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Seasonal Shifts of Photosynthesis in *Portulacaria afra* (L.) Jacq.[1]

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ABSTRACT

Portulacaria afra (L.) Jacq., a perennial facultative Crassulacean acid metabolism (CAM) species, was studied under natural photoperiods and temperatures in San Diego, California. The plants were irrigated every fourth day throughout the study period. Measurements of $^{14}CO_2$ uptake, stomatal resistance, and titratable acidity were made periodically from July 1981 through May 1982. _P. afra_ maintained C_3 photosynthesis during the winter and the spring. Diurnal acid fluctuations were low and maximal $^{14}CO_2$ uptake occurred during the day. The day/night ratio of carbon uptake varied from 5 to 10 and indicated little nocturnal CO_2 uptake. CAM photosynthesis occurred during the summer and a mixture of both C_3 and CAM during the fall. Large acid fluctuations of 100 to 200 microequivalents per gram fresh weight were observed and maximal $^{14}CO_2$ uptake shifted to the late night and early morning hours. Daytime stomatal closure was evident. A reduction in the day/night ratio of carbon uptake to 2 indicated a significant contribution of nocturnal CO_2 uptake to the overall carbon gain of the plant. The seasonal shift from C_3 to CAM was facilitated by increasing daytime temperature and accompanied by reduced daytime CO_2 uptake despite irrigation.

The CAM pathway of photosynthesis is characterized by nocturnal CO_2 fixation and stomatal opening which is accompanied by a diurnal organic acid fluctuation (8, 15). CAM plants have been classified as being either obligatory CAM or facultative CAM (11). Obligate CAM species maintain CAM photosynthesis under nearly all environmental conditions including irrigation (6, 13) and may shift to what is referred to as CAM-idling under extreme drought (7, 12). During CAM-idling, the stomata remain closed day and night, but a dampened organic acid fluctuation is still observed (7, 12).

Facultative CAM plants are capable of shifting their photosynthetic mode reversibly from C_3 to CAM in response to the environment (11). There have been few reports of seasonal studies utilizing facultative CAM species. Early in the season, nighttime CO_2 fixation in *Dudley farinosa* contributed approximately 50% to the overall carbon uptake of the plant, yet with increasing drought, only nocturnal CO_2 fixation was observed (1). A seasonal shift from C_3 to CAM in the annual *Mesembryanthemum crystallinum* occurred as soil water potential decreased (3, 18). This was evidenced by increasing diurnal acid fluctuations and a shift of $^{13}C/^{12}C$ isotope composition from −26‰ to −16‰ (3, 18). A similar observation has also been reported for *M. nodiflorum* (17).

Portulacaria afra is a perennial with succulent leaves native to South Africa and is reportedly a facultative CAM plant. When in the C_3 mode, it exhibits primarily daytime CO_2 fixation and stomatal opening with little diurnal organic acid fluctuation (7, 16). *P. afra* responds to water stress or irrigation with 2% NaCl by switching to nocturnal CO_2 fixation accompanied by a large diurnal organic acid fluctuation (7, 16). Recent research with *P. afra* has indicated that induction of CAM is related to leaf age (5). This study was done simultaneously with previously reported growth chamber experiments (5). Its objectives were to determine the effect of ambient conditions of light, temperature, humidity, and gradual shifts of photoperiod on the C_3-to-CAM and CAM-to-C_3 shifts in irrigated *P. afra*.

MATERIALS AND METHODS

Plant Material. Mature leaf tissue was taken from a large *Portulacaria afra* (L.) Jacq. shrub, 2.5 to 3 m in height, growing under natural light and temperatures outside the San Diego State University greenhouse. The shrub was on a drip irrigation system and was watered every 4th d throughout the study period. The plant was fertilized monthly, but was not fertilized during the three winter months. In addition, periodic sampling of smaller potted shrubs, clones of the larger shrub, were done to compare results.

