THE ECO-PHYSIOLOGY OF NEW ZEALAND FOREST LICHENS WITH SPECIAL REFERENCE TO CARBON DIOXIDE EXCHANGE

by

WILLIAM PATRICK SNELGAR

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ABSTRACT

This study of lichen ecological physiology was carried out in four parts:

(i) The growth rates of specimens of *Pseudocyphellaria homoeophylla* and *Sticta caperata* growing in the Urewera National Park (New Zealand) were measured photographically. The mean radial increase of *P. homoeophylla* was related to thallus diameter and rates as high 27 mm yr^{-1} (32 cm diameter) were recorded. The growth of *S. caperata* was not influenced by thallus size and the mean radial increase was 7.0 mm yr^{-1}.

(ii) The intraspecific morphological variations of *Pseudocyphellaria dissimilis* populations are assessed and are related to the nitrogenase activity and water relations of each population.

The effect of thallus water content on CO_2 exchange in eight lichen and one bryophyte species was studied using an infrared gas analyser operating as a discrete sample analyser. Species from moist habitats showed a net loss of CO_2 at low thallus water contents whilst some other species showed a depression of net photosynthesis (NP) at high thallus water contents. In *Sticta latifrons* this depression was less apparent when the lower surface was dried. Experiments with a split chamber demonstrated that virtually all CO_2 uptake occurs through the lower, cyphellate surface. These results suggest that the cyphellae of *S. latifrons* act as air pores and that blockage of these pores with water results in a depression of NP.

(iii) Low oxygen concentrations (1% v/v) were shown to increase NP in *S. latifrons*, *P. homoeophylla* and *P. billardierii* by up to

55%. This stimulation was considered to result from the repression of photorespiration. The oxygen effect was separated into two components; (a) the sensitivity of the carboxylation system and (b), the rate of CO_2 evolution in the light at zero CO_2. Of the lichen species studied all but *P. billardierii* had a carboxylation sensitivity typical of the values expected in C3 plants. *P. billardierii* had an unusually low carboxylation sensitivity. The rate of CO_2 evolution in the light showed large inter and intraspecific variations. Low rates of CO_2 evolution were often associated with a high CO_2 refixation ability.

(iv) Total CO_2 diffusion resistance - water content curves for six lichen species are presented. All species show increased resistances at low thallus water contents and some also show high resistances at high thallus water contents. The total CO_2 resistances of *S. latifrons* and *P. amphisticta* are separated into transport and carboxylation components. Cyphella , pseudocyphella , and medulla resistances are calculated from morphological data. Although transport resistances are often larger than carboxylation resistances the results suggest that at ambient CO_2 levels carboxylation processes limit photosynthesis. The relationship between resistance to water vapour loss and resistance to CO_2 uptake in *P. homoeophylla* and *S. latifrons* is established and the ecological implications are discussed.

In a general summary, the size of the internal and external water holding capacities of several lichen species is estimated. The effectiveness of these reserves in maintaining lichens in a moist condition in the field is calculated and the results are related to

the ecology of the species. Morphological adaptations of Stictaceae lichens which maximise water holding capacity yet minimise CO_2 diffusion resistances are considered. It is suggested that lichen water contents should be related to thallus area, rather than thallus weight, as the former parameter appears to be of greater physiological significance.

The relevance of this work to that of previous authors is discussed, particularly in regard to photorespiration, CO_2 uptake, and thallus water content in lichens.

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FOREWORD

Chapters IV to IX and XI - XIV of this thesis have been published, or accepted for publication in various journals, (see References under Green, and Snelgar). Due to the different requirements of these journals the chapters vary slightly in diagram layout, style, and nomenclature. In particular it should be noted that net photosynthesis is referred to as NP, NPR, and net assimilation rate (NAR). Figures and Tables are numbered from one onwards in each chapter, rather than consecutively throughout the thesis. In chapter II materials and methods commonly used during this work, and the results of preliminary experiments, are described in detail. Briefer accounts of the relevant materials and methods are included in each chapter.

CHAPTER I

INTRODUCTION - GENERAL

LICHEN RESEARCH

During this century there has been a substantial shift of emphasis in the topics studied by lichenologists. Initially the primary concern was the taxonomy of lichens. Some of the earlier attempts at classification and description are recounted by Richardson (1975) while Poelt (1973) provides a summary of the current taxonomic system. The discovery that lichens produce a wide range of secondary metabolic products provided a stimulus for further research leading to a new branch of lichenology, chemotaxonomy. The distribution patterns of lichen species is another area of interest. Many authors have compiled species lists and in some instances these have been collated and distribution maps produced (e.g. Rose 1973). Others have studied lichen growth rates (Armstrong 1973), or the influence of substrate on lichen ecology (Brodo 1973). However, during the last two decades, there has been an increased interest in the physiology of lichens. One of the first aspects to be investigated was the exchange of substances between the symbionts (e.g. Drew 1966, Richardson 1967, Hill 1970, Green 1970, Farrar 1973). Further topics of particular interest have been; the relationship between thallus water content, photosynthesis, and ecology; the effects of SO_2; and nitrogenase activity in lichens. There is an abundance of literature available on selected aspects of lichenology. Topics recently reviewed include: Nitrogen fixation - Stewart (1966), Millbank and Kershaw (1973), Millbank (1974, 1976); Growth - Hale (1973, 1974), Armstrong (1976), Topham (1977); CO_2 exchange and water relations - Kallio and Karenlampi (1975), Harris (1976); Physiology of symbiosis - Richardson (1973), Hill (1976); General physiology - Farrar (1973). Information

on many other features of lichenology may be found in Ahmadjian and Hale (1973), Ferry, Baddeley and Hawksworth (1973), Richardson (1975), Brown, Hawksworth and Bailey (1976), and Seaward (1977).

LICHENOLOGY IN NEW ZEALAND

Galloway (1974) has compiled a comprehensive bibliography of New Zealand lichenology during the period 1783 - 1973. The guide to contents provided indicates that of the 477 publications cited 6 are bibliographical, 26 are concerned with lichen chemistry, 130 with distribution (primarily species lists), 77 are of an ecological nature (this includes plant geography and sociology), and 292 are taxonomic. (Some references are classified as fitting more than one of these categories). In a more recent publication Galloway (1979) gives a brief summary of lichenology in New Zealand and details the biogeographical affinities of the New Zealand lichen flora. He notes that although only 1 - 2% of New Zealand's macrolichen flora is endemic (cf 78% of the vascular flora) there is a high degree of endemism in some genera. (For instance of the 40 species of *Pseudocyphellaria*, 17 are endemic).

Other publications produced since 1973 include; Green and Snelgar (1977), Grace and Hayward (1978) - growth rates; Wilkins and James (1979), Galloway and James (1980) - taxonomy; Cowan *et al* (1979a, 1979b) - lichen metabolism; and Green *et al* (1980) - nitrogen fixation. In addition species and distribution lists for several sites have been produced by Hayward and Hayward (1974a, 1974b, 1978, 1979), Hayward *et al* (1975, 1976) and Hayward and Wright (1977).

The paucity of research on lichen physiology in New Zealand is emphasised by the fact that Galloway (1974) did not classify any of the pre-1974 publications as physiological. In fact the papers by Cowan *et al* (1979a, 1979b) and Green *et al* (1980) appear to be

the only published works concerned with physiological aspects of New Zealand lichens. Similarly, although some data is available on lichen growth rates on glacial moraines (Burrows and Orwin, 1971), coastal rocks (Grace and Hayward, 1978), and glass (Green and Snelgar, 1977), there is no published information on the growth rates of New Zealand forest lichens.

A MISSING LINK

From the brief summary of lichenology presented above, a trend is apparent. Research on lichens has progressed from taxonomy and chemotaxonomy through distribution, ecology, growth, and the biology of symbiosis to the present stage of whole-plant physiology. Within this latter topic studies of CO_2 exchange have been of particular interest. Many authors have produced a large amount of data on the relationship between CO_2 exchange and thallus water content. Others have provided information on the effects of temperature, light intensity, and pretreatment. In some instances models of lichen CO_2 exchange have been developed and used for predictive purposes (Harris, 1972). In view of the large amount of information available on photosynthesis and respiratory rates it is surprising that so little is known about CO_2 metabolism and CO_2 exchange pathways in lichens. Although the δ^{13} ratios found by Shomer-Ilan *et al* (1979) indicate that CO_2 uptake occurs via ribulose biphosphate carboxylase there are few short term studies on primary photosynthetic products, (e.g. Bednar and Smith, 1966). Photosynthesis and respiratory rates are both known to be affected by thallus water content, yet at present there is no satisfactory technique for determining the precise location of the water within the lichen thallus. The results of Smith (1962) suggest that this water is not evenly distributed. Experimental measurements of light respiration in lichens are entirely lacking. Although the

phenomenon of dark CO_2 fixation was reported several years ago (Drew 1966) it is only recently that more detailed work has been undertaken (Kershaw *et al* 1979).

It is clear that net photosynthesis in lichens is the product of several CO_2 exchange processes, none of which are completely understood. This has meant that analysis of such results as net photosynthesis - water content graphs has generally been confined to a description of curve shapes. The factors affecting these shapes have long been a subject of conjecture (Lange 1980). One of the aims of this thesis is to attempt to provide a better basis for the interpretation of the lichen response to environmental factors, especially water.

SOME FACTORS WHICH AFFECT CO_2 EXCHANGE IN LICHENS

THE LICHEN AS A POIKILOHYDRIC PLANT

(a) *Introduction.* Unlike vascular plants lichens are not able to physiologically control the uptake or loss of water (Blum, 1973). The water content of lichen thalli therefore fluctuates with the water availability and drying stress of the environment. Water relations of this type are termed poikiliohydric. Despite this lack of control the thallus water content has a profound effect on lichen metabolism. In most species the rate of net photosynthesis increases with water content until an optimum level is attained. Further increases in water content have little effect on photosynthesis in some species, while in others photosynthesis is depressed. The optimal water content value for net photosynthesis, and the extent of the depression at supra optimal values combine to produce a relationship between water content and photosynthesis which is characteristic for each lichen species. Detailed work by Harris (1971) has

indicated that the shape of this response may be related to the environment of the lichen, and may show interspecific variations.

Thallus water content also affects dark respiration rates. Often respiration first increases with increasing thallus water content, then remains constant at higher saturation levels (Hale, 1974). In some instances respiration has been reported to continuously increase over the entire range of water contents studied (Kershaw 1977a).

(b) *Desiccation effects*. In most of the lichen species so far examined CO_2 exchange ceases when lichens are dried below a certain critical water content value. The extent to which lichens can tolerate long periods of dryness appears to be related to the habitat of each species or, in some instances, ecotype (Bewley 1979). Aquatic lichens cannot tolerate desiccation (Bewley op. cit.) but species from desert habitats can regain photosynthetic ability after 34 weeks of desiccation (water content 1.1 mg water per mg dry weight, Lange *et al* 1969). The ability to withstand dry storage is greatest at very low water contents, possibly because the enzyme systems are maintained in a protected form under these conditions.

Recent work by Cowan *et al* (1979a) has shown that the Calvin cycle is more sensitive to desiccation than the Tricarboxylic acid cycle. This difference in sensitivity implies that at a certain state of dryness the photosynthetic processes would be completely inactivated, but dark respiration could continue. At lower water contents both processes would cease.

(c) *Rewetting physiology.* Experimental work has shown that lichens can absorb sufficient water from moist air to recommence CO_2 fixation. The immediate effects of this rehydration on respiration are not known (Farrar 1973). However field experiments by Lange (1969) indicates that the rewetting of *Ramilina maciformis* by morning dew results in a net uptake of CO_2. In contrast, rewetting a lichen with liquid water produces a very rapid rate of respiration. Farrar (1973) has separated this response into three components:

(a) Wetting burst - A large release of CO_2 (and other gases) which is a purely physical phenomenon.

(b) Resaturation respiration - This is cyanide and azide sensitive (Smith and Molesworth, 1973) and lasts for one to nine hours.

(c) Basal respiration - This is cyanide and azide insensitive.

According to Smith and Molesworth (1973), the rates of resaturation respiration of lichens from moist environments may be greater than those of lichens from drier habitats. The degree of dessication necessary to induce resaturation respiration (upon rewetting) is thought to vary between lichen species. In *Peltigera polydactyla* this critical level is 40 - 50% of the saturated water content (Smith and Molesworth, 1973).

(d) *Rewetting physiology in the field.* Under natural conditions lichens are subjected to frequent wetting and drying cycles (Kappen *et al*, 1975; Rundel, 1974). Several authors (e.g. Ahmadjian, 1973; Farrar, 1973) have suggested that these cycles are necessary for the maintenance of the symbiotic state.

The lack or stability, or regularity, in lichen water relations in the field creates problems in the use of field-collected material. Unless the recent history of the lichen is known (and this is rare), there is no practical means of ensuring that resaturation respiration is not affecting CO_2 exchange. Frequently the lichens collected must be rewetted by either mist spraying or immersion in water (a highly unnatural event for many species). Rates of CO_2 exchange are likely to be affected for some hours following such treatment. Furthermore as differences in lichen microhabitat will affect wetting and drying rates it is unlikely that all of the lichens collected will be at a similar water content. Thus the magnitude of resaturation effects (if any) would be expected to vary between thalli. Although some workers have demonstrated a seasonal variation in lichen photosynthesis (see later) the results of Kershaw (1977a) and Kershaw and MacFarlane (1980) imply that these variations could be related to the climatic conditions of the previous few days, rather than to an annual cycle. If rates of lichen photosynthesis do respond to short-term climatic changes then field-collected samples are likely to exhibit photosynthetic response patterns which vary from week to week.

Since any researcher working on lichen physiology must use either fresh or stored field-collected samples it is necessary to consider seasonal and storage effects in some detail.

SEASONAL CHANGES

In a review of the early literature on seasonal changes, Smith (1962) concluded that the level of physiological activity of lichens

is higher in winter than in summer. The work of Stafelt (1939) which demonstrated that lichen photosynthesis is generally higher in winter than in summer supports this conclusion. It was considered that the low rates of photosynthesis during summer may have been a result of low chlorophyll contents (Wilhelmson, 1959). The increases in dry weight per unit area of thallus, and in glucose absorption capacity of *Peltigera polydactyla* during winter (Smith, 1961) were interpreted as further evidence of an increased rate of metabolic activity during this season.

Larson (1980) has summarised much of the more recent literature on seasonal changes in the CO_2 exchange rate of lichens. The combined results of several studies show that seasonal trends in photosynthesis and dark respiration vary greatly between species. These responses were classified as 'acclimation' or 'seasonal changes'. The definition of acclimation used was that of Prosser (1955) and includes a requirement for the homeostatic control of some vital process. Thus lichens having a low photosynthetic temperature optimum in winter, and a high optimum in summer would be considered to acclimate only if the photosynthetic rates in winter (at optimum temperature) are as high as those of summer (at optimum temperature). Using this criterion only three lichen species [*Alectoria ochroleuca*, *Cetraria nivalis* (Larson and Kershaw, 1975a); *Bryoria nituclula*, (Kershaw 1975)] are said to acclimate. However 'seasonal changes' in optimal conditions for photosynthesis which appear to be correlated with environmental conditions have been observed in other species (e.g. *Peltigera canina* var *praetextata* and *Peltigera polydactyla*, Kershaw, 1977a). Lichenologists generally refer to such changes as acclimation. Throughout the present work the term acclimation will be used in the latter context.

STORAGE CONDITIONS

For physiological studies of 'higher' plant species experimental material is commonly grown in controlled environment cabinets, or glasshouses. Some similar attempts at growing, or at least maintaining specimens have been made using lichens (Pearson and Benson, 1977; Kershaw and Millbank, 1969). However since such experiments require a considerable amount of equipment, and have been of limited success, this approach is rarely used. Instead lichens required for physiological studies are collected from the field and used immediately (on the day of collection) where this is feasible or, more commonly, they are stored. The storage conditions used during a number of studies are given in Table 1. Since the transport of specimens from the field to the laboratory can take several days (e.g. Eickmeyer and Adams, 1973) comment on this has been included where possible. When lichens are stored in a dry condition some type of rehydration routine is used prior to experimental use. Information on this procedure is also presented in Table 1. The range and variety of climate conditions given in Table 1 is striking, although it should be noted that some of the more extreme conditions (e.g. Larson 1978) were used during investigations of storage effects. Unfortunately another feature of the table is the incomplete nature of the information supplied by some authors. Frequently such important details as thallus water content, light intensity, photoperiod or temperature are not given, thus making it impossible for other workers to replicate the storage conditions, or to assess the likelihood of acclimation occuring during storage. Further comment on lichen storage conditions and the likely effects on subsequent metabolism can be found in Larson (1978).

TABLE 1 Lichen Storage Conditions

Reference	Transport	Storage	Reactivation
Larson, D. W. (1978)	< 2 days, air dry	3.5 years at -60°C, 20% water content	12 h distilled water at 6°C.
Larson, D. W. (1980)	dry	> 3 weeks Summer 14°C 350 μE $m^{-2}s^{-1}$ 14 h day 10°C night Winter > 2 weeks -10°C dark	Soaked 1 h tap water at experimental temperature
Kershaw, K. A., Smith, M. M. (1978)	> 2 days dry over silica gel.	air dry, 300 μE $m^{-2}s^{-1}$ Temp. and day length equivalent to current field conditions.	Various
Kershaw, K. A. (1972)		< 2 days In growth chamber with periodic watering.	Soaked at 20°C for 15 min, then blotted.
Tegler, B., Kershaw K. A. (1980)	dry	> 4 days Summer 15 h day at 20°C, 300 μE $m^{-2}s^{-1}$ Winter 9 h day at 0°C, 300 μE $m^{-2}s^{-1}$	Soaked overnight at storage temperature in distilled water.
Tysiaczny, M. J., Kershaw, K. A. (1979)		Stored at temperature and photoperiod characteristic of field conditions.	Soaked in distilled water for 4 h at 300 μE $m^{-2}s^{-1}$
Farrar, J. F. (1976)		< 24 h dry in polyethylene bag.	Washed with distilled water.
Eickmeier, W. A., Adams, M. S. (1973)	< 4 days, dry	Frozen	Placed in distilled water at room temperature for 10 m
Hallgren, J. E., Huss, K. (1975)		< 1 week. Dry in desiccator at 4°C in the dark.	Soaked in distilled water for 15 min. Put in moist chamber at room temp., 22 W m^{-2} for 16 h.
Harris, G. P. (1971)		Air dry in controlled environment cabinet.	Soaked in distilled water for periods ranging from 1 h to overnight.
Rundel, P. W., Bratt, G. C., and Lange, O. L. (1979)	Air mailed air dry	> 7 days 10°C, 12 h photoperiod of 10,000 lux. Sprayed with deionized water once daily.	
Nash, T. H., Moser, T. J., and Link, S. O. (1980)	Air mailed air dry	Several weeks dry in a freezer.	10°C, 12 h photoperiod for 5 days, periodically moistened.
Lechowicz, M. J., Adams, M. S. (1973)		2 weeks, dry at 3°C.	Immersed in distilled water at room temperature for 15 - 30 mins.
Pearson, L. C., Brammer, E. (1978)		2 - 3 weeks in a growth chamber.	
Lechowicz, M. J. (1978)	Air mailed in dormant condition	0°C.	Soaked 1 - 2 h in distilled water, stored 12 - 16 h in dark at 20 - 25°C. Resoaked 0.5 - 1.0 h.

STORAGE EFFECTS

There have been few comprehensive studies on the alterations of lichen CO_2 exchange rates following storage. As noted by Larson (1978), most authors have limited such investigations to single checks on the viability of the species concerned. The experiments of Kershaw (1977b) are a notable exception. In this study *Peltigera* control material collected from the field was stored in a growth cabinet at current field temperature and daylength, while experimental material was stored moist at higher or lower temperatures. The results show that thalli collected during late October had low (winter) rates of net photosynthesis which could be increased to high (summer) rates by two days of high temperature storage. However these rates declined to the original levels during further high temperature storage. Conversely *Peltigera* species collected during April showed a high temperature induced increase in net photosynthesis which was stable during the experimental period.

Specimens collected during July showed high rates of net photosynthesis which were temporarily depressed by storage at 5° for one day. Thalli collected during October showed a permanent cold induced depression of photosynthesis. Parallel checks on dark respiration rates during all of the above experiments showed no significant changes.

Further studies on the sensitivity to thermal stress of air-dry thalli during long term storage (MacFarlane and Kershaw, 1980) have shown that thermal sensitivity is correlated with the ecology of lichen populations. Dark respiration rates were not affected by thermal stress.

Light acclimation responses of *Peltigera* species have been investigated by Kershaw and MacFarlane (1980). Specimens of *Peltigera scabrosa* collected from under a deciduous tree canopy showed an

ability to acclimate to high or low light levels, even when stored in an air dry condition (7 - 12 mg water per 100 mg dry weight). The increases in net photosynthesis under low light conditions were accompanied by an increase in dark respiration. Some populations of this species were adversely affected by air dry storage at high light levels. Moist-stored specimens of *Peltigera canina* var. *praetextata* were also able to acclimate to low light conditions, but in this instance increases in net photosynthesis occurred in conjunction with a decrease in dark respiration. Continuous light, and variations in photoperiod during moist-storage depressed net photosynthesis in this species. It is interesting that temperature acclimation is achieved by changes in photosynthesis only, while light acclimation is a result of changes in both photosynthesis and dark respiration.

Another study of storage effects has been carried out by Larson (1978). It was shown that after 3.5 years storage at -60°C, 20% water content, the photosynthetic and respiratory responses of *Alectoria ochroleuca* are similar to those of freshly collected material. However under some conditions (e.g. 21°C, 150 μE m^{-2} s^{-1}) the actual rates of net photosynthesis in stored material was markedly lower than those of fresh specimens. Interpretation of these results is complicated by the use of specimens collected at different times, in different years.

Farrar (1976a, 1976b) has examined some of the effects of constant thallus saturation, and wetting and drying cycles on *Hypogymnia physodes*. Constant saturation for 7 days at 20°C or 5°C in continuous light or darkness resulted in reduced photosynthetic rates. This reduction was greatest at 20°C in the dark. In contrast, the photosynthetic rate of lichens stored under 'mild' wetting and drying cycles increased during the first 16 days of

storage.

Consideration of the above results shows that acclimation in lichens is a complex phenomenon. It is influenced by the environmental conditions prior to the time of collection, and by the microclimate of the habitat. In some instances acclimation responses are stable for experimental periods of several weeks, at other times it is a transient condition lasting only days. Acclimation can occur in air dry lichens in response to changes in temperature, the length of the photoperiod, or light level. The changes in respiratory and/or photosynthesis rates during acclimation can be very rapid, on occasions being completed in less than one day. Due to the obvious complexity of these responses, and the present lack of information on underlying mechanisms, the prediction of acclimation trends under experimental conditions can only be speculative.

INTRODUCTION - THIS STUDY

WHY THE STICTACEAE?

Rundel *et al* (1979) observed that the cool temperate rainforests of the Southern Hemisphere possess a rich epiphytic flora which is dominated by large foliose macrolichens of the genera *Sticta* and *Pseudocyphellaria*. Although the abundance and floristic importance of these lichens have been noted by others (e.g. Cockayne, 1910; Galloway, 1979; Green *et al*, 1980) the work by Rundel *et al* (1979) is the first on the physiology of these lichens.

Sticta and *Pseudocyphellaria* are closely allied genera belonging to the family *Stictaceae*. All species have numerous small circular pits on the upper, the lower, or both surfaces. In species of *Sticta* these pits, *cyphellae*, are bordered by a raised rim and have a smooth lining. In contrast the *pseudocyphellae* of the genus *Pseudocyphellaria* are more similar to breaks in the lichen cortex

through which the hyphae of the medulla can be seen. Although all *Sticta* and *Pseudocyphellaria* species are foliose macrolichens (some attain a diameter of 50 cm; Martin and Child, 1972) the species exhibit a range of morphologies and ecologies. Thallus structures vary from the broad complete lobes of *Sticta latifrons* to the much-divided thalli of *Sticta filix*. Several species are scrobiculate or faveolate, and the latter frequently have long narrow lobes of a divaricating form (e.g. *Pseudocyphellaria billardierii*). Attachment of the thallus to the substrate is normally by rhizines or a tomentum, but some species possess a basal stalk. The density and thickness of the tomentum is highly variable, both within and between species. The primary phycobiont may be a green or blue-green alga, however all species with a green primary phycobiont have cephalodia (James and Henssen, 1976). Of the 29 species assayed in New Zealand, all showed nitrogenase activity (Green *et al* 1980).

Most species of *Sticta* and *Pseudocyphellaria* are epiphytic, the most common habitat being the lower trunks of *Nothofagus* trees, however some can be saxicolous or terricolous, while others are normally found in the canopy or subcanopy of *Nothofagus* forests. The greatest abundance is in mountain rainforests with some species being restricted to such habitats. Fortunately others (e.g. *Pseudocyphellaria billardierii, P. dissimilis*) are relatively common in lowland forested areas where a high incidence of mist or rainfall reduces the drying stress of the environment.

The large size, the availability of quantities of specimens, the floristic importance and the variety of the *Stictaceae* render them a group worthy of study. In addition the postulated function of the cyphellae and pseudocyphellae as aerating organs (Rundel *et al* 1979) is an intriguing subject for research. In some experiments species from other genera (e.g. *Peltigera, Stereocaulon, Usnea*)

were included to provide a contrast in morphology or physiology with the *Stictaceae*.

SECTION A - GROWTH

This study was initiated in order to provide basic data on the growth rates of some of the dominant species found in New Zealand forests. When physiological studies are sufficiently advanced to enable prediction of net carbon gain over long periods growth data will provide a means of checking the accuracy of such models. Growth data can also be used to estimate the gross productivity of lichens. These estimates, in conjunction with physiological data on nitrogenase activity, can be used to predict the nitrogen input of lichens into forest ecosystems (e.g. Denison, 1973).

SECTION B - PHYSIOLOGY AND ECOLOGY

Intraspecific variations in the morphology and physiology of lichens have been reported by several authors (e.g. Hill and Woolhouse, 1966). In Chapter IV the water relations and the nitrogenase activity of three populations of *Pseudocyphellaria dissimilis* are related to the anatomy and ecology of the populations.

As noted previously there is a complete lack of information on the CO_2 exchange characteristics of New Zealand lichens. Chapter V is a study of the relationship between thallus water content and CO_2 exchange in several species and was carried out using the injection IRGA (Infra red gas analyser) technique of Larson and Kershaw (1975b). Subsequent observations of the effects of differential drying (upper or lower surface) of *Sticta latifrons* suggested that CO_2 uptake may be mainly via the lower, cyphellate surface. In Chapter VI the proportion of net CO_2 uptake occurring through each surface of the lichen *S. latifrons* is measured using a divided CO_2 exchange cuvette.

The injection IRGA method of Larson and Kershaw (1975b) is based on the assumptions that the rate of photosynthesis is independent of CO_2 concentration over the range 150 to 350 µl CO_2 l^{-1}, and ventilation. In view of the work with 'higher' plants (Jarvis *et al* 1971), these assumptions are unusual, therefore the effects of these variables are investigated in Chapter VII.

SECTION C - PHOTORESPIRATION

Photorespiration is the process whereby CO_2 is evolved from photosynthetic tissue in the light (Zelitch 1979). This net loss of CO_2 causes a considerable depression of net photosynthesis and consequently, a reduction in plant productivity. Photorespiration is caused by the oxygenase activity of ribulose biphosphate carboxylase/oxygenase. The normal product is glycolate which is subsequently oxidised to serine, thereby releasing CO_2.

Precise measurements of photorespiration (PR) are difficult to obtain due to the necessary occurrence of other CO_2 exchange processes such as photosynthesis, and possibly dark respiration. Since there have been many articles and monographs published in recent years which deal with various aspects of photorespiration (Canvin, 1979; Canvin and Fock, 1972; Ludlow and Jarvis, 1971; Zelitch, 1971, 1979) only a brief summary of the techniques used to measure PR will be presented here. This summary is based on Zelitch (1979).

(i) When a photosynthesising leaf is removed from the light a high rate of CO_2 evolution is often observed during the first few minutes. This CO_2 evolution, which can be several fold the steady state dark respiration rate, is considered to result from PR. Although this phenomenon is easily demonstrated,its transience and lack of stability make accurate

measurement difficult. The changes in metabolism and stomatal resistance that may occur when a leaf is moved from the light to darkness are other sources of error.

(ii) If a leaf is photorespiring reducing the oxygen concentration from 21% to 1 - 3% increases the net photosynthetic rate by about 30 - 50%. This increase is regarded as a measure of PR. D'Aoust and Canvin (1973) have suggested that this method overestimates PR, since lowering the O_2 concentration affects both the synthesis and the oxidation of glycolate. However Zelitch (1979) considers that this method is more likely to underestimate PR since glycolate metabolism occurs even at low O_2 concentrations. He further notes that this method has given similar results to that of method (iii), which is predicted to underestimate PR.

(iii) The rate of CO_2 efflux from a leaf into CO_2 - free air in the light is a measure of PR. However if some of the photorespired CO_2 is refixed PR will be underestimated. This method is sometimes used with leaves prelabelled with $^{14}CO_2$. In this manner $^{14}CO_2$ efflux and $^{12}CO_2$ influx can be measured simultaneously. Although this method is more useful it too is affected by refixation of respired CO_2, and also by the internal recycling of CO_2 which can alter the specific activity of the $^{14}CO_2$.

(iv) The net rate of CO_2 uptake (net photosynthesis) of a leaf photosynthesising under steady-state conditions is a balance of CO_2 uptake (gross photosynthesis) and CO_2 release (photorespiration). If $^{14}CO_2$ is suddenly supplied the rate of $^{14}CO_2$ uptake will give a measure of gross photosynthesis. The PR rate can then be calculated as the difference between net and gross photosynthesis. Unfortunately experiments by Ludwig and Krotkov (1967) have shown that $^{14}CO_2$ fixed by a leaf, then respired, can

be detected outside the leaf in as little as 15 seconds. Clearly experiments must be of very short duration if recycling problems are to be avoided.

To summarise, although all four methods have often been used to demonstrate the existence of PR, all are prone to error. The most common source of error is the underestimation caused by the possible refixation of respired CO_2. Zelitch (1979) also notes that such refixation is an even greater problem when experiments are carried out in an aqueous medium, due to the low diffusivity of CO_2 in water. At the present time saturated lichen thalli are known to contain large volumes of water, but whether the algae are sited in an aqueous or a gaseous medium is not yet known.

Some initial observations of photorespiration and CO_2 compensation points in lichens are presented in Chapter VIII. These results lead to a more detailed study of the relationship between thallus water content and photorespiration in three lichen species (Chapter IX). In a further survey of the relationship between CO_2 compensation point, PR, and carboxylation efficiency (Chapter X) the results for *Stictaceae* lichens are grouped into three response patterns. The similarities and differences between lichen and 'higher' plant results are discussed.

SECTION D - GAS EXCHANGE RESISTANCES

The total resistance of a leaf to CO_2 uptake can be calculated from the linear slope of a photosynthesis - CO_2 concentration graph at low CO_2 levels. This total resistance can be subdivided into several components by a variety of methods (Gaastra, 1959; Jones and Slatyer, 1972). The values and ratios of these components have proved useful in our understanding of the factors limiting photosynthesis (e.g. Jarvis, 1971; Körner *et al* 1979).

Since the diffusion of CO_2 in water is about 10^4 fold slower than diffusion in air the depression of photosynthesis sometimes observed in lichens at high water contents has often been attributed to the high CO_2 resistances predicted to occur in a saturated thallus (Reid, 1960; Lange, 1980). However, although some authors (e.g. Collins and Farrar, 1978; Lange, 1980) have attempted to develop electrical analogue CO_2 resistance models for lichens, these attempts have been largely theoretical. Only one estimate of the CO_2 resistance of a lichen has been published (Collins and Farrar, 1978) and the methodology of these authors has been criticised. (See Chapter XI).

The relationship between total CO_2 resistance (Σr) and thallus water content in six lichen species is investigated in Chapter XI. In Chapter XII Σr is separated into carboxylation and transport components. The transport resistances of the lichen medulla, cyphellae and pseudocyphellae are calculated in Chapter XIII. Some authors (e.g. Collins and Farrar, 1978) have inferred a correspondence between resistance to water vapour loss and resistance to CO_2 uptake in lichens. Measurements of both parameters were made at several thallus water contents (Chapter XIV) and the relationship between water vapour resistance, Σr, and net photosynthesis is described.

MATERIALS AND METHODS

NOMENCLATURE

The taxonomy of New Zealand lichens is currently under review (Galloway, pers. com.). The nomenclature used in the present work is from Galloway (pers. com.) and Martin and Child (1972), as used by Green *et al*, (1980). However, in a recent publication (Galloway and James 1980), the nomenclature of some species of *Pseudocyphellaria* has been redefined. A comparative list of the nomenclature used in the present study and that of Galloway and James (1980) is presented below.

Present study	Galloway and James (1980)
Peltigera dolichorhiza (Nyl.) Nyl.	-
Pseudocyphellaria amphisticta Kremp.	*P. lividofusca* (Krempelh) Galloway and P. James comb. nov.
P. billardierii (Del.) Ras.	*P. billardierii* (Delise) Räsänen
P. colensoi (Bub in Hook F.) Vain	*P. colensoi* (Church Bab.) Vaino
P. delisea (Fee in Del.) D. Gall and P. James *in litt*	*P. delisea* (Fee) Galloway and P. James comb. nov.
P. dissimilis (Nyl.) D. Gall and P. James *in litt*	*P. dissimilis* (Nyl.) Galloway and P. James comb. nov.
P. faveolata (Del.) Malme	*P. faveolata* (Delise) Malme
P. homoeophylla (Nyl.) Dodge	*P. homoeophylla* (Nyl.) Dodge
Stereocaulon ramulosum (Sw.) Rausch	-
Sticta caperata Bory. in Nyl.	-
S. latifrons Rich.	-

COLLECTION

(a) *Sites.* Lichens were collected from localities in the central North Island of New Zealand listed in Table 1. The majority of the species used are normally corticolous and

TABLE 1. Collection Sites

Location	New Zealand Map Series Reference (NZMS 1)	Altitude (m.a.s.l)	Vegetation description
Hakirimata	N56 645595	90	Lowland valley forest dominated by *Beilschmiedia tawa*. Other species include *Weinmannia racemosa*, *Leptospermum scoparium*, and *Melicytus ramiflorus*.
Kauaeranga	N49 140315	120	Heterogenous cut over lowland forest. Common species are; *B. tawa*, *L. scoparium* and *Pittosporum* species.
Rangataua	N121 985500	700	Beech forest. *Nothofagus menziesii*, *N. fusca*, *N. solandri* var *cliffortioides*.
Mnt. Te Aroha	N57 233777	950	Mainly stunted *W. racemosa*. Surrounding forest contains *N. menziesii* and *Ixerba brexioides*.
Lake Waikareiti	N96 565330	900	Beech forest. *N. menziesii* - *N. fusca* association.
Lake Waikaremoana	N105 580296	700	Podocarp - beech forest. Dominant species are; *N. fusca*, *N. menziesii*, *Dacrydium cupressinum*, *Podocarpus spicatus*, *P. ferrugineus*.

Pseudocyphellaria homoeophylla, P. amphisticta, P. colensoi, P. delisea, Sticta caperata, and *S. latifrons* were usually collected from the lower 2-3 metres of tree trunks, or from tree buttresses. However *P. homoeophylla* from Mount Te Aroha was found growing on the ground beneath low *Weinmannia* shrubs. *P. billardierii* and *Usnea* were also epiphytic and were generally collected from small branches, the former in the understorey or in the subcanopy and the latter in the exposed outer canopy. In the areas studied *P. dissimilis* shows less specific substrate preferences than the above species and can be found growing on the ground, tree roots, and tree trunks. Specimens were taken from terricolous populations unless otherwise stated. *Peltigera dolichorhiza* was invariably taken from moist ground in relatively open sites. *Stereocaulon ramulosum* is commonly found in exposed situations on steep clay and rock banks and the specimens used in this work were collected from such a site at the summit of Mount Te Aroha.

(b) *Transport.* Specimens to be used on the day of collection were placed in polyethylene bags for transport to the laboratory. Lichens collected from more distant sites were air dried then transported in insulated, darkened, containers. The period between collection and arrival at the laboratory was never longer than two days. Prior to experimental use the specimens were treated as described in storage and pretreatment.

STANDARD EXPERIMENTAL PROCEDURES

(a) *Water contents - alteration before or during experiments.* The water contents of lichen thalli were adjusted by mist spraying with distilled water, blotting with paper towels

(or tissues), and drying in a stream of compressed air. In some instances thalli were immersed in distilled water to ensure complete saturation. Water contents are expressed as gram water per gram dry weight or as milligram water per milligram dry weight, both values being numerically identical. Dry weights were obtained by drying to a constant weight at 100°C.

(b) *Production of constant water content by use of constant humidity.* These were generated by filling the bottom of glass desiccators with saturated solutions of the compounds listed below (Slavick 1974). All desiccators were kept in the dark at 20 ± 0.5°C in a walk-in constant temperature room.

Compound	Relative humidity (at 20°C)	Water potential (bars at 20°C)
$Na_2 SO_3 7H_2O$	95	- 69.3
$Zn SO_4 7H_2O$	90	- 14.2
NH_4Cl	79.2	- 319
$Na NO_2$	66	- 560

Moistened lichens were placed in the desiccators and left until a constant thallus weight was reached. This normally took between two and four days.

(c) *Area Measurement.* In this study area measurement were made only on large lobed, foliose lichens. A Koisumi compensating planimeter (Type KP-27) was used. Thalli were held flat by a sheet of 1.5 mm clear glass. Thalli which naturally flattened in a multilayered arrangement, were cut into separate pieces and the areas were summed. To obtain accurate estimates, particularly of small specimens, all thalli were traced repeatedly until the total area measured was at least 10 cm^2.

The area measured by this method is that of the 'shadow area' and is equivalent to only one side of the lichen. The use of this area in photosynthesis and respiration measurements is suggested to be valid since CO_2 exchange occurs predominantly through one surface (Chapter VI).

(d) *Anatomical Measurements*. Thin sections were hand cut from moistened thalli about 1 cm from the lobe tips. Thallus layer depths were measured using a binocular microscope fitted with a calibrated eyepiece. Some vertical and horizontal sections were viewed with a Joel-JSM 35 scanning electron microscope. Thalli used for this purpose were prepared for sectioning by either air drying or hydrating in distilled water, fixing in 4% glutaraldehyde (buffered with 0.025 M phosphate buffer, pH 7.0) for 12 h, dehydrating through an ethanol series, then critical point drying with CO_2. All specimens were coated with 50 nm of gold and palladium. Anatomical measurements were made from scanning electron micrographs.

MEASUREMENT OF CO_2 EXCHANGE

(a) *Introduction*. Lichen photosynthesis and respiration rates can be measured by several techniques including; O_2 evolution or uptake, ^{14}C exchange, and CO_2 exchange. The former technique (e.g. Smythe, 1934; Pearson and Skye, 1965) is now used infrequently since it involves subjecting the lichen to unnatural (and often undefined) O_2 and CO_2 concentrations. The ^{14}C method has been more commonly used (e.g. Hill, 1971; Hallgren and Huss, 1975; Farrar, 1976a, 1976b) but this too is not satisfactory in that the CO_2 concentrations used may not approximate ambient levels. The practice of floating lichen

discs on ^{14}C labelled bicarbonate solutions further exacerbates this problem by supplying ^{14}C both as $^{14}CO_2$ and $H^{14}CO_3^-$. Since variations in O_2 and CO_2 levels are known to affect net photosynthesis in 'higher' plants (e.g. Brown, 1980), the use of experimental techniques in which the concentrations of these gases are varied, or not monitored, appears to be unwise.

The methods used for determining CO_2 concentrations in air have been reviewed by Šesták *et al* (1971). For botanical work the instrument most frequently used for this purpose is the infra red gas analyser (IRGA). Such instruments accurately measure CO_2 concentrations by assessing the amount of infra red radiation absorbed by the CO_2 in a gas mixture (Janáč *et al*, 1971). Continuous monitoring of CO_2 concentrations can be achieved by pumping air through the IRGA analysis tube. The incubation chamber - IRGA gas exchange system may be arranged in the following ways:

(1) Closed system.

Air is continuously recirculated through the IRGA and the incubation chamber and the decrease in CO_2 concentration is recorded. This method has practical advantages in that the system is simple, accurate measurements of flow rates are not necessary, and the dehydrating effect of the air flow is limited since water lost from the plant increases the humidity of the air. A disadvantage of the system is the lack of a stable CO_2 concentration.

(2) Semi-closed system.

As above except that CO_2 is added to (or removed from) the system in such a way as to maintain a near-constant CO_2 concentration. For accurate control of CO_2 fluctuations very precise recording flow meters are required.

(3) Open system.

Air of known CO_2 concentration flows through the incubation chamber, then the IRGA, then to waste. A second gas line through only the IRGA provides a reference CO_2 concentration. This method allows rapid assessment of CO_2 exchange rates under steady state conditions. However photosynthesis rates must be high enough to measurably alter the CO_2 concentration of the air passing through the chamber. At the same time flow rates and the size of the chamber must be balanced in order to maintain short gas turnover times. (This is less critical when chambers are adequately ventilated). In the past the low photosynthetic rates of lichens have resulted in some workers using flow rates so low that turnover times were over five hours (Larson and Kershaw 1975b).

(4) The IRGA can also be used as a discrete sample analyser. A carrier gas is pumped through the IRGA analysis cell and small gas samples (usually 1.0 - 3.0 cm^3) are injected directly into the analysis cell. The CO_2 content of the sample is estimated from the momentary deflection of the IRGA meter as the sample passes through the analysis tube. Plant samples are incubated in individual cuvettes and CO_2 exchange rates are calculated from periodic determinations of the CO_2 concentration. The main advantage of this system is that many cuvettes may be run simultaneously using only one IRGA. Also cuvettes may be simple in design since Larson and Kershaw (1975b)report that rates of lichen photosynthesis are not measurably affected by fluctuations in CO_2 concentration between 150 and 350 $\mu l\ CO_2\ l^{-1}$ or by the absence of ventilation. This contrasts with the strong requirement for ventilation and the CO_2 concentration dependance reported

for higher plants (Jarvis 1971).

(b) *This Work.* In this investigation rates of CO_2 exchange were measured using an Analytical Development Company (ADC) infra red gas analyser operating in one of the following ways:

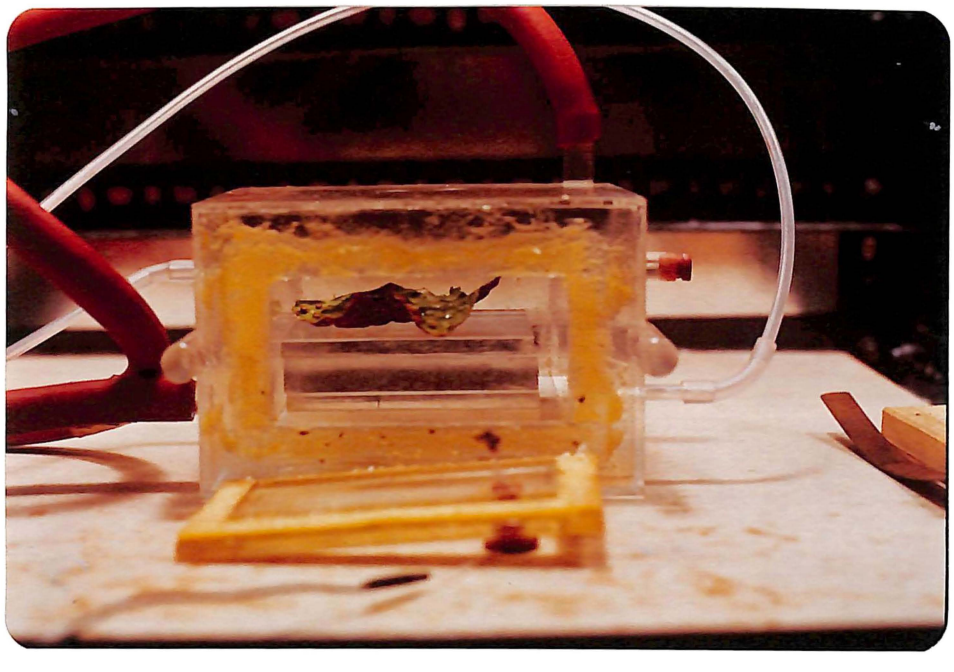
(1) As a discrete sample analyser of 1 cm^3 gas samples withdrawn from a 30 cm^3 incubation chamber.

(2) In a closed loop system.

Method (1) is used only in Chapters II, V and VIII and is fully described in Chapter V. Method (2) a standard technique used throughout this work and is therefore described here in some detail. A variation of this method using a divided incubation chamber (Figure 1b) is presented in Chapter VI.

The perspex chamber used as a flow through cuvette is shown in Figure 1a. A 2 cm thick water jacket surrounds five sides of the cuvette and water pumped through this jacket controls the air temperature within the cuvette. The door of the cuvette was edged with foam plastic impregnated with petroleum jelly and was held firmly in position by a high tensile copper bar. Air was circulated from the pump fitted to the IRGA through the cuvette and flow gauge (Marconi 0-1 $\ell\ min^{-1}$) to the 0-500 $\mu l\ l^{-1}$ CO_2 analysis cell at a rate of 0.5 $\ell\ min^{-1}$. From there it returned to the pump, thus forming a closed loop system. High density nylon tubing and polypropylene tubing were used for all gas lines. The total internal volume of the system, including the cuvette, was 550 cm^3. However this was normally reduced to 370 cm^3 by inserting solid perspex blocks as shown in Figure 1a. The system was

A



B

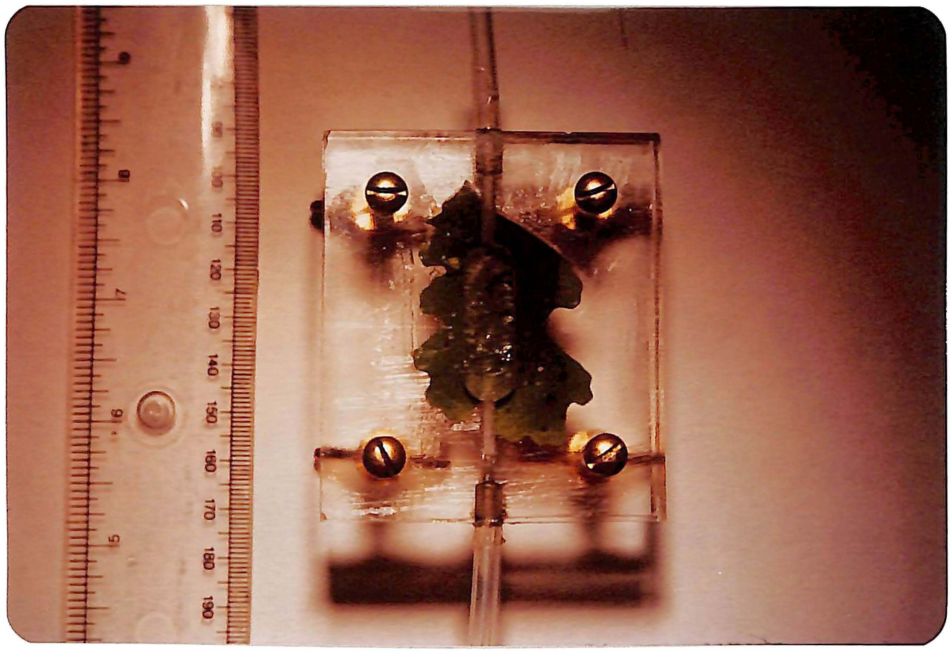


FIGURE 1 A. CO_2 exchange cuvette with lichen in place on perspex blocks. Door in foreground.

1 B. Top view of split chamber with lichen in place. Scale divisions in inches and centimetres.

periodically tested for CO_2 leakage by running it empty at a CO_2 concentration below 150 µl l^{-1}.

Plant temperature within the cuvette was maintained at 16 ± 0.5°C and was monitored directly with a thermistor probe. Early experiments showed that thallus temperature could be more conveniently assessed by covering the thermistor probe with a heat-killed, moistened, lichen and this procedure was used throughout.

Most experiments were carried out at about ambient CO_2 and O_2 levels (350 µl CO_2 l^{-1}, 21% O_2). When low CO_2 concentrations were required the system was purged with CO_2 free air for several minutes, thereby reducing the CO_2 levels to near Zero. The CO_2 concentration could then be adjusted to the required value by injecting small volumes of a 20,000 µl CO_2 l^{-1} standard gas mixture. Low O_2 levels were created by purging the system with O_2 - free nitrogen for 3 minutes at a flow rate of 0.7 ℓ min^{-1}. Analysis of gas samples by thermal conductivity gas chromatography showed that this consistently reduced O_2 levels to about 1%, yet did not result in levels so low that dark respiration was affected (see Chapter IX). CO_2 concentrations were then adjusted by injection of high concentration CO_2 mixtures (in nitrogen) as described previously.

The IRGA reading was amplified (two to five fold) and continuously graphed on a servoscribe chart recorder. Photosynthetic (or respiratory) rates were obtained from the slope of the CO_2 - time curve at chosen CO_2 concentrations. Incubation periods were generally between 10 and 30 minutes, although some much longer periods were used when lichens were left until the CO_2 compensation point was reached. (Chapters VIII and XI).

Closed loop flow through systems can be criticised on the grounds that CO_2 exchange rates are not measured under steady state conditions, since CO_2 concentrations are constantly changing. In order to evaluate any systematic changes in net photosynthesis (NP) three successive incubations under identical experimental conditions were run using the same specimen of *S. latifrons*. Further NP measurements were then made under changed conditions. The results are presented below in chronological order. All rates are in mg CO_2 g^{-1} dry weight h^{-1}.

Series	Experimental conditions	NP			Mean	Standard error of Mean
1	wc* = 1.34, 21% O_2, 350 µl CO_2 l^{-1}	0.84	0.84	0.86	0.85	0.01
2	wc = 1.35, 1% O_2, 350 µl CO_2 l^{-1}	1.24	1.33	1.31	1.29	0.05
3	wc = 1.23, 21% O_2, 800 µl CO_2 l^{-1}	1.46	1.46	1.44	1.45	0.01

* wc = water content.

It is clear that the precision of this method is high. There are no indications of systematic changes in NP with repeated measurements, even following large changes in O_2 and CO_2 levels. The rapidity of the NP response may be due to the lack of a variable, physical control of gas exchange, such as the stomata of higher plants.

