CAM-cycling in the cycad *Dioon edule* Lindl. in its natural tropical deciduous forest habitat in central Veracruz, Mexico

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The cycad *Dioon edule* Lindl. inhabits a seasonally-dry tropical forest along with associated CAM plants such as bromeliads and cacti. To test the hypothesis that *D. edule* might also be a CAM plant, diel total-acid fluctuation was measured through the dry to wet seasons of 4 consecutive years on adult *D. edule* plants in their natural forest habitat in Veracruz, Mexico. Correlations between acid fluctuation index and climatic data, and also soil water potential were determined over this period. Laboratory trials were followed up to estimate diel patterns of CO_2 exchange and estimation of $\delta^{13}C$ value. A comparison of stomatal density cm^{-2} with other C_3, CAM and CAM-facultative plants was made. The diel total titratable-acid fluctuation values, although variable, were found to be consistent and significant for the dry season. Carbon dioxide exchange was found to be typical of C_3 plants when hydrated but when the plant was stressed by withholding water, although the leaf remained healthy, there was no significant dark-period CO_2 output. Stomatal density was comparable to other CAM and CAM-facultative plants. It was concluded that *D. edule* is a C_3 plant that shows CAM-cycling metabolism when water stressed. © 2002 The Linnean Society of London, *Botanical Journal of the Linnean Society*, **138**, 155–161.

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INTRODUCTION

It is widely believed that CAM (crassulacean acid metabolism) is a metabolic sequence related to adaptation to arid environments and, on the whole, CAM plants are commonly found in these environments mostly concentrated within the tropics and subtropics. Among species of tropical deciduous forests and thorn-brush-succulent forests many are CAM plants. These include all the cacti of the Americas, the succulent euphorbias of the Old World and many rosette plants in the Agavaceae, Bromeliaceae and Liliaceae, and also members of the Aizoaceae, Oxalidaceae, Piperaceae and Portulacaceae (Kluge & Ting, 1978; Osmond, 1978; Osmond *et al.*, 1982; Lüttge, 1997). CAM has also been observed in aquatic vascular plants that grow in environments where there is high carbon dioxide and/or bicarbonate competition during daylight hours, especially in moderately fertile shallow temporary pools that experience extreme diel fluctuations in inorganic carbon availability. *Isoëtes* is an example of an aquatic CAM plant reported by Keeley (1981, 1983) and Keeley *et al.* (1983). Although at first this met with scepticism and was considered an oxymoron by some, it has since

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been shown that some aquatic species do indeed possess CAM photosynthesis (Keeley, 1998). The common feature for both arid environment and aquatic CAM plants is the restricted availability of inorganic carbon. This can be via the dependence of atmospheric CO_2 acquisition on water vapour loss as a result of aridity on land (hence CO_2 uptake at night when H_2O evaporation is at a minimum) or actual inorganic carbon limitation in the daytime in the water column in some freshwater bodies.

It has been found that certain species of C_3 (Calvin cycle) plants under conditions of water stress or salinity shift from C_3 photosynthesis to CAM and back to C_3 when the stress is removed. These have been denominated 'facultative-CAM' plants (Kluge & Ting, 1978; Ting, 1985; Ting *et al.*, 1985a,b) or C_3–CAM intermediates (Griffiths, 1989). The shift in the mechanism from C_3 to CAM has been suggested as the result of stomatal closure in response to water stress. Examples of facultative-CAM plants are *Mesembryanthemum crystallinum*, *Peperomia obtusifolia* and *Ananas comosus* (Kluge & Ting, 1978).

