Drought Adaptation in *Opuntia basilaris*

SIGNIFICANCE OF RECYCLING CARBON THROUGH CRASSULACEAN ACID METABOLISM[1]

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ABSTRACT

Contrasting metabolic regimes operate in *Opuntia basilaris* Engelm. and Bigelov, before and after precipitation. During periods of drought, atmospheric CO_2 exchange and transpiration are greatly reduced throughout the day/night cycle by stomatal closure and a highly impervious cuticle. The hypothesis is that endogenously produced CO_2 is retained and recycled through dark CO_2 fixation, organic acid transformations, photosynthesis, and respiration. Immediately following precipitation, nighttime stomatal opening is initiated, permitting increased atmospheric CO_2 assimilation and organic acid synthesis.

A variety of photosynthetic adaptations to arid environments have been reported. A few desert perennials remain photosynthetically active throughout a wide range of environmental temperatures and are capable of rapidly responding to precipitation (1, 6, 10). Others are photosynthetically inactive during some portion of the year and become active during periods of low water stress at moderate air temperatures (5, 8, 11) or high air temperature (4). We report here the results of a field study with a desert cactus, indicating not only the persistence of photosynthetic activity during periods of drought, but a dramatic, rapid shift in the level of metabolic activity in response to precipitation.

MATERIALS AND METHODS

The goal of our research was to gain an understanding of the gas exchange characteristics of *Opuntia* spp. under field conditions. Therefore, methodologies and equipment were adopted which could efficiently be utilized for *in situ* measurements. Plants of *Opuntia basilaris* Engelm. and Bigelov, used in this study were native to the University of California's Philip L. Boyd Deep Canyon Desert Research Center, near Palm Desert, California (116° 07' W, 33° 30' N).

Carbon dioxide assimilation was monitored with a portable $^{14}CO_2$ porometer, similar to those described elsewhere (2, 16). The total acidity of stem tissue was estimated by titration, utilizing a technique modified from Sideris *et al.* (18) by Ting

and Dugger (20). Gas diffusion resistances were determined with a diffusion resistance hygrometer, similar to those described earlier (22, 23). Organic acids were separated with TLC methods (19). Cuticular resistances to water vapor loss were determined gravimetrically with severed stems which did not show patterns of stomatally regulated water vapor loss. Severed stems, assayed for acid accumulation under various experimental conditions, were kept in temperature- and light-controlled growth chambers. Tissue water potential was estimated by thermocouple psychrometry, using subepidermal stem tissue.

RESULTS AND DISCUSSION

Crassulacean acid metabolism is a term applied to a series of distinctive metabolic events associated with the flow of carbon in succulent plants (15, 21). The following criteria were used to establish CAM[2] in plants of *O. basilaris*: (*a*) stomata are more often open at night than during the day; (*b*) *in situ* $^{14}CO_2$ labeling experiments confirm dark CO_2 fixation into organic acids (Table I); (*c*) total titratable acidity of the stem tissue fluctuates diurnally; and (*d*) the major acid fluctuation occurs in subepidermal, chloroplast-containing cells. Throughout the following discussion, a direct stoichiometric relationship between organic acid fluctuations and CO_2 uptake and release is presumed.

During periods of high water stress, the level of maximum dark acidity is low, fluctuating diurnally from 10 to 35 $\mu eq/g$ fresh weight (Fig. 1A, day 0). The maximum acidity usually persists beyond sunrise, although the subsequent rate of acid depletion is rapid. Stem tissue water potentials are characteristically -12 to -16 bars, and stomata do not open during the day/night cycle (Fig. 1B, day 0). The cuticular resistance to water vapor loss is very high, averaging over 600 sec/cm for mature, turgid stems. Nighttime assimilation of exogenous CO_2 is insignificant (Table II, day 0), even with elevated concentrations of CO_2, suggesting the diffusive resistance of the cuticle to CO_2 may be higher than might be anticipated from the transpiration resistance measurements. High gas diffusion resistances, which limit atmospheric CO_2 uptake, must also restrict the leakage of endogenously produced CO_2 during periods similar to day 0. Dark respiration rates at nighttime temperatures similar to those of day 0 approach 0.6 mg CO_2/g dry weight·hr, and in the light at 40 C net CO_2 evolution continues (Szarek, unpublished results). The total carbon balance is probably negative, although the loss of endogenous CO_2 on a short term basis is not as significant as it might be if stomata

[2] Abbreviations: CAM: Crassulacean acid metabolism; PEP: phosphoenolpyruvate.

were opening. During such periods of stomatal closure any residual CO_2, which is not photosynthetically assimilated, may, nevertheless, be conserved.

