

CRASSULACEAN ACID METABOLISM IN THE ZZ PLANT, *ZAMIOCVLCAS ZAMIIFOLIA* (ARACEAE)¹

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Zamioculcas zamiifolia (Araceae), a terrestrial East African aroid, with two defining attributes of crassulacean acid metabolism (CAM) (net CO₂ uptake in the dark and diel fluctuations of titratable acidity) is the only CAM plant described within the Araceae, a mainly tropical taxon that contains the second largest number of epiphytes of any vascular plant family. Within the Alismatales, the order to which the Araceae belong, *Z. zamiifolia* is the only documented nonaquatic CAM species. *Zamioculcas zamiifolia* has weak CAM that is upregulated in response to water stress. In well-watered plants, day–night fluctuations in titratable acidity were 2.5 μmol H⁺·(g fresh mass)⁻¹, and net CO₂ uptake in the dark contributed less than 1% to daily carbon gain. Following 10 d of water stress, net CO₂ uptake in the light fell 94% and net CO₂ uptake in the dark increased 7.5-fold, such that its contribution increased to 19% of daily carbon gain. Following rewatering, dark CO₂ uptake returned to within 5% of prestressed levels. We postulate that CAM assists survival of *Z. zamiifolia* by reducing water loss and maintaining carbon gain during seasonal droughts characteristic of its natural habitat.

Key words: Araceae; CO₂ exchange; crassulacean acid metabolism; drought stress; photosynthesis; *Zamioculcas*.

Crassulacean acid metabolism (CAM) is the second most common pathway of photosynthesis in vascular plants (Winter and Smith, 1996). CAM has evolved often, with species distributed in 29 families and 338 genera of flowering plants (Smith and Winter, 1996; Silvera et al., 2005; Liu and Wang, 2006), two families of gymnosperms (Vovides et al., 2002; von Willert et al., 2005), two families of leptosporangiate ferns (Hew and Wong, 1974; Carter and Martin, 1994; Holtum and Winter, 1999), and one family of lycophytes (Keeley, 1981). Initially considered primarily a water-conserving adaptation of terrestrial succulent plants to hot, semi-arid environments, CAM assists species in a diverse range of habitats (Skillman et al., 2005). The majority of CAM plants are probably epiphytes in tropical and subtropical forests (Crayn et al., 2004; Holtum and Winter, 2005; Silvera et al., 2005), but CAM has also been reported in tropical trees (Gehrig et al., 2003; Holtum et al., 2004; Lüttge, 2006), halophytes (Winter and Holtum, 2005, 2007), alpine succulents (Osmond et al., 1975), aquatic plants of oligotrophic lakes or seasonal pools (Keeley, 1981, 1996), and in plants without stomata that obtain CO₂ via their roots (Keeley et al., 1984).

The CAM pathway, which enables plants to successfully live in such a range of environments, involves the ability to fix CO₂ during the dark, storing the carbon as malic acid in vacuoles. In the light, the malic acid is decarboxylated, and the CO₂ evolved is refixed by Rubisco and used for growth and maintenance (Holtum et al., 2005). CAM species with functional stomata are highly water-use efficient because decarboxylation and CO₂ refixation are accompanied by reduced stomatal aperture and, consequently, lower rates of transpiration (Winter et al., 2005). In aquatic plants, CAM is an adaptation to CO₂-limited

environments in which the levels of dissolved CO₂ are either permanently low or low during the light (Keeley, 1996).

Unlike C₃ or C₄ photosynthesis, the phenotypic expression of CAM is not an all-or-nothing phenomenon. Some CAM species obtain virtually all of their CO₂ during the dark, most species obtain CO₂ during the dark and the light, and in some species dark CO₂ fixation is restricted to the refixation of respiratory CO₂. Such variety of expression increases the utilitarian nature of CAM (Osmond, 2007). The ecological adaptability of CAM plants is often enhanced by an ability to modify, in response to environmental conditions, the relative amounts of CO₂ assimilated in the light via the less water-use efficient C₃ photosynthesis vs. CO₂ assimilated in the dark by the more water-use efficient CAM process. The ability to express CAM may be constitutive or facultative, developmentally programmed, or induced in response to stress.

The majority (>90%) of epiphytes with CAM are in the monocotyledonous families Bromeliaceae (Poales) and Orchidaceae (Asparagales). In 1989, Kress calculated that of an estimated 23 466 species of vascular plants that are epiphytes (16 610 monocots and magnoliids, 4 253 eudicots, four gymnosperms, and 2 599 ferns or fern allies; roughly 10% of vascular plants in toto), 74% of the species are in five families: the Orchidaceae (13 951 species), the Araceae (1 349 species), the Bromeliaceae (1 145 species), the Polypodiaceae (1 029 species), and the Piperaceae (710 species). Though CAM is well represented in the Orchidaceae and the Bromeliaceae and moderately represented in the Piperaceae, few species have been described in the Polypodiaceae, and CAM has never been reported in the Araceae (Smith and Winter, 1996), the family with the second highest number of epiphytic species. The absence of documented CAM species from the Araceae is surprising because it is large (at least 4 025 species and 106 genera; Croat [2004]; Stevens [2006]), mainly tropical, and epiphyte-rich. Moreover, some epiphytes, particularly in the genus *Anthurium*, sport succulent leaves so characteristic of plants with CAM.

