

Gas Exchange Characteristics of the Submerged Aquatic Crassulacean Acid Metabolism Plant, *Isoetes howellii*¹

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JON E. KEELEY AND GEORGE BOWES

Department of Biology, Occidental College, Los Angeles, California 90041 (J. E. K.); and Department of Botany, University of Florida, Gainesville, Florida 32611 (G. B.)

ABSTRACT

The submerged aquatic plant *Isoetes howellii* Engelmann possesses Crassulacean acid metabolism (CAM) comparable to that known from terrestrial CAM plants. Infrared gas analysis of submerged leaves showed *Isoetes* was capable of net CO₂ uptake in both light and dark. CO₂ uptake rates were a function of CO₂ levels in the medium. At 2,500 microliters CO₂ per liter (gas phase, equivalent to 1.79 milligrams per liter aqueous phase), *Isoetes* leaves showed continuous uptake in both the light and dark. At this CO₂ level, photosynthetic rates were light saturated at about 10% full sunlight and were about 3-fold greater than dark CO₂ uptake rates. In the dark, CO₂ uptake rates were also a function of length of time in the night period. Measurements of dark CO₂ uptake showed that, at both 2,500 and 500 microliters CO₂ per liter, rates declined during the night period. At the higher CO₂ level, dark CO₂ uptake rates at 0600 h were 75% less than at 1800 h. At 500 microliters CO₂ per liter, net CO₂ uptake in the dark at 1800 h was replaced by net CO₂ evolution in the dark at 0600 h. At both CO₂ levels, the overnight decline in net CO₂ uptake was marked by periodic bursts of accelerated CO₂ uptake. CO₂ uptake in the light was similar at 1% and 21% O₂, and this held for leaves intact as well as leaves split longitudinally. Estimating the contribution of light versus dark CO₂ uptake to the total carbon gain is complicated by the diurnal flux in CO₂ availability under field conditions.

Dark fixation of CO₂ is widespread in plants, having been reported from leaves, stems, and roots in both terrestrial and aquatic plants (10, 11, 25). However, only in CAM plants does dark fixation substantially contribute to the total carbon gain (18). Until recently, CAM was known only from terrestrial plants where, in many cases, it contributes to greatly enhanced water use efficiency.

The submerged aquatic, *Isoetes howellii* (*Isoetaceae*) possesses CAM comparable to that found in terrestrial CAM plants (12; J. Keeley, unpublished data). CO₂ uptake occurs in the dark and is stored as malic acid. Overnight acidification is followed by daytime deacidification resulting in a diurnal flux of 100 to 300 $\mu\text{eq g}^{-1}$ fresh weight. Maximum malic acid production rates can be accommodated by the measured PEP² carboxylase activities. Tracer studies show that dark-fixed carbon moves in the light from organic acids to 3-phosphoglyceric acid and phosphorylated

sugars. Assimilation of CO₂ occurs in the light through C₃ type reactions.

Day and night CO₂ uptake is known from terrestrial CAM plants though the prototype (super-CAM of Kluge and Ting [18]) has a diurnal pattern of changes in stomatal conductance which results in the bulk of the carbon gain occurring at night. *I. howellii* has stomata, though Sculthorpe (21) contends that stomata on submerged aquatics are nonfunctional because of a wax occlusion and, based on light microscopy examination, *I. howellii* appears to be no exception.

Evidence to date indicates that the CAM pathway is common in the genus *Isoetes*; it has been found in 12 of the over 60 species in the genus (13, 17). The aquatic species surveyed include ones from oligotrophic lakes, seasonal pools, and tidal creeks. Some have stomata but others do not. All of these aquatic *Isoetes* show diurnal acid fluctuations while submerged, but upon emergence acid metabolism dampens out. This correlates with the observation that the terrestrial *Isoetes nuttallii* and *Isoetes butleri* lack acid metabolism even if artificially submerged (15).

