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Light-Inhibition of Dark Respiration in *Lemna minor* L.¹⁾

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Abstract

Fuhrer, J. 1983. Light-inhibition of dark respiration in *Lemna minor* L. Bot. Helv. 93: 67-75. Based on the oxygen-dependence of mesophyll resistance and carbon dioxide compensation concentration, the fraction of dark respiration not inhibited by light was derived for *Lemna minor* L. It was observed that approximately 77% of dark respiration was inhibited at light intensities saturating for net photosynthesis at low carbon dioxide concentration. The corresponding rates of «day» respiration were 13.8 and 12.4 $\mu\text{g CO}_2 \text{ m}^{-2} \text{ sec}^{-1}$ at 21 and 1% oxygen, respectively. The rate at 1% oxygen (under non-photorespiratory conditions) was in good agreement with the rate of CO_2 -release into CO_2 -free air. Rates of «day» respiration were nearly constant at light intensities above 100 $\mu\text{E m}^{-2} \text{ sec}^{-1}$, but increased rapidly as the light intensity decreased to lower values.

Introduction

Much of the work carried out in this laboratory has dealt with the regulation of carbon assimilation in C_3 plants. Most recently it was observed that beans growing on ammonium as N-source show faster ^{14}C -labelling in intermediates of the photosynthetic carbon oxidation (PCO) cycle than those growing on nitrate (Marques et al. 1983). This stimulation of the C-flux to glycollate by ammonium most likely also occurs in other plants, such as *L. minor*, where an enhanced activity of glycollate oxidase was found under the same conditions (Emes and Erismann 1982). Currently, an attempt is being made to predict quantitatively carbon flux rates between pools of major intermediates in PCO and PCR (photosynthetic carbon reduction) cycles based on the measured rate of net C-assimilation and the CO_2 compensation concentration (Fuhrer and Erismann, in preparation). These calculations are based on a model of PCO and PCR cycles which integrates the current knowledge of the biochemistry involved (Farquhar et al., 1980). It is the main idea of this model to express C-fluxes on the basis of the amount of C fixed by ribulose-bisphosphate (RuBP) carboxylase, F_{IN} , and the properties of RuBP carboxylase/oxygenase (RUBISCO).

¹⁾ This work is dedicated to Prof. Dr. K.H. Erismann, in honor of his 60th birthday.

The reaction of RuBP with one mol of O₂ leads to the release of 0.5 mol of CO₂ through glycine decarboxylation. Therefore, net photosynthesis, F_{Net}, is given by

$$F_{\text{Net}} = F_{\text{IN}} - 0.5\alpha F_{\text{IN}} - R_{\text{D}} \quad (1a)$$

where α is the ratio between oxygenation and carboxylation of RuBP and R_D denotes CO₂ evolution in the light («day» respiration) due to processes other than glycine decarboxylation. Dark respiratory processes are known to occur in the light, although at a lower rate than in the dark (Mangat et al. 1974).

Equation (1a) can be rearranged to calculate F_{IN} from F_{Net}, R_D and α .

$$F_{\text{IN}} = \frac{F_{\text{Net}} + R_{\text{D}}}{1 - 0.5\alpha} \quad (1b)$$

To be able to use accurate rates of F_{IN} as the model's input variable, values for R_D have to be known. In this paper, the fraction of dark respiration not inhibited by light is calculated from the O₂-dependence of both, the mesophyll resistance and the CO₂ compensation concentration. Rates were determined at different light intensities to examine whether R_D forms an increasing portion of C-fluxes with decreasing light intensity, as predicted by Farquhar's model.

Theoretical considerations for the calculation of «day» respiration rates

The calculation of R_D was based on a simple model of CO₂ exchange presented by Peisker and Apel (1980). According to this model, R_D can be calculated from the O₂-dependence of (i) the mesophyll resistance, r_m, and (ii) the CO₂ compensation concentration, τ .

