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Carbon Metabolism in Two Species of *Pereskia* (Cactaceae)[1]

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ABSTRACT

The *Pereskia* are morphologically primitive, leafed members of the Cactaceae. Gas exchange characteristics using a dual isotope porometer to monitor $^{14}CO_2$ and tritiated water uptake, diurnal malic acid fluctuations, phosphoenolpyruvate carboxylase, and malate dehydrogenase activities were examined in two species of the genus *Pereskia*, *Pereskia grandifolia* and *Pereskia aculeata*. Investigations were done on well watered (control) and water-stressed plants. Nonstressed plants showed a CO_2 uptake pattern indicating C_3 carbon metabolism. However, diurnal fluctuations in titratable acidity were observed similar to Crassulacean acid metabolism. Plants exposed to 10 days of water stress exhibited stomatal opening only during an early morning period. Titratable acidity, phosphoenolpyruvate carboxylase activity, and malate dehydrogenase activity fluctuations were magnified in the stressed plants, but showed the same diurnal pattern as controls. Water stress causes these cacti to shift to an internal CO_2 recycling ("idling") that has all attributes of Crassulacean acid metabolism except nocturnal stomata opening and CO_2 uptake. The consequences of this shift, which has been observed in other succulents, are unknown, and some possibilities are suggested.

Evidence suggests that some succulent plants exhibit a shift in their pattern of carbon metabolism. This phenomenon has generally been observed as a change from C_3-type photosynthesis to CAM in response to a variety of environmental conditions (1, 3, 9, 10, 13). Recent literature has also examined the possible occurrence of CAM in a succulent C_4-type plant exposed to a short photoperiod and water stress (8), and in a submersed aquatic macrophyte (4). The final CAM mode of carbon fixation, however, is variously defined in these reports. Some researchers classify plants as CAM based only on the observed patterns of gas exchange. Others classify plants in which diurnal changes in titratable acidity exist as CAM, regardless of the observed gas exchange patterns. We believe that to be classified as having CAM photosynthesis, a plant should exhibit the gas exchange patterns typical of CAM, *i.e.* nighttime stomatal opening and daytime closure, and a diurnal change in titratable acidity. One characteristic alone is insufficient to warrant CAM classification.

The Cactaceae have been described as an exclusive CAM family, *i.e.* all members investigated show CAM (7, 12, 13). In this report, we extend studies on the Cactaceae by showing that a morphologically primitive, leafed genus of the family, the *Pereskia*, preferentially exhibits C_3-type photosynthesis, and that even under conditions of extreme water stress, CAM photosynthesis as defined above is not observed. However, there is a shift from C_3 photosynthesis to a modification of CAM previously called "idling." Here, there is a measurable diurnal fluctuation of organic acid, but no measurable gas exchange. Evidently, internally produced CO_2 is recycled through the CAM pathway. In this paper, we describe this unique response and speculate on its adaptive significance.

MATERIALS AND METHODS

Plants. *Pereskia aculeata* plants were propagated by cuttings from a parent plant grown in the University of California, Berkeley Botanic Gardens. *Pereskia grandifolia* cuttings were propagated from a parent plant growing on the University of California, Riverside campus. Cuttings were rooted in sand and grown in a glasshouse. Plants were repotted into a peat:sand:sandy loam (1:2:2) soil mixture in gallon pots, and experimentation was conducted when plants had been in pots for 6 months. Control plants were well watered. Water-stressed plants were well watered for 3 weeks, then watered weekly for 5 weeks, after which water was withheld for 10 days prior to experimentation. Water potential measurements were not made due to the lack of an appropriate technique. The plants tested have extremely short petioles and so the Scholander bomb could not be used. Water stressed plants showed visible wilting and chlorosis at the time of experimentation. Photoperiod was allowed to vary as it would under natural conditions. Table I describes environmental conditions during the duration of the experiment.

Acid Titrations. Leaf samples were punched with a No. 10 cork borer (area = 2.405 cm^2) and collected in triplicate. They were frozen until assayed. Individual samples were ground in glass-distilled H_2O with a mortar and pestle. They were then titrated to a pH 7 endpoint with 0.01 N KOH. Data are expressed as μeq acid/g fresh weight.

