Plant Physiol. (1984) 76, 525–530
0032-0889/84/76/0525/06/$01.00/0

Carbon Assimilation Characteristics of the Aquatic CAM Plant, *Isoetes howellii*[1]

Received for publication March 15, 1984 and in revised form June 12, 1984

JON E. KEELEY* AND GEOFF BUSCH
Department of Biology, Occidental College, Los Angeles, California 90041

ABSTRACT

The relationship between malic acid production and carbon assimilation was examined in the submerged aquatic Crassulacean acid metabolism (CAM) plant, *Isoetes howellii* Engelmann. Under natural conditions free-CO_2 level in the water was highest at 0600 hours and $^{14}CO_2$ assimilation rates in *I. howellii* were also highest at this time. After 0900 hours there was a similar pattern in (a) rate of free-CO_2 depletion from the water, (b) reduction of carbon assimilation rates, and (c) rate of deacidification in leaves. Rates of daytime deacidification increased under CO_2-free conditions and as irradiance intensity increased. Nighttime CO_2 uptake was estimated to contribute one-third to one-half of the total daily gross carbon assimilation. CO_2 uptake, however, accounted for only one-third to one-half of the overnight malic acid accumulation. Internal respiratory CO_2 may be a substrate for a large portion of overnight acid accumulation as leaves incubated overnight without CO_2 accumulated substantial levels of malic acid. Loss of CAM occurred in emergent leaf tips even though submerged bases continued CAM. Associated with loss of CAM in aerial leaves was an increase in total chlorophyll, a/b ratio, and carotenoids, and a decrease in leaf succulence. $\delta^{13}C$ values of *I. howellii* were not clearly distinguishable from those for associated non-CAM submerged macrophytes.

Carbon assimilation in the dark occurs in many plants but only in species with CAM does it contribute significantly to the carbon economy of the plant (10). CAM occurs largely in xeric-adapted succulents where it may play a major role in the water-use efficiency of the plant. The discovery of CAM in the submerged aquatic fern ally *Isoetes howellii* (Isoetaceae) (5) and other aquatics (6, 9) adds a new dimension to CAM.

In *Isoetes howellii*, CAM is indicated by the following (5, 8; J. E. Keeley, unpublished data): (a) in the dark, but not in the light, CO_2 is fixed into malic acid in photosynthetic tissues but not in other tissues, (b) malic acid accumulates in these tissues overnight, (c) there is a diurnal cycle of nighttime acidification/daytime deacidification of 50 to 300 μeq g^{-1} fresh weight, (d) PEP[2]case activity is sufficient to account for observed rates of acid accumulation, (e) PEPcarboxykinase (ATP dependent) activities are sufficient to account for decarboxylation of malic acid (malate enzyme activity is low), and (f) carbon released from malic acid in light is incorporated in phosphoglycerate and phosphorylated sugars.

It has been hypothesized that CAM is of selective value to aquatic species of *Isoetes* because it enhances carbon uptake capacity under carbon-limiting conditions (5, 7). Though aquatic environments commonly have higher than air saturated CO_2 levels, inorganic carbon may be limiting due to the fact that not all carbon is in an available form (*e.g.* some species do not take up HCO_3^-), plus the diffusive resistance to CO_2 in water is orders of magnitude greater than in air.

Although *Isoetes howellii* possesses stomata, they are nonfunctional while the plants are submerged (5). Carbon assimilation occurs via passive diffusion of CO_2, and possibly a low level of active uptake of HCO_3^- (7), across the epidermis. Previous studies showed that under the same pH and inorganic carbon conditions, gross and net CO_2 uptake rates in the light are many times greater than uptake rates in the dark (7, 8). Thus, the preliminary conclusion was that CAM contributes relatively little to the total carbon gain. However, carbon assimilation in both the light and dark are functions of ambient pH and total inorganic carbon level and these parameters fluctuate diurnally under natural conditions (7). Based on this information, it was predicted that in nature CAM would contribute significantly to the carbon gain in *Isoetes howellii*. We report here a test of that prediction.

MATERIALS AND METHODS

Isoetes howellii Engelmann was studied in a large (2 ha) seasonal pool on Mesa de Colorado, Riverside Co., CA (elevation 675 m). This pool filled to a depth of 300 to 400 mm during the winter and spring rainy season and dried out in late spring. The field studies reported here were done over a 48-h period on three separate occasions from early to late spring. Every 3 h, from 0600 to 2400 h, leaf and water samples were collected for analysis.

Acidity. Leaf samples (<0.5 g fresh weight) were washed, blotted with tissue paper, and weighed. After grinding with 15.0 ml cold CO_2-free deionized H_2O, a 10.0-ml sample was immediately titrated with CO_2-free 0.01 N NaOH to pH 6.4 and a 1-ml sample was deproteinized with an equal volume of 0.6 N $HClO_4$ and returned to the lab for enzymic determination of malic acid (3).

