Effects of Thallus Size on Morphology and Physiology of Foliose Lichens: New Findings with a New Approach

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Abstract

We present a new approach to the study of the functional implications of thallus size and intrathalline variability in foliose lichens that combines quantification of structural features by means of stereology coupled with a range of physiological techniques (gas exchange, chlorophyll fluorescence and carbon isotope discrimination). The percentage of the thallus of *Lobaria scrobiculata* occupied by photobiont cells was significantly larger in marginal than in central thallus zones. Central zones were twice as thick as marginal zones but, surprisingly, their density was lower than that of the marginal zones. We developed a new and simple system to compare gas diffusion in marginal and central thallus zones. Large thalli of *L. scrobiculata* were exposed to different gases (ambient air and nitrogen) and the time response of the photobiont of different zones were followed *in situ* by chlorophyll fluorescence. Marginal zones responded faster indicating lesser diffusion resistances than in central zones. However, carbon isotope discrimination was similar in the two zones. This fact suggested that the activity of a carbon concentrating mechanism (CCM) in *L. scrobiculata* overrode the limitations to CO_2 fixation imposed by large diffusion

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resistances. Photosynthetic lichen water use efficiency (LWUE) of marginal zones was significantly higher than that of central zones due to the combination of long water retention times with high photosynthetic rates. It is suggested that both water storage and gas exchange (usually opposing strategies in lichens) were maximized in the marginal zones of *L. scrobiculata* due to the operation of a CCM, which created a homogeneous CO_2 environment across the thallus counterbalancing some of the structural intrathalline differences found in large individuals of this lichen.

1. Introduction

Morphology, mass allocation and physiology of the lichen thallus vary with size (Nash et al., 1980; Lechowicz, 1983; Larson, 1984). As a foliose thallus increases in size, its surface to volume ratio decreases and its structural and physiological intrathalline variability becomes larger. Water relations and gas diffusion become increasingly different between zones within the same thallus that are of different age or of different location (i.e. proximity to the margins of the thallus), as has been reported in large thalli of Umbilicariaceae (Larson, 1984; Larson and Carey, 1986; Valladares et al., 1994).

It is generally accepted that foliose and fruticose lichens possess an apical or marginal growth, and therefore, the marginal tips are very active in comparison to the inner parts (Hale, 1973; Hill, 1985). Intercalary or diffuse growth have been reported in several foliose lichen species, as in *Umbilicaria* spp. and *Lobaria* spp., although thallus margins seem to be the most actively growing zones (Honegger, 1991; Valladares et al., 1994). This common growth pattern together with the large structural and functional intrathalline differences found between marginal and central zones in large thalli led to the proposal of a certain distribution of functions within the thallus: marginal zones would maximize gas exchange while central zones would maximize water storage since both strategies are usually antagonistic in lichens (Valladares et al., 1994).

The study of water relations and gas exchange of lichens requires a solid understanding of thallus anatomy and both ecophysiological aspects have been previously linked to thallus structure (e.g. Larson, 1983; Jahns, 1984; Sancho and Kappen, 1989; Palmer and Friedman, 1990). However, thallus structure has been quantified very rarely and usually with inappropriate methods (see discussion in Valladares and Ascaso, 1992; Ascaso and Valladares, 1994). This scarcity of

reliable quantitative data regarding thallus structure has made the linkage between structure and function very weak. For instance, the evaluation of CO_2 resistances within the lichen thallus has been very little explored in combination with structural studies (Snelgar et al., 1981; Cowan et al., 1992) despite its central role in lichen ecophysiology.

