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Functional Analysis of the Intrathalline and Intracellular Chlorophyll Concentrations in the Lichen Family Umbilicariaceae

FERNANDO VALLADARES*†, LEOPOLDO G. SANCHO‡ and CARMEN ASCASO*

** Centro de Ciencias Medioambientales, CSIC. Serrano 115 dpdo. 28006 Madrid and ‡ Departamento de Biología Vegetal II, Facultad de Farmacia, Universidad Complutense de Madrid, 28040 Madrid, Spain*

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Intrathalline and intracellular chlorophyll concentrations together with thallus structure were studied in 12 species of the lichen family Umbilicariaceae in order to explore pigment limitations for light absorption and for maximum rates of net photosynthesis (A_{max}). Species from high light environments tended to have relatively low chlorophyll concentrations and vice versa. Chlorophyll concentration on a surface area basis of all species studied was sufficient to absorb 80–99% of the incident light, which led us to believe that chlorophyll concentration was not a limiting factor for A_{max}. A_{max} of Umbilicariaceae was smaller than A_{max} of leaves of vascular plants, both on a dry weight and on a surface area basis. These differences slightly decreased but did not disappear when referred to the chlorophyll concentration of the photosynthetic tissues, but reference units had a confounding effect in the comparisons. Differences in A_{max} on a dry weight basis between Umbilicariaceae and vascular plants disappeared when comparisons were made with the whole plant and not simply with the leaf. We found a significant, inverse relationship between amount of alga and chlorophyll concentration within the algal cells in the lichens studied. This was interpreted as a mechanism to avoid pigment self-shading within the algal layer, distributing more efficiently the photosynthetic pigments over a given surface area, and is the first indication of such a mechanism in lichens. It is suggested that thallus structure and physiology have a larger influence on the observed low A_{max} of the lichens studied than chlorophyll content.

Key words: Algal cells, chlorophyll concentration, lichens, light harvesting, net photosynthesis, stereology, thallus structure, Umbilicariaceae.

INTRODUCTION

Photosynthesis is driven by the energy of light, which is collected by the photosynthetic pigments. Chlorophylls are the only pigments essential for photosynthesis because the photochemical reactions involve exclusively special types of chlorophyll *a* for electron transfer (Lawlor, 1993). The role of chlorophylls in light capture has sustained a remarkable interest since plants may increase light absorption by increasing their chlorophyll *a* density (Björkman, 1981; Scheer, 1991). Pigments packaged within photosynthetic organisms exhibit a reduced light absorption efficiency (Kirk, 1983). Light harvested by individual pigment molecules decreases with increasing chlorophyll density due to self-shading, and, in the same way, light harvested by individual photosynthetic cells or organs (such as leaves) decreases with increasing cell or organ densities. Despite structural differences among photosynthetic organisms, Agustí *et al.* (1994) determined that a single asymptotic relationship between light absorption and chlorophyll density applied equally well to different photosynthetic organisms, and predicted that photosynthetic tissues of 1000 mg chlorophyll *a* m^{-2} absorb 99% of incident light.

Light harvesting characteristics are not well known for several groups of terrestrial photosynthetic organisms. In spite of the fact that lichens are dominant in about 8% of terrestrial ecosystems (Larson, 1987), many aspects of their ecological physiology, such as their light absorption efficiency, are poorly understood. Lichens are photosynthetic, symbiotic organisms composed of a fungal, heterotrophic partner, the mycobiont, and one or more autotrophic partners, the photobionts, that are frequently unicellular, green algae (Hale, 1983). As poikilohydric plants, lichens live opportunistically, resting when it is dry and being active only when moisture is available from the environment. Consequently they grow slowly in comparison with cormophytic plants (e.g. Hale, 1973; Sancho and Valladares, 1993). Some physiological features such as low chlorophyll contents and low maximum rates of net photosynthesis (A_{max}) have also been considered in the explanation for the low growth rates observed in lichens (Green and Lange, 1994; Green, Lange and Cowan, 1994). But it is still unclear how chlorophyll content and A_{max} of lichens compare with their vascular plant counterparts. This is due to a scarcity of comparative studies and to the fact that the choice of the basis for both parameters (dry weight, surface area, tissue volume, etc.) affects interpretation of results. Effective light absorption, that is, light absorbed by the photobiont cells, is difficult to estimate in lichens due to thallus structural heterogeneity, and it has never been documented. In order to explore the possibility that the low growth rates of lichens can be in part caused by an inefficient harvesting of light we must assume that the

† Present address: Departamento de Biología Vegetal II, Facultad de Farmacia, Universidad Complutense de Madrid, 28040 Madrid, Spain.

