Plant Physiol. (1982) 70, 1455–1458
0032-0889/82/70/1455/04/$00.50/0

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

Received for publication April 26, 1982 and in revised form June 8, 1982

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 CO_2 uptake in both light and dark. CO_2 uptake rates were a function of CO_2 levels in the medium. At 2,500 microliters CO_2 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 CO_2 level, photosynthetic rates were light saturated at about 10% full sunlight and were about 3-fold greater than dark CO_2 uptake rates. In the dark, CO_2 uptake rates were also a function of length of time in the night period. Measurements of dark CO_2 uptake showed that, at both 2,500 and 500 microliters CO_2 per liter, rates declined during the night period. At the higher CO_2 level, dark CO_2 uptake rates at 0600 h were 75% less than at 1800 h. At 500 microliters CO_2 per liter, net CO_2 uptake in the dark at 1800 h was replaced by net CO_2 evolution in the dark at 0600 h. At both CO_2 levels, the overnight decline in net CO_2 uptake was marked by periodic bursts of accelerated CO_2 uptake. CO_2 uptake in the light was similar at 1% and 21% O_2, and this held for leaves intact as well as leaves split longitudinally. Estimating the contribution of light *versus* dark CO_2 uptake to the total carbon gain is complicated by the diurnal flux in CO_2 availability under field conditions.

Dark fixation of CO_2 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). CO_2 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 μeq g^{-1} fresh weight. Maximum malic acid production rates can be accomodated by the measured PEP[2] 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 CO_2 occurs in the light through C_3 type reactions.

Day and night CO_2 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 CO_2 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 CO_2 exchange

[1] Supported by American Philosophical Society Grant 8662 and National Science Foundation Grant DEB-8004615 to J. E. K. and United States Department of Agriculture/Science and Education Administration Competitive Research Grant 78-59-2121-0-0-082-1 to G. B.

[2] Abbreviations: PEP, phosphoenolpyruvate; Γ, CO_2 compensation point.

from changes in the CO_2 content of the gas stream. Unless indicated otherwise, all experiments were run at 21% O_2. 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 CO_2 l^{-1} (in the gas phase which was equivalent to 0.23 and 1.79 mg CO_2 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 CO_2, there was a light compensation point at ~125 μE $m^{-2}s^{-1}$, whereas at 2,500 μl CO_2 l^{-1} there was no light compensation point due to net CO_2 uptake in the dark. The light-saturated CO_2 uptake rate was over an order of magnitude greater at 2,500 μl CO_2 l^{-1} than at 340 μl l^{-1}. At the high CO_2 level, net CO_2 uptake was 3-fold greater in the light than in the dark.

At 2,500 μl CO_2 l^{-1}, CO_2 uptake was continuous during the transition from the light to the dark period (Fig. 2). During the first 5 min in the dark, the CO_2 uptake rate was approximately half of the maximum dark CO_2 uptake rate which was reached within 30 min. Continuous net CO_2 uptake was likewise characteristic of the converse transition from dark to light (Fig. 3).

The CO_2 response curve for net CO_2 uptake in the light showed that uptake was a linear function of CO_2 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 CO_2 l^{-1}. Figure 5 shows dark CO_2 uptake was saturated at 2,000 μl CO_2 l^{-1} and the Γ value was > 100 μl l^{-1}.

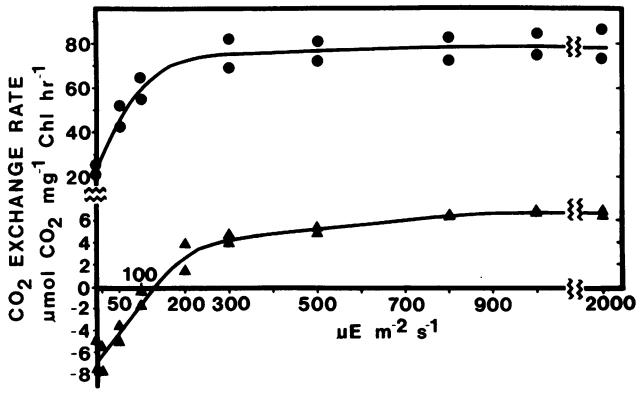

FIG. 1. Light response curves for *I. howellii* leaves at 2,500 (●) and 340 (▲) μl CO_2 l^{-1} measured during the day.

