Carbon isotope composition and water-use efficiency in plants with crassulacean acid metabolism

Klaus Winter[A,C], *Jorge Aranda*[A] *and Joseph A. M. Holtum*[A,B]

[A]Smithsonian Tropical Research Institute, P.O. Box 2072, Balboa, Ancón, Republic of Panama.
[B]School of Tropical Biology, James Cook University, Townsville, Qld 4811, Australia.
[C]Corresponding author. Email: winterk@tivoli.si.edu

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Abstract. The relationship between water-use efficiency, measured as the transpiration ratio (g H_2O transpired g^{-1} above- plus below-ground dry mass accumulated), and $^{13}C / ^{12}C$ ratio (expressed as $\delta^{13}C$ value) of bulk biomass carbon was compared in 15 plant species growing under tropical conditions at two field sites in the Republic of Panama. The species included five constitutive crassulacean acid metabolism (CAM) species [*Aloe vera* (L.) Webb & Berth., *Ananas comosus* (L.) Merr., *Euphorbia tirucalli* L., *Kalanchoë daigremontiana* Hamet et Perr., *Kalanchoë pinnata* (Lam.) Pers.], two species of tropical C_3 trees (*Tectona grandis* Linn. f. and *Swietenia macrophylla* King), one C_4 species (*Zea mays* L.), and seven arborescent species of the neotropical genus *Clusia*, of which two exhibited pronounced CAM. The transpiration ratios of the C_3 and CAM species, which ranged between 496 g H_2O g^{-1} dry mass in the C_3–CAM species *Clusia pratensis* Seeman to 54 g H_2O g^{-1} dry mass in the constitutive CAM species *Aloe vera*, correlated strongly with $\delta^{13}C$ values and nocturnal CO_2 gain suggesting that $\delta^{13}C$ value can be used to estimate both water-use efficiency and the proportion of CO_2 gained by CAM species during the light and the dark integrated over the lifetime of the tissues.

Keywords: C_3 photosynthesis, C_4 photosynthesis, carbon-isotope ratio, crassulacean acid metabolism, transpiration ratio, water-use efficiency.

Introduction

A capacity to conserve water while assimilating CO_2 is generally recognised as the most important functional attribute of CAM photosynthesis in terrestrial plants (Winter and Smith 1996). The minimisation of water expenditure results from the ability of CAM plants to fix atmospheric CO_2 at night when the driving forces for transpirational water loss are low, and to close stomata during the middle of the day when the driving forces for transpirational water loss are high. Quantification of nocturnal and diurnal net CO_2 exchange and transpiration of leaves or photosynthesising stems enclosed in gas-exchange cuvettes has provided ample evidence for a high instantaneous water-use efficiency (WUE) of CAM plants compared with C_3 plants (Neales 1973; Osmond *et al.* 1979; Nobel 1988; Woerner and Martin 1999), although there may be exceptions (Griffiths *et al.* 1986; Eller and Ferrari 1997). Despite the widely accepted reputation of the CAM pathway as a water-conserving

mechanism and its estimated presence in ∼6% of higher plant species (Smith and Winter 1996; Crayn *et al.* 2004), few comparative data exist on the WUE of CAM plants at the whole-organism level and integrated over prolonged periods (e.g. Silva and Acevedo 1995; Han and Felker 1997).

We have conducted an outdoor study on the transpiration ratio (TR), a long-term measure of the amount of water lost through transpiration per amount of dry mass produced (Ehrler 1969), in a range of CAM species that differ in their capacity for nocturnal CO_2 fixation, and compared their responses with those of C_3 plants and a C_4 plant. The experiments were performed in a relatively humid tropical environment where CAM species are abundant (Winter *et al.* 1983; Earnshaw *et al.* 1987; Holtum and Winter 1999; Gehrig *et al.* 2003; Crayn *et al.* 2004; Holtum *et al.* 2004), but where investigations on water relations of these plants have been few in comparison to those on CAM succulents from semi-deserts (Nobel 1988).