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relationship between light absorption and chlorophyll concentration observed by Agustí *et al.* (1994) can be applied to lichens as well. We must also assume that light scattering within the thallus is relatively constant because light scattering increases the probability for absorption at wavelengths between 400–700 nm where cholorophyll absorption is weak (Vögelmann, pers. comm.). This aspect is technically difficult to tackle because light absorbance measurements with an integrating sphere are affected by secondary pigments that absorb in the visible and that are particularly abundant in lichens. Therefore, chlorophyll concentration becomes the only available estimator for lichens of light absorption by the photosynthetic units. In fact, chlorophyll concentration has been suggested as the limiting factor for A_{max} in lichens because, on a dry weight basis, it was apparently very low compared to vascular plants (Tretiach and Carpanelli, 1992; Green and Lange, 1994).

We wanted to investigate the limitation imposed by chlorophyll concentration on A_{max} in a group of lichen species of similar growth form from different environments. Within this objective, we also wanted to assess how general the correlation was between chlorophyll content and A_{max} found for some lichens (Nash, Moser and Link, 1980; Tretiach and Carpanelli, 1992). Chlorophyll concentration on a thallus dry weight or surface area basis *per se* might not be directly related to the light absorption and the consequent A_{max} due to pigment packaging within the cells, as has been reported for algae and cyanobacteria of phytoplankton (Agustí, 1991; Agustí and Phlips, 1992). For that reason, a second goal of the present paper was to study variations in chlorophyll concentration at the intracellular level in order to elucidate the existence in lichens of mechanisms for avoiding pigment self-shading like those reported for photosynthetic organisms of phytoplankton.

We chose the lichen family Umbilicariaceae for the present study because there were available data on net photosynthetic rates from an earlier work (Sancho and Kappen, 1989) and because the family was well known from structural and ecophysiological points of view (Larson, 1979*a*, *b*, 1981, 1983, 1984*a*; Larson and Carey, 1986; Sancho and Crespo, 1989; Valladares, Wierzchos and Ascaso, 1993; Sancho, Valladares and Ascaso, 1994; Valladares, 1994*a*, *b*; Valladares and Ascaso, 1994; Valladares, Ascaso and Sancho, 1994; Valladares and Sancho, 1995; Sancho, Schroeter and Valladares, 1996). We supplemented the structural information available for this lichen family with a stereological study of the thallus of the 12 selected species with the aim of getting a more complete interpretation of the chlorophyll data.

MATERIALS AND METHODS

The Umbilicariaceae studied were collected in the Spanish Sistema Central (Sierra de Guadarrama, Madrid and Segovia, and Sierra de Gredos, Avila, Spain) from 1989 to 1991. Collection sites ranged from 650 to 2200 m above sea level and from understorey boulders to montane and alpine overhanging walls and runoffs. Complete lists of locations together with information regarding the autecology of the species are given in Sancho (1986), Sancho and Kappen (1989) and Valladares (1993). The 12 species considered were: *Lasallia hispanica* (Frey) Sancho & Crespo, *L. pustulata* (L.) Mérat, *Umbilicaria carpetana* prov., *U. cinereorufescens* (Schaer.) Frey, *U. cylindrica* (L.) Del. ex Duby, *U. freyi* Codogno, Poelt & Puntillo, *U. grisea* Hoffm., *U. hirsuta* (Sw. ex Westr.) Hoffm., *U. spodochroa* (Hoffm.) DC. in Lam. & DC., *U. polyphylla* (L.) Baumg., *U. polyrrhiza* (L.) Fr., *U. vellea* (L.) Ach. *U. carpetana* is a provisional name for *U. spodochroa* var. *carpetana* described in Sancho (1986); a detailed description of this taxon is in preparation. The size of the collected specimens was the most representative for each species and both very large and very small thalli were discarded to avoid the effects of thallus size, which can alter the results in comparative studies of lichens belonging to this family (Larson, 1984*b*).