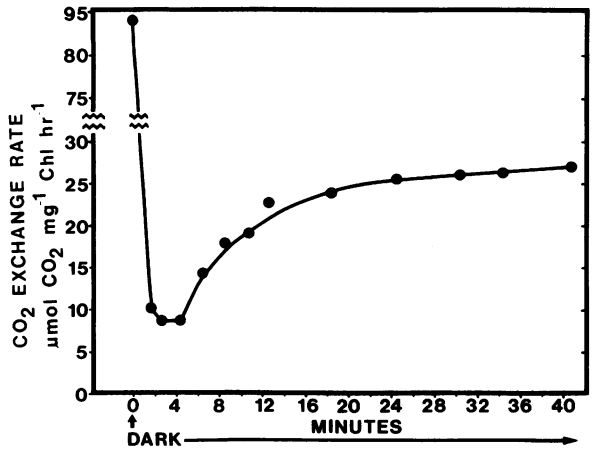

FIG. 2. Time course of net CO_2 uptake during the transition from the light to the dark period at 2,500 μl CO_2 l^{-1}.

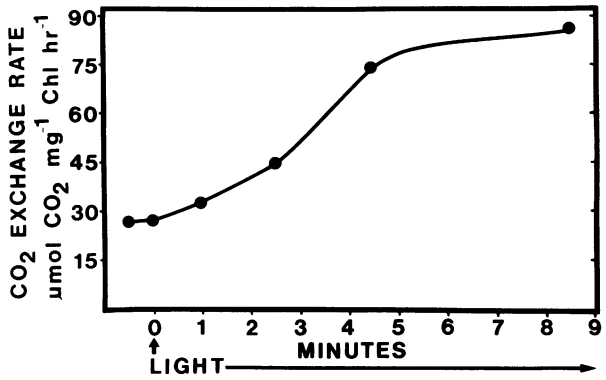

FIG. 3. Time course of net CO_2 uptake during the transition from the dark to the light at 2,500 μl CO_2 l^{-1} and 1,000 μE m^{-2} s^{-1}.

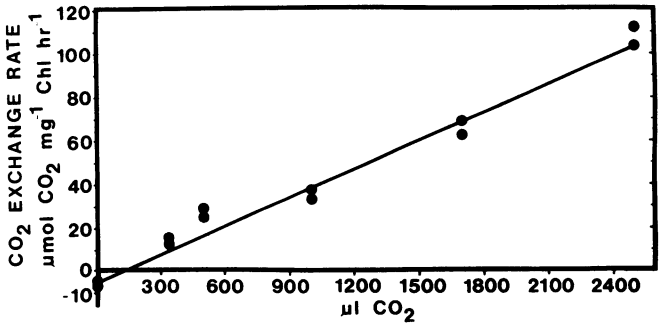

FIG. 4. CO_2 response curve for net CO_2 exchange in the light (1,000 μE m^{-2} s^{-1}), measured during the day.

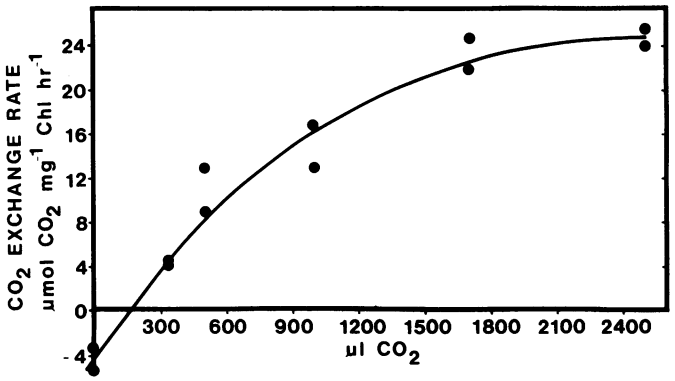

FIG. 5. CO_2 response curve for net CO_2 exchange in the dark, measured during the night.

