Carbon Isotope Ratios in Crassulacean Acid Metabolism Plants

SEASONAL PATTERNS FROM PLANTS IN NATURAL STANDS

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ABSTRACT

A year round study of photosynthesis and carbon isotope fractionation was conducted with plants of Opuntia phaeacantha Engelm. and Yucca baccata Torr. occurring in natural stands at elevations of 525, 970, 1450 and 1900 m. Plant water potentials and the daytime pattern of 14CO2 photosynthesis were similar for all cacti along the elevational gradient, despite significant differences in temperature regime and soil water status. Carbon isotope ratios of total tissue and soluble extract fractions were relatively constant throughout the entire year. Additionally, the 13C values were similar in all plants of the same species along the elevational gradient, i.e., -12.5 ± 0.86 %/o for O. phaeacantha and -15.7 ± 0.95 %/o for Y. baccata. The results of this study indicate Crassulacean acid metabolism predominates as the major carbon pathway of these plants, which do not facultatively utilize the reductive pentose phosphate cycle of photosynthesis as the primary carboxylation reaction.

 Succulent plants capable of CAM3 have the enzymic potential for the assimilation of CO2 by different photosynthetic pathways. This option distinguishes CAM plants from both C3 and C4 plants, which have obligate photosynthetic pathways. Additionally, gas exchange studies with CAM plants have shown either C3 photosynthesis or CAM may be induced by regulation of physicochemical growth conditions (1, 13). Based upon these earlier studies it has been suggested that CAM plants are environmentally sensitive, and may facultatively employ different photosynthetic carbon pathways according to environmental growth conditions (5, 15).

Secondly, succulent plants suspected of being CAM show a wide range of carbon isotope ratios, with 13C values ranging from -14 to -33 %/o (1, 3). The analysis of carbon isotope ratios permits an assessment of photosynthetic CO2 assimilation, such that the wide range of 13C values for succulent plants is suspected to be due to the facultative use of different carboxylation reactions. Thus, succulent plants are further distinguished from both C3 and C4 plants, the latter having 13C values within more narrow ranges that are largely insensitive to environmental growth conditions (4, 19). Examples of the range of 13C values for C3 and C4 plants are -23 to -34 %/o; and -10 to -18 %/o, respectively (3).

In the context of these studies the facultative nature of the light versus dark CO2 assimilation exhibited by CAM plants is worthy of additional investigation. Laboratory studies have shown the carbon isotope ratio of a single species of CAM plant undergoes an actual shift in value in response to controlled growth conditions (1, 3, 9, 12, 15). Field studies with succulent plants suspected of being CAM have shown a relationship between environmental conditions and carbon isotope ratio (10, 11, 14, 16). In general, succulent plants from more arid environments have C4-like 13C values, and succulent plants from more mesic environments have C3-like 13C values. Other field studies investigating the gas exchange patterns of CAM plants have shown daytime CO2 assimilation is regulated by environmental conditions (2, 18). However, seasonal field studies documenting an actual shift in carbon isotope ratio within a single species of CAM plant have yet to be reported.

The following study was initiated to determine whether the facultative nature of light versus dark CO2 assimilation exhibited by CAM plants under laboratory conditions also occurs under field conditions. Two plants were selected for this study, Opuntia phaeacantha, a stem succulent cactus, and Yucca baccata, a leaf succulent plant. Both occur throughout a large elevational and climatic gradient, with significant environmental differences in water status and temperature regime.

MATERIALS AND METHODS

Plant Material. Plants of Opuntia phaeacantha Engelm. and Yucca baccata Torr. were in natural stands at elevations of 525, 970, 1450, and 1900 m. The four study sites occurred along an elevational gradient from near Tempe, Arizona (33° 37'N, 111° 37'W) to Strawberry, Arizona (34° 25'N, 111° 30'W), covering a distance of nearly 100 km by air. Individual plants of each species were marked at each study site and sampled repeatedly throughout 1975. In all experiments young plant material was utilized, i.e. less than 1 year in age and free from tissue deterioration. The Opuntia stem material and the Yucca leaf material collected in the field were immediately stored under dry ice until returned to the laboratory. All plant material remained frozen until being processed in the laboratory.

Titratable Acidity. Tissue acidity was determined by titration of aqueous extracts of plant material. Three g fresh weight of tissue were homogenized in 100 ml of H2O by high speed blending for 3 min. All samples were titrated to pH 7 with NaOH. Plant material was collected at different times of the day and stored under dry ice until being processed in the laboratory.