Carbon Assimilation. Two-cm leaf sections (about 0.25 g fresh weight) were incubated in 25-ml vials filled (and free of gas bubbles) with water collected from the pool at the time of sampling. After 10 min preincubation in a water bath at ambient light and temperature, experiments were initiated by injection of 2.5 μmol $NaH^{14}CO_3$ (4 μCi $vial^{-1}$) and then incubated for 30 min. Tests showed this level of $NaH^{14}CO_3$ altered the pH of the medium <0.1 unit. At 0600 and 1800 h, dark uptake vials were covered with foil. Experiments were terminated by addition of boiling 80% methanol. Samples were returned to the laboratory, ground, centrifuged for 20 min at 11,870*g* and the supernatant, plus that from a methanol wash and a deionized H_2O wash of the pellet, were dried in an oven. The residue was resuspended in deionized H_2O and the samples were counted in a 1:10 volume of Brays scintillation fluid. Late in the season, emergent plants

[1] Supported by National Science Foundation Grants DEB-82-06887, CDP-80-01910, and TFI-81-00529.

[2] Abbreviation: PEP, phosphoenolpyruvate.

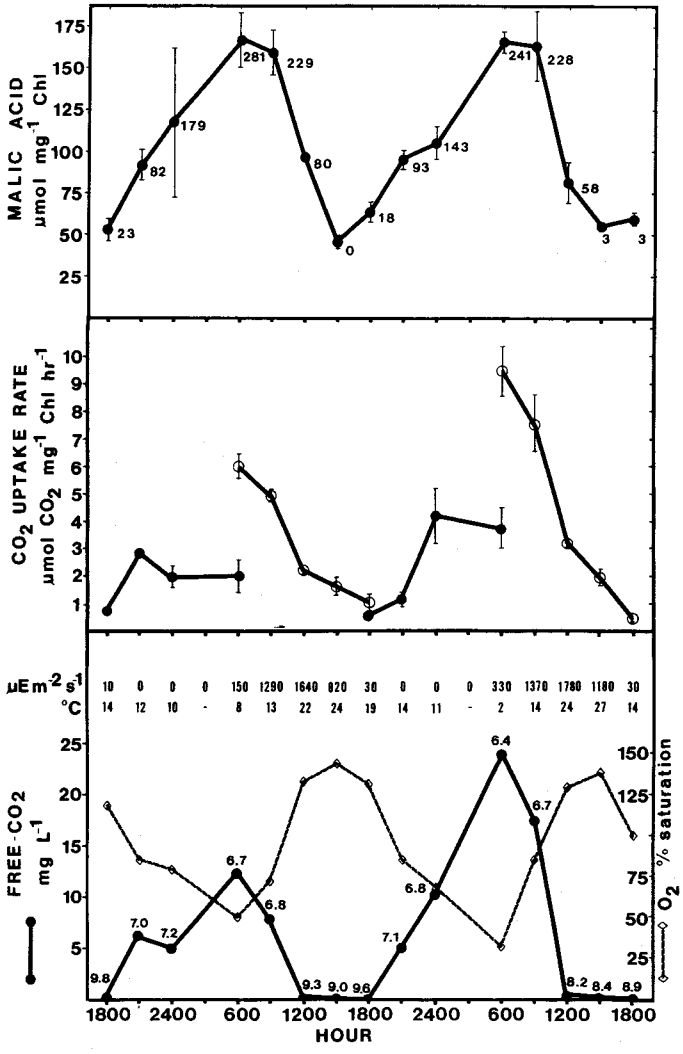

FIG. 1. Malic acid levels (number indicates titratable acidity) and carbon assimilation rates in the dark (●) and in the light (O) for submerged *I. howellii* leaves in Mesa de Colorado pool April 12–14, 1983 (bars indicate ±1 SE, $n = 3$). Environmental measurements include photosynthetic photon flux density at the water surface, temperature, free CO_2 (number indicates pH) and O_2 level for the water. Total alkalinity ranged from 31.3 to 31.6 (mg l^{-1} as $CaCO_3$) at 0600 h to 23.8 to 29.6 mg l^{-1} at 1800 h. Specific conductance was 43 µmho cm^{-1}.

