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Very high CO_2 partially restores photosynthesis in sunflower at low water potentials

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Abstract. We re-examined the question of whether the stomata limit photosynthesis in dehydrated sunflower (*Helianthus annuus* L.) plants having low leaf water potentials. A gas-exchange apparatus was modified to operate at external CO_2 partial pressures as high as 3000 Pa (3%), which were much higher than previously achieved. This allowed photosynthesis and stomatal behavior to be monitored simultaneously at very high CO_2 in the same leaf. The data were compared with those from leaves treated with abscisic acid (ABA) where effects on photosynthesis are entirely stomatal. Photosynthesis was inhibited at low water potential and was only slightly enhanced by increasing the external CO_2 partial pressure from 34 Pa (normal air) to 300 Pa. Photosynthesis in ABA-treated leaves was similarly inhibited but recovered fully at 300 Pa. In both cases, the stomata closed to the same extent as judged from the average conductance of the leaves. Because the ABA effect resulted from diffusion limitation for CO_2 caused by stomatal closure, the contrasting data show that most of the dehydration effect was nonstomatal at low water potentials. When CO_2 partial pressures were raised further to 3000 Pa, photosynthesis increased somewhat at low water potentials but not in ABA-treated leaves. This indicates that some nonstomatal component of photosynthesis responded differently in leaves at low water potential and leaves treated with ABA. Because this component was only partially restored by very high CO_2, it was likely to be metabolic and was an important source of photosynthetic inhibition.

Key words: Abscisic acid and photosynthesis – Carbon dioxide pressures and photosynthesis – Gas exchange – *Helianthus* – Photosynthesis and dehydration

Introduction

The inhibition of whole-plant photosynthesis by drought is one of the greatest effects that water deficits can have on the growth and metabolism of mesophytic plant species. Limiting supplies of water lead to low water potentials (low ψ_w) and can result in stomatal closure, loss of turgor, accumulation of abscisic acid (ABA), and concentration of solutes. Any or all of these factors may have to be included in a detailed, quantitative description of the mechanism responsible for the diminished photosynthetic performance (for review, see Kaiser 1987).

Stomatal closure is such a common response to water shortage that it was often considered to cause the decrease in photosynthesis by starving the leaf interior for CO_2. However, losses in chloroplast activity also were implicated because 1) increasing the CO_2 partial pressure outside the leaf did not relieve the inhibition (Boyer 1971; Sharkey and Badger 1982; Forseth and Ehleringer 1983; Ehleringer and Cook 1984; Matthews and Boyer 1984; Sharp and Boyer 1986; Vassey and Sharkey 1989); 2) calculated CO_2 partial pressures inside the leaf did not decrease (Radin and Ackerson 1981; Forseth and Ehleringer 1983; Ehleringer and Cook 1984; Sharp and Boyer 1986); 3) chloroplasts isolated from the leaves showed losses in activity of the photosynthetic partial reactions (Boyer and Bowen 1970; Fry 1970, 1972; Potter and Boyer 1973; Keck and Boyer 1974; Mohanty and Boyer 1976; Fellows and Boyer 1976; Mayoral et al. 1981; Matthews and Boyer 1984); and 4) chloroplasts in vivo showed substantial inhibition of quantum yields and CO_2-saturated rates of photosynthesis (Mohanty and Boyer 1976; Sharp and Boyer 1986; Rao et al. 1987). The altered chloroplast metabolism was considered to decrease the demand for CO_2. The reduced demand accounted for the apparent high internal CO_2 partial pressures and for the inability of high CO_2 to relieve the inhibition (Matthews and Boyer 1984).

These results indicate that stomatal closure was not the only cause of the inhibition of photosynthesis at low ψ_w at least in mesophytic species (however, see Sharkey and Seemann (1989) for an alternate interpretation). This conclusion needs further investigation in view of findings with ABA-feeding of leaves. In leaves at low ψ_w endogenous ABA accumulates and there is considerable evidence that elevated ABA results in stomatal closure (for review, see Raschke 1975). In hydrated leaves fed ABA, internal CO_2 partial pressures remained high

Abbreviations and symbol: ABA = abscisic acid; Chl = chlorophyll; p_a = external partial pressure of CO_2; p_i = intercellular partial pressure of CO_2; ψ_w = water potential

(Cornic and Miginiac 1983; Raschke and Hedrich 1985). Although this has been considered evidence for metabolic effects of ABA, the stomata in some species closed in a patchy manner (Farquhar et al. 1987; Ward and Drake 1988; Terashima et al. 1988; Downton et al. 1988). This can cause the CO_2 partial pressures calculated for the leaf interior to be artifactual (Farquhar et al. 1987; Terashima et al. 1988; Downton et al. 1988). When the epidermis was removed from leaf disks or the external CO_2 was elevated, the inhibition of photosynthesis was completely relieved (Terashima et al. 1988). These results conclusively showed that the inhibition of photosynthesis in ABA-treated leaves was entirely caused by stomatal closure.

Likewise, in leaves subjected to dehydration, the internal CO_2 appeared high (Radin and Ackerson 1981; Forseth and Ehleringer 1983; Ehleringer and Cook 1984; Sharp and Boyer 1986). This parallel response in ABA-treated and dehydrated leaves implied that the mechanism of inhibition could be similar in both treatments (Bunce 1987). Indeed, Downton et al. (1988) proposed that patchy stomatal closure may fully account for the inhibition of photosynthesis at low ψ_w.