Here we report the presence of CAM in a terrestrial aroid, *Zamioculcas zamiifolia* Schott (Araceae). Apart from having CAM, *Z. zamiifolia* is an atypical aroid in several taxonomic

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TABLE 1. Day–night variation in titratable acidity, expressed as $\mu\text{mol H}^+(\text{g fresh mass})^{-1}$, of leaflets from well-watered *Zamioculcas zamiifolia* in comparison to two C_3 aroids, *Epipremnum aureum* and *Anthurium michelii*, and known CAM species. Values are the means of three replicates ($\pm\text{SE}$) with the exception of the values for *Platynerium veitchii* for which $N = 5$. The significance of dawn–dusk differences in titratable acidity was tested using unpaired one-tailed t tests.

Photosynthetic pathway and plant species	Dusk $\mu\text{mol H}^+(\text{g fresh mass})^{-1}$ ($\pm\text{SE}$)	Dawn $\mu\text{mol H}^+(\text{g fresh mass})^{-1}$ ($\pm\text{SE}$)	Dawn–dusk $\mu\text{mol H}^+(\text{g fresh mass})^{-1}$	df	t	P
C_3						
<i>Epipremnum aureum</i>	5.2 \pm 0.2	5.4 \pm 0.3	0.2	4	0.51	>0.05
<i>Anthurium michelii</i>	6.8 \pm 0.9	6.6 \pm 0.2	–0.2	4	0.36	>0.05
CAM						
<i>Zamioculcas zamiifolia</i>	3.5 \pm 0.2	6.0 \pm 0.5	2.5	4	8.24	≤ 0.05
<i>Platynerium veitchii</i> ^a	9.8 \pm 1.0	13.9 \pm 1.5	4.1	8	2.34	≤ 0.05
<i>Agave attenuata</i>	5.9 \pm 0.5	53.5 \pm 5.1	47.6	4	9.32	≤ 0.05
<i>Cissus rotundifolia</i>	18.5 \pm 5.0	111.4 \pm 7.4	93.0	4	10.46	≤ 0.05
<i>Kalanchoe pinnata</i>	11.6 \pm 2.2	152.6 \pm 18.5	141.0	4	5.69	≤ 0.05

^a The values for *Platynerium veitchii*, a weak CAM plant, are from Holtum and Winter (1999) and are included for comparison.

and ecological respects. Within the Araceae, *Zamioculcas* is a monotypic genus in the Zamioculcadeae, a tribe basal to the subfamily Aroideae (Hesse et al., 2001; Tam et al., 2004; Bogner and Hesse, 2005). Unique in the Araceae, the Zamioculcadeae can propagate vegetatively from fallen leaflets. Not a wet rainforest species like most aroids, *Z. zamiifolia* inhabits humid to seasonally dry forests, open bushland, and savannas in tropical east and subtropical southeast Africa (Peter, 1929; Mayo et al., 1997; Newton, 1997), where it is seldom found above 800 m a.s.l. Its ability to tolerate water stress and low light has elevated *Z. zamiifolia* to international horticultural importance (the “ZZ plant”; Chen and Henny, 2003). Belowground, plants consist of a large tuber and a short, thick underground stem from which arise compound leaves. Aboveground, each compound leaf consists of 4–8 pairs of oblong–elliptic, glabrous, coriaceous, slightly succulent leaflets borne on an elongate rachis attached to a succulent petiole (Mayo et al., 1997).

MATERIALS AND METHODS

Plant material—Plants from the family Araceae and known CAM species from other plant families were cultivated in pots, with regular watering and periodic provision of slow-release complete fertilizer at the Smithsonian Tropical Research Institute, Panama City, Republic of Panama [*Anthurium michelii* Guillaumin (Araceae) and *Z. zamiifolia*]; the Department of Plant Sciences, Oxford, UK [*A. harrisii* (Graham) G. Don (Araceae), *Kalanchoe daigremontiana* Raym.-Hamet & H. Perrier (Crassulaceae) and *Z. zamiifolia*]; and the School of Marine and Tropical Biology, James Cook University, Australia [*Agave attenuata* Salm-Dyck (Agavaceae), *Cissus rotundifolia* Vahl (Vitaceae), *Epipremnum aureum* (Linden & André) G. S. Bunting (Araceae), *Kalanchoe pinnata* (Lam.) Pers. (Crassulaceae) and *Z. zamiifolia*].

