CO₂ and O₂ Exchange in Two Mosses, *Hypnum cupressiforme* and *Dicranum scoparium*

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ABSTRACT

Photosynthetic CO₂ and O₂ exchange was studied in two moss species, *Hypnum cupressiforme* Hedw. and *Dicranum scoparium* Hedw. Most experiments were made during steady state of photosynthesis, using ¹⁸O₂ to trace O₂ uptake. In standard experimental conditions (photoperiod 12 h, 135 micromoles photons per square meter per second, 18°C, 330 micromoles per liter CO₂ 21% O₂) the net photosynthetic rate was around 40 micromoles CO₂ per gram dry weight per hour in *H. cupressiforme* and 50 micromoles CO₂ per gram dry weight per hour in *D. scoparium*. The CO₂ compensation point lay between 45 and 55 micromoles per liter CO₂ and the enhancement of net photosynthesis by 3% O₂ versus 21% O₂ was 40 to 45%. The ratio of O₂ uptake to net photosynthesis was 0.8 to 0.9 irrespective of the light intensity. The response of net photosynthesis to CO₂ showed a high apparent K₅ (CO₂) even in nonsaturating light. On the other hand, O₂ uptake in standard conditions was not far from saturation. It could be enhanced by only 25% by increasing the O₂ concentration (saturating level as low as 30% O₂) and by 65% by decreasing the CO₂ concentration to the compensation point. Although O₂ is a competitive inhibitor of CO₂ uptake it did not replace CO₂ completely as an electron acceptor, and electron flow, expressed as gross O₂ production, was inhibited by both high O₂ and low CO₂ levels. At high CO₂, O₂ uptake was 70% lower than the maximum at the CO₂ compensation point. The remaining activity (30%) can be attributed to dark respiration and the Mehler reaction.

Extensive ecological and ecophysiological research has been made on the photosynthesis and productivity of mosses (12, 14). Typical mosses are their low photosynthetic rate, usually less than 10% of that in higher C₃ plants, and their tolerance of many environmental stresses, which is due mainly to their poikilohydric water economy. On the other hand, little attention has been paid to the physiological background of their photosynthesis and to the amount of photosynthesis. The values obtained for carbon discrimination ratios (18, 21) and the CO₂ compensation points (4, 7) confirm that in mosses CO₂ fixation follows the C₃ pathway as in other lower plants. However, among the mosses wide variation has been observed in light. CO₂ tensions has also been reported to vary from a value close to 0% up to 200%, depending on the moss species (19). The rates of ¹⁴CO₂ release in light and darkness give reason to suspect that mosses behave more like green algae than C₃ terrestrial plants in this respect and that they also have a reduced rate of glycolate oxidation compared with higher C₃ plants (7).

It has been suggested that lower plants probably had a high photosynthetic rate in the past, when the atmosphere was rich in CO₂. The present low rate could be attributed to a limitation of photosynthesis by the current CO₂ concentration and, in fact, there is some indication that the photosynthetic rates of mosses have very high CO₂ saturation levels (16). It was of interest to elucidate this point and to study whether the low rate of CO₂ fixation in mosses is accompanied by a proportionately high level of photorespiration. The photosynthesis of two moss species, *Hypnum cupressiforme* and *Dicranum scoparium*, was measured directly by using ¹⁸O₂ to trace O₂ uptake. The aim was to establish the level of O₂ uptake in these mosses at normal atmospheric gas concentrations and, also, by studying the response of O₂ uptake and photosynthesis to different CO₂ and O₂ concentrations, to clarify more precisely the *in vivo* exchange of these gases, which has been observed not always to match the *in vitro* properties of RuBP²-carboxylase/oxygenase alone (8).

