Plant Physiol. (1981) 67, 335–340
0032-0889/81/67/0335/06/$00.50/0

Induction of Reduced Photorespiratory Activity in Submersed and Amphibious Aquatic Macrophytes[1]

Received for publication May 13, 1980 and in revised form September 10, 1980

MICHAEL E. SALVUCCI AND GEORGE BOWES
Department of Botany, University of Florida, Gainesville, Florida 32611

ABSTRACT

Incubation under water in a 30 C/14-hour or 12 C/10-hour photoperiod caused the CO_2 compensation points of 10 aquatic macrophytes to decrease below 25 or increase above 50 microliters CO_2 per liter, respectively. Submerged and aerial leaves of two amphibious angiosperms (*Myriophyllum brasiliense* and *Proserpinaca palustris*) maintained high compensation points when incubated in air but, when the submerged or aerial leaves of *Proserpinaca* were incubated under water, the compensation points dropped as low as 10. This suggests that, in addition to temperature and photoperiod, some factor associated with submergence regulates the compensation point of aquatic plants. In the high-compensation point plants, photorespiration, as a percentage of net photosynthesis, was equivalent to that in terrestrial C_3 plants. For *Hydrilla verticillata*, the decreasing CO_2 compensation points (110, 40, and 10) were associated with reduced photorespiration, as indicated by decreased O_2 inhibition, decreased rates of CO_2 evolution into CO_2-free air, and increased net photosynthetic rates.

The decrease in the CO_2 compensation points of *Hydrilla*, *Egeria densa*, and *Cabomba caroliniana* was accompanied by an increase in the activity of phosphoenolpyruvate, but not of ribulose bisphosphate, carboxylase. In *Hydrilla*, several C_4 enzymes also increased in activity to the following levels (micromoles per gram fresh weight per hour): pyruvate Pi dikinase (35), pyrophosphatase (716), adenylate kinase (525), NAD and NADP malate dehydrogenase (6565 and 30), NAD and NADP malic enzymes (239 and 44), and aspartate and alanine aminotransferases (357 and 85), whereas glycolate oxidase (6) and phosphoglycolate and phosphoglycerate phosphatases (76 and 32) showed no change. Glycolate dehydrogenase and phosphoenolpyruvate carboxykinase were undetectable. The reduced photorespiration in these plants may be due to increased CO_2 fixation via a C_4 acid pathway. However, for three *Myriophyllum* species, some other mechanism appears operative, as phosphoenolpyruvate carboxylase was not increased in the low compensation point state, and ribulose bisphosphate carboxylase remained the predominant carboxylation enzyme.

There is some confusion in the literature as to the level of photorespiratory activity in submersed freshwater macrophytes (5, 19, 27, 28). High Γ[2] values are one indicator of photorespiratory activity (7), but both high and low values have been reported for submersed aquatic plants (5, 17, 19, 28, 30) and even for the same species (19, 28). Recent studies with *Hydrilla* and other aquatic angiosperms (4, 16) have demonstrated that the Γ values of these plants vary markedly in response to growth conditions. The magnitude of the change in the light-dependent Γ values of submersed aquatic plants is unprecedented among higher plants, such as the C_3, C_4, and C_3-C_4 intermediate species (7, 10, 26). Differences in the photosynthetic and photorespiratory characteristics of *Hydrilla* in the high- and low-Γ states have been reported previously (4, 16), but the presence and characteristics of intermediate-Γ conditions have not been evaluated.

Although it is known that photorespiration can be reduced in submersed macrophytes, it is not known whether a similar reduction can be observed with emergent species. Emergent aquatic species, such as *Myriophyllum brasiliense* and *Proserpinaca palustris*, are amphibious and heterophyllic, that is, they possess both submerged and aerial leaves together on the same plant, either as different parts of the same stem or on different stems. The submerged leaves, which are usually only two or three cells thick, are similar to the leaves of submersed species, whereas the aerial leaves are morphologically similar to terrestrial C_3 leaves and possess functional stomates (25). These amphibious plants provide an interesting natural system for comparing, on the same plant, photosynthetic and photorespiratory metabolism in a terrestrial *versus* aquatic environment. The aerial leaves of amphibious plants are frequently inundated under natural conditions, but the effect of this immersion on the photosynthesis and photorespiration of these morphologically terrestrial leaves is unknown.

Efforts in this laboratory have recently focused on identifying the metabolic characteristics which enable aquatic macrophytes to reduce photorespiration. It has been shown that reduced photorespiration in *Hydrilla* is correlated with increased PEP carboxylase activity (4) and possibly a change in the kinetic properties of this enzyme (2). Holaday and Bowes (16) measured considerable dark fixation, diurnal fluctuation in titratable acidity levels and substantial photosynthetic malate and aspartate synthesis in low-Γ *Hydrilla* plants. Similarly, high levels of C_4 acid synthesis have been reported for other aquatic macrophyte species (5, 8). It is unknown whether increased PEP carboxylase is a general characteristic associated with the inducible reduction in photorespiratory activity in other aquatic species. It is also not known whether changes in the activities of other C_4 and photorespiratory enzymes play a role in the reduced photorespiration of low-Γ aquatic plants.