Five thalli of each species and three fragments from each thallus were taken for systematic measurements. The three fragments per thallus were taken from three different thallus zones (marginal, close to the umbilicus and intermediate, as in Valladares *et al.*, 1994) in an attempt to get more realistic average values for thalli that have been shown to be of a great intrathalline variability (Larson, 1983; Larson and Carey, 1986; Posner, Feige and Huneck, 1990; Posner, Feige and Leuckert, 1991; Seriñá, Arroyo and Manrique, 1991; Valladares *et al.*, 1994). Lichen fragments of around 4 mm² were embedded in Spurr's resin (Spurr, 1969) and cut in 0·8 μm thick sections for light microscopy study and for the estimation of the stereological parameters (as in Ascaso and Valladares, 1994; Valladares *et al.*, 1994). The sections were stained with Methylene Blue and examined with a Zeiss Axiophot light microscope. The percentage of thallus volume occupied by the fungal cells of each layer and by the algal cells (the stereological parameter volume density, Vv) was calculated by point counting on a grid test system (Gundersen *et al.*, 1988; Ascaso and Valladares, 1994) applied over microscopical images of the sections obtained from the thallus fragments embedded in Spurr's resin. The volume of algal cells corresponding to a certain thallus surface area (the stereological parameter $Vs_{algal\ cells,\ thallus}$) was calculated from vertical sections (Baddeley, Gundersen and Cruz-Orive, 1986; Ascaso and Valladares, 1994).

Three thallus fragments of 20 mg were taken from each of five thalli per species for chlorophyll measurements. Dimethyl sulphoxide (DMSO) was used for chlorophyll extractions (Barnes *et al.*, 1992). DMSO was reported to extract chlorophyll succesfully from lichens (Ronen and Galun, 1984; Harrison, Walton and Rothery, 1989; Manrique *et al.*, 1989; Balaguer and Manrique, 1991; Boonpragob and Nash, 1991; Valladares *et al.*, 1994). The chlorophyll content of the algal cells (expressed as μg Chl mm^{-3} algal cell) was calculated from the chlorophyll concentration on a dry weight basis, the stereological data, which allowed the estimation of the volume of the photobiont per thallus weight, and estimations of thallus density. Thallus density was calculated from thallus volume estimations (estimated surface area multiplied by thallus thickness) and oven dry weight.

Linear regression analysis was used to explore the relationships between the different parameters considered.

Sixty points (average of each of the five thalli of the 12 species) were used in the analyses, although only the average for each species is plotted in the figures together with the regression curve and the correlation coefficient obtained.

RESULTS AND DISCUSSION

Thallus structure of the species studied was characterized by a thick medulla with a relatively small percentage of its volume occupied by protoplasts of the fungal cells ($Vv_{\text{fungal cells, thallus}}$), and thin cortices and algal layer with a relatively large percentage of their volumes occupied by the protoplasts of the symbiont cells (Table 1). Nevertheless, within this general pattern for the family Umbilicariaceae that agreed with previous observations (Scott and Larson, 1984; Valladares and Sancho, 1995), we found significant differences among the species studied both in thickness and in $Vv_{\text{fungal cells, thallus}}$ of the different layers of the thallus.

