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Photosynthesis and Growth of Water Hyacinth under CO_2 Enrichment[1]

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ABSTRACT

Water hyacinth (*Eichhornia crassipes* [Mart.] Solms) plants were grown in environmental chambers at ambient and enriched CO_2 levels (330 and 600 microliters CO_2 per liter). Daughter plants (ramets) produced in the enriched CO_2 gained 39% greater dry weight than those at ambient CO_2, but the original mother plants did not. The CO_2 enrichment increased the number of leaves per ramet and leaf area index, but did not significantly increase leaf size or the number of ramets formed. Flower production was increased 147%. The elevated CO_2 increased the net photosynthetic rate of the mother plants by 40%, but this was not maintained as the plants acclimated to the higher CO_2 level. After 14 days at the elevated CO_2, leaf resistance increased and transpiration decreased, especially from the adaxial leaf surface. After 4 weeks in elevated as compared to ambient CO_2, ribulose bisphosphate carboxylase activity was 40% less, soluble protein content 49% less, and chlorophyll content 26% less; whereas starch content was 40% greater. Although at a given CO_2 level the enriched CO_2 plants had only half the net photosynthetic rate of their counterparts grown at ambient CO_2, they showed similar internal CO_2 concentrations. This suggested that the decreased supply of CO_2 to the mesophyll, as a result of the increased stomatal resistance, was counterbalanced by a decreased utilization of CO_2. Photorespiration and dark respiration were lower, such that the CO_2 compensation point was not altered. The photosynthetic light and CO_2 saturation points were not greatly changed, nor was the O_2 inhibition of photosynthesis (measured at 330 microliters CO_2 per liter). It appears that with CO_2 enrichment the temporary increase in net photosynthesis produced larger ramets. After acclimation, the greater total ramet leaf area more than compensated for the lower net photosynthetic rate on a unit leaf area basis, and resulted in a sustained improvement in dry weight gain.

For many plants, especially those with C_3 photosynthesis, an increase in CO_2 produces an increase in net photosynthetic rate (9). This is largely attributable to the elevated CO_2 competing with O_2 to promote the activity of RuBP carboxylase but inhibit that of RuBP oxygenase (30). Although there are notable examples where these short-term increases in photosynthetic efficiency are directly extrapolated into long-term growth improvements (6, 22, 28, 33), this is not always the case (20, 21, 24). Many plants exhibit some form of acclimation to high CO_2, such that the initial increase in net photosynthesis is moderated or even lost, and the expected gains in productivity are not realized (10, 16, 25). The nature of this acclimation has not been fully elucidated, and it differs among species and developmental stages (11, 13, 21). It does not seem to affect directly the competitive interaction between CO_2 and O_2 for fixation by RuBPCase,[2] but may be mediated by more indirect effects, such as increased stomatal resistance or starch production (7, 13, 17).

This study was designed to evaluate the photosynthetic and growth responses of water hyacinth to CO_2 enrichment and to characterize the nature of any acclimations. Water hyacinth is a fresh-water aquatic angiosperm usually occurring as a floating plant, with its roots submersed but its leaves completely aerial. It is most likely a C_3 plant (23). Unlike submersed macrophytes which are shade plants (3), water hyacinth leaves growing in exposed locations can utilize full sunlight for photosynthesis (23). Despite C_3 characteristics, water hyacinth exhibits prolific growth in tropical and subtropical regions, with an annual biomass production of up to 250 tonnes dry weight ha^{-1} (2), and it is now regarded as one of the foremost aquatic weed problems in the world (14). However, because of its ability to scavenge nutrients from water and its high biomass production rate, it has been used experimentally in secondary waste-water treatment facilities, and also as a biomass source for methane generation (18). For use as an energy crop, besides its high productivity, water-hyacinth has the advantages that it is not a food commodity and it does not compete for land space with agricultural food and fiber production.

