Effect of Elevated Carbon Dioxide on the Growth and Physiological Responses of Pineapple, A Species with Crassulacean Acid Metabolism

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ABSTRACT. Despite the potential impact of rising global CO₂ levels, only a limited number of studies have been conducted on the effects of ambient and elevated CO₂ on plants having Crassulacean acid metabolism (CAM). To our knowledge, there are no studies for pineapple [*Ananas comosus* (L.) Merr.], the most commercially important CAM plant. Pineapple plants were grown at CO₂ levels of ≈330 (ambient) and ≈730 (elevated) μmol·mol⁻¹ in open-top chambers for 4 months. The mean air temperature in the chambers was ≈39 °C day/24 °C night. Average plant dry mass at harvest was 180 g per plant at elevated CO₂ and 146 g per plant at ambient CO₂. More biomass was partitioned to stem and root but less to leaf for plants grown at elevated CO₂; leaf thickness was 11% greater at elevated than at ambient CO₂. The diurnal difference in leaf titratable acidity (H⁺) at elevated CO₂ reached 347 mmol·m⁻², which was up to 42% greater than levels in plants grown in ambient CO₂. Carbon isotopic discrimination (Δ) of plants was 3.75‰ at ambient CO₂ and 3.17‰ at elevated CO₂, indicating that CO₂ uptake via the CAM pathway was enhanced more by elevated CO₂ than uptake via the C₃ pathway. The nonphotochemical quenching coefficient (q_N) of leaves was ≈45% lower in the early morning for plants grown at elevated than at ambient CO₂, while afternoon values were comparable. The q_N data suggested that the fixation of external CO₂ was enhanced by elevated CO₂ in the morning but not in the afternoon when leaf temperature was ≥40 °C. We found no effect of CO₂ levels on leaf N or chlorophyll content. Pineapple dry matter gain was enhanced by elevated CO₂, mainly due to increased CO₂ dark fixation in environments with day temperatures high enough to suppress C₃ photosynthesis.

Atmospheric CO₂ levels have steadily increased from 275 to 350 μmol·mol⁻¹ since the beginning of the industrial revolution (Sarmiento and Bender, 1994), and the CO₂ level is predicted to double by the middle of the next century. The growth of many species having the reductive pentose phosphate pathway (C₃ plants) is significantly increased when the CO₂ level is doubled while plants fixing CO₂ by the C₄-dicarboxylic acid pathway (C₄ plants) show little or no response when the CO₂ level is doubled, (Acock, 1990). This study was designed to investigate the growth and photosynthetic responses of pineapple, a plant having Crassulacean acid metabolism (CAM), to near ambient and elevated CO₂ in open-top chambers. We hypothesized that dry matter accumulation would be enhanced, primarily by increased C₃ photosynthesis, when plants were grown in elevated CO₂.

The environmental physiology of pineapple was recently reviewed (Bartholomew and Malézieux, 1994), and the available data show that the greatest percentage of CO₂ is fixed at night. This CO₂ is incorporated into C₄ acids, primarily malate (Carnal and Black, 1989), followed by massive accumulation in the vacuole (phase I) (Osmond, 1978). The accumulated acids are decarboxylated during the day (phase III), and the released CO₂ is incorporated into carbohydrate by the C₃ pathway. Once stored acids are depleted, significant quantities of CO₂ are assimilated from the air

by the C₃ pathway (phase IV). Phase II (Osmond, 1978) in pineapple is a transitory period between phase I and III that occurs at the beginning of the light period.

Gas exchange studies with CAM plants show that elevated CO₂ primarily enhances CO₂ fixation during phase IV (Nobel and Hartsock, 1986; Szarek et al., 1987), although some species also have increased CO₂ uptake (Black, 1986; Nobel and Israel, 1994; Raveh et al., 1995; Winter, 1985) or increased malate accumulation (Nowak and Martin, 1995) during phase I. Only a few studies of the effects of elevated CO₂ on CAM plant growth and physiol-

Table 1. Growth responses of pineapple after 123 d at two CO₂ levels in open-top chambers. Data are means of six plants per treatment.