Acid Titrations. During the sampling periods, three sets of three leaves were randomly collected from the large shrub every 3 to 4 h, weighed, and quickly placed on dry ice. The samples were lyophilized to dryness, then ground to a fine powder and homogenized with 20 ml of glass-distilled H_2O. The samples were titrated with 0.01 N KOH to a pH 7.0 endpoint. Four leaves were taken from the smaller clones for acid samples by the same methods.

Gas Exchange Measurements. Stomatal resistance to water vapor was measured using an autoporometer (Li-Cor, LI-65) according to Guralnick *et al.* (5). The sensor was placed on the abaxial surface of each of three intact leaves at each sampling interval. The $^{14}CO_2$ was measured on detached leaves using a modified system of Oechel and Mustafa (10) described by Guralnick *et al.* (5). Day, night, and total $^{14}CO_2$ uptake was calculated from the integrated areas under the curves. The day/night ratios of carbon uptake were then computed from these results.

RESULTS

The results from the large potted shrub and the clones (data not shown) did not show any significant differences in $^{14}CO_2$ uptake and acid fluctuations. Therefore, only results from the large shrub are presented. On August 11, large diurnal acid

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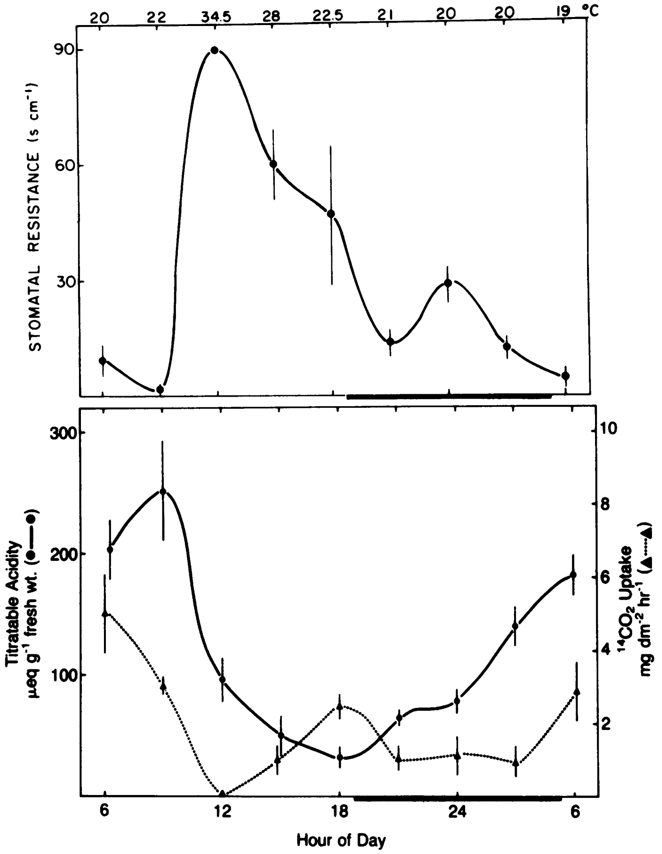

FIG. 1. Diurnal variation top: stomatal resistance and bottom: $^{14}CO_2$ uptake (▲---▲) and titratable acidity (●——●) of *P. afra* on August 11-12, 1981. Black bar indicates darkness. Each point represents the mean ($n = 3$ for stomatal resistance, $n = 6$ for $^{14}CO_2$ uptake, and $n = 3$ for acidity determinations for all figures) ± SE. Environmental conditions were: RH maximum/minimum, 85/44%, PAR was 2070 µmol m^{-2} s^{-1}.