The relationship between r_m and the O₂ concentration can be written as,

$$\beta = \frac{\Delta r_m}{\Delta [\text{O}_2]} \quad (2)$$

and was calculated from values of r_m obtained at 1% and 21% O₂. The mesophyll resistance at each O₂ concentration was derived as the slope of the relationship between F_{Net} and the intercellular CO₂ concentration.

$$r_m = \frac{[\text{CO}_2] - \tau - r_s}{F_{\text{Net}}} \quad (3)$$

Resistance to CO₂ exchange, r_s, (commonly called «stomatal resistance», but gas exchange in *Lemna minor* is likely to occur through the cuticle as well) was taken as a constant value of 1.6 cm/sec (unpublished observation). Intercellular CO₂ concentrations were calculated according to von Caemmerer and Farquhar (1981).

The CO₂ compensation concentration, τ , depends on the O₂ concentration in a linear way (Forrester et al. 1966),

$$\gamma' = \frac{\Delta \tau}{\Delta [\text{O}_2]} \quad (4)$$

and the whole O₂ dependence of γ' is given by

$$\gamma' = \gamma + \mu \cdot \beta \cdot R_{\text{N}}$$

where R_N is the rate of dark respiration.

This relationship is linear when β , μ and R_N are constant, and γ , the part of the O_2 -dependence which only depends on the kinetic properties of RUBISCO, can be derived as intersection with the ordinate axis (Peisker et al. 1981). Under the different light conditions used in the present study, however, γ , the fraction of R_N not inhibited by light, becomes variable. Therefore, γ had to be derived by extrapolation of the non-linear relationship (see Fig. 2).

For each treatment, $\beta \cdot R_N$ and γ' were determined and used together with the constant value for γ in Eq. (5b) to calculate μ :

$$\mu = \frac{\gamma' - \gamma}{\beta \cdot R_N} \quad (5b)$$

γ , which did not depend on R_D , was also used to determine the CO_2 compensation concentration in the absence of R_D , τ^* , using Eq. (6) according to Farquhar et al. (1980).

$$\tau^* = [O_2] \cdot \gamma \quad (6)$$

Materials and Methods

Lemna minor L. were cultivated in aerated Fernbach flasks at 20 °C and ambient CO_2 for seven days on a modified Hutner medium containing 7 mM NO_3^- as nitrogen source (Emes and Erismann 1982). Light intensity was $250 \mu E m^{-2} sec^{-1}$ provided by fluorescence bulbs placed beneath and above the culture flasks.

Rates of net photosynthesis (F_{Net}) and dark respiration (R_N) were determined after transfer of the culture to a round, stainless steel cuvette (with a glass window build into the removable cover) which was part of an open fumigation system (designed by Prof. K.H. Erismann). Air flow through the cuvette was $1 l min^{-1}$. Carbon dioxide from a pressure tank was added to CO_2 -free and dried ambient air through permeation tubes placed in the gas stream. Individual CO_2 concentrations were obtained by adjusting the length of the permeation tube. Air entering or leaving the cuvette was analyzed for CO_2 with a Siemens IRGA (Ultramat 32) after passing through a condenser at 4 °C. Oxygen concentrations other than that of ambient air (21%) were obtained by using commercially purchased O_2/N_2 mixtures (Carba Gas, Liebefeld-Bern) instead of ambient air.

Quantum yield of net photosynthesis under different CO_2 and O_2 conditions was determined according to Ehleringer and Björkman (1977). Total leaf area of a culture was estimated on a photographic picture with a planimeter.

The open IRGA system was adapted to measure the CO_2 compensation concentration, τ , at 25 °C. The gas from the sample cell of the IRGA was recycled through the plant cuvette back to the IRGA by a diaphragm pump to create a closed system. Before closure, the system was flushed with the appropriate O_2/N_2 mixture and $578 ng cm^{-3} CO_2$.

Light ($1200 \mu E m^{-2} sec^{-1}$ PHAR, 400-750 nm) provided by a 1000 W quartz iodide lamp (Philips 12013 R) placed above a tank containing circulating tap water. The cuvette was covered with a plastic bowl containing a 2 cm deep layer of a $CuSO_4$ solution (10 g/500 ml) to reduce heat transfer into the cuvette. Various light intensities were obtained by placing metal screens with different size holes above the cuvette. Light intensities were measured with a Quanta spectrometer (Tehtum QSM 2500). The temperature in the cuvette was adjusted to 25 °C by circulating water from a temperature-controlled water bath through an inner compartment in the stainless steel body.

Carbon dioxide evolution in the dark (R_N) was measured after a 20 min light period followed by a 20 min dark period.