A low amplitude diurnal fluctuation of stem tissue acidity is typical of periods when precipitation is absent. The amplitude of 25 μeq/g fresh weight is predictable and persists during the winter and summer months, suggesting the persistence of enzymatic activity, *i.e.* PEP carboxylase, throughout the entire year. Other workers have demonstrated that organic acids fluctuate reciprocally with photosynthetic products in CAM

Table I. *Distribution of ^{14}C after in Situ Incubation with $^{14}CO_2$ Following Precipitation*

Intact stems were incubated with 25 μc of $^{14}CO_2$ at 19 C for 1 hr in the dark. Total activity of 222,156 dpm/sample.

Sample Fraction	Radioactivity in Fractions
	dpm/fraction
Lipid	0
Carbohydrate	0
Amino acid	3750
Organic acid	87171
Malic	74793
Citric	7804
Others	4574

plants (12), so that perhaps the diurnal fluctuation of total acidity for day 0 may also reflect the persistence of photosynthetic activity during periods of high water stress. The hypothesis is that during periods of stomatal closure, CO_2 available through the endogenous supply, is recycled and metabolic energy continually supplied from the flow of carbon through dark CO_2 fixation, organic acid transformations, photosynthesis, and respiration.

In *Opuntia* stems with closed stomata most of the respiratory CO_2 would be retained in the stems. Stem tissue respiration rates at 32 C are approximately twice as great as those measured at 15 C (Szarek, unpublished results), and so would

Table II. *Nighttime Exogenous CO_2 Fixation Rates Preceding and Following Precipitation*

Day 0 is prior to precipitation, day 1 is 1st day following, and day 3 is 3rd day after precipitation.

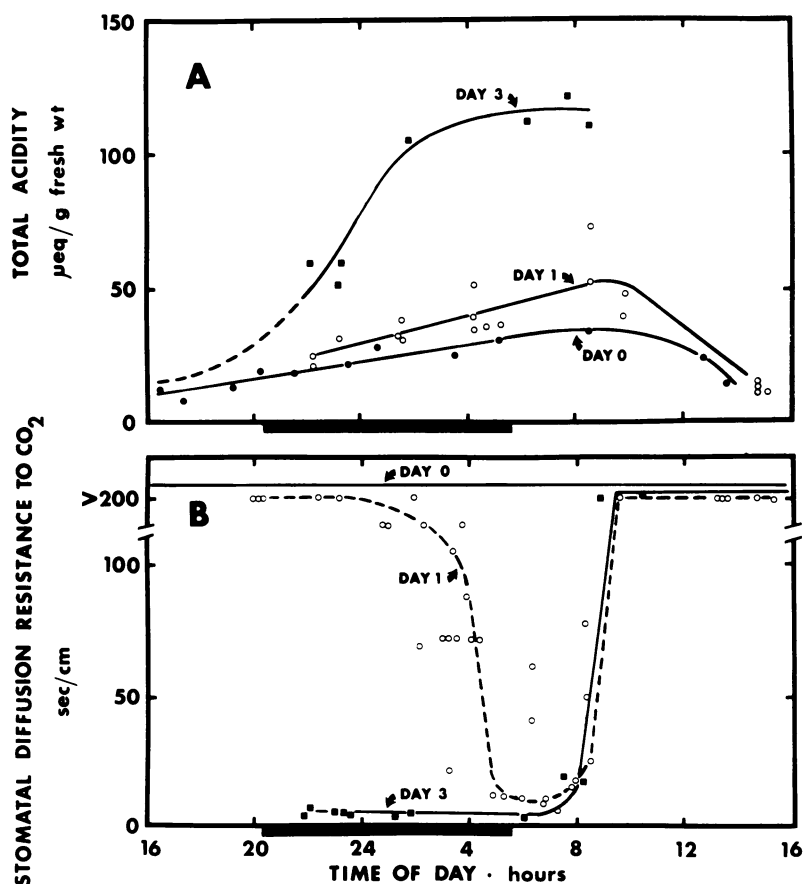| Day	CO_2 (μl/l)		
	240	286	800
	mg CO_2/dm^2·hr		
0	0	0	0
1	0.1	0.4	3.1
3	0.2	1.3	21.1

