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Ecophysiological Significance of CO_2-Recycling via Crassulacean Acid Metabolism in *Talinum calycinum* Engelm. (Portulacaceae)[1]

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ABSTRACT

High levels of variability in gas exchange characteristics and degree of CAM-cycling were found in the same and different individuals of *Talinum calycinum* Engelm. collected from rock outcrops in Missouri. Differences in CO_2 assimilation were mostly correlated with differences in shoot conductance to CO_2 not shoot internal CO_2 concentration. As found previously, CAM acid fluctuations were evident in well-watered plants exhibiting C_3 gas exchange patterns (CAM-cycling) and also in drought-stressed plants with stomata closed, or nearly so, day and night (CAM-idling). Drought stress also resulted in rapid stomatal closure, conserving water during droughts. Maximal CO_2 uptake rates occurred below 35°C; higher temperatures induced decreases in CO_2 assimilation and conductance while shoot internal CO_2 concentrations remained similar. Plant water-use-efficiency was severely curtailed at temperatures above 30°C. Tissue acid fluctuations were the result of changes in malic acid concentrations. Calculations of the amount of water potentially conserved by CAM-cycling yielded values of approximately 5 to 44% of daytime water loss. Thus, CAM-cycling may be an important adaptation minimizing water loss by perennial succulents growing in shallow soil on rock outcrops.

The ecophysiological significance of the nocturnal assimilation of atmospheric CO_2 via CAM in many plants inhabiting xeric macro- or microhabitats is now quite clear—conservation of water (11). Since nights are nearly always characterized by lower temperatures and higher relative humidities, compared with days, fewer moles of water are lost by CAM plants per mole of CO_2 fixed at night, relative to non-CAM plants (10, 11). Of course, this higher WUE[3] is only true if the stomata of CAM plants remain closed for most of the day.

Recently, several species of plants have been found having characteristics of both C_3 and CAM plants. A now-classic example is that of *Mesembryanthemum crystallinum* which is a C_3 plant when well-hydrated yet converts to CAM when drought- or salt-stressed (39, 40). The ecophysiological significance of such C_3-CAM intermediacy is obvious: growth is maximized via the C_3 pathway when water is plentiful, while water conservation is maximized via CAM during droughts. On the other hand, the ecophysiological significance of another form of C_3-CAM intermediacy is less clear. Many species are now known which exhibit C_3 gas exchange patterns accompanied by CAM-like tissue acid fluctuations (Table I). When stomata are closed at night, tissue acidity increases, presumably a result of the fixation of respiratory CO_2 by PEP carboxylase (27, 28, 41). Upon illumination, the stomata open and atmospheric CO_2 is fixed in addition to CO_2 arising from decarboxylation of acid. Thus, tissue acidity decreases throughout the day. Because all atmospheric CO_2 is fixed by RuBP carboxylase/oxygenase, these plants exhibit C_3-like stable carbon isotope ratios (16, 18, 26, 29). This phenomenon has been termed 'CAM-cycling' (26) and has been reported in 43 species in 15 families (Table I)

Thus far, three hypotheses have been forwarded regarding the potential benefits of CAM-cycling: (a) Ting *et al.* (21, 23, 34) have suggested that this process is simply a precursor to CAM-idling (described below). It is clear, however, that CAM-cycling is not a prerequisite for CAM-idling since some plants lack entirely acid fluctuations when well-watered, yet undergo CAM-idling when drought-stressed (7); (b) Martin *et al.* (16–18) have hypothesized that plants with CAM-cycling will benefit from the conservation of CO_2 otherwise presumably lost through the cuticle or incompletely closed stomata at night. However, this hypothesis, strictly interpreted, is untenable since a plant must expend the same amount of energy to fix internally produced CO_2 as for atmospheric CO_2; and (c) on the other hand, Cockburn (2) has suggested that such refixation should conserve water. This assumes, of course, that the plant would fix more atmospheric CO_2 if internal CO_2 were not available. Unfortunately, no data are available estimating the amounts of water potentially conserved.

CAM-idling is similar to CAM-cycling in that nocturnal increases in acidity are also observed without concomitant stomatal opening (27, 28). Unlike CAM-cycling, however, CAM-idling occurs only under severe drought stress. Thus, stomata remain closed throughout the day and night. This process has been detected in plants with C_3, CAM, and CAM-cycling modes of photosynthesis (7, 16, 18, 21, 23, 28). The ecophysiological significance of CAM-idling is presumably the maintenance of an active metabolic state during severe droughts, allowing rapid recovery of photosynthetic gas exchange after minimal precipitation events (27, 28).