Results and Discussion

Net CO₂ uptake rate of *L. minor*, F_{Net} , depended on the light intensity (I) and the CO₂ and O₂ concentrations as shown in Fig. 1A. Table 1 contains the quantum yields determined from the data presented in Fig. 1A. They were in agreement with those from other C₃ plants (Ehleringer and Björkmann 1977).

Table 1: Quantum yields for *Lemna minor* L. at different CO₂ and O₂ concentrations.

	CO ₂ concentration (ng cm ⁻³)	
	150	478
O ₂ concentration (%): 21	0.032	0.068
1	0.080	0.080

A rapid decrease of F_{Net} occurred as I decreased from about 100 $\mu\text{E m}^{-2} \text{sec}^{-1}$ to the light compensation point. On the other hand, an increase of τ over the same range of I was observed (Fig. 1B). At I saturating for F_{Net} at low CO₂ concentration, τ was approximately 90 ng cm⁻³ at 21% O₂ and 19 ng cm⁻³ at 1% O₂. The value of τ at 21% O₂ was slightly higher than of wheat leaves (Feller and Erismann 1978, Peisker and Apel 1975), but lower than that of adult bean leaves (Peisker et al. 1981). A similar increase of τ below 260 $\mu\text{E m}^{-2} \text{sec}^{-1}$ (equivalent to 84 $\mu\text{E m}^{-2} \text{sec}^{-1}$ when using an IR-filter as in this study) was reported for bean leaves of different age (Catský and Tichá 1979). The observed increase of τ under condition of inhibited photorespiration (1% O₂) indicated the apparent effect of «day» respiration, R_D . According to Farquhar et al. (1980), τ depends on the properties of RUBISCO, the O₂ concentration and the rate of R_D . A linear correlation between τ and $R_D / V_{c,\text{max}}$, with $V_{c,\text{max}}$ as maximal carboxylation velocity, can be observed (Peisker et al. 1981).

Table 2: Mean values for r_m at 21% and 1% O₂, respectively, the O₂-dependence of the carboxylation resistance, β , the product of β and the rate of dark respiration, R_N , the O₂-dependence of τ , γ' , and the difference between γ' and the O₂-dependence of the CO₂ compensation concentration in the absence of «day» respiration, γ , in *Lemna minor* L. at different light intensities. Standard errors were generally within $\pm 10\%$ of the mean value (not shown).

I ($\mu\text{E m}^{-2} \text{sec}^{-1}$)	r_m (21%) (sec m^{-1})	r_m (1%) (sec m^{-1})	β ($\text{sec m}^{-2} \text{kg}^{-1}$)	$\beta \cdot R_N$ (g kg^{-1})	γ' (g kg^{-1})	$\gamma' - \gamma$ (g kg^{-1})
380	1313	1255	215	0.0129	0.270	0.003
150	1710	1571	515	0.0309	0.276	0.009
90	2461	2238	826	0.0496	0.290	0.023
60	4000	3724	1022	0.0613	0.301	0.038
50	5299	4892	1248	0.0749	0.316	0.049

