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Influence of Drought Acclimation and CO_2 Enrichment on Osmotic Adjustment and Chlorophyll *a* Fluorescence of Sunflower during Drought

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ABSTRACT

Osmotic adjustment occurred during drought in expanded leaves of sunflowers (*Helianthus annuus* var Hysun 30) which had been continuously exposed to 660 microliters CO_2 per liter or had been previously acclimated to drought. The effect was greatest when the treatments were combined and was negligible in nonacclimated plants grown at 340 microliters CO_2 per liter. The concentrations of ethanol soluble sugars and potassium increased during drought but they did not account for the osmotic adjustment. The delay in the decline in conductance and relative water content and in the loss of structural integrity with increasing drought was dependent on the degree of osmotic adjustment. Where it was greatest, conductance fell from 5.8 millimeters per second on the first day of drought to 1.3 millimeters per second on the fourth day and was at approximately the same level on the eighth day. The relative water content remained constant at 85% for three days and fell to 36% on the sixth day. There was no evidence of leaf desiccation even on the eighth day. In contrast, the conductance of leaves showing minimal adjustment fell rapidly after the first day of drought and was negligible after the fourth, at which time the relative water content was 36%. By the sixth day of drought, areas near the margins of the leaves were desiccating and the plants did not recover upon rewatering. Despite the differences in the rate of change of conductance and relative water content during drought, photosynthetic electron transport activity, inferred from measurements of chlorophyll *a* fluorescence *in vivo* and PSII activity of isolated thylakoids, remained functional until desiccation occurred.

Plants are more tolerant of drought when water is withheld under conditions that favor osmotic adjustment, namely: after previous acclimation to drought (13, 30); when water deficits are slowly imposed (6, 9); or, when the CO_2 concentration is higher than 340 μl L^{-1} (5, 28). As water deficits increase, both leaf conductance to CO_2 and the capacity of the mesophyll to fix CO_2 decline. The decline is delayed in osmotically adjusted plants (9) but it is unclear whether electron transport capacity or other mesophyll processes are affected. When drought was imposed under conditions not favoring osmotic adjustment, the PSII electron transport activities of thylakoids isolated from *Picea sitchensis* and *Phaseolus vulgaris* were fully functional even when leaf photosynthesis was too low to measure (1, 3). However, in another study, the PSII activity of thylakoids isolated from *Helianthus annuus* declined in parallel with leaf photosynthesis, the rate of decline being slower in drought acclimated plants (13). These inconsistencies may result from differential loss of PSII activity during chloroplast isolation or from effects of other environmental factors such as high leaf temperature or light intensity, which could occur concurrently with drought (12), *e.g.* the PSII electron transport capacity of *Macroptilium atropurpureum* was reduced by drought at 31°C when the photon flux density was 1500 μmol m^{-2} s^{-1} (12). These conditions were optimum for photosynthesis in well watered plants.

There are several mechanisms by which osmotic adjustment could delay the effects of drought on photosynthetic electron transport capacity, *e.g.* the slower decline in cell volume caused by the accumulation of solutes could reduce conformational changes in the thylakoid membranes. Accumulated solutes may also ensure the maintenance of the structural integrity of the thylakoid membranes during drought and it has been suggested that solutes such as proline and betaine can associate with hydrophobic groups thus enhancing the stability of macromolecules under drought stress (26). However, both these effects are likely to be negated by isolation procedures if the chemical composition of the isolation medium does not closely match that of the chloroplast stroma. Osmotic adjustment may also delay stomatal closure and consequently the depletion of intercellular CO_2 thereby prolonging CO_2 fixation. It has been suggested that depletion of intercellular CO_2 in some species may lead to photoinhibition of PSII at high light intensities (22). However, Sharp and Boyer (27) concluded that there was no photoinhibition in droughted sunflower at a photon flux density of 900 μmol m^{-2} s^{-1}.

This study was conducted to evaluate the effect of osmotic adjustment on electron transport capacity of sunflower during drought, under conditions where neither heat inactivation nor photoinhibition were likely. The degree of osmotic adjustment was varied by drought acclimating the plants, by growing them at 660 μl CO_2 L^{-1}, or by exposing them to both treatments. Chl *a* fluorescence of intact leaf tissue and PSII activity of isolated thylakoids under hypotonic osmotic conditions were used to assess the status of photosynthetic electron transport during drought. The changes in leaf water status, conductance, osmotic volume, and the concentrations of K and soluble sugars were monitored throughout the droughting cycle.

MATERIALS AND METHODS

Plant Growth. One hundred and twenty sunflower (*Helianthus annuus* var Hysun 30) seedlings were grown individually in 1.5 L pots containing 1300 g of sand/peat/soil mix. The water potential of the soil was restored to −0.03 MPa daily. A solution of a complete fertilizer was applied twice weekly to ensure adequate nutrition. Plants were grown in one of two, matched, controlled environment cabinets both of which were maintained

at 25°C for the 16 h light period and 18°C for the 8 h dark period. The relative humidity was approximately 50% and the photosynthetic photon flux density at the top of the plants was 450 μmol m^{-2} s^{-1}. One cabinet was at the ambient CO_2 concentration of 340 μl CO_2 L^{-1} and the other was maintained at 660 μl CO_2 L^{-1} by continuous injection of CO_2.