CAM-cycling is found in several species of succulents growing in shallow soil overlying rock outcrops in the USA (16–18). Although one such plant, *Talinum calycinum*, has been investigated previously and can undergo both CAM-cycling and CAM-idling (18), many questions regarding these processes remain unanswered. It has yet to be shown that the predominant acid involved in CAM-cycling in this species is malic acid. Also, levels of variability in this process have been ignored. Finally, though Martin and Zee (18) calculated the amount of CO_2 conserved by CAM-cycling in *T. calycinum*, no attempts were made to estimate the amount of water potentially conserved as a result of refixing internal CO_2 at night. Thus, it was the purpose of this paper to investigate further the process of CAM-cycling in this species, focusing on the potential conservation of water resulting

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[3] Abbreviations: WUE, water use efficiency; DW, dry weight; FW, fresh weight; PPFD, photosynthetic photon flux density.

Table I. *Species Exhibiting CAM-Cycling, i.e., CAM-like Diurnal Acid Fluctuations Concomitant with C_3 Gas Exchange Patterns*

Also included are several species having CAM-like acid fluctuations and C_3-like stable carbon isotope ratios.

Species	Family	Reference
Sesuvium maritimum	Aizoaceae	17
Senecio mikaniodes	Asteraceae	26
Adenia keramanthus	Asteraceae	26
Catopsis nutans	Bromeliaceae	20
Guzmania monostachia	Bromeliaceae	13
Puya floccosa	Bromeliaceae	20
Tillandsia adpressiflora	Bromeliaceae	20
Vriesea platynema	Bromeliaceae	20
Pereskia grandifolia	Cactaceae	21
Pereskia aculeata	Cactaceae	21
Pereskiopsis velutina	Cactaceae	26
Aellenia subaphylla	Chenopodiaceae	42
Horaninowia ulicina	Chenopodiaceae	42
Salsola richteri	Chenopodiaceae	42
Salsola praecox	Chenopodiaceae	42
Diamorpha cymosa	Crassulaceae	31
Diamorpha smallii	Crassulaceae	17
Dudleya blochmanae	Crassulaceae	30
Sedum acre	Crassulaceae	22
Sedum album	Crassulaceae	4
Sedum greggii	Crassulaceae	29
Sedum mite	Crassulaceae	22
Sedum nuttallianum	Crassulaceae	16
Sedum pulchellum	Crassulaceae	—[a]
Sedum rubrotinctum	Crassulaceae	32
Sedum telephioides	Crassulaceae	17
Sedum ternatum	Crassulaceae	17
Euphorbia milii	Euphorbiaceae	19
Pedilanthus tithymaloides	Euphorbiaceae	19
Pelargonium crassicaule	Geraniaceae	26
Stylites andicola	Isoetaceae	9
Yucca elata	Liliaceae	24
Yucca gloriosa	Liliaceae	17
Drosanthemum hispidum	Mesembryanthemaceae	15
Peperomia camptotricha	Piperaceae	23
Peperomia orba	Piperaceae	33
Portulaca oleracea	Portulacaceae	12
Talinum calycinum	Portulacaceae	18
Talinum parviflorum	Portulacaceae	—[a]
Talinum teretifolium	Portulacaceae	17
Cissus quadrangularis	Vitaceae	36
Cissus tuberosa	Vitaceae	26
Welwitschia mirabilis	Welwitschiaceae	34

[a] C. E. Martin (unpublished data).

from recycling CO_2.

MATERIALS AND METHODS

Plant Material. Plants of *T. calycinum* Engelm. were collected from shallow soil overlying limestone along Shoal Creek, 1 km south of Joplin, Newton County, MO and in the town of Pioneer in the same county. Plants were potted in soil (3 parts sand/topsoil, 2 parts peat, 1 part vermiculite) in styrofoam cups (6 cm diameter), placed in the University of Kansas greenhouse, and watered and fertilized regularly. Environmental conditions in the greenhouse approximated those outside in the summer; additional heat was provided in the winter. After death of the shoots, rhizomes were cold-treated (4°C) for approximately 1 month, then returned to the greenhouse. Subsequently produced shoots were also used in the experiments. All plants were either flowering or with flower buds when used for measurements.

