

# Minor Physiological Response to Elevated CO<sub>2</sub> by the CAM Plant *Agave vilmoriniana*<sup>1</sup>

Received for publication August 13, 1986 and in revised form November 24, 1986

STAN R. SZAREK\*, PETER A. HOLTHE, AND IRWIN P. TING

Department of Botany and Plant Sciences, University of California, Riverside, California 92521 (P.A.H., I.P.T.); and Department of Botany and Microbiology, Arizona State University, Tempe, Arizona 85287 (S.R.S.)

## ABSTRACT

One-year-old plants of the CAM leaf succulent *Agave vilmoriniana* Berger were grown outdoors at Riverside, California. Potted plants were acclimated to CO<sub>2</sub>-enrichment (about 750 microliters per liter) by growth for 2 weeks in an open-top polyethylene chamber. Control plants were grown nearby where the ambient CO<sub>2</sub> concentration was about 370 microliters per liter. When the plants were well watered, CO<sub>2</sub>-induced differences in stomatal conductances and CO<sub>2</sub> assimilation rates over the entire 24-hour period were not large. There was a large nocturnal acidification in both CO<sub>2</sub> treatments and insignificant differences in leaf chlorophyll content. Well watered plants maintained water potentials of -0.3 to -0.4 megapascals. When other plants were allowed to dry to water potentials of -1.2 to -1.7 megapascals, stomatal conductances and CO<sub>2</sub> uptake rates were reduced in magnitude, with the biggest difference in Phase IV photosynthesis. The minor nocturnal response to CO<sub>2</sub> by this species is interpreted to indicate saturated, or nearly saturated, phosphoenolpyruvate carboxylase activity at current atmospheric CO<sub>2</sub> concentrations. CO<sub>2</sub>-enhanced diurnal activity of ribulose biphosphate carboxylase activity remains a possibility.

The bulk of the world's vegetation belongs to the C<sub>3</sub> photosynthetic group and will likely respond to atmospheric CO<sub>2</sub> enrichment with a moderate increase in growth and yield (9, 21). However, it is clear that some C<sub>3</sub> plants will not respond to CO<sub>2</sub> enrichment (17, 18). Furthermore, short- versus long-term studies of a single species may yield different experimental results (19). Photosynthetic and growth responses to CO<sub>2</sub>-enriched atmospheres have also been reported for numerous C<sub>4</sub> species (3, 6, 10, 16, 20, 24), which change their photosynthetic capacity less when grown with elevated CO<sub>2</sub>. This diminished response must be biochemically based since stomata of C<sub>3</sub> and C<sub>4</sub> plants are equally sensitive to CO<sub>2</sub> concentration changes (12, 24). As with C<sub>3</sub> plants, differential responses to CO<sub>2</sub> enrichment occur between the various C<sub>4</sub> plants species (20).

The physiological response of CAM plants to CO<sub>2</sub>-enriched atmospheres is less clear since studies are few and, at present, contradictory. Bonner and Bonner (4) reported a sharp increase in the rate of acid synthesis in *Bryophyllum crenatum* leaves after a 2-d period of CO<sub>2</sub> enrichment at partial pressures ranging from 300 to 1000  $\mu\text{L L}^{-1}$ . Subsequent work with the same species suggests phosphoenolpyruvate carboxylase may play a role in this response at CO<sub>2</sub> partial pressures below 5000  $\mu\text{L L}^{-1}$  (22).

CO<sub>2</sub> enrichment caused an immediate increase in the photosynthesis rate of *Kalanchoë daigremontiana* during Phase IV gas exchange (1). In this study, the photosynthetic response to CO<sub>2</sub> concentration was linear over a wide range and saturated at about 1650  $\mu\text{L L}^{-1}$ . A similar response was reported during short-term exposure of *Ananas comosus* leaves to elevated CO<sub>2</sub> (11). In contrast, Holtum *et al.* (7) reported little, or even no, change in both nocturnal and diurnal CO<sub>2</sub> uptake during enrichment of leaves of *K. daigremontiana*. The reason for this contradiction with the earlier *Kalanchoë* study is not clear but may be the result of different procedures being used.