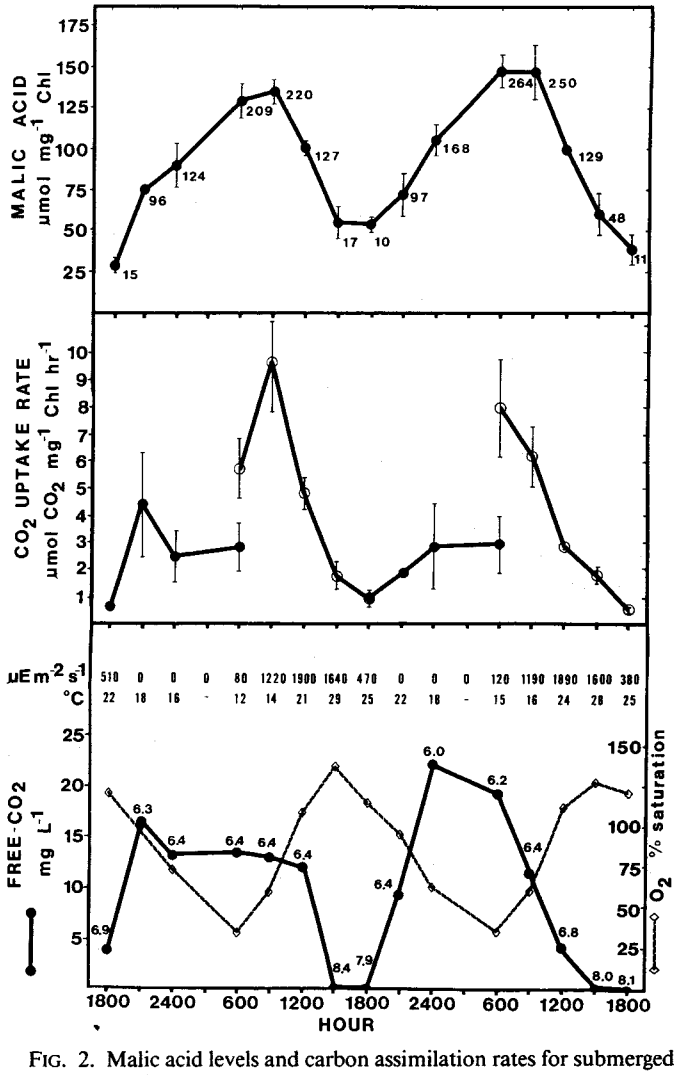

FIG. 2. Malic acid levels and carbon assimilation rates for submerged *I. howellii* leaves May 10–12, 1983. Details as in Figure 1. Alkalinity ranged from 15.7 to 17.2 mg l^{-1} (0600 h) to 11.0 to 15.7 mg l^{-1} (1800 h). Specific conductance was 56 µmho cm^{-1}.

Table I. *Day and Night Carbon Assimilation for I. howellii Leaves in Mesa de Colorado Vernal Pool Estimated from CO_2 Uptake Curves in Figures 1 to 3*

Values were used to calculate the proportion of the 24-h CO_2 uptake contributed by dark CO_2 uptake and the proportion of overnight malic acid accumulation accounted for by dark CO_2 uptake.

24 h Period	Light CO_2 Uptake		Total Dark CO_2 Uptake 1800–0600 h	% of 24-h CO_2 Uptake Due to Dark Uptake	% of Overnight Malic Acid Accumulation Accounted for by Dark CO_2 Uptake
	0600–1800 h	0900–1800 h			
	µmol mg⁻¹ Chl				
April 12–13	39.4	22.5	24.7	39	22
April 13–14	57.3	30.9	34.5	38	31
May 10–11	61.6	39.3	34.4	36	35
May 11–12	48.8	26.6	29.0	37	31
May 23–24	67.5	34.6	59.0	47	48
May 24–25	68.5	33.1	66.4	49	35

