Variable Photosynthetic Metabolism in Leaves and Stems of *Cissus quadrangularis* L.  

IRWIN P. TING, LEONEL O. STERNBERG, AND MICHAEL J. DE NIRO  
Department of Botany and Plant Sciences, University of California, Riverside, California 92521 (I.P.T.);  
and Department of Earth and Space Sciences (L. O. S., M. J. D.) and Program in Archaeology (M. J. D.),  
University of California, Los Angeles, California 90024

**ABSTRACT**

By measuring titratable acidity, gas exchange parameters, mesophyll succulence, and 13C/12C ratios, we have shown that *Cissus quadrangularis* L. has C3-like leaves and stems with Crassulacean acid metabolism (CAM). In addition, the nonsucculent leaves show the diurnal fluctuations in organic acids termed recycling despite the fact that all CO₂ uptake and stomatal opening occurs during the day. Young succulent stems have more C₃ photosynthesis than older stems, but both have characteristics of CAM. The genus *Cissus* will be a fruitful group to study the physiology, ecology, and evolution of C₃ and CAM since species occur that exhibit characteristics of both photosynthetic pathways.

Mesophyll succulence was calculated from the ratio of tissue water to total Chl (2) in fresh leaf and stem samples. Total water content was determined by comparing the weights of fresh samples and samples dried in a microwave oven for 10 min. Titratable acidity was determined by grinding tissue with a hand tissue grinder and titrating to pH 7.0 with 0.01 N KOH solution using an automatic titrator. Diurnal CO₂ uptake and stomatal conductance were measured under greenhouse conditions with a dual isotope porometer (3).

Whole tissue samples were prepared for carbon isotope ratio determination by grinding in liquid N₂ and freeze drying. Starch was extracted from the ground samples by the method of Pucher *et al.* (9). Powdered tissue and starch samples were combusted by a modified version (8) of the Stump and Frazer method (10). CO₂ was purified from the combustion products by cryogenic distillation and its 13C/12C ratio determined by mass spectrometry. Carbon isotope ratios are expressed as δ¹³C values, where

\[ δ^{13}C = \left( \frac{(13C/12C)_{sample}}{(13C/12C)_{standard}} - 1 \right) \times 1000% \]

The standard is the PeeDee belemnite carbonate. Precision of the δ¹³C measurements was ±0.2‰.

**RESULTS**

Organic acid fluctuations (Fig. 1a) of about 100 μeq titratable acidity per g fresh weight in the stem of *C. quadrangularis* were quite marked and typical of CAM plants (4). The leaves also showed some acid fluctuation, but the magnitude was only about 30 μeq/g fresh weight. This amount of organic acid fluctuation has been observed in succulent plants not showing CAM and can be explained by recycling of respired CO₂ during the night period (13).

Leaves of *C. quadrangularis* did not fix CO₂ (Fig. 1b) and had negligible stomatal conductance (Fig. 1c) during the night. Stems, however, had considerable CO₂ uptake and stomatal conductance during the day and night period.

Previous work has shown that the leaves of *C₃* species have mesophyll succulence (g H₂O/mg Chl) less than 1.0 whereas the green photosynthetic tissues of CAM plants have mesophyll succulence between 1.0 and 10.0 (4). Based on analysis of mesophyll succulence, the leaves of *C. quadrangularis* are typical of *C₃* plants and the stems are typical of CAM species (Table I).

Carbon isotopic ratios of different tissues of *C. quadrangularis* are shown in Table II. Isotope ratios of whole leaves are typical of *C₃* plants, while those of stems are typical of CAM plants (4, 7).

Younger stems had lower mesophyll succulence (Table I) and δ¹³C values for total organic carbon than older stems (Table II). This suggests that younger stems have a higher proportion of *C₃* metabolism to CAM than older stems. Isotopic ratios of total

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1 Supported in part by National Science Foundation grants PCM 82-00366 and ATM 79-24591.
organic matter record the cumulative photosynthetic history of a tissue, which for the stem may have included considerable amounts of C3 metabolism. Under the assumption that starch is newly synthesized 

\textit{in situ} during the day, we decided to investigate the current photosynthetic status of each tissue by measuring the isotopic ratios of starch extracted at the end of the light period. The $\delta^{13}C$ value of starch in older stems is about 2% more positive than the $\delta^{13}C$ value of starch from the younger stems. If our assumption that starch is synthesized \textit{in situ} is correct, this observation indicates that there is a shift towards more CAM as stems of \textit{C. quadrangularis} mature.

**DISCUSSION**

\textit{C. quadrangularis} illustrates another variation in the photosynthetic metabolism of facultative CAM plants. The leaves have C3 photosynthesis with recycling of respired CO$_2$ during the night. The stems show progressively more CAM as they mature, as evidenced by the increase in mesophyll succulence and the increase in tissue and starch $^{13}C/^{12}C$ ratios.

The genus \textit{Cissus} has about 300 species native to southern and tropical Africa. We suspect, based on the observations presented here and on preliminary unpublished experiments with other species of \textit{Cissus}, that the genus will have all combinations of C3 and CAM metabolism.

Our results are similar to those of Lange and Zuber (5) who found C3 leaf activity and CAM stem activity in \textit{Freesia}, the only difference being that in \textit{C. quadrangularis} the leaves show recycling.

Just how the observation of facultative CAM (11) fits into the observations presented here with \textit{Cissus} is not clear. We have not investigated the possibility that water stress may shift the C$_3$-like leaves of \textit{C. quadrangularis} to CAM in a manner similar to \textit{Portulacaria} and \textit{Mesembryanthemum} (12, 14).

**Acknowledgements**—We thank Dr. Winter for assistance in making the isotopic measurements.

**LITERATURE CITED**


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**Table II. Carbon Isotopic Composition of \textit{C. quadrangularis} Tissues and Extracted Starch**

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Total Carbon $\delta^{13}C$</th>
<th>Starch $\delta^{13}C$</th>
</tr>
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<tbody>
<tr>
<td>Leaf</td>
<td>-25.3</td>
<td>-25.9</td>
</tr>
<tr>
<td>Young stem</td>
<td>-19.4</td>
<td>-17.6</td>
</tr>
<tr>
<td>Old stem</td>
<td>-17.8</td>
<td>-15.4</td>
</tr>
</tbody>
</table>

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**Table I. Mesophyll Succulence of Different Tissues of \textit{C. quadrangularis}**

| Tissue          | Mesophyll Succulence $g$ water/mg Ch
<table>
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<tbody>
<tr>
<td>Leaf</td>
<td>0.72 ± 0.12</td>
</tr>
<tr>
<td>Young stem*</td>
<td>3.54 ± 0.13</td>
</tr>
<tr>
<td>Old stem*</td>
<td>6.86 ± 1.63</td>
</tr>
</tbody>
</table>

*Young stems were just developing and about 2 weeks old. Old stems were mature, without leaves.

*Data are means ± SD of three replicates.
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and nitrogen in organic compounds. Nucl Sci Abstr 28: 746