MATERIALS AND METHODS

Mosses, *Hypnum cupressiforme* Hedw. and *Dicranum scoparium* Hedw., were collected in an oakwood on the north-facing slope of the hill near Manosque in France, between January and July 1983. The shoots were cut off about 1.5 cm from the tip and included all the green parts, probably representing several years' growth. The shoots were sprayed with water and arranged on the grid at the bottom of the assimilation chamber (volume 600 ml). After a stabilization period of 1 to 2 d in standard conditions (temperature 18°C, photon fluence rate 135 μmol m⁻² s⁻¹, photoperiod 12 h), the photosynthesis of the mosses remained relatively constant for up to 10 d (with linear growth), during which time different experiments were conducted, with 1 d in standard conditions between the experimental days. The temperature was controlled by circulating cold water through the body of the chamber. This caused some condensation in the bottom of the chamber, ensuring water uptake of the mosses by capillarity during the experiments. The water content of the shoots was kept at the optimal level for photosynthesis (around 400% of dry weight) and boundary layer resistance was minimized by effective ventilation inside the chamber. The relative humidity was about 70%. Light was provided by 1 to 5 Osram HQI 250-w lamps.

The gas exchange measurements were made in an airtight closed circuit system, similar to that used by Gerbaud and André (10), modified for use in a smaller chamber. The ventilation inside the chamber was generated by a pump and the air flow was enhanced by using an air injector. A loop with a low flow

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² Abbreviation: RuBP, ribulose 1,5-bisphosphate.
rate branching from the ventilation circuit contained the gas analysis and regulation systems. The CO₂ concentration was continuously monitored with an ADC-Mark II IR gas analyzer, and the other gases and also CO₂ above 1000 μl l⁻¹ gas were monitored with a RIBER QMN 17 quadrupolar mass spectrometer 4 times an h. The level of CO₂ was regulated with a computer by injecting pulses of CO₂ (10.6 μl per pulse) or by opening a CO₂ trap (trapping efficiency 1.2 μl s⁻¹) to compensate for the current level of photosynthesis or respiration and to maintain the CO₂ level at the set point selected for the experiment. A normal O₂ level was maintained by simultaneous injections of N₂ and CO₂, to dilute the O₂ produced by photosynthesis.

Net photosynthetic CO₂ uptake during the day and the respiration during the following night were calculated from the number of pulses and seconds of trapping, respectively. Photosynthetic (or respiratory) O₂ uptake was measured by using ^18O₂ as a tracer and neon as a reference gas (initial concentrations about 1%, injected at the beginning of each day). The calculations of O₂ uptake and net O₂ photosynthesis are based on differences in the decrease of ^18O₂ and variations in the level of ^16O₂, as previously described in detail (10). The gross O₂ evolution is the sum of the net photosynthetic production of O₂ and the O₂ uptake.

Different O₂ levels were obtained by injecting pure O₂ into the chamber or by flushing the chamber with N₂ at the beginning of the day and giving extra injections of N₂ during the day of the experiment in low O₂. The change in the O₂ level during the days of experiments in nonstandard O₂ levels was usually 1 to 2%.

All raw data, including the gas measurements, temperatures, and light intensities, were collected and stored in real time by a Telemeacanique 1600 minicomputer on an hourly basis. The results, if not otherwise mentioned, are the mean values of the day or night and represent the activities during steady state of photosynthesis.

RESULTS

Gas Exchange at Normal CO₂ and O₂ Levels. The absolute values of CO₂ and O₂ exchange in H. cupressiforme and D. scoparium at normal atmospheric gas concentrations are given in Table 1. During the course of the experiments, the photosynthetic rate in standard conditions (18°C, 135 μmol photons m⁻² s⁻¹) varied from 31 to 45 μmol CO₂ g dry weight⁻¹ h⁻¹ in H. cupressiforme and from 49 to 58 μmol CO₂ g dry weight⁻¹ h⁻¹ in D. scoparium. Some seasonal changes were evident in these rates, with decreases toward the summer. It is generally considered that no real seasonal trends exist in mosses (20), but that the rate varies with the amount of new photosynthetically active tissue. Almost the same values were obtained when net photosynthesis was measured as net CO₂ uptake or net O₂ production (Table 1). The photosynthetic rate as high as 68 μmol CO₂ g dry weight⁻¹ h⁻¹ was measured for H. cupressiforme at 670 μmol photons m⁻² s⁻¹. However, photoinhibition occurred within a few hours resulting in a 40 to 50% decrease in the photosynthetic rate which did not recover next day. Some photoinhibition (about 20%) in net photosynthesis was also observed in D. scoparium during the first hours in strong light (670 μmol photons m⁻² s⁻¹), mean CO₂ uptake later being 78 μmol g dry weight⁻¹ h⁻¹.