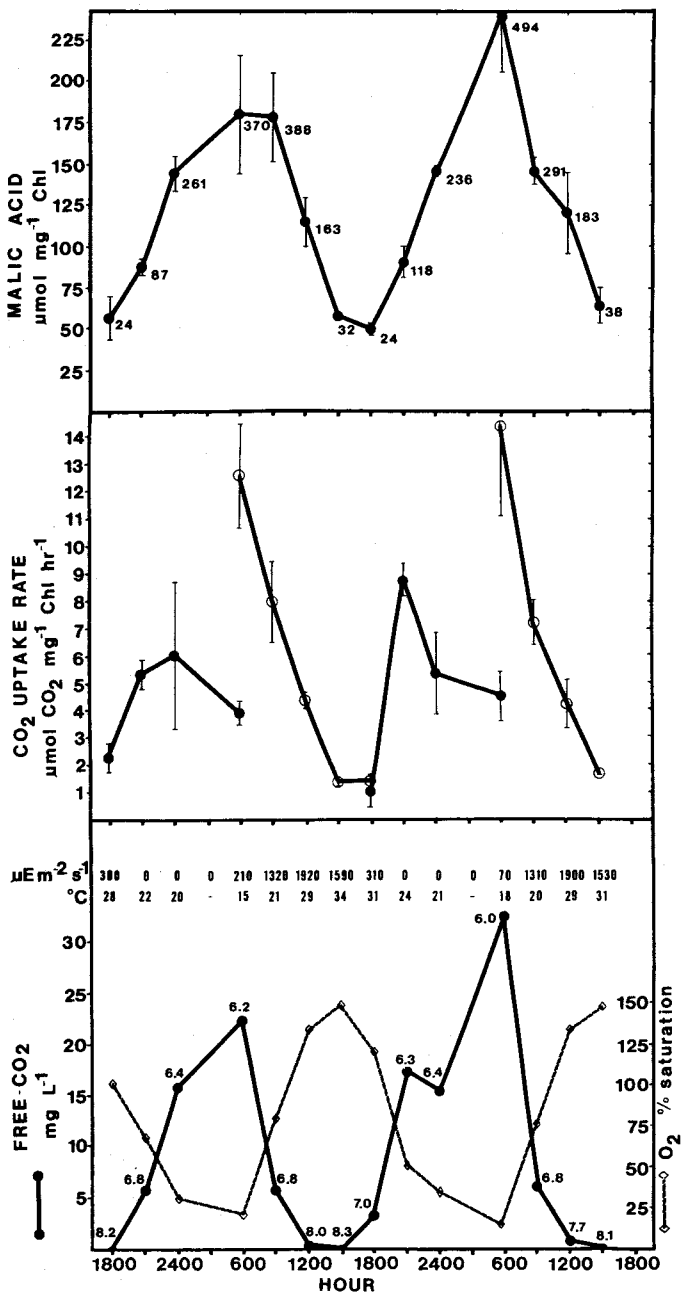

FIG. 3. Malic acid levels and carbon assimilation rates for submerged *I. howellii* leaves May 23–25, 1983. Details as in Figure 1. Alkalinity ranged from 16.9 to 18.4 mg l^{-1} (0600 h) to 15.5 to 16.7 mg l^{-1} (1800 h). Specific conductance was 101 µmho cm^{-1}.

were sampled in air as follows: leaf sections were placed on small screens in vials and 0.25 µmol $NaH^{14}CO_3$ (0.4 µCi $vial^{-1}$) was injected into 1 ml of 0.1 N HCl beneath the screen. Separate leaf samples were returned on ice for Chl determination. Leaves were ground on ice in 80% acetone and centrifuged for 20 min at 11,870*g*. *A* at 710 nm was subtracted from absorbances at other wavelengths (17) prior to calculating Chl *a* and *b* (2). Carotenoids were calculated from an equation given in (12).

Environmental Measurements. Photosynthetic photon flux density was measured with a LiCor LI-188B integrating meter with a LI-190SB quantum sensor at the water surface. Temperature and oxygen in the water was determined with a YSI-5700 meter and conductivity was measured with a YSI-33 meter at

Table II. *Overnight Changes in Titratable Acidity and Malic Acid in I. howellii Leaves Sparged from 1800 to 0600 h with either CO_2-Free Gas or 1% CO_2 Gas*

This experiment was repeated on separate days. Chl content was 0.46 ± 0.05 mg g^{-1} fresh weight.

Hour	Acidity		Malic Acid	
	Exp 1	Exp 2	Exp 1	Exp 2
	µeq mg^{-1} Chl		*µmol mg^{-1} Chl*	
1800	9 ± 2[a]	9 ± 9	46 ± 9	44 ± 7
0600				
Sparged overnight with 1% CO_2 (21% O_2)	343 ± 108	325 ± 18	187 ± 53	171 ± 9
Sparged overnight with CO_2-free gas (21% O_2)	149 ± 31	174 ± 40	92 ± 20	99 ± 18
P	<0.05	<0.01	<0.05	<0.01

[a] Mean ± SD (n = 3). P is for the 1-tailed t test between 1% CO_2 and CO_2-free treatments.

25° C. Water samples were collected and stored (usually less than 1 h) on ice for alkalinity and pH. Alkalinity was determined on water samples by titrating to pH 4.5 with 0.02 N H_2SO_4 (1). Earlier trials showed this endpoint to be the inflection point for alkalinity titrations of these waters. Free-CO_2 was calculated from pH and alkalinity (13).

CO_2 Manipulations. In the laboratory, the effect of CO_2 levels on acid production and rates of deacidification were determined. Plants were transplanted and maintained as described earlier (7). Leaves were severed from the corm and grouped together by tightly stretched parafilm around the base. These were submerged in 125-ml gas washing bottles fitted with a glass gas filter and three-quarters filled with pool water. Plants were incubated in solution with a 100 ml min^{-1} nitrogen gas stream containing either 1% CO_2 plus 21% O_2 or 0% CO_2 plus 21% O_2. Gas washing bottles were maintained in a 25°C water bath. Depending upon the experiment, the bottles were either covered with a dark cloth or exposed to a 1000-w quartz-halogen lamp.

Mass spectrophotometer determinations of the stable carbon isotopes ^{13}C and ^{12}C were made from dried tissue samples and for the inorganic carbon from the pool collected in early and late spring of 1981. These were processed in the laboratory of C. B. Osmond (Australian National University, Canberra) with methods described in (14).

RESULTS

Early in the season, there was an approximately linear increase in nocturnal malic acid accumulation in submerged *I. howellii* leaves (Fig. 1). During the day, between 0600 and 0900, there was a very slight drop in acid content in contrast to a very marked drop between 0900 and 1200 h.

Maximum carbon assimilation rates were observed in the light at 0600 h but rates dropped rapidly through the day. Throughout much of the night, maximum carbon assimilation rates were greater than light CO_2 uptake rates during the latter half of the day.