Measurements of CO_2 exchange—Net CO_2 exchange by *Z. zamiifolia* was measured for distal sections of compound leaves containing eight leaflets and associated rachis. For *A. michelii*, a single attached fully expanded leaf was measured. Leaves, still attached to the parent plant, were sealed with Terostat VII (Henkel-Teroson, Düsseldorf, Germany), inside a gas-exchange cuvette (GWK-3M, Walz, Effeltrich, Germany) in a controlled-environment chamber operating under 12 h light (28°C , $350 \mu\text{mol photon}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$)/12 h dark (22°C) cycles. Dew point of air entering the chamber was 18°C . Net CO_2 exchange was measured using a LI-6252 CO_2 analyzer (LI-COR, Lincoln, Nebraska, USA) in a flow-through gas-exchange system (Holtum and Winter, 2003) operating at $2.38 \text{ L air}\cdot\text{min}^{-1}$. Air was sourced 16 m above ground level and passed through a 1-m^3 buffer.

The CO_2 exchange of a succulent petiole of *Z. zamiifolia* was quantified for two day–night cycles in the gas-exchange system described. The petiole, 4.9

cm high and 1.9 cm diameter, was detached from the stem under water and was sealed into a beaker containing water. The detached petiole and beaker were placed in the gas-exchange system.

Extraction and assay of PEP carboxylase (PEPC)—Deacidified tissue, frozen in liquid N_2 , was extracted in 10 volumes of ice-cold buffer containing 200 mM tricine-KOH pH 8.0, 10% (v/v) ethanediol, 5 mM EGTA, 5 mM MgCl_2 , 1% (w/v) BSA, 5 mM dithiothreitol (DTT), 0.1% (v/v) Triton X-100, and insoluble polyvinylpyrrolidone (PVPP) of a mass equal to that of the tissue. The extracts were filtered through two layers of cheesecloth, centrifuged at $120000 \times g$ for 10 min, and desalted through Sephadex G-25 (PD-10 column; GE Life Sciences, NSW, Australia) with grinding medium minus Triton-100, PVPP, and BSA.

PEPC was assayed at 30°C according to Kluge et al. (1981).

Titratable acidity—Titratable acidity ($\mu\text{mol H}^+$) was determined by measuring the volume of 5 or 10 mM NaOH required to neutralize to pH 7.0 extracts of leaves of known masses that had been frozen in liquid N_2 and then boiled sequentially in 50% ethanol and water.

RESULTS

***Zamioculcas zamiifolia* has day–night fluctuations in titratable acidity**—Well-watered *Z. zamiifolia* accumulated H^+ in leaflets during the dark (Table 1). In comparison, leaves of *E. aureum*, a C_3 aroid climber, and *A. michelii*, an epiphytic aroid with thick leaves, did not accumulate H^+ during the dark. The day–night fluctuations of H^+ of $2.5 \mu\text{mol H}^+(\text{g fresh mass})^{-1}$ in *Z. zamiifolia* were small compared to those of three species with strongly expressed CAM in which maximal diel acidity changes ranged between 48 and $141 \mu\text{mol H}^+(\text{g fresh mass})^{-1}$.

PEPC activity from *Z. zamiifolia* is greater than in two C_3 aroids—In a pattern similar to that observed for H^+ fluctuations, the extractable activity of PEPC from *Z. zamiifolia* was greater than the activities from two C_3 members of the Araceae, 8.5-fold greater than that from *E. aureum* and 2.7-fold greater than that from *A. harrisii*, but was only one-eighth that of a strong-CAM plant, *Kalanchoe daigremontiana* (Table 2). The PEPC activities in the two CAM plants differed significantly from that of the C_3 species (Mann–Whitney test, $Z = 2.88$, $\text{df} = 6.6$, $P < 0.01$).

Well-watered *Z. zamiifolia* has net CO_2 uptake in the dark—Well-watered *Z. zamiifolia* had net CO_2 uptake in the dark, a defining characteristic of CAM (Fig. 1). The small amount of net CO_2 uptake in the dark, about 0.2% of that observed in the

TABLE 2. Comparison of the extractable activity, expressed as $\mu\text{mol}\cdot\text{min}^{-1}\cdot(\text{g fresh mass})^{-1}$, of PEPC from three members of the Araceae and a strong CAM plant, *Kalanchoe daigremontiana*. Values are means of three extractions \pm SE. The PEPC activities in the two CAM species differed significantly (asterisks) from those of the two C_3 species (Mann-Whitney test, $Z = 2.88$, $\text{df} = 6.6$, $P \leq 0.01$).