Ting (1985) reported the following variants of CAM: (i) CAM-idling, where there is low diel acid fluctuation and respiratory CO_2 is cycled through the CAM pathway when stomata are closed; and (ii) CAM-cycling, where plants show organic acid fluctuation with little or no exogenous nocturnal CO_2 fixation at night which may be related to water balance. Within the Crassulaceae, there are plants that show 100% C_3 photosynthesis with no cycling of organic acids, others that show 100% C_3 type gas exchange but cycling is present, and those that show full CAM. *Mesembryanthemum crystallinum* and other species of the Aizoaceae as well as *Peperomia* spp. are induced to CAM by irrigation with salt water or by withholding water. Most cacti tend to maintain the C_3 photosynthetic metabolism during early ontogeny of seedlings, turning typically CAM when adult (Altesor *et al.*, 1992). Rayder & Ting (1981) and Altesor *et al.* (1992) reported CAM-cycling in the cactus genus *Pereskia* Lüttge (1997) mentions the nocturnal recycling of respiratory CO_2 in the cactus *Subpilocereus ottonis* under extreme water stress. *Welwitschia mirabilis* is an example of a CAM-cycling gymnosperm. Here organic acid fluctuation is observed but little or no exogenous nocturnal CO_2 fixation occurs (Ting & Burk, 1983; Ting, 1985).

The cycad *Dioon edule* has a tropical deciduous forest habitat which is subjected to relatively long dry periods and much heat during the early part of the year, and several other known CAM plants occupy the same habitat. These include bromeliads, opuntias, agaves and members of the Crassulaceae (Vovides, 1990). To test the hypothesis that *D. edule* might also be a CAM plant, diel total-acid fluctuation was measured during the dry, 'winter' and wet seasons of the years 1983–86 in adult *D. edule* plants in their natural habitat at Corral Falso (south-east of the City of Xalapa). The field trials were also backed up with laboratory tests to detect diel patterns of CO_2 exchange and carbon isotopic discrimination. In addition, a correlation was determined between field diel total-acid fluctuation and climatic data obtained during the period of field trials.

MATERIAL AND METHODS

DIEL FLUCTUATION OF TOTAL-TITRATABLE ACIDS

A known weight of fresh leaf tissue was taken (2–5 g) whilst still in the dark (at break of dawn in the case of field trials) and at the end of the light period (at dusk in the field). 10–12 leaflets from mid-way up a healthy leaf were sampled. Leaflets from along one side of the rachis were taken for the light titration and those along the opposite side of the rachis of the same leaf were taken for the corresponding dark titration. This was done in order to eliminate any variation, which may be encountered in widely separated leaflets. In the field, the leaf tissue was cut into small squares and immediately placed into absolute alcohol then stored in a freezer until use. For the titration, the leaflet pieces were boiled in distilled water (including the alcohol they were stored in) for five minutes to soften the tough tissues then ground for 2–3 min in a blender. The macerate was then boiled down to about 20 mL. This was centrifuged at 3000 r.p.m. for 10 min and the supernatant collected for titration. Titration was done against a standard solution of 0.02 N sodium hydroxide using a 10 mL microburette. The titration was taken to an end point of pH 8.0 using a pH meter whilst constantly agitating the solution. The NaOH standard was made up from a 2.0 N stock solution by dilution with distilled water. This standard was checked daily against a 0.02 N solution of hydrochloric acid. The titration was taken to an end-point of pH 8.0 (the average plant acid has two carboxyl groups and these are neutralized at pH 8.0).

The high fibre content of *Dioon* leaflets makes it desirable to express the acidity in terms of mesophyll fresh weight to allow comparison with fleshy succulent CAM plants such as *Sedum*. The calculation of the percentage of fibre contained in an average leaflet was done by making a low power map of a transverse section of the mid-portion of a leaflet, using a *camera lucida*. The total area of the section mesophyll (spongy and palisade) and the fibres, including the vascular bundles, was computed by digitizing the map. The area occupied by the mesophyll was thus easily calculated. The acid values were expressed as microequivalents (μEq) per gram fresh weight of mesophyll tissue. The mean of three titrations per individual and
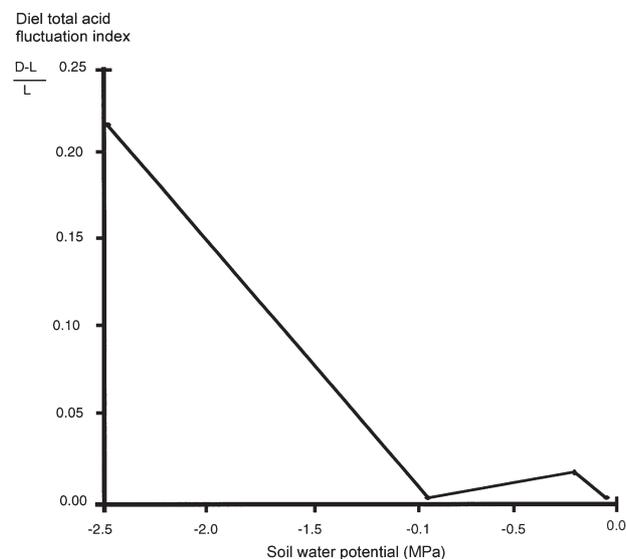
its standard error were calculated. The titration values between light and dark were subjected to a one-tailed Student's *t*-test. The means of the difference in acid fluctuation were subjected to a one way analysis of variance in order to detect any significant difference between the values obtained during the different seasons of sampling. The relative water content of three to five leaflets taken at random from each individual was measured according to Etherington (1982).

