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THE OCCURRENCE OF CRASSULACEAN ACID METABOLISM AMONG EPIPHYTES IN A HIGH-RAINFALL REGION OF COSTA RICA

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ABSTRACT. Epiphyte species were examined in Golfito, a high-rainfall region of southwestern Costa Rica. Six out of the 15 species examined exhibited significant diurnal increases in tissue acidity. This study constitutes the first report of Crassulacean acid metabolism (CAM) in the orchid genus *Caularthron* and in the species *Oncidium ascendens, Epidendrum isomerum,* and *Epidendrum nocturnum.* The finding of CAM in the epiphyte *Vittaria lineata* constitutes the first report of a CAM fern outside the family Polypodiaceae. The ecological significance of CAM in a region of high-rainfall is discussed in light of drought tolerance and the availability of CO_2.

INTRODUCTION

The photosynthetic pathway Crassulacean acid metabolism (CAM) is most common in plants that grow in arid environments (Osmond 1978, Kluge & Ting 1978, Winter 1985). This type of metabolism results in a higher water-use efficiency in comparison with C_3 and C_4 plants. Plants with CAM open their stomata and absorb CO_2 at night when the air is cooler and more humid, relative to daytime conditions. Such plants characteristically exhibit large diurnal fluctuations in tissue acidity, the detection of which provides a simple diagnostic tool to identify CAM plants.

Since their initial discovery in terrestrial plants growing in arid environments, e.g., deserts, many CAM species have been reported in non-arid environments such as tropical rainforests. Many, if not most, of the CAM species in tropical rainforests are epiphytes, which experience occasional drought stress between precipitation events (Osmond *et al.* 1982, Winter 1985, Martin *et al.* 1990, Martin 1994).

Because CAM is an adaptation that minimizes drought stress, it seems unlikely that CAM would be prevalent in tropical rainforests with exceptionally heavy rainfall. Therefore, the purpose of this study was to investigate the potential for CAM in selected epiphytes, as well as two vines and a terrestrial herb, in Golfito, Costa Rica, a tropical region that receives up to five meters of rain each year.

MATERIALS AND METHODS

All epiphytes and the vine *Codonanthe* sp. were growing in canopies of several species of trees within the town of Golfito, Costa Rica (elev. ~ 10 m). Most, if not all, of these epiphytes were also found in the nearby rainforest as well. *Tripogandra multiflora* and *Peperomia rotundifolia* were growing at the base of several tree species. The species examined, their families, and their life-forms are given in Table 1. The average annual rainfall in this region ranges from four to five meters with the heaviest amounts of rainfall occurring from September to November (Janzen 1983). The annual daily temperatures range from 22.4 to 32.3 degrees with a mean of 27.4 degrees (Coen 1983).

A leaf section (0.13 g) was excised in situ within one hour of sunset, and another was taken within one hour of sunrise. Plants were sampled from 27 September to 27 October 1993 and only on days with a minimum of eight hours of sunlight and minimal or no nightly rainfall. Plant tissue was placed in plastic bags and frozen within 5–10 minutes of collection. Titratable acidity was measured within one hour after collection.

Frozen tissue was thawed, weighed, and ground in distilled water with a mortar and pestle. The resultant slurry was titrated to pH 7.0 with 0.01 N NaOH. The pH meter was calibrated between every measurement with a standard buffer.

Morning and evening means were tested for significant differences using the Mann-Whitney *U*-test (Sokal and Rohlf 1981). Differences between means were considered significant when $P \leq 0.05$.

RESULTS AND DISCUSSION

The species included in this study were selected according to some or all of the following criteria: 1) accessibility, 2) succulence, and 3) previous reports of CAM in the family. Six of the 15 species examined exhibited significant diurnal increases in tissue acidity (Table 1). No significant changes in titratable acidity were found in leaves of the terrestrial herb *Tripogandra mul-*

TABLE 1. Species, family, life-form, and mean (standard deviation in parentheses) evening and morning titratable acidities of leaves of selected species in Golfito, Costa Rica. Measurements were taken from 27 September to 27 October 1993. Data are expressed on a fresh weight basis. Probabilities indicate results of Mann-Whitney *U*-test comparing evening and morning means. NS = not significant ($P > 0.05$). $N = 5$.