Recently, long-term studies of CAM plant growth during CO<sub>2</sub> enrichment have been conducted (8, 23). *Agave vilmoriniana* was selected for one investigation since it demonstrated both daytime and nighttime <sup>14</sup>CO<sub>2</sub> assimilation (SR Szarek, unpublished data). It was expected that CO<sub>2</sub> enrichment would enhance the extent of CO<sub>2</sub> assimilation in the light more than for nocturnal CO<sub>2</sub> assimilation (23). During the day, Phase II and Phase IV periods of gas exchange occur, periods when ribulose biphosphate carboxylase activity plays a role in the total daily carbon acquisition (14). In CAM plants, this enzyme saturates at a higher partial pressure of CO<sub>2</sub> than does phosphoenolpyruvate carboxylase (15). Plants of *Agave* were grown outdoors at four CO<sub>2</sub> concentrations and two water treatments (8). Unexpectedly, well watered plants responded less to CO<sub>2</sub> enrichment than water-limited plants. Second, CO<sub>2</sub>-induced differences in growth were only statistically significant between the extreme CO<sub>2</sub> concentrations. The study reported here was designed to investigate the photosynthetic physiology of plants of *Agave vilmoriniana* during long-term growth under CO<sub>2</sub> enrichment and two contrasting water treatments.

## MATERIALS AND METHODS

**Plant Material.** One-year-old plants of the leaf succulent *Agave vilmoriniana* Berger were grown outdoors at Riverside, CA in an open-top chamber constructed of clear polyethylene. The cylindrical chamber was 0.7 m high and had a diameter of 0.65 m. This chamber was positioned below a polyethylene roof which diverted rain. The roofing reduced midday PAR by 0.25 mmol m<sup>-2</sup> s<sup>-1</sup>. Individual plants were placed in 1.5-L pots containing a 1:1 mixture of vermiculite and UC potting mixture. All were watered on alternate days and one-quarter strength Hoagland solution was applied weekly. Two weeks prior to each experiment, watering was stopped for the "dry treatment" plants. The "wet treatment" plants were watered as before. A CO<sub>2</sub>-enriched atmosphere was produced by pumping ambient air through the chamber after CO<sub>2</sub> had been added from a cylinder of liquid CO<sub>2</sub> known to be free of ethylene contamination (13). The air exchange rate through the chamber was about 22 L min<sup>-1</sup> and the

<sup>1</sup> Supported by National Science Foundation grant DMB 84-16981 to I.P.T.