Free-CO_2 levels in the water paralleled the changes in carbon assimilation, highest at 0600 h but dropping rapidly during the morning. By noon, free-CO_2 in the pool was largely depleted. During the first half of the night, free-CO_2 levels increased, as did dark CO_2 uptake rates in *I. howellii* leaves, although between 2400 and 0600 h further increases in free-CO_2 were not accompanied by increases in the rate of dark CO_2 uptake.

The patterns observed at the second sampling date (Fig. 2), midway through the season, were similar in many respects to the

Table III. *Daytime Deacidification Rates in I. howellii Leaves*

The leaves were sparged from 0600 to 0900 h with either 1% CO_2 or CO_2-free gas at two light intensities (a constant 1000 $\mu E\ m^{-2}\ s^{-1}$ or a stepwise increase of 100 (0600–0700 h), 300 (0700–0800 h), and 500 (0800–0900 h) $\mu E\ m^{-2}\ s^{-1}$. Chl content was 0.46 ± 0.05 mg g^{-1} fresh weight.

Hour	Exp 1[a]		Exp 2[a]		Exp 1[b]		Exp 2[b]	
	Acidity	Malic acid	Acidity	Malic acid	Acidity	Malic acid	Acidity	Malic acid
	μeq mg⁻¹ Chl	*μmol mg⁻¹ Chl*	*μeq mg⁻¹ Chl*	*μmol mg⁻¹ Chl*	*μeq mg⁻¹ Chl*	*μmol mg⁻¹ Chl*	*μeq mg⁻¹ Chl*	*μmol mg⁻¹ Chl*
0600	305 ± 18[c]	147 ± 9	242 ± 20	147 ± 33	363 ± 44	207 ± 20	325 ± 29	187 ± 22
0900								
Sparged with 1% CO_2 (21% O_2)	152 ± 29	112 ± 7	130 ± 20	90 ± 11	284 ± 13	153 ± 18	248 ± 9	154 ± 10
Sparged with CO_2-free gas (21% O_2)	77 ± 29	75 ± 13	70 ± 7	59 ± 9	229 ± 40	119 ± 15	185 ± 48	117 ± 22
P	<0.05	<0.01	<0.01	<0.01	<0.05	<0.05	<0.05	<0.05

[a] Light intensity, a constant 1000 $\mu E\ m^{-2}\ s^{-1}$.
[b] Light intensity, a stepwise increase of 100, 300, and 500 $\mu E\ m^{-2}\ s^{-1}$.
[c] See footnote, Table II.

Table IV. *Comparison of Titratable Acidity and Malic Acid Levels in Submerged and Emergent I. howellii Leaves in Mesa de Colorado Pool, May 23–25, 1983*

Chl levels are given in Table VI.

	Submerged Leaves		Emergent Leaves	
	Acidity	Malic acid	Acidity	Malic acid
	μeq mg⁻¹ Chl	*μmol mg⁻¹ Chl*	*μeq mg⁻¹ Chl*	*μmol mg⁻¹ Chl*
May 23–24				
1800 h	24 ± 1[a]	57 ± 13	12 ± 3	42 ± 4
0600 h	370 ± 66	180 ± 36	26 ± 5	45 ± 6
P	<0.01	<0.01	<0.01	>0.05
May 24–25				
1800 h	24 ± 13	51 ± 3	5 ± 5	40 ± 6
0600 h	494 ± 20	243 ± 37	24 ± 7	52 ± 6
P	<0.01	<0.01	<0.01	>0.05

[a] Mean ± SD ($n = 3$). P is for the 1-tailed *t* test between 1800 and 0600 h.

Table V. *Carbon Assimilation Rates by Emergent Leaves of I. howellii from Margin of Mesa de Colorado Pool, May 23–24 1983*

PAR values at 3-h intervals on these 2 days are shown in Figure 3.

Time	CO_2 Uptake Rate
h	*μmol mg⁻¹ Chl h⁻¹*
2100	0.46 ± 0.04[a]
2400	0.66 ± 0.15
0900	6.68 ± 1.18
1200	8.01 ± 1.07
1500	5.86 ± 0.74
2100	0.39 ± 0.23
2400	0.27 ± 0.10
0900	9.13 ± 1.17
1200	5.45 ± 2.45
1500	8.63 ± 0.04

[a] Mean ± SD ($n = 3$). On both days CO_2 uptake rates in the light at all hours (0900, 1200, 1500 h) were significantly greater than dark (2100, 2400 h) CO_2 uptake rates ($P < 0.01$ with 2-tailed *t* test). Uptake rates in the light were not significantly different between hours ($P > 0.05$).

results obtained earlier. One difference was that early in the morning on the first day it was overcast and windy. These conditions resulted in low light intensity and strong mixing of the air-water interface, and relatively little change in levels of free-CO_2 in the water during the morning. As a consequence, the carbon uptake pattern differed from that observed during other sampling periods. On this first day carbon assimilation rates increased during the early morning reaching a peak at 0900 h.

