thallus centres from 5 thalli. Thalli were removed carefully from the rock surface with a scalpel and adhering rock, soil and vegetation removed. Unwashed thalli were air-dried on paper towels and ground with a pestle and mortar. Subsamples of 0.5 gm were then digested with a mixture of concentrated nitric and perchloric acids (Iskander and Syers, 1972). Total ion levels (extracellular plus intracellular) were measured by atomic absorption spectroscopy (Pye Unicam SP90A) (Armstrong 1990). CsCl and $LaCl_3$ were added to samples and standards to suppress interference effects. Data analysis was carried out using a three-factor (split-split plot) ANOVA (SuperAnova software, Abacus concepts), thallus size as the major factor and thallus location (margin and centre) and ion levels as the subplot factors.

Analysis of carbohydrates

On 24, Sep 1994, four replicate samples of the marginal lobes and centres of large and small *P. conspersa* thalli were collected from a large population on a south facing rock surface as described above (Analysis 1). A second set of samples (Analysis 2) was collected from a separate south facing site on 10, Sep 1995. Samples were stored in 80% ethanol in a refrigerator for analysis of carbohydrates. Carbohydrates were analysed within a week of collection by gas chromatography using the methods described previously (Armstrong and Smith, 1987). Essentially, carbohydrates were extracted from each sample by refluxing in 80% (v/v) ethanol. Subsequently, extracts were silylated and then characterised by capillary gas chromatography. The levels of ribitol, arabitol and mannitol, the major carbohydrates present in *P. conspersa* (Armstrong and Smith, 1994), were determined by reference to known carbohydrate standards added at the initial extraction stage. This was done to compensate for any losses of carbohydrate resulting from extraction or incomplete derivatisation during chromatography. Levels of carbohydrates were expressed as mg g biomass^{-1}. The data for each carbohydrate were analysed separately by a two-factor (split-plot) ANOVA (SuperAnova), thallus size as the major factor and thallus location (margin and centre) as the subplot factor.

3. Results

The size frequency distribution of entire thalli and thalli with 'windows' in a single population (PG1) is shown in Fig. 1. The proportion of thalli with 'windows' increased with thallus size, all thalli in the larger size classes showing some evidence of degeneration. The relationship between percent thalli with 'windows' and mid-point of size class was fitted by a third-order (cubic) polynomial ($r=0.99$, $P<0.001$). All populations studied showed essentially the same pattern.

A summary of the data from the 20 populations is shown in Table 1. The data suggest: 1) the size class at which 50% and 100% of thalli had 'windows' varied between populations but not between species, 2) these variations were not significantly correlated with distance from the sea, aspect, slope, porosity or the number of associated species and 3) the size class at which 50% of thalli had 'windows' was negatively correlated with the degree of lichen cover of the rock surface ($r=-0.61$, $P<0.01$), i.e., a higher percentage of smaller thalli had 'windows' on rock surfaces with a greater lichen cover.