Given that methane digesters produce CO_2 as a by-product, together with the predicted doubling of atmospheric CO_2 levels in the next century, it was of interest to determine both short- and long-term effects of a relatively small CO_2 enhancement (doubling of atmospheric levels) on the growth of this plant. The data presented here indicate that significant and sustained dry weight increases are attainable, even though the plant exhibits several acclimations to CO_2 enrichment which result in a reduced net photosynthetic rate on a unit leaf area basis.

MATERIALS AND METHODS

Plant Material. Four plants of *Eichhornia crassipes* (Mart.) Solms, each approximately 215 g fresh weight, were placed into a 20 L plastic tub filled with one-third strength macronutrient and full strength micronutrient Hoagland solution (12), which was replaced weekly. Two Conviron E-15 growth chambers (Controlled Environments Inc.) each received four replicate tubs. The quantum irradiance at the plant level was 650 μmol m^{-2} s^{-1} (400–700 nm) and the plants were exposed to a 12-h, 25°C photoperiod and a 20°C scotoperiod. Growth levels of CO_2 in

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[2] Abbreviations: RuBPCase, ribulose bisphosphate carboxylase-oxygenase; PEPCase, phosphoenolpyruvate carboxylase.

the ambient CO_2 chamber were maintained at approximately 330 μl CO_2 L^{-1} by a flow-through system open to outside air. For the enriched-CO_2 chamber, an IR gas analyzer and an input solenoid system connected to a cylinder of ethylene-free liquid CO_2 maintained the CO_2 level in the air at 600 ± 20 μl CO_2 L^{-1} both day and night. The fresh weight, number of daughter plants (ramets), and flowers produced were recorded weekly. After 4 weeks, the plants were separated into mother plants and ramets, weighed, their leaf area and number of leaves determined, and they were dried to constant weight at 60°C. The complete experiment was performed twice, and the data are expressed as the means of the two experiments (8 replicates) ± the standard deviations. Statistical analysis was performed using a paired *t* test.

Gas Exchange Measurements. Transpiration rates and resistances for the adaxial and abaxial leaf surfaces were determined *in situ* with a portable LI-COR porometer model LI-1600 at weeks, 0, 1, 2, and 3 after the start of the experiment. At the start and end of the 4 week growth period, gas exchange measurements were made on excised leaves in a Plexiglas leaf chamber. Ancillary experiments showed that leaf excision had little effect on the net photosynthetic rate, apart from a transitory reduction of up to 16%. Photorespiratory CO_2 release rates into CO_2-free air and CO_2 compensation points were determined in a closed system similar to that described by Van *et al.* (31), using an ADC (Analytical Development Company Ltd.) series 225-Mk3 IR gas analyzer. Net photosynthetic and dark respiration rates were determined with the system in an open mode. Transpiration rates were determined with an in-line EG&G Dew-All humidity analyzer model 911, from dew point measurements of the gas at the inlet and outlet of the leaf chamber. Leaf internal CO_2 concentrations were calculated as in Farquhar and Sharkey (8). Unless otherwise stated, all measurements were made at 30°C, and a quantum irradiance of 600 μmol m^{-2} s^{-1} (400–700 nm).

Leaf Extraction and Analyses. At the end of the experimental period, leaf samples were harvested in the light and stored in liquid N_2 until analysis as described previously (32). Leaf extracts were prepared by grinding to a powder approximately 1 g of frozen material in a mortar with liquid N_2. The frozen powder was then extracted at 4°C in a Ten Broeck homogenizer containing 10 ml of 50 mM Tris-HCl, 10 mM $MgCl_2$, 0.1 mM EDTA, 5 mM isoascorbate, and 1% w/v PVP-40 at pH 8.0. Aliquots were removed for Chl (1), soluble protein (4), and starch (5) determinations. The glucose released from starch (5) was measured using a Yellow Springs Instruments Company glucose analyzer model 27. RuBPCase and PEPCase assays were performed at 30°C. RuBPCase activity was assayed in the active form as described by Vu *et al.* (32), and PEPCase activity as in Van *et al.* (31).