Included in our study was an investigation of TR in seven species of *Clusia*, a genus of ∼300 species native to wet and seasonally dry tropics and sub-tropics in the New World (Hammel 1986; Pipoly *et al*. 1998). The genus harbours an exceptional diversity of life forms, which include terrestrial trees and shrubs, lianas, hemi-epiphytes and epiphytes (Zotz and Winter 1994*a*, *b*; Lüttge 1996). *Clusia* is singular in that not only do many species have the ability to grow as hemi-epiphytes, epiphytes or as terrestrial plants but the genus contains C_3 species and species that exhibit weak, inducible or obligate CAM (Franco *et al*. 1990; Winter *et al*. 1990, 1992; Borland *et al*. 1992; Zotz and Winter 1993; Lüttge 1999), including the only known dicotyledonous CAM plants that exhibit a classic tree-like arborescent habit.

Our study also considered the possible use of the $^{13}C : {}^{12}C$ ratio of plant organic matter as a surrogate for measurements of WUE in CAM plants. In plant leaves, carbon isotope discrimination is related to p_i / p_a, the ratio of internal to external CO_2 partial pressure, via the ratio of CO_2 to water vapour fluxes across stomatal pores (Farquhar *et al*. 1982, 1988; Máguas and Griffiths 2003). As a consequence, $\delta^{13}C$ values have been extensively used to screen for genotypes of C_3 and C_4 crop species with beneficial WUE (Condon *et al*. 1993, 2002; Wright *et al*. 1994; Henderson *et al*. 1998). When growing in the same environment, the interspecific variation in $\delta^{13}C$ values of C_3 and C_4 plants is nonetheless relatively small in comparison to isotopic variation in CAM plants. In CAM plants, $\delta^{13}C$ values correlate linearly with the proportions of CO_2 derived during the light and dark (Winter and Holtum 2002, 2005) and the isotopic composition varies markedly between species that rely to different degrees on the CAM cycle for carbon acquisition. Since dark CO_2 fixation is associated with lower transpiration rates than CO_2 fixation in the light, one might predict a strong link between $\delta^{13}C$ value and WUE in CAM plants, a hypothesis that was supported in the present study.

Materials and methods

Experiment 1

This experiment was performed in a large forest clearing of the seasonally dry tropical forest of Parque Natural Metropolitano, Panama City, Republic of Panama (Winter *et al*. 2001*a*). Three plants of each species were grown at one plant per pot in 38-L Rubbermaid Round Brute containers (upper diameter 36.5 cm; lower diameter 32 cm; height 44 cm; Consolidated, Twinsburg, OH). Pots contained: a layer of charcoal at the base to improve drainage, a 1 : 1 mixture of soil and leaf litter saturated with water, and a surface layer of gravel (particle size 1.1 ± 0.6 cm ($n = 13$) to reduce evaporation. Pots were covered with lids (see Winter *et al*. 2001*a*), which had either a 3.4-cm or a 7.4-cm diameter hole in the center, through which the plants grew. Initial plant dry mass ranged between 0.04 and 1.43 g.

Tectona grandis Linn. f. was grown from 5 June 2000 to 23 October 2000, *Swietenia macrophylla* King from 8 May 2000 to 2 November 2000, *Zea mays* L. from 18 September 2000 to 23 October 2000,

Ananas comosus (L.) Merr. from 25 April 2000 to 12 February 2001, *Aloe vera* (L.) Webb. & Berth. from 25 April 2000 to 19 February 2001, *Euphorbia tirucalli* L. from 25 April 2000 to 15 January 2001, *Kalanchoë pinnata* (Lam.) Pers. from 25 April 2000 to 18 December 2000 and *Kalanchoë daigremontiana* Hamet et Perr. from 25 April 2000 to 08 January 2001.

Plants were maintained beneath six rain shelters made of aluminum frames that supported translucent shields of plexiglas at a height of 2.5 m. In previous studies, these same aluminum frames had been used to provide support for plastic film side walls of octagonally shaped open-top chambers 2 m across (Winter *et al*. 2000, 2001*b*). The side walls were removed in the present study.

