PHYSIOL. PLANT. 52: 417–422. Copenhagen 1981

Carbon dioxide exchange in lichens: Estimation of internal thallus CO_2 transport resistances

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Snelgar, W. P., Green, T. G. A. and Beltz, C. K. 1981. Carbon dioxide exchange in lichens: Estimation of internal thallus CO_2 transport resistances. – Physiol. Plant. 52: 417–422.

The gaseous exchange pathways of *Sticta latifrons* Rich. and *Pseudocyphellaria amphisticta* Kremp. were examined using both light and scanning electron microscopes. The size and frequency of the pores in the gas exchange structures (cyphellae and pseudocyphellae) and in the medulla were measured and from these CO_2 diffusion resistances were calculated. Pseudocyphellae were found to be smaller and more widely spaced than cyphellae, consequently the resistance of the pseudocyphellae, was much greater than that of the cyphellae. Medulla resistances were low in both lichens and are probably unimportant, even at high water contents. No evidence of hyphal swelling was found. Gas exchange structure resistances were more than five-fold greater than medulla resistances. It is suggested that this arrangement of resistances may simultaneously encourage refixation of respired CO_2 and maintain a non desiccating environment for the lichen algae. The internal transport resistances calculated in this work approximate experimentally obtained values.

Key-words: Sticta latifrons, Pseudocyphellaria amphisticta, cyphellae, pseudocyphellae, recycling, photorespiration, photosynthesis, water relations, hyphae, alga.

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Introduction

Previous studies of total resistances to CO_2 uptake (Σr) in six species of lichens (Snelgar *et al.* 1981) demonstrated that variations in Σr with thallus water content of each species could be fitted into three categories:

1. Lichens with low maximum water contents [*circa* 2.0 g water (g dry weight)$^{-1}$] and which exhibited low Σr values at water contents producing optimal net photosynthetic rates. The Σr increased markedly at low water contents and to a much smaller degree at supra optimal water contents.

2. Lichens having a higher maximum water holding capacity (about 3.5 g water g^{-1}) and which showed similar trends as mentioned above at water contents of 2.0 and below, but at high water contents Σr increased rapidly, in a response similar to that observed at low water contents.

3. Lichens with a very large water holding capacity (*circa* 5.0 g water g^{-1}) and which again showed similar trends at low water contents to the previous groups, but had very little increase in Σr at supra optimal water levels, even at water contents as high as 5.0.

Further work (Green and Snelgar 1981) which subdivided Σr of *Pseudocyphellaria amphisticta* (group 3) and *Sticta latifrons* (group 2) confirmed that the resistance observed at high thallus water contents was caused by internal transport resistances (r_{ti}). In the present investigation, path lengths and CO_2 resistances within the thalli of these two species are estimated from measurements of the vertical thickness of thallus tissue layers, and from the results of scanning electron microscope studies.

The lichens used in this investigation are members of the Stictaceae, a group characterised by the presence of 'aeration' structures in their dense lower cortex. These circular structures may represent breaks in the lower cortex through which the loosely packed fungal hyphae of the medulla protrude (pseudocyphellae). Alternatively they may have a smooth lining which is distinct, both in structure and in thickness, from the cortex

Received 18 November, 1980; revised 3 March, 1981

 0031-9317/81/080417-06 $03.00/0

(cyphellae). *P. amphisticta* is unusual in possessing pseudocyphellae on both upper and lower surfaces. A diagrammatic representation of the structure of these lichens is given in Figure 1.

List of symbols:

Σr = total CO_2 resistance, s cm^{-1}
r_t = CO_2 transport resistance ($r_{ti} + r_a$), s cm^{-1}
r_{ti} = internal CO_2 transport resistance, s cm^{-1}
r_e = CO_2 carboxylation resistance, s cm^{-1}
r_a = CO_2 boundary layer resistance, s cm^{-1}
r_g = CO_2 resistance of gas exchange structure (cyphellae or pseudocyphellae), s cm^{-1}
r_{pore} = CO_2 resistance of any pore system, excluding end corrections, s cm^{-1}
r_{end} = end correction CO_2 resistance for a single pore, s cm^{-1}
r_{pe} = CO_2 resistance of pore system including an end correction, s cm^{-1}
D_{CO_2} = diffusivity of CO_2 in appropriate medium (air or water), $cm^2\ s^{-1}$
l = length of pore, cm
d = diameter of pore, cm
n = number of pores per square centimetre
d' = diameter of gas exchange structure, cm
n' = number of gas exchange structures per square centimetre
L_m = medulla CO_2 diffusion pathway, cm
SE = standard error of the mean.

