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The role of CAM in the carbon economy of the submerged-aquatic *Isoetes howellii*

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With 1 figure in the text

Carbon assimilation in the dark occurs in many plants, but only in species with Crassulacean Acid Metabolism (CAM) does it play a major role in the carbon economy of the plant (KLUGE & TING 1978). CAM occurs largely in xeric adapted succulents where it plays a major role in the water-use efficiency of the plant. Thus, the recent discovery of CAM in the submerged aquatic *Isoetes howellii* (Isoetaceae) was unexpected (Keeley 1981).

The following characteristics of *I. howellii* are taken as evidence of CAM: 1) Dark CO_2-fixation occurs in photosynthetic tissues but not in corms; 2) CO_2 is fixed into malic acid in the dark but not in the light; 3) malic acid accumulates in the leaves overnight; 4) there is a diurnal cycle of nighttime acidification/daytime deacidification involving a fluctuation of up to 300 μequivalents $\cdot g^{-1}$ fresh weight; 5) PEP carboxylase activities are sufficient to account for observed rates of acid accumulation; and 6) during daytime decarboxylation CO_2 fixed in the dark enters the C_3 cycle (KEELEY 1981 and unpubl.; KEELEY & BOWES 1982).

In terrestrial species the role of CAM in the carbon economy of the plant is variable. Carbon assimilation is dependent in large part upon stomatal conductance to CO_2. In the prototype CAM plant conductance is low during the day and highest at night and thus CAM contributes the major portion of the net carbon gain. In other CAM species daytime stomatal conductance may equal or exceed nighttime conductance. In these plants CAM contributes a lesser proportion of the net carbon assimilation. Still other CAM species have little or no CO_2 uptake in the dark, yet substantial CO_2 fixation and acid accumulation occurs from refixation of internally derived CO_2. In these species CAM does not contribute to the net carbon gain yet it plays a role in recapturing CO_2 and affects the overall carbon economy.

In aquatic plants stomata are absent or nonfunctional and carbon assimilation occurs via passive diffusion of CO_2 or active uptake of HCO_3^-. The role of CAM in the carbon economy of *Isoetes howellii* is unclear. Previous studies showed that under similar pH and carbon conditions CO_2 uptake rates in the light are several times greater than uptake rates in the dark (KEELEY & BOWES 1982). However, carbon assimilation in both the light and dark are functions of pH and total inorganic carbon level (KEELEY 1983). Since the pH and carbon concentration under field conditions fluctuates diurnally (KEELEY 1983) it would seem that levels of light and dark CO_2 uptake likewise would fluctuate. I report here field studies of light and dark CO_2 uptake rates through a 48 hr period and an estimate of the contribution of CAM to the carbon economy of *I. howellii*.

Methods

Field studies were carried out 12—15 April 1983 in a large temporary (vernal) pool on Mesa de Colorado, Riverside Co., California, U. S. A. Methods were largely as described in KEELEY (1983) except that carbon assimilation rates were determined on ~0.25 g samples of leaves cut into 2 cm sections. These were incubated in 25 ml vials filled with water taken from the pools at the time of sampling. Experiments were initiated by addition of 25 μmol $H_2{}^{14}CO_3$ (40 μCi $\cdot$ vial^{-1}) and terminated after 30 min with boiling 80 % methanol. CO_2 uptake in the light was measured every 3 hr from 06.00 to 18.00 hr. Dark CO_2 uptake was measured every 3 hr from 18.00 hr to 06.00 hr, with the exception of 03.00 hr. At the two extreme time periods dark uptake was insured by covering the vials with foil.

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Results

Fig. 1 shows the changes in malic acid level and carbon assimilation rates as well as physical and chemical characteristics of the pool over the 48 hr period.