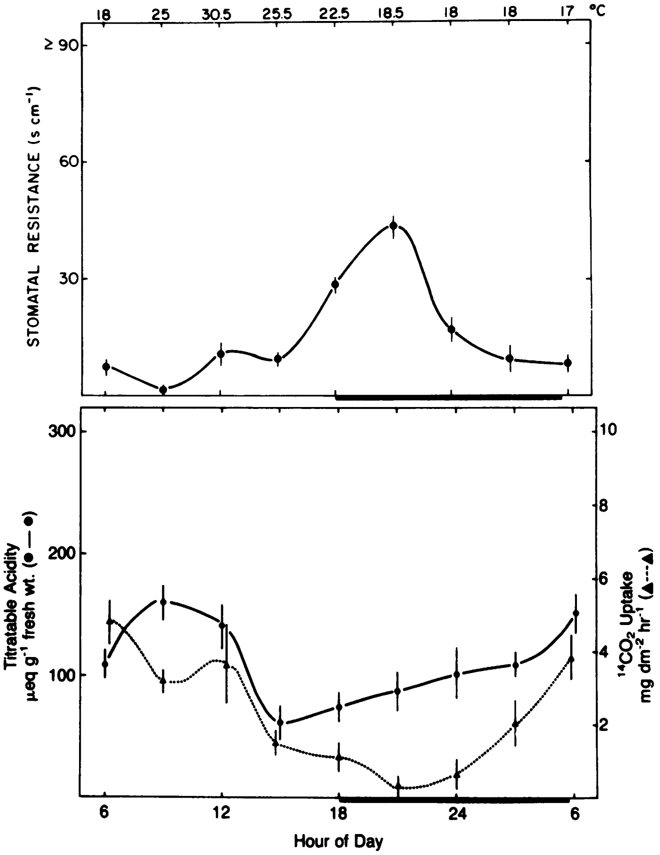

FIG. 2. Diurnal variation top: stomatal resistance and bottom: $^{14}CO_2$ uptake (▲---▲) and titratable acidity (●——●) of *P. afra* on September 10-11, 1981. Black bar indicates darkness. Environmental conditions were: RH maximum/minimum, 62/56%, PAR was 2280 µmol m^{-2} s^{-1}.

fluctuations of 200 µeq g^{-1} FW[2] were accompanied by early morning $^{14}CO_2$ uptake rates of 2 to 4 mg CO_2 dm^{-2} h^{-1}, minimal uptake at noon, and greater CO_2 uptake rates in the late afternoon (Fig. 1). Similar results were also obtained in July 1981 (data not shown). Stomatal resistance on August 11 was at a minimum in the early morning hours, but increased to a maximum at noon (Fig. 1). Resistance decreased slowly in the afternoon and reached a minimum at the start of the night period.

On September 10, the diurnal acid fluctuation was only 100 µeq g^{-1} FW and noon CO_2 uptake was higher than that observed on August 11 (Fig. 2). Early morning CO_2 uptake rates were still on the order of 2 to 4 mg CO_2 dm^{-1} h^{-1}. During the first 6 h of the night period, CO_2 uptake was low. Minimum stomatal resistance, 2 to 10 s cm^{-1}, occurred in the early morning, and maximum resistance, 40 s cm^{-1}, was observed at the beginning of the night (Fig. 2). After midnight, resistance decreased to less than 15 s cm^{-1}.

On February 3, no diurnal acid fluctuation was observed, and the tissue acid levels had risen to 150 µeq g^{-1} FW (Fig. 3). These results were similar to those observed in December with the exception that the baseline acid levels, 130 µeq g^{-1} FW, were significantly lower than observed on February 3 (data not shown). Maximum CO_2 uptake rates of 7 mg CO_2 dm^{-1} h^{-1} occurred during the daytime with very little nocturnal uptake (Fig. 3). Stomatal resistance increased throughout the day to a maximum of 50 s cm^{-1} at the beginning of the night period (Fig. 3). Nighttime resistance decreased to 10 to 25 s cm^{-1}.

On April 21, CO_2 uptake occurred primarily in the daytime with little or no nocturnal uptake (Fig. 4). This pattern was similar to that observed in February except that maximal CO_2 uptake was higher in April. No diurnal acid fluctuation was observed, but the baseline organic acid levels had risen to 500 µeq g^{-1} FW (Fig. 4). Stomatal resistance during the early afternoon was less than 2 s cm^{-1}, and a maximum resistance of 35 s cm^{-1} was observed at the end of the day (Fig. 4).