Photosynthesis. Carbon dioxide-14C uptake was measured

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2 Abbreviations: CAM: Crassulacean acid metabolism; C3: reductive pentose phosphate cycle; C4: dicarboxylic acid cycle; ρ: water potential.
with a portable porometric system as previously described (17).

**Stomatal Conductance.** Stomatal diffusive conductance was measured with a portable, commercially available transpiration rate hygrometer (Ennis and Associates). Direct transpiration measurements on a flat Opuntia stem could be obtained within a few min, although the instrument could not be used on the curved Yucca leaf. The Opuntia stems were shaded during the actual transpiration measurement. Stem temperatures were measured by IR thermometry (model PRT-10, Barnes Engineering Co.).

**Water Potentials.** Plant and soil ψ were determined with a portable, commercially available dewpoint microvoltmeter (model HR-33, Wescor, Inc.). One soil hygrometer probe was permanently placed at each study site at a depth of 20 cm, with the sensor axis positioned parallel to the soil surface. The probes were calibrated by equilibration with solutions of NaCl of known molality. Plant ψ was determined with detached segments of subepidermal tissue, using a thermocouple hygrometer sample chamber (model C-51, Wescor, Inc.). The detached tissue segments were equilibrated for 1 hr in an insulated chamber prior to the measurement of ψ.

**Carbon Isotope Analysis.** Stom and leaf material was separated into duplicate samples, one used for the total sample fraction and one used for the sample extract fraction. The tissue for the total sample fraction was oven-dried at 100 C for 24 hr, and ground to a fine powder with a mortar and pestle. The tissue for the sample extract fraction was boiled for 2 hr in 400 ml of H2O, and the supernatant suction filtered. The filtered liquid was evaporated to dryness in a mutating evaporimeter (model 1200 A, Zymel Corp.), and ground to a fine powder with a mortar and pestle.

The δ13C analysis was measured following the technique of Craig (6). The plant material was placed in a platinum boat and combusted in an atmosphere of O2 at 900 C over copper oxide. The CO2 was collected in a liquid O2 trap and freed from contaminants by alcohol/dry ice traps. The samples were analyzed with a Nucleotide Corporation R.M.S. 6 to 60° sector double collector, double inlet ratio mass spectrometer. The 13C to 12C standard used was the Wellington (Rafter) Te Kuiti limestone standard. The results are corrected for the PDB standard and expressed as δ13C, in units of ‰. Craig (7) compares the isotopic standards and his corrections were utilized throughout the study.

**RESULTS**

**Determination of CAM.** The total titratable acidity of stem and leaf material, which follows a circadian fluctuation, was used to establish CAM in *O. phaeacantha* and *Y. baccata*. Plants of *O. phaeacantha* at the four study sites demonstrate a persistent fluctuation in titratable stem tissue acidity, with an average day to night amplitude of 43 μeq/g fresh weight. The variation in acidity between individual plants at any one site was as large as the variation between sites. Plants of *Y. baccata* at the four study sites also demonstrate a persistent fluctuation in titratable leaf tissue acidity, with an average day to night amplitude of 35 μeq/g fresh weight. The variation in acidity between individual plants at any one site was as large as the variation between sites.

**Environmental Conditions.** The four study sites can be distinguished by significant differences in environmental conditions (Table 1). A climatic gradient occurs along the elevational gradient, with a continuous transition in temperature regime and soil water status from site 1 to site 4. The lowest elevation site is characterized by hot summers and mild winters, with only 12 days/year with nighttime minimum temperatures below 0 C. The annual mean precipitation is low, and contributes to year round arid conditions. Soil ψ is low throughout most of the year, and minima ψ values were often below the detection level of the soil hygrometer probes. The highest elevation site is characterized by mild summers and severe winters, with 176 days/year with nighttime minimum temperatures below 0 C. The annual mean precipitation is 3-fold greater than precipitation at site 1, and coupled with the lower temperature regime, the soil ψ was high throughout the year. The annual mean soil ψ was nearly an order of magnitude higher than the soil ψ at site 1, and minimum values were not below -16 bars.

**Plant Water Potentials.** The plant ψ of *O. phaeacantha* at the four study sites parallels the gradient of soil ψ, although the difference between sites is not as marked (Table II). The plant ψ at the lowest elevation site reaches minima values of -19 bars during three sampling periods, and the annual mean plant ψ is the lowest of all the sites. Minima plant ψ at sites 2 and 3 are -19 and -20 bars, respectively, with similar annual mean values at both sites. The highest plant ψ occurs at the highest elevation site, with minima values of -15 bars. The annual mean plant ψ for the four sites are not significantly different despite significant site variations in soil ψ. The plant ψ of *Y. baccata* was similar to the values of *O. phaeacantha*, and the annual mean values from site 1 to site 4 were -17, -15, -14, and -14 bars, respectively (data not shown).