SOIL WATER POTENTIAL

The percent dry weight soil moisture content was determined by bringing a soil sample in a sealed plastic bag back to the laboratory, where it was immediately weighed then oven dried at 98 °C overnight and re-weighed. The soil water potential was determined at five different water-percent-dry-weight values using the pressure membrane technique described by Etherington (1982) and Bannister (1986). The water potentials (mega-Pascal, MPa) were plotted against soil water content (% dry weight). Soil water potential values were derived from the relationship thus obtained, and a graph was plotted of diel acid fluctuation index expressed as: $(D - L)/L$ (D = mean dark period acid value; L = mean light period acid value) against soil water potential (Fig. 1).

STOMATAL DENSITY

Stomatal densities (stomata cm^{-2}) of *D. edule* and other Mexican cycads were investigated for comparison with that of a known CAM plant. Non-CAM and facultative-CAM plants were also investigated. Abaxial epidermal strips were taken from the mid-portion of leaflets taken from the mid-portion of the leaf in the case of the cycads, and the abaxial surface of the mid-portion of the leaves of the other species. Maceration of the mesophyll tissues and hypodermal fibres was carried out using a modified version of Jeffrey's technique (Stace, 1965), samples were stained with Bismark brown and permanent preparations made. Stomata were counted in six random fields aided by an eyepiece grid and intervein distance measured from three leaflets of *D. edule*. Average stomatal density and intervein distance were calculated with corresponding standard errors.

DIEL PATTERNS OF CO$_2$ EXCHANGE

The diel variation of CO_2 exchange in *D. edule* was studied under controlled environment conditions. Illumination used was that of metal halide lamps giving 100 W m^{-2} (400–700 nm) and photoperiod of 16 h. Temperature was set at 15 °C night and 25 °C day. Individual attached leaves of potted *D. edule* plants were enclosed in a cylindrical Perspex chamber through which ambient air was pumped. The air stream was then passed through an ADC Series 225 infra-red gas analyser, operating in the differential mode to detect carbon dioxide. Ambient air was pumped from the roof of the laboratory building to avoid excessive CO_2 enrichment from automobile exhausts. The air stream was split, half through the leaf chamber, and half to serve as a reference. Depletion of CO_2 by photosynthesis or dark-fixation and enrichment by respiratory output could be detected. Except for the details of the leaf chamber and gas analyser, the system was as described by Etherington (1967).

ESTIMATION OF δ^{13}C VALUE OF *DIOON EDULE*

In the free atmosphere, the concentration of the carbon isotopes ^{13}C and ^{12}C is about 1.11% ^{13}C and 98.9% of ^{12}C and it is known that C$_3$ plants discriminate against the heavier isotope. The carboxylating enzymes phosphoenol pyruvate carboxylase (PEPC) and ribulose bisphosphate carboxylase-oxygenase (Rubisco) are responsible for a large part of the fractionation of the carbon isotopes in atmospheric carbon dioxide. Rubisco in the Calvin (C$_3$) pathway discriminates against the heavier ^{13}C isotope (Ting *et al.*, 1985b). In C$_4$ and CAM plants PEPC discriminates less against ^{13}C than the other carboxylating enzymes and both ^{12}C and ^{13}C isotopes of atmospheric carbon dioxide are used, resulting in a higher accumulation of ^{13}C relative to the Calvin pathway. The isotope composition ratio is usually expressed as a δ^{13}C