Fig. 1. A: Diurnal course of the fluctuation in total acidity in stem tissue of *O. basilaris* preceding and following precipitation; B: diurnal course of the stomatal diffusion resistance to CO_2 in stems of *O. basilaris* preceding and following precipitation. Data for day 0 from 14–15 July 1971, following a period of high water stress; daytime temperature maximum of 40 C and nighttime temperature minimum of 27 C. Data for day 1 from 20–21 June 1972, following a summer rainfall on June 19, 1972; daytime temperature maximum of 40 C and nighttime temperature minimum of 23 C. Data for day 3 from 22–23 June 1972, during a period of low water stress; daytime temperature maximum of 32 C and nighttime temperature minimum of 15 C.

generate twice the amount of available endogenous substrate for dark acidification at the higher nighttime temperature. Preliminary results with *O. basilaris* support this hypothesis, as the amount of acid accumulated in stems with closed stomata following 12 hr of darkness at 32 C was 34.2 ± 4.1 μeq/g fresh weight, while at 15 C the acid accumulation was 16.4 ± 4.5 μeq/g fresh weight. These results contrast with those of Bennett-Clark (3), who found greater dark acidification with a decreased respiration rate. However, water stress in this earlier work was most likely low, and endogenously produced respiratory CO_2 might have been able to diffuse out of the succulent tissue through open stomata. In experiments with *Opuntia versicolor* (17), high nighttime temperatures have been shown to reduce stomatal apertures, which would restrict atmospheric gas exchange. Indeed, the more commonly observed enhancement of acid accumulation at lower nighttime temperatures (3, 13) may also be related to the stomatal response in low water stress experiments. Generally, low nighttime temperatures (15–20 C) enhance stomatal opening (9, 22) and enzymatic reactions that favor acid accumulation (13). During periods of low water stress and low nighttime temperatures, the atmospheric supply of CO_2 available through open stomata, may exceed the available endogenous supply of CO_2, such that greater acid accumulation could result in succulent plants which show atmospheric gas exchange. Dark acidification in *O. basilaris*, as will be pointed out below, is enhanced when atmospheric gas exchange can proceed.

In the field following 1 cm of rain in midsummer 1972, the stem tissue water potentials of −12 to −16 bars in plants of *O. basilaris* increased to −6 to −7 bars, confirming the absorption of available soil water. Night and early morning opening of stomata and an increased CO_2 assimilation resulted in magnified diurnal fluctuations of acidity (Fig. 1, A and B, Table II, days 1 and 3). Stomatal diffusion resistances on day 1 decreased as the night progressed, with minima near the end of the dark period. Stomata closed within a few hours after sunrise, concomitantly restricting atmospheric gas exchange. Thus, the first night after precipitation, stomata opened, atmospheric CO_2 assimilation was initiated, and the corresponding level of maximum stem tissue acidity increased 45%. Three days after precipitation (day 3) minimum nighttime temperatures decreased to 15 C and minimum stomatal resistances were recorded earlier in the night, while stomata continued to close early in the morning. Though the minimum stomatal resistances of day 3 were similar to those of day 1, the observed short time CO_2 fixation rates increased further, and stem tissue acidity increased 200% relative to day 0. The CO_2 assimilation data suggest other residual resistances also decreased during this same period, which may represent enzymatic (14) or other, short term nonstomatal, rhythmic fluctuations of CO_2 uptake previously suggested to occur in CAM plants (7).

Within 2 weeks, the diurnal fluctuation of stem tissue acidity was re-established at a level similar to that of day 0. Atmospheric gas exchange was again restricted throughout the day/night cycle, stabilizing the total endogenous supply of CO_2. Similar responses to precipitation have been measured throughout the entire year, although in the winter months the periods of atmospheric gas exchange are usually of longer duration. Plants of *O. basilaris* experience net gains in carbon only during

periods of low water stress. The rest of the time the plants persist in a reduced, but moderately active metabolic state, by recycling endogenously produced CO_2. Thus, a moderate energy state is maintained, which enables a rapid response to small amounts of precipitation by assimilation of atmospheric CO_2.

