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Short Communication

Moisture Content and CO_2 Exchange of Lichens. II. Depression of Net Photosynthesis in *Ramalina maciformis* at High Water Content is Caused by Increased Thallus Carbon Dioxide Diffusion Resistance

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Summary. Thalli of *Ramalina maciformis* were moistened to their maximal water holding capacity, thus, simulating actual conditions following a heavy rainfall. Time courses of net photosynthesis at 17° C and 750 $\mu E\ m^{-2}\ s^{-1}$ light intensity (PAR) were obtained during drying of the thalli. At ambient CO_2 concentrations from 200 to 1,000 ppm, CO_2 uptake of the moist lichens was depressed at high water content. After a certain water loss, net photosynthesis increased to a maximal value and decreased again with further drying of the thalli. The degree of initial depression of photosynthesis decreased with increasing ambient CO_2 concentration, and it was fully absent at 1,600 ppm ambient CO_2. Under these conditions of CO_2 saturation, net photosynthesis remained constant at maximum for many hours and decreased only when substantial amounts of water had been lost. We conclude that the carboxylation capacity of the lichen is not affected by high contents of liquid water. Therefore, the depression of CO_2 uptake of the water saturated lichen at lower (e.g. natural) ambient CO_2 must be due exclusively to increased resistance to CO_2 diffusion from the external air to the sites of carboxylation.

Introduction

In many lichens, net photosynthesis increases as water content increases, reaches an optimum and then decreases with further moistening of the thallus (see literature review by Lange and Matthes 1981). Already in 1927 Stocker made the assumption that the decrease in CO_2 uptake at high water content occurs because CO_2 diffusion is impeded in water saturated thalli. However, those data of CO_2 exchange as a function of thallus water content which have been reported repeatedly by Kershaw and his co-workers (e.g. Kershaw 1977) do not support this suggestion. As discussed previously in this series (Lange 1980) and by Lange and Matthes (1981), their results indicate the possible existence of a metabolic component which influences lichen photosynthetic gas exchange and decreases photosynthesis at high contents of liquid water. In those cases where depression of net CO_2 uptake has been reported in water saturated lichen thalli, no attempt has been made to determine whether the decrease was caused by an increase in physical CO_2 diffusion resistance or whether changes in the biochemical carboxylation resistance were also involved. Such a differentiation of causal factors is important when considering how the gas exchange of lichens is affected by superoptimal saturation with water occurring commonly in nature after rainfall and whether some lichens are especially adapted to this condition.

Lichen photosynthetic CO_2 influx (NP) can be described at steady-state by the Fick's Law equation:

$$NP = \frac{C_A - C_P}{R_T + R_C}$$

in which C_A and C_P are the partial pressures of CO_2 in the external air and at the site of carboxylation in the phycobionts respectively, R_T is the diffusion resistance for CO_2 transport from the ambient air to the site of carboxylation, and R_C is the biochemical carboxylation resistance (see Šestác et al. 1972; Collins and Farrar 1978). Increasing external CO_2 concentration may result in increasing net photosynthesis, but only until the carboxylation reaction is saturated. At CO_2 saturation the magnitude of transport resistance is no longer important in determining the actual rate of net photosynthesis, rather this is then determined exclusively by the photosynthetic capacity. We have measured the rate of net CO_2 exchange in *Ramalina maciformis* thalli of different water contents and at different ambient CO_2 concentrations. These experiments allow us to determine whether physical diffusion or biochemical processes limit CO_2 uptake in this lichen at high water contents.

