

INFORMATION TO USERS

This manuscript has been reproduced from the microfilm master. UMI films the text directly from the original or copy submitted. Thus, some thesis and dissertation copies are in typewriter face, while others may be from any type of computer printer.

The quality of this reproduction is dependent upon the quality of the copy submitted. Broken or indistinct print, colored or poor quality illustrations and photographs, print bleedthrough, substandard margins, and improper alignment can adversely affect reproduction.

In the unlikely event that the author did not send UMI a complete manuscript and there are missing pages, these will be noted. Also, if unauthorized copyright material had to be removed, a note will indicate the deletion.

Oversize materials (e.g., maps, drawings, charts) are reproduced by sectioning the original, beginning at the upper left-hand corner and continuing from left to right in equal sections with small overlaps. Each original is also photographed in one exposure and is included in reduced form at the back of the book.

Photographs included in the original manuscript have been reproduced xerographically in this copy. Higher quality 6" x 9" black and white photographic prints are available for any photographs or illustrations appearing in this copy for an additional charge. Contact UMI directly to order.

UMI

A Bell & Howell Information Company
300 North Zeeb Road, Ann Arbor MI 48106-1346 USA
313/761-4700 800/521-0600

PHYSIOLOGICAL RESPONSES OF PINEAPPLE (*ANANAS COMOSUS* (L.) MERR.)
TO CO₂ ENRICHMENT, TEMPERATURES AND WATER DEFICIT

A DISSERTATION SUBMITTED TO THE GRADUATE DIVISION OF THE
UNIVERSITY OF HAWAII IN PARTIAL FULFILLMENT OF THE
REQUIREMENTS FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

IN

AGRONOMY AND SOIL SCIENCE

MAY 1996

By

Jun Zhu

Dissertation Committee:

Duane P. Bartholomew, Chairperson
Guillermo H. Goldstein
Frederick C. Meinzer
James A. Silva
Goro Uehara

UMI Number: 9629870

UMI Microform 9629870
Copyright 1996, by UMI Company. All rights reserved.

**This microform edition is protected against unauthorized
copying under Title 17, United States Code.**

UMI
300 North Zeeb Road
Ann Arbor, MI 48103

ACKNOWLEDGEMENTS

I wish to express my deepest gratitude to my advisors Dr. Duane P. Bartholomew and Dr. Guillermo H. Goldstein for their invaluable guidance, warm encouragement, and tremendous help during my educational career in the USA. This project received a award of NRI Competitive Grants Program/USDA due to their dedicated effort on the proposal, wide knowledge in environmental plant physiology and well-known reputation in this scientific field, which not only made this research possible, but also provided me with financial support. Both my family and myself greatly appreciated their enthusiastic help and support, which will be kept in my heart for forever.

Great thanks are also due to Drs. Frederick C. Meinzer, James A. Silva and Goro Uehara for serving as the members of the dissertation committee, and their valuable help during my graduate program.

I am also grateful to Dr. Elizabeth A. Graser, for her various help and financial support during early part of my graduate program.

Sincere thanks are also due to Dr. Robert Caldwell for his enthusiastic help and encouragement during my graduate program.

I also want to express my great appreciation to all the staff in the Department office for their kind assistance.

Financial support provided by NRI Competitive Grants Program/USDA is gratefully acknowledged.

ABSTRACT

The over goal of this project was to understand the physiological mechanisms by which pineapple (*Ananas comosus* [L.] Merr.), a species having Crassulacean acid metabolism (CAM), will respond to the increase in atmospheric CO₂ and environmental temperatures projected to occur over the next century. The treatments in present study consisted of CO₂ concentrations of near ambient 350 μmol mol⁻¹ and twice ambient (700 μmol mol⁻¹), and day/night temperatures of 30/20, 30/25 and 35/35°C. Experiments were conducted by growing plants in these environments for six months or more and measuring the physiological responses when plants were exposed to the two CO₂ levels, with long-term as plants grown at ambient/elevated CO₂ and measured at ambient/elevated CO₂, and short-term as plants grown at ambient/elevated CO₂ and measured at elevated/ambient CO₂ environment. The specific objectives were designed to 1) study the responses in leaf gas exchange and stomatal conductance to CO₂ and temperatures, 2) quantify the effects of CO₂ and temperatures on biomass accumulation and partitioning, 3) investigate the some physiological responses, including accumulation of organic acids, nitrogen and chlorophyll contents, chlorophyll fluorescence and carboxylating enzyme activities, and 4) examine the effects of prolonged water deficit to leaf water relations, gas exchange and acidification under ambient and elevated CO₂ and three day/night temperatures.

Two major experiments were conducted. In the first experiment, plants were grown at ambient CO₂, while in the second experiment, the responses of plants to

elevated CO₂ was examined. After pineapple was adapted to CO₂ and temperatures in growth chambers for six months, a water deficit was imposed on some of the plants by withholding irrigation for two months to study the effect of water deficit. An additional experiment was conducted in open-top chambers to examine the response of pineapple to ambient and elevated CO₂.

CAM activity of pineapple was intensified at a day/night temperature differential of 10 °C, while the relative contribution of the C₃-type photosynthetic pathway to carbon assimilation was enhanced where the daily temperature range was 5°C and night temperature of 25 °C. Elevated CO₂ enhanced daily CO₂ fixation, but reduced stomatal conductance, thus increasing water use efficiency, and the effect was greatest during light period. Carbon isotopic discrimination data indicated that the relative contribution of C₃ pathway to CO₂ fixation was enhanced by elevated CO₂ at all temperatures. There was a significant temperature by CO₂ interaction on leaf gas exchange. Daytime CO₂ fixation was greatly increased by elevated CO₂, and nocturnal fixation and acidification were also enhanced, which was in contrast to some studies on CAM species. Elevated CO₂ promoted biomass accumulation in pineapple due to increased net assimilation rate, with the greatest effect at smaller daily temperature differential of 5 °C. At twice ambient CO₂, more biomass was partitioned to stem and root, but less to leaf. Elevated CO₂ also enhanced stem and leaf dry matter contents, and increased leaf thickness and rate of surface area expansion. Leaf nitrogen and chlorophyll contents were reduced at elevated CO₂ for plants grown in growth chambers, but there was no such response for plants grown in open-top chambers due

to improved nutrient management. Prolonged drought reduced leaf water content and water potential components, with greater effect on plants grown at night temperature of 25°C. Diurnal gas exchange and stomatal conductance also decreased, and the effect was greater in the light period. Therefore, the reduction in CO₂ fixation due to stomatal closure was greater for plants grown at elevated CO₂, especially, early in the drought. Reduced nocturnal acidification resulted in CAM-idling for plants grown at night temperature of 25°C. The decrease in tissue water content and water potential components were relatively lower in higher CO₂ environment. Lower night temperature also help to sustain relative higher leaf water status and accumulation of organic acids as drought became severe.

TABLE OF CONTENTS

ACKNOWLEDGEMENTS.....	iii
ABSTRACT.....	iv
LIST OF TABLES.....	ix
LIST OF FIGURES.....	xi
CHAPTER 1 INTRODUCTION.....	1
CHAPTER 2 LITERATURE REVIEW.....	5
CHAPTER 3 GAS EXCHANGE OF PINEAPPLE GROWN AT AMBIENT AND ELEVATED CO ₂ AND THREE TEMPERATURE REGIMES.....	16
ABSTRACT.....	16
INTRODUCTION	17
MATERIALS AND METHODS.....	20
RESULTS.....	25
DISCUSSION.....	31
CHAPTER 4 BIOMASS ACCUMULATION AND PHOTOSYNTHETIC CHARACTERISTICS OF PINEAPPLE IN RESPONSES TO ELEVATED CO ₂ AND TEMPERATURES.....	50
ABSTRACT.....	50
INTRODUCTION.....	51
MATERIALS AND METHODS.....	52
RESULTS.....	58