Drought acclimation was commenced after 3 weeks growth when there were four to six leaves per plant. For half the plants in each cabinet the watering was continued as before. For the other half, water was withheld until the soil water potential reached −1.5 MPa. The soil water potential was subsequently restored to −0.03 MPa then droughting was repeated. After the end of the second droughting cycle the soil water potential was raised to and maintained at −0.03 MPa for 2 d. Water was then withheld from all pots. This resulted in four treatments each on 30 plants, namely: ± drought acclimation at both CO_2 concentrations.

Measurements of leaf conductance, water status, soluble sugar and K content, and Chl fluorescence were made 6 h after the commencement of the light period on a destructive basis, *i.e.* no plant was sampled more than once. Where LSDs are reported, analysis of variance was performed.

Leaf Conductance. Measurements of attached leaves were made using a Delta T (mark 3) diffusion porometer while the plants were in the cabinets in which they had been grown. Measurements were made on the lower side of the leaves only, because there is little difference between the conductance of the upper and lower sides of sunflower leaves (16). A single measurement was made per plant on one leaf of the most recently fully expanded pair of leaves (*i.e.* the third above the cotyledons of the drought acclimated plants or the fourth of nonacclimated plants). The measurements were made on five plants from each treatment on d 1, 2, 3, 4, 6, and 8 of the drought period.

Leaf Water Status. Immediately after measurement of leaf conductance the leaves on which the conductance mesurements had been made were detached and two adjoining 10 × 10 mm sections were cut from one side of the midvein. The water potential of one section was measured using a thermocouple psychrometer then the osmotic potential was measured after the same sample had been frozen and thawed in the chamber. Turgor was calculated as the difference between the water and the osmotic potentials. The other section was weighed then floated on distilled water for 4 h at 25°C at the light compensation point. This treatment had been shown to result in full hydration of the leaf. The turgid section was weighed, dried at 70°C, and weighed again. The *RWC*[1] was calculated from:

$$RWC = \frac{\text{Fresh weight} - \text{dry weight}}{\text{Turgid weight} - \text{dry weight}}$$

Logarithmic plots of *RWC* against osmotic potential were analyzed by the method of Pollard (21) to determine the best single or two line fit to the data.

Chl *a* Fluorescence. The leaf portions remaining after sampling for measurement of water status were immediately wrapped in cling wrap and aluminum foil. After 1 h of dark adaptation, one disc (16 mm diameter) was cut from each leaf near the midvein but on the opposite side of the vein from the area sampled for water status. On d 6, additional samples were also taken from desiccated areas nearer the leaf margin.

A plexiglass light mixing rod connected to a bifurcated light pipe was positioned over the center of each disc. The disc, at 23°C, was irradiated through one arm of the pipe by a regulated tungsten light source filtered through a Corning 4-96 filter to give a photosynthetic photon flux density of 22.5 μmol m^{-2} s^{-1} at the leaf surface. The resulting Chl fluorescence was directed via the second arm of the pipe onto a photomultiplier tube protected by a Bausch and Lomb 693 nm interference filter and a Corning 2-64 red cutoff filter. The output from the photomultiplier was interfaced to a HP-9826 computer (Hewlett-Packard, Sydney). The magnitude of F_V/F_O was used to evaluate PSII electron transport activity. F_O describes the initial rise occurring after illumination, F_V the subsequent rise to the maximum (F_P), and R_O the maximal rate of decline after F_P (19).

Chloroplast Isolation and Assay. The five discs per treatment which had been used for Chl fluorescence measurements were homogenized for 5 s using an Ultra-Turrax (at half-line voltage) in 8 ml of medium containing 50 mM NaCl, 5 mM KH_2PO_4, and 15 mM Na_2HPO_4 (pH 7.5). The homogenate was filtered through Miracloth and 0.1 ml of the filtrate containing approximately 0.5 μg Chl was immediately transferred to 1.5 ml of reaction mixture 50 mM NaCl, 5 mM KH_2PO_4, 15 mM Na_2HPO_4 (pH 7.5), 0.1 mM *p*-phenylenediamine, 0.7 mM $K_3Fe(CN)_6$. Electron flow through PSII was measured at 23°C as ferricyanide reduction with *p*-phenylenediamine as the primary electron acceptor. An Aminco model DW-2 dual wavelength spectrophotometer (American Instrument Co., Silver Spring, MD) was used to follow the difference in adsorption between 420 and 450 nm as ferricyanide was reduced. The reaction mix was side-illuminated with tungsten light that had passed through a Corning 2-60 red cutoff filter. The photomultiplier tube was protected by a Corning 4-96 blue filter.

Soluble Sugars and Potassium. On d 2, 4 and 6 leaves opposite to those sampled for water status were removed and frozen. After thawing they were extracted twice in 80% ethanol at 80°C. The extract was evaporated to dryness after which the remaining material was dissolved in water. The sugar concentration of the extract was measured by the anthrone method (20) and the K concentration by atomic absorption spectrophotometry. The mM concentrations in the total leaf water were calculated.