The object of the study presented here was to investigate further the photorespiration-reducing mechanism in aquatic plants by examining the involvement of various C_4 and photorespiratory enzymes, determining the extent to which changing carboxylase activity can be associated with Γ reduction in aquatic plants other than *Hydrilla*, and identifying whether amphibious plants have the potential for reducing their Γ values.

MATERIALS AND METHODS

Plant Material. *Hydrilla verticillata* (L.F.) Royal, *Ceratophyllum demersum* L., *Myriophyllum brasiliense* Camb., *Egeria densa*

[1] This research was supported by Grant 5901-0410-8-0082-0 from the Competitive Research Grants Office of the United States Department of Agriculture-the Science and Education Administration and by Grant 249-K17 from the Bureau of Aquatic Plant Research and Control, Florida Department of Natural Resources. This is Florida Agricultural Experiment Station Journal Series No. *2459*.

[2] Abbreviations: Γ, CO_2 compensation point; PEP, phosphoenolpyruvate; RuBP, ribulose 1,5-bisphosphate.

Planch., *Cabomba caroliniana* Gray, and *Nitella* sp. were collected from Orange Lake, Cross Creek, or Lake Lochloosa, and *Myriophyllum spicatum* was collected from Crystal River, FL. *Myriophyllum heterophyllum* Michx. and *Fissidens cf. manateensis* Grout *ex.* Holz. were obtained from the Ichetucknee and Santa Fe Rivers, respectively, and *Proserpinaca palustris* L. was gathered from roadside ditches near Cross Creek, FL. All plants were washed repeatedly to remove epiphytes. Apical segments, 10 cm long, were cut under water and incubated for 6 to 12 days under either a 30 C/14-h photoperiod or a 12 C/10-h photoperiod to induce low- and high Γ-values, respectively (4). Leaves from *Sorghum bicolor* (L.) Moench were obtained from greenhouse-grown plants and spinach (*Spinacia oleracea* L.) was field-grown.

IR Gas Analyzer Measurements. Gas-exchange measurements in the light and dark were determined with an ADC (Analytical Development Company Ltd.) series 225 gas analyzer incorporated into a closed system similar to that described by Van *et al.* (30). Net photosynthesis and dark respiration rates were determined from the time required for the plants to decrease or increase the CO_2 concentration in the circulating gas mixture between 327 and 317 μl CO_2/l (gas phase). This concentration gave a dissolved free-CO_2 level of 9.2 μM, a value similar to that found in Florida lakes (30). Photorespiratory CO_2 evolution in the light was determined from the time required for the plant material to increase the CO_2 concentration from 5 to 10 μl CO_2/l (gas phase). All gas exchange measurements were made at 30 C and at a saturating irradiance of 1000 $\mu E/m^2 \cdot s$. The Γ values were determined in a closed system as described by Van *et al.* (30) with 21% O_2 in the gas phase, unless otherwise indicated.

Enzyme Extraction. Plant extracts were prepared by grinding 1.0 g leaf material from the aquatic plants or 0.5 g sorghum or spinach leaves in a TenBroeck homogenizer at 4 C. The extraction medium, which consisted of 50 mM Tris-HCl, 10 mM $MgCl_2$, 0.1 mM EDTA, 5 mM isoascorbate, and 1% w/v PVP-40 (pH 8), was supplemented with 2.5 mM $MnCl_2$ for the malate dehydrogenase, malic enzyme, and PEP carboxykinase extractions. The buffer was changed to 50 mM Hepes (pH 7) for PEP carboxykinase extractions and to Tris-HCl (pH 8.5) for RuBP carboxylase, malic enzyme, and malate dehydrogenase. Aliquots of the homogenates were taken for Chl determinations (1); the remainder was centrifuged at 10,000*g* for 5 min and the supernatant was used for the assay. For measurements of pyruvate Pi dikinase activity, the following precautions were taken to ensure activation (14): the leaves were harvested during their light period and ground under a N_2 atmosphere, and the supernatant was preincubated at 30 C for 15 min in the presence of DTT and Pi.

Enzyme Assays. All assays were performed at 30 C according to previously established procedures. RuBP carboxylase was assayed in the active form as described by Lorimer *et al.* (20). PEP carboxylase was measured as $^{14}CO_2$ fixation into acid stable products according to the procedure employed by Van *et al.* (30). Adenylate kinase (15), aspartate and alanine aminotransferases (13), and malate dehydrogenases (18) were assayed spectophotometrically by the decrease in *A* at 340 nm due to the oxidation of NADH or NADPH. Malic enzyme activities were determined from the increase in *A* at 340 nm due to the reduction of NAD or NADP (12). Oxalate (1 mM), an inhibitor of NADP malic enzyme (24), was added to the malic enzyme assays to verify that the activity was NADP-specific. The ATP-exchange reaction as described by Mazelis and Vennesland (21), was used to measure PEP carboxykinase activity. Pyruvate Pi dikinase activity was determined by the method of Hatch and Slack (14), modified as previously described (16). Glycolate oxidase and dehydrogenase were assayed polarographically (9) in a Rank O_2 electrode system; they were also measured spectrophotometrically by following the decrease in *A* at 600 nm due to the anaerobic reduction of 2,6-dichlorophenolindophenol in the presence of glycolate, L-lactate, or D-lactate (9). The activities of P-glycerate phosphatase (23), P-glycolate phosphatase (23), and pyrophosphatase (15) were determined from the rate of Pi formation. The Pi released was measured by a modified Fiske-Subarrow method (23). The results presented for all the enzyme assays represent the mean of triplicate determinations.