STORAGE AND PRETREATMENT

(a) *Preliminary Studies.* Some initial studies of the changes in dark respiration rate following storage were made using *P. dissimilis* and the injection IRGA technique (see Chapter V for method). Results for freshly collected samples are given in Figure 2. Storage trials were carried out under the following conditions: 53% RH in continual darkness at 16°C, 100% RH in

continual darkness at 16°C, 100% RH on a 12 h dark/12 h light cycle (70 μE m^{-2} s^{-1}) at 16°C. The results of this series are shown in Figures 3 and 4. For clarity of presentation only the hand fitted curves are presented but the number and scatter of data points for each curve were similar to those of Figure 3a. It is evident that the respiration rate of wet stored specimens continued to change during the seven days of storage. These changes were most obvious at high thallus water contents. In the dry stored series (53% RH) a substantial increase in respiration rate occurred during the first day but after this changes were minor and no trend was apparent. An interesting feature of these results is the rapid change that occurred in the five hours between the first and second experimental runs. During this time thalli were stored in a wet condition in polyethylene bags at 20 μE m^{-2} s^{-1}, 16°C. The reason for this change is not known.

Further investigations of storage effects with *P. billardierii* and *P. dissimilis* showed that both photosynthesis and respiratory rates changed markedly during seven days light or dark storage at 100% RH. (Figures 5 and 6). Moreover it was notable that the photosynthesis rate of *P. dissimilis*, a species usually collected from moist habitats, tended to increase during storage in the light while that of *P. billardierii* (normally found in a drier environment) decreased during the same treatment. The observed differences in thallus water contents during measurements of CO_2 exchange rates (see Figures 5 and 6) could not account for these changes (Chapter V).

(b) *Standard pretreatment routine.* The results of other workers (see Introduction) and the above results indicate that the maintenance of stable rates of CO_2 exchange during storage is

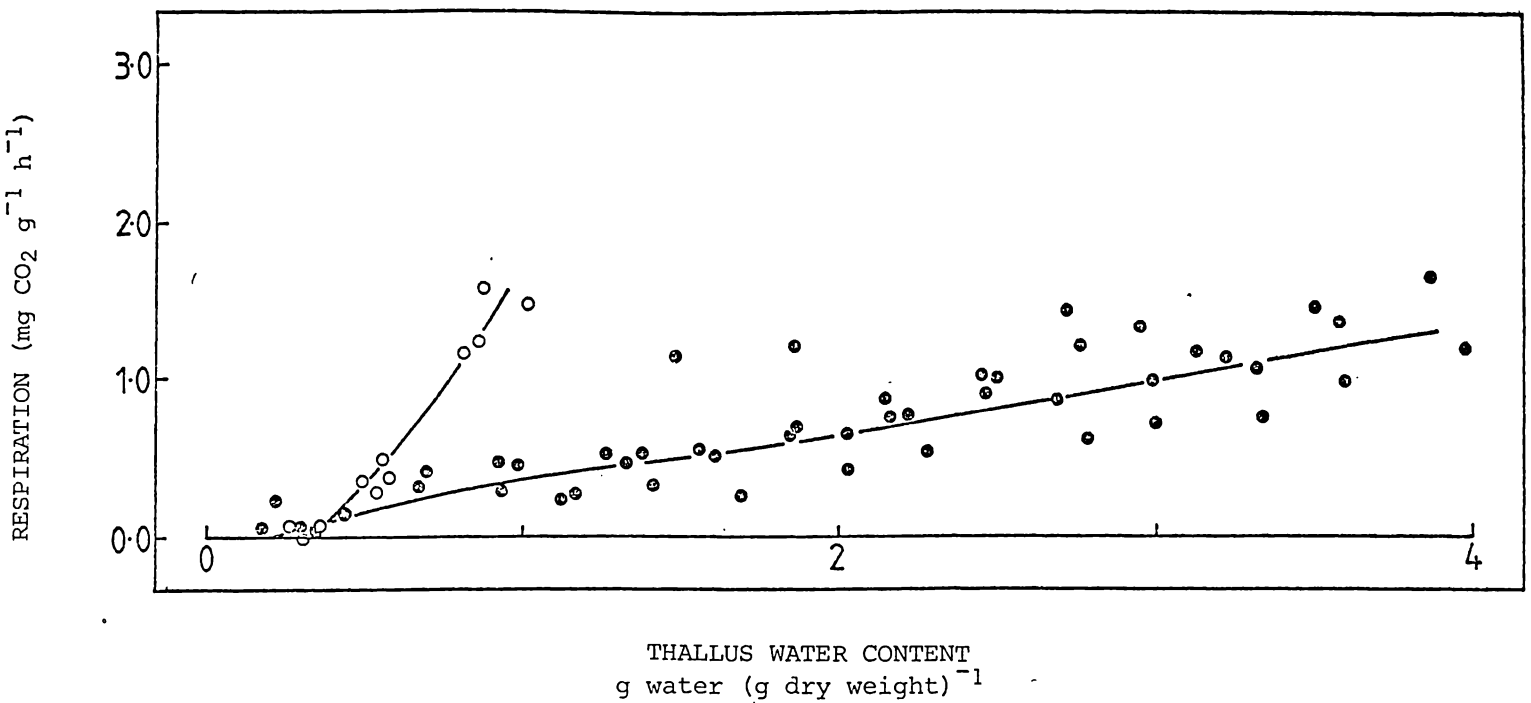


FIGURE 2. Relationship between dark respiration rate and thallus water content for freshly collected (⊙) *Pseudocyphellaria dissimilis*. (o) = second experiment using specimens which had been air-dried, then rewetted. All measurements made at 16°C, in darkness, using the injection IRGA technique.

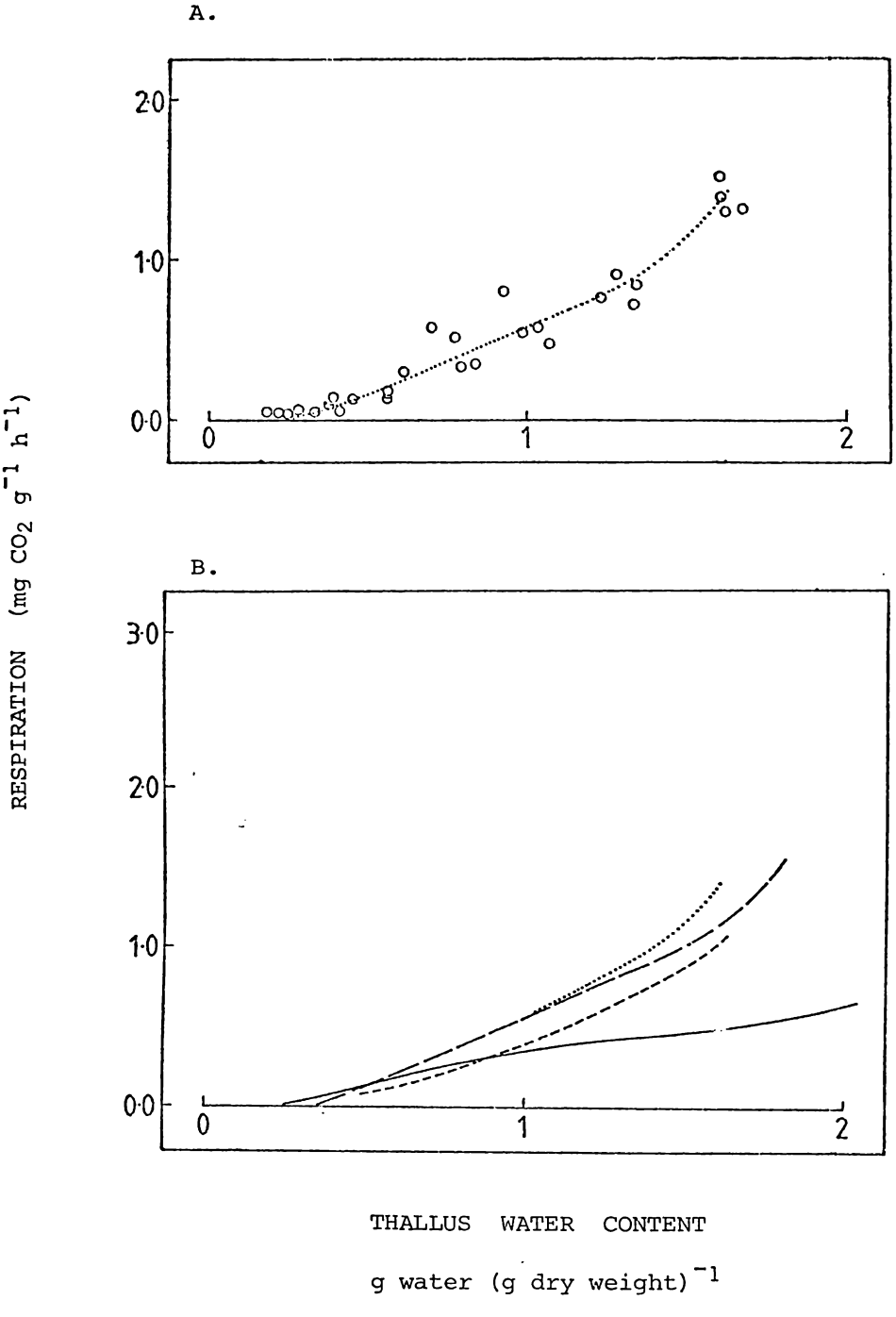


FIGURE 3. Relationship between dark respiration rate and thallus water content for *Pseudocyphellaria dissimilis* following storage at 53% RH in continuous darkness.

A - Result of 2 days storage showing data points and hand fitted curve.

B - Result of 0(——), 0.2(--), 1(---) and 2(···) days storage.

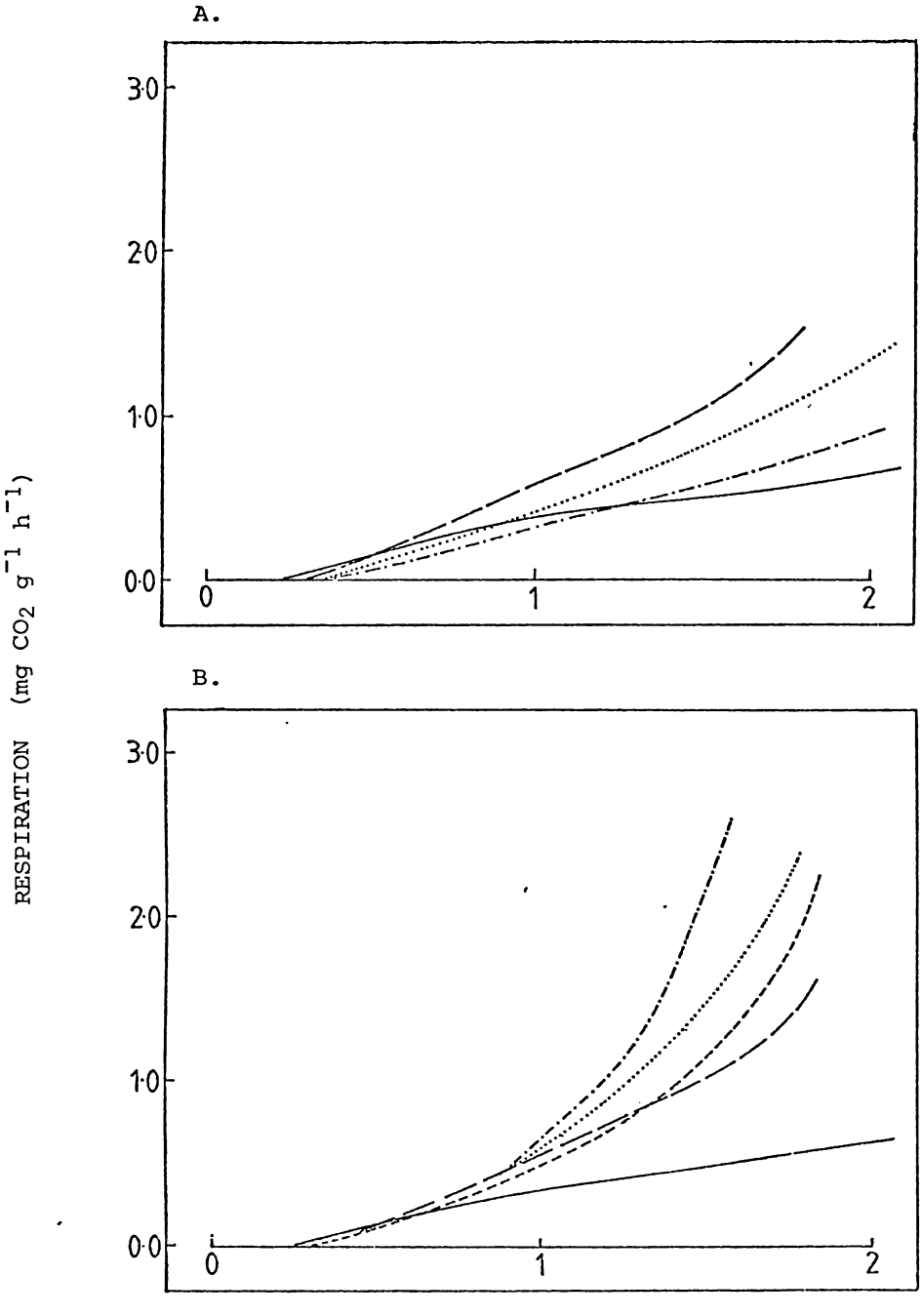


FIGURE 4. Relationship between dark respiration and thallus water content for *Pseudocyphellaria dissimilis* following storage at 100% RH in (A) darkness, and (B) on a 12 h dark/12 h light cycle. Duration of storage in days was; 0(——), 0.2(— —), 1(--), 2(···) and 7(·—·—).

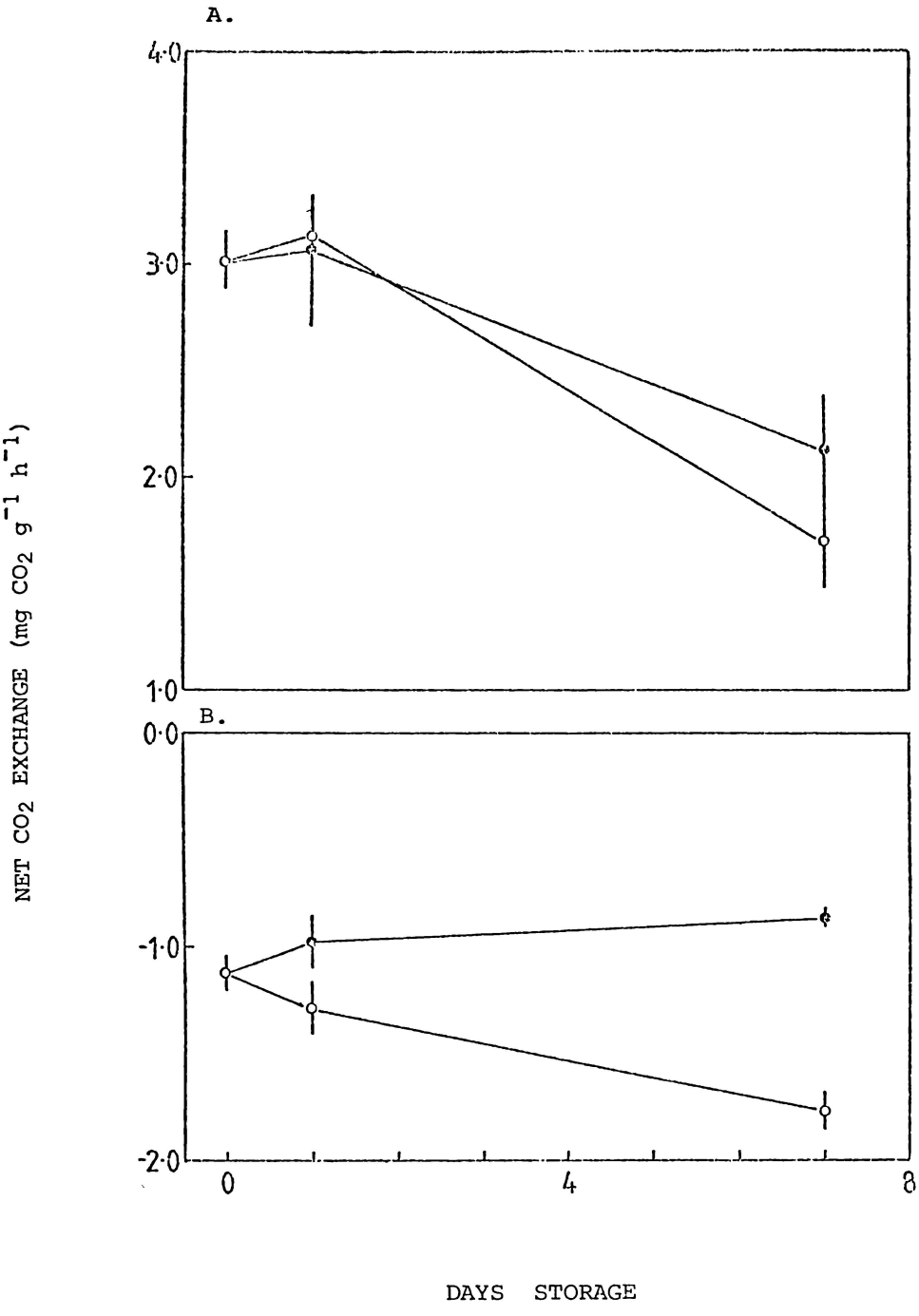


FIGURE 5. Net photosynthesis (A) and dark respiration rates (B) of *Pseudocyphellaria billardierii* following storage at 70 µE $m^{-2}s^{-1}$on a 12 h light/12 h dark cycle (O) or in the dark (⊘). During storage all thalli were kept moist and at 16°C. Rates of CO_2 exchange were measured at 16°C in darkness or at 70 µE m^{-2} s^{-1} using the injection IRGA technique. Bars represent the standard error of the mean. During CO_2 exchange measurements thallus water contents ranged from 1.3 - 1.9 g g^{-1}.

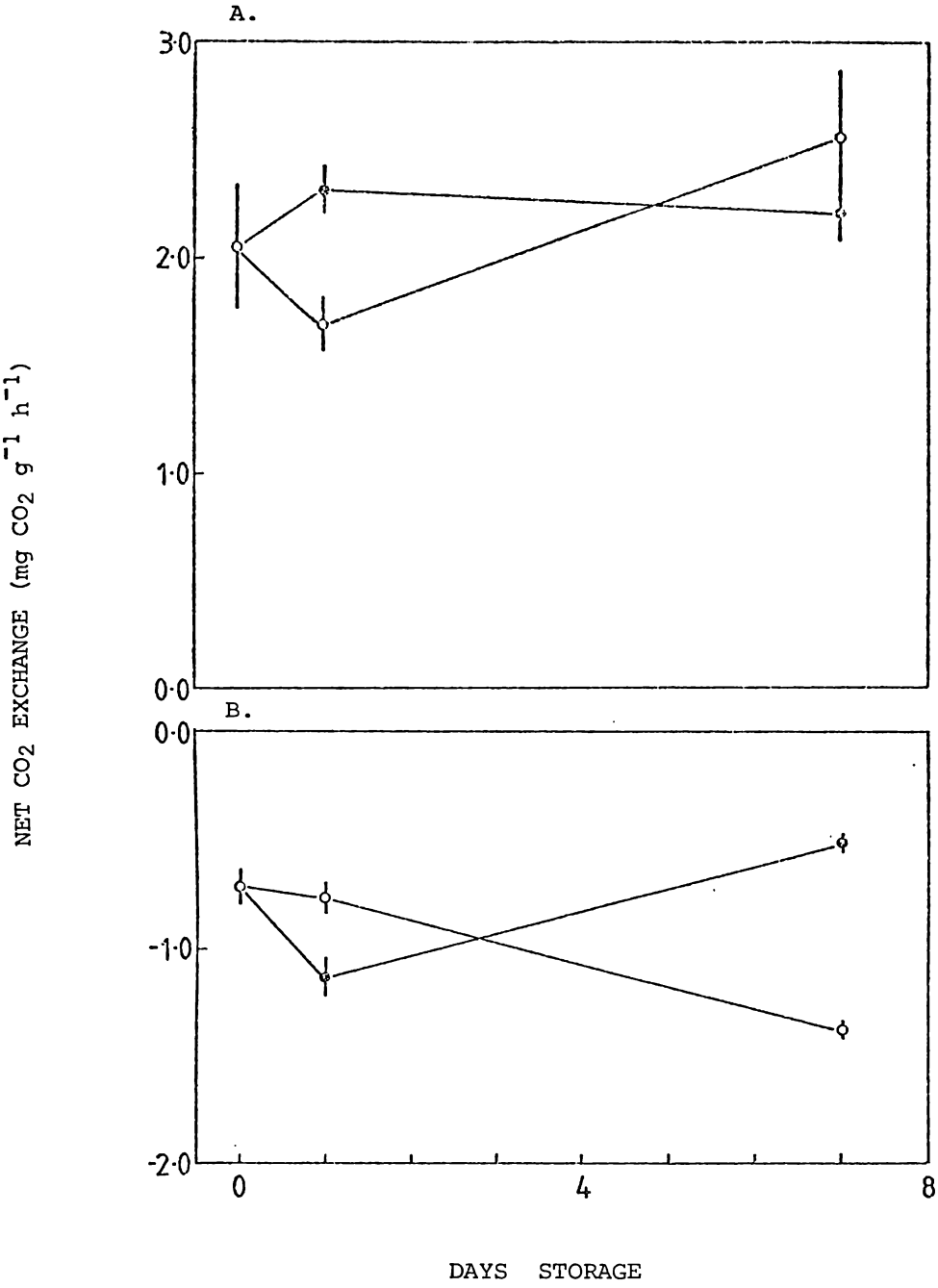


FIGURE 6. Net photosynthesis (A) and dark respiration rates (B) of *Pseudocyphellaria dissimilis* following storage at 70 µE m^{-2} s^{-1} on a 12 h light/12 h dark cycle (O) or in the dark (●). During storage all thalli were kept moist at at 16°C. Rates of CO_2 exchange were measured at 16°C in darkness or at 70 µE m^{-2} s^{-1} using the injection IRGA technique. Bars represent the standard error of the mean. During CO_2 exchange measurements thallus water contents ranged from 1.6 to 2.1 g g^{-1} except for the light stored, day one photosynthesis determination which was 1.4 g g^{-1}.

a complex exercise, especially when lichens are collected from diverse habitats. Stability could be verified only by conducting an extensive series of multifactorial experiments designed to elucidate the effects of thallus water content, season, climate, light intensity and temperature for each species used. Because such an exercise is beyond the scope of the present work the following standard routines were adopted:

(1) Where possible only fresh specimens (used on the day of collection) were used. These thalli were stored in a moist condition at 100% RH, 50 $\mu E\ m^{-2}\ s^{-1}$, 16°C until required.

(2) Thalli not able to be used immediately were stored dry over silica gel at 16°C in darkness. This treatment resulted in very low and uniform levels of thallus water content, typical mean values ($\pm$ standard error of the mean) being; 0.030 $\pm$ 0.006 (*S. latifrons*), 0.021 $\pm$ 0.001 (*P. colensoi*), 0.031 $\pm$ 0.004 (*P. amphisticta*) and 0.033 $\pm$ 0.004 (*P. homoeophylla*). The day prior to experimental use, the lichens were moistened by spraying with distilled water and stored at 16°C, 100% RH 50 $\mu E\ m^{-2}\ s^{-1}$ on a 12 h light/12 h dark cycle until required. This overnight 'reactivation period' was used to eliminate the resaturation respiration commonly observed when dry lichens are rewetted (Smith and Molesworth, 1973; Farrar and Smith, 1976). Larson (1979b) measured the net photosynthetic rate of rewetted *Umbilicaria* species and concluded that steady state CO_2 exchange was attained within 60 minutes of rewetting but the rates of some species decreased after being held in a moist condition for more than 10 hours. Similar decreases may have occurred during the present investigation. The rates of photosynthesis of freshly collected material (Chapter V) were

often higher than those of stored material (Chapter IX). Generally, stored lichens were used only in experiments designed to examine the effects of O_2 and CO_2 concentrations, rather than in experiments where the absolute rates were of significance.

LIGHT RESPONSES

(a) *Introduction and methods.* Information on the light responses of members of the Stictaceae was required both as an aid for ecological interpretation of data, and as a basis for laboratory work. Fresh material was used wherever possible (Table 2) as MacFarlane and Kershaw (1980) and Kershaw and MacFarlane (1980) have demonstrated that light acclimation can occur in air-dry lichens. Because field collected lichens were sometimes dry all specimens were moistened by mist spraying with distilled water at least two hours prior to experimental use. During experiments thallus water contents were maintained (as described previously) at values expected to produce optimal, or near optimal, rates of photosynthesis (cf Chapter V). An additional series of curves at several thallus water contents was obtained for *S. latifrons*. All CO_2 exchange rates were measured at 16°C, 350 µl CO_2 l^{-1} in the 370 cm^3 flow-through perspex cuvette. Light levels (Photosynthetically Active Radiation, PhAR) within the 400 - 700 nm waveband were measured inside the cuvette using a Licor quantum probe (190 s). Light was provided by two 'Atlas' white 20 watt fluorescent tubes (colour number 35) or a Philips 1000 watt 12013 R tungsten halogen lamp. When using the latter two 10 cm deep water baths were placed between the lamp and the cuvette to reduce heat input, (Figure 7). Light intensities were altered by moving the light source or inserting shade cloth (70% and 50%

TABLE 2. Collection data for specimens used in light intensity experiments

Species	Collection Date	Site[1]	Days Storage
Pseudocyphellaria amphisticta	19.3.80	W	5
Pseudocyphellaria billardierii	14.2.80	H	0
Pseudocyphellaria colensoi	24.11.79	R	0
Pseudocyphellaria delisea	30.11.79	R	0
Pseudocyphellaria homoeophylla	23.11.79	R	0
Peltigera dolichorhiza	19.3.80	H	1
Sticta latifrons	26.3.79	R	0
Sticta latifrons[2]	16.9.80	H	2

[1] W = Lake Waikareiti, R = Rangataua, H = Hakirimata.

[2] This collection used for light intensity - water content work. (Figure 12)

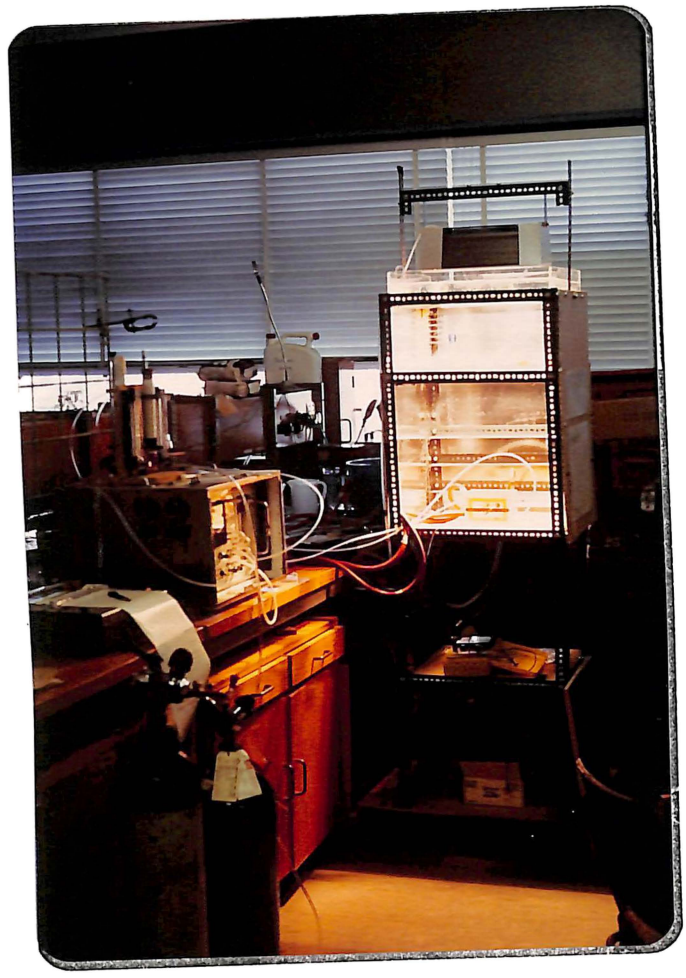


FIGURE 7. General view showing the arrangement of the IRGA, cuvette, tungsten halogen lamp, and water baths.

transmission, Sarlon). Dark respiration rates were measured after covering the cuvette with several layers of black cloth. The sequence of light levels used during experiments was varied. However high levels (>200 μE m^{-2} s^{-1}) were always used last in order to avoid possible light induced damage to the lichens (Kershaw and MacFarlane, 1980).

(b) *Results.* Net photosynthesis versus light intensity curves for *Pseudocyphellaria amphisticta, P. billardierii, P. delisea, P. homoeophylla, Peltigera dolichorhiza, Sticta filix* and *S. latifrons* are presented in Figures 8 to 11. The response curves for all species are extremely similar with NP increasing rapidly with PhAR so that photosynthetic rates are generally maximal, or near maximal, at 100 μE m^{-2} s^{-1}. At higher light levels NP remains approximately constant, although at the highest levels used there is some indication of an NP decrease for *P. billardierii* and an increase for *Peltigera dolichorhiza*. Light compensation points were in the range 6 - 12 μE m^{-2} s^{-1}. The effect of thallus water content on the NP - PhAR response of *S. latifrons* is shown in Figure 12. At a water content of 1.4 the overall response is similar to that of Figure 9B. The other curves for *S. latifrons* generally show that at high thallus water contents NP is depressed and dark respiration increased. The effect of suboptimal water contents is to decrease both NP and dark respiration. These changes decrease the initial slope of the NP - PhAR curve, but have little effect on the compensation point. Both sub-optimal and supra-optimal water contents tend to lower the light saturation point.

(c) *Discussion.* In a review of the literature on the light responses of lichens Kallio and Karenlampi (1975) stated that most species are light saturated at between 10 and 20 klux

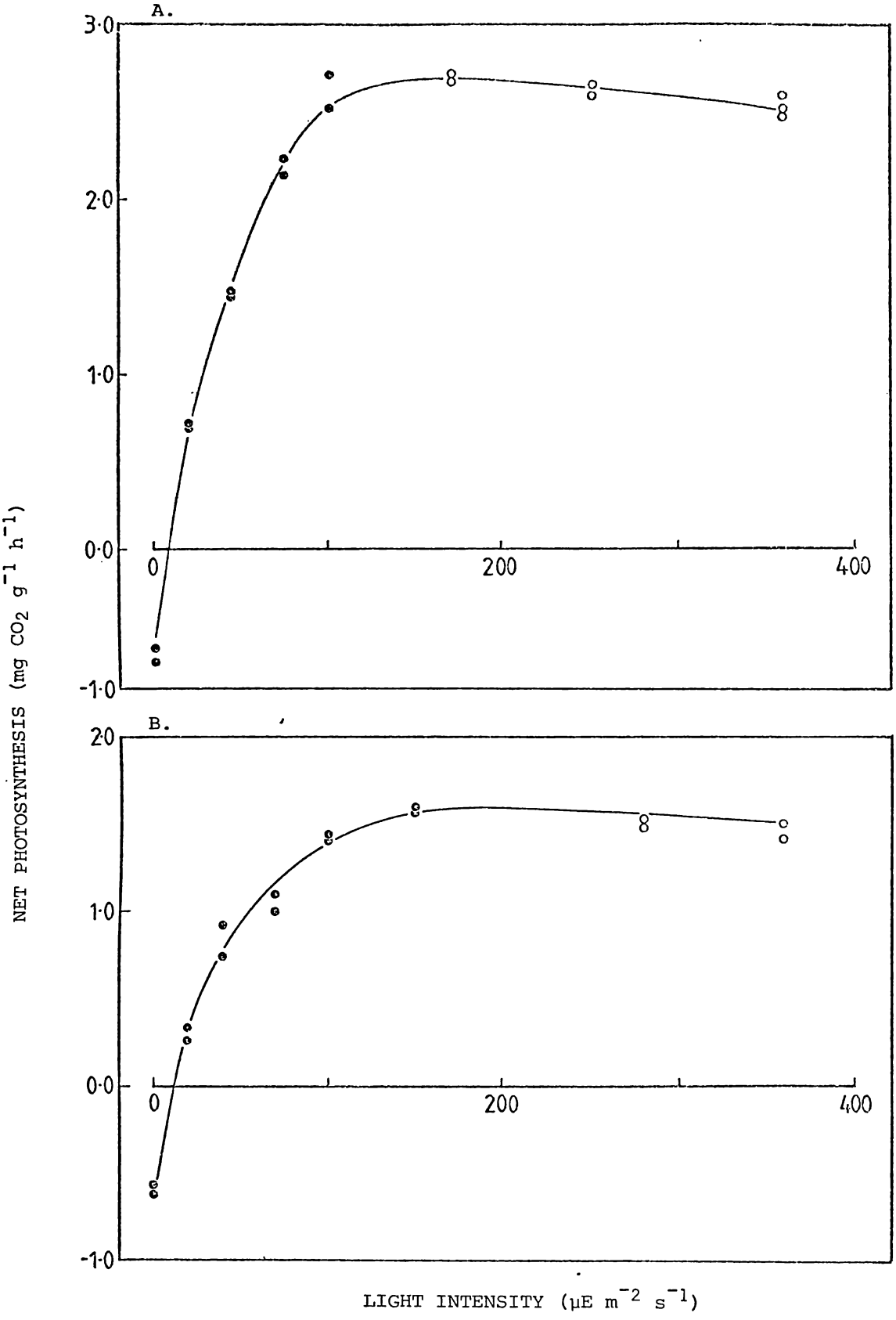


FIGURE 8. Net photosynthesis versus light intensity.
A = *Pseudocyphellaria homoeophylla*, thallus water content 2.5 - 3.0 g g^{-1}
B = *Pseudocyphellaria delisea*, thallus water content 1.2 - 1.6 g g^{-1}
Data obtained using a tungsten halogen lamp (o) or fluorescent tubes (⊗).

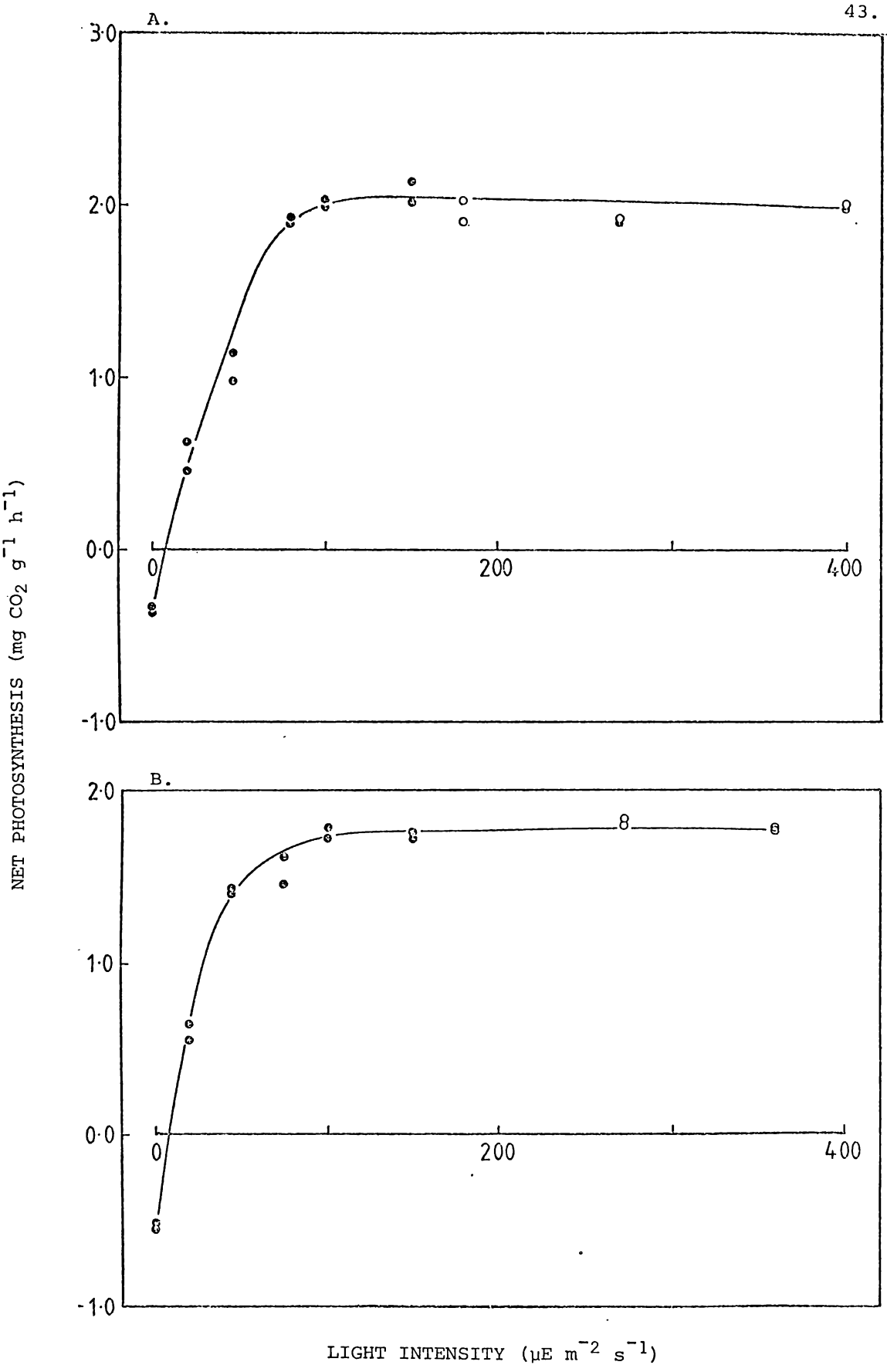


FIGURE 9. Net photosynthesis versus light intensity.
A = *Pseudocyphellaria colensoi*, thallus water content 0.9 - 1.0 g g^{-1}
B = *Sticta latifrons*, thallus water content 1.4 - 1.6 g g^{-1}
Data obtained using a tungsten halogen lamp (o) or fluorescent tubes (●)

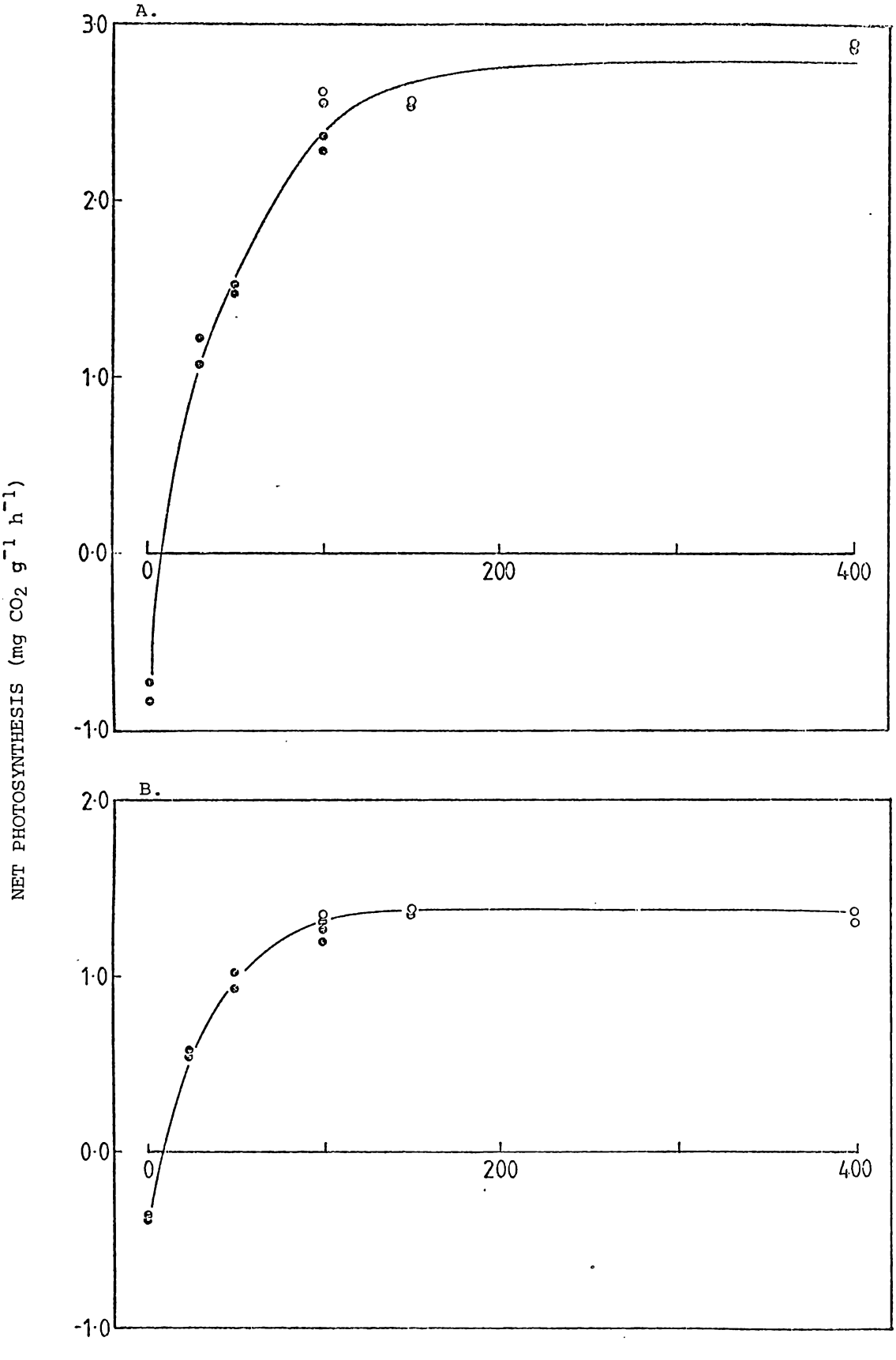


FIGURE 10. Net photosynthesis versus light intensity.
A = *Peltigera dolichorhiza*, thallus water content 4.4 - 5.2 g g^{-1}
B = *Pseudocyphellaria amphisticta*, thallus water content 2.3 - 2.6 g g^{-1}
Data obtained using tungsten halogen lamp (o) or fluorescent tubes (●).

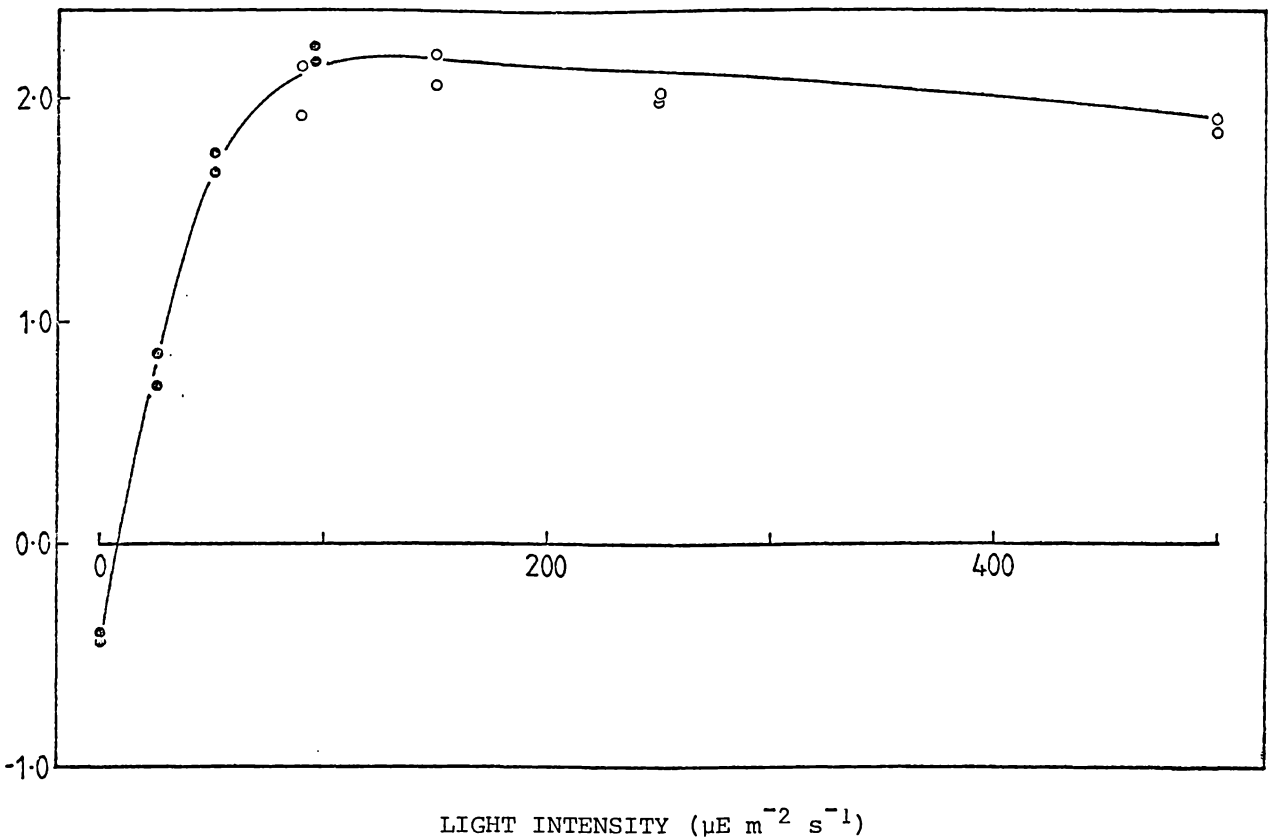


FIGURE 11. Net photosynthesis versus light intensity. *Pseudocyphellaria billardierii*, thalli water content 1.1 - 1.2 g g^{-1}. Data obtained using a tungsten halogen lamp (o) or fluorescent tubes (⊕).

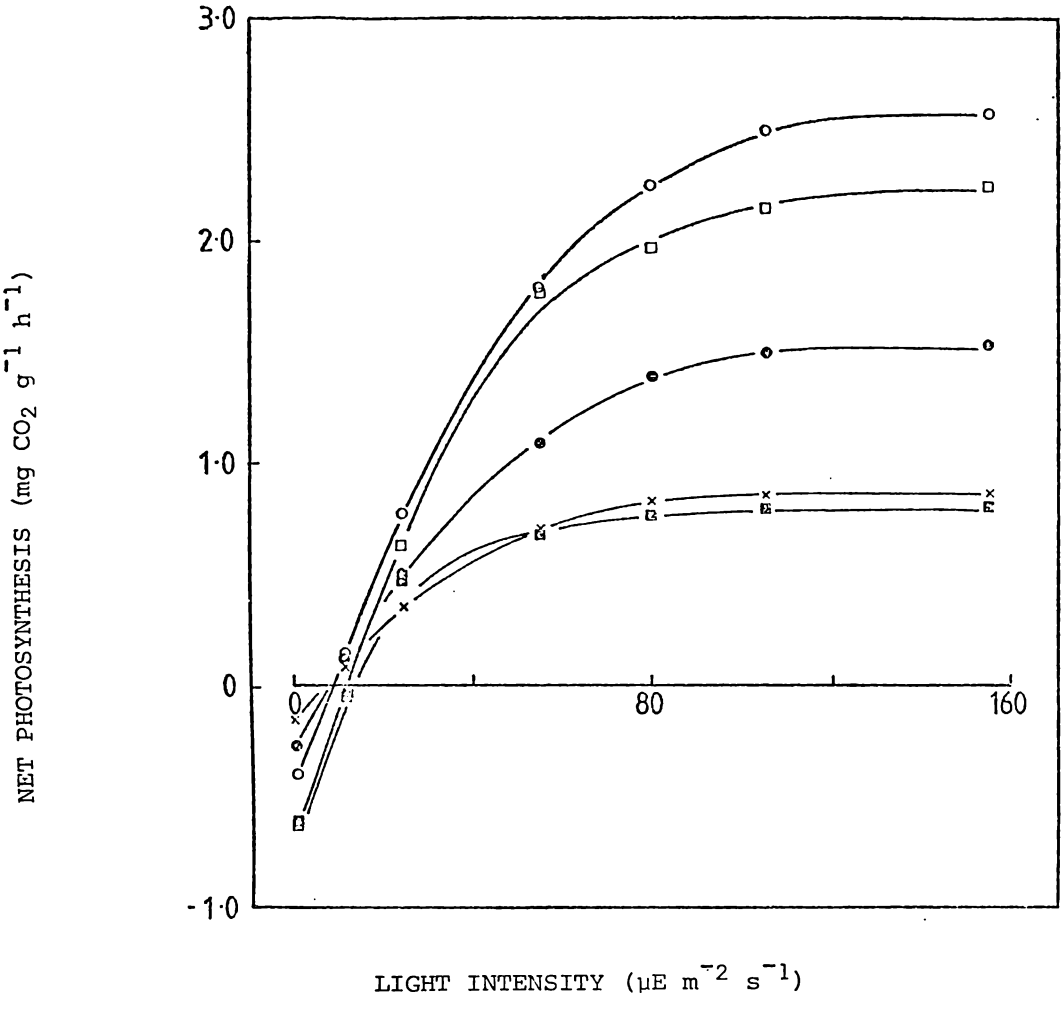


FIGURE 12. The effect of light intensity on net photosynthesis in *S. latifrons* at several thallus water contents. (o) = 1.4, (□) = 2.05, (⊙) = 0.71, (x) = 0.47, (⊠) = 2.83. All values in g water (g dry weight)$^{-1}$. All data obtained using fluorescent tubes.

(10 - 20% full sunlight), although some species from open habitats require over 48 klux (45% f.s.). Reported light compensation points range from 150 - 2000 lux (0.15 - 2.0% f.s.). The light compensation point results of the present study fall within this range, but the light saturation values are rather lower (5 - 7.5% f.s.), probably because the lichens were collected from low light environments. Information on the light responses of lichens from temperate forests is sparse. The only equivalent work was carried out by Rundel *et al* (1979) and they found higher light saturation values of 400 $\mu E\ m^{-2}\ s^{-1}$ for *Sticta filix* and 500 $\mu E\ m^{-2}\ s^{-1}$ for *Pseudocyphellaria delisei* at 20°C. (The latter appears to be a synonym of *P. delisea*, see Galloway and James, 1980). Some of the differences between these results and those of the present study may have been caused by the seven day (minimum) acclimation period used by Rundel *et al* (1979). Furthermore *P. delisei* is described as being characteristic of forest edges whereas the *P. delisea* used in the present study was always collected from the interior of *Nothofagus* forests where light intensities are low (about 5% incident light, see Chapter V).