The day/night ratio of carbon uptake varied in the winter and spring, but there was very little contribution of nocturnal CO_2 uptake to the total carbon uptake (Table I). In March, there was a substantial reduction of total carbon uptake due to very cloudy weather. During August and September, the day/night ratio decreased and was due to a decrease in total CO_2 uptake and an increase of nocturnal CO_2 uptake. On May 19, the day/night ratio was intermediate between the summer and winter values.

DISCUSSION

Portulacaria afra grown outdoors in southern California and continuously irrigated, displayed a shift in the temporal pattern of CO_2 uptake from summer to spring. Under relatively cool conditions in the spring, CO_2 uptake occurred primarily during the day, while nocturnal CO_2 uptake was minimal. Acid levels fluctuated slightly, indicative of little CAM activity. In the spring, *P. afra* fixes CO_2 through the C_3 pathway almost exclusively. The CAM pathway may function slightly by recycling respiratory CO_2 to increase baseline acid levels though there is no evidence to support this point. Other facultative CAM species do not

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[2] Abbreviations: FW, fresh weight; PEP, phosphoenolpyruvate.

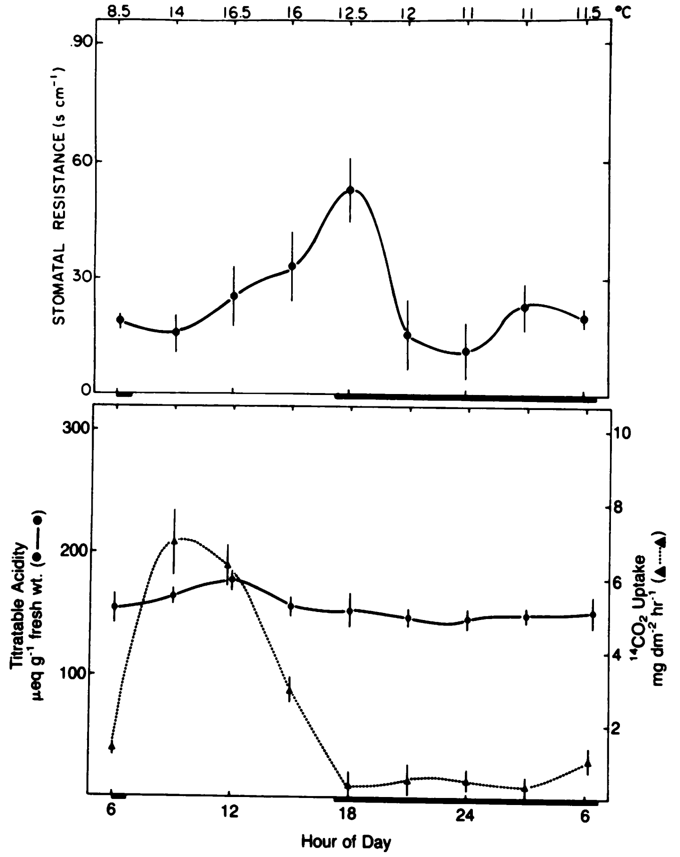

FIG. 3. Diurnal variation top: stomatal resistance and bottom: $^{14}CO_2$ uptake (▲---▲) and titratable acidity (●——●) of *P. afra* on February 3–4, 1982. Black bar indicates darkness. Environmental conditions were: RH maximum/minimum, 61/39%, PAR was 1350 μmol m^{-2} s^{-1}.

