

C₃ Photosynthesis and Crassulacean Acid Metabolism in a Kansas Rock Outcrop Succulent, *Talinum calycinum* Engelm. (Portulacaceae)¹

Received for publication April 27, 1983 and in revised form July 5, 1983

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ABSTRACT

The potential for Crassulacean acid metabolism (CAM) was investigated in the sandstone outcrop succulent *Talinum calycinum* in central Kansas. Field studies revealed CAM-like diurnal acid fluctuations in these plants. These fluctuations persisted under all moisture and temperature regimes in the laboratory. Despite this CAM-like acid metabolism, simultaneous gravimetric determinations of day- and nighttime transpiration rates indicated the presence of a C₃ gas exchange pattern. Subsequent analyses of diurnal CO₂ and H₂O exchange patterns under well-watered conditions and after 3, 5, and 7 days of drought confirmed these findings, though low rates of nocturnal CO₂ uptake were observed on the fifth night after continuous drought. Finally, the $\delta^{13}\text{C}/^{12}\text{C}$ value of this succulent, -27.8‰ , emphasizes the insignificance of any nocturnal CO₂ uptake in the lifelong accumulation of carbon in this species. Thus, it is proposed that *T. calycinum* is a C₃ plant with some CAM characteristics, including the ability to re-fix respiratory CO₂ at night under all moisture regimes, potentially resulting in a conservation of carbon, and occasionally to fix atmospheric CO₂ at night. These findings may prove to be common among rock outcrop succulents.

CO₂ (23). The latter phenomenon was first found in the desert cactus *Opuntia basilaris* (24), an obligate CAM plant (4), and subsequently has been reported in several other species (3, 11, 16, 23). Aside from one report of diurnal acid fluctuations in *Talinum guadalupense* (6), and the above-mentioned study (11), members of this genus have not been investigated with regard to their photosynthetic pathway.

The North Carolina succulent survey (11), in addition to at least one other study of the ecophysiology of a rock outcrop succulent (20), indicates that the operation of both the C₃ and CAM pathways, either simultaneously or not, may be a common phenomenon among outcrop succulents, though too few detailed studies exist to warrant such a generalization. It was the purpose of this study to determine the photosynthetic pathway or variant thereof, and its response to water stress, in the Kansas rock outcrop succulent *Talinum calycinum* (rock-pink fameflower).

MATERIALS AND METHODS

Field Studies. All field studies were carried out on a population of *Talinum calycinum* growing on an exposed sandstone outcrop at the southern tip of Lucas Park at Wilson Lake, Russell County, approximately 22 km north of Wilson, KS. On two dates in June 1982, five plants were detached from their rhizomes at 3- to 4-h intervals over a 24-h period, placed in plastic bags, and immediately frozen on dry ice. Air temperature and RH were continuously recorded by a Kahl Scientific Instrument Co. hygromograph in a ground-level ventilated instrument shelter. Irradiance was measured with a hand-held quantum (PAR) sensor (Li-Cor LI-190SB) and meter (Li-Cor LI-185B). Upon return to the laboratory, plants were analyzed for titratable acidity as in Martin *et al.* (10).

Plant Material for Laboratory Studies. Individuals of *T. calycinum* were collected during the summers of 1981 and 1982 from two locations: sandstone outcrops above Hell Creek near Kansas Highway 232, Lincoln County, about 10 km north of Wilson, KS; and sandstone outcrops near U. S. Highway 166 about 3 km east of Sedan, KS, in Chautauqua County. Plants were potted in sand in styrofoam cups (6 cm diameter) and watered irregularly under greenhouse conditions. At least 1 week prior to each experiment, plants were transferred to a growth chamber with 25 (± 2)°C, 1.49 (± 0.15) kPa vpd² daytime and 18 (± 2)°C, 1.46 (± 0.15) kPa vpd nighttime conditions and irradiance (PAR) at plant height of 300 $\mu\text{mol m}^{-2} \text{s}^{-1}$ on a 12-h photoperiod.