However, the photosynthetic inhibition of ABA-treated leaves has not been directly compared with that of leaves having low ψ_w at various CO_2 levels. In principle, a high CO_2 partial pressure should relieve the photosynthetic inhibition at low ψ_w exactly as it does during ABA treatment if the cause of the inhibition is the same in both conditions. The following experiments were undertaken to make this comparison. To strengthen the comparison, a gas-exchange apparatus was modified so that both photosynthesis and stomatal conductance could be monitored simultaneously at very high CO_2 partial pressures.

Material and methods

Plant material and growth conditions. Sunflower (*Helianthus annuus* L. cv. IS897; seeds from Interstate Seed Co., Fargo, ND, USA) plants were grown in a controlled-environment chamber (day/night temperature, 25/20±1 °C; day/night relative humidity 70/100± 5%; photon flux density, 900 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of photosynthetically active radiation (PAR) from cool-white fluorescent lamps (General Electric, Cleveland, Oh., USA; light period, 14 h). Seeds were germinated in vermiculite moistened with 0.5-strength nutrient solution (see solution composition below). After 4 d, seedlings were transplanted and grown singly in 2-l opaque plastic containers having fitted lids and filled with an aerated nutrient solution. Fresh solution was supplied twice per week and had the following composition (modified from Epstein 1972): 6 mM $Ca(NO_3)_2$, 4 mM KNO_3, 10 mM $MgCl_2$, 2 mM KH_2PO_4, 1 mM $(NH_4)_2SO_4$, 1 mM 2-(N-morpholino)ethanesulfonic acid (Mes), 100 µM FeNa-ethylenediaminetetraacetic acid (FeNa-EDTA), 25 µM H_3BO_3, 10 µM $MnSO_4$, 2 µM $ZnSO_4$, 0.5 µM $CuSO_4$ and 0.5 µM H_2MoO_4. The pH of the solution was initially adjusted to 6.2 with KOH. Water was replenished and the pH was readjusted to 6.2 as necessary. After 14 d the plants were transferred to 8-l containers. Experiments were performed with leaves five to seven nodes above the cotyledons 21–25 d after planting. Individual leaves had an area of 180–240 cm^2. Plants were deprived of their water supply by removing the nutrient solution. The dehydration of the experimental leaf thus depended on water stored in the plant and occurred in 8–40 h.

Measurements of photosynthesis. Photosynthetic CO_2 consumption and transpiration were measured on entire single attached leaves with a compensating infrared gas-analyzer that maintained air temperature, CO_2 and H_2O vapor constant (Martin et al. 1981; Matthews and Boyer 1984). We employed a CO_2 analyzer (Model LI-6251; LI-COR, Lincoln, Neb., USA) that enabled measurements at very high CO_2 levels (up to 3000 Pa). Measurements at these levels were achieved by using the analyzer only for controlling the CO_2 partial pressure in the assimilation chamber and not for making differential measurements. As long as the partial pressure of reference gas was constant and the analyzer output was constant, the gas partial pressure in the sample cell and assimilation chamber also was constant. The rate of photosynthesis was determined from the rate of CO_2 injection necessary to maintain the CO_2 partial pressure constant. This approach had the advantage that the measurement of photosynthesis was relatively unaffected by the sensitivity of the analyzer to CO_2, which becomes small at very high CO_2 partial pressures.

During the experiments the air temperature was 25.0 °C, the relative humidity was 80%, the O_2 partial pressure was 21000 Pa, and the photon flux density was 2050 $\mu mol \cdot m^{-2} \cdot s^{-1}$. The air was stirred rapidly to maintain a high boundary-layer conductance to CO_2 diffusion (6.73 $mol \cdot m^{-2} \cdot s^{-1}$) and a leaf temperature within 2.5 °C of air temperature at the lowest rate of transpiration observed during these experiments. Leaf temperature was monitored with a custom-made thermocouple (75 µm diameter) pressed against the underside of the leaf. Stomatal conductance and internal CO_2 partial pressure (p_i) were calculated according to von Caemmerer and Farquhar (1981). Photosynthetic measurements were made during the normal light period and the plants were returned to the growth chamber at the end of each measurement. Photosynthetic rates are reported on a chlorophyll basis in all cases in order to eliminate the effect of leaf shrinkage. Chlorophyll (Chl) contents of sunflower do not change at the low ψ_w and short times used in this study (Mohanty and Boyer 1976).

Photosynthesis was also measured with a leaf-disk O_1-electrode system (Model LD-2; Hansatech Limited, Norfolk, UK). The leaf disks had an area of 4 cm^2 or 10 cm^2 depending on whether they would be used later in the psychrometer (4 cm^2) or not (10 cm^2). They were illuminated at a photon flux density of 2100 $\mu mol \cdot m^{-2} \cdot s^{-1}$ in the presence of humidified gas flowing at 1 $ml \cdot s^{-1}$ for at least 10 min prior to sealing the leaf disk in the chamber and measuring O_2 evolution. Because tissue temperatures rose when the disks were illuminated, the temperature of the gas and chamber walls was kept at 21 °C during the preillumination and the tissue temperature rose to 26±1 °C after sealing.

Leaf water status. Except as noted, measurements of ψ_w were made in the same leaf or leaf disk immediately following measurements of photosynthesis. A 4-cm^2 disk was taken from interveinal tissue to determine leaf ψ_w by isopiestic thermocouple psychrometry (Boyer and Knipling 1965; Boyer 1966) using chambers coated with melted and resolidified petrolatum (vaseline; Boyer 1967). Measurements were corrected for heat of respiration (Barrs 1965).