From consideration of the results a light level of 150 $\mu E\ m^{-2}\ s^{-1}$ was chosen as an adequate and convenient saturating light intensity for future work with these species.

CARBON DIOXIDE DIFFUSION RESISTANCES

(a) *Total resistance*. Total CO_2 diffusion resistances (ΣR) in lichens were calculated from the initial linear slope of NP - CO_2 curves (e.g. Figure 13A) at 16°C, >1% O_2, 150 $\mu E\ m^{-2}\ s^{-1}$. The low O_2 concentration served to inhibit photorespiration which would otherwise depress NP, thereby resulting in an over-estimate of Σr. The Σr value included the boundary layer,

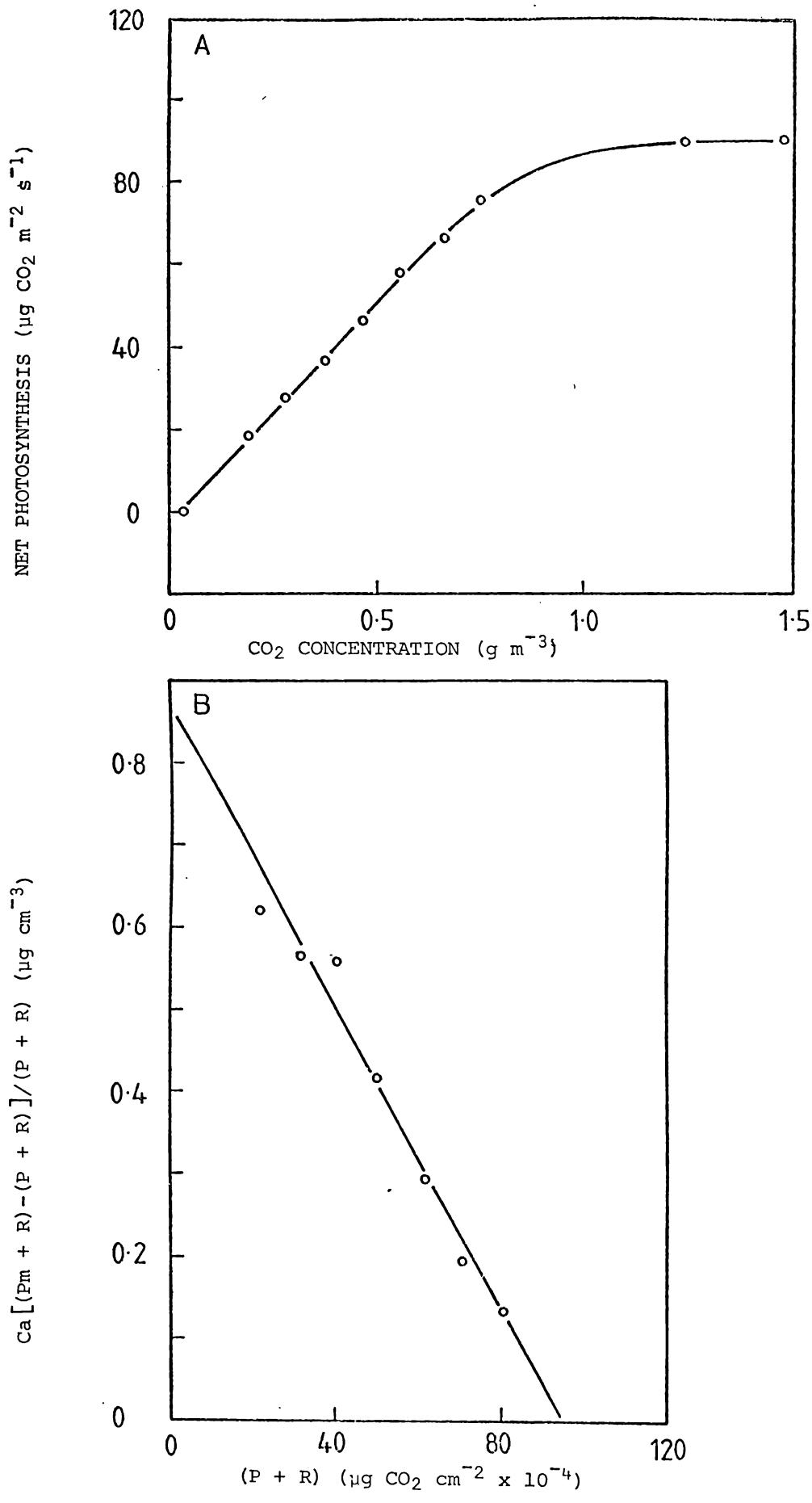


FIGURE 13. A. NP - CO_2 response of *Pseudocyphellaria amphisticta* at a thallus water content of 3.24 g g^{-1}

B. The same data transformed to a linear form see text for explanation.

internal transport, and carboxylation resistances. Σr were calculated as

$$\Sigma r = \frac{(Ca - \tau)}{P} 1.874 \times 10^{-3}$$

where Ca = ambient CO_2 concentration (µl CO_2 l^{-1})

τ = CO_2 compensation point (µl CO_2 l^{-1})

P = NP at Ca (µg CO_2 m^{-2} s^{-1})

1.874×10^{-3} converted µl CO_2 l^{-1} to g m^{-3}.

The values so calculated were expressed in units of seconds per metre, although for convenience these were usually converted to seconds per centimetre.

(b) *Boundary layer resistances* (r_a). These were estimated by the following methods:

(1) When the windspeed is known r_a can be calculated from

$$r_a \text{ (s cm}^{-1}) = 1.3\sqrt{\frac{u}{d}}$$ Monteith (1965)

where d = the characteristic dimension of the lichen (e.g. diameter) in cm.

u = windspeed in cm s^{-1}

(2) In nominally still air the depth of the boundary layer (D) can be calculated as

$$D \text{ (cm)} = \frac{\pi d^{0.6}}{8}$$ Meidner and Mansfield (1968)

where d = the characteristic dimension of the lichen in cm.

From this r_a can be derived as:

$$r_a \text{ (s cm}^{-1}) = \frac{D}{D_{CO_2}}$$

where D_{CO_2} = the diffusivity of CO_2 in air (0.147 cm^2 s^{-1} at 15°C, Šesták *et al* 1971)

(3) The r_a of filter paper models (7.0 cm diameter discs) was measured as:

$$r_{a(H_2O)} \quad (s\ cm^{-1}) = \frac{q_s - q_a}{E}$$ Lansberg and Ludlow (1970)

where q_s = water vapour concentration (g cm^{-3}) at the liquid-air interface

q_a = water vapour concentration (g cm^{-3}) of the ambient air.

E = the evaporation rate from the filter paper in g cm^{-2} s^{-1}.

This $r_{a(H_2O)}$ was converted to $r_{a(CO_2)}$ by multiplying by 1.37 (Chartier *et al* 1970). Details of experimental techniques can be found in Chapter XII.

(c) *Subdivision of* Σr. Σr was divided into transport (r_t) and carboxylation (r_e) resistances by a modification of the method of Jones and Slatyer (1972). This method is based on the transformation of an NP - CO_2 curve (e.g. Figure 13) into the linear form:

$$\frac{Ca[(P_m + R) - (P + R)]}{(P + R)} \quad vs \ (P + R)$$

where Ca = ambient CO_2 concentration

P = NP at Ca

R = rate of CO_2 release at Zero CO_2 concentration

The derivation of this formula and the means by which r_t and r_e are estimated are fully explained in Chapter XII.

PHOTORESPIRATION

Rates of photorespiration were measured by the following techniques:

(i) NP were measured at 16°C, 150 μE m^{-2} s^{-1}, 350 μl CO_2 l^{-1} under low (<1%) and ambient (21%) O_2 concentrations. The effect of these variations in O_2 level on dark respiratory rates was checked at 16°C, 350 μl CO_2 l^{-1} in a darkened cuvette. Photorespiratory rates were calculated as the difference in NP at <1% and 21% O_2. Care was taken to ensure that thallus water contents were identical at both O_2 concentrations.

(ii) The rate of CO_2 evolution into CO_2 - free air at 16°C, 150 μE m^{-2} s^{-1}, 21% O_2 was estimated from a linear extrapolation of NP rates at low CO_2 levels (10 - 50 μl CO_2 l^{-1}).

SECTION A

GROWTH

CHAPTER III

Lichen growth rates

INTRODUCTION

The slow growth and longevity of lichens (Smith, 1973) have long been subjects of interest to lichenologists. Progress in this area has recently been reviewed in a number of lichenological texts. (Hale, 1973; Armstrong, 1976; Richardson, 1975; Topham, 1977). A survey of these reviews shows that although there is a considerable amount of information available on the ecology and growth of lichens in temperate and boreal regions, there is little on the lichens of tropical and subtropical forests (Seaward, 1977). A note by Green and Snelgar (1977) has indicated that lichen growth may be unusually rapid in the humid conditions of a warm temperate climate.

The existence of different growth phases during the life of a lichen has been acknowledged by several authors, however there is incomplete agreement on the definition of these phases. Hale (1973) separates growth into three periods: (a) A juvenile period of establishment and slow growth, (b) A 'great' period (the use of this terminology is criticised by Topham, 1977) lasting 2-10 years during which growth is rapid, (c) Maturity and senescence. During this phase growth is linear until maturity is reached, then slows down or ceases. At this point some disintegration of the thallus may occur. Armstrong (1976) in a more detailed, diagrammatic summary describes analogous prelinear and linear growth phases but states that at least for *Parmelia glabratula* subsp *fuliginosa* there is no evidence of a post-linear growth phase. While Topham (1977) notes that the evidence, particularly for *Parmelia* species fits the model proposed by Armstrong she also cites other workers who have found a gradual decline in growth without distinct phases.

In the majority of studies on lichen growth rates researchers have expressed growth in terms of radial increase [mm(unit time)$^{-1}$]. However, as noted above, radial growth rates are sometimes influenced by the original thallus size. To compensate for this effect Woolhouse (1968) has suggested the use of Mean Relative Rate of Thallus Growth (RGR). This is derived from the compound interest law in relation to the growth of higher plants (Blackman, 1919). The units used are $cm^2\ cm^{-2}$ (unit time)$^{-1}$ thus effectively relating growth to original size. This approach has been used successfully by Rhoades (1977) for the estimation of growth of *Lobaria oregana*. Armstrong has noted that the use of RGR is valid only if all carbon fixed by the lichen is available for radial growth. This implies that fixed carbon can be translocated from the centre of the thallus to the lobes. Such movement is as yet unproven, in fact recent results (Armstrong, 1979) suggest that movement of fixed carbon for radial growth may occur mainly in a narrow anulus at the perimeter of foliose lichens. Furthermore the use of RGR is complicated by such effects as; the disintegration of the centre of the lichen, variations in thallus thickness, and diversion of carbon for reproductive purposes. Asplin and Hill (1979) concluded that the RGR model did not describe lichen growth as observed in nature since in larger thalli the rate of area increase does not proceed exponentially.

Direct measurement of radial increase from either an internal thallus marker (Hale, 1970) or an external marker (Armstrong, 1973) over a period of time is the most simple method of measuring lichen growth rates. As rates of radial increase are often low (0.7 to 5.62 mm yr^{-1} for foliose lichens; Hale, 1973) these measurements must be accurate, hence Armstrong (1973) has described a technique whereby measurements are made using a low power binocular microscope. Although this method is both accurate and inexpensive the necessity for the

substrate to be both hard and smooth, limits the practicality of this technique. Tracing the outline of thalli on transparent plastic sheets is another direct method which has been employed by some lichenologists, (Hale, 1954; Brodo, 1964, 1965). This technique has never been widely used as it is said to be both tedious (Hale, 1973) and inaccurate over short time intervals (Armstrong, 1976). In recent years the most popular method of measuring lichen growth has been that of close-up photography (Philips, 1962; Showman, 1976; Rhoades, 1977). This offers the advantages of a permanent record and extreme precision as photographs can be enlarged much beyond life size either as prints (Hale, 1970) or as colour transparencies (Showman, 1976). Also measurements may be made rapidly in the field, an attribute which should not be overlooked when measurements are to be made in a remote field situation, regardless of weather conditions. A detailed investigation of the accuracy of this technique, and of the mathematical adjustments which can be applied to correct for parallax and magnification errors can be found in Hooker and Brown (1977).

MATERIALS AND METHODS

Studies of growth rates were made at five sites in the Lake Wakareiti region of the Urewera National Park. The lower two sites (700 m.a.s.l.) were in rimu-beech forest (McKelvey, 1973) dominated by *Dacrydium cupressinum*, *Podocarpus spicatus*, *P. ferrugineus*, *Nothofagus menziesii* and *N. fusca*. The remaining three sites were all near Lake Wakareiti at about 900 m.a.s.l. The forest is principally an *N. menziesii - N. fusca* association, although some podocarps are present (Grant, 1963). Further details of forest types and the geology and topography of the area can be found in Grant (1963), James and Wallis (1969) and McKelvey (1973).

In order to allow easy access only corticolous lichens less than 2 m above ground level were used in this study. Initially the lichens

selected were labelled by nailing a numbered 25 mm diameter stainless steel disc near each thallus. Colour transparencies of each thallus were taken with an Olympus OM 1 camera fitted with a 50 mm lens and an electronic flash unit. A perspex set square (85 x 85 mm) was included in each frame as an internal scale. Study of trial photographs showed that the set square was not always orientated in an identical plane in successive photographs, and this variation created errors in measurements. Consequently a set square was permanently fixed beside each thallus using shortened plastic golf tees. (Preliminary tests using galvanised nails resulted in the discolouration of adjacent thalli, presumably because of the toxic effect of Zinc ions distributed by the impact of rain droplets).

Philips (1962) and Showman (1976) have demonstrated that dry lichen thalli increase in size when moistened with water. Unfortunately, neither author attempted to measure the effect of subsaturating water contents on thallus size nor did they define the term dry in absolute terms (mg water per mg thallus dry weight). In order to always measure lichens at a constant water content some authors (e.g. Showman, 1976) have elected to photograph lichens only when they are dry. This routine may not completely eliminate errors due to the expansion and contraction of thalli as personal observations of *Parmelia scabrosa* growing on clay roof tiles have shown that visibly dry lichens can also fluctuate in size. This effect is probably a result of lichens becoming visibly dry at water contents greater than those of 'air dry' thalli. Thus these lichens could still lose water and contract. In this context it is of interest that, at least in the Stictaceae, the water content at which thalli become visibly dry varies between species. [*P. colensoi* 0.2 g water (g d.w)$^{-1}$, *S. latifrons* 0.5 g g^{-1}]. Since the state of dryness of lichens is difficult to assess in the field the alternative method

of photographing lichens only when wet (Rhoades, 1977) was used for the present investigation. Photographs were taken at about three monthly intervals and thalli which were not already wet were moistened by mist spraying with lake water. (Lake Waikareiti is an oligotrophic lake and unpublished results by Brown, Green and Snelgar indicate that nutrient levels are extremely low - total P 7 mg m^{-3}, NO_3^- 5 mg m^{-3})

Growth measurements were made on images projected onto a screen constructed of 'Permatrace' draughting paper. As this paper functions as a back projection screen measurements could be made without the problems of self-shading encountered in the use of an orthodox screen. The projector used was a Leitz Pradovit 250 fitted with a Leitz Wetzler 90 mm lens. The screen-projector distance was adjusted to give an image of about 3 x life size. Each transparency was checked for angular distortion by comparing the scales on each arm of the set square. If these scales differed by more than 5% either the transparency was discarded or only measurements parallel to the set square scales were used. Linear distortion in most transparencies was less than 2%. Radial growth rates were assessed by measuring the distance between lobe tips and fixed points (Stainless steel pegs, set square, stainless steel discs) using a pair of dividers. These distances were converted to millimetres using the internal scale. Wherever possible measurements were made on several lobes of each thallus.

RESULTS

Growth rate estimates were obtained for a total of 33 individual thalli over periods of 386 - 733 days. The number of lobes measured on each thallus ranged from one to six. Many of the records are incomplete for a variety of reasons including; initial problems with the orientation of the internal scale, overgrowth of markers, death

or damage of thalli, and changes in the direction of growth of lobes. The majority of the lichens studied were either *Sticta caperata* (13) or *Pseudocyphellaria homoeophylla* (12) and the mean annual growth rates of individual lobes of these two species are given in Figure 1. Rates are plotted against the mean initial diameter of each thallus. Annual growth rates were calculated from the total growth observed during the growth study and since this rarely approximated a one (or two) year period the results could be biased by seasonal fluctuations in growth rate, if such fluctuations occur.

The least squares regression fitted to Figure 1a (*S. caperata*) is described by the equation $y = 5.1 + 0.14\ x$. The correlation coefficient was not significant indicating that the growth of this species is independent of thallus size over the range 1.5 - 20 cm thallus diameter. The mean radial growth rate of *S. caperata* was 7.0 mm yr^{-1}. Maximum and minimum growth rates were 3.0 and 16.7 mm yr^{-1} respectively.

Growth rates of *P. homoeophylla* were positively correlated with thallus diameter ($y = 5.3 + 0.54\ x$). The correlation coefficient ($r = 0.606$, $n = 23$) is significant ($P<0.01$). Using this regression equation the calculated growth rates for thalli of 5 cm and 10 cm diameter are 8.0 and 10.7 mm yr^{-1} respectively. The measured growth rates were highly variable with some data points deviating from the fitted regression by as much as 143%. All mean annual growth rates were in the range 3.0 - 27.0 mm yr^{-1}.

The pattern of seasonal variation in growth of *S. caperata* and *P. homoeophylla* over two years has been plotted in Figure 2. Each of the data points is the average growth rate of between 2 and 11 lobes. Only a general interpretation of the results is possible since the number of growth determinations was small, and no correction

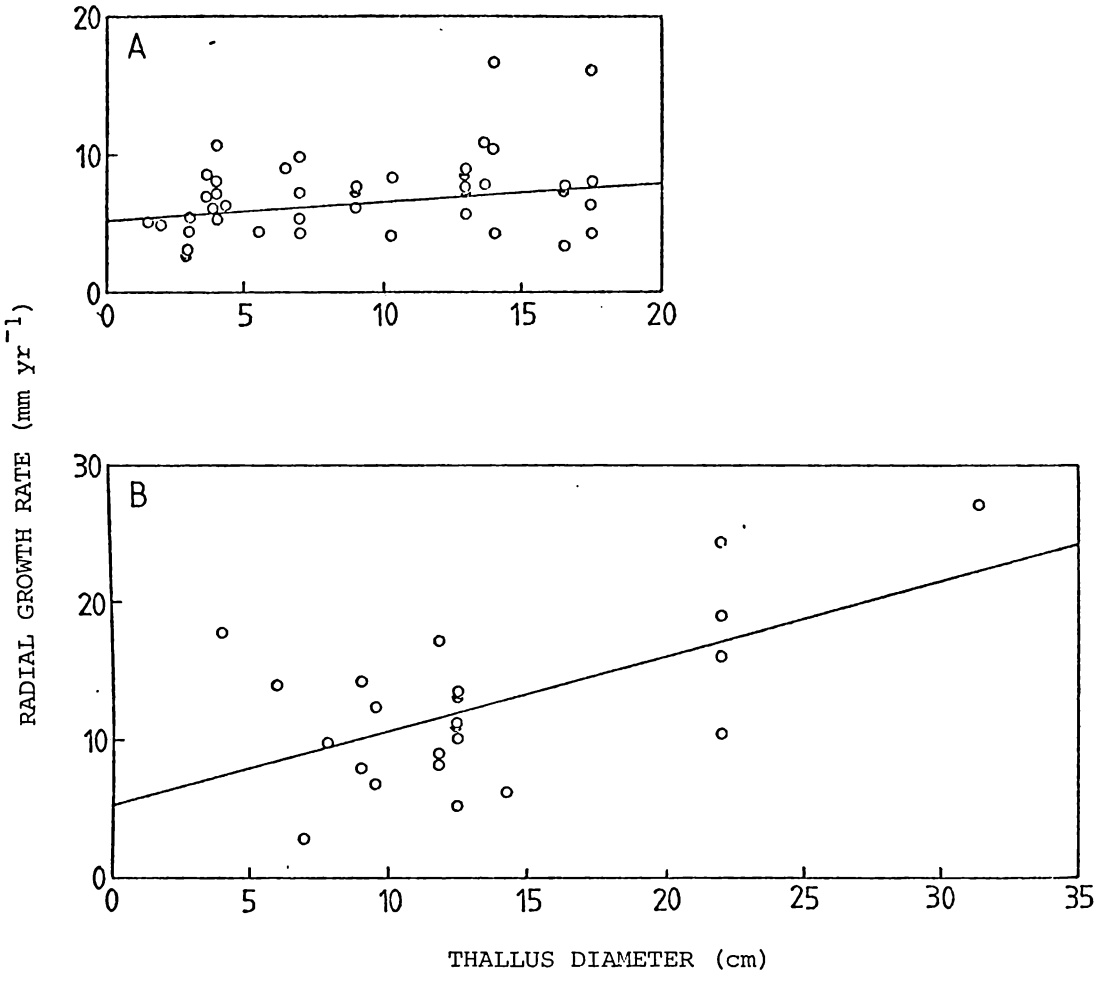


FIGURE 1. Relationship between mean annual radial growth rate and initial thallus diameter for A, *Sticta caperata*, and B, *Pseudocyphellaria homoeophylla*.

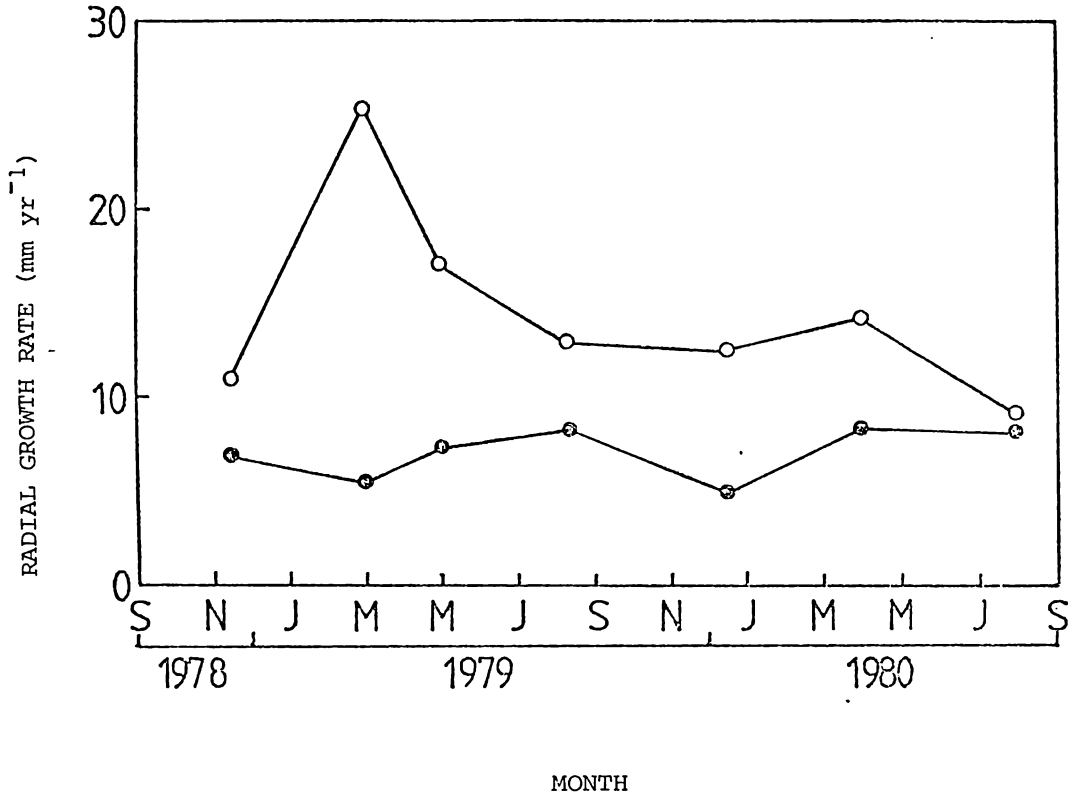


FIGURE 2. Seasonal variation in radial growth rate of *Pseudocyphellaria homoeophylla* (o), and *Sticta caperata* (⊙).

was made for thallus size. For *P. homoeophylla* growth rates were high in the late summer - autumn period and lower in the winter. *S. caperata* shows a different pattern with rates being higher in the winter and lower in the summer. The seasonal growth rates of individual lobes of both species were markedly more variable than the mean annual growth rates. For *P. homoeophylla* values as high as 70.4 mm yr^{-1} (data not shown) were recorded while other lobes showed no measurable growth during periods as long as four months. Similarly *S. caperata* seasonal growth rates ranged from 0 to 38.0 mm yr^{-1}.

Growth rates for four other lichen species are presented in Table 1. Thalli from site 102 are of particular interest in that they were growing on the upper branches of a small shrub (*Myrsine divaricata*) approximately one metre tall. In the area studied many lichens grow in such habitats.

DISCUSSION

The mean annual growth rates of *S. caperata* and *P. homoeophylla* were high in comparison with previously reported results for foliose lichens (Rhoades, 1977; Armstrong, 1973; Hale, 1973). This may be at least partly due to the high rainfall in the Urewera National Park (about 100" per annum; Grant, 1963) coupled with a high incidence of mist and cloud in the Lake Waikareiti area.

The results obtained in the present study of *S. caperata* appear to fit the model of Hale (1973) in that the radial growth rate of thalli greater than 1.5 cm diameter was constant. However the data for *P. homoeophylla* show a linear relationship between thallus diameter and radial growth rate. Although such trends are generally found only in small thalli (Topham, 1977), Rhoades (1977), working on *Lobaria* species, also found that the radial growth rate increases with thallus diameter. The existence of two different growth patterns in lichen species growing in the same area seems to be a

TABLE 1. Growth rates of lichens.

Species	Site number	Mean diameter of thallus (cm)	Mean annual growth rate of individual lobes (mm)
P. amphisticta	19	3.8	9.6, 4.6, 4.9
P. colensoi	102	1.3	8.6
P. faveolata	102	11.3	13.1, 12.1
	102	0.3	3.7
	102	0.2	6.6
	28	7.8	5.7, 7.0
S. filix	13	7.2	14.1, -20.0
	14	2.5	9.3, 11.5

clear indication that one lichen growth model cannot be expected to adequately describe the growth of all species. Further work aimed at elucidating the causes of these differences could be most informative.

When making growth measurements of lichens there are several possible sources of error apart from the obvious problems of photographic distortion. During the present study the divaricating nature of lobe growth in *S. caperata* and particularly in *P. homoeophylla* frequently altered the direction of marginal growth. This resulted in inaccuracies when growth was measured from a single fixed point. The movement of lobe tips due to hydration and desiccation stresses created similar difficulties. A source of systematic bias which is rarely mentioned in the literature is that of substrate choice. The lower plant flora of the beech forests studies is profuse and this often produces situations where loosely attached lichens grow over a thick moss turf either on a tree trunk, or on the ground. In such a situation attachment of fixed markers is extremely difficult. As the above features are likely to result in inaccurate growth estimates thalli growing on these substrates tend to be avoided. Lichens which have a fruticose growth habit, or which grow on small shrubs are similarly avoided. The few thalli sampled in the latter habitat (Table 1) exhibited relatively high growth rates, thus the possibility of markedly different growth rates due to microclimate variations should be considered. The dense flora previously mentioned also creates such a profusion of species and thalli that it can be difficult to delineate individual thalli. Senescence of central portions of a thallus can also complicate matters since outer lobes are sometimes completely separated from the rest of the thallus.

All of the characteristics listed above contribute errors and bias to estimates of lichen growth. The use of colour transparencies

to record lichen size has proven to be rapid in the field, accurate, and relatively inexpensive. The suitability of this technique allows a larger, more varied sample of thalli to be used and this should partially compensate for the errors listed above.

The biomass of lichens within two metres of the forest floor has been estimated at 100 kg dry weight per hectare (Green *et al*, 1980). The bulk of this was *P. homoeophylla* (80%). There are no estimates of lichen biomass above the 2 metre line, but observations indicate that it may be considerable. The quantity of lichens present in this area, together with the relatively high growth rates reported here, and the nitrogen fixation rates found by Green *et al*, (1980) all suggest that the Stictaceae lichens may play an important role in the nitrogen budget of these forests.

SECTION B

PHYSIOLOGY AND ECOLOGY

CHAPTER IV

Ecologically-linked variation in morphology, acetylene reduction and water relations in *Pseudocyphellaria dissimilis*

SUMMARY

Pseudocyphellaria dissimilis (Nyl.) D. Gall and P. James *in litt* populations were shown to possess different morphologies which correlated with the evaporative demand of their environment. The variations in morphology consisted of increased thallus thickness, mostly from increased medulla thickness, and increased rhizine length in the more exposed population. These changes confer a decreased surface/volume ratio and increased water storage in the form of external water that could be removed by blotting but not shaking. A consequence of these changes was a slower drying down rate. No differences in water loss rate per unit area were found between populations indicating that no morphological modifications exist to decrease water loss. Preliminary work on the acetylene reduction rates of the populations demonstrated a considerable difference in rates between the individual populations' and indicated that acetylene reduction could be another useful indicator of physiological adaptation.

INTRODUCTION

The existence of chemical and morphological races in lichen species has been known for some time and a summary of much of the more recent work in this field can be found in Lechowicz and Adams (1973) and Larson and Kershaw (1976).

Differences in carbon dioxide exchange rates have been demonstrated between sun and shade races of *Cladonia subtenuis* (Rundel, 1972) and *Parmelia physodes* (Harris, 1971), although only minor differences have been found for *Cladonia mitis* (Lechowicz and Adams, 1973). It is unfortunate that in many instances there is very little available information on the water relations and morphological variations of these races. Conversely, a study of *Xanthoria parietina* by Hill and Woolhouse (1966) produced considerable data on morphological differences but none on the water relations or physiology of the species.

Until recently it has been axiomatic in the literature that lichens lack morphological mechanisms for the control, in the sense of affecting rate, of water uptake and loss, (Harris, 1976; Farrar, 1973; Blum, 1973; Kershaw and Rouse, 1971). Thus the water relations of lichens were said to be purely physical and to resemble those of a gel. This view was so widely accepted that artificial models designed to simulate the water relations of lichens were simply portions of filter paper (Kershaw and Rouse, 1971; Harris, 1972). More recent work has demonstrated an intraspecific variation in morphology and water relations for *Alectoria ochroleuca* and *Cladina alpestris* (Larson and Kershaw, 1976), whilst Larson (1979a) has shown interspecific differences in water relations of *Umbilicaria* species. Larson (1979a) provides a review of lichen water relations and makes the important point that even if a lichen behaves exactly as a gel, the water relations could still be affected by variations in the three-dimensional shape of the gel. It thus appears that the area of lichen water relations could be more complex than previously thought.

During field work studying *Pseudocyphellaria dissimilis*, a lichen with a blue-green phycobiont, a wide range of thallus morphologies was observed and a superficial study indicated that these thallus types may be related to

habitat differences. Subsequently a laboratory study of this species was initiated in order to ascertain how much of the variation in acetylene reduction rates was attributable to these variations in thallus type. As unpublished data indicates that there is a positive linear relationship between acetylene reduction rate and thallus water content the rates of water loss from thalli were also assessed.

METHODS

All samples of *P. dissimilis* were collected from the same valley within the Hakirimata reserve, Ngaruawahia, Central North Island, New Zealand. The valley is narrow, steep sided, containing a permanent stream running through forest with a canopy at about 10m. The forest is composed mainly of deciduous *Fuschia excorticata* near the stream and evergreen species (including *Podocarpus* spp., *Weinmannia racemosa, Macropiper excelsa*) elsewhere. The presence of *Elatostema rugosum* and the large liverwort *Monoclea forsteri* indicate that conditions were very moist.

Selections from three distinct habitats were made and these were subjectively classified as:

P I. 'Shade' population. Thalli were growing between and over mosses and tree roots on a wet bank facing south-west. The area was shaded by the canopy and subcanopy; thalli were only loosely attached and often standing out from the substrate.

P II. 'Mesic' population. These lichens were growing on the vertical trunk (breast height diameter of 20cm) of a *Beilschmeidia tawa* tree, approximately one metre above the ground. Light intensity varied throughout the day as a result of sun flecking and the habitat was more exposed to wind than the shady bank.

P III. Sun population. This was situated approximately three metres above ground level on the trunk of a *W. racemosa*. The tree was inclined over a small stream with only a sparse forest canopy above it so that direct sunshine reached the plants for a short period. Consequently the habitat were considered to be rather more exposed than either I or II. These thalli were closely attached to the substrate with only the lobe tips being completely free.

In all cases only the terminal 2-3cm of healthy lobes were collected and fertile lobes were normally avoided. After collection lichens were transported to the laboratory (0.5 hrs), sprayed with distilled water and stored in a glass topped chamber on damp filter paper until required. Storage was always in a walk-in temperature controlled room (16°C) at 50μE m^{-2} sec^{-1} (fluorescent tubes, colour number 35, white) on a 12 hour day, 12 hour night cycle. Light intensities were measured with a Licor quantum meter model LI 185A.

Acetylene reduction rates were used as an assay of nitrogenase activity and these were carried out in 7ml glass vials capped with a butyl rubber seal. Specimens were first soaked in distilled water for 2 minutes, blotted dry, then incubated in an atmosphere of 10% acetylene (V/V) at 16°C, 100μE m^{-2} sec^{-1} for approximately one hour. Gas samples were then assayed by gas chromatography using the methods and equipment described by Green *et al* (1980).

Rates of water loss for each of the three populations were measured by soaking lobes for 2 minutes in distilled water, blotting or shaking dry and following the rate of weight change by weighing on a torsion balance at 5.- 20 minute intervals. Between weighings lichens were placed on a laboratory bench at 21°C, 40% R.H. (saturation deficit = 14.9 mb). The light intensity was

5μE m^{-2} sec^{-1} from cool white (colour number 33) fluorescent tubes. Wind speed was zero.

Chlorophyll a content was measured using the method of Hill and Woolhouse (1966) modified by the addition of 6 pre-washes of the air dry thalli in 1ml aliquots of absolute acetone for one minute in order to remove acidic lichen substances, as recommended by Brown and Hooker (1977).

Area estimates of samples were obtained by tracing the outline of the thalli with a Koizumu compensating planimeter (Type KP-27). Lobes were held flat by a 1.5mm sheet of glass and in order to increase the accuracy of measurements each lobe was traced repeatedly until a total area of at least 10cm^2 had been obtained.

Heterocyst frequencies were determined by digestion of thalli in 10% w/v chromium trioxide solution for at least 20 hours (Hitch and Millbank,1975) followed with maceration by being drawn several times through a fine pasteur pipette. Microscope counts were made of vegetative and heterocyst cells with only cells in long filaments being used and a total of 1000 cells per population were counted.

Gross anatomical measurements were made in a manner similar to that of Hill and Woolhouse (1966). Lobes from each population were hand sectioned approximately 1cm from the lobe tip, then the vertical thickness of each layer was measured under a binocular microscope using a calibrated eyepiece.

Dry weights were obtained after samples had been dried to constant weight at 100°C.

The evaporation demand of each of the three habitats was assessed by installing matched Piche evaporimeters for a period of 12 days, with all three habitats being assessed simultaneously. Evaporimeters were hung vertically with the evaporation pad as close as possible to the lichens.

Wherever practical all measurements were replicated (5) and the results are presented as a mean plus or minus the standard error of the mean. Student's t-tests between pairs were used to define differences between means.

RESULTS

Acetylene reduction rates of the three populations following overnight storage were not significantly different when expressed on a dry weight basis (Table 1). Chlorophyll a content and heterocyst frequencies were also not significantly different between populations although the variability of the chlorophyll estimates was high (Table 2). However, on an area basis, the acetylene reduction rate for the 'sun' population was significantly higher. Acetylene reduction rates were also measured for thalli that had been stored for six days at 100% humidity under standard conditions. Compared to fresh thalli (100%), the six day rates (on an area basis) were 'shade', 215%; mesic, 61%; and 'sun', 2%. Water contents show similar variations to those found for acetylene reduction rates when expressed on a dry weight or area basis. In both cases the variations can be explained by the significantly greater dry weight per unit area of the 'sun' population. A measure of the density of the thalli obtained by calculating the ratio of total vertical thickness of the thallus to the weight per unit area shows no difference between the populations with values of 481, 488 and 474mg cm^{-3} for 'shade', 'mesic' and 'sun' populations respectively, indicating that thallus thickness is proportional to thallus weight.

Population	Acetylene reduction		Water Content		Weight/unit area
	nmol g^{-1} min^{-1}	nmol cm^{-2} min^{-1} x 10^3	mg mg^{-1}	mg cm^{-2}	mg cm^{-2}
Shade	28.5±3.4 a	164±16 a e	2.02±.07 a	11.8±0.9 a e	5.9±0.5 a e
Mesic	24.6±1.0 a	193±19 a ef	2.05±.03 a	15.1±0.7 a ef	7.4±0.4 a e
Sun	29.4±3.5 a	262±20 f	2.10±.07 a	19.2±1.4 f	9.1±0.6

Table 1 Acetylene reduction rates of *P. dissimilis* at 16°C, 100μE m^2 sec^{-1}. Thalli were stored for one night. Means followed by the same letter (a-d) or combination of letters are not significantly different at P=0.05. Similarly letters e-g represent P=0.01 level.

Population	Chlorophyll *a* content mg g^{-1}	Heterocyst frequency (percentage)
Shade	0.24±0.04 a	4.6±0.3 a
Mesic	0.26±0.08 a	4.8±0.6 a
Sun	0.18±0.02 a	4.9±0.1 a

Table 2 Chlorophyll contents and heterocyst percentages of the three populations of *P. dissimilis*. Methods as in text. (For subscripts see Table 1).

Population	Upper cortex	Algal layer	Medulla	Lower cortex	Total thickness of thallus (excludes rhizines)	Rhizines
Shade	31±1 a	38±1 e	40±4 a	26±2	135	55±4 a
Mesic	35±3 a	48±1 a ef	46±5 a	31±2 a	160	76±10 a
Sun	32±2 a	50±4 a f	82±16 a	30±0 a	194	202±22 a

Table 3 Vertical thickness of tissue layers in microns; details of measurement methods in text. (For subscripts see Table 1)

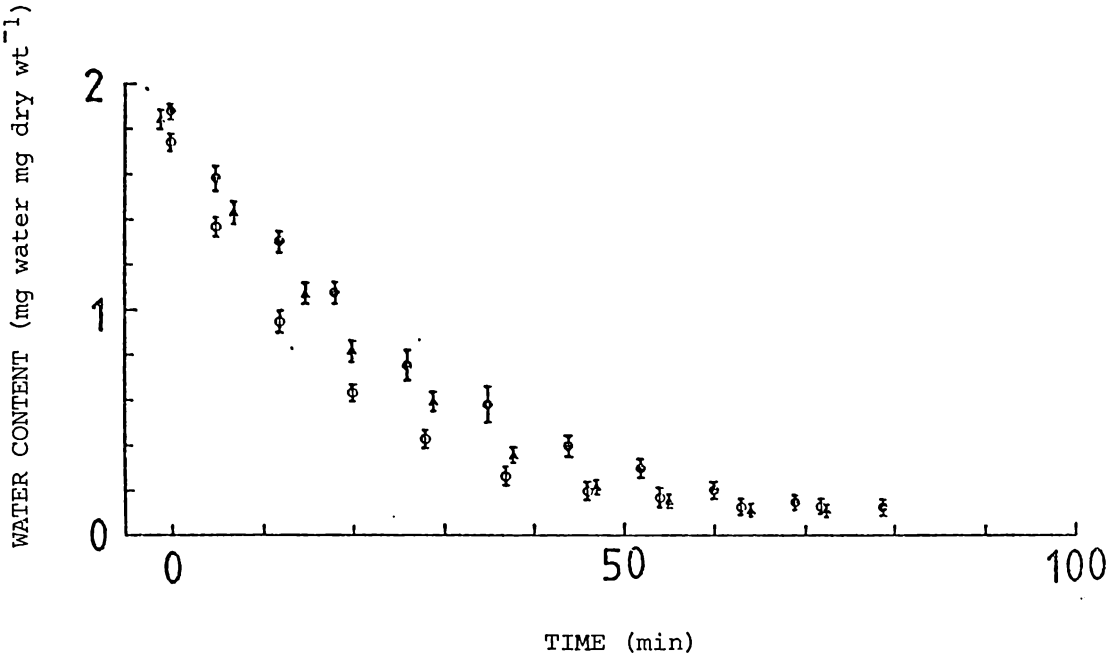


FIGURE 1. Loss of water from *blotted* samples of three populations of *P. dissimilis* against time of drying. Population description and sample treatment in text. ▲ , shade population; o, mesic population; ●, sun population; bars indicate onestandard error of mean on either side of mean value.

The upper cortex of all three populations is equally thick but 'mesic' and 'sun' have a thicker algal layer and lower cortex whilst 'sun' has a thicker medulla and rhizine layer. The rhizines, in particular, are much longer in the 'sun' population, (four times 'shade', three times 'mesic'). The rates at which thalli from each population dried out are shown in Figure 1. Only small absolute differences between populations were found for initial water contents and time to reach 50% blotted weight. The 'mesic' population sample took least time to reach 50% blotted weight whilst the 'sun' and 'shade' populations were very similar. When repeated with water content expressed as mg cm^{-2} even smaller differences were found, (Table 4). The experiment was repeated with thalli only shaken dry rather than blotted, a situation thought to be more analogous to that found in the field where the rhizines would hold a large amount of water that would be removed by blotting. Using shaken specimens clear differences were found between the populations, (Figures 2A, 2B, Table 5) with the 'sun' population having the highest initial water content and also taking longer to dry down. The almost identical water contents when blotted dry for the three populations (Table 4) indicate that the extra water in the shaken thalli must be external and easily available for removal by blotting.

A comparison of the rate of water loss per unit dry weight of the two most different populations ('shade' and 'sun') showed identical rates of water loss at low water contents but differences at higher water contents where the 'shade' population lost water faster, (Figure 3A). This reflects the smaller surface to volume ratio of the 'sun' population and when water loss rates are plotted on a unit area basis no difference between the populations is found (Figure 3B) suggesting that there are no morphological differences to control water loss from the thallus.

Population	Initial water content		50% saturation water content		Minutes to reach 50% saturation	
	mg mg^{-1}	mg cm^{-2}	mg mg^{-1}	mg cm^{-2}	mg mg^{-1}	mg cm^{-2}
Shade	1.86±0.07	10.8±0.8	0.93	5.4	18	18
Mesic	1.70±0.04	12.4±0.3	0.85	6.2	15	17
Sun	1.88±0.02	17.4±1.3	0.94	8.7	21	17

Table 4 Drying times of blotted dry lichen thalli. Lichen thalli are those from which data for Table 1 were obtained.

Population	Initial water content		50% saturation water content (from Table 1)		Minutes to reach 50% saturation		Dry weight unit area
	mg mg^{-1}	mg cm^{-2}	mg mg^{-1}	mg cm^{-2}	mg mg^{-1}	mg cm^{-2}	mg cm^{-2}
Shade	3.0±0.08	19.5±1.1	1.01	5.9	43	45	6.5±0.2
Mesic	3.6±0.12	28.1±1.8	1.02	7.6	49	51	7.8±0.4
Dry	3.9±0.32	35.5±1.9	1.05	9.6	67	66	9.2±0.6

Table 5 Drying down times of shaken dry lichens thalli from the three populations. Conditions of experiment in text.

Population	Evaporation over 12 days (cm^3)	Substrate	Height above ground (m)
Shade	1.6	Mossy bank	0
Mesic	3.9	*Beilschmeidia tawa* trunk	1
Sun	10.1	*Weinmannia racemosa* trunk	3

Table 6 Habitat description, including evaporation rates of the three populations.

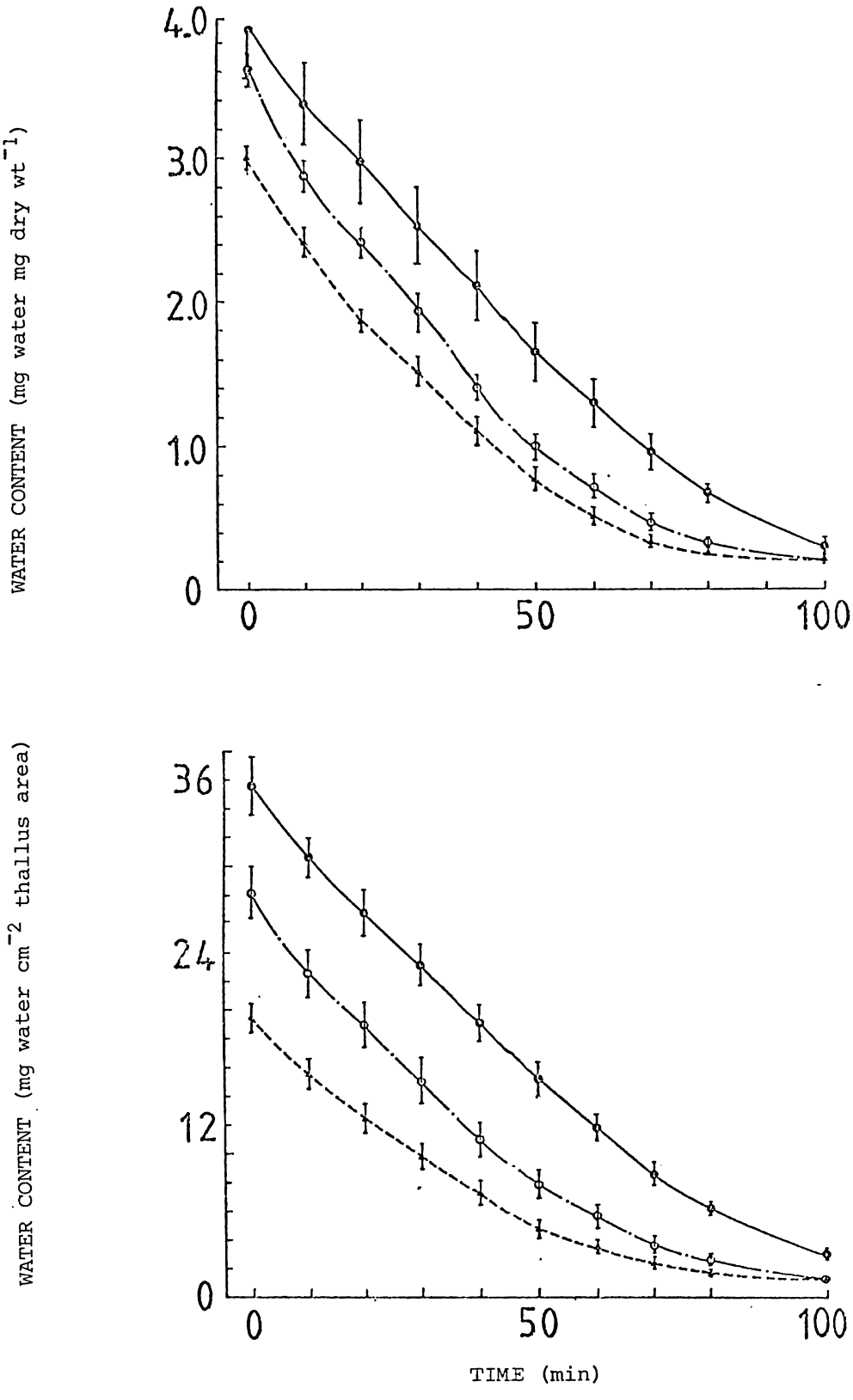


FIGURE 2. Loss of water from *shaken* samples of three populations of *P. dissimilis* against time of drying. Population description and sample treatment described in text; Δ --- Δ, shade population; o-·-o, mesic population; ⊘ - ⊘, sun population; bars indicate one standard error of mean on either side of mean value.

Population	Acetylene reduction rate		Water content		Dry weight/ unit area
	nmol g^{-1} min^{-1}	nmol cm^{-2} min^{-1} $x10^3$	mg mg^{-1}	mg cm^{-2}	mg cm^{-2}
Shade	61.2±5.6 a	437±19 a e	1.58±0.09	11.3±0.2	6.7±0.2
Mesic	15.0±2.3 b e	97±15 b f	1.88±0.08	12.0±0.7	7.0±0.5
Sun	0.5±0.1 c e	4.8±1.1 c g	2.10±0.14	15.6±1.0	7.5±0.3

Table 7 Acetylene reduction rates of *P. dissimilis* at 16°C, 100μE m^{-2} sec^{-1} after 6 days wet storage; storage conditions as in methods.

Population	Blotted water storage		External water storage		Total water		Total thickness
	mg mg^{-1}	mg cm^{-2}	mg mg^{-1}	mg cm^{-2}	mg mg^{-1}	mg cm^{-2}	
Shade	1.9	10.8	1.1	7.7	3.0	18.5	190
Mesic	1.7	12.4	1.5	12.9	3.2	25.3	236
Sun	1.9	17.4	1.8	16.3	3.7	33.7	396

Table 8 Summary of water storage characteristics of the three populations of *P. dissimilis*. Water retained by shaken soaked thalli *after* blotting is "Blotted Water Storage"; water *removed* by the blotting of shaken soaked thalli is "External Water storage". 'Total Water' is the sum of 'Blotted Water storage' and "External Water storage". Data represents an amalgamation of water contents from Tables 4 and 5, and thallus thickness from Table 3.

