Amer. J. Bot. 69(2): 254–257. 1982.

DISTRIBUTION OF DIURNAL ACID METABOLISM IN THE GENUS ISOETES[1]

JON E. KEELEY

Department of Biology, Occidental College, Los Angeles, California 90041

ABSTRACT

Previous studies have revealed a crassulacean acid metabolism-like pathway in the submerged aquatic *Isoetes howellii* (Isoetaceae). A survey of 11 other species in this largely aquatic genus showed all had diurnal fluctuations in titratable acidity and malic acid concentration. The magnitude of the diurnal fluctuation in titratable acidity varied from 119 (μeq g^{-1} fresh weight) for *I. bolanderi* to 44 for *I. engelmanni*. Although these species were grown under similar conditions in a greenhouse, it is questionable whether or not any significance can be attached to observed species specific differences in magnitude of diurnal acid fluctuation. This is suggested by a single species comparison of plants in the field with greenhouse grown plants which indicated nearly as much variation in magnitude of acid fluctuations as observed across all species in the greenhouse. Much of this variation may be due to seasonal differences. Substrate appears to have very little effect on magnitude of acid fluctuations. Since this survey covered most of the range of the latitudinal, elevational, habitat, and morphological variation in the genus, it is likely that diurnal acid metabolism will prove to be common in the genus *Isoetes*.

RECENT EVIDENCE has been presented that the submerged aquatic *Isoetes howellii* Engelmann (Isoetaceae) has a diurnal acidification/deacidification cycle similar to crassulacean acid metabolism (CAM) (Keeley, 1981a). At night, submerged leaves of *I. howellii* fix CO_2 into malic acid and this product accumulates overnight. The malic acid is broken down during the day resulting in a diurnal fluctuation of 100–200 μeq titratable acidity per g fresh weight. *Isoetes howellii* differs from terrestrial CAM plants in stomatal behavior. The prototype CAM plant closes stomates much of the day thus restricting CO_2 uptake to the night. *Isoetes howellii* stomata are apparently nonfunctional while submerged and CO_2 uptake occurs in the light and the dark (Keeley, 1981a). Gas exchange studies indicate there is net CO_2 uptake at night and under certain conditions it is more significant than daytime uptake (Keeley and Bowes, unpubl. data). It has been 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.

The purpose of this study was to determine if diurnal acid metabolism is widely distributed in the genus *Isoetes*.

MATERIAL AND METHODS—*The genus Isoetes*—*Isoetes* includes over 60 species distributed worldwide, usually in "temperate" climates and in freshwater aquatic or seasonally wet habitats (Pfeiffer, 1922). Morphologically, *Isoetes* species are quite similar. All have a rosette of quill-like leaves arising from an underground corm. Each leaf has four longitudinal air canals, separated from one another by septa and surrounded by a peripheral wall of green tissue (Pfeiffer, 1922). A few species show a tendency for branching of the corm and this is highly developed in *I. tegetiformans* Rury (Rury, 1978). Another major difference between species is that perennially submersed ones lack stomata, unlike periodically inundated species such as *I. howellii*. Sculthorp (1967) contends that stomata on submersed aquatics are functionless because of the occlusion of the stomatal aperture by cuticular waxes. Submersed *Isoetes howellii* apparently are no exception, as evidenced by the thick wax which lines the stomatal aperture of submerged leaves (Keeley, 1981b).

The species selected for this study were chosen so as to represent the range of variation in the genus, with respect to presence or absence of stomata and distribution with respect to: 1) latitude; 2) elevation; 3) habitat; and 4) substrate. This information is given in Table 1 and indicates a wide range in stomatal presence (absent to abundant), latitude (10° to 53°),

[1] Received for publication 20 October 1980; revision accepted 7 January 1981.

I gratefully acknowledge the following persons for assistance in collecting *Isoetes*: Janis Antonovics, Steve Bostic, Sterling Keeley, Julian Priddle, Philip Rury, Carl Taylor, and Paul Zedler. Carl Taylor assisted in identification, Julius Bochantin in analysis and Susan Penn and Lori Lowe in manuscript preparation. This research was supported by NSF grants CDP8001910 and DEB8004614.