Chlorophyll determination. For each experimental leaf or leaf disk a sample of interveinal tissue was homogenized in cold 80% acetone, and total Chl was determined using the specific absorption coefficients determined by Ziegler and Egle (1965) as described in Graan and Ort (1984).

Chemicals. ACS Reagent-grade chemicals were used in solution culture whenever possible. ±*cis-trans* Abscisic acid was obtained from Sigma Chemical Co. (St. Louis, Mo., USA), dissolved in methanol, and diluted 2000-fold to give a concentration of 50 µM before supplying to the petiole of detached leaves.

Each experiment was repeated at least three times. Data presented are the results of typical experiments.

Results

Intact sunflower plants deprived of water showed a virtual cessation of light-saturated CO_2 fixation during the subsequent 8–10 h. The data in Fig. 1 A represent a typical response in normal air and show that photosynthesis decreased at leaf ψ_w below -0.8 to -1.0 MPa and became less than 10% of the initial rate at ψ_w of -1.6 MPa. Although the stomatal conductance decreased between ψ_w of -0.8 and -1.0 MPa, the calculated p_i did not (Fig. 1 B). This type of response has been considered evidence for a nonstomatal inhibition of photosynthesis (Radin and Ackerson 1981; Forseth and Ehleringer 1983; Sharp and Boyer 1986).

Because the validity of this evidence is in question if patchy stomatal closure occurred (Terashima et al. 1988; Downton et al. 1988), we measured photosynthesis at various external CO_2 partial pressures (p_a). In principle, high p_a should cause photosynthetic activity to return to normal levels by driving more CO_2 into the leaf if photosynthesis is inhibited entirely by CO_2 starvation resulting from stomatal closure. In leaves at high ψ_w, CO_2 fixation was rapid and increased only moderately when p_a was raised from 34 Pa (normal air) to 300 Pa (Fig. 2A, -0.43 to -0.45 MPa). There was no further increase at very high p_a to 3000 Pa which indicates that the leaf was saturated with CO_2. The stomatal conductance was high and unchanged by the varying CO_2, which is commonly observed at irradiances as high as were used in these experiments (Boyer 1971; Laisk et al. 1989). When the ψ_w of the same leaves were lowered to -1.33 to -1.44 MPa, CO_2 fixation decreased to 15% of the initial rate. There was a slight increase when p_a increased from 34 Pa to 300 Pa (Fig. 2A), but as partial pressures increased further to 3000 Pa, CO_2 fixation increased significantly to a maximum of 71% of the initial rate. The stomatal conductances were low and remained unchanged as the p_a changed (Fig. 2B).

By using the O_2 electrode, we tested whether increasing p_a even further would increase photosynthesis at low ψ_w. However, regardless of ψ_w, a p_a of 3000 Pa was always saturating. Instead, higher CO_2 partial pressures

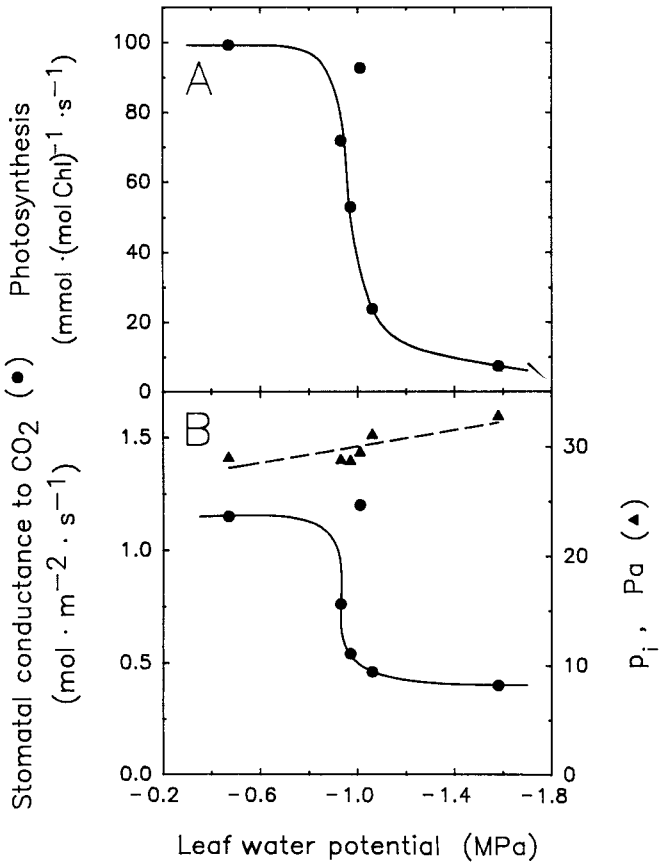

Fig. 1 A, B. Photosynthesis, stomatal conductance, and internal partial pressure of CO_2 (p_i) at various leaf water potentials in an attached sunflower leaf in normal air (34 Pa CO_2). **A** Photosynthetic CO_2 assimilation; **B** calculated p_i and stomatal conductance to CO_2 for the leaf in **A**. Assimilation was monitored in a single attached leaf in a gas-exchange apparatus before and after removal of the nutrient solution around the roots. Assimilation of 100 mmol·(mol Chl)$^{-1}$·s^{-1} was equivalent to 60 μmol·m^{-2}·s^{-1}. The water potentials were measured in the same leaf, and the progress from high to low potential occurred over a period of 10 h