Theory

The resistance to CO_2 diffusion (r_{pe}) of any set of pores which are widely spaced in comparison with the pore diameter can be calculated using the formula of Monteith (1973).

$$r_{pe} = \frac{4(l + \frac{\pi d}{8})}{\pi n d^2 D_{CO_2}} \quad (1)$$

This equation incorporates one end correction which takes into account the diffusion pattern around pores of diameter d, which are widely separated. The use of this end correction for the results obtained in this study is probably untenable as the pores are tightly clustered within the gas exchange structures (cyphellae or pseudocyphellae) rather than being arranged on the entire thallus surface at large interpore spacings as the model of Monteith (1973) presupposes. An alternative method of analysis is to calculate the resistance of the pore system without including any end correction by

$$r_{pore} = \frac{4l}{\pi n d^2 D_{CO_2}} \quad (2)$$

The end correction for the gas exchange structures (rather than for the individual pores) can then be calculated separately by

$$r_{end} = \frac{1}{2n'd'D_{CO_2}} \quad (3)$$

This end correction may be an overestimate as the gas exchange structures are not a pore but are a cluster of small pores. End corrections were calculated by both of these methods in order to obtain probable maximum and minimum values. The mean length (L_m) of the medulla CO_2 diffusion pathways for each species was estimated as the arithmetic mean of the minimum and maximum distances from the internal surface of the gas exchange structures to the algal layer, assuming that CO_2 entered the thallus only through cyphellae or pseudocyphellae. Previous experimental results (Green *et al.* 1981) lend support to this assumption. Unpublished data (Green) on the mass flow of air through thalli of *S. latifrons* indicates that flow is virtually undetectable until the upper cortex is visibly ruptured. Entry of CO_2 only through gas exchange pores also seems a reasonable assumption (at least at medium and low water contents) in view of the small magnitude of internal transport CO_2 resistances compared with the values predicted from calculations of CO_2 exchange through the cortices of these lichens (Snelgar *et al.* 1981). *P. amphisticta* is a lichen which has pseudocyphellae on both the upper and lower surfaces; however the thalli used in studies carried out in this laboratory have a lower surface which is densely tomentose and possesses few pseudocyphellae. As unpublished results (see Green *et al.* 1981 for method) have repeatedly indicated that CO_2 uptake occurs only through the upper surface, the pseudocyphellae of the lower surface were not included in CO_2 uptake models.

Materials and methods

Specimens of *Pseudocyphellaria amphisticta* Kremp., and *Sticta latifrons* Rich., were collected and stored as described previously (Green and Snelgar 1981). Only terminal 3–5 cm of healthy lobes were used. The vertical thicknesses of tissue layers of each species were determined from hand cut transverse sections of moistened thalli using a stereoscopic microscope fitted with a calibrated eye piece.

Thalli sectioned for scanning electron microscopy were prepared by one of the following methods:

1. Thalli were air dried.
2. Thalli were completely hydrated in distilled water, fixed in 4% glutaraldehyde buffered with 0.025 *M* phosphate buffer, ($KH_2PO_4 - Na_2HPO_4$) pH 7.0 for 12 h, dehydrated through a graded ethanol series, and then critical point dried with CO_2.

After this preparation thalli were hand sectioned under a stereoscopic microscope in both transverse and horizontal planes. Sections were mounted on stubs using double sided sellotape coated with 50 nm of gold and palladium, and viewed with a JEOL–JSM 35 scanning electron microscope operated at 25 KV.

Measurements of hyphal diameter, pore size and tissue thickness were made from electron micrographs using vernier calipers. Results are presented as the mean of a number of measurements ($\bar{x}$) together with

the standard error of the mean (SE) where appropriate. It did not prove possible to cut horizontal sections through cyphellae or pseudocyphellae, so the number of pores per unit area was determined from transverse sections by assuming a depth of field of 5 μm in pseudocyphellae and cyphellae. This figure approximated the diameter of the pores found within these structures. Hyphal diameters could be measured accurately, but the assessment of pore diameter (i.e. the space between fungal hyphae) and the number of these pores per unit area was necessarily more arbitrary, so that these figures should be regarded as estimates.