Gas Exchange and Acid Fluctuations. Rates of CO_2 and H_2O vapor exchange of each of 13 plants were monitored continuously for 2 to 4 d after sealing the shoot in the gas exchange chamber; data from d 1 were never used. Plants were kept well watered. Description of the chambers, gas exchange system, calculations of gas exchange, and determinations of tissue dry weight have been described previously (16, 18). Whole-shoot conductances to CO_2 and internal CO_2 concentrations were calculated using equations from Farquhar and Sharkey (5). WUE was calculated by dividing a single CO_2 uptake rate by the simultaneous transpiration rate, or integrated WUE were derived from CO_2 and H_2O exchange data throughout an entire day. Chamber environmental conditions were: PPFD inside the chamber of approximately 1500 μmol m^{-2} s^{-1} (14-h photoperiod), day/night temperatures of 30.0/20.0°C, and a 24-h dew point of 13.0°C. Daytime leaf temperatures varied depending on placement of the thermocouple but were typically 1 to 2°C above air temperature. Chamber CO_2 concentrations were usually within the range 340 to 360 μl L^{-1}.

Simultaneously with monitoring gas exchange with one plant, two other plants (one in one experiment) were placed in identical chambers under the same environmental conditions. These plants were not monitored for gas exchange but instead were sampled for titratable acidity. This was necessary given the small size of the plants, *i.e.* gas exchange rates were too low after removal of tissue for acidity determinations. During the final 24-h period of gas exchange measurements, these plants were sampled for tissue titratable acidity shortly before lights-on and again before lights-out. Acid content of the gas exchange plants was also determined when plants were removed from the chambers.

Nine individuals of *T. calycinum* were allowed to dry under metal halide lamps for 24 h which was sufficient to induce leaf wilting and shrinkage. Three were then placed in gas exchange chambers while the others were used for determinations of tissue acid fluctuations as described above. Chamber environmental conditions were also as above. Attempts to measure water potentials of the leaves or shoot proved fruitless with either a pressure chamber or the Shardakov dye method, presumably a result of the succulent nature of the tissue as well as its high mucilage content. Regardless, plants for these experiments were clearly drought-stressed as indicated above (also see Table II).

Effects of Temperature on Gas Exchange. Rates of CO_2 and H_2O vapor exchange of each of three plants were monitored continuously for 9 d after shoots were sealed in the gas exchange chamber. Plants were kept well watered throughout. PPFD inside the chamber was 1500 to 1700 μmol m^{-2} s^{-1}. Day/night air temperatures were changed day to day in the following sequence: 30/20, 30/20, 35/25, 40/30, 30/20, 25/15. 20/10, 45/35, and 30°C the day of plant removal. Air dew point was 15.5°C except when day/night air temperature was 25/15 and 20/10°C; the respective day/night dew points were then 15.5/10.0°C and 9.0/5.5°C. These changes were made to avoid water condensation inside the chambers. The replicate 30/20°C regime on d 5 allowed comparison of photosynthetic rates with those on the first 2 d. No substantial differences were observed.

Tissue Acidity and Malate Content. Tissue titratable acidity was measured as described previously (14). Tissue malate content was determined according to Hohorst (8) following grinding with a mortar and pestle. The same extract was used for each plant in the acidity-malate comparison. Overnight (or nocturnal) accumulation of acid refers to the difference between morning and late afternoon acidity levels.

Statistical Analyses. Where appropriate, data were analyzed with a Student's *t*-test, a Pearson rank correlation, or an analysis of variance. Differences between means were considered significant when $P < 0.05$.

RESULTS

Under well watered conditions, none of the 13 individuals of *T. calycinum* exhibited nocturnal CO_2 uptake (results of two plants given in Fig. 1A). All plants responded to light by opening their stomata (Fig. 1B), actively absorbing CO_2, and displaying lower shoot internal CO_2 concentrations (Fig. 1C). In 25 plants kept under identical conditions, the average overnight accumulation of acid was 348.59 μeq g^{-1} DW (SD = 333.43); only one plant did not exhibit an acid accumulation. Thus, it is most

Table II. *FW/DW Ratios for 45 Well Watered and 9 Drought-Stressed Individuals of Talinum calycinum under Identical Environmental Conditions*

Tissue samples were taken simultaneously with collections for titratable acidity (see "Materials and Methods"). Most plants were sampled twice and some plants were used for experiments not reported in this study.

Treatment	FW/DW Ratio		N	Probability[a]
	Mean	SD		
Well watered	12.39	3.30	76	$P < 0.01$
Drought stressed	9.34	2.88	15	

[a] Result of *t*-test for significance of difference between the means.