LITERATURE CITED

1. ADAMS, M. S. AND B. R. STRAIN. 1969. Seasonal photosynthetic rates in stems of *Cercidium floridum* Benth. Photosynthetica 3 : 55–62.
2. AUSTIN, R. B. AND P. C. LONGDEN. 1967. A rapid method for the measurement of rates of photosynthesis using $^{14}CO_2$. Ann. Bot. 31 : 245–253.
3. BENNETT-CLARK, T. A. 1933. The role of organic acids in plant metabolism. Part II. New Phytol. 32 : 128–161.
4. BJÖRKMAN, O., R. W. PEARCY, A. T. HARRISON, AND H. MOONEY. 1972. Photosynthetic adaptation to high temperatures: a field study in Death Valley, California. Science 175 : 786–789.
5. CUNNINGHAM, G. L. AND B. R. STRAIN. 1969. Ecological significance of seasonal leaf variability in a desert shrub. Ecology 50 : 400–408.
6. HIKMAT, A. A., B. R. STRAIN, AND H. A. MOONEY. 1972. The physiological ecology of diverse populations of the desert shrub *Simmondsia chinensis*. J. Ecol. 60 : 41–57.
7. JONES, M. B. AND T. A. MANSFIELD. 1972. A circadian rhythm in the level of carbon dioxide compensation in *Bryophyllum fedtschenkoi* with zero values during the transient. Planta 103 : 134–146.
8. MOONEY, H. A. AND B. R. STRAIN. 1964. Bark photosynthesis in ocotillo. Madrono 17 : 230–233.
9. NISHIDA, K. 1963. Studies on stomatal movements of crassulacean plants in relation to the acid metabolism. Physiol. Plant. 16 : 281–298.
10. OECHEL, W. C., B. R. STRAIN, AND W. R. ODENING. 1972. Phtosynthetic rates of a desert shrub, *Larrea divaricata* Cav., under field conditions. Photosynthetica 6 : 183–188.
11. OWOSEYE, J. A. AND W. W. SANFORD. 1972. An ecological study of *Vellozia schnitzleinia*, a drought-enduring plant of northern Nigeria. J. Ecol. 60 : 807–817.
12. PUCHER, G. W., H. B. VICKERY, M. D. ABRAHAMS, AND C. S. LEAVENWORTH. 1949. Studies in the metabolism of crassulacean plants: diurnal variation in organic acids and starch in excised plants of *Bryophyllum calycinum*. Plant Physiol. 24 : 610–620.
13. QUEIROZ, O. 1965. Sur le métabolisme acide des crassulacées action à long terme de la température de nuit sur la synthèse d'acide malique par *Kalanchoe blossfeldiana* var. Tom Thumb placée en jours courts. Physiol. Veg. 3 : 203–213.
14. QUEIROZ, O. 1968. Sur le métabolisme acide des crassulacées. III. Variations d'activité enzymatique sous l'action du photopériodisme et du thermopériodisme. Physiol. Veg. 6 : 117–136.
15. RANSON, S. L. AND M. THOMAS. 1960. Crassulacean acid metabolism. Annu. Rev. Plant Physiol. 11 : 81–110.
16. SHIMSHI, D. 1969. A rapid method for measuring photosynthesis with labelled carbon dioxide. J. Exp. Bot. 20 : 381–401.
17. SHREVE, E. B. 1916. An analysis of the causes of variations in the transpiring power of cacti. Physiol. Res. 2 : 73–127.
18. SIDERIS, C. P., H. Y. YOUNG, AND H. H. Q. CHUN. 1948. Diurnal changes and growth rates as associated with ascorbic acid, titratable acidity, carbohydrate and nitrogenous fractions in the leaves of *Ananas comosus* (L) Merr. Plant Physiol. 23 : 38–69.
19. TING, I. P. AND W. M. DUGGER. 1965. Separation and detection of organic acids on silica gel. Anal. Biochem. 12 : 571–578.
20. TING, I. P. AND W. M. DUGGER. 1968. Non-autotrophic carbon dioxide metabolism in cacti. Bot. Gaz. 129 : 9–15.
21. TING, I. P., H. B. JOHNSON, AND S. R. SZAREK. 1972. Net CO_2 fixation in crassulacean acid metabolism plants. *In:* C. C. Black, ed., Net Carbon Dioxide Assimilation in Higher Plants. Cotton Inc., North Carolina. pp. 26–53.
22. TING, I. P., M. D. THOMPSON, AND W. M. DUGGER. 1967. Leaf resistance to water vapor transfer in succulents: effect of thermoperiod. Amer. J. Bot. 54 : 245–251.
23. VAN BAVEL, C. H. M., F. S. NAKAYAMA, AND W. L. EHRLER. 1965. Measuring transpiration resistances of leaves. Plant Physiol. 40 : 535–540.