The diameter of cyphellae and pseudocyphellae and number per cm^2 of thallus were measured at low magnification under a stereoscopic microscope. The saturated water content of lichen thalli was determined by immersing thalli that had been moistened and held at 100% RH for several hours in distilled water for two minutes, blotting off excess water with tissues, then weighing. Infiltrated water content was estimated in a similar manner following immersion of thalli in distilled water under three cycles of vacuum for 80 min. Following either of these treatments thalli were reimmersed in distilled water for 30 seconds, blotted and weighed to check for any variation in blotting efficiency, the mean figure being used in all further calculations. Dry weights of thalli were determined after drying to a constant weight at 100°C. All water contents are expressed as g water per g thallus dry weight.

Results

A summary of data is presented in Table 1. The pathways used for calculation of the medulla CO_2 resistances are indicated by lines (L_m) in Figure 1. These are likely to be underestimated as no allowance has been made for the probable, twisting nature of the diffusion pathway.

The dense nature of the cell layer lining the cyphellae can be seen in Figure 2B and has been described previously (Henssen and Jahns 1974). In contrast the fungal material within pseudocyphellae (Fig. 2C) is loosely arranged. A consequence of this difference in structure is a smaller number of pores per unit area of gas exchange structure for cyphellae than pseudocyphellae. However, when the greater number and size of cyphellae, in comparison to pseudocyphellae, is taken into account there is a larger number of pores per square centimetre of lichen thallus in S. latifrons than in P. amphisticta. The pores in the cyphellae are smaller in diameter. The extremely dense nature of the cortices of both lichens, and the lack of any cortical air pores, is apparent in Figure 2.

Sticta latifrons

An estimate of total internal transport resistance (r_{ti}) to CO_2 uptake in S. latifrons can be made by summing the resistance of the cyphellae (r_g) and the medulla (r_m).

Tab. 1. Morphological data used to calculate the CO_2 diffusive resistances and medulla air volume for *Sticta latifrons* and *Pseudocyphellaria amphisticta*. Unless otherwise noted all dimensions are μm. Where appropriate the standard error of of the mean, and the number of measurements (brackets) are given.

System	Measurement	*Sticta latifrons*	*Pseudocyphellaria amphisticta*
Vertical thickness of thallus layers	upper cortex	55 ± 13(5)	58 ± 10(5)
	algal layer	70 ± 10(5)	68 ± 15(5)
	medulla	278 ± 68(5)	235 ± 78(5)
	lower cortex	63 ± 13(5)	35 ± 5(5)
	total	466	396
	rhizines	83 ± 30(5)	183 ± 60(5)
Weight per unit area density	mg dw cm^{-2}	16.7 ± 2.5(5)	9.7 ± 1.3(5)
	g dw cm^{-3}	0.358	0.245
Cyphellae/ pseudocyphellae	diameter	259 ± 45(10)	127 ± 12(10)
	No. cm^{-2} thallus	47 ± 4(5)	26 ± 4(5)
	depth	20 − 30	40 − 60
Pore system within cyphellae/pseudocyphellae	diameter of pores	3.6 ± 0.2(7)	5.1 ± 0.8(6)
	distance between pores	15	7
	No. cm^{-2} thallus	149 × 10^2	92 × 10^2
	No. cm^{-2} in cyphellae/ pseudocyphellae	0.6 × 10^6	2.8 × 10^6
Pore system within medulla	pore diameter (wet)	5.8 ± 0.7(12)	5.2 ± 0.6(7)
	pore diameter (dry)	5.5 ± 0.4(9)	5.0 ± 0.4(10)
	No. cm^{-2} thallus	4.6 × 10^5	4.9 × 10^5
	mean path length	400	500
	hyphal diameter (wet)	3.3 ± 0.2(10)	3.2 ± 0.3(9)
	hyphal diameter (dry)	3.3 ± 0.2(9)	3.3 ± 0.2(9)

