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Carbon dioxide gas exchange and the energy status of leaves of *Primula palinuri* under water stress

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Abstract. The photosynthetic rate of water-stressed leaves of *Primula palinuri* was reduced drastically by stomatal closure, not by limitations imposed on the capacity of the photosynthetic apparatus, when water loss exceeded 20% of the water content of turgid leaves. The sudden decrease in phtosynthesis was not observed when the lower epidermis of the leaves had been removed. In these 'stripped' leaves, inhibition of photosynthesis increased only gradually during the wilting caused by increasing water stress and was complete when the relative water content was as low as 20% compared with the initial value. This corresponded to a water potential of about −40 bar. The light intensity at which half-maximum rates of photosynthesis were observed decreased as stress increased. In intact leaves photosynthesizing in the presence of CO_2, light scattering, which is a measure of thylakoid energization, increased steeply during stomatal closure. The observed increase corresponded to the light scattering level measured in the absence of CO_2. When the lower epidermis was removed, no sudden increase in thylakoid energization could be observed during dehydration. Thylakoid energization remained high even at low water potentials. It decreased drastically only below a relative water content of 20%. Irrespective of the extent of water stress, CO_2 fixation of stripped leaves increased when the oxygen content of air was reduced from 21% to 2%. Usually the transition from 21 to 2% O_2 was accompanied by increased thylakoid energization. The increase in energization was more pronounced below than above a relative water content of 50%. The data show that energy-dissipating photorespiratory CO_2 turnover in the intercellular space of water-stressed leaves whose stomata are closed decreases only slowly as water stress increases. Respiratory CO_2 production by leaves in the dark was even more resistant to water stress than photosynthesis. It was still significant at water potentials as low as −80 bar.

Key words: Leaf (CO_2 exchange) – Light scattering (leaf) – Photosynthesis (water stress) – Photorespiration – *Primula* – Water stress (photosynthesis).

Introduction

During water stress, photosynthetic carbon assimilation is known to be reduced. There are different possibilities to explain this inhibition of photosynthesis. In leaves, water stress results in partial or complete closure of stomata. In consequence, the CO_2-diffusion resistance is increased. In air and under high-intensity illumination photosynthesis is CO_2-limited even when the stomata are open. While improving the water economy of the leaves, stomatal closure must therefore decrease photosynthesis (Moldau 1973; O'Toole et al. 1977; Settler et al. 1980). However, from calculations of the CO_2 concentration in the intercellular space, it has been suggested that the rate of photosynthesis may in some cases control stomatal aperture rather than reverse (for review see Farquhar and Sharkey 1982). If this were correct, water stress might affect photosynthesis directly and stomatal closure would be the secondary phenomenon.

In previous work with isolated spinach chloroplasts, spinach protoplasts and thin leaf slices from different plants which were suspended in media of different osmotic potentials, it could be demonstrated that osmotic dehydration indeed reduced photosynthesis and that the extent of inhibition depended on the extent of water loss. However,

Abbreviation: RWC = relative water content

inhibition was severe only after considerable dehydration. While the resistance to water loss differed in different plant species, comparable water loss produced comparable inhibition of photosynthesis in hygro-, meso- and xerophytes (Kaiser et al. 1981a, b; Kaiser and Heber 1981; Kaiser 1982). In the present study, we were interested to know whether the observations made on osmotically dehydrated organelles or cells were valid also for leaves subjected to dehydration in air. Furthermore, we wished to know whether photorespiration, a process known to dissipate energy rather than generating it, was affected by dehydration to a similar extent as photosynthesis. Photorespiration has been proposed to play a role in preventing photooxidative damage to the photosynthetic apparatus (Heber and Krause 1980; Osmond 1981). Such a role would be particularly important in water-stressed leaves in which stomatal closure precludes the dissipation of light energy accompanying a net gain in reduced carbon.

For our work, we have used excised leaves of *Primula palinuri*, a mediterranean mesophyte, whose lower epidermis can be easily removed (Bertsch 1969). We have examined light scattering and gas exchange by epidermis-free and intact leaves under conditions of water stress. Light-induced changes in light scattering by the leaves are an indicator of chloroplast energization in the light (Heber 1969; Krause 1973). They reveal changes in the chloroplastic ATP/ADP ratio (Köster and Heber 1982; Kobayashi et al. 1982) which is an important factor in the driving force for carbon reduction.

Material and methods

Primula palinuri L. was grown in a greenhouse. Leaves were harvested in the morning. Leaf fragments were cut out with razor blades. If desired, the lower epidermis was removed and the fresh weight of the leaf fragment was immediately determined. Gas exchange and light scattering were measured simultaneously in a plexiglas cuvette of volume 7 ml. Gases were taken from cylinders and, if necessary, mixed with a Wösthoff pump. The gas stream was divided into a reference and a measuring stream, which passed through the cuvette. The gas was moistened in a long, thin, glass tube with wet filter paper before entering the cuvette. After passage, the water content of the air was reduced by $CaCl_2$ and CO_2-concentration changes between the reference and the measuring stream were recorded by an infrared gas analyzer (BINOS 1 type Leybold-Heraeus, Köln, FRG). The flow rate was 15 l h^{-1}.