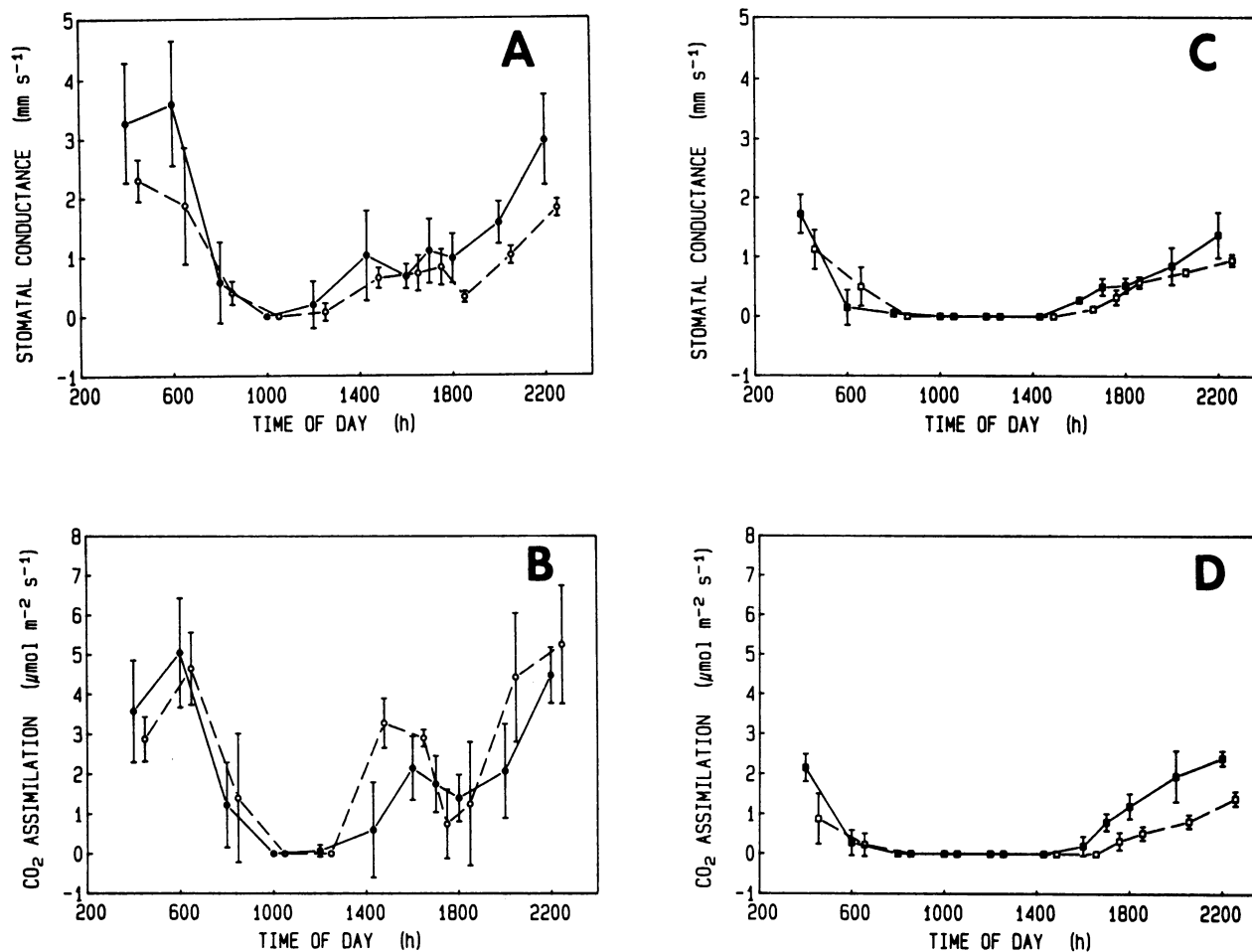


FIG. 1. Stomatal conductance and CO<sub>2</sub> assimilation rates for plants of *A. vilmoriniana* grown in elevated (closed symbols) and ambient (open symbols) CO<sub>2</sub> with two water treatments: wet (circles) and dry (squares). Symbols depict mean values for  $n = 4$  while error bars depict the standard deviation. On this date, dawn was at 0505 h and dusk was at 1829 h.

Table I. Nocturnal Acidification of *Agave vilmoriniana* Leaves from Plants Acclimated to Ambient and Elevated CO<sub>2</sub> Concentrations

Water Treatment	Nocturnal Acidity Increase <sup>a</sup>	
	Ambient versus Elevated CO <sub>2</sub>	
	$\mu\text{eq g}^{-1}$ fresh wt	
Wet	205 ± 33 A	230 ± 30 A
Dry	161 ± 28 A	173 ± 43 A

<sup>a</sup> Each value is the mean of 24 leaf discs ± the SD. Acidity differences between the CO<sub>2</sub> treatments followed by the same letter were not significantly different at  $P = 0.05$ .

CO<sub>2</sub> concentration within it was determined with a portable IR gas analyzer (described below). Plants which were not CO<sub>2</sub>-enriched were grown nearby. Average CO<sub>2</sub> concentrations during the experiments were  $752 \pm 87 \mu\text{l L}^{-1}$  and  $367 \pm 14 \mu\text{l L}^{-1}$ . These experiments were conducted in the months of April to June 1986 since this species is quiescent over the winter.

**Acclimation Period.** A 2 week period of acclimation to the elevated CO<sub>2</sub> concentration preceded each experiment. In other plants, photosynthesis reversibly acclimates to different CO<sub>2</sub> concentrations within 3 to 4 d (19). Winter (23) did not observe an acclimation response in his unpublished studies with CAM plants and CO<sub>2</sub> enrichment.