RESULTS AND DISCUSSION

Osmotic Adjustment. Logarithmic plots of *RWC* against osmotic potential were biphasic for all treatments except the 340 μl CO_2 L^{-1} nonacclimated treatment (Fig. 1). Above the inflection points the *RWC* was almost constant while the osmotic potential fell. This is characteristic of osmotic adjustment where an accumulation of solutes results in a greater reduction in osmotic potential than can be accounted for by the concentrating effects of water loss (14, 15). Below the inflection points the slopes were lower and were approximately the same for all three treatments. In this region the decrease in osmotic potential was primarily attributable to water loss. Both drought acclimation and high CO_2 resulted in some degree of osmotic adjustment as compared to the 340 μl CO_2 L^{-1} nonacclimated treatment (Fig. 1). Drought acclimation had a greater effect than high CO_2 and the combination, if anything, produced an even bigger effect.

The degree of osmotic adjustment depends on the rate of decrease in leaf water potential (9). In sorghum the degree of adjustment was the same when the water potential decreased at either 0.15 or 0.7 MPa/d and it was only when the rate was increased to 1.2 MPa/d that osmotic adjustment became negligible (9). Therefore, it is unlikely that the differences in the rate of decline in water potential between the treatments in our experiment (Fig. 2), which varied over a narrow range (0.45–0.58 MPa/d between d 2 and 8) would have contributed substantially to the differences in osmotic adjustment.

The increase in the concentration of K and 80% ethanol soluble sugars during drought (Table I) was not large enough to

[1] Abbreviations: *RWC*, relative water content; F_O, constant yield Chl fluorescence; F_V, variable yield Chl fluorescence; F_P, maximum Chl fluorescence (constant plus variable yield fluorescence); R_Q, maximal rate of Chl fluoresence quenching; Q_A, primary electron acceptor of PSII.

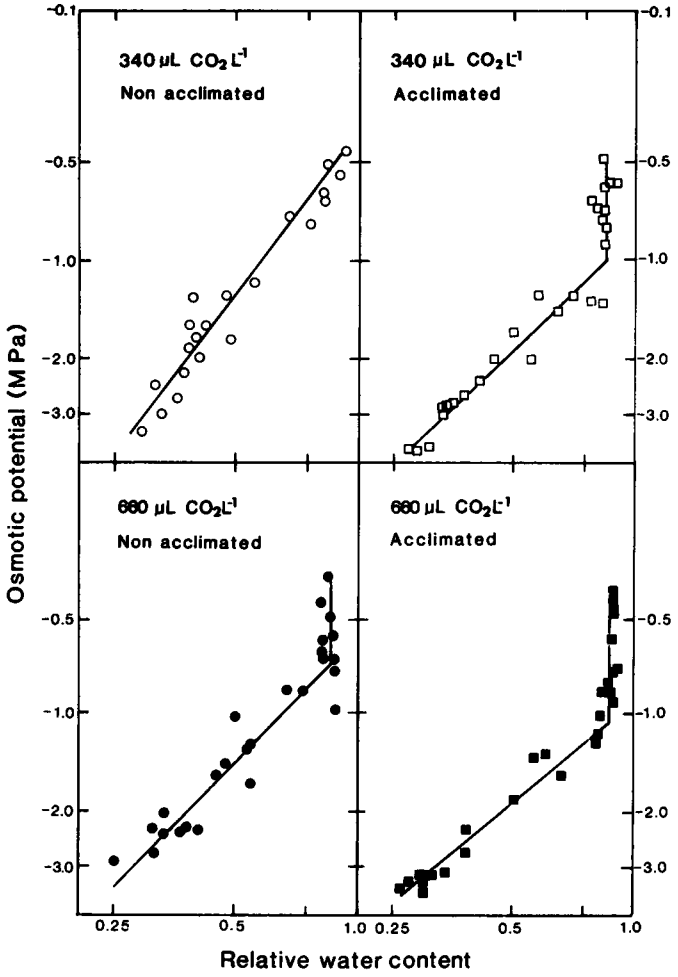

FIG. 1. Influence of drought acclimation and CO_2 enrichment on osmotic adjustment of expanded sunflower leaves during drought. Slopes of greater than plus one in the logarithmic plot of minus *RWC* against osmotic potential indicate that osmotic adjustment has occurred (16). Plants were continuously exposed to either 340 (open symbols) or 660 (closed symbols) μl CO_2 L^{-1}. The plants were well watered for 3 weeks after which half of them at each CO_2 concentration were drought acclimated. The other half were watered daily. Water was then withheld from all the plants. During the ensuing drought period, water status was measured on d 1, 2, 4, 6, and 8 using, on each occasion, one leaf per plant selected from the most recently expanded leaf pair, on each of the five plants per treatment.