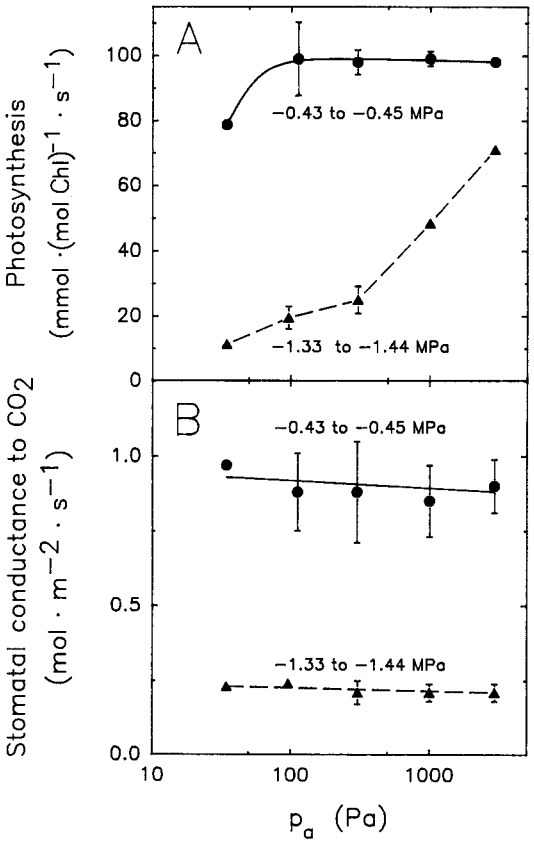

Fig. 2A, B. Photosynthesis and stomatal conductance in an attached sunflower leaf at various partial pressures of external CO_2 (p_a). **A** Photosynthetic CO_2 assimilation; **B** stomatal conductance to CO_2 for the leaf in **A**. Assimilation was monitored in single attached leaves in a gas-exchange apparatus before and after removal of the nutrient solution around the roots. The water potentials were measured in the same leaf, and the progress from high (-0.43 to -0.45 MPa) to low (-1.33 to -1.44 MPa) water potential occurred over a period of 40 h. Note logarithmic scale on the *horizontal axis*. Data are means ± 1 SD (for some data points, SD is smaller than the symbol)

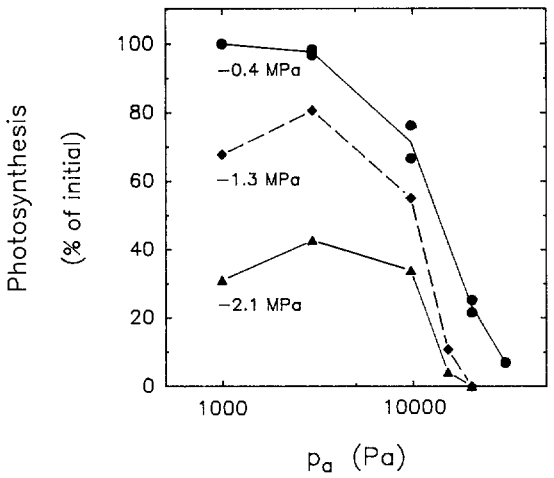

Fig. 3. Photosynthesis at various leaf water potentials and external partial pressures of CO_2 (p_a) in sunflower leaf disks. Photosynthesis was measured with the leaf-disk O_2-electrode using 4-cm^2 disks. Control rates for the two leaves used for the experiments were 71 and 83 mmol $O_2 \cdot$(mol Chl)$^{-1} \cdot s^{-1}$. Each CO_2 response was obtained from a single leaf and, after a measurement at high potential (−0.4 MPa), the low water potentials were obtained from a leaf on the same plant (−2.1 MPa) or the same leaf (−1.3 MPa). Illumination of the leaf disk resulted in a decrease in the leaf water potential of 0.3 to 0.6 MPa in the electrode. For this experiment only, quoted water potentials were determined at the beginning of the photosynthesis measurement and thus were maximum water potentials. Note the logarithmic scale on the *horizontal axis*

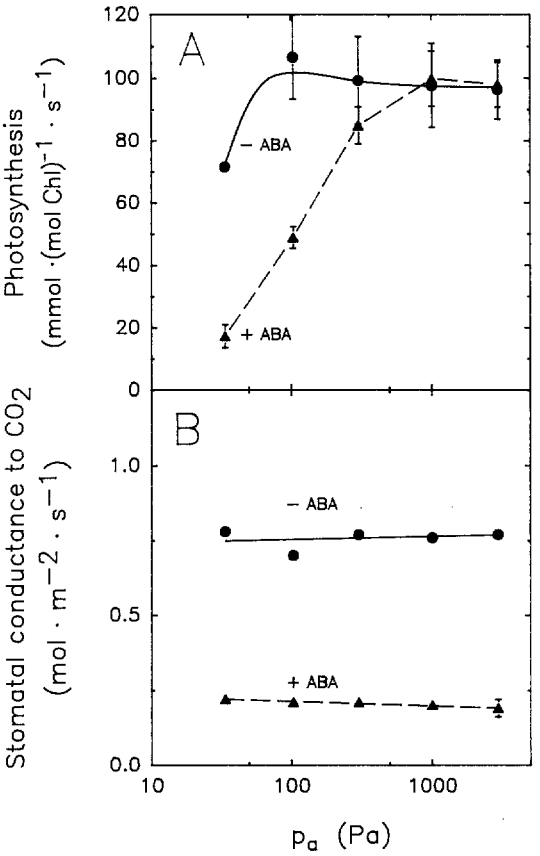

Fig. 4A, B. Photosynthesis in a detached sunflower leaf at various external partial pressures of CO_2 (p_a) with and without ABA fed through the petiole. **A** Photosynthetic CO_2 assimilation; **B** stomatal conductance to CO_2. The photosynthetic response to p_a was determined in the absence of exogenous ABA, then an aqueous solution of ABA (50 μM) was supplied to the cut petiole. Photosynthesis was monitored in the steady-state. Note logarithmic scale on the *horizontal axis*. Data are means ± 1 SD (for some data points, SD is smaller than the symbol) except stomatal conductance at −ABA, which is for a single leaf

