

Responses of Succulents to Plant Water Stress¹

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ABSTRACT

Experiments were performed to test the hypothesis that succulents "shift" their method of photosynthetic metabolism in response to environmental change. Our data showed that there were at least three different responses of succulents to plant water status. When plant water status of *Portulacaria afra* (L.) Jacq. was lowered either by withholding water or by irrigating with 2% NaCl, a change from C₃-photosynthesis to Crassulacean acid metabolism (CAM) occurred. Fluctuation of titratable acidity and nocturnal CO₂ uptake was induced in the stressed plants. Stressed *Peperomia obtusifolia* A. Dietr. plants showed a change from C₃-photosynthesis to internal cycling of CO₂. Acid fluctuation commenced in response to stress but exogenous CO₂ uptake did not occur. *Zygocactus truncatus* Haworth plants showed a pattern of acid fluctuation and nocturnal CO₂ uptake typical of CAM even when well irrigated. The cacti converted from CAM to an internal CO₂ cycle similar to *Peperomia* when plants were water-stressed. Reverse phase gas exchange in succulents results in low water loss to carbon gain. Water is conserved and low levels of metabolic activity are maintained during drought periods by complete stomatal closure and continual fluctuation of organic acids.

Crassulacean acid metabolism is a term applied to the unique sequence of carbon uptake and acid metabolism in some succulents. The most important diagnostic feature of CAM³ is the massive (100-200 $\mu\text{eq g}^{-1}$ fresh wt) diurnal fluctuation of organic acids, resulting from the nighttime carboxylation and daytime decarboxylation processes (18). This concept, along with the ever growing body of literature concerning "shifts" (4) of photosynthetic metabolism in succulents, prompted this study of acid metabolism and gas exchange in succulents. In studies similar to ours, Nishida (11) tested a variety of CAM species in the greenhouse and found that stomata may be open during the day or night.

Studies which have the greatest significance to ours are those that relate CO₂ uptake in succulents to the plant water regime (1, 4) or to some form of water stress (20). Studies from our laboratory have shown that acid metabolism and gas exchange in desert cacti are regulated primarily by the water status of the plant, with conditions of drought leading to complete stomatal closure (13) and continual irrigation leading to stomatal opening which may extend into the daylight hours (3). We report here on three succulent species within three plant families which respond differently to water stresses.

MATERIALS AND METHODS

Plants. *Portulacaria afra* (L.) Jacq. and *Peperomia jerreyrae* A.

Dietr. were propagated by cuttings from parent plants growing in the U.C. Riverside greenhouse. *Zygocactus truncatus* Haworth plants were purchased from the Desert Nursery, Riverside, Calif. Cuttings were rooted in Vermiculite or potting mix and irrigated regularly with water plus nutrient solution. After rooting, non-stressed (control) plants were watered at least every 3 days to keep tissue water potentials high. Plants were stressed by withholding water or by weekly irrigation with 2% NaCl solution.

Acid Titrations. Tissue samples for acid titrations were collected in triplicate and frozen on dry ice until assayed. The samples were extracted by grinding with a TenBroeck tissue grinder in distilled H₂O followed by boiling. After debris removal by centrifugation, samples were titrated to a pH 7 end point. Data are expressed as microequivalent (μeq) of acid g^{-1} fresh wt of tissue.

Gas Exchange Studies. Gas exchange parameters were estimated with a dual-isotope (THO and ¹⁴CO₂) diffusion porometer. An air stream of ¹⁴CO₂ was passed through THO of known specific radioactivity for humidification and then through a small chamber clamped to the plant surface. Isotope radioactivity in the plant samples was determined by liquid scintillation methods and analyzed by a computer program. The assumption was made that diffusion of THO vapor into the plant followed the same physical pathway as water vapor leaving, and hence encountered the same resistances. The dual-isotope porometer allowed simultaneous measurement of gross ¹⁴CO₂ uptake and transpiration from the same plant sample.