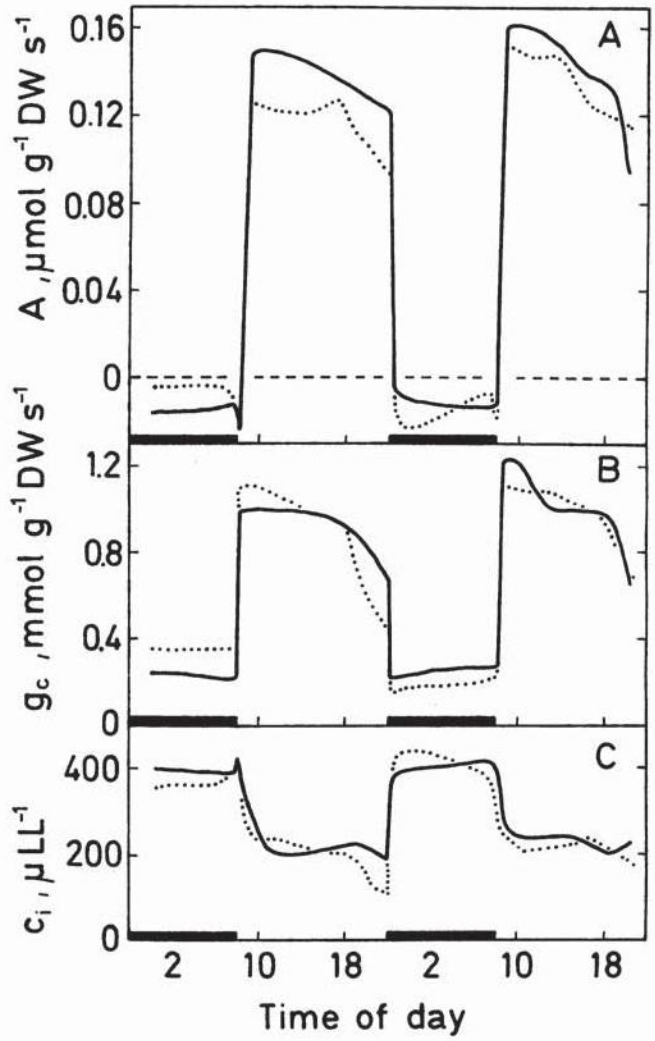

FIG. 1. CO_2 exchange (A; A), shoot conductance to CO_2 (g_c; B), and shoot internal CO_2 concentration (c_i; C) in two well-watered individuals of *T. calycinum*. Environmental conditions within the gas exchange chamber are given in "Materials and Methods." Black bars indicate darkness.

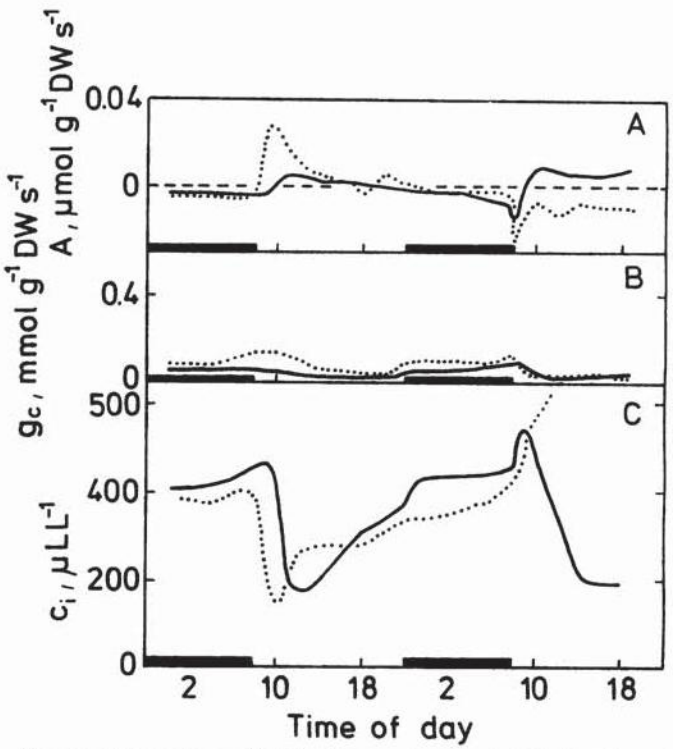

FIG. 2. CO_2 exchange (A; A), shoot conductance to CO_2 (g_c; B), and shoot internal CO_2 concentration (c_i; C) in two desiccated individuals of *T. calycinum*. Environmental conditions within the gas exchange chamber as in Figure 1 (see "Materials and Methods"). Black bars indicate darkness.