Experiment 2

The second experiment was conducted with seven species of the neotropical genus *Clusia*: *C. cylindrica* Hammel, *C. odorata* Seeman, *C. pratensis* Seeman, *C. rosea* Jacq., *C. uvitana* Pittier, *C. valerioi* Standl. and *C*. sp. E [an identified but yet to be named species that corresponds to *Clusia* sp. E in Gehrig *et al*. (2003) and Holtum *et al*. (2004)], which were grown at the Smithsonian Tropical Research Institute Santa Cruz Experimental Field Facility in Gamboa, Republic of Panama. Plants were maintained under a permanent rain shelter consisting of a 4×8-m concrete floor and a 6×10-m translucent glass roof at 3–3.5 m height. There were no side walls. Soil containers were as described for experiment 1, except that pots were not covered with a lid. The initial dry masses of plants ranged between 1.8 and 7.8 g.

Microclimate

Climate data for Parque Natural Metropolitano and the rain shelters are provided by Winter *et al*. (2001*a*). Climate data for Santa Cruz, Gamboa, are available from the Smithsonian Web Site (http: //striweb.si.edu/esp/physical_monitoring/download_gamboa.htm; verified 24 March 2005). PFD was reduced by ∼10% underneath the Gamboa rain shelter, and maximum temperatures were increased up to ∼1°C. Free water evaporation was measured during experimental periods at the two sites using Etgage Model A evaporimeters (Etgage Co., Loveland, CO), which closely simulated evaporation from the water surface of a standard class A evaporation pan (Winter *et al*. 2001*a*; Table 1).

Transpiration ratio

The weight of plant containers was determined initially at 7-d intervals, and later at 2–3-d intervals with a 64-kg capacity balance with a resolution of 5 g (Sartorius model QS64B, Thomas, Swedesboro, NJ). Lost water was replaced after each measuring cycle to bring the pots to their initial weight (44 kg for experiment 1, 46 kg for experiment 2). Transpiration was calculated from the difference in weight loss of pots plus plants and control pots without plants, and corrected for biomass (fresh mass) increase. At the conclusion of experiments, plants were divided into roots, stems and leaves and dried at 65°C for dry mass determination. Leaf areas were measured with a LI-3100 Area Meter (Li-Cor, Lincoln, NE). The transpiration ratio, TR, was calculated as the ratio of cumulative water transpired to dry matter produced (g H_2O g^{-1} dry mass), a high TR ratio indicating low water-use efficiency and a low TR indicating high water-use efficiency.

Regressions between TR and $\delta^{13}C$ value or nocturnal CO_2 gain were fitted with Tablecurve 2D version 5.01 (SYSTAT Software Inc., CA).

Titratable acidity

At least four leaf discs, each of 1.4 cm^2, were excised from mature leaves at dusk and dawn, pooled, weighed and immediately frozen in liquid nitrogen. The discs were boiled sequentially in 50% methanol and H_2O, and titratable acidity was determined subsequently

Table 1. Evaporation measured at plant height under rain shelters at Parque Natural Metropolitano and at Santa Cruz, and measured in the open at Santa Cruz

Dry season is from January to April

Experiment 1, Parque Natural Metropolitano		Experiment 2, Santa Cruz, Gamboa		
	Evaporation (mm)		Evaporation (mm)	
2000 / 2001	Rain shelter	2002 / 2003	Rain shelter	In open
May	109	August	121	
June	76	September	113	95
July	86	October	104	95
August	86	November	93	69
September	89	December	152	132
October	76	January	172	147
November	87	February	169	164
December	103	March	214	206
January	156	April	157	143
February	171			

from the volume of 5 mM NaOH required to titrate the resulting extracts to pH 7.0.

Carbon-isotope ratio

Unless specified otherwise, oven-dried whole shoots (leaves and stems) were homogenised to a fine powder with a Tecator Cyclotec mill (Fisher Scientific, PA). Carbon-isotope ratios were determined for CO_2 derived from 3-mg samples of plant powder (Crayn *et al.* 2001; Pierce *et al.* 2002; Winter and Holtum 2002). Samples were analysed at the Analytical Chemistry Laboratory, Institute of Ecology, University of Georgia, Athens, GA, by isotope-ratio mass spectrometry. Following the appropriate corrections for other isotopes, the abundance of ^{13}C in each sample was calculated relative to the abundance of ^{13}C in standard CO_2 that had been calibrated against Pee Dee belemnite (*Belemnitella americana*). Relative abundance was determined by the relationship:

$$\delta^{13}C (‰) = [(^{13}C / ^{12}C \text{ of sample}) / (^{13}C / ^{12}C \text{ of standard}) - 1]$$

$$\times 1000.$$

Results and discussion

TR and δ^{13}C values of CAM, C_4 and C_3 species (experiment 1)