**Gas Exchange Measurements.** The second and third fully expanded leaves on plants from each treatment were chosen for measurement. A terminal section of each leaf was sealed in a

Table II. Chlorophyll Content of *Agave vilmoriniana* Leaves from Plants Acclimated to Ambient and Elevated CO<sub>2</sub> Concentrations

Water Treatment	Parameter	Ambient versus Elevated CO <sub>2</sub> <sup>a</sup>	
		Ambient	Elevated
Wet	Total Chl ( $\mu\text{g g}^{-1}$ fresh wt)	149 ± 6 A <sup>b</sup>	131 ± 26 A
Dry		132 ± 23 A	116 ± 23 A
Wet	Chl <i>a</i> ( $\mu\text{mol m}^{-2}$ )	237 ± 30 A	190 ± 32 A
Dry		169 ± 34 A	149 ± 19 A
Wet	Chl <i>b</i> ( $\mu\text{mol m}^{-2}$ )	96 ± 18 A	66 ± 8 B
Dry		68 ± 4 A	68 ± 4 A
Wet	Chl <i>a</i> /Chl <i>b</i>	2.5 A	2.8 A
Dry		2.5 A	2.2 A

<sup>a</sup> Each value is the mean of 24 leaf discs ± SD. <sup>b</sup> Chl contents between CO<sub>2</sub> treatments followed by the same letter are not significantly different at  $P = 0.05$ .

polycarbonate chamber and its gas exchange measured with a LI-COR model LI-6000 Portable Photosynthesis System. Each measurement period was for 90 s before the CO<sub>2</sub> assimilation rate and stomatal conductance were computed by the instrument. The system was calibrated before each experiment and was zeroed on an alternate hour basis during each 24-h experiment.

**Titrate Acids.** The same leaves used in the gas exchange measurements were harvested and placed on dry ice until they were brought to the laboratory. Leaf discs, 7 mm in diameter,

were weighed and then ground in glass-distilled H<sub>2</sub>O with a Potter-type tissue homogenizer. Samples were titrated to a pH 7.0 endpoint using 0.01 N KOH and a Brinkmann Auto-titrator.

**Chl Content.** Other discs from the leaves used above were weighed and then ground with sea sand in a chilled mortar and pestle. Pigments were extracted in 80% acetone (v/v) and about 10 mg of L-ascorbic acid (sodium salt) added to each sample. The preparation was cleared by centrifugation at 1500g for 10 min at room temperature. *A*<sub>645</sub> and *A*<sub>663</sub> were measured with a Beckman model DU-7 Spectrophotometer and the Chl components determined by the formulae of Arnon (2).

**Water Potentials.** Single discs were placed in Wescor model C-52 Sample Chambers and equilibrated for 2 h at a constant temperature. The water potential was then measured with a model HR 33 Dewpoint Microvoltmeter. Duplicate samples were measured for each leaf.

**Statistical Analyses.** Titratable acids and Chl contents were analyzed to determine which means were greater than that predicted by the *F* value. This was done using a Tukey's least statistical difference test by microcomputer.

## RESULTS AND DISCUSSION

Well watered plants of *Agave vilmoriniana* did not exhibit a large difference in gas exchange between the two CO<sub>2</sub> treatments when compared over the entire 24-h period (Fig. 1, A and B). These results are similar to the investigation of Holtum *et al.* (7) who also found small variations in the CO<sub>2</sub> uptake rate of *K. daigremontiana* at elevated CO<sub>2</sub>. The predominant period for fixing atmospheric CO<sub>2</sub> is the nighttime. Stomata close within a few hours after dawn, allowing for about 20% of the daily gas exchange during Phase II. A Phase IV period of CO<sub>2</sub> assimilation commences a few hours after midday and contributes about 15% of the daily total gas exchange. Diurnal CO<sub>2</sub> assimilation occurred in both CO<sub>2</sub> treatments, indicating the feedback inhibition of ribulose biphosphate carboxylase by long-term CO<sub>2</sub> enrichment, sometimes reported in other studies (*e.g.* 18), is negligible in this CAM plant. Phase IV CO<sub>2</sub> assimilation was consistently higher in the elevated CO<sub>2</sub>, dry treatment plants (Fig. 1D).