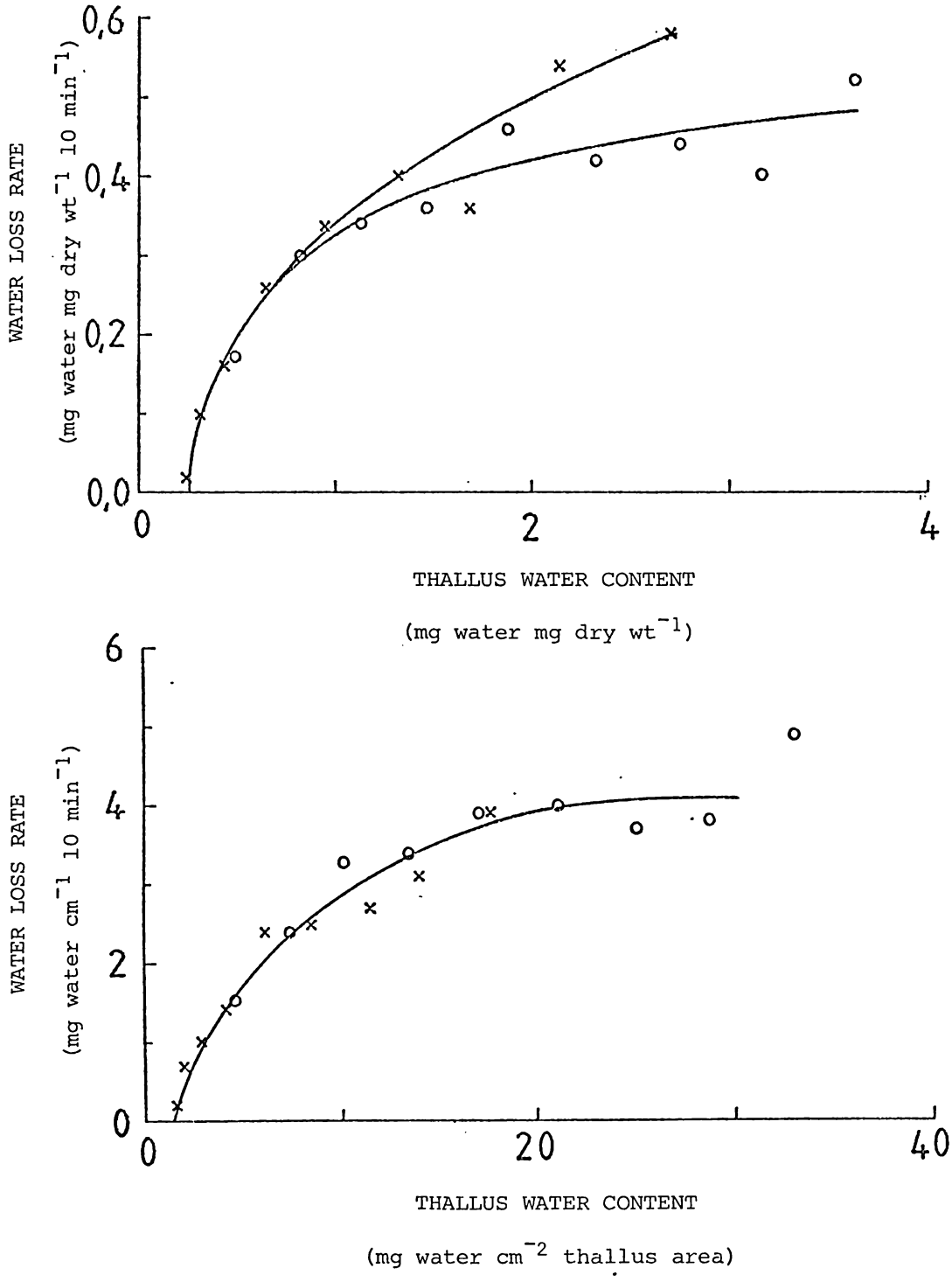


FIGURE 3. Rate of water loss from sun and shade populations of *P. dissimilis* with respect to thallus water content. Population description and experimental details in text; o, sun population; x, shade population.

The evaporation rates, assessed with a Piche evaporimeter were very low in all three areas with a mean rate of $0.13cm^{-3}$ day^{-1} for the 'shade' population and considerable differences were found between the three locations. Evaporation at the 'sun' population site is six times greater than at the 'shade' site and nearly three times that of the 'mesic' site.

DISCUSSION

The three populations of *P. dissimilis* studied show differences in thallus morphology which are correlated with evaporative demand of their environment The major morphological differences of thallus thickness and rhizine development act to change the water relations of the thalli. Increased thickness of a foliose lichen produces a reduced surface/volume ratio which leads to a decreased water loss when expressed as a proportion of the total water in the thallus and also allows a greater internal water reservoir to be held. As the internal water holding capacity is likely to be limited by the volume of intercellular space, the similar density found for the three populations suggests a similar water storage capacity per unit weight. Water in excess of this figure would therefore be expected to be external to the thallus forming a surface layer on the cortex or within the rhizine layer. Increase in rhizine length would be expected to allow increased external water storage since,in the genus *Pseudocyphellaria,* rhizines tend to form a tomentum. Certainly the quantities of water that can be removed by blotting rank the three populations in the same order as rhizine length but the differences are not of the same magnitude. (Table 8). Field observations suggest that the growth form of the plant would also aid in water retention since the 'sun' population tends to be appressed to the tree trunk whilst the 'shade' plants project from the substrate and are only loosely attached.

In summary, the 'sun' population of *P. dissimilis* possesses three adaptations which help maintain the lichen in a moist condition.

1. The thallus has the classic xerophyte characteristic of reduced surface area to volume ratio.
2. An increased water storage capacity is attained by increased thallus thickness and a more developed rhizine layer.
3. The thalli are appressed to the substrate thereby aiding water storage by the rhizines and reducing the area exposed to water loss by evaporation.

These adaptations parallel those described by Larson and Kershaw (1976) for fruticose lichens growing in an exposed environment; and by Larson (1979a) for more temperate lichens. The results presented here demonstrate that morphological adaptation controlling water loss can be found within a species confined to the moister, shadier environments of temperate/sub-tropical rain forest. Such adaptations can now be reasonably expected in the majority of lichen species. At this stage it is unclear whether these adaptations are the result of acclimation during the growth of the lichen or are genetically determined. Detailed and difficult transplant experiments would be required to provide conclusive results. This study also demonstrates the importance of 'external' water, that is water retained on shaken lichens but removed by blotting, in the water relations of lichens. External water has been shown to be an important water reservoir in bryophytes, (Busby and Whitfield, 1978; Snelgar *et al*, 1980) and morphological adaptions exist to control its location (Dilks and Proctor, 1979). Physiological studies where the lichen has been blotted dry may not have covered the full physiological range of the plant.

The large differences in acetylene reduction rates per unit area between the populations is principally produced by a change in algal layer depth which is deeper in the exposed 'sun' thalli and thinner in the 'shade' thalli. The thinner algal layer of the more shaded population, and the identical chlorophyll contents of all populations (Table 3) is in contrast to the results of Hill and Woolhouse (1966) who reported an increase in chlorophyll content and algal layer thickness in a 'shade' population of *Xanthoria parietina*. The lack of such differences in the present investigation is probably a result of the very low light regime of all three populations.

Storage for six days at 100% relative humidity results in large changes of acetylene reduction activity (Tables 1 and 7). The 'shade' population shows a large increase in activity whilst the 'mesic' population declines slightly and the 'sun' population massively. It is of interest that the variations in morphology between population which prompted this investigation were not large in this experiment yet the differences in the physiological response of nitrogenase activity to storage were enormous. No physiological interpretation of these changes can be made although unpublished results indicate that net carbon balance effects may be important. However, where previous investigations of lichen races have usually concentrated on differences in carbon dioxide exchange (Lechowicz and Adams, 1973; Rundel, 1972) morphological features (Hill and Woolhouse, 1966), or water loss (Larson and Kershaw, 1976; Larson 1979a) it appears that nitrogenase activity may be an extremely sensitive technique for detecting population differences in lichens.

CHAPTER V

A provisional survey of the interaction between net photosynthetic rate, respiratory rate, and thallus water content in some New Zealand cryptogams

INTRODUCTION

In the last decade considerable advances have been made in the understanding of gas exchange and productivity of lichens and bryophytes. Research has concentrated on species from relatively open habitats such as Canadian open woodland (Kershaw 1972, 1977a, 1977b; Kershaw and Smith 1978; Harris, 1971; Busby and Whitfield, 1978), deserts (Lange 1969), maritime Antarctica (Collins 1976), Canadian tundra (Kershaw and Rouse, 1971), and English woodlands (Dilks and Proctor 1979). There is a paucity of research on species from dense forest, particularly rain forest in subtropical and tropical areas. In New Zealand a considerable biomass of lichens and bryophytes exists in rain forests and, to date, there appear to have been no physiological studies on the productivity or gas exchange of these plants. An initial study of nitrogen fixation by New Zealand lichens has been carried out (Green *et al.* 1980) and demonstrates the suitability of the plants for physiological studies.

In this work the relationship between carbon dioxide exchange and water content of eight lichens and one bryophyte was studied using the injection infrared gas analyser technique pioneered by Larson and Kershaw (1975b). This technique provides considerable time advantages over a gas flow system when many samples are involved.

Seven of the lichens studied are members of the Peltigerinae (six in the Stictaceae) and were chosen to represent the ecological range found within this group. One lichen species (*Usnea* sp.) was included to provide the contrast of a lichen that does not fix

nitrogen and occurs in an exposed habitat. Similarly, one species of bryophyte was included so that a comparison of the water relations of a different plant type growing in the same area could be made. It was considered that these comparisons would be of interest in this preliminary study of New Zealand species.

The lichens forming the central part of this study may be of great importance in New Zealand forests, particularly in their ability to fix nitrogen. This work is part of a major study on the productivity and ecology of this group in an attempt to assess their role in the nutrient cycles of forests.

MATERIALS AND METHODS

Lichens and bryophytes were collected from the localities listed in Table 1. The date of collection is also given and all plants, except *Pseudocyphellaria dissimilis*, were assayed on the same day. *P. dissimilis* was stored for 24 hr at 16°C, 100% r.h. 12 - hour day at 70 $\mu E\ m^{-2}\ s^{-1}$ before assay.

Plant material was thoroughly moistened with a distilled water spray and stored in polythene bags at ambient temperature until required. Healthy terminal portions of thalli were selected and excess surface moisture removed by gentle shaking or blotting. With *Sticta latifrons* older non-fertile portions of the thalli were used. Samples were placed in small, pre-weighed aluminium-foil boats to enable easy handling, then assayed.

Assays of both photosynthesis and respiration were carried out using an ADC series 225 infrared gas analyser (IRGA) operating on a sample injection system as shown in Fig. 1, which was similar to that described by Clegg *et al.* (1978) and Larson & Kershaw (1975b). Modifications to previous systems included the use of CO_2 - free air as carrier gas and a smaller injection volume of 1.0 ml. Signal output from the IRGA on 100 m V range was fed to a Servoscribe chart

recorder set on 20 m V range (x 5 amplification). The IRGA was initially set up to produce full scale deflections when 500 ppm CO_2 were passed through the 5% analysis cell. The flow system was then altered to pass the CO_2-free carrier gas through the full 100% analysis cell as shown in Fig. 1. Injection of 1 ml of 203 ppm CO_2 produced a mean peak height of 39 chart units (recorder full scale = 100 chart units) so that 1 chart unit = 5.2 ppm CO_2. For CO_2 concentrations >500 ppm the zero substraction on the recorder of 100 - 400% could be used so that samples of up to 2500 ppm could be measured without loss of accuracy. Samples could be assayed at the rate of 2 per min and incubation times depended on the rate of gas exchange, varying between 3.5 and 20 min. Separate CO_2-free gas supplies to analysis and reference cells were used so that loss of CO_2 absorbancy could be rapidly detected. A major advantage of the system is that no gas cylinder is needed to provide the carrier gas. Gas flow rates were 900 ml min^{-1} analysis cell, 180 ml min^{-1} reference cell. Calibration was by injection of 1 ml volumes of known CO_2 mixtures (N.Z. Industrial Gases Ltd). Precision was estimated to be $\pm$ 2 ppm CO_2.

Plant samples were incubated in 30 ml universal bottles mounted by rubber O rings in a stainless steel water bath as shown in Fig. 2. Plant sample temperatures were monitored using a hypodermic thermistor probe attached to a plant sample in an extra incubation chamber. Temperature was maintained at 16 $\pm$ 0.5°C by circulating the water over a cooling coil and thermostatically controlled heater. Light was provided by two No. 35 white 20 W fluorescent tubes suspended above the bath and intensity was measured using a Licor Quantum meter Model LI 185A. A light intensity of 70 $\mu E\ m^{-2}\ s^{-1}$ was routinely used. Respiration assays were obtained with the tubes blacked out by placing a lid over the steel chamber. In some cases temperature

TABLE 1. Location and description of collection sites

Species	Date of collection	Locality	Locality description
Peltigera dolichorhiza (Nyl.) Nyl.	4.7.79 5.7.79	A	Ground, moist bank at side of track.
Pseudocyphellaria billardieri (Del.) Ras.	21.6.79	C	Branches of *Weinmannia racemosa*.
Pseudocyphellaria colensoi (Bab. in Hook f.) Vain	3.7.79 1.6.79	A	*Nothofagus menziesii* trunk, 2 m above ground.
Pseudocyphellaria dissimilis (Nyl.) D. Gall. et P. James *in litt.*	22.5.79	C	Ground, mossy wet bank.
Pseudocyphellaria homoeophylla (Nyl.) Dodge	31.5.79 2.6.79	A	*N. menziesii* trunk, 1 m above ground.
Sticta caperata Bory. in Nyl.	31.5.79 2.6.79	A	*N. menziesii* trunk, 1 m above ground.
Sticta latifrons Rich.	5.7.79	B	Hinerau's track, *N. menziesii*, 2 m above ground.
Usnea sp.	4.7.79 5.7.79	A	*Coprosma* scrub, exposed branches 2 m above ground.
Weymouthia mollis (Hedw.) Broth.	4.7.79 5.7.79	A	Small *N. menziesii* by side of track, 2 m above ground.

[1]A: Clearing ("Paradise clearing") near Lake Waikareiti, altitude 900 m. The clearing is bordered with *Coprosma* scrub, and surrounded by mature forest dominated by *Nothofagus menziesii* and *N. fusca*. Map reference: NZMS 1 N96: 565330.

B: Headquarters of Urewera National Park, Waikaremoana, altitude 620 m, forest of *N. fusca*, *N. menziesii*, and *Dacrydium cupressinum* emergents. Map reference: NZMS 1 N105: 580295.

C: Hakarimata Range, Ngaruawahia, adjacent to water supply dam in moist valley; altitude 60 m. Major trees are *Weinmannia racemosa*, *Fuchsia excorticata*, *Leptospermum scoparium*. Map reference: NZMS 1 N56: 645622.

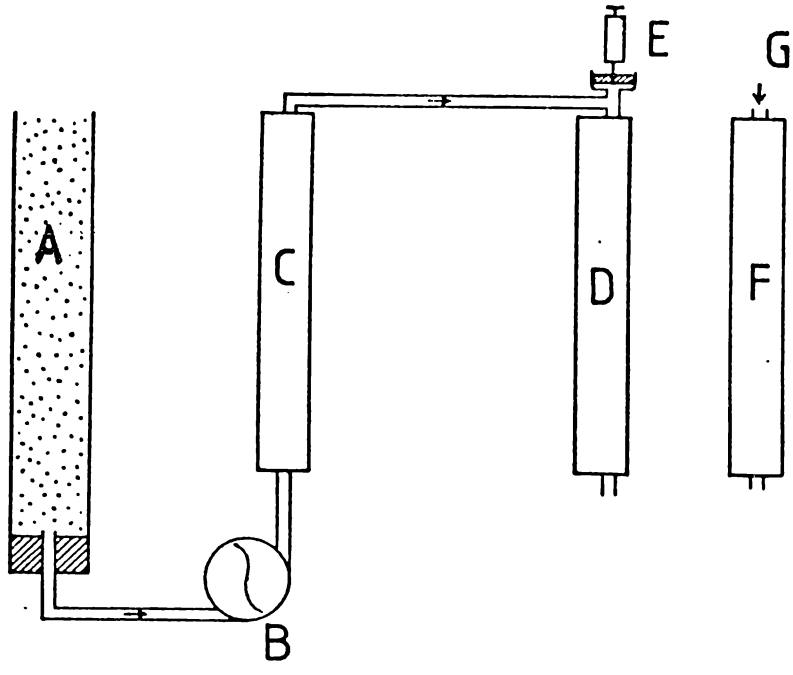


FIGURE 1. Diagramatic representation of gas flow system. A: carbasorb column; B: air pump; C: gas flow meter; D: analysis cell of IRGA; E: gas sample injection point through suba seal; F: reference cell of IRGA; G: CO_2-free air from internal supply in IRGA. Gas flow rates were 900 ml min^{-1} through analysis cell, 180 ml min^{-1} through reference cell.

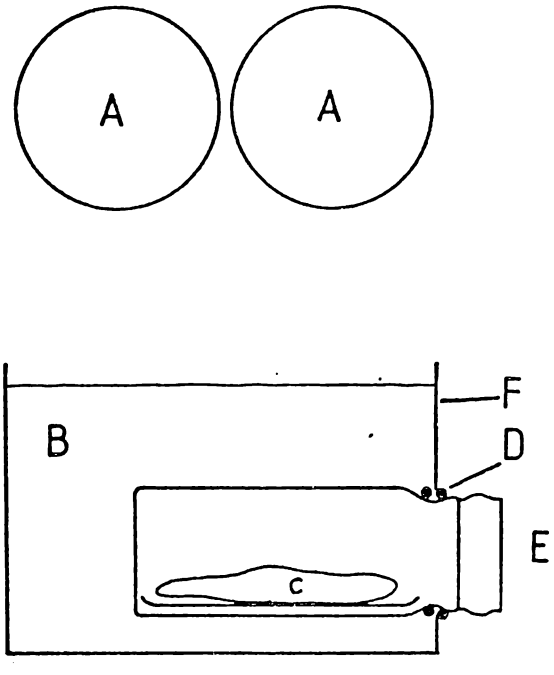


FIGURE 2. Photosynthetic incubation vial positioned in temperature controlled stainless-steel water bath. A: fluorescent tubes above water bath; B: circulated water at constant temperature; C: plant sample on aluminium-foil boat; D: rubber O-ring seals; E: screw cap with butyl rubber septum-seal on universal bottle; F: stainless-steel water bath.

was regulated by carrying out the assays in a 16°C controlled temperature room.

The standard incubation routine (prepared samples were assayed as shown in Fig. 3) used was a modification of Larson and Kershaw (1975b) who demonstrated that under the conditions shown CO_2 concentration and ventilation had a negligible effect on the rates obtained.

Dry weights of samples were obtained by drying to a constant weight of 100°C. In all instances CO_2 exchange rates are given in units of mg CO_2/g dry weight/hr. Similarly, all water contents are in mg water/mg dry weight.

RESULTS

Peltigera dolichorhiza (Fig. 4a)

This species was commonly found on the wet banks of tracks or where there was an ample ground-water supply as seepage and also a low to moderate light level. Saturated plants had a very high water content (maximum *c.* 6.0) and showed no clear depression of net assimilation rate (NAR) at these levels. Maximum NAR (6.5 mg CO_2 g^{-1} hr^{-1}) was high compared with other species tested. NAR was negative below a water content of 1.8 and remained so until gas exchange ceased at a water content of *c.* 0.4. Respiration rates remained nearly constant at *c.* 2.5 mg CO_2 g^{-1} hr^{-1} over a wide range of water contents from 2.5 to 6, with a steady decline with water content below 2.5. In comparison with other lichen assayed, the thallus of *P. dolichorhiza* appeared to be much more delicate and less robust.

Pseudocyphellaria billardierii (Fig. 4b)

The species is normally epiphytic, growing horizontally as large thalli across small branches in the lower canopy or subcanopy; relatively open forest areas appear to be favoured. Maximum water

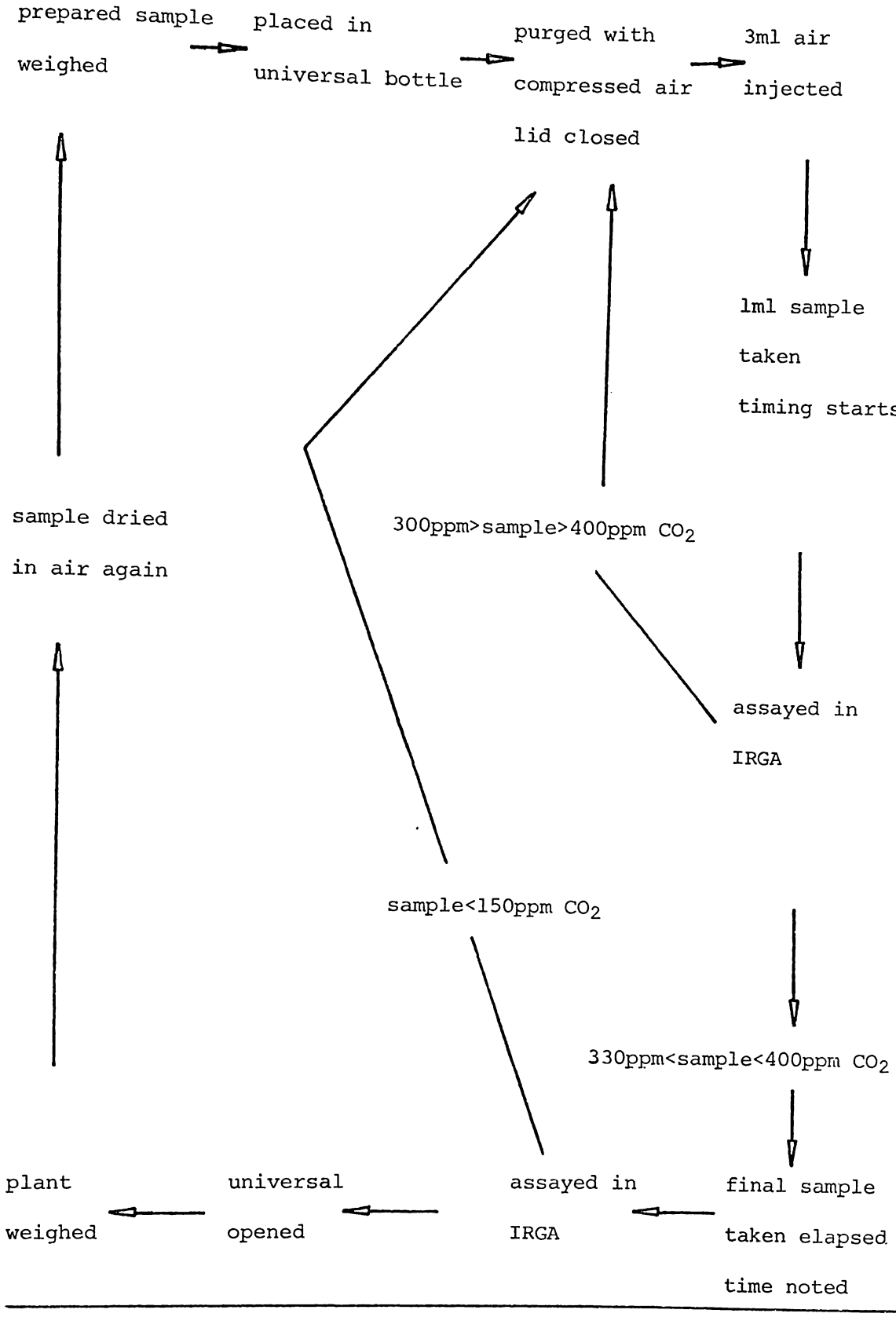


FIGURE 3. Standard assay routine indicating decision points necessary to maintain a constant CO_2 concentration range. The decision points do not need to be applied during respiration assays.

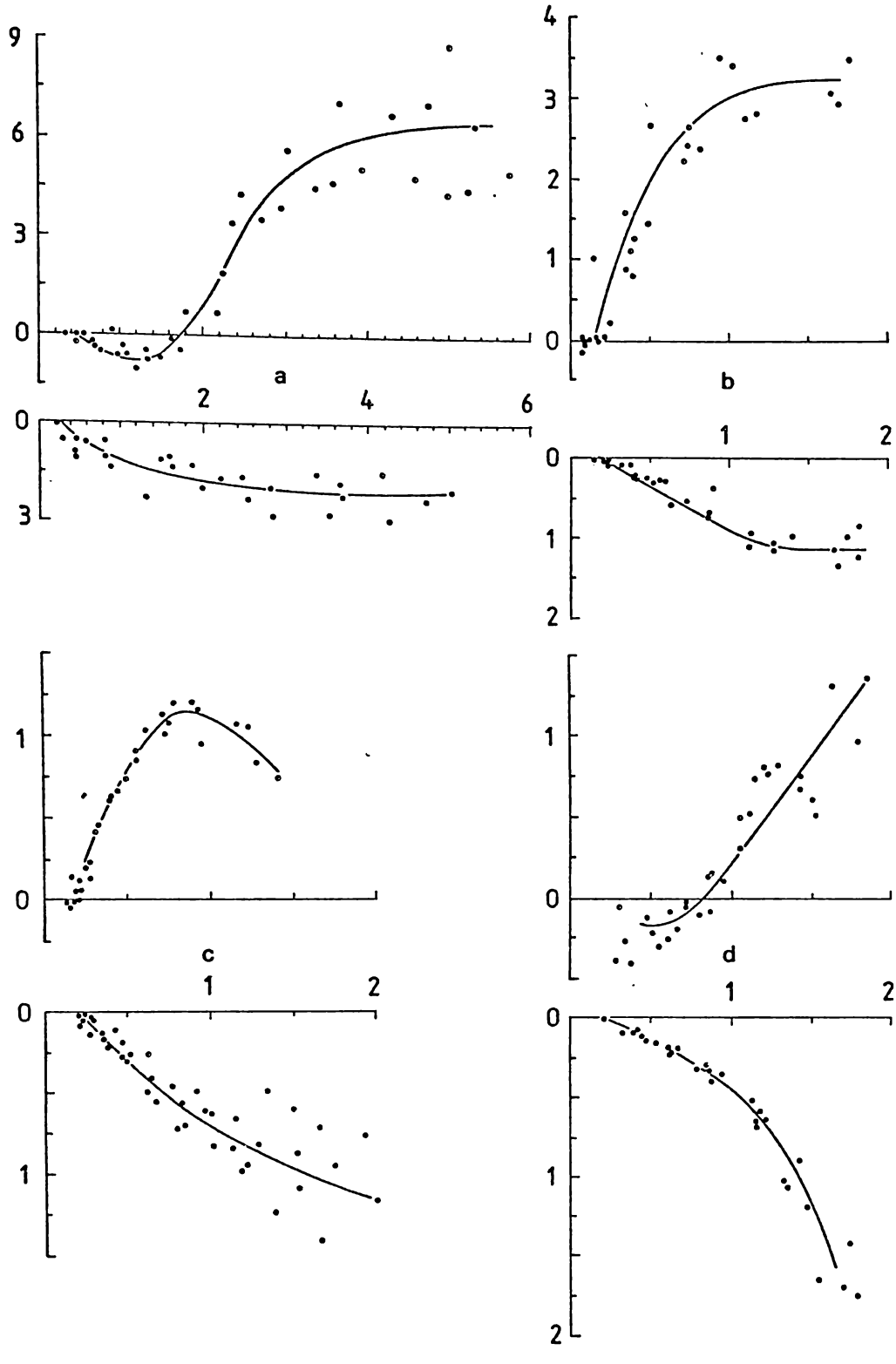


FIGURE 4. Net assimilation rates (upper graph) and respiration rate (lower graph) in mg CO_2 g^{-1} hr^{-1} with respect to water content for (a) *Peltigera dolichorhiza*, (b) *Pseudocyphellaria billardierii*, (c) *Pseudocyphellaria colensoi*, (d) *Pseudocyphellaria dissimilis*. Experimental details in text. Horizontal axis of all graphs is water content in mg water/ mg dry weight.

content (*c.* 1.8) is much lower than that of *Peltigera dolichorhiza* but approximates that of the other lichen species. The NAR shows a much higher rate of increase with water content and appears to approach a maximum rate of *c.* 3.0 mg CO_2 g^{-1} hr^{-1} at a water content of 1.0. Positive NAR was attained at a water content as low as 0.15 and this, together with a high NAR value at low water contents, indicates a tolerance of drying conditions.

Pseudocyphellaria colensoi (Fig. 4c)

P. colensoi is typically found on branches in the tree canopy, occurring high up tree trunks but lower down on trees fringing open areas. Such an environment would have reasonably high light intensities and drying conditions. Respiration rate increased steadily with water content and the maximum rate occurred at the maximum water content studied. NAR, however, increased to a maximum value of 1.2 mg CO_2 g^{-1} hr^{-1} at a water content of 0.9 and declined at higher water contents. At the highest water content the NAR was *c.* 60% of the maximum rate.

Pseudocyphellaria dissimilis (Fig. 4d)

P. dissimilis is normally found growing on the forest floor in very low light regimes. It is generally epiphytic on the exposed roots or lower trunks of trees, although it is sometimes found growing on rocks and mosses in very moist habitats.

The NAR graph for this species is unusual in that the increase is almost linear with water contents up to saturation point. This is the only species which did not show a levelling off or a decrease in NAR at high water content. At water contents below 0.8 NAR is negative and has a response similar to that of *Peltigera dolichorhiza*. The respiratory response is also unusual in that it appears to be asymptotic rather than linear, and again no obvious maximum was found.

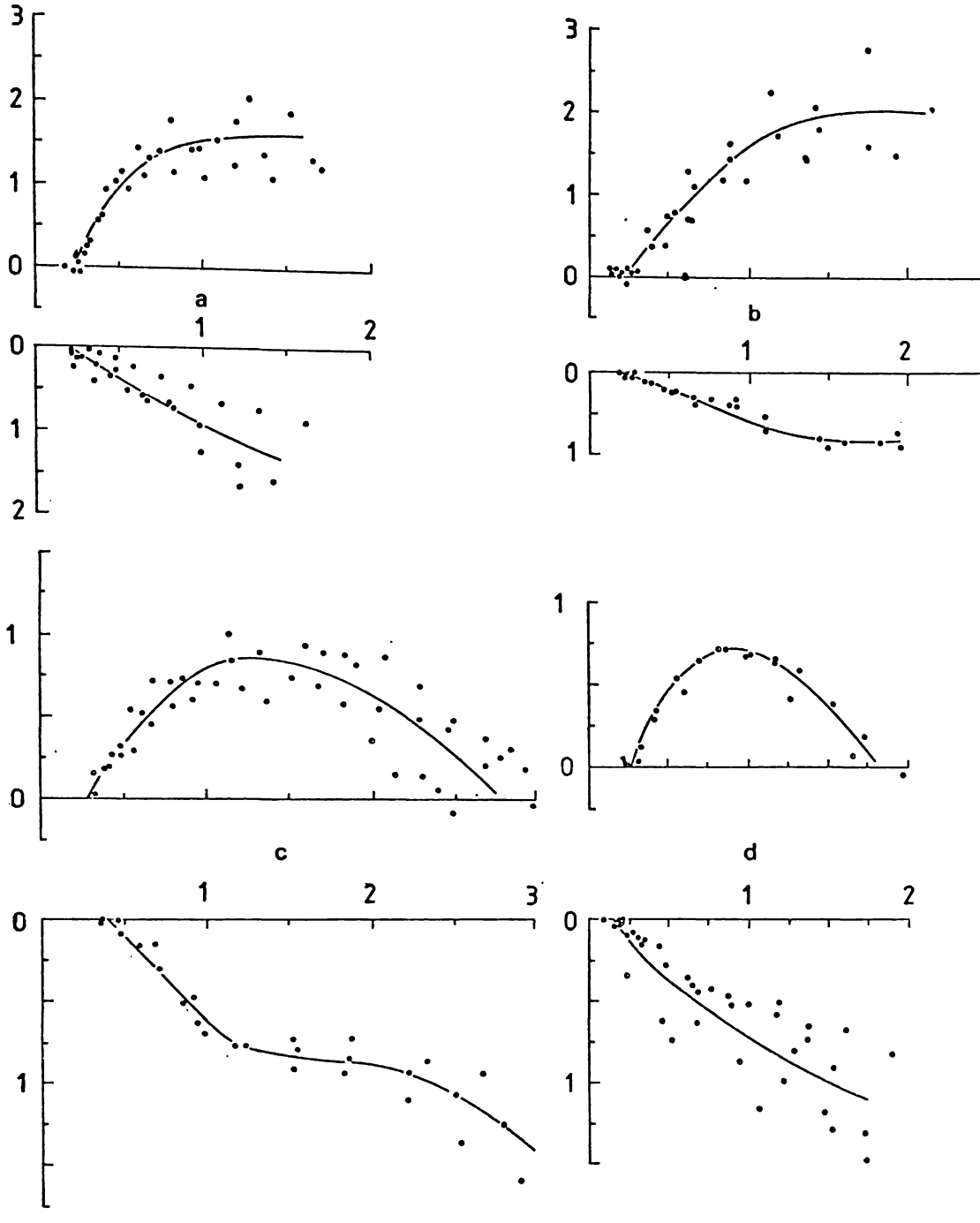


FIGURE 5. Net assimilation rates (upper graph) and respiration rate (lower graph) in mg CO_2 g^{-1} hr^{-1} with respect to water content for (a) *Pseudocyphellaria homoeophylla*, (b) *Sticta caperata*, (c) *Sticta latifrons*, (d) *Usnea* sp. Experimental details in text. Horizontal axis of all graphs is water content in mg water/mg dry weight.

Asymptotic respiratory responses have also been found by Kershaw (1977a, b) for *Peltigera canina* var. *praetextata* and *P. polydactyla*.

Pseudocyphellaria homoeophylla (Fig. 5a)

This is by far the most abundant lichen found in the mixed beech forest surrounding Lake Waikareiti. It is invariably epiphytic on the lower trunks of large trees (most often *Nothofagus menziesii*) or on the well developed buttresses of *Nothofagus fusca*. In these positions the plants receive *c*. 5% of the incident light and an ample water supply from rain and stem flow. In these forests *P. homoeophylla* is rare at altitudes below 900 m, being replaced by *P. delisia*, a similar, but isidiate, lichen.

The NAR increases rapidly with water content and reaches a constant rate of 1.6 mg CO_2 gm^{-1} hr^{-1} at a water content of *c*. 1.2. There appears to be little evidence for negative NAR at very low water contents, indicating simultaneous cessation of respiration and photosynthesis.

The respiratory rate increases steadily with water content over the range investigated, with some indication of a slowing in the rate of increase at the higher water contents.

Sticta caperata (Fig. 5b)

This species appears to occupy an almost identical habitat to that of *P. homoeophylla*. In this investigation both species were collected from the same tree, within 30 cm of one another. The response of NAR to water content is very similar to that of *P. homoeophylla*, with the main difference being a slightly higher maximum rate, 2.0 mg CO_2 g^{-1} hr^{-1}, which is attained at a higher water content of 1.5. Negative NAR values were rarely obtained at low water contents. Respiration rate increases steadily with water content, reaching a maximum rate of 0.9 mg CO_2 g^{-1} hr^{-1} at water contents of 1.5 and higher.

Sticta latifrons (Fig. 5c)

S. latifrons differs from the other lichen species studied in possessing a stalk and in growing out horizontally, particularly when young, on tree trunks normally some metres above the ground. Also, in contrast with the other species, older sections of the thalli were studied to avoid inclusion of ascocarps which commonly crowd the upper surface. NAR was zero at both high and low water contents, with a maximum rate of 0.8 mg CO_2 g^{-1} hr^{-1} at the intermediate water content of 1.3. Respiration rate shows a biphasic response to water content, increasing rapidly between water contents of 0.4 - 1.5 and 2.0 - 3.0 but remaining nearly constant between water contents of 1.5 and 2.0. *S. latifrons* and *Pseudocyphellaria dissimilis* are the only two species studied which show an asymptotic respiration increase at high water contents.

Usnea sp. (Fig. 5d)

Usnea sp. was the only lichen studied from outside the Peltigerineae, and was thus the only nitrogen non-fixer (Green *et al.* 1980). Plants are found on the exposed terminal twigs of trees and shrubs and are clearly visible from outside the canopy, obviously growing at a higher light level than any of the other species studied. NAR rises from nil at a water content of *c.* 0.25 to a maximum rate of 0.7 mg CO_2 g^{-1} hr^{-1} at a water content of 0.9 and declines to zero or slightly negative values at water contents >1.9. No evidence was found of negative NAR values at low water contents. The respiration response to water contents is almost identical to that found for *Pseudocyphellaria colensoi*, reaching a maximum value of 1.1 mg CO_2 g^{-1} hr^{-1} at the highest water contents studied.

Weymouthia mollis (Fig. 6)

W. mollis was the only bryophyte studied and was chosen because of its widespread occurrence in close proximity to many of the lichen

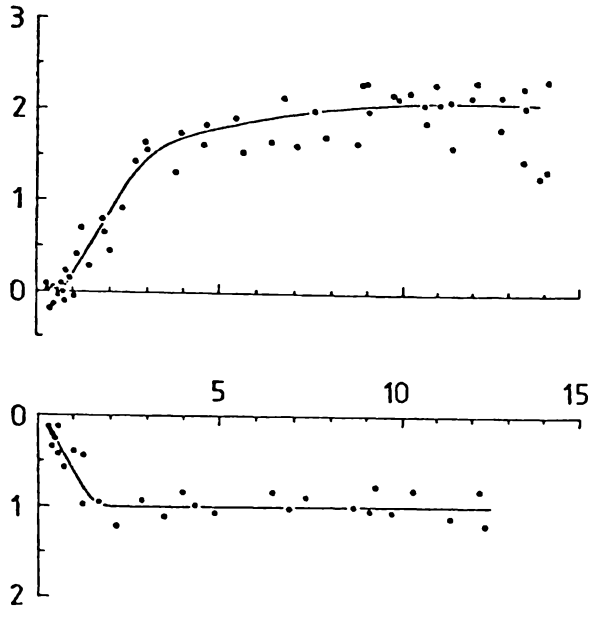


FIGURE 6. Net assimilation rate (upper graph) and respiration rate (lower graph) in mg CO_2 g^{-1} hr^{-1} with respect to water content for *Weymouthia mollis*. Experimental details in text. Horizontal axis is water content in mg water/mg dry weight.

species in the forests. Plants of *W. mollis* hang vertically from horizontal branches and twigs and are typical of many bryophytes in possessing a far greater water content at saturation than lichens. Both NAR and respiration rate show almost constant rates over most of the range of water contents (respiration above 2.0 and NAR above 4.0). Below these levels both NAR and respiration rates decline rapidly with lowering of water content. Maximal rates of NAR (2.0 mg CO_2 g^{-1} hr^{-1} at 10.0) and respiration (1.0 mg CO_2 g^{-1} hr^{-1} to all water contents above 2.0) are similar to those obtained for the lichens studied.

Effect of light intensity

Certain species, *Sticta latifrons, Pseudocyphellaria colensoi,* and *Usnea* sp. were found to show decreased NAR at the higher water contents. This effect is most marked in *Usnea* sp. and *S. latifrons* where slightly negative NAR was found. Since the NAR is the sum of photosynthetic CO_2 uptake by the alga and CO_2 release by the alga and fungus, it is possible that this decrease is a result of photosynthesis reaching maximal values at moderate to high water contents whilst respiration continues to increase with water content. A possible photosynthetic limitation was the light level used, set at 70 μE m^{-2} s^{-1} for all assays, which may be suboptimal for these three species, all of which come from moderate to high light areas. To investigate this possibility *Usnea* sp. was assayed at a higher light level, 280 μE m^{-2} s^{-1}, and a lower level, 37 μE m^{-2} s^{-1}. *P. colensoi* was also assayed at 280 μE m^{-2} s^{-1}.

Comparison of the NAR response of *Usnea* at the three light levels shows that -

(a) increasing light produces an increased maximum NAR.

(b) the water content at which NAR was maximal is similar at all three light levels.

(c) the depression of NAR at high water contents is decreased by increasing light level. At 37 μE m^{-2} s^{-1} there is considerable negative NAR at water contents >1.4 (note that results are variable) whereas at 280 μE m^{-2} s^{-1} no negative NAR was recorded; NAR at the highest water content exceeded the maximum NAR at 70 μE m^{-2} s^{-1} (Fig. 7 a, b, c).

The same features are found for *P. colensoi* when comparing NAR at 70 μE m^{-2} s^{-1} and 280 μE m^{-2} s^{-1}. However, when the maximal NAR values obtained at each light level are compared the two species differed in the size of increase. NAR (max.) of *Usnea* sp. was directly proportional to the light level (i.e., 400% increase in light gives a 400% increase in NAR (max) whereas the same increase in light level for *P. colensoi* gives a smaller increase in NAR (max.) (400% increase in light gives a 200% increase in NAR (max.)).

DISCUSSION

All species investigated showed a strong relationship between NAR and water content. The form of the responses of the lichen species, as shown in Figs. 4-6, can be divided into three main groups.

Group 1: *Peltigera dolichorhiza, Pseudocyphellaria dissimilis:* species showing pronounced negative NAR at low water contents, a high minimum water content for positive NAR, and no depression of NAR at high water contents. The responses of this group correlate with the observed ecology. These species are found in constantly wet conditions (though not necessarily low light) where very low water contents would be infrequent. *Peltigera dolichorhiza* may be capable of tolerating more open areas by means of a water supply from the soil through

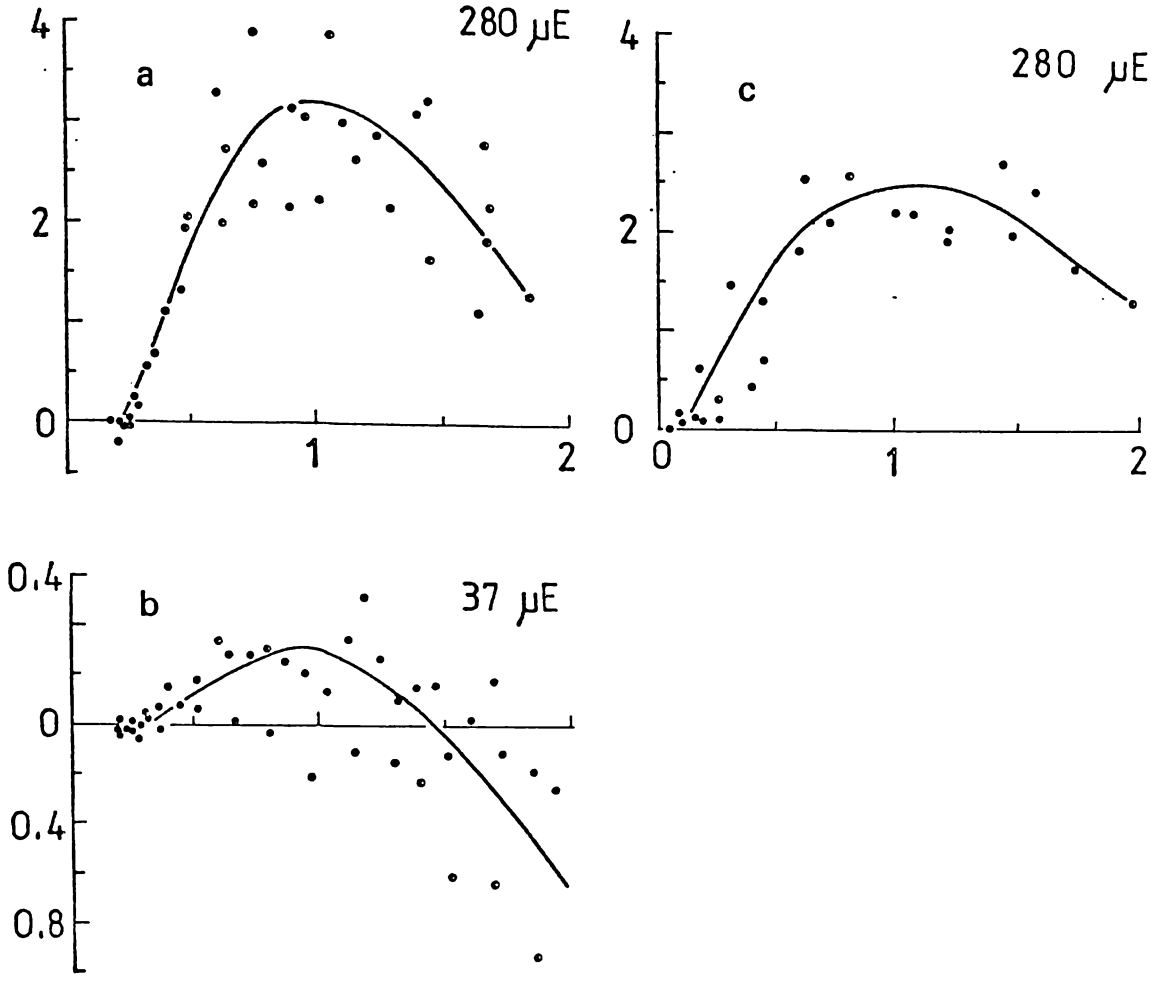


FIGURE 7. Net assimilation rate (mg CO_2 g^{-1} hr^{-1}) with respect to water content for (a) *Usnea* sp. at 280 µE m^{-2} s^{-1}; (b) *Usnea* sp. at 37 µE m^{-2} s^{-1}; (c) *Pseudocyphellaria colensoi* at 280 µE m^{-2} s^{-1}. Experimental details in text. Horizontal axis is water content in mg water/ mg dry weight.

the rhizines, although no direct evidence is available to support this suggestion.

Group 2: *Pseudocyphellaria homoeophylla, Sticta caperata, Pseudocyphellaria billardierii:* species showing just detectable negative NAR at low water contents, low minimum water contents for positive NAR, and a constant NAR at moderate to high water contents. *P. homoeophylla* and *S. caperata* are both found at the base of tree trunks whereas *P. billardierii* grows on branches. All receive low light levels and a greater and more frequent drying stress than do Group 1 species. *P. billardierii*, in particular, grows in more exposed conditions, and is almost equivalent to a fruticose lichen in being free from the substrate, and has a very low minimum water content for positive NAR.

Group 3: *Pseudocyphellaria colensoi, Sticta latifrons, Usnea* sp.: none or very little negative NAR at low water contents, low minimum water content for positive NAR, depression of NAR at high water contents. These species grow in exposed conditions of moderate to high light and consequent higher desiccation stress. Maximum NAR is reached at low water contents and is depressed at high water content.

There is also a trend for water contents at which maximum NAR occurs to be in the order Group 1>Group 2>Group 3.

The negative NAR of *Usnea* sp. at high water contents is a result of the failure of photosynthesis to counter the effects of increasing respiration, (Harris 1976). This effect can be alleviated by increasing the light intensity and thus increasing photosynthesis, although it is possible that the depression may not be completely

removed. This alleviation indicates that the Group 3 species are being assayed under sub-optimal conditions with regard to light, which again correlates well with the observed ecology.

A similar alleviation of depression at high water content by high light intensity is also shown for *Stereocaulon paschale* by Kershaw and Smith (1978). Kershaw (1977a, b), however, shows this NAR depression for *Peltigera polydactyla* and *P. canina* var. *praetextata* and with no alleviation at higher light intensities, although maximum water contents are lower than those found for *P. dolichorhiza* in the present investigation. Comparison of NAR and respiration responses with water content (Kershaw 1977a, b) show that in this case NAR depression is not a result of increased respiration and must result from some other factor which affects photosynthesis, possibly carbon dioxide diffusion (Harris, 1976). The maximum NAR values obtained by Kershaw (1977a, b) of 2.0 mg CO_2 g^{-1} hr^{-1} at 100 µE m^{-2} s^{-1}, 15°C are much less than those obtained here (6.5 mg CO_2 g^{-1} hr^{-1} at 70 µE m^{-2} s^{-1}, 16°C). This, together with the enhancement with higher light intensities obtained by Kershaw suggests that the species which he trested were from more open environments than those of *P. dolichorhiza*.

The maximum NAR values obtained for lichens in the present investigation agree well with maximal rates published elsewhere (Kershaw 1977a, b; Larson and Kershaw 1975b, Kershaw and Smith 1978), indicating the validity of the present technique. Respiration values obtained are also comparable. In assays of both NAR and respiration a noticeably greater variability between replicates was found at the higher water contents. This may reflect uneven distribution of liquid water content and location at these water contents (Smith, 1962).

The fall in NAR at low water contents has been accepted to be a result of physiological changes in the lichen. However, it is difficult to understand why respiration (negative NAR) should continue to much lower water contents in *Peltigera dolichorhiza* and *Pseudocyphellaria dissimilis*. It is known that the photosynthetic pathways are more sensitive to decreasing water content than respiratory pathways (Cowan *et al.* 1979a),however, such differences are not obvious for the Group 2 and Group 3 lichens. A further constraint that could apply to photosynthesis is the increasing opacity of the lichen cortex to light at low water contents. Evidence that this might be important is present in the data of Kershaw (1977b) where increasing light intensity lowered the minimum water content for positive NAR in *Peltigera polydactyla* and *P. praetextata*.

Weymouthia mollis was the sole bryophyte studied and clearly demonstrates the major differences between bryophytes and lichens in water relations. *W. mollis* had a far greater water content at saturation than any of the lichens (15.0 against 6.0 for *Peltigera dolichorhiza*). Most of the water is held externally on the surface of the leaves, so that NAR and respiration behave independently of water content until external and internal liquid water reserves are lost. This agrees with the results of Busby and Whitfield (1978) for four bryophyte species. *Ptychomnion aciculare* also shows this photosynthetic pattern (unpublished data). Similar external storage of water may occur in some species of *Pseudocyphellaria* (Snelgar and Green 1981b).

The results demonstrate that New Zealand species of cryptogams provide excellent experimental material for gas exchange studies. Interpretations of the responses to water content changes are similar to those proposed in published overseas work. Certain

differences are, however, very clear; these include the abundance of species from low light, moist habitats in contrast with those of more open areas described elsewhere. Also, the genus *Pseudocyphellaria* contains species which collectively span an extremely wide ecological range and thus provides material for comparative ecophysiological studies not available elsewhere.

CHAPTER VI

Carbon dioxide exchange in lichens: CO_2 exchange through the cyphellate lower cortex of *Sticta latifrons* Rich.

INTRODUCTION

There have been many studies on the net photosynthetic rate (NPR) of lichens, particularly with respect to thallus water content. There appears, however, to have been little research into the actual details of the path of carbon dioxide diffusion into the thallus. Collins and Farrar (1978) investigated the resistances to carbon dioxide movement in *Xanthoria parietina* and concluded that resistance values were far higher than those found in higher plants and that the increase could be explained by the dense upper cortex. The possibility that the upper cortex could be limiting to photosynthesis was originally suggested by Hill (1976) although no actual evidence was presented. Lichens in the family Stictaceae possess either cyphellae or pseudocyphellae on the underside and in the past these have been suggested to have a role in gas exchange although, again, no actual evidence is available (Henssen and Jahns, 1974; Hale, 1974; Rúndel *et al.* 1979; Ahmadjian and Hale, 1973). Finally Scott (1960) proposed from growth studies that gas exchange in *Peltigera praetextata* occurred entirely through the fungal medulla and that excess water would diminish the rate of exchange.