The TRs of species with differing pathways of photosynthesis were distinct. In experiment 1, the two C_3 tree species, *T. grandis* and *S. macrophylla*, exhibited ratios of 373 and 256 g H_2O g^{-1} dry mass, respectively, which set them clearly apart from the C_4 grass, *Zea mays*, with a ratio of 132 g H_2O g^{-1} dry mass and the group of CAM species with low TR values of between 109 and 54 g H_2O g^{-1} dry mass (Table 2). The 50% higher water use of *T. grandis* compared with *S. macrophylla* is consistent with differences in leaf orientation. The leaflets of *S. macrophylla* subtended the rachis assuming a semi-vertical position, whereas the much larger leaves of *T. grandis* were horizontally oriented and intercepted higher radiation loads. In a previous study at

Table 2. Final dry mass (above- plus below-ground), amount of water transpired per unit dry mass produced and δ^{13}C value of the above-ground biomass of seedlings of two tropical C_3 trees, a C_4 grass, and five species exhibiting crassulacean acid metabolism

Values are means $\pm$ s.d. ($n = 3$)

Species	Final dry mass (g)	Final / initial	Transpiration ratio (g H_2O g^{-1} dry mass)	δ^{13}C (‰)
C_3 species				
Swietenia macrophylla	31 $\pm$ 5	222 $\pm$ 34	256 $\pm$ 9	−28.7 $\pm$ 0.5
Tectona grandis	28 $\pm$ 5	165 $\pm$ 29	373 $\pm$ 18	−27.8 $\pm$ 0.3
C_4 species				
Zea mays	36 $\pm$ 6	96 $\pm$ 15	132 $\pm$ 9	−10.6 $\pm$ 0.1
CAM species				
Aloe vera	125 $\pm$ 22	219 $\pm$ 38	54 $\pm$ 6	−14.8 $\pm$ 0.0
Ananas comosus	167 $\pm$ 51	116 $\pm$ 36	63 $\pm$ 10	−13.5 $\pm$ 0.3
Euphorbia tirucalli	73 $\pm$ 8	146 $\pm$ 16	105 $\pm$ 6	−16.5 $\pm$ 0.5
Kalanchoë daigremontiana	68 $\pm$ 12	>500	109 $\pm$ 8	−16.3 $\pm$ 0.3
Kalanchoë pinnata	88 $\pm$ 20	>500	106 $\pm$ 14	−19.3 $\pm$ 0.2

the same site, comparable seasonally dependent TRs ranging between 229 and 309 g H_2O g^{-1} dry mass were measured for *Ficus insipida* (Winter *et al.* 2001*a*), a fast-growing C$_3$ pioneer tree capable of extremely high rates of photosynthetic net CO_2 uptake (Zotz *et al.* 1995).

The five CAM species studied in experiment 1 had δ^{13}C values of −13.5 to −19.3‰ indicating that in all of them the CAM cycle was well expressed, albeit to different degrees (Winter and Holtum 2002). Their low TRs are similar to TRs reported in the literature for a range of CAM plants that include *Ananas comosus* (55 g H_2O g^{-1} dry mass, Sideris and Krauss 1955; 50 g H_2O g^{-1} dry mass, Joshi *et al.* 1965), *Agave americana* (47–68 g H_2O g^{-1} dry mass, Neales *et al.* 1968; 71 g H_2O g^{-1} dry mass, Ehrler 1969), and *Echeveria pumila* (135 g H_2O g^{-1} dry mass, Meinzer and Rundel 1973), despite marked differences in plant size, pot size and growth conditions. *Aloe vera*, with a TR of 54 g H_2O g^{-1} dry mass, was the most water-use efficient species in our study. Even lower TRs, ranging from 19 to 53 g H_2O g^{-1} dry mass, were reported for 10 species of *Opuntia* cultivated in the arid Mediterranean region of Chile (Silva and Acevedo 1995). In contrast, a relatively high TR of 162 g H_2O g^{-1} dry mass was reported for *Opuntia ellisiana* growing in plantations in south Texas (Han and Felker 1997). In the latter study, the dry mass of roots was not included in the estimation of TR, and transpiration was derived from meteorological and soil water-balance determinations rather than measured gravimetrically.