A recent study of carbon isotope discrimination (Δ) revealed large intrathalline variations in certain *Lobaria* species that could be related to anatomical changes responsible for varying gas resistances across the thallus (Máguas and Brugnoli, 1996). The fractionation of stable carbon isotopes, observed in all photosynthetic organisms, is determined by factors such as: i) carboxylation enzyme activity (Rubisco/PEPcarboxylase) ii) the activity of a biophysical CO_2 concentrating mechanism (CCM), iii) CO_2 transfer resistance between the atmosphere and the sites of carboxylation and iv) isotopic composition of the source air. A wide range of Δ has been observed in lichens (Lange et al., 1988; Máguas et al., 1993; Máguas et al., 1995) which was mainly ascribed to the activity of a CCM in cyanobiont and some phycobiont lichens (Palmqvist et al., 1994; Máguas et al., 1995; Smith and Griffiths, 1996). The effect of a CCM induction has been primarily shown to influence instantaneous Δ in free-living algae, with Δ declining from 28‰ to 4‰ following transfer from elevated CO_2 to air-equilibrated supply (Sharkey and Berry, 1985). The CCM may in fact confer a particular advantage to lichens, since the resistances to CO_2 diffusion in the hydrated fungal matrix may be high (Lange et al., 1988; Cowan et al., 1992; Lange et al., 1996). The activity of a CCM would temper the effect of large CO_2 diffusion resistances within the thallus, since it increases the CO_2 concentration at the carboxylation site (Farquhar et al., 1989). CO_2 is accumulated within a partially closed compartment, rising the CO_2 concentration at the carboxylation sites. One of the implications of such photosynthetic CO_2 concentrating mechanism is a reduced discrimination against $^{13}CO_2$, which has been reported for both C_4-plants (Farquhar, 1983; Farquhar et al., 1989), CAM-plants (Griffiths et al., 1990) and by algae possessing the CCM (Beardall et al., 1982; Sharkey and Berry, 1985). This is shown by the isotopic composition of these plants which are enriched in $^{13}CO_2$ in comparison to C_3-plants. However, the importance of internal gas diffusion resistances for carbon gain and its structural basis remains to be elucidated in lichens. There have been several attempts to measure internal resistances (Green and Snelgar, 1981; 1982; Coxson and Lancaster, 1988) but it was not until the work of Cowan et al. (1992) using helox that it has been possible to reliably quantify diffusion resistances in lichens. Nevertheless, the effect of thallus size in gas diffusion and the intrathalline variability of diffusion in large individuals remained unexplored together with their structural basis and their implications on water relations.

Structural and physiological strategies that allow for enhanced gas exchange are usually not compatible with those aimed to reduce water loss by evaporation. The tradeoff between carbon gain and water loss is addressed in ecophysiological studies by the calculation of the photosynthetic water use efficiency (WUE), a parameter that is missing in the lichen literature. A possible explanation for the absence of WUE analyses in studies of lichen ecophysiology could be the fact that CO_2 and water vapour fluxes cannot be controlled by the stomata, as in vascular plants, and water evaporates from the entire surface of the lichen thallus. However, since the duration of a period of hydration can be controlled to some extent by thallus structure and density (Valladares et al., 1997) and thallus structure and density have a direct effect on gas diffusion and consequently in photosynthetic gas exchange, we propose a new term LWUE (lichen water use efficiency). This term, can provide an information in lichen studies equivalent to that usually obtained in ecophysiological studies of other photosynthetic organisms, knowing that the meaning of controlling or changing WUE of higher plants is different from what we called here LWUE.

In this study we propose a new methodological approach, consisting of quantification of both structural and physiological features of the thallus, to explore the effect of thallus size on gas exchange and water relations and on the intrathalline differentiation in structurally and functionally heterogeneous zones in foliose lichens. We wanted to confirm the possible existence of a certain intrathalline distribution of functions within the thallus in large individuals of *Lobaria scrobiculata*, as has been suggested for *Lasallia* spp. (Valladares et al., 1994). Quantification of structure was carried out by stereology (Ascaso and Valladares, 1991; 1994) and thallus physiology was studied by means of gas exchange, chlorophyll fluorescence and carbon isotope techniques (Brugnoli and Lauteri, 1991; Máguas and Brugnoli, 1996). In this article we calculated LWUE for different zones of the lichen thallus since it was our aim to distinguish between different parts of the thallus in terms of water loss. Also we describe a new and very simple system to compare gas diffusion resistances of different thallus zones. Effects of varying thallus structure on gas exchange and water relations are discussed together with some possible functional consequences of a CCM activity in *L. scrobiculata*.