RESULTS

Water hyacinth grown under enriched-CO_2 conditions (600 μl CO_2 L^{-1}) showed a 32% increase in dry matter production as compared to the plants grown at ambient CO_2 levels (Table I). Most of this increase was due to an increase in ramets. The dry matter production of the ramets from the enriched-CO_2 treatments was 39% greater than that of the ambient grown ramets, but the mother plants showed little difference (Table 1). The dry weight of the individual ramets was significantly greater in the enriched-CO_2 treatment, but the number of ramets produced per m^2 did not change (Table I). There was also a large increase in the number of flowers formed in the enriched-CO_2 treatment (Table I).

As shown in Table II, the number of leaves and the leaf area per plant increased under the elevated CO_2, resulting in a 46% increase in the total leaf area index. However, the area of individual leaves exhibited only a slight increase. The dry weight per g fresh weight was higher in ramets from the enriched-CO_2 treatment, as was the starch content of the leaves (Table III); but both soluble protein and Chl were substantially decreased by the CO_2 enrichment (Table III).

Net photosynthetic rates of the enriched-CO_2 and ambient grown leaves, measured at 330 μl CO_2 L^{-1}, saturated at a quantum irradiance of approximately 900 and 1100 μmol m^{-2} s^{-1} (400–700 nm), respectively. Light compensation points of 16 and 14 μmol m^{-2} s^{-1} (400–700 nm), respectively, also were comparable (Fig. 1). Net photosynthetic rates of the enriched-CO_2 grown leaves, when measured at 330 μl CO_2 L^{-1}, were considerably less at all irradiances than those of the ambient grown plants (Fig. 1).

With respect to external CO_2 concentration, the light-saturated net photosynthetic rate of the mother plants at the start of the experiment was saturated at approximately 1100 μl CO_2 L^{-1}. Increasing the CO_2 level from ambient to 600 μl CO_2 L^{-1} resulted in a 42% increase in net photosynthetic rate (data not shown). Similar results were obtained at the conclusion of the experiment for the ramets produced in the growth chamber at ambient levels of CO_2 (Fig. 2). The leaves of the enriched-CO_2 grown ramets required less CO_2 to achieve saturation (about 800 μl CO_2 L^{-1}). Also, they had a lower net photosynthetic rate (expressed on a Chl basis) at each CO_2 concentration than the leaves of ambient grown plants (Fig. 2). However, when measured at the CO_2 level under which they were grown, net photosynthetic rates of leaves from the enriched-CO_2 and ambient growth treatments were similar (68 *versus* 64 μmol mg^{-1} Chl h^{-1}, respectively; Fig. 2). When net photosynthetic rates were expressed on a per unit leaf area basis (Table IV) the enriched plants showed a rate that was about half that of the ambient grown plants, measured at either growth CO_2 level.

Leaves of the enriched-CO_2 grown plants had lower net photosynthetic rates for a given internal CO_2 concentration than their ambient grown counterparts (Fig. 3). Calculated leaf internal CO_2 levels were almost linearly related to the external CO_2 level between 100 and 900 μl CO_2 L^{-1} (Fig. 4). However, for a given external CO_2 concentration, leaves grown under the two

Table I. *Increase in Dry Weight, Number of Flowers, and Ramet Production of Water Hyacinth Grown under 330 and 600 μl CO_2 L^{-1} over a 4 Week Period*

	Increase in Wt			Individual Ramet Wt	Ramet Production	Flower Production
	Total Plants	Mother Plants	Ramets			
	g dry wt·m^{-2}			*g dry wt*	*number·m^{-2}*	
Grown at 330 μl CO_2 L^{-1}	122.2[a] ± 14.2	44.4 ± 14.5	108.1 ± 7.1	2.3 ± 0.4	46.6 ± 5.4	1.9 ± 1.6
Grown at 600 μl CO_2 L^{-1}	161.0 ± 14.6	47.0 ± 9.2	150.0 ± 11.4	3.1 ± 0.3	48.1 ± 6.1	4.7 ± 1.1
% change relative to 330 μl CO_2 L^{-1}	32[b]	6	39[b]	35[b]	3	147[b]

[a] Each value is the mean of 8 replications ± SD. [b] Difference between the means significant at the $\alpha = 0.05$ level as determined by a paired *t* test.

