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# Correlation between CAM-Cycling and Photosynthetic Gas Exchange in Five Species of *Talinum* (Portulacaceae)[1]

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## ABSTRACT

**Photosynthetic gas exchange and malic acid fluctuations were monitored in 69 well-watered plants from five morphologically similar species of *Talinum* in an investigation of the ecophysiological significance of the Crassulacean acid metabolism (CAM)-cycling mode of photosynthesis. Unlike CAM, atmospheric $CO_2$ uptake in CAM-cycling occurs exclusively during the day; at night, the stomata are closed and respiratory $CO_2$ is recaptured to form malic acid. All species showed similar patterns of day-night gas exchange and overnight malic acid accumulation, confirming the presence of CAM-cycling. Species averages for gas exchange parameters and malic acid fluctuation were significantly different such that the species with the highest daytime gas exchange had the lowest malic acid accumulation and *vice versa*. Also, daytime $CO_2$ exchange and transpiration were negatively correlated with overnight malic acid fluctuation for all individuals examined together, as well as within one species. This suggests that malic acid may effect reductions in both atmospheric $CO_2$ uptake and transpiration during the day. No significant correlation between malic acid fluctuation and water-use efficiency was found, although a nonsignificant trend of increasing water-use efficiency with increasing malic acid fluctuation was observed among species averages. This study provides evidence that $CO_2$ recycling via malic acid is negatively correlated with daytime transpirational water losses in well-watered plants. Thus, CAM-cycling could be important for survival in the thin, frequently desiccated soils of rock outcrops on which these plants occur.**

CAM-cycling is a variant of CAM in which the CAM pathway is limited primarily to refixing respiratory $CO_2$, resulting in an overnight accumulation of malic acid that is decarboxylated during the subsequent day (7, 10, 18). Unlike obligate CAM plants, plants with CAM-cycling keep their stomata closed at night and open during the day under well-watered conditions, with atmospheric $CO_2$ fixation occurring via the $C_3$ pathway (29). Stable carbon isotope ratios of CAM-cycling plants are, therefore, within the range of $C_3$ plants (8, 14, 25, 28). CAM-cycling is a widespread phenomenon, having been described in plants from 15 families (10), the majority of which are succulents that occur in microenvironments subject to recurrent drought.

The ecophysiological significance of $CO_2$ recycling is less clear than that of CAM because the former does not involve the high WUE[2] associated with the nocturnal uptake of atmospheric $CO_2$. Beneficial consequences of CAM-cycling may be indirect, as in ensuring the capability of $CO_2$ recycling during 24-h stomatal closure and drought stress. The latter phenomenon has been termed "CAM-idling" (26, 27, 30). By this cycling of $CO_2$, CAM-idling may maintain photosynthetic enzyme activities for the duration of a drought and, thereby, enable a rapid response of photosynthesis when tissue is rehydrated (26, 27). In species of *Peperomia* (21, 30) and in *Sedum pulchellum* (11), for example, CAM-cycling preceded CAM-idling. In these species, levels of CAM acid fluctuations increased during drought stress.

In addition to the above, CAM-cycling may have important consequences for the carbon and water balance of such plants during well-watered conditions. In certain species, *e.g. Talinum calycinum* and *Sedum nuttallianum*, CAM-cycling may be less important as a precursor for CAM-idling because CAM activity is substantially reduced during severe drought stress (12, 14). Instead, as initially hypothesized by Cockburn (2), CAM-cycling may effect an increase in the WUE of plants by increasing daytime $C_i$ in the leaves, as a result of the decarboxylation of malic acid, thus reducing stomatal conductance and transpiration. Martin *et al.* (10) estimated that CAM-cycling in *T. calycinum* could potentially result in daily water savings of up to 43% compared to hypothetical plants lacking $CO_2$ recycling, although this figure is probably lower in most individuals of this species. With the exception of *S. pulchellum* (11), no other studies have provided evidence for or against this hypothetical relationship.

