

# Nonstomatal Inhibition of Photosynthesis in Sunflower at Low Leaf Water Potentials and High Light Intensities<sup>1</sup>

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## ABSTRACT

The inhibition of photosynthesis at low leaf water potentials was studied in soil-grown sunflower to determine the degree to which photosynthesis under high light was affected by stomatal and nonstomatal factors. Below leaf water potentials of  $-11$  to  $-12$  bars, rates of photosynthesis at high light intensities were insensitive to external concentrations of  $\text{CO}_2$  between 200 and 400 microliters per liter. Photosynthesis also was largely insensitive to leaf temperature between 10 and 30 C. Changes in  $\text{CO}_2$  concentration and temperature had negligible effect on leaf diffusive resistance. The lack of  $\text{CO}_2$  and temperature response for both photosynthesis and leaf diffuse resistance indicates that rates of photosynthesis were not limited by either  $\text{CO}_2$  diffusion or a photosynthetic enzyme. It was concluded that photosynthesis under high light was probably limited by reduced photochemical activity of the leaves at water potentials below  $-11$  to  $-12$  bars.

This work was done to compare the effects of stomatal closure and chloroplast activity on photosynthetic response to low leaf water potentials. In higher plants, decreasing leaf water potentials ( $\psi_w$ ) are followed ultimately by decreasing rates of photosynthesis. The photosynthetic decline is usually attributed to stomatal closure (4, 7, 9, 15, 16, 19, 20), although it has occasionally been suggested that the diffusive resistance of the mesophyll cells increases (9, 16, 18), decreasing the  $\text{CO}_2$  flux from the cell surfaces to the interior of the chloroplasts. Recently, we have shown (5) that chloroplast activity, measured as oxygen evolution in the presence of 88  $\mu\text{M}$  dichloroindophenol as an electron acceptor, is inhibited when the chloroplasts are isolated from sunflower leaves having low  $\psi_w$ . The inhibition parallels that occurring when photosynthesis is measured in intact sunflower leaves under high light intensities and is of a magnitude which could account for the inhibition of photosynthesis *in vivo*. However, stomatal apertures also become progressively smaller as  $\psi_w$  decreases in sunflower (5). Thus, from these data, it was not possible to distinguish whether stomatal closure or chloroplast activity limited photosynthesis.

At high light intensities, rates of photosynthesis are often considered to be limited by either the diffusive resistance to  $\text{CO}_2$  entry into the leaf (9) or the activity of the carboxylating enzymes associated with  $\text{CO}_2$  fixation (8, 13, 14). For limitation due to  $\text{CO}_2$  diffusion, the rate of photosynthesis ( $P$ ,  $\text{g sec}^{-1} \text{cm}^{-2}$  of leaf

area measured from the leaf outline) responds to changes in the external concentration of  $\text{CO}_2$  according to (9):

$$P = \frac{-(C_c - C_a)}{\Sigma r} \quad (1)$$

where  $C_c$  is the effective concentration of  $\text{CO}_2$  at the site of  $\text{CO}_2$  fixation within the chloroplasts ( $\text{g cm}^{-3}$ ),  $C_a$  is the  $\text{CO}_2$  concentration of the bulk air, and  $\Sigma r$  is the diffusive resistance of the entire diffusion pathway for  $\text{CO}_2$ : the boundary layer around the leaf, the gas phase within the leaf (including the stomatal resistance), and the so-called mesophyll resistance to  $\text{CO}_2$  diffusion ( $\text{sec cm}^{-1}$ ). According to equation 1, if the inhibition of photosynthesis at low  $\psi_w$  is associated with an increased diffusive resistance for  $\text{CO}_2$ , it should be possible to overcome the inhibition by increasing the external concentration of  $\text{CO}_2$ . This approach was used in the following work at  $\text{CO}_2$  concentrations around those occurring naturally in air (300  $\mu\text{l/liter}$ ).

## MATERIALS AND METHODS

Sunflower (*Helianthus annuus* L. var. Russian Mammoth) plants were grown in soil in a constant environment chamber (temperature,  $29 \pm 0.5$  C day and  $23 \pm 0.5$  C night; relative humidity,  $70 \pm 5\%$ ; light intensity,  $0.15 \text{ cal cm}^{-2} \text{ min}^{-1}$  (fluorescent); photoperiod, 14 hr). After 5 weeks, they had reached a height of 45 cm and various leaf water potentials were produced by withholding water from the soil for as long as 4 days.