As predicted from theory (Farquhar *et al.* 1989), the TR of the C$_4$ grass *Zea mays* was between 35 and 52% of the TR for the two C$_3$ species, but higher than the TRs of all five CAM species. The TR value for well watered *Zea mays* was similar to values reported for other C$_4$ species (Ghannoum *et al.* 2002).

TR and δ^{13}C values of Clusia species (experiment 2)

A similar relationship to that determined for TR and δ^{13}C value in C$_3$ and constitutive CAM species was observed for 7 species of *Clusia* grown in a terrestrial mode (Table 3). The two CAM species examined, *C. rosea* and *C. uvitana*, with the largest nocturnal acid accumulation in the leaf tissue and the least negative δ^{13}C values, expressed the lowest TRs. *Clusia pratensis*, at the other extreme, had the highest TR of 496 g H_2O g^{-1} dry mass and the most negative δ^{13}C value. Plants of *C. pratensis* were fast growing and attained the second-highest total plant biomass of all species. Although *C. pratensis* had high background levels of titratable acidity, it did not exhibit significant nocturnal acid accumulation in this experiment (Fig. 1); in other experiments it has been shown to have the ability to express weak CAM (Holtum *et al.* 2004). In general, the δ^{13}C values of individual representative mature leaves of the *Clusia* species were similar to δ^{13}C values of the bulk dry mass of whole shoots, suggesting that the δ^{13}C value of representative mature leaves can be a reasonable indicator of whole-plant CAM expression (Table 3). However, for *C. rosea*, which exhibited the largest capacity for nocturnal accumulation of titratable acidity, the selected leaf was significantly less negative, by 1.1‰, than the bulk dry mass of whole shoots, and for *C. pratensis*, which appeared to be operating primarily as a C$_3$ plant, the selected leaf was significantly more negative, by −1.1‰.

## Correlations between TR, δ^{13}C and nocturnal CO$_2$ gain

The relationship between TR and δ^{13}C for the five species of constitutive CAM plants, the seven species of *Clusia*, and the two species of obligate C$_3$ species examined over a wide range of values between −13.5‰

Table 3. Final dry mass (above- plus below-ground), amount of water transpired per unit dry mass produced and δ^{13}C value of the above-ground biomass of seven species of *Clusia*, in two of which crassulacean acid metabolism was well expressed (*C. rosea* and *C. uvitana*)

Values are means ± s.d. (*n* = 5). Experimental period: 15 July 2002–28 April 2003. The δ^{13}C values for leaves and stems were calculated from values measured separately for 'all leaves' and stems of plants and their leaf / stem dry mass ratio

Species	Final dry mass (g)	Final / initial	Transpiration ratio (g H_2O g^{-1} dry mass)	δ^{13}C (‰)			
				Mature leaf	All leaves	Stems	Leaves and stems
Clusia cylindrica	115 ± 32	40 ± 11	297 ± 33	−25.3 ± 1.1	−26.4 ± 0.9	−26.4 ± 0.7	−26.4 ± 0.8
Clusia odorata	52 ± 10	29 ± 5	335 ± 29	−26.3 ± 0.5	−25.7 ± 0.3	−26.4 ± 0.2	−25.9 ± 0.3
Clusia pratensis	105 ± 7	24 ± 2	496 ± 43	−27.9 ± 0.5	−27.1 ± 0.2	−26.3 ± 0.2	−26.8 ± 0.2
Clusia rosea	66 ± 27	34 ± 14	84 ± 17	−16.1 ± 0.3	−17.4 ± 0.4	−15.9 ± 0.5	−17.2 ± 0.4
Clusia uvitana	90 ± 21	22 ± 5	227 ± 14	−22.9 ± 1.5	−22.6 ± 0.7	−21.5 ± 0.7	−22.3 ± 0.7
Clusia valerioi	56 ± 28	8 ± 4	291 ± 24	−26.4 ± 0.4	−26.0 ± 0.5	−24.7 ± 0.4	−25.6 ± 0.4
Clusia sp.[A]	82 ± 24	111 ± 32	335 ± 29	−26.4 ± 0.4	−26.5 ± 0.4	−26.0 ± 0.5	−26.3 ± 0.4