Drought Effects on Acidity and Transpiration. Immediately before and daily after cessation of watering, one of two shoots

Within the past several years, it has become increasingly clear that many plants do not fit neatly into the three categories of photosynthetic carbon reduction pathways - C₃, C₄, and CAM (3, 5, 7, 11, 12, 16, 18, 20, 26, 27, 30). This is particularly true of plants showing characteristics of the C₃ and CAM pathways. In some, *e.g.* *Mesembryanthemum* species, water stress induces a shift from C₃ to CAM photosynthesis (3, 5, 18, 30). In others, temperature appears to be a more important factor regulating the mode of photosynthesis (27). The stem succulent *Frerea indica* has a CAM stem and, when well-watered, C₃ leaves (8). Furthermore, several species apparently utilize the CAM biochemical pathway when water-stressed without concomitant nocturnal stomatal opening (3, 11, 16, 23). A recent survey (11) has emphasized the variability in photosynthetic pathways present in succulent plants of North Carolina. One of the species included in this study was *Talinum teretifolium* (Portulacaceae), which grows in shallow soil on exposed rock outcrops. The results indicated that this succulent may have a very limited ability to fix CO₂ at night, yet undergoes substantial CAM-like tissue acid fluctuations—suggestive of nocturnal recycling of respiratory

¹ Supported by University of Kansas General Research allocation 3255-X038. The carbon isotope determinations were funded by the National Science Foundation (DEB 8021270 to Dr. J. A. Teeri, University of Chicago).

² Abbreviations: vpd, vapor pressure deficit; DW, dry weight; FW, fresh weight; WUE, water use efficiency.

from each of five plants was excised at the end of the day and the other at the end of the night, placed in a plastic bag, weighed, and frozen for subsequent titratable acidity and DW determinations (10). All titratable acidity values in this and other experiments were expressed on a DW basis. Though this is not the convention for the expression of such values for CAM plants, it was felt that a FW basis would bias the results since the FW changed during the drought. The soil and root systems from another ten plants were sealed in plastic bags and the plants were weighed at mid-day, the end of the day, and the end of the night. With no water loss directly from the soil, these plants remained hydrated longer than did the others. Resultant transpiration rates were substantially higher than those measured in the gas exchange system (see below). This may be related to the fact that the plants utilized in this experiment were much younger than those used in the gas exchange system. Also, it is possible that the plastic bags did not totally prevent water loss from the soil. At the end of the experiment, DWs of these shoots were obtained. Measurements of *T. calycinum* tissue water potential using a Scholander pressure chamber proved unreliable due to the presence of large amounts of mucilage in the leaves and stems of these plants. In all DW determinations, plant shoots were heated in an oven at 85°C for several days (until no further weight loss), cooled in a desiccator, and weighed. Upon harvest, the soil was dried and weighed in a similar fashion.

With the exception of the transpiration determinations, the above procedures were repeated with less frequent sampling and greater sample sizes. Instead of daily during the drought, plants were sampled under well-watered conditions ($n = 20$) and after 5 d ($n = 10$) and 9 d ($n = 10$) of drought.

Temperature Effects on Acidity. After several weeks in the growth chamber described above (25/18°C), five plants were transferred to another chamber for an experimental temperature treatment, while five controls remained in the first chamber. Control plants were sampled simultaneously with each set of experimental plants since the latter were sampled on different days. After 24 h, plants were sampled as described above—in the morning and at the end of the 2nd d. The experimental growth chamber differed from the control only in temperature and humidity. Day/night air temperatures are given in Figure 4. From low to high temperature regimes, the corresponding day/night vpd regimes were: 1.07/0.68 kPa, 1.16/0.66 kPa, 0.70/1.07 kPa, <0.63/0.94 kPa, <0.85/<0.63 0.63 kPa, and <1.12/<0.90 kPa. Daytime leaf temperatures were typically 1 to 3°C higher than air temperatures, all measured with a thermistor and Yellow Springs Instrument telethermometer.