Rates of photosynthesis and transpiration were measured with an infrared gas analyzer as previously described (5), except that determinations were made with recently fully expanded, single leaves while intact on the plant in order to avoid variability which occurred when the whole shoot was used. (Conditions in the assimilation chamber: air temperature,  $25 \pm 0.25$  C unless otherwise specified; leaf temperature was measured with a thermocouple inserted within the leaf blade; relative humidity,  $77 \pm 2\%$ ; wind speed,  $1.7 \text{ m sec}^{-1}$ ;  $\text{CO}_2$  concentration,  $300 \pm 1 \mu\text{l/liter}$  unless otherwise specified.) Incandescent light was filtered through 10 cm of water and supplied at intensities measured by a Moll thermopile at leaf height.

Immediately following measurements of photosynthesis,  $\psi_w$  was measured with an excised leaf thermocouple psychrometer (6). The leaf was sampled by removing a disc from interveinal tissue which had been washed with distilled water and permitted to dry before measurements of photosynthesis. Isopiestic technique was used (2, 6), psychrometer chambers were coated with melted and resolidified petrolatum (3), and measurements were corrected for heat of respiration (1).

Because photosynthesis and transpiration were measured simultaneously in the assimilation chamber, diffusive resistances to  $\text{CO}_2$  entry into the leaf could be calculated. The resistance of the boundary layer outside the leaf ( $r_a$ ) was determined from the

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thermal resistance to heat transfer between the leaf and the chamber air, which in turn was calculated from measurements of the sensible heat flux, and the air and leaf temperature within the assimilation chamber (17). The diffusive resistance of the gas phase within the leaf ( $r_l$ ) was calculated from (9):

$$r_l = \frac{-1.7 (C_i' - C_a')}{T} - r_a \quad (2)$$

where  $T$  is the transpirational flux ( $\text{g sec}^{-1} \text{cm}^{-2}$  of leaf area measured from the leaf outline),  $C_i'$  is the concentration ( $\text{g cm}^{-3}$ ) of water vapor at the evaporating surface (assumed to be saturated at the temperature of the leaf),  $C_a'$  is the concentration of water vapor in the external air, and the coefficient 1.7 converts diffusive resistances for water vapor to those for  $\text{CO}_2$ .

## RESULTS

In order to determine the most satisfactory method of calculating the rate of total photosynthesis, rates of respiration in the dark were compared with rates of respiration in the light. Figure 1 shows that rates of respiration in the light measured as  $\text{CO}_2$  release in  $\text{CO}_2$ -free air were slightly higher than rates of dark respiration in well watered plants and slightly lower than rates of dark respiration in desiccated plants. This measurement probably underestimated the amount of respiration in the light because of recycling of  $\text{CO}_2$  within the leaf. Measurements of respiration in the light were also made in  $\text{O}_2$ -free air (as the rate of net photosynthesis in  $\text{N}_2$  and  $300 \mu\text{l/liter CO}_2$  minus the rate of net photosynthesis in air) and averaged  $3.3 \text{ mg hr}^{-1} \text{dm}^{-2}$  of leaf area in well watered plants,  $2.5 \text{ mg hr}^{-1} \text{dm}^{-2}$  in plants having  $\psi_w$  of  $-10$  bars, and  $1.5 \text{ mg hr}^{-1} \text{dm}^{-2}$  in plants with  $\psi_w$  of  $-18$  bars. The measurements made in  $\text{O}_2$ -free air should have inhibited respiration in the light (19) and probably provided better estimates of respiration in the light than those measured in  $\text{CO}_2$ -free air. However, since rates of respiration in the light in  $\text{O}_2$ -free air were approximately the same as rates of dark respiration at  $\psi_w$  below  $-10$  bars, and since the effect of long exposures to  $\text{O}_2$ -free air in the following experiments would be uncertain, total photosynthesis was calculated as the rate of net photosynthesis in air plus the rate of dark respiration.