[A]Corresponds to *Clusia* sp. E in Gehrig *et al.* (2003) and Holtum *et al.* (2004).
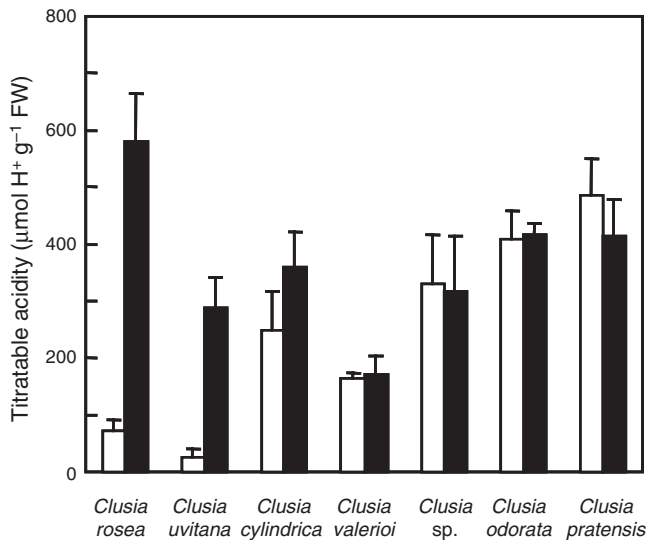
Fig. 1. Titratable acidity in mature leaves of seven species of *Clusia* on 23 and 24 April 2003, 5 d before plants were harvested for biomass and determination of transpiration ratios (mean ± s.d., $n = 3$). *Clusia* sp., an identified but yet to be named species that corresponds to *Clusia* sp. E in Gehrig *et al.* (2003) and Holtum *et al.* (2004), was titrated to pH 7.5 whereas all other species were titrated to pH 7.0. Open bars, dusk; closed bars, dawn. Differences between dusk and dawn were significantly different ($P < 0.01$) in *C. rosea* and *C. uvitana*.
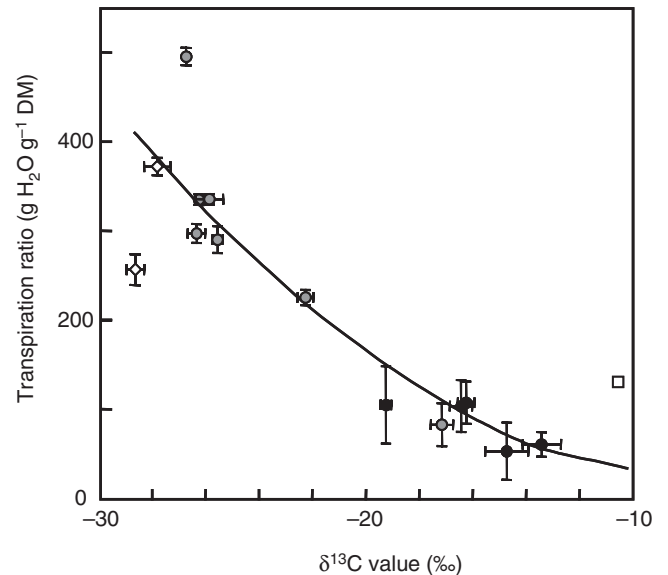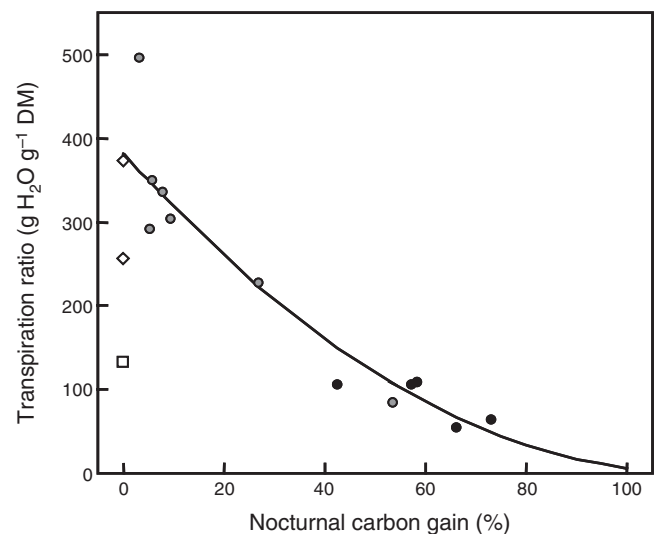