Diurnal fluxes in titratable acidity or malic acid have recently been reported for several submerged aquatic plants. Browse *et al.* (9) reported a small daytime accumulation of malate in *Egeria densa*. A similar phenomenon has been observed for several aquatics from a eutrophic lake in northern California (16), but this phenomenon obviously is not CAM. The aquatics, *Crassula aquatica* and *Littorella uniflora*, show diurnal acid fluctuations comparable to *Isoetes* (16), and *Hydrilla verticillata* (10) and *Scirpus subterminalis* (4) show a limited capacity for overnight acid accumulation of approximately an order of magnitude less than what is typically observed in terrestrial CAM plants or *Isoetes*.

The significance of CAM in *Isoetes* remains to be determined. The present study examines net light and dark CO₂ exchange characteristics of *I. howellii* with an IR gas analysis system designed for aquatic studies.

MATERIALS AND METHODS

Plant Material. *Isoetes howellii* Engelmann was collected from seasonal pools on Mesa de Colorado, Riverside Co., CA, and maintained submerged in growth chambers on a 12-h photoperiod (0600–1800 h) with light/dark temperature of 25°C/15°C.

IR Gas Analysis. Prior to analysis, leaves were severed from the corm and bound together at the base with parafilm. These were submerged in 200-ml gas washing bottles fitted with a fritted glass gas filter and containing 150 ml of 10 mM Mes-NaOH (pH 5.5) and 5% (v/v) Hoagland solution. The bottle was immersed in a glass-sided water bath maintained at 25 ± 0.5°C. The quantum flux density was provided by a 1,000-w quartz-halogen lamp. The IR analysis was performed in a closed system as described previously (10, 20, 27) and consisted of bubbling gas through the chamber solution (at 1 L min⁻¹) and determining CO₂ exchange

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²Abbreviations: PEP, phosphoenolpyruvate; Γ , CO₂ compensation point.

from changes in the CO₂ content of the gas stream. Unless indicated otherwise, all experiments were run at 21% O₂. Chl was determined by the procedure of Arnon (2) with correction for small *A* at 710 nm as suggested by Sestak *et al.* (22).

RESULTS

Figure 1 shows light response curves at 340 and 2,500 μl CO₂ l⁻¹ (in the gas phase which was equivalent to 0.23 and 1.79 mg CO₂ l⁻¹ in the aqueous phase). Leaves were largely light saturated at low quantum flux densities of 200 to 300 μE m⁻² s⁻¹. At air levels of CO₂, there was a light compensation point at ~125 μE m⁻² s⁻¹, whereas at 2,500 μl CO₂ l⁻¹ there was no light compensation point due to net CO₂ uptake in the dark. The light-saturated CO₂ uptake rate was over an order of magnitude greater at 2,500 μl CO₂ l⁻¹ than at 340 μl l⁻¹. At the high CO₂ level, net CO₂ uptake was 3-fold greater in the light than in the dark.

At 2,500 μl CO₂ l⁻¹, CO₂ uptake was continuous during the transition from the light to the dark period (Fig. 2). During the first 5 min in the dark, the CO₂ uptake rate was approximately half of the maximum dark CO₂ uptake rate which was reached within 30 min. Continuous net CO₂ uptake was likewise characteristic of the converse transition from dark to light (Fig. 3).

The CO₂ response curve for net CO₂ uptake in the light showed that uptake was a linear function of CO₂ concentration up through 2,500 μl l⁻¹ (Fig. 4). The Γ value was above 100 μl l⁻¹. Subsequent experiments have shown that Γ , in the light, is dependent upon prior incubation conditions. Plants maintained for more than a week on a 14-h photoperiod at 30°C day/22°C night showed Γ values ~30 μl CO₂ l⁻¹. Figure 5 shows dark CO₂ uptake was

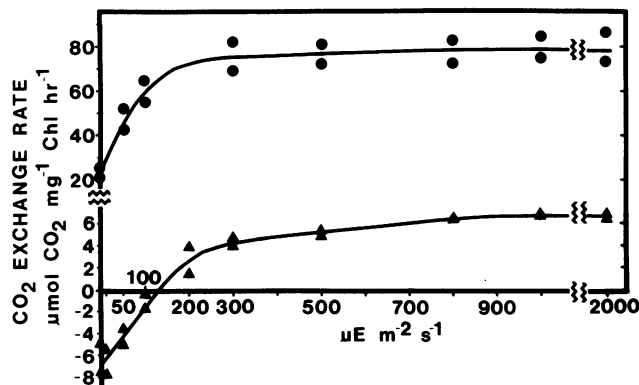


FIG. 1. Light response curves for *I. howellii* leaves at 2,500 (●) and 340 (▲) μl CO₂ l⁻¹ measured during the day.