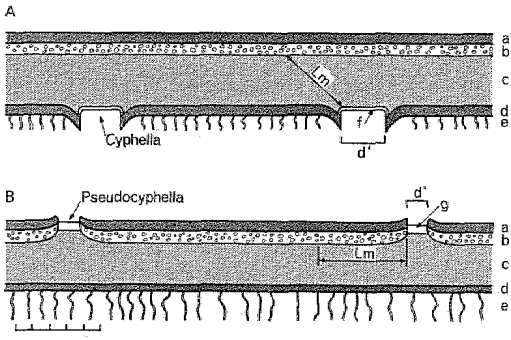
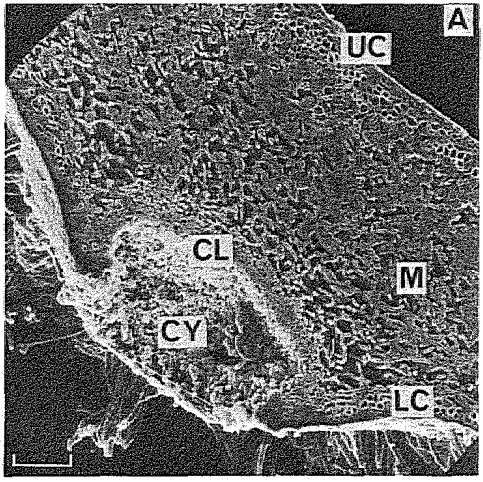
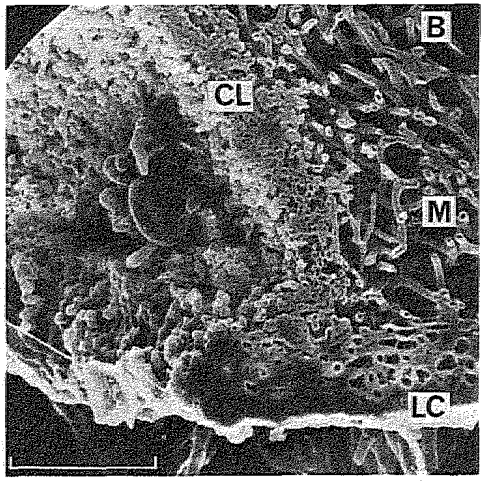


Fig. 1. Diagram of vertical sections across the thalli. A = *Sticta latifrons*; B = *Pseudocyphellaria amphisticta*. Thallus layer thicknesses are to scale: (A) Upper cortex, (b) algal layer, (c) medulla, (d) lower cortex, (e) rhizine tomentum, (f) cyphellal lining depth, (g) pseudocyphellal depth. Also to scale are: (L_m) the mean length of the CO_2 diffusion pathway in the medulla and (d') the diameter of the cyphellae/pseudocyphellae. The bar at the bottom of the figure corresponds to 500 µm.





Using the data of Table 1 and assuming that all pores are filled with air at a temperature of 15°C, then

r_g (total of pores plus pore end correction) = 14.0 s cm^{-1} Equation (1)

r_{pore} (without any end correction) = 13.5 s cm^{-1} Equation (2)

r_{end} (single end correction of whole cyphella) = 2.8 s cm^{-1} Equation (3)

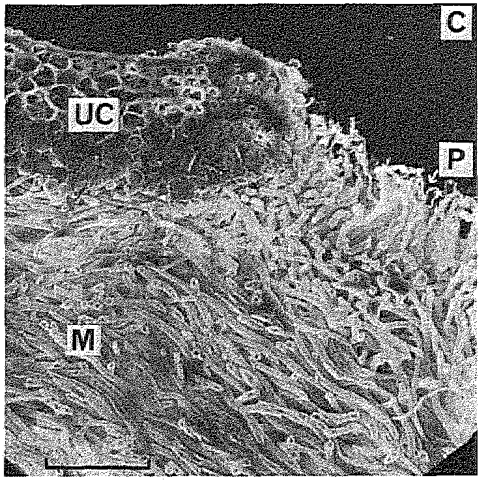
r_m (medulla resistance) = 2.2 s cm^{-1} Equation (2)

In making the above calculation the pore length (l) has been estimated as 1.5 times the depth of the cell layer lining the cyphellae. This adjustment was made because of the tortuous nature of the pores as is visible in Figure 2B.