Drought Effects on Gas Exchange. Plants were maintained in the growth chamber as above for several weeks before use and were subjected to varying degrees of drought. The entire shoot was then sealed in a Plexiglas, water-jacketed chamber (the empty chamber exhibited no detectable gas exchange) with an internal volume of 140 ml and a magnet-driven fan for increasing turbulence within. The root system and soil remained outside the chamber. Outside air (mean CO₂ concentration was 336 $\mu\text{l l}^{-1}$ (SD = 17, $n = 108$ determinations)) was pumped through two gas mixing bottles, a humidifier, and the chamber at a flow rate of approximately 1.5 l min⁻¹. After removal of water with ZnCl₂ (19), the air was analyzed for CO₂ concentration by a differential Anarad AR500R IR gas analyzer, calibrated at 2- to 3-h intervals (one 8-h interval at night) throughout each experiment, using bottled gas of known CO₂ concentrations (Linde Division, Union Carbide Corp.). Barometric pressure was measured during calibrations with a standard mercury barometer. Prior to dehydration, the airstream dew point was analyzed with two EG & G model 911 Dew-All analyzers. All pertinent temperatures (chamber air, leaf, and flowmeter) were monitored with copper-constantan thermocouples (0.17 mm diameter) and an Omega model

410A temperature meter. Leaf temperatures were measured with a thermocouple affixed to the underside of a leaf with a small amount of chamber sealant (Fun-Tak, International Adhesives Corp.). Chamber conditions were: mean day temperature of 30.0°C (SD = 0.8, $n = 101$), mean night temperature of 19.9°C (SD = 0.4, $n = 46$), mean day/night vpd of 2.6 (SD = 0.3, $n = 102$)/0.7 (SD = 0.1, $n = 45$) kPa, 12-h photoperiod with a range of 1500 to 2350 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PAR across the top of the chamber, and leaf temperatures approximately the same as air temperatures at night and a mean of 4.5°C (SD = 1.0, $n = 80$) higher than air temperatures during the day. Following gas exchange measurements, the entire shoot was removed from the chamber, severed from its rhizome, ground in 80% acetone for 2 min, soaked 2 h, vacuum-filtered, and the Chl content of the extract was determined spectrophotometrically, using the equations of Arnon (1). All gas exchange calculations were made utilizing equations in Šesták *et al.* (19). Gas exchange data are presented as mean rates of two to three plants at 15-min intervals.

Statistical Analyses. Where appropriate, data were analyzed using a *t* test (TWO-SAMPLE T) or analysis of variance (AOV ONEWAY) as performed by the Minitab computing system (Pennsylvania State University, University Park, PA). Statistical significance was inferred when $P \leq 0.05$.

$\delta^{13}\text{C}/^{12}\text{C}$ Analyses. Five field-collected, greenhouse-grown individuals of *T. calycinum* were oven-dried and analyzed for their stable carbon isotope ratios according to the procedures described in Teeri *et al.* (25).

RESULTS

On both dates in June 1982, tissue acidity of *Talinum calycinum* plants collected at Wilson Lake decreased during the day and increased the following night in a typical CAM fashion (Fig. 1). Dampened acid fluctuations on June 1–2, 1982, are probably attributable to the cloudy and rainy conditions at this time (15, 17).

Under laboratory conditions, tissue water content decreased after 3 d of drought, then remained relatively constant, while soil water content rapidly dropped to very low levels (Fig. 2). Daytime transpiration rates always exceeded nighttime rates and decreased in a similar fashion as did plant water content (Fig. 3). Afternoon transpiration rates responded more quickly to the drought than did morning rates of transpiration.