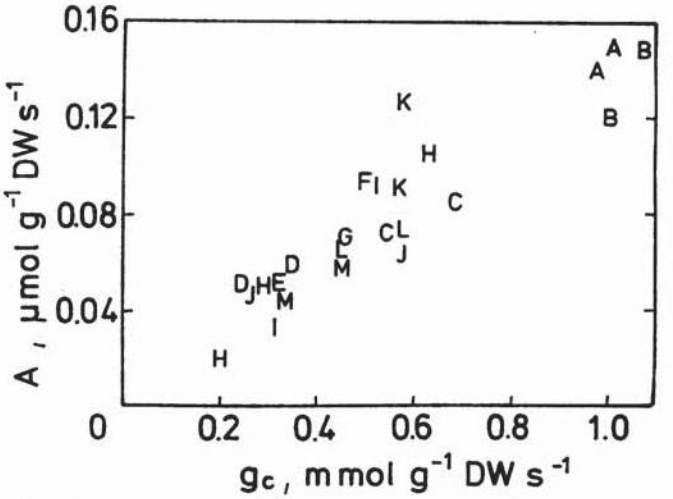

FIG. 3. Relationship between CO_2 exchange rate (A) and shoot conductance to CO_2 (g_c) at 1400 h in 13 individuals of *T. calycinum*. Measurements were conducted for 1 to 3 d with each individual. Different plants are denoted by different letters. Correlation analysis yields: $y = 0.1311x + 0.0085$; $r = 0.919$ ($P < 0.001$).

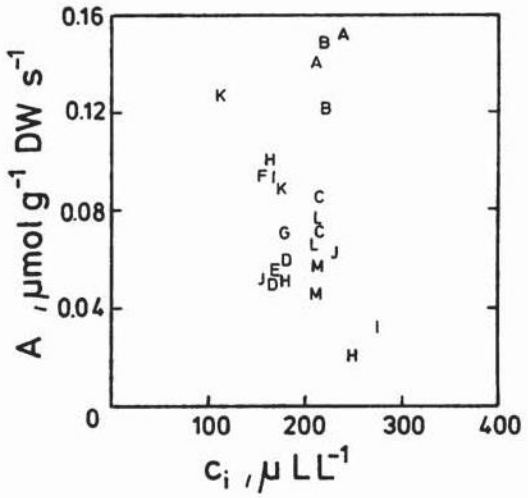

FIG. 4. Relationship between CO_2 exchange rate (A) and shoot internal CO_2 concentration (c_i) at 1400 h in 13 individuals of *T. calycinum*. Measurements were conducted for 1 to 3 d with each individual. Different plants are denoted by different letters. Correlation analysis yields: $x = -148.36y + 210.62$; $r = -0.151$ (not significant).

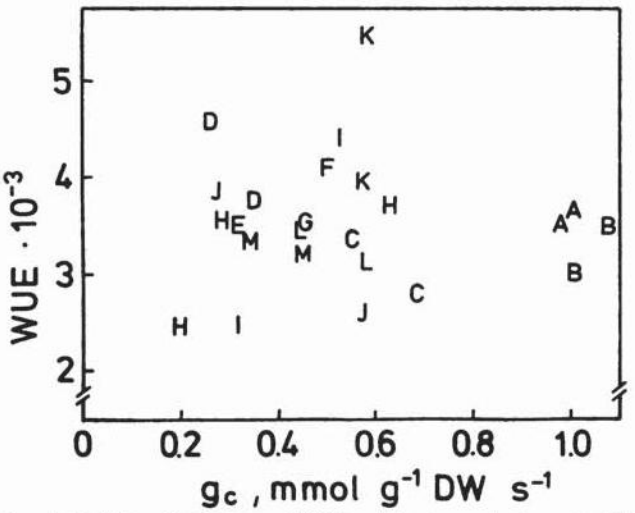

FIG. 5. Relationship between WUE and shoot conductance to CO_2 (g_c) at 1400 h in 13 individuals of *T. calycinum*. Measurements were conducted for 1 to 3 d with each individual. Different plants are denoted by different letters. Correlation analysis yields: $y = -0.0128x + 3.5311$; $r = -0.005$ (not significant).

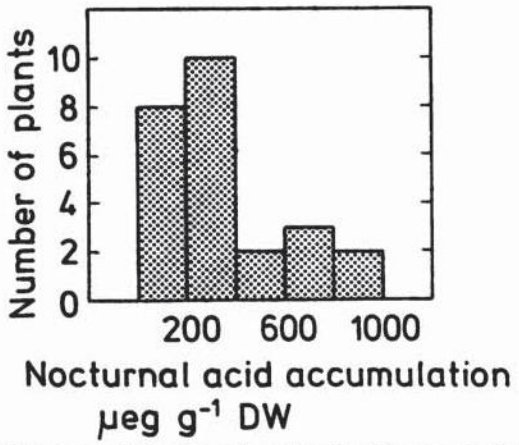

FIG. 6. Frequency histogram of nocturnal acid accumulation in 25 well watered individuals of *T. calycinum* under environmental conditions identical with those in Figure 1 (see "Materials and Methods").