DISCUSSION	63
CHAPTER 5 WATER RELATIONS, GAS EXCHANGE AND ACIDIFICATION	
DURING DROUGHT FOR PINEAPPLE GROWN AT AMBIENT AND	
ELEVATED CO₂ AND THREE TEMPERATURES.....	76
ABSTRACT.....	76
INTRODUCTION.....	77
MATERIALS AND METHODS.....	79
RESULTS.....	83
DISCUSSION	86
CHAPTER 6 PHYSIOLOGICAL RESPONSES TO AMBIENT AND ELEVATED	
CO₂ IN PINEAPPLE GROWN IN OPEN-TOP CHAMBERS.....	100
ABSTRACT.....	100
INTRODUCTION.....	101
MATERIALS AND METHODS.....	103
RESULTS AND DISCUSSION.....	108
CHAPTER 7 SUMMARY.....	118
APPENDIX A INTEGRATED CO₂ UPTAKE BY YOUNGEST	
PHYSIOLOGICALLY MATURE LEAVES OF PINEAPPLE	
GROWN AT AMBIENT AND ELEVATED CO₂ AND THREE	
DAY/NIGHT TEMPERATURES.....	122
REFERENCES.....	125

LIST OF TABLES

<u>Table</u>	<u>Page</u>
3.1. Integrated net CO ₂ uptake over day, night and 24-h periods by the youngest physiologically mature leaves of pineapple grown at two CO ₂ levels and three day/night temperatures.....	40
3.2. Mean ratio of intercellular space CO ₂ (c _i) to ambient CO ₂ (c _a) concentration (c _i /c _a) and mean c _i in the dark (phase I) and afternoon (phase IV) of the youngest physiologically mature leaf of pineapple grown at two CO ₂ levels and three day/night temperature	41
3.3. Mean water use efficiency (WUE) for the youngest physiologically mature leaves of pineapple grown at two CO ₂ levels and three different day/night temperatures.....	42
4.1. Mean values of relative growth rate (RGR), net assimilation rate (NAR) and leaf expansion rate (LER) of pineapple grown six months at two CO ₂ levels and three day/night temperatures	68
4.2. Mean leaf and stem dry matter contents (DMC) of pineapple plants grown six months at two CO ₂ levels and three day/night temperatures.....	69
4.3. Stem weight ratio (SWR), root weight ratio (RWR) and leaf weight ratio (LWR) of pineapple grown six months at two CO ₂ levels and three day/night temperatures	70
5.1. Responses of integrated net CO ₂ uptake and H ₂ O vapor exchange to water deficit during the indicated period for the youngest physiologically mature leaves of pineapple grown at two CO ₂ levels and three day/night temperatures.....	92
6.1. Chlorophyll fluorescence kinetics coefficients of the youngest physiologically mature leaves of pineapple grown at ambient (330 μmol mol ⁻¹) and elevated (730 μmol mol ⁻¹) CO ₂ in open-top chambers.....	115
6.2. Titratable acidity and osmolarity of the youngest physiologically mature leaves of pineapple grown at ambient (330 μmol mol ⁻¹) and elevated (730 μmol mol ⁻¹) CO ₂ in open top chambers.....	116

6.3.	Growth responses to CO ₂ levels for pineapple grown at ambient (330 μmol mol ⁻¹) and elevated (730 μmol mol ⁻¹) CO ₂ in open top chambers	117
6.4.	Chlorophyll and nitrogen contents in the youngest physiologically mature leaves of pineapple grown four months at near ambient (330 μmol mol ⁻¹) and elevated (730 μmol mol ⁻¹) CO ₂ in open top chambers	117

LIST OF FIGURES

<u>Figure</u>	<u>Page</u>
3.1. Net CO ₂ exchange rates of the youngest physiological mature leaves of pineapple grown at two CO ₂ levels and three day/night temperatures.....	43
3.2. Intercellular space CO ₂ concentrations (c _i) of the youngest physiological mature leaves of pineapple grown at two CO ₂ levels and three day/night temperatures.....	44
3.3. Stomatal conductances (g _s) of the youngest physiologically mature leaves of pineapple grown at two CO ₂ levels and three day/night temperatures.....	45
3.4. H ₂ O exchange rates of the youngest physiologically mature leaves of pineapple grown at two CO ₂ levels and three day/night temperatures.....	46
3.5. Sensitivity (β) of total net CO ₂ uptake to an increase in CO ₂ level from 350 to 700 $\mu\text{mol mol}^{-1}$ during the day, night and 24-h periods for the youngest physiologically mature leaves of pineapple grown at three day/night temperatures	47
3.6. Carbon isotopic discrimination (Δ) in the youngest physiologically mature leaves of pineapple grown at two CO ₂ levels and three day/night temperatures	48
3.7. Change in mean carbon isotopic discrimination (Δ) with the mean ratio of night/day net CO ₂ uptake by the youngest physiologically mature leaves of pineapple grown at two CO ₂ levels and three different day/night temperatures	49
4.1. Plant dry weight (W) and leaf area (A) of pineapple during six months of growth at two CO ₂ levels and three day/night temperatures.....	71
4.2. Specific leaf weight (SLW) of pineapple grown at two CO ₂ levels and three day/night temperatures during six months of growth.....	72
4.3. Effect of CO ₂ levels on the titratable acidity (TA) of the youngest physiologically mature leaves of pineapple grown at three day/night temperature during six months of growth.....	73

4.4.	Lead sap osmolality of the youngest physiologically mature leaves of pineapple grown at two CO ₂ levels and three day/night temperature during six months of growth.....	74
4.5.	Chlorophyll content of the youngest physiologically mature leaves of pineapple grown at two CO ₂ levels and three day/night temperatures during six months of growth.....	75
5.1	Relative water content (RWC) of the youngest physiologically mature leaves of pineapple grown at two CO ₂ levels and three day/night temperatures during a 70-day period of drought	93
5.2	Predawn water (ψ_w), osmotic (ψ_π) and turgor (ψ_p) potentials of the youngest physiologically mature leaves of pineapple grown at two CO ₂ levels and three day/night temperatures during a 60-day drought period.....	94
5.3.	CO ₂ exchange rates of the youngest physiologically mature leaves of pineapple grown at two CO ₂ levels and three day/night temperatures, measured on 5, 10, 15, 20 and 30 days after withholding irrigation.....	95
5.4.	Stomatal conductance (g_s) of the youngest physiologically mature leaves of pineapple grown at two CO ₂ levels and three day/night temperatures, measured on 5, 10, 15, 20 and 30 days after withholding irrigation.....	96
5.5.	H ₂ O vapor exchange rates for the youngest physiologically mature leaves of pineapple grown at two CO ₂ levels and three day/night temperatures, measured on 5, 10, 15, 20 and 30 days after withholding irrigation	97
5.6	Titrateable acidity (TA) during a 60-day drought period for the youngest physiologically mature leaves of pineapple grown at two CO ₂ levels and three day/night temperatures	98
5.7	Chlorophyll fluorescence (F_v/F_m) during a 70-day drought period for the youngest physiologically mature leaves of pineapple grown at two CO ₂ levels and three day/night temperatures	99
6.1	Carbon isotopic discrimination (Δ) of the youngest physiological mature leaves of pineapple grown at near ambient (330 $\mu\text{mol mol}^{-1}$) and elevated (730 $\mu\text{mol mol}^{-1}$) CO ₂ in open-top chambers	114

CHAPTER 1

INTRODUCTION

The atmospheric CO₂ concentration has steadily increased since the beginning of industrial revolution (Sarmiento and Bender, 1994), largely reflecting the ever-increasing consumption of fossil fuels. Consequently, air temperature is predicted to increase as result of the so-called greenhouse effect induced by elevated CO₂ concentration (Porter and Carter, 1991). The increased air temperature is expected to affect world agricultural production (Acock and Allen, 1985; Strain and Cure, 1985). Considerable progress has been made in understanding the responses of agronomic crops, specifically the C₃ species, to the climatic change projected to occur in the next century (Acock and Allen, 1985; Strain and Cure, 1985). Only a few studies were found of the physiological responses of CAM species to increasing atmospheric CO₂ (Nobel and Hartsock, 1986; Nobel, 1991; Cui et al., 1993). Despite the fact that it is the most important commercial CAM plant, pineapple has attracted very little attention. No study was found that examined response of the plants to the combined effects of CO₂ and temperature.