Taxon	PEPC activity $\mu\text{mol}\cdot\text{min}^{-1}\cdot(\text{g fresh mass})^{-1}$
Araceae	
<i>Zamioculcas zamiifolia</i> (CAM)	$0.93 \pm 0.14^*$
<i>Anthurium harrisii</i> (C_3)	0.34 ± 0.06
<i>Epipremnum aureum</i> (C_3)	0.11 ± 0.04
Crassulaceae	
<i>Kalanchoe daigremontiana</i> (CAM)	$7.5 \pm 0.43^*$

light, was insufficient to completely offset respiratory CO_2 lost in the dark. As a result, carbon balance in the dark was negative overall (Fig. 2). The maximal rate of CO_2 uptake in the light, generally observed about 1 h after illumination of the leaflets, was 135-fold greater than the maximal dark rate, which occurred 3 to 4 h after the onset of darkness.

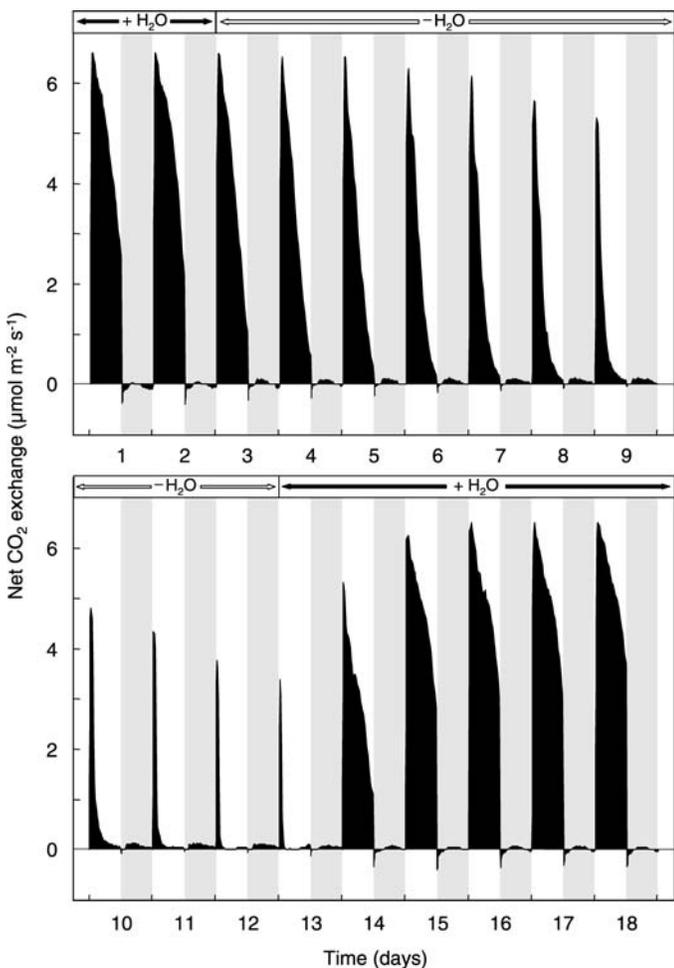


Fig. 1. Net CO_2 exchange by *Zamioculcas zamiifolia* during an 18-d drying-rewetting cycle. Plants were grown under 12 h light (28°C , $350 \mu\text{mol photon}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$)/12 h dark (22°C) cycles. Watering ceased on day 3 and was reinitiated after the onset of the light period on day 13. Darkness is indicated by stippling, watering periods by closed arrows, and drought by open arrows.