In spite of some seasonal variation in the net photosynthetic rates, the ratio of O₂ uptake to net photosynthesis remained more constant throughout the experiments. In nonsaturating light intensity (135 μmol photons m⁻² s⁻¹), when there is competition between CO₂ and O₂ for reducing equivalents, this ratio was 0.88 for H. cupressiforme and 0.79 for D. scoparium (Table 1) and did not increase at saturating light intensity (Table II).

CO₂ Compensation Point. The CO₂ compensation point at normal O₂ levels was around 50 μl l⁻¹ in both H. cupressiforme (Fig. 1) and D. scoparium. These values are typical of plants that fix CO₂ by C₃ metabolism. The CO₂ compensation point was independent of light intensity, but very sensitive to variations in the O₂ concentration (Fig. 1). In 40% O₂ the compensation point was 100 μl l⁻¹ CO₂, and in 3% O₂ it was only 14 μl l⁻¹ CO₂ in both mosses. Extrapolation of the CO₂ compensation point in H. cupressiforme to zero O₂ (Fig. 1) indicates the presence of CO₂ evolution in the light other than photosynthesis (2). Using the coefficients of André and Massimino (1) for this moss, a value as low as 0.1 times net photosynthesis was obtained for this residual dark respiration.

Effect of CO₂ Concentration on Gas Exchange at 21% O₂. The curves of the short-term response to CO₂ (Fig. 2) show a remarkable increase in net photosynthesis with rising CO₂, especially at saturating light intensities. However, even in nonsaturating light of 135 μmol photons m⁻² s⁻¹, used as a standard light intensity in the present experiments, the CO₂ fixation increased with increasing CO₂ concentration up to about 2000 μl l⁻¹ CO₂ (Fig. 3). CO₂ uptake then being 2.5 times that at a normal CO₂ level. A CO₂ concentration as high as 1.5% for 1 d had no inhibitory effect on photosynthesis (results not shown). Gross O₂ production increased with the CO₂ level in both saturating and nonsaturating light (Fig. 3; Table II), which is evidence that in both cases the rate of gas exchange at low CO₂ levels is restricted by the lack of electron acceptors. Accordingly, it seems that O₂ cannot completely replace CO₂ as an electron acceptor and therefore is not fully competitive with CO₂ as an acceptor. Although the highest level of O₂ uptake was observed at the CO₂ compensation point (Fig. 3), it did not reach the level of the maximum CO₂ uptake observed at saturating CO₂ (Fig. 3), as is the case in higher C₃ plants even in moderate light (6, 11). As CO₂ rose above the normal level, the O₂ uptake slowly decreased although, within the same range of CO₂ concentrations, there was a considerable increase in net photosynthesis (Fig. 3; Table II). At saturating CO₂, the O₂ uptake was depressed about 70% of the maximum observed around the CO₂ compensation point.

Effect of O₂ Concentration on Gas Exchange. A clear Warburg effect was observed in both mosses. In H. cupressiforme at normal CO₂ net photosynthesis was enhanced by 40 ± 3% when the O₂ level was reduced from 21 to 3%, and this response was relatively independent of the light level. The corresponding value for D. scoparium was 45 ± 2%. Almost the same results were obtained in the short-term experiments (Fig. 4) and in steady state experiments lasting 1 d (Fig. 5). Study of the Warburg effect in relation to the CO₂ concentration showed that CO₂ levels up to 900 μl l⁻¹ did not overcome the inhibitory effect of O₂. This is consistent with the fact that CO₂ levels between 300 and 900 μl l⁻¹ did not have much effect on O₂ uptake (Fig. 3). At 35% O₂, the inhibition

Table 1. Mean Rates of CO₂ and O₂ Exchange in H. cupressiforme and D. scoparium during the Experiments