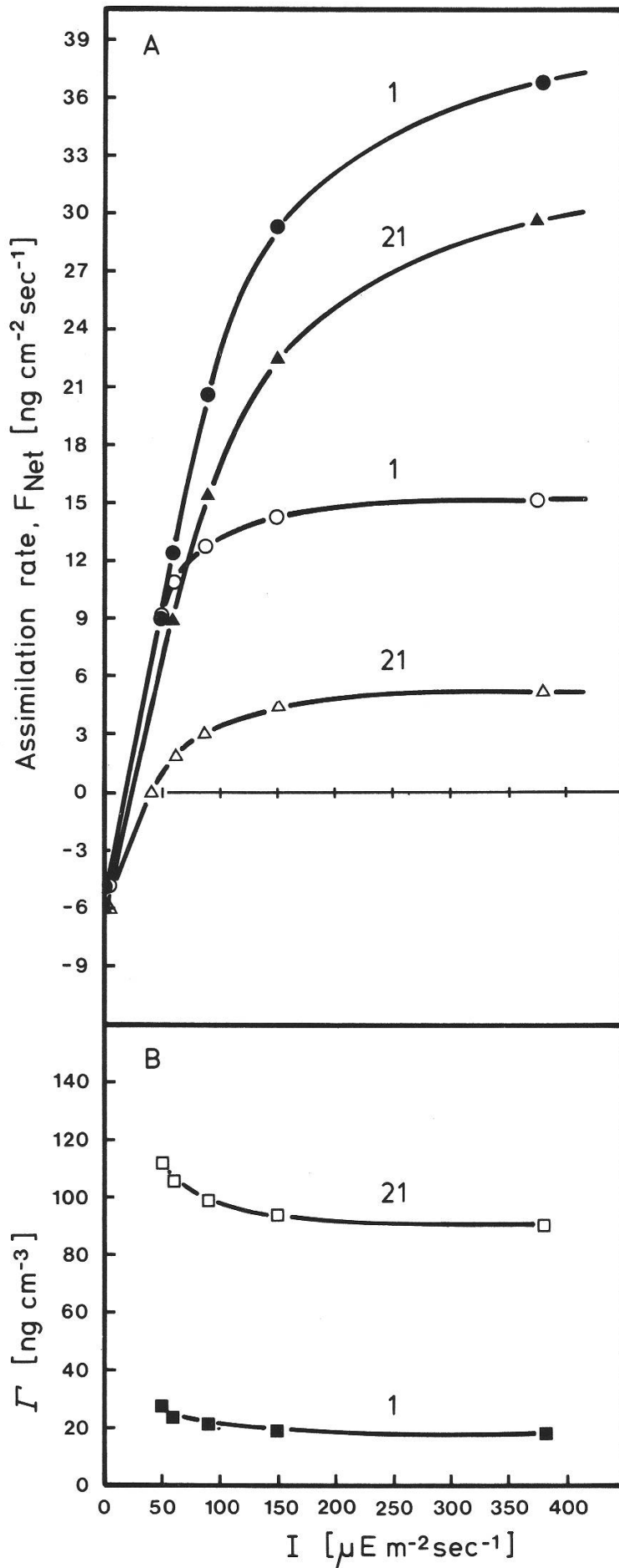


Fig.1: A. Rate of net CO_2 assimilation, F_{Net} , versus light intensity, I , in *L. minor* L. measured at 528 $ng\ cm^{-3}$ (closed symbols) or 150 $ng\ cm^{-3}$ (open symbols) external CO_2 concentration, and at either 21% or 1% O_2 . Negative values of F_{Net} indicate CO_2 evolution. B. Carbon dioxide compensation concentration, Γ , versus light intensity, I , in *L. minor* L. at 21% or 1% O_2 .

The initial slope of the relationship between F_{Net} and the intercellular CO_2 concentration was used as a measure for the mesophyll resistance to CO_2 , r_m (Table 2). Values obtained with air containing 1 % and 21 % O_2 were used to derive the slope of the dependence of r_m on O_2 , β . Values for β largely depended on I (Table 2).

The rate of dark respiration, R_N , measured after a 20 min dark period following 20 min of light, was $60 \pm 10 \mu\text{g CO}_2 \text{ m}^{-2} \text{ sec}^{-1}$ at 21 % O_2 , similar to the rate determined for wheat leaves under comparable experimental conditions (Peisker and Apel 1975), and $50 \pm 6 \mu\text{g CO}_2 \text{ m}^{-2} \text{ sec}^{-1}$ at 1 % O_2 . The calculated factor $\beta \cdot R_N$ is listed in Table 2 for the different light intensities used. This factor was lowest at light intensities saturating for F_{Net} at low CO_2 concentrations (see Fig. 1A).