Characteristics ^a	CO ₂ level (μmol·mol ⁻¹)		t test
	330	730	
Wt (g/plant)	146.0	180.1	**
AL (cm ² /plant)	6404	6746	NS
NAR (g·m ⁻² ·d ⁻¹)	1.64	2.23	---
RGR (g·kg ⁻¹ ·d ⁻¹)	7.08	8.83	---
SMR (%)	7.2	9.8	*
RMR (%)	8.7	11.6	**
LMR (%)	84.1	78.7	**
SLM (g·cm ⁻²)	0.019	0.021	**
dmc (stem) (%)	11.20	13.12	**
dmc (leaf) (%)	11.35	11.21	NS

^aWt = plant dry mass; AL = plant leaf area; NAR = net assimilation rate; RGR = relative growth rate; SMR = stem mass/total mass; RMR = root mass/total mass; LMR = leaf mass/total mass; SLM = specific leaf mass; dmc = dry matter content. Data for SMR, RMR, and LMR are expressed as the percentage.

^bThese growth characteristics were calculated from mean values for plants harvested at the beginning and end of the study so no statistical analysis could be performed.

NS, *, ** Nonsignificant or significant at *P* = 0.05 or 0.01, respectively.

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ogy were found (Cui and Nobel, 1994; Cui et al., 1993; Idso et al., 1986; Nobel and Hartsock, 1986; Raveh et al., 1995), and we could find no studies for pineapple, the most commercially important crop plant having CAM.

Our objectives were to examine the effects of CO₂ levels on some growth and physiological aspects of pineapple. The relative contributions of the C₃ and CAM pathways to growth were assessed by measuring leaf titratable acidity, leaf osmolarity, and stable carbon isotope discrimination (Δ). Smaller values indicate enrichment in ¹³C relative to ¹²C in leaf tissues, reflecting higher CAM activity, while higher Δ values indicate relatively greater C₃-type activity (Griffiths, 1992). Chlorophyll fluorescence induction kinetics ('Kautsky' effect) were measured during phase II and IV to evaluate the effect of ambient and elevated CO₂ levels on photochemical (q_p) and nonphotochemical (q_N) quenching coefficients and the photochemical efficiency of photosystem II (PSII) (Jones, 1992; Krause, 1991). Such data, which to our knowledge, have not previously been published for pineapple, may help to interpret the results of the effects of environmental factors on plant photosynthesis (Guralnick et al., 1992).

Materials and Methods

PLANT MATERIALS AND CHAMBER CONDITIONS. Thirty fruit tops (crowns) of the Smooth Cayenne pineapple clone Champaka 153 obtained from a local pineapple company were planted in $\approx$ 4-L pots in 1 black cinder : 1 horticultural vermiculite (v/v) on 1 Jan. 1995. After a 4-month establishment period, six plants of uniform size were selected and placed in each of two open-topped chambers. The number of plants used per treatment was limited by chamber size. The plants were grown with CO₂ at 330 $\pm$ 20 (ambient) and 730 $\pm$ 35 (elevated) μ mol·mol⁻¹ from 1 May to 31 Aug. 1995 in a greenhouse at the Univ. of Hawaii at Manoa. The elevated CO₂ level was achieved by bleeding pure CO₂ through a control valve, flow meter, and tubing into the base of the open-top chamber. The CO₂ concentration in the chambers was measured with an IR gas analyzer (Li-6262, LI-COR, Lincoln, Neb.) and the flow rate was adjusted over a few days until the desired set point was reached. The CO₂ concentration then was monitored periodically to assure that the elevated CO₂ level was maintained at the desired set point.