RESULTS

Submersed Plants. Incubation of several submersed angiosperms, the moss *Fissidens*, and the alga *Nitella* in a growth chamber at 30 C with a 14-h photoperiod caused a decrease in their Γ values to less than 26 μl CO_2/l (Table I). These same species, when incubated under winter-like conditions (12 C/10-h photoperiod) increased their Γ values, generally to above 50 μl CO_2/l (Table I). Similar variations in Γ values also were observed in the field. Plants collected during the winter months, without prior incubation typically exhibited high-Γ values, whereas, during the summer months, these same species possessed low-Γ values. Thus, for unincubated *H. verticillata* and *M. spicatum* plants, freshly collected in August, the values were 28 and 27 μl CO_2/l, respectively, whereas the Γ value measured for both species in February was 110 μl CO_2/l.

Table II shows that, for *Hydrilla*, the growth chamber-induced variations in Γ values were accompanied by changes in the rates of net photosynthesis, in CO_2 evolution in the light and dark, and in the O_2 inhibition of photosynthesis. The low-Γ condition was characterized by a greatly increased rate of net photosynthesis and by a decreased O_2 inhibition of photosynthesis, to about 5% (Table II). The rate of CO_2 evolution into CO_2-free air in the light (an estimate of photorespiration) was almost totally suppressed in the low-Γ plants, being reduced as a per cent of the net photosynthetic rate to 1% (Table II). Dark CO_2 evolution (respiration) was also reduced in the low-Γ plants (Table II).

In addition to altered gas-exchange characteristics, *Hydrilla* and two other submersed aquatic angiosperms, *Egeria* and *Cabomba*, exhibited increased levels of PEP carboxylase activity and, to a far lesser extent, reduced RuBP carboxylase activity, associated with their change from high- to low-Γ values (Table III). PEP carboxylase levels for *Hydrilla* and *Egeria* fell between the 1617 and 75 μmol/mgChl·h values found for a C_4 plant (sorghum) and a C_3 plant (spinach), respectively. The shift in the activities of the carboxylase enzymes was reflected in a lower ratio of RuBP/PEP carboxylases in the low-Γ plants and a higher ratio in the high-Γ plants (Table III). A similarly low RuBP/PEP carboxylase ratio

Table I. *Effect of Incubation on Γ Values of Several Submersed Aquatic Macrophytes*

Plant Species	Γ	
	30 C/14-h photoperiod	12 C/10-h photoperiod
	μl CO₂/l	
Angiosperms		
C. caroliniana	10	82
C. demersum	26	82
E. densa	17	43
H. verticillata	10	84
M. brasiliense	11	62
M. heterophyllum	10	
M. spicatum	12	83
P. palustris	24	58
Moss		
F. cf. manateensis	16	50
Alga		
Nitella sp.	6	110

Table II. *A Comparison of Gas-exchange Characteristics of Hydrilla Plants with Low- and High-Γ Values*

Plants	Γ		Net Photosynthetic Rate		Inhibition by 21% O_2	CO_2 Evolution into CO_2-free Air	Dark CO_2 Evolution
	21% O_2	1% O_2	21% O_2	1% O_2			
	μl CO_2/l		*μmol CO_2/mg Chl·h*		*%*	*μmol/mg Chl·h*	
Low Γ	10	9	14.5 ± 2.4[a]	15.7 ± 2.5	4.8	0.15 ± 0.05	3.1 ± 1.0
High Γ	40	21	5.0 ± 0.6	5.8 ± 0.4	13.5		5.4 ± 1.4
	110		1.5 ± 0.6	2.1 ± 0.6	28.5	2.68 ± 0.45	8.7 ± 2.2

[a] Mean of three replicates ± SD.

Table III. *RuBP and PEP Carboxylase Activity in Relation to Γ Values of Three Submersed Aquatic Angiosperms*

Plant Species	Γ	Carboxylase Activity		
		RuBP	PEP	RuBP/PEP
	μl CO_2/l	*μmol/mg Chl·h*		*ratio*
H. verticillata	24	33.7 ± 1.6[a]	116.2 ± 8.0	0.29
	76	44.2 ± 1.6	31.6 ± 0.6	1.41
E. densa	26	70.6 ± 9.0	130.4 ± 17.9	0.54
	43	76.3 ± 2.6	104.0 ± 2.0	0.73
C. caroliniana	26	24.6 ± 0.6	22.3 ± 1.2	1.10
	150	27.8 ± 0.8	15.2 ± 0.8	1.83

[a] Mean of three replicates ± SD.