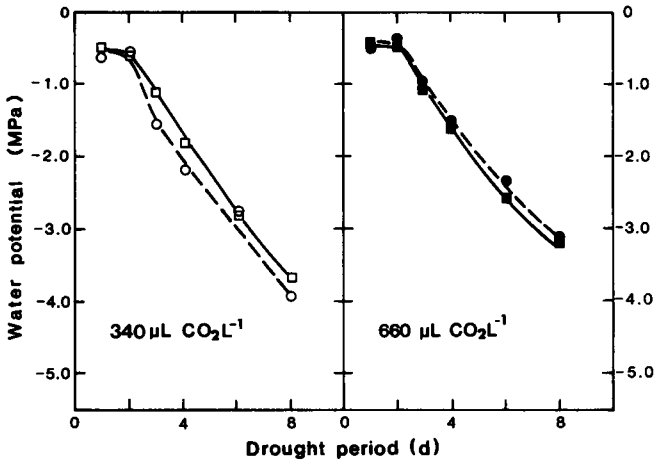

FIG. 2. Effect of drought acclimation and CO_2 enrichment on the water potentials of expanded sunflower leaves during drought. Plants were continuosly exposed to either 340 (open symbols) or 660 (closed symbols) μl CO_2 L^{-1}. The plants were well watered for 3 weeks after which half of them at each CO_2 concentration were drought acclimated (solid lines). The other half were watered daily (dashed lines). Water was then withheld from all the plants. During the ensuing drought period, measurements were made on d 1, 2, 4, 6, and 8 using, on each occasion, one leaf per plant selected from the most recently expanded leaf pair, on each of the five plants per treatment. Each data point represents the mean of five measurements, LSD = 0.19, $P \leq 0.05$.

account for the osmotic adjustment. Osmotic potentials were calculated from the soluble sugar and K concentrations, on the assumption that K was accompanied by an equivalent concentration of a univalent anion. On d 2 these solutes accounted for 70 to 90% of the osmotic potential but even though their concentrations increased during the drought period (Table I), they accounted for progressively less of the osmotic potential and at d 6 accounted for only 28 to 38%. Thus it is unlikely that these solutes were the major contributors to osmotic adjustment. It has been reported previously that soluble sugars were not responsible for osmotic adjustment in sunflowers (10).

Ratio of Turgid Weight to Dry Weight. There was a sudden increase in the turgid weight to dry weight ratio in all treatments during the drought period (Table II). This occurred shortly after the *RWC* began to decline and so was evident first in the leaves with least osmotic adjustment (Figs. 1 and 3). There was no change in leaf dry weight (data not shown); therefore, the increases in the ratio must have been due to increases in the uptake of water when the droughted leaf sections were rehydrated. Changes in the elastic properties of the cell walls due to cell division or elongation were not responsible because the leaves were fully expanded before the droughting was commenced. Further, drought does change the elastic modulus of fully expanded sunflower leaves (29) and it is unlikely that CO_2 enrichment would modify this response. Thus the sudden increases in the turgid to dry weight ratio of our sunflowers during drought are likely to be related to changes in the apoplastic and/or symplastic water in the rehydrated leaf sections. The cells of the droughted leaves would only have expanded to larger volumes than those of the undroughted leaves in treatments where osmotic adjustment had occurred. However, if an elastic modulus of 5 MPa (29) and an average solute molecular weight of 100 is assumed, then less than 50% of the change in the turgid to dry weight ratio can be accounted for. Furthermore, in this study and that of Flower and Ludlow (6) with *Cajanus cajan*, the degree of osmotic adjustment was not closely related to the changes in the turgid to dry weight ratio. Consequently, it appears that changes in the amount of apoplastic water may have contributed to the observed increase in the ratio.

The turgid weight to dry weight ratio was always lower in the leaves exposed to high CO_2 (Table II). We have previously shown that the density of *Pinus radiata* needles increased at elevated CO_2 concentrations and that this was not due to anatomical changes (4) but was caused by an accumulation of starch (JP Conroy, unpublished data). The lower turgid weight to dry weight ratio of the sunflower leaves from the high CO_2 treatment could also have been due to the presence of starch.

***RWC*, Turgor, and Conductance.** Osmotic adjustment delayed the decline in *RWC* and conductance during drought, and as with the turgid weight to dry weight ratio, the length of the delay was dependent on the degree of adjustment (Fig. 3). Loss of turgor was also delayed (data not shown). The turgor of leaves from the nonacclimated 340 μl CO_2 L^{-1} treatment was zero on d 3 and on d 4 the *RWC* had fallen to 36% of its maximum. On

Table I. *Influence of CO_2 Enrichment and Drought Acclimation on the Concentration of K and Soluble Sugars in Sunflower Leaves during Drought*

The plants were well watered for 3 weeks after which half of them at each CO_2 concentration were drought acclimated. Water was then withheld from all the plants. During the ensuing drought period, one leaf per plant was selected from the most recently expanded leaf pair, on each of the five plants per treatment, on d 2, 4, and 6 and the resulting five leaves per treatment were pooled for chemical analysis.

Drought period	Solute	Solute Concentration/CO_2 Concentration			
		340 μl L^{-1}		660 μl L^{-1}	
		Nonacclimated	Acclimated	Nonacclimated	Acclimated
d		*mM*			
2	K	88	141	70	84
	Sugars	59	35	119	49
4	K	153	189	112	124
	Sugars	87	45	210	122
6	K	156	241	110	159
	Sugars	104	69	209	124

Table II. *Influence of Drought Acclimation on the Ratio of Turgid Weight to Dry Weight of Sunflower Leaves Exposed Continuously to Either 340 or 660 μl CO_2 L^{-1}*

The plants were well watered for 3 weeks after which half of them were drought acclimated. During the droughting period water status was measured on d 1, 2, 3, 4, 6, and 8 using on each occasion, one leaf per plant selected from the most recently expanded leaf pair, on each of the five plants per treatment.