Rates of total photosynthesis were lower in desiccated leaves than in the same leaves when well watered at all light intensities (Fig. 2). The data are similar to those found earlier with whole plants (5), except that photosynthesis is greater for a given light intensity with the single leaves. Although light saturation appeared to occur regardless of  $\psi_w$ , it occurred at higher light intensities when  $\psi_w$  was high than when  $\psi_w$  was low. All subse-

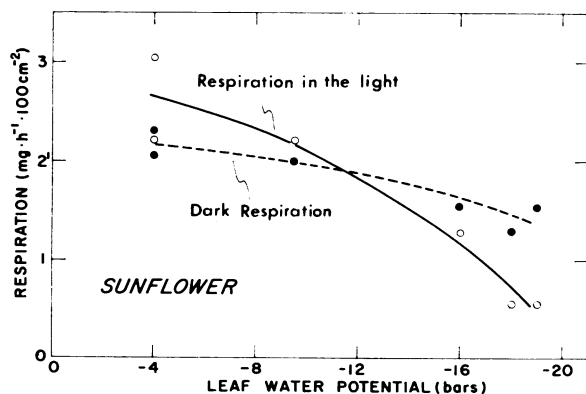


FIG. 1. Rates of respiration in the dark and in the light at saturating light intensities in intact sunflower leaves having various water potentials. Respiration in the light was measured in  $\text{CO}_2$ -free air.

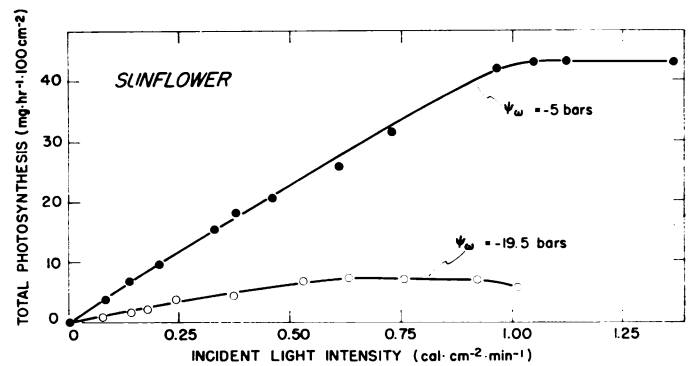


FIG. 2. Rates of total photosynthesis at various light intensities in an intact sunflower leaf. Photosynthesis was measured when the plant was well watered and the measurement was repeated after water had been withheld from the plant for 3 days.

quent measurements of photosynthesis in high light were made at saturation ( $1.4$ – $1.6 \text{ cal cm}^{-2} \text{ min}^{-1}$  for well watered plants,  $1.0$ – $1.4 \text{ cal cm}^{-2} \text{ min}^{-1}$  for plants having  $\psi_w$  of  $-10$  to  $-14$  bars, and  $0.7$ – $0.9 \text{ cal cm}^{-2} \text{ min}^{-1}$  for plants having  $\psi_w$  below  $-14$  bars). In order to estimate the photochemical activity of leaves, measurements of photosynthesis were made at low (limiting) light intensities on the linear portion of the photosynthesis-intensity curve ( $0.20 \text{ cal cm}^{-2} \text{ min}^{-1}$  and less).

For photosynthesis that is limited by the rate of  $\text{CO}_2$  diffusion, doubling the  $\text{CO}_2$  concentration difference between the fixation sites and the external air should approximately double the rate of photosynthesis. Concentration  $C_c$  (equation 1) was considered to be zero, since measurements in the light in  $\text{O}_2$ -free air indicated that the  $\text{CO}_2$  compensation point was less than  $1 \mu\text{l/liter}$  and was independent of  $\psi_w$  (19). In well watered sunflower under high light, doubling the  $\text{CO}_2$  gradient led to an increase in the rate of total photosynthesis, but not to the extent expected if only  $\text{CO}_2$  concentration limited photosynthesis (Fig. 3A). After desiccation to  $\psi_w$  of  $-11$  to  $-12$  bars, the response of photosynthesis to  $\text{CO}_2$  concentration was almost negligible. After desiccation to  $\psi_w$  of  $-18$  to  $-19$  bars, there was no response of photosynthesis to  $\text{CO}_2$  concentration. The resistance,  $r_a$ , was negligibly small ( $0.11 \text{ sec cm}^{-1}$ ) and constant for all  $\psi_w$  because of similar positioning of the leaf and stirring of the air during each measurement. Carbon dioxide concentration had a negligible effect on  $r_l$  at all  $\psi_w$  (Fig. 3B).