Diel total acid fluctuation index

Figure 1. Correlation of diel total acid fluctuation index in *Dioon edule* with soil water potential at Corral Falso, Veracruz.

value, in parts per thousand, after reference to a limestone standard:

$$\delta^{13}C(‰) = \left[\frac{^{13}C/^{12}C \text{ Sample}}{^{13}C/^{12}C \text{ Standard}} - 1 \right] \times 10^3$$

(Osmond *et al.* 1982). The standard used was NBS Solenhofen limestone reference standard No. 20 which gave $\delta^{13}C$ PDB = $-23.23‰ \pm 0.09$. Referring to this PDB standard, C_3 plants have a mean $\delta^{13}C$ of $-27‰$, C_4 plants a mode of $-11‰$ and CAM plants have been reported to vary between -14 to $-31‰$ (Kluge & Ting, 1978).

The $\delta^{13}C$ trials were run on *D. edule* as an alternative method that may support the hypothesis that this species presents CAM, although this method alone cannot disprove the presence of CAM (Osmond *et al.*, 1982). The $\delta^{13}C$ assay was also carried out on three other cycad species. The samples were oven dried at $90–100\,°C$ and then finely ground. $3–6\,mg$ of material was used from three replicates per species. The ground leaf material was converted into carbon dioxide using a standard combustion train system. The gas was trapped and passed through a VG Micromass 602C dual inlet stable isotope mass spectrometer with precision usually better than $\pm 0.05‰$.

COMPARISON OF CLIMATIC DATA WITH FLUCTUATION OF TOTAL TITRATABLE ACIDS

Climatic data for the periods 1983–86 were obtained from the SARH (Secretaría de Agricultura y Recursos Hidraulicos) from a meteorological station near the study site. Mean, maximum, and minimum temperatures and total precipitation were obtained for each of 30 days before leaf tissue sampling. Mean diel acid fluctuation index was plotted against precipitation/mean temperature quotient for each of the sampling periods (Fig. 2).

RESULTS

DIEL TOTAL-ACID FLUCTUATION

During the 1983 and 1984 dry seasons a total of 35 separate titrations of leaf extracts from five plants showed in every case an increase of titratable acidity during the night, and this was statistically significance using a one-tailed Student's *t*-test ($P < 0.05$, and $P < 0.01$, 2 d.f., respectively). At the beginning of the wet season of 1983 diel acid fluctuation was still occurring in two plants ($P < 0.01$, 3 d.f.). During the wet seasons of both years 24 separate titrations of leaf extracts from eight different plants showed variable values of diel acid fluctuation, none of which showed statistical significance in night acid accumulation (data not shown). During the 1984 'winter' season

Diel total acid fluctuation index
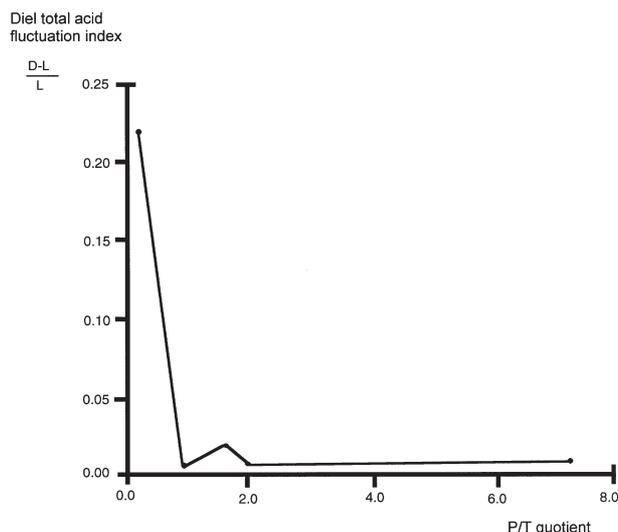
Figure 2. Correlation of diel total acid fluctuation index in *Dioon edule* with precipitation/temperature quotient (P/T) at Corral Falso, Veracruz.

(cooler part of the dry season) 27 separate titrations of leaf extracts from three different plants showed no statistical significance in diel acid fluctuation. This was probably due to lower temperatures, lower water stress and lower light intensity.

