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5. Stable Carbon Isotopes in Vernal Pool Aquatics of Differing Photosynthetic Pathways

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Introduction

Studies of photosynthetic pathways in submerged aquatic macrophytes have shown that the aquatic environment has selected for a number of surprising characteristics. Examples include Crassulacean acid metabolism (CAM, a pathway typically restricted to xerophytes) in submerged aquatic species of *Isoetes* and other aquatic macrophytes (Keeley 1981; Keeley and Morton 1982) and the unusual combination of C₃ and C₄ carboxylation reactions within the same cells in leaves of *Hydrilla verticillata* (Bowes and Salvucci 1984).

Stable carbon isotopes have been shown to be useful indicators of photosynthetic pathways in terrestrial plants. C₃ species are distinguished from C₄ species by the $\delta^{13}\text{C}$ ratio; it is in the range of -28‰ or lower for the former group and -12‰ to -14‰ for C₄ species. This technique, however, is not always capable of distinguishing Crassulacean acid metabolism in terrestrial plants. Species that obtain most of their carbon by uptake and fixation at night have $\delta^{13}\text{C}$ ratios similar to C₄ plants. Many species with the CAM pathway can couple dark CO₂ uptake with CO₂ uptake in the light or, in some seasons, can rely totally on light uptake, and thus $\delta^{13}\text{C}$ ratios will span the entire range from -12 to -30‰ (Teeri 1982).

Here I examine the relationship between photosynthetic pathway, in particular CAM and non-CAM plants, and the stable carbon isotope ratios of sub-

merged aquatic macrophytes coexisting in shallow seasonal (vernal) pools in southern California.

Methods

Plants were collected from a seasonal pool on the Santa Rosa Plateau (610 m), Riverside County, California. This “vernal” pool is filled in most years between January and May and has been studied in detail (Keeley and Busch 1984).

Plants were tested for the presence of CAM by measuring the titratable acidity to pH 6.4 and malic acid content of photosynthetic tissues at 0600–0700 h and 1700–1800 h. Techniques used were described in Keeley and Busch (1984).

The carbon isotopes ^{13}C and ^{12}C were determined on plant and water samples as described by Sternberg et al. (1984). These were expressed as $\delta(\text{‰}) = [(\text{isotope ratio of sample/isotope ratio of standard}) - 1] \times 1000$ relative to the common standard for this isotope.

The initial carboxylation products were determined for selected species with ^{14}C tracer. Leaves were incubated in 10 mM morpholinoethanesulfonic acid (MES)–NaOH (pH 5.5) with 1 mM $\text{NaH}^{14}\text{CO}_3$ (25 μCi) with 1000 $\mu\text{mol m}^{-2} \text{s}^{-1}$ irradiance. After a brief exposure, tissues were killed in boiling methanol, homogenized, and centrifuged. After drying, samples were resuspended in water, and products were separated with two-dimensional thin-layer electrophoresis and chromatography followed by autoradiography as described by Morton (1984).

Results and Discussion

Photosynthetic Pathways

For the vernal pool species tested, only two showed evidence of CAM activity (Table 5.1). The *Isoetes* species had high CAM activity as do all other aquatic species in that genus (Keeley 1982, unpublished data). The only other vernal pool species with overnight acid accumulation of the order of magnitude observed for these *Isoetes* are *Crassula aquatica* and other aquatic species in that genus (Keeley and Morton 1982, unpublished data). However, evidence of CAM activity at much reduced levels is known from several other submerged aquatic species, e.g., *Hydrilla verticillata* (Holaday and Bowes 1980), *Scirpus subterminalis* (Beer and Wetzel 1981), and *Orcuttia californica* (Keeley, unpublished data).