Table II. *Number of Leaves and Leaf Area of Ramets Produced by Water Hyacinth Grown under 330 and 600 μl CO_2 L^{-1} over a 4 Week Period*

	Leaves Per Plant	Leaf Area Per Plant	Area Per Leaf	Leaf Area Index
	number	*dm^2*		*$dm^2 \cdot dm^{-2}$*
Grown at 330 μl CO_2 L^{-1}	5.9[a] ± 0.4	6.0 ± 0.5	1.0 ± 0.4	2.8 ± 0.2
Grown at 600 μl CO_2 L^{-1}	7.5 ± 0.8	8.4 ± 0.9	1.1 ± 0.9	4.1 ± 0.5
% change relative to 330 μl CO_2 L^{-1}	27[b]	40[b]	10	46[b]

[a] Each value is the mean of 7 replications ± SD. [b] Difference between the means significant at the α = 0.05 level as determined by a paired *t* test.

Table III. *Dry:Fresh Weight Ratio, and Starch, Soluble Protein, and Chlorophyll Content of Ramets Produced by Water Hyacinth Grown under 330 and 600 μl CO_2 L^{-1} over a 4 Week Period*

	Dry Wt:Fresh Wt Ratio	Starch	Protein	Chlorophyll
	$g \cdot g^{-1}$		*$mg \cdot g^{-1}$ fresh wt*	
Grown at 330 μl CO_2 L^{-1}	0.039[a] ± 0.004	7.0 ± 0.9	7.8 ± 0.6	1.9 ± 0.01
Grown at 600 μl CO_2 L^{-1}	0.049 ± 0.001	9.8 ± 1.3	4.0 ± 0.3	1.4 ± 0.05
% change relative to 330 μl CO_2 L^{-1}	26	40[b]	−49[b]	−26[b]

[a] Each value is the mean of 3 replications ± SD. [b] Difference between the means significant at the α = 0.05 level as determined by a paired *t* test.

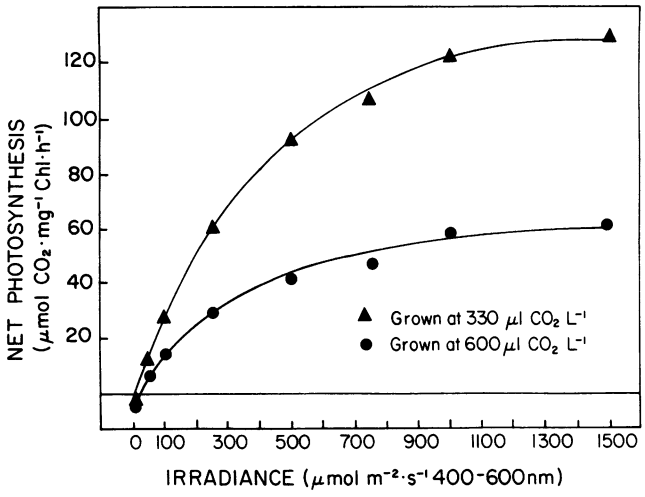

FIG. 1. Photosynthetic response to irradiance of water hyacinth grown under 330 and 600 μl CO_2 L^{-1} for a 4 week period. Measurements were made at 330 μl CO_2 L^{-1}.