In a recent study of the NPR with respect to water content of some New Zealand lichens (Snelgar *et al.* 1980) several members of the Stictaceae were surveyed using a drying down method that involved directing a jet of air onto the lichen for a known time. The possibility existed that the lichens could respond differently if the top or bottom surface was selectively dried and, in particular, it was

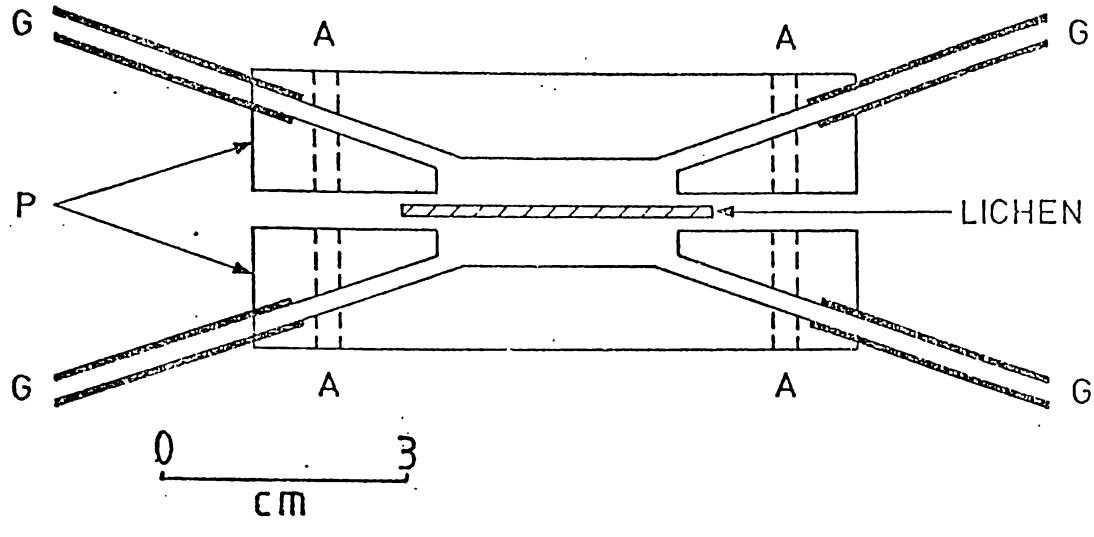
thought that drying down of the upper surface could lead to selective desiccation of the algal layer compared to the rest of the thallus. In this paper it is reported that differences do occur if the top and bottom surfaces are selectively dried but that these most probably result from changes in the resistance to carbon dioxide exchange.

MATERIALS AND METHODS

Large specimens of *Sticta latifrons*, Rich. a stipitate species, were collected from *Nothofagus* forest at Park Headquarters, Urewera National Park, North Island, New Zealand (NZMS 1 N96 575 430). Plants were used immediately or within two weeks after dry storage over silica gel at 16°C. Thalli were often large, up to 20 cm in length and breadth, but only younger portions, approximately 5 cm by 2 cm,free from damage and apothecia were used in experiments. Thalli that had been stored were remoistened and held at 16°C, 50 $\mu E\ m^{-2}\ s^{-1}$ and 100% RH for at least six hours before use in order to minimise the effects of resaturation respiration.

In drying down experiments specimens were incubated in 30 ml glass vials at 70 $\mu E\ m^{-2}\ s^{-1}$, 16°C with 1ml gas samples being removed at the start and finish of incubation. The gas samples were analysed on an ADC series 225 infra red gas analysers (IRGA) modified to allow sample injection (Larson and Kershaw, 1975b; Snelgar *et al.* 1980). Specimens were dried for a short time with a jet of air between incubations and were weighed before and after incubation. Thallus water contents are expressed as mg water per mg thallus dry weight and represents the mean value for each incubation.

Split chamber experiments were carried out with a simple perspex chamber into which a thallus could be clamped so that it acted as a partition between the two halves of the chamber (Figure 1).



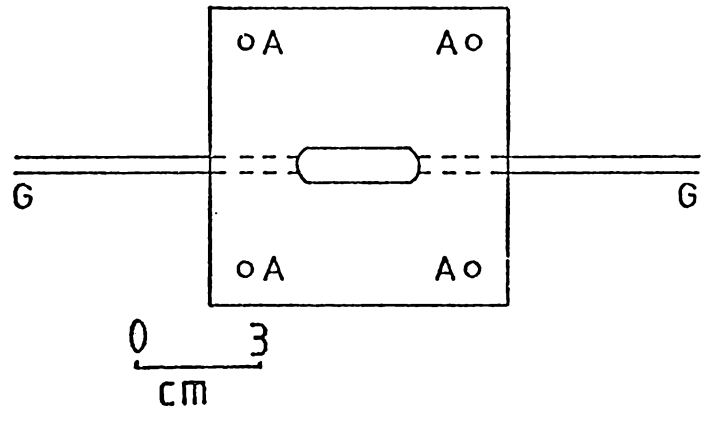


FIGURE 1. Details of Split Chamber: Upper diagram - section across the chamber showing the two perspex halves (P) with central chamber and gas line connections (G). A lichen thallus is shown dividing the chamber and is sealed in place by petroleum jelly whilst the two chamber halves are held together by four bolts through the holes marked A. Lower diagram - plan view of bottom of chamber half; note central chamber and four securing bolt positions (A). Chamber dimensions are approximately 30 mm long, 10 mm wide and 5 mm deep (in each chamber half). Total area of lichen exposed to each gas stream was 2.5 cm^2.

The edges of the chamber were sealed with petroleum jelly. A stream of air (approx. 340 µl CO_2 l^{-1}) was supplied to one side of the lichen whilst the other side of the chamber formed part of a closed loop that included the IRGA analyser column. When the chamber was sealed 2.5 cm^2 of each lichen surface was exposed to the air flow. Gas flows were normally 2.5 cm^3 s^{-1} and incubations were at a thallus temperature of 16 - 20°C and 150 µE m^{-2} s^{-1} (saturating light).

RESULTS

The change of net photosynthetic rate as the thallus dried is shown in Figure 2. Initially all thalli showed a low NPR at high water content, a feature consistently found for this species. During drying bottom dried thalli showed a rapid increase in NPR to a maximum at a water content of 1.5 followed by a decline with further drying. Top dried thalli showed a similar pattern but the rise in NPR from the low levels at high water content occurred at slightly lower water contents than found for bottom dried lichens. Although there was variation between specimens this delay in the rise of NPR appeared to be a consistent effect. This difference could not be explained on the basis of increased desiccation of the algae since, with further drying down, both top and bottom dried specimens showed increased NPR. Neither could the differences be explained by changes in the respiration rate since these were similar for bottom and top dried lichens (Figure 2). Top dried thalli do show a slightly lower value for maximum NPR suggesting that there could be a slightly increased dessication effect on the algae although the results presented are not conclusive (Figure 2).

Typical results from the use of the split chamber are shown in Table 1. There was minimal carbon dioxide exchange through the top cortex whilst the rate through the lower cortex approximated to that

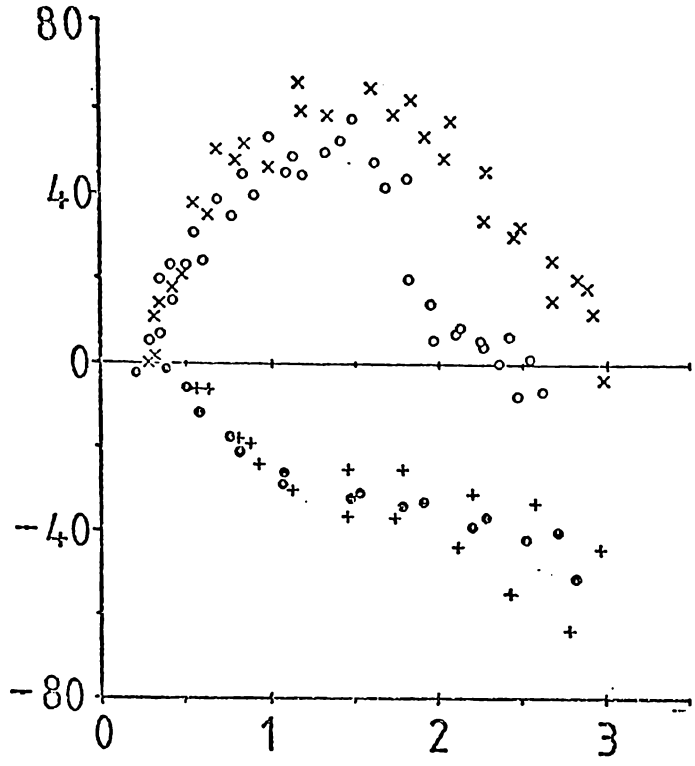


FIGURE 2. NPR and respiration rate for top and bottom dried thalli of *Sticta latifrons*. Methods are given in text; incubations at 16°C, 70 μE m^{-2} s^{-1} using the injection technique. Vertical axis is carbon dioxide exchange in μg m^{-2} s^{-1} (negative values indicate net release of carbon dioxide). Horizontal axis is water content as mg water per mg thallus dry weight. Top dried thalli: O, NPR, ⊕, respiration. Bottom dried thalli: ×, NPR, +, respiration.

TABLE 1. CO_2 exchange of *Sticta latifrons* complete thalli, and separately through top or bottom surfaces, during incubations at 16 - 20 C 150 μE m^{-2} s^{-1} (saturating intensity). Values for individual lichen surfaces were obtained using the split chamber described in Figure 1 whilst values for complete thalli were obtained using the injection technique (see methods). Values for complete lichen thalli are from different thalli to those used in the split chamber.

		NPR			RESPIRATION		
	Thallus Surface assayed	μg CO_2 m^{-2} s^{-1}	Mean	%†	μg CO_2 m^{-2} s^{-1}	Mean	%†
INTERMEDIATE WATER CONTENT (0.8 to 1.0)	Top	-1.9, -2.8, 4.9	0.1	0.2	-6.6, -28.5, -5.7	-13.6	19
	Bottom	62.2, 59.9, 56.5	59.5	99.8	-72.3, -42.9	-57.6	81
	Complete Thallus	40 to 60			-30		
HIGH WATER CONTENT (1.7 to 2.0)	Top	0, 0, 0, -4.3	-1.1	0	-13.6, -8.5, -18.4, -19.8	-15.1	16
	Bottom	17, 11.3, 4.8, 22.6	13.9	100	-72.3, -84.8,-63.6, -107.4	-82.0	84
	Complete Thallus	15 to 40			-40 to -50		

† Percentage CO_2 exchange occurring through each surface

obtained from studies on a complete thallus. The thallus water contents were such that near maximal NPR would be expected. Soaking of the thallus so that the lower cortex carried a film of water resulted in depressed CO_2 uptake through the lower cortex, similar to the NAR depression found for complete thalli at high water contents, and only minimal exchange occurred through the upper cortex. Respiratory gas exchange was detectable through the upper cortex but at a much lower rate than through the lower lichen surface.

DISCUSSION

The split chamber experiments clearly show that minimal detectable carbon dioxide exchange occurs through the upper cortex even when NAR is close to the maximum value. The initial results obtained by drying down *S. latifrons* on either top or bottom surface can be explained on the basis that the majority of the carbon dioxide exchange occurs through the lichen undersurface. At high water contents the film of water covering the lower cortex will act as a barrier to carbon dioxide diffusion since the rate in solution is approximately 10^4 times slower than in air. Drying down of the lower surface only would rapidly remove the water film and allow gas exchange to proceed. Drying down of the top surface would leave the water film intact on the lower surface for a longer period and cause the period of low NPR to extend to lower thallus water contents. Support is thus given to the proposal of Scott (1960) that lichen medullas may be important for gas exchange.

The results provide strong circumstantial evidence that the cyphellae present on the lower surface of *S. latifrons* do act as air pores. The possibility is made even more likely by the occurrence of a cortex on both upper and lower surfaces of the species with the

lower cortex being penetrated by the cyphellae, (Henssen and Jahns, 1974). It is possible that the cyphellae could also be important with respect to the water relations of the species. The high water contents used in this investigation were obtained by spraying water on both sides of the thalli so that the entire underside is soaked. Such conditions are not likely to be common in nature. Lichen thalli collected in the field after three days continuous rain during which a Piche evaporimeter in the open recorded zero evaporation had water contents from 2.02 to 2.57 whereas thalli collected early in the morning after dew formation during a dry night had water contents from 0.89 to 1.01. *S. latifrons* hangs from vertical tree trunks by single holdfast and presents only its upper surface to rain or mist. Wetting of the exposed upper surface will have no effect on gas exchange whilst the under surface is protected from direct water impact. Studies on another member of the Stictaceae, *Pseudocyphellari dissimilis* (Nyl.) D. Gall, and P. James *in litt.*, (Snelgar and Green, 1980b) have shown that the tomentum on the lower surface of the species acts as a reservoir of water. A similar situation is suggested for *S. latifrons* where the tomentum would hold water on the lower surface whilst the cyphellae, which are free of tomentum, would allow free gas exchange.

There has been only one published study on resistances to carbon dioxide movement in lichens (Collins and Farrar, 1978). In that study it was assumed that all gas exchange occurred through the upper cortex and equivalent water path lengths were calculated on that basis. The results from this study cast doubt on that assumption since gas exchange could easily have been occurring through the more loosely arranged medulla. The results presented here, taken with those of Snelgar and Green (1980b), indicate that the Stictaceae will prove to be an excellent group for the study of lichen water relations because of the wide range of ecology and morphology in the group.

CHAPTER VII

Carbon dioxide exchange in lichens: Relationship between net photosynthesis and CO_2 concentration

INTRODUCTION

Studies of photosynthesis in lichens have been made by a variety of techniques including the measurement of ^{14}C uptake (Cowan *et al*, 1979a; Farrar, 1976a; Hallgren and Huss, 1975), changes in gas volume both by manometry (Smythe, 1934) and a cartesian diver system (Pearson and Brammer, 1978) and O_2 concentration by O_2 electrode (Baddely *et al*, 1971). Most of these methods have the disadvantage that the lichens are subjected to highly unnatural conditions, such as immersion in buffer solutions and exposure to CO_2 and O_2 concentrations which differ markedly from ambient levels. The results of ^{14}C uptake experiments also present interpretation difficulties as the gross photosynthetic rate of lichens is substantially greater than the net photosynthetic rate (NPR). More recently most studies of the rates of CO_2 exchange have used an infra red gas analyser (IRGA) system, sometimes, with an adaptation of a flow through cell (Harris, 1971) to allow simultaneous determination of CO_2 exchange and water content. However, the low resistance to water loss common to all lichens (Blum, 1973),coupled with the close interdependence of gas exchange rates and thallus water content, led to the use of low flow rates and, in some instances, this resulted in substantial systematic errors (see Larson and Kershaw (1975b for discussion).

Several authors (Atkins and Pate, 1977; Clegg *et al*, 1978; Larson and Kershaw,1975b) have independently described a method of adapting IRGAs for use as discrete sample analysers whereby small volumes of gas samples (1-3 ml) can be injected, via a carrier gas, directly into the analysis tube of an IRGA. The maximum deflection

of the IRGA is found to be proportional to the quantity of CO_2 injected, thus, for constant injection volume, it is proportional to the CO_2 concentration of the injected gas. Recently this technique has been adopted by some lichenologists (Kershaw and Smith, 1978; Larson,1979b; Snelgar *et al*, 1980), for routine assays of photosynthetic and respiratory rates. Thalli are incubated in a small, temperature controlled, glass cuvette for a period of 5-30 min. Initial and final gas sampling allows determination of the rate of decrease of CO_2 concentration within the cuvette and from this rates of CO_2 exchange are easily calculated. The major advantage of this technique over conventional gas flow methods is the large number of low cost cuvettes that can be used simultaneously, thus allowing the use of more replicates and more comprehensive, multifactorial, experimental design. The advantages are further detailed by Larson and Kershaw (1975b).

Use of such simple, unventilated, cuvettes is possible only when CO_2 exchange rates are independent of both CO_2 concentration, over the range used, and ventilation. Larson and Kershaw (1975b) and Larson (1979b) have presented results which indicate that neither of these factors may have any measurable effect on NPR at CO_2 concentrations between 150 and 350 $\mu l\ l^{-1}$ for a variety of lichen species. Further it has been stated that water content has no effect on the relationship between NPR and CO_2 concentration and also that lichens differ from higher plants in that NPR is CO_2 saturated at low (150-200 $\mu l\ l^{-1}$) CO_2 concentrations (Larson and Kershaw, 1975b). Surprisingly it has been found that no published data exist for the relationship between NPR of lichens and CO_2 concentration other than that given in support of the IRGA injection technique. Recent work in this laboratory concerned with the measurement of CO_2 resistance in several species of Stictaceæ has

indicated that these lichens often require very high CO_2 concentrations in order to saturate photosynthesis. These results imply that lichen photosynthesis may be affected by variations in CO_2 concentration at near ambient levels, therefore a study of the relationship between CO_2 concentration, ventilation and NPR was considered necessary.

MATERIALS AND METHODS

Healthy lichen thalli were collected from Hakarimata Scenic Reserve, Ngaruawahia (NZMS 1, N56 645622), from the Urewera National Park (NZMS 1, N96 619437) and from Mount Te Aroha (NZMS 1, N57 233776) all localities being in the central North Island of New Zealand. Only the terminal 3-8 cm of foliose lichen thalli was used, whilst for *Stereocaulon ramulosum* 8-20 cm^2 central portion of the lichen mat were selected at random. Thalli not used on the day of collection were stored air dry over silica gel at 16°C in darkness for not more than two weeks. Prior to experimental use stored thalli were moistened by mist spraying with distilled water and held at 100% RH 16°C, 50 $\mu E\ m^2 s^{-1}$ for at least 10 hours to eliminate rehydration effects.

Measurements of NPR were carried out in a water jacketed perspex cuvette using an ADC series 225 infra red gas analyser (IRGA) operating in a closed loop mode. The total volume of the system was 370 cm^3, flow rate 0.5 l min^{-1} giving a calculated mean windspeed of 0.28 cm s^{-1} within the cuvette. Thallus temperature was maintained at 16.0 $\pm$ 0.5 C and monitored by thermistor probe. Quantum flux density was 150 $\mu E\ m^{-2} s^{-1}$ at the lichen surface. The output of the IRGA was amplified and continuously graphed on a servoscribe recorder. NPR was calculated as the slope of the tangent to the CO_2 concentration against time curve for chosen CO_2 levels. The

precision of a single measurement has been estimated by repeated measurement to be better than ± 3% for any specimen. The CO_2 concentrations required were generated by flushing the cuvette with CO_2 free air and injecting small volumes CO_2 standards (20,000 µl l^{-1}). During NPR measurements on any thallus CO_2 concentrations were first decreased from 400 µl l^{-1} CO_2 to near zero, then progressively increased from 400 µl l^{-1} to the highest levels used. Slight water loss occurred from thalli during each incubation and water contents are expressed as the mean of the initial and final water content in units of g water per g dry weight thallus (obtained by drying to a constant dry weight at 100°C). Water content was adjusted to the desired value by a combination of mist spraying and blotting thalli with paper towels.

RESULTS

Relationship between NPR and CO_2 Concentration. Figures 1A and 1B show the relationship between NPR and CO_2 concentration for two thalli of *Sticta latifrons* and represent the most extreme between thalli differences found. These differences probably result from variations in morphology between thalli such as the unusually thick tomentum of thallus B. Thallus A, at intermediate and low water contents, shows a typical linear response of NPR to CO_2 at low CO_2 concentrations with CO_2 saturation being reached at about 400 µl l^{-1}. At high water contents, however, the NPR response was still almost linear at 1000 µl l^{-1} CO_2. Unpublished work, under microaerobic (<1% O_2) conditions, has shown that maximal NPR can be attained at high thallus water contents but that saturating CO_2 concentrations may be in excess of 2000 µl l^{-1}. At low water content the maximum NPR is depressed probably as a result of the effect of decreasing water potential on the carboxylation system. Thallus B

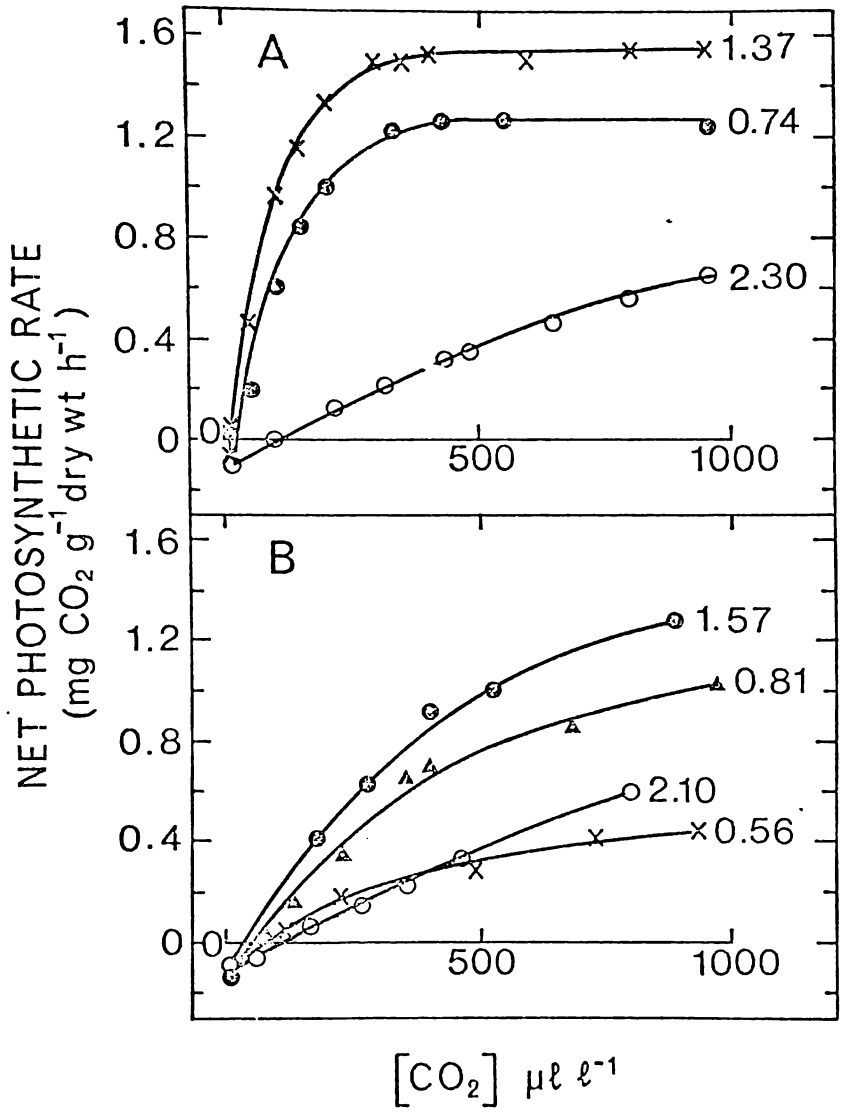


FIGURE 1. Variation of NPR with CO_2 concentration for two thalli (A and B) of *Sticta latifrons*. Each curve is labelled with the thallus water content at which the determination was made.

showed a similar result with the difference that saturation was reached at about 1000 µl l^{-1} CO_2. Blotted thalli of *Peltigera dolichorhiza*, *Pseudocyphellaria billardierii* and *Stereocaulon ramulosum* showed almost identical responses of NPR to CO_2 concentration (the results are transformed to percentage maximum NPR) with saturation at about 450 µl l^{-1} (Fig. 2).

These results do not agree with those of Larson and Kershaw (1975b) and Larson (1979b) (Fig. 2), diverging markedly between 70 and 400 µl l^{-1} CO_2 and also in the CO_2 concentration at which NPR is CO_2 saturated. At 200 µl l^{-1} CO_2 Larson and Kershaw (1975b) suggest that NPR was still maximal whilst in this investigation NPR had declined to about 68% of maximum. To make a comparison of the two methods used to obtain these results a complete graph of decline in CO_2 concentration in the closed IRGA system against time was obtained for *P. billardierii* and NPR was calculated from this graph by the following two methods:

(1) The 'instantaneous' NPR was measured at the slope of the tangent to the curve at any particular CO_2 concentration.

(2) The time taken for the CO_2 concentration to drop from 350 µl l^{-1} to *x* µl l^{-1} was measured and the mean NPR calculated. This rate was then plotted against the final CO_2 concentration (*x*). This method appears to be analogous to that of Larson and Kershaw (1975b) and Larson (1979b) in that NPR was determined from the initial and final CO_2 concentrations, then plotted against the final CO_2 concentration.

The results are shown in Figure 3, together with those obtained by Larson and Kershaw (1975b). The curve produced by method 2 has a closer resemblance to that obtained by Larson and Kershaw. The practice of plotting NPR against final CO_2 concentration is an

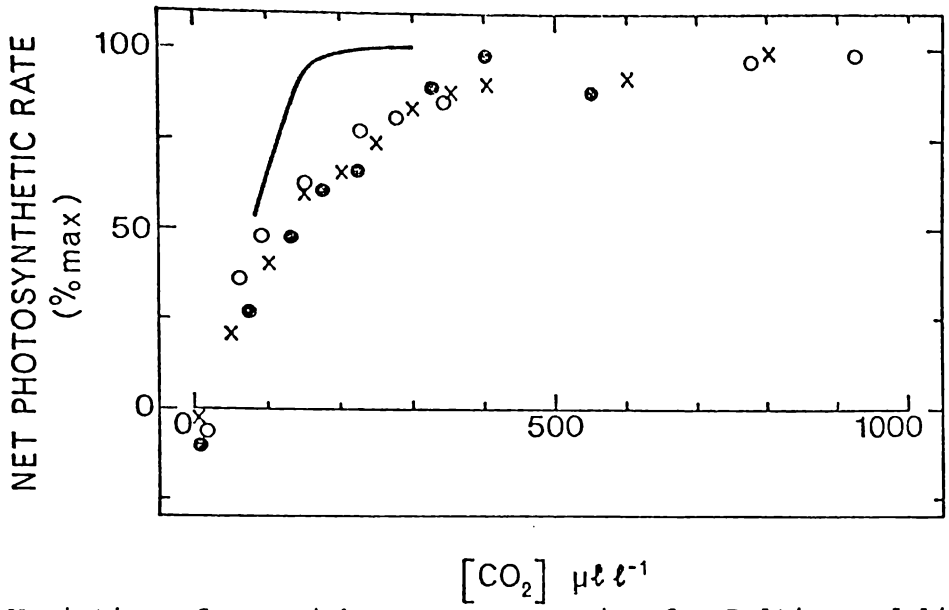


FIGURE 2. Variation of NPR with CO_2 concentration for *Peltigera dolichorhiza* (x, water content 3.21), *Pseudocyphellaria billardierii* (o, water content 1.34) and *Stereocaulon ramulosum* (⊙, water content 1.90). Solid line represents the results obtained by Larson and Kershaw (1975b).

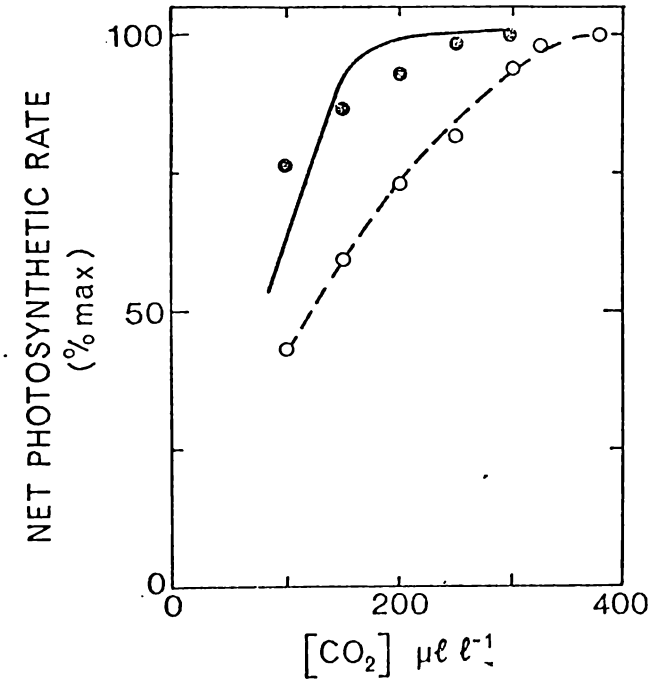


FIGURE 3. Variation of NPR of *Pseudocyphellaria billardierii* with CO_2 concentration as analysed by methods 1 (o) and 2 (⊙). See text for details. Solid line represents the results obtained by Larson and Kershaw (1975b).

important factor in producing this resemblance. This type of response curve is more properly a check on the minimum CO_2 concentrations to which incubations can be taken without measurably affecting NPR rather than an accurate representation of the relationship between NPR and CO_2 concentration.

Ventilation of Cuvette. Jarvis (1971) stressed the importance of adequate ventilation within curvettes in order to minimise boundary layer CO_2 resistance (r_a). In well stirred cuvettes typical r_a values of 0.5 to 2.0 s cm^{-1} are attained and are reasonable in comparison to field values of 0.1 to 0.3 s cm^{-1} and the total resistance (Σr) of a plant, with open stomata, to CO_2 uptake.

The lichen r_a in the ventilated cuvette of Larson (1979b) can be calculated by the equation of Monteith (1965).

$$r_a = 1.3 \sqrt{\frac{d}{u}} \qquad (1)$$

r_a is 0.15 s cm^{-1} where d (characteristic dimension of the lichen) is 5 cm and u (windspeed) is 400 cm s^{-1}.

In unstirred cuvettes, as normally used in the discrete sampling injection IRGA technique, the effective depth of the boundary layer (D), in nominally still air, can be calculated (Meidner and Mansfield, 1968), from

$$D = \frac{\pi d^{0.6}}{8}$$

For the same dimensions used in solving equation 1 the boundary layer depth becomes 1.03 cm giving r_a = 7.0 s cm^{-1}.

Larson (1979b) found no detectable difference in NPR at ambient CO_2 levels in ventilated and non-ventilated cuvettes although r_a would have increased significantly, as calculated above, from 0.15 s cm^{-1} to 7.0 s cm^{-1}. It is probable that the explanation of

this result lies in the unusually high total resistance (Σr) reported for lichens: 50 to 300 s cm^{-1} (Snelgar, Green and Wilkins, 1981b); 136 s cm^{-1} (Collins and Farrar, 1978). When Σr are of this magnitude an increase in r_a of 6.85 s cm^{-1} would increase Σr by 2-14%. If the relationship between NPR and (CO_2) is linear this would result in a 2-14% reduction in NPR. However at ambient CO_2 levels factors other than CO_2 concentration may be limiting NPR and under these conditions the increase in r_a would have less effect.

CO_2 compensation value (τ). The results given in Figures 1A and 1B and 2 confirm previous preliminary studies that lichens often have unexpectedly low CO_2 compensation values (Snelgar and Green, 1980). The present results indicate that τ may vary depending on thallus water content with intermediate water contents having the lowest values and τ being considerably larger at high water contents (Fig. 1A). Comparison of Figures 1A and 1B clearly demonstrates that τ may also show large variations between thalli.

DISCUSSION

In many ways the response of the NPR of lichens to CO_2 concentration is similar to that of higher plants. Lower CO_2 saturation levels, as suggested by Larson and Kershaw (1975b) were not found and CO_2 saturation was normally more than 400 μl l^{-1} CO_2 although there are differences between thalli of the same species. Thallus water content is a major factor affecting the response to CO_2 and at high water contents in *Sticta latifrons* it is apparent that saturating CO_2 levels around 2000 μl l^{-1} may be expected and the response to changes in CO_2 concentration is linear at ambient levels. At low and intermediate thallus water contents the saturating CO_2 concentration was reduced and maximal values of NPR are depressed as the thallus becomes drier.

These results complicate the use and accuracy of the discrete sampling technique as pioneered by Larson and Kershaw (1975b). Clearly it is important that initial and final CO_2 levels be as uniform as possible, and that the mean CO_2 concentration be as near ambient as is practical. Under these conditions the limitations of the technique due to CO_2 concentration changes would be minimised and allow the benefits of multiple replication to be exploited. Calculations of r_a confirm that ventilation of the cuvette is not necessary for lichens, however it is recommended that a comparison of ventilated and non-ventilated cuvettes be carried out where accurate determination of NPR are required. It should be noted that ventilation effects could be important if this technique is adapted for use with plants which have lower CO_2 resistances than lichens.

It is now apparent that lichens must be added to the growing list of plants that show low CO_2 compensation values. However, the results suggest that in lichens τ is variable, being affected by thallus water content and possibly thallus structure. The influence of these factors may be at least partially due to the depression of NPR resulting from the large CO_2 diffusion resistances found at high thallus water contents. Further work is being carried out to elucidate this phenomenon.

SECTION C

PHOTORESPIRATION

CHAPTER VIII

Carbon dioxide exchange in lichens: Low carbon dioxide compensation levels and lack of apparent photorespiratory activity in some lichens

It has been accepted that the majority of higher plants can be classified, in terms of photosynthetic physiology, as either C4, with low CO_2 compensation levels, low apparent photorespiratory activity and PEP carboxylase as primary CO_2 acceptor or C3, with high CO_2 compensation levels (>50 µl CO_2 l^{-1}), photorespiratory activity and RuBP carboxylase as primary CO_2 acceptor. It is apparent from the literature that lichens have been considered to be normal C3 plants (Hill, 1976). In fact, although there have been many studies of gross carbon dioxide exchange, particularly with respect to water relations (Bewley, 1979), there is a shortage of physiologically or biochemically orientated work. To date only one estimate of CO_2 compensation level appears to have been published and that was a high 170 µl CO_2 l^{-1} for *Xanthoria parietina* (Collins and Farrar, 1978). In this study we are able to show that some lichens do not behave as typical C3 plants but appear to have no, or very low, apparent photorespiratory activity. The results come from studies of carbon dioxide compensation levels and carbon dioxide exchange in the presence and absence of oxygen. In many ways the lichens behave similarly to some algal species which also show low compensation levels (Birmingham and Colman, 1979).

An assessment of the relationship between net carbon dioxide exchange and external carbon dioxide concentration was made using an ADC series 225 infra red gas analyser (IRGA) and a closed loop gas circulation system. Experiments were carried out at a thallus temperature of 20 $\pm$ 1 C and a saturating irradiance of 150 µE m^{-2} s^{-1}.

Lichen thalli were blotted before use and had been held moist at 100% RH, 16°C and 70 μE m^{-2} s^{-1} for at least six hours to minimise any resaturation respiration. A rapid survey technique was also used in which lichens, collected wet from the field, were incubated in a 30 cm^3 vessel kept at a thallus temperature of 20°C by a water bath. One cm^3 gas samples were taken at the start of incubation and again after two hours and analysed on the IRGA which had been modified to take injection samples (Larson and Kershaw 1975b; Snelgar *et al.* 1980).

Initial results using *Pseudocyphellaria billardierii*, a green algal lichen with cephalodia, demonstrated that the lichen had a carbon dioxide compensation level consistently below 15 μl CO_2 l^{-1} and occasionally approaching 0 μl CO_2 l^{-1}. Further, the lowering of the oxygen level from 20% to about 1% had little effect on net photosynthesis (Table 1). A survey of several species showed many to have low compensation levels but a few to have levels expected of a C3 plant (Table 2). Detailed work using *P. delisea*, a high compensation level species showed that net photosynthesis was markedly sensitive to the presence of oxygen being 60% greater at 1% oxygen than at 20% oxygen (Table 1). This is an expected result for plants showing photorespiratory activity. There appears to be no correspondence between primary phycobiont type and compensation level although at this point only a small number of species have been surveyed.

Both a low carbon dioxide compensation level and the lack of an oxygen effect on net photosynthesis are normally taken to indicate an absence of photorespiration. Photorespiration is a normal result of the functioning of ribulose biphosphate carboxylase/oxygenase as primary acceptor in the Calvin cycle. The results of one short-term fixation study (Bednar and Smith, 1966), the fixation of ^{14}C as $^{14}CO_2$

TABLE 1. CO_2 compensation level and net photosynthetic rates (in the presence and absence of oxygen) of *P. billardierii* and *P. delisea*. All experiments carried out at saturating light and 20°C. Net photosynthesis measurements made at 350 µl CO_2 l^{-1}. Lichens were collected wet from the field and stored at 70 µE m^{-2} s^{-1}, 16°C before use.

Lichen species †	CO_2 compensation level (µl CO_2 l^{-1})	Photosynthetic rate (mg CO_2 g^{-1} hr^{-1}) 20% O_2	1% O_2	Increase in photosynthetic rate at low O_2 %
Pseudocyphellaria billardierii	15	2.60	2.68	3
Pseudocyphellaria delisea	98	1.47	2.34	59

† Both lichen species have green primary phycobionts and blue-green algae in cephalodia.

TABLE 2. Carbon dioxide compensation levels of a variety of lichens all collected in the same location. All measurements were at 150 μE m^{-2} s^{-1}, 20°C using an incubation time of 120 minutes in a sealed 30 cm^3 vial. Carbon dioxide measurements were by an injection sampling technique; at low carbon dioxide levels (<25 μl CO_2 l^{-1}) vibration errors lead to a consistent over-estimation of carbon dioxide concentration. All lichens were collected wet from the field and used immediately.

Lichen species	Primary phycobiont[1] [2]	CO_2 Compensation level μl CO_2 l^{-1}	Compensation level (rating)
Nephroma rufum	bg	14, 22, 17	low
Peltigera horizontalis	bg	10, 8, 8	low
Pseudocyphellaria amphisticta	g	19, 15, 8	low
P. billardierii	g	17, 17, 18	low
P. colensoi	g	62, 65	high
P. delisea	g	68, 68, 76	high
P. dissimilis	bg	21, 27, 20, 25	low
P. hookeri	bg	19, 14, 17	low
P. psilophylla	g	11, 14, 14	low
Sticta filix	g	57, 53, 49	high
S. fuliginosa	bg	22, 20	low
S. latifrons	g	14, 11, 17, 18, 19	low
Stereocaulon ramulosum	g	21, 20	low

[1] bg: blue-green primary phycobiont;

g: green primary phycobiont.

[2] all lichens with green primary phycobiont contain blue-green algae in cephalodia.

gas (Cowan *et al.*1979a) and $^{13}C/^{12}C$ discrimination ratio (Shomer-Ilan *et al.* 1979) all indicate that ribulose biphosphate carboxylase is the primary acceptor in lichens. The lack of apparent photorespiratory activity most probably indicates a lack of the metabolic pathways resulting in carbon dioxide release that are found in higher plants. A similar lack of carbon dioxide evolution in the light with no oxygen effect has been found for free-living algae (Lloyd *et al.* 1977; Birmingham and Colman, 1979) and no explanation for the phenomenon is apparently available. The situation is clearly more complex in lichens since some species do show classic photorespiratory effects and, in the absence of cultural data, it is uncertain whether this indicates different species of phycobiont.

The pseudoparenchymatous cortex and thick fungal medulla could result in a higher resistance to carbon dioxide diffusion than found in the leaves of higher plants (Hill, 1976; Collins and Farrar, 1978). A high affinity fixation system resulting from the lack of photorespiration would help overcome this resistance and could be advantageous particularly since oxygen levels in the thallus can exceed normal atmospheric levels (Millbank, 1977). Certainly lichens could prove to be much more interesting for detailed photosynthetic studies than the quantity of past research would suggest.

CHAPTER IX

Carbon dioxide exchange in lichens: Apparent photorespiration and possible role of CO_2 refixation in some members of the Stictaceae (Lichenes)

INTRODUCTION

Photorespiration (PR) has been defined as the evolution of CO_2 from photosynthetic tissue in the light (Kumar and Singh, 1979). It is a process which has been widely studied and reviewed (Zelitch, 1971; Gibbs and Latzko, 1979) but which is still incompletely understood, partly because of the lack of a satisfactory method for the measurement of photorespiratory rates, (Ludlow and Jarvis, 1971). When estimates of the net photosynthetic rate (NP) of plants are required then knowledge of the magnitude of the photorespiratory rate is unnecessary, although it may well have a direct bearing on NP. However, when studies involve an estimation of the efficiency of light utilisation (quantum yield), or the resistance to CO_2 uptake then prevention of photorespiration or some measurement of its rate is essential in order to obtain an accurate assessment of the true photosynthetic rate.

Pathways of CO_2 exchange in lichens are unusually complex because the thallus contains both a mycobiont and a phycobiont; thus the overall process of CO_2 exchange in the light can be summarised by:

NP = True photosynthetic rate - (algal photorespiration + fungal dark respiration).

Richardson (1973) observed that while fungal respiration is known to be large under some conditions the extent of algal photorespiration is unknown. Farrar (1973) commented that although photorespiration was recognised for some plants it was still an area of controversy, hence the term was best forgotten.

Neither of the published attempts at developing electrical analogue CO_2 resistance models for lichens (Collins and Farrar, 1978; Lange, 1980) have included a photorespiratory component in the models. More recently Sigfridsson (1980) has noted this lack of information on photorespiration in lichens and has suggested the use of luminescence and fluorescence techniques as a means of circumventing the problems created by respiration. It has been previously demonstrated that lichens may have low CO_2 compensation values (< 20 $\mu\ell\ \ell^{-1}$ CO_2) and that the NP of *P. billardierii* appeared insensitive to oxygen concentration, both factors suggesting low photorespiratory activity (Snelgar and Green 1980). In view of the almost complete lack of data on photorespiration in lichens and the possible importance of this phenomenon more information in this field was considered necessary.

Techniques for measuring photorespiration (PR) and the problems involved with each have been intensively reviewed by Ludlow and Jarvis *(op. cit.)*. The more commonly used methods can be summarised as:

1. Measurement of post illumination CO_2 burst.
2. ^{14}C efflux from prelabelled plants.
3. CO_2 efflux into CO_2 free air.
4. Varying the oxygen concentration and measuring the effect on NP (Warburg effect).

Methods 1 and 2 are considered inaccurate because PR is thought to preferentially utilise recently assimilated products and stomatal resistance (and hence rate of CO_2 release) is likely to vary when an illuminated plant is removed from the light. Release of CO_2 into CO_2 free air is likely to be a more suitable method but would be misleading if PR was linked to the true photosynthetic rate (TP). Recent studies of

CO_2 exchange in lichens (Snelgar, Green and Wilkins 1981b) have demonstrated that large resistances to CO_2 uptake occur in many species. Further studies recently carried out in this laboratory have indicated that a large CO_2 transport resistance is located within the cortex of some species, thus creating a situation where it is probable that CO_2 transport resistances between respiratory sites (both light and dark) and photosynthetic sites are considerably lower than the resistances between respiratory sites and the external atmosphere. Under these conditions it is likely that a large amount of refixation of respired CO_2 might occur, a situation similar to that postulated for plants possessing Krantz anatomy. A consequence of this recycling is that any method of estimating PR from CO_2 effluxes in the light would be likely to be highly misleading. Method 4 was therefore considered to be the only acceptable technique available for work with these lichens but method 3 was also included for purposes of comparison. Rates of light and dark CO_2 evolution under microaerobic (1% O_2) conditions were also measured in order to obtain estimates of the magnitude of CO_2 recycling in lichens.

METHODS

Terminal portions of lobes from healthy lichen thalli of *Pseudocyphellaria billardierii* (Del.) Ras, *P. amphisticta* Kremp, *P. colensoi* (Bab. in Hook F.) Vain, *P. homoeophylla* (Nyl.) Dodge, and *Sticta latifrons* Rich. were collected from the Urewera National Park (NZMS 1 N96 619437) and the Hakirimata Scenic Reserve (NZMS 1 N56 645622). Both areas are forest covered and are situated in the central North Island of New Zealand.

Lichens were stored air dry in darkness over silica gel for a maximum of three weeks. When required thalli were mist sprayed with distilled water and maintained at 16°C, 50 μE m^{-2} s^{-1}, 100% RH for a minimum of 10 hours prior to use in experiments. CO_2 exchange rates were measured using an ADC series 225 infra red gas analyser operating in a closed system which included a 370 cm^3 water jacketed perspex cuvette. Thallus temperature was maintained at 16.0±0.5°C as measured by a miniature thermistor probe; quantum flux density was either 150 μE m^{-2} s^{-1} (saturating for these species, Snelgar unpublished data) or zero. The oxygen concentration within the cuvette was adjusted to 21% by flushing with air and to 1% by flushing with oxygen free nitrogen for a fixed time period determined by experimentation in which the oxygen concentration was measured by a thermal conductivity gas chromatogram. Oxygen levels were never low enough to affect dark respiration rates. Respiration rates in the light were obtained from a linear extrapolation to zero CO_2 concentration of NP measurements made at 10 - 100 μℓ $ℓ^{-1}$ CO_2. Dark respiration rates were measured at 350 μℓ $ℓ^{-1}$ CO_2 unless otherwise specified. The use of a closed gas system minimised water loss from the lichen thalli but slight loss did occur and water contents are expressed as the mean of the initial and final values for each incubation in units of g water per g thallus dry weight (obtained by drying to a constant weight at 100°C). The maximum water contents found in this investigation were obtained by mist spraying the thalli on both sides with distilled water so that they appeared visibly wet. Thalli were only lightly shaken before incubation and were not blotted thus ensuring retention of any external water store (Snelgar and Green 1981b). A Koizumi compensating planimeter was used to measure the area of thalli.

RESULTS

The variation of NP and dark respiration with water content, under both 21% and 1% oxygen, for *S. latifrons* is shown in Figure 1A and, although it is evident that oxygen concentration has no effect on the dark respiratory rate, there is a marked increase in NP at the lower oxygen concentration. This increase is largest at optimal water contents and apparently diminishes at both high and low water contents. The dark respiratory rate remains constant at supra optimal water contents, with no indication of a respiration increase at thallus saturation (cf. Snelgar, Brown and Green 1980). The results for *P. homoeophylla* (Fig. 1B) are similar to those of *S. latifrons*, although the depression of NP at medium to high water contents is not so marked as found for *S. latifrons*. There was increased variability of NP determination at high water contents (Fig. 1B) as noted previously (Snelgar, Brown and Green 1980). The NP of *P. billardierii* (Fig. 1C) is stimulated by low oxygen concentrations but to a considerably lesser extent than either of the previous species. Again the dark respiratory rate is unaffected by oxygen concentration and is constant at supra-optimal water contents.

A summary of the results from Figure 1, together with estimates of the percentage stimulation of both NP and TP is presented in Table 1. At most water contents *S. latifrons* and *P. homoeophylla* showed a consistent increase in NP and TP at low oxygen levels of about 40 - 50% and 30 - 40 % respectively. The increase appeared to be more constant when expressed on a TP basis. In both lichens no stimulation was found at the highest water content studied. With respect to this stimulation it should be noted that although the NP curves at 21% and 1% oxygen appear to converge at both high and low water contents, it is only at high water contents that the stimulation markedly decreases. *P. billardierii* shows a different pattern of stimulation at low oxygen with low and

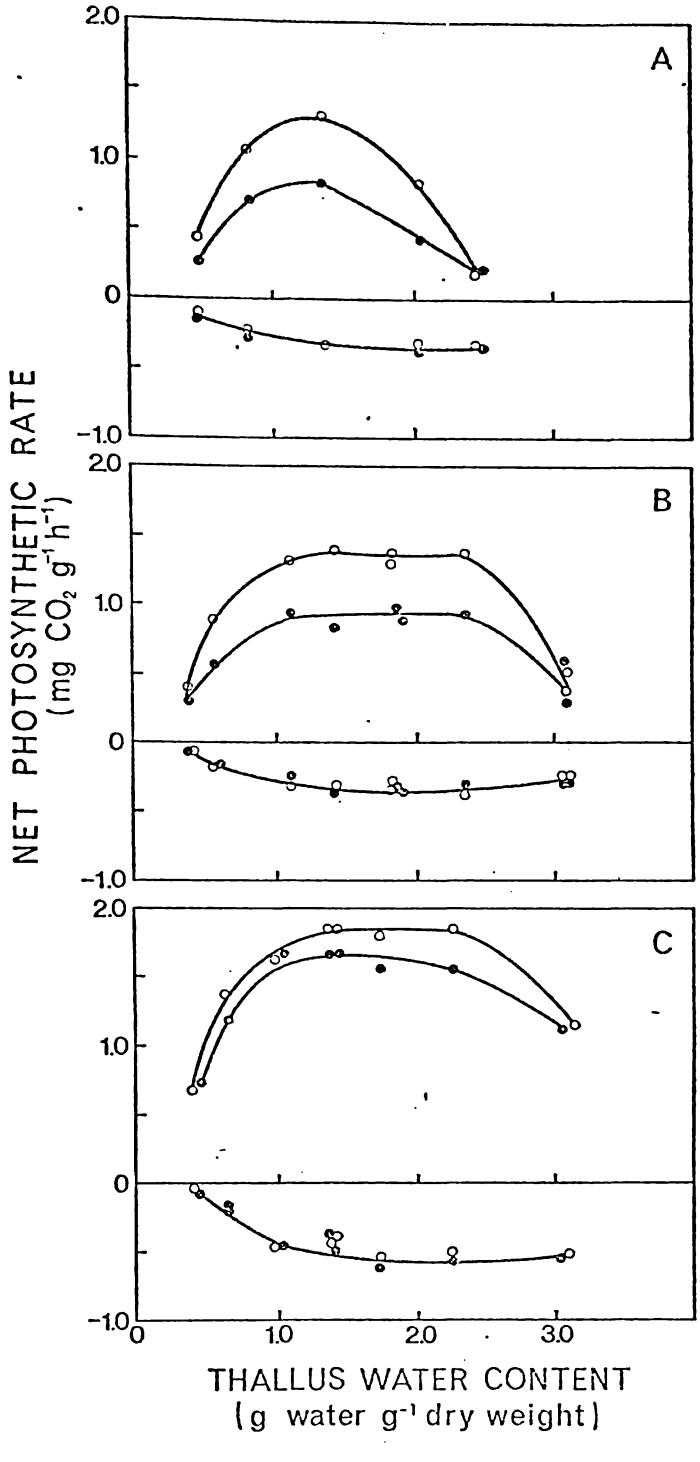


FIGURE 1. The effect of oxygen concentration on NP and dark respiration in *S. latifrons* (A) *P. homoeophylla* (B) and *P. billardierii* (C) o = 1% oxygen, ● = 21% oxygen.