TABLE 1. Isoetes *species used in this survey*

Species[a]	Collection locality	Latitude	Elevation	Habitat	Substrate	Habit	Stomata
I. bolanderi Engelm.	Siesta Lake, Sierra Nevada, California	38°	2,450 m	shallow oligotrophic lake	loam-clay	perennially submerged	absent
I. echinospora ssp. *maritima* (Underw.) Love	Somas River, Alberni, Vancouver Island, British Columbia	49°	~sea-level	fresh water tidal creek	clay-loam	diurnally submerged	abundant
I. engelmanni A. Br.	Riopel Pond, Mountain Lake Biological Station, Pembroke, Virginia	37°	1,160 m	pond	clay	seasonally submerged	abundant
I. howellii Engelm.	Miramar Mesa, San Diego, California	33°	125 m	vernal pool	clay	seasonally submerged	present
I. lacustris L.	Llynnau Mumbr, Caernarvon, Wales	53°	180 m	oligotrophic lake	gravel-coarse sand	perennially submerged	absent
I. lithophila Pfeiffer	Enchanted Rock State Park, Llano Co., Texas	31°	555 m	granite outcrop pool	decomposed granite	seasonally submerged	abundant
I. mexicana Underw.	26.0 km northwest of Quetzaltenango, Guatemala	15°	2,850 m	shallow pool	sand-loam	seasonally submerged	present
I. occidentalis Hend.	Alice Lake, Garibaldi Provencial Park, British Columbia	50°	~200 m	oligotrophic lake	sand	perennially submerged	absent
I. orcuttii Eaton	Santa Rosa Plateau, Riverside Co., California	33°	610 m	vernal pool	clay	seasonally submerged	present
I. riparia Engelm. A. Br.	Chesapeake Bay, Havre de Grace, Hartford Co., Maryland	40°	~sea-level	freshwater tidal bay	gravel-coarse sand	diurnally submerged	present
I. storkii Palmer	Lagunna del Poas, Parque Volcan Poas, Costa Rica	10°	2,600 m	oligotrophic lake	coarse sand	±perennially submerged	present
I. tegetiformans Rury	Heggies Rock, Columbia Co., Georgia	34°	110 m	granite outcrop pool	decomposed granite	seasonally submerged	present

[a] Vouchers are deposited in LOC.

TABLE 2. *Morning and afternoon fluctuations in titratable acidity (to pH 6.4) and malic acid concentration for greenhouse maintained* Isoetes *species* ($\bar{x} \pm 1$ *S.D.*, $n = 2$)

Species	Titratable acidity (μeq g^{-1} fr wt)			Malic acid (mg g^{-1} fr wt)	
	0700 hr	1500 hr	Diurnal change	0700 hr	1500 hr
I. bolanderi	138 ± 2	19 ± 1	7.3	8.9 ± 0.5	0.9 ± 0.2
I. echinospora ssp. *maritima*	136 ± 2	21 ± 7	6.5	9.8 ± 0.4	2.6 ± 0.5
I. engelmanni	52 ± 2	8 ± 4	6.5	4.8 ± 0.1	1.9 ± 0.2
I. howellii	67 ± 29	14 ± 1	4.8	8.7 ± 0.6	2.0 ± 0.1
I. lacustris	108 ± 23	24 ± 13	4.5	7.4 ± 0.8	2.2 ± 1.1
I. lithophila	163 ± 23	40 ± 19	4.1	12.1 ± 0.3	5.6 ± 2.3
I. mexicana	88 ± 12	15 ± 8	5.9	8.1 ± 0.4	1.5 ± 0.2
I. occidentalis	93 ± 9	38 ± 15	2.4	7.3 ± 0.4	3.3 ± 0.9
I. orcuttii	85 ± 7	20 ± 16	4.2	7.0 ± 1.2	2.1 ± 1.0
I. riparia	117 ± 59	33 ± 1	3.5	10.5 ± 2.3	1.8 ± 0.4
I. storkii	99 ± 11	11 ± 1	9.0	11.1 ± 0.7	1.1 ± 0.2
I. tegetiformans[a]	95	51	1.9	7.9	2.5

[a] Due to insufficient material, $n = 1$.

TABLE 3. *A comparison of diurnal malic acid fluctuations in* Isoetes orcuttii *leaves in the spring in the field with plants sampled in summer in the greenhouse in either sand or natural substrate* ($\bar{x} \pm 1$ *S.D.*, $n = 2$)

Date and location	Malic acid (mg g^{-1} fresh wt) 0700 hr	1500 hr
April in the field	13.1 ± 2.6	3.7 ± 1.4
August in the greenhouse		
—substrate sand	7.0 ± 1.2	2.1 ± 1.0
—substrate clay from field site	6.8 ± 0.8	2.3 ± 0.1

altitude (sea level to 2,850 m), substrate (gravel to clay), and in habitat. Included are species from oligotrophic lakes where the plants are submerged permanently throughout the year, species from seasonal pools where the plants are submerged part of the year and emergent or dormant during dry parts of the year, and species indigenous to tidal flats where they are submerged part of the day and emergent at other times of the day.