Resistances to water vapor and CO₂ were derived directly from THO vapor uptake and ¹⁴CO₂ uptake. Stomatal resistance to CO₂ was calculated from the product of THO vapor uptake and the ratio of the diffusion coefficients of H₂O and CO₂ in air as described previously (3). CO₂ uptake rates ($\text{mg of CO}_2 \text{ uptake dm}^{-2} \text{ hr}^{-1}$) were calculated from the ambient CO₂ concentration (300 $\mu\text{l liter}^{-1}$) and the combined stomatal and mesophyll resistance to ¹⁴CO₂, assuming the internal CO₂ concentrations were zero. Transpiration rates ($\text{g of H}_2\text{O loss dm}^{-2} \text{ hr}^{-1}$) were calculated from the water vapor gradient and the calculated resistance to THO.

Tissue water potentials were estimated with a pressure bomb (17) or hydraulic jack apparatus obtained from Campbell Scientific, Inc., Logan, Utah. Important microclimate measurements were determined with portable thermocouple sensors and IR thermometers.

RESULTS AND DISCUSSION

Review articles have recently related CO₂ assimilation in succulents to variations of gas exchange parameters (9) and to the stability and/or increase of the enzymes involved in the CO₂ fixation process (12). Several questions concerning acid metabolism and gas exchange in succulents, however, still need to be answered. For instance, Osmond leaves open the question whether "obligate" CAM plants (*i.e.* succulent plants which have the enzyme systems for CAM at all times) can be subdivided into species in which CO₂ uptake occurs in the light or dark, and species in which CO₂ uptake is limited to the dark only. Secondly, to what extent are responses of "inducible" CAM in succulent plants, in which CAM activity is triggered by environmental

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³ Abbreviation: CAM: Crassulacean acid metabolism.

factors, genetic? Finally, how do changes in the gas exchange, acid metabolism, and enzymic machinery of succulents relate to the over-all water economy of this group of plants that are often found in arid and semiarid habitats of the world?

We initiated a series of studies which may eventually result in partial or complete answers to these and other questions. A method of studying gas exchange in leaves and stems of succulent plants utilizing THO vapor as a tracer for transpiration has been developed in our laboratory, and recently used to study succulent plants in the field (3) and in the greenhouse (17). This gas exchange technique, coupled with our knowledge of acid titration and metabolism in succulents (18), was used to test a variety of succulent plants growing under irrigated and stressed ($-H_2O$ and NaCl) conditions. Our initial studies indicate that at least three succulent plants respond in a different manner to plant water stresses.

Portulacaria afra. The titratable acidity data of *P. afra* (Fig. 1) show that well watered (control) plants which had leaf water potentials of greater than -10 bars did not have a large diurnal fluctuation of organic acids.⁴ However, when leaf water potentials were depressed below -10 bars either by withholding water or by irrigating with 2% NaCl, *Portulacaria* plants developed a diurnal fluctuation of organic acids typical of CAM succulents (Fig. 1). There was no apparent difference in the fluctuation of acid levels among water-stressed and salt-stressed plants (data not shown).

Our gas exchange data for *P. afra* reported here (Figs. 2-4) and previously (17) support the hypothesis developed from acid titration studies that carbon uptake occurred primarily during the day when plants were well watered and that CAM (*i.e.* nighttime CO_2 uptake) was initiated when the plants were water- or salt-stressed. Stomatal resistance to CO_2 uptake in leaves of well watered *Portulacaria* plants, computed from THO uptake, was low during the day and maximal at night. Minimum values during the daylight period were on the order of 2 to 10 $sec\ cm^{-1}$ with nighttime values 80 $sec\ cm^{-1}$ or more. Stomatal resistance patterns changed when plants were water- or salt-stressed. The resistance values in stressed plants exceeded 80 $sec\ cm^{-1}$ throughout the day and minima of 10 to 15 $sec\ cm^{-1}$ were recorded toward the end of the dark period. Laboratories other than ours have also recorded variable stomatal responses in leaf succulents which were growing under stressed conditions (4, 7, 19). This pattern of stomatal opening has an effect on the water economy of the plant, which can be seen in the transpiration data for *Portulacaria* shown in Figure 3. Daytime stomatal opening, and the accompanying increase in the temperature-related vapor pressure gradient resulted in a large net transpiration of water. Conversely, a low net transpiration of water occurred when stomata were open during the relatively cooler nighttime, which can be seen in the stressed *Portulacaria* plants.