Thus $r_{ti} = r_m + r_g = 2.2 + (14.0 \text{ to } 16.3) = 16.2$ to 18.5 s cm^{-1} depending on end correction used (Tab. 2). These values closely correspond to the experimentally determined minimum r_{ti} (15.5 s cm^{-1}) of previous work (Green and Snelgar 1981) but as the Σr values in that study were unusually low (cf. Snelgar *et al.* 1981) then an r_{ti} of 15.5 s cm^{-1} may also be underestimated.

In the preceding calculations it has been assumed that no water was present in the interhyphal pores. The percentage thallus volume occupied by the medulla pore system can be calculated from the mean diameter of the pores, and the number per square cm (Tab. 1) as 0.12 cm^2 pore area per cm^2 thallus cross sectional area or 12% of the thallus volume. Only the larger pores were considered when compiling data on pore size and number, thus the volume of this system has probably

Fig. 2. Scanning electron micrographs of transverse sections of lichen thalli. A = *Sticta latifrons*; B = cyphella of *Sticta latifrons*; C = *Pseudocyphellaria amphisticta*. (UC) upper cortex, (LC) lower cortex, (M) medulla, (CY) cyphella, (CL) cyphellal lining, (P) pseudocyphella. The bar in each plate represents 50 µm.



been underestimated and consequently r_m overestimated.

The volume air space within S. latifrons at thallus saturation can be estimated from the differences in water content of 'blotted' and saturated thalli, (± SE, n = 5):

mean infiltrated water content
= 1.64 ± 0.07 g water (g dry weight)$^{-1}$
mean saturated water content
= 1.23 ± 0.08 g water (g dry weight)$^{-1}$
difference = 0.41 ± 0.04 g water (g dry weight)$^{-1}$

As S. latifrons has a density of 0.358 g cm^{-3} (Tab. 1) then 0.41 g water g^{-1} is equivalent to 0.147 g cm^{-3}. Since 1 g water = 1 cm^3 then 0.147 g cm^{-3} = 147 cm^3/1000 cm or 14.7% of thallus volume. Thus both theoretical calculations and experimental measurements indicate that an air filled pore system of approximately 12–15% of thallus volume exists in S. latifrons.

Increases in internal water content (as opposed to water held in the tomentum) could be expected to decrease the volume of internal air space. If each pore in the medulla was half filled with water then the effective pore diameter would be 4.1 μm and r_m would increase to 4.5 s cm^{-1}. A similar situation in the cyphellae would double r_g.

Pseudocyphellaria amphisticta

The internal transport resistances of P. amphisticta with an air filled pore system can be calculated in a similar manner to those of S. latifrons (Tab. 2). The r_{ti} obtained by summing these components varies from 21.6 to 31.5 s cm^{-1} depending on the end correction used. Both figures underestimate the minimum r_{ti} value (46 s cm^{-1}) previously reported (Green and Snelgar 1981). The result of halving pore areas (e.g. due to increased water content) would be to increase r_{ti} to 42.9–52.5 s cm^{-1}. The maximum r_{ti} measured at high water content during previous work was 81 s cm^{-1} (Green and Snelgar 1981).

Discussion

The values for r_{ti} obtained by calculation from thallus dimensions (summarised in Tab. 2) show a close approximation to those obtained by experimentation. In the case of S. latifrons the degree of agreement of the two values was very good and choice of end correction was of minimal importance since this part of the total resistance is small. Calculated and experimental results are not in such close agreement for P. amphisticta, and choice of end correction is important. An extra resistance of 10.0 s cm^{-1} is added if the end correction is applied to the pseudocyphellae rather than the pseudocyphellal pores. Because the pores are aggregated little more than one pore diameter apart the application of the end correction on the basis of the whole pseudocyphella being treated as one pore would seem more correct. This will give a higher calculated total resistance, and better agreement with experimental values.