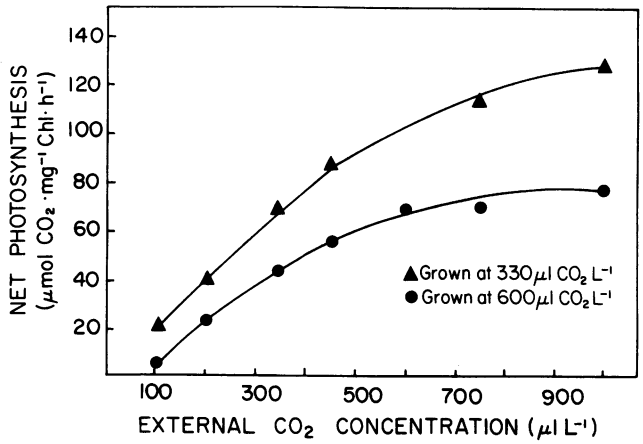

FIG. 2. Photosynthetic response to external CO_2 concentration of water hyacinth grown under 330 and 600 μl CO_2 L^{-1} for a 4 week period.

different CO_2 regimes exhibited almost identical internal CO_2 levels.

The CO_2 compensation points for leaves of the enriched and ambient grown plants were similar (Table V), and did not change from the initially measured values. The degree of inhibition of photosynthesis by 21% O_2, although slightly less for the enriched as compared to the ambient grown ramets, was not statistically different. In contrast, estimated photorespiratory rates, measured as CO_2 release into CO_2-free air in the light, were almost halved following growth at the enriched-CO_2 level (Table V). Dark respiration rates (measured at 330 μl CO_2 L^{-1}) of the leaves were reduced by about one-third in the enriched plants, as compared with those grown at ambient CO_2 levels (Table V).

Transpiration and total leaf resistance were measured on leaves in the growth chambers throughout the experiment (Table VI). The transpiration rate of enriched-CO_2 plants declined over the course of the experiment. This was especially evident for the adaxial leaf surfaces. After 21 d, adaxial transpiration from the enriched-CO_2 leaves was only 15% of that of the ambient grown plants, while for the abaxial surfaces it was 67%. Concomitantly, total leaf resistance increased during the same time period in the enriched-CO_2 plants (Table VI). When enriched-CO_2 plants were

Table IV. *Comparison of the Photosynthetic Rates of Air- and Enriched-CO_2 Grown Water Hyacinth Ramets on a Unit Leaf Area Basis*

CO_2 Concentration Used for Plant Growth	Net Photosynthetic Rate	
	Measured at 330 μl CO_2 L^{-1}	Measured at 600 μl CO_2 L^{-1}
μl L^{-1}	*μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$*	
330	11.4[a] ± 1.7	16.7 ± 1.9
600	5.8 ± 2.7	8.9 ± 4.3

[a] Each value is the mean of three replications ± SD.

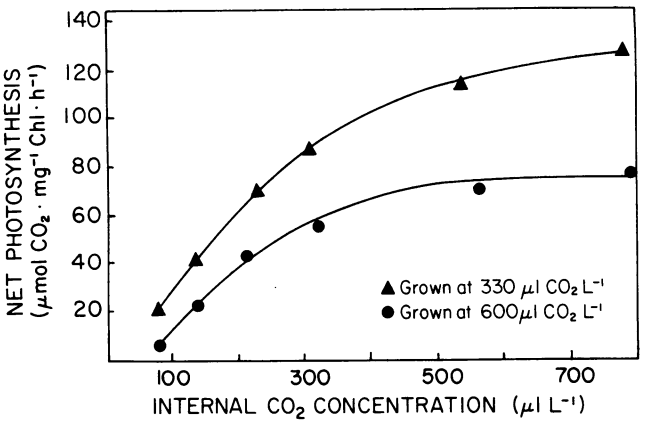

FIG. 3. Photosynthetic response to internal CO_2 concentration of water hyacinth grown under 330 and 600 μl CO_2 L^{-1} for a 4 week period.