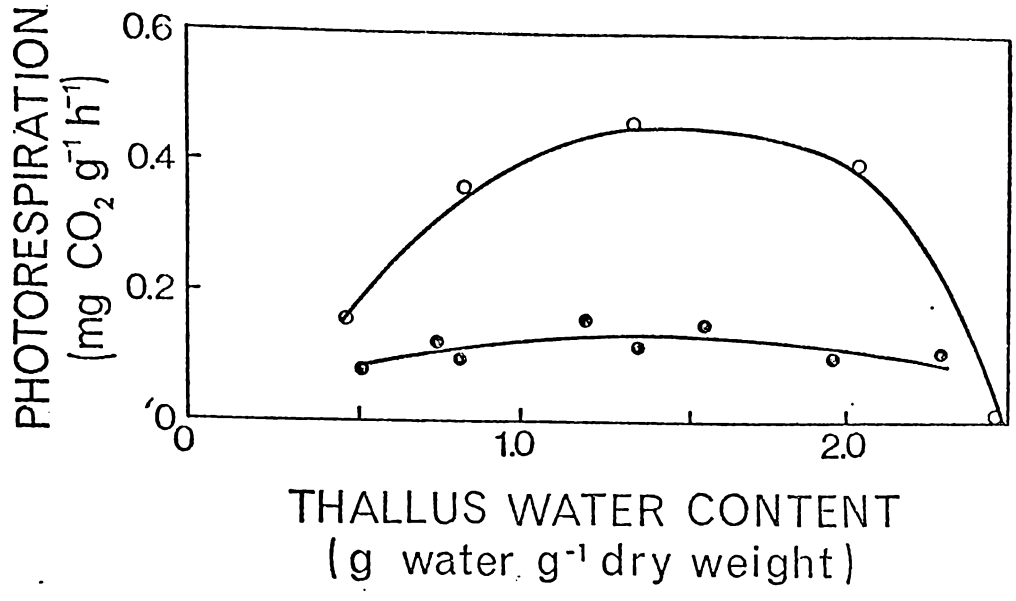


FIGURE 2. Rate of photorespiration for *S. latifrons* at several thallus water contents. Rates were measured by extrapolation of NP to zero CO_2 (●), or as the difference in NP at 21% and 1% O_2 (○).

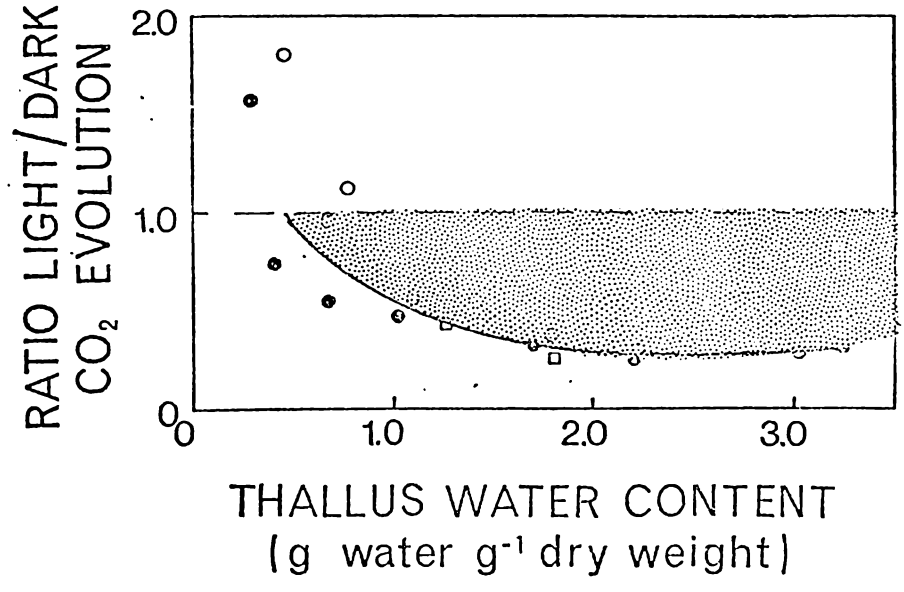


FIGURE 3. Ratio of light to dark CO_2 evolution at 0 μℓ $ℓ^{-1}$ CO_2 and 1% O_2 contents for *P. homoeophylla* (○), *P. colensoi* (●), and *P. amphisticta* (▫). The shaded area indicates the magnitude of refixation of respired CO_2.

TABLE 1. Effect of oxygen on NP and TP for *S. latifrons*, *P. homoeophylla* and *P. billardierii*. All photosynthetic rates are expressed as mg CO_2 g^{-1} h^{-1}. Water content values (g water per g thallus dry weight) are shown as the range of mean values found during CO_2 exchange incubations.

	Water Content	NP (mg CO_2 g^{-1} h^{-1}) 21% O_2	NP (mg CO_2 g^{-1} h^{-1}) 1% O_2	TP (mg CO_2 g^{-1} h^{-1}) 21% O_2	TP (mg CO_2 g^{-1} h^{-1}) 1% O_2	% Increase NP	% Increase TP
S. latifrons	2.45-2.49	0.21	0.19	0.56	0.52	-10	-7
	2.03-2.04	0.43	0.83	0.80	1.14	93	43
	1.34-1.35	0.84	1.30	-	-	55	-
	0.81-0.83	0.71	1.07	0.97	1.30	51	34
	0.47	0.28	0.44	0.41	0.54	57	32
P. homoeophylla	3.07-3.09	0.44	0.44	0.72	0.70	0	-3
	2.35-2.36	0.93	1.37	1.24	1.65	47	33
	1.89-1.94	0.94	1.34	1.28	1.66	43	30
	1.41-1.42	0.84	1.39	1.20	1.72	65	43
	1.10	0.94	1.32	1.20	1.63	40	36
	0.55-0.56	0.57	0.88	0.75	1.06	54	41
	0.38	0.31	0.40	0.38	0.47	29	24
P. billardierii	3.05-3.10	1.12	1.14	1.67	1.65	2	-1
	2.26	1.56	1.85	2.13	2.37	19	11
	1.73	1.56	1.80	2.17	2.34	15	8
	1.39-1.43	1.66	1.85	2.10	2.25	11	7
	0.99-1.03	1.66	1.63	2.13	2.10	-2	-1
	0.63	1.19	1.37	1.40	1.55	15	11
	0.40-0.43	0.73	0.68	0.81	0.72	-6	-11

variable values on a NP basis and a more consistent value (about 9%) on a TP basis. The rates of photorespiration of *S. latifrons*, defined as PR = NP (1% O_2) - NP (21% O_2) are presented in Figure 2. Photorespiratory rates, calculated by extrapolation of NP to 0 $\mu\ell\ \ell^{-1}$ CO_2 (method 3), are also included in Figure 2 and are lower and less affected by thallus water content than those obtained by method 2. The light/dark CO_2 evolution ratios (defined as CO_2 release in the light divided by CO_2 release in the dark at 0 $\mu\ell\ \ell^{-1}$ CO_2 and 1% oxygen to eliminate photorespiratory effects) are given for different thallus water contents in Figure 3. The low values of this ratio (about 0.3) at intermediate to high water contents show that around 70% of respired CO_2 is refixed by the photosynthetic activity of the phycobiont, assuming that there is little affect of light on the respiration rate. The light/dark CO_2 evolution ratio increases at low water contents, implying less recycling, possibly because of a proportionately greater effect of low water potential on photosynthesis than respiration. Ratios greater than unity at low water contents are difficult to explain but may be the result of inaccuracies in the measurement of the very small respiratory rates; and small fluctuations in thallus water content.

DISCUSSION

The results presented here confirm previous studies (Snelgar and Green 1980) which indicated that the NP of some lichens was stimulated by low oxygen in a manner typical of the presence of photorespiratory activity. *S. latifrons* and *P. homoeophylla* showed increases of over 40% for both NP and TP a value similar to the theoretical 43% calculated by Schrader (1975) and in the range expected for a higher plant with the C3 photosynthetic pathway. The stimulation of NP by decreased oxygen is relatively constant at all water contents except the highest studied where no stimulation was found. Previous work (Snelgar, Green and Wilkins 1981b) has shown that

these lichens possess increased resistances (about 300 s cm^{-1}) to CO_2 diffusion at high thallus water contents. Various lines of evidence including differential drying of upper and lower surfaces (Green, Snelgar and Brown 1981) removal of the resistance by blotting (Snelgar, Green and Wilkins 1981b) and calculation of resistance location from morphological data (Snelgar, Green and Beltz 1981a) all suggest that the extra resistance is located at the surface of the lichen, probably in the cyphellae or pseudocyphellae. The gas diffusion path within the thallus from phycobiont to gas exchange structures can be calculated to have a much lower value than the surface resistance. Under these conditions refixation of photorespired CO_2 would be enhanced and could result in no apparent affect of oxygen concentration on NP (Fig. 1). This situation appears to be analogous to that of aquatic plants where the surrounding water confers a very high resistance to CO_2 diffusion and the low PR rates of these plants has been attributed to a high rate of CO_2 refixation (Canvin 1979).

The response of NP of *P. billardierii* to decreased oxygen is markedly different from that of the previous two species and in fact closely resembles a C4 higher plant. There was little stimulation of NP or TP at any thallus water content and τ (CO_2 compensation point) was consistently very low (<10 $\mu\ell\ \ell^{-1}$ CO_2). Reduced rates of PR have been predicted to result in increased rates of NP provided CO_2 resistances are not affected (Zelitch 1971). Maximum TP and NP were both appreciably higher in *P. billardierii* than in *S. latifrons* or *P. homoeophylla* (Table 1). As yet it has not proved possible to explain these

results. *P. billardierii* does not have a higher total CO_2 diffusion resistance neither is any unusual morphological feature obvious in scanning electron micrographs. The possibility exists that *P. billardierii* may possess a different species of phycobiont to the other lichen species but unfortunately no published details on phycobiont taxonomy are available.

Recent investigations of the PR activity of free living algae have yielded variable results. When assayed in an aqueous environment several species of algae have shown a post-illumination CO_2 burst typical of PR. However work using algae placed in thin layers on nylon cloth or filter paper in a gaseous medium has demonstrated a lack of PR in many species (Zelitch 1979). In this latter technique the arrangement of algae into thin layers exposed to a gaseous atmosphere has a distinct resemblance to the manner in which algae are organised in the lichen thalli studied here. It is unfortunate that no data appears to exist on the water relations of the lichen algae within the thallus and it is uncertain whether they are actually immersed in a liquid medium. It has also been suggested that the formation of high CO_2 concentrations within algal cells could suppress the activity of ribulose biphosphate oxygenase (Canvin 1979).

Brown (1980) working on higher plants with photosynthetic mechanisms that were either C3, C4 or C3/C4 intermediate has suggested that refixation of photorespired CO_2 could explain why some plants demonstrate a sensitivity of NP to oxygen (i.e. photorespire) yet have a low τ. The low ratio of light to dark CO_2 evolution (Fig. 3) measured under conditions which repressed photorespiration indicates that refixation of respired CO_2 occurs

at all but the lowest water content. At intermediate and high water contents around 70% of respired CO_2 was refixed and and data from split chambers (Green, Snelgar and Brown 1981 for method) indicate that the remaining CO_2 could be lost directly to the outside via the lichen cortex, rather than through the exchange pores. Variation in CO_2 refixation rates, possibly related to changes in CO_2 diffusion resistance, could explain the range of τ observed in different lichen species (Snelgar and Green 1980). It should also be noted that an increased CO_2 diffusion resistance would depress the gradient of the linear NP response to CO_2 at low CO_2 concentration. Consequently, at a constant respiratory rate, this effect would automatically result in a higher value of τ. Such a response could explain the variations of τ (10 - 50 $\mu\ell\ \ell^{-1}$) for *S. latifrons* (Green and Snelgar 1981a). Thus changes in CO_2 diffusion resistances may have both an indirect (refixation) and direct (NP gradient) effect on τ.

Lichens have also been shown to possess significant dark fixation processes (Kershaw, Morris, Tysiaczny and MacFarlane 1979) and it is uncertain how this may affect the PR response or τ.

The CO_2 compensation value and oxygen sensitivity of NP of lichens appears to be an area of diverse results with variation between and within species. An important factor appears to be thallus water content and the related effects of high surface diffusive resistances. The results presented here relate solely to the large foliose lichens of the Stictaceae which possess gas exchange pores. It would prove interesting to see how the CO_2 exchange of other groups of lichens fit in with the above patterns.

CHAPTER X

Carbon dioxide exchange in lichens: Analysis of the photorespiratory response.

INTRODUCTION

In previous work, data were presented which showed low rates of photorespiration (PR) and low CO_2 compensation points (τ) in some lichen species (Chapter VIII). Other experiments, using *S. latifrons*, revealed that this compensation point can vary with thallus water content and between different thalli (Chapter VII). A detailed investigation on the effect of thallus water content on PR in *S. latifrons*, *P. homoeophylla* and *P. billardierii* established that although PR (as a percentage of net photosynthesis) is relatively constant at most water contents, extreme values result in marked PR changes (Chapter IX). The depression of PR at high water contents was considered to result from high rates of refixation of respired CO_2 caused by the large external CO_2 resistances found in these lichens (Chapter XIII). It was suggested that changes in either PR or CO_2 resistance, or both, could account for some of the observed variations in τ.

The factors which have been postulated as possibly influencing τ have generally been evaluated in separate experiments due to the time involved in making these measurements. Unfortunately this approach produces results which are difficult to interpret, since collation of data from separate experiments would be unwise, considering the intraspecific variability which has been demonstrated (Chapter VII). In this study a modified experimental technique which produces simultaneous estimates of PR, CO_2 resistance, τ, and the O_2 sensitivity of the carboxylation system was used.

MATERIALS AND METHODS

Lichen thalli were collected from the Hakirimata reserve and the Urewera National Park and stored as described in Chapter II. Rates of CO_2 exchange were measured in a 370 cm^3 perspex cuvette at 16°C, 150 μE m^{-2} s^{-1}. Net photosynthesis - CO_2 responses for each speciman were obtained under both aerobic (21% O_2) and microaerobic (<1% O_2) conditions. The CO_2 concentrations required were generated by flushing the cuvette with nitrogen or CO_2- free air, then injecting small volumes of CO_2 standards. The order of NP measurements was always from the lowest to high CO_2 concentrations. Dark respiration rates at low CO_2 levels (0-50 μl l^{-1}) were measured during this sequence.

The total diffusive resistance to CO_2 was calculated from the initial linear gradient of the NP-CO_2 curve in <1% O_2. At ambient O_2 levels this slope is generally depressed. Although the total resistance of these lichens is composed of several components, including the boundary layer, gas exchange pores, medulla, and carboxylation resistances (Chapter XIII) only the latter component is considered to be O_2 sensitive. In view of this the O_2 sensitivity of the total CO_2 resistance can be regarded as principally a carboxylation effect (Morgan and Brown, 1980). The increase in slope in <1% O_2 is expressed as a percentage of the slope in 21% O_2 and referred to as α. Carbon dioxide compensation points were obtained from NP-CO_2 graphs at both O_2 tensions. Photorespiratory rates were estimated as the difference in NP at 150 μl CO_2 l^{-1} under aerobic and microaerobic conditions.

RESULTS

Figure 1 depicts the range of NP-CO_2 responses observed in this study. Table 1 presents a summary of the data.

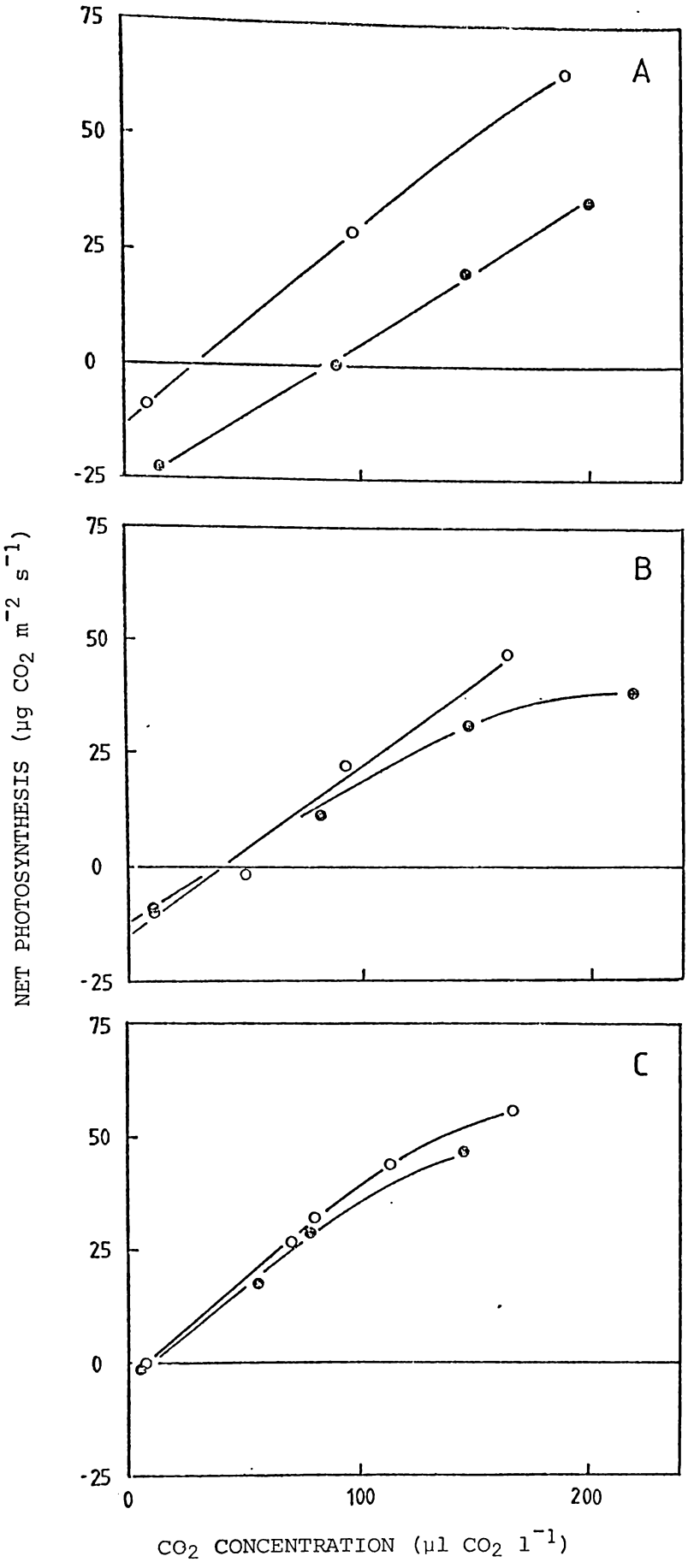


FIGURE 1. Relationship between net photosynthesis and CO_2 concentration for *Pseudocyphellaria homoeophylla* (A), *Peltigera dolichorhiza* (B), and *Pseudocyphellaria billardierii* (C). (o) = <1% O_2, (⊕) = 21% O_2.

TABLE 1. The effect of O_2 concentration on CO_2 exchange and τ in several lichen species. (See text for details)

	Sticta latifrons	*Sticta latifrons*	*Sticta latifrons*	*Pseudocyphellaria amphisticta*	*Pseudocyphellaria colensoi*	*Pseudocyphellaria homoeophylla*	*Pseudocyphellaria billardierii*	*Peltigera dolichorhiza*
Collection Site [1]	H	W	W	W	W	W	H	H
Storage time (days)	1	20	7	20	6	7	1	1
Thallus water content (g g^{-1})	1.61	1.52	1.43	2.71	0.98	1.68	1.86	1.90
mg dry weight cm^{-2} thallus	12.0	22.8	16.9	9.8	17.1	12.7	7.4	6.6
τ at <1% O_2 (μl CO_2 l^{-1})	14	23	18	33	23	31	7	38
τ at <1% O_2, R corrected (μl CO_2 l^{-1})	18	31	34	46	42	47	4	35
τ at 21% O_2 (μl CO_2 l^{-1})	10	56	50	74	86	86	8	40
τ from previous results, 21% O_2, (μl CO_2 l^{-1})	11-19	11-19	11-19	8-19	62-65	60-72	17-18	-
NP at 150 μl CO_2 l^{-1}, <1% O_2 (μg CO_2 m^{-2} s^{-1})	66	49	50	41	35	51	62	42
NP at 150 μl CO_2 l^{-1}, 21% O_2 (μg CO_2 m^{-2} s^{-1})	53	26	29	19	15	21	48	32
NP at 150 μl CO_2 l^{-1}, 21% O_2 (mg CO_2 g^{-1} h^{-1})	1.59	0.41	0.61	0.69	0.32	0.59	2.28	1.74
PR, R corrected, (μg CO_2 m^{-2} s^{-1})	-10	-19	-15	-17	-16	-23	- 7	-11
PR, R corrected, (mg CO_2 g^{-1} h^{-1})	-0.30	-0.30	-0.32	-0.62	-0.33	-0.65	-0.31	-0.57
R^2 at <1% O_2 (μg CO_2 m^{-2} s^{-1})	-20	-20	-17	-21	-17	-24	-14	-20
R at 21% O_2 (μg CO_2 m^{-2} s^{-1})	-23	-24	-23	-26	-21	-31	-16	-19
R at 21% O_2 (mg CO_2 g^{-1} h^{-1})	-0.69	-0.38	-0.49	-0.95	-0.45	-0.88	-0.77	-1.03
Initial NP - CO_2 slope <1% O_2 (s cm^{-1})	28	46	47	56	58	44	43	51
Initial NP - CO_2 slope 21% O_2 (s cm^{-1})	34	55	62	75	72	56	46	64
Percentage increase in slope at <1% O_2 (a)	18	16	24	25	19	21	7	20
y intercept at <1% O_2 (μg CO_2 m^{-2} s^{-1})	- 9	-10	- 8	-13	- 9	-14	- 4	-15
y intercept at 21% O_2 (μg CO_2 m^{-2} s^{-1})	- 6	-19	-16	-19	-23	-24	- 4	-12
Difference in y intercepts, R corrected (d) (μg CO_2 m^{-2} s^{-1})	- 6	- 5	- 2	- 1	-10	- 8	- 2	- 2
Refixation ratio: y intercept, <1% O_2/R, <1% O_2	0.45	0.48	0.44	0.60	0.53	0.56	0.25	0.75

[1] H = Hakirimata, W = Lake Waikareiti

[2] R = dark respiration rate

In Chapter IX it was demonstrated that dark respiration in *P. homoeophylla*, *P. billardierii* and *S. latifrons* is not affected by decreasing the O_2 concentration from 21% to <1%. The present results are at variance with this finding as several species exhibited a lower rate of dark respiration in <1% O_2. This depression may have been caused by unusually low oxygen concentrations within the experimental cuvette created by excessive flushing with nitrogen in an attempt to lower CO_2 concentrations to near zero. The use of 1% O_2 in nitrogen as a purging gase, or standard purge times as in previous work (Chapter IX) would eliminate this problem. Provided that dark respiration in <1% O_2 was constant for any incubation series (and this seems likely as O_2 levels would not alter substantially) then this depression of dark respiration would not affect the initial NP-CO_2 slopes or the evaluation of carboxylation O_2 sensitivity. However τ in <1% O_2 may be underestimated and PR overestimated, therefore 'respiration corrected' values have been included in Table 1. This correction was achieved by subtracting from all NP measurements obtained at <1% O_2 a value equivalent to the difference in dark respiration rates under 21% and <1% O_2.

The total CO_2 resistance values and the PR estimates are in reasonable agreement with previously published figures (Chapters VIII, XI). The O_2 sensitivity of carboxylation was within the range 16% - 25% for all species except *P. billardierii*. The latter had a much lower sensitivity of about 7%.

In Chapter IX a technique was described for estimating the degree of photosynthetic refixation of respired CO_2 based on the ratio of CO_2 evolution at zero CO_2, <1% O_2 in the light, and in darkness. For all species except *P. amphisticta* and *Peltigera dolichorhiza* the values calculated from the present data (Table 1) closely correspond to previous estimates. The ratios for

TABLE 2. Correlation of various parameters with τ. See text for details. NS = Not significant.

Linear regression	Correlation coefficient (r)	Probability (P)
Carboxylation sensitivity *vs* τ	0.61	NS
Total CO_2 resistance *vs* τ	0.77	<0.05
mg dry weight cm^{-2} thallus *vs* τ	0.39	NS
CO_2 refixation ratio *vs* τ	0.50	NS
Net photosynthesis $\mu g\ CO_2\ m^{-2}s^{-1}$ (at 150 $\mu l\ CO_2\ l^{-1}$) *vs* τ	-0.97	<0.01
Net photosynthesis mg $CO_2\ g^{-1}h^{-1}$ (at 150 $\mu l\ CO_2\ l^{-1}$) *vs* τ	-0.86	<0.01
Dark respiration $\mu g\ CO_2\ m^{-2}s^{-1}$ *vs* τ	0.65	NS
Dark respiration mg $CO_2\ g^{-1}h^{-1}$ *vs* τ	-0.08	NS
Photorespiration $\mu g\ CO_2\ m^{-2}s^{-1}$ *vs* τ	0.85	<0.01
Photorespiration mg $CO_2\ m^{-2}s^{-1}$ *vs* τ	0.61	NS

P. amphisticta and *Peltigera dolichorhiza* are unusually high, indicating a low amount of CO_2 refixation.

Carbon dioxide compensation points at ambient oxygen levels can be grouped into low (<25 µl CO_2 l^{-1}) and high (≥25 µl CO_2 l^{-1}) categories. Although both species previously described as having high τ values (Chapter VIII) showed similarly high values, other species previously found to have low τ exhibited high values. Furthermore both high and low τ were obtained for different specimens of *S. latifrons*. Collation of the data in Table 1 and in Chapter VII reveals that lichens which have low τ have invariably been collected from low altitude sites (Hakirimata 90 m.a.s.l., Kauaeranga Valley 120 m.a.s.l.) and used fresh or after only one days storage. (Specimens stored for more than one day are dried over silica gel). Conversely although lichens collected from low altitude sites may have low (e.g. *S. latifrons*) or high (e.g. *P. colensoi*) τ, specimens from high altitude sites (Mt. Te Aroha 950 m.a.s.l., Waikaremoana 700 m.a.s.l.) have always had high τ. (e.g. *P. colensoi, P. amphisticta, P. homoeophylla, S. latifrons*). The importance of pretreatment conditions (i.e. dry stored or fresh) is not known. Since fresh high altitude specimens of *P. homoeophylla* and *S. latifrons* have high τ dry storage may not affect these species. However there is no data available on the effects of dry storage on low τ specimens.

The CO_2 compensation point of a lichen represents an equilibrium between many CO_2 exchange processes (algal photosynthesis, photo-respiration, dark respiration, fungal dark respiration, heterotrophic dark fixation), and alterations in any one of these processes chould affect τ. Additionally, as noted in Chapter VII, any variation in total CO_2 resistance could also shift τ. As an initial attempt to evaluate the importance of some of the above factors linear regressions

of lichen dry weight per unit area, dark respiration, CO_2 refixation, O_2 sensitivity of the carboxylation system, net photosynthesis, total CO_2 resistance and PR were made against τ (at 21% O_2). Only the latter three parameters were significantly correlated with τ (Table 2). It is interesting that the PR correlation is significant only when PR is expressed on an area basis, rather than a dry weight basis. Further regressions using only *S. latifrons* data (from the present work and from Chapter VII) showed that τ is positively correlated with the weight per unit area of the lichen ($r = 0.86$, $n = 5$) and that the weight per unit area is also correlated with the total CO_2 resistance ($r = 0.72$, $n = 5$). Although neither of these correlations are significant, the results do suggest that high resistance values tend to occur in lichens which are thick and/or dense, and that these lichens have high τ. As these large intra-specific variations in τ appear to be habitat related it seems likely that discrete populations, such as those described in Chapter IV could be involved. If this is so then the use of simple linear regression correlations may be inappropriate.

DISCUSSION

The results summarised in Table 1 can be classified into three groups on the basis of carboxylation sensitivity to O_2 and τ.

(i) Group one lichens have a high carboxylation sensitivity to O_2 (16% - 25%) and high ($\geq$25 µl CO_2 l^{-1}) τ in air. In <1% O_2 τ is markedly lower. Examples are; *S. latifrons*, *P. amphisticta*, *P. colensoi* and *P. homoeophylla*. (Figure 1A).

(ii) Group two have a high carboxylation sensitivity to O_2 (18% - 20%). τ may be low or high and is little affected by changes in O_2 concentration. Examples are; *S. latifrons* and *Peltigera dolichorhiza*. (Figure 1B).

(iii) Group three consists of only one species, *P. billardierii*. This species exhibits a low carboxylation sensitivity to O_2 (7%) and low τ in both 21% and <1% O_2. (Figure 1C).

These variations in response can be separated into two components. Firstly if Line A (Figure 2) represents the response of NP to CO_2 at <1% O_2 then angle α is a measure of the depression in slope of this regression at 21% O_2 (Line B). Line C lies parallel to Line B and is separated from B by a constant distance (d) which represents the depression of NP in 21% O_2. The value of d is not affected by NP or CO_2 concentration. Assessed in these terms Group One, above, has a large α and a large d, Group two has a large α and Zero d, Group three has a small α and Zero d. These responses closely correspond to those described by Brown (1980) for C3, C3-C4 intermediates and C4 *Panicum* species. Brown interprets α as being indicative of the extent of O_2 inhibition of ribulose biphosphate carboxylase activity. Since species having a complete C4 cycle should have near Zero α values the present results are not indicative of C4 activity in these lichens. The low α of *P. billardierii* is of particular interest as it seems to be well below the values expected in C3 plants yet is substantially above the Zero value reported for C4 species. Kennedy *et al* (1980) have also reported low α values in a plant (*Mollugo nudicaulis*) which does not possess a complete C4 anatomy and physiology. In this instance the reduced O_2 sensitivity was attributed to the CO_2 concentrating activity of phosphoenol pyruvate carboxylase and malic enzyme. It seems likely that the low O_2 sensitivity of *P. billardierii* is also the result of some CO_2 concentrating mechanism. An active inward transport of bicarbonate, coupled with a high carbonic anhydrase activity could fulfill this requirement.

As the α values of groups one and two are similar, the response

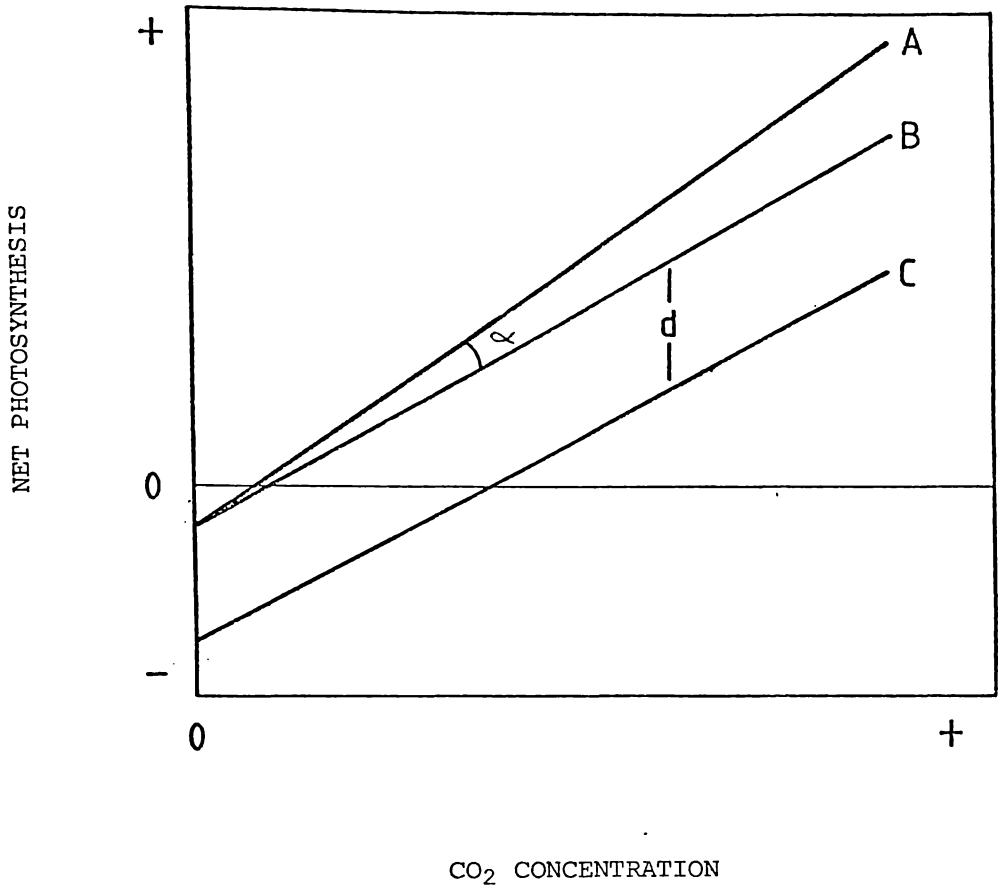


FIGURE 2. Components of the interaction between Net Photosynthesis, O_2 level, and CO_2 concentration. (A) response at <1% O_2, (B & C) possible responses at 21% O_2. (α) represents the effect of O_2 on carboxylation efficiency and (d) the rate of photorespiratory release of CO_2.

differences between these groups is largely attributable to variations in the d parameter. Values of d, calculated as the difference between 21% O_2 and <1% O_2 regression intercepts on the y axis and corrected for dark respiration variations, range from 1 to 10 for group one. Values for the other two groups vary between -2 and -6, the negative values resulting from lines A and B (Figure 2) intercepting at a low CO_2 concentration, rather than at Zero as depicted in Figure 2. Brown (1980) considered d to be a measure of the photorespiratory loss of previously fixed CO_2. He further suggested that near Zero values of d in species with a high α were due to the refixation of photorespired CO_2, this refixation being enhanced by low CO_2 diffusion resistances between release and refixation sites. A similar explanation could apply to the present results, especially in view of the high CO_2 resistances of many lichens (Chapter XI). However previous results have indicated that up to 70% of dark respired CO_2 is refixed in the light and similar refixation ratios are presented in Table 1. If members of the Stictaceae can refix CO_2 this efficiently at ambient O_2 levels then a lack of recycling ability is unlikely to be the cause of the high τ values reported here. However the possibilities that either photorespired CO_2 is not readily available for refixation, or that refixation is affected by O_2 (as is carboxylation efficiency) should be considered, particularly in view of the significant correlation between photorespiratory rate and τ.

SECTION D

GAS EXCHANGE RESISTANCES

CHAPTER XI

Carbon dioxide exchange in lichens: Resistances to CO_2 uptake at different thallus water contents

SUMMARY

CO_2 resistance and water content curves are presented for *Pseudocyphallaria amphisticta*, *P. homoeophylla*, *P. billardierii*, *P. colensoi*, *Sticta latifrons* and *Peltigera polydactyla*. In all cases the curves are triphasic with a high resistance at low and high water contents and a low resistance at intermediate water contents. The exact water contents at which these changes in resistance occur, and the rate at which the resistance increases at the higher water contents appear to be species specific. The depressed NAR often found at higher water contents is shown to be caused in part by the increased resistance since it occurs under conditions where there are no changes in respiratory rate. The increase in resistance at higher water contents is suggested to be a result of water on the lower cortex.

INTRODUCTION

Although there have been many studies of the relationship between the net assimilation rate (NAR) of lichens and thallus water content (Bewley 1979), there have been no detailed investigations of the causes of the changes in NAR at different water contents. Bewley *(op. cit)* in a review has again drawn attention to the common feature of depressed NAR at high thallus water contents. The usual explanations for this depression are either increased respiration and/or increased resistance to gas diffusion. The increased resistance is suggested to result from diffusion pathway constriction either by liquid water or by cell wall expansion.

In studies on the CO_2 exchange of higher plant leaves measurements of the resistance to carbon dioxide diffusion have proved very useful. It is probable that determination of these resistances in lichen thalli at different water contents could provide more useful information on NAR depression at high water contents. The only published value for the CO_2 resistance of a lichen is that of Collins and Farrar (1978). The total resistance found(Σr = 13.8 x 10^3 s m^{-1}) was far higher than typical values for higher plants. Collins and Farrar further subdivided the total resistance into boundary layer resistance (r_a); cortex resistance (r_c), and carboxylation resistance (r_e). It is unfortunate that, except for r_a, the calculations are open to criticism. It is uncertain whether the thalli studied were incubated at saturating light intensity with carbon dioxide as the sole factor limiting photosynthesis. A linear relationship between CO_2 concentration and photosynthesis has been assumed, rather than demonstrated, and consequently the resistance could well be overestimated (Jarvis 1971). Furthermore, no allowance was made for photorespiration and it is possible that photosynthesis could be depressed to 60% of levels at 1% oxygen again leading to a possible over-estimate of resistance (Snelgar and Green 1980). The value for r_e (carboxylation resistance) is based on a value for internal thallus CO_2 concentration which was taken to be identical to the CO_2 compensation value (Γ), an assumption that appears not to be made for higher plant leaves (Jarvis 1971). The value of Γ is unusually high, about 170µl CO_2 l^{-1} compared to a normal 50 to 100µl CO_2 l^{-1} for C3 plants and 10 to 50 µl CO_2 l^{-1} for some lichens (Snelgar and Green 1980), particularly when other authors (see Larson and Kershaw 1975b) claim that many lichen species reach CO_2 saturation at 150µl CO_2 l^{-1}. Finally, there is now evidence that most CO_2 exchange may be through the lower cortex and if

this occurs to any extent in *Xanthoria parietina* it would invalidate calculations of diffusion path (Green unpublished data).

In this investigation CO_2 resistances have been determined for a number of species using the standard techniques applied to higher plant leaves (Jarvis 1971). No attempt is made to subdivide the total CO_2 resistance since no estimate was able to be made for thallus internal CO_2 concentration.

MATERIALS AND METHODS

Thalli of *Pseudocyphellaria homoeophylla* (Nyl.) Dodge, *P. amphisticta* Kremp., *P. billardierii* (Del.) Ras, *P. colensoi* (Bab. in Hook f.) Vain, *Sticta latifrons* Rich., and *Peltigera dolichorhiza* (Nyl.) Nyl. were collected from *Nothofagus* forest near Kaipo Lagoon, Urewera National Park, North Island, New Zealand (NZMS 1 N96 619437). Air dried thalli were stored in the dark over silica gel at 16°C for not more than two weeks. Prior to use a 20-50 cm^2 portion of thallus was mist sprayed with distilled water and held at 100% R.H., 16°C, 50μE m^{-2} s^{-1} for at least ten hours in order to avoid the effects of resaturation respiration. Repeat experiments confirmed that NAR and respiration rate were stable after this period of pretreatment. Net assimilative rates (NAR) were assayed on a water jacketed perspex cuvette using an A.D.C. series 225 infra red gas analyser (IRGA) operating in a closed loop mode. The total volume of the system was 370 cm^3 and flow rates were set at 8.3cm^3 s^{-1}, giving a turnover period of 45 seconds. The output of the IRGA was amplified and graphed on a servoscribe recorder. Thallus temperature was maintained at 16.0 $\pm$ 0.5°C and was monitored using a thermistor probe. Lighting was by a single 1000 watt quartz halogen lamp filtered through two 10cm deep water baths and arranged to give light intensity of 150μE m^{-2} s^{-1}

(Licor Quantum meter Model LI 185A) inside the cuvette. Light response curves at optimal water content, 350µl l^{-1} CO_2 concentration and 20% oxygen were constructed for all species. In all cases light saturation was reached at 100 to 150µE $m^{-2}s^{-1}$ and only *P. billardierii* showed any depression of NAR at high light levels (14% depression at 500µE $m^{-2}s^{-1}$). Frequent checks during experimental runs at 1% oxygen and particularly at low water contents confirmed that the lichens were light saturated. NAR were measured at CO_2 concentrations of 0-150µl l^{-1} under micro-aerobic conditions (< 1% O_2) and over a wide range of thallus water contents. The precision of the determination as estimated from repeated measurements was better than $\pm$ 3% for a single specimen. A dark respiration rate under the same gaseous atmosphere was obtained at the end of each NAR experiment. Subsequent experiments have shown no difference in the relationship between respiration rate and water content at ambient and microaerobic (< 1%) oxygen concentrations. Slight water loss occurred from thalli during each (0.5 - 1.5 hr) experiment and water contents are expressed as the mean of the initial and final values for each incubation in units of mg water per mg thallus dry weight (obtained by drying to a constant weight at 100°C). The maximum water contents found in this investigation probably represent the highest likely to be reached in nature since thalli were sprayed with water on both sides and were visibly wet. Thalli were only lightly shaken before incubation and were not blotted thus ensuring retention of any external water store (Snelgar and Green 1981b). A Koizumi compensating planimeter was used to measure the area of each thallus. The resistance to carbon dioxide uptake was calculated from the slope of the linear portion of the N.A.R. against CO_2 concentration graph at each thallus water content, using the equation:

$$\Sigma r = \frac{Ca - \Gamma}{P} \qquad \text{(Jarvis 1971)}$$

where Γ = CO_2 compensation point ($g\ m^{-3}$),

Ca = ambient CO_2 concentration ($g\ m^{-3}$), and

P = NAR at Ca ($g\ m^{-2}s^{-1}$).

Σr is the total resistance to CO_2 uptake ($s\ m^{-1}$) and includes the boundary layer, internal transport and carboxylation resistances. An estimate of the boundary layer resistance calculated from the mean flow rate in the chamber indicates a maximum value of 0.8×10^3 $s\ m^{-1}$. It should be noted that as the dark respiration rate was constant at each water content it will not affect the slope of the NAR against CO_2 concentration plot used to calculate the total resistance. Repeat experiments demonstrated that resistance curve of identical form could be obtained for different specimens of a particular species. However at the highest water contents absolute values for resistances often varied between specimens probably as a result of slight differences in water location in the thalli.

Dimensions of the thalli were measured from hand cut thin sections using a calibrated microscope.

RESULTS

Graphs of the relationship between total CO_2 resistance and thallus water content for all six species are presented in Figures 1, 2 and 3.

All species show higher CO_2 resistance at low water contents although the exact water content at which the resistance starts to rise during drying differs between species. However, differences in resistance changes at high water contents allow the lichens to be divided into three groups each containing two species. Group 1 (Figure 1) is

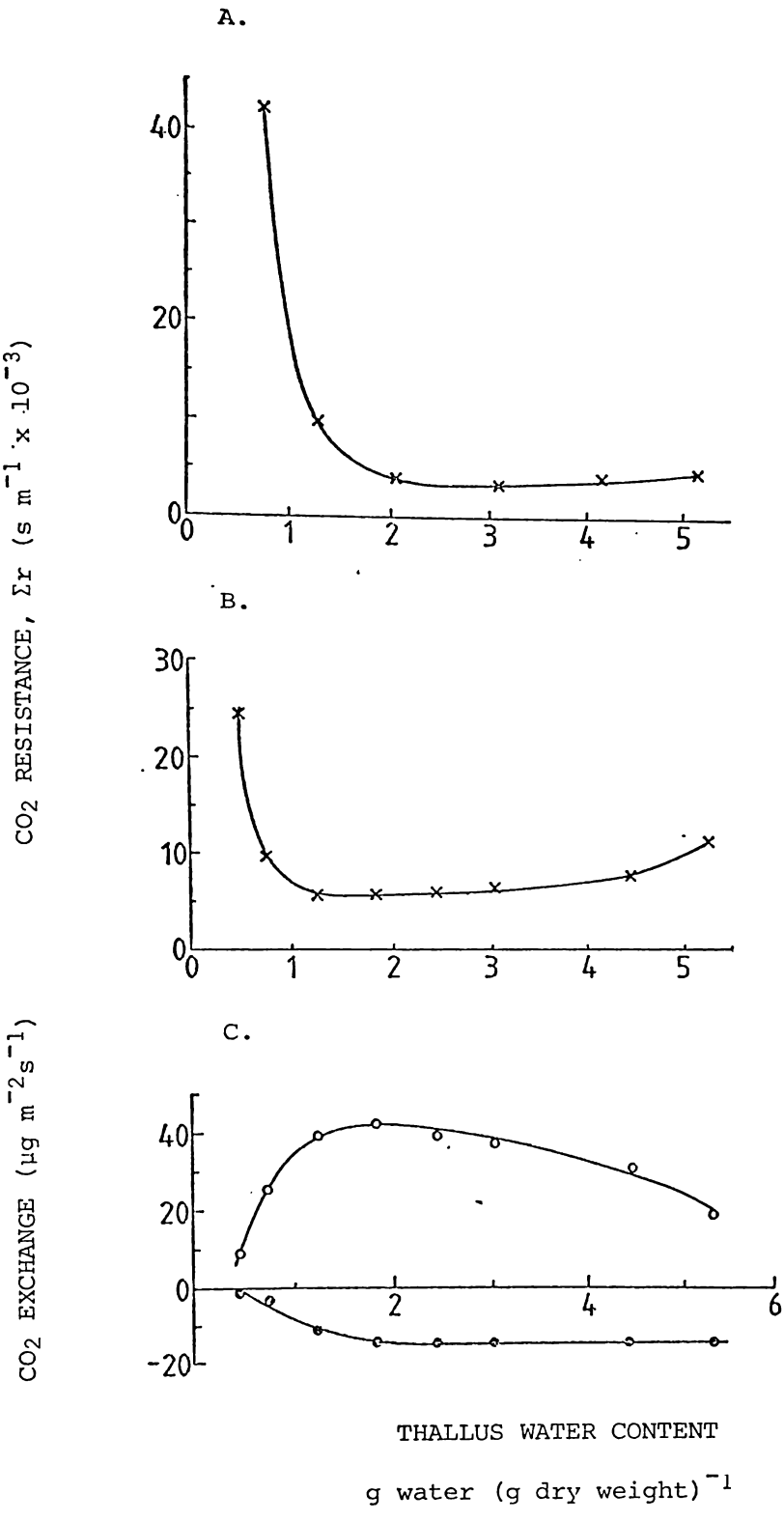


FIGURE 1. Group I. CO_2 resistance versus water content for (A) *Peltigera dolichorhiza* and (B) *Pseudocyphellaria amphisticta*. (C) NAR (o) and (●) respiratory rate versus water content for *P. amphisticta*.

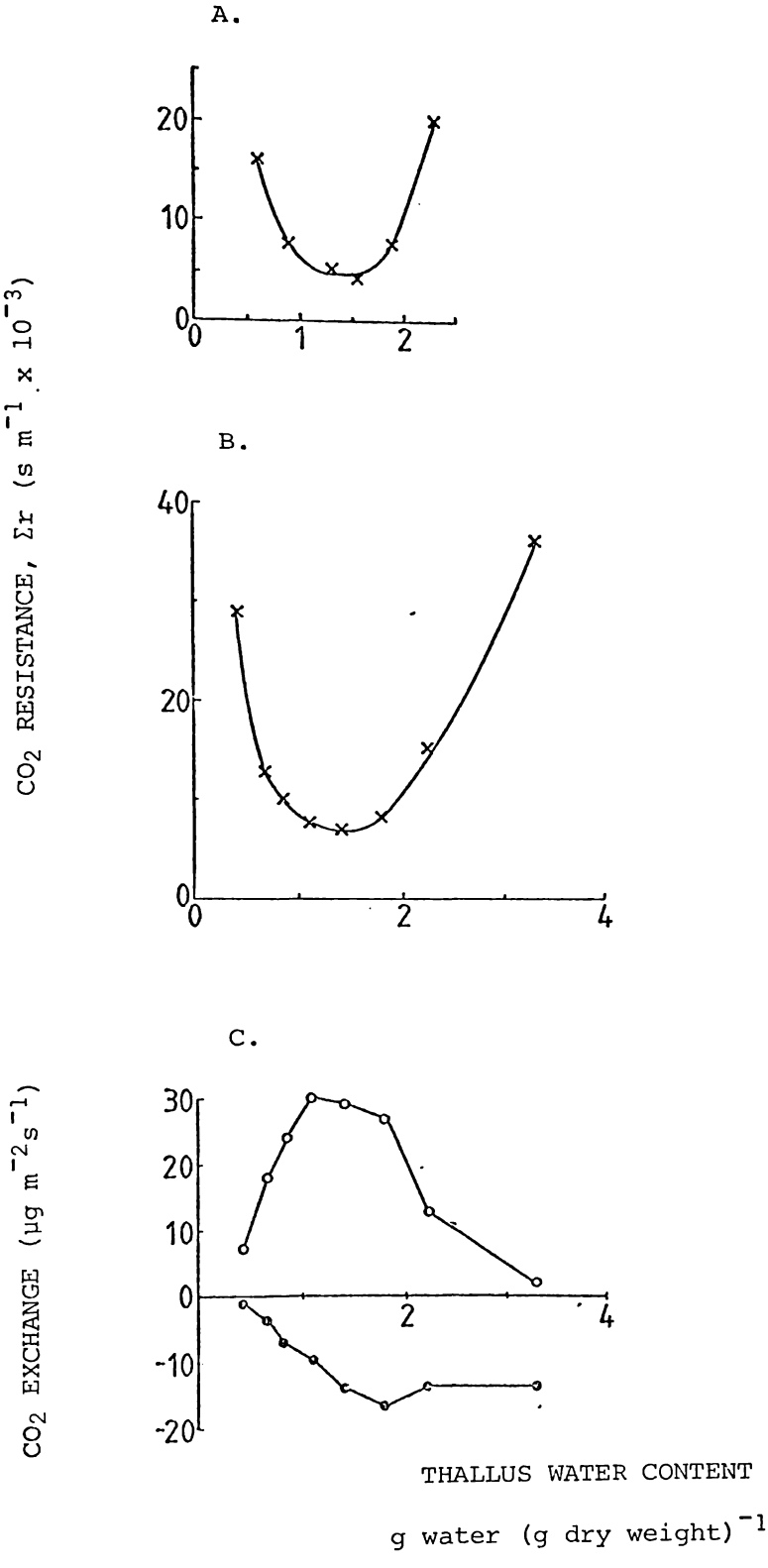


FIGURE 2. Group II species. CO_2 resistance versus water content for (A) *Sticta latifrons* and (B) *Pseudocyphellaria homoeophylla*. (C) NAR (o) and (●) respiratory rate versus water content for *P. homoeophylla*.