(to 30000 Pa) caused photosynthesis to decrease (Fig. 3). At CO_2 partial pressures around 30000 Pa, photosynthesis approached zero. Thus, CO_2 had penetrated the entire leaf and all the photosynthetic tissue appeared CO_2 saturated even at the lowest ψ_w (−2.1 MPa). It is noteworthy that the leaves at high ψ_w had the most open stomata but required the highest p_a for complete inhibition. Stomatal closure at low ψ_w did not protect the photosynthetic apparatus from the high-CO_2 inhibition.

We compared these results with those from leaves treated with ABA and maintained at high ψ_w (−0.5 MPa to −0.7 MPa). At a p_a of 34 Pa, CO_2 fixation decreased to 14% of the initial rate as a result of the ABA treatment (Fig. 4A). The stomatal conductance became 0.2 mmol$\cdot$m$^{-2}\cdot$s^{-1} under these conditions, which virtually duplicated the CO_2 fixation and stomatal conductance at low ψ_w (compare Figs. 2 and 4). The calculated p_i was unaffected by the decreased stomatal conductance (data not shown), as others have observed (Bunce 1987; Ward and Bunce 1987; Robinson et al. 1988). However, in contrast to leaves having low ψ_w, CO_2 fixation was completely restored at p_a of 300 Pa (Fig. 4A). The large response of the ABA-treated leaf but small response of the low-ψ_w leaf at these CO_2 levels indicates that there was a major difference in the effect of the ABA and ψ_w treatments on photosynthesis.

In order to determine whether this difference operated over a range of ψ_w, we measured photosynthesis in leaves saturated with CO_2 and having various leaf ψ_w. The CO_2 saturation should have eliminated any stomatal effects. Figure 5A shows that at CO_2 partial pressures of 3000 Pa in the assimilation chamber or 5000 Pa in the leaf-disk O_2-electrode, the same results were obtained and CO_2 fixation remained below control rates at low ψ_w (below about −1.2 MPa). At ψ_w of −1.9 MPa, photosynthesis was about 65% of the rate at high ψ_w. Although this inhibition was less than in normal air (e.g. see performance at 34 Pa from Fig. 1 shown as dashed line in Fig. 5), this result when taken together with the results in Figs. 2 and 4 indicates that a nonstomatal inhibition existed at all ψ_w below about −1.2 MPa. In support of this point, photosynthesis was independent of stomatal conductance (compare Fig. 5A, solid line, and B) and remained high even though the stomata closed in a fashion similar to that in normal air (compare Figs. 1B and 5B).

Discussion

The barriers to CO_2 diffusion are substantial when stomata close but they are not insurmountable. In principle

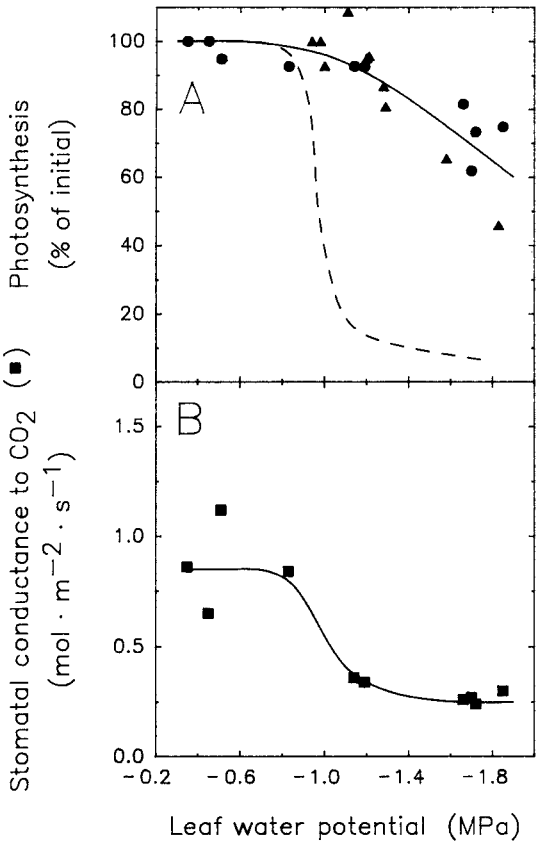

Fig. 5A, B. Photosynthesis in sunflower at various leaf water potentials in saturating CO_2. **A** Photosynthetic CO_2 assimilation by two single attached leaves at 3000 Pa CO_2 measured in the gas-exchange apparatus. The low water potentials were achieved while the leaf was photosynthesizing at elevated CO_2. The initial rates were 134 and 96 mmol CO_2 fixed·(mol Chl)$^{-1}$·s^{-1}. ▲ Photosynthetic O_2 evolution by leaf disks (10 cm^2) from two single attached leaves. The measurements of O_2 evolution were performed in a leaf-disk O_2-electrode at a CO_2 partial pressure of 5000 Pa. Low leaf water potentials were developed at ambient CO_2 where photosynthesis is largely inhibited (see Fig. 1). The control rates of photosynthesis were 70 and 74 mmol O_2 evolved·(mol Chl)$^{-1}$·s^{-1}. For comparison, the *dashed line* shows the response of photosynthesis to low water potentials at ambient CO_2 and is taken from Fig. 1A. **B** Calculated stomatal conductances for the two leaves used to measure gas exchange in **A**