Fig. 2. Relationship between transpiration ratio and δ^{13}C value for two species of C_3 tropical trees ($\diamond$), one C_4 grass species ($\square$), five constitutive CAM species ($\bullet$) and seven species of *Clusia* ($\bullet$). The bars indicate the standard deviation; where no bars are present the variation is smaller than the size of the symbol. The values are described by the equation $Y^{0.5} = a + bx$ ($r^2 = 0.791$), where $a = -3.95797$ and $b = -0.84487$.

and $-28.7‰$ could be described by curvilinear function, $Y^{0.5} = a + bx$ (for which $a = -3.9580$ and $b = -0.84487$), with an r^2 of 0.791, which suggests that δ^{13}C value is a reasonable predictor of TR (Fig. 2). The departure from the fitted curve was greatest for plants with δ^{13}C values of approximately -27 to $-30‰$ where plants exhibited large variation in TR over a small δ^{13}C range. The relationship between TR and δ^{13}C in the C_4 species differed from the CAM and C_3 species in that the TR for *Zea mays* was greater than predicted by the calibration curve for the δ^{13}C value measured (Fig. 2). Although fractionation during CO_2 uptake in C_4 and CAM plants is dictated by the characteristics of PEPC, CAM plants lose less H_2O because CO_2 fixation occurs during the night when the driving forces for transpirational water loss are lower than during the day. In our study TR in the C_4 species was equivalent to that observed for a CAM plant that exhibited ∼50% nocturnal CO_2 gain.

Although the curvilinear function $Y^{0.5} = a + bx$ was fitted to the relationship between TR and δ^{13}C for the C_3 and CAM plants, a straight line regression produced a marginally better fit ($r^2 = 0.804$) but by intercepting the axis at $-12.1‰$ the linear regression violated the assumption that transpiration should be small but positive at δ^{13}C values expected for 100% dark CO_2 gain.

The predictive ability of δ^{13}C content for TR was poorest for *S. macrophylla* and *C. pratensis*, two of the species that exhibited C_3-like δ^{13}C values (Figs 2 and 3). The TRs of

Fig. 3. Relationship between transpiration ratio and the proportion of CO_2 gain accrued during the dark for two species of C_3 tropical trees ($\diamond$), one C_4 grass species ($\square$), five constitutive CAM species ($\bullet$) and seven species of *Clusia* ($\bullet$). The proportion of CO_2 gain accrued during the dark was obtained by applying the δ^{13}C values shown in Fig. 1 to the calibration established by Winter and Holtum (2002) for the relationship between CO_2 gain in the light and the dark and δ^{13}C value for well watered CAM plants. The relationship between transpiration ratio and the proportion of CO_2 gain accrued is described by the equation $Y^{0.5} = a + bx$ ($r^2 = 0.837$), where $a = 19.55549$ and $b = -0.17257$.

S. macrophylla and *C. pratensis* ranged between 256 and 496 g H_2O g^{-1} dry mass, i.e. a 1.9-fold difference, but their $\delta^{13}C$ values differed by only 1.9‰. In comparison, the intraspecific variation over a similar isotopic range for 16 genotypes of wheat was between 196 and 238 g H_2O g^{-1} dry mass, i.e. 1.2-fold (Condon and Richards 1993), and was between 200 and 285 g H_2O g^{-1} dry mass, i.e. 1.4-fold, for 10 genotypes of barley (Acevedo 1993).

The model overestimated TR in *S. macrophylla*, probably because its leaf morphology and habit favours lower average leaf temperatures and smaller boundary layers. The driving forces for transpiration would be expected to be lower in smaller, subtended leaves than in the large horizontal leaves of *T. grandis* and the more succulent leaves of *C. pratensis*.