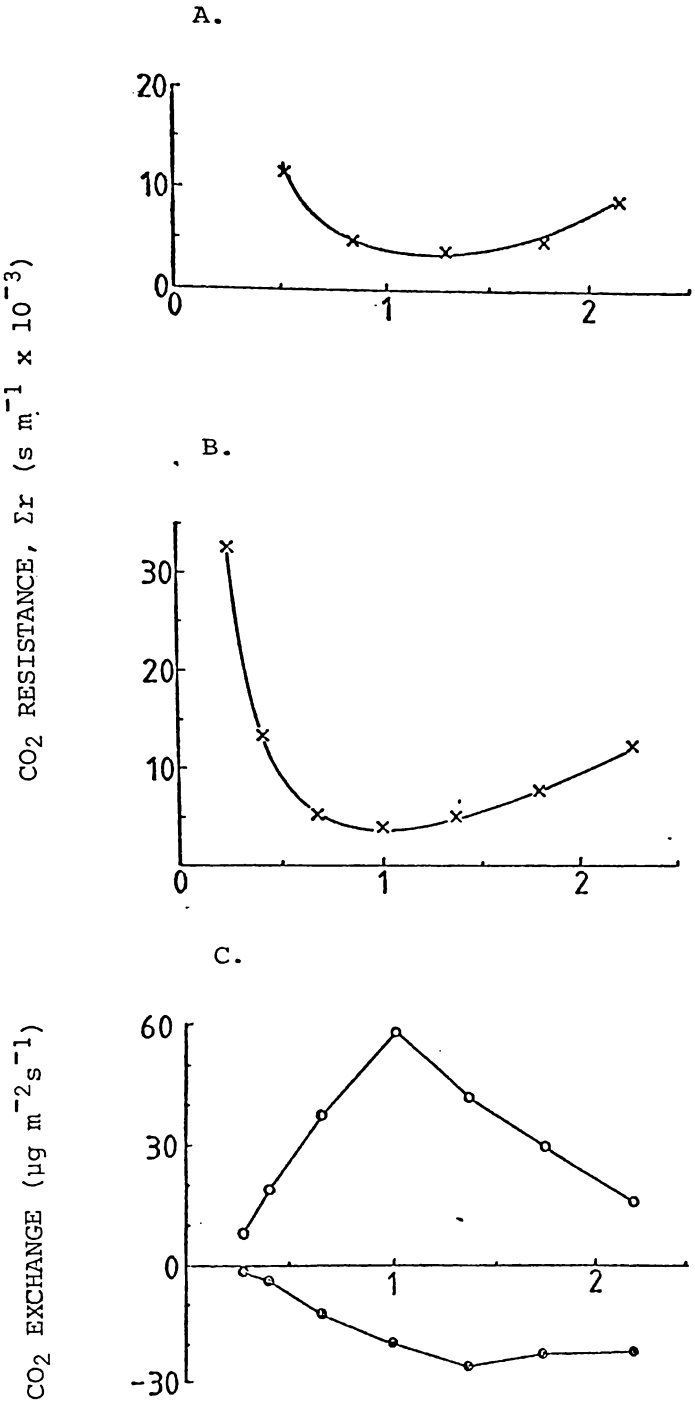


FIGURE 3. Group III. CO_2 resistance versus water content for (A) *Pseudocyphellaria billardierii* and (B) *Pseudocyphellaria colensoi*. (C) NAR (o) and (●) respiratory rate versus water content for *P. colensoi*.

TABLE 1. Summary of the principal features of Figures 1, 2 and 3 together with details of thallus morphology, blotted water content and a short ecological summary. The ecological details are from non-quantitative observations. Water contents are in mg water per mg thallus dry weight; thallus thickness is total thallus thickness excluding rhizines in μm.

Group	Species	Water content below which Σr rises rapidly	Low resistance phase values	Water content above which Σr rises	Water content of blotted thalli	Gradient of Σr rise at high water contents	Lower surface detail	Cyphellae (C) or Pseudocyphellae (P)	Thallus thickness (μm)	Ecology
1	Peltigera polydactyla	1.6	3.0×10^3	Very little increase	3.27	almost nil	rhizinae	none	299	moist ground, moderately open forest, medium light.
1	Pseudocyphellaria amplisticta	1.0	5.6×10^3	4.5	2.23	slight	well developed tomentum	P (top and bottom)	396	forest margin or sub-canopy, medium light.
2	P. homoeophylla	1.0	7.0×10^3	1.7	1.68	steep	slight tomentum	P	613	forest floor - lower tree trunk, low light.
2	Sticta latifrons	1.0	5.0×10^3	1.6	1.49	steep	slight tomentum	C	466	lower tree trunks, low light
3	P. billardierii	0.7	4.0×10^3	1.8	1.78	moderate	slight tomentum on older parts	P	424	tree branches in open areas, high light.
3	P. colensoi	0.6	3.8×10^3	1.4	1.26	moderate	nude	P	411	forest margins and canopy, high light.

composed of *Peltigera dolichorhiza* and *Pseudocyphellaria amphisticta* both species which show very high thallus water contents (up to 5) with only a small increase in resistance. The remaining four species all have a lower maximum water content (about 3.3) but differ in the magnitude of the resistance change at the higher water contents. Group 2, *P. homoeophylla* and *S. latifrons*, show very large increases in resistance at the higher water contents with the resistance reaching levels similar to those at low water contents. Group 3, *P. billardierii* and *P. colensoi*, show a small rise in resistance at high water content but much lower than those found for Group 2.

The responses of NAR and respiration to water content for *P. amphisticta*, *P. homoeophylla* and *P. colensoi* are shown in Figures 1C, 2C and 3C respectively. In each case there is a reduction in NAR at the lower water contents. All three also show reduced NAR at higher water contents but to differing extents, a result that has been previously obtained for *P. homoeophylla* and *P. colensoi* at ambient oxygen levels (Snelgar *et al.* 1980). *P. amphisticta* shows the least reduction with the NAR close to maximum values over most of the range of thallus water content. *P. homoeophylla* shows a severe decline in NAR at the higher water contents with values close to zero being reached. *P. colensoi* shows an intermediate response. All three species show an almost linear rise in respiration with increase in water content at low water contents but a plateau value is reached and approximately maintained at medium to high water contents.

DISCUSSION

The total resistance to CO_2 diffusion was shown to change considerably with thallus water content. However, all six species had three

identifiable phases in the pattern of the changes: very high resistance at low water contents; low resistance over a range of intermediate water content and an increase in resistance at high water contents. The water contents at which each of the three phases occur appear to be species specific and are summarised in Table 1. The lowest resistance values are all around $5 \times 10^3 \times m^{-1}$, considerably lower than the value found by Collins and Farrar (1978) for *X. patietina* (13.8×10^3 s m^{-1}) but still higher than a normal leaf with open stomata (about 1×10^3 s m^{-1}). The depressed NAR at high water contents found here for *P. amphisticta*, *P. colensoi* and *P. homoeophylla* clearly correlate well with the increase in total CO_2 resistance. There is no correlation with respiration rate which in all cases remains almost constant, or even declines slightly, over the water content range where NAR decreases. The experimental routine used here effectively prevents any respiration effect from interfering. Also, all measurements were made under limiting CO_2 concentrations thus any variations in respiration rate could be absorbed by the carboxylation reaction without affecting NAR. In general there can be little doubt that, when a lichen is close to CO_2 saturation, then increased respiration must depress NAR, however, it is now clear that CO_2 resistance increases will also contribute to the changes in NAR.

Although the location of this extra CO_2 resistance at higher water contents is uncertain, it is probable that the presence of liquid water on the undersurface is at least partly responsible. Previous work by Green (unpublished data) demonstrated that the lower surface and probably the cyphellae, was the major site of CO_2 exchange in *S. latifrons* and it is very likely that this result is also applicable to other species in *Sticta* and *Pseudocyphellaria*. It was also shown that the presence of excessive water on the lower surface strongly reduced

CO_2 exchange. The values for the water contents of blotted thalli, i.e. with surface water removed, for the lichens of groups II and III agree exceptionally well with the water content values at which the CO_2 resistance starts to rise (Table 1). It is clear that the presence of any external water leads to an increased resistance.

P. homoeophylla and *S. latifrons* (group II) have slightly tomentose undersurfaces that can hold water and these species show a large increase in resistance at higher water contents. *P. colensoi* and *P. billardierii* (group III) have a nude underside and/or weak tomentum development respectively, which would hold little excess water and showed only a moderate resistance increase at higher water contents. As described in the methods section, the lichens were wetted by spraying with water which is not a natural situation. It is interesting that the group III lichens which have poorly developed or no tomentum, occur in more open areas exposed to direct rainfall or mist. The group II lichens are found in the base of the forest where only leaf drip falling vertically would strike them on the upper surface.

The group 1 lichens, *Peltigera dolichorhiza* and *Pseudocyphellaria amphisticta* are exceptions to the above discussion. *P. amphistica* has a very well developed tomentum which almost completely obscures the pseudocyphellae and would thus be expected to have a high CO_2 resistance at higher water contents. There is very little effect of high water content on CO_2 resistance and it seems that this is a result of the CO_2 exchanging through the pseudocyphellae on the upper surface. Unpublished results using a split chamber (Green) confirmed that CO_2 does exchange almost entirely through the upper surface of *P. amphisticta*. *Peltigera dolichorhiza* is also exceptional in the

small increase in CO_2 resistance with water content and possession of the lowest total resistance value for the species surveyed. It is possible that this results from (a) a lack of a lower cortex so that the entire lower surface is available for CO_2 exchange, (b) no tomentum, (c) a thinner thallus than the other lichens, (d) a very open medulla structure and (e) the thinnest cortex of the lichens surveyed through which some CO_2 may exchange. It is unfortunate that the fragility of the thallus prevented use in the split chamber.

The resistance values obtained here confirm that CO_2 exchange must take place almost entirely through the gas phase in the lichen medulla. As an example, a water diffusion path of about 8μm would account for the lowest resistance found for *S. latifrons*, and a water path of 35μm for the highest, yet the distance from lower surface to algal cells is about 340μm.

The increased CO_2 resistance found at lower water contents is most easily explained by increased carboxylation resistance, however it is possible that drying of the thallus could cause increased diffusion resistances from contraction of the extra cellular polysaccharides. Sub division of the total resistance into carboxylation, cortical and medulla resistances would add considerably to our understanding of lichen photosynthesis, unfortunately, considerable technical difficulties exist in obtaining values for the internal CO_2 concentration particularly because of the more fragile nature of lichen thalli in comparison to leaves, and a lack of correlation between water and CO_2 diffusion resistances (Snelgar and Green unpublished results). The results of this paper further emphasise the degree of ecophysiological adaptation found in the lichens of temperate rainforests and further studies of the link between morphology and physiology are continuing.

CHAPTER XII

Carbon dioxide exchange in lichens: Partition of total CO_2 resistances at different thallus water contents into transport and carboxylation components

Introduction

The identification and quantification of the various resistances to CO_2 uptake within higher plant leaves has proved most useful in our understanding of the factors limiting rates of photosynthesis. Resistance analogues describing the control of CO_2 uptake have been constructed and used both to explain the experimental observations and to predict the probable effects of untried experiments such as the removal of stomatal resistances (Körner *et al.* 1979). Although attempts have been made to construct similar resistance analogue models for lichens (Collins and Farrar 1978, Lange 1980), these have necessarily been largely theoretical owing to the lack of relevant experimental data.

The first estimation of CO_2 resistances in a lichen (Collins and Farrar, 1978) has been criticised on several points of experimental technique (Snelgar *et al.* 1981b) and was based on assumptions regarding the diffusion pathway of CO_2 in lichens which may not be correct (Green *et al.* 1981). As noted previously (Snelgar *et al.* 1981b), estimation of the various components of total CO_2 resistance (Σr) is also complicated by the difficulties of calculating or measuring the CO_2 concentrations within the lichen thallus.

In this paper an alternative approach is used in which net photosynthesis rates at various CO_2 concentrations are used to construct a linear graph from which transport (r_t) and carboxylation (r_e) resistances may be calculated. This model was developed by Jones and Slatyer (1972) for the partition of intracellular resistances of cotton leaves (*Gossypium hirsutum*), and the theory and methodology

of the technique are fully described in that paper. Although internal CO_2 concentrations were used by Jones and Slatyer (1972), Browse *et al.* (1979) have adapted the method for use with external CO_2 concentrations in the case of a submerged aquatic (*Egeria densa*). A similar adaptation was used during this investigation.

List of Symbols: Σr = total CO_2 resistance ($r_t + r_e$), s cm^{-1}; r_e = CO_2 carboxylation 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_a = boundary layer resistance for CO_2, s cm^{-1}; $r_{a\ H_2O}$ = boundary layer resistance for water vapour, s cm^{-1}; k' = Michaelis constant for the carboxylation system, g cm^{-3}; c_w = CO_2 concentration at mesophyll cell wall, g cm^{-3}; c_a = ambient CO_2 concentration, g cm^{-3}; c_i = CO_2 concentration at carboxylation site, g cm^{-3}; τ = CO_2 compensation point, g cm^{-3}; P = net photosynthetic rate at c_a, g cm^{-2} s^{-1}; P_m = net photosynthetic rate at saturating CO_2, g cm^{-2} s^{-1}; R = rate of CO_2 release at zero CO_2 concentration and quantum flux density of 150 μE m^{-2} s^{-1}, g cm^{-2} s^{-1}; q_a = water vapour concentration in ambient air, g cm^{-3}; q_s = water vapour concentration at liquid-air interface, g cm^{-3}; E = evaporation rate from filter paper surface, g cm^{-2} s^{-1}; D = mean diameter of evaporation surface (either lichen thallus or filter paper disc), cm; u = windspeed, cm s^{-1}; L = depth of boundary layer, cm.

Theory

Σr at any water content was calculated from the equation of Jarvis (1971).

$$\Sigma r = \frac{c_a - \tau}{P} \qquad (1)$$

under conditions of low (1%) oxygen and limiting CO_2 (Snelgar *et al.* 1981).

r_a was calculated using the equation of Monteith (1965)

$$r_a = 1.3\sqrt{\frac{d}{u}} \qquad (2)$$

and was further checked by experimental measurement using the equation of Landsberg and Ludlow (1970).

$$r_{a_{H_2O}} = \frac{q_s - q_a}{E} \qquad (3)$$

$r_{a_{H_2O}}$ was converted to r_a by multiplying by 1.37 (Chartier *et al.* 1970).

The depth of the boundary layer in nominally still air was determined using the equation of Meidner and Mansfield (1968).

$$L = \frac{\pi D^{0.6}}{8} \qquad (4)$$

Σr may be mathematically subdivided into r_t and r_e using the model developed by Jones and Slatyer (1972)

$$c_w \frac{(Pm-P)}{P} = -r_t P + r_t P_m + k' \qquad (5)$$

(After equation 13, Jones and Slatyer, 1972)

This model is based on the following assumptions. (a) Photosynthesis is light saturated. (b) Photosynthesis is proportional to c_w. (c) Photorespiration is not detectable. (d) The overall kinetics of the carboxylation system fit Michaelis Menton kinetics with respect to CO_2 as the substrate, $P = P_m\, c_i/(k' + c_i)$. (e) Transport resistance is independant of CO_2 concentration.

In the present work c_w cannot be assessed by the methods normally used on higher plants. Calculation of c_w from measurements of the resistance to water vapour loss is not possible since there is no simple correlation between this parameter and resistance to CO_2 uptake in these lichens (Green unpublished data). However, because lichens lack a physiologically controlled variable resistance to CO_2 uptake such as the stomata found in higher plant leaves c_w can be replaced by c_a. The transport resistance (r_t) then includes all resistances to CO_2 diffusion except r_e, ($r_t = \Sigma r - r_e$). The assumption is made that at all points of the pathway of CO_2 from the outside of the lichen to the carboxylation site the rate of diffusion is proportional to the difference in CO_2 concentration regardless as to whether the CO_2 is diffusing in air or water. Although photorespiration was suppressed by conducting all experiments at 1% oxygen, a small respiratory component remained, probably due to continued 'basal' respiration of the lichen mycobiont in the light. This could be corrected by subtracting τ from each value of c_a so that the corrected line passes through the origin (Jones and Slatyer 1972). However, in this instance an alternative correction of adding R to all values of P was preferred, as this results in all data points (including those below τ) being included in the linear regression. Equation (5) becomes:

$$c_a\frac{[(P_m + R) - (P + R)]}{P + R} = -r_t(P + R) + r_t(P_m + R) + k' \qquad (6)$$

From Jones and Slatyer, if the proposed model fits the experimental data, a plot of $c_a[(P_m + R) - (P + R)]/(P + R)$ against $(P + R)$ should be a straight line of slope $-r_t$ and y intercept $[(P_m + R)r_t + k']$. Hence, k' may be calculated from the y intercept value and then r_e from

$$r_e = \frac{k'}{(P_m + R)} \qquad (7)$$

(After equation 8, Jones and Slatyer, 1972)

The relative importance of internal transport and carboxylation processes at high values of c_a (where P may not be directly proportional to c_a) may be calculated from the ratio of the change in $(c_a - c_i)$ to the change in c_i for any given small change in c_a or P. This ratio may be calculated as:

$$\frac{\delta(c_a - c_i)}{\delta c_i} = \frac{r_{ti}}{k'} \frac{[(P_m + R) - (P + R)]^2}{(P_m + R)} \qquad (8)$$

(After equation 14, Jones and Slatyer, 1972)

This reduces to r_{ti}/r_e at low values of P (or c_a). Values of r_{ti} are used in equation (8) in preference to r_t in order to eliminate the effect of r_a which was a product of the experimental system used.

Materials and Methods

Specimens of *Pseudocyphellaria amphisticta* Kremp. were collected from *Nothofagus* forest near Kaipo Lagoon, Urewera National Park, North Island, New Zealand (New Zealand Map Series 1 Sheet N96 grid reference 619437). *Sticta latifrons* Rich. was

collected from the Hakirimata reserve, Ngaruawahia, Central North Island, New Zealand (NZMS 1 N56 645622). Air dried thalli were stored in the dark over silica gel at 16°C for not more than two weeks. Prior to use a 10 - 50 cm^2 portion of thallus was moistened by mist spraying with distilled water, then held at 100% RH, 16°C, 50 $\mu E\ m^{-2}\ s^{-1}$ for at least ten hours in order to avoid resaturation effects. The light source was two Atlas 20 watt Colour number 35, white fluorescent tubes. All radiation measurements were made with a Licor Quantum meter, Model LI 185A, measuring photosynthetically active radiation between 400 and 700 nm. Net photosynthesis rates were assayed in a water cooled perspex cuvette using an A.D.C. series 225 infra red gas analyser operating in a closed loop mode. Experimental conditions were: Quantum flux density 150 $\mu E\ m^{-2}\ s^{-1}$ (saturating), the light source was Phillips 1000 watt 12013 R tungsten halogen above two 10 cm deep water filters, temperature 16°C, flow rate 0.5ℓ min^{-1} oxygen concentration 1% v/v, carbon dioxide concentration 0 - 2500 $\mu\ell\ \ell^{-1}$. Lichens were positioned on small stands within the photosynthetic chamber, so that air flowed over both surfaces of the thallus. Dry weights were determined after drying to a constant weight at 100°C. A Koizumi compensating planimeter was used to measure thallus area.

The boundary layer resistance (r_a) was estimated from the mean flow rate through the chamber and the dimensions of the thallus by using equation (2). This was further checked by measuring evaporation from 7.0 cm diameter filter paper discs and calculating $r_{a\ H_2O}$ using equation (3). Evaporation rates were determined as the weight loss of filter paper discs during

5-15 minute incubations. The water vapour concentrations of the air entering and leaving the chamber were obtained from measurements of humidity, and air temperature, using matched Vaisala humidity sensors and thermistor probes; q_a was taken to be the arithmetic mean of these concentrations. The values of q_s were calculated from surface temperature measurements (thermistor probe) of the filter paper discs.

Results

Comparison of estimates of r_a obtained by calculation or by direct measurement of water loss from a filter paper (Figure 1) shows that both methods gave similar results at intermediate flow rates, but differences were obvious at both high (10ℓ min^{-1}) and low (0.5ℓ min^{-1}) flow rates. Using equation (4) and a filter paper diameter of 7.0 cm the effective maximum depth of the boundary layer in nominally still air can be calculated as 1.26 cm and from the coefficient of diffusion of CO_2 in air, 0.147 cm^2 s^{-1} , r_a is 9 s cm^{-1}. This value was used in all further calculations concerned with the subdivision of Σr because it represents both a predicted maximum and the mean of the two estimates, although it is possible that this is an over estimate of r_a. The differences in calculated and measured r_a values at the high flow rates were probably due to the poor aerodynamics of the experimental cuvette. This would result in actual mean air speed being lower than those calculated from the flow rates.

The P *vs* CO_2 response curves for *S. latifrons* and *P. amphisticta* (Figure 2) all show an initial linear phase followed by a decreasing response until CO_2 saturation is attained. The CO_2

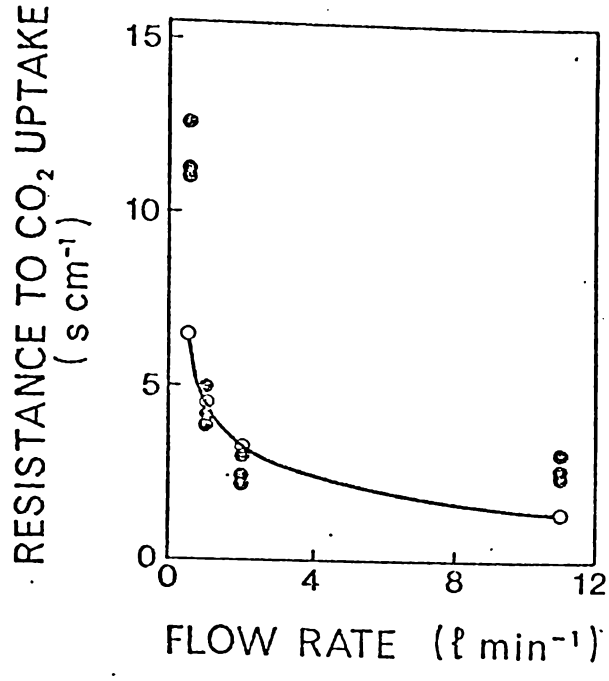


FIGURE 1. Effect of flow rate on boundary layer resistance. Data points were obtained by experimental (●) and theoretical (o) methods.

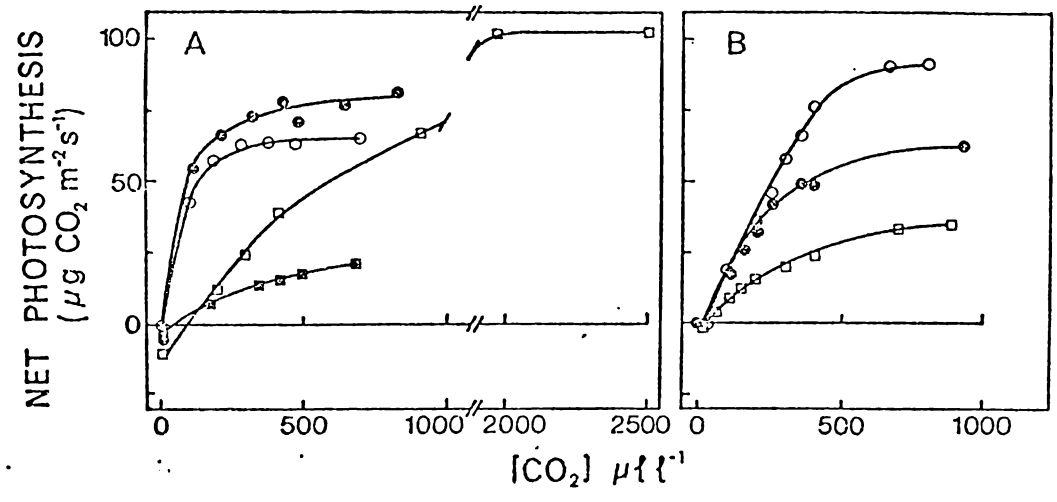


FIGURE 2. Effect of CO_2 concentration on net photosynthesis at several water contents. A = *Sticta latifrons*; B = *Pseudocyphellaria amphisticta*. The water contents [g water (g dry weight)$^{-1}$] were: A, (●) 1.47, (o) 0.87, (□) 2.41, (■) 0.41; B, (o) 3.24, (●) 0.79, (□) 0.51.

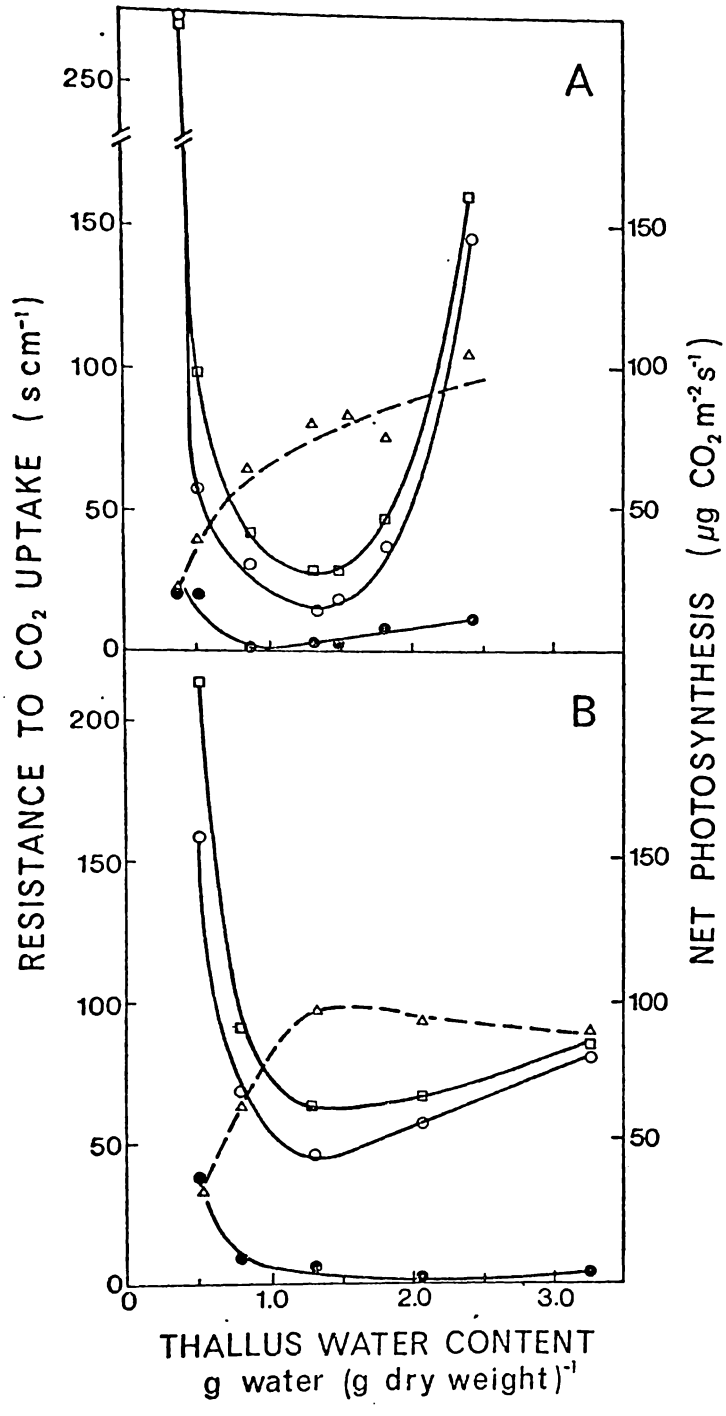


FIGURE 3. Relationship between carbon dioxide resistances, photosynthesis and thallus water content. A = *Sticta latifrons;* B = *Pseudocyphellaria amphisticta*. (□) total CO_2 resistance, Σr (equation 1); (o) internal transport resistance, r_{ti} (equation 6, $r_{ti} = r_t - r_a$); (●) carboxylation resistance, r_e (equation 7); (Δ) net photosynthesis rate at saturating c_a (P_m).

concentrations required to saturate photosynthesis were normally about 400 - 500 $\mu\ell\ \ell^{-1}$, but at high thallus water contents values of up to 2000 $\mu\ell\ \ell^{-1}$ were necessary. The gradient of the linear portion of these curves was used to construct Σr curves (Figure 3). Although this gradient decreases at both high and low water contents a decreased maximum photosynthesis rate that is not alleviated by higher CO_2 concentrations occurs only at low water contents (Figure 2A). Linear transformations (see methods) of the complete P against CO_2 concentration curves were used to obtain the r_t and r_e components of Σr (Figure 3).

The correlation coefficients of the linear regressions made on transformed data (Table 1) show a high degree of fit. This, together with the close correspondence of Σr, obtained using equation (1) and $(r_t + r_e)$ values, obtained from the partition model (Table 1), is a strong indication that this transformation technique is valid under the experimental conditions used.

From Figure 3 it can be seen that r_e is a small component of Σr at all water contents. The very large resistances found in *S. latifrons* at high water contents appear to be due to increases in transport resistances. Both lichen species exhibit extremely high Σr values at low water contents, as found in previous investigations (Snelgar *et al.* 1981b) and, although there is a marked rise in r_e, r_{ti} is still the major component. At optimal water contents for photosynthesis r_{ti} for *S. latifrons* reaches a minimum value of 15 s cm^{-1} (by subtraction of r_a, 9 s cm^{-1}, from r_t, 24 s cm^{-1}). Similarly r_{ti} of *P. amphisticta* reaches a minimum of 46 s cm^{-1} at a water content of 1.30.

Table 1. *Total resistance (Σr), internal transport resistance (r_{ti}) carboxylation resistance (r_e) and relative contribution of internal transport and carboxylation processes as limiting factors to photosynthesis at several water contents for* Sticta latifrons *and* Pseudocyphellaria amphisticta. Σr were obtained by the use of equation (1). r_{ti} and r_e were obtained from linear transformations of P vs c_a curves. Correlation coefficients (cc) and number of data points (n) are given. r_{ti} is $(r_t - r_a)$ and r_a has a constant value of 9.0 s cm^{-1}. All resistance values are in s cm^{-1}. The relative contribution of internal transport and carboxylation processes in limiting net photosynthesis is given as r_{ti}/r_e at low c_a and by $\left[\frac{\delta(c_a-c_i)}{\delta c_i}\right]_{330}$ (from equation 8) at 330 µl l^{-1} CO_2. All water contents are in g water (g dry weight)$^{-1}$.

Water Content	Σr	r_{ti}	r_e	n	cc	$\frac{r_{ti}}{r_e}$	$\left[\frac{\delta(c_a-c_i)}{\delta c_i}\right]_{330}$
S. latifrons							
0.40	273	275	21	5	-0.980	13.4	1.8
0.51	99	58	21	6	-0.998	2.8	0.24
0.87	41	31	1.2	6	-0.999	25.8	0.17
1.32	29	15	3.2	7	-0.975	4.7	0.14
1.47	29	19	3.3	7	-0.968	5.8	0.24
1.82	48	38	9.2	6	-0.991	4.1	0.43
2.41	162	147	11.7	6	-0.977	12.6	7.1
P. amphisticta							
0.51	214	159	36.7	6	-0.971	4.3	0.97
0.79	92	69	9.8	5	-0.992	7.0	0.48
1.30	64	46	6.7	7	-0.992	6.9	0.64
2.05	67	57	1.9	5	-0.996	30.0	1.90
3.24	85	81	4.1	7	-0.985	19.8	24.0

Calculation of $\frac{r_{ti}}{r_e}$ ratios (Table 1) at low CO_2 concentrations demonstrates that r_{ti} is always the larger resistance in both species at all of the water contents studied. This effect is most obvious at high water contents where substantial r_{ti} increases occur. However when the relative contributions of internal transport and carboxylation processes are calculated at 330 $\mu\ell\ \ell^{-1}$ CO_2 (equation 8) it is evident that at intermediate water contents carboxylation is the more important limiting factor although transport limitations are still dominant at higher water contents.

Discussion

Extremely large carbon dioxide diffusion resistances have again been demonstrated in lichens at low thallus water contents, and in some instances at very high water contents, thus confirming previous results (Snelgar *et al.* 1981b). Partition analysis indicates that at low CO_2 concentrations r_{ti} is the major component of Σr at all thallus water contents. The large r_{ti} found in *S. latifrons* at high water contents is most plausibly explained by a decrease in the availability of air diffusion pathways within the lichen thallus due to infilling with water. This could be coupled with an increase in the length of the CO_2 diffusion pathway in water and, since CO_2 diffuses 10^4 times faster in air than in water, even a very small increase in the length of this pathway would create a large resistance. The effect of increased r_{ti} has been previously suggested by other authors (Collins and Farrar 1978, Lange 1980) after observations of decreased net photosynthesis at high water contents. During

the present work r_{ti} for *S. latifrons* and *P. amphisticta* was found to vary between 15 and 275 s cm^{-1}. These resistances may be totally accounted for by water diffusion paths of 2.4 and 44 μm, respectively. As these lichens are 396 - 466 μm thick and have cortices of 35 - 63 μm depth, it seems likely that even at high water contents the majority of CO_2 diffusion to the photosynthetic cells takes place in air.

The large r_{ti} resistances at low thallus water contents are more difficult to explain, although contraction of extra-cellular polysaccharides has been suggested (Snelgar *et al.* 1981b). However Troughton (1969), working on cotton leaves, also reported increases in transport resistance (mesophyll) from 2.5 to 12 s cm^{-1} during extreme water stress (75 - 56% relative water content). It is possible that both these findings are the result of the decreased water potential at low water contents inhibiting the enzymatic systems of photosynthesis, including carbonic anhydrase which may have an important role in CO_2/HCO_3^- equilibria (Bird *et al.* 1980).

When thalli of *S. latifrons* or *P. amphisticta* are at intermediate water content and 330 μl l^{-1} CO_2 then the extremely low $\frac{\delta(c_a-c_i)}{\delta c_i}$ ratios indicate that carboxylation is the major factor limiting photosynthesis. An interesting implication of this result is that under these conditions of normal ambient CO_2 levels the high internal transport resistances found in lichens have little effect on net photosynthesis. The carboxylation restraint on net photosynthesis could result from a limitation at any of the steps of the photosynthetic process. It has been previously suggested that chlorophyll content could be the factor

limiting lichen photosynthesis (Richardson 1973). However, results from higher plant studies indicate that this would be an unusual situation, and it is more often found that either ribulose biphosphate carboxylase content or the electron transport pathway is the controlling factor (Boardman 1977).

It is important to realise that, at ambient CO_2 levels, carboxylation processes are the dominant limiting factors only at intermediate water contents (0.51 to 1.82, *S. latifrons*; 0.51 to 2.05, *P. amphisticta*). At lower water content in *S. latifrons* and, particularly at higher water contents in both species, limitation by internal transport resistances is more important. Consequently the lower rates of net photosynthesis at high thallus water contents and ambient c_a can be eliminated by increasing CO_2 concentration. This may be of importance since Daubenmire (1959) has reported CO_2 levels in the lower layers of forests up to six fold normal. If such levels are found when lichens are water saturated, then transport resistances would have little effect on net photosynthesis. The reduced maximal net photosynthetic rates found at low water contents may have an effect on the physiological ecology of the species since the rates are not increased by high CO_2 levels. Unfortunately there is a lack of data on the frequency and length of occurrence of water contents of lichens in the field so that it is impossible to assess the importance of restriction of photosynthesis by low water contents.

Prioul *et al.* (1975) indicate the need for caution in the use of mathematical models in the study of complex biological processes and have suggested that the use of the Jones and Slatyer (1972) model

will automatically lead to high r_{ti}/r_e ratios. High ratios have been found in this investigation however there is also evidence that the ratios change with thallus water content. The r_e values rise at low water contents, particularly in *P. amphisticta* (Table 1) at the same time as P_m is lowered. This is an expected result if the carboxylation pathways are sensitive to decreasing water potential. The large internal transport resistances also found at low water contents are, however, difficult to explain.

Chartier and Čatský (1975) noted that many of the methods which have been developed for the study of photosynthesis have not been fully utilised for studies of lower plants. The present study illustrates how some of these techniques can be adapted for work with lichens and shows that the results can be of aid in clarifying our understanding of lichen photosynthesis.

CHAPTER XIII

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

Introduction

Previous studies of total resistances to CO_2 uptake (Σr) in six species of lichens (Snelgar *et al.* 1981b) 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 1981b) 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' pores in their dense lower cortex. These circular pores may represent breaks in the lower cortex through which the loosely packed fungal hyphae of the medulla protrude (pseudocyphellae). Alternatively the pores have a smooth lining which is distinct, both in structure and in thickness, from the cortex (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).

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

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

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

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

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

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.* 1981b). *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 & Snelgar 1981b). 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 3 cycles of vacuum for 80 minutes. 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

Table 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 x 10^2	92 x 10^2
	No. cm^{-2} in cyphellae / pseudocyphellae	0.6 x 10^6	2.8 x 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 x 10^5	4.9 x 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)

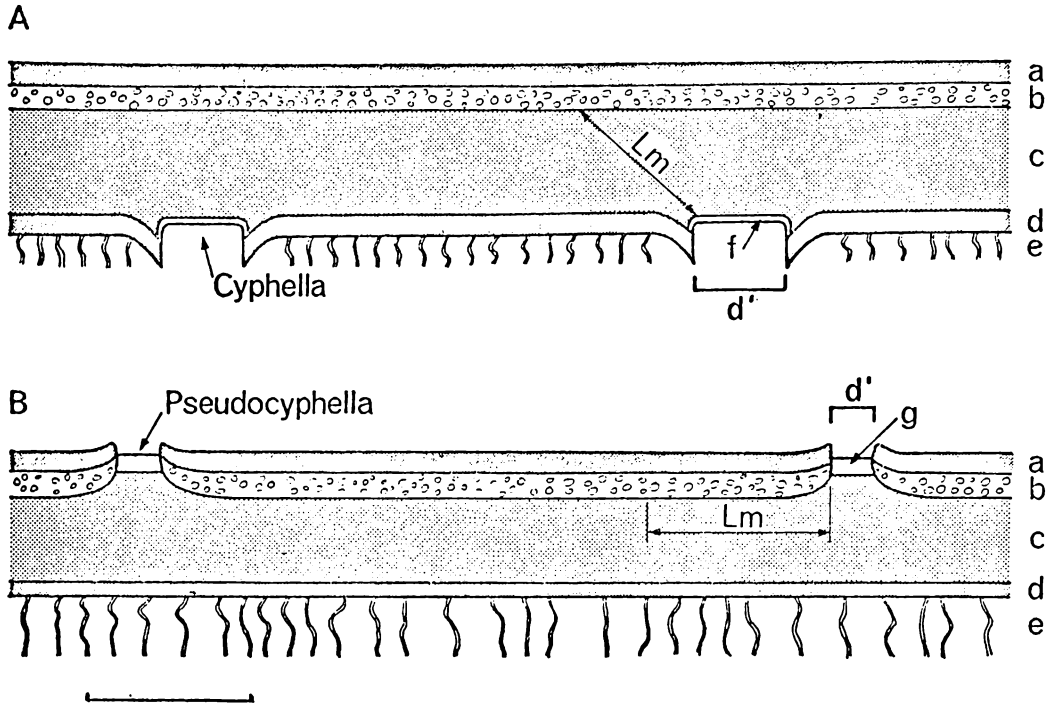
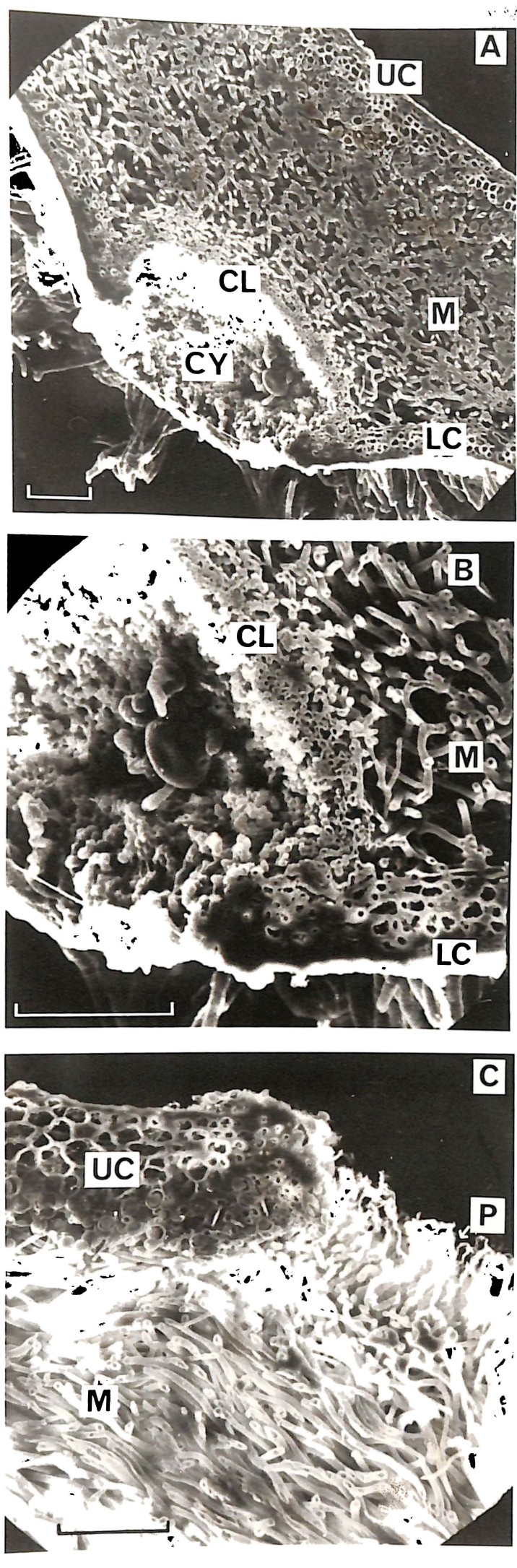


FIGURE 1. Diagram of vertical sections across the thalli. A = *Sticta latifrons;* B = *Pseudocyphellaria amphisticta*. Thallus layer thicknesses are to scale: (a) upper cortex, (b) algal layer, (c) medulla, (d) lower cortex, (e) rhizine tomentum, (f) cyphellal lining depth, (g) pseudocyphellal depth. 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.

FIGURE 2. (Opposite). 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.


A
UC
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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 (Figure 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 cyphellal pores 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). 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.

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

	Water† Content	Σr_{CO_2}†	r_{ti}†	r_a†	r_{pore}	r_{pe}	r_{end}	r_m	r_e†
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)	

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

In the preceeding 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 (Table 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 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} (Table 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* (Table 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 & Snelgar 1981b). 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 & Snelgar, 1981b).

Discussion

The values for r_{ti} obtained by calculation from thallus dimensions (summarised in Table 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, in fact pseudocyphellae are little more than an extension of the medulla (Figure 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 (Figure 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 (Green and Snelgar 1981b), 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 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 (Table 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 empty 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 1981a) 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.

CHAPTER XIV

Carbon dioxide exchange in lichens: Relationship between the diffusive resistances of carbon dioxide and water

Introduction

In higher plants, particularly angiosperm leaves, diffusive resistances have proved to be of considerable significance in the study of photosynthesis and water relations (Jarvis 1971). It is unfortunate that the study of carbon dioxide and water diffusive resistances has been given little emphasis in lichen physiology. Published estimates of CO_2 diffusion resistances have only recently appeared for lichens the first being that of Collins and Farrar (1978) who obtained a value of 138 s cm^{-1} for *Xanthoria parietina*. Studies on large foliose members of the New Zealand Stictaceae have shown that minimum carbon dioxide resistances varied from 30 to 70 s cm^{-1} and that increases may occur at both high and low water contents (Snelgar *et al.* 1981b). Diffusive resistances to water loss have rarely been calculated but very low values in the range of 0.035 to 0.40 s cm^{-1} at medium thallus saturation found by Larson (1979a) would be typical (Harris 1976, Larson and Kershaw 1976).

There have been no published comparisons of the magnitudes of the carbon dioxide and water diffusive resistances at different thallus water contents. Collins and Farrar (1978) make the point that "one consequence of a high r_c (cortex resistance) for carbon dioxide is a similarly high resistance to water loss and hence, during desiccation, the rate of loss of water from algal

cells will be reduced". This is a view that originates from studies of higher plant stomatal diffusion resistances where there is a simple linear relationship between the two resistances. Such a relation holds only where both the carbon dioxide and water are diffusing in air and would not be expected where carbon dioxide is moving in solution. The latter situation is found in the cuticular layers of leaves where the water resistance is considerably different to the carbon dioxide resistance because the water is moving by mass flow to the surface where it evaporates whilst the carbon dioxide has to diffuse slowly through the water to reach the surface (Jarvis, 1971). A similar situation might be expected to occur in the cortical layers of lichens where few gaseous pathways appear to exist (Collins and Farrar 1978, Lange 1980). In this study a comparison of the water and carbon dioxide diffusion resistances was made at various water contents in an attempt to clarify the relationship between the two.

Abbreviations

Σr_{CO_2}, total CO_2 diffusion resistance, s cm^{-1}; Σr_{H_2O}, total diffusion resistance for water loss, s cm^{-1}; r_c, part of Σr_{CO_2} attributed to cortex of lichen, s cm^{-1}; RH, relative humidity, %; ψ, water potential, bar; NP, net photosynthesis; mg CO_2 per gram dry weight per hour.

Materials and Methods

Specimens of *Sticta latifrons* Rich., *Pseudocyphellaria colensoi* (Bab. in Hook.f.) Vain, *P. billardierii* (Del.) Ras. and *P. homoeophylla* (Nyl.) Dodge were collected from the Waikareiti area of the Urewera National Park (NZMS 1 N96 619437), North Island, New Zealand. Lichens were stored air dry in the dark over silica gel for a maximum of three weeks.

CO_2 resistance determinations: these were carried out as described in Snelgar *et al.* (1981b) and involved the determination of the slope of net photosynthesis rate against CO_2 concentration at several water contents. All measurements were made at a saturating light intensity of 150 $\mu E\ m^{-2}\ s^{-1}$ and under microaerobic conditions (1% oxygen), in order to minimise photorespiratory activity.

Water diffusion resistance: this was determined with a ΔT series 2 automatic porometer, using the standard methods of measurement and calibration as described by the manufacturer. Lichen thalli were allowed to dry down under normal laboratory conditions or were equilibrated at fixed relative humidities (RH) generated by saturated salt solutions in a desiccator at 20°C. Measurements were limited to low and medium water contents since the porometer is not designed for use where surface water is present. Water potentials were obtained from standard tables (Slavík, 1974), and water contents are expressed as mg water per mg thallus dry weight.

Photosynthesis rates: NP determinations were made using an ADC series 225 infra red gas analyser in a closed loop system described in Snelgar, *et al.* (1981b). All determinations were at 350 $\mu\ell\ CO_2\ \ell^{-1}$, 16°C and were carried out at several thallus water contents starting with a saturated lichen thallus which was dried slightly between each determination. All results are expressed as mg CO_2 fixed per gram dry weight per hour (mg $CO_2\ g^{-1}\ h^{-1}$).

Results

The relationships between water potential (ψ) and water content for *S. latifrons, P. colensoi, P. billardierii* and

P. homoeophylla all show a similar pattern in which there is at first a slow decrease in ψ with decrease in thallus water content changing to a more rapid decrease below a water content of about 0.5 (Fig. 1). *P. colensoi* is distinguished from the other species by a lower water content for any particular ψ; this is clearly seen at ψ= -50 bar where it has a water content of about 60% of the other species (Fig. 1B). A similar relationship is found between Σr_{H_2O} and thallus water content for *S. latifrons* and *P. homoeophylla* (Fig. 2). Figure 2A was constructed from Σr_{H_2O} values obtained separately from the top and bottom surfaces of thalli of *S. latifrons* that were allowed to dry in air and measured at various water contents, and also from the top surface of thalli equilibrated to constant water content at several values of RH. A single line is a good fit for all data points and no detectable differences in Σr_{H_2O} between top and bottom thallus surfaces or between thalli of similar water content obtained by drying or equilibration. The porometer could be used only at low and medium water contents since at higher water contents liquid water was present. However, for this reason, at high water contents Σr_{H_2O} would be expected to be identical to, or lower than, the values at the highest water content measured. The results of Harris (1976), Larson and Kershaw (1976) and Larson (1979a) tend to substantiate this extrapolation.

Figures 3A and 3B are a summary for *S. latifrons* and *P. homoeophylla* of the relationships between net photosynthesis (NP), Σr_{CO_2} Σr_{H_2O} ψ and thallus water content. Both graphs show very similar patterns with - ψ and Σr_{H_2O} increasing steeply

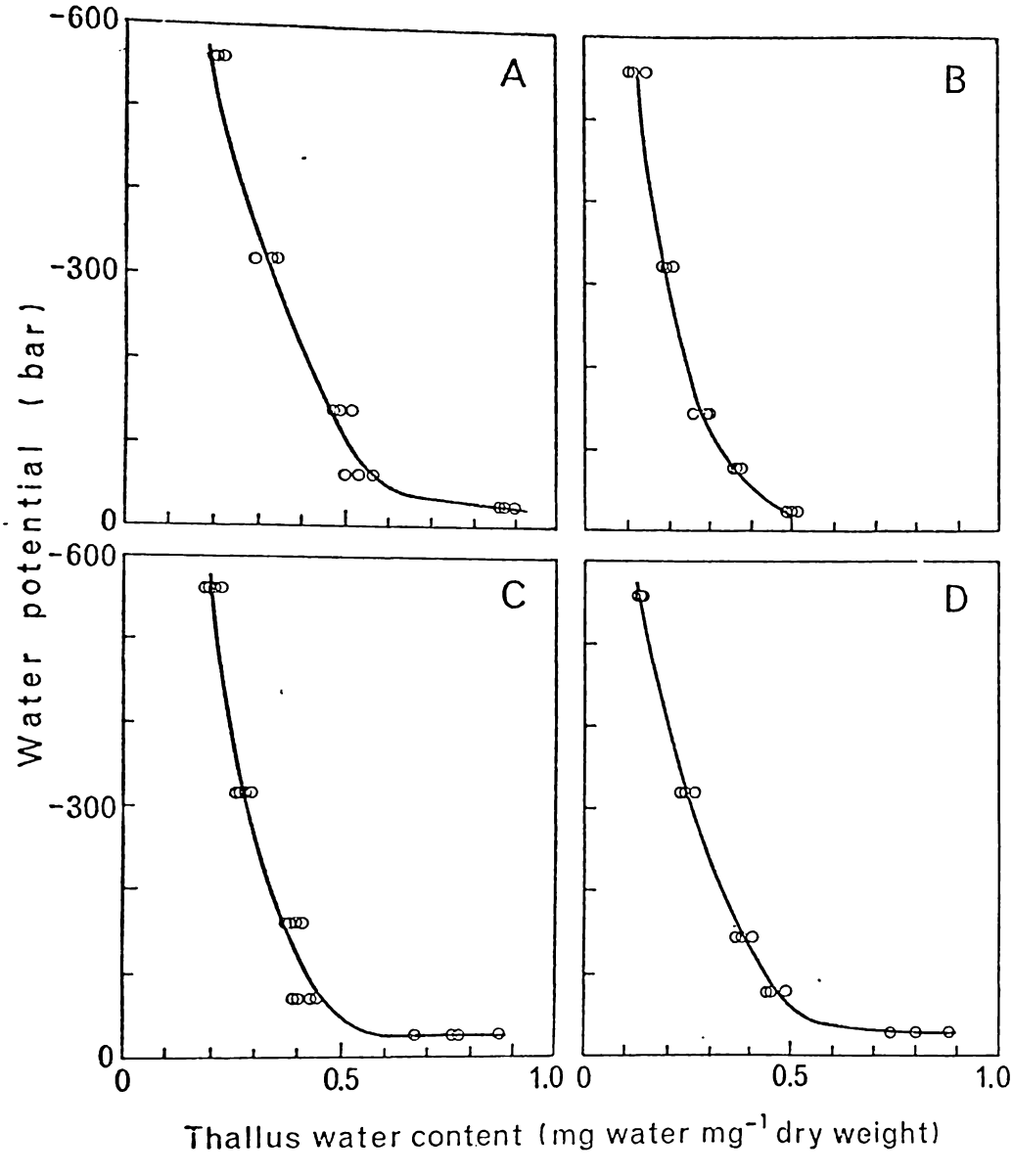

FIGURE 1. Relationship between water potential and thallus water content for: A, *Sticta latifrons;* B, *Pseudocyphellaria colensoi;* C, *P. billardierii;* and D, *P. homoeophylla*. Thalli were equilibrated to constant thallus water content at several relative humidities.