On the last sampling date late in the season the patterns were similar to those observed during the first sampling period, except that peak free-CO_2 levels in the water were greater, as were peak carbon assimilation rates, both in the light and the dark (Fig. 3).

Combining all of the data from the season, a highly significant correlation ($r = 0.91$ with the Pearson Product correlation, $P < 0.01$, $n = 29$) was found between CO_2 uptake in the light and free-CO_2 level in the water. Dark CO_2 uptake rates were less strongly correlated with free CO_2 levels ($r = 0.58$, $P < 0.01$, $n = 24$).

Gross carbon assimilation was estimated by intergrating the areas under the curves in Figures 1 to 3. Through the season, dark carbon uptake comprised one-third to one-half of the total carbon gain of *I. howellii* (Table I). Generally, the total carbon assimilated overnight was equal to, or greater than, the total amount of carbon taken up from 0900 h through the rest of the day. Assuming an equal molar relationship between dark CO_2 uptake and acid accumulation, it appears that only about one-third to one-half of the malic acid accumulation derives from dark CO_2 uptake.

Experimental evidence that all of the overnight acid accumulation is not dependent on CO_2 assimilation from the ambient environment is shown in Table II. Leaves, sparged overnight with CO_2-free gas, still accumulated substantial levels of malic acid, half of the level under the 1% CO_2 treatment or in the field (Figs. 1–3).

In general there was little deacidification in leaves prior to 0900 h. It was hypothesized that deacidification was environmentally regulated to some extent by ambient CO_2 levels. Experimental evidence of this environmental control of deacidification rates is shown in Table III. Leaves in solution sparged with CO_2-free gas, deacidified at a much faster rate than leaves sparged with 1% CO_2 gas. It is also clear from Table III that the rate of deacidification is a function of light intensity.

By the last sampling date many of the plants near the periphery of the pool were entirely emergent. Overnight accumulation was greatly decreased in these leaves (Table IV). Relative to submerged leaves on this date (Fig. 3), very little CO_2 uptake occurred in the dark, and during the day CO_2 uptake rates did not show a daytime decline (Table V).

Throughout the season CHl levels changed as leaves became emergent (Table VI); total Chl and percentage Chl *a* increased. Relative to the total Chl carotenoid levels were significantly greater in the upper half of the leaf.

The leaves produced first in the rosette are generally submerged

Table VI. *Chl Concentration in I. howellii Leaves from Mesa de Colorado Pool*

	Total Chl	Chl *a*	Carotenoids / Total Chl
	mg Chl g^{-1} fresh wt	%	
April 12–14			
Submerged leaves	0.67 ± 0.16[a]	73.4 ± 0.4	0.17 ± 0.01
May 10–12			
Completely submerged			
Leaf bases	0.62 ± 0.19	72.9 ± 0.7	0.17 ± 0.01
Leaf tips	0.59 ± 0.10	74.7 ± 0.6	0.25 ± 0.01
Partially submerged			
Submerged leaf bases	0.38 ± 0.03	75.4 ± 0.4	0.19 ± 0.00
Emergent leaf tips	0.75 ± 0.09	78.6 ± 0.4	0.27 ± 0.03
May 23–25			
Completely submerged leaves	0.50 ± 0.04	73.5 ± 0.8	0.18 ± 0.01
Completely emergent leaves	1.00 ± 0.03	78.5 ± 0.7	0.25 ± 0.01
P[b]	<0.01	<0.01	<0.01
LSD[c] (0.01)	0.23	1.4	0.03

[a] Mean ± SD ($n = 3$).
[b] Significance level for one-way analysis of variance.
[c] Difference necessary between means to be considered statistically different.

Table VII. *Comparison of Outer Leaves of I. howellii Rosette (Which are Usually Submerged) with Inner Leaves (Which Are Usually Emergent)*

Collected May 2, 1983 from Mesa de Colorado.

	X-Sectional Diameter		Fresh Wt / Dry Wt	Surface Area (cm) / g Dry Wt	Chl / g Dry Wt	Sm[a]
	Leaf	Lacunae				
	mm			*ratio*		
Outer leaves	2.4 ± 0.1[b]	1.0 ± 0.1	18.3 ± 0.5	8247	10.9	1.66
Inner leaves	1.2 ± 0.1	0.4 ± 0.0	10.7 ± 0.4	7330	10.7	1.00
P	<0.01	<0.01	<0.01			

[a] Sm, mesophyll succulence, defined by (10) as mg Chl g^{-1} H_2O.
[b] Mean ± SD ($n = 3$). P is for the 2-tailed t test.