The basic goal of this research was to understand the physiological mechanisms by which species having Crassulacean acid metabolism (CAM), namely pineapple (*Ananas comosus* (L.) Merr.), respond to the increased atmospheric CO₂ concentration and environmental temperature projected to occur over the next century. Since water deficit is the most common environmental stress that occurs in the arid

and semi-arid tropics and water status is considered as one of a few environmental factors that play a dominant role in productivity of CAM crops (Nobel, 1991), the impact of soil water deficit will also be examined. This research is expected to provide data that will help to understand how environment influences the growth and productivity of pineapple in the different environments where it is grown. The major objective is to quantify the long- and short-term effects of doubled CO₂ level on the gas exchange, physiological activities and biomass accumulation of pineapple in three temperature regimes. In support of these goals, the following specific objectives have been proposed: 1) measure gas exchange (CO₂ and H₂O, and stomatal conductance) and the changes in ribulosebiphosphate carboxylase/oxygenase (Rubisco) and phosphoenolpyruvate carboxylase (PEPCase) activities in response to ambient and elevated CO₂; 2) examine the change in relative amounts of carbon fixation during the day and at night at varying day/night temperatures that include regimes with temperature elevated above current ambient levels; 3) quantify the effects of CO₂ concentrations and day/night temperature regimes on the relative C₃ photosynthetic activity during the day and biomass accumulation and partitioning, and 4) investigate the effects of soil water deficit on leaf gas exchange, acidification and water potentials at two CO₂ levels and three day/night temperature combinations.

For this study, the basic predications are: 1) elevated atmospheric CO₂ will increase biomass production in pineapple mainly by increasing the amount of daytime CO₂ assimilation via the C₃ photosynthetic pathway, 2) a reduced diurnal temperature fluctuation and the higher night temperatures associated with global warming will

reduce CO₂ dark fixation, and hence increase the relative amount of C₃-type CO₂ fixation, and 3) a higher atmospheric CO₂ concentration and increased air temperatures will result in more negative carbon isotope composition (higher carbon isotope discrimination against ¹³C) as a consequence of increased C₃-type activity and reduced CO₂ dark fixation.

This research will identify some of the physiological regulatory mechanisms that will increase our ability to predict the responses of pineapple to increased atmospheric CO₂, day/night temperature variations and soil water deficit. Diurnal CO₂ fixation and organic acidity measurements were used to investigate the effect of CO₂ and temperature on the fixation of carbon into organic acids in the dark. Growth analysis was needed to evaluate the plant biomass accumulation and partitioning. Analyses of carbon isotope composition and integrated gas exchange were utilized to characterize the relative importance of the CAM and C₃ pathways over the long-term on plant growth and the fraction of the dry matter fixed by the CAM and C₃ pathway. Chlorophyll fluorescence measurements were used to investigate some of C₃-type photosynthetic characteristics during the light period. At a more biochemical level, the activities of Rubisco and PEPCase were assessed.

Insight into all of the above factors will improve our understanding of the physiological regulatory mechanisms of CAM in pineapple, and therefore aid in the prediction of changes in the carbon economy and primary productivity resulting from the projected increases in atmospheric CO₂ level and global temperature. The research will thus benefit crop production in arid and semi-arid regions where

agriculture is more sensitive to climate change because of low rainfall and high evapotranspiration rates. Cultivation of CAM plants with their high water use efficiencies have been increasingly recognized as appropriate in those regions, especially when water for irrigation is not available or salinization caused by previous irrigation becomes so severe that continued irrigation for growing C₃ or C₄ crops becomes unfeasible (Marzola and Bartholomew, 1979; Cortàzar and Nobel, 1990). Increased knowledge of the behavior of pineapple in a CO₂-enriched environment will make it possible to more fully exploit the economic potential of pineapple.

CHAPTER 2

LITERATURE REVIEW

General View

In the span of a few human generations, the life support system of the earth is expected to change more rapidly than it has over any comparable period of human history. Much of this change will be of our own making. In particular, the atmospheric levels of CO₂ have steadily increased from 275 to 350 $\mu\text{mol mol}^{-1}$ over last two centuries, and this 27% increase has occurred since the beginning of the industrial revolution (Sarmiento and Bender, 1994). This increase largely reflects the increased consumption of fossil fuels and other human activities. It is predicted that the CO₂ level will double by the middle of next century (Cure and Acock, 1986).

Carbon dioxide is important not only as one of the raw materials of photosynthesis in green plants, but also as a greenhouse gas that traps infrared radiation, thus warming the earth's surface. Some sates that there has been an increase in mean annual global temperature of approximately 0.3-0.7 °C over last century (Porter and Carter, 1991). The increased temperature is predicted to affect world agricultural production (Acock and Allen, 1985; Strain and Cure, 1985). Besides its projected effects on global climate change, elevated atmospheric CO₂ has important direct effects on the physiology and growth of plants. At elevated atmospheric CO₂ levels, the net CO₂ fixation, biomass accumulation and water use

efficiency of plants having the C₃ photosynthetic pathway will increase (Acock and Allen, 1985; Cure, 1986). The increases for C₄ species expected to be less than for C₃ species (Strain and Cure, 1985; Acock, 1990). Very little work has been done so far on the responses of the plants having Crassulacean acid metabolism (CAM) to elevated CO₂, even though 10% of the earth's vascular plant species have the CAM pathway, and some of them, such as pineapple, are cultivated throughout the tropics and subtropics on a commercial scale.

Pineapple (*Ananas comosus* [L.] Merr.), a crop native to the tropical Americas, is grown commercially over a wide range of latitudes from approximately 30° N in India and the Canary Islands to 33° 55' in the southern hemisphere (Bartholomew and Malézieux, 1994). Total world production of pineapple in 1990 was estimated to be 9,652,000 metric tons with 20 countries producing more than 100,00 MT each (Anon., 1993).

Pineapple apparently is the most important food crop among the few commercial CAM plants cultivated in the tropics. The plant is a succulent, herbaceous, perennial plant with its leaves arranged in a dense rosette pattern (Bartholomew and Malézieux, 1994). There are relatively few stomates per unit leaf area located only on the abaxial side of the leaves (Krauss, 1949). The primary pathway of carbon assimilation in pineapple is via CAM and the leaves have a conspicuous water storage parenchyma (Bartholomew and Kadzimin, 1977) and a chlorenchyma with vacuoles that occupy approximately 85% of the cell volume (Black et al., 1982). The presence of CAM contributes to the high water use efficiency of

pineapple. Depending on growing conditions, and particularly the temperature regime (Neales et al., 1980; Bartholomew, 1982), a substantial amount of CO₂ may be assimilated during the day via the C₃ pathway. The prevailing temperature influences the relative amount of nocturnal and diurnal CO₂ fixation, and this effect is reflected in the wide variation in carbon isotope ratios of leaf samples obtained from plants grown at different temperatures in controlled environments (Bartholomew and Malézieux, 1994). Quantitative data on the effects of solar radiation, water deficit and temperature on the environmental biology of pineapple are relatively sparse (Bartholomew and Malézieux, 1994). CO₂ assimilation by detached pineapple leaves in light and 21% O₂ increased with increasing CO₂ concentration to 540 μmol mol⁻¹ (Moradshahi et al., 1977). No data were found on the combined effects of CO₂ and temperature on carbon assimilation of pineapple.

Over the last two decades, substantial progress has been made in understanding the responses of agricultural crops to elevated atmospheric CO₂ and induced climatic changes. So far, at least 8 important C₃ crops (wheat, barley, rice, soybean, cotton, alfalfa, potato and sweet potato) and 2 major C₄ species (corn and sorghum) have been intensively studied (Acock and Allen, 1985; Cure and Acock, 1986). These studies contribute tremendously to our understanding of the responses of agricultural crops to climate change. Only a few studies, however, have been done so far on the responses of CAM species to increasing CO₂. Those species include important crops in the arid and semi-arid tropics that contribute significantly to the dry matter production and food supply on about one-third of the earth's land area (Nobel and

Cortàzar, 1991). Various species of agaves and cacti, for example, have been used as human food for more than 9000 years, and also have been bred, sold and collected throughout the world as ornamental plants (Nobel, 1993). Pineapple, the most widely cultivated CAM crop, makes a considerable contribution to the economy of many areas of the tropics (Anon., 1990), including Hawaii. Eighteen pineapple varieties were widely cultivated as major local crops in northern South America (Medina et al., 1993). *Opuntia ficus-indica* (prickly pear cactus), another important CAM crop, is cultivated for its fruits in approximately 25 countries and its young cladodes (stem segments) are used as a human food and mature cladodes are used as animal forage or fodder (Cortazar and Nobel; 1990, Nobel, 1993).