2. Materials and Methods

Lichen material and sample preparation

Lichen thalli of *Lobaria scrobiculata* (Scop.) DC. were collected in Serra de S. Mamede, 250 km NW of Lisbon. The lichen samples used in gas exchange experiments were re-wetted by spraying with distilled water for 2 or 3 days in

ambient air (35–37 Pa of CO_2 partial pressure) at 12 ± 2°C and a photosynthetic photon flux density (PPFD) of 15–25 µmol m^2/s with a 12 h photoperiod. Two different zones were distinguished within individual lichen thalli: i) marginal zones, 0 to 1.5 cm from lobe tip, and ii) central zones, located further than 1.5 cm away from the margins of the thallus. In order to reduce possible errors due to changes in the boundary layer we used big thalli where the cut samples were large enough (average 10 cm long) and the exposure of new surfaces were relatively small.

Stereological study

Pieces of around 6 mm^2 were taken from each thallus zone (marginal and central zones) and cut in 5 µm thick sections with a freezing microtome (Slee Mainz MTC). The sections were stained with lactophenol cotton-blue and observed with a Carl Zeiss Jenaval light microscope provided with a camera lucida. From these sections, the thickness of each thallus layer and the percentage of the section surface area occupied by the photobiont cells were measured. The percentage of the section surface area occupied by the photobiont cells was measured by counting points of a grid test system superimposed on the image by a camera lucida. This parameter is an unbiased estimate of the percentage of thallus volume occupied by the photobiont cells (the stereological parameter volume density, Vv Ascaso and Valladares, 1994). To avoid errors due to overprojections, the microscopic sections have to be as thin as possible. By considering only those photobiont cells that were sharply focused, with the diagram of the microscope totally open, we avoided this source of error, since the actual section thickness that we were considering was less than 1 µm. Thallus density was calculated as in Valladares et al. (1994).

Carbon isotope discrimination analysis

Lichen samples were combusted in a dumas-combustion elemental analyser (model Na-1500, Carlo Erba, Milan, Italy) as described by Brugnoli and Lauteri (1991). CO_2 was purified on cryogenic traps and then analysed by a dual-inlet isotope ratio mass spectrometer (Model SIRA II Series, VG Isotech, Middlewich, UK). The carbon isotope ratio of the sample CO_2 was compared with that of a reference CO_2 calibrated against PDB. Internal precision of individual measurements was always greater than 0.01‰. Carbon isotope discrimination was determined from the known carbon isotope composition of plant material (δ_p) and source air CO_2 (δ_a) according to Farquhar *et al.* (1989).

$$\Delta = (\delta_a - \delta_p) / (1 + \delta_p)$$

where δ_a is the carbon isotope composition of source air (typically $-8‰$) and δ_p the carbon isotope composition of plant material.

Evaluation of diffusion resistances

The system showed in Fig. 1 was used in order to assess differences in gas diffusion resistance in various zones of the lichen thallus. A hydrated lichen thallus was placed on wet filter paper in a Petri-dish (ca 63 ml capacity), which was sealed once the thallus was correctly positioned for fluorescence measurements. Lichen thalli inside the Petri dish were exposed to two different gases, normal ambient air (36 Pa CO_2 and 21 Kpa O_2) and nitrogen. The flows of the two gases passing through the system were set at about $1,500\pm50$ ml/min, and a Y-valve allowed to switch very rapidly from normal air to nitrogen and vice versa. With this flow rate the Petri-dish atmosphere was renewed about 23 times per minute. The initial water content of *L. scrobiculata* was 250 ± 12 (% of dry weight), and thallus water content did not vary during the experiment because the gas flow passed through a humidifier before entering the Petri dish (Fig. 1). All measurements were performed at room temperature. Incident light was measured with a Li-Cor 185B Photometer, and a constant PPFD of 5 ± 2 μmol/m^2/s was maintained throughout the experiments. Variable fluorescence was measured with a PAM 103 (WALZ, GmbH, Germany) and care was taken to follow the same thallus zones by means of a grid drawn on the Petri-dish cover. We followed the kinetics of photochemical efficiency of PSII (Φ_{PSII} = (Fm' − Fo')/Fm', quantum yield of photosynthetic electron transport *sensu* Genty, et al., 1989) for each specific point of the thallus. Because electron transport rate = quantum yield PSII x PPFD (incident on PSII), at constant light the quantum yield of PSII is proportional to the electron transport rate. Decrease or increase of Φ_{PSII} after exposure to nitrogen or upon return to normal air was plotted against time and the linear part of each curve was fitted using SIGMAPLOT (Jandel, USA). Differences between fitted lines were checked by ANOVA using SIGMASTAT (Jandel, USA) following the procedure of Wardlaw (1987) for comparisons between fitted curves.