Drought Period	Ratio of Turgid Weight to Dry Weight[a]/CO_2 concentration			
	340 μl L^{-1}		660 μl L^{-1}	
	Nonacclimated	Acclimated	Nonacclimated	Acclimated
d				
1	7.9	8.6	7.0	7.1
2	7.7	8.4	6.6	7.0
3	9.0	8.4	7.8	7.6
4	9.1	9.2	7.6	7.5
6	9.9	9.9	8.8	9.8
8		10.7	9.0	9.2

[a] Values are the means of five measurements. LSD = 0.85, $P \leq 0.05$.

d 4 and thereafter, conductance and presumably CO_2 assimilation, was negligible. In contrast, leaves from the high CO_2, drought acclimated treatment did not lose turgor until d 4, their *RWC* did not reach 36% until d 6, and their conductance was still measureable at d 8.

It appears that plants that regularly wilt can continue to function at *RWC* as low as 36%. *C. cajan* can also dehydrate to a *RWC* of 36% without permanent injury (6). Wilted sunflower leaves can maintain 50% of their photosynthetic capacity at water potentials as low as −2.2 MPa (23). Although we have no microscopic evidence, the morphology of the mesophyll cells within the leaf when it is at a *RWC* of 36% is likely to be very different from that of cells in a fully hydrated leaf and it is probable that deformation of the organelles and the membranes contained therein will occur. Metabolic function could be substantially altered under these conditions.

Chl *a* Fluorescence of Detached Leaves and PSII Activity of Isolated Thylakoids. During the early stages of drought (d 1–d 4) F_V/F_O increased in all treatments (Fig. 4). This coincided with decreases in *RWC* rather than water potential (Figs. 2 and 3). The changes in F_V/F_O were due to changes in both F_O and F_V (Table III) with F_O initially decreasing (d 1–d 2) and F_V subsequently increasing (d 2–d 3 for nonacclimated leaves and d 3–d 4 for acclimated leaves). These changes are likely to have resulted from altered photochemistry rather than from physical changes. While shinkage of the leaves would increase the number of fluorescing centers per unit area, this would be counteracted by decreased light penetration and increased absorption of fluorescence within the leaf. Optical changes would in any event affect F_O and F_V equally and this was not observed.

The main source of the F_O emission is energy trapped within the photon harvesting system which cannot be used in photochemistry (19). It hence provides a measure of the fraction of the light energy distributed to PSII. Thus a possible interpretation of the decreases in F_O, which were observed early in the drought period (Table III) after only small changes in water potential and *RWC* had occurred (Figs. 2 and 3), is that an increase in the efficiency of excitation trapping at the active centers of PSII had taken place.

The rise in fluorescence emission from F_O to F_P (see inset to Fig. 4) parallels increases in the ratio of the reduced to oxidized state of Q_A, the primary acceptor of PSII (19). In dark adapted leaf tissue, Q_A is in the oxidized state and is able to accept electrons. Energy loss via F_V is therefore minimal. Upon irradiation, Q_A becomes reduced via PSII and the F_V emission increases. The subsequent decrease or quenching of F_V, which occurs immediately after F_P, can mainly be attributed to oxidation of Q_A by electron transfer reactions of the pathway after PSII. Other quenching processes, notably those associated with energization of the thylakoid membranes can contribute to the total quenching. The level of F_V thus reflects the balance between the rate of PSII activity, *i.e.* the rate of reduction of Q_A and the

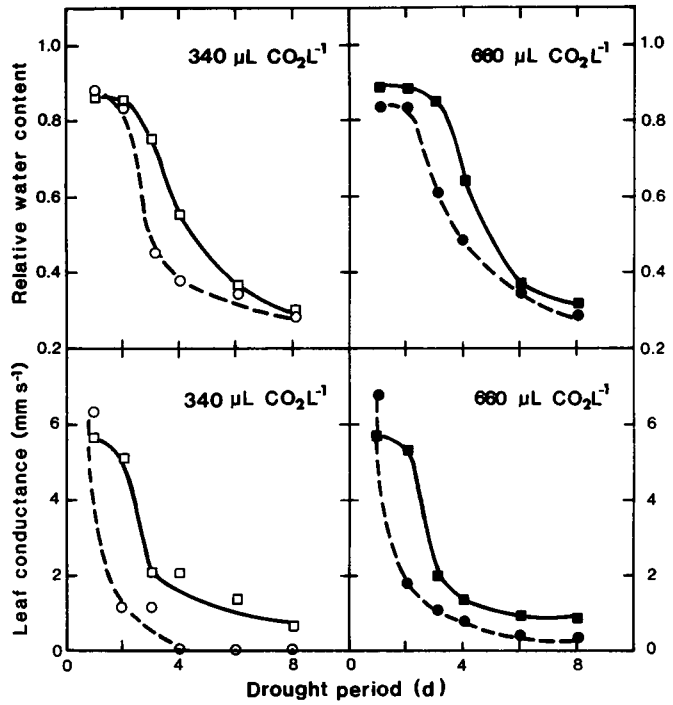

FIG. 3. *RWC* and conductance of expanded sunflower leaves during drought stress. Details of growth, acclimation, sampling, and symbols are as in Figure 2. Each data point represents the mean of five measurements. For *RWC* the LSD = 0.09 and for conductance LSD = 1.0, $P \leq 0.05$.