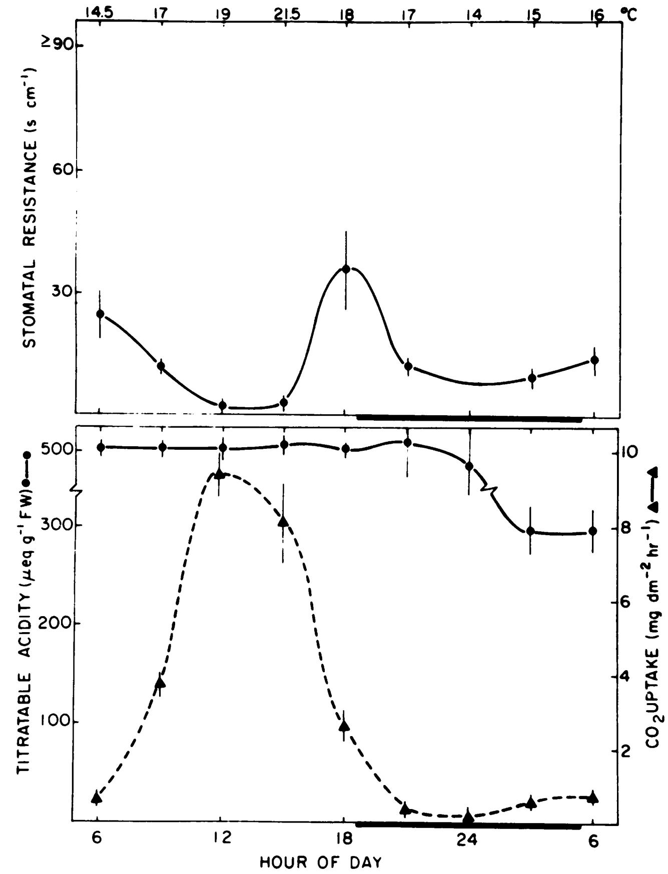

FIG. 4. Diurnal variation top: stomatal resistance and bottom: $^{14}CO_2$ uptake (▲---▲) and titratable acidity (●——●) of *P. afra* on April 28–29, 1982. Black bar indicates darkness. Environmental conditions were: RH maximum/minimum, 85/55%, PAR was 1710 μmol m^{-2} s^{-1}.

Table I. *Integrated Day and Night* $^{14}CO_2$ *Uptake (mg* $^{14}CO_2$ dm^{-2} h^{-1}*) in P. afra*

Date	Day	Night	Total	Day/Night
Aug. 11, 1981[a]	26.4	15.2	41.6	1.7
Sep. 10, 1981[a]	31.9	15.6	47.5	2.0
Feb. 3, 1982[a]	45.7	9.0	54.7	5.1
Mar. 25, 1982[b]	30.2	3.2	33.4	9.4
Apr. 21, 1982[a]	68.8	6.6	75.4	10.4
May 19, 1982[c]	30.3	7.5	37.8	4.0

[a] See appropriate figure legends for relevant environmental data.

[b] In March, environmental conditions were: irradiance of 300 PAR μmol m^{-2} s^{-1}, 20/11°C day/night air temperatures, 95/42% max/min RH.

[c] In May, environmental conditions were: irradiance of 1920 PAR μmol m^{-2} s^{-1}, 25/12°C day/night temperatures, 81/46% max/min RH.

maintain high organic acid levels, while the C_3 pathway is operating (8, 14).

The shift from C_3 to CAM appears to commence in May when *P. afra* is grown outdoors in San Diego. Acid fluctuations greater than 100 μeq g^{-1} FW were first observed in May 1981 (4, 5). In May 1982, a marked reduction in daytime CO_2 uptake was observed, while nocturnal CO_2 uptake remained at low levels. During summer, daytime temperatures above 30°C were common and midday stomatal closure occurred. Maximal rates of CO_2 uptake shifted from the daytime to the night and early morning hours. A larger diurnal acid fluctuation, increased daytime stomatal resistance, and increased nocturnal CO_2 uptake indicated the operation of CAM photosynthesis. These data are similar to those found for *P. afra* when water- or salt-stressed (7, 16).