Species	Family	Life-form	Titratable acidity, mmol kg^{-1}		Probability
			Evening	Morning	
Vittaria lineata (L.) J. E. Smith	Vittariaceae	epiphytic fern	0.0	24.6 (15.9)	$P < 0.05$
Microgramma percussa (Cavanilles) de la Sota	Polypodiaceae	epiphytic fern	4.3 (9.7)	0.0	NS
Catopsis sessiliflora (Ruiz et Par.) Mez.	Bromeliaceae	epiphyte	0.0	0.0	NS
Tillandsia balbisiana Schult.	Bromeliaceae	epiphyte	0.0	247.8 (94.5)	$P < 0.02$
Tripogandra multiflora (Sw.) Raf.	Commelinaceae	terrestrial herb	0.0	0.0	NS
Codonanthe species	Gesneriaceae	vine	0.8 (1.7)	0.0	NS
Caularthron bilamellatum (Rchb. f.) R. E. Schultes	Orchidaceae	epiphyte	26.1 (44.0)	107.2 (30.9)	$P < 0.05$
Dimerandra emarginata (C. F. W. Meyer) Hoehne[1]	Orchidaceae	epiphyte	0.0	2.8 (3.9)	NS
Epidendrum isomerum Schltr.[1]	Orchidaceae	epiphyte	0.0	86.9 (14.5)	$P < 0.02$
Epidendrum nocturnum Jacq.[1]	Orchidaceae	epiphyte	43.5 (21.1)	105.8 (21.5)	$P < 0.02$
Maxillaria uncata Lindl.[1]	Orchidaceae	epiphyte	0.0	4.3 (6.5)	NS
Nidema boothii Schltr.	Orchidaceae	epiphyte	1.4 (3.2)	5.8 (9.4)	NS
Oncidium ascendens Lindl.[1]	Orchidaceae	epiphyte	0.0	272.4 (79.8)	$P < 0.02$
Scaphyglottis prolifera (Lindl.) Cogn.[1]	Orchidaceae	epiphyte	0.0	1.4 (3.2)	NS
Peperomia rotundifolia (L.) Kunth	Piperaceae	vine	0.0	5.8 (7.9)	NS

[1] Species identification based on sterile specimens, thus, should be considered tentative.

tiflora. The latter result corroborates previous findings with well-watered *Tripogandra multiflora* (Martin *et al.* in press). Drought-stressed individuals of this species exhibit low levels of CAM. In this study *Tillandsia balbisiana* displayed significant diurnal changes in tissue acidity levels, in agreement with previous reports of CAM in this epiphytic bromeliad (Martin 1994).

The results of this study report CAM for the first time in several genera and species. Previously, CAM has been reported in the orchid genera *Oncidium* and *Epidendrum* but not in the species *O. ascendens, E. isomerum,* and *E. nocturnum* (Neales *et al.* 1975, Arditti 1979, Avadhani *et al.* 1982). Furthermore, CAM has not been reported in the genus *Caularthron* (Neales *et al.* 1975, Arditti 1979, Avadhani *et al.* 1982). Thus, the finding of significant diurnal acidity fluctuations in *C. bilamellatum* in this study constitutes the first report of CAM in this orchid genus. This study reports C_3 photosynthesis for the first time in the orchid genus *Nidema* and also confirms past reports of C_3 photosynthesis in the genera *Maxillaria* and *Scaphyglottis* (Neales *et al.* 1975, Arditti 1979, Avadhani *et al.* 1982).

No diurnal acid fluctuations were observed in fronds of the epiphytic fern *Microgramma percussa*. In contrast, *Vittaria lineata* exhibited a small but significant nocturnal increase in tissue acidity (Table 1). Previously, CAM in ferns has been found only in species of *Pyrrosia* (Kluge *et al.* 1989). Thus, this constitutes the first report of CAM in a fern outside the family Polypodiaceae.

Crassulacean acid metabolism is considered an adaptation of plants to arid environments where minimization of water loss plays an important role in the survival of the plants (Kluge & Ting 1978). The area under investigation in this study, however, is a humid coastal tropical rainforest that receives large amounts of rainfall, especially at the time of this study. It is possible that some CAM epiphytes might experience stress even after short rainless periods. In fact, CAM ferns in southeast Asia showed evidence of drought stress after the absence of rain for one day (Ong *et al.* 1986). CAM might benefit these ferns by conserving water as in drier habitats. It is also conceivable that CAM may provide an additional advantage unrelated to water conservation. Crassulacean acid metabolism might allow the epiphytes to capitalize on elevated CO_2 levels at night, a consequence of forest respiration, and to avoid competition for CO_2 during

the day when the tree is photosynthetically active (Knauft & Arditti 1969, Benzing 1990). More research is needed to further investigate this possibility in epiphytic CAM plants growing in humid tropical environments.

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