As expected, the chlorophyll concentration of the species was correlated with their natural light environment. For example, *Lasallia hispanica*, a species from relatively high light environments had lower chlorophyll concentrations than the other Spanish species of the genus, *L. pustulata*, which is more frequent in understorey, shaded boulders (Fig. 1). The same trend was observed in other groups of species, such as *Umbilicaria hirsuta* agg. (Codogno, Poelt and Puntillo, 1989), where the higher the light environment (*U. grisea* > *U. freyi* > *U. hirsuta*) corresponded with the lower the chlorophyll content (*U. grisea* < *U. freyi* < *U. hirsuta*). These differences were true not only for chlorophyll concentration on a dry weight basis, in agreement with what has been frequently described for sun and shade vascular plants (Evans, von Caemmerer and Adams, 1988; Larcher, 1995) and lichens (Tretiach and Brown, 1995), but also on a thallus surface area basis. Regardless of the light environment, chlorophyll content of all the species studied was enough to absorb 80% of the incident light according to Agustí *et al.* (1994). In many species, the chlorophyll concentration was close to that required to absorb 99% of the incident light (Fig. 1). Besides, light scattering within the thallus (not estimated here) could increase light absorption at wavelengths where absorption by chlorophylls is weak (in the green). These results led us to believe that chlorophyll concentration was not a limiting factor for maximum rates of net photosynthesis (A_{max}) in the lichens studied.

Chlorophyll contents were considered to be lower in lichens than in vascular plants, especially when expressed on a dry weight basis (Green and Lange, 1994). The comparison of the values of chlorophyll content for a wide range of wild, terrestrial, vascular plants with the Umbilicariaceae species studied here showed no differences when the values were expressed on a surface area basis (Table 2). Chlorophyll content on a dry weight basis of leaves of vascular plants was up to three-fold larger than that of the thalli studied here. But when the values for the leaves of vascular plants were compared with the values for the algal layer of the lichens studied, instead of with the whole thallus, the ranges for the two groups of organisms overlapped almost completely. And the same was true when the comparison

TABLE 1. *Thickness* (*Th*, μm) *and volume density* (*Vv*, %) *of the protoplast of the fungal cells of each thallus layer in relation to the volume of the thallus for the 12 species of Umbilicariaceae studied. Each value is the average of five independent thalli. The standard deviation is shown in brackets*

	Upper cortex		Algal layer		Medulla		Lower cortex		TOTAL	
Species	Th	Vv	Th	Vv	Th	Vv	Th	Vv	Th	Vv
L. hispanica	82	26	40	9	135	32	45	14	303	71
	(7)	(3)	(6)	(3)	(16)	(4)	(5)	(3)	(24)	(15)
L. pustulata	70	19	41	11	123	28	52	14	290	72
	(6)	(2)	(6)	(3)	(16)	(3)	(7)	(3)	(26)	(12)
U. carpetana	37	5	60	9	241	29	37	7	325	52
	(5)	(2)	(7)	(2)	(24)	(4)	(4)	(2)	(36)	(7)
U. cinereorufescens	35	8	39	9	129	12	27	10	238	38
	(4)	(3)	(5)	(2)	(16)	(3)	(4)	(3)	(28)	(6)
U. cylindrica	59	7	77	12	133	15	28	5	276	41
	(7)	(2)	(8)	(2)	(12)	(4)	(5)	(2)	(25)	(8)
U. freyi	22	4	56	11	176	24	40	5	291	44
	(5)	(2)	(7)	(3)	(16)	(8)	(5)	(2)	(31)	(6)
U. grisea	29	7	35	10	134	19	28	7	210	42
	(7)	(2)	(6)	(3)	(15)	(5)	(5)	(2)	(23)	(9)
U. hirsuta	25	5	73	14	140	18	25	7	250	44
	(3)	(2)	(9)	(3)	(14)	(4)	(4)	(3)	(20)	(8)
U. polyphylla	19	9	34	14	105	18	22	7	167	49
	(3)	(2)	(5)	(4)	(11)	(3)	(5)	(2)	(18)	(9)
U. polyrrhiza	18	11	37	13	87	28	32	11	176	63
	(3)	(3)	(5)	(2)	(12)	(5)	(8)	(3)	(43)	(8)
U. spodochroa	28	10	45	8	92	16	26	6	198	41
	(5)	(2)	(4)	(2)	(11)	(3)	(6)	(2)	(17)	(8)
U. vellea	29	7	63	16	166	19	22	7	263	49
	(6)	(2)	(9)	(3)	(16)	(3)	(6)	(3)	(22)	(8)