The photosynthetic characteristics of the non-CAM species shown in Table 5.1 have not been studied for all species, although some information is available. No submerged aquatic species has kranz anatomy which would suggest C_4 photosynthesis (Hough and Wetzel 1977; Keeley, unpublished data). However, as shown for *Hydrilla verticillata* (Bowes and Salvucci 1984), kranz anatomy is

Table 5.1. Evidence of Crassulacean Acid Metabolism in Submerged Aquatic Plants from a Vernal Pool on Santa Rosa Plateau, Riverside County, California

Species	Overnight Increase (per g Fresh Weight)	
	$\mu\text{mol H}^+$ (Mean \pm SD)	$\mu\text{mol Malic Acid}$ (Mean \pm SD) ^a
<i>Callitriche longipedunculata</i> Moron. (Callitricaceae)	1 \pm 1	1 \pm 1 (3)
<i>Chara contraria</i> Braun ex. Kutzing (Characeae)	0 \pm 0	1 \pm 2 (3)
<i>Eleocharis acicularis</i> (L.) R. & S. (Cyperaceae)	6 \pm 3	5 \pm 3 (6)
<i>E. macrostachya</i> Britton in Small (Cyperaceae)	0 \pm 0	0 \pm 0 (3)
<i>Isoetes howellii</i> Englemann (Isoetaceae)	245 \pm 9	109 \pm 4 (10)
<i>I. orcuttii</i> A. A. Eaton (Isoetaceae)	152 \pm 5	70 \pm 7 (6)
<i>Lythrium hyssopifolium</i> L. (Lythraceae)	0 \pm 0	0 \pm 0 (3)
<i>Plagiobothrys undulatus</i> (Piper) Jtn (Boraginaceae)	0 \pm 0	0 \pm 0 (6)
<i>Ranunculus aquatilis</i> L. (Ranunculaceae)	2 \pm 3	1 \pm 3 (6)

^a Value in parentheses is *n*.

not a prerequisite for the C₄ metabolic pathway of photosynthesis. *Eleocharis acicularis* seems to fit the *Hydrilla* pattern as seen in Table 5.2; the initial products of CO₂ fixation in the light are about equally divided between phosphoglycerate (PGA) and the organic acids malate plus aspartate. Other species in vernal pools appear to possess relatively straightforward C₃-type photosynthesis. For example, *Plagiobothrys undulatus* does not have nighttime CO₂ uptake or acid accumulation, and the early products of light fixation indicate ribulose biphosphate (RuBP) carboxylase fixation (Table 5.2).

Carbon Isotope Ratios

$\delta^{13}\text{C}$ ratios for some of the submerged aquatic vernal pool species are shown in Table 5.3. There was no obvious difference in $\delta^{13}\text{C}$ value between CAM, C₃, and C₃-C₄ intermediates.

The $\delta^{13}\text{C}$ value of the water inorganic carbon was markedly more negative than the atmospheric level of -7‰ . In addition, by comparing these numbers with values from an earlier study on the Santa Rosa Plateau vernal pool, it is evident that the $\delta^{13}\text{C}$ value of the water becomes progressively more negative from early to late spring; $\delta^{13}\text{C}_{\text{water}}(\text{‰}) = -16.5, -18.5, -20.3$, respectively, for 4 April 1981, 3 May 1981 (Keeley and Busch 1984), and 25 May 1983 (Table 5.3). The very negative $\delta^{13}\text{C}$ values for the pool water can be accounted for by heterotrophic release of previously fractionated carbon, via decomposition of organic material and respiration by the pool flora. This pool, as well as seasonal pools in general, exhibits marked diurnal changes in CO₂ level due to daytime photosynthetic depletion and overnight respiratory input (Keeley and Busch 1984; Keeley et al. 1983). Because the ambient carbon source for CAM is largely respiratory CO₂ from the pool flora (and invertebrate fauna), it reflects previous fractionation events. This would account for the similar $\delta^{13}\text{C}$ values for CAM and non-CAM species in this pool, despite the fact that half of the carbon uptake

Table 5.2. Light Fixation Products with 5- and 30-s steady-state C^{14} Labeling for CAM and Non-CAM Vernal Pool Species