(0.63) was obtained with low-Γ *Egeria* plants that were assayed after collection in July, without prior incubation.

To determine whether *Hydrilla* plants with high and low levels of PEP carboxylase had different potentials for metabolizing the C_4 acids that would be produced as a result of the PEP carboxylase reaction, a number of enzymes known to be involved in the C_4 metabolic pathway were assayed. A comparison of the activities of the enzymes in low- and high-Γ plants is shown in Table IV. The enzyme activities were expressed on both a Chl and a fresh weight basis for evaluation because the Chl (but not the fresh or dry weight) tended to decrease in the 12 C/10-h photoperiod incubation treatment. For malate production, NAD and NADP malate dehydrogenases were present, although the NAD-dependent type was the predominant dehydrogenase (Table IV). Of the decarboxylases known to be active in C_4 and CAM plants, a CoA-activated, NAD malic enzyme was measured in *Hydrilla* and, on a fresh weight basis, its activity in low-Γ plants was over 2-fold higher than in high-Γ plants (Table IV). Oxalate-inhibited NADP malic enzyme was also detected, with higher activity in the low-Γ than in the high-Γ plants. There was no evidence of PEP carboxykinase activity (Table IV). To determine the potential for the interconversion of aspartate and oxaloacetate, aspartate aminotransferase was measured in *Hydrilla* (Table IV). The level of this enzyme in low-Γ plants was over twice that found in the high-Γ plants or in spinach (165 μmol/g fresh weight·h). It was not as high as that found in sorghum (942 μmol/g fresh weight·h). In contrast, alanine aminotransferase activity (Table IV), on a fresh weight basis, was only slightly higher in the low-Γ *Hydrilla* plants and comparable to activities found in spinach and sorghum (135 and 87 μmol/g fresh weight·h, respectively).

Table V shows, for high- and low-Γ *Hydrilla* plants, the activities of three enzymes known to be required for the regeneration of PEP in C_4 plants. Pyruvate Pi dikinase activity was detected in *Hydrilla* (Table V), and by far the highest activity occurred in the low-Γ plants. Somewhat lower levels of this enzyme were also found in *Egeria* (data not shown). As has been previously reported (11), pyruvate Pi dikinase was not present in the leaves of the C_3 plant spinach. Pyrophosphatase and adenylate kinase, which provide for the removal of the metabolic products formed in the pyruvate Pi dikinase reaction, were also present in *Hydrilla* (Table V). The activities of these two enzymes in low-Γ *Hydrilla* plants were higher than those found in the high-Γ plants or in spinach (Table V).

The activities of several enzymes normally associated with the photorespiratory pathway, and also P-glycerate phosphatase, were measured in high- and low-Γ *Hydrilla* plants (Table VI). The levels of P-glycolate phosphatase activity were similar in low- and high-Γ plants as were the levels P-glycerate phosphatase; the P-glycerate phosphatase activity was half that of the P-glycolate phosphatase. The glycolate oxidase activity, determined by monitoring O_2 uptake due to the oxidation of glycolate, was also similar in low- and high-Γ plants (Table VI). O_2 uptake (6.3 μmol/mg Chl·h) could also be measured when L-lactate was substituted for glycolate. Glycolate oxidase activity in low-Γ plants, determined by a spectrophotometric method, was 7.2 μmol/mg Chl·h with glycolate as the substrate. Glycolate dehydrogenase activity, however, could not be detected in the low-Γ plants since D-lactate did not substitute for glycolate in either the polarographic or spectrophotometric assays (Table VI).

Amphibious Plants. *M. brasiliense* and *P. palustris* are amphibious species with emergent and submersed forms or a combination of leaf types on one plant. When emergent and submersed parts were incubated for 10 days under a 30 C/14-h photoperiod (which induces a low-Γ value in submersed plants), either in air or under water, only the parts incubated under water decreased their Γ

Table IV. *A Comparison of Activities of Various C_4 Enzymes in Low- and High-Γ Hydrilla Plants*

Enzyme	Low Γ	High Γ	Low Γ	High Γ
	μmol/g fresh wt·h		*μmol/mg Chl·h*	
NAD malate dehydrogenase	6459 ± 1147[a]	5445 ± 448	4844 ± 860	6247 ± 515
NADP malate dehydrogenase	30.8 ± 3.7	21.7 ± 2.7	22.9 ± 2.8	31.5 ± 8.5
NAD malic enzyme	257.3 ± 32.8	87.8 ± 6.5	174.7 ± 22.2	143.6 ± 10.6
NADP malic enzyme	44.1 ± 0.7	16.7 ± 0.7	25.8 ± 0.4	27.3 ± 1.2
PEP carboxykinase	ND[b]		ND	
Aspartate aminotransferase	357.1 ± 23.8	148.5 ± 7.4	292.8 ± 19.6	189.7 ± 9.5
Alanine aminotransferase	84.6 ± 4.3	56.3 ± 11.2	46.2 ± 2.4	72.0 ± 14.4

[a] Mean of three replicates ± SD.
[b] ND, not detected.