Table VIII. *Comparison of Overnight Acidity Changes between Completely and Partially Submerged Isoetes howellii Leaves Maintained for 1 Month under Such Conditions in Artificial Pools*

	Chl	Acidity	Malic Acid
	mg g^{-1} fresh wt	*μeq mg^{-1} Chl*	*μmol mg^{-1} Chl*
Completely submerged	0.46 ± 0.05[a]		
1800 h		9 ± 2	46 ± 9
0600 h		303 ± 26	171 ± 19
P		<0.01	<0.01
Partially submerged			
Submerged bases	0.16 ± 0.02		
1800 h		19 ± 13	57 ± 13
0600 h		643 ± 178	382 ± 70
P		<0.01	<0.01
Emergent tips	1.14 ± 0.09		
1800 h		3 ± 2	6 ± 3
0600 h		28 ± 6	34 ± 1
P		<0.01	<0.01

[a] Mean ± SD ($n = 3$). P is for the 1-tailed t test between 1800 and 0600 h.

for the majority of their lifespan, whereas the inner rosette leaves spend the bulk of their lifespan emergent. The outer rosette leaves are structurally quite different from the inner rosette leaves (Table VII).

Overnight acid accumulation in leaves maintained either completely or partially submerged is shown in Table VIII. Leaf tips, maintained out of water for 1 month, largely lost CAM whereas their submerged bases continued to accumulate acids overnight.

$\delta^{13}C$ values of leaves were −10 to −12‰ more negative than the water carbonate and did not differ greatly between submerged and emergent plants (Table IX).

DISCUSSION

A major role of CAM is the daytime generation of an internal CO_2 source for photosynthesis. Two very different ecological situations have put a premium on this ability: certain xeric environments where daytime stomatal closure limits CO_2 availability and certain aquatic habitats where daytime ambient CO_2 levels are limiting to photosynthesis.

Isoetes howellii occupies shallow pools which are densely vegetated. Plant cover is over 75% of the ground surface (11); however, *Isoetes howellii*, and the very similar CAM species *I. orcuttii* (6), constitute less than one-fourth of the cover. Only one of the other six common vascular plant species is CAM (9); it is *Crassula aquatica*, a diminutive annual which constitutes a minor part of the total plant cover. Little is known about the photosynthetic characteristics of the other (non-CAM) species. In these pools CO_2 levels are highest in the early morning. As light intensity and temperature increase, photosynthetic consumption of CO_2 by the pool flora, coupled with the lower solubility of CO_2 at higher temperatures and low rate of mixing at the air-water interface (4), results in a depletion of free-CO_2.

Table IX. *$\delta^{13}C$ for I. howellii Leaves and Roots from Mesa de Colorado Pool*

Pondwater Carbonate	Submerged		Emergent	
	Leaves	Roots	Leaves	Roots
		‰		
−15.5 to −18.6	−27.9 to −29.4	−28.5 to −28.8	−29.4 to −30.1	−29.0 to −29.8

Carbon assimilation by *I. howellii* closely parallels this daytime depletion of free-CO_2 (Figs. 1–3).

Under natural conditions, early morning photosynthesis is fed by ambient CO_2 and consequently very little of the internal malic acid pool in the leaf is consumed during this time. The rate of deacidification accelerates as the ambient CO_2 supply diminishes and as light intensity increases the photosynthetic demand for CO_2.

Overnight, CO_2 levels build up in the pool due in large part to respiration by the total pool flora and invertebrate fauna. Carbon assimilation rates by *I. howellii* leaves increase during the first half of the night although, generally, dark CO_2 uptake rates at 0600 h were equal to or less than midnight even though CO_2 levels in the water were always higher at 0600 h. High internal acid levels near the end of the dark period may play a role in dampening dark CO_2 uptake as is the case in terrestrial CAM plants (10).

Our estimates indicate that dark CO_2 uptake contributes substantially to the total gross carbon assimilation in *I. howellii* (Table I). It is clear, however, that a substantial portion of the overnight acid accumulation derives from internal CO_2 sources (Tables I and II). Sources of internal CO_2 include CO_2 evolved from dark respiration and CO_2 remaining at the end of the day in the lacunal gas space. This latter source may not represent a large pool of CO_2 since free-CO_2 sources in the water are largely depleted during the latter half of the day. Consistent with the hypothesis of refixation of respiratory CO_2 is the fact that *I. howellii* shows no net CO_2 evolution in the dark at CO_2 levels close to natural conditions (8). Recycling of respiratory CO_2 through CAM is also known from cacti under severe drought conditions when stomatal resistance remains high both day and night (19). Such recycling of CO_2 may also account for the discrepancy between levels of overnight acid accumulation and dark CO_2 uptake observed for *I. lacustris* in oligotrophic lakes (16).

The gradual loss of CAM upon exposure to the atmosphere is consistent with our hypothesized role for CAM in aquatic plants. Also, since the ambient CO_2 level of the air is not depleted during the day, it is to be expected that daytime CO_2 uptake by emergent leaves would not decrease during the day (Table V) as is the case with submerged leaves (Figs. 1–3).