In spite of the above results, all indicative of a C₃ gas exchange pattern, substantial diurnal acid fluctuations occurred in the

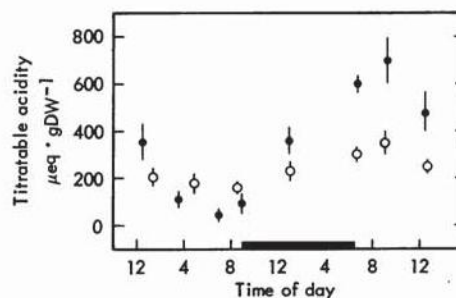


FIG. 1. Diurnal changes in tissue acidity of *Talinum calycinum* growing near Wilson Lake, Kansas on June 1–2, 1982 (○) and June 29–30, 1982 (●). Values are means (and SD) for five individuals. Time is Central Daylight Savings; black bar indicates darkness. Environmental conditions on June 1–2: overcast with rain on June 2 morning, maximum air temperature 27°C, minimum 14°C, maximum vpd 2.07 kPa, minimum 0 kPa; and on June 29–30: partly cloudy, irradiance to 2200 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PAR, maximum air temperature 38°C, minimum 23°C, maximum vpd 4.11 kPa, minimum 0.56 kPa. For each date, data are significantly different at the 0.001 level (one-way analysis of variance).

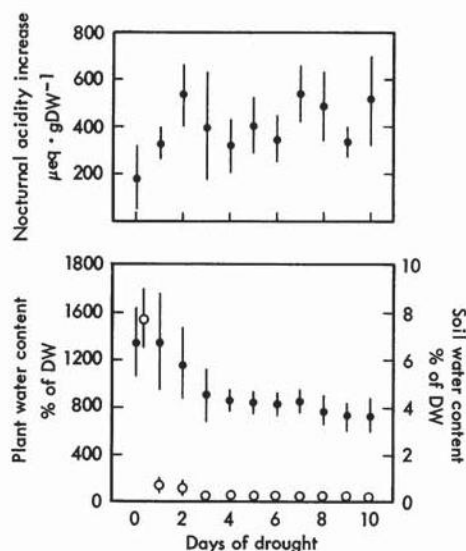


FIG. 2. Responses of soil water content (lower graph, O), plant water content (lower graph, ●), and tissue titratable acidity fluctuations (upper graph) in *Talinum calycinum* to increasing drought. Plants were watered on day 0 only. Values are means (and SD when large enough to be plotted) of five samples. Water content = $(\text{FW}-\text{DW})(\text{DW})^{-1} \times 100$. See text for further details. For each set of data, the means are significantly different at the 0.005 level (one-way analysis of variance).

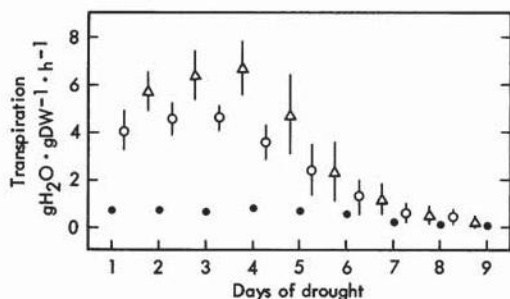


FIG. 3. Response of *Talinum calycinum* transpiration to increasing drought. Plants were watered on day 0 only. Values are means (and SD when large enough to be plotted) of ten samples. Nocturnal (●), morning (○), and afternoon (Δ) rates are given for each day. See text for further details. For each set of data (night, AM, PM), the means are significantly different at the 0.001 level (one-way analysis of variance).

Table I. Effects of Drought on Soil Water Content, Tissue Water Content, and Tissue Acid Fluctuations in *Talinum calycinum* under Growth Chamber Conditions

Sample sizes are 20 for 0 d of drought and 10 for both 5 and 9 d of drought. Per cent H_2O calculated as $(\text{FW}-\text{DW})(\text{DW})^{-1} \times 100$. Plant per cent H_2O means are not significantly different; soil per cent H_2O and acidity increase means are different at $P < 0.025$ (one-way analyses of variance).

Time of Drought	Soil H_2O	Plant H_2O	Nocturnal Acidity Increase
<i>d</i>	%	%	$\mu\text{eq g}^{-1} \text{DW}$
0	10.6 (2.1) ^a	1269 (516) ^a	457 (197) ^a
5	0.2 (0.04)	1190 (228)	623 (87)
9	1.0 (1.6)	1240 (320)	648 (187)

^a Mean (SD).