Maximum CO_2 uptake in well watered *Portulacaria* plants occurred during the day (Fig. 4). There was little CO_2 uptake during the beginning of the dark period in *Portulacaria*, but by the end of the dark period CO_2 uptake approached the levels of nocturnal uptake in stressed plants. The data are consistent with those of Neales (9) for *Portulacaria* which showed some late night CO_2 uptake. In experiments with water-stressed plants, a partial change to daytime CO_2 uptake as water became available was reported for *Agave americana* (10) and *Dudleya farinosa* (1). In our experiments, in which gas exchange was monitored as the plants were stressed by withholding water or by irrigation with NaCl, a change to nighttime CO_2 uptake occurred in *Portulacaria* (Fig. 4). Studies previously reported from this laboratory established that this process was reversible (17).

Peperomia obtusifolia. The acid metabolism and gas exchange

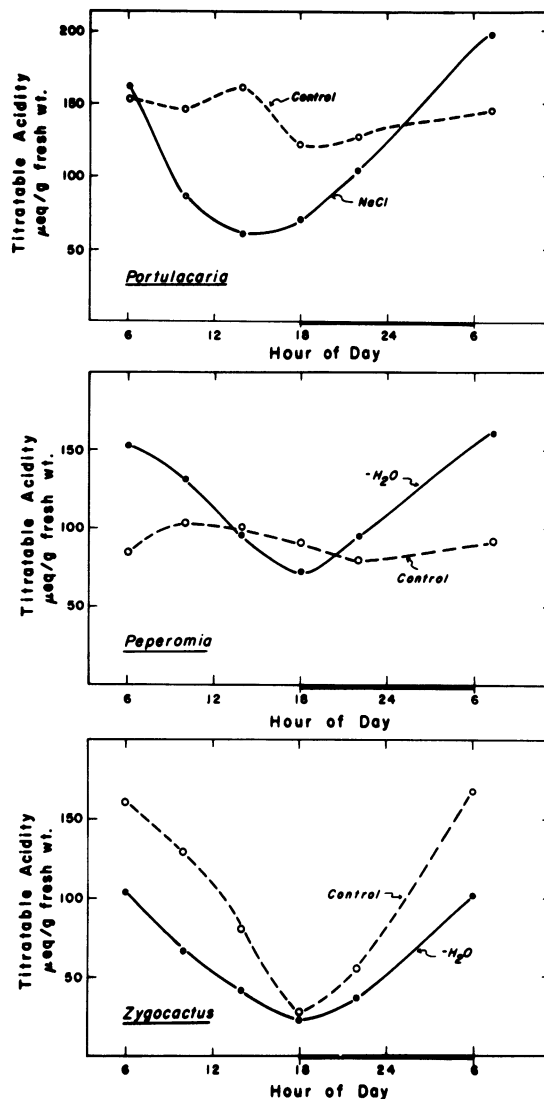


FIG. 1. Diurnal variation in titratable acidity of well watered, unstressed (○—○) *Portulacaria afra*, *Peperomia obtusifolia*, and *Zygocactus truncatus* and stressed (●—●) plants. Acid determined by duplicate titration of samples collected from three different plants. The dark bar along the abscissa indicates the dark period.

data of *P. obtusifolia* show that C_3 -photosynthesis is the apparent metabolic sequence when the plants are irrigated regularly, and plants convert to a phase of internal cycling of CO_2 when plant water potential is lowered by withholding water or by 2% NaCl irrigation. Our acid titrations of leaves from well watered (control) plants, with leaf water potentials of greater than -5 bars, established the fact that an appreciable diurnal fluctuation of organic acids did not normally occur (Fig. 1). When, however, leaf water potential was lowered to less than -10 bars, either by withholding H_2O or by irrigating with 2% NaCl, a large diurnal fluctuation of organic acids was induced. Again, plants which were water-stressed were not significantly different from salt-stressed plants (data not shown).