Enzyme Assays.

Preparation of Extract. One g leaf tissue was ground at 0 C in 9 ml extraction medium consisting of 0.1 M Hepes-NaOH (pH = 7.5), 2 mM $MgCl_2$, 2 mM EDTA, and 10 mM DTT. Polyclar AT (0.5 g/g leaf tissue) was added before grinding each sample to aid in mucilage removal. The homogenate was squeezed through one layer of Miracloth and a small sample was removed for Chl determination. The extract was then centrifuged at 10,000 rpm and 4 C for 10 to 20 min before being passed through a small Sephadex G-25 column previously equilibrated with extraction buffer adjusted to pH 7.5. Each point on the figure indicates the mean of three samples.

Assay of PEP[2] Carboxylase. PEP carboxylase was assayed spectrophotometrically by following the oxidation of NADH at 340 nm and 27 C. The reaction mixture contained 50 mM Hepes-NaOH (pH = 7.8), 5 mM $MgCl_2$, 2 mM PEP, 1 mM $NaHCO_3$, 0.2 mM NADH, and 100 μl extract. Enzyme activity is expressed as μmol min^{-1} mg Chl^{-1} (16).

Assay of Malate Dehydrogenase. Malate dehydrogenase was assayed spectrophotometrically by following the oxidation of

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[2] Abbreviations: PEP, phosphoenolpyruvate; MDH, malate dehydrogenase; THO, tritiated water.

Table I. *Environmental Conditions in Glasshouse during Course of the Experiment*

Time	T_{air}	RH	ΔH_2O	Irradiance
	C	%	mg cm^{-3}	$\mu E\ m^{-2}\ s^{-1}$
1700	25.5	48.75	12.15	270
2000	23.5	44	11.86	5
2300	22.0	41	11.46	0
0500	20.5	48	9.26	0
0800	26.5	37	15.79	200
1100	35.0	23	30.52	630
1400	35.0	28	28.53	525

NADH at 340 nm and 27 C. The reaction mixture contained 50 mM Hepes-NaOH (pH = 7.8), 1 mM oxaloacetate, 0.1 mM NADH, and 50 μl extract. Activity is expressed as μmol min^{-1} mg Chl^{-1} (17).

Gas Exchange Studies. Gas exchange parameters were determined with a dual isotope porometer (6). The porometer passes an air stream of $^{14}CO_2$ (~300 μl/l in an 80:20 mixture of N_2:O_2) through tritiated water of known specific radioactivity for humidification. The abaxial leaf surface was exposed for 20 s to the gases via a small chamber clamped on the leaf. Isotope radioactivity in the leaf samples was determined by liquid scintillation counting. The resistances to water vapor transfer and CO_2 uptake were derived directly from THO vapor uptake and $^{14}CO_2$ uptake according to the basic gas exchange equation as we have previously applied it (6). Values for resistances (s cm^{-1}), transpiration rates (g water loss dm^{-2} h^{-1}) and CO_2 uptake rates (mg CO_2 uptake dm^{-2} h^{-1}) were also calculated from the gas exchange data. Datum points represent the average of six samples.

RESULTS

Diurnal Acidity. Well watered *P. grandifolia* plants showed little diurnal fluctuation of acidity and low average acid levels. Plants which were stressed by withholding water showed a more typical CAM-like diurnal fluctuation of acidity with maximum levels of organic acids present at the end of the dark period, or slightly thereafter (Fig. 1). Maximum values of titratable acidity were not high for this plant, even under conditions of water stress, but stress did increase the magnitude of the fluctuations.

The situation in the other cactus, *P. aculeata*, is much more dramatic. Control plants did show a diurnal fluctuation in titratable acidity common to CAM. Acid levels were fairly high throughout the cycle. Water-stressed plants showed much larger diurnal fluctuations in acidity (103.8 μeq g^{-1}), and the pattern followed that of a typical CAM plant (Fig. 1). In the stressed plants, acidity levels remained high throughout most of the morning hours before dropping at around 11:00 AM which is not typical of CAM.