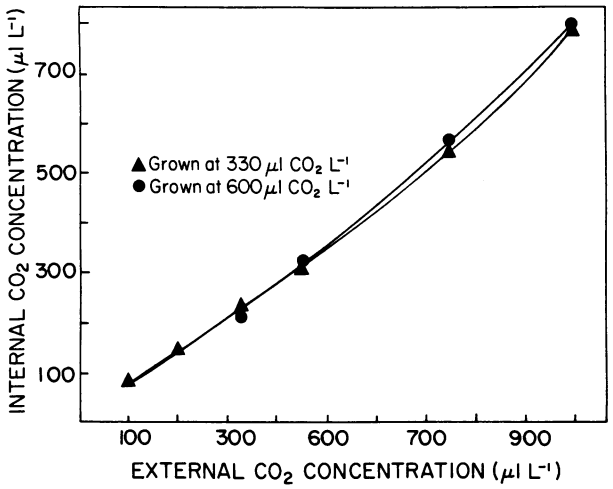

FIG. 4. Internal CO_2 concentration *versus* external CO_2 concentration of water hyacinth grown under 330 and 600 μl CO_2 L^{-1} for a 4 week period.

returned to ambient air, the leaf resistances and adaxial transpiration rates did not return to initial levels, even after several days (data not shown).

Maximum PEPCase activities measured in water hyacinth leaves were about 6 to 7% of the maximum RuBPCase values (Table VII). RuBPCase was the primary carboxylation enzyme and its activity on a Chl basis declined by approximately 20% during growth at enriched as compared to ambient levels (Table VII). This decline was even more evident when the activity was expressed on a fresh weight basis. PEPCase activity showed less difference between the ambient- and enriched-CO_2 grown plants.

DISCUSSION

The growth enhancement of water hyacinth due to CO_2 enrichment was largely restricted to the ramets. A number of workers have reported that immature tissue is more responsive to CO_2 (11, 21, 28) but this cannot fully explain the current findings, since the mother plants did show a large, temporary, enhancement of photosynthetic rate when initially exposed to high CO_2. Presumably, much of the increase in assimilate formed by the mother plants during the temporary photosynthetic rate enhancement was allocated to the ramets. The temporary rate enhancement for the mother plants in enriched CO_2 did not increase the number of ramets produced, but did increase the dry weight, number of leaves, leaf area per ramet, and leaf area index.

Water hyacinth growth is indeterminate, and the major method of reproduction is vegetative by ramet production (26). The results presented here indicate that CO_2 enrichment led to an enhancement of leaf meristem initiation, rather than an enhancement of ramet-producing meristems whose numbers may be more limited. This contrasts with observations for wheat, in which tiller production is greater under elevated CO_2 (29) and soybean, where dry weight increases have been attributed to greater specific leaf weights and not the initiation of new leaves or greater leaf area (6). For some species, CO_2 enrichment has been reported to delay flower production (19). This is not the case for water hyacinth which showed a substantial increase in flower production at 600 μl CO_2 L^{-1}, a response similar to that reported for greenhouse-grown tomato and cucumber (33). Thus, CO_2 enrichment could increase the potential for sexual reproduction in water hyacinth, but this is still an uncertain conclusion because seed production was not determined. Seed production is not necessary for the maintenance of water hyacinth in a nonfluctuating water-body (26), as regrowth is usually initiated vegetatively by plants that have over-wintered. However, in ephemeral water bodies where vegetative material desiccates, regeneration from seed becomes important (26).