The purpose of this study was to examine the relationship between CAM-cycling and $CO_2$ and water vapor exchange in plants collected from numerous populations of several species of *Talinum* which exhibit different degrees of $CO_2$ recycling. The objective was to test the hypothesis that high levels of CAM-cycling result in decreases in transpiration and increases in WUE in well-watered plants. Correlations among malic acid accumulation, $CO_2$ assimilation, transpiration, and WUE were examined in 69 plants of five species of *Talinum* (Portulacaceae). Photosynthesis was examined previously in *T. calycinum*, which exhibited $C_3$ gas exchange in addition to overnight malic acid accumulation (10, 14). Carbon metabolism has not been investigated in the other four species of *Talinum*, except for reports of acid fluctuations in *Talinum teretifolium* (13) and *Talinum parviflorum* (10). All five spe-

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[2] Abbreviations: WUE, water-use efficiency; $C_i$, intercellular $CO_2$ concentration; $C_a$, ambient $CO_2$ concentration; $g_c$, shoot conductance to $CO_2$.

cies are small perennials that are nearly indistinguishable morphologically, with terete, succulent leaves (6, 15, 31). All occur in shallow soils subject to frequent and severe desiccation on exposed rock outcrops throughout the southeastern and midwestern United States.

## MATERIALS AND METHODS

### Study Species and Growth Conditions

Plants were collected from 23 populations of five species of *Talinum* in the eastern and midwestern United States during the summer of 1987. Three of the species occur only in the eastern United States: *Talinum mengesii* Wolf occurs on sandstone and granite outcrops in northern Alabama and Georgia (19); *T. teretifolium* Pursh. occurs on sandstone and granite outcrops throughout the Piedmont plateau and on serpentine outcrops in southeastern Pennsylvania and Maryland (15); and *Talinum calcaricum* Ware occurs on limestone glades in central Tennessee and northern Alabama (31). The other two species occur in the midwestern United States: *T. calycinum* Engelm. occurs on limestone, chert, granite, and sandstone outcrops in Missouri and Arkansas and the eastern Great Plains (5); and *T. parviflorum* Nutt. occupies granite and sandstone outcrops throughout the same regions as *T. calycinum* but extends farther south and west in the Great Plains to the Rocky Mountains (5). Five populations of each species were sampled, except four populations for *T. calcaricum* and *T. calycinum.* Locations of the 23 populations sampled in the study are given in the paper by Harris and Martin (8).

During the summer of 1987, 10 whole plants were collected from unshaded locations at each site and transported to the University of Kansas greenhouse where they were potted individually in Styrofoam cups (6 cm diameter) with 100 mL of soil (3 parts sand, 1 part each of peat, topsoil, vermiculite). The plants were watered three times a week with deionized water and fertilized once per week with a standard greenhouse fertilizer (20:10:20 N:P:K; with trace elements). Following the onset of dormancy in September 1987, the plants were cold treated 3.5 to 5 months at 4°C and then regrown in a growth chamber. The plants were then watered daily with deionized water and fertilized once per week (18% each of total N, $P_2O_5$, $K_2O$; with trace elements). The regrowth of plants was staggered so that gas exchange measurements were made on shoots of approximately the same age (1.5 months). Environmental conditions in the growth chamber, determined with a LI-COR (Lincoln, NE) LI-6000 portable photosynthesis system, were: 14-h photoperiod with a photosynthetic photon flux density at a plant height of 670 $\mu$mol $m^{-2}$ $s^{-1}$; day/night air temperatures of 30/20°C (±1.5°C); day/night relative humidities of 40/58% (±5%). All shoots were flowering when used.

### Gas Exchange and Malic Acid Measurements

Gas exchange of two potted plants was monitored simultaneously with shoots sealed in two water-jacketed polycarbonate chambers, beginning in mid-February 1988. The soil in the pots, which remained outside the chambers, was kept moist throughout the gas exchange measurements. After passing through three large gas-mixing bottles, the air before and after passing through the gas exchange chambers was monitored for $CO_2$ concentration using an Anarad (Santa Barbara, CA) model AR500R differential infrared gas analyzer and for dew points using EG & G (Waltham, MA) model 911 Dew-All analyzers. Leaf and chamber temperatures were monitored with Cu-constantan thermocouples (0.17 mm diameter) and an Omega (Stamford, CT) model 410A temperature meter. The $C_a$ used in the study varied from 340 to 400 $\mu$L $L^{-1}$ $CO_2$ but was typically near 355 $\mu$L $L^{-1}$. Day/night chamber temperatures were 30/20°C with a 24-h dew point of 13.7°C. A 14-h photoperiod was used with a photosynthetic photon flux density of 750 (±50) $\mu$mol $m^{-2}$ $s^{-1}$ inside the chambers.