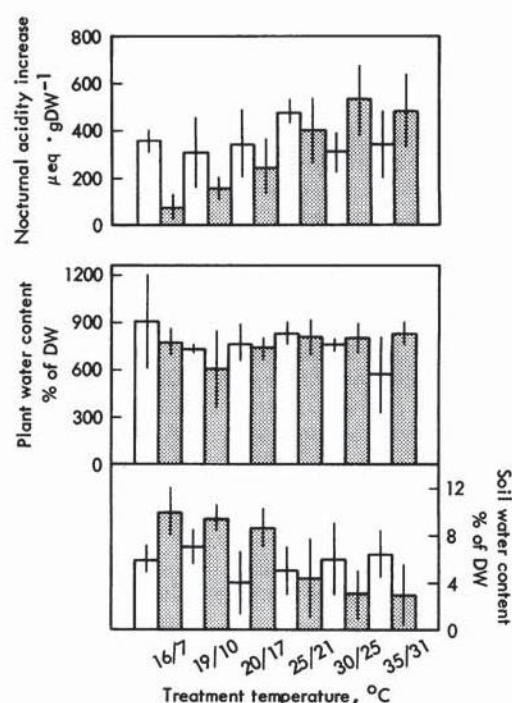


FIG. 4. Effects of temperature on soil water content (lower graph), plant water content (center graph), and tissue nocturnal increase in acidity (upper graph) in *Talinum calycinum*. Unshaded bars represent controls at 27/17°C day/night air temperature regime; shaded bars for experimental plants at day/night air temperatures indicated. Bars represent means (and SD) of five samples. See text for further details. Control means are not significantly different; experimental means are different at the 0.001 level (one-way analysis of variance).

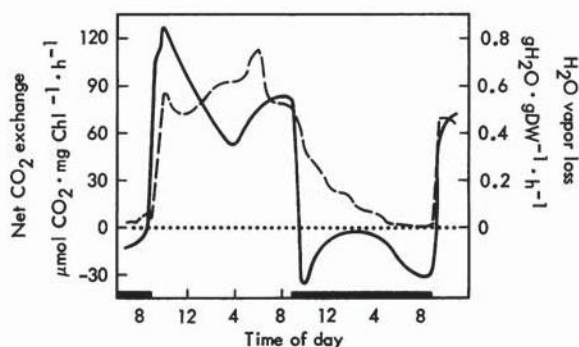


FIG. 5. Mean diurnal patterns of CO_2 (—) and H_2O vapor (---) exchange for three individuals of *Talinum calycinum* kept under well-watered conditions. Positive CO_2 exchange values indicate uptake by the plant. Black bar indicates darkness. Chamber conditions were: irradiance of 1500–2350 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PAR, 30/19.9°C day/night air temperatures (leaf temperature was approximately 4.5°C higher during the day), and 2.6/0.7 kPa day/night vpd. Mean SD of CO_2 exchange data = 14 (range 1–96; $n = 108$), of H_2O loss data = 0.3 (range 0.01–0.6; $n = 95$).

plants throughout the water stress experiment (Fig. 2). Maximum acid fluctuations were similar to those observed in the field. Tissue acid fluctuations were lower under well-watered conditions, but did not appear to change under increasing levels of water stress. To check these findings, the experiment was repeated with a greater sample size. Again, well-watered plants exhibited a smaller nocturnal increase in tissue acid content relative to those plants under 5- and 9-d drought conditions (Table I).

Growth chamber temperature had a marked effect on *T.*

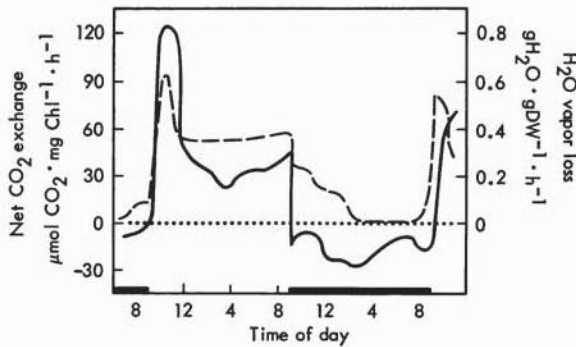


FIG. 6. Mean diurnal patterns of CO_2 (—) and H_2O vapor (---) exchange for two individuals of *Talinum calycinum* after 3 d with no water. Mean SD of CO_2 exchange data = 16 (range 0.2–160; $n = 98$), of H_2O loss data = 0.07 (range 0.01–0.6; $n = 80$). See Figure 5 legend for further information.