it should be possible to use high enough CO_2 partial pressures to penetrate them diffusively. A good illustration is the diffusion of CO_2 out of the leaf in the dark when stomata are closed. Such diffusion can be detected at very low ψ_w and indicates that CO_2 penetrates from the leaf interior to the outside air under these conditions (Boyer 1970, 1971). In the present experiments, we demonstrated this penetration in another way, by increasing the CO_2 partial pressures around the leaf whereupon the photosynthetic activity increased. The activity increased until so much CO_2 entered the leaf that the activity began to be inhibited. At still higher partial pressures, it approached zero indicating that all barriers to CO_2 penetration had been overcome. Thus, the diffusion barriers were penetrable and could be extensively studied in ABA-treated and dehydrated leaves.

The results showed that leaves responded differently to the two treatments. Abscisic-acid treated leaves recovered completely in moderately high CO_2 partial pressures. The ABA caused only stomatal closure (Terashima et al. 1988; Downton et al. 1988) and thus this experiment measured the effects of stomatal closure. In dehydrated leaves, the same degree of stomatal closure was achieved, judging from the average conductance of the leaves. Although photosynthesis was somewhat restored at high CO_2, it did not return to the rates in hydrated leaves no matter how high the external CO_2 became. The inability of high CO_2 to cause full recovery of photosynthesis indicates that dehydrated leaves suffered from more than a stomatal limitation to photosynthesis.

The dehydrated leaves probably contained diffusional barriers in addition to those imposed by the stomata because the intercellular spaces collapsed. In sunflower, electron-microscope evidence has shown that shrinkage is extensive and results in a diminished and more tortuous intercellular space system than at high ψ_w (Fellows and Boyer 1978). This additional barrier to CO_2 not only would add directly to stomatal effects but would limit the lateral diffusion of CO_2 between possible patches of closed stomata, effectively sealing regions of the leaf even more than in ABA-treated leaves. Sharkey and Seemann (1989) demonstrated patchy photosynthesis in *Phaseolus* having low ψ_w. On the other hand, because the diffusive conductances were similar for ABA-treated and dehydrated leaves, feeding the same levels of CO_2 should have similarly overcome the diffusion barriers whether they were stomatal or intercellular, patchy or not.

Further to ensure that these barriers were indeed penetrated we fed CO_2 at much higher partial pressures than were required for ABA-treated leaves. These partial pressures did not restore photosynthesis to the level in hydrated leaves. If this was effect caused by patches because CO_2 was less able to penetrate, the diminished penetration should have protected photosynthesis from inhibition at very high CO_2 partial pressures. The reverse occurred, however. Leaves at low ψ_w did not require as much CO_2 for inhibition as did the nonpatchy controls. This result taken together with the similarity in diffusive conductance of the leaves indicates that patchiness was unlikely to have caused the CO_2 response.

The only remaining possibility is that a nondiffusional, that is metabolic, inhibition of photosynthesis occurred at low ψ_w (below about -1.2 MPa). The lack of ability to recover photosynthetic activity fully, regardless of the CO_2 level provides strong evidence in favor of this interpretation and affirms earlier work conducted with isolated thylakoid membranes (Boyer and Bowen 1970; Fry 1970; 1972; Potter and Boyer 1973; Keck and Boyer 1974; Mohanty and Boyer 1976; Fellows and Boyer 1976; Mayoral et al. 1981), chloroplasts (Berkowitz and Whalen 1985; Kaiser 1987) and intact leaves (Mohanty and Boyer 1976; Matthews and Boyer 1984; Sharp and Boyer 1986), showing that substantial changes can occur in chloroplast metabolism at low ψ_w. It also is consistent with reports of losses in photosyn-

thetic activity without stomatal closure at low ψ_w (Ackerson et al. 1977; Bunce 1988).

If the limitation was metabolic it also appeared to be CO_2-responsive. Several hypothetical mechanisms are suggested: 1) elevated CO_2 levels might have had a direct effect on the pH of the photosynthetic tissue leading to increased activity; 2) high CO_2 might have supplied increased substrate that overcame a decreased affinity of an enzyme for CO_2 (e.g. carbonic anhydrase, ribulose-1,5-bisphosphate carboxylase); 3) CO_2 might have activated an enzymatic reaction that was inhibited (e.g. ribulose-1,5-bisphosphate carboxylase). An enzyme reported to change with CO_2 is sucrose-phosphate synthase (SPS), although there is disagreement about how the activities were affected. Quick et al. (1989) observed increased SPS activities at low ψ_w whereas Vassey and Sharkey (1989) saw decreased activities. Quick et al. (1989) observed little effect of CO_2 on SPS activities at low ψ_w but Vassey and Sharkey (1989) saw a CO_2 effect.

It also may be important that, in *Phaseolus* leaves having low ψ_w Vassey and Sharkey (1989) observed no recovery in photosynthetic activity at moderately elevated levels of CO_2 for short times, in agreement with the present work. However, they report a partial recovery after long times (5 h). Thus, time might have been a factor. They interpreted this behavior to have been caused by stomatal patchiness, which they detected with $^{14}CO_2$. However, patchiness detected by this technique could as well indicate metabolic inhibition because of the dependency of $^{14}CO_2$ fixation on photosynthetic metabolism. As a consequence, the significance of stomatal patchiness on photosynthetic behavior at low ψ_w probably needs further investigation.