Greenhouse—Plants of *Isoetes* were transplanted to aquaria with a substrate of 5 parts sand to 1 part potting soil and filled with deionized water. Plants were maintained submerged at all times. Collections were made between December and August and all plants were assayed in August or September. A separate aquarium of a local species, *I. orcuttii,* was maintained in its natural substrate, a slightly acidic lateritic clay (Rosario, 1979), to examine the effect of substrate.

Laboratory analysis—Green leaf material was collected at 0700 hr and 1500 hr (±30 min), washed, blotted dry and weighed. Leaves were ground in a Ten Broeck homogenizer with distilled water, filtered through four layers of cheesecloth and centrifuged at 2,400 g for 10 min. The supernatant was partitioned into two samples. One sample was deproteinized with an equal volume of 1 N perchloric acid and assayed enzymatically for malic acid (Bergmeyer, 1974). The other sample was titrated with 0.01 N (CO_2-free) NaOH to a pH of 6.4, 7.0, and 8.3, which represents the range of values utilized in previous studies on CAM. Each species was sampled twice in the morning and twice in the afternoon.

RESULTS AND DISCUSSION—All *Isoetes* species surveyed showed a marked drop in titratable acidity and malic acid between 0700 hr and 1500 hr (Table 2). The titratable acidity is presented for pH 6.4 (90–100% of the pH 8.3 diurnal acid change was detected at the pH 6.4 endpoint).

Diurnal fluctuation in titratable acidity varied from 119 μeq g^{-1} fresh weight in *Isoetes bolanderi* to only 44 μeq g^{-1} fresh weight in *I. engelmannii* and *I. tegitiformans*. Nine species had four-fold or greater changes in titratable acidity between morning and afternoon. Diurnal fluctuations in malic acid likewise varied across species from 10.0 mg g^{-1} FW in *I. storkii* to 2.9 mg g^{-1} FW in *I. engelmannii*.

To determine whether or not the magnitude of diurnal acid fluctuation varied with conditions, the locally available *I. orcuttii* was studied in the field and greenhouse (Table 3). It is apparent that the magnitude of overnight acid accumulation is variable. Since the field and greenhouse samples were taken at different times of the year, it is unknown to what extent these differences reflect seasonal variability. It appears, though, that substrate (clay vs. sand) has little effect on the magnitude of diurnal acid fluctuation. These data warn against attempting, at this point, any generalizations about species specific differences in magnitude of acid fluctuations.

Within the genus *Isoetes* it is apparent (Table 1, 2) that diurnal acid metabolism occurs in species distributed across a wide range of latitude, elevation, habitats, and substrates. It is present in species lacking stomata and in perennially, seasonally, or diurnally submerged species. With regards to this last point it should be kept in mind that the data in Table 2 are based on submerged plants. Data on *I. howellii* (Keeley, 1981b) indicate that diurnal acid fluctuations dampen out as the plants become emergent.

The *Isoetes* species included in this survey represent much of the range of variability in latitude, elevation, habitat, substrate, habit, and stomatal presence (Table 1) found in the genus (Pfeiffer, 1922). It seems likely that diurnal acid metabolism will prove to be common in the genus *Isoetes*. This should have a significant impact on studies of photosynthesis in *Isoetes* since recent work on *I. howellii* indicates overnight acid accumulation is associated with substantial net CO_2 uptake (Keeley and Bowes, unpubl. data).

LITERATURE CITED

BERGMEYER, H. U. (ed.) 1974. Methods of enzymatic analysis. 4 vol. Academic Press, New York.

KEELEY, J. E. 1981a. *Isoetes howellii*: A submerged aquatic CAM plant? Amer. J. Bot. 8: 420–424.

———. 1981b. Diurnal acid metabolism in vernal pool *Isoetes*. Madroño 28: 167–171.

PFEIFFER, N. E. 1922. Monograph of the Isoetaceae. Ann. Mo. Bot. Gard. 9: 79–103.

ROSARIO, J. A. 1979. Distributional ecology of vegetation in the vernal pools of the Santa Rosa Plateau, California. Ph.D. thesis. Loma Linda University, Loma Linda, Calif.

RURY, P. M. 1978. A new and unique, mat-forming merlin's-grass (*Isoetes*) from Georgia. Amer. Fern. J. 68: 99–108.

SCULTHORPE, C. D. 1967. The biology of aquatic vascular plants. E. Arnold Publ., London.