The rest of 1984 and all of 1985 were exceptionally wet with no real dry period during 1985. During this period, 24 separate titrations of leaf extracts from four different plants showed no statistical significance in diel acid fluctuation.

During the dry season of 1986, 18 separate titrations of leaf extracts from three different plants showed a small but consistent increase of titratable acidity during the night ($P = 0.03$, 2 d.f.). The average range of acid values for the light and dark periods, and acid fluctuation in $\mu Eq\ g^{-1}$ Fwt mesophyll tissue is given in Table 1.

One way ANOVA tests on the means of the difference of percent change in acid fluctuation between the wet, winter and dry seasons (1983–86) have shown a significant difference between the dry and winter seasons ($P = 0.02$, 2 d.f.).

CO$_2$ EXCHANGE IN A CONTROLLED ENVIRONMENT AND $^{13}C/^{12}C$ DISCRIMINATION

Unstressed leaves showed a pattern of CO_2 exchange typical of plants with the C_3 mode of photosynthesis. Photosynthetic CO_2 assimilation commenced when the lights came on, rising to a transient peak and then settling to a lower steady state during the day. During darkness the leaf showed a net respiratory output of

Table 1. Seasonal mean total-acid values and diel acid fluctuation ($\mu Eq\,g^{-1}$ Fwt mesophyll tissue ± SE) in *Dioon edule* in its natural habitat at Corral Falso

Season	Light	Dark	Fluctuation
Dry 1983	12.1–21.4 ± 0.8	14.9–35.5 ± 1.4	2.5–12.4*
Dry 1984	61.0–107 ± 4.0	75.0–178 ± 7.0	8.2–89.0**
'Winter' 1984	14.7–36.7 ± 2.5	16.3–33.2 ± 1.7	0.6–1.6†
'Dry' 1985	24.7–27.6 ± 1.3	21.9–27.8 ± 1.5	0.3–3.1†
Dry 1986	33.0–40.8 ± 4.7	38.3–43.6 ± 3.0	2.8–5.3*

*$P < 0.05$; **$P < 0.01$; †$P > 0.05$

Table 2. Carbon isotopic discrimination by cycads ($\delta^{13}C$)

Species	$\delta^{13}C$ (% ± SE)
Ceratozamia mexicana	−28.4 ± 0.2
Dioon edule	−26.3 ± 0.4
D. purpusii	−24.6 ± 0.8
Zamia furfuracea	−27.3 ± 0.2

Table 3. Stomatal density cm^{-2} ($n = 6$) calculated for *Dioon edule* and seven additional cycad species compared with that of known CAM, CAM-facultative and C_3 plants

Species	No. stomata cm^{-2} (± SE)
Ficus sp. *	27 671 ± 715
Philodendron sp. †?	6599 ± 225
Peperomia obtusifolia †	5502 ± 226
Ananas comosus ††	7032 ± 115
Nolina parviflora ††	10 845 ± 484
Ceratozamia mexicana ‡?	2009 ± 231
C. miqueliana ‡?	1370 ± 94
Dioon edule ‡	7374 ± 544
D. purpusii ‡?	4430 ± 353
D. spinulosum ‡?	4133 ± 238
Zamia loddigesii ‡?	5320 ± 307
Z. furfuracea × loddigesii ‡?	6575 ± 141
Z. furfuracea ‡?	8699 ± 617

*C_3; † CAM-facultative; †† CAM; ‡ CAM-cycling

CO_2 which declined slightly during the first hour and then settled to a steady state during the dark period. Quantification of CO_2 exchange was not made since the investigation was aimed only at establishing a diel pattern. In a number of similar trials it was never possible to observe any dark period CO_2 uptake. However, when the plant was stressed by withholding water, the daytime photosynthetic assimilation de-

clined over a few days to zero at which time, though the leaf remained healthy, there was no significant dark-period CO_2 output. Attempts to measure diel acid fluctuation of leaf tissues under laboratory conditions failed to indicate significant dark acidification.