The very weak 535-nm light needed to measure light-scattering changes was recorded by a photomultiplier, which was protected against actinic light (half band width 625 to 675 nm, RG 610 (Schott, Mainz, FRG) + KG 65 (Balzers, Liechtenstein) + heat filter (Toshiba, Japan)) by a suitable filter combination (Corning filter 9780, 9782, Corning, New York, N.Y., USA; BG 18, Schott, Mainz, FRG). The difference between light scattering in light and in dark was taken as a relative measure for the energization of the photosynthetic apparatus (Heber 1969; Kobayashi et al. 1982).

Standard series of measurements were performed in five different gas mixtures; air (21% O_2) +380 µl l^{-1} CO_2; air+500 µl l^{-1} CO_2; air−CO_2; 2% O_2−CO_2; and 2% O_2+500 µl l^{-1} CO_2. Light (5 min) was followed by darkness (5 min), during which the gas composition was changed. During one series of measurements the epidermis-free leaf fragments lost about 10% of their cellular water, as determined by weighing at the end. When gas-exchange and light-scattering data were plotted as a function of water loss, it was assumed that dehydration was linear with time between the first and the second weighing procedure. Between the measurements, leaves lost water in air of a relative humidity between 40 and 55% at 20° C. Relative water contents (RWC) were calculated according to

$$RWC = (w_a - w_d)/(w_f - w_d),$$

where w_a, w_f and w_d are the weights of leaves after partial water loss, of fully turgescent leaves and of dried leaves respectively. Dry weights (w_d) were determined after 24 h at 95–100° C.

Osmotic potentials were calculated from the freezing-point depression of cell saps as determined with a Knauer semi-micro osmometer type M 2120 (Knauer, Berlin).

Results

Figure 1 shows simultaneous recordings of CO_2 gas exchange (*A*) and of the apparent absorbance (*B*) of a leaf, whose lower epidermis had been removed, during a standard series of measurements (see Material and methods) under different atmospheric conditions. Changes in the apparent absorbance of a green measuring beam by a leaf illuminated with (red) actinic light gives information on both the light-generated membrane potential and on the transthylakoid proton gradient which are believed to drive light-dependant ATP synthesis in chloroplasts. The membrane potential gives rise to the rapid absorption changes seen on illumination and darkening. They peak at 518 nm but are still clearly visible at 535 nm (for review see Junge 1977).

Slow changes in the apparent absorbance are caused by changes in light scattering which indicate formation or breakdown of the transthylakoid proton gradient. Both components of the signal are kinetically distinguishable as can be seen from the traces of Fig. 1.

When the leaf was illuminated in air containing CO_2, massive CO_2 uptake replaced the respiratory CO_2 production observed in the dark. The highest assimilation rate was found in 2% O_2 + 500 µl l^{-1} CO_2. Under these conditions, photorespiration does not decrease net CO_2 uptake in the light. In a stream of air from which CO_2 had been removed, respiratory CO_2 production was decreased in the

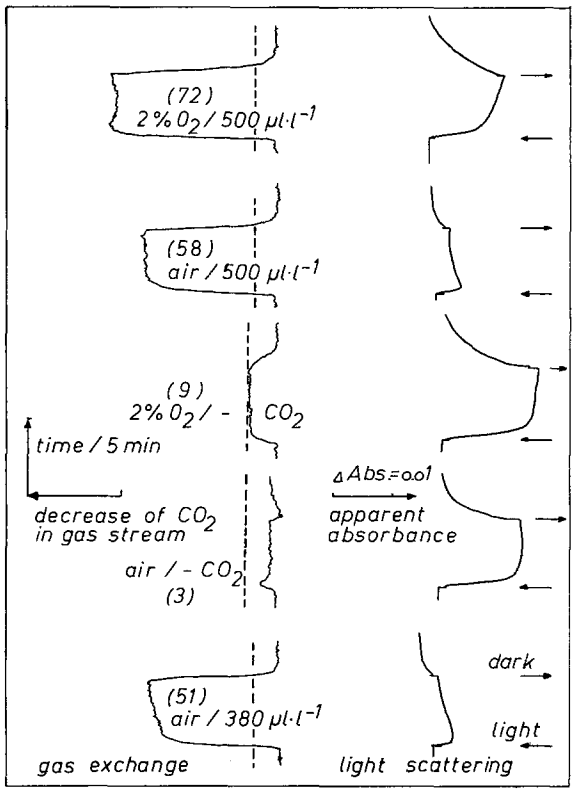

Fig. 1. Simultaneous recordings of light scattering and CO_2 gas exchange by a *Primula* leaf, whose lower epidermis had been removed, in five different gas mixtures at approx. 100% RWC. It should be noted that the sensitivity of the BINOS gas analyzer to different CO_2 concentrations is not linear. Dashed line referes to the signal obtained in the absence of the leaf; to the left of dashed line: net CO_2 uptake; to the right of dashed line: net CO_2 release. Numbers in brackets: difference between CO_2 gas exchange in dark and light in µmol CO_2 mg^{-1} Chlorophyll h^{-1}