One month after treatments were started, plants were transferred to $\approx$ 8-L pots for the remainder of the experiment to assure that rooting volume did not limit growth. Plants were irrigated twice weekly with a nutrient solution specifically developed for pineapple (W.G. Sanford, unpublished data), which consisted of

(in mmol·L⁻¹) 0.5 KH₂PO₄, 0.82 MgSO₄·7H₂O, 0.5 K₂SO₄, 2.5 NH₄NO₃, and 0.5 CaCl₂ (anhydrous) and (in μ mol·L⁻¹) 11.5 H₃BO₃, 2.3 MnCl₂·4H₂O, 0.3 ZnSO₄·7H₂O, 0.08 CuSO₄·5H₂O, 0.03 H₂MoO₄·H₂O, and 9.0 Fe as the chelate. Pots were flushed with tap water monthly to prevent nutrients from accumulating in the media.

The two open-top chambers were 1.5 m long, 1.3 m wide, and 1.4 m tall and were constructed of a frame covered with mylar film. A table fan ($\approx$ 20 cm in diameter) was used in each chamber to circulate the air. The two chambers were adjacent to each other on one greenhouse bench and perpendicular to the prevailing air flow across the bench. Because air temperature in the chambers was not controllable, they were covered with 30% neutral-density shade cloth to reduce heat build-up. Air temperatures at the midplant height in the two chambers were measured with thermocouples shaded with aluminum foil. Leaf temperatures were measured periodically by inserting fine-wire thermocouples into the tissue of the tallest leaf, which is identified by bunching the uppermost leaves on the plant together and selecting the one whose leaf tip extends above all the others. With normal growth the tallest leaf, termed the "D" leaf in the pineapple literature (Bartholomew and Kadzimin, 1977), is about the youngest physiologically mature leaf on the plant. The D leaf has been used to index plant nutrient levels and evaluate the effects of environmental factors on plant growth and water status (Bartholomew and Kadzimin, 1977). All thermocouple data were collected using a datalogger (21X, Campbell Scientific, Logan, Utah). The mean air temperatures inside the chambers during the day (7:30 AM to 6:30 PM) were 39.0 °C in the ambient and 39.5 °C in the elevated CO₂ treatments, while the mean temperature at night was 24.1 °C in both chambers. Reducing the radiation load with shade cloth resulted in mean leaf temperatures that were only $\approx$ 1.0 °C above mean air temperature. In Hawaii, leaf temperatures of pineapple can be several degrees above air temperature (Noffsinger, 1961), and temperatures as high as 50 °C have been measured in the field with no physiological injury (Aubert and Bartholomew, 1973). The average midday maximum photosynthetic photon flux (PPF) measured by the quantum sensor (LI-COR) was $\approx$ 500 μ mol·m⁻²·s⁻¹ at midplant height inside the chambers.

GROWTH ANALYSIS. The mean initial fresh and dry mass of leaves, stems, and roots was calculated from measurements made at the beginning of the experiment on six 4-month-old plants comparable to those used in the study. Final data on the previously described parameters were obtained by destructive harvest of the experimental plants after 4 months of treatment. Dry mass was measured after oven-drying at 70 °C for 2 weeks. Green leaf area was measured using a leaf area meter (LI-3100; LI-COR). Dry mass partitioning was assessed by calculating the ratios of stem (SMR), root (RMR), or leaf (LMR) to whole-plant dry mass. Relative growth rate (RGR) and net assimilation rate (NAR) (Chiariello et al. 1989) were calculated as described by the following equations:

$$RGR = \frac{\ln w_2 - \ln w_1}{t_2 - t_1} \quad [1]$$

and

$$NAR = \frac{2(w_2 - w_1)}{(A_2 + A_1)(t_2 - t_1)} \quad [2]$$

where W is total plant dry mass, A is green leaf area, t is time, and the subscripts 1 and 2 represent samples collected on 1 May and 31 Aug., respectively. Specific leaf mass (SLM), the leaf dry mass : green leaf area ratio, and tissue dry matter contents also were calculated.