The diurnal titration curves for *Peperomia* were strikingly similar to those of *Portulacaria* (Fig. 1) and would support the hypothesis of a change from C_3 -photosynthesis to CAM. The stomatal resistance data (Fig. 2), however, do not support the proposed change, but do support the hypothesis that this plant is capable of a rapid conversion from C_3 -photosynthesis to a phase in which organic acids fluctuate internally without exogenous CO_2 uptake. Repeated experiments in the U.C. Riverside green-

⁴ Data from (17) are redrawn and reproduced here for comparative purposes.

house, at 17 and 27 C, and at all stages of water and salt stress, failed to detect stomatal opening once the plants were stressed enough to initiate acid fluctuation.

The consequence of complete stomatal closure during the 24-hr cycle is obvious. Little, if any, transpiration occurs under these conditions (Fig. 3). Thus, the plants are capable of withstanding extended periods of drought in much the same way as *Opuntia basilaris* during periods between rainfalls (18). Although water is conserved, the complete stomatal closure results in the lack of exogenous CO_2 uptake (Fig. 4). Our experiments showed that CO_2 uptake occurred only when plants were irrigated, and took place only during the daylight hours. Continual experiments to detect nighttime CO_2 uptake during the same time that organic acids were fluctuating in a diurnal manner were unsuccessful. We interpret our data to mean that *Peperomia* is capable of a change from a typical diurnal mode of gas exchange to a mode in which internal CO_2 pools are continually fluctuating, presum-

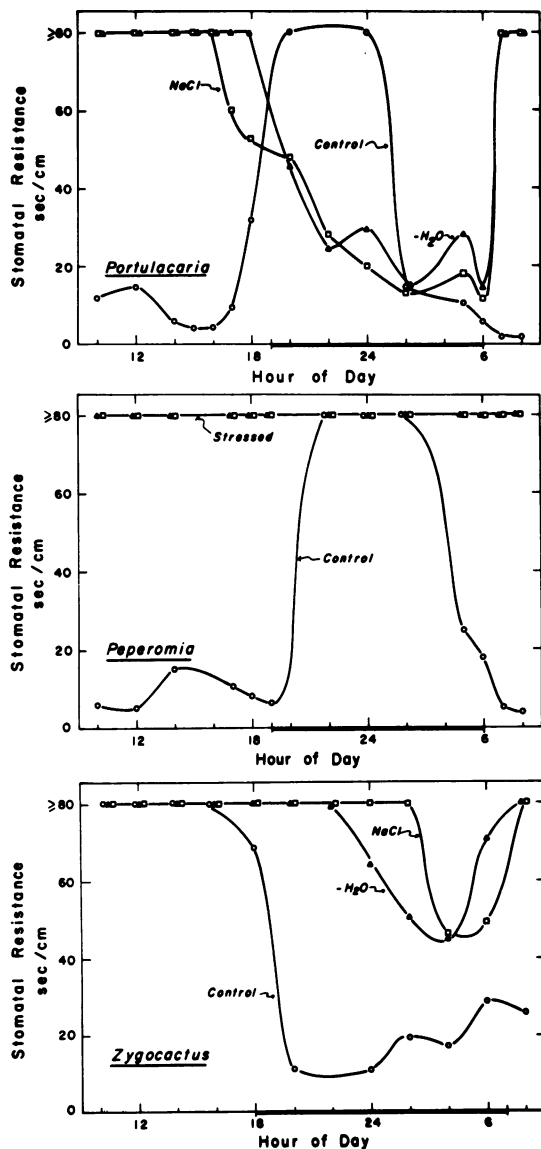


FIG. 2. Diurnal variation in stomatal resistance to CO_2 transfer (sec cm^{-1}) for control, unstressed plants (O—O), and water-stressed ($-\text{H}_2\text{O}$, \blacktriangle — \blacktriangle) and salt-stressed (NaCl, \square — \square) plants. The unstressed plants were watered no less frequently than every 3rd day. The water-stressed plants were watered weekly, and salt-stressed plants were irrigated with 2% NaCl weekly. Resistance to $^{14}\text{CO}_2$ uptake was determined with a dual-isotope (THO and $^{14}\text{CO}_2$) porometer.

