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Research Notes

Light/Dark Control of Diurnal Acid Metabolism in the Submerged Aquatic *Isoetes howellii*

It has been demonstrated recently that the leaves of the submerged aquatic *Isoetes howellii* Engelmann (Isoetaceae) possess a diurnal acidification-deacidification cycle strongly resembling that observed in terrestrial CAM (Crassulacean Acid Metabolism) plants (Keeley 1981a). In *I. howellii* leaves there is an overnight increase of 100–200 μequivalents titratable acidity per gram fresh weight, the bulk of which is due to malic acid. It was also demonstrated that these leaves are capable of substantial CO_2-fixation in the dark, the primary product being malic acid. More recent studies show that these plants have a net uptake of CO_2 at night and, depending upon conditions, this can exceed daytime CO_2 uptake (Keeley and Bowes 1982). It was suggested (Keeley 1981a) that diurnal acid metabolism may have been selected for in this aquatic plant because of low availability of CO_2 for photosynthesis during the day and there is some evidence for this (Keeley 1981b). Studies of other *Isoetes* species, representing a range of habitats, indicates that diurnal acid metabolism is common in the genus (Keeley 1982).

The purpose of this study was to determine the extent of light/dark control on malic acid accumulation in submerged leaves of *Isoetes howellii.*

I. howellii is an aquatic or amphibious lower vascular plant with quill-like leaves (15–30 cm) arising from an underground corm. It is distributed throughout California and other western states in temporary pools (Munz 1959). The individuals utilized in this study were obtained in the spring of 1979 from "vernal pools" on Miramar Naval Air Station, San Diego County, California, USA.

Mature *I. howellii* were transplanted, along with substrate, to an aquarium where the water level was maintained above the tips of the leaves. The aquarium was kept in an environmental chamber with a 12 hr photoperiod and 30°C light/20°C dark air temperature. Photon flux density of 400 $\mu E\ m^{-2}\ sec^{-1}$ (PAR) was provided with a combination of incandescent and fluorescent bulbs. IRGA studies show CO^2-uptake in the light by *I. howellii* is light saturated at $<400\ \mu E\ m^{-2}\ sec^{-1}$ (Keeley and Bowes 1982). All experiments were initiated with the photo period running from 0600 hr (Pacific Standard Time) to 1800 hr or 1800 hr to 0600 hr.

Every 12 hours, at 0600 hr (AM) and 1800 hr (PM), two samples of several leaves each were washed with distilled water, blotted dry, weighed, and ground in a mortar with cold distilled water. This extract was filtered through cheesecloth, deproteinized with 1 N perchloric acid and centrifuged at low speed. The supernatant was assayed spectrophotometrically using an enzymatic end-product assay of Gutmann and Wahlefeld (1974).

The normal nighttime acidification and daytime deacidification cycle in *I. howellii* involved a diurnal fluctuation of >100 μeq/g FW. This pattern changed abruptly when the light/dark cycle was disturbed. Figure 1 shows that, under continuous light (beginning at the end of the normal light period), the malic acid concentration rose to a level intermediate to control AM and PM levels. Over

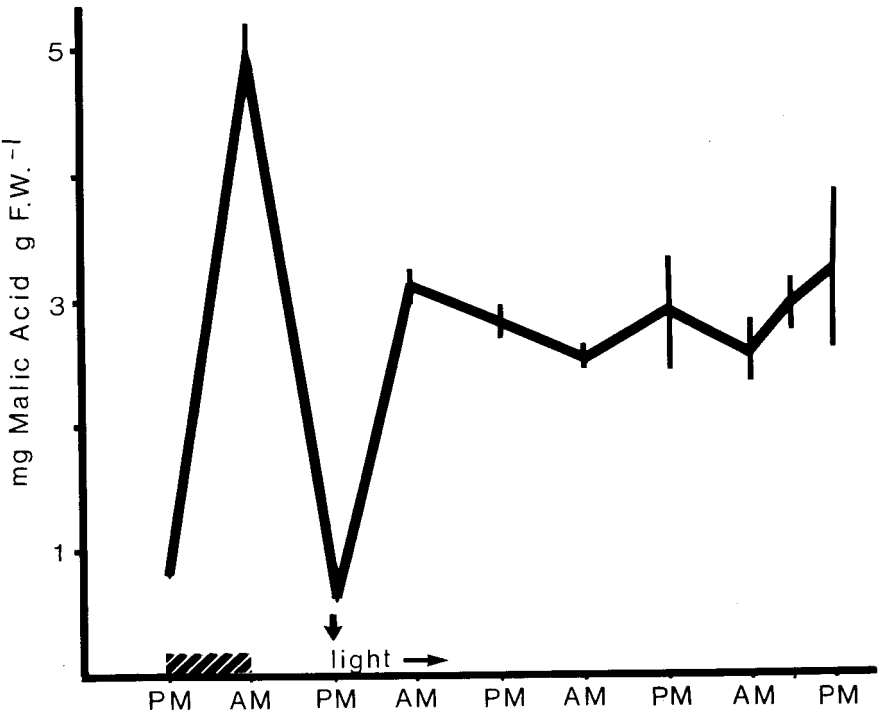

Fig. 1. Effect of continuous light on malic acid concentration (mg per gm fresh weight) in leaves of *I. howellii* at 0600 hr (AM) and 1800 hr (PM). Striped lines indicate darkness. Controlled conditions were disrupted at the arrow by continuous light. Points are the mean of 2 replicates, vertical lines indicate ±1 S.E. of the mean.

the 72 hr period of continuous light, malic acid concentration fluctuated very little around this intermediate level with no diurnal rhythm.