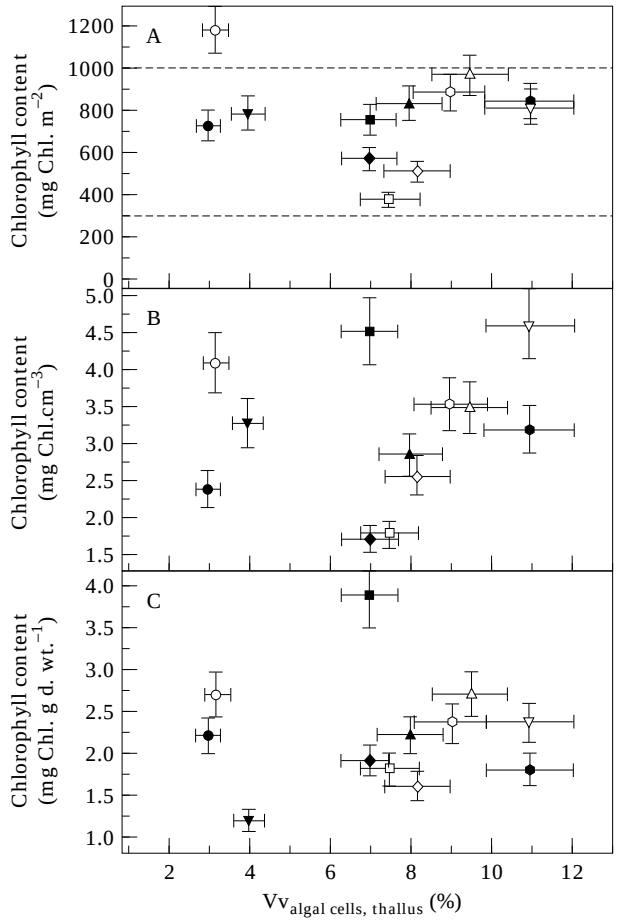

FIG. 1. Chlorophyll content *vs*. percentage of thallus volume occupied by algal cells in the 12 Umbilicariaceae species studied. Chlorophyll content is expressed on a dry weight (C), thallus volume (B) and thallus surface area (A) bases. The dotted line in the upper graph indicates the chlorophyll concentration found to absorb 80% (lower line) and 99% (upper line) of the incident light for a wide range of photosynthetic organisms (after Agustí *et al*., 1994). Each point is the average of five independent thalli and the bars represent the standard error. ●, *L. hispanica*; ○, *L. pustulata*; ■, *U. polyphylla*; □, *U. grisea*; ▲, *U. freyi*; △, *U. cylindrica*; ▼, *U. cinereorufescens*; ▽, *U. polyrrhiza*; ◆, *U. carpetana*; ◇, *U. spodochroa*; ⬢, *U. vellea*; ⬡, *U. hirsuta*.

was made between the whole lichen thallus and the whole above ground plant including petioles, branches and stems (Table 2).

Since chlorophyll concentrations were not very different between the lichen family studied and terrestrial, vascular plants, we wanted to scale up to photosynthetic utilization of the light absorbed. For that purpose, we compared A_{max} obtained in an earlier work for the lichens studied here (Sancho and Kappen, 1989) with A_{max} data for vascular plants available in the literature. We used our chlorophyll and structural data to do the required transformations to express A_{max} on a dry weight, surface area, or chlorophyll basis, and we re-calculated the data from the literature to make the units uniform. A_{max} on a dry weight basis was up to ten-fold smaller in Umbilicariaceae lichens than in vascular plants when the algal layer was compared with the leaf (Table 2). But this difference reduced down to two-fold when the A_{max} on a dry weight basis was compared between the lichen thallus and the whole vascular plant, including the dry weight of all non-photosynthetic tissues and structures above ground and considering the actual light absorbed by the plant canopy architecture.