likely that plants in the gas exchange chambers were accumulating acid at night when the stomata were closed, or nearly so (Fig. 1A). Withholding water for 3 d in the laboratory resulted in nearly complete 24-h stomatal closure in three individuals of *T. calycinum* (Fig. 2). Very low rates of nocturnal CO_2 assimilation were observed in only one of the plants (data not shown). The mean nocturnal acid accumulation in six individuals treated identically was 246.95 μeq g^{-1} DW (SD = 107.16), which is not significantly different from the value for the well watered plants (Student's *t*-test; $P < 0.05$). In two of the three plants, this acid accumulation necessarily resulted from refixation of internally produced CO_2; in the third, enough atmospheric CO_2 was assimilated at night (180 μmol CO_2 g^{-1} DW) to account for an acid accumulation of this magnitude, assuming one CO_2 yields one malic acid and one malic acid yields two titratable protons.

Although the two plants in Figure 1 were selected for their uniformity in photosynthetic characteristics, a high level of variability was a more common finding (Figs. 3–5). Variability between days with the same plant can be small (see plants "A" and "B" in Figs. 3–5) or rather large (see plants "H" and "I" in Figs. 3–5). Environmental conditions were identical during all measurements. Between-plant variability in CO_2 assimilation rates was also very high and was highly correlated with differences in shoot conductance to CO_2 (Fig. 3); shoot internal CO_2 concentrations did not differ substantially between plants (Fig. 4). No correlation was found between WUE and plant conductance in 13 individuals of *T. calycinum* (Fig. 5). High levels of variability were also encountered in nocturnal acid accumulation in 25 plants under identical conditions (Fig. 6). The range of nocturnal acid increase was 0 to 1262.18 μeq g^{-1} DW; the mean and SD were given above.

Air temperatures less than 40°C had little effect on CO_2 assimilation by *T. calycinum*, but higher temperatures decreased CO_2 uptake rates (Fig. 7A). Correlated with the high temperature-induced decline in CO_2 uptake rates was a similar decline in shoot conductance to CO_2 (Fig. 7B). Internal CO_2 concentrations did not change between 20 and 45°C (Fig. 7C). Maximum WUE were observed at 20 and 25°C; high temperatures resulted in a drastic decline in WUE (Fig. 7D). Although high levels of variability were encountered, the proton concentrations in plant extracts were usually twice the malate concentrations in the same extracts (Fig. 8). This relationship was clearer at high acid contents than low.

DISCUSSION

Under well watered conditions, plants of *T. calycinum* undergo CAM-cycling, *i.e.* they exhibit C_3 gas exchange patterns and CAM tissue acid fluctuations. Without water, *T. calycinum* maintains acid fluctuations while exhibiting little or no CO_2 uptake. Similar results were reported previously (18). Some individuals of this species apparently can exhibit CAM under drought stress (one plant in this study; see also Martin and Zee [18]).

Levels of variability in all photosynthetic parameters were very high. This includes day to day intraplant variability as well as that between individuals. Differences in photosynthesis may result from differences in past history before collection, different rhizome sizes, dissimilar developmental stages of the shoots, and differential treatment prior to experimentation, although attempts were made to minimize all these differences. Given the latter, it appears likely that much of the variability encountered