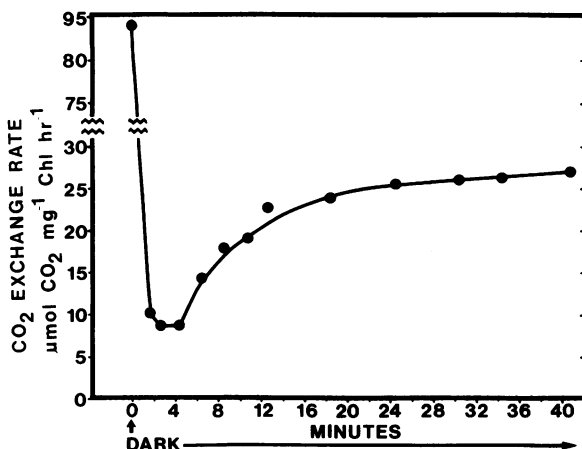


FIG. 2. Time course of net CO₂ uptake during the transition from the light to the dark period at 2,500 μl CO₂ l⁻¹.

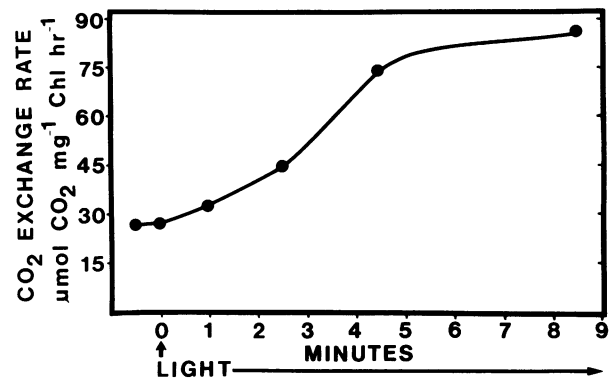


FIG. 3. Time course of net CO₂ uptake during the transition from the dark to the light at 2,500 μl CO₂ l⁻¹ and 1,000 μE m⁻² s⁻¹.

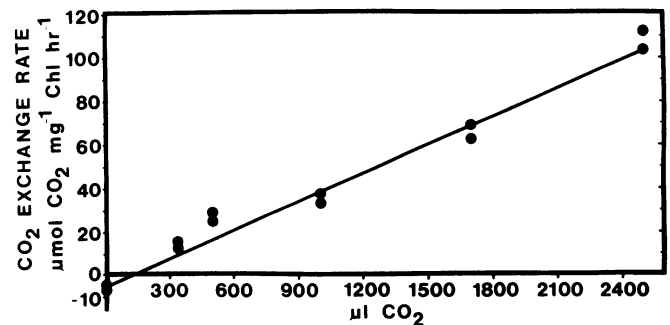


FIG. 4. CO₂ response curve for net CO₂ exchange in the light (1,000 μE m⁻² s⁻¹), measured during the day.

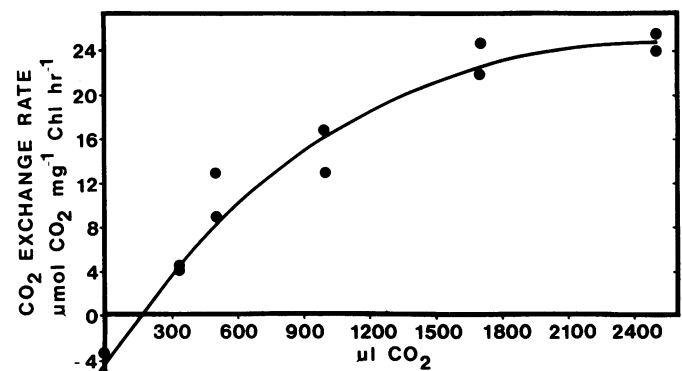


FIG. 5. CO₂ response curve for net CO₂ exchange in the dark, measured during the night.

saturated at 2,000 μl CO₂ l⁻¹ and the Γ value was > 100 μl l⁻¹.