Table V. *A Comparison of Activities of Enzymes Involved in Regeneration of PEP in Low- and High-Γ Hydrilla Plants, Spinach, and Sorghum*

Plant	Pyruvate Pi Dikinase	Pyrophosphatase	Adenylate Kinase	Pyruvate Pi Dikinase	Pyrophosphatase	Adenylate Kinase
		μmol/g fresh wt·h			*μmol/mg Chl·h*	
Hydrilla						
Low Γ	35.0 ± 1.6[a]	716.3 ± 4.9	525.1 ± 22.2	41.4 ± 1.8	532.7 ± 3.6	282.0 ± 12.8
High Γ	3.1 ± 0.5	140.3 ± 13.7	68.6 ± 17.2	2.9 ± 0.6	232.4 ± 22.7	81.0 ± 20.2
Spinach	ND[b]	111.5 ± 13.2	393.4 ± 0.0	ND	69.2 ± 8.2	173.1 ± 0.0
Sorghum	229.2 ± 0.8	16,361 ± 4,328	6,063 ± 597	75 ± 0.2	3,462 ± 940	2,094 ± 205

[a] Mean of three replicates ± SD.
[b] ND, not detected.

Table VI. *Activities of Three Photorespiratory Enzymes and P-glycerate Phosphatase in Extracts of Low- and High-Γ Hydrilla Plants*

Enzyme	Low Γ	High Γ
	μmol/mg Chl·h	
P-glycolate phosphatase	75.6 ± 7.8[a]	73.4 ± 3.8
P-glycerate phosphatase	33.8 ± 3.2	34.4 ± 6.2
Glycolate oxidase	6.6 ± 1.4	8.4 ± 0.8
Glycolate dehydrogenase	ND[b]	

[a] Mean of three replicates ± SD.
[b] ND, not detected.

Table VII. *Effect of Incubation in Air or under Water on Γ Values of Submersed and Emergent Forms of Two Amphibious Species*

All plant parts were incubated in a 30 C/14-h photoperiod.

Plant Species and Form	Incubation Medium	Γ	
		Measured in air	Measured under water
		μl CO_2/l	
Submersed form			
M. brasiliense	Air	77	82
	Water		13
P. palustris	Air	58	
	Water		24
Emergent form			
M. brasiliense	Air	59	64
P. palustris	Air	60	
	Water	10	18

values (Table VII). The decrease in the Γ value of *M. brasiliense* and *P. palustris* appeared to be dependent on the plants being held under water inasmuch as the normally submersed forms that were incubated in air maintained high-Γ values even in the appropriate low-Γ-inducing temperature and photoperiod regime (Table VII). The effect of submergence was not restricted to the submersed forms of the plants because the emergent form of *P. palustris* also showed a decreased Γ value after being immersed during incubation (Table VII). The medium (air or water) used during measurement of the Γ values did not appreciably affect the observed results (Table VII).

Similar results were obtained in a further experiment in which *M. brasiliense* plants, with a combination of aerial and submerged leaves each comprising half of the plant body, were incubated with the lower submerged leaves immersed and the upper aerial leaves in a water-saturated atmosphere (air). The Γ values after incubation were 13 and 60 μl CO_2/l for the submersed and emergent portions of the same plant, respectively.

For *Hydrilla*, *Egeria*, and *Cabomba*, the decrease in Γ as a result of incubation was accompanied by a shift in the carboxylase enzymes in favor of PEP carboxylase (Table III). However, this was not found to be the case with *M. brasiliense* and a closely related species, *M. spicatum*. Table VIII shows that the activity of PEP carboxylase remained low, and that of RuBP carboxylase remained high, in both high- and low-Γ forms of these two species. The emergent form of *M brasiliense* with a high-Γ value also showed similar results (Table VIII). A third species, *M. heterophyllum*, in the low-Γ condition (10 μl CO_2/l), was also found to possess low PEP carboxylase activity (4.4 μmol/mg Chl·h). Thus, in all *Myriophyllum* species examined, the RuBP/PEP carboxylase ratio remained high (Table VIII) and did not change with the differing incubation regimes or Γ values.

DISCUSSION

In terrestrial plants, the Γ value for a particular species is relatively constant (7, 26) and has proven to be a good indicator of photorespiratory activity (7, 10). Although, for certain terrestrial C_3 species, age-, seasonal-, and chemical-dependent variations in Γ have been reported (26), none of these factors caused Γ to drop into the C_4 or even intermediate range (7, 10). In contrast, for freshwater submersed aquatic macrophytes, we have found that the relationship among photosynthetic, photorespiratory, and respiratory activities was not constant but varied markedly, generating a graduation of Γ values from less than 10 to over 100 μl CO_2/l for each species. The Γ values for each could be manipulated to give high-Γ values, or what are normally considered C_3, at one extreme through low-Γ values, approaching those of C_4 plants, at the other. Several investigators have reported either high (5, 17, 19, 30)- or low (28, 30)-Γ values for submersed aquatic macrophytes, but only recently has it been shown that variations actually take place in the natural environment (4, 16) and can be induced by growth chamber conditions (2, 4, 16). From the study presented here, it seems that this may be general phenomenon in aquatic macrophytes, both angiosperms and nonangiosperms.