**Photosynthesis.** The diurnal pattern of gas exchange was similar for all plants at the different study sites throughout the course of the year. The typical patterns of stomatal conductance and photosynthesis are presented in Figure 1. The data represent the maximum observed daytime period of gas exchange for *O. phaeacantha*, since during most sampling periods the only period of gas exchange occurred during the initial hours of daylight. The first period of photosynthetic assimilation of 14CO2 occurs following dawn when maximum stomatal conductances were recorded. The rate of photosynthesis decreases rapidly in the 1st hr, and falls to zero before stomata close. Throughout most of the day photosynthesis is restricted due to stomatal closure.

**Table I. Environmental Data of Study Sites**

<table>
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<th>Site Number</th>
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<th>2</th>
<th>3</th>
<th>4</th>
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<tr>
<td>Elevation (m)</td>
<td>525</td>
<td>970</td>
<td>1450</td>
<td>1900</td>
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<tr>
<td>Annual Mean Temperature (C)</td>
<td>21</td>
<td>18</td>
<td>13</td>
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<td>Monthly Mean Minimum Temperature (C)</td>
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<td>7</td>
<td>3</td>
<td>2</td>
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<tr>
<td>Monthly Mean Maximum Temperature (C)</td>
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<td>31</td>
<td>24</td>
<td>21</td>
</tr>
<tr>
<td>Annual Mean Precipitation (cm)</td>
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<td>46</td>
<td>53</td>
<td>70</td>
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<tr>
<td>Annual Mean Soil Water Potential (bars)</td>
<td>-48</td>
<td>-36</td>
<td>-25</td>
<td>-5</td>
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<tr>
<td>Minimum Soil Water Potential Observed (bars)</td>
<td>&lt;-90</td>
<td>-88</td>
<td>-77</td>
<td>-16</td>
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**Table II. Plant Water Potentials of Opuntia phaeacantha at Study Sites.**

<table>
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<th>Site Number</th>
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<tr>
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second period of photosynthetic assimilation of $^{14}$CO$_2$ occurs prior to sunset, and initiates with stomatal opening. Unlike the earlier gas exchange period, this second period appears to be limited solely by stomatal conductance. At all sites the maximum photosynthetic rate was measured in the postdawn period of gas exchange, with an absolute maximum value of 8 mg CO$_2$/dm$^2$·hr.

**Carbon Isotope Ratios.** The pattern of the seasonal variation in carbon isotope ratios for *O. phaeacantha* is presented in Figure 2. These results are similar to the seasonal patterns of the carbon isotope ratios for *Y. baccata* at each elevation (data not shown). Leaf and stem material was collected monthly from one plant at each site, and all monthly samples were taken from the same plant. Since tissue age influences the carbon isotope ratio (8) only young material was collected in order to standardize age effects.

The carbon isotope ratios for *O. phaeacantha* do not shift significantly throughout the course of the year (Fig. 2). At the lowest elevation site the $\delta^{13}$C values become less negative at the end of the year, and may represent a slight response to the arid fall season conditions of the site. The seasonal patterns from the other sites are irregular, which are probably due to the differences in $\delta^{13}$C values between different plant parts and not an irregular physiological response. The annual mean $\delta^{13}$C values of the sample extract fractions are slightly less negative than the values of the total sample fraction, although the relationship is variable through the year. The largest difference between the two fractions occurs at the lowest elevation site, where the annual mean $\delta^{13}$C value of the sample extract is 0.8% less negative. The annual mean carbon isotope ratios for the total sample fraction become slightly less negative as site elevation increases, i.e., $-13.5 \pm 1.08$%oo, $-12.7 \pm 0.79$%oo, $-12.4 \pm 0.79$%oo, and $-12.5 \pm 0.67$%oo, respectively. At all sites the plants have C$_4$-like $\delta^{13}$C values averaging $-12.5 \pm 0.86$%oo for both sample fractions throughout the year.

The carbon isotope ratios for *Y. baccata* also do not shift significantly throughout the course of the year. Moreover, the annual mean $\delta^{13}$C values for the sample extract fraction are also less negative than the values of the total sample fraction at each elevation. The annual mean $\delta^{13}$C values for the total sample fraction become slightly more negative as site elevation increases, i.e., $-14.5 \pm 1.14$%oo, $-15.3 \pm 0.71$%oo, $-15.6 \pm 0.82$%oo, and $-16.1 \pm 1.01$%oo, respectively. At all sites the plants have C$_4$-like $\delta^{13}$C values averaging $-15.7 \pm 0.95$%oo for both sample fractions throughout the year.