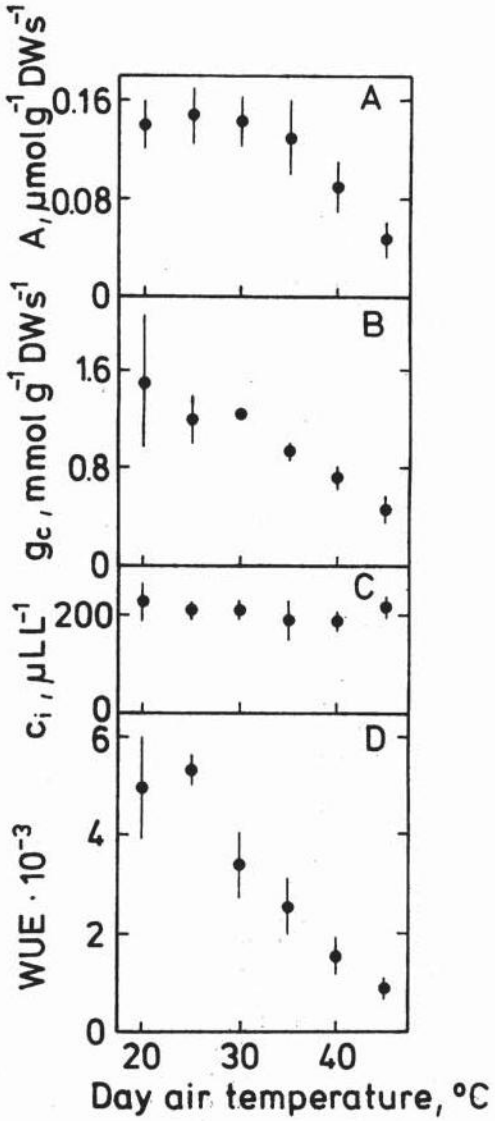

FIG. 7. Mean responses of CO_2 exchange (A; A), shoot conductance to CO_2 (g_c; B), shoot internal CO_2 concentration (c_i; C), and WUE (D) to air temperature in 3 individuals of *T. calycinum*. Bars indicate standard deviations. All but c_i means are significantly different at different temperatures (ANOVA; $P < 0.01$). All but WUE values taken at 1430 h; data for WUE were integrated for an entire day.

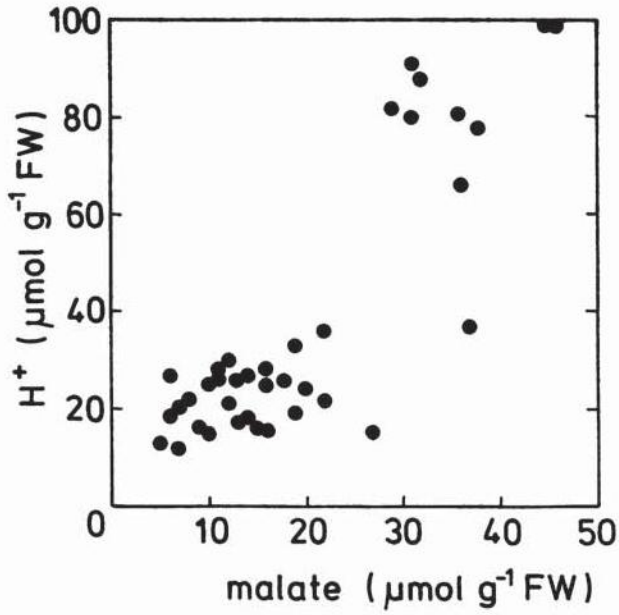

FIG. 8. Relationship between H^+ and malic acid content in 19 individuals of *T. calycinum* sampled twice on one day. Correlation analysis yields: $y = 2.0723x - 2.8377$: $r = 0.858$ ($P < 0.001$).

Table III. *Comparison of Mean Gas Exchange Parameters at 1000 h and 1500 h in 13 Individuals of Talinum calycinum Measured for a Total of 24 d under Identical Environmental Conditions*

Standard deviations in parentheses. No means were significantly different between the two times (*t*-test; $P > 0.05$).

Parameter	1000 h	1500 h
A, μmol g^{-1} DWs^{-1}	0.084 (0.038)	0.080 (0.037)
g_c, mmol g^{-1} DWs^{-1}	0.554 (0.297)	0.544 (0.257)
c_i, μl l^{-1}	200.2 (31.5)	198.8 (36.1)

results from genotypic differences. This does little, however, to explain differences in day to day responses with the same plant.

Without detailed analyses of photosynthetic responses to manipulated differences in internal CO_2 concentrations, it is not possible to accurately differentiate stomatal effects from biochemical factors in interpreting differences in CO_2 assimilation rates (5). On the other hand, given the consistent results obtained here, it appears likely that differences in assimilation between and within well watered individuals of *T. calycinum* measured under identical environmental conditions are a result of differences in metabolic efficiencies of CO_2 utilization. Since calculated shoot internal CO_2 concentrations were remarkably similar between days and individuals, in spite of widely varying conductances, stomatal factors alone are probably not the main determinants of photosynthetic rates (1, 5). It is not known to what extent differences in tissue acid content may contribute to differences in the biochemical capacity for atmospheric CO_2 fixation.

Drought stress, even after 24 h, resulted in stomatal closure. Although drought stress conditions imposed in the laboratory were probably more severe than those typically encountered in the field, it can be predicted that *T. calycinum* reduces water loss relatively quickly when droughts occur by closing its stomata. This rapid response, coupled with metabolic maintenance through CAM-idling, will undoubtedly conserve water during droughts and should prove beneficial for survival during frequent droughts which occur in the shallow soil overlying rock outcrops.