In a few cases, very high-Γ values, equivalent to between 300 and 960 μl CO_2/l in the gas phase, have been reported for some submersed macrophytes (17, 27). These high values are somewhat

Table VIII. *RuBP and PEP Carboxylase Activities in Two Myriophyllum Species, Each with Low- and High-Γ Values*

Plant Species	Γ	Carboxylase Activity		
		RuBP	PEP	RuBP/PEP
	μl CO_2/l	*μmol/mg Chl·h*		*ratio*
M. brasiliense	13	77.8 ± 2.1[a]	4.0 ± 0.2	19.45
	62	71.8 ± 0.9	5.6 ± 0.4	12.82
M. brasiliense (emergent form)	59	154.3 ± 2.7	2.7 ± 0.4	57.15
	27	47.1 ± 1.6	8.5 ± 0.7	5.54
M. spicatum	79	55.8 ± 0.3	8.6 ± 0.4	6.49

[a] Mean of three replicates ± SD.

questionable. For *Najas* (17), the $^{14}CO_2$ uptake method that was used required long incubation periods and apparently was not corrected for possible specific radioactivity changes. Furthermore, under some growth and/or manipulation conditions, submersed macrophytes may exhibit a large and long-lived efflux of CO_2 (G. Bowes, unpublished data; see also ref. 29), which can give anomalously high Γ values.

For *Hydrilla*, low-Γ values were reliable indicators of reduced O_2 inhibition of photosynthesis, suppressed CO_2 evolution in the light, and increased net photosynthesis. In contrast to most terrestrial plants (7), dark respiration appeared to be a component of Γ since in 1% O_2, the Γ values for *Hydrilla* plants were greater than zero. Even so, the observed changes in net photosynthesis and photorespiration could not be explained solely by the reduction in dark respiration that occurred in low-Γ plants because the decreased rate of dark CO_2 evolution was not sufficient to account for the greatly increased rate of net photosynthesis. It is likely that the increased net photosynthesis is due to additional CO_2 fixation in the light via the higher PEP carboxylase activity of low-Γ plants. In addition, it has been shown that low-Γ *Hydrilla* plants are capable of considerable CO_2 fixation in the dark (16). It is thus uncertain whether the decreased rate of apparent dark respiration in low Γ plants is due to a direct reduction in respiratory CO_2 production or to increased dark refixation of respired CO_2, via PEP carboxylase. The increased dark respiration rates of the high-Γ plants grown at 12 C may be partially attributable to the fact that they were measured at 30 C.

It is likely that increased PEP carboxylase activities of the low-Γ *Hydrilla*, *Egeria*, and *Cabomba* plants caused a shift in the carboxylating potential, so that PEP carboxylase became a predominant carboxylation enzyme. This enzyme activity shift lowered the RuBP/PEP carboxylase ratio toward a C_4-type ratio (10). In low-Γ *Hydrilla* plants, the greatly increased activities of NAD malate dehydrogenase and aspartate aminotransferase would facilitate the interconversion of oxaloacetate (produced by the high PEP carboxylase activity) with malate and aspartate. Substantial malate and aspartate production has been demonstrated by pulse-chase labeling studies with low-Γ *Hydrilla* plants: these acids comprised 60% of the initial products of carbon fixation (16). The greatly increased level of NAD malic enzyme in low-Γ plants represents circumstantial evidence that the photosynthetically derived malate is, at some state, decarboxylated. This is further supported by the observation that the addition of exogenous malate to low-Γ *Hydrilla* plants evoked a rapid efflux of CO_2 (M. E. Salvucci and G. Bowes, unpublished data).

In C_4 and some CAM plants (14, 22), pyruvate produced by the decarboxylation of malate is converted to PEP by the enzyme pyruvate Pi dikinase, and this enzyme is considered to be a major control point (11, 14, 15). To date, pyruvate Pi dikinase has not been reported in the leaves of C_3 plants (11). Its presence in *Hydrilla* and *Egeria* leaves suggests that these plants are not C_3. Considering the maximum photosynthetic rate of *Hydrilla* (30), this enzyme may be a rate-limiting factor in the low-Γ plants. The increase in this and other C_4 enzymes is consistent with the substantial ability of low-Γ *Hydrilla* plants to form C_4 acids. Whether the much lower levels of C_4 enzymes in high-Γ plants cause a concomitant shift towards C_3 photosynthetic products is currently being investigated. Pulse-chase labeling data from *Egeria* plants support the possibility because the labeling pattern of the photosynthetic intermediates was similar to that of terrestrial C_3 plants (6).