Ben et al. (1987) also measured CO_2 fixation in sunflower at low ψ_w and very high CO_2 and similarly observed an inhibition that they attributed to nonstomatal effects. However, the inhibition was larger than we observed perhaps because they dehydrated the plants over longer times. It is not possible from their data to determine whether very high CO_2 partially reversed the inhibition.

It is noteworthy that in ABA-treated leaves, we were able to return photosynthesis to control rates at lower CO_2 partial pressures than were reported earlier (Terashima et al. 1988; Robinson et al. 1988). We suggest that this difference may be attributable to the different methods used to expose the leaves to CO_2. The leaf-disk O_2-electrode (Terashima et al. 1988; Robinson et al. 1988) contained unstirred air and held the disk against the solid window of the electrode. This arrangement caused large boundary layers around the disk, which were confirmed by the rapid 5 °C jump in disk temperature when the tissue was illuminated. As a result, larger external CO_2 partial pressures were needed to see the same effect as in stirred systems. The air was rapidly stirred in the gas-exchange system and it seems that the stomata may not present as large barriers to diffusion as has been suggested from O_2-electrode data (Terashima et al. 1988; Robinson et al. 1988) in ABA-treated leaves.

It is concluded that leaves treated with ABA probably reproduce the stomatal closure that is associated with low ψ_w, but there are other changes occurring at low ψ_w that are not present in ABA-treated leaves. The nature of these changes is not known but appears to involve an altered but CO_2-sensitive photosynthetic metabolism. In view of the results, the exploration of the altered metabolism will require the use of much higher CO_2 partial pressures than is common practice, and will need to rely on evidence other than calculated internal CO_2 levels. We recommend the modified gas-exchange system as a means for identifying nonstomatal effects because it is less affected by boundary layers and temperature drifts than the leaf-disk O_2-electrode, it allows stomatal behavior to be monitored simultaneously, and it can supply very high CO_2 partial pressures. However, until additional understanding of stomatal patchiness and the calculation of internal CO_2 partial pressure is available for gas-exchange studies, results based on relationships between photosynthesis and internal partial pressures of CO_2 must be viewed with caution.

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References

Ackerson, R.C., Krieg, D.R., Haring, C.L., Chang, N. (1977) Effects of plant water status on stomatal activity, photosynthesis, and nitrate reductase activity of field grown cotton. Crop Sci. **17**, 81–84

Barrs, H.D. (1965) Comparison of water potentials in leaves as measured by two types of thermocouple psychrometers. Aust. J. Biol. Sci. **18**, 36–52

Ben, G.Y., Osmond C.B., Sharkey, T.D. (1987) Comparisons of photosynthetic responses of *Xanthium strumarium* and *Helianthus annuus* to chronic and acute water stress in sun and shade. Plant Physiol. **84**, 476–482

Berkowitz, G.A., Whalen, C. (1985) Leaf K^+ interaction with water stress inhibition of nonstomatal-controlled photosynthesis. Plant Physiol. **79**, 189–195

Boyer, J.S. (1966) Isopiestic technique: measurement of accurate leaf water potentials. Science **154**, 1459–1460

Boyer, J.S. (1967) Leaf water potentials as measured with a pressure chamber. Plant Physiol. **42**, 133–137

Boyer, J.S. (1970) Leaf enlargement and metabolic rates in corn, soybean, and sunflower at various leaf water potentials. Plant Physiol. **46**, 233–235

Boyer, J.S. (1971) Nonstomatal inhibition of photosynthesis in sunflower at low leaf water potential and high light intensities. Plant Physiol. **48**, 532–536

Boyer, J.S., Bowen, B.L. (1970) Inhibition of oxygen evolution in chloroplasts isolated from leaves with low water potentials. Plant Physiol. **45**, 612–615

Boyer, J.S., Knipling, E.B. (1965) Isopiestic technique for measuring leaf water potentials with a thermocouple psychrometer. Proc. Natl. Acad. Sci. USA **54**, 1044–1051

Bunce, J.A. (1987) Species-specific responses to water stress of gas exchange parameters mimicked by applied abscisic acid. Can. J. Bot. **65**, 103–106

Bunce, J.A. (1988) Nonstomatal inhibition of photosynthesis by water stress. Reduction in photosynthesis at high transpiration rate without stomatal closure in field-grown tomato. Photosynth. Res. **18**, 357–362

Cornic, G., Miginiac, E. (1983) Non-stomatal inhibition of net CO_2 uptake by abscisic acid in *Pharbitis nil*. Plant Physiol. **73**, 529–533

Downton, W.J.S., Loveys, B.R., Grant, W.J.R. (1988) Stomatal closure fully accounts for the inhibition of photosynthesis by abscisic acid. New Phytol. **108**, 263–266

Ehleringer, J.R., Cook, C.S. (1984) Photosynthesis in *Encelia farinosa* Gray in response to decreasing leaf water potential. Plant Physiol. **75**, 688–693

Epstein, E.E. (1972) Mineral nutrition of plants: principles and perspectives. Wiley, New York

Farquhar, G.D., Hubick, K.T., Terashima, I., Condon, A.G., Richards, R.A. (1987) Genetic variation in the relationship between photosynthetic CO_2 assimilation rate and stomatal conductance to water loss. In: Progress in photosynthesis research, vol. IV, pp. 209–212, Biggins, J., ed. Nijhoff, Dordrecht

Fellows, R.J., Boyer, J.S. (1976) Structure and activity of chloroplasts of sunflower leaves having various water potentials. Planta **132**, 229–239

Fellows, R.J., Boyer, J.S., (1978) Altered ultrastructure of cells of sunflower leaves having low water potentials. Protoplasma **93**, 381–395