Comparisons of A_{max} made on a surface area basis showed similar trends (Table 2). A_{max} on a chlorophyll basis was up to seven-fold larger in vascular plants than in the lichens studied. Consequently, differences in A_{max} between Umbilicariaceae lichens and vascular plants slightly decreased but did not disappear when referred to chlorophyll concentration, despite the general trend suggested by Green and Lange (1994) of a similar photosynthetic performance on a chlorophyll basis of lichens and vascular plants. The choice of the reference units has a profound influence on the comparisons of chlorophyll concentration and A_{max} between plants and lichens. In particular, the consideration of the dry weight of non-photosynthetic tissues, and of the limitations in light harvesting imposed by the architecture of the plant canopy, dramatically reduced the differences in A_{max} between the two groups of photosynthetic organisms.

A_{max} was not correlated with the chlorophyll concentration in species belonging to the family Umbilicariaceae (see Table 1 in Sancho and Kappen, 1989). The correlation

TABLE 2. *Chlorophyll concentration and net photosynthesis (A_{max}) of wild, terrestrial, vascular plants from different environments and of foliose lichens (Umbilicariaceae). A_{max} on a surface area basis for the whole canopy is $\leqslant A_{max}$ for the leaf since it reflects light harvesting limitations imposed by plant canopy architecture (mostly self-shading among leaves)*

Parameter	Wild, terrestrial, vascular plants	Foliose lichens (Umbilicariaceae)
Chlorophyll on a dry weight basis (mg g^{-1})	Leaf: 1·2–15·6 Above ground plant*: 0·07–3·20	Algal layer: 2–18 Whole thallus: 0·4–3·7
Chlorophyll on an area basis (mg gm^{-2})	200–1800	380–1200
A_{max} on a dry weight basis (mg CO_2 g^{-1} h^{-1})	Leaf: 1·8–109·5 Above ground plant*: 0·3–2·3	Algal layer: 1·0–12·5 Whole thallus: 0·1–1·2
A_{max} on an area basis (mg CO_2dm^{-2} h^{-1})	Leaf: 5·0–40·5 Whole canopy* 1·1–6·5	Algal layer: — Whole thallus: 0·4–5·7
A_{max} on a chlorophyll basis (mg CO_2 mg^{-1} Chl h^{-1})	1·5–7·0	0·27–1·01

Data for vascular plants from Larcher, 1995; Maslova and Popova, 1993; Augustí *et al*., 1994; Green and Lange, 1994; Green *et al*., 1994, data for Umbilicariaceae from our own measurements and from Sancho and Kappen (1989).

* Data calculated for tropical and temperate evergreen shrubs from Pearcy and Yang (1996) and Valladares and Pearcy (unpubl. res.).