Gas exchange and photosynthetic lichen water use efficiency

The photosynthetic water use efficiency of the thallus (LWUE, photosynthetic carbon gain divided by the water loss due to evaporation) was evaluated under controlled conditions in a minicuvette system model CMS 400 (WALZ, GmbH, Germany). Net photosynthesis was measured throughout a drying course under saturating light (570 μmol/m^2/s of photosynthetically active radiation), ambient CO_2 (35–37 Pa partial pressure), and at 15°C and 90% relative humidity. Carbon
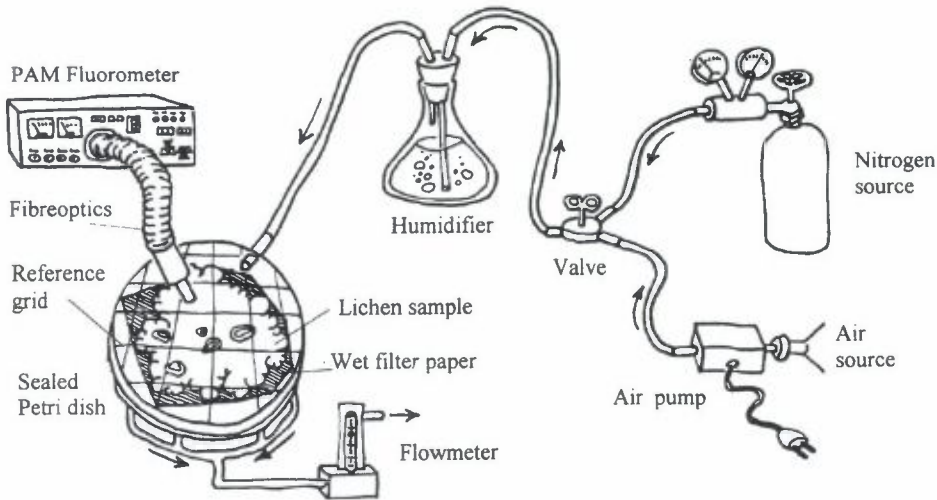
Figure 1. System developed to assess gas diffusion resistances within the lichen thallus. One lichen thallus is maintained wet within a sealed Petri-dish. Thallus water content was checked to be relatively constant once equilibrium with saturated air was reached. The gas within the Petri-dish is renewed ca 23 times per minute (flow of $1,500\pm50$ ml/min). The source gas used was either ambient air or nitrogen. Variable chlorophyll fluorescence was measured periodically under low light in different thallus zones with a PAM fluorometer. The decrease (when nitrogen is provided instead of ambient air) or increase (when ambient air substitutes nitrogen) of the variable fluorescence is recorded for each zone and differences in its time evolution are used as indications of differences in gas diffusion resistances.

assimilation along the drying cycle was integrated for samples of the two different thallus zones and divided by the amount of water loss by the sample since the beginning of the measurements (thallus fully saturated with water, water content above 250% dry weight) until no gas exchange was detected by the system (water content below 60%). The water loss was calculated as the difference between the water contents at the beginning and at the end of the cycle. Lichen water use efficiency (LWUE) of marginal and central zones were expressed as mmol CO_2/g H_2O. LWUE was expressed on the basis of: thallus dry weight, thallus surface area, photobiont volume and chlorophyll content. Chlorophyll data were taken from Máguas and Brugnoli (1996).