Physiology of CAM Plants

Species showing CAM have an inverted pattern of stomatal opening as well as low stomatal conductance, features that are considered to be of adaptive value to plants growing in arid and semi-arid environments. CAM is characterized by a massive diurnal variation in titratable acidity, accounted for mainly by malic acid accumulation in cell vacuoles in the leaves of most CAM plants (Kenyon et al., 1985; Klug and Ting, 1978). During nighttime malate accumulation, carbon is mobilized from a carbohydrate pool, primarily sucrose in pineapple (Borland and Griffiths, 1989; Carnal and Black, 1990), and oxidized via glycolysis to phosphoenolpyruvate (PEP). The PEP is carboxylated via phosphoenolpyruvate carboxylase (PEPCase) to form oxaloacetate (OAA), which is reduced to form malate (Klug and Ting, 1978).

Recently, Borland and Griffiths (1989) reported that citrate can account for a significant fraction of the acidity in pineapple supplied with adequate nitrogen.

The generalized pattern of carbon fixation by CAM plants over 24 hours has been characterized as consisting of four phases (Osmond, 1978). **Phase I** is the nocturnal period of carbon fixation and organic acid synthesis and accumulation. **Phase II** occurs at the beginning of the light period when CO₂ fixation gradually shifts from carboxylation of PEP to carboxylation of ribulosebisphosphate and is characterized by a burst of CO₂ uptake at the onset of illumination. During **Phase III**, photosynthesis occurs when malate is transported from the vacuole, decarboxylated, and the CO₂ thus released is refixed by ribulosebisphosphate carboxylase/oxygenase (Rubisco) (Klug and Ting, 1978). The stomates are closed during this phase when malate is degraded because of a high intercellular space CO₂ concentration. **Phase IV** begins when the malate pool becomes depleted, and CO₂ fixation exceeds the rate of internal CO₂ production. As a result, the intercellular CO₂ partial pressure declines and the stomates open. Carbon assimilation during **Phase IV** is primarily by Rubisco via the C₃ pathway, although recent evidence indicates that PEPCase may become active towards the end of the phase (Cote et al., 1989; Winter, 1985; Griffiths, et al., 1990). The bulk of carbon reduction to carbohydrate occurs during **Phase III** and **IV** (Winter, 1985). Carbon fixation in the four phases is closely related with irradiance and temperature in **Phase III** and **IV** (light period) which affect carbohydrate accumulation and thus the availability of substrate for acid synthesis in **Phase I** (dark period) (Kaplan et al., 1976).

Effects of Elevated CO₂ and Induced Temperature Changes on CAM Plants

The carboxylation of PEP approaches saturation at present atmospheric CO₂ levels and CAM plants have a finite capacity to fix CO₂ into organic acids at night (Ting, 1993), so elevated CO₂ values are not expected to significantly affect CO₂ assimilation during phase I. The reaction of CO₂ with Rubisco is not saturated at ambient CO₂ levels. This suggests that elevated atmospheric CO₂ will increase CO₂ assimilation of CAM species primarily during Phase IV. When ambient CO₂ was increased from 350 to 650 $\mu\text{mol mol}^{-1}$, net CO₂ fixation of the leaf succulent *Agave deserti* and the stem succulent *Ferocactus acanthodes* were increased by 30% during the light period with little effect at night (Nobel and Hartsock, 1986). A few studies indicated that elevated CO₂ concentrations increased dry matter production by CAM plants (Idso et al., 1986; Black, 1986; Nobel and Hartsock, 1986). In arid and semiarid climates, growth of *Agave vilmoriniana* was enhanced under CO₂ enrichment (Idso et al., 1986). Increasing CO₂ from 350 to 650 $\mu\text{L L}^{-1}$ over one year enhanced day matter accumulation by about 30% in *A. deserti* and *F. acanthodes* (Nobel and Hartsock, 1986). At elevated CO₂ levels, net CO₂ uptake, water use efficiency and biomass production of *Opuntia ficus-indica* grown in open-top chambers were significantly increased during 23-week period (Cui et al., 1993).

The activities of the carboxylating enzymes Rubisco and PEPcase decreased after long-term exposure to elevated CO₂ (Stitt, 1991; Arp, 1991; Rowland-Bamford et al., 1991; Hogan et al., 1991). Similar results were obtained from the CAM species *Opuntia ficus-indica* (Cui and Nobel, 1993; Israel and Nobel, 1994). At a

doubled CO₂ concentration, nitrogen content in cotton leaves was lowered (Wong, 1990), and both leaf nitrogen and chlorophyll contents declined at elevated CO₂ in *Opuntia ficus-indica* (Cui and Nobel, 1993). Those phenomenon may relate to the so-called 'non-adaptive' or 'adaptive' responses in photosynthetic capacity to elevated CO₂ (Stitt, 1991). A non-adaptive response was viewed as a direct inhibition due to the accumulation of starch or sucrose at higher ambient CO₂ level, which minimized or prevented further accumulation of carbohydrate through 1) physical disruption of the chloroplast due to large starch grains, and 2) an increase the length of the diffusion path for CO₂ in the chloroplast (Arp, 1991; Stitt, 1991). The adaptive response was considered to be an indirect inhibition of some of physiological processes (Arp, 1991; Stitt, 1991), which readjusts the sink-source balance by allowing nitrogen and other components to be re-mobilized from the leaves and invested in sink growth. For example, decrease in protein content may lead to a reduction in Rubisco activity (Rowland-Bamford et al., 1991). In some studies, the photosynthetic capacity of C₃ plants increased at elevated CO₂ (Long, 1991; Mott, 1991), while others reported that the capacity was reduced (Wong, 1990; Cui and Nobel, 1993; Israel and Nobel, 1994). Detailed biochemical and physiological studies are therefore needed to clarify those contradictions.

Increased temperature due to accumulation of greenhouse gases are predicted to increase evaporation and absolute humidity. This, in turn, will increase nighttime temperature and decrease the diurnal temperature variation as a result of increased cloudiness (Taylor and MacCracken, 1990). The temperature change induced by CO₂

and other trace gases could increase the duration of phase IV of CAM plants, and thus intensify C₃ fixation relative to CAM fixation. Pineapple, for example, shows more C₃-type fixation when the diurnal temperature variation is small and dark temperatures are high (Bartholomew and Kadzimin, 1977; Bartholomew, 1982; Neales et al., 1980). As the day/night temperature differential increases and the night temperature decreases, dark acidification and nighttime CO₂ fixation approach their maximum rates.

Most pineapple is grown in or near coastal or island areas where the climates tend to be more oceanic than continental, and temperature and humidity extremes are less severe than they would be for a continental climate at a comparable latitude (Bartholomew and Kadzimin 1977). A specific feature of the coastal and island areas is a small differential between maximum and minimum temperature, though there are still relatively large temporal and spatial variations in temperature due to local variations in elevation, slope and aspect. Also, there are quite large differences in mean annual temperature for the areas where pineapple is grown on a commercial scale (Bartholomew and Kadzimin, 1977). Even though few data are available on the effects of temperature on pineapple growth and development, temperature appears to be one of the most important environmental factors determining pineapple distribution and productivity in the world (Bartholomew and Malézieux, 1994).

Irradiance primarily affects the quantity of CO₂ assimilated by pineapple while temperature and its range affects both total assimilation and the proportion fixed in the dark (Bartholomew and Malézieux, 1994). Pineapple will not tolerate temperatures

near 0 °C for prolonged periods. High day and low night temperatures favor acid accumulation and CAM metabolism, and small diurnal temperature variations and a high nocturnal temperature result in more C₃-type carbon fixation in pineapple (Bartholomew, 1982). Leaf conductance of pineapple, by day and by night, was strongly influenced by ambient temperature, with cool conditions favoring stomatal opening (Neales et al., 1980). CAM activity and total carbon assimilation approaches its maximum at a day/night temperature of about 30/22-24 °C (Bartholomew, 1982). The optimum day/night temperature for pineapple was considered to be close to 30/20 °C, with an optimum mean temperature around 23 to 24 °C (Neild and Boshell 1976).

An important effect of increased temperature could be stomatal closure because higher temperature could increase leaf photo and dark respirations, thus elevating the intercellular space CO₂ concentration. Increased leaf temperature may raise the vapor pressure gradient between the leaves and the ambient air, thereby resulting in stomatal closure (Choudhury and Monteith, 1986; Schulze, 1986). Although no data are known to exist for pineapple, the stomata of some CAM plants do not seem to be as sensitive to CO₂ at night as they are during the day (Ting, 1993).