In contrast to *S. macrophylla*, *C. pratensis* exhibited a TR that, at 496 g H_2O g^{-1} dry mass, was ~30% higher than predicted by the model. In comparison to the obligate C$_3$ species, *T. grandis* and *S. macrophylla*, which were grown during the wet season in experiment 1, *C. pratensis* was grown during the wet and dry season and accumulated much of its carbon during the dry season when the rates of evaporation were about twice those during the wet season (Table 1). However, the high TR in *C. pratensis* cannot be explained by differences in evaporative demand alone as the other *Clusia* species studied also completed much of their growth during the same period as *C. pratensis*, yet exhibited TRs that were well predicted by $\delta^{13}C$ value. Clearly *C. pratensis* warrants further detailed study of how growth and isotopic complement are related to CO_2 and H_2O-vapour exchange.

With the exception of the petroleum plant, *E. tirrucalli* (Maugh 1976; Calvin 1980), a largely stem-succulent species, the CAM species included in our study were leaf succulents. It is not known whether the TR–$\delta^{13}C$ relationship differs between terrestrial leaf and stem succulents and epiphytic CAM species or between groupings of CAM species based on other criteria such as growth altitude, taxonomic affiliation or CAM metabolic variant (Christopher and Holtum 1996). For example, in C$_4$ species TR differed significantly between 9 NAD-ME and 9 NADP-ME species when plants were water-stressed but not when well watered (Ghannoum *et al.* 2002).

The derivation of the relationship between TR and percentage CO_2 gain during the dark (Fig. 3), obtained by transforming $\delta^{13}C$ values to percentage CO_2 gain during the dark using the calibration established for well watered CAM and C$_3$ species (Winter and Holtum 2002, 2005), was well described for the C$_3$ and CAM species but not the C$_4$ species by the curvilinear function, $Y^{0.5} = a + bx$ (for which a = 19.55549 and b = −0.17257; r^2=0.837). As with the fit between TR and $\delta^{13}C$ values (Fig. 2), curvilinear and linear regressions produced similar fits (r^2=0.837 *v.* r^2=0.832 respectively) for the relationship between TR and percentage

CO_2 gain during the dark but once again the linear regression violated the assumption that transpiration must be positive at 100% dark CO_2 gain.

The TR–CO_2 gain relationship predicted extensive contribution of nocturnal CO_2 gain to growth in *C. rosea* (43%) and *C. uvitana* (27%), two species in which significant nocturnal increases in titratable acidity were determined 5 d before the plants were harvested (Figs 1 and 3). For the remaining five *Clusia* species tested, no significant nocturnal increases in titratable acidity were detected 5 d before the plants were harvested, but the model predicted small contributions of nocturnal CO_2 gain to the carbon budget of between 3 and 9% (Fig. 3). Indeed, each of the five species has been reported to exhibit a capacity for C$_3$ photosynthesis and weak CAM on the basis of C$_3$-like $\delta^{13}C$ values and seasonally dependant expression of nocturnal increases in titratable acidity (Wanek *et al.* 2002, Holtum *et al.* 2004). Although our calibrations are not yet robust enough to detect with confidence contributions of nocturnal CO_2 gain of less than ~10%, the predictive value of the relationships between TR and $\delta^{13}C$ or dark CO_2 gain illustrated in Figs 2 and 3 should improve once a larger number of C$_3$ and weak CAM species with $\delta^{13}C$ values more negative than −26‰ have been assessed and the effects of environmental and biological variables analysed in greater detail.

Conclusions

This study broadens the previously small database on whole-plant WUE in CAM plants, and consistently demonstrates a lower water-requirement for biomass accumulation in CAM plants than in C$_3$ plants grown at the same site. The WUE of CAM species increases with increasing expression of CAM as indicated by a close relationship between TR and $\delta^{13}C$ value, a relationship that is broadly similar for CAM plants with differing leaf morphologies. $\delta^{13}C$ value can therefore be used as an indicator of the expression of CAM and also as an indicator of WUE. The example of *Clusia pratensis* demonstrates that the potential to exhibit weak CAM under stressful conditions does not preclude high transpirational water loss in relation to dry mass production when water supply is abundant and the plants operate in the C$_3$ mode. The observation that $\delta^{13}C$ measurements of representative mature *Clusia* leaves provide reasonable estimates of the $\delta^{13}C$ value of whole shoots should facilitate more detailed analyses of the relationships between WUE and photosynthetic pathway options in this physiologically plastic genus.

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