3. Results

Marginal and central zones of thalli of *L. scrobiculata* exhibited remarkable

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Table 1.   Percentage of thallus volume occupied by photobiont cells, thallus thickness
and thallus density in the two different thallus zones (marginal and central
zones) studied in *Lobaria scrobiculata*. Differences between the two zones were
statistically significant for the three parameters (ANOVA, p<0.05). Values are
mean of five samples ± SD. A decrease in the amount of algae (% of thallus
volume) from the margin towards the center was observed.

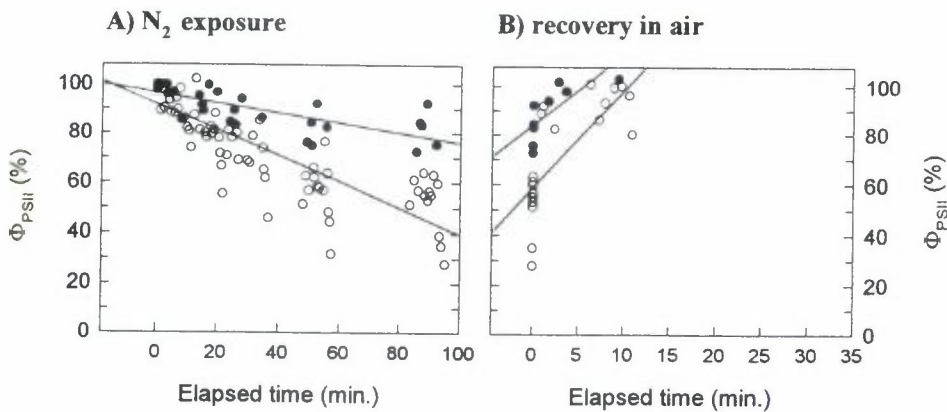|	Marginal zone	Central zone
Percentage of thallus volume occupied by photobiont cells	14.1 ± 1.3	10.6 ± 0.5
Thallus thickness (μm)	221 ± 28	422 ± 47
Thallus density (g/cm^3)	0.76 ± 0.05	0.58 ± 0.07

Figure 2.   Kinetics of PSII quantum yield (ΦPSII) of marginal and central zones of a thallus
of *Lobaria scrobiculata* following gaseous nitrogen exposure (A) and recovery
under normal, ambient air after the exposure to nitrogen (B) using the system
described in Fig. 1. The same thallus points were followed throughout the
whole process and afterwards they were arranged according to their proximity
to the margins in marginal zones (open symbols) and central zones (closed
symbols). Exposure to nitrogen atmosphere caused a decline in PSII quantum
yield and subsequent air exposure caused a recovery of the initial PSII
quantum yield values. The slopes in the two graphs differed significantly for
the two thallus zones (P<0.01). Changes in fluorescence during recovery were
faster than changes during nitrogen exposure. Slopes were –0.52 (marginal
zones) and –0.20 (central zones) in graph A and 3.97 (marginal zones) and 2.92
(central zones) in graph B. Differences between fitted lines were checked
following the method of Wardlaw (1987).

morphological and physiological differences. The stereological study revealed that the percentage of the volume of the thallus occupied by photobiont cells was significantly larger in the marginal zones than in the central zones (Table 1). Besides, central zones were twice as thick as marginal zones but, surprisingly, their density was lower than that of the marginal zones (Table 1). Since we consider that gas diffusion should be addressed in three dimensions, it is important to retain that the amount of photobiont cells should be considered in relation to thallus volume, rather than in relation to thallus surface area.