Carbon Stable Isotopes of CAM

Measurement of the stable carbon isotopic composition is a powerful technique to distinguish between plants having the C₃ and C₄ photosynthetic pathways (Smith and Epstein, 1971). Plants fixing CO₂ by the CAM pathway often have $\Delta^{13}\text{C}$ values intermediate between C₃ and C₄ plants (Bender et al., 1973; Bartholomew and

Malézieux, 1994). Farquhar et al. (1982) proposed that variation in $\delta^{13}\text{C}$ should be dependent upon the ratio of intercellular CO_2 concentrations (c_i) to ambient CO_2 concentrations (c_a) as described by the equation:

$$\delta^{13}\text{C}_{\text{leaf}} = \delta^{13}\text{C}_{\text{air}} - a - (b-a)c_i/c_a$$

where a is the biophysical discrimination factor arising from differential diffusion of ^{13}C over ^{12}C (4.4 ‰) into the leaf, and b is the biochemical discrimination factor due to Rubisco activity (27 ‰). The primary carboxylase of C_4 photosynthesis, PEPCase, discriminates much less strongly against ^{13}C (Reibach and Benedict, 1977). The resulting effect is that the isotopic signature of plants utilizing the C_3 pathway for carbon fixation will differ from those utilizing PEPCase as the primary CO_2 fixing enzyme. The carbon isotope ratio of plants can vary from -7 to -35 ‰ with values for C_4 plants ranging from -7 to -15 ‰, and values for C_3 plants range from -20 to -35 ‰ (Ehleringer and Osmond, 1989; Ting, 1985). In CAM plants, the proportion of carbon uptake in the dark (by PEPCase) or in the light (by Rubisco) is directly indicated by the variation in carbon isotopic composition (Bender et al., 1973; Osmond, et al., 1973). If growing condition alter the relative proportion of nocturnal and diurnal CO_2 assimilation of CAM plants, the isotopic composition of the leaf will change. The isotopic composition of CAM plants ranges from -10 to -20 ‰ (Ehleringer and Osmond, 1989; Ting, 1985). The isotopic composition of pineapple leaves ranged from -11 ‰ to about -19 ‰, depending largely on the day/night temperature regimes, particularly nocturnal temperatures (Bartholomew and Malézieux, 1994). The more negative carbon isotopic composition corresponded to

higher night temperatures, thereby suggesting that high night temperature enhanced C₃-type carbon assimilation during phase IV. It is clear that carbon isotope ratios are a powerful tool to understand the long-term change in the relative proportion of C₃ vs C₄ fixation in CAM plants brought about by variations in CO₂ levels and temperatures.

CHAPTER 3

GAS EXCHANGE OF PINEAPPLE GROWN AT AMBIENT AND ELEVATED CO₂ AND THREE TEMPERATURE REGIMES

ABSTRACT

Pineapple (*Ananas comosus* (L.) Merr.), a species having crassulacean acid metabolism (CAM), was grown in controlled environment chambers for six months at day/night temperatures of 35/25, 30/25 and 30/20°C, and near ambient (350 μmol mol⁻¹) and elevated CO₂ (700 μmol mol⁻¹) levels. The effects of CO₂ and temperatures on diurnal change in stomatal conductance, CO₂ and H₂O exchange rates, and water use efficiency (WUE) were studied. Stable carbon isotopic discrimination was analyzed to identify the relative contributions of the C₃ and CAM pathway to total carbon accumulation. Gas exchange of the youngest physiologically mature leaf was measured at low or high CO₂ for 24 hours every six weeks for 24 weeks. Gas exchange was also measured on leaves of plants grown at low or high CO₂ and exposed for 24 hours to the opposite CO₂ level to determine the effect of short-term exposure to CO₂ levels. Total daily CO₂ uptake was 306 and 352 μmol m⁻² day⁻¹, respectively at low and high CO₂ for plants grown at 30/20 °C, while the uptake was 187 and 343 μmol m⁻² day⁻¹ at low and high CO₂ in 35/25 °C regime, and 175 and 346 μmol m⁻² day⁻¹ at low and high CO₂ in 30/25 °C regime. Therefore, effect of CO₂ enrichment was greater for plants grown at 35/25 and 30/25 °C. Doubling of the CO₂ concentration increased daytime CO₂ uptake by 4.6, 2.3 and 2.0 times, respectively,

at 35/25, 30/25 and 30/20°C, but only enhanced nocturnal CO₂ uptake by about 44% at 35/25°C and 80% at 30/25°C, resulting in a significant CO₂ by temperature interaction on total daily CO₂ uptake. Stomatal conductance and H₂O vapor exchange rate were lower, and WUE was higher for plants grown at high CO₂. The intercellular space CO₂ concentration (c_i) was about 100 μmol mol⁻¹ in late afternoon and ranged from 170 to 365 μmol mol⁻¹ in the night at both low and high CO₂. Carbon isotopic discrimination of leaf tissue increased by 1.84‰ at 35/25°C, 1.56‰ at 30/25 °C and 0.87‰ at 30/20°C as CO₂ was doubled. Both the CO₂ uptake and carbon isotopic discrimination data indicated that 5 °C day/night temperature range decreased CAM activity, while a 10 °C diurnal temperature differential increased the actual and relative contribution via CAM pathway.

INTRODUCTION

Pineapple is a species having Crassulacean acid metabolism (CAM). The diel cycle of CO₂ fixation in CAM plants is characterized by four phases (Osmond, 1978). Phase I is a nocturnal period of CO₂ assimilation catalyzed by the enzyme phosphoenolpyruvate (PEP) carboxylase (PEPCase), with malic acid being the primary product accumulated in cell vacuoles. Phase II occurs at the beginning of the light period when there is a transition from carboxylation of PEP by PEPCase to carboxylation of ribulosebisphosphate (RuBp) by ribulosebisphosphate carboxylase/oxygenase (Rubisco). This phase is marked by a rapid increase in CO₂ influx upon illumination. During phase III, the stomata are closed, malic acid is

transported from the cell vacuoles, decarboxylated, and the CO₂ thus released is assimilated by Rubisco into carbohydrates. Phase IV occurs late in the light period and is typical of C₃-type photosynthesis with direct CO₂ fixation via Rubisco. Carbon assimilation during phase III and IV is almost exclusively via the C₃ photosynthetic pathway. Some evidence indicates that PEPCase becomes active towards the end of phase IV when malate reaches a minimum level (Cote et al., 1989; Winter, 1985; Griffiths et al., 1990).

Carboxylation of PEP approaches saturation at ambient CO₂, while the carboxylation of RuBP is not saturated at these levels (Ting, 1993). In some CAM species the capacity to utilize elevated atmospheric CO₂ is limited during phase I and enhancement occurs primarily during Phase IV (Nobel and Hartsock, 1986; Cui, et al., 1993). But enhanced CO₂ uptake during phase I was also observed for some CAM species grown at elevated CO₂ (Winter, 1985; Nobel and Israel, 1994; Raveh et al., 1995). Short-term CO₂ fixation by pineapple was increased at elevated CO₂ during phase IV (Cote et al., 1992), but the long-term impact on diurnal carbon assimilation was not studied.

Temperature is one of the most important environmental factors influencing CO₂ fixation and water use efficiency of pineapple (Bartholomew and Malézieux, 1994). As atmospheric CO₂ concentration increases, global circulation models (GCMs) predict that temperature will increase concurrently (White, 1990). In pineapple, warm night temperature (about 25°C) not only decreases total daily carbon assimilation, but also reduces the relative amount of fixation during phase I and IV of

diel CO₂ uptake (Bartholomew and Kadzimin, 1977; Neales et al., 1980; Bartholomew, 1982). The interactive effects of CO₂ and temperature are also of considerable interest (Hogan et al., 1991; Eamus, 1991), because CO₂ and temperatures are expected to increase concomitantly under most global climate change scenarios. Their combined influence on pineapple physiology is expected to differ from their separate effects. No reports were found on the interactive effects of CO₂ levels and temperatures on gas exchange rates for any tropical plants (Hogan et al., 1991).