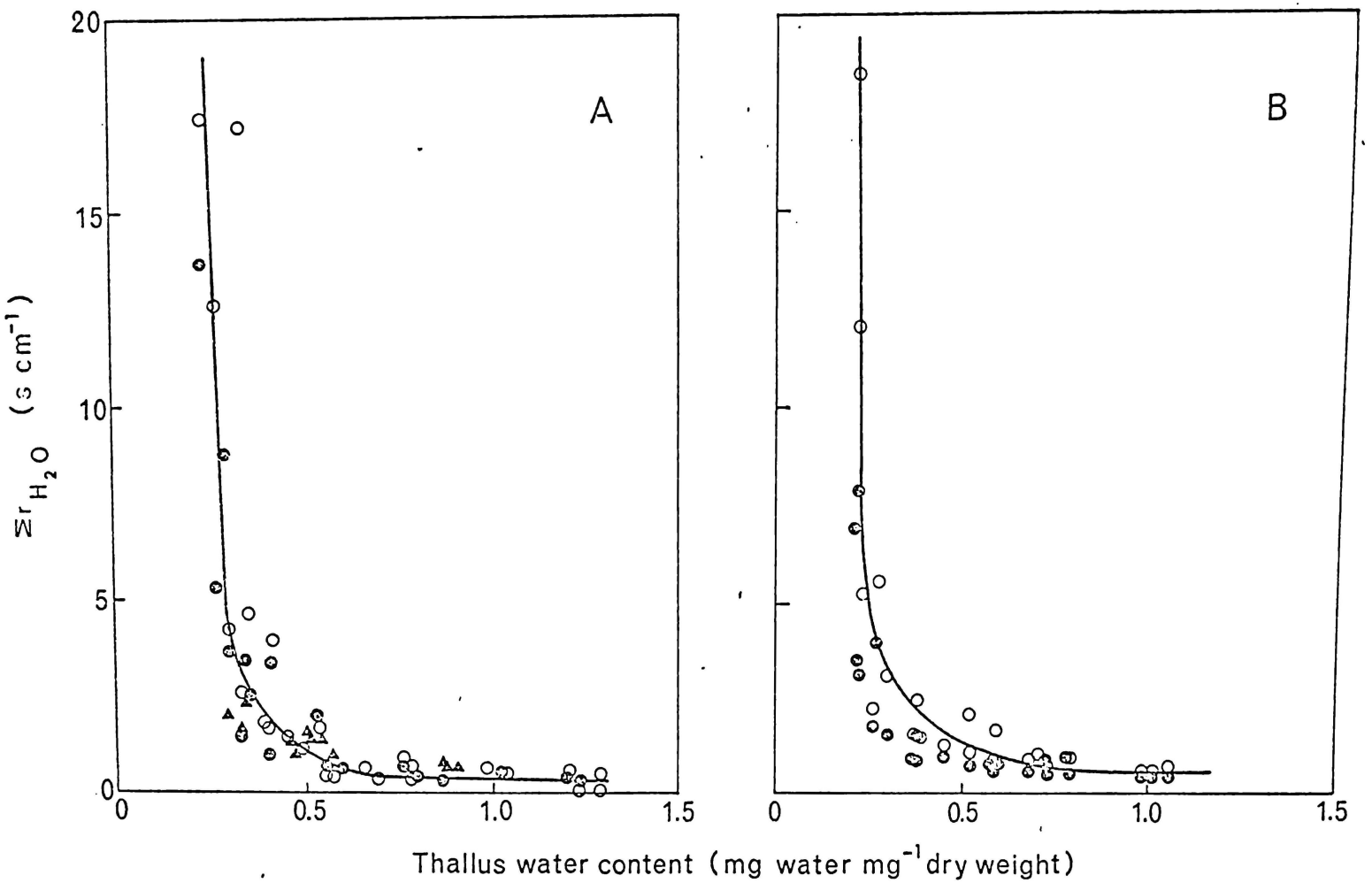

FIGURE 2. Relationship between total water diffusion resistance (Σr_{H_2O}) and thallus water content for: A, *Sticta latifrons;* and B, *Pseudocyphellaria homoeophylla*. Σr_{H_2O} was measured on the upper (⊙) and lower (o) surfaces of thalli dried in air; or on the upper surface (▲) of thalli equilibrated to constant thallus water content at a fixed RH.

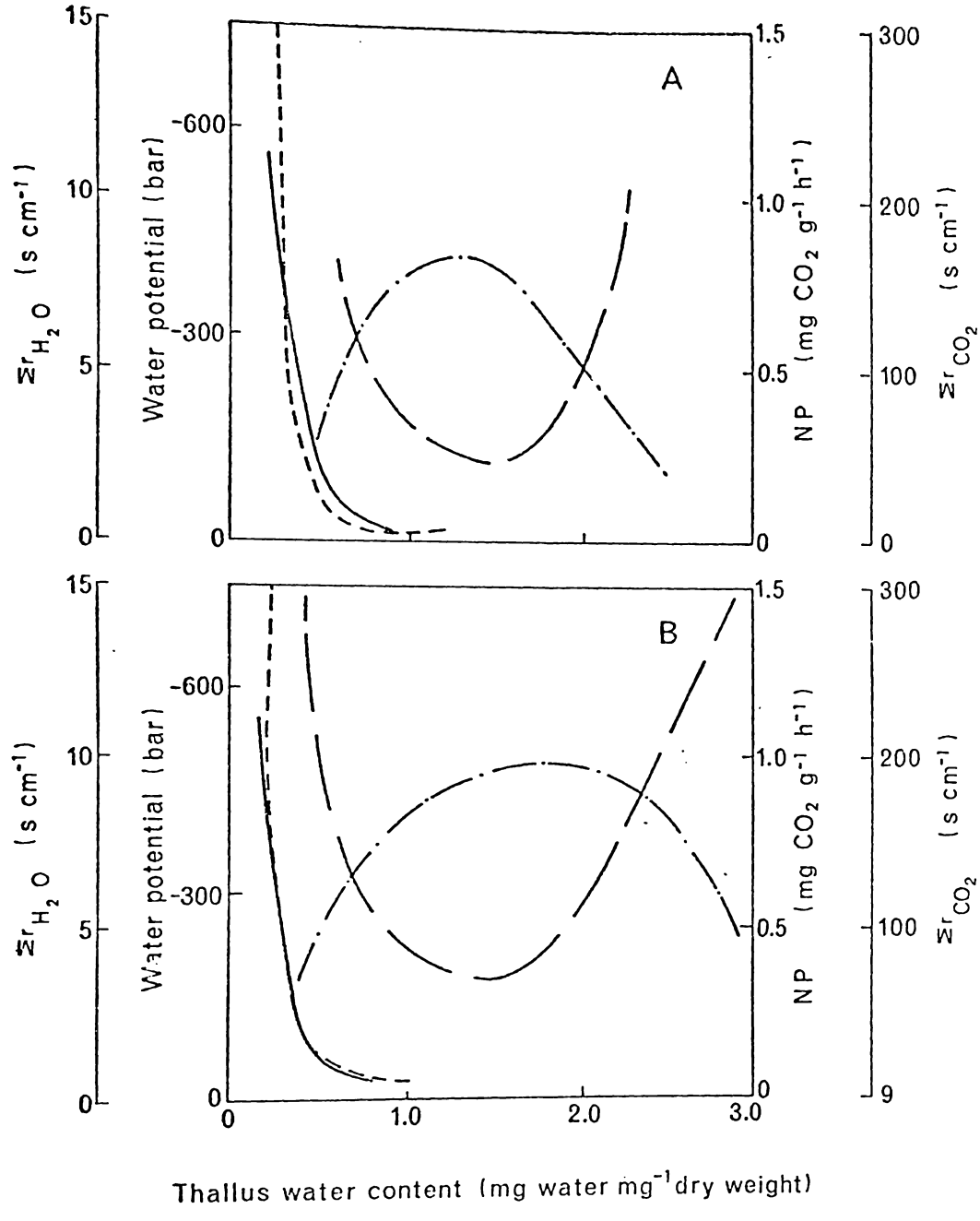

FIGURE 3: Summary of the relationship between water potential (ψ), total water diffusion resistance (Σr_{H_2O}), total CO_2 diffusion resistance (Σr_{CO_2}), net photosynthesis (NP) and thallus water content for: A, *Sticta latifrons*; B, *Pseudocyphellaria homoeophylla*. ψ: ——; Σr_{H_2O}:----; Σr_{CO_2}:— —; NP: ·-·- .

at low water contents, Σr_{CO_2} increasing at both low and high water contents and NP almost an inverse of Σr_{CO_2}. When comparing Σr_{H_2O} and Σr_{CO_2} it is important to note that they differ markedly at high water contents where Σr_{H_2O} is low but Σr_{CO_2} rises rapidly, and that Σr_{CO_2} has considerably higher values than Σr_{H_2O} at any particular water content. At water contents of 1.0 Σr_{CO_2} is 180 times greater than Σr_{H_2O}. At no water content is Σr_{CO_2} less than a factor of ten greater than Σr_{H_2O}.

Discussion

The results summarised in Figure 3 indicate that the resistance to water loss is high only at low water contents and then relates most closely to the water potential of the thallus. This suggests that at low water contents the resistance to water loss is determined by the physical properties of the thallus, in particular the thallus water potential. This result is in accord with those of Harris (1976), and Snelgar and Green (1981b) which indicate that there are no modifications to limit surface water loss in lichens. A similar direct relationship between thallus water potential and the resistance to water loss has been suggested for intertidal algae (Jones and Norton 1979).

The data on Σr_{CO_2} shows a more complex situation with high resistances occurring at both low and high water contents (Figs 3A and 3B), a pattern that has been reported previously (Snelgar, *et al.* 1981b). The increased resistances at low water contents may be attributed to a biochemical inhibition resulting from the low water potentials as indicated by the results of Cowan *et al.* (1979a) and Green and Snelgar (1981b).

High Σr_{CO_2} at high water contents are probably caused by a partial decrease in volume of the gaseous CO_2 diffusion pathways by water infiltration. (Green and Snelgar, 1981b; Snelgar *et al.* 1981b). Carbon dioxide diffusion in water is so much slower than diffusion in air that even a short water diffusion path results in a marked resistance increase. The results demonstrate that the relationship between Σr_{CO_2} and Σr_{H_2O} in lichens is complex and cannot be interpreted as a simple linear relationship as found for the stomata of higher plants. Even at low water contents where the responses of both parameters are similar Σr_{CO_2} values are up to 160 fold higher than Σr_{H_2O} values. Further evidence of the lack of correspondence is found at higher water contents where Σr_{CO_2} increases drastically when Σr_{H_2O} must be low. These marked differences between Σr_{CO_2} and Σr_{H_2O} strongly suggest that CO_2 and water follow different diffusion pathways in the lichen. Circumstantial evidence obtained by using a split cuvette (Green *et al.* 1981) suggests that CO_2 diffuses through the cyphellae or pseudocyphellae of the lower surface of these lichens with the major diffusion resistance lying in the gas exchange structure itself (Snelgar *et al.* 1981a).

The porometer evidence presented here indicates that water loss may occur at identical rates by evaporation from both upper and lower surfaces with the water moving by mass flow through the cortex. The situation would be analagous to a higher plant leaf that exchanged carbon dioxide through the stomatal pores of the lower surface but lost water from its entire surface. Normally such a leaf would have a 'waterproof' cuticle that prevented surface water loss and confined it also to the stomatal

pores. Such a situation is not feasible for lichens which do not have a continuous internal supply of water from a vascular system. Lichens must also be capable of being moistened by rain or mist and may need to utilise external water reserves (Snelgar and Green 1981b. Such a low resistance to water loss also means that there is little advantage, in terms of water loss rate, between internal and external water storage.

The stomata of higher plant leaves perform both carbon dioxide exchange and water conservation functions with a consequent close relation between carbon dioxide and water diffusion resistances. The evidence presented indicates that this could not be so for the lichens studied. At all thallus water contents Σr_{CO_2} was many times greater than Σr_{H_2O} suggesting that water and CO do not follow the same diffusive pathway. Further, changes in Σr_{CO_2} were not always reflected by changes in Σr_{H_2O} so that increased resistance to CO_2 exchange did not mean a similar increase in desiccation resistance. This is an unfortunate situation since the simple linear relationship between the two resistances found for higher plants allows calculation of leaf internal CO_2 concentrations from water loss rates. This calculation is therefore not possible for lichens and no simple method appears to exist for obtaining thallus internal CO_2 concentration.

CHAPTER XV - DISCUSSION

WATER STORAGE IN LICHENS

(a) *Introduction.* The productivity of lichens is considerably affected by the water relations of the thallus (Harris, 1976). In view of this it is surprising that the subject is so little studied. Early workers were quick to realise that uptake and loss of water from lichens is a passive process, and therefore not linked to the physiology of the organism. Only recently have researchers studied the effects of lichen morphology on water loss. Following the results of Larson and Kershaw (1976), Larson (1979a) and those presented in Chapter IV it is now clear that some lichens possess the following adaptations which help to maintain the thallus in a moist condition.

(1) Thalli may have a reduced surface area to volume ratio, thereby maximising the volume of water within the thallus and reducing the surface area from which it can evaporate.

(2) Many lichens have a well developed tomentum or rhizine layer which can function as a water storage area, (Chapter IV). The low resistance of lichens to water loss (Chapter XIV) means that there is no disadvantage in storing water externally.

(3) Some lichens are closely appressed to the substrate, thus aiding the retention of water in the tomentum and reducing the surface area exposed to evaporative water loss.

(b) *Water storage location.* In a recent review of the physiological ecology of bryophytes Dilks and Proctor (1979) noted that these plants hold water within the cell, in the cell wall, and in external capillary spaces. Some of this capillary water is

held external to the bryophyte thallus. A reappraisal of structure - function relationships (Proctor, 1979) led to the conclusion that most (though not all) bryophytes rely on a rapid conduction (internal or external) of water, rather than on water storage, as a means of alleviating desiccation stress. The relative ineffectiveness of water storage in a leafy liverwort was emphasised by the estimated water storage capacity of 1700 µg water cm^{-2} of *Lejeunea ulicina*. At an evaporation rate of 1 µg cm^{-2} s^{-1} this entire water supply would be depleted in about 30 minutes.

It seems likely that water within the lichen thallus is similarly distributed to that within bryophytes. However lichens appear to have adopted a strategy of water storage, rather than water conduction, as a means of extending the periods during which thalli remain moist (and therefore metabolically active). The magnitude of lichen water storage systems is illustrated by the data of Table 1. The amount of water held within the thallus (internal water) is relatively similar in all of the species studied, except for the shade and mesic populations of *P. dissimilis*. These had notably low water contents. In contrast the external (blottable) water content was more variable with values ranging from 23 mg cm^{-2} for *P. homoeophylla* to 5 mg cm^{-2} for *P. billardierii*.

(c) *Water storage effectiveness.* It is possible to calculate the length of time for which a lichen might maintain a positive rate of NP from the minimum water content at which NP is positive, the initial water content of the thallus, and the evaporative demand of the environment. Field measurements of water loss from Piche evaporimeters (Chapter IV) indicate that evaporation rates in the natural habitat are low

TABLE 1. Water holding capacity and drying times for some lichens. Results were calculated using data from Chapters IV, V and XI. Drying times for species marked † were calculated assuming water was lost from both sides of the thallus. For all other species evaporation was assumed to occur from only the upper surface since the lower cortex is generally in close contact with the substrate.

Species	Water content ($mg\ cm^{-2}$)						Time to reach critical water content (h)			
	Internal (Thallus blotted)	External	Total	Critical water content (NP=0)	Internal - critical	Total - critical	Evaporation rate ($\mu g\ cm^{-2}\ s^{-1}$) 0.14		0.88	
					A	B	A	B	A	B
Peltigera dolichorhiza	22	13	35	12	10	23	20	46	3.2	7.3
Pseudocyphellaria amphisticta	22	19	51	5	17	46	34	91	5.4	15
P. homoeophylla	25	23	48	4	21	44	42	87	6.6	14
Sticta latifrons †	25	13	38	5	20	33	19	31	3.2	5.2
Pseudocyphellaria billardierii †	22	5	27	2	20	25	19	23	3.2	3.9
P. colensoi †	18	13	31	3	15	28	14	26	2.4	4.4
P. dissimilis (shade) †	11	8	19	5	6	13	5.6	12	0.9	2.1
P. dissimilis (mesic)	12	13	25	6	6	19	12	38	1.9	6.0
P. dissimilis (sun)	17	16	33	7	10	25	20	50	3.2	7.9

(0.14 - 0.88 μg cm^{-2} s^{-1}). Other data, obtained in the Lake Waikareiti region during winter, confirm these observations (0.42 - 0.63 μg cm^{-2} s^{-1}). Calculations (Table 1) show that at the lower evaporation rate the internal water supply of all seven species is sufficient to maintain positive photosynthesis for at least 5.6 hours. This period is reduced to as little as 0.9 hours under the higher evaporative stress. However in *P. dissimilis* the interaction between morphology and environment results in the shade population maintaining a net CO_2 uptake for up to 12 hours in the natural habitat (i.e. a low evaporative stress environment) while net CO_2 uptake in the sun population remains positive for up to 7.9 hours in the sun habitat (high evaporative stress). It is clear that the morphological adaptations previously described (Chapter IV) greatly enhance the ability of *P. dissimilis* to exist in the more xeric habitat. The relationship between the predicted period of CO_2 uptake and ecology is less clear for other lichen species. In particular those lichens which have an ascending growth habit and/or which grow in the sub-canopy (e.g. *P. billardierii, P. colensoi, S. latifrons*) seem to have inadequate water reserves. The pools of water observed on the upper surfaces of scrobiculate/faveolate species (see following paragraph), which were not taken into account in the above calculations, may partially explain this discrepancy. Nevertheless all of the species listed in Table 1 are capable of remaining moist for long periods. Considering the rainfall of the Urewera National Park (about 100" per annum; Grant, 1963) and the frequency of dew and mist, it is likely that lichens are often sufficiently moist to maintain photosynthesis.

(d) *Water storage - CO_2 exchange; a compromise.* A further point of comparison between bryophytes and lichens is the spatial separation of water supply and gas exchange sites. Proctor (1979) observed that the concave inner surfaces of *Myurium hochstetteri* leaves each hold a drop of water following mist spraying while surface tension keeps the outer (convex) surface free of surplus water. This arrangement thus allows free exchange of CO_2 through the low resistance pathway of the outer leaf surface even when external water is held in the leaf axil. A somewhat analogous situation is found in *P. amphisticta* as this species stores water externally on the tomentose undersurface, and exchanges CO_2 through the pseudocyphellate upper surface. However in most *Sticta* and *Pseudocyphellaria* species the 'aeration pores' are situated in the lower, tomentose, surface. Although in this instance water storage and gas exchange facilities are adjacent there is some circumstantial evidence that interference between the two is minimal. Observations of the lower surface of *S. latifrons* show the cyphellae to be free of water. The measured CO_2 resistance values confirm this (Chapter XI). Further, the raised rim of the cyphellae (Figure 1, Chapter XIII) and hydrophobic substances on the cyphellal hyphae (see following paragraph) may help to exclude water. Lastly, because the tomentum of most species is protected from direct rain water uptake must normally be from stem flow, rain splash, fog, or mist. Such indirect uptake would probably limit the degree of inundation of the lower cortex.

Several species of *Pseudocyphellaria* (e.g. *P. hirsutula, P. pubescens, P. hamata, P. greta*) have a tomentose upper surface. Here the arrangement found in *P. amphisticta* is

reversed. Water could be held on the upper surface and CO_2 taken up through the lower, pseudocyphellate, cortex. A variation on this theme is the faveolate upper surface of *P. billardierii* which could also function as a water storage facility. Laboratory experiments have shown that up to 49 mg water cm^{-2} can be held in this manner.

(e) *Distribution of water within the thallus.* The distribution of water within the lichen thallus is an aspect of lichen physiology that has received little attention. The few results so far available are contradictory. Smith (1962) maintains that the medulla of *Peltigera polydactyla* contains 25% more water (on a dry weight basis) than the algal layer. Others, (Tobler, 1925; Showman and Rudolf, 1971) using different species, have found that the algal layer and cortex hold most of the water. Since the physiological responses of a lichen to alterations in thallus water content are likely to be related to the water availability at metabolically active sites it is unfortunate that these results are so inconclusive.

In this regard it may be significant that scanning electron micrographs of *P. colensoi* (T.G.A. Green, unpublished data) have revealed the presence of a band of hyphae, coated with what is probably a triterpene, directly beneath the algal layer. This coating may render the hyphae hydrophobic (A. L. Wilkins, pers. com.). Scanning electron micrographs of acetone washed specimens (i.e. with the triterpene removed) show that this substance coats (or fills) what appears to be an extensive lacunal system. There are also indications of some coating on bands of hyphae which extend from the

pseudocyphellae to the lacunal system. It is tempting to postulate that these bands are hydrophobic and are a means of keeping CO_2 exchange pathways between the algal layer and the external atmosphere free of water. Such a suggestion is not new. Gobel (1926; reported in Smith, 1962) has previously noted the existence of heavy encrustations of unwettable substances beneath the algal layer and in the cyphellae and pseudocyphellae of some lichens.

Development of a method which is capable of rapidly, and non destructively, measuring the location and quantity of water within the lichen thallus would be a major aid in interpreting lichen CO_2 exchange - water content responses.

(f) *Modes of expression and consequences.* As noted elsewhere, (Farrar, 1973; Kershaw, 1972), the water content of lichens is commonly expressed in several different ways. The method favoured by some workers (Harris, 1971; Kershaw, 1972) has been to express the degree of hydration as a percentage of thallus saturation. The use of this technique is unfortunate as Blum (1973) has presented data which shows that the amount of water held by a 'saturated' thallus often increases following long periods of immersion in water. Also, studies carried out using several lichen species (Chapter XI) have shown that in lichens a considerable amount of water is held external to the thallus. This water can be removed by blotting but the final 'blotted' weight depends on the efficiency, or otherwise, of the blotting technique. Thus it can be seen that the 'saturated' water content is in fact a highly subjective measure. Although it is quite possible that individual operators could produce uniform results, the correlation of results with

those of other workers would be a dubious proposition. Lastly, the absolute quantity of water held by a lichen has been found to vary, even between populations of the same species (Chapter IV). If such variations occurred in the specimens used by Harris (1971) then the results could be biased and some correction would have to be made before different populations could be compared.

The most widely used method of expressing thallus water content has been mg water per unit dry weight of thallus. Although there have been variations in the means of determining thallus dry weight, some authors using air dry weight while others prefer oven dry weight, the latter now appears to be the more widely accepted method. The advantages of this technique, in yielding absolute figures which are suitable for inter and intraspecific comparisons are obvious. However this method too produces biased data in that the weight of the thallus varies with thallus thickness. This may or may not be paralleled by an increase in the volume of water held. In view of this problem perhaps the most meaningful, though less convenient, way of presenting water contents is as weight per unit area (mg cm^{-2}). Since the number of algae (and hence NP; Harris, 1971) and the evaporation rate of water from a thallus are both closely related to thallus area, rather than thallus weight, the former is likely to be of greater physiological significance. Conversion of results to this form has already been shown to eliminate many anomalies. (See Table 1). When Σr - water content curves are expressed in this manner (Figure 1) it is apparent that at low water contents the responses of the three species are almost identical. Thus the 'species specific' water content

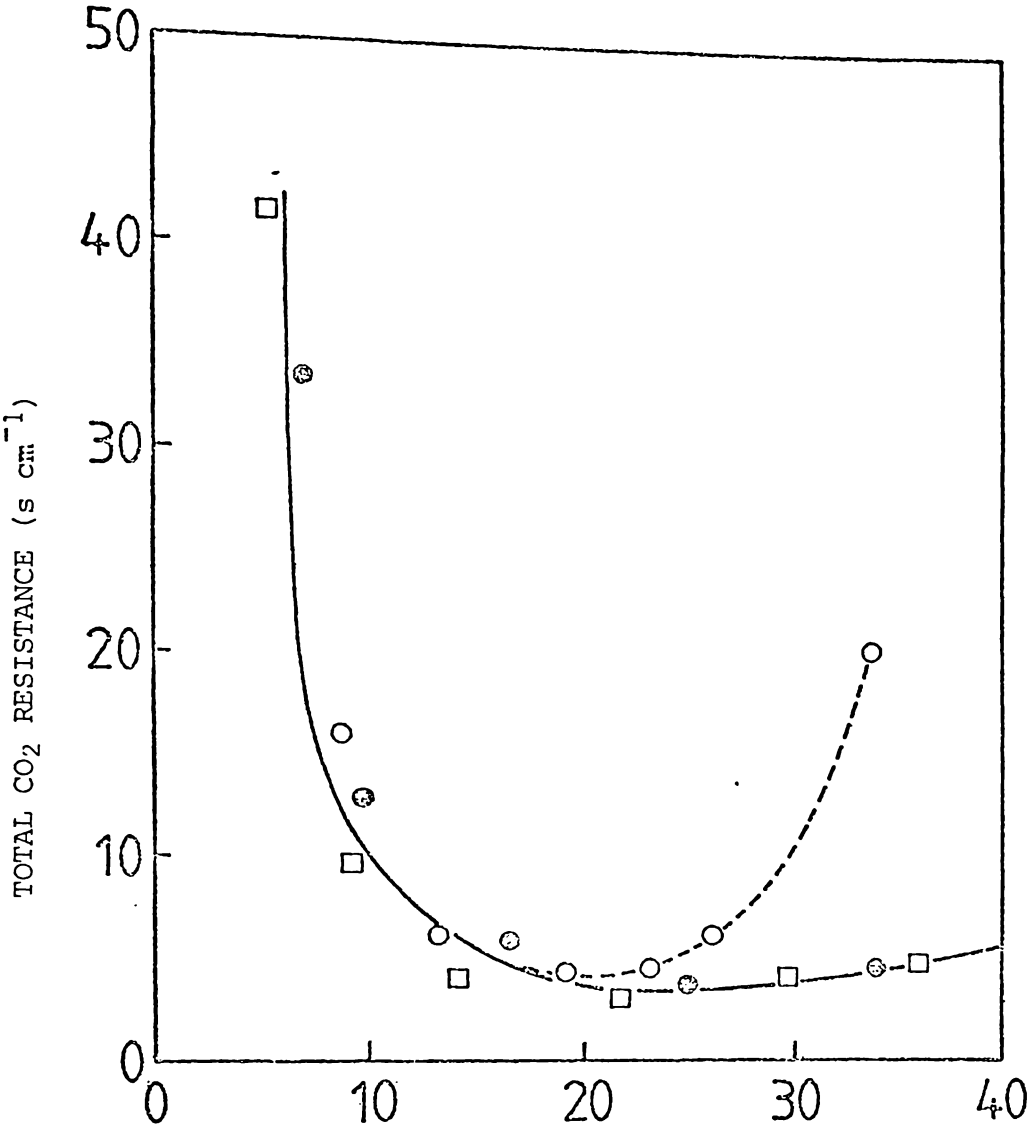

FIGURE 1. Total CO_2 resistance versus thallus water content for *Sticta latifrons* (o), *Pseudocyphellaria colensoi* (⊙), and *Peltigera dolichorhiza* (□).

at which Σr rises sharply (Chapter XI) may reflect differences in thallus density and thallus water holding capacity, rather than differences in physiology as inferred in Chapter XI. It is also likely that many of the variations in water potential (Chapter XIV) and photosynthesis/respiration response (Chapter V), with thallus water content can be similarly accounted for.

PHOTORESPIRATION

As outlined in the introduction the measurement of photorespiration is plagued by experimental difficulties. None of the techniques in current use are entirely satisfactory. Initial experiments based on the Warburg effect (Chapter IX) showed high photorespiratory rates in *S. latifrons* and *P. homoeophylla,* and a much lesser rate in *P. billardierii*. In subsequent experiments the photorespiratory response was subdivided into two components consisting of:

(i) The oxygen sensitivity of the carboxylation system. This decreases NP by a constant percentage and is depicted as α in Figure 2, Chapter X.

(ii) The rate of CO_2 evolution in the light at Zero CO_2. This is presumed to be unaffected by CO_2 concentration and is shown as 'd' in Figure 2, Chapter X.

Some workers (e.g. Brown, 1980) define only (ii) as photorespiration *per se*. Component (i) is then classified as a direct effect of O_2 on NP.

The results of Chapter X show that of the lichen species studied all but *P. billardierii* had a similar, large, α effect (16 - 25%) which was comparable to those measured in C3 plants (Kennedy *et al,* 1980). The exceptionally low value of α found in *P. billardierii* (7%) is most interesting as it seems to imply that this species possesses either a photosynthetic pathway which is different from

that found in C3 plants or, some means of excluding O_2 and/or concentrating CO_2 within the algal layer. Low α values in higher plants are commonly the result of high internal CO_2 concentrations caused by the activity of phosphoenol pyruvate carboxylase. In lichens it is more likely that some other form of CO_2 pump (e.g. active bicarbonate uptake coupled with carbonic anhydrase activity) is involved. In view of the existing interest in angiosperm C3 - C4 intermediates (e.g. Raghavendra, 1980) the biochemistry of photosynthesis in *P. billardierii* warrants further investigation.

The 'd' values (rate of CO_2 evolution in the light) of lichens show a large inter and intraspecific variability with values ranging from less than 0 to 10 $\mu g\ CO_2\ m^{-2}\ s^{-1}$. In the past very low values of d have been thought to be associated with the C4 syndrome. However recent work by Brown (1980) has shown that some *Panicum* species which have anatomical characteristics intermediate between those of C3 and C4 plants can also have low d values. It is interesting that intraspecific variability of d, as found in *S. latifrons*, has also been reported in *Panicum* species (Brown, 1980). At present the cause of this variability is not known, but in the lichens studied it may be related to some aspect of thallus structure (Chapter X).

Perhaps the most striking feature of photorespiration in the *Stictaceae* is the range of responses observed, and the manner in which these closely mimic the results found in some groups of angiosperms. Responses which approximate those of C3, C3 - C4 intermediates and C4 plants have all been demonstrated. The most obvious conclusion to be drawn from these observations is that it is not essential for plants to possess a C4 metabolism and Krantz anatomy in order to achieve low photorespiratory rates.

Photorespiration in lichens, is a complex phenomenon. The parallels observed between lichens and angiosperms suggest that an

understanding of lichen photorespiration could aid interpretation of angiosperm results.

COMPENSATION POINTS

Although many lichen species have high CO_2 compensation points (τ > 50 µl CO_2 l^{-1}) as expected in C3 plants, several species exhibit low values of τ (about 10 µl CO_2 l^{-1}) which are more typical of C4 plants. However determinations of the oxygen sensitivity of the carboxylation system in lichens suggest that they do not operate a C4 metabolism.

The CO_2 compensation point found in free living algae is currently a topic of some interest. Black (1973) asserted that the ability of algae to remove virtually all of the CO_2 from a sealed chamber is well known. Recent results, mainly obtained using algae suspended in a thin layer, (e.g. Lloyde *et al* 1977) have confirmed this view. However other workers have found the τ of algae to be variable (Shelp and Canvin, 1980) and Bidwell (1977) has shown τ to be affected by the CO_2 concentration during pretreatment. Birmingham and Colman (1979) have suggested that active uptake of bicarbonate by algae could produce C4 physiological characteristics even though the primary carboxylation system is C3. Disparities in the efficiency of bicarbonate transport could account for inter-specific variations of τ.

It is possible that the low τ in lichens could result from bicarbonate uptake by lichen algae. However such an explanation does not explain the variation in τ found in different specimens of *S. latifrons*. Since all thalli were subjected to a similar experimental procedure it is also unlikely that short term pretreatment effects caused this variation. Clearly other factors are involved. Differences in photorespiratory rate, CO_2 refixation ability, dark

CO_2 fixation, CO_2 resistance, and fungal respiration rate could all affect τ. Regardless of the mechanisms involved it is remarkable that plants with a limited photosynthetic rate, and a large basal respiration load, can attain low τ. As a consequence of these results the view that low τ values are linked to a high photosynthetic efficiency must be reconsidered. Certainly the τ reported in this work emphasise the sophistry of interpreting lower plant responses in higher plant terms.

RECYCLING OF CARBON DIOXIDE

(a) *At low CO_2 and O_2 levels.* Comparison of rates of CO_2 evolution in the light and in the dark at < 1% O_2 and Zero CO_2 (Chapter IX) led to the suggestion that about 70% of respired CO_2 is refixed, the remaining 30% being released to the external atmosphere. Dark respiration measurements made with a split chamber (Chapter VI) have shown that 16 - 19% of the CO_2 evolved is released from the upper, non cyphellate, cortex. In view of the high cortical resistances of these lichens (Chapter XI) this could be construed as measure of upper cortex respiration (See Figure 2). Assuming that respiration in the lower cortex proceeds at a similar rate, and diffuses along similar pathways, the total cortex respiration could be equivalent to the non-refixed fraction of lichen respiration, the implication being that all CO_2 evolved into the internal atmosphere of the lichen is refixed.

(b) *At ambient CO_2 and O_2 levels.* Under ambient conditions refixation could be influenced by the following factors:

(i) The high ambient CO_2 levels would favour inward diffusion of respired CO_2.

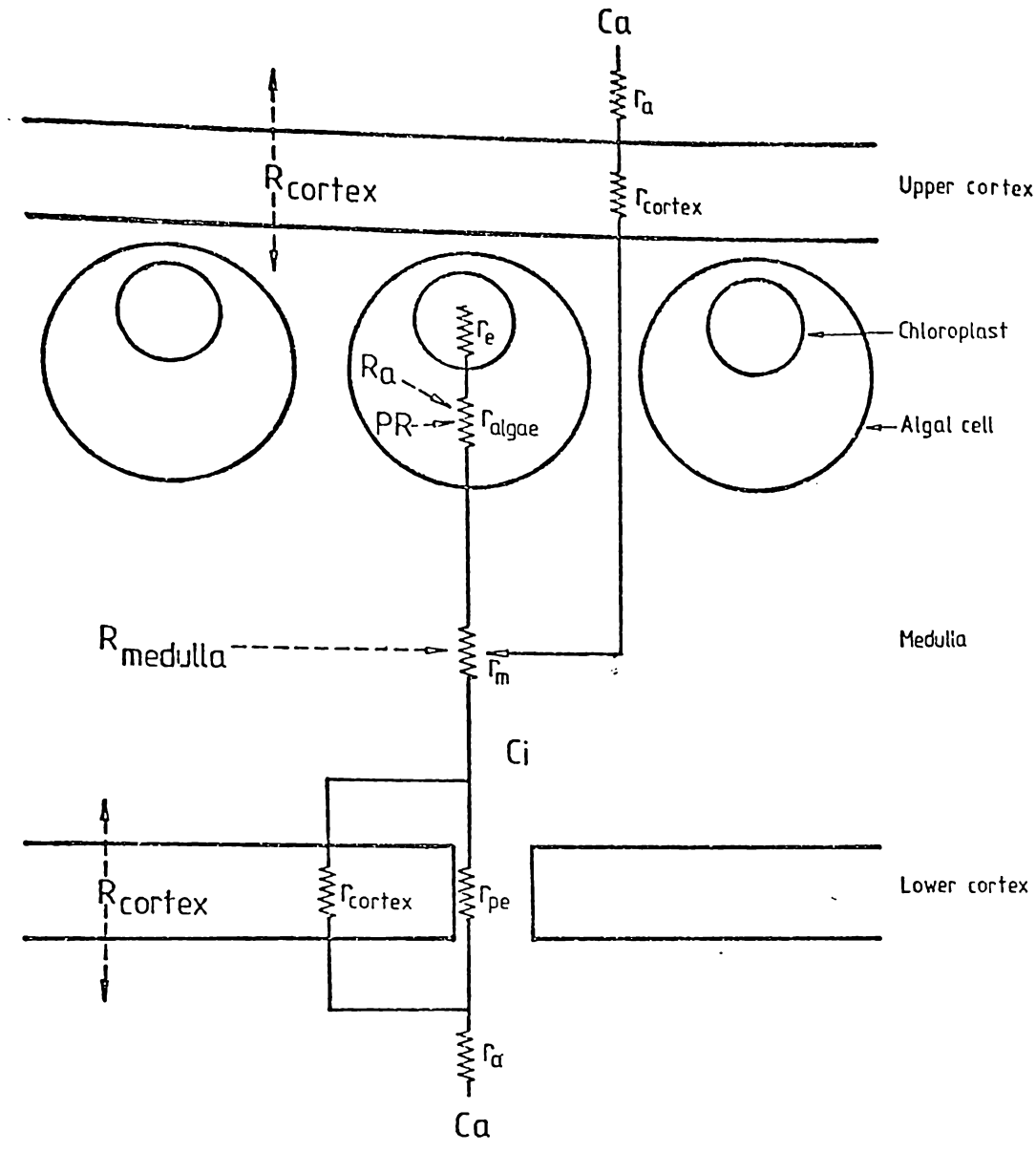

FIGURE 2. Diagrammatic summary of the CO_2 diffusion resistances (r) of a lichen with a cyphellate (or pseudocyphellate) lower cortex. Sites of CO_2 evolution (R) are also shown. (r_a), boundary layer resistance; (r_{pe}), resistance of cyphellae (or pseudocyphellae); (r_{cortex}), cortex resistance; (r_m), medulla resistance; (r_{algae}), resistance of pathway through algal cell; (r_e), carboxylation resistance; (R_{cortex}), cortex dark respiration; ($R_{medulla}$), medulla dark respiration, (R_a), algal dark respiration; (PR), algal photorespiration; (C_a), external CO_2 concentration; (C_i), internal CO_2 concentration.

(ii) The rate of CO_2 evolution would be much greater due to the combined effects of dark respiration and algal photorespiration. The rate of photorespiratory CO_2 evolution for *S. latifrons* (at Zero CO_2, Chapter X) can be as high as 5 μg CO_2 m^{-2} s^{-1}. If this represents only the 30% fraction which is not refixed then actual photorespiratory CO_2 evolution could be as high as 17 μg CO_2 m^{-2} s^{-1}. (cf NP of 60 μg CO_2 m^{-2} s^{-1}, Chapter VI).

(iii) The lichen carboxylation system may be at, or near, CO_2 saturation (Chapter XII).

The large CO_2 gradient and the high rate of NP at ambient CO_2 levels would probably result in rates of CO_2 refixation at least as high as those reported in Chapter IX, (e.g. Split chamber experiments in the light at 350 μl CO_2 l^{-2} show no loss of CO_2 from the upper cortex, Chapter VI). However if photosynthesis is limited by the carboxylation capacity of the algal cells then the release and refixation of photorespiratory CO_2 would still affect NP. Every molecule of CO_2 refixed would exclude a molecule of external CO_2 which could otherwise have been fixed, the net result would still be a depression of NP. Nevertheless at very high thallus water contents, when NP is limited by CO_2 transport resistances, refixation of CO_2 may not be counter productive since under these conditions there would be an excess of carboxylation capacity. This may explain the lack of photorespiration noted at high water contents in Chapter IX.

CARBON DIOXIDE RESISTANCES

(a) *In the Stictaceae.* In moving from the external atmosphere to the chloroplast CO_2 is subject to five principal diffusion

resistances. These are summarised in Figure 2.

The first is the boundary layer resistance (r_a) which, in the present work, had a value of 9 s cm^{-1}. This value is a product of the low gas flow rates used and the results of other workers (e.g. Nobel, 1974) indicate that under field conditions the boundary layer resistance is probably much lower (0.1 - 1.0 s cm^{-1}).

The major site of CO_2 entry to the thallus appears to be the cyphellae (or pseudocyphellae) and the calculated cyphellal resistance of *S. latifrons* is 14.0 - 17.3 s cm^{-1}. The estimated resistances for water-filled upper and lower cortices are 390 and 340 s cm^{-1} respectively. As they are arranged in parallel the combined resistance is 180 s cm^{-1}. Providing CO_2 transport into the lichen is a passive process and occurs only by gaseous diffusion then it is likely that uptake through the cortices is a minor pathway.

The third resistance is located in the fungal medulla (r_m). Although this is a long pathway the very open nature of the medulla results in the resistance being low in both of the species studies (2.2 s cm^{-1} in *S. latifrons*). Experiments with *S. latifrons* show that even at high water contents a significant volume of the pore system within the thallus remains free of water. It is therefore concluded that, at least in *S. latifrons*, the depression of photosynthesis at high water contents is unlikely to be due to the infilling of the medulla with water. From theoretical considerations it seems more likely that this depression is a result of pore blockage in the cyphellae (or pseudocyphellae). The maintainance of an aerated medullary pore system would clearly be of ecological advantage to lichens which grow in wet environments.

From the medulla CO_2 diffuses through the algal cell wall and across the cytoplasm to the chloroplast. The resistance of this process has not been calculated in the present work. However since the algal cells are of small diameter (4 - 8 μm in *S. latifrons*) the diffusion pathway should be short, and the resistance low. Collins and Farrar (1978) report that the algae of *Xanthoria parietina* have a diameter of 7 μm and the mean distance from the algal cell wall to the chloroplast is 0.53 μm. The CO_2 resistance was estimated to be 3 s cm^{-1}. If the ratio of algal cell wall area to lichen thallus area (4.6:1) is taken into account this value is further reduced (0.7 s cm^{-1}). In the absence of experimental data it seems reasonable to assume that the algal resistance of *S. latifrons* is of this magnitude.

The fifth resistance which may affect the rate of CO_2 diffusion is the rate of carboxylation at the chloroplast. When the CO_2 supply is adequate and the light intensity saturating then the rate of photosynthesis is governed by the CO_2 fixation process (Jarvis, 1971). Although this is not a CO_2 resistance *per se*, for comparative purposes it can be calculated as such and is known as the carboxylation or excitation resistance (r_e). In *S. latifrons* (and *P. amphisticta*) this resistance increases at suboptimal and supra-optimal thallus water contents. At optimal thallus water content (for photosynthesis) the carboxylation resistance of *S. latifrons* is 3.3 c cm^{-1}.

To summarise, estimates derived from experimental measurements and morphological calculations indicate that the sum of the CO_2 resistances within *S. latifrons* can be accounted for by the resistances of the diffusion pathway shown in

Figure 2. It should be noted that, apart from diffusion through the algal cell, all of the pathway appears to be via an air-filled pore system. Although transport resistances (particularly those of the cyphellae) form by far the largest portion of the resistances depicted in Figure 2 estimates of photosynthesis limitations at 330 µl CO_2 l^{-1} (Chapter XII) show that for the most part carboxylation resistances limit photosynthesis.

In view of the above results cyphellae (and pseudocyphellae) can be regarded as pores which function as a buffer between the humid environment of the algal layer and the external atmosphere. The resistance of this buffer appears to be adjusted to an optimal value whereby the loss of water vapour is minimised but the rate of photosynthesis is not affected.

(b) *Application to other species.* Since cyphellae or pseudocyphellae are common to all members of the *Sticta* and *Pseudocyphellaria* genera the preceding discussion is probably applicable to all of these lichens. The low minimum total resistance observed in *Peltigera dolichorhiza* (Chapter XI) can be attributed to the lack of a lower cortex in this species. (In this instance excessive dehydration of the algal layer may be alleviated by the moist conditions in which this species usually grows). Pores which may facilitate gas exchange have also been noted in several other lichen genera. Hale (1974) reports that they are conspiciously developed in the genera *Cetraria*, *Cetrelia* and *Parmelia*. A pored epicortex has been observed in *Coccocarpia* and *Physma* species (Hale, 1976). Hawker (1968) states that cracks in the thallus of some crustose lichens (aereolae) achieve a similar purpose, as do the papillae of *Parmelia* species, and the breaks in the lower

cortex of *Lobaria*. Collins and Farrar (1978) suggest that the pores in the upper cortex of *Xanthoria parietina* could reduce CO_2 uptake resistances. Clearly then, many lichen genera possess some form of discontinuity of either the upper or lower cortex which could function as a CO_2 uptake site. However it is also apparent that in the majority of lichen genera no such pores have been observed. If lichens lack a lower cortex (e.g. *Peltigera*) or have a cortex only one cell thick (e.g. *Leptogium*) CO_2 resistances need not be excessive. On the other hand those species which have entire cortices which are not unusually thin would be expected to have very large CO_2 resistances and accordingly, low rates of net photosynthesis. Although some low rates of photosynthesis have been reported (e.g. Kershaw, 1972) these were later found to be an artifact of the experimental system (Larson and Kershaw, 1975b). Other reports of lichen photosynthesis (e.g. Kallio and Karenlampi, 1975; Carstairs and Oechel, 1978; Lechowicz, 1978) indicate that rates are similar to those found in the present study. It is possible that CO_2 uptake in these species occurs via a different pathway. Active transport, bicarbonate utilisation, and morphological adaptations could be involved. Alternatively cortical pores may be more common than previously thought.

(c) *Relevance to other work.* The suggestion that CO_2 diffuses into lichens through a high resistance air-filled pore system has some important implications for studies of lichen physiology. The most obvious of these is the doubt cast on previous experiments in which lichens were submerged in, or floated on, aqueous media. Such experimental techniques have been commonly used (see review by Farrar, 1973) and may well have resulted

in atypical pathways of CO_2 uptake. Further, in aqueous media much of the carbon source is likely to be in the form of bicarbonate. This may act only as a reservoir in supplying CO_2 where local deficiencies occur (Raven, 1970). However, since the precise location of water within the lichen thallus is not known, the possibility of direct utilisation of bicarbonate cannot be disregarded.

According to O'Leary and Osmond (1980), the δ^{13} values of plants can be affected by discrimination prior to carboxylation, as well as by the carboxylation process itself. Thus the diffusive resistances of lichens would be expected to contribute to the discrimination against ^{13}C observed in lichens (Shomer - Ilan *et al*, 1979). This effect, which would vary with thallus water content, would be most important at extreme water content values where diffusive resistances, rather than carboxylation processes, limit uptake of CO_2.

FUTURE WORK

(a) *Biochemistry.* One of the most consistent problems encountered during the present work has been the paucity of information on biochemical pathways in lichens. In several instances an attempt has been made to study and interpret CO_2 exchange on a whole plant level, rather than at a cellular, biochemical level. Since CO_2 exchange may be controlled by any of the several biochemical steps which occur between diffusion of CO_2 into the thallus and carboxylation within the chloroplast, the measurement of net CO_2 exchange is a rather indirect method of studying photosynthesis limitations. It is therefore not surprising that some of the phenomena observed have not been comprehensively explained.

Short term studies of initial photosynthetic products are obviously required. The effect of carbon source (CO_2 or HCO_3^-) on photosynthetic pathways should also be determined. Parallel experiments on isolated lichen algae could be used to define the effects (if any) of the internal atmosphere of the lichen thallus. Responses to O_2 and CO_2 levels would establish whether or not *P. billardierii* is able to exclude O_2 or concentrate CO_2. Measurements of the amount and activity of the carboxylating enzyme within the lichen would also be useful for assessing photosynthetic capacity and limitations. It is perhaps appropriate at this point to emphasise that in any such study the collection, storage, and experimental use of lichens must be carried out under defined, reproducable conditions since intraspecific physiological variations are not uncommon.

(b) *CO_2 resistances.* The data presented in this work has been most useful in forming a theoretical model of CO_2 uptake by cyphellate and pseudocyphellate lichens. By using this model the importance of the various CO_2 resistances in limiting photosynthesis have been quantified. It would now be most informative to apply these methods to other lichen or bryophyte species. In particular the enigma of CO_2 uptake by lichens with complete cortices needs to be resolved. The influence of thallus form on CO_2 resistance in fruticose genera such as *Cladonia, Usnea* and *Cladia* would also be an interesting research topic.

(c) *Photorespiration.* Photorespiration has proved to be a major, but variable component of CO_2 exchange in lichens. While the results presented here have demonstrated the magnitude of

photorespiration, and a certain interspecific variability, they must be regarded as only an initial survey. More data is needed on the effects of temperature, carbon source, and light intensity. As noted earlier, the low rates of photorespiration, particularly the low α value, of *P. billardierii* have yet to be satisfactorily explained.

(d) *Growth studies.* The discovery that the radial growth rate of *P. homoeophylla* increases with thallus size while that of *S. caperata* does not,raises a multitude of questions. Since the CO_2 exchange rates, and the response to thallus water content of both species is similar it seems the reason for this growth difference must be elsewhere. The first point that needs to be established is the relationship between area and dry weight in each species. If *P. homoeophylla* is in fact more productive than *S. latifrons* this could be related to a superior water storage system in the former, or perhaps to a greater diversion of carbon for reproductive purposes in the latter. A lesser facility for carbon transport, or differences in photosynthetic activity in various parts of the thallus are other possibilities which could affect growth rates.

Despite these differences the growth rates of both lichens are high in comparison with those reported elsewhere. Meaningful estimates of net nitrogen input to the ecosystem by this extensive and productive lichen flora have not yet been made.

(e) *Final note.* In conclusion it is appropriate to reiterate the comment of Chartier and Catsky (1975); 'many of the methods which have been developed for the study of photosynthesis have not yet been fully utilised for studies of lower plants'. Application of these methods both clarifies our

understanding of CO_2 exchange in lower plants and, at times, provides a broader perspective for the interpretation of higher plant results.

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