Table 2. Photochemical (q_p) and nonphotochemical (q_N) quenching and photochemical efficiency (F_v : F_m) (n = six plants) of the youngest physiologically mature leaves of pineapple grown at two CO₂ levels in open-top chambers.

Parameters	Phase ^a	CO ₂ level (μ mol·mol ⁻¹)		t test
		330	730	
q _p (morning)	II	0.727	0.841	NS
q _p (afternoon)	IV	0.809	0.897	NS
q _N (morning)	II	0.681	0.377	*
q _N (afternoon)	IV	0.643	0.651	NS
F _v : F _m (morning)	II	0.831	0.864	NS
F _v : F _m (afternoon)	IV	0.790	0.833	NS

^aMeasured at the beginning (phase II) and end (phase IV) of the light period.

NS,*Nonsignificant or significant at P = 0.05, respectively.

CARBON ISOTOPE DISCRIMINATION. Relative natural abundance of the stable carbon isotopes ^{13}C and ^{12}C ($\delta^{13}\text{C}$) in leaf tissue was determined on a subsample of the D leaf. Leaves were oven-dried at 70 °C and finely ground. The $^{13}\text{C} : ^{12}\text{C}$ ratio of the leaf tissue was measured on a SIRA series II isotope ratio mass spectrometer (VG Isotech, Middleswich, U.K.) at the Duke Univ. Phytotron. The $^{13}\text{C} : ^{12}\text{C}$ ratio of source air in the chambers was measured by collecting samples in 250-mL pyrex gas collecting tubes. The air samples were analyzed using a mass spectrometer (Finnigan MAT 252; Finnigan Corp., Germany) in the Institute of Geophysics, Univ. of Hawaii. The $\delta^{13}\text{C}$ data were used to calculate carbon isotope discrimination (Δ) by the following equation:

$$\Delta = \frac{\delta_a - \delta_p}{1 + \delta_p} \quad [3]$$

where "a" is the isotopic composition of air, and "p" is that of the leaves.

CHLOROPHYLL FLUORESCENCE KINETICS. Fluorescence induction kinetics were measured biweekly with a chlorophyll fluorometer (PAM 101; Walz, Effeltrich, Germany) beginning 1 month after starting the CO_2 treatments. Measurements were made on D leaves at $\approx 8:00$ AM (phase II) and 5:00 PM (near the end of phase IV) when C_3 -type photosynthesis would be high (Zhu, 1996). The plants were dark-acclimated for ≈ 30 min, and the fluorescence intensity of the D leaves was detected following the procedure described by Guralnick et al. (1992) and Jones (1992). Leaves were illuminated with modulated light ($\text{PPF} \approx 2$ to $4 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) to induce the initial fluorescence yield (F_0), followed by a saturating pulse ($\text{PPF} \approx 2200 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) to create maximum fluorescence (F_m , dark adapted) and the peak fluorescence [$F_m(t)$, light adapted] and further by actinic light ($\text{PPF} \approx 350 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) to drive photosynthesis and, thus, increase fluorescence yield. Variable fluorescence (F_v) was calculated as $F_m - F_0$ and the photochemical efficiency of PSII was estimated as F_v/F_m (Jones, 1992). The values for q_p and q_N were calculated using the equations described by Jones (1992) and Guralnick et al. (1992).

TITRATABLE ACIDITY AND OSMOLARITY. Leaf titratable acidity (TA) and osmolarity were measured on one D leaf from each of six plants per chamber collected at the beginning and end of the light period. The D leaf was split longitudinally and one-half was removed at dawn and frozen at -75 °C to disrupt the cell membranes. The second leaf segment was harvested in the late afternoon and similarly frozen. To determine TA, two leaf disks (1.3 cm in diameter) were cut from the middle one-third of each frozen leaf segment and ground in a mortar with 20 mL distilled water; the total volume titrated to pH 7.2 using 0.01 N NaOH. Osmolarity was measured on leaf sap extracted from frozen-thawed tissues with a vapor pressure osmometer (5500S, Wescor, Logan, Utah).