Stable carbon isotopic composition ($\delta^{13}\text{C}$) or discrimination (Δ) estimates the relative intensity of C₃ and CAM photosynthetic pathways in CAM species (Ehleringer and Osmond, 1989; Griffiths, 1992). A lower Δ value indicates that tissues are enriched in ¹³C, reflecting a greater relative contribution by CAM to total CO₂ fixation, while larger Δ values indicate that the relative contribution of C₃-type photosynthetic activity is greater (Ehleringer and Osmond, 1989; Griffiths, 1992). Environmental temperature regulates the relative intensities of C₃ and CAM photosynthetic activities in the daily cycle of CO₂ assimilation, and thus alters isotopic discrimination values (Holtum, 1983; Griffiths, 1992; Bartholomew and Malézieux, 1994). Analysis of carbon isotopic discrimination in pineapple, therefore, reflects the relative contribution of the C₃ and CAM pathways to total carbon gain.

Pineapple is a major crop grown in the tropics and subtropics and is the most important food crop among the few CAM plants of commercial importance. Knowledge of the effects of elevated CO₂ and temperature on pineapple will enhance

our knowledge of the behavior of this CAM species in a CO₂-enriched environment, expand our understanding of how the crop will respond to global climate change and make it possible to more fully exploit its economic potential. The objectives of the study were to examine the short- and long-term responses of pineapple to ambient and elevated CO₂ levels in three temperature regimes by: 1) determining the diurnal oscillation of CO₂ and H₂O vapor fluxes, stomatal conductance and WUE, 2) comparing integrated CO₂ fixation and mean WUE between ambient and elevated CO₂ at different temperatures, and 3) examining the interactive effects of CO₂ and temperature on gas exchange.

MATERIALS and METHODS

Treatment Design and Environmental Conditions

The effects of CO₂ levels of 355 ± 20 (low) and $710 \pm 50 \mu\text{mol mol}^{-1}$ (high) CO₂ and day/night temperatures of 30/20, 30/25 and 35/25 °C on the growth of pineapple were studied in controlled environment chambers. The photosynthetic photon flux density in the growth chambers was maintained at about $400 \mu\text{mol m}^{-2} \text{s}^{-1}$ at the mid-plant height during a 12 h photoperiod. Humidity in the chambers was not controlled.

Because only three chambers were available, the effects of temperature on pineapple were studied at low CO₂ from November, 1993 to May, 1994, followed by the study at high CO₂, which was begun in August 1994 and ended in February, 1995. The temperature treatments were based on the changes that might be expected to occur at Wahiawa, Hawaii, USA, one of the better pineapple producing areas of the

world. The mean monthly temperature at Wahiawa ranges from 20.1 °C in February to 24.3 °C in August (data for 1953-1962). February temperatures are too cool for optimum growth so fruit tops (crowns) are planted through black plastic mulch to take advantage of the increased soil temperature under the mulch (Ekern, 1969). Thus, 30/20 °C was considered to be near the optimum for pineapple growth and was used as the control in the current study.

In both experiments, pineapple crowns from the Smooth Cayenne clone Champaka 153 were obtained from a local pineapple company and grown for three months in ~4 liter pots containing a 1:1 (V:V) mixture of Sunshine #4 (a commercial potting media) and horticultural perlite. The plants were watered once every five days to keep the media moist, and fertilized once every two weeks with a dilute nutrient solution containing 0.4% (w/v) urea and 0.5% (w/v) Gaviota Foliar 62 (Brewer Environmental Industries, Honolulu), a commercial soluble fertilizer containing 12% N, 24% P₂O₅, 24% K₂O, 0.04% Mg, 0.10% Fe, 0.013% Cu, 0.01% B, 0.02% Mo, 0.012% Mn, 0.014% Zn and 0.0005% Vitamin B1.

After the initial 3-month establishment period, about 22 plants were placed in each of the controlled environment chambers. The low CO₂ level was maintained at near the ambient value by adjusting the rate of air exchange between the growth chambers and the outside. The high CO₂ level was established by bleeding pure CO₂ from a gas cylinder into the growth chambers. The CO₂ concentration was established by adjusting the flow rate of the pure CO₂ into the chambers and the rate of air exchange between the growth chambers and the outside. The CO₂

concentration in the chambers was measured frequently with a Li-Cor 6262 gas analyzer (Li-Cor, Inc., Lincoln, NB) until the desired set point was established. At six months after planting, all plants were repotted into ~8 L pots for the remainder of the experiment to minimize the effects of limited rooting volume on photosynthesis and growth.

Gas Exchange Measurements

Gas exchange measurements were made on the youngest fully expanded and the tallest leaf on the plant from the ground level, termed the 'D' leaf in the literature (Bartholomew and Kadzimin, 1977). The 'D' leaf has been used to index plant nutrient levels and evaluate the effects of environmental factors on plant water status and growth of pineapple (Bartholomew and Kadzimin, 1977). Diurnal gas exchange data were collected on 'D' leaves about once every six weeks during the six-month period. Near the end of each study, 'D' leaves of plants grown at low or high CO₂ were exposed to the opposite CO₂ levels for 24 h and gas exchange data were collected.

Diurnal CO₂ and H₂O vapor exchanges were measured using a gas exchange system consisting of a CO₂ and H₂O IR gas analyzer (Li-6262, Li-Cor, Inc., Lincoln, Nebraska, USA), a dew point generator (Li-610, Li-Cor, Inc.), and a 1.57-liter cuvette specifically designed to fit the pineapple leaf. The cuvette had a recirculating fan and water jacket to control leaf temperature using a temperature-controlled water bath. A datalogger (21X, Campbell Scientific Inc., Logan, Utah, USA) was used to

record data and control the operation of the gas exchange system, via solid state relays and solenoid valves. The diurnal cycle of gas exchange was monitored with a 5-minute averaging interval over 24-h periods and the measurements were made at approximately 1.5, 3, 4.5 and 5.5 months after planting. At each measurement data were collected from one 'D' leaf per plant in each treatment. The CO₂ source for gas exchange measurements at low CO₂ was the ambient air pumped from outside the building through a 35 L bottle, which served to stabilize fluctuations in the atmospheric CO₂ concentration. The elevated CO₂ source was from a compressed air cylinder containing 700 μmol mol⁻¹ CO₂ (balance air) for short-term enrichment, and ambient air plus pure CO₂ to average 710 ± 50 μmol mol⁻¹ CO₂ for the long-term treatments.

Carbon Isotope Discrimination

Leaf carbon isotope discrimination was determined on a subsample of a whole 'D' leaf that had been oven dried at 70°C and finely-ground. The carbon isotopic composition of the source air was also measured periodically by collecting air samples in the growth chambers in 0.5-liter glass ampules that were flame-sealed. The natural abundance ¹³C/¹²C ratios were measured on a SIRA Series II isotope ratio mass spectrometer (VG Isotech, Middlewich, UK) at the Duke University Phytotron. The isotopic discrimination (Δ) was calculated from stable isotope composition (δ) as

$$\Delta = \frac{\delta_a - \delta_p}{1 + \delta_p} \quad (3.1)$$

where δ_a is the isotopic composition of air and δ_p is that of the leaves.

Data Analysis

Instantaneous net CO₂ and H₂O exchanges, stomatal conductance (g_s) and intercellular CO₂ concentration (c_i) were calculated according to Long and Hallgren (1985) and Christopher et al. (1989). Instantaneous water use efficiency was calculated as the ratio of CO₂ assimilated to H₂O vapor transpired (Jones, 1992). Total net CO₂ uptake in light, dark and 24 hours were calculated by integrating the 5 minute average values over those periods. Mean WUE was calculated by averaging values over light, dark and 24-hour periods. Since there were no trends in rates of CO₂ fixation with increasing age, all the values calculated were from the data collected at about 3 months in growth chambers when plants were assumed to fully adapt to treatments. Sensitivity of carbon fixation to CO₂ (β) was calculated using a formula modified from Kirschbaum (1994):

$$\beta = \frac{\Delta A / A_m}{\Delta c / c_m} \quad (3.2)$$

where A is the daily mean net CO₂ uptake and c is the CO₂ concentration in air. $\Delta A = A_{700} - A_{350}$ and $\Delta c = c_{700} - c_{350}$, $A_m = (A_{700} + A_{350})/2$, and $c_m = (c_{700} + c_{350})/2$. The subscripts 350 and 700 refer to low and high CO₂ levels. The CO₂ and WUE data for the day, night and 24-h period were analyzed by two-way ANOVA and least significant difference (LSD) by standard methods (Little and Hill, 1977).