Material and Methods

The origin and treatment of the *Ramalina maciformis* samples have been described by Lange (1980). As explained in detail in that paper, CO_2 exchange of the thalli was measured in small cuvettes submerged in a water bath for temperature control. The thallus temperature in all experiments reported was maintained at 17° C, and incident light intensity was 750 $\mu E\ m^{-2}\ s^{-1}$ PAR (corresponding to 50,000 Lux). In addition to experiments using outside air (varying in its CO_2 content between 330 and 360 ppm), measurements were conducted with air of controlled CO_2 concentration. To obtain the concentrations used, a stream of outside air was passed through a column of soda lime (BDH Chemicals, Poole, England) to effectively remove all CO_2 and this stream of CO_2 free air then entered a large vessel where it was mixed with a stream of pure CO_2 of a set flow rate. Both gas streams were held constant and were regulated with mass flow controllers (Brooks Instruments, USA; the gas mixing instrumentation was built by H. Walz, Mess- und Regeltechnik, Effeltrich, FRG). The absolute CO_2 concentration of the air entering the gas exchange chamber was measured (ranges 0–400 or 0–1,600 ppm CO_2) with a BINOS I infrared gas analyser (Leybold Heraeus, Hanau, FRG). A second channel of the analyser (range $-50 \ldots \pm \ldots +50$ ppm CO_2 differential at 300 ppm CO_2 ground level) recorded the differential in CO_2 concentration

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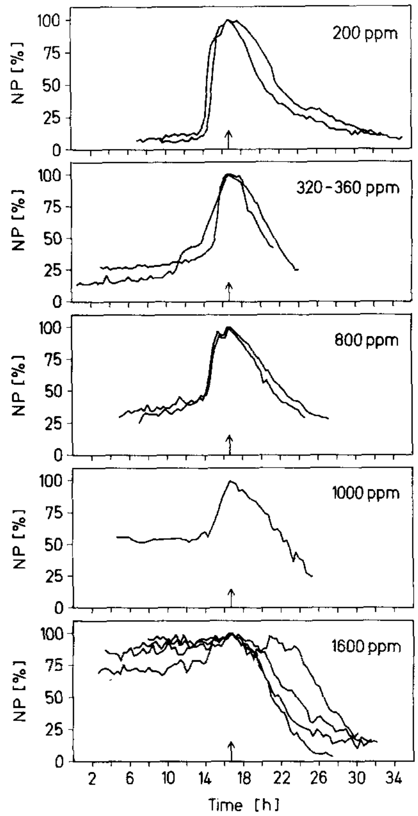

Fig. 1. Time courses of net photosynthetic CO_2 uptake (*NP*, in percent of maximal rates) of drying thalli of *Ramalina maciformis* (17° C and 750 $\mu E\ m^{-2}\ s^{-1}$ PAR). The lichens were initially moistened to maximal water holding capacity simulating a situation attained after heavy rain (beginning of each individual curve). Subsequently thalli lost water. The response curves have been synchronized with respect to the time of maximal rate of CO_2 uptake (*arrows*). Experiments were conducted at different ambient CO_2 concentrations, which are indicated in ppm

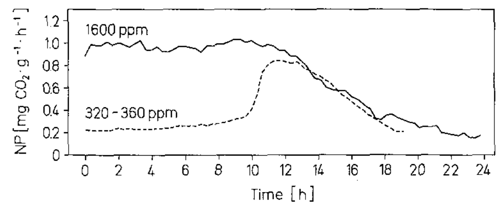

Fig. 2. As Fig. 1, however, with net photosynthetic CO_2 uptake given as the absolute rate

occurring across the gas exchange chamber (inlet versus outlet) due to CO_2 exchange of the enclosed plant material. The differential analyser sensitivity was established for ten background CO_2 concentrations between 0 and 1,600 ppm CO_2. Two plant cuvettes operating in parallel allowed replication of each experiment. The light intensity (essentially constant in these experiments), CO_2 exchange of the lichen, absolute CO_2 concentration, and lichen temperature were recorded at two minute intervals. The data were plotted on analog recorders, were simultaneously digitalized, were punched on paper tape, and then processed in the computing center of The University of Würzburg. In Figs. 1 and 2, data were averaged over 10 min periods.

The lichen thalli were soaked in water for several hours. They were then placed in the cuvettes and thoroughly sprayed with water. The degree of moistening was then similar to that achieved after an extensive rainfall in nature (and was higher than that used for the experiments of Lange 1980). Subsequently, the lichens were illuminated and CO_2 exchange measurements were begun. The thalli slowly lost water to the nearly water saturated air flowing through the cuvettes. They were almost dry after a period of 20 to 30 h. The time course of CO_2 exchange (as depicted in Figs. 1 and 2), indicates net photosynthesis of the lichen as water content decreases. At the beginning of each individual curve the maximum water possible is held by the thallus while at the end of the curve a water content of about 20% in relation to dry weight of the thallus was found.