CHLOROPHYLL AND N CONTENTS. Leaf chlorophyll content was determined on 1.3-cm disks from the middle one-third of the D leaf preserved by freezing as previously described. The frozen disks were ground to a powder in liquid N, and a 200-mg sample was extracted by grinding it with 80% (v/v) acetone, which was brought to a final volume of 20 mL with 80% acetone. The mixture was incubated overnight at room temperature (22 °C) and centrifuged at $12000\times g$ for 1 min, and the absorbance of the supernatant was read at 647- and 664-nm wavelengths with a spectrophotometer (Spectronic 21, Bausch & Lomb, New York). Chlorophyll content was calculated according to Coombs et al. (1985).

Leaf total N content was determined using the same leaf tissue powder used for the chlorophyll measurement by the micro-kjeldahl technique at the Agricultural Diagnostic Service Center,

Table 3. Titratable acidity (TA) and osmolarity of the youngest physiologically mature leaves ($n = \text{six plants}$) of pineapple grown at two CO_2 levels in open-top chambers.

Characteristics	Phase ^z	CO ₂ level ($\mu\text{mol}\cdot\text{mol}^{-1}$)		<i>t</i> test
		330	730	
TA, H^+ ($\text{mmol}\cdot\text{m}^{-2}$)				
30 June 1995	I	225.5	293.2	**
31 Aug. 1995	I	278.2	384.3	**
30 June 1995	IV	31.6	32.9	*
31 Aug. 1995	IV	35.4	37.4	*
ΔTA^y				
30 June 1995		194.0	260.3	**
31 Aug. 1995		242.8	347.0	**
Osmolarity ($\text{mmol}\cdot\text{kg}^{-1}$)				
31 Aug. 1995	I	418.4	504.5	**
31 Aug. 1995	IV	441.2	484.0	*

^zMeasured at the end of the dark (phase I) and light (phase IV) periods.

^yDifference between TA in phase I and IV.

*,**Significant at $P = 0.05$, or 0.01 , respectively.

Univ. of Hawaii at Manoa. The data were reported as the percentage N in the leaf on a dry mass basis (m/m, percent).

Results and Discussion

Mean D leaf temperature during the study was 40.4 °C day/23.9 °C night in elevated and 40.0 °C day/24.2 °C night in the ambient CO_2 chambers. Despite the high average day temperature, growth was normal, and there was no evidence of heat injury to the plants. The leaf temperatures that we encountered also occur in the field in Hawaii (Aubert and Bartholomew, 1973).

Biomass accumulation over the 4-month growing period was significantly greater at elevated than at ambient CO_2 (Table 1). This result confirmed those of a previous study (Zhu, 1996) where pineapple dry mass of plants grown 6 months in controlled environments at 30 °C day/20 °C night, 30 °C day/25 °C night, and 35 °C/25 °C night was 23% to 42% greater at $700 \mu\text{mol}\cdot\text{mol}^{-1}$ CO_2 than at $350 \mu\text{mol}\cdot\text{mol}^{-1}$. However, the CO_2 treatments in that study were conducted at different times because only three controlled environment chambers were available. There also was enhanced plant growth at elevated CO_2 for the CAM species *Agave deserti* Engelm. and *Ferocactus acanthodes* (Lem.) Britt. and Rose (Nobel and Hartsock, 1986), *Agave vilmoriniana* A. Berger (Idso et al., 1986), and *Opuntia ficus-indica* (L.) Mill. (Cui and Nobel, 1994; Cui et al., 1993).