Well watered plants of *A. vilmoriniana* maintained water potentials of -0.3 to -0.4 MPa. When other plants were allowed to dry to water potentials of -1.2 to -1.7 MPa, gas exchange patterns were similar but reduced in magnitude. Hence, well watered plants exhibited a large nocturnal acidification in both CO<sub>2</sub> treatment (Table I). During the three replications of these experiments, the acidity flux measured on each increased with day lengths and ambient temperatures. In June, the maximum acid content at dawn was about 360 μeq g<sup>-1</sup> fresh weight. Leaves of the same plant had a minimum acid content at dusk of about 40 μeq g<sup>-1</sup> fresh weight. However, the approximate percentage difference between the water and CO<sub>2</sub> treatments remained the same in each replication. In comparison with other CAM plants, *A. vilmoriniana* appears to have high total activity of phosphoenolpyruvate carboxylase (14). Elevated CO<sub>2</sub> did not, in these experiments, alter Chl parameters relative to plants grown in ambient CO<sub>2</sub> (Table II). In other studies, CO<sub>2</sub> enrichment caused small decreases in the Chl concentration (5).

The lack of a nocturnal response to CO<sub>2</sub> enrichment indicates saturated, or nearly saturated, activity of phosphoenolpyruvate carboxylase at current atmospheric CO<sub>2</sub> concentrations. This is concluded from the two recent gas exchange studies with plants of *Kalanchoe* which did not show enhanced rates of CO<sub>2</sub> uptake when exposed to supranormal concentrations of CO<sub>2</sub> (7, 15). The importance of ribulose biphosphate carboxylase activity upon total daily carbon gain is more evident in the water-limited plants, although the well-watered plants also fixed CO<sub>2</sub> during the daytime. CO<sub>2</sub>-enhanced growth may be more important in

plants that have a greater period of daytime CO<sub>2</sub> assimilation. A previous growth study with *A. vilmoriniana* indicated a 28% enhancement with a doubled atmospheric CO<sub>2</sub> concentration for large, dry plants (8). After the current study, we have to conclude that the 3-fold increase in growth was overestimated for juvenile plants and may be the result of water stress effects leading to slow or no growth in some of these plants.

**Acknowledgments**—Plants for this study were provided by the Desert Botanical Garden, Phoenix, AZ. The technical and computer graphics assistance of Ms. Janet Hann was greatly appreciated.