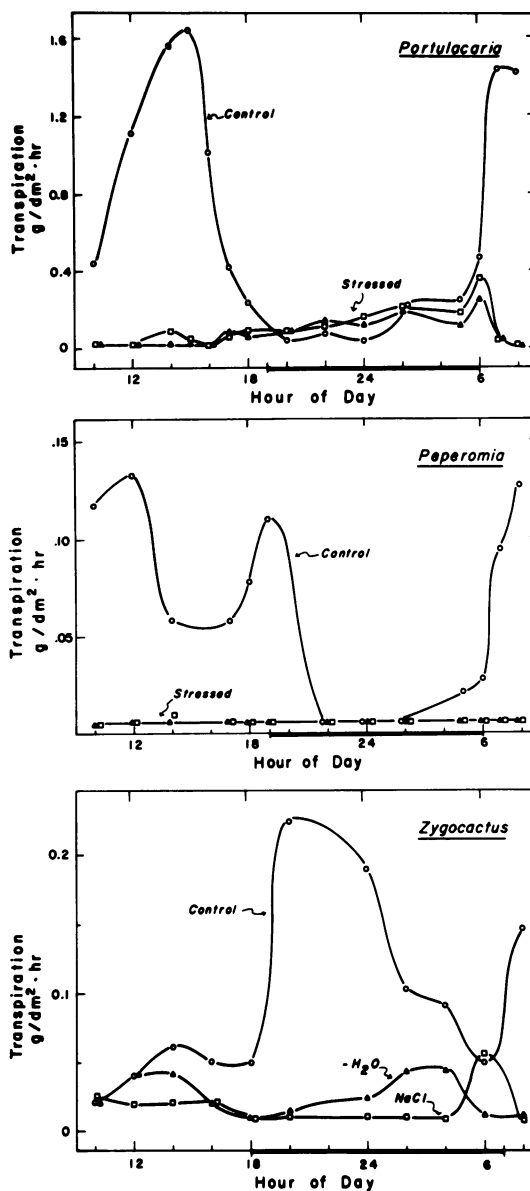


FIG. 3. Calculated diurnal transpiration rates for unstressed (control, O—O), water-stressed ($-\text{H}_2\text{O}$, \blacktriangle — \blacktriangle), and salt-stressed (NaCl, \square — \square) plants. Data are $\text{g dm}^{-2} \text{ hr}^{-1}$. Transpiration rates were calculated directly from THO uptake.

ably for maintenance of moderate energy levels during periods of environmental stress, as has been reported for *O. basilaris* (13). This pattern of acid metabolism and gas exchange is unusual and unique for a leaf succulent. Studies are presently underway to develop a clearer understanding of the carboxylating and decarboxylating enzymes which may control this process.

Zygocactus truncatus. Stems of *Z. truncatus* plants had a maximum accumulation of titratable acids when the plants were continuously irrigated and nourished (Fig. 1). Water- and salt-stressed plants were characterized by diminished levels of titratable acids. Nocturnal acid fluctuations in succulents have long been studied and in the past have been associated with increased P-enolpyruvate carboxylase activity (8) or cell vacuole size (5). More recently, water and salt stress (4, 17) were shown to increase titratable acid levels in leaf succulents. The pattern of acid accumulation and depletion in *Zygocactus* stems was found to be in opposition to the pattern observed in the leaf succulents mentioned above, and strikingly similar to the diurnal acid curves titrated