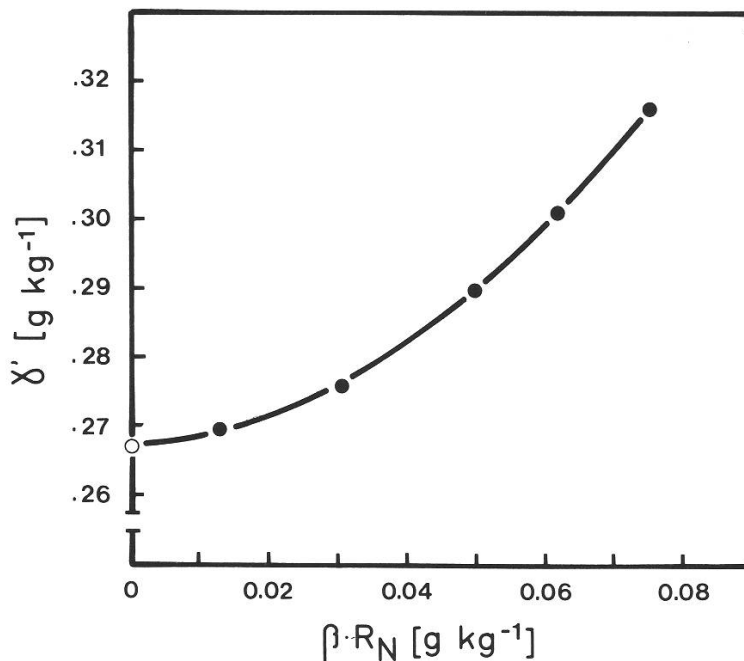


Fig. 2: Relationship between the O_2 -dependence of the CO_2 compensation concentration, γ' , and the product of the O_2 -dependence of the carboxylation resistance, β , and the CO_2 evolution in darkness, R_N , in *L. minor* L. The intersection with the ordinate axis, (open circle), γ , was found by extrapolation.

Values for $\beta \cdot R_N$ were plotted against those found for γ' at the different light intensities (Fig. 2). The non-linear regression line was extrapolated to $\beta \cdot R_N = 0$. At this point, γ' equaled γ (0.276 g kg^{-1}). γ should not differ greatly between C_3 species and should not be influenced by varying I . In fact, Peisker and Apel (1980) reported 0.26 g kg^{-1} for wheat leaves at 23°C and Peisker et al. (1981) found 0.308 g kg^{-1} for primary leaves of beans at 28°C . Charles-Edwards (1978) listed a series of values around 0.24 g kg^{-1} for a variety of C_3 plants at 25°C . The accuracy of the value for *L. minor* reported here is therefore satisfactory, considering the possible limitations for the model of Peisker and Apel (1980) at high CO_2 concentrations and the uncertainty in deriving γ by extrapolation.

The value for γ was used in Eq. (6) to calculate τ^* , the CO_2 compensation concentration in the absence of «day» respiration. At 21 % O_2 , τ^* was 71.8 ng cm^{-3} . This concentration only depends upon the characteristics of RUBISCO (Farquhar et al. 1980). A similar value for different C_3 species could be expected. Farquhar et al. (1980) calculated 55 ng cm^{-3} based on *in vitro* data from the spinach chloroplast enzyme, and data from Badger and Andrews (1974) suggest 81 ng cm^{-3} . The difference might be due to differences in the *in vitro* assays used by these authors.