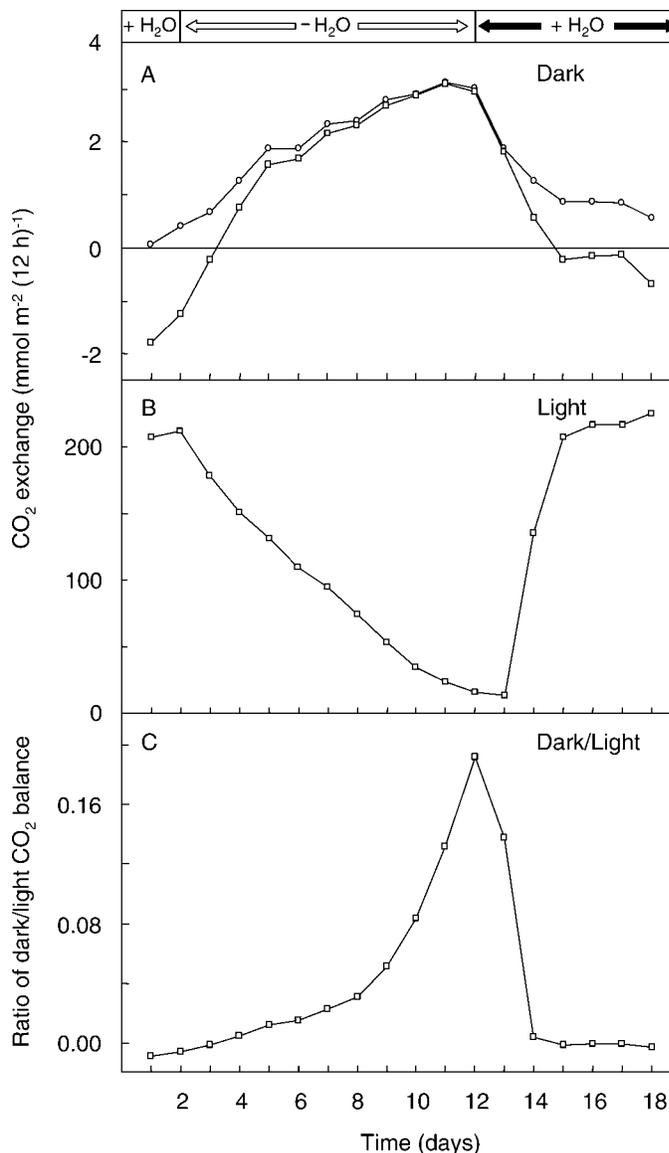


Fig. 2. (A) CO_2 balance (squares) and net CO_2 uptake (circles) during the dark, (B) CO_2 balance in the light, and (C) the ratio dark : light CO_2 balance by *Zamioculcas zamiifolia* during the 18-d drying-rewetting cycle shown in Fig. 1. Watering periods are indicated by closed arrows and drought by open arrows.

Net CO_2 uptake in the dark was a leaf blade phenomenon. The detached petiole had net CO_2 loss during the light and the dark. The rate of CO_2 loss in the dark averaged $0.5 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ($\approx 0.08 \mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{s}^{-1}$), whereas in the light the loss was 60% less, averaging approximately $0.2 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ($\approx 0.03 \mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{s}^{-1}$).

Dark CO_2 uptake is upregulated by water stress in *Z. zamiifolia*—Following the imposition of water stress, carbon gain during the dark increased and carbon gain during the light decreased, such that the proportion of carbon fixed during the dark rose relative to the light (Fig. 2). The reduction in CO_2 uptake during the light was not uniform. Initially, the rate of CO_2 uptake decreased late in the light period but not

TABLE 3. Day–night variation in titratable acidity, expressed as $\mu\text{mol H}^+(\text{g fresh mass})^{-1}$, in leaflets of *Zamioculcas zamiifolia* that were well watered and then grown without watering for 10 d. Plants were cultivated in a growth cabinet under conditions described in the Materials and Methods. Values are the means of four replicate leaves ($\pm\text{SE}$). Dawn values are significantly greater (one-way *t* test) than dusk values for well-watered ($df = 6$, $t = 5.9$, $P \leq 0.001$) and drought-treated leaves ($df = 6$, $t = 4.9$, $P \leq 0.001$). The dawn–dusk titratable acidities of well-watered and drought-treated leaves differed significantly ($df = 6$, $t = 2.5$, $P \leq 0.05$; two-way *t* test).

Treatment	Dusk $\mu\text{mol H}^+(\text{g fresh mass})^{-1}$ ($\pm\text{SE}$)	Dawn $\mu\text{mol H}^+(\text{g fresh mass})^{-1}$ ($\pm\text{SE}$)	Dawn–dusk $\mu\text{mol H}^+(\text{g fresh mass})^{-1}$
<i>Zamioculcas zamiifolia</i>			
Well-watered	3.8 \pm 0.2	7.0 \pm 0.5	3.2
Drought-treated	4.6 \pm 0.5	9.5 \pm 0.6	5.0

during the early light period. Subsequently, the rates of uptake in the early light period also decreased. During the imposition of stress, the enhanced carbon gain during the dark resulted from higher rates of dark CO_2 uptake and longer periods during which CO_2 exchange was positive. Net carbon exchange during the dark became positive after 1 d without watering and remained so for the 10 d without watering. Rewatering of drought-stressed plants was accompanied by a reduction in CO_2 gain during the dark, which was initially observed during the first night after rewatering, and an increase in CO_2 gain during the light (Figs. 1 and 2).

The increase in dark CO_2 gain in response to drought was accompanied by a 56% increase in H^+ accumulation in comparison to well-watered plants (Table 3).

***Anthurium michelii* had C_3 -like day–night CO_2 exchange**—*Anthurium michelii*, an epiphytic Panamanian rain-forest epiphyte with slightly succulent leaves (leaf thickness of 0.50 ± 0.01 mm SE in comparison to 0.70 ± 0.01 SE mm for *Z. zamiifolia*) did not have net CO_2 uptake during the dark during 2 d under well-watered conditions or 4 d of drought (Fig. 3). During the drought treatment, the rate of dark respiration in *A. michelii* fell by 25%, from -0.12 to $-0.09 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$.