As opposed to the effects of  $\text{CO}_2$  concentration, temperature should have little effect on total photosynthesis if  $\text{CO}_2$  diffusion is limiting. In well watered plants at high light, the rate of photosynthesis increased 1.6-fold when leaf temperature increased from  $15$  to  $25 \text{ C}$  (Fig. 4A). Under desiccated conditions, temperature had no effect on photosynthesis, except at  $30 \text{ C}$  and  $\psi_w$  of  $-18$  bars. In general, resistance  $r_l$  did not change with temperature (Fig. 4B), except at  $30 \text{ C}$  and  $\psi_w$  of  $-18$  bars. The increase in  $\Sigma r$  as a result of the increased  $r_l$  was not sufficient to account for the drop in photosynthesis noted at  $30 \text{ C}$  and  $\psi_w$  of  $-18$  bars.

Under low light intensities, where the rate of photochemical reactions limits the rate of photosynthesis (13, 14), total photosynthesis in sunflower was insensitive to  $\text{CO}_2$  concentration and temperature (Fig. 5). As leaf water potential dropped, photochemical activity measured at limiting light intensities was inhibited by the same amount as the rate of photosynthesis at high light intensities (Fig. 6).

## DISCUSSION

At external  $\text{CO}_2$  concentrations around those which occur naturally in air, rates of photosynthesis did not respond to

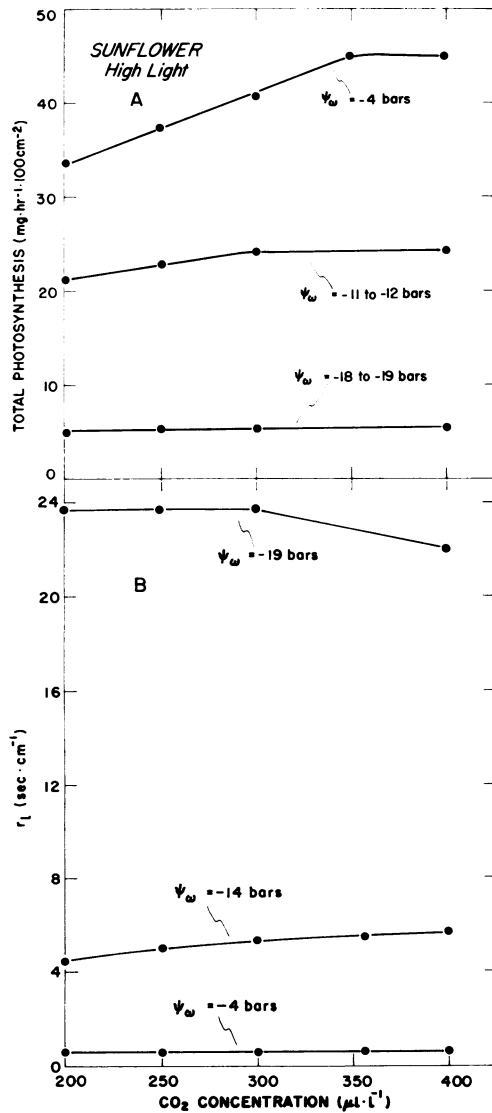


FIG. 3. Rates of total photosynthesis (A) and leaf diffusive resistances (B) at various external concentrations of CO<sub>2</sub> under high (saturating) light and at three leaf water potentials.

changes in external CO<sub>2</sub> concentrations when  $\psi_w$  was below  $-11$  bars. The lack of response could not be attributed to compensating changes in  $r_l$ . Others have also observed that  $r_l$  is insensitive to CO<sub>2</sub> under high light when concentrations are varied around 300  $\mu\text{l/liter}$  (9, 10), presumably because stomatal apertures are unaffected (under low light, however, I observed that  $r_l$  became sensitive to CO<sub>2</sub> in sunflower, which has also been noted in other species [9, 10]). Resistance  $r_a$  was not altered by CO<sub>2</sub>, and the physical resistance between the surface of the mesophyll cells and the interior of the chloroplasts has been shown to be constant over a range of CO<sub>2</sub> concentrations as well (19). In any case, both  $r_a$  and this latter resistance would have been required to change more than 10-fold to account for the CO<sub>2</sub> data. Changes of this magnitude are unlikely. Thus, photosynthesis was clearly not limited by the rate of CO<sub>2</sub> diffusion when  $\psi_w$  was below  $-11$  bars in sunflower and light intensities were high.

Above  $\psi_w$  of  $-11$  bars, photosynthesis was affected by external CO<sub>2</sub> concentrations and, therefore, CO<sub>2</sub> diffusion may have been a factor in the response of photosynthesis to this level of desiccation. Nevertheless, photosynthesis was affected

less by external CO<sub>2</sub> levels than would have been the case if CO<sub>2</sub> diffusion were solely limiting. Thus, photosynthesis still appeared to be limited in part by a nondiffusional factor. This is supported by the temperature response of the nondesiccated plants, which represented a  $Q_{10}$  of 1.6 for photosynthesis between 15 and 25 C. A  $Q_{10}$  of this magnitude is considerably larger than expected for a diffusion-limited process.