Sixty-nine plants were used in the study: three plants per population. $g_c$, $C_i$, net $CO_2$ assimilation, and transpiration rates were calculated for each plant according to equations in the works by Šesták *et al.* (20) and Farquhar and Sharkey (4). Carbon dioxide and water vapor exchange rates were recorded at 5-min intervals for 48 h, but only data from the second day and night of measurements were used in the analysis. The plants were kept in the same conditions in the cuvettes during the third day, and several leaves were removed at "lights-on" and again at "lights-off" and were immediately frozen at −65°C for malic acid analyses. Malic acid concentrations were determined from leaf sap extracted from plant tissue samples following the procedure of Smith and Lüttge (22). These extracts were then analyzed spectrophotometrically by enzymatic analysis, according to the method of Hohorst (9), using standards of known malic acid concentrations. Dry weight of the tissue recovered after extraction of sap was determined after drying in an oven at 65°C for 3 d.

### Statistical Analyses

Species averages for malic acid fluctuations and gas exchange measurements were analyzed for significant differences using the Kruskal-Wallis test and a nonparametric multiple comparison of means test based on average ranks in the Kruskal-Wallis test (3). Correlation coefficients were determined for correlations between gas exchange measurements and malic acid fluctuations for individual plants and species averages. All statistical tests were performed using the Statview 512+ statistics package (Brainpower Inc., Ventura, CA), except for significance tests of correlation coefficients, which were determined according to the methods of Sokal and Rohlf (24).

## RESULTS

Nearly all plants showed typical $C_3$ gas exchange patterns, with net atmospheric $CO_2$ uptake only during the day (Fig. 1A). Although six plants (of 69) showed some net $CO_2$ uptake at night, the amounts were small, ranging from 0.6 to 4.9% of total daytime assimilation (Fig. 1A). In most plants, $g_c$ values were high during the day and decreased to near zero at the beginning of the night, with a gradual increase near the end of the night (Fig. 1B). Calculated values of $C_i/C_a$ were much higher at night than during the day in all plants (Fig. 1C).

Instantaneous rates of $CO_2$ assimilation at 1500 h on the

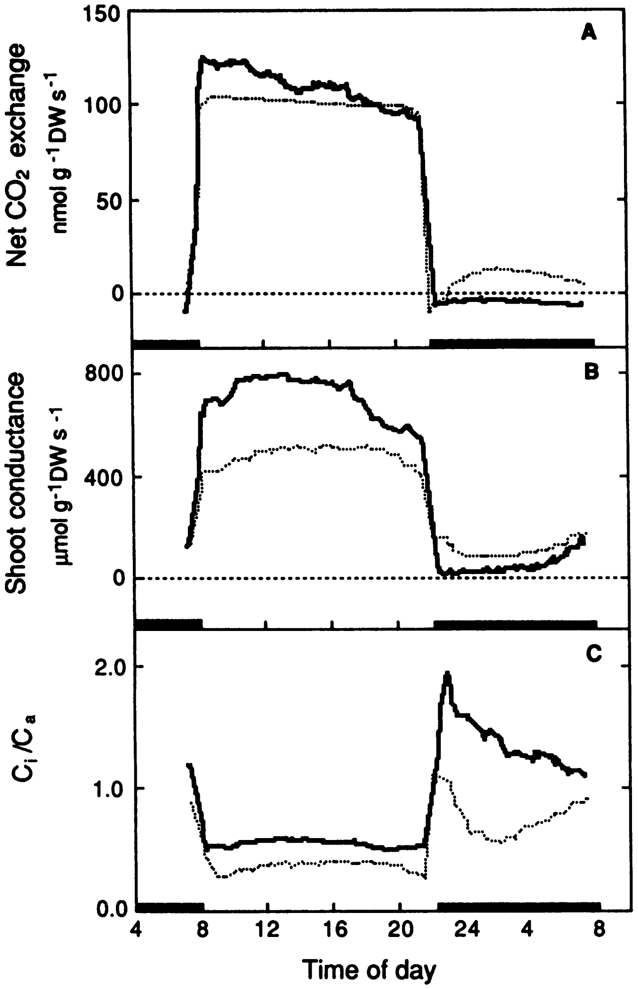

**Figure 1.** Representative examples of gas exchange characteristics in *Talinum* under well-watered conditions: net $CO_2$ exchange (A), $g_c$ (B), and $C_i/C_a$ (C) during a 24-h period for a plant with a net loss of $CO_2$ at night (solid line; *Talinum mengesii*) and a plant with a net uptake of $CO_2$ at night (dotted line; *Talinum calcaricum*). Black bars on horizontal axes indicate night. DW, dry weight.