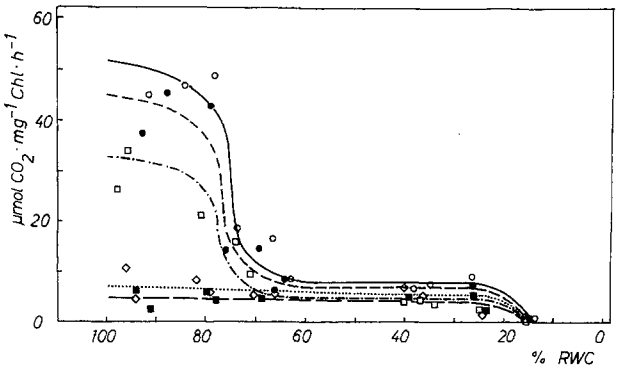

Fig. 2. CO_2 uptake by intact leaves in ○, 2% O_2+500 µl l^{-1} CO_2; ●, 21% O_2+500 µl l^{-1} CO_2; □, 21% O_2+380 µl l^{-1} CO_2; ◇, 2% O_2−CO_2; and ■, 21% O_2−CO_2 as a function of water loss (0% RWC = complete water loss). Light intensity: 35 W m^{-2}; the rate of CO_2 production by respiration in the dark represented by zero on the ordinate. *Chl*, chlorophyll

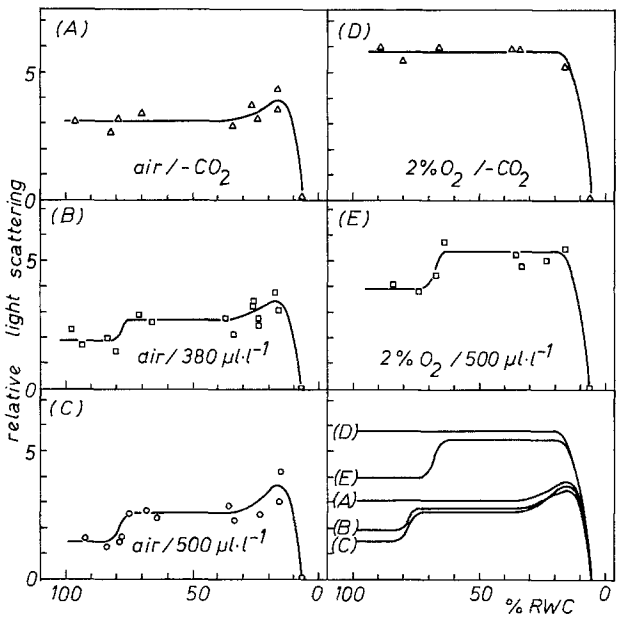

Fig. 3A–E. Light scattering by intact leaves as a function of RWC in different gas mixtures (**A–E**); in **F** the curves of **A** to **E** are combined to show the effects of alterations in gas composition. Light intensity: 35 W m^{-2}

light, and it was almost completely suppressed when the O_2 concentration was reduced to 2%.

The slow light-scattering changes and the fast electrochromic shifts between light and dark were large in the absence of CO_2 and in low O_2; they were small in the presence of 21% O_2 (air), when CO_2 was also available. This indicates the decrease of thylakoid energization (or of the proton motive force) during rapid ATP consumption in photosynthesis (Köster and Heber 1982; Kobayashi et al. 1982). The decrease in energization observed in 21% O_2 compared with that in 2% O_2 is interpreted to be caused by photorespiratory energy consumption in 21% O_2. It is often more clearly seen that in the experiments of Fig. 1 (compare Fig. 3 and Heber 1969).

In Fig. 2, the CO_2 gas exchange of intact *Primula* leaves is shown as a function of the relative water content (RWC). Between 100 and about 75% RWC, photosynthetic gas exchange followed the pattern shown in Fig. 1. Below 75% RWC, the CO_2 fixation was diminished in all gas mixtures containing CO_2. Near 20% RWC, the transition from dark to light no longer led to observable changes in CO_2 gas exchange, and both in the dark and in the light only CO_2 production by dark respiration could be seen (see also Fig. 9).

Light-dependent changes in light scattering measured simultaneously with gas exchange are shown in Fig. 3. In the absence of CO_2 and in the presence of low oxygen concentration, fully turgescent leaves exhibited large scattering changes

as a result of illumination (Fig. 3D). The light-scattering changes were small when CO_2 (380 and 500 µl l^{-1}) was present and the oxygen concentration was high (Fig. 3B, C). In 21% O_2-CO_2 and in 2% O_2+CO_2 the changes were between both extremes. The well-known decrease of light scattering by CO_2 (Heber 1969) was clearly visible between 80% and 100% RWC. Below 80% RWC, light scattering by leaves in atmospheres containing CO_2 increased to the levels which were close to those observed in the absence of CO_2. It should be noted that this increase coincided with stomatal closure as apparent from the gas exchange measurements of Fig. 2. In water-stressed intact leaves, light scattering in 21% O_2+380 µl l^{-1} or 500 µl l^{-1} CO_2 was similar to that observed in air from which CO_2 had been removed. Likewise, light scattering in 2% O_2+500 µl l^{-1} CO_2 was not much different from that measured in 2% O_2 in the absence of CO_2. Below 30% RWC, light scattering tended to increase before extreme water loss prevented energization of the photosynthetic apparatus in the light. Generally, light scattering remained high even in severely wilted leaves until RWC values dropped below 20%. After such extreme dehydration, *Primula* leaves did not recover when supplied with water.