If the acid accumulated overnight is decarboxylated quickly the next day, as is the case with species of *Opuntia* and *Sedum* (3, 25), one might expect a plant with a high acid content to exhibit a high shoot internal CO_2 concentration and, hence, a low CO_2 assimilation rate. A comparison of photosynthetic parameters in *T. calycinum* at 1000 h and at 1500 h indicates that, although acid levels are higher earlier in the morning (18), shoot assimilation rates and internal CO_2 concentrations do not differ (Table III). Thus, either tissue acid contents in this succulent seldom reach levels high enough to influence photosynthetic gas exchange, internally produced CO_2 is reassimilated at high rates, or acid decarboxylation is slow, as is apparently the case with other CAM species (6).

Air temperatures exceeding 35°C diminished CO_2 uptake, apparently by altering the biochemical capacity for CO_2 fixation. Since the plant to air vapor pressure difference also increased with increasing temperature this is not an unusual response (1). Assimilation of CO_2 and WUE were maximal at 25°C. In a pre-

Table IV. *Calculations of Potential Water Conservation Resulting from CAM-Cycling in Talinum calycinum*

Acid accumulation data from Figure 6; WUE and total daytime water loss data calculated as mean daytime integrations of gas exchange for 13 plants. Extreme values are maximum acid accumulation, minimum WUE, and minimum daytime water loss. Recycled CO_2 calculated from acid accumulation values assuming 2 mol acid = 1 mol malate = 1 mol CO_2 fixed. Water conservation data calculated by dividing CO_2 recycled values by WUE.

	Nocturnal Acid Accumulation	Nocturnal CO_2 Recycled by CAM-cycling	WUE	Water Conserved by CAM-Cycling	Daytime Water Loss	Water Conserved as Percent of Daytime Loss
	$\mu eq\ g^{-1}\ DW$	$\mu mol\ g^{-1}\ DW$	$\times 10^{-3}$	$mmol\ g^{-1}\ DW$		%
Mean	348.59	174.30	3.20	54.49	1122.36	4.85
Extreme	1262.18	631.09	2.24	282.37	642.24	43.97

vious study with *T. calycinum* (18), maximum nocturnal acid accumulations were measured following day temperatures of 25, 35, and 40°C. Thus, based on results of both studies, a daytime air temperature of 25°C is apparently optimal for *T. calycinum*. Based on these findings, it is not surprising that maximal growth in this species occurs in the spring *in situ* since summer daytime air temperatures typically exceed 25°C. It should be noted, however, that CO_2 uptake and nocturnal acid accumulation were both quite high at daytime air temperatures of 35°C. Furthermore, although most growth *in situ* occurs in the spring, flowering and seed production occur throughout the summer (CE Martin, personal observation; also see [38]).

Although the data are quite variable, it is likely that the major acid involved in the fluctuations of tissue acidity measured in *T. calycinum* is malic acid. The poor correlation between proton and malate concentrations at low tissue acid content is currently unexplained, yet such discrepancies have also been reported in other species (35). Also, it is possible that other acids, *e.g.* citric acid, contribute to fluctuations in titratable acidity as has been found with other species (37).

Martin and Zee (18) estimated that nocturnal respiration rates in *T. calycinum* would double if CAM-cycling did not occur. Since CO_2 release from the shoots is observed even with CAM-cycling (Fig. 1), this appears to be a reasonable assumption. Theoretically, if a plant did not recycle respiratory CO_2 in this way, yet were to maintain identical growth rates as a plant having CAM-cycling, the plant lacking CAM-cycling must increase its daytime uptake of atmospheric CO_2 (or decrease its requirements of reduced carbon substrates and, hence, slow growth). Of course, the latter results in increased water loss by the plant—the amount dependent on the WUE at the time. With the results of this study, it is possible, therefore, to estimate the amount of water potentially conserved by CAM-cycling in *T. calycinum*. Using overall average values for nocturnal acid accumulation, WUE, and daily integrated water loss, the amount of water potentially conserved over a 24-h period amounts to 197.28 mmol $H_2O\ g^{-1}$ DW, or almost 5% of the total daily water loss (Table IV). Substituting the maximum acid accumulation observed in this study into the calculations, this value becomes approximately 18%. If a best case is calculated, *i.e.* using the maximum acid accumulation, minimum WUE, and smallest amount of water lost in a day, then the amount of water potentially conserved by CAM-cycling is almost 44% of total daily water loss. For all these calculations, only values from plants examined under identical conditions were used. Given the large changes in WUE evident at different temperatures, this 'water conservation' value will decrease below 20°C and increase above 30°C. Thus, under midsummer conditions on rock outcrops throughout the central USA, the savings of water attributable to CAM-cycling may well prove of vital importance to the ecophysiology, if not survival, of plants having this variant of photosynthesis.

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