These data are in contrast to those found for species such as turnip and cucumber, which have linear responses to external CO<sub>2</sub> concentrations of about 300  $\mu\text{l/liter}$  and little sensitivity to temperature under well watered conditions (9). The difference is probably associated with species differences (9, 11) in  $\Sigma r$ . In well watered sunflower,  $\Sigma r$  was 4.5 sec cm<sup>-1</sup>, whereas in turnip and cucumber it was 10 sec cm<sup>-1</sup> and 16 sec cm<sup>-1</sup>, respectively (calculated from Gaastra [9]). The low resistance of sunflower probably permits a high enough flux of CO<sub>2</sub> to the chloroplasts so that the enzymes for CO<sub>2</sub> fixation operate on the nonlinear portion of the substrate-velocity curve at CO<sub>2</sub> concentrations of 300  $\mu\text{l/liter}$ . Thus, under well watered conditions, photosynthetic rate could be affected by both enzymatic activity and CO<sub>2</sub> diffusion (4, 19) and photosynthesis would have a large sensitivity to temperature. Photosynthetic limitation at high light has often been attributed to enzymatic activity (13, 14). This suggests

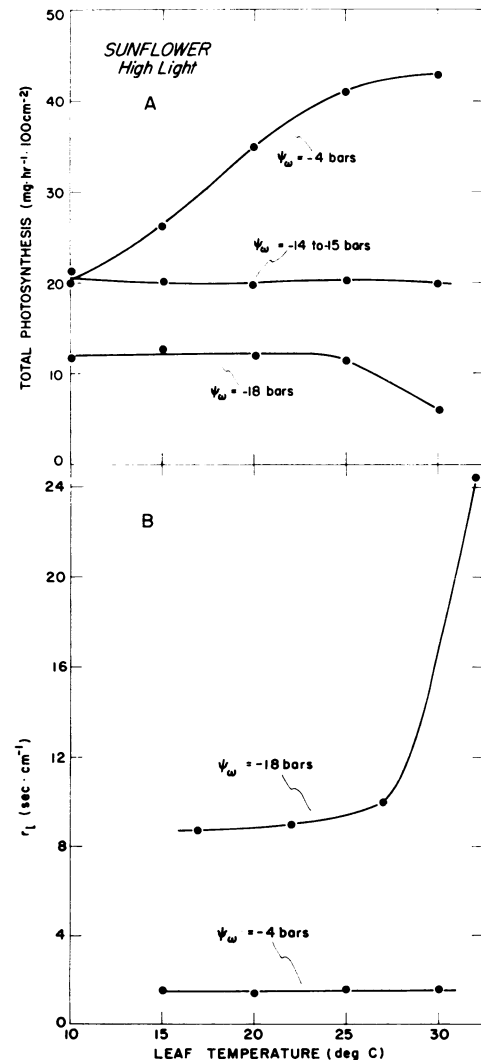


FIG. 4. Rates of total photosynthesis (A) and leaf diffusive resistances (B) at various leaf temperatures under high (saturating) light and at various leaf water potentials.

that those instances (16, 18) in which photosynthetic effects have been ascribed to changes in mesophyll resistance may have involved changes in some aspect of chloroplast activity as well, particularly when limitation by  $\text{CO}_2$  diffusion has not been rigorously demonstrated.

Since photosynthesis did not respond to changes in external  $\text{CO}_2$  concentrations during moderate to severe desiccation in sunflower, its rate must have been limited by the activity of photosynthetic enzymes or by photochemical activity. For enzyme limitation, photosynthesis should have been affected by temperature to a degree similar to that found for well watered conditions. The reverse was the case (Fig. 4A), however. The sensitivity to temperature seen in well watered plants disappeared when leaf  $\psi_w$  was below  $-11$  bars. Apart from the small anomaly at  $30^\circ\text{C}$  and  $-18$  bars (Fig. 4A and 4B), the constancy of the temperature data suggests that photosynthesis was inhibited by a nonenzymatic factor when  $\psi_w$  was below  $-11$  bars. This is in agreement with the work of Huffaker *et al.* (12) who showed little effect of leaf desiccation on the activity of enzymes of the carboxylative phase of photosynthesis.