Seasonal shifts from C_3 to CAM have been reported for *Mesembryanthemum crystallinum* and *M. nodiflorum* and were correlated with decreasing soil water potential and presumably plant water potential (3, 17, 18). In *P. afra*, the seasonally induced increase in diurnal acid fluctuations were similar to those reported for the *Mesembryanthemum* species, but they were not correlated with decreasing soil water potential since the plants were irrigated. The increasing diurnal acid fluctuations appeared to be related to increasing daytime temperatures and decreasing daytime CO_2 uptake. Increased evaporative demand and low transient plant water potentials could initiate the process of CAM induction in *P. afra*. In addition, the seasonal acid fluctuations indicate that *p. afra* shifts from the C_3 pathway to CAM with a return to the C_3 pathway. This differs from the *Mesembryanthemum* species, which end their life cycle in the CAM pathway (3, 17, 18).

Induction of CAM in *P. afra* is correlated with daytime stomatal closure (14). In the latter study, the stomatal resistance pattern of *P. afra* was observed to be closely linked to the CAM response. Similar results were found in the present study. When *P. afra* used the C_3 pathway, maximum daytime stomatal resistance was one-half to one-third that found when the CAM pathway was being utilized. Stomatal closure alone, however, cannot account for the induction of CAM in *P. afra*. Previously, it was shown that water stress or closure of stomata by abscisic acid induced a large diurnal acid fluctuation as well as increased PEP carboxylase activity in *P. afra* (14).

The increased CAM activity observed in the present study may be the result of decreasing daytime CO_2 uptake. In May 1982, the day/night ratio of carbon uptake was reduced from that observed in April 1982 from 10.4 to 4.0. This response was primarily due to a reduction of daytime CO_2 uptake. Yet, the daytime CO_2 uptake was similar to that observed in the summer months, but nocturnal uptake was only half that of the summer months. This indicates that the CAM pathway was not fully functional despite the reduced daytime CO_2 uptake. Nocturnal temperatures were lower in May than in the summer, which might lower CAM activity. However, similar nocturnal temperatures were observed in October 1983 which showed significant nocturnal CO_2 uptake (L. J. Guralnick, unpublished data). Thus, induction of CAM photosynthesis may require an increasing number of days of reduced daytime CO_2 uptake to become operational.

Results reported here may help to explain the effect of leaf aging and LD photoperiods on CAM induction in *P. afra* (5). During the spring, when *P. afra* was utilizing the C_3 pathway, daytime temperatures were low and in the range reported to be optimal for C_3 plants (2). The shift to CAM occurred when daytime temperatures increased under long days. The day and night temperatures observed under long days, 30 to 35°C during the day and 17 to 20°C at night in August and September, are in the range reported to be optimal for CAM photosynthesis (2, 8). The shift to CAM might enable *P. afra* to maintain photosynthetic activity while reducing water loss during periods of high daytime temperatures and evaporative demand.

P. afra is endemic to South Africa and can be a dominant or subdominant shrub associated with sclerophyllous shrubs (Richard Cowling, personal communication). Plants occur in the karoo of the western Cape and to the east in the dry river valley of Natal. The climate is typically Mediterranean, with rainfall occurring primarily in the spring, winter, and autumn, with a distinct summer drought (Richard Cowling, personal communication). Mooney *et al.* (9) have found that *P. afra*, *in situ* in South Africa, has a $\delta^{13}C$ value of $-17.5‰$ which is intermediate between that of a strict CAM plant and C_3 plant. This suggests that *P. afra* shifts between C_3 and CAM with a tendency toward the CAM metabolic pathway and could be an adaptation to the summer drought in South Africa.

P. afra does show a seasonal shift from C_3 to CAM and back to the C_3 pathway under irrigated conditions. Preliminary work with stressed *P. afra* has shown that the switch from CAM to C_3 in the late fall and early winter is influenced by decreasing daytime temperatures. However, more work is needed to resolve the questions of what may control the switch from C_3 to CAM and CAM to C_3 in perennial facultative CAM plants under field and laboratory conditions.

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