	Percentage of Label					
	5 s			30 s		
	Phosphoglycerate	Organic Acids	Other	Phosphoglycerate	Organic Acids	Other
<i>Isoetes howellii</i> (CAM species)	64	24	10	42	33	22
<i>Eleocharis acicularis</i> (non-CAM species)	44	42	14	40	36	28
<i>Plagiothyris undulatus</i> (non-CAM species)	68	23	9	27	22	51

Table 5.3. $\delta^{13}\text{C}$ Isotope Ratios for Submerged Aquatic Species from Santa Rosa Plateau Vernal Pool

Species	$\delta^{13}\text{C}$ (‰)	
	April	May
<i>Chara contraria</i>	-15.8	-25.1
<i>Isoetes howellii</i>	-29.1	-28.4
<i>I. orcuttii</i>	-24.0	-27.6
<i>Eleocharis acicularis</i>	-25.0	-28.9
<i>E. macrostachya</i>		-28.6
<i>Lythrium hyssopifolium</i>		-30.7
<i>Plagiobothrys undulatus</i>		-27.4
<i>Ranunculus aquatilis</i>	-14.5	-20.7
Water (Inorganic carbon)	—	-20.4 (A.M.) -21.2 (P.M.)

in *Isoetes* species is initially fixed in the dark via phosphoenolpyruvate (PEP) carboxylase (Keeley and Busch 1984).

Other Fractionation Effects

Thus, regardless of photosynthetic pathway, vernal pool macrophytes have $\delta^{13}\text{C}$ values ranging from 0 to 10‰ more negative than the source carbon in the water. Such values are not readily explained. C_3 species such as *Plagiobothrys undulatus* that utilize RuBP carboxylase would be expected to have a $\delta^{13}\text{C}$ value at least 27‰ more negative than the source carbon due to fractionation by that enzyme (Osmond et al. 1981). This species is largely restricted to the use of free- CO_2 (Keeley, unpublished data), as is the case with *Isoetes howellii* and *Eleocharis acicularis* (Keeley and Busch 1983; Morton 1984); therefore, there should be an additional -8‰ fractionation due to the equilibrium fractionation between HCO_3^- and CO_2 (Raven et al. 1982). Osmond et al. (1981) argue that for aquatic plants that utilize CO_2 and rely on RuBP carboxylase, as the resistances (internal and external) to CO_2 diffusion increase, the $\delta^{13}\text{C}$ value of the biomass should approach that of the source carbon.

Diffusional resistances probably play an important role in the Santa Rosa Plateau pool. For species in that pool that rely entirely on CO_2 , the source carbon would have a $\delta^{13}\text{C}$ value of -23‰ (including the -8‰ correction) early in the season and -28‰ late in the season, values that are very similar to the $\delta^{13}\text{C}$ values for plant material of most species. The fact that throughout the season a couple of species, e.g., *Ranunculus aquatilis*, had $\delta^{13}\text{C}$ values 8‰ more positive than these values suggests dependence on bicarbonate uptake.

Summary

The ratio of $^{13}\text{C}/^{12}\text{C}$ for photosynthetic tissues of seasonal pool aquatic species is unrelated to photosynthetic pathway. CAM and non-CAM species have sim-

ilar $\delta^{13}\text{C}$ values. Despite the fact that these CAM species derive up to half of their net carbon through dark fixation, their $\delta^{13}\text{C}$ values are similar to associated non-CAM species. This is, in part, because the ambient carbon source for dark CO_2 uptake is CO_2 released from organic carbon, either from respiration or decomposition. Thus, the carbon source for CAM reflects previous isotopic discrimination events. Although carbon isotopes are unable to distinguish photosynthetic pathways, there is good evidence that they may prove invaluable in the study of diffusional resistances to photosynthesis. Such evaluations require careful analysis of photosynthetic pathway, carbon species utilized, and $\delta^{13}\text{C}$ value of the source carbon.

Note added in proof: Recent anatomical studies reveal that the submerged leaves of the vernal pool aquatic grass, *Neostapfia colusana*, have kranz anatomy (Keeley, unpublished data).

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