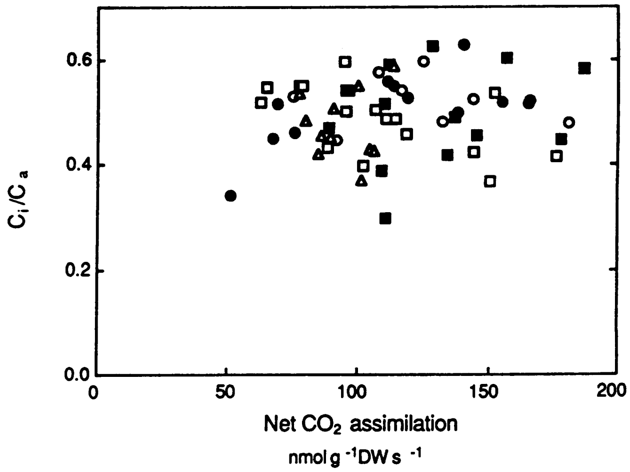

**Figure 2.** Relationship between calculated $C_i/C_a$ and instantaneous net $CO_2$ assimilation for individual plants of five species of *Talinum* under well-watered conditions at 1500 h (midday) on the second day of gas exchange measurements. r = 0.04, NS. △, *T. calcaricum*; ●, *T. calycinum*; ○, *T. mengesii*; □, *T. parviflorum*; ■, *T. teretifolium*.

second day of measurements varied considerably among the plants and species in the study (Fig. 2). The calculated internal $CO_2$ concentration corresponding to $CO_2$ assimilation measurements at the same time varied from 99.8 to 223.0 $\mu$L L$^{-1}$, with an average of 173.8 $\mu$L L$^{-1}$. Although the $C_i/C_a$ values were not correlated with $CO_2$ assimilation rates (r = 0.04; Fig. 2), $CO_2$ assimilation rates and $g_c$ values were positively correlated (r = 0.88, $P < 0.01$; Fig. 3). Integrated, net $CO_2$ exchange during the night for all plants was negatively correlated (r = −0.66, $P < 0.01$) with daytime $CO_2$ exchange such that those plants with the highest loss of $CO_2$ at night had the greatest daytime $CO_2$ assimilation (Fig. 4).

Malic acid fluctuations varied from zero (eight individuals, representing four species) to nearly 500 $\mu$mol g$^{-1}$ dry weight among the individuals in the study and were also positively correlated with net overnight $CO_2$ exchange among all individuals (r = 0.58, $P < 0.01$) and within three species (Fig. 5). Unlike the majority of plants, atmospheric $CO_2$ uptake accounted for nearly all malic acid fluctuation in three of the six plants that exhibited a net uptake of $CO_2$ at night.

Integrated, net $CO_2$ uptake during the day was negatively correlated with day-night malic acid fluctuation for all plants (r = −0.41, $P < 0.01$; Fig. 6A). When examined by species, however, this correlation was significant within only *T. parviflorum*. $g_c$ showed the same relationship with malic acid fluctuation (data not shown), which was expected given the close correlation between $CO_2$ assimilation and conductance in Figure 2. Likewise, integrated daytime transpiration showed a correlation with malic acid fluctuation similar to that of net $CO_2$ assimilation (Fig. 6B). The correlation coefficient was again significant when all individuals were grouped

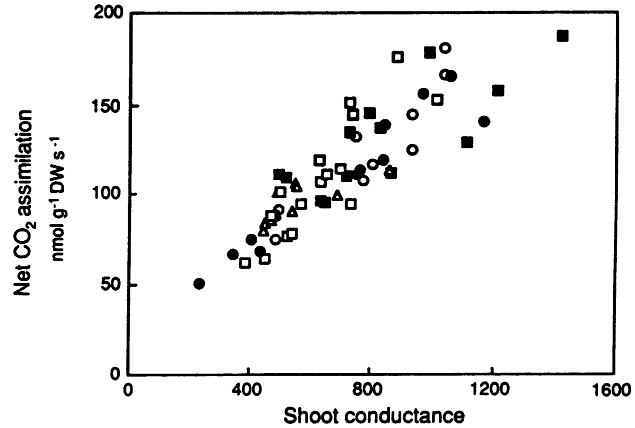

**Figure 3.** Relationship between instantaneous net $CO_2$ assimilation rate and shoot conductance for individual plants of five species of *Talinum* under well-watered conditions at 1500 h (midday) on the second day of gas exchange measurements. r = 0.88, $P < 0.01$. △, *T. calcaricum*; ●, *T. calycinum*; ○, *T. mengesii*; □, *T. parviflorum*; ■, *T. teretifolium*.