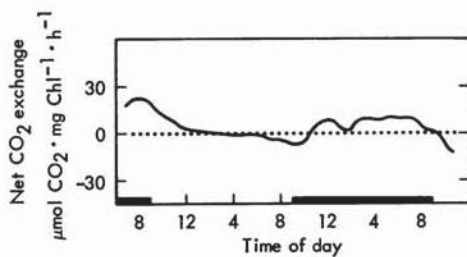


FIG. 7. Mean diurnal patterns of CO_2 exchange for two individuals of *Talinum calycinum* after 5 d with no water. Mean SD = 4.2 (range 0.03–12.6; $n = 64$). See Figure 5 legend for further information.

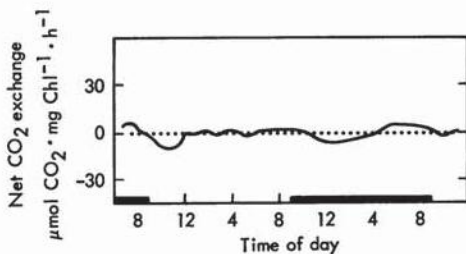


FIG. 8. Mean diurnal patterns of CO_2 exchange for two individuals of *Talinum calycinum* after 7 d with no water. Mean SD = 3.8 (range 0.01–10.2; $n = 78$). See Figure 5 legend for further information.

calycinum diurnal acid fluctuations (Fig. 4). Very little increase in nighttime tissue acid content was observed at a 16/7°C day/night temperature regime, whereas maximal fluctuations occurred at 30/25°C temperatures. Even at 35/31°C day/night temperatures, acid fluctuations were large. Despite decreasing soil water content over the range of temperatures in this experiment, plant water content did not significantly change (Fig. 4).

CO_2 and water vapor exchange patterns of potted *T. calycinum* individuals under increasing water stress are shown in Figures 5 through 8. Under well-watered conditions, high rates of CO_2 uptake and water loss were measured during the day, followed by CO_2 loss and some water loss at night (Fig. 5). After 3 d of drought, early morning CO_2 uptake rates remained high but quickly dropped by noon, followed by loss during the night (Fig. 6). Water loss patterns were similarly depressed during the day. Following 5 d of drought, little or no CO_2 uptake occurred during the day while some low-level uptake was noted at night (Fig. 7). Water loss rates were below detectable limits. Even more reduced rates of gas exchange were observed after 7 d of continuous drought (Fig. 8).

DISCUSSION

Under all water stress conditions—high and low, in the field and in the laboratory—tissue acidity in *Talinum calycinum* increased at night and decreased during the day in a typical CAM pattern (6). Diurnal acid changes observed in this study, up to $500 \mu\text{eq g}^{-1} \text{DW}$, were nearly identical with those observed in *T. teretifolium* in North Carolina (11) and in *T. guadalupense* from the Berkeley Botanic Garden (6), after conversion to a fresh weight basis in the latter case ($500 \mu\text{eq g}^{-1} \text{DW}$ for *T. calycinum* is approximately equal to $50 \mu\text{eq g}^{-1} \text{FW}$). There was no indication of greater acid fluctuations in more hydrated tissue, as has been reported for some desert cacti (4, 22, 24). Quite the contrary, in two instances (Fig. 2; Table I), diurnal acid fluctuations were lower under well-watered conditions. The depressed acid fluctuations observed *in situ* on June 1–2, 1982, may also support these findings; however, decreased irradiance may have contributed to this reduction of CAM acid fluctuation (15, 17). Reasons for the larger variability in acid fluctuations observed under identical conditions (Fig. 2; Table I) are unknown but are possibly related to different histories of the two sets of plants or different ages (though all appeared to be at the same stage of development).