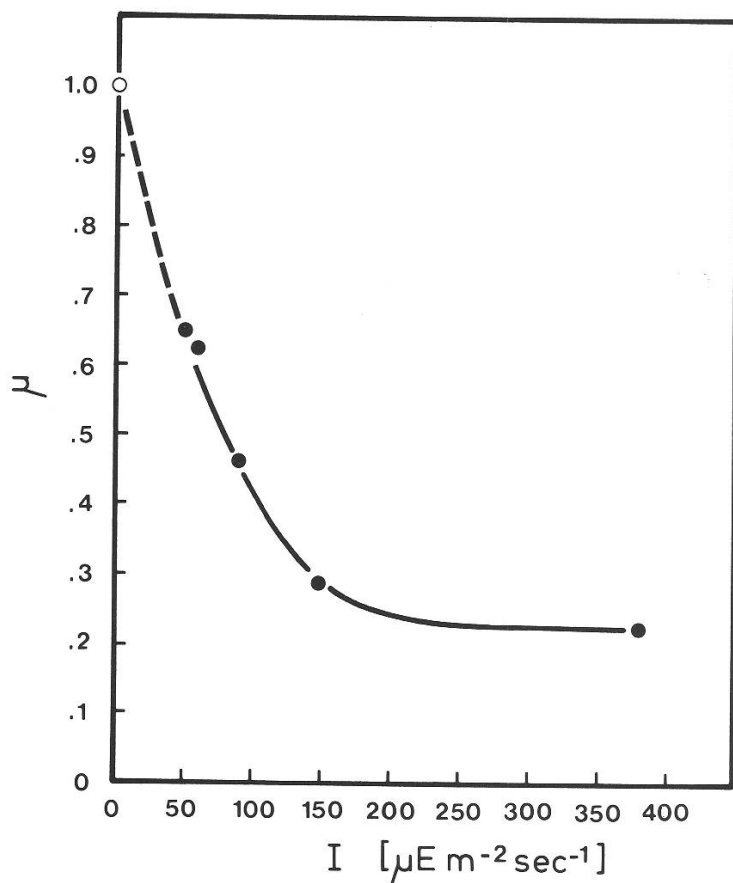


Fig. 3: The fraction of dark respiration not inhibited by light, μ , versus light intensity, I , in *L. minor* L.

Based on the values for $\beta \cdot R_N$ and $\gamma' - \gamma$ given in Table 2, the fraction of R_N not inhibited by light, μ , was calculated according to Eq. (5b). Values obtained at each light intensity are plotted in Fig. 3. It was found that μ was about 0.23 at high light intensity, which means that 77% of R_N was inhibited by light, leaving a rate of R_D of $13.8 \mu\text{g CO}_2 \text{ m}^{-2} \text{ sec}^{-1}$. This equaled 7.7% of F_{Net} at ambient CO_2 and O_2 concentrations. Farquhar et al. (1980) assumed a comparable value of 5.8%. A smaller light inhibition of R_N (66%) was observed in wheat leaves at $500 \mu\text{E m}^{-2} \text{ sec}^{-1}$ (Peisker and Apel 1980). Values for bean leaves fluctuated between 75% and 20% during ontogenesis (Peisker et al. 1981). Mangat et al. (1974) derived 75% inhibition for bean leaves based on ^{14}C labelling experiments.

At I limiting for F_{Net} , μ increased. A value of $39.2 \mu\text{g CO}_2 \text{ m}^{-2} \text{ sec}^{-1}$ for R_D was calculated from μ ($= 0.654$) at $50 \mu\text{E m}^{-2} \text{ sec}^{-1}$. The broken line in Fig. 3 indicates a possible extrapolation below the light compensation point to complete darkness where R_D equals R_N . The increase of μ with decreasing I agrees with the prediction by the model of Farquhar et al. (1980). This observation underlines the possibility that «day» respiration depends on ATP supplied by photophosphorylation which would be low at light intensities limiting for photosynthesis (Mangat et al. 1974).

Assuming that R_D was inhibited by low O_2 concentration to a similar extent as R_N (10%), R_D at 1% O_2 was $12.4 \mu\text{g CO}_2 \text{ m}^{-2} \text{ sec}^{-1}$. On the other hand, photorespiratory CO_2 -release is likely to be very small at 1% O_2 . Therefore, the rate of CO_2 -release into CO_2 -free air at 1% O_2 should be similar to the rate of R_D . In fact, a value of 12.6 ± 3.5 (S.E.) $\mu\text{g CO}_2 \text{ m}^{-2} \text{ sec}^{-1}$ was determined at high light intensities. This agreement indicates that R_D can be estimated in CO_2 gas exchange experiments from the CO_2 release into CO_2 -free air under non-photorespiratory conditions.

Zusammenfassung:

Auf der Grundlage der Sauerstoffabhängigkeit des Mesophyllwiderstandes und der Kohlendioxid-Kompensationskonzentration wurde der Anteil der Dunkelatmung von *Lemna minor* bestimmt, der nicht durch Licht gehemmt wird. Es wurde beobachtet, daß ungefähr 77% der Dunkelatmung durch Lichtintensitäten, die für die Netto-Photosynthese bei geringer CO₂-Konzentration sättigend sind, gehemmt werden. Die entsprechenden Raten der «Licht»-Atmung betrugen 13,8 und 12,4 µg CO₂ m⁻² sec⁻¹ bei 21%, bzw. 1% Sauerstoff. Die Rate bei 1% Sauerstoff (bei gehemmter Photorespiration) stimmte gut mit der Rate der CO₂-Abgabe in CO₂-freie Luft überein. Unter Lichtintensitäten über 100 µE m⁻² sec⁻¹ waren die Raten der «Licht»-Atmung nahezu konstant, stiegen aber an, wenn die Lichtintensität unter diesen Wert absank.

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