To investigate nighttime changes in dark CO_2 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 CO_2 uptake rates decreased through the night at both 500 and 2,500 μl CO_2 l^{-1}. This overnight decline was marked by periodic short-term increases in dark CO_2 uptake for plants at both the low and the high CO_2 levels. At the end of the dark period (0600 h), plants maintained at 2,500 μl CO_2 l^{-1} showed net CO_2 uptake, but about 75% lower than at the beginning of the dark period. Plants maintained at 500 μl CO_2 l^{-1} showed net CO_2 evolution at 0600 h. Perhaps related to this overnight decline in dark CO_2 uptake is the observation that net CO_2 evolution into a CO_2-free medium in the dark increased from 0.92 μmol CO_2 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% O_2 (in the gas phase

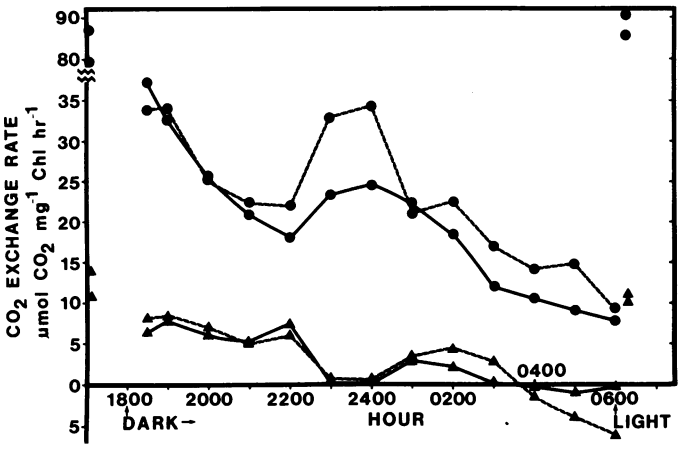

FIG. 6. Overnight time course of net CO_2 exchange in the dark at 2,500 (●) and 500 (▲) μl CO_2 l^{-1}. Each line represents a single plant. Points for CO_2 uptake in the light (at 1,000 μE m^{-2} s^{-1}), before and after the dark period, are not connected to the dark CO_2 exchange curves.

Table I. *Net CO_2 Uptake Rates in the Light at Air Levels of CO_2 and 1% and 21% O_2 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 CO_2 uptake in the light at air levels of CO_2. Under these conditions, light CO_2 uptake appeared to be insensitive to O_2 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 CO_2 are substantially lower than rates for terrestrial C_3 plants. Van *et al.* (27) reported that, for *Hydrilla verticillata*, *Ceratophyllum demersum*, and *Myriophyllum spicatum*, CO_2-saturated photosynthetic rates were an order of magnitude greater than rates at air levels of CO_2. At the highest CO_2 level tested (2,500 μl CO_2 l^{-1}), *Isoetes* also had light CO_2 uptake rates which were an order of magnitude greater than rates at air levels of CO_2. 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 CO_2 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 CO_2 levels in the pools are always much higher than mid-day CO_2 levels; typically, the free CO_2 levels at 0600 h will be several times higher than the highest CO_2 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 CO_2 levels but air levels of CO_2 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 O_2 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 O_2 with increased photorespiratory CO_2 evolution, this was only for intact leaves. Once the leaves of these species were split open, increased O_2 produced markedly increased CO_2 evolution. The lack of O_2 inhibition by some aquatic species has been attributed to extensive refixation of photorespiratory CO_2 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 O_2 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 CO_2 uptake in the dark distinguished *Isoetes* from most other aquatics. Dark CO_2 uptake rates are clearly sensitive to the time at which they are measured (Fig. 6). The overnight decline in net CO_2 uptake was observed at both high and low CO_2 levels. Terrestrial CAM plants exhibit a decline in CO_2 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 CO_2 uptake rates are greater after 12 h at 2,500 μl CO_2 l^{-1} than at 500 μl CO_2 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 CO_2 uptake (Fig. 6) probably would not be observed under field conditions where free CO_2 levels are zero during the early evening. It may be significant that the free CO_2 levels in the pools approach their maximum levels at approximately the time accelerated CO_2 uptake was observed (2200–2300 h) in Figure 6.

Under similar conditions, it appears that dark CO_2 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 CO_2 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 CO_2 uptake rates are a function of CO_2 concentration and under natural conditions in the aquatic environment the CO_2 level is a function of the time of the day. This is unlike the situation in an aerial environment where the CO_2 concentration is relatively constant. Consequently, even though *Isoetes* may have the potential for substantial carbon gain during the day, the low environmental daytime CO_2 levels may preclude this and result in the bulk of the carbon gain coming from dark CO_2 uptake.