As is commonly found with C_3 plants, doubling the ambient CO_2 concentration during short-term photosynthesis measurements resulted in an immediate 40 to 60% increase in the net photosynthetic rate of water hyacinth. However, the initial increase per unit leaf area of water hyacinth was not maintained. After 4 weeks, the net photosynthetic rate of the enriched plants was approximately half that of ambient grown plants when both were measured at the same external CO_2 concentration. As might be anticipated from the lack of any major change in the CO_2 compensation point and O_2 inhibition of photosynthesis, the reduced net photosynthetic rate of the enriched-CO_2 plants was accompanied by a concomitant reduction in the estimated rate

Table V. *CO_2 Compensation Point, O_2 Inhibition of Photosynthesis, Photorespiratory CO_2 Release, and Dark Respiration of Ramets Produced by Water Hyacinth Grown under 330 and 600 μl CO_2 L^{-1} for 4 Weeks*

	CO_2 Compensation Point	O_2 Inhibition[a]	CO_2 Release into CO_2 Free Air	Dark Respiration
	μl L^{-1}	%	μmol $CO_2 \cdot mg^{-1}$ Chl $\cdot h^{-1}$	
Grown at 330 μl CO_2 L^{-1}	51.7[b] ± 3.2	41.0 ± 4.8	11.0 ± 3.5	5.0 ± 0.3
Grown at 600 μl CO_2 L^{-1}	55.5 ± 2.6	33.7 ± 7.4	5.6 ± 0.6	3.2 ± 1.7
% change relative to 330 μl CO_2 L^{-1}	7	−18	−49[c]	−36

[a] Measured at 21% *versus* 1% O_2 and at 330 μl CO_2 L^{-1}. [b] Each value is the mean of 3 replications ± SD. [c] Difference between the means significant at the $\alpha = 0.05$ level as determined by a paired t test.

Table VI. *Transpiration Rates and Leaf Resistances of Ramets Produced by Water Hyacinth Grown in Growth Chambers at 330 and 600 μl CO_2 L^{-1} over a 3 Week Period*

Time	Grown at 330 μl CO_2 L^{-1}			Grown at 600 μl CO_2 L^{-1}		
	Transpiration		Total Leaf Resistance	Transpiration		Total Leaf Resistance
	Adaxial	Abaxial		Adaxial	Abaxial	
d	*mmol H_2O m^{-2} s^{-1}*		*s m^{-1}*	*mmol H_2O m^{-2} s^{-1}*		*s m^{-1}*
2	8.1[a]	6.3	48	6.7	7.1	49
7	9.3	8.4	19	6.2	7.5	60
14	6.2	3.4	72	0.6	4.1	378
21	7.6	8.2	66	1.2	5.5	115

[a] Each value is the mean of 3 measurements.

Table VII. *RuBPCase and PEPCase Activities of Ramets Produced by Water Hyacinth Grown under 330 and 600 μl CO_2 L^{-1} over a 4 Week Period*

	RuBPCase	PEPCase	RuBPCase	PEPCase
	μmol·mg^{-1} Chl·h^{-1}		*μmol·g^{-1} fresh wt·h^{-1}*	
Grown at 330 μl CO_2 L^{-1}	380 ± 11[a]	23 ± 0.3	703 ± 20.3	43 ± 0.6
Grown at 600 μl CO_2 L^{-1}	301 ± 12	22 ± 1.2	424 ± 16.9	31 ± 1.7
% change relative to 330 μl CO_2 L^{-1}	−21[b]	−4	−40[b]	−28[b]

[a] Each value is the mean of 3 replications ± the SD. [b] Difference between the means significant at the $\alpha = 0.05$ level as determined by a paired *t* test.

of photorespiration. The dark respiration rate also decreased. Although the lower dark respiration rate was probably a factor in achieving the higher dry weight of the enriched-CO_2 plants, by itself it was of insufficient magnitude to account for the phenomenon.

The increase in leaf resistance and decrease in transpiration, together with the decreased photosynthetic rate, all point to a leaf acclimation to elevated CO_2, which occurred for water hyacinth after 7 to 14 d of growth at 600 μl CO_2 L^{-1}. The adaxial and abaxial stomates responded differently to the increased CO_2, such that stomatal closure on the adaxial side appeared to be the major cause of the increased leaf resistance measurements. This stomatal acclimation was not a transitory effect, as it did not disappear when the plants were returned to ambient CO_2 levels. These data, together with the increase in starch content, and decreases in levels of soluble protein, RuBPCase activity, and Chl indicate that a variety of adaptations occur during growth at elevated CO_2 levels. Thus, the observed growth response cannot be attributed simply to a lessening of photorespiration, as is the case for short-term photosynthesis measurements at high CO_2 levels.