The strong control of submergence on CAM is illustrated by the fact that the emergent leaf tips largely lose CAM even though their submerged bases are still undergoing CAM. Also associated with emergence is an increase in total Chl and a decrease in leaf succulence. It is unknown if reduced succulence is due to the loss of CAM, although the two are commonly associated, and it has been suggested that succulence is an inevitable result of a large vacuole for malic acid storage (10).

The $\delta^{13}C$ values reported here for *I. howellii* fall within the range of values noted for six other non-CAM species in the pool (−25 to −30‰, J. E. Keeley, Sternberg, and DeNiro, unpublished data). Clearly, the stable carbon isotope ratio is not a good indicator of photosynthetic pathway for these aquatic plants. Recent studies (14–16, 18) have shown that $\delta^{13}C$ values for aquatic plants are affected by factors not encountered by terrestrial species, *e.g.* diffusional resistances of the water, gas buildup in lacunae, and HCO_3^- uptake (in some species). In the case of *I. howellii*, interpretation of $\delta^{13}C$ values is further complicated by the fact that the elevated nighttime free-CO_2 level in the water is undoubtedly derived in large part from respiration of the associated flora and fauna. Consequently, the $\delta^{13}C$ of the carbon source for the CAM pathway in *I. howellii* reflects previous fractionation events by associated species. This may explain why the $\delta^{13}C$ for *I. howellii* is indistinguishable from associated non-CAM species.

Acknowledgment—We thank C. B. Osmond for providing the stable carbon isotope analysis.

LITERATURE CITED

1. American Public Health Association 1976 Standard Methods for the Examination of Water and Wastewater. Ed 14. American Public Health Association. Washington. DC
2. Arnon DI 1949 Copper enzymes in isolated chloroplasts. Plant Physiol 24: 1–15
3. Gutmann I. AW Wahlefeld 1974 L-Malate: Determination with malate dehydrogenase and NAD. *In* HU Bergmeyer. ed. Methods of Enzymatic Analysis. Vol 4. Academic Press. New York. pp 1585–1589
4. Hutchinson GE 1975 A Treatise on Limnology. Vol I Geography. Physics. and Chemistry. Part 2 Chemistry of Lakes. John Wiley. New York
5. Keeley JE 1981 *Isoetes howellii*: A submerged aquatic CAM plant? Am J Bot 68: 420–424
6. Keeley JE 1982 Distribution of diurnal acid metabolism in the genus *Isoetes*. Am J Bot 69: 254–257
7. Keeley JE 1983 Crassulacean acid metabolism in the seasonally submerged aquatic *Isoetes howellii*. Oecologia 58: 57–62
8. Keeley JE. G Bowes 1982 Gas exchange characteristics of the submerged aquatic Crassulacean acid metabolism plant. *Isoetes howellii*. Plant Physiol 70: 1455–1458
9. Keeley JE. BA Morton 1982 Distribution of diurnal acid metabolism in submerged aquatic plants outside the genus *Isoetes*. Photosynthetica 16: 546–553
10. Kluge M. IP Ting 1978 Crassulacean Acid Metabolism: Analysis of an Ecological Adaptation. Springer-Verlag. Berlin
11. Kopecko KJP. EW Lathrop 1975 Vegetation zonation in a vernal marsh on the Santa Rosa Plateau of Riverside County. California. Aliso 8: 281–288
12. Lewandowska M. PG Jarvis 1977 Changes in chlorophyll and carotenoid content. specific leaf area and dry weight fraction in sitka spruce. in response to shading and season. New Phytol 79: 247–256
13. Lind OT 1979 Handbook of Common Methods in Limnology. CV Mosby. St. Louis
14. Osmond CB. N Valaane. SM Hasland. P Uotila. Z Roksandic 1981 Comparisons of $\delta^{13}C$ values in leaves of aquatic macrophytes from different habitats in Britain and Finland: Some implications for photosynthetic processes in aquatic plants. Oecologia 50: 117–124
15. Raven JA. J Beardall. H Griffiths 1982 Inogranic C-sources for *Lemanea*. *Cladophora* and *Ranunculus* in a fast flowing stream: Measurements of gas exchange and of carbon isotope ratio and their ecological implications. Oecologia 53: 68–78
16. Richardson K. H Griffiths. ML Reed. JA Raven. NM Griffiths 1984 Inorganic carbon assmilation in the isoetids. *Isoetes lacustris* L. and *Lobelia dortmanna* L. Oecologia 61: 115–121
17. Sestak Z. J Katsky. PG Jarvis. eds 1971 Plant Photosynthesis Production. Manual of Methods. Dr W Junk. The Hague
18. Smith FA. NA Walker 1980 Photosynthesis by aquatic plants: Effects of unstirred layers in relation to assimilation of CO_2 and HCO_3^- and to carbon isotope discrimination. New Phytol 86: 245–259
19. Szarek SR. HB Johnson. IP Ting 1973 Drought adaptation in *Opuntia basilaris*: significance of recycling carbon through Crassulacean acid metabolism. Plant Physiol 52: 539–541