The $\delta^{13}C$ values for *D. edule* and three other cycad species were found to lie between −23.8 to −28.6 % which is within the range for C_3 plants; however, this does not disprove the presence of CAM (Table 2). Plants do not assimilate carbon isotopes equally, and discriminate against the heavier ^{13}C and ^{12}C (Kluge & Ting 1978; Ting *et al*., 1985b). Carbon assimilation results in carbon fractionation because PEPC trapping of CO_2 shows less discrimination or fractionation of ^{13}C (and ^{14}C) than the C_3 path. This is shown by tissue differences in $\delta^{13}C$ values in C_4 plants that use this enzyme system, which discriminates less against ^{13}C than the C_3 plants' Rubisco system. This is not necessarily so in facultative-CAM plants, although CAM and C_4 plants use Rubisco but not under conditions in which its discrimination is expressed only weakly. Research has shown that CAM plants have a variable $\delta^{13}C$ value despite the relative constancy for C_3 and C_4 plants, with means being −27% and −11%, respectively (Kluge & Ting, 1978). Vogel (1980) found the $\delta^{13}C$ values of certain facultative-CAM *Aloe* spp. to lie within the range of C_3 plants (−22% to −34%).

STOMATAL DENSITY

On the whole, CAM plants have fewer stomata cm^{-2} than most mesophytic plants: Kluge & Ting (1978) and Osmond *et al*. (1982) reported that CAM species have approximately 2500 stomata cm^{-2}, while C_3 and C_4 plants have around 20 000 stomata cm^{-2}. Stomatal density could be a useful indicator of CAM. A survey of stomatal density of a few selected cycads compared to *Ficus* sp., a C_3 plant, and other plants having CAM or CAM-facultative metabolism is presented in Table 3. Intervein distance for *D. edule* ranged between 250 and 500 μm (mean = 356 ± 0.02, $N = 18$).

CORRELATION OF DIEL TOTAL-ACID FLUCTUATION WITH CLIMATE DATA

Figure 2 shows the diel acid fluctuation expressed as the diel acid fluctuation index against the total precipitation/temperature quotient for the periods of leaf tissue sampling. It can be seen that there is a threshold value of P/T quotient of approximately 0.85 below which there is a sharp increase in diel fluctuation of titratable acids. Figure 1 shows the relationship between the diel acid fluctuation index and the soil water potential. A threshold of -0.95 MPa can be seen below which there is an increase in diel fluctuation of titratable acids. Since there was no soil water content data for the May 1983 trials, the soil water potential was extrapolated by plotting the four water potential values against the P/T quotients for the five periods of sampling (not shown). This gave an exponential curve with $r^2 = 0.98$, and the P/T quotient for May 1983 was 0.2 which gave an estimated soil water potential of -2.5 MPa.

DISCUSSION

The range of total-acid values in *Dioon edule*, though variable, were consistent and cover the range of that found for *Sedum telephium*, a recognized facultative-CAM plant (Kluge & Ting, 1978) that gave 20–28 μEq g^{-1} Fwt for the light period and 22–78 μg^{-1} Fwt for the dark period (A. Groenhof, pers. comm.). Liu & Chen (1999) found acid values for the cycad *Cycas miquelii* to be 20 μEq g^{-1} Fwt for the light period and 22.5 μg^{-1} Fwt for the dark period. Although they ruled out C_4 and full CAM for this species and concluded that *C. miquellii* is a C_3 plant, they did not consider the possibilities of facultative-CAM.

Carbon dioxide exchange pattern in *D. edule* was found to be similar to C_3 plants when hydrated, where CO_2 uptake was detected during the light period and CO_2 output, though of a lesser amount, during the dark period. However, when water-stressed there was no dark-period CO_2 output detected. Other workers have interpreted this as being carbon-conservative in drought, in that the photosynthetic machinery is maintained when stomata are closed by recycling of respiratory CO_2 through the CAM pathway, and when hydrated the plant shows typical C_3 gas exchange (Ting 1985; Griffiths 1989). Because the gas exchange pattern was not quantified during this study and $^{14}CO_2$ incorporation experiments were not carried out we cannot, at this time, give an estimate of the contribution of CAM to the carbon budget of *D. edule*. Research has shown that CAM plants have a variable $\delta^{13}C$ value (-10% to -33%) despite the relative constancy for C_3 and C_4 plants (-27% and -11%, respectively; Kluge & Ting, 1978; Ting, 1985). Vogel (1980) found the $\delta^{13}C$ values of certain facultative-CAM *Aloe* spp. to lie within the range of C_3 plants (-22% to -34%). These findings are very comparable to that of *D. edule*, which showed a $\delta^{13}C$ value of -25.9% to -26.7%.