The water content of the thalli, however, did not decrease proportionally with time, rather a higher rate of water loss from the enclosed plant material occurred at the beginning of the experiment when the thalli were moist, than toward the end when the lichens were almost dry. Furthermore, the individual samples dried out at different rates due to differences in the shape of the thalli which determined the turbulence in the cuvette and light energy absorption. In Fig. 1, therefore, the individual response curves were synchronized with respect to the time at which maximal net photosynthesis was reached (arrows). In parallel measurements, we determined that the photosynthetic capacity of *Ramalina maciformis* at constant water content and illumination does not change during a period of at least 30 h. Thus changes in CO_2 uptake during the experiments may be attributed to the changes in water content of the thalli.

Results

At a natural ambient CO_2 concentration of 320–360 ppm, the relative (%) net photosynthesis of water saturated *Ramalina maciformis* thalli was initially low (Fig. 1). It increased only slightly during the first 10 to 14 h of the experiment. Subsequently, CO_2 uptake increased rapidly and reached the maximum rate within a short period of time. The maximum level was maintained for no longer than about two hours after which net photosynthesis decreased with further drying of the thallus. The initial depression of CO_2 uptake in the fully saturated thalli is approximately 75% to 90% when related to maximal net photosynthesis reached at optimal water content. At a lower ambient CO_2 concentration of 200 ppm, this depression is even larger. However, it decreases with increasing ambient CO_2 concentration (ca. 70% at 800 ppm and ca. 50% at 1,000 ppm). At 1,600 ppm, the highest CO_2 concentration used, a depression was in most cases fully absent. In this situation, the thalli already showed maximal rates of net photosynthesis at full water holding capacity. A similar pattern of response also occurred with respect to the absolute rates of net photosynthesis measured (Fig. 2). A large difference was found in the gas exchange rates in water saturated thalli occurring at natural CO_2 concentration (320–360 ppm CO_2) and those at high CO_2 concentration (1,600 ppm). However, the difference in CO_2 uptake was small when the maximal rates at optimal water content are considered.

Discussion

The gas exchange of lichens under varying external CO_2 conditions has been studied only seldomly. Smyth (1934) measured the net photosynthesis of *Peltigera praetextata* at several levels of ambient CO_2 partial pressure and found CO_2 saturation occurring between 3 and 4 mg CO_2 per 1 air (corresponding to between 1,500 and 2,000 ppm CO_2). Green and Snelgar (1981) investigated CO_2 responses in *Stereocaulon ramulosum, Sticta latifrons, Peltigera dolichorhiza,* and *Pseudocyphellaria billardierii.* All species showed a linear relationship between net photosynthesis and external CO_2 at low concentrations, with CO_2 saturation levels being in excess of 400 ppm. At high thallus water content net photosynthetic response was still nearly linear at 1,000 ppm in *Sticta latifrons*. On the other hand, Larson and Kershaw (1975) reported CO_2 saturation of net photosynthesis for three different lichen species (*Alectoria ochroleuca, Cetraria nivalis, Parmelia caperata*) at any given thallus water content at a CO_2 concentration far below the natural ambient level, namely at 150 ppm. In the present investigations *Ramalina maciformis* reached CO_2 saturation only at or above 1,600 ppm at high thallus moisture content. In agreement with the results of Green and Snelgar (1981) the characteristics of the CO_2-dependence of photosynthesis in our study were highly influenced by the actual water status of the lichen. It seems unlikely that the discrepancies between these findings and those of Larson and Kershaw (1975) regarding CO_2 saturation can be explained by species-specific peculiarities alone. While more information is needed concerning the response of the different lichen types to changes in external CO_2 conditions, the results obtained by Larson and Kershaw may be influenced by their particular method of measuring lichen CO_2 exchange (as has been discussed in detail by Green and Snelgar 1981).