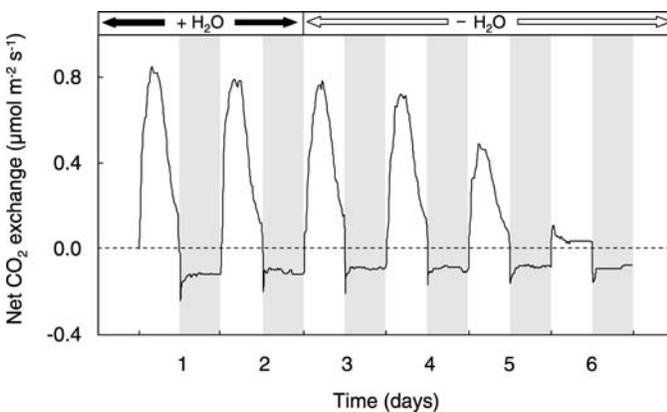


Fig. 3. Net CO_2 exchange by *Anthurium michelii* during a 6-day wetting and drought treatment. The plant, grown under 12 h light (28°C , $350 \mu\text{mol photon}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$)/12 h dark (22°C) cycles, was not watered after day 2. Darkness is indicated by stippling, watering periods by closed arrows, and drought by open arrows.

DISCUSSION

***Z. zamiifolia* has CAM**—The essential criteria that define CAM include an ability to fix CO_2 during the dark and to store the carbon fixed in the vacuole as an organic acid, generally malic acid. Both well-watered and water-stressed *Z. zamiifolia* had net CO_2 uptake during the dark and day–night fluctuations in titratable acidity (Fig. 1, Table 1). Thus, *Z. zamiifolia* can be classified as a CAM plant. Within the Araceae, it is the only CAM species yet reported, and within the Alismatales it is the only documented terrestrial species with CAM.

CAM in *Z. zamiifolia* is upregulated in response to water stress—The expression of CAM increased in response to water stress. After only 2 d without watering, both the rate and extent of dark CO_2 uptake increased such that CO_2 balance in the dark shifted from negative to positive. After 10 d without watering, net dark CO_2 uptake increased 7.5-fold (Figs. 1 and 2).

Although CO_2 uptake in the dark increased in response to water stress, the overall response to the 10 d drought treatment was a reduction by 93% of the total day–night CO_2 gain (Figs. 1 and 2). This decrease in CO_2 balance involved a 94% reduction in CO_2 gain during the light that was offset by a small increase in CO_2 uptake in the dark. Initially, the reduction in CO_2 uptake in the light was confined to midday and afternoon CO_2 fixation, but CO_2 uptake during the morning began to decrease after 4 d of water stress.

The stimulation of CAM in response to water stress of *Z. zamiifolia* was reversible (Figs. 1 and 2). Upon rewatering, CO_2 uptake during the light reverted to its prestress levels and dark CO_2 uptake was reduced, with net CO_2 uptake in the dark decreasing by 95% and CO_2 balance in the dark changing from positive to negative.

The increase in net CO_2 uptake in the dark that accompanied drought was most likely not solely due to an increase in PEPC-catalyzed uptake. The 56% greater diel fluctuation in titratable acidity in the water-stressed plants was consistent with an increase in net flux of carbon from CO_2 to malic acid, but the rise was less than the 7.5-fold predicted from measurements of net dark CO_2 uptake (Fig. 2; Table 3). Although fluctuations in titratable acidity were not measured for the leaves inside the gas-exchange cuvette (they were measured in leaves from a companion plant that grew in the growth chamber), it is probable that not all of the net increase in carbon flux into the plant was sequestered in organic acid. A component of the net increase in CO_2 flux into the water-stressed tissue in the dark may have been the result of a reduction in respiratory carbon loss rather than increased assimilation per se. Stress-related

decreases in CO₂ uptake in the light are often accompanied by reductions in respiratory CO₂ loss in the dark, a phenomenon exemplified by *A. michelii*, which has a 25% reduction in rates of CO₂ loss in the dark after 4 d without water (Fig. 3). It is also possible that some of the CO₂ fixed in the dark may have been converted to nonacidic metabolites. However, the major routes by which the early products of PEPC-mediated dark CO₂ uptake are metabolized involve passage through the Krebs cycle, which results in the loss of the CO₂ originally assimilated (Holtum et al., 2005). We conclude that the net transfer of dark-fixed carbon to non-acid components is likely to be small.