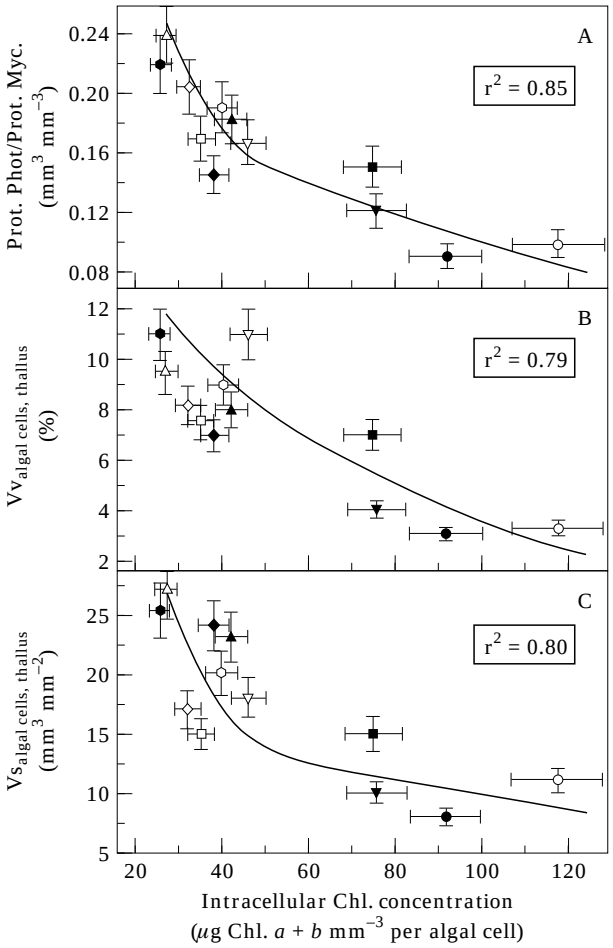

FIG. 2. Volume of algal cells per thallus surface area (C) and thallus volume (B), and protoplast of photobiont cells/protoplast of mycobiont cells ratio (A) *vs.* intracellular chlorophyll concentration in the 12 Umbilicariaceae species studied. Each point is the average of five independent thalli and the bars represent the standard error. ●, *L. hispanica*; ○, *L. pustulata*; ■, *U. polyphylla*; □, *U. grisea*; ▲, *U. freyi*; △, *U. cylindrica*; ▼, *U. cinereorufescens*; ▽, *U. polyrrhiza*; ◆, *U. carpetana*; ◇, *U. spodochroa*; ⬢, *U. vellea*; ⬡, *U. hirsuta*.

between chlorophyll concentration and A_{max} found before in some lichens seems to be true only for different individuals of the same species (Tretiach and Carpanelli, 1992; Tretiach and Brown, 1995) or for different zones of the same thallus (Nash *et al.*, 1980). This lack of correlation at the species level adds further support to the idea that the structure (resistances to gas diffusion, Cowan, Lange and Green, 1992) and the physiology (respiration rates of the mycobiont, Kershaw, 1985) of the thallus have a larger influence on the net assimilation rate than the chlorophyll content.

The amount of algal cells per thallus volume was not related to the amount of chlorophylls expressed in any units (Fig. 1). This independence of these two parameters was due to changes in the intracellular chlorophyll concentration. In fact, we found a significant, inverse relationship between the amount of algae and the intracellular chlorophyll concentration, so thalli richer in algae had algal cells with lower chlorophyll concentration and vice versa (Fig. 2). This implies that the algal layer inside the lichens studied acted as a distinct optical unit, rather than as the sum of their component cells, in analogy to cyanobacteria colonies *vs.* single-celled cyanobacteria (Agustí and Phlips, 1992). The avoidance of self-shading by reducing the intracellular chlorophyll concentration as a way of distributing more efficiently the photosynthetic pigments over a given surface area has not been reported in lichens before, despite its presence in free-living, unicellular algae (Agustí, 1991). We observed the same trend (large volume of the thallus occupied by algal cells associated with low intracellular chlorophyll concentrations) in a comparative study of different zones of the umbilicate thallus (Valladares *et al.*, 1994), but we interpreted it as a result of a different algal age and metabolic activity in the different zones rather than a self-shading avoidance phenomenon. Chlorophyll concentration cannot be used as an estimation of the ratio between photo- and mycobiont (as assumed by Tretiach and Carpanelli, 1992) because, as we have found here, the chlorophyll concentration of the thallus may not be directly related to the amount of algae due to changes in the intracellular chlorophyll concentration (Figs 1 and 2).

Thallus structure and internal organization are expected to play a role in light absorption properties of a lichen. For instance, upper cortex structure influenced albedo in some Umbilicariaceae (Sancho *et al.*, 1994). The dense upper cortex of many lichens, sometimes densely pigmented (Hale, 1983), may limit light penetration, so the light absorbed by the chlorophylls of the photobiont cells could be a relatively small fraction of the incident light as shown for dark thalli of *Peltula* (Büdel, 1987). On the other hand, light scattering inside the lichen thallus could contribute significantly to the quantity of light reaching the photosynthetic units, as is the case for many multicellular photosynthetic tissues (Vögelmann and Björn, 1986; Vögelmann, 1989, 1993; Ramus, 1990). For example, crystals of secondary metabolites that tend to accumulate in the medulla (Honegger, 1991) could act as internal reflectors that bounce light back into the algal layer where it can be absorbed for photosynthesis. Our results pointed to the chlorophyll concentration and its potential in light harvesting as not being a limiting factor for A_{max} in certain lichens, but the real light being harvested by the photosynthetic pigments of these lichens cannot be ruled out as a limiting factor for A_{max} until it is measured.

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