The removal of the lower epidermis of the leaves (which, for reasons of simplification, will be called stripped leaves) led to an altered response of CO_2 gas exchange and light scattering to water stress. This is shown in Figs. 4 and 5. Photosynthesis gradually declined as the stripped leaves lost water. The differences in gas exchange between the different gas mixtures were maintained in stripped leaves until photosynthesis was completely inhibited at RWC values below 20% (Fig. 4). The highest rates of photosynthesis were observed in 2% O_2+500 µl l^{-1} CO_2. Oxygen inhibition of photosynthesis and photorespiratory processes decreased net CO_2 uptake in 21% O_2+380 or 500 µl l^{-1} CO_2. The drastic restriction of CO_2 assimilation observed with intact leaves at RWC values below 80% (Fig. 2) was not seen in stripped leaves. However, as in intact leaves, light-dependent changes in the CO_2 gas exchange were absent below 20% RWC.

While photosynthetic CO_2 uptake by stripped leaves was inhibited during progressive dehydration, CO_2 lost the ability to lower the energy status of the water-stressed leaves. This is shown in Fig. 5 where the ratio of light scattering observed in the presence of 380 µl l^{-1} CO_2 to that measured in its absence is plotted as a function of RWC. Between 100 and 60% RWC, the ratio remained es-

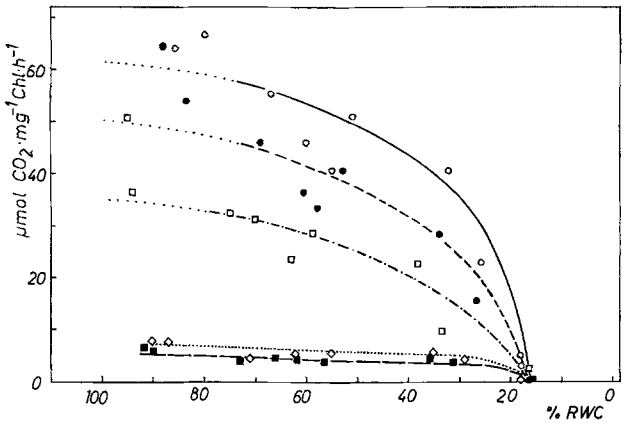

Fig. 4. CO_2 uptake by 'stripped' leaves in ○, 2% O_2+500 µl l^{-1} CO_2; ●, 21% O_2+500 µl l^{-1} CO_2; □, 21% O_2+380 µl l^{-1} CO_2; ◇, 2% O_2-CO_2; ■, 21% O_2-CO_2. See also legend to Fig. 2

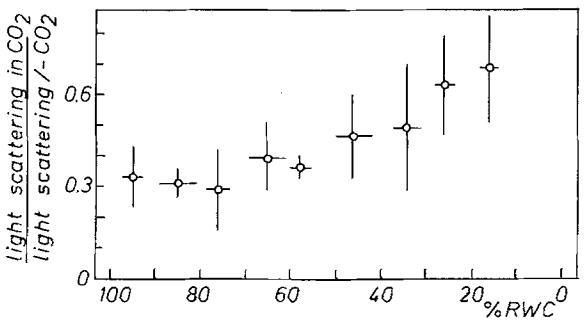

Fig. 5. Effect of water stress on the ratio of light scattering with and without CO_2 in the gas stream. All points represent the mean±SD (N=3–5). The ascent of the curve was because of an increase of the light-scattering change in air+380 µl l^{-1} CO_2

sentially unchanged. Further water loss increased it significantly from 0.3 to 0.7.

In Fig. 6, CO_2 assimilation by stripped leaves is shown as a function of oxygen partial pressure at approx. 100 and 25% RWC. The response of photosynthesis to oxygen and to anaerobiosis was similar in turgescent leaves and in water-stressed leaves. Net photosynthesis was maximal at 2% O_2. It was suppressed in nitrogen+500 µl l^{-1} CO_2 and by high O_2 concentrations.

In Fig. 7 the relative increase in CO_2 uptake following a transition from 21 to 2% O_2 is plotted against the RWC of stripped leaves. Between 100 and about 50%, the ratio of photosynthesis in 2% O_2 to that measured in 21% O_2 remained essentially constant. Assimilation increased by about 15% when the O_2 content of an atmosphere containing 500 µl l^{-1} CO_2 was decreased from 21 to 2%. Below 50% RWC, it increased more.