| Gas exchange rates (μmol g dry wt⁻¹ h⁻¹) were measured during steady state of photosynthesis (photoperiod 12 h), at 18°C, 135 μmol photons m⁻² s⁻¹, 330 μl l⁻¹ CO₂, 21% O₂. Dark O₂ uptake was a mean of the following night. |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| H. cupressiforme| D. scoparium |
| Net CO₂ uptake | 39 ± 4.9* | 52 ± 4.9 | 38 ± 4.5 | 50 ± 4.5 | 34 ± 2.2 | 41 ± 1.8 | 72 ± 5.8 | 92 ± 4.9 | 13 ± 1.8 | 12 ± 1.8 | 0.88 ± 0.06 | 0.79 ± 0.06 |
| Net O₂ production | 38 ± 4.5 | 50 ± 4.5 | 34 ± 2.2 | 41 ± 1.8 | 72 ± 5.8 | 92 ± 4.9 | 13 ± 1.8 | 12 ± 1.8 | 0.88 ± 0.06 | 0.79 ± 0.06 |
| O₂ uptake | 34 ± 2.2 | 41 ± 1.8 | 72 ± 5.8 | 92 ± 4.9 | 13 ± 1.8 | 12 ± 1.8 | 0.88 ± 0.06 | 0.79 ± 0.06 |

* Mean ± sd.
of photosynthesis was increased by about 30% at 330 μl l⁻¹ CO₂ as compared with the inhibition at the normal O₂ level (Figs. 4 and 5).

Measurements of the effect of O₂ in steady state conditions, which allowed determination of O₂ uptake (Fig. 5), support the conclusion that the increase of photosynthesis at low O₂ tensions can be ascribed to inhibition of photorespiration. Gross O₂ production was constant when the O₂ level was reduced from 21 to 2% showing that CO₂ can completely replace O₂ as an electron acceptor. On the other hand, a decrease in gross O₂ production was observed at high O₂ levels. At all O₂ concentrations (Fig. 5) the level of gross O₂ production was lower than that observed at saturating CO₂ and normal O₂ (Fig. 3). O₂ uptake was saturated at as low an O₂ level as about 30%, but this saturated O₂ uptake rate was lower than the highest rate observed at the CO₂ compensation point and 21% O₂. So, not only is O₂ unable completely to replace CO₂ as an electron acceptor when CO₂ concentration is low (Fig. 3), but even a saturating O₂ concentration (Fig. 5) did not allow O₂ uptake to reach its maximum rate in the presence of normal CO₂, and even at 50% O₂ considerable CO₂ uptake took place.

FIG. 1. Effect of O₂ concentration on the CO₂ compensation point in H. cupressiforme. The points are the results of independent experiments at 135 (○) and 670 (■) μmol photons m⁻² s⁻¹. Temperature was 18°C.

FIG. 2. Short-term response to CO₂ of net CO₂ uptake by H. cupressiforme at 18°C, 21% O₂, and different quantum fluence rates. 670 (△), 400 (□), 260 (△), 135 (●), and 60 (○) μmol photons m⁻² s⁻¹. The shape of the corresponding curves by D. scoparium did not differ notably from those in this figure.

**DISCUSSION**

The rates of photosynthesis in H. cupressiforme and D. scoparium are low compared with those in higher C₃ plants (13) but correspond well to the mean published maximal activities for mosses, which usually vary between 10 and 90 μmol CO₂ g dry weight⁻¹ h⁻¹ (12, 19), depending on the species but being relatively independent of their geographic distribution.

The curves of the response to CO₂ suggest relatively high apparent Kₐ (CO₂) values for photosynthesis in these mosses as compared, for example, with wheat (11). However, according to the literature (5, 23) no great differences exist between the Kₐ (CO₂) values for RuBP-carboxylase in mosses and higher C₃
FIG. 3. Effect of CO₂ concentration during steady state of photosynthesis on gross O₂ production (●), net CO₂ uptake (○), net O₂ production (▲), O₂ uptake (▲), and dark O₂ uptake during the following night (●) in *H. cupressiforme* at 21% O₂, 135 μmol photons m⁻² s⁻¹, 18°C. The results of different experiments have been standardized to give net CO₂ uptake of 40 μmol h⁻¹ at 330 μl l⁻¹ CO₂, net O₂ production then being 39 ± 1.2 μmol h⁻¹ and O₂ uptake 35 ± 1.2 μmol h⁻¹. The results in nonstandard CO₂ concentrations are the mean values of two to five independent experiments. All results are the mean values for a day (or night).

FIG. 4. Short-term response to CO₂ of net CO₂ uptake by *H. cupressiforme* at 3% (▲), 21% (○), and 36% (●) oxygen. Temperature was 18°C and light intensity 135 μmol photons m⁻² s⁻¹.

plants and, therefore, this apparently low affinity of mosses for CO₂ is probably mostly due to their higher resistance to diffusion of CO₂, as mosses have no stomata and their cell walls are very thick. This interpretation is supported by the modeling experiments of André and Massimino (1).