The optimum temperature regime for maximal nocturnal acidification in *T. calycinum* was high (day/night air temperature of 30/25°C), especially when daytime leaf temperatures, approximately 33 to 35°C, are considered. This temperature optimum is higher than those of most other CAM plants (6, 27) and may reflect an adaptation to the hot, mid-summer conditions on sandstone outcrops in central Kansas. For example, the maximum air temperature measured on June 29 was 38°C.

In spite of the CAM-like acid fluctuations observed in *T. calycinum* under well-watered conditions, laboratory analyses of CO_2 uptake and water vapor loss patterns indicate that this succulent is a C_3 plant. Daytime water loss rates always exceeded nighttime losses, when detectable.

Although lower than many C_3 plants, maximal CO_2 uptake rates in *T. calycinum* were similar to those of other succulents (9, 18, 27) when compared on the same basis (for *T. calycinum*, $100 \mu\text{mol CO}_2 \text{ mg}^{-1} \text{Chl h}^{-1}$ is approximately equal to $150 \mu\text{mol CO}_2 \text{ g}^{-1} \text{DW h}^{-1}$). The mid-day depression in CO_2 uptake observed under well-watered conditions and after 3 d of drought in *T. calycinum* was also seen in the facultative CAM succulents *Sedum acre* (18) and *Sempervivum montanum* (27). Water use efficiencies (calculated as $\text{g CO}_2 \text{ uptake} [\text{g H}_2\text{O loss}]^{-1}$) under these two hydration regimes ranged from 0.005 to 0.008 (Table II), slightly lower than those of other succulents (12, 14, 24) and other xerophytic C_3 plants (21).

After 5 d of withholding water, low-level rates of CO_2 uptake were measured at night in *T. calycinum*. The integrated amount of CO_2 taken up nocturnally was only about 10% of total daytime uptake under well-watered conditions (Table II). Although apparently CAM-like CO_2 uptake, the stomata must have been barely open, since there was no greater water loss at night after 5 d of drought relative to well-watered plants in the previous water stress experiment (Figs. 2 and 3) and no water loss could be detected with the IR gas analysis system in this experiment. This lack of detectable water loss is supported further by the insignificant difference in plant water content during the gas analysis measurements throughout the increasing drought (Table II). This puzzling phenomenon was also observed in *Sedum acre* (18).

The amount of CO_2 taken up during the night after 5 d of water stress could potentially account for $100 \mu\text{mol malate mg}^{-1} \text{Chl}$ ($\approx 150 \mu\text{mol malate g}^{-1} \text{DW}$), or $200 \mu\text{eq acid mg}^{-1} \text{Chl}$ ($300 \mu\text{eq acid g}^{-1} \text{DW}$), which is only slightly less than the acid accumulation observed in the plants on the 5th d of drought in the previous experiment (Fig. 2). Although it is probable that

Table II. Integrated Day and Night Gas Exchange, Water Use Efficiency, and Tissue Water Content for *Talinum calycinum* under Well-Watered and Drought Conditions

Chamber conditions during gas exchange measurements were: 30/19.9°C day/night air temperature, 2.6/0.7 kPa day/night vpd, and daytime (12 h) irradiance of 1500 to 2350 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PAR. All values are means of three plants at 0 d of drought and two plants at 3, 5, and 7 d. Plant percent $\text{H}_2\text{O} = (\text{FW} - \text{DW}) / \text{DW} \times 100$. Remaining data were taken from Figures 5 through 8 by integrating the appropriate areas under the curves. Negative values represent CO_2 release. WUE was calculated as g CO_2 uptake $\text{g}^{-1} \text{DW d}^{-1}$ divided by $\text{g H}_2\text{O}$ lost $\text{g}^{-1} \text{DW d}^{-1}$.