rate of photooxidation of Q_A^- ultimately via PSI. Consequently, any imbalance which may arise during droughting might be expected to also alter the pattern of induced Chl fluorescence and the level of F_V. Specifically, a loss of PSII activity or, less likely, a marked increase in the rate of photooxidation of Q_A^- would result in a decrease in F_V. However, in our droughted sunflowers, the level of F_V actually increased up to d 4 and remained high throughout the experiment in all treatments with the exception of the 340 μl CO_2 L^{-1} non-acclimated treatment (Fig. 4). This coupled with the evidence that there were only minor changes in the PSII activity of thylakoids isolated from droughted plants (Fig. 5) points to the preservation rather than the inhibition of PSII activity *in vivo* coincidental with substantial decreases in *RWC*.

Increases in F_V associated with dehydration have been previously observed. Leaves of the desert plant *Borya nitida*, which had been slowly dehydrated to initiate degreening, showed a 160% stimulation of F_V (7). Degreening is a reversible mechanism by which the damaging effects of high insolation and drought are avoided, but like osmotic adjustment, it occurs only if dehydration is slow enough to allow for metabolic adjustments. Thus, when the rates of dehydration in *B. nitida* were rapid, degreening was inhibited and the stimulation of F_V was abolished (8). F_V also increased in willow leaves as the *RWC* declined (18). These results and the observation that maximum photosynthetic rates occurred in *Commenlina africana*, *Spinacia oleracea*, and *Zebrina pendula* when the leaf tissues were at about 75% of their turgid volumes (11), suggest that the concentration of solutes in fully hydrated cells may be too low to maintain the thylakoid membranes in a conformation which optimizes electron transport through PSII.

The PSII activity of isolated thylakoids under hypotonic osmotic conditions did not change in parallel with changes in conductance or *RWC* from d 1 to d 4 (Fig. 5). Activities remained constant or declined slightly, thus confirming the inferences from the Chl fluorescence data that no major decreases in PSII activity accompanied decreases in *RWC*. The increases observed in Chl fluorescence *in vivo* were not paralleled by increases in PSII activity *in vitro*, but the latter may not accurately reflect those found *in vivo* because the osmotic potential of the isolation and measurement media (−0.45 MPa) did not match that of the leaves which varied from −0.7 to −1.6 MPa between d 1 and d 4.

Electron transfer activity on the photooxidizing side of PSI also appeared to be maintained between d 1 and d 4. As PSII remained active during this period (Fig. 4 and 5), a drought-induced inhibition of the photooxidation of Q_A^- should have led to a decrease in R_Q (see inset to Fig. 4) but this did not occur (Table IV). In the early stages of drought, R_Q was lower in the acclimated leaves but increased as droughting progressed so that by d 4 the values were similar in both acclimated and nonacclimated treatments (Table IV).

By d 6, conductance was markedly reduced in all treatments and had been negligible for 2 d in leaves from the 340 μl CO_2 L^{-1} nonacclimated treatment (Fig. 3). This caused no loss of F_V *in vivo* and no appreciable decline in PSII activity of isolated thylakoids (Figs. 4 and 5). In contrast, when stomatal closure was simulated by applying aluminum-foil adhesive tape to the lower leaf surfaces of well watered *Nerium oleander*, some loss of PSII electron transport activity occurred (2). The experiment was conducted in full sunlight and therefore photoinhibition may have contributed to the loss of activity.

The *RWC* was about 35% in all treatments by d 6 (Fig. 3). Even if there were some change in the apoplastic volume with drought, the symplastic volume must have been reduced by at least 50%. If decreases in chloroplast volume paralleled those of the symplast, then reductions in photosynthesis could have been expected because the CO_2 dependent O_2 evolution of intact isolated chloroplasts is very sensitive to osmotically induced reductions in volume (24). The osmotic potential of the chloroplast stroma in an intact leaf is likely to be similar to leaf osmotic potential, otherwise the outer membrane of the chloroplast would rupture (17). However, the changes in the osmotic volume of the leaf would only be paralleled by changes in chloroplast volume if the relative concentration of solutes in each compartment remained equal. If the chloroplast is able to preferentially accumulate solutes, then its volume will not decrease as rapidly as the cell osmotic volume. In our experiment no inactivation of electron transport capacity occurred until the *RWC* was below 30%. There are two possible explanations for this: first, a constant chloroplast volume may have been maintained despite the reduction in cell volume or second, electron transport capacity may have been unaffected by changes in chloroplast volume.