Estimates of photorespiration for *Hydrilla* and other aquatics (27) are low in comparison with terrestrial C_3 plants, but this can be misleading inasmuch as, for high-Γ *Hydrilla* plants, the rates of CO_2 evolution into CO_2-free air are quite high in comparison with their net photosynthetic rate. Similar C_3-type Γ values indicate that the photosynthesis/photorespiration ratio is similar in high-Γ aquatics and terrestrial C_3 plants. Van *et al.* (30) considered the reduced photosynthesis and photorespiration to be at least partly a consequence of generally low enzyme activities in submersed aquatic macrophytes. This is substantiated by the work reported here.

In contrast to the high-Γ plants, photorespiration in low-Γ plants was very low when compared to the photosynthetic rate, but the activities of glycolate oxidase and P-glycolate phosphatase were not decreased in the low-Γ plants. It is therefore unlikely that the reduced photorespiratory activity was due to a direct reduction in the capacity of the photorespiratory pathway. An altered photorespiratory pathway, such as that reported for certain green algal species (9), or glycolate excretion are also unlikely to be factors contributing to reduced photorespiratory CO_2 production, as we could find no evidence for glycolate dehydrogenase activity and as low-Γ *Hydrilla* plants excrete very little organic carbon (A. S. Holaday and G. Bowes, manuscript in preparation).

For aquatic plants such as *Hydrilla*, *Egeria*, and possibly *Cabomba*, the increased activities of C_4 enzymes may be responsible for reducing Γ, photorespiratory activity, and the O_2 inhibition of photosynthesis by one or all of the following mechanisms.

1. Dark fixation via PEP carboxylase. Dark fixation reduces respiratory CO_2 loss at night (16) and, with malate decarboxylation during the day, it could provide an internal level of CO_2 to reduce photorespiration, even when CO_2 is of limited availability in the environment.

2. Light fixation via PEP carboxylase. An increased amount of fixation via PEP carboxylase would add an additional, O_2-insensitive component to CO_2 uptake, thereby decreasing the effect of O_2 on total fixation and increasing net photosynthesis.

3. Refixation via PEP carboxylase. Refixation of photorespired CO_2 would reduce the observed rate of CO_2 evolution into CO_2-free air and the Γ value; such refixation would be aided by the CO_2 diffusion resistance of water.

The decreased Γ values measured for the three *Myriophyllum* species were not associated with increased PEP carboxylase activity. It appears that, for aquatic angiosperms, the environmentally induced change in photosynthetic and photorespiratory activities occurs by some mechanism other than a shift in the RuBP/PEP carboxylase ratio. Thus, *Myriophyllum* species differ from other higher plants with low- or intermediate-Γ values, namely C_4 (7, 11) and C_3-C_4 intermediates (10), as well as *Hydrilla*-like aquatics, which rely on high PEP carboxylase activity as part of their mechanism to reduce Γ. It is unlikely that the low-Γ value of *Myriophyllum* can be ascribed to a HCO_3^--pumping mechanism as may occur in certain unicellular algae (3) since low-Γ values for normally submersed plants could be measured in air and in solution at pH 5.5; both conditions where external HCO_3^- ions are virtually absent.

From the incubation experiments with the two amphibious species, it seems that only leaves actually immersed under water, and not those incubated in air, decrease their Γ values. There are thus three known factors which affect the photosynthetic and photorespiratory metabolism of aquatic plants: photoperiod (4), temperature (4), and submergence. It is not known what aspects of submergence provide the stimulus for these changes, but whatever triggers the appropriate low-Γ-producing mechanism in *Hydrilla* and *Myriophyllum* also apparently affects aerial parts of *Proserpinaca*. The significance of this response in *Proserpinaca* under natural conditions is uncertain, but immersion is a periodic occurrence for this species in its ditch habitat. It is possible that the characteristics associated with the low-Γ state enable plants, or plant parts, that are submerged to reduce CO_2 loss when CO_2 is of limited availability (30).

Acknowledgments—We wish to thank W. T. Haller, G. Buckingham, and M. Glenn for their assistance in locating and collecting plants used here.