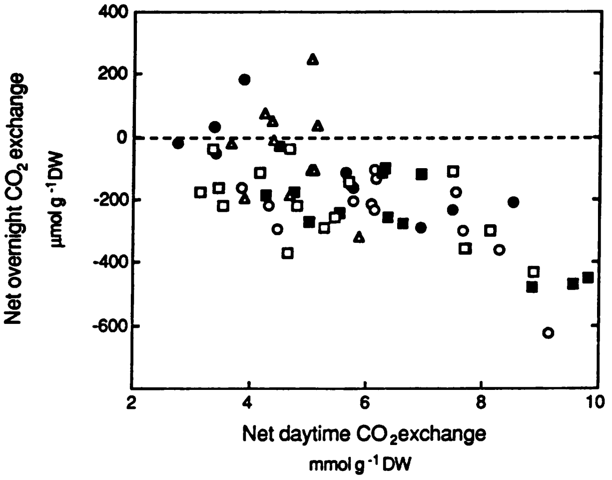

**Figure 4.** Relationship between integrated, net overnight $CO_2$ exchange and integrated, net daytime $CO_2$ exchange for individual plants of five species of *Talinum* under well-watered conditions. Overall r = −0.66**. Symbols and r values: △, *T. calcaricum*(−0.23); ●, *T. calycinum* (−0.79*); ○, *T. mengesii* (−0.62*); □, *T. parviflorum* (−0.56*); ■, *T. teretifolium* (−0.74**). * and **, r values significantly different from 0 at $P < 0.05$ and $P < 0.01$, respectively.

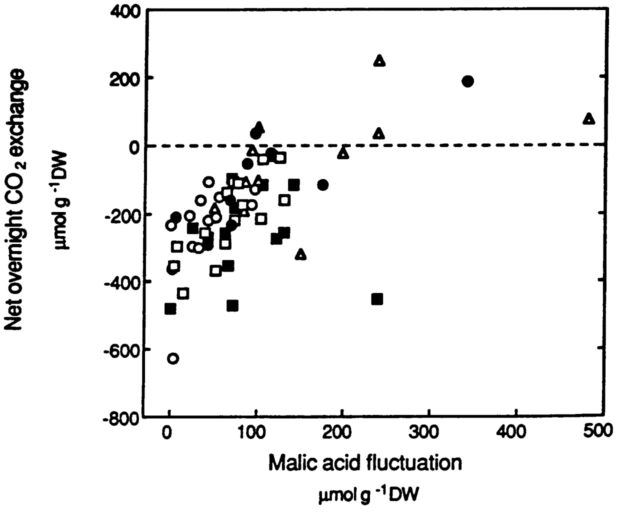

**Figure 5.** Relationship between integrated net overnight $CO_2$ exchange and malic acid fluctuation for individual plants of five species of *Talinum* under well-watered conditions. Overall r = 0.58**. Symbols and r values: △, *T. calcaricum* (0.52); ●, *T. calycinum* (0.81**); ○, *T. mengesii* (0.60*); □, *T. parviflorum* (0.78**); ■, *T. teretifolium* (0.05). * and **, r values significantly different from 0 at $P < 0.05$ and $P < 0.01$, respectively.