The main functional implications of the observed structural differences between the two thallus zones concerned photosynthesis (gas exchange) and water relations (water storage and holding capacities). Different approaches were followed to explore these functional implications in *L. scrobiculata*. We studied intrathalline differences in gas diffusion resistances by means of a very simple device that allowed for *in situ* measurements of photobiont activity under different gases (Fig. 1). *In vivo* chlorophyll fluorescence (quantum yield of photosynthetic electron transport, Φ_{PSII}) measured at low light (10 μmol/m^2/s), was followed once ambient air was replaced by nitrogen. Φ_{PSII} linearly decreased with increasing time of exposure to gaseous nitrogen and rapidly recovered when nitrogen was replaced by ambient air (Fig. 2). The speed of both processes was higher in marginal zones than in central zones as revealed by the significant differences in the slopes of the fitted lines (Fig. 2). As it was mentioned, the fluorescence measurements were carried out at low light in order to prevent any kind of photoinhibition process in the absence of CO_2. This fact may contribute to a slow response to N_2, since at low light the CO_2 requirements of the photobionts are also low. Nevertheless, the differences presented in Fig. 2 may clearly be related to differences in the time required by each gas to reach the target (photobiont cells) and pointed to the existence of larger gas diffusion resistances in the central zones than in the marginal zones. The results actually indicate that are no major alternative pathways for electrons, so that electron transport can be considered to be representative for photosynthetic carbon fixation.

Another approach to the study of intrathalline differences in gas diffusion resistance was carried out by means of carbon isotope discrimination (Δ). Carbon isotope discrimination in dry thalli of *L. scrobiculata* was on average 13.5% and the differences between marginal zones and central zones were not statistically significant (Table 2).

Finally, we compared certain aspects of photosynthesis and water relations in the two zones of thalli of *L. scrobiculata*. Marginal zones stored similar amounts of water on a dry weight basis than central zones (Table 3) but they retained water for longer periods of time under the same environmental conditions compared to central zones. Photosynthetic rates on a dry weight basis were also

Table 2.   Carbon isotope discrimination (Δ, ‰) measured on dry material of different lichen thallus zones (marginal and central zones). Data are means of three samples ± SD.

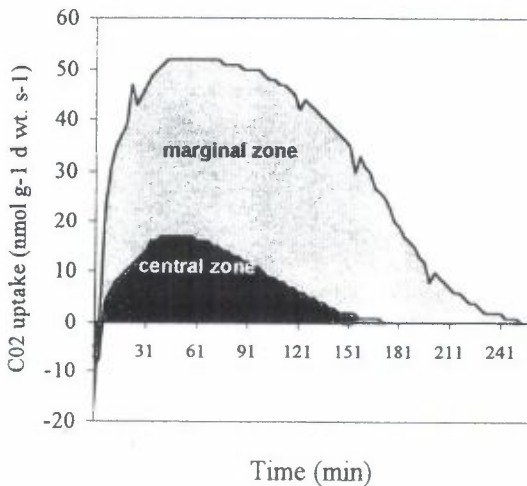|	Δ Marginal zones	Δ Central zones	Δ Whole thallus
Lobaria scrobiculata	13.7 ± 0.2	13.1 ± 0.3	14.0 ± 0.4

Figure 3. Kinetics of net photosynthetic CO_2 uptake during a drying course under controlled conditions of marginal and central thallus zones of *Lobaria scrobiculata*. Photosynthetic CO_2 uptake was measured under saturating light (570 μmol/m^2/s of photosynthetically active radiation), ambient CO_2 concentration, 90% of relative humidity and 15°C. The total carbon gain throughout the process was calculated integrating the two curves. The values obtained were used for the calculation of the lichen photosynthetic water use efficiency presented in Table 3.

higher in marginal zones than in central zones (Fig. 3) and this difference slightly decreased but did not disappear when expressed on a surface area basis (data not shown). The photosynthetic lichen water use efficiency (LWUE, carbon gain vs water loss) of the marginal zones was significantly higher than that of the central zones due to the combination of long water retention times with high photosynthetic rates (Table 3). LWUE of marginal zones was between 6 and 16 times larger than LWUE off central zones depending on the reference units (Table 3). The largest difference in LWUE between the two zones was obtained on a photobiont volume basis. Although it may be true that the use of cut parts

of a lichen thallus may introduce errors due to changes in the boundary layer, it was our intention to compare and to characterize different parts of the thalli which might not be equal to LWUE of intact thalli.