A double reciprocal plot of the light dependence of photosynthesis in 2% O_2 at three different

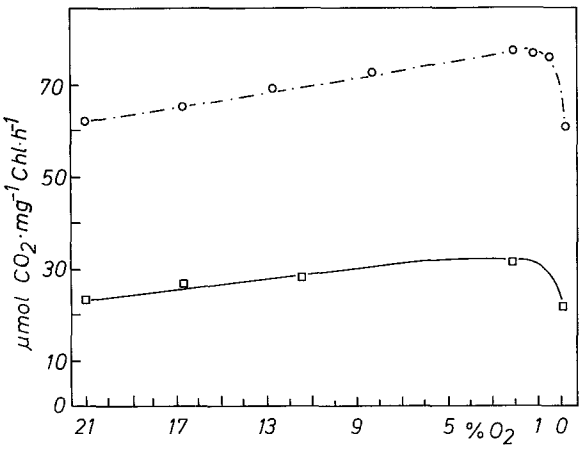

Fig. 6. CO_2 uptake by a *Primula* leaf, from which the lower epidermis was removed at different O_2 concentrations. Light intensity: 35 W m^{-2}; CO_2 content: 500 µl l^{-1}. o, 100% RWC; □, 25% RWC. *Chl*, chlorophyll

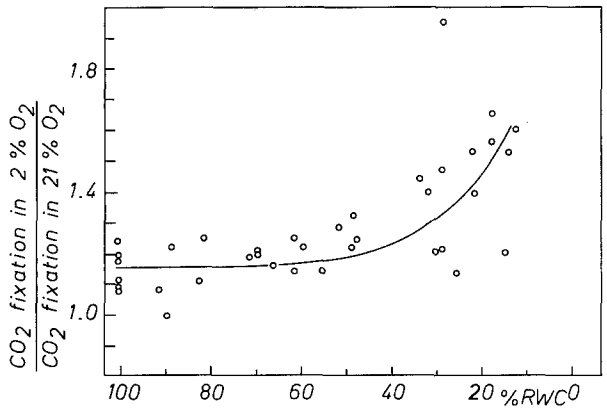

Fig. 7. Percent increase of CO_2 uptake by stripped *Primula* leaves as measured during the transition from 21 to 2.1% O_2 as a function of RWC. Light intensity: 35 W m^{-2}; CO_2 concentration in the gas stream: 500 µl l^{-1}

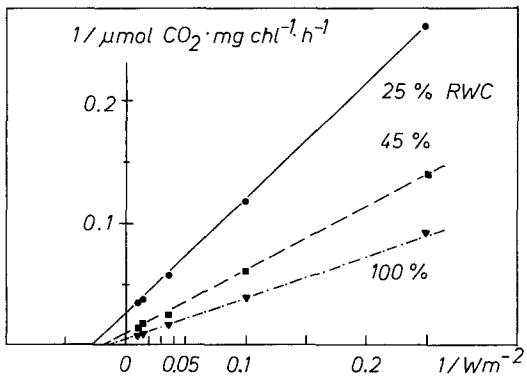

Fig. 8. Light intensity dependence of CO_2 fixation by a stripped *Primula palinuri* leaf at approximately 100% (▼), 45% (■), 25% (●) RWC as double reciprocal plot

water states of a leaf is shown in Fig. 8. The maximum rate of CO_2 fixation as well as the light intensities that led to half maximal photosynthesis were reduced by water stress (Table 1).

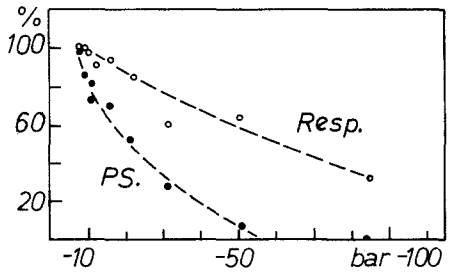

Fig. 9. Dark respiration and photosynthetic CO_2 uptake of a stripped *Primula* leaf under water stress as a function of water potential: the rates at 100% RWC (=7.5 bar osmotic potential) were set to 100%. Light intensity: 35 W m^{-2}; CO_2 concentration: 380 µl l^{-1} CO_2

Table 1. Maximal CO_2 fixation rate and light intensities that lead to half-maximum activities of the photosynthetic apparatus of *Primula* leaves at 100, 45, 25% RWC in 2% O_2 + 500 µl l^{-1} CO_2 as taken from the double reciprocal plot in Fig. 8. k_1 = light intensity; f_{max} = maximum rate of CO_2-fixation

RWC (%)	k_1 (W m^{-2})	f_{max} (µmol CO_2/mg^{-1} chl·h^{-1})
100	71	200
45	48	91
25	35	37

Figure 9 compares the sensitivity of dark respiration and photosynthesis to increasing osmotic potentials of the leaf cell sap. At 100%, RWC *Primula palinuri* leaves usually had an osmotic potential of about 7.5 bar. At 20 bar, photosynthesis was reduced to 50% of the control; at 50 bar, inhibition was nearly complete. In contrast, dark respiration was reduced by only one half at 60 bar and there was still respiratory activity left at 80 bar.