It is surprising, however, that the higher diffusion resistance,

FIG. 5. Effect of O₂ concentration during steady state of photosynthesis on gross O₂ production (●), net CO₂ uptake (○), O₂ uptake (▲), and dark O₂ uptake during the following night (●) in *H. cupressiforme* at 330 μl l⁻¹ CO₂, 135 μmol photons m⁻² s⁻¹, 18°C. The results have been standardized as in Figure 3 to give 40 μmol h⁻¹ CO₂ uptake in standard conditions of 21% O₂, O₂ uptake then being 36 ± 1.2 μmol h⁻¹.

which causes a lower internal CO₂ concentration in mosses than in higher C₃ plants, does not result in a higher ratio of O₂ uptake to net photosynthesis. This ratio was well below one at ambient external CO₂ and O₂ levels in nonsaturating light (Fig. 1), which is at the same level or even lower than the corresponding ratios obtained for higher C₃ plants (3, 6, 10, 11). If in normal air the internal CO₂ concentration in mosses is about 100 μl l⁻¹ lower than, for example, in wheat (1) and if the RuBP-carboxylase/oxygenase kinetics is the same then, according to curves published for higher C₃ plants (3, 6, 10, 11), the ratio of O₂ uptake to net photosynthesis in mosses would be expected to be well above 1. Moreover, this ratio did not increase in mosses at saturating light intensity (Table II), in contrast to its behavior in higher C₃ plants in which the proportion of electrons transported to O₂ increases with increasing light intensity (6). This suggests that the oxygenase activity in relation to the carboxylase activity is lower in mosses than in higher C₃ plants.

In *H. cupressiforme* the curves of CO₂ and O₂ uptake plotted against CO₂ concentration (Fig. 3) are not the mirror image of each other as in higher C₃ plants at low or moderate light (3, 6, 11). However, the maximum O₂ uptake around the CO₂ compensation point and also the 70% depression of O₂ uptake at high CO₂ in *H. cupressiforme* are seen only at nonsaturating light in higher C₃ plants, too. The ratio of maximum O₂ uptake around the CO₂ compensation point to maximum net photosynthesis at high CO₂ is, in *H. cupressiforme* (Fig. 3), much lower than in higher C₃ plants at nonsaturating light (3, 6, 11). In *H. cupressiforme* this value is intermediate between the corresponding ratios for wheat at 21 and 2% O₂ (11), providing further support for the hypothesis that in mosses oxygenase activity, at least *in vivo*, is lower in relation to the carboxylase activity than in higher C₃ plants. Moreover, the ratio of the maximal activity of RuBP-oxygenase to RuBP-carboxylase *in vitro* in the protonemata of the moss *Ceratodon purpureus* has been observed to be lower than in higher C₃ plants (17) and also the glycolate oxidase activity of this moss is low (22).

If the purpose of photorespiration is to protect plants against photoinhibition (15), the low capacity of O₂ to accept electrons may be a reason for the sensitivity of many mosses to photoinhibition in strong light.

There is indirect evidence that the O₂ uptake measured here is
not entirely due to photorespiration. For example, at high CO₂ when oxygenase activity is mainly inhibited, there is still considerable O₂ uptake in *H. cupressiforme* (Fig. 3). In experiments of a similar type with spinach chloroplasts and isolated *Xanthium* cells (9), the O₂ uptake persisting at a high level of CO₂ was attributed to direct photoreduction of O₂ via a Mehler reaction. However, the level of dark respiration, at least in darkness, is higher in *H. cupressiforme*. Although there seems to be about 70% inhibition of dark respiration in the light in *H. cupressiforme* at zero CO₂ (1), it is impossible to say how the rate of dark respiration is affected by increasing CO₂ levels. As it was not possible to measure photorespiration, dark reaction, and the Mehler reaction separately from each other, the exact share of these reactions of the total O₂ uptake in different experimental conditions is difficult to interpret, but according to the modeling experiments of André and Massimino (1), the photosynthetic gas exchanges of *H. cupressiforme* are best explained supposing also the existence of the Mehler reaction.

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