Table 3. Lichen photosynthetic water use efficiency (LWUE, the ratio of carbon gain to water loss) of the two thallus zones studied in *Lobaria scrobiculata* (marginal and central zones). Water content (% dry weight) at the beginning (WCi) and the end (WCf) of a drying course were used to determine water loss during the experiment. LWUE was calculated on the basis of four different reference units (photobiont volume, thallus dry weight, chlorophyll content and thallus surface area) and they are presented in descending order of the ratio marginal zone/central zone.

	Marginal zone	Central zone	Ratio
LWUE – photobiont volume basis (μmol CO_2/cm^3 photobiont cells/g H_2O)	1.41	0.09	15.7
LWUE – dry weight basis (μmol CO_2/g dry weight/g H_2O)	13.2	1.5	8.8
LWUE – chlorophyll basis (μmol CO_2/mg chlorophyll/g H_2O)	6.2	0.8	7.7
LWUE – surface area basis (μmol CO_2/cm^2 thallus surface area/g H_2O)	0.23	0.04	5.7
WCf (percentage thallus dry weight)	55	32	1.7
WCi (percentage thallus dry weight)	258	267	0.9

4. Discussion

In this paper we present a stereological study of *L. scrobiculata* in the line of previous studies with other lichen species (e.g. Valladares et al., 1994) together with a suite of physiological results, to understand intrathalline variations in gas diffusion limitations, gas exchange and water relations.

The internal diffusion paths for CO_2 are relevant for photosynthetic organisms because i) Rubisco has poor affinity for CO_2 so CO_2 gradient affects the overall nitrogen use efficiency, ii) prediction of photosynthetic rates from the biochemical properties of the tissues requires a good estimate of the partial pressure of CO_2 at the carboxylation site, iii) internal resistance to CO_2 reduces carbon gain relative to water loss (Evans and Caemmerer, 1996). As water

evaporates from the entire surface of the lichen thallus, measurements of water loss cannot be used as with leaves of higher plants, to assess the diffusion of CO_2 into the photosynthetic tissues. Instead of a numeric estimation of resistances to CO_2 diffusion (Cowan et al., 1992) we wanted to compare the functional implications of diffusion resistances in structurally different thallus zones. We designed a very simple system to compare the time response of the photochemical activity of photobiont populations from different thallus zones to very fast environmental changes (Fig. 1) and we obtained significant differences in the kinetics of PSII quantum yield (Fig. 2). These differences were mostly due to differences in the time required by each gas to reach the carboxylation sites (photobiont cells) and also in the time required for respired CO_2 to leave the thalline interior (Fig. 2). These results pointed out to the existence of larger gas diffusion resistances in the central zones than in the marginal zones. However, other causes for this differential response (e.g. different physiological properties of the photobiont cells of each zone, differences in the respiration rates of both symbionts) cannot be completely ruled out.

The chlorophyll fluorescence parameter used, quantum yield of photosynthetic electron transport, at constant light is a good measure of linear electron transport through PSII (Genty et al., 1989) and may reflect the internal CO_2 concentration at the photobiont (Lange et al., 1996). Therefore, the observed decrease of PSII quantum yield when lichens were exposed to nitrogen may be related to a decrease in carbon concentration at the photobiont site. Nevertheless, the slow reduction in PSII quantum yield (Fig. 2) upon switching to N_2 is surely not only connected to diffusion processes, as CO_2 recycling may be an additional explanation for the delay in response. Recovery of variable fluorescence in ambient air after exposure was faster than the decrease upon exposure to nitrogen (Fig. 2). One possibility is that the increase in CO_2 pressure at the carboxylation site, may be faster upon return to normal air than its decrease after exposure to N_2 because of respiration and recycling of CO_2. However, the role of structural characteristics, such as gas storage and diffusion within the pore system, and of physiological features, such as carboxylation and oxygenation in the photobiont, and dark respiration of both symbionts, in the explanation of these differences requires further study.