RESULTS

Diel Cycle of Leaf Gas Exchange Rates

Although gas exchange data were collected several times over the six-month period, there were no consistent changes in leaf gas exchange associated with growth. Therefore, representative curves for a single 24-h period are presented. Net CO₂ uptake occurred predominately during phase I and phase IV in all treatments. CO₂ uptake in the light reached a maximum within 120 to 150 minutes after phase IV started, and in most case declined thereafter. CO₂ uptake in phase I was greater than any other phase and approached a peak 70 to 90 minutes after the onset of phase I in all treatments (Fig. 3.1). Nocturnal CO₂ uptake decreased steeply after the peak was reached, after which the rates of uptake remained relatively constant rate until the end of dark period for plants grown at 35/25 and 30/25 °C, or decreased steadily towards the end of the dark period for plants at 30/20 °C (Fig. 3.1). Phase II was characterized by short marked increase in CO₂ fixation immediately after the onset of the light period, with greater response for plants grown at high CO₂ (Fig. 3.1). Phase II was followed by a 'quiescent period' designated as phase III (Osmond, 1978), during which stomata were basically closed and measurable net CO₂ uptake was absent (Fig. 3.1).

Intercellular space CO₂ concentration (c_i) was on average 2 to 3-fold greater in phase I than in phase IV, and remained relatively constant over the entire dark period (Fig. 3.2). Values of c_i early in phase IV were very high and dropped rapidly as CO₂ assimilation from external environmental CO₂ increased. Late in phase IV, c_i

gradually decreased towards the end of phase IV for plants grown at 35/25 and 30/20 °C, or remained relatively constant at 30/25 °C (Fig. 3.2).

The diurnal course of stomatal conductance (g_s) basically paralleled that of net CO₂ uptake rate, being lower in the light and much greater in the dark (Fig. 3.3). The g_s values were generally 2 to 3-fold greater for plants grown at low CO₂ than for plants at high CO₂ in the three temperature regimes, with a greater effect occurring during the early part of phase I (Fig. 3.3). The decrease in g_s at high CO₂ in phase I was not as great at 30/20 °C as it was at the other two temperatures. H₂O vapor exchange rates (E) paralleled g_s and were lower during the 24 h period for plants grown at high than at low CO₂ (Fig. 3.4). The E values in phase IV were greater than might be expected from the lower g_s values during that phase (Fig. 3.4).

Integrated Gas Exchange, Mean c_i and c_i/c_a Ratios

As mentioned previously, CO₂ uptake by pineapple 'D' leaves was measured during the six-month growth period. The data were integrated for the day, night and 24-h periods, and combined to illustrate the effect of treatments on the CO₂ assimilation by pineapple (Table 3.1). For plants grown at low CO₂, CO₂ uptake in the light was significantly lower at 35/25°C than in the 30/25 and 30/20 °C treatments, which were not different (Table 3.1). Nocturnal CO₂ uptake was significantly greater at 30/20 °C than at 30/25 and 35/25 °C and the latter rates were not different (Table 3.1). Therefore, the total daily assimilation over the 24-h period was also significantly higher at 30/20°C, and then at 30/25 or 35/25 °C, primarily as

a result of higher nocturnal CO₂ uptake. The percentage of CO₂ uptake in the dark relative to the total daily value was 87.4 and 81.4 at 35/25 and 30/20 °C, and 68.6 at 30/25 °C for plants grown at low CO₂. For plants grown at high CO₂, CO₂ uptake in the light, dark and 24-h periods was similar at all three temperatures (Table 3.1). CO₂ fixation in the dark was about 68% of the total value for the 24-h period at 35/25 and 30/20 °C, and about 63% at 30/25 °C for plants grown at high CO₂.

CO₂ uptake in the light at high CO₂ was 4.6, 2.3 and 2.0 times greater than at low CO₂, respectively, at 35/25, 30/25 and 30/20°C ($p < 0.01$), with no CO₂ by temperature interaction (ANOVA not shown). Nocturnal CO₂ uptake was significantly greater at high CO₂ with a 44% increase at 35/25°C and an 80% increase at 30/25°C ($p < 0.05$). There was no CO₂ enhancement at 30/20 °C, resulting in a significant CO₂-temperature interaction on nocturnal CO₂ uptake ($p < 0.01$; ANOVA not shown). The enhancement in CO₂ uptake by elevated CO₂ over the 24-h period was 84% at 35/25 °C, 97% at 30/25 °C and 15% in 30/20 °C. Relative to the low CO₂ by temperature treatments, a simultaneous increase in CO₂ level and temperature from 30/20 to 35/25°C or 30/25°C enhanced CO₂ uptake in the light by 1.9 and 2.3 times ($p < 0.001$), respectively, but reduced nocturnal CO₂ uptake by 5.5 ($p < 0.05$) and 13.1% ($p < 0.01$) (Table 1). Thus, the average enhancement in CO₂ uptake over 24-h was about 12.5% ($p < 0.01$) when both CO₂ and temperature were increased.

The short term response to both low and high CO₂ were very consistent among the three temperature regimes (Table 3.1). Plants grown at high CO₂ and measured at low CO₂ had higher CO₂ uptake than plants grown and measured at low CO₂,

particularly for plants grown at 35/25 and 30/25 °C (Table 3.1). Plants grown at low CO₂ and measured at high CO₂ had higher CO₂ uptake in the light, dark and 24-h period than plants measured and grown at low CO₂ (Table 3.1). Plants grown and measured at high CO₂ had higher CO₂ assimilation than did plants grown at the low CO₂ whether measured at high or low CO₂, indicating there was no decrease in the rate of CO₂ assimilation for pineapple due to long-term acclimation to elevated CO₂.

The sensitivity of net CO₂ uptake to CO₂ levels (β) characterizes the enhancement in CO₂ assimilation due to the increase in CO₂ concentration. The higher the sensitivity, the greater the enhancement in CO₂ assimilation by elevated CO₂. A value of 1.0 indicates doubling the CO₂ assimilation as environmental CO₂ levels were doubled, while a value of zero indicates that the CO₂ assimilation is not affected by an increase in CO₂ concentration. The value of β was greater in the light than in the dark period (Fig. 3.5). Daytime sensitivity was greatest at 35/25 °C, and nocturnal sensitivity was greatest at 35/25 and 30/25 °C, with near zero nocturnal sensitivity at 30/20 °C (Fig. 3.5). Therefore, the sensitivities over 24-h were much greater for plants grown at 35/25 and 30/25°C (Fig. 3.5).

For plants grown at low CO₂, the mean c_i/c_a ratios in phase IV were basically not affected by temperature treatments, while the values in phase I were 2-3 times higher and more variable than those in phase IV (Table 3.2). The greatest c_i/c_a in phase I occurred at 30/20 °C and smallest value occurred at 30/25 °C. At high CO₂, the mean c_i/c_a in phase IV was greatest at 30/20 °C and smallest at 30/25°C. The values in phase I responded to temperature in a manner similar to that for plants

grown at low CO₂ (Table 3.2). The c_i/c_a values were lower in high than in low CO₂ in both phases IV and I, with greater differences observed at 35/25 and 30/25 °C (Table 3.2).

Intercellular space CO₂ concentration (c_i) was approximately 200 $\mu\text{mol mol}^{-1}$ in phase I and 100 $\mu\text{mol mol}^{-1}$ in phase IV and for plants grown at low CO₂ (Table 3.2). At high CO₂, c_i was highest in phase I and phase IV at 30/20°C, while the values in the other temperature regimes were greater in phase I and similar in phase IV compared to those at low CO₂ (Table 3.2). The temperature response of c_i was basically similar with the that of c_i/c_a ratio (Table 3.2).