Discussion

1. CO_2 fixation. When initially water-saturated leaves of *Primula palinuri* lost more than 20% of their cellular water, closure of the stomata lead to decreased CO_2-fixation rates (Fig. 2). Experiments with stripped leaves made it unlikely that the drastic response observed was caused by a primary inhibition of photosynthesis by water stress. Rather, the capacity of the photosynthetic apparatus for CO_2 reduction was only gradually diminished (Fig. 4). The removal of the lower epidermis by stripping reduced the weight of the leaves by about 6% and the chlorophyll content by about 0.1%. Apparently, there was no substantial destruction of mesophyll cells during stripping (Bertsch 1969).

If the assimilation rate of leaves which no longer exhibit a substantial resistance to CO_2 diffusion (e.g. stripped leaves) is termed A_0, and that of leaves after a stress treatment which limits CO_2 fixation is termed A, $(A_0-A)/A_0$ is a measure of stomatal limitations on photosynthesis (Farquhar and Sharkey 1982). On the basis of the results shown in Figs. 2 and 4, one can compute that at an RWC of 80% the still-open stomata reduce photosynthesis by only about 20%. The values obtained for 70, 50 and 30% RWC are comparable and indicate an inhibition of photosynthesis by stomata of about 90%.

Responses of CO_2 fixation to reduced water potentials similar to those observed in stripped leaves of *P. palinuri* (Fig. 4) were also described by Kaiser et al. (1981a, b), Kaiser and Heber (1981) and Kaiser (1982), who bypassed stomatal diffusion resistance by using thin leaf slices suspended in media of different osmotic potentials. In spinach, 50% inhibition of oxygen production or $^{14}CO_2$ fixation in the light could be observed at osmotic potentials as high as 25 and 30 bar, and complete inhibition at about 50 bar (Kaiser et al. 1981b). Cell saps of well watered *P. palinuri* and spinach leaves have comparable osmotic potentials (7–11 bar). Relating the osmotic inhibition of photosynthesis to the total protoplast volume of vacuum-infiltrated leaf discs as measured by the difference between ^3H_2O-labeled space and ^{14}[C]sorbitol-labeled space, Kaiser (1982) found a similar response of photosynthesis to water loss in xerophytes, hygrophytes and mesophytes. Complete inhibition of CO_2 fixation was observed at 20% relative protoplast volume. These results, which were obtained in an osmotic system, are very similar to our data with *Primula* leaves subjected to water stress by exposure to dry air. Also, when leaf slices of *Commelina africana, Nerium oleander* and *Spinacia oleracea* were gradually dried in air, severe inhibition of CO_2 fixation was measurable at 60% RWC and was complete at 20% RWC (data not shown).

2. Thylakoid energization. Changes in light scattering on illumination and darkening of chloroplasts are related to the formation and decay of the transthylakoid proton gradient (Köster and Heber 1982). In the absence of amines such as NH_4Cl, increases in ATP/ADP ratios accompanied increases in light scattering not only in chloroplasts but also in leaves (Kobayashi et al. 1982). In Fig. 3, an increase in light scattering was observed when stomatal closure decreased photosynthesis. This may indicate increased leaf phosphorylation potentials as a consequence of decreased ATP consumption. Light scattering remained high in intact leaves between 80 and 20% RWC. This indicates a high energization of the photosynthetic apparatus even during extensive wilting. In stripped leaves illuminated in air, light scattering increased during dehydration while photosynthesis declined (Fig. 5). It was always high when ATP demand was low in the absence of CO_2 (Fig. 1).

A comparison of the light scattering data (Fig. 3) with CO_2 reduction by stripped *Primula* leaves (Fig. 4) indicates that leaf energization, which is based on electron transport, is more resistant to water stress than CO_2 reduction. The data in Fig. 8 are consistant with the view that dark reactions limit photosynthesis under water stress. The light intensities needed for half-saturation of CO_2 reduction decreased with increasing water stress. Apparently, formation of the light products NADPH and ATP limited photosynthesis less than the dark reactions did. Still, it should be noted that high thylakoid energization at low water potentials, as shown in Fig. 3, does not necessarily indicate a particular resistance of ATP synthesis to water stress. Inhibition of ATP synthase (Younis et al. 1979) might decrease the supply of ATP without decreasing the proton gradient which gives rise to increased light scattering.

3. Energy dissipation and CO_2 turnover by photorespiration. In intact leaves, a large difference between the light-scattering levels in air and in 2% O_2 were maintained at RWC values ranging between 80 and 30%. Although stomata are closed in these leaves it must be emphasized that this does not stabilize intercellular oxygen levels during large changes in the external oxygen concentration. Closed stomata effectively control CO_2 fluxes, because diffusion gradients for CO_2 are small. They exert poor control on oxygen diffusion because of much larger diffusion gradients when the gas composition is changed from 21 to 2% O_2 or vice versa. In 2% O_2, photorespiration is known to be largely inhibited, while significant photorespiration can proceed in 21% O_2. The complete oxidation of a sugar or sugar phosphate to CO_2 does not yield but consumes energy in the form of ATP and NADPH. The CO_2 liberated by photorespiration into the intercellular space of water-stressed leaves can be reassimilated. This process also consumes energy. The difference in energization of water-stressed leaves in 21 and 2% O_2, therefore, indicates energy dissipation by CO_2 turnover.