Between d 6 and d 8 the drought effects became so severe in nonacclimated plants that areas near their leaf margins became desiccated. These symptoms were first apparent on leaves from the nonacclimated, 340 μl CO_2 L^{-1} treatment. No loss of Chl occurred in the desiccated areas (data not shown) but Chl fluorescence of intact leaves and PSII activity of thylakoids isolated under hypotonic osmotic conditions indicated that electron transport was severely impaired (Table V). However, it was still functional in nondesiccated adjacent areas nearer the midvein (Table V). This suggests that loss of electron transport function is not gradual but occurs after some catastrophic event. Plants exhibiting these symptoms did not recover upon rewatering. By d 8, desiccation of the leaves from the nonacclimated 340 μl CO_2 L^{-1} treatment was widespread and there was little measurable photosynthetic electron transport activity. In the other treatments electron transport remained functional (Figs. 4 and 5; Table IV).

Concluding Remarks. Under conditions where photoinhibition and heat inactivation were unlikely, the photosynthetic electron

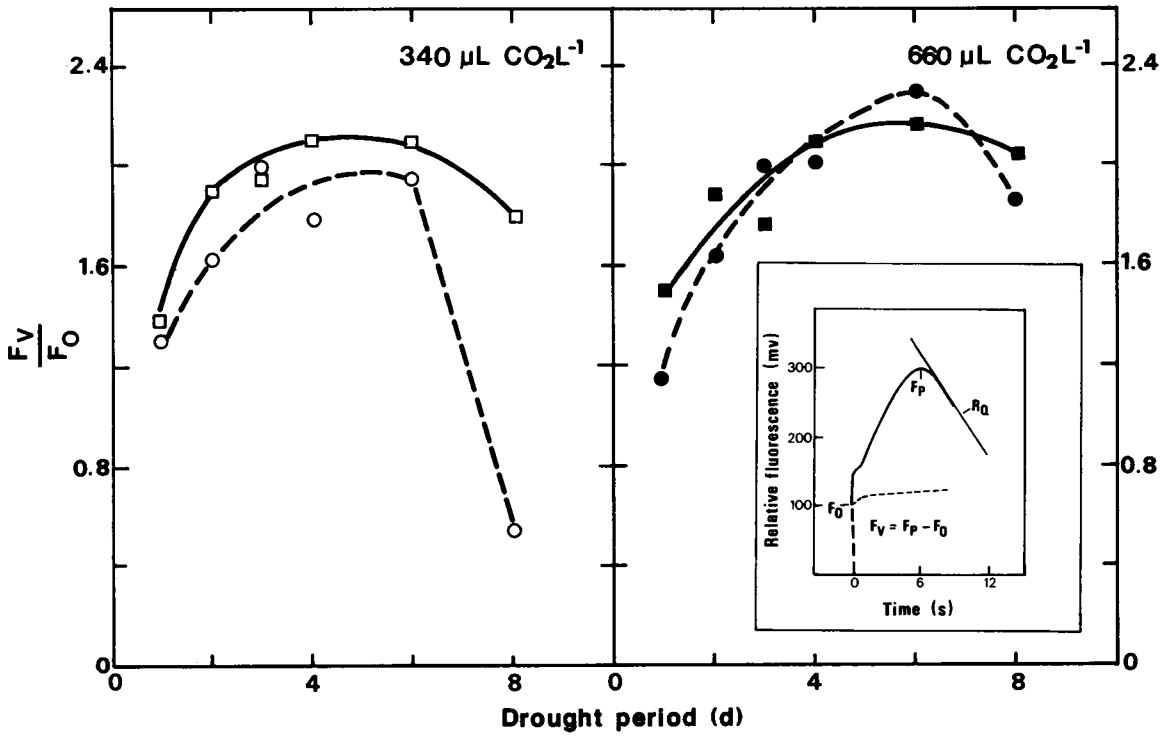

FIG. 4. Chl *a* fluorescence of leaves from acclimated and nonacclimated plants grown at either 340 or 660 μl CO_2 L^{-1}. Details of growth, acclimation, sampling, and symbols are as in Figure 2. Each data point represents the mean of five measurements. LSD = 0.28, $P \leq 0.05$. Inset: Typical fluorescence induction kinetics obtained for turgid and wilted portions of the leaves (solid line) and for desiccated portions of the leaves (dashed line).

Table III. *Constant Yield and Variable Chl Fluorescence of Leaves from Drought Acclimated and Nonacclimated Sunflower during Drought*

The plants were well watered for 3 weeks after which half of them at each CO_2 concentration were drought acclimated. Water was then withheld from all the plants. During the ensuing drought period water status and Chl *a* fluorescence were measured on d 1, 2, 3, 4, 6, and 8 using, on each occasion, one leaf per plant selected from the most recently expanded leaf pair, on each of the five plants per treatment.