Time of Drought	Plant H_2O	12-h Integrated CO_2 Exchange		24-h Integrated CO_2 Exchange	24-h Integrated H_2O Loss	WUE
		Daytime	Nighttime			
<i>d</i>	%	$\mu\text{mol mg}^{-1} \text{Chl}$		$\mu\text{mol mg}^{-1} \text{Chl}$	$\text{g g}^{-1} \text{DW}$	
0	1213 (240) ^a	1020	-181	839	8.34	0.008
3	1368 (161)	594	-200	394	5.50	0.005
5	1447 (60)	0	104	104	ND ^b	ND
7	1848 (341)	-40	-20	-60	ND	ND

^a Mean (SD).

^b Not detectable.

this nocturnal CO_2 uptake resulted in malate formation, it is clear from the other results that such acid fluctuations can occur without concomitant nocturnal atmospheric CO_2 uptake. For example, after 7 d of drought, essentially no gas exchange occurred (nocturnal CO_2 uptake of 15 $\mu\text{mol CO}_2 \text{ mg}^{-1} \text{Chl}$), yet large acid fluctuations were observed (over 500 $\mu\text{eq acid g}^{-1} \text{DW}$). This phenomenon has been termed 'idling' and has been reported for other succulents (3, 16, 23, 26).

The results of this study indicate that *T. calycinum* is a C_3 plant in most respects, yet undergoes CAM-like acid fluctuations under all watering regimes, in addition to occasional, low-level nocturnal CO_2 uptake. The small amounts of nocturnal CO_2 uptake, presumably involving phosphoenolpyruvate carboxylase, were inconsequential or infrequent enough so as to have no effect on the $\delta^{13}\text{C}/^{12}\text{C}$ value of *T. calycinum* tissue: -27.8‰ (mean of five plants, SD = 0.7), a value typical of many C_3 species (2). Such a low carbon isotope value reflects little, if any, participation by phosphoenolpyruvate carboxylase in the fixation of atmospheric CO_2 ; however, this enzyme is presumably involved in the nocturnal accumulations of acid observed in this study, through the fixation of internal, respiratory CO_2 .

Given the apparent minor importance of nocturnal CO_2 uptake, it would seem inappropriate to refer to *T. calycinum* as a facultative CAM plant. Its physiology more closely resembles that of several plants which undergo 'idling' (3, 16, 23, 26). As suggested for these species, this nocturnal acid accumulation without concomitant atmospheric CO_2 uptake is probably indicative of recycling of respiratory CO_2 . It is clear that this CAM-like physiology will result in a conservation of carbon, assuming this carbon is not lost the following day. Note that an acid accumulation of 500 $\mu\text{eq g}^{-1} \text{DW}$ in *T. calycinum*, representing a potential 250 $\mu\text{mol CO}_2 \text{ g}^{-1} \text{DW}$ fixed during the night, is similar to observed respiratory loss rates under well-watered conditions (Table II). Thus, without this recycling of CO_2 , it is possible that nocturnal respiratory losses might be twice those observed.

Stomatal closure, full or partial, during both day and night under severe water stress, may help to explain why tissue water content remained high throughout these experiments. The results of this study may also enhance our understanding of the ability of related species of *Talinum* to withstand severe water stress (13, 28, 29).

The above findings of acid fluctuations with little, if any, nocturnal atmospheric CO_2 fixation may be a common feature among non-desert rock outcrop succulents (11, 20; C. E. Martin, J. L. Jackson, manuscript in preparation). Further investigations of such species are necessary before a complete understanding of the significance and prevalence of this physiology is obtained.

Acknowledgments—We would like to express our gratitude to the following

people for helpful assistance with various aspects of this study. W. Adams, R. Brooks, K. Gemucio, J. Harland, J. Jackson, F. Norman, M. J. Poehler, J. Sheinbein, M. Smith, and D. Warner. We appreciate the excellent help of the Division of Biological Sciences Word Processing staff and S. Hagen in the preparation of the manuscript, as well as that of Dr. C. Haufler in criticizing it. Special thanks to Mr. T. Cannon, U. S. Army Corps of Engineers, for permission to collect and do research at Wilson Lake and to Dr. J. Teeri for the carbon isotope analyses.

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