Stomatal density of the cycads investigated range from around 1400–8700 stomata cm^{-2} with *D. edule* showing about 7400 cm^{-2}, which lies within the range found for the two known facultative CAM species. Liu & Chen (1999) reported a stomatal density of 4200 stomata cm^{-2} for the cycad *Cycas miquelii* which is within the range reported for cycads during our research. Intervein distance of *D. edule* lies within the range found for leaf succulent dicots (180–1000 μm). The range of stomatal density values and intervein distance appear to be a promising avenue for future research that might help distinguish CAM from C_3 plants (Gibson, 1982).

Correlation with climatic data (Figs 1 and 2) for the sampling period has shown two interesting thresholds where diel acid fluctuation appears to be triggered by *D. edule*: (i) at a P/T quotient of approximately 0.85, and (ii) at a soil water potential below approximately -1.0 MPa. Acid fluctuation appears to be related to water balance and is suggestive of CAM-cycling activity *sensu* Ting (1985). When water-stressed (under laboratory conditions) the resultant lack of dark period CO_2 output suggests that *D. edule* shifts into a CAM-cycling mode, and when hydrated a typical C_3 gas exchange is observed.

It is interesting to note that it is the more primitive forms of the Crassulaceae that show CAM-cycling in which there is organic acid fluctuation with no apparent nocturnal net CO_2 fixation (Ting, 1985). Some primitive species of the Crassulaceae (Ting, 1985; Ting *et al.*, 1985b) as well as the primitive cactus genus *Pereskia* (Altesor *et al.*, 1992) and *Subpilocereus ottonis* under water stress (Lüttge, 1997) show CAM-cycling. Greenhouse-grown plants of *Welwitschia mirabilis* (a CAM-cycling gymnosperm) showed $\delta^{13}C$ values ranging from -24.4 to -25.8%, and in young plants diel fluctuation of organic acids was evident yet no nocturnal gas exchange was measured (Ting, 1985).

Griffiths (1989) suggests that CAM evolution proceeded from dark fixation of respiratory carbon to dark CO_2 uptake and that cladistic analysis supports an origin of CAM in seasonal wetlands, thence on into oligotrophic lakes and eventually terrestrial habitats (Keeley, 1998). Keeley (1998) has observed that *Isoëtes* (Lycophyta) is the oldest lineage of CAM plants whose origins may be traced to the Carboniferous (DiMichele & Bateman, 1996). Cycads also appear to have a Palaeozoic origin (Worsdell, 1901; Mamay, 1976; Crane, 1988; Zhifeng & Thomas, 1989) and gymnosperms were already numerous during the Carboniferous (Norstog & Nicholls, 1997). It is well known that during the Permo-Trias mass-extinction there was a drying of climates and widespread desertifica-

tion which marked the transition from the Palaeozoic to the Mesozoic, causing extinction well into the Triassic (Wilson 1992). It is therefore not unreasonable to speculate that those plants that had already developed a 'precursory' variant of CAM such as respiratory CO_2 recycling or CAM-cycling by late Palaeozoic times would have had a selective advantage during the drier climates. Hence, perhaps CAM photosynthesis could be an important factor that contributed to the success and abundance of cycads during the Mesozoic.

SUGGESTIONS FOR FURTHER RESEARCH AND CONCLUSIONS

The quantification of gas exchange patterns as well as $^{14}CO_2$ incorporation experiments to give some estimate of the contribution of CAM to the carbon budget in cycads should be interesting lines of future research. A survey on stomatal density ranges and interveinal distance of cycads for screening promising species for laboratory research is desirable. Assays of organic acids in cycads, such as malate and citrate, as well as PEPC and Rubisco activity should also be considered.

We conclude that *D. edule* is a C_3 plant that appears to present CAM-cycling under certain climatic conditions. *Dioon edule* is thus another possible gymnosperm that presents a variant of CAM metabolism, which may be the case for most cycads.

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