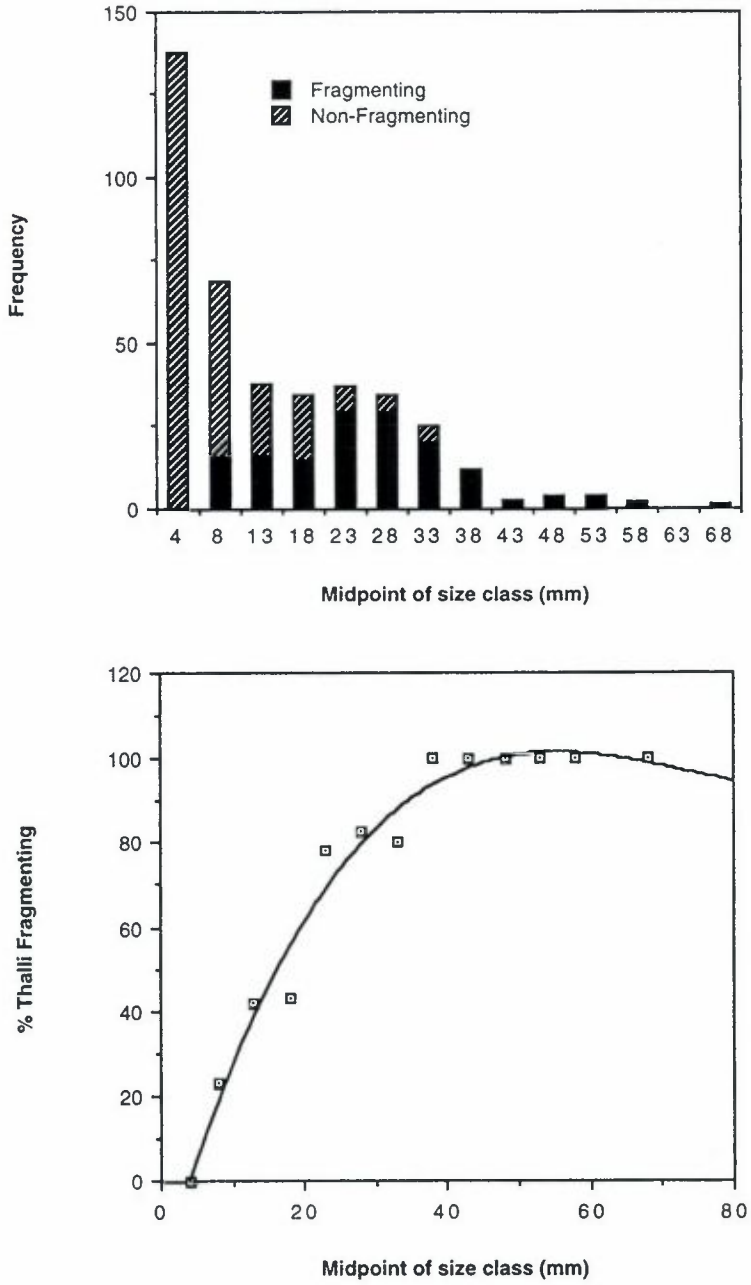

Figure 1. Size class frequency distribution of a typical population of *Parmelia glabratula* ssp. *fuliginosa* showing A) the frequency of entire thalli and thalli with 'windows' in each size class and B) the percentage thalli with 'windows' (Y) in each size class (X) (Polynomial fit of Y to the mid-point of the size class: $Y=-19.68 + 5.54X - 0.081X^2 + 3.71X^3$; $r=0.99$, $P<0.001$).

Table 1. Thallus degeneration (the mid-point of the size class in which 100% and 50% of thalli had 'windows') in relation to the environment of the rock surface (Dist = Distance from the sea, Sp = Species) in twenty populations (P) of foliose saxicolous lichens (PG = *Parmelia glabratula* ssp. *fuliginosa*, PC = *Parmelia conspersa*, PO = *Physcia orbicularis*, PS = *Parmelia saxatilis*).

'Windows'			Rock surface environment					
P	100%	50%	Dist (m)	Aspect (°)	Slope (°)	Rock porosity	No. of Sp.	Lichen cover (%)
PG1	37.5	17.5	400	180	63	1	12	38
PG2	37.5	17.5	600	320	90	3	23	76
PG3	43.5	23.5	400	320	25	1	13	36
PG4	27.5	13.5	600	130	53	1	16	84
PG5	27.5	7.5	800	320	50	2	13	86
PG6	43.5	17.5	400	270	80	2	14	60
PG7	43.5	23.5	800	320	50	2	14	58
PG8	43.5	17.5	1400	130	90	1	11	93
PG9	27.5	13.5	1400	130	90	1	12	70
PG10	7.5	13.5	600	130	52	4	12	79
PC1	33.5	13.5	600	130	64	4	14	72
PC2	33.5	23.5	400	130	74	1	12	38
PO1	27.5	17.5	600	130	64	4	14	72
PO2	27.5	13.5	600	130	53	1	12	84
PO3	43.5	37.5	400	130	74	1	12	38
PS1	22.5	13.5	600	320	90	3	24	76
PS2	43.5	17.5	800	320	50	2	15	81
PS3	33.5	17.5	800	320	60	4	11	81
PS4	33.5	17.5	1400	130	90	1	12	70
PS5	37.5	22.5	1000	320	90	5	12	92

Significant correlation: Size class at which 50% of thalli had 'windows' vs lichen cover ($r=-0.61$, $P<0.01$). ANOVA for comparisons between aspect groups: 100% 'windows' $F=0.96$ ($P>0.05$), 50% 'windows' $F=0.99$ ($P>0.05$).

The concentrations of inorganic ions in marginal lobes and thallus centres of large and small thalli of *P. conspersa* is shown in Table 2. Magnesium was the most abundant ion followed by zinc, calcium and copper. None of the main effects or interactions were significant suggesting that levels of individual ions were similar in large and small thalli and in the centre and marginal lobes.

Table 2. Levels of Ca, Mg, Cu and Zn (μm g dry weight^{-1}) in the thallus centres and margins of large (>4 cm) and small (<2 cm) thalli of the lichen *Parmelia conspersa*.

Cation	Thallus size and location			
	Small, edge	Small, centre	Large, edge	Large, centre
Calcium	80	90	80	110
Magnesium	1210	1560	1520	1460
Copper	60	70	60	70
Zinc	240	260	250	260

Analysis of variance (Three-factor, split-split plot): Thallus diameter $F=1.59$ ($P>0.05$), Thallus location $F=3.11$ ($P>0.05$), Thallus diameter x Location $F=4.05$ ($P>0.05$), Ions $F=10.07$ ($P<0.001$), Thallus diameter x Ions $F=1.52$ ($P>0.05$), Thallus location x Ions $F=2.57$ ($P>0.05$).

The concentration of ribitol, arabitol and mannitol in the marginal lobes and centres of large and small thalli of *P. conspersa* is shown in Fig. 2. The proportions of the three carbohydrates vary in the two analyses, the proportion of arabitol to mannitol being greater in analysis 1. However, differences between centres and margins within large and small thalli were similar in both analyses. The data suggest a reduction in the level of carbohydrates in the centre of large thalli compared with the margin of large thalli and the centre of small thalli. In addition, levels in the centre of large thalli were similar to levels in the margin of small thalli.