LITERATURE CITED

1. AGAMI M, S BEER, Y WAISEL 1980 Growth and photosynthesis of *Najas marina* L. as effected by light intensity. Aquat Bot 9: 285–289
2. ARNON DI 1949 Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. Plant Physiol 24: 1–15
3. BARKO JW, RM SMART 1981 Comparative influences of light and temperature on the growth and metabolism of selected submerged freshwater macrophytes. Ecol Monogr 51: 219–235
4. BEER S, RG WETZEL 1981 Photosynthetic carbon metabolism in the submerged aquatic angiosperm *Scirpus subterminalis*. Plant Sci Lett 21: 199–207
5. 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. Proceedings of the Fourth International Congress on Photosynthesis. The Biochemical Society, London, pp 289–298
6. BOWES G, TK VAN, LA GARRARD, WT HALLER 1977 Adaptation to low light

levels by *Hydrilla*. J Aquat Plant Managem 15: 32–35
7. BOYLEN CW, RB SHELDON 1976 Submerged macrophytes: growth under winter ice cover. Science (Wash DC) 182: 841–842
8. BROWN JMA, FI DROMOGOOLE, 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
9. BROWSE JA, JMA BROWN, FI DROMGOOLE 1980 Malate synthesis and metabolism during photosynthesis in *Egeria densa* Planch. Aquat Bot 8: 295–305
10. HOLADAY AS, G BOWES1980 C_4 acid metabolism and dark CO_2 fixation in a submersed aquatic macrophyte *(Hydrilla verticillata)*. Plant Physiol 65: 331–335
11. JACOBSON L 1965 Carbon dioxide fixation and ion absorption in barley roots. Plant Physiol 40: 264–269
12. KEELEY JE 1981 *Isoetes howellii*: a submerged aquatic CAM plant? Am J Bot 68: 420–424
13. KEELEY JE 1982 Distribution of diurnal acid metabolism in the genus *Isoetes*. Am J Bot 69: 254–257
14. KEELEY JE 1982 Crassulacean acid metabolism in the seasonally submerged aquatic *Isoetes howellii*. Oecologia. In press
15. KEELEY JE 1983 Lack of diurnal acid metabolism in terrestrial *Isoetes* (Isoetaceae). Photosynthetica. In press
16. KEELEY JE, BA MORTON 1982 Distribution of diurnal acid metabolism in submerged aquatic plants outside the genus *Isoetes*. Photosynthetica. In press
17. KEELEY JE, B MORTON, B BABCOCK, P CASTILLO, B FISH, E JERAULD, B JOHNSON, L LANDRE, H LUM, C MILLER, A PARKER, G VAN STEENWYK 1981 Dark CO_2 fixation and diurnal malic acid fluctuations in the submerged aquatic *Isoetes storkii*. Oecologia 48: 332–333
18. KLUGE M, IP TING 1978 Crassulacean Acid Metabolism. Springer-Verlag, Heidelburg
19. RAVEN JA, SM GLIDEWELL 1975 Photosynthesis, respiration and growth in the shade alga *Hydrodictyon africanum*. Photosynthetica. 9: 361–371
20. SALVUCCI ME, G BOWES 1981 Indication of reduced photorespiratory activity in submersed and amphibious aquatic marcophytes. Plant Physiol 67: 335–340
21. SCULTHORPE CD 1967 The Biology of Aquatic Vascular Plants. E. Arnold, London
22. SESTAK Z, J KATSKY, PG JARVIS, eds 1971 Plant Photosynthetic Production. Manual of Methods. W. Junk, The Hague
23. SÖNDERGAARD 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
24. SØNDERGAARD M, RG WETZEL 1980 Photorespiration and internal recycling of CO_2 in the submersed angiosperm *Scirpus suberminalis*. Can J Bot 58: 591–598
25. TING IP, WM DUGGER 1966 CO_2 fixation in *Opuntia* roots. Plant Physiol 41: 500–505
26. TITUS JE, MS ADAMS 1970 Coexistence and the comparative light relations of the submerged macrophytes *Myriophyllum spicatum* L. and *Vallisneria americana* Michx. Oecologia 40: 273–286
27. VAN TK, WT HALLER, G BOWES 1976 Comparison of the photosynthetic characteristics of three submersed aquatic plants. Plant Physiol 58: 761–768