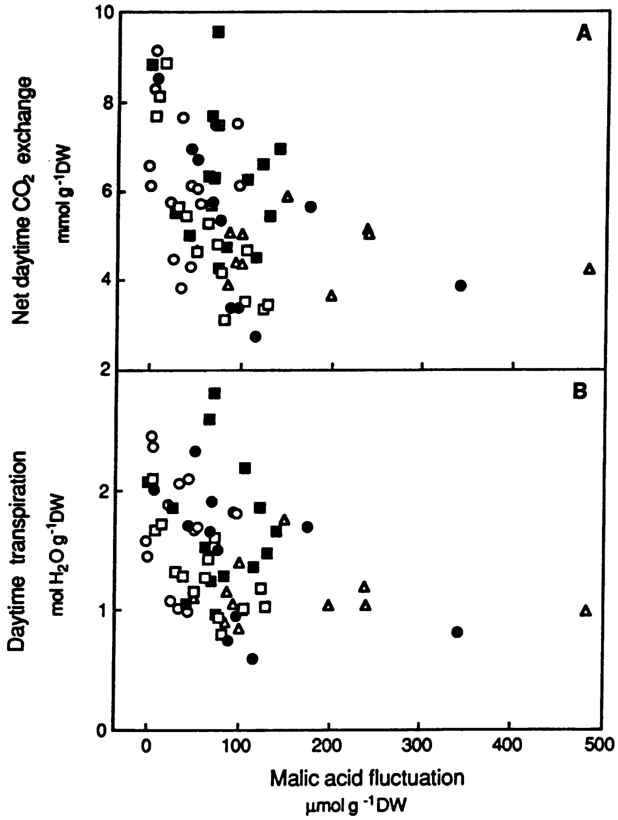

**Figure 6.** Relationship between malic acid fluctuation and integrated daytime net $CO_2$ exchange (A) and integrated daytime transpiration (B) for individual plants of five species of *Talinum* under well-watered conditions. Overall r values for A/B are −0.41**/−0.37**. Symbols and A/B r values: △, *T. calcaricum* (−0.16/−0.10); ●, *T. calycinum* (−0.25/−0.11); ○, *T. mengesii* (−0.11/−0.11); □, *T. parviflorum* (−0.81**/−0.72**); ■, *T. teretifolium* (−0.52/−0.52). * and **, r values significantly different from 0 at $P < 0.05$ and $P < 0.01$, respectively.

together (r = −0.37, $P < 0.01$), yet, it was significant within only *T. parviflorum*, when examined by species.

Integrated WUE was not correlated with malic acid fluctuation among all plants (r = 0.08, NS; Fig. 7). On the other hand, when examined by species, *T. parviflorum* showed a significant negative correlation between WUE and malic acid fluctuation (r = −0.56, $P < 0.05$), whereas none of the other species showed significant correlations. *T. calcaricum* appeared to have the highest average WUE of all species, although none of the species-average WUE values were significantly different (Fig. 8). In spite of the latter, the average WUE per species appeared positively correlated with species-average malic acid fluctuation (r = 0.78; Fig. 8).

The five species differed significantly ($P < 0.05$) in their average day-night malic acid fluctuation as well as in integrated gas exchange characteristics (Table I). For example, *T. calcaricum* had significantly higher average acid fluctuations than all other species, as well as lower average $CO_2$ assimilation and transpiration than *T. mengesii* and *T. teretifolium.* Furthermore, the latter two species had the highest $CO_2$ assimilation and transpiration and among the lowest malic acid fluctuations. On the other hand, *T. parviflorum* showed

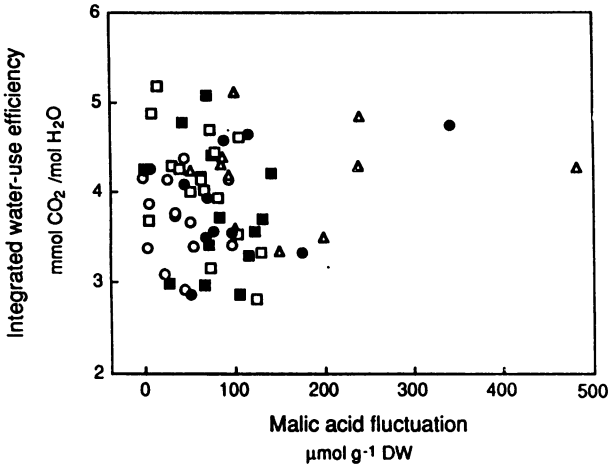

**Figure 7.** Relationship between integrated WUE and malic acid fluctuation for individual plants of five species of *Talinum* under well-watered conditions. Overall r = 0.08, NS. Symbols and r values: △, *T. calcaricum* (0.15); ●, *T. calycinum* (−0.19); ○, *T. mengesii* (0.03); □, *T. parviflorum* (−0.56*); ■, *T. teretifolium* (0.13). *, r value significantly different from 0 at $P < 0.05$.

low transpiration together with low malic acid fluctuations, which did not follow the inverse relationship between malic acid fluctuation and gas exchange during the day found in the other species (Table I; also see Fig. 6).

## DISCUSSION

Most individuals of all five species of *Talinum* exhibited similar $C_3$-like patterns of gas exchange, with net uptake of atmospheric $CO_2$ occurring solely during the day coupled with low rates of net $CO_2$ release at night. In addition, nearly all plants showed overnight accumulations of malic acid. Thus, these results confirm the occurrence of CAM-cycling in *T. calycinum* (10, 14), *T. parviflorum* (10), and *T. teretifolium* (13) and demonstrate, for the first time, this phenomenon in *T. calcaricum* and *T. mengesii*. This overall physiological similarity among the species is not surprising given their high degree of morphological similarity. There were significant differences in the rates of gas exchange, however, as well as average malic acid fluctuations between the species. For example, *T. calcaricum* exhibited nearly five times the acid fluctuation as *T. mengesii*. The amount of $CO_2$ recycled as malic acid amounted to low proportions of total $CO_2$ assimilation in all species (Table I) and was comparable to levels found in other studies of *Talinum* and *Sedum* (10, 11), another genus with species typically found on rock outcrops.