While CO_2 fixation by stripped leaves was decreased with increasing water stress both in 21%

$O_2 + 500\ \mu l\ l^{-1}$ CO_2 and in 2% $O_2 + 500\ \mu l\ l^{-1}$ CO_2, inhibition was less pronounced in 2% O_2 at very low water potentials (Fig. 7). This indicates increased oxygenation of ribulose-1,5-bisphosphate (RuBP) relative to carboxylation under very severe water stress, although the solubility of O_2 is decreased more than that of CO_2 when solutes such as KCl are concentrated by loss of water (May 1967). Increased oxygenation of RuBP under heat stress has been reported by Laing et al. (1974). Vapaavuori and Valanne (1982) failed to observe changes in the ratio of oxygenase to carboxylase activity of RuBP carboxylase (from *Salix* spp.) which was inhibited at low water potentials. In wilted leaves, the rate of intercellular CO_2 turnover is determined by the intercellular CO_2 concentration and by the effectiveness of the cells to produce CO_2 by photorespiration and to refix it by photosynthesis. Dark respiration which is very resistant to water stress (Fig. 9) could also be a potential source for CO_2, if the suppression of dark respiration by light is relieved under conditions of water stress.

In a simplified calculation, it is possible to get an impression of the role photorespiration plays in dissipating light energy by permitting CO_2 turnover in wilted leaves. Table 2 shows CO_2 turnover rates in wilted leaves as calculated on the assumption (not really correct) that the CO_2 dependence of photosynthesis of a leaf is adequately described by Michaelis-Menten kinetics. In 21% oxygen, half-maximum photosynthesis of spinach protoplasts was observed at CO_2 concentrations between 8 and 12 µM or 220 to 320 $\mu l\ l^{-1}$ in the gas phase (Kaiser and Heber 1983). If 300 $\mu l\ l^{-1}$ is used together with the values for the maximum rate of CO_2-fixation for photosynthesis in 21% O_2 (data not shown, but similar to those of Table 1), CO_2-turnover rates can be calculated for the different CO_2 compensation concentrations listed in Table 2. Plaut and Bravdo (1973) and Lawlor (1976) have measured CO_2 compensation concentrations in water-stressed leaves. At an RWC of about 50%, the CO_2 compensation concentration of spinach leaves was increased by about 30% compared with that of turgescent leaves (Plaut and Bravdo 1973). In wheat, Lawlor (1976) observed a similar increase; at 23% RWC, the CO_2 compensation concentration even reached 300 $\mu l\ l^{-1}$. For the different RWC values listed in Table 2, CO_2 turnover was therefore calculated for three different assumptions of intercellular CO_2 concentrations.

Since production of 1 mol CO_2 in the photorespiratory carbon cycle consumes about twice as much ATP (and NADPH) (Heber and Krause 1980) as its refixation in photosynthesis, ATP turnover rates can also be estimated. They turn out to be very appreciable (Table 2). It should be noted that alternative calculations not based on leaf cell parameters as in Table 2 but on known k_{mCO_2} (17 µM CO_2; Badger and Andrews 1974), k_{IO_2} (350 µM O_2; Badger and Andrews 1974) and V_{max} (1,000 µmol CO_2 mg^{-1} chl h^{-1}; Lilley and Walker 1975) values for ribulose bisphosphate carboxylase activity yield turnover rates which were even higher than those given in Table 2.

The CO_2 turnover indicated in Table 2 permits dissipation of light energy and may be one of the means to prevent photooxidative damage to the photosynthetic apparatus when net photosynthesis is not possible.

Table 2. Internal CO_2 turnover (and associated ATP consumption) during water stress as calculated by $v = v_{max}/(1 + k_m\ s^{-1})$. v is the turnover rate in µmol CO_2 mg chl$^{-1} \cdot h^{-1}$, v_{max} is derived from the f_{max} values given in Table 1 which were corrected for the 15% inhibition of photosynthesis observed when 2% O_2 + 500 $\mu l\ l^{-1}$ CO_2 were substituted by 21% $O_2 + 500\ \mu l\ l^{-1}$ CO_2. Corrected values were extrapolated from 500 $\mu l\ l^{-1}$ CO_2 to saturating CO_2. k_m is the CO_2 concentration (300 $\mu l\ l^{-1}$) at which half-maximum rates of photosynthesis were observed in spinach protoplasts at 20° C and 0.28 mM O_2 (=21% O_2). The calculations of internal ATP turnover were based on the assumption that photosynthetic fixation of 1 CO_2 consumes 3 ATP and photorespiratory production of 1 CO_2 consumes 6 ATP

RWC (%)	Compensation concentration ($\mu l\ l^{-1}$)	v_{CO_2}	v_{ATP}
		(µmol mg^{-1} chl h^{-1})	
100	30	25	225
	50	39	351
	80	57	513
45	50	18	162
	80	26	234
	150	41	369
25	50	8	72
	150	20	180
	300	30	270