Drought Period	Fluorescence Variable[a]			
	F_O		F_V	
	Water pretreatment			
	Nonacclimated	Acclimated	Nonacclimated	Acclimated
d	*mV*			
1	158	138	190	199
2	118	104	190	195
3	108	103	218	194
4	119	119	226	221
6	110	109	231	229
8	135	125		237

[a] CO_2 had no significant effect on either fluorescence variable except at d 8 in the nonacclimated plants grown at 340 μl CO_2 L^{-1} and therefore values on each day except d 8 are means of 10 measurements (5 replicates at each CO_2 level). For F_O LSD = 9 and F_V LSD = 18, $P \leq 0.05$.

transport activity of droughted sunflower leaves remained resilient until the leaves began to die. Death occurred first in leaves with minimal osmotic adjustment when the *RWC* fell below 30%. This is consistent with the recent observation that leaves of *C. cajan* plants, subjected to a range of osmotic adjustment treatments, died at a critical *RWC* (35–36%) rather than at a critical water potential (6). These results emphasize the need for the maintenance of structural integrity within the cell during drought. Although the critical *RWC* for *C. cajan* was similar to that for sunflower, it is unlikely that the figure has any fundamental significance, because anatomically different leaves would be expected to have different critical *RWC*. The stability of the electron transport activity of sunflowers during drought may reflect the ability of the chloroplast to maintain volume by synthesizing solutes which are compatible with reactions of the electron transport system. This was recently demonstrated *in vivo* in chloroplasts of salt stressed *Spinacia oleracea* leaves (25). The significance of osmotic adjustment by chloroplasts to the maintenance of carbon fixation and cycling within the leaf during drought remains to be investigated.

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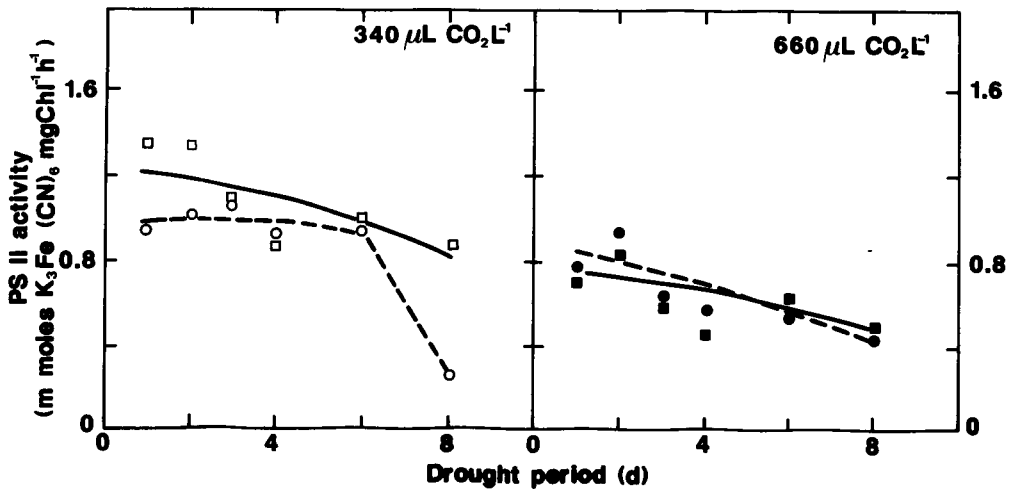

FIG. 5. PSII activity of chloroplast fragments isolated from the five leaf discs per treatment which were used for the Chl *a* fluorescence measurements. Details of growth, acclimation, sampling, and symbols are as in Figure 2.

Table IV. *Effect of CO_2 Enrichment and Drought Acclimation on the Maximal Rate of Quenching of Chl Fluorescence*

The plants were well watered for 3 weeks after which half of them at each CO_2 concentration were drought acclimated. Water was then withheld from all the plants. During the ensuing drought period water status and Chl *a* fluorsecence were measured on d 1, 2, 3, 4, 6, and 8 using on each occasion, one leaf per plant selected from the most recently expanded leaf pair, on each of the five plants per treatment.

Drought Period	R_Q[a] at CO_2 Concentration			
	340 μl L^{-1}		660 μl L^{-1}	
	Nonacclimated	Acclimated	Nonacclimated	Acclimated
d		*mV* s^{-1}		
1	3.1	2.1	3.5	1.9
2	3.7	2.9	4.3	2.9
3	4.4	2.8	3.2	2.1
4	3.8	3.9	3.2	4.1
6	2.5	2.9	3.6	3.1
8	0.2	2.3	3.1	3.6

[a] Values are the means of five measurements. LSD = 0.7, $P \leq 0.05$.

Table V. *Water Status, Chl a Fluorescence and PSII Activity of Isolated Chloroplasts of Turgid, Wilted, and Desiccated Portions of Leaves from Nonacclimated Plants Grown at 340 μl CO_2 L^{-1}*

Plants were watered daily for 4.5 weeks. Water was then withheld. For the single leaf measurements, the wilted samples were taken from areas close to the midrib and the desiccated samples from the margins. For comparison, the mean values measured at d 1 and d 6 are shown.

Drought Period		Condition of Leaf Sample	Water Potential	*RWC*	F_V/F_O	R_Q	PSII Activity
	d		*MPa*			*mV* s^{-1}	*mmol* K_3 *Fe(CN)*$_6$ *mg*$^{-1}$ *Chl* h^{-1}
Single leaf	6	Wilted	−3.4	0.29	1.7	2.6	ND[c]
		Desiccated	ND	ND	0.6	0.0	0.12
Daily[b]	1	Turgid	−0.6	0.87	1.3	3.1	0.94
Mean	6	Wilted	−2.8	0.34	1.9	2.5	0.94

[a] Mean of two measurements for all parameters except for PSII activity which was measured on a pooled sample. [b] Mean of five measurements except for PII activity which was measured on a pooled sample. [c] Not determined.

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