Active gas exchange and reduction of water loss through evaporation are usually opposing problems in photosynthetic organisms. This tradeoff is commonly estimated in vascular plants by the photosynthetic water use efficiency (WUE, carbon gain vs water loss). However, it is the combination of stomatal resistance and boundary layer resistances that are important to influencing gas exchange. In the case of lichens we just have boundary layer resistances. As a direct factor in natural selection, WUE is probably of evolutionary significance especially for CAM and poikilohydric plants. For most

vascular plants, the strategy of having a higher WUE and "saving water for the future" is not necessarily successful if water saved in the soil can be used by other neighboring plants (James Ehleringer, personal communication). However, in the case of CAM plants water taken up can be stored and then lost more slowly over time, allowing a plant with higher WUE to gain carbon for a longer time during the drought period. A similar argument might be true for lichens. Variations in morphology and/or interior-to-exterior structural differences could lead to differences in lichen water use efficiency (LWUE) and to the length of time while a lichen can remain photosynthetically active, albeit that the time scale is minutes or hours instead of days-or-weeks as for CAM plants. Thallus density, in particular, seems to play an important role in controlling water retention capacity, as shown for 12 Umbilicariaceae species (Valladares et al., 1997) and as found here for *L. scrobiculata* (Table 1).

Remarkable intrathalline variability in growth, metabolic activity and carbon isotope discrimination has been reported in certain large foliose thalli (e.g. Larson and Carey, 1986; Máguas and Brugnoli, 1996) and different roles have been suggested for the different zones of these thalli. Marginal zones exhibited higher LWUE than the central zones due to the combined effect of higher net photosynthetic rates and longer water retention times. Interestingly, the differences in net photosynthesis and LWUE observed between the two zones not only did not disappear when expressed on a photobiont volume basis but were even larger than when expressed on the basis of thallus volume, area or dry weight (Table 3). Consequently, marginal zones of *L. scrobiculata* were able to simultaneously maximize water storage and gas exchange. This result was surprising because water storage and gas exchange in lichens are usually opposing problems due to the blocking of pores by water and by the fungal hyphae that swell when hydrated. One possible explanation for this result is the existence of a CO_2 concentrating mechanism (CCM) in *L. scrobiculata* (Badger et al., 1993; Palmqvist, 1993; Máguas et al., 1993, 1995; Smith and Griffiths, 1996). The problems of large diffusion resistances to CO_2 due to high thallus density and the large associated water retention capacity would be overriden by the increased CO_2 concentration at the site of carboxylation, made possible by the CCM.

Another piece of evidence for the functional relevance of the CCM in *L. scrobiculata* was obtained from the results of carbon isotope discrimination (Δ). No significant differences were observed in Δ between the two zones (Table 2). This result was in apparent contradiction with the differences found between the two zones regarding thallus density and gas diffusion (Table 1, Fig. 2). Carbon isotope discrimination (Δ) was expected to be lower in zones of larger CO_2 resistances, such as the central zones of *L. scrobiculata*, since a decrease in CO_2 concentration at the site of carboxylation leads to a lower discrimination of $^{13}CO_2$

by Rubisco. The lack of differences in Δ could be related to the CCM activity, which would maintain relatively high and constant CO_2 concentrations throughout the thallus. In other words, actively growing marginal zones of *L. scrobiculata* were denser but the disadvantages of this (increased CO_2 limitations) were compensated by the CCM activity, which allowed for enhanced water storage with apparently no cost in carbon gain. We are currently extending our study to other foliose lichens lacking a CCM in order to test the hypothesis that having a CCM is one of the very few ways of affording enhancement of water storage with no reduction in the potential net photosynthesis and carbon gain.

In fact, rather than drawing to many conclusions, the work presented here aims to explore the combination of different techniques and approaches to study the complex interactions between thallus size, structure and gas diffusion properties.

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