Six plants showed net $CO_2$ uptake at night, although the rates were low in comparison with daytime $CO_2$ uptake. These were individuals of *T. calcaricum* and *T. calycinum*, species that had the highest average levels of malic acid fluctuation of all species in the study. Most individuals of these species, however, had net losses of $CO_2$ at night as in the other species. Only three of these plants showed malic acid fluctuations that accounted for nearly all nighttime $CO_2$ uptake, indicating that they performed $C_3$ photosynthesis with some CAM rather than CAM-cycling.

Increasing $g_c$ near the end of the night, indicative of stomatal opening, was observed in most of the plants in this study regardless of the amount of malic acid accumulated. This phenomenon could be explained by accelerated rates of malic acid formation toward the end of the night, which would decrease the $C_i$ and increase stomatal conductance. Similar patterns of nighttime gas exchange and malic acid formation were reported by Patel and Ting (17) for *Peperomia camptotricha* which also undergoes CAM-cycling.

Plants with high net $CO_2$ assimilation rates during the day also exhibited high rates of $CO_2$ release at night, which may be related to relatively high rates of respiration and/or low rates of nighttime $CO_2$ fixation. All but six plants (see above) showed net losses of $CO_2$ at night, indicating that the rate of $CO_2$ release by respiration exceeded the rate of $CO_2$ recapture resulting in the formation of malic acid. This would suggest that the amounts of malic acid accumulated during CAM-cycling were not limited by the amounts of $CO_2$ released by respiration but by some other factor such as the activity of phospho*enol*pyruvate carboxylase. In apparent contrast, previous work with CAM plants has shown that nighttime $CO_2$ recycling may be greater in plants with higher respiration rates (32).

Although net $CO_2$ assimilation rates varied greatly, all plants exhibited a similar range in $C_i/C_a$ values over the range of $CO_2$ assimilation rates. In addition, shoot conductances were highly correlated with $CO_2$ assimilation rates, as was found in a previous study of *T. calycinum* (10). According to theoretical considerations of plant gas exchange (4), such differences in net $CO_2$ assimilation among the plants are most likely due to differences in the biochemistry of $CO_2$ fixation rather than the degree of stomatal opening. Thus, these findings suggest that the internal release of $CO_2$ from the decarboxylation of malic acid most likely contributes to differences in stomatal conductance as well as $CO_2$ exchange rates. Further evidence in support of this is found in the negative correlation between malic acid fluctuations and net daytime

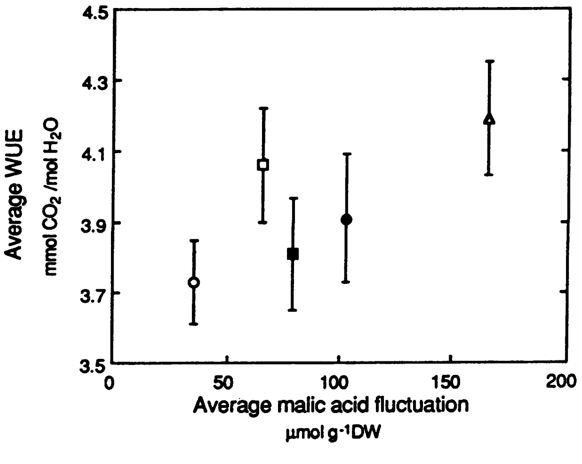

**Figure 8.** Relationship between average WUE and average malic acid fluctuation for five species of *Talinum* under well-watered conditions. r = 0.78, NS. △, *T. calcaricum*; ●, *T. calycinum*; ○, *T. mengesii*; □, *T. parviflorum*; ■, *T. teretifolium*. Error bars, SE. WUE averages were not significantly different.