Enzyme Assays. Enzyme assays were simultaneous with titratable acidity measurements. Control plants of *P. grandifolia* showed some variation in PEP carboxylase and MDH activities during the diurnal cycle, but fluctuations were not great (Fig. 2). Activities of the two enzymes seem to parallel each other in almost all treatments and time periods. Stressed plants of *P. grandifolia* showed a wide fluctuation of enzyme activity during the diurnal cycle, and these fluctuations were concomitant with changes in pH. *P. aculeata* plants also exhibited changes in enzyme activity between control and stressed plants. Well watered plants showed little variation in activity of PEP carboxylase and MDH during the cycle whereas stressed plants showed a diurnal fluctuation, although not as large as that observed in *P. grandifolia* (Fig. 3). The sample taken at 7:00 PM from stressed treatments of both species was noteworthy, because it was the only time when PEP carboxylase and MDH activities were not coordinated, and may indicate relative sensitivity differences of the enzymes to inhibition or regulation.

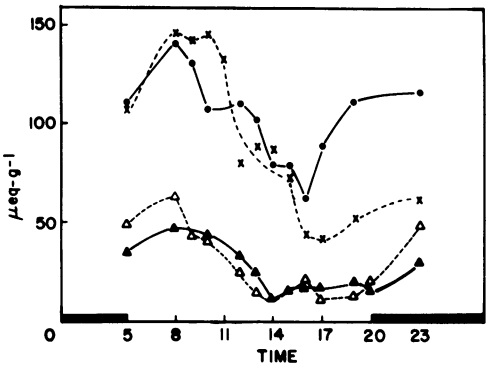

FIG. 1. Diurnal variation in titratable acidity of well watered, control (●——●) and water-stressed (×- - -×) leaves of *P. aculeata*, and control (▲——▲) and water-stressed (△- - -△) leaves of *P. grandifolia*. The dark bar along the abscissa indicates the dark period.

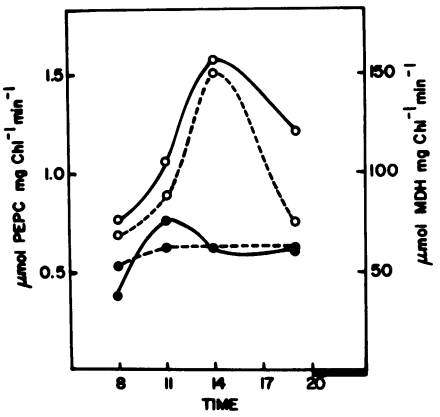

FIG. 2. Activity of PEP carboxylase (PEPC) (——) and MDH (- - -) in well watered control (●) and water-stressed (○) leaves of *P. grandifolia* over a diurnal cycle. The dark bar along the abscissa indicates the dark period.

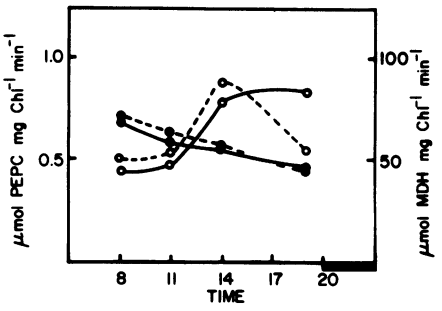

FIG. 3. Activity of PEP carboxylase (PEPC) (——) and MDH (- - -) in well watered control (●) and water-stressed (○) leaves of *P. aculeata* over a diurnal cycle. The dark bar along the abscissa indicates the dark period.

Gas Exchange Studies. Under favorable water conditions, both *P. aculeata* and *P. grandifolia* showed some stomatal opening during the day and none at night as judged from the estimations of leaf resistance to gas exchange (Fig. 4). When stressed by withholding water, there was an increase in stomatal resistance to gas exchange during the day indicating daytime stomatal closure. In both well watered and water-stressed condition, leaf resistances to gas exchange were high indicating stomatal closure at night.

The well watered *P. grandifolia* plants showed a typical C_3-type diurnal transpiration curve with maximum predicted water loss

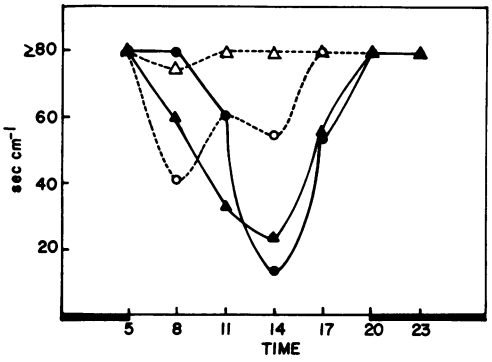

FIG. 4. Diurnal variation in stomatal resistance to $^{14}CO_2$ transfer in well watered, control (●——●) and water-stressed (○- - -○) leaves of *P. aculeata*, and control (▲——▲) and water-stressed (△- - -△) leaves of *P. grandifolia*. The dark bar along the abscissa indicates the dark period.

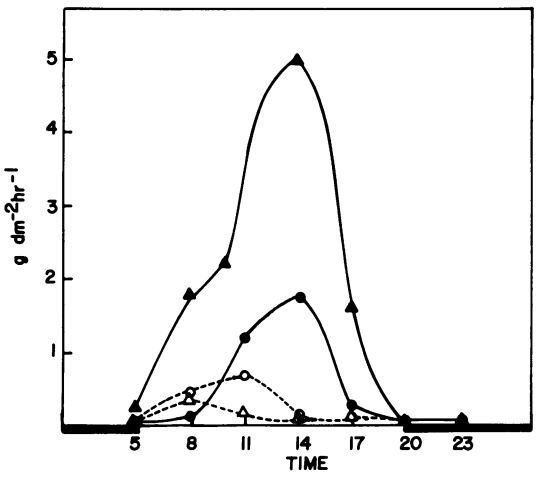

FIG. 5. Calculated diurnal transpiration rates for well watered control (●——●) and water-stressed (○- - -○) leaves of *P. aculeata* and control (▲——▲) and water-stressed (△- - -△) leaves of *P. grandifolia*. Data are given as g dm^{-2} h^{-1}. Transpiration rates were calculated from resistances to uptake. The dark bar along the abscissa indicates the dark period.

rates of 5 to 6 g dm^{-2} h^{-1} occurring at midday (computed from the resistance to THO uptake). Water-stressed plants showed little transpiration although slight amounts of water loss were observed in the early morning and late afternoon (Fig. 5).

P. aculeata plants exhibited a different pattern of gas exchange than *P. grandifolia*. Control plants of both species show typical C_3 patterns of gas exchange. Stomata were open during the light hours and closed in the dark. Water-stressed *P. aculeata* plants showed a midday stomatal closure, but the closure was evidently not complete. The most striking difference between stressed plants of the two species was observed in the early morning responses of the stomata. Stomata of stressed *P. aculeata* showed less resistance than control plants at 8:00 AM, *i.e.* they appeared to open more rapidly. The stomata of these stressed plants remained slightly open throughout the morning hours which accounted for relatively high transpiration values (Fig. 5) observed during the first half of the light period.

CO_2 uptake calculated from the resistance to $^{14}CO_2$ uptake showed patterns similar to the transpiration curves (Fig. 6). CO_2 uptake was predicted during the day for both species when under good water status, but when stressed CO_2 uptake was greatly reduced. There was no indication of any CO_2 uptake at night.

DISCUSSION

The following criteria are used to establish CAM in succulent plants: (a) stomata are more open at night than during the day

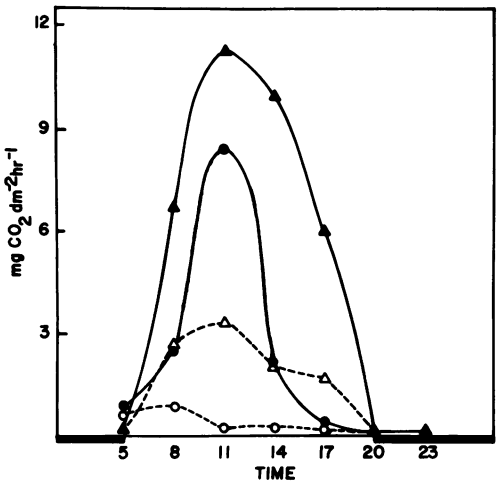

FIG. 6. Calculated diurnal CO_2 uptake rates for well watered control (●——●) and water-stressed (○- - -○) leaves of *P. aculeata*, and control (▲——▲) and water-stressed (△- - -△) leaves of *P. grandifolia*. CO_2 uptake rates were calculated from resistances to $^{14}CO_2$ uptake and are expressed as mg CO_2 dm^{-2} h^{-1}. The dark bar along the abscissa indicates the dark period.

and plants show net CO_2 uptake at night with little or reduced uptake during the day, and (b) total titratable acidity (mostly because of malic acid) of photosynthetic tissue fluctuates diurnally with reciprocal fluctuations in storage carbohydrate (11). CAM plants show diurnal fluctuations in enzyme activity such that PEP carboxylase activity is high when malic acid is low. However, enzyme activity has not been integrated into the definition of CAM.

Our data obtained here for the two *Pereskia* species indicate that organic acids fluctuate diurnally typical of the CAM pattern. Under water stress conditions, the magnitude of fluctuation is somewhat greater, but fluctuation occurs in both well watered and water-stressed plants. In the case of extractable activity of PEP carboxylase and malate dehydrogenase, water stress is accompanied by an increase with a distinct diurnal fluctuation, there being more measureable activity at the end of the light period when the concentration of organic acids reaches a low value. This pattern of fluctuation when the plants are water-stressed is typical of CAM plants when performing CAM (7). Unlike CAM plants, however, there is no measureable stomatal opening at night, and consequently, no night gas exchange. In some species, such as *Portulacaria afra* (13) and others, there is a typical C_3 photosynthetic carbon metabolism during favorable water conditions, but a shift to all the attributes of CAM when stressed. It is clear from our data, that under favorable water conditions *P. aculeata* and *P. grandifolia* show C_3 photosynthesis in terms of gas exchange, but not organic acid fluctuation. When water-stressed, the organic acid fluctuation increases somewhat and the extractable activity of PEP carboxylase and malate dehydrogenase increases, but there is no shift in gas exchange except for an increase in daytime stomatal resistance and a reduction in daytime gas exchange.

Previously, the genus *Pereskia* was classified as CAM based solely on acidity measurements (7, 12). However, the plants are not CAM when assessed with the criteria used here, but show a modification of CAM previously called "idling" (11). The fact that organic acids fluctuate even when not water-stressed with typical C_3-type gas exchange is truly enigmatic.

The development of CAM depends on interactions between the environment and the metabolism of the plant. It was anticipated that *Pereskia* would shift from C_3 to CAM when water-stressed especially since there was previously evidence (7, 12) that the plants showed high acidity levels that could fluctuate diurnally.

The plants, however, are native only to the tropical regions of the New World, and thus probably have not experienced low humidity and dry soil conditions during much of their evolutionary history (5). Perhaps their history can serve to explain both the leafed habit and different pattern of carbon metabolism in contrast to other genera of the Cactaceae.

CO_2 fixation in CAM and probably in "idling" plants is mediated at the carboxylation of P-enolpyruvate to oxaloacetate via PEP carboxylase with subsequent reduction of oxaloacetate to malate by MDH (1, 14, 15). Levels of MDH in most plant tissues are quite high, and therefore control of the pathway presumably is at PEP carboxylase. PEP carboxylase is inhibited by malate (14), and as titratable acidity increases PEP carboxylase activity decreases. This correlation occurs in the *Pereskia* species investigated and PEP carboxylase levels are lowest at the end of the dark period when acidity is highest. Greenway *et al.* (1) perceive that malic acid fluctuations develop in a C_3 to CAM shift after PEP carboxylase fluctuations are fairly well established, and that PEP carboxylase in mature CAM plants may exist in two distinct forms. Because our assays were conducted at pH 7.5, we may have only measured one form of the enzyme.

The recycling of internal CO_2 theoretically contributes to plant survival under arid conditions. Consequently, a moderate energy state is maintained, and a rapid reponse to small amounts of precipitation by assimilation of atmospheric CO_2 would be possible. This unique modification of CAM or "idling" may be regulated by stomatal closure, enzyme activity, a combination of both or some other internal factor. The exact mechanisms and conditions required for this shift to "idling" will require further study.

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