From the viewpoint of using water hyacinth as an energy crop (27), the reallocation of fixed carbon at elevated CO_2 levels to favor carbohydrates, rather than protein, would be beneficial; conversely this effect would be detrimental if the plant were to be used as an animal feed supplement (18). A similar redistribution of carbon to favor starch formation is a common phenomenon among CO_2-enriched plants (15, 25).

Plants whose growth responds positively to CO_2 enrichment seem to exhibit two general photosynthetic patterns. In some the net photosynthetic rate per unit leaf area is increased at the elevated CO_2 and usually remains so throughout the growth cycle, although the magnitude of the response may be modified at different developmental stages (11, 15). Thus, the improvement in total dry weight is largely due to a permanent increase in photosynthetic efficiency, as a result of the reduced O_2 effects on RuBP carboxylase-oxygenase (30). In these plants, RuBPCase shows little reduction in total activity by growth at up to 1000 μl CO_2 L^{-1} (11, 32).

The more usual pattern of response to elevated CO_2 is that shown by water hyacinth in this study. There is an initial, but temporary, enhancement in net photosynthesis per unit leaf area, which leads to an increased production of biomass. In the case of water hyacinth, the increase in biomass was most apparent as an increase in the number of leaves, which resulted in an increase in leaf area index. Following the rate enhancement, the plants acclimate to the high CO_2 and the photosynthetic rate diminishes, sometimes even below that of the plants at ambient levels (7, 25). For water hyacinth the sustained total dry weight increase appeared to be due to the greater leaf area index, rather than an improved photosynthetic efficiency per unit leaf area. Although CO_2 enrichment reduced the competitive inhibition of RuBPCase by O_2, this advantage was more than offset by the eventual large decrease in RuBPCase activity, which must have been a factor in the reduced overall photosynthetic efficiency per unit leaf area (25). Crowding of water hyacinth ramets and thus maximizing the leaf area index, could be expected to reduce the CO_2-enrichment effects on dry weight, though the yield increase should be self-sustaining if the plants were harvested or thinned-out at appropriate intervals to prevent overcrowding.

The cause of the acclimation and eventual diminution of photosynthetic rate seen in many plants grown at elevated CO_2 levels has been the subject of debate (11, 16, 25). For CO_2-enriched water hyacinth, the increase in leaf resistance after 14 d, due to the closure of adaxial stomates, could not by itself explain the reduced photosynthetic rate. This is because at any given external CO_2 level the internal CO_2 concentrations of the ambient grown and enriched plants were similar, despite the high leaf resistances of the enriched plants. This indicates that not only was the supply of CO_2 to the mesophyll cells restricted in the enriched plants, but that utilization of the internal CO_2 was also reduced, counterbalancing the restricted supply.

Starch accumulation, leading to some form of feedback inhibition or chloroplast disruption, has been most frequently postulated as responsible for the reduced photosynthetic rates in CO_2 enriched plants (13). Certainly in water hyacinth the carbon allocated to starch was much greater in the enriched-CO_2 grown plants. However, the redistribution of carbon was paralleled by a substantial reduction in extractable RuBPCase activity and Chl. All of these factors potentially impinge on the photosyn-

thetic rate, and at this stage it would be premature to single out any one as being the primary cause of the reduced rate in water hyacinth. Until a more detailed kinetic analysis of these parameters is undertaken for water hyacinth during the acclimation period, it can only be speculated as to whether the increased leaf resistance precedes, or is subsequent to, the long-term changes in the mesophyll cells. The data are suggestive, however, that long-term stomatal behavior and the photosynthetic metabolism of the underlying mesophyll cells may be closely regulated in concert.

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