When lights were left off at the end of the dark period, malic acid concentrations dropped in the first 12 hrs (Fig. 2). Over the 12 hr dark period malic acid levels continued to drop to a level approximating PM levels under controlled conditions.

Figure 3 shows the effect of shifting the light/dark cycle 12 hr. This was accomplished by interjecting a 24 hr dark period followed by a return to the 12 hr light/12 hr dark cycle but offset such that the end of the light period occurred at 0600 hr and the dark period ended at 1800 hr. Malic acid concentration immediately started "tracking" the photo period; acidification during the dark (0600 hr to 1800 hr) and deacidification during the light (1800 hr to 0600 hr). During the first 72 hr after the photo period was shifted, the malic acid fluctuations were 40% lower than controls. This experiment was continued for another 72 hrs during which measurements were not made. When measurements were resumed it was observed that within 144 hrs after the original shift in the light/dark cycle the malic acid fluctuations approached control levels.

Conclusions

It is apparent that the acidification/deacidification cycle in *Isoetes howellii* leaves is not an endogenous rhythm. Continuous light or dark immediately disrupts the

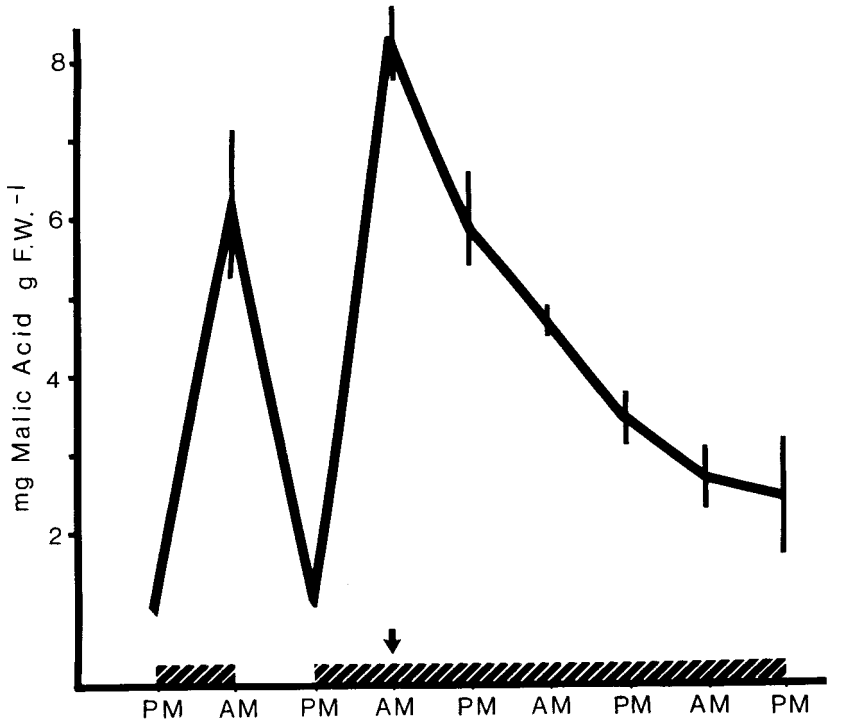

Fig. 2. Effect of continuous dark on malic acid concentration in leaves of *I. howellii* at 0600 hr (AM) and 1800 hr (PM). Striped lines indicate darkness. Controlled conditions were distrupted at the arrow by continuous dark. Points are the mean of 2 replicates, vertical lines indicate ±1 S.E. of the mean.

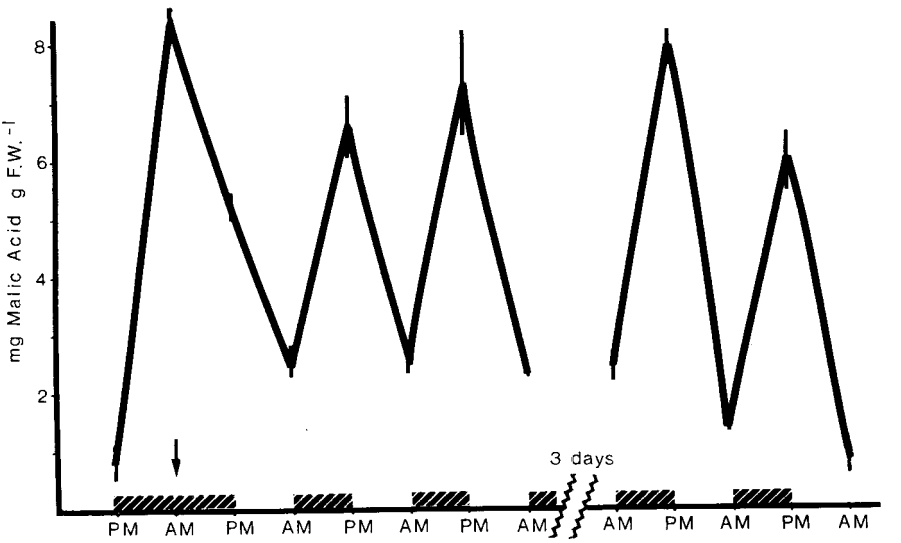

Fig. 3. Effect of reversed light/dark cycle on malic acid concentration in *I. howellii* leaves. Striped lines indicate darkness. Light/dark cycle was reversed at the arrow so that dark began at 0600 hr (AM) and light began at 1800 hr (PM). Points are the mean of 2 replicates, vertical lines indicate ±1 S.E. of the mean.

normal pattern of malic acid build-up and breakdown. The strong control exerted by the light/dark cycle is evidenced by the abrupt reversal of acidification/deacidification after a 12 hr shift in the photo period.

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