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References

Badger, M.R., Andrews, T.J. (1974) Effects of CO_2, O_2 and temperature on a high-affinity form of ribulose diphosphate carboxylase-oxygenase from spinach. Biochem. Biophys. Res. Commun. **60**, 204–210

Bertsch, A. (1969) Der Diffusionswiderstand der Spaltöffnungen: ein Vergleich des CO_2-Gaswechsels von Blättern mit und ohne Epidermis. Planta **87**, 102–109

Farquhar, G.D., Sharkey, T.D. (1982) Stomatal conductance and photosynthesis. Annu. Rev. Plant Physiol. **33**, 317–345

Heber, U. (1969) Conformational changes of chloroplasts induced by illumination of leaves in vivo. Biochim. Biophys. Acta **180**, 302–319

Heber, U., Krause, G.H. (1980) What is the physiological role of photorespiration. Trends Biochem. Sci. **5**, 32–34

Junge, W. (1977) Membrane potentials in photosynthesis. Annu. Rev. Plant Physiol. **28**, 503–536

Kaiser, G., Heber, U. (1983) Photosynthesis of leaf cell protoplasts and permeability of the plasmalemma to some solutes. Planta **157**, 462–470

Kaiser, W.M., Kaiser, G., Schöner, S., Neimanis, S. (1981a) Photosynthesis under osmotic stress. Inhibition of photosynthesis of intact chloroplasts, protoplasts, and leaf slices at high osmotic potentials. Planta **153**, 430–435

Kaiser, W.M., Kaiser, G., Prachuab, P.K., Wildman, S.G., Heber, U. (1981b) Photosynthesis under osmotic stress. Inhibition of photosynthesis of intact chloroplats, protoplasts, and leaf slices at high osmotic potentials. Planta **153**, 416–422

Kaiser, W.M., Heber, U. (1981) Photosynthesis under osmotic stress. Effect of high solute concentrations on the permeability properties of the chloroplast envelope and on the activity of stroma enzymes. Planta **153**, 423–429

Kaiser, W.M. (1982) Correlation between changes in photosynthetic activity and changes in total protoplast volume in leaf tissue from hygro-, meso-, xerophytes under osmotic stress. Planta **154**, 538–545

Kobayashi, Y., Köster, S., Heber, U. (1982) Light scattering, chlorophyll fluorescence and state of the adenylate system in illuminated spinach leaves. Biochim. Biophys. Acta **682**, 44–54

Köster, S., Heber, U. (1982) Light scattering and quenching of 9-aminoacridine fluorescence as indicators of the phosphorylation state of the adenylate system in intact spinach chloroplasts. Biochim. Biophys. Acta **680**, 88–94

Krause, G.H. (1973) The high-energy state of the thylakoid system as indicated by chlorophyll fluorescence and chloroplast shrinkage. Biochim. Biophys. Acta **292**, 715–728

Laing, W.A., Ogren, W.L., Hageman, R.H. (1974) Regulation of soybean net photosynthetic CO_2-fixation by the interaction of CO_2, O_2 and ribulose-1,5-diphosphate carboxylase. Plant Physiol. **54**, 678–685

Lawlor, D.W. (1976) Water stress induced changes in photosynthesis, photorespiration, Respiration and CO_2 compensation concentration of wheat. Photosynthetica **10**, 378–387

Lilley, R. McC., Walker, D.A. (1975) Carbon dioxide assimilation by leaves, isolated chloroplasts, and ribulose bisphosphate carboxylase from spinach. Plant Physiol. **55**, 1087–1092

May, A. (1967) Löslichkeit von Gasen in Flüssigkeiten. In: Handbuch für Chemiker und Physiker, vol. 1: Makroskopische physikalisch-chemische Eigenschaften, pp. 1203–1232, Lax, E., ed. Springer, Berlin Heidelberg New York

Moldau, H. (1973) Effects of various water regimes on stomatal and mesophyll conductances of bean leaves. Photosynthetica **7**, 1–7

Osmond, C.B. (1981) Photorespiration and photoinhibition, some implications for the energetics of photosynthesis. Biochim. Biophys. Acta **639**, 77–98

O'Toole, J.C., Ozbun, J.L., Wallace, D.H. (1977) Photosynthetic response to water stress in Phaseolus vulgaris. Physiol. Plant. **40**, 111–114

Plaut, Z., Bravdo, B. (1973) Response of carbon dioxide fixation to water stress. Plant Physiol. **52**, 28–32

Settler, T.L., Brun, W.A., Brenner, M.L. (1980) Stomatal closure and photosynthetic inhibition in soybean leaves induced by petiole girdling and pod removal. Plant Physiol. **65**, 884–887

Vapaavuori, E.M., Valanne, N.K.S. (1982) Activities of ribulose-1,5-biphosphate carboxylase-oxygenase in *Salix* sp. during water stress. Photosynthetica **16**, 1–6

Younis, H.M., Bayer, J.S., Govindjee (1979) Conformation and activity of chloroplast coupling factor exposed to low chemical potential of water in cells. Biochim. Biophys. Acta **548**, 328–340

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