Carbon Isotopic Discrimination

For plants grown at low CO₂, the carbon isotopic discrimination (Δ) of 'D' leaf tissues was significantly greater at 30/25 °C than at 35/25 and 30/20 °C ($p < 0.01$) and the latter two values were not different (Fig. 3.6). At high CO₂, the Δ value at 30/25 °C was also significantly greater than the values at 35/25 and 30/20 °C ($p < 0.001$), while the Δ values for the 35/25 and 30/20 °C treatments were not different. Elevated CO₂ significantly increased the Δ values ($p < 0.001$) by 0.87‰ at 30/20 °C, 1.56‰ at 30/25°C and 1.84‰ at 35/25°C. Plants grown in an environment with a daily temperature range of 5°C had the highest Δ , while plants grown at a day/night differential of 10°C had relatively lower Δ values (Fig. 3.6).

Carbon isotopic discrimination tended to decrease as the ratio of night/day CO₂ uptake increased (Fig. 3.7), indicating that discrimination against the heavier ¹³C

increased as the relative contribution to total CO₂ uptake via C₃ pathway increased. At low CO₂, a greater change in night/day CO₂ uptake ratio resulted in a relatively small change in Δ values, while at high CO₂, a slight increase in the ratio resulted in a large decrease in Δ values (Fig. 3.7).

Mean Water Use Efficiency

Mean WUE calculated from net CO₂ and H₂O vapor exchange data of the 'D' leaves was higher in the dark and lower in the light (Table 3.3). At low CO₂, daytime WUE was significantly lower at 35/25 °C than at 30/25 and 30/20 °C, while nocturnal WUE was significantly greater at 30/20 °C than at 35/25 and 30/25 °C, resulting in the highest daily WUE at 30/20 °C, while values at 35/25 and 30/25 °C were comparable (Table 3.3). At high CO₂, the effects of temperature on WUE were consistent with those at low CO₂, but the values were greater than those observed at low CO₂, especially in the light (Table 3.3). A simultaneous increase in CO₂ level and temperature from 30/20 to 35/25 °C, and 30/20 °C to 30/25 °C significantly enhanced daytime WUE by 0.81 and 1.32 times, respectively. WUE in the dark decreased slightly with a simultaneous increase in CO₂ and temperature, so daily mean WUE was only increased by 6.3% at 35/25 °C and by 15.8% at 30/25 °C (Table 3.3).

Short-term response in WUE to CO₂ levels was consistent among the three temperature regimes. Plants grown at high CO₂ but measured at low CO₂ for 24 hours had similar daytime WUE, but lower nocturnal and total daily values compared to that for plants grown and measured at low CO₂ (Table 3.3). Plants grown at low CO₂ but

measured at high CO₂ for 24 hours exhibited much greater WUE in the light, dark and 24-h period than for plants grown and measured at both low and high CO₂ (Table 3.3).

DISCUSSION

The diel rhythms of CO₂ exchange rate in pineapple observed in this study were consistent with the results of previous studies for pineapple reviewed by Bartholomew and Malézieux (1994). For plants grown at ambient CO₂, maximum CO₂ uptake rates in the dark were about 2.5-fold greater than the values observed by Bartholomew (1982) and approximately 2 μmol m⁻² s⁻¹ greater than those obtained by Cote et al. (1992), while the maximum rates in the light were about 1.5 μmol m⁻² s⁻¹ greater than those observed by Bartholomew (1982) and comparable with the values obtained by Cote et al. (1992). The difference in maximum CO₂ uptake rates observed in those studies may be attributable to different day/night temperature regimes and photoperiod under which pineapple was grown, or growth conditions. No detailed data on CO₂ uptake rates were found for pineapple grown long-term at a doubled CO₂ level.

The diurnal oscillation in net CO₂ uptake rate for pineapple exhibited the four basic phases common to CAM species as described by Osmond (1978). Increasing the ambient CO₂ level from 350 to 700 μmol mol⁻¹ and altering day/night temperatures

had little effect on the basic pattern of leaf gas exchange. The sharp peak of net CO₂ uptake during phase II presumably reflected the combination of continued CO₂ uptake via the CAM pathway and direct fixation of external CO₂ via the C₃ pathway (Kluge, et al., 1982; Winter, 1985; Lüttge, 1987). The quick decline in net CO₂ uptake at the onset of dark period represents the transition from light-driven C₃ photosynthesis during IV to mobilization of reserves required for the synthesis of PEP. It was unclear why there was a sharp drop in nocturnal CO₂ uptake after the peak values were reached for plants grown in 35/25 and 30/25 °C, while lack of such response for plants grown at 30/20 °C. Elevated dark respiration at higher temperature might be partially responsible for this decrease, which could also account for the greater CO₂ uptake in the dark for plants at 30/20 °C. It is assumed that the decrease in net CO₂ uptake late in the dark reflected partial saturation of the acid storage capacity in mesophyll vacuoles as has been previously reported (Kluge and Ting, 1978) or utilization of the substrate for production of PEP, or both.

Relatively constant c_i values during phase I for pineapple grown in both low and high CO₂ levels and three temperature regimes were consistent with the change in c_i during that phase for the CAM species *Kalanchoë daigremontiana* temporarily exposed to 100, 330 and 1000 $\mu\text{mol mol}^{-1}$ CO₂ levels (Holtum et al., 1983). Higher c_i in phase I than phase IV for pineapple might be due in part to greater stomatal conductance that prevailed during that phase, which could allow a greater inflow of external CO₂. The lower c_i during phase IV might be the combination of 1) a lower stomatal conductance in the light common to CAM species, 2) direct fixation of

external CO₂ via C₃-type photosynthetic pathway, and 3) gradual increase in carboxylation of CO₂ into malate by PEPCase after the initiation of phase IV (Kluge, et al., 1982; Winter, 1985). The progressive decline in c_i during phase IV for plants grown at 35/25 and 30/20°C was in agreement with the result obtained for the CAM species *Kalanchoë pinnàta* (Winter, 1980), and is thought to be consistent with the increase in external CO₂ fixation by PEPCase towards the latter part of light period (Winter, 1980, 1985; Cote et al., 1989). The lower c_i early in phase IV for plants grown in a 30/25°C regime might be an indication of earlier incorporation of CO₂ into malate by PEPCase or greater CO₂ fixation via the C₃ pathway, which might account for the greater contribution of CO₂ uptake in the light in that temperature regime. A higher c_i at elevated CO₂ could account for reduced g_s in those treatments (Farquhar and Sharkey, 1982; Mansfield et al., 1990), which in turn decreased H₂O vapor exchange rate. This result is in good agreement with the data for some C₃ species (Kimball and Idso, 1983).

The greatest total daily CO₂ uptake at 30/20 °C confirmed that this temperature regimes near the optimum thermal condition for pineapple growth. The result was consistent with data obtained for the CAM species *Opuntia ficus-indica*, *Hylocereus undatus*, *Agave deserti* and *Ferocactus acanthodes* (Nobel and Hartsock, 1986; Nobel and Israel, 1994; Raveh et al., 1995), which indicated that total CO₂ fixation over 24 h was greatest at 25/15 °C, a temperature regime near the optimum for total daily CO₂ uptake by these species (Nobel, 1994), for plants grown at both ambient and twice ambient CO₂ levels. The data in this study clearly showed that among the three

temperature regimes, 30/20 °C was the optimal temperature for carbon assimilation of pineapple grown at both ambient and elevated CO₂ environment.

The proportion of CO₂ uptake in the dark is closely related to the day/night temperature regime: (Bartholomew, 1982; Cote et al., 1992; Bartholomew and Malézieux, 1994). The values obtained in this study were similar to those reported by Bartholomew (1982) for pineapple grown at ambient CO₂. In this study, CO₂ fixation at ambient CO₂ was high in both the light and dark at 30/20 °C. Raising the night temperature of 5 °C reduced CO₂ dark fixation, while raising the temperature in the light by 5 °C reduced the fixation in the light. The net effect of a 5 °C diurnal differential was to reduce the fraction of CO₂ fixed by CAM while a 10 °C differential maintained higher fraction of CO₂ fixed in the dark, but the higher temperature reduced the total quantity of CO₂ fixed. It is unclear why elevating day temperature from 30/25 to 35/25 °C produced higher nocturnal CO₂ uptake than occurred at 30/25 °C, especially for plants grown at low CO₂. A temperature differential of 10 °C might promote nocturnal uptake, and thus compensate for the lower uptake in the light, but more detailed biochemical and physiological studies are needed before further interpretation can be made. The higher CO₂ uptake in the light by pineapple at high CO₂ is consistent with the response of C₃ plants to elevated CO₂. The increased Δ values at high CO₂ confirms that elevated CO₂ enