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Short Communication

Induction of Acid Metabolism in *Portulacaria afra*[1]

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ABSTRACT

***Portulacaria afra*, a succulent plant, shifts from a predominantly C_3 mode of gas exchange to a typical Crassulacean acid metabolism type CO_2 uptake in response to water or NaCl stress. Control plants in the absence of water stress assimilated CO_2 during the light (about 7-8 mg CO_2 dm^{-2} hr^{-1}), transpiration (about 1.5 g dm^{-2} hr^{-1}) was predominantly during the day, stomates were open during the day, and there was little diurnal organic acid fluctuation. Stressed plants showed only dark CO_2 uptake and dark water loss, nocturnal stomatal opening, and an increased diurnal fluctuation of titratable acidity. Within 2 weeks after rewatering, stressed plants returned to the control acid fluctuation levels indicating that the response to stress was reversible.**

Crassulacean acid metabolism is known to occur in at least 18 different flowering plant families (13). These succulent plants showing the CAM[2] metabolism have become important experimental objects because of the evidence suggesting that they have the capability to shift from a CAM-type carbon metabolism to a C_3 type (7). In the former (*i.e.* CAM), CO_2 is fixed initially into 4-carbon acids, *viz.* malic acid, during the dark period when stomates are open resulting in a large accumulation of acid of up to 200 μeq g^{-1} fresh weight. During the subsequent light period, stomates are closed, the malic acid is decarboxylated, and CO_2 is assimilated by carboxylation through ribulose biphosphate carboxylase not unlike the C_3 pathway. Glucose or other carbohydrate fluctuates reciprocally with acid (12, 16).

A shift to the C_3 pathway presumes carboxylation through ribulose biphosphate carboxylase during the light period when stomates are open and little or no malic acid fluctuation and/or accumulation.

Many of the plant families showing CAM appear to be exclusive, *e.g.* Cactaceae and Crassulaceae, in that all members investigated show CAM. Others such as the Orchidaceae and Bromeliaceae (5), and the Mesembryantheae (15) are variable as to their species. Another succulent family, the Portulacaceae, has species with the C_4 and CAM carbon metabolism pathways. For example, *Portulacea oleracea* is C_4 and succulent genera such as *Anacampseros*, *Thallinum*, and *Portulacaria* show a variable, diurnal acid fluctuation (unpublished).

Much of the variability and uncertainty with CAM may be the result of reversible shifts from CAM to C_3 as was shown for the Mesembryantheae (15). In confirmation of such a hypothesis, we report here that this capability to shift from C_3 to CAM in response to stress (water or salt) is true for *Portulacaria afra*.

MATERIALS AND METHODS

Plants. *P. afra* plants were propagated by cuttings from a parent plant grown on the UC Riverside campus. Cuttings were rooted in vermiculite and irrigated regularly with water or water plus nutrient solution. After rooting, control, nonstressed plants were watered at no less than 3-day intervals to keep leaf water potentials high. Plants stressed with NaCl were irrigated with 2% NaCl at weekly intervals and water-stressed plants were irrigated with water at weekly intervals. NaCl and water-stressed plants had leaf water potentials consistently less then -10 bars after treatment whereas the control, nonstressed plants had leaf water potentials greater than -10 bars.

Acid Titrations. Leaf samples were collected in triplicate and frozen in dry ice until assayed. Duplicate samples were extracted by grinding with a TenBroeck tissue grinder in distilled H_2O followed by boiling. After debris removal by centrifugation, samples were titrated to pH 7 with 0.01 N NaOH. Data are expressed as μeq acid/g of tissue, fresh weight.

Gas Exchange Studies. CO_2 uptake and transpiration were estimated on the same plant samples with the use of a double isotope (THO and $^{14}CO_2$) diffusion porometer. CO_2 uptake in mg dm^{-2} hr^{-1}, transpiration in g dm^{-2} hr^{-1}, leaf resistance to gas exchange in sec cm^{-1}, and T/P were calculated by computer program from dpm uptake of THO vapor and $^{14}CO_2$ plus known flow rates, specific activity of supplied isotopes, temperature, and vapor pressure data.

The double isotope porometer has not yet been described by publication but the procedure is essentially as follows.

While in a small chamber, the tissue was exposed for 20 sec to a stream of air containing THO vapor and $^{14}CO_2$. The THO vapor portion of the porometer was based on the assumption that the diffusion of THO vapor into the leaf would follow the same physical pathway as H_2O leaving the plant. The concentration of THO vapor in the air stream was maintained at a constant known level by bubbling air from the supply tank through a liquid THO source having a known specific radioactivity (5.55×10^9 dpm/ml of stock solution) and known temperature. The $^{14}CO_2$ component of the porometer was a modification of that described by Shimshi (10) and estimates gross CO_2 uptake. The $^{14}CO_2$ (3.8×10^4 dpm/cm^3 air) diffuses into the leaf along the same path as the THO vapor but in addition must pass to the chloroplast and through the biochemical events of carboxylation. Thus, leaves exposed in the chamber assimilate the two isotopes quantitatively with respect to diffusion resistance to water vapor and the total resistance to photosynthesis.

The resistance of exposed plants was quantified using the gas exchange equation, $r = \Delta/f$, where f is the rate of isotope uptake in dpm cm^{-2} sec^{-1} and Δ is the isotope diffusion gradient in dpm cm^{-3}. The estimate r is the usual sec cm^{-1}. Dpm are used in the formulation since they represent the basic units of measurement and provide the most direct route for obtaining the resistance to gas exchange. Transpiration and CO_2 uptake were calculated directly from dpm THO vapor or $^{14}CO_2$ uptake.

Data obtained are comparable to those published for similar

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[2] Abbreviations: CAM: Crassulacean acid metabolism; THO: tritiated water; T/P: transpiration ratio.

succulent plants. For *P. afra*, calculated CO_2 uptake rates are 7 to 8 mg CO_2 dm^{-2} hr^{-1}, transpiration rates are 1 to 1.5 g dm^{-2} hr^{-1}, leaf resistances to gas transfer are as low as 2 to 10 sec cm^{-1}, and transpiration ratios are 100 to 200 (*cf.* 2).

Water Potential. Water potential was estimated with a pressure bomb (9) or hydraulic jack apparatus obtained from Campbell Scientific, Logan, Utah.

RESULTS

Diurnal Acidity. Plants which are well watered with leaf water potentials greater than −10 bars show little or no diurnal fluctuation of acidity despite having fairly high acid levels (Fig. 1). Once stressed by either witholding water to depress leaf water potentials below −10 bars or irrigating weekly with 2% NaCl, a typical CAM-type diurnal fluctuation of acidity commenced (Fig. 1). Whereas control plants rarely had a diurnal fluctuation, stressed plants frequently showed 100 to 150 μeq g^{-1} titratable acidity fluctuation.

A time course for diurnal acid fluctuation induction with NaCl-stressed plants is shown in Figure 2. After 3 to 6 days, diurnal acid fluctuation was induced due to both a greater night accumulation and day depletion of acid relative to controls. The watered control had some acid fluctuation, but this was variable on a day to day basis (Fig. 2). The fluctuation could be a response to an infrequent watering pattern of the controls. Being succulent, the well watered controls were irrigated only every 3rd day or so depending on the glasshouse temperature. The over-all fluctuation could be the result of periodic drying during the variable watering cycle.

There are no apparent differences in the increase in acid levels between water-stressed and NaCl-stressed plants (Table I).

Figure 3 shows a time course for diurnal acid fluctuation induction by 2% NaCl irrigation. The data (Δ/Δ μeq g^{-1}) are total diurnal acid fluctuation of the NaCl-treated plant less the control plant fluctuation. There was a steady increase to nearly 100 μeq g^{-1} excess over the control for up to a 3-week period. After 3 weeks, the NaCl-treated plants were irrigated with low salt tap water and they returned to the control acid fluctuation level within 2 weeks.

Transpiration. The well watered control plants showed a normal type diurnal transpiration curve reaching rates as high as 1.1 g dm^{-2} hr^{-1} during the day. The only unexpected anomaly was an increase toward the end of the dark period (Fig. 4). These data, however, are entirely consistent with those of Neales (6)

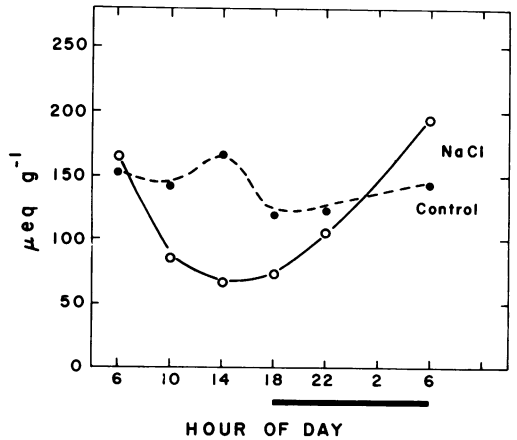

FIG. 1. Diurnal variation in titratable acidity of well watered, unstressed *P. afra* (control) and NaCl-stressed plants by weekly irrigation with 2% NaCl for 3 weeks. Acid determined by duplicate titration of samples collected from three different plants. Dark bar below the abscissa indicates the dark period.

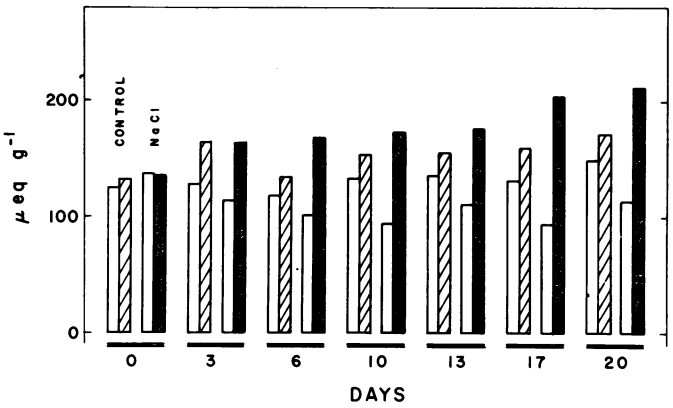

FIG. 2. Daily time course of change in maximum and minimum acidity in control and NaCl-stressed plants (see Fig. 1). (□): end of the day minimum acid levels; (▨ ▩): end of the night maximum acid levels for control and NaCl-stressed plants, respectively.

Table I. Titratable acidity for watered control plants, water-stressed, and NaCl irrigated plants.

	Acidity[1] 6 am	6 pm	Water potential
	μeq g^{-1} fresh weight		bars
CONTROL	142	141	- 7.9
$-H_2O$	178	113	-12.5
2% NaCl	188	80	-10.9

1 2 separate determinations.

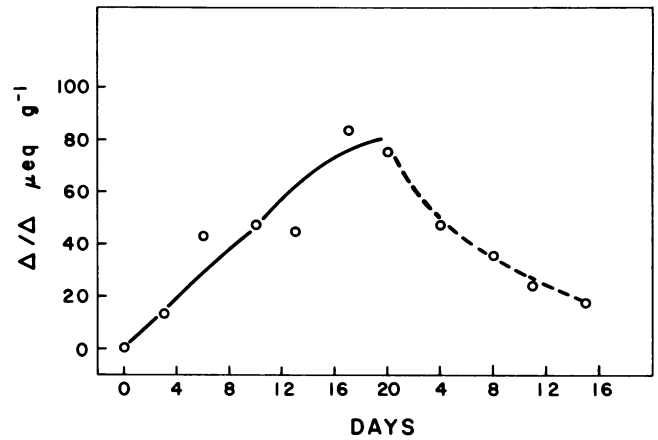

FIG. 3. Time course of change in maximum diurnal acid fluctuation after onset of 2% NaCl irrigation (day 0). Data (Δ/Δ μeq g^{-1}) are the difference in the diurnal fluctuation of acid between stressed and control plants. At day 20, plants were irrigated with water to relieve the stress. The data show a reversible response to NaCl stress.

for *P. afra* showing some late night period CO_2 uptake.

The water- and NaCl-stressed plants showed nearly a complete cessation of transpiration during the day period and a small but significant transpiration at night, particularly at the end of the night period (Fig. 4).

Leaf Resistance. Leaf resistance to gas exchange computed from leaf and air temperature, vapor pressures, and THO uptake, showed the expected curve for the watered, control plants (Fig. 4). Minimum values during the daylight period were on the order of 2 to 5 sec cm^{-1} with nighttime values of 80 sec cm^{-1} or more. These data clearly indicate day stomatal opening and night closing. Consistent with the transpiration curve, leaf resistance decreased toward the end of the dark period allowing some

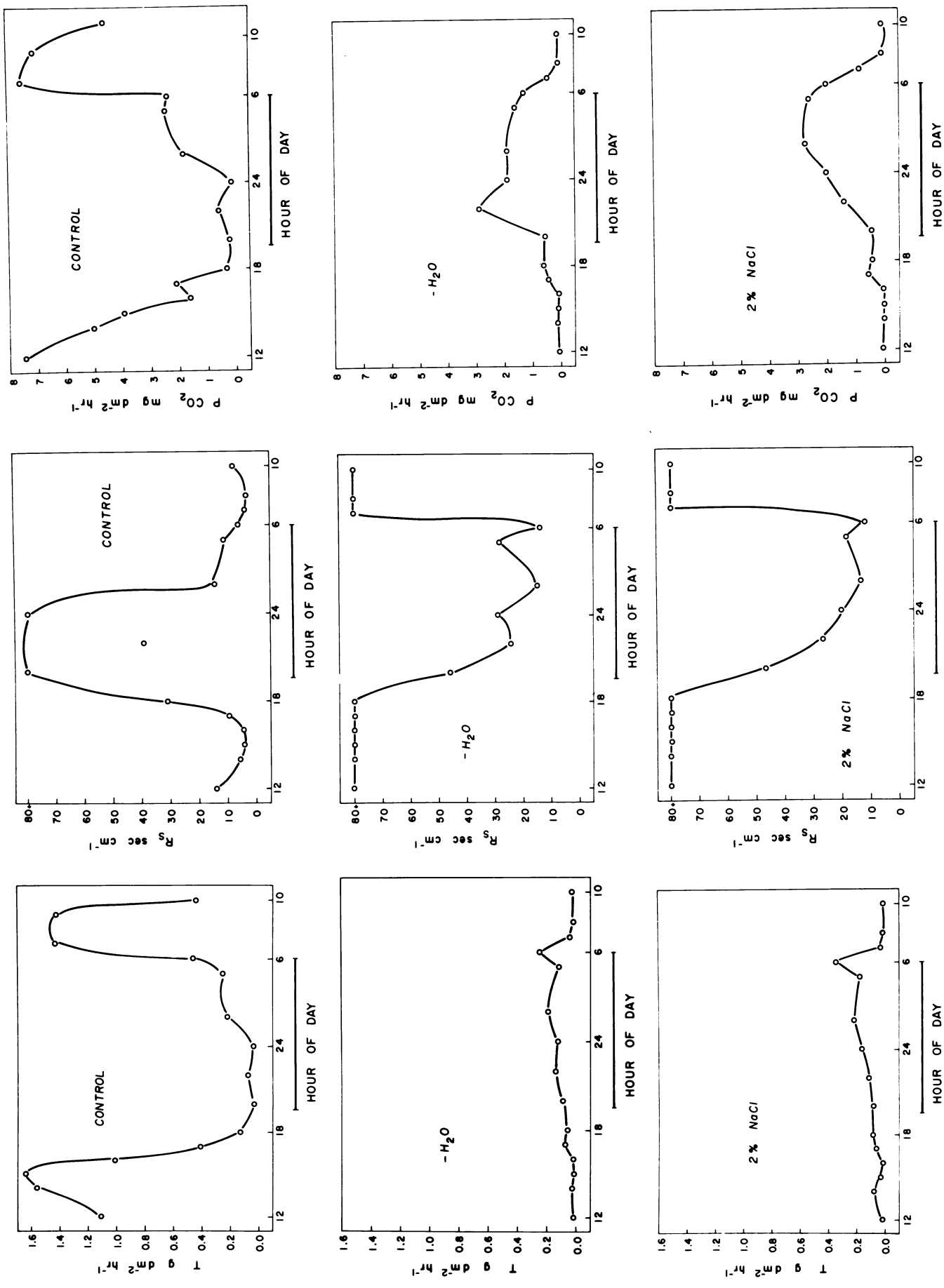

FIG. 4. Calculated diurnal transpiration rates (T), leaf resistance to gas transfer (R_s), and CO_2 uptake rates (P), for unstressed, watered plants water-stressed plants, and NaCl-stressed plants. The unstressed plants were watered no less frequently than every 3rd day. The water-stressed plants were watered weekly and NaCl-stressed plants were irrigated with 2% NaCl weekly. Transpiration and CO_2 uptake rates were calculated from THO and $^{14}CO_2$ uptake rates with the use of a double isotope (THO and $^{14}CO_2$) porometer. Dark bar below the abscissa is the dark period.

gas exchange. Estimates reached lows of 10 sec cm^{-1}.

The water- and salt-stressed plants showed almost the reverse of the watered controls. Leaf resistance exceeded 80 sec cm^{-1} throughout the daylight period and after dark, leaf resistance began to decrease until minima of 10 to 15 sec cm^{-1} were recorded toward the end of the dark period. The dark period data for the stressed plants are similar to the dark period data of the control plants.

CO_2 Uptake. The watered control plants showed maximum CO_2 uptake during the light period with values as high as 7.5 mg dm^{-2} hr^{-1} (Fig. 4). There was little CO_2 uptake during the beginning of the dark period, but by the end of the dark period, as much as 2.5 mg dm^{-2} hr^{-1} CO_2 was being taken up.

Except for the very beginning and very end of the light period, the stressed plants showed only CO_2 uptake at night. Average values for dark CO_2 uptake, mostly toward the end of the dark period, were 2 to 3 mg dm^{-2} hr^{-1}.

Once again, the water-stressed and NaCl-stressed treatments were similar.

Transpiration Ratio. Transpiration ratios computed for all data shown in Figure 4 were extremely variable and not clearly interpretable. When, however, the T/P was integrated over a 24-hr period for those times when stomates were open (arbitrarily set at 30 sec cm^{-1} leaf resistance), then the control plants had a T/P nearly twice the stressed plants (control = 193, $-H_2O$ = 83, 2% NaCl = 101). Since gas exchange was so low at R_s estimates above 30 sec cm^{-1}, we felt justified in not using such data.

DISCUSSION

Much of the evidence that CAM plants are variable or have the capability to shift photosynthetic pathways comes from ^{13}C studies showing that unlike C_3 and C_4 plants which have a predictable estimate (a mode of about $-12‰$ for C_4 and $-27‰$ for C_3; see ref. 3), CAM succulents may vary from -11 to $-30‰$. Apparently the variability results from the mode of CO_2 carboxylation, *i.e.* through the CAM or C_3 pathways exclusively or some combination. Further, many have now shown that environmental growth conditions will shift these $\delta^{13}C$ estimates from C_3-like to CAM-like (1, 4, 8).

In an attempt to categorize succulents, Neales (6) classified according to dark *versus* light CO_2 uptake, either "weak-CAM," "full-CAM," or "super-CAM." Weak-CAM plants, including *P. afra*, showed slight CO_2 uptake at the end of the dark period with mostly light CO_2 assimilation.

Evidently, *P. afra* is only of the weak-CAM-type when grown under nonstressed conditions. Our gas exchange data for well watered *P. afra* appear identical to Neales (6, his Fig. 3), and the stressed plants shift to a pattern not too unlike his reported full-CAM. We interpret these data to mean that Neales' categories may be environmental and not necessarily genetic.

The shift reported here for C_3-like or weak-CAM to CAM could be explained completely on the basis of stomatal changes responding to water stress. Nevertheless, we have measured (not shown in text) a somewhat elevated level of P-enolpyruvate carboxylase in the stressed plants consistent with reports of Treichel *et al.* (14) for *Carpobrotus* and *Mesembryanthemum*. We suspect that in *P. afra*, metabolic changes other than those directly associated with stomatal changes are occurring in response to water stress.

These data support the hypothesis that certain succulent plants shift their mode of photosynthetic carbon metabolism in response to environmental change. To date, there is no evidence that all succulents shift. Studies with cactus in the United States and in Australia have yielded little evidence of any significant diurnal CO_2 uptake in the absence of dark CO_2 uptake (7, 11).

The T/P calculations clearly suggest the "ecological advantage" of such a shift from C_3-like or daytime CO_2 assimilation to dark uptake of CO_2. The reverse phase stomatal opening and hence reverse phase gas exchange results in low water loss to carbon gain in the stressed plants effectively conserving water. In the water-stressed plants, gas exchange takes place largely at night when the evaporative demand is low.

LITERATURE CITED

1. Bender MM, I Rouhani, HM Vines, CC Black 1973 $^{12}C/^{13}C$ ratio changes in Crassulacean acid metabolism plants. Plant Physiol 52: 427-430
2. Black CC 1973 Photosynthetic carbon fixation in relation to net CO_2 uptake. Annu Rev Plant Physiol 24: 253-286
3. Lerman JC 1975 How to interpret variations in the carbon isotope ratio of plants: biologic and environmental effects. *In* R Marcelle, ed, Environmental and Biological Control of Photosynthesis. W Junk, The Hague pp 323-335
4. Lerman JC, O Queiroz 1974 Carbon fixation and isotope discrimination by a Crassulacean plant: dependence on the photoperiod. Science 183: 1201-1209
5. Medina Z, JH Troughton 1974 Dark CO_2 fixation and the carbon isotope ratio in Bromeliaceae. Plant Sci Lett 2: 357-362
6. Neales TF 1975 The gas exchange patterns of CAM plants. *In* R Marcelle, ed, Environmental and Biological Control of Photosynthesis. W Junk, The Hague pp 299-310
7. Osmond CB 1975 Environmental control of photosynthetic options in Crassulacean plants. *In* R Marcelle, ed, Environmental and Biological Control of Photosynthesis. W Junk, The Hague pp 311-321
8. Osmond CB, WG Allaway, BG Sutton, JH Troughton, O Queiroz, U Lüttge, K Winter 1973 Carbon isotope discrimination in photosynthesis of CAM plants. Nature 246: 41-42
9. Scholander PF, HT Hammel, EO Bradstreet, EA Hemmingsen 1965 Sap pressure in vascular plants. Science 148: 339-346
10. Shimshi D 1969 A rapid field method for measuring photosynthesis with labelled carbon dioxide. J Exp Bot 20: 381-401
11. Szarek SR, IP Ting 1974 Seasonal patterns of acid metabolism and gas exchange in *Opuntia basilaris*. Plant Physiol 54: 76-81
12. Ting IP 1971 Nonautotropic CO_2 fixtion and Crassulacean acid metabolism. *In* MD Hatch, CB Osmond, RO Slatyer, eds, Photosynthesis and Photorespiration. Wiley-Interscience, New York pp 169-185
13. Ting IP 1976 Crassulacean acid metabolism in natural ecosystems in relation to annual CO_2 uptake patterns and water utilization. *In* RH Burris, CC Black, eds, CO_2 Metabolism and Plant Productivity. University Park Press, Baltimore pp 251-268
14. Treichel SP, GO Kust, DJ von Willert 1974 Veranderung der Activitat der Phosphoenolpyruvat-Carboxylase durch NaCl bei Halophyten verschiedener Biotope. Z Pflanzenphysiol 71: 437-449
15. Winter K 1973 CO_2-Fixierungsreaktionen bei der Salzpflange Mesembryanthemum crystallinum unter variierten Aussenbeding ungen. Planta 114: 75-85
16. Wolf J 1960 Der diurnale Saurerhythmus. Handbuch der Pflanzenphysiologie 12 Part II Springer-Verlag, Berlin pp 809-889