Thus, neither  $\text{CO}_2$  diffusion nor the activity of photosynthetic enzymes appears to be a factor determining photosynthetic response to  $\psi_w$  below  $-11$  bars in sunflower. The possibility remains, however, that the inhibition was due to reduced photochemical activity, where I use the term photochemical activity to mean the general complex of nonenzymatic reactions associated with the light reactions and measured at limiting light intensities. The following evidence supports this suggestion: photochemical activity *in vivo* (Fig. 6) and oxygen evolution *in vitro* (5) are inhibited by desiccation to a degree which could limit photosynthesis under high light. Light-limited photosynthesis shows the same temperature and  $\text{CO}_2$  response (Fig. 5) as observed in leaves having  $\psi_w$  below  $-11$  bars at nonlimiting light intensities (Figs. 3 and 4). It has previously been suggested that photosynthesis at low  $\psi_w$  would be limited by photochemical activity only if light intensities were low (5). However, the present data indicate that the photochemical activity of the chloroplasts probably limited photosynthesis over the entire range of light intensities when  $\psi_w$  was below  $-11$  bars.

It is possible that limited diffusion of a photosynthetic intermediate other than  $\text{CO}_2$  could also cause the responses to  $\text{CO}_2$  and temperature that were observed in the present study at  $\psi_w$  below  $-11$  bars. However, it is unlikely that diffusion of an

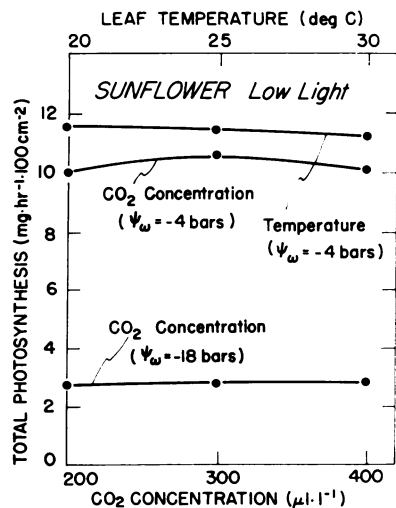


FIG. 5. Rates of total photosynthesis at various  $\text{CO}_2$  concentrations and leaf temperatures under low (limiting) light intensities and two leaf water potentials.

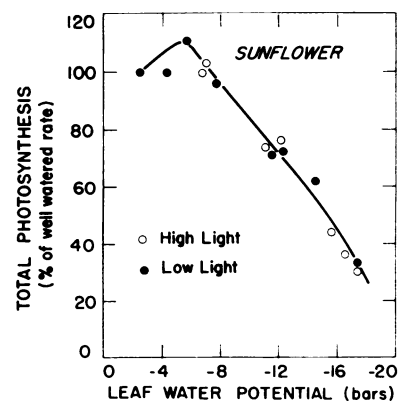


FIG. 6. Rates of total photosynthesis at high (saturating) and low (limiting) light intensities in intact leaves having various water potentials. The low light data were obtained under conditions of linearity between photosynthesis and light intensity and consequently represent a measure of photochemical activity. Rates are expressed as a percentage of the well watered rate before desiccation. Rates in well watered plants were: high light,  $45 \text{ mg hr}^{-1} \text{ dm}^{-2}$ ; low light,  $12 \text{ mg hr}^{-1} \text{ dm}^{-2}$ . Data represent individual measurements from two plants at high light and two different plants at low light intensities.

intermediate would be as strongly inhibited as rates of photosynthesis (to 10% of the well watered rate at  $\psi_w$  of  $-20$  bars) without similar effects on the diffusion of other metabolites. There is no evidence that such a general reduction in diffusion of metabolites occurs during desiccation.

In the absence of the limitation of photosynthesis by  $\text{CO}_2$  diffusion or enzymatic activity, it is surprising that light saturation was observed in desiccated leaves (Fig. 2). The presence of light saturation can be interpreted to mean that whole photosynthetic units were inhibited during desiccation (13). It is also possible that the high leaf temperature under saturating light (as much as  $2^\circ\text{C}$  above air temperature in this study) was inhibitory to photosynthesis (Fig. 4) and may have interacted with light intensity to cause an apparent light saturation. This apparent saturation would have resulted if an increase in the rate of photochemically limited photosynthesis were opposed by an inhibition of photosynthesis by rising leaf temperature.

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