Forseth, I.N., Ehleringer, J.R. (1983) Ecophysiology of two solar tracking desert winter annuals. III. Gas exchange responses to light, CO_2 and VPD in relation to long-term drought. Oecologia **57**, 344–351

Fry, K.E. (1970) Some factors affecting the Hill reaction activity in cotton chloroplasts. Plant Physiol. **45**, 465–469

Fry, K.E. (1972) Inhibition of ferricyanide reduction in chloroplasts prepared from water-stressed cotton leaves. Crop Sci. **12**, 698–701

Graan, T., Ort, D.R. (1984) Quantitation of the rapid electron donors to P700, the functional plastoquinone pool, and the ratio of the photosystems in spinach chloroplasts. J. Biol. Chem. **259**, 14003–14010

Kaiser, W.M. (1987) Effect of water deficit on photosynthetic capacity. Physiol. Plant. **71**, 142–149

Keck, R.W., Boyer, J.S. (1974) Chloroplast response to low leaf water potentials. III. Differing inhibition of electron transport and photophosphorylation. Plant Physiol. **53**, 474–479

Laisk, A., Oja, V., Kiirats, O., Raschke, K., Heber, U. (1989) The state of the photosynthetic apparatus in leaves as analyzed by rapid gas exchange and optical methods: the pH of the chloroplast stroma and activation of enzymes in vivo. Planta **177**, 350–358

Martin, B., Ort, D.R., Boyer, J.S. (1981) Impairment of photosynthesis by chilling temperatures in tomato. Plant Physiol. **68**, 329–334

Matthews, M.A., Boyer, J.S. (1984) Acclimation of photosynthesis to low leaf water potentials. Plant Physiol. **74**, 161–166

Mayoral, M.L., Atsmon, D., Gromet-Elhanan, Z., Shimshi, D. (1981) The effect of water stress on various enzyme activities in wheat and related wild species: carboxylase activity, electron transport, and photophosphorylation in isolated chloroplasts. Aust. J. Plant Physiol. **8**, 385–394

Mohanty, P., Boyer, J.S. (1976) Chloroplast response to low leaf water potentials. IV. Quantum yield is reduced. Plant Physiol. **57**, 704–709

Potter, J.R., Boyer, J.S. (1973) Chloroplast response to low leaf water potentials. II. Role of osmotic potential. Plant Physiol. **51**, 993–997

Quick, P., Siegl, G., Neuhaus, E., Feil, R., Stitt, M. (1989) Short-term water stress leads to a stimulation of sucrose synthesis by activating sucrose-phosphate synthase. Planta **177**, 535–546

Radin, J.W., Ackerson, R.C. (1981) Water relations of cotton plants under nitrogen deficiency. III. Stomatal conductance, photosynthesis, and abscisic acid accumulation during drought. Plant Physiol. **67**, 115–119

Rao, I.M., Sharp, R.E., Boyer, J.S. (1987) Leaf magnesium alters photosynthetic response to low water potentials in sunflower. Plant Physiol. **84**, 1214–1219

Raschke, K. (1975) Stomatal action. Annu. Rev. Plant Physiol. **26**, 309–340

Raschke K., Hedrich, R. (1985) Simultaneous and independent effects of abscisic acid on stomata and the photosynthetic apparatus in whole leaves. Planta **163**, 105–118

Robinson, S.P., Grant, W.J.R., Loveys, B.R. (1988) Stomatal limitation of photosynthesis in abscisic acid-treated and in water-stressed leaves measured at elevated CO_2. Aust. J. Plant Physiol. **15**, 495–503

Sharkey, T.D., Badger, M.R. (1982) Effects of water stress on photosynthetic electron transport, photophosphorylation, and metabolite levels of *Xanthium strumarium* mesophyll cells. Planta **156**, 199–206

Sharkey, T.D., Seemann, J.R. (1989) Mild water stress effects on carbon-reduction-cycle intermediates, RuBP carboxylase activity, and spatial homogeneity of photosynthesis in intact leaves. Plant Physiol. **89**, 1060–1065

Sharp, R.E., Boyer, J.S. (1986) Photosynthesis at low water potentials in sunflower: lack of photoinhibitory effects. Plant Physiol. **82**, 90–92

Terashima, I., Wong, S.C., Osmond, C.B., Farquhar, G.D. (1988) Characterization of non-uniform photosynthesis induced by abscisic acid in leaves having different mesophyll anatomies. Plant Cell Physiol. **29**, 385–394

Vassey, T.L., Sharkey, T.D. (1989) Mild water stress of *Phaseolus vulgaris* plants leads to reduced starch synthesis and extractable sucrose phosphate synthase activity. Plant Physiol. **89**, 1066–1070

von Caemmerer, S., Farquhar, G.D. (1981) Some relationships between the biochemistry of photosynthesis and gas exchange of leaves. Planta **153**, 376–387

Ward, D.A., Bunce, J.A. (1987) Abscisic acid simultaneously decreases carboxylation efficiency and quantum yield in attached soybean leaves. J. Exp. Bot. **38**, 1182–1192

Ward, D.A., Drake, B.G. (1988) Osmotic stress temporarily reverses the inhibitions of photosynthesis and stomatal conductance by abscisic acid – Evidence that abscisic acid induces a localized closure of stomata in intact, detached leaves. J. Exp. Bot. **39**, 147–155

Ziegler, R., Egle, K. (1965) Zur quantitativen Analyse der Chloroplastenpigmente. Beitr. Biol. Pflanz. **41**, 11–37

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