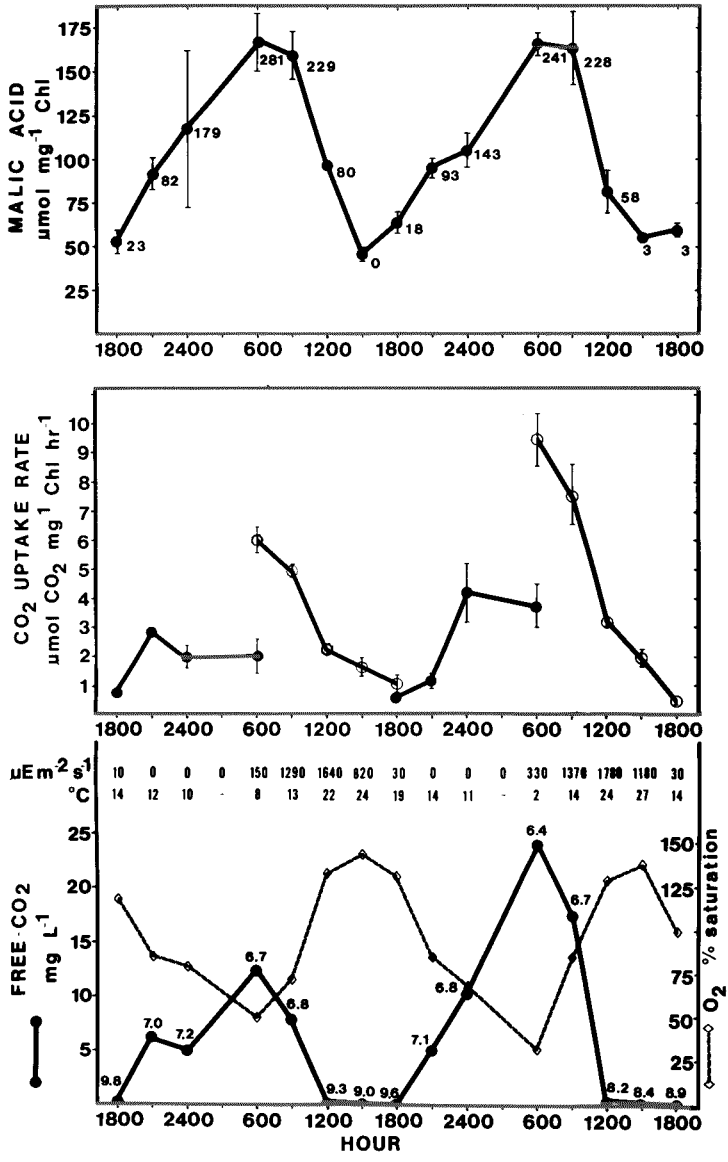

Fig. 1. (Top) Malic acid levels (± S. E.) in submerged *Isoetes howellii* leaves (titratable acidity to pH 6.4 in μequivalents · mg^{-1} Chl given adjacent to each point). (Middle) Carbon assimilation rates by *I. howellii* leaves: closed circles in the dark and open circles in the light. (Bottom) Free-CO_2 (pH given adjacent to each point) and O_2 levels in the pool. Solar radiation at the water surface and water temperature given above. Total alkalinity of the water during this time period varied from 31 (06.00 hr) to 28 (18.00 hr) mg · l^{-1} as $CaCO_3$. Specific conductance of the water was 43 μmhos · cm^{-1}.

There was a diurnal fluctuation of malic acid and titratable acidity of ~120 μmol · mg^{-1} Chl (~80 μmol · g^{-1} fresh weight) and ~250 μequivalents · mg^{-1} Chl (~167 μeq · g^{-1} FW). During daytime deacidification there was no significant change between 06.00 and 09.00 hrs, but a rapid drop occurred between 09.00 and 12.00 hrs.

Over the 48 hr period maximum CO_2 uptake rates were observed at 06.00 hr, but by noon rates were ⅓ this level and they continued to drop through the rest of the day. Maximum nighttime carbon uptake rates were approximately equal to noontime CO_2 uptake rates.

Free CO_2 levels in the pool fluctuated markedly. They increased overnight reaching their highest levels by 06.00 hr. Between 06.00 hr and noon, photosynthesis by the pool flora contributed to substantial increases in oxygen and decreases in free-CO_2.

Discussion

Estimates of gross carbon gain by *Isoetes howellii* can be made by calculating the areas under the dark and light uptake curves. During the first 24 hr period total carbon gain was 64 μmol CO_2 · mg^{-1} Chl and dark uptake contributed 39 %. For the second 24 hr period the total was 92 μmol and dark uptake accounted for 38 %. It appears that well over ⅓ of the total carbon gain is contributed by CO_2 uptake at night. Studies are presently underway to evaluate the magnitude of mitochondrial respiration and photorespiration. These are needed before we can say what proportion of the net carbon gain is contributed by dark CO_2 uptake.

Although dark CO_2 uptake contributes substantially to the carbon economy of *Isoetes howellii* it only accounts for a fraction of the total acid accumulated overnight; for the 2 nights under study CO_2 uptake accounted for only 22 % and 31 % of the malic acid accumulated. Thus, CAM in addition to contributing to the net carbon gain of the plant also affects the carbon economy substantially by apparently refixing internally derived "respiratory" CO_2.

The marked drop in daytime CO_2 uptake during the first part of the day concomitant with the rapid depletion of free CO_2 in the pool is consistent with the hypothesis that daytime CO_2 limitation has selected for nighttime carbon uptake.

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