For both lichens, the calculations demonstrate quite clearly that the main CO_2 diffusive resistances are located in the gas exchange structures, the cyphellae or pseudocyphellae. Although the pore size of the pseudocyphellae in P. amphisticta is very similar to that of the medulla and, in fact, pseudocyphellae are little more than an extension of the medulla (Fig. 2C), the high resistance is created by the small number and area of the pseudocyphellae. Conversely the medulla of both lichens has a low resistance, less than one tenth of the total, even though diffusion path lengths were long. This finding emphasises the very open nature of the medulla (Fig. 2) and indicates that medulla resistance (r_m) is unlikely to be large even at very high thallus water contents. The hypothesised effect of hyphal swelling at high water contents (Lange 1980) was not detected in either of the species examined.

Previous studies (Snelgar *et al.* 1981) have shown that the change in r_{ti} at high water contents is quite different for these two lichens. S. latifrons shows a large increase in r_{ti} (up to 147 s cm^{-1}) whilst P. amphisticta shows less change (71 s cm^{-1}). This difference is apparently the result of P. amphisticta carrying out CO_2 exchange solely through the pseudocyphellae of the upper cortex, whilst the lower cortex is highly tomentose and can hold quantities of water. S. latifrons has cyphellae only on the lower surface surrounded by the tomentum. The holding of water on this surface could impede gas exchange since the diffusion of CO_2 through water is 10^{-4} of the rate in air. It is possible to calculate the effect of a thin layer of water covering the cyphellal pores. For instance if a 1.0 μm layer of water covers all pores in the cyphellae then, at a temperature of 15°C, the resistance of the water pathway is: r_g = 471 s cm^{-1} according to

Tab. 2. Summary of calculated and experimentally obtained resistances for *Sticta latifrons* and *Pseudocyphellaria amphisticta*. Experimental values (†) are from Green and Snelgar (1981). Calculated values were obtained using morphological data (Tab. 1). All resistance values are s cm^{-1}. Water content is g water (g dry weight)$^{-1}$.

Species	Water	Σr_{CO_2}†	r_{ti}†	r_a†	r_{pore}	r_{pe}	r_{end}	r_m	r_c†
S. latifrons	1.47	29	19	9	13.5	14.0	2.8	2.2	3.3
P. amphisticta	1.30	64	46	9	17.4	18.1	10.6	3.5	7.0
Equation used					(2)	(1)	(3)	(2)	

equation (2). The creation of such a large resistance by a thin layer of water is a result of the narrow size of the pores and their small area in relation to the total surface area. At such high resistances diffusion of CO_2 through the 55 µm thick upper cortex (Tab. 1) could become important. If the diffusivity of CO_2 in the cortex is equivalent to that in water the cortex resistance would be 390 s cm^{-1}. Complete or partial blockage of pores in the cyphellae would account for the highest transport resistance found for S. latifrons.

The experimental results have shown that the large internal transport resistance of both lichens are composed of two components; one located in the fungal medulla and the other, up to eight-fold larger, in the cyphellae or pseudocyphellae. Both experimental measurements and theoretical calculations indicate that CO_2 uptake in these lichens takes place via an air filled pore system which remains almost completely free of water even after the immersion of thalli in water.

One consequence of the arrangement of resistances is that CO_2 exchange between symbionts within the lichen takes place in a pathway of much lower resistance than CO_2 exchange between either of the symbionts and the external atmosphere. Under these conditions it is likely that refixation of respired CO_2 would be encouraged (Snelgar and Green 1981) and this could be one explanation of the very low CO_2 compensation values reported for lichens (Snelgar and Green 1980). A further consequence of the high cyphellal/pseudocyphellal resistance would be to hinder water loss to the outside from the internal atmosphere of the lichen, hence the algal layer would be maintained in a high humidity atmosphere, which is at least partially buffered from external desiccating influences.

Thus it seems that although the large resistance to CO_2 uptake would be expected to limit net photosynthesis, this is not normally the case in these lichens. Instead it can be argued that these resistances confer certain advantages in the area of conservation of water vapour and respired CO_2. This in turn implies a higher degree of relationship between structure and physiology in lichens than has previously been considered.

Acknowledgement – The University Grants Committee is thanked for the provision of a post-graduate studentship to WPS and equipment funds to TGAG. The Board and Staff of the Urewera National Park are thanked for assistance in the field and granting permission to collect in the Park. Lands and Survey are thanked for permission to collect in reserve areas. Mr K. Stewart is thanked for the preparation of the Figures.

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Edited by A. K.