Zamioculcas zamiifolia is a weak CAM plant. The fluctuations in titratable acidity of 3–5 μmol H⁺·(g fresh mass)⁻¹, the extractable activity of PEPC, and the carbon gain in the dark were all low in comparison to levels in well-documented strong CAM species (Fig. 2; Tables 1–3). Why call *Z. zamiifolia* a CAM plant when, under well-watered conditions, it obtains 0.2% of its daily carbon during the dark, and even when stressed and CO₂ uptake in the dark increased 7.5-fold in response to stress, dark CO₂ uptake was only 1.5% of CO₂ uptake in the light under well-watered conditions? *Zamioculcas zamiifolia* could be categorized as a C₃ plant with a small capacity for CAM if the term CAM is applied in the narrow way that the terms C₃ and C₄ are generally employed, to simply describe the major pathway of photosynthesis by which the plant gains carbon throughout its lifetime. However, no plant species known is exclusively CAM, and the expression of CAM is rarely constant throughout the developmental and environmental lifetime of a plant. As a result of this flexibility of expression of CAM, the current, widely used definition of a CAM phenotype is a plant that, at any time during its lifecycle, irrespective of the amount of CO₂ uptake in the light, assimilates CO₂ in the dark and temporarily stores the carbon as organic acids in the vacuole, thus having diel fluctuations in titratable acidity. Ultimately, the presence or absence of the CAM cycle must be defined at the molecular level. There is, as yet, no consensus of what are the fundamental molecular markers of CAM. Because most components of the CAM cycle are also functionally present in C₃ plants, the most promising candidates for markers are CAM-specific isogenes, the best studied of which is PEPC (Taybi et al., 2004; Gehrig et al., 2005). However, because CAM appears to have evolved often, it will be necessary to verify that marker isogenes are expressed in species across diverse taxa.

What is the advantage of CAM for *Z. zamiifolia*?—Although information on the ecology of *Z. zamiifolia* is scarce, it appears that in east Africa *Z. zamiifolia* is a plant of rocky, partially shaded sites in habitats subject to prolonged seasonal drought (Mayo et al., 1997; Newton, 1997). *Zamioculcas zamiifolia* in its natural habitat of high evaporative demand probably has a positive dark CO₂ balance throughout much of the year as long as leaves are present. In situ water stress is likely to be more prolonged and severe than that imposed by us experimentally. In our experiments, the decrease in CO₂ uptake during the light had not stabilized after 10 d of water stress (Figs. 1 and 2) and could be expected to fall further. At times, the contribution of CAM to 24-h carbon gain in *Z. zamiifolia* in its natural habitat is thus likely to exceed the 19% we observed. The role of CAM in the survival of *Z. zamiifolia* is that it contributes to the maintenance of a positive carbon balance while the plant reduces water loss by reducing CO₂ uptake in the light. Water-use efficiency is thereby increased, and the

period of net carbon gain is prolonged. Eventually, if stress persists, the rachis and leaflets abscise, reducing the above-ground plant to an apparently dormant cluster of erect, succulent, petiole bases (Newton, 1997).

CAM is not the only water-conserving feature of *Z. zamiifolia*. Radiative load and transpirational water loss are reduced by erect, coriaceous, reflective, succulent leaflets with abaxial stomata, and by the erect, cylindrical, succulent petiole and rachis. The leaflets and tuber are rarely subject to herbivory, an observation consistent with the presence of chemical defenses, a feature common in the Araceae (Dring et al., 1995).

Why is CAM apparently rare in the Araceae, particularly in epiphytes?—CAM is a derived condition that has evolved repeatedly in response to selection imposed by dry conditions and/or selection driven by low levels of dissolved carbon (Griffiths, 1989; Keeley, 1998). CAM is uncommon in the basal angiosperms, where it is present only in the most derived order, the Piperales (*Peperomia* spp.; Holthe et al., 1992). Similarly, in the basal monocots, CAM is absent (Acorales, Petrosaviales) or rare (Alismatales). Many species in the basal monocots, particularly in the Alismatales, the order in which the Araceae are located, inhabit aquatic, marine, or mesic swampy areas. It is therefore perhaps not surprising that both known CAM species in the Alismatales, *Sagittaria subulata* (L.) Buchenau (Alismataceae) and *Vallisneria americana* Michx. (Hydrocharitaceae), are aquatic (Keeley, 1981, 1996; Webb et al., 1988).

Fossil Araceae are associated with lacustrine deposits and generally moist habitats (Friis et al., 2004; Wilde et al., 2005). Indeed, the Araceae have not radiated extensively into water-limited environments (Mayo et al., 1997). The few Araceae that inhabit deserts and semiarid regions, or grow at high altitude, tend to be geophytes that are characteristically deciduous or seasonally dormant. Modern Araceae are most abundant and diverse in the humid tropics. The epiphytic Araceae are principally wet forest species, generally inhabiting sites less exposed than those of most bromeliads and orchids. Benzing (1989) notes that although many Araceae are secondary hemiepiphytes, a life form that might be expected to be well adapted to intermittent water stress, they characteristically exhibit fewer epiphytic specializations for coping with water stress than do bromeliads and orchids. As a result, they are typically restricted to more moist habitats where selection pressure for CAM may not be as pronounced.