Collins and Farrar (1978) determined the CO_2 compensation point of net photosynthesis for the lichen *Xanthoria parietina*. They used this value along with measured rates of net photosynthesis at 330 ppm CO_2 to calculate the total resistance to CO_2 flux during photosynthesis. Further CO_2 compensation levels of gas exchange in a variety of species have been reported by Snelgar and Green (1980). In recent investigations Snelgar et al. (1981) have estimated the total resistance (transport plus carboxylation) for photosynthetic CO_2 uptake in several lichens as affected by thallus water content. Their calculations were based on photosynthesis measurements at CO_2 concentrations of 0 to 150 ppm. In most cases, three resistance phases are found with high resistance values at low and high water contents and with low values at intermediate water contents. They conclude further that the depression of net photosynthesis at high water content is caused in part by an increase in physical diffusion resistance.

Data from the present investigations with *Ramalina maciformis* allow a clear determination of the role of diffusion resistance in limiting gas exchange. At 1,600 ppm ambient CO_2, the depression of net photosynthesis in the moisture saturated lichen is overcome. Then, as water is lost, photosynthetic CO_2 uptake of the moistened lichen remains almost constant until it is restricted at low water content. Consequently, high water content does not affect the carboxylating capacity of the lichen photosynthetic apparatus (at least not within the time span of the experiment). Under the condition of CO_2 saturation, net photosynthesis is only reduced when a certain level of dehydration is reached and biochemical and biophysical reactions are affected by a decrease in water potential (Bewley 1979).

On the other hand, at lower ambient CO_2 concentration, net photosynthesis in the water saturated lichen is obviously determined by the supply of CO_2 to the sites of carboxylation and this in turn depends on the physical diffusion resistance of the thallus. As a first approximation, we may assume that the diffusion resistance of a well-moistened thallus changes proportionally with water content, since water content probably determines the length of the diffusion pathway for CO_2 in the liquid phase. Water content also directly influences the volume of air spaces in the lichen cortex and medulla. The photosynthetic flux of CO_2, which is proportional to the conductance for CO_2 transport, is therefore approximately inversely related to water content. This may explain the exponential nature of the first part of the response curve found with drying at nonsaturating ambient CO_2. The steep increase in photosynthesis may be even more abrupt if during the process of drying, a water film covering the lichen thallus externally 'breaks', suddenly facilitating gas exchange.

Under natural ambient CO_2 conditions, the changes in photosynthetic CO_2 uptake in the initially maximally wetted and subsequently drying lichen, are determined at first primarily by the diffusion resistances of the thallus. Later the carboxylation resistances become predominant and govern the acutal rates of net photosynthesis. At those water contents of the lichen which allow high rates of net photosynthesis, both resistances are effective and interact to determine the actual rate of CO_2 uptake. The magnitude of the individual resistances then depends not only on the water content of the thallus but also on other parameters, e.g. temperature affects R_T and temperature and light intensity affect R_C. The degree of hydration optimal for maximal net photosynthesis of a lichen is, therefore, not constant but varies with other environmental factors (see for instance Fig. 4 in Lange 1980) which must be taken into account when considering species ecology.

Depression of net photosynthesis at high water content may well restrict the primary production of a lichen in nature. This disadvantage, a necessary consequence of the poikilohydric structure of a terrestrial thallophyte, is not shared by cormophytes where intercellular air spaces in the leaves provide effective diffusion pathways for CO_2. Despite the general constraints inherent in their poikilohydric structure, however, we may certainly expect that there are lichen species that have evolved mechanisms which enable them, at least to some extent, to overcome CO_2 diffusion problems at high water contents. In order to investigate this, we must in the future quantify the water content dependent diffusion resistances of the different types of lichen thalli and determine whether any variations in resistance due to growth form are correlated with types of microenvironment. Further, we must determine whether special anatomical and morphological features in the structure of the cortex, cyphellae and pseudocyphellae or impregnation of the mycobiont with unwettable crystals of lichen substances (Goebel 1926) can facilitate gas diffusion in the wet lichen thallus and thus be interpreted as adaptations to moisture conditions of specific habitats.

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Erratum

C.E. Martin, N.L. Christensen, B.R. Strain: Seasonal Patterns of Growth Tissue and Fluctuations and $^{14}CO_2$ Uptake in the Crassulacean Acid Metabolism Epiphyte *Tillandsia usneioides* L (Spanish Moss) 49:322–328 (1981) There was a printing error in the title. The genus was spelled "Tjllandsia" and should have been "Tillandsia"