LITERATURE CITED

1. Arnon DI 1949 Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. Plant Physiol 24: 1–15
2. Ascencio JA 1979 Phosphoenolpyruvate carboxylase kinetics and the CO_2 requirement of photosynthesis in *Hydrilla verticillata*. PhD dissertation. University of Florida, Gainesville
3. Berry J, J Boynton, A Kaplan, M Badger 1975 Growth and photosynthesis of *Chlamydomonas reinhardtii* as a function of CO_2 concentration. Carnegie Inst Year Book 75: 423–432
4. Bowes G, AS Holaday, TK Van, WT Haller 1978 Photosynthetic and photorespiratory carbon metabolism in aquatic plants. *In* DO Hall, J Coombs, TW Goodwin, eds, Photosynthesis 77. Proc Fourth Int Congr Photosynthesis. The Biochemical Society, London, pp 289–298
5. Brown JMA, FI Dromgoole, MW Towsey, J Browse 1974 Photosynthesis and photorespiration in aquatic macrophytes. *In* RL Bieleski, AR Ferguson, MM Cresswell, eds, Mechanisms of Regulation of Plant Growth. The Royal Society of New Zealand, Wellington, pp 243–249
6. Browse JA, FI Dromgoole, JMA Brown 1977 Photosynthesis in the aquatic macrophyte *Egeria densa*. I. $^{14}CO_2$ fixation at natural CO_2 concentrations. Aust J Plant Physiol 4: 169–176
7. Chollet R, WL Ogren 1975 Regulation of photorespiration in C_3 and C_4 species. Bot Review 41: 147–179
8. DeGroote D, RA Kennedy 1977 Photosynthesis in *Elodea canadensis* Michx. Four-carbon acid synthesis. Plant Physiol 59: 1133–1135
9. Frederick SE, PJ Gruber, NE Tolbert 1973 The occurrence of glycolate dehydrogenase and glycolate oxidase in green plants. An evolutionary survey. Plant Physiol 52: 318–323
10. Goldstein LD, TE Ray, DP Kestler, BC Mayne, RH Brown, CC Black 1976 Biochemical characterization of *Panicum* species which are intermediate between C_3 and C_4 photosynthesis plants. Plant Sci Lett 6: 85–90
11. Hatch MD 1976 Photosynthesis: the path of carbon. *In* J Bonner, JE Varner, eds, Plant Biochemistry, Ed 3 Chap 24. Academic Press, New York, pp 797–843
12. Hatch MD, T Kagawa 1974 Activity, location, and role of NAD malic enzyme in leaves with C_4-pathway photosynthesis. Aust J Plant Physiol 1: 357–369
13. Hatch MD, SL Mau 1973 Activity, location, and role of aspartate aminotransferase and alanine aminotransferase isoenzymes in leaves with C_4-pathway photosynthesis. Arch Biochem Biophys 156: 195–206
14. Hatch MD, CR Slack 1969 Studies on the mechanism of activation and inactivation of pyruvate Pi dikinase. Possible regulatory role for the enzyme in the C_4 dicarboxylic acid pathway of photosynthesis. Biochem J 112: 549–558
15. Hatch MD, CR Slack, TA Bull 1969 Light-induced changes in the content of some enzymes of the C_4-dicarboxylic acid pathway of photosynthesis and its effect on other characteristics of photosynthesis. Phytochemistry 8: 697–706
16. Holaday AS, G Bowes 1980 C_4 acid metabolism and dark CO_2 fixation in a submersed aquatic macrophyte (*Hydrilla verticillata*). Plant Physiol 65: 331–335
17. Hough RA, RG Wetzel 1978 Photorespiration and CO_2 compensation point in *Najas flexilis* Limnol Oceanogr 23: 719–724
18. Johnson HS, MD Slack 1970 Properties and regulation of leaf nicotinamide-adenine dinucleotide phosphate-malate dehydrogenase and malic enzyme in plants with the C_4 dicarboxylic acid pathway of photosynthesis. Biochem J 119: 273–280
19. Lloyd NDH, DT Canvin, JM Bristow 1977 Photosynthesis and photorespiration in submerged aquatic vascular plants. Can J Bot 55: 3001–3005
20. Lorimer GH, MR Badger, TJ Andrews 1977 D-Ribulose-1,5-bisphosphate carboxylase-oxygenase. Improved methods for the activation and assay of catalytic activities. Anal Biochem 78: 66–75
21. Mazelis M, B Vennesland 1975 Carbon dioxide fixation into oxaloacetate in higher plants. Plant Physiol 32: 591–600
22. Osmond CB 1978 Crassulacean acid metabolism: a curiosity in context. Annu Rev Plant Physiol 29: 379–414
23. Randall DD, NE Tolbert 1971 3-Phosphoglycerate phosphatase in plants. I. Isolation and characterization from sugarcane leaves. J Biol Chem 246: 5510–5517
24. Ratham CKM, GE Edwards 1977 Use of inhibitors to distinguish between C_4 acid decarboxylation mechanisms in bundle sheath cells of C_4 plants. Plant Cell Physiol 18: 963–968
25. Sculthorpe CD 1971 The Biology of Aquatic Vascular Plants. E. Arnold, London
26. Smith EW, NE Tolbert, HS Ku 1976 Variables affecting the CO_2 compensation point. Plant Physiol 58: 143–146
27. Søndergaard M 1979 Light and dark respiration and the effect of the lacunal system on refixation of CO_2 in submerged aquatic plants. Aquat Bot 6: 269–283
28. Stanley RA, AW Naylor 1972 Photosynthesis in Eurasian watermilfoil (*Myriophyllum spicatum* L.) Plant Physiol 50: 149–151
29. Tolbert NE, W Garey 1976 Apparent total CO_2 equilibrium point in marine algae during photosynthesis in sea water. Aust J Plant Physiol 3: 69–72
30. Van TK, WT Haller, G Bowes 1976 Comparison of the photosynthetic characteristics of three submersed aquatic plants. Plant Physiol 58: 761–768