## LITERATURE CITED

1. ALLAWAY WG, B AUSTIN, RO SLATYER 1974 Carbon dioxide and water vapor exchange parameters of photosynthesis in a Crassulacean plant, *Kalanchoe daigremontiana*. Aust J Plant Physiol 1: 397-405
2. ARNON D 1949 Cooper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. Plant Physiol 24: 1-15
3. BAZZAZ FA, RW CARLSON 1984 The response of plants to elevated CO<sub>2</sub>. I. Competition among the assemblage of annuals at two levels of soil moisture. Oecologia 62: 196-198
4. BONNER W, J BONNER 1948 The role of carbon dioxide in acid formation by succulent plants. Am J Bot 35: 113-117
5. DELUCIA EH, TW SASEK, BR STRAIN 1985 Photosynthetic inhibition after long-term exposure to elevated levels of atmospheric carbon dioxide. Photosynth Res 7: 175-184
6. GIFFORD RM, JIL MORISON 1985 Photosynthesis, water use and growth of a C<sub>4</sub> grass stand at high CO<sub>2</sub> concentration. Photosynth Res 7: 69-76
7. HOLTUM JAM, MH O'LEARY, CB OSMOND 1983 Effect of varying CO<sub>2</sub> partial pressure on photosynthesis and on carbon isotope composition of carbon-4 of malate from the Crassulacean acid metabolism plant *Kalanchoe daigremontiana* Hamet et Perr. Plant Physiol 71: 602-609
8. IDSO SB, BA KIMBALL, MG ANDERSON, SR SZAREK 1986 Growth response of a succulent plant, *Agave vilmoriniana*, to elevated CO<sub>2</sub>. Plant Physiol 80: 796-797
9. KIMBALL BA 1983 Carbon dioxide and agricultural yield: an assemblage and analysis of 430 prior observations. Agron J 75: 779-788
10. MAUNEY JR, G GUINN, KE FRY, JD HESKETH 1979 Correlation of photosynthetic carbon dioxide uptake and carbohydrate accumulation in cotton, soybean, sunflower and sorghum. Photosynthetica 13: 260-266
11. MORADSHAHI A, HM VINES, CC BLACK 1977 CO<sub>2</sub> exchange and acidity levels in detached pineapple, *Ananas comosus* (L.) Merr., leaves during the day at various temperatures, O<sub>2</sub> and CO<sub>2</sub> concentrations. Plant Physiol 59: 274-278
12. MORISON JIL, RM GIFFORD 1983 Stomatal sensitivity of carbon dioxide and humidity: a comparison of two C<sub>3</sub> and two C<sub>4</sub> grass species. Plant Physiol 71: 789-796
13. MORISON JIL, RM GIFFORD 1984 Ethylene contamination of CO<sub>2</sub> cylinders: effects on plant growth in CO<sub>2</sub> enrichment studies. Plant Physiol 75: 275-277
14. OSMOND CB 1978 Crassulacean acid metabolism: a curiosity in context. Annu Rev Plant Physiol 29: 379-414
15. OSMOND CB, O BJORKMAN 1975 Pathways of CO<sub>2</sub> fixation in the CAM plant *Kalanchoe daigremontiana*. II. Effects of O<sub>2</sub> and CO<sub>2</sub> concentration on light and dark CO<sub>2</sub> fixation. Aust J Plant Physiol 2: 155-162
16. PATTERSON DT, EP FLINT 1980 Potential effects of global atmospheric CO<sub>2</sub> enrichment on the growth and competitiveness of C<sub>3</sub> and C<sub>4</sub> weed and crop plants. Weed Sci 28: 71-75
17. PEET MM 1986 Acclimation to high CO<sub>2</sub> in monoecious cucumbers I. Vegetative and reproductive growth. Plant Physiol 80: 59-62
18. PEET MM, SC HUBER, DT PATTERSON 1986 Acclimation to high CO<sub>2</sub> in monoecious cucumbers. II. Carbon exchange rates, enzyme activities, and starch and nutrient concentrations. Plant Physiol 80: 63-67
19. SASEK TW, EH DELUCIA, BR STRAIN 1985 Reversibility of photosynthetic inhibition in cotton after long-term exposure to elevated CO<sub>2</sub>. Plant Physiol 78: 619-622
20. SIONIT N, DT PATTERSON 1984 Responses of C<sub>4</sub> grasses to atmospheric CO<sub>2</sub> enrichment. I. Effect of irradiance. Oecologia 65: 30-34
21. STRAIN BR, F BAZZAZ 1983 Terrestrial communities. In E Lemon, ed, CO<sub>2</sub> and Plants: The Response of Plants to Rising Levels of Atmospheric Carbon Dioxide. Westview Press, Boulder, CO, pp 177-222
22. WALKER DA, JMA BROWN 1957 Physiological studies on acid metabolism. 5. Effects of carbon dioxide concentration on phosphoenolpyruvate carboxylase activity. Biochem J 67: 79-83
23. WINTER K 1985 Crassulacean acid metabolism. In J Barber, NR Baker, eds, Photosynthetic Mechanisms and the Environment. Elsevier Science Publishers B.V., Amsterdam, pp 329-387
24. WONG SC 1979 Elevated atmospheric partial pressure of CO<sub>2</sub> and plant growth. I. Interaction of nitrogen nutrition and photosynthetic capacity in C<sub>3</sub> and C<sub>4</sub> plants. Oecologia 44: 68-74