The mean RGR and NAR values also were higher for plants grown at elevated than at ambient CO_2 . Since mean plant leaf areas in the two treatments were not significantly different (Table 1), we conclude that the growth response to elevated CO_2 was due to the greater NAR. The mean NAR value for plants grown in ambient CO_2 in this study was $\approx 20\%$ greater than those obtain in controlled environments (Zhu et al., 1996), while the NAR value at elevated CO_2 in this study was $\approx 8\%$ smaller than that obtained previously (Zhu, 1996). This result indicates that plant growth in this study was not suppressed by the high daytime temperature. Our results also are consistent with previous studies of leaf gas exchange (Zhu, 1996) that showed that elevated CO_2 significantly enhanced diurnal CO_2 fixation. No CO_2 exchange data were obtained for this study because equipment to continuously measure CO_2 exchange rates of pineapple leaves was unavailable and attempts to seal a

portable leaf chamber on the sharply curved pineapple leaves were unsuccessful.

At elevated CO_2 , more biomass was partitioned to root and stem and less to leaf, thus accounting for the significantly greater fraction of plant biomass incorporated into stem (SMR) and root (RMR) and the smaller portion partitioned to leaf (LMR) (Table 1). Leaf thickness (SLM) was significantly greater at elevated than at ambient CO_2 (Table 1). Cladodes of the CAM species *O. ficus-indica* also were thicker when plants were grown at elevated CO_2 (Nobel and Israel, 1994). Stem dry matter content was significantly greater for plants grown at elevated than at ambient CO_2 , but there was no effect on the leaf dry matter content (Table 1). Pineapple stem dry matter and starch contents are positively correlated (Bartholomew and Paull, 1986). Thus, the greater stem dry matter content may be due in part to greater stem starch content at elevated CO_2 .

The q_p values of plants grown at elevated CO_2 were not significantly higher than those for plants grown at ambient CO_2 (Table 2). During phase II, the q_N values for plants grown at high CO_2 averaged 45% less than for plants grown at ambient CO_2 , but the values during phase IV were similar (Table 2). High rates of CO_2 fixation result in lower q_N and higher q_p values because the pH gradient across the thylakoid membranes is low due to faster consumption of ATP and NADPH (Guralnick et al., 1992). Thus the q_N and q_p data obtained in this study indicate that the fixation of external CO_2 via the C_3 pathway was significantly greater for pineapple plants grown at elevated CO_2 in phase II but not in phase IV. In other studies with pineapple at day temperatures of $\leq 35^\circ\text{C}$, net CO_2 assimilation via the C_3 pathway occurred mainly in phase IV (Bartholomew, 1982; Cote et al., 1993; Zhu, 1996), and uptake during that phase was significantly enhanced in elevated CO_2 (Zhu, 1996). The lack of any effect of CO_2 level on q_N or q_p in phase IV in our study made it appear likely that CO_2 uptake during this phase was suppressed, possibly by the high day temperature prevailing in this study. The F_v and F_m values at elevated CO_2 were slightly but not significantly greater than those at ambient CO_2 in

phase II and IV (Table 3), indicating that the photochemical efficiency of plants grown at ambient CO_2 (Fig. 1) was significantly greater than that for plants grown at elevated CO_2 . The assimilation of a greater fraction of the CO_2 via the C_3 pathway by plants grown at ambient CO_2 in this study is in contrast to the results obtained in an earlier growth-chamber study with day temperatures of $\leq 35^\circ\text{C}$ (Zhu, 1996). In that study, the Δ values of D-leaf tissue from plants grown in elevated CO_2 were significantly greater than those for plants grown at ambient CO_2 (Zhu, 1996). The Δ values for our study indicate that at an average day temperature near 40°C , the relative contribution of the CAM pathway to total CO_2 uptake, was greater for pineapple grown at elevated than at ambient CO_2 .