**Table I.** *Species Averages (SE in Parentheses) for Malic Acid Fluctuation (Δ Malic Acid), Net $CO_2$ Exchange, Transpiration, and Range of Percentage Net Daytime $CO_2$ Uptake Recycled as Malic Acid (Assuming All Malic Acid Resulted from Refixation of Respiratory $CO_2$) for Five Species of Talinum under Well-Watered Conditions*

Different letters in a column denote significantly different means at $P < 0.05$.

| Species | No. | Δ Malic Acid | Net $CO_2$ Exchange | Transpiration | % $CO_2$ Recycled |
|---|---|---|---|---|---|
| | | *μmol g⁻¹ dry wt* | *mmol g⁻¹ dry wt* | *mol g⁻¹ dry wt* | |
| *T. calcaricum* | 12 | 165.5 (37.1)a | 4.67 (0.64)a | 1.14 (0.08)a | 1.1–11.4 |
| *T. calycinum* | 11 | 102.7 (27.1)b | 5.43 (0.57)a,b | 1.45 (0.17)a,b | 0.1–8.8 |
| *T. mengesii* | 14 | 35.4 (8.4)c | 6.27 (0.40)b | 1.71 (0.12)b | 0.0–1.5 |
| *T. parviflorum* | 15 | 65.0 (9.8)b,c | 5.38 (0.45)a,b | 1.31 (0.09)a | 0.1–3.8 |
| *T. teretifolium* | 14 | 79.2 (10.9)b,c | 6.29 (0.42)b | 1.71 (0.15)b | 0.0–2.6 |

$CO_2$ assimilation and transpiration rates observed among all plants grouped together, as well as between species averages. In other words, daytime decarboxylation of malic acid may decrease shoot conductance, through internal release of $CO_2$, which would decrease the net uptake of atmospheric $CO_2$ and loss of water vapor. Other factors potentially contributing to such findings include inversely proportional activities of ribulose-1,5-bisphosphate carboxylase/oxygenase and phospho*enol*pyruvate carboxylase, which would also result in a negative correlation between daytime $CO_2$ uptake and nighttime accumulation of malic acid. At this time, these possibilities cannot be differentiated.

WUEs observed in this study were similar to values found in *T. calycinum* (10), as well as other succulents and $C_3$ xerophytes (12, 23), but were not as high as in obligate CAM plants (1, 16). WUE was not correlated with the level of malic acid fluctuation when all plants were examined together, probably because both $CO_2$ assimilation and transpiration rates varied similarly. On the other hand, there was a trend toward a positive correlation between WUE and malic acid fluctuation among the species averages, which was not statistically significant because of the low number of means in the correlation. Such small differences in WUE, if real, may amount to more substantial differences in carbon assimilation throughout an entire growing season, especially during more water-limited conditions typically encountered *in situ.*

In comparison with the results for the other four species in this study, *T. parviflorum* showed unexpectedly low average malic acid fluctuations, given its gas exchange rates. In a study of the comparative drought tolerance of these species (8), *T. parviflorum* collected *in situ* exhibited the least negative stable carbon isotope ratios ($\delta^{13}C$), indicating that it was the most drought-stressed in the field, given the relationship between drought-imposed reductions in stomatal conductance and $\delta^{13}C$ in $C_3$ plants (8). Furthermore, this species, together with *T. calcaricum*, exhibited more negative stable carbon isotope ratios than the three other species under identical conditions in a growth chamber, indicating that *T. parviflorum* and *T. calcaricum* were less drought-stressed in the growth chamber. Thus, it was concluded that *T. parviflorum* is among the most drought-tolerant species of the five examined (8). *T. parviflorum* grows throughout the typically hot summer in the southern and western Great Plains (5) and may exhibit additional, *e.g.* morphological and anatomical, adaptations to severe drought that might account for its relatively high WUE.

In a parallel study of the same populations and species of *Talinum* (8), all populations showed malic acid fluctuations that were at least twice as high in the field as in this study. Furthermore, in the same study (8), the magnitude of overnight malic acid fluctuations in all species grown under controlled conditions increased significantly with moderate levels of drought stress. $CO_2$ recycling should, therefore, constitute a much greater proportion of daytime $CO_2$ exchange in the more drought-stressed conditions of the field. During drought stress, plant gas exchange should decrease, in response to lower shoot water potentials, concomitant with an increase in malic acid fluctuation. The greater degree of CAM-cycling observed in the field might, therefore, result in greater WUEs than observed in plants grown under well-watered conditions in this study.

In summary, the results of this study demonstrate that daytime rates of $CO_2$ and water vapor exchange correlated negatively with levels of overnight malic acid accumulation in individuals of five species of *Talinum* measured under well-watered conditions. Such findings would be expected if daytime decarboxylation of malic acid effected decreased gas exchange in these plants. On the other hand, few corresponding differences in plant WUEs were observed. Nevertheless, plants with greater degrees of CAM-cycling exhibited lower daytime rates of transpiration, which should result in a valuable savings of water for such plants growing throughout the summer in the frequently desiccated soils of exposed rock outcrops.

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