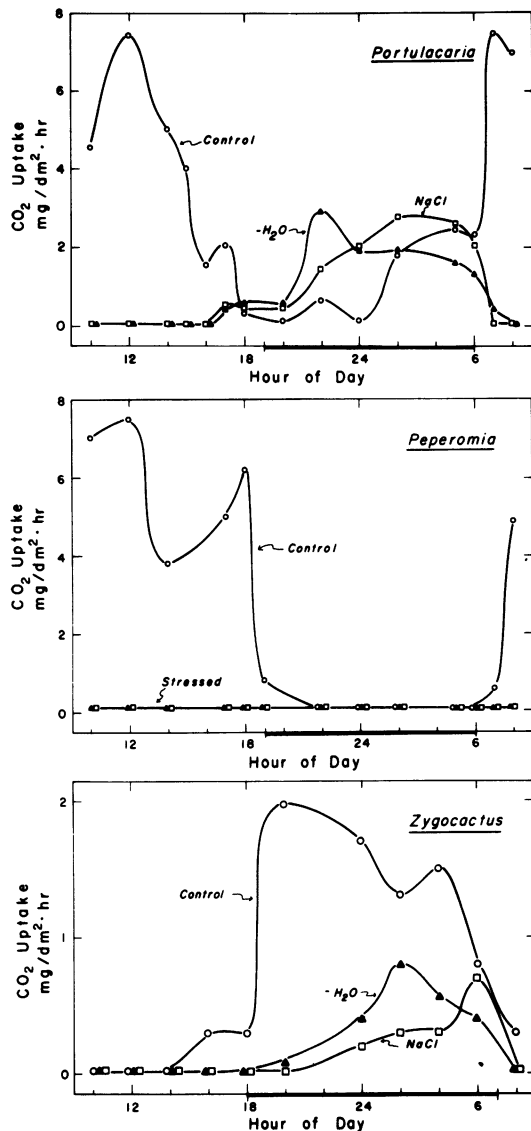


FIG. 4. Calculated diurnal carbon uptake rates for unstressed and stressed plants. Data, $\text{mg dm}^{-2} \text{hr}^{-2}$, were calculated directly from total $^{14}\text{CO}_2$ uptake.

from the stem succulent *O. basilaris* after precipitation (16) and after extensive irrigation (3).

Stomatal resistance to CO_2 uptake in irrigated *Zygocactus* stems, like the diurnal fluctuation of organic acids, was typical of CAM succulents (18), and markedly different from *Portulacaria* and *Peperomia* (Fig. 2). Minimum daytime values were in excess of 80 sec cm^{-1} (our arbitrary maximum), and nighttime values were as low as 10 sec cm^{-1} . Nighttime gas exchange in stressed ($-\text{H}_2\text{O}$ and/or NaCl) *Zygocactus* stems was suppressed. Minimal stomatal resistances to CO_2 uptake on the order of 40 to 50 sec cm^{-1} were recorded in the experiments reported here (Fig. 2) near the end of the dark period. The water and salt stress eventually resulted in complete stomatal closure and a continual but somewhat reduced fluctuation of organic acids (data not shown).

As would be expected, irrigated *Zygocactus* stems, in contrast to *Portulacaria* and *Peperomia*, transpired during the night when stomatal resistance to gas exchange was minimal (Fig 3). In addition, the reduction of transpiration in water- and salt-stressed

plants was typical of the pattern observed by others in stressed stem succulents (2, 6).

The preservation in stem succulents of H_2O by complete stomatal closure and with the absence of CO_2 uptake (Fig. 4) is well documented (14, 15). The fact that daytime CO_2 uptake in irrigated *Zygocactus* was minor, and that daytime CO_2 uptake in irrigated *Zygocactus* was minor, and that daytime maximum uptake of CO_2 occurred at night shows that CAM is the metabolic sequence for carbon uptake in this group of plants under ideal physiological conditions, i.e. when an exogenous water supply is abundant. Likewise, C_3 -photosynthesis is the metabolic sequence in *Portulacaria* and *Peperomia*. All three species, in addition to the metabolic sequence operative in situations of abundant water supply, have evolved means of withstanding varying degrees of water (salt) stress.

Since most succulents capable of CAM are found in arid and semiarid habitats with infrequent rainfall patterns, it becomes apparent that an important survival mechanism of the three plant species outlined above is the maintenance of moderate energy levels during periods of moisture stress, possibly by the internal cycling of CO_2 accompanied by complete stomatal closure. Gas exchange and exogenous CO_2 uptake in an arid environment are limited to the periods immediately following precipitation. Reverse phase gas exchange results in low water loss to carbon gain. Water conservation and maintenance of moderate energy states during periods of drought are important to the survival of succulents, and clearly require additional study.

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