The TA values obtained at the end of phases I and IV were significantly greater for plants grown at elevated than at ambient CO_2 , but the differences in the values for phase IV were small (Table 3). The diurnal difference in TA (ΔTA) between phase I and IV, a measure of net CO_2 dark fixation (Griffiths, 1988), also was significantly greater for plants grown at elevated than at ambient CO_2 (Table 2). Thus, the TA data are completely consistent with the data for carbon isotope discrimination (Δ) (Fig. 1). The results of this study are not consistent with those for pineapple grown at lower temperatures (Zhu, 1996), and some other species having CAM, which showed that elevated CO_2 enhances CO_2 fixation primarily via C_3 pathway (Nobel and Hartsock, 1986; Szarek et al., 1987). Nocturnal carbon assimilation mediated by phosphoenolpyruvate carboxylase (PEPCase) was reported to be insensitive to CO_2 enrichment (Bowes, 1991; Winter, 1985) because it was assumed that carboxylation by PEPCase was saturated at ambient CO_2 levels. However, it is clear from this and some other studies (Nowak and Martin, 1995; Zhu, 1996) that phase-I CO_2 assimilation by PEPCase in pineapple leaves was not CO_2 -saturated at ambient CO_2 levels. The enhancement of CO_2 dark fixation by elevated CO_2 could be due to one or more factors: 1) elevated CO_2 might help to offset the effect of low leaf conductance, which could limit the rate of CO_2 transport to the site of fixation, 2) PEPCase in pineapple might not be CO_2 saturated at ambient CO_2 concentrations, or 3) dark respiration could be reduced at elevated CO_2 (Amthor, 1991; Nobel and Israel, 1994).

Osmolarity at the end of phases I and IV were significantly higher at elevated than at ambient CO_2 , with a greater effect for the phase I measurement (Table 3). The greater osmolarity at elevated CO_2 in phase I is attributable primarily to the higher nocturnal organic acid levels (Medina et al., 1991, 1993; Zhu, 1996). The greater osmolarity in phase IV for plants grown at elevated CO_2 may result from a greater accumulation of assimilates, such as soluble sugars, in leaf tissues (Medina et al., 1993). Soluble sugars are a major carbohydrate storage pool in pineapple leaves and reach their maximum level near the end of phase IV (Carnal and Black, 1989).

The percent total N in D leaves was not significantly different between treatments (1.28 in ambient and 1.20 in elevated CO_2), and D-leaf chlorophyll in the same two treatments was 3.83 and $4.03 \mu\text{mol}\cdot\text{m}^{-2}$ (difference also was not significant). The lack of a significant effect of elevated CO_2 on the leaf N and chlorophyll contents in this experiment was in contrast to the results obtained earlier (Zhu, 1996). We attribute the different responses in the two studies to improved nutrient management in this study, as a result of using a specially prepared nutrient solution and more frequent application of nutrients to the plants. Leaf chlorophyll and N contents decreased for *O. ficus-indica* after plants had been grown at elevated CO_2 for ≈ 3 months (Cui and Nobel, 1994). Leaf N content also declined as CO_2 increased for some C_3 plants (Rowland-

Fig. 1. Carbon isotopic discrimination (Δ) of the youngest physiologically mature leaf of pineapple grown at two CO_2 levels in open-top chambers.

Bamford et al., 1991; Tissue et al., 1993; Wong, 1990) and the C_4 species *Amaranthus retroflexus* (Coleman et al., 1991). Most of those reports clearly stated that the nutrients applied were sufficient to prevent N deficiency. Our results indicate that, with careful nutrient management and adequate rooting volume, long-term exposure to elevated CO_2 did not reduce leaf N or chlorophyll contents if complete nutrients were applied twice weekly. Further investigation may be needed to understand the fundamental cause of the decrease in tissue N and chlorophyll contents in response to elevated CO_2 reported in some studies.

Our results show that pineapple growth was enhanced after 4 months of exposure to elevated CO_2 , even with a high day temperature. Furthermore, the Δ and TA data demonstrated unequivocally that this enhancement was mainly the result of greater nocturnal CO_2 fixation. These data and those obtained previously in controlled environments (Zhu, 1996) indicate that even if global warming results in a significant temperature increase in the tropics, dry matter accumulation by pineapple should be significantly enhanced by elevated CO_2 .

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