To investigate nighttime changes in dark CO₂ uptake, individual plants were monitored 30 min after the light was turned off (at 1830 h) and then every hour through the night. Figure 6 shows that dark CO₂ uptake rates decreased through the night at both 500 and 2,500 μl CO₂ l⁻¹. This overnight decline was marked by periodic short-term increases in dark CO₂ uptake for plants at both the low and the high CO₂ levels. At the end of the dark period (0600 h), plants maintained at 2,500 μl CO₂ l⁻¹ showed net CO₂ uptake, but about 75% lower than at the beginning of the dark period. Plants maintained at 500 μl CO₂ l⁻¹ showed net CO₂ evolution at 0600 h. Perhaps related to this overnight decline in dark CO₂ uptake is the observation that net CO₂ evolution into a CO₂-free medium in the dark increased from 0.92 μmol CO₂ mg⁻¹ Chl h⁻¹ (SD = 0.44; *n* = 2) at 1900 h to 4.87 (± 0.25) at 0600 h.

Table I shows the effect of 1% and 21% O₂ (in the gas phase

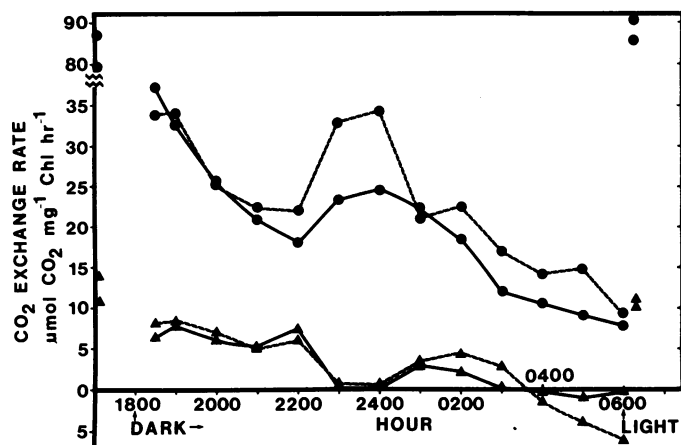


FIG. 6. Overnight time course of net CO₂ exchange in the dark at 2,500 (●) and 500 (▲) μl CO₂ l⁻¹. Each line represents a single plant. Points for CO₂ uptake in the light (at 1,000 μE m⁻² s⁻¹), before and after the dark period, are not connected to the dark CO₂ exchange curves.

Table I. Net CO₂ Uptake Rates in the Light at Air Levels of CO₂ and 1% and 21% O₂ for Intact and Longitudinally Split *I. howellii* Leaves

O ₂ Concn.	Intact Leaves	Split Leaves
%	μmol CO ₂ mg ⁻¹ Chl h ⁻¹	
1	10.95 ± 2.84 ^a	20.78 ± 3.54
21	10.45 ± 3.17	19.00 ± 1.76

^a Mean ± SD (n = 2).

which is equivalent to 0.4 and 7.9 mg l⁻¹ in the aqueous phase) on CO₂ uptake in the light at air levels of CO₂. Under these conditions, light CO₂ uptake appeared to be insensitive to O₂ concentration and this was true whether the leaves were intact or longitudinally split. Cutting open the leaves approximately doubled the uptake rates (Table I). This cutting procedure apparently had two major effects; one was to destroy the extensive lacunal gas space and the other was to expose more photosynthetic tissue to the medium. Wound responses could not be ruled out, though these uptake rates remained quite stable for more than 1 d.