If ancient Araceae and *Isoetes*, the oldest lineage of plants with CAM, were plants of wetlands, why is CAM common in extant *Isoetes* but not in the Araceae? A selection pressure for the retention of CAM in *Isoetes* may have been their poor ability to compete with faster-growing radiating flowering plants (Keeley, 1998). Presumably by retaining CAM, *Isoetes* could reduce competition for inorganic carbon and thus could grow in the presence of more vigorous species in temporally carbon-infertile lakes. CAM also provided a mechanism for survival in permanently carbon-infertile waters and enabled them to utilize locally high concentrations of CO₂ in muds. In contrast, the more rapid growth rates of the Araceae may have remained to be associated with C₃ because they gleaned sufficient inorganic carbon in lacustrine environments. Presumably the lacustrine Araceae adopted amphibian habits that provided access to atmospheric CO₂. The selection pressures for CAM in terrestrial plants living in extremely moist soils were unlikely to be strong. It may be relevant that many extant

TABLE 4. List of 20 orders and 34 families in which CAM species have been reported. The list is based upon that of Smith and Winter (1996), which has been updated using the phylogeny compiled by Stevens (2006). Superscripts denote families in which CAM has been detected since Smith and Winter (1996).

Phylum (group)	Order	Family
Lycopodiophyta	Isoetales	Isoetaceae
Peridophyta	Polypodiales	Polypodiaceae Vittariaceae
Cycadophyta	Cycadales	Zamiaceae ^a
Gnetophyta	Gnetales	Welwitschiaceae
Magnoliophyta		
Magnoliids	Piperales	Piperaceae
Monocotyledons	Alismatales	Araceae ^b Hydrocharitaceae Alismataceae
	Asparagales	Orchidaceae Asphodelaceae Agavaceae Ruscaceae
	Poales	Bromeliaceae
	Commelinales	Commelinaceae
Eudicotyledons	Caryophyllales	Aizoaceae Cactaceae Portulacaceae Didiereaceae Crassulaceae
	Saxifragales	Vitaceae
	Vitales	Geraniaceae
	Geraniales	Passifloraceae Clusiaceae Euphorbiaceae
	Malpighiales	Oxalidaceae Cucurbitaceae Rubiaceae Apocynaceae Lamiaceae Gesneriaceae Plantaginaceae
	Oxalidales	Apiaceae
	Cucurbitales	Asteraceae
	Gentianales	
	Lamiales	
	Apiales	
	Asterales	

^a Vovides et al. (2002)

^b This study.

lacustrine *Isoetes* are amphibious species in which the submerged leaves express CAM but the emerged leaves are C₃ (Keeley, 1996, 1998).

It is also possible that CAM may be more common in the Araceae than we are aware. Certainly, Araceae are often under-represented in isotopic surveys of epiphytes (Winter et al., 1983; Earnshaw et al., 1987; Carter and Martin, 1994), possibly because the surveyors tend to collect species that have recognizably succulent leaves and tend to ignore hemi-epiphytes. The most likely araceous candidates for CAM, on the basis of epiphytic habitat and succulence of leaves, are in the sections *Leptanthurium* and *Porphyrochitonium* of the genus *Anthurium* (subfamily Pothoideae), which contains over 800 species (Mayo et al., 1997). To date, the few *Anthurium* spp. examined in carbon isotope surveys have C₃-like δ¹³C values, e.g., nine *Anthurium* species on Barro Colorado Island, Panama had δ¹³C values between -28.1‰ and -33.7‰ (Zotz and Ziegler, 1997). However, carbon isotope surveys that measure the integrated day-night carbon uptake signal over the life of the organ sampled rarely have the resolution required to detect weak CAM in plants that acquire only a small proportion of their carbon during the dark (Winter and Holtum, 2002).

How prevalent is CAM in vascular plants?—The most recent estimate of the prevalence of CAM in vascular plants is that of Smith and Winter (1996), who documented CAM in 33 families and 328 genera containing about 16 000 species. The species number was based on the estimation that 50% of tropical epiphytic orchids and bromeliads are CAM. The 1996 census requires updating to accommodate reassessments of vascular plant taxonomy (e.g., Asclepiadaceae are now in the Apocynaceae, and Dracaenaceae are now in the Ruscaceae; Chase et al., 2000; Stevens, 2006), the report of CAM in a cycad (Vovides et al., 2002), an extensive survey of bromeliad carbon isotope compositions (Crayn et al., 2004), the discovery of new CAM genera in the Crassulaceae and Orchidaceae (Silvera et al., 2005; Liu and Wang, 2006), and this communication of CAM in the Araceae. We report that CAM is present in 34 families and 343 genera of vascular plants (Table 4). The great uncertainty is still the total number of CAM species. An improved estimate of the number of species requires extensive carbon isotope surveys of, in particular, the orchids. We suspect, however, that there are many species like *Z. zamiifolia*, in which the contribution of dark CO₂ uptake to daily carbon gain is so small that it would not produce an identifiable isotopic signal (Winter and Holtum, 2002; Silvera et al., 2005). Discovery of these CAM species requires a more labor-intensive quantification of H⁺ fluctuations.

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