Drought Adaptation in *Opuntia basilaris*

**SIGNIFICANCE OF RECYCLING CARBON THROUGH CRASSULACEAN ACID METABOLISM**

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**ABSTRACT**

Contrasting metabolic regimes operate in *Opuntia basilaris* Engelm. and Bigelow, before and after precipitation. During periods of drought, atmospheric CO₂ 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 CO₂ is retained and recycled through dark CO₂ fixation, organic acid transformations, photosynthesis, and respiration. Immediately following precipitation, nighttime stomatal opening is initiated, permitting increased atmospheric CO₂ 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 Bigelow, 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 ³¹CO₂ 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). The diffusion resistances to water vapor loss were determined gravimetrically with severed stems which did not show patterns of stomata 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 in plants of *O. basilaris*: (a) stomata are more often open at night than during the day; (b) *in situ* CO₂ labeling experiments confirm dark CO₂ 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 CO₂ 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 μeq/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 CO₂ is insignificant (Table II, day 0), with elevated concentrations of CO₂, suggesting the diffusive resistance of the cuticle to CO₂ may be higher than might be anticipated from the transpiration resistance measurements. High gas diffusion resistances, which limit atmospheric CO₂ uptake, must also restrict the leakage of endogenously produced CO₂ during periods similar to day 0. Dark respiration rates at nighttime temperatures similar to those of day 0 approach 0.6 mg CO₂/g dry weight-hr, and in the light at 40 C net CO₂ evolution continues (Szarek, unpublished results). The total carbon balance is probably negative, although the loss of endogenous CO₂ on a short-term basis is not as significant as it might be if stomata

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2 Abbreviations: CAM: Crassulacean acid metabolism; PEP: phosphoenolpyruvate.
were opening. During such periods of stomatal closure any residual CO₂, 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 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, CO₂ available through the endogenous supply, is recycled and metabolic energy continually supplied from the flow of carbon through dark CO₂ fixation, organic acid transformations, photosynthesis, and respiration.

In Opuntia stems with closed stomata most of the respiratory CO₂ 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 1. Distribution of ¹⁴C after in Situ Incubation with ¹⁴CO₂ Following Precipitation

Intact stems were incubated with 25 μc of ¹⁴CO₂ at 19 C for 1 hr in the dark. Total activity of 222,156 dpm/sample.

<table>
<thead>
<tr>
<th>Sample Fraction</th>
<th>Radioactivity in Fractions (dpm/fraction)</th>
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<tbody>
<tr>
<td>Lipid</td>
<td>0</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>0</td>
</tr>
<tr>
<td>Amino acid</td>
<td>3750</td>
</tr>
<tr>
<td>Organic acid</td>
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</tr>
<tr>
<td>Malic</td>
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</tr>
<tr>
<td>Citric</td>
<td>7804</td>
</tr>
<tr>
<td>Others</td>
<td>4574</td>
</tr>
</tbody>
</table>

Table 2. Nighttime Exogenous CO₂ 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.

<table>
<thead>
<tr>
<th>Day</th>
<th>CO₂ (μL/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>240</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>0.1</td>
</tr>
<tr>
<td>3</td>
<td>0.2</td>
</tr>
</tbody>
</table>

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 CO₂ 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 CO₂ 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 night-time 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 CO₂ available through open stomata, may exceed the available endogenous supply of CO₂, 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 CO₂ 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 CO₂ assimilation was initiated, and the corresponding level of maximum stem tissue acidity increased 45%. Three days after precipitation (day 3) minimum night-time 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 CO₂ fixation rates increased further, and stem tissue acidity increased 200% relative to day 0. The CO₂ 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 CO₂ 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 CO₂. 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 CO₂. Thus, a moderate energy state is maintained, which enables a rapid response to small amounts of precipitation by assimilation of atmospheric CO₂.

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**LITERATURE CITED**