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Variable Photosynthetic Metabolism in Leaves and Stems of *Cissus quadrangularis* L.[1]

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ABSTRACT

By measuring titratable acidity, gas exchange parameters, mesophyll succulence, and $^{13}C/^{12}C$ ratios, we have shown that *Cissus quadrangularis* L. has C_3-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 CO_2 uptake and stomatal opening occurs during the day. Young succulent stems have more C_3 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 C_3 and CAM since species occur that exhibit characteristics of both photosynthetic pathways.

CAM is the photosynthetic mechanism in which CO_2 uptake and stomatal opening occur at night. The CO_2 is fixed initially into malic acid which is decarboxylated during the following day. The CO_2 is then fixed into carbohydrate via the C_3 cycle (4). It has been shown that CAM is an adaptation to arid environments, since the ratio of water transpired relative to CO_2 absorbed is very small (4). Several combinations of C_3 and CAM photosynthesis have been reported. Old leaves of *Kalanchoë* show more CAM and less C_3 photosynthesis than do young leaves (4). CAM in *Kalanchoë blossfeldiana* increases relative to C_3 metabolism when grown on SD (6). *Membryanthemum crystallinum* (14) and *Portulacaria afra* (12) are examples of plants which can shift from C_3 to CAM when water stressed. *M. crystallinum* has a high level of acidity and shows acid flux only when water stressed (14). *P. afra*, however, sustains a high level of acidity even when well watered, although acid flux occurs only when it is water stressed (12). *Frerea indica* is the only reported case in which different photosynthetic pathways are segregated in different anatomical structures of the plant (5). Deciduous leaves of *F. indica* show C_3 metabolism, while the succulent stems have CAM (5).

We report here yet another variation between C_3 and CAM in *Cissus quadrangularis* (Vitaceae). The succulent stem shows increased CAM photosynthesis with age while the leaves have the C_3 mode with some recycling of respired CO_2.

MATERIALS AND METHODS

Plant cuttings of *Cissus quadrangularis* L. were grown in a sandy loam soil in a greenhouse. Plants were watered as needed to avoid stress.

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 CO_2 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 N_2 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). CO_2 was purified from the combustion products by cryogenic distillation and its $^{13}C/^{12}C$ ratio determined by mass spectrometry. Carbon isotope ratios are expressed as $\delta^{13}C$ values, where

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

The standard is the Peedee belemnite carbonate. Precision of the $\delta^{13}C$ 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 CO_2 during the night period (13).

Leaves of *C. quadrangularis* did not fix CO_2 (Fig. 1b) and had negligible stomatal conductance (Fig. 1c) during the night. Stems, however, had considerable CO_2 uptake and stomatal conductance during the day and night period.

Previous work has shown that the leaves of C_3 species have mesophyll succulence (g H_2O/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 C_3 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 C_3 plants, while those of stems are typical of CAM plants (1, 7).

Younger stems had lower mesophyll succulence (Table I) and $\delta^{13}C$ values for total organic carbon than older stems (Table II). This suggests that younger stems have a higher proportion of C_3 metabolism to CAM than older stems. Isotopic ratios of total

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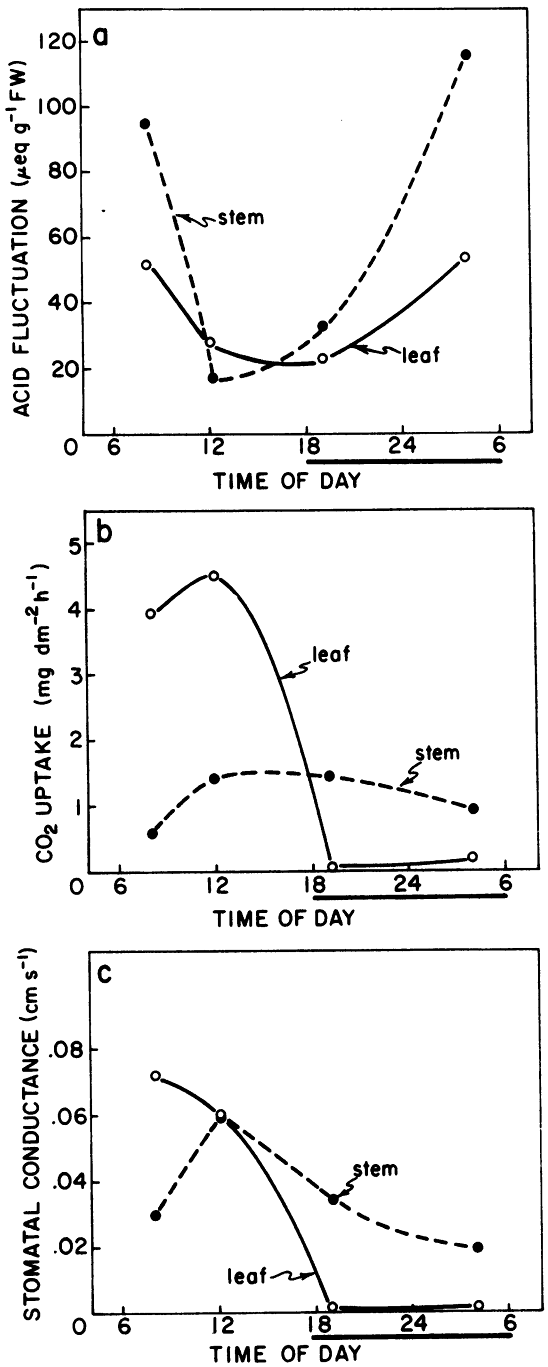

FIG. 1. Diurnal variation in: a, titratable acidity; b, CO_2 uptake; and c, stomatal conductance for young stems and leaves of *C. quadrangularis*. The solid bar indicates the dark period. Measurements were taken in a greenhouse. Each point indicates the mean of three measurements.

Table I. *Mesophyll Succulence of Different Tissues of C. quadrangularis*

Tissue	Mesophyll Succulence
	g water/mg Chl[b]
Leaf	0.72 ± 0.12
Young stem[a]	3.54 ± 0.13
Old stem[a]	6.86 ± 1.63

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

[b] Data are means ± SD of three replicates.

Table II. *Carbon Isotopic Composition of C. quadrangularis Tissues and Extracted Starch*

Tissue	Total Carbon $\delta^{13}C$	Starch $\delta^{13}C$
	‰	
Leaf	−25.3	−25.9
Young stem	−19.4	−17.6
Old stem	−17.8	−15.4

organic matter record the cumulative photosynthetic history of a tissue, which for the stem may have included considerable amounts of C_3 metabolism. Under the assumption that starch is newly synthesized *in situ* during the day, we decided to investigate the current photosynthetic status of each tissue by measuring the isotope 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 *in situ* is correct, this observation indicates that there is a shift towards more CAM as stems of *C. quadrangularis* mature.

DISCUSSION

C. quadrangularis illustrates another variation in the photosynthetic metabolism of facultative CAM plants. The leaves have C_3 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 *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 *Cissus*, that the genus will have all combinations of C_3 and CAM metabolism.

Our results are similar to those of Lange and Zuber (5) who found C_3 leaf activity and CAM stem activity in *Frerea*, the only difference being that in *C. quadrangularis* the leaves show recycling.

Just how the observation of facultative CAM (11) fits into the observations presented here with *Cissus* is not clear. We have not investigated the possibility that water stress may shift the C_3-like leaves of *C. quadrangularis* to CAM in a manner similar to *Portulacaria* and *Mesembryanthemum* (12, 14).

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