4. Discussion

The proportion of thalli with 'windows' increased with thallus size consistent with an age-related degenerative process. There was no evidence for significant variation between species but individual populations differed considerably in the size classes at which 50% and 100% of thalli possessed 'windows'. These variations were not correlated with distance from the sea, aspect, slope, or porosity of the substrate. However, the size class at which 50% of thalli had 'windows' was negatively correlated with the degree of lichen cover. This suggests that the intensity of competition could be a factor associated with degeneration (Armstrong, 1982).

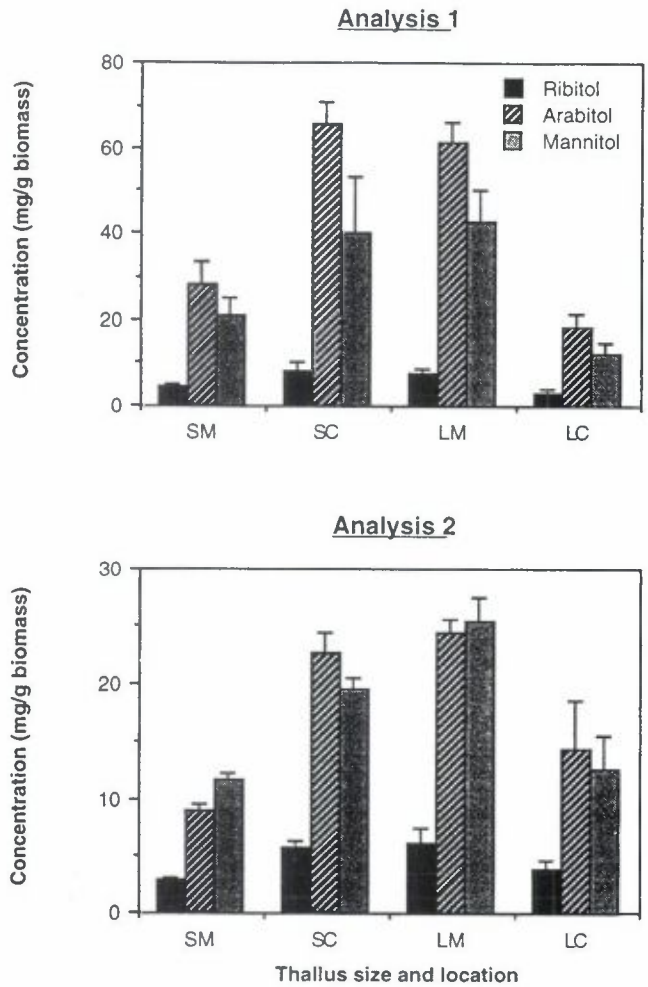

Figure 2. The concentration (mg g biomass^{-1}) of ribitol, arabitol and mannitol in the marginal lobes (M) and centres (C) of large (L) and small (S) thalli of the lichen *Parmelia conspersa*. Least significant differences (LSD) A) for comparing thallus regions within a thallus size; B) for comparing thallus sizes within a thallus region: Analysis 1, Ribitol A=4.8, B=5.1; Arabitol A=36.5, B=40.2; Mannitol A=24.3, B=25.1. Analysis 2, Ribitol A=1.8, B=1.2; Arabitol A=7.6, B=6.5, Mannitol Ribitol A=5.8, B=6.7.

The levels of Mg and Cu were higher and Ca lower than expected while the levels of Zn were within published values (Nieboer et al., 1978). The level of Ca in the substrate was low relative to the level of Mg (Armstrong, 1990) and the

level of Mg was also high in rock surface runoff at the site (Armstrong, 1997) which could explain these differences. There was no evidence that degeneration of the centre was associated with either a reduction of calcium/magnesium or an accumulation of copper/zinc in the centres of larger thalli.

Levels of carbohydrates were reduced in the centre of large thalli compared with the margin of large thalli and the centre of small thalli. Decreased photosynthesis has been observed in the older parts of fruticose thalli (Nash et al., 1980; Lechowicz, 1983) which could reflect a reduction in chlorophyll concentration (Karenlampi, 1970) as a result of the senescence of algal cells (Greenhalgh and Anglesea, 1979). A reduction in the soluble carbohydrate pool in the centre could deplete the levels of arabitol and mannitol required for tissue maintenance under stress (Farrar, 1973; 1976) and lead to degeneration. However, levels of carbohydrate in the centre of large thalli were similar to levels at the margin of small thalli, a region not associated with significant degeneration. This could reflect lower densities of algal cells or transport from edge to centre in small thalli. There could be two explanations for this result. First, higher levels of arabitol and mannitol may be required for maintenance of older central tissue compared with younger marginal tissue. Second, a reduction in carbohydrate in the centre of large thalli is an effect rather than a cause of the degenerative process in the centre. More detailed studies of carbohydrate metabolism and algal cell densities from margin to centre in large and small thalli may help to elucidate the factors involved in the formation of 'windows'.

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