DISCUSSION

Photosynthesis in *Isoetes* is similar in some respects to photosynthesis in other submerged aquatic plants. Light-saturated photosynthetic rates are comparable to those reported for species from subtropical lakes (5, 27). In these species, as well as in *Isoetes*, photosynthetic rates at air levels of CO₂ are substantially lower than rates for terrestrial C₃ plants. Van *et al.* (27) reported that, for *Hydrilla verticillata*, *Ceratophyllum demersum*, and *Myriophyllum spicatum*, CO₂-saturated photosynthetic rates were an order of magnitude greater than rates at air levels of CO₂. At the highest CO₂ level tested (2,500 μl CO₂ l⁻¹), *Isoetes* also had light CO₂ uptake rates which were an order of magnitude greater than rates at air levels of CO₂. The maximum photosynthetic rates observed for *Isoetes* were double those observed for the three species studied by Van *et al.* (27).

Light saturation at a low quantum flux density as for *Isoetes*, is likewise common in aquatics (3, 6, 19, 26). This is particularly striking in *Isoetes* where, at high CO₂ levels, light saturation occurs at only 10% of full sunlight. Even though *I. howellii* grows in shallow pools where noontime light intensity is generally 75% of surface light intensity (14), light saturation at very low irradiance levels may still be very significant under field conditions. This is because early morning CO₂ levels in the pools are always much higher than mid-day CO₂ levels; typically, the free CO₂ levels at

0600 h will be several times higher than the highest CO₂ level used in these IR gas analysis studies (2,500 μl l⁻¹), but drop to zero by noon (14).

Light compensation points have been reported as low as 5 μE m⁻² s⁻¹ in *Najas marina* (1). *Isoetes* showed no light compensation point at high CO₂ levels but air levels of CO₂ had a light compensation point ~125 μE m⁻² s⁻¹ (Fig. 1). However, it is clear from Figure 6, this value would likely be lower if measured in the late afternoon rather than, as was the case, in the early morning.

The insensitivity to O₂ exhibited by intact or split *Isoetes* leaves (Table I) is contrary to what has been observed for other aquatics (8, 23, 24, 27). Although Søndergaard reported some species that did not respond to increased O₂ with increased photorespiratory CO₂ evolution, this was only for intact leaves. Once the leaves of these species were split open, increased O₂ produced markedly increased CO₂ evolution. The lack of O₂ inhibition by some aquatic species has been attributed to extensive refixation of photorespiratory CO₂ from the lacunal air spaces (23, 24). At least for *Isoetes*, this explanation does not seem to be adequate, as the split leaves showed no O₂ inhibition. A more likely explanation would be that some fixation occurs via residual PEP carboxylase activity in the light, as is found in low Γ *Hydrilla* plants (10).

Net CO₂ uptake in the dark distinguished *Isoetes* from most other aquatics. Dark CO₂ uptake rates are clearly sensitive to the time at which they are measured (Fig. 6). The overnight decline in net CO₂ uptake was observed at both high and low CO₂ levels. Terrestrial CAM plants exhibit a decline in CO₂ uptake near the end of the dark period which has been attributed to reduced vacuolar storage capacity for malic acid (18). If this explanation was applicable to *Isoetes*, it remains to be explained why dark CO₂ uptake rates are greater after 12 h at 2,500 μl CO₂ l⁻¹ than at 500 μl CO₂ l⁻¹. Perhaps the decline in rate is a consequence of some endogenous rhythm, as occurs in terrestrial CAM plants (18). The patterns observed for overnight CO₂ uptake (Fig. 6) probably would not be observed under field conditions where free CO₂ levels are zero during the early evening. It may be significant that the free CO₂ levels in the pools approach their maximum levels at approximately the time accelerated CO₂ uptake was observed (2200–2300 h) in Figure 6.

Under similar conditions, it appears that dark CO₂ uptake may contribute less than a quarter to the total carbon gain in *Isoetes*. However, there are a number of factors which could affect the contribution of dark versus light CO₂ uptake. For example, *Isoetes* may have some capacity for bicarbonate assimilation, though it appears to be quantitatively unimportant in both the dark and light (14). A major complication is that both dark and light CO₂ uptake rates are a function of CO₂ concentration and under natural conditions in the aquatic environment the CO₂ level is a function of the time of the day. This is unlike the situation in an aerial environment where the CO₂ concentration is relatively constant. Consequently, even though *Isoetes* may have the potential for substantial carbon gain during the day, the low environmental daytime CO₂ levels may preclude this and result in the bulk of the carbon gain coming from dark CO₂ uptake.

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