**DISCUSSION**

These seasonal measurements of photosynthesis and carbon isotope fractionation are from CAM plants growing under significantly different environmental conditions and permit an assessment of the environmental influence of CAM. The physiological responses were similar in both plant species, despite one being a stem succulent CAM plant and the other being a leaf succulent CAM plant. Such results appear to be related to the similarity in plant $\psi$ between the four study sites.

Previous seasonal studies with a CAM plant growing *in situ* have documented the regulation of metabolic activity due to plant $\psi$ (17). Despite significant differences between sites in temperature regime and soil $\psi$, plants of *O. phaeacantha* and *Y. baccata* maintain similar plant $\psi$ values. The annual mean values were moderate in both species and neither experiences severe plant water stress at any of the sites. The maintenance of similar plant $\psi$ may be due to different levels of tissue matric and osmotic potentials at the study sites. Nevertheless, the similarity in plant $\psi$ effects a similarity in photosynthetic gas exchange at each site.

The daytime gas exchange pattern of *O. phaeacantha* is typical of CAM plants growing *in situ* (2, 18), and corresponds to the full CAM pattern of gas exchange recently described by Neales (13). Water stress affects daytime gas exchange by reducing stomatal conductance throughout most of the day. The potential period for light CO$_2$ assimilation is only a few hours of the day under the most optimal environmental conditions. The domi-
nance of the nocturnal CO₂ assimilation of CAM is evident from the σ₁³C values of O. phaeacantha and Y. baccata. Both of these plants have σ₁³C values of the two tissue fractions near the upper limit for C₄-like metabolism (3). Since the soluble extract fraction has been suggested to represent the recent metabolism of the plant (9), it appears these plants solely utilize CAM as the primary carboxylation reaction. Finally, the similarities of the carbon isotope ratios between study sites indicates a common carboxylation reaction, since the isotopic composition of the atmosphere is nearly constant. Along the elevational gradient of this study the atmospheric σ₁³C values have been estimated to range from −7 %₀₀ at site 1 to −9 %₀₀ at site 4 (J. C. Lerman, personal communication).

The results of this study indicate a year round constancy of photosynthetic carbon pathway in CAM plants occurring along a climatic gradient. Our results are in agreement with those of Mooney et al. (11), whose data indicates no consistent shift in carbon isotope ratio of CAM plants with changes in aridity. Additionally, the magnitude of the variation in σ₁³C values between sites is within the range of values reported from other CAM plants growing in situ. Osmond et al. (16) have reported a variation near 3 %₀₀ for total tissue samples of three Semprevi- vum species occurring from habitats with different water supply. Within single species of CAM plants the variation in total tissue σ₁³C values from plant to plant may range from 3 to 6 %₀₀ (11, 14), which is probably effected by differences in age of tissue (8).

The hypothesis that CAM plants may facultatively utilize different photosynthetic carbon pathways arises from the assumption that the succulent growth form occurs concomitantly with CAM. It appears that succulent plants capable of CAM have only C₄-like σ₁³C values, while others which are not CAM plants have only C₃-like σ₁³C values (10, 13). With the exception of the CAM plant Guzmania monostachia (10) all succulent species which show a predominance of nocturnal CO₂ assimilation have σ₁³C values within the narrow range of C₄-like metabolism. Nevertheless, an induction of different photosynthetic pathways within a single species of CAM plant may occur in habitats with greater seasonal fluctuations in plant ψ. A CAM plant growing in a coastal environment has already been shown to rely mainly upon daytime gas exchange during periods of optimum plant moisture (2).

In laboratory studies with CAM plants maintained with an adequate water supply, light versus dark CO₂ assimilation may be induced by growth conditions. The photosynthetic metabolism can be shifted from CAM to C₄ metabolism (1, 3), with a mean shift in the total tissue σ₁³C value ranging from 3 to 4 %₀₀ (our calculation from original data). Additionally, the photosynthetic metabolism can be shifted from C₄ to CAM metabolism (9, 15) with a mean shift in the total tissue σ₁³C value ranging from 6 to 8 %₀₀ (our calculation from original data). The effect of droughting increases the magnitude of the shift in the total tissue σ₁³C value, and overrides the effects of thermoperiodic (1) and photoperiodic growth conditions (12). By depriving the plants of daily watering a reliance on nocturnal CO₂ assimilation was induced, although the levels of plant and soil ψ were not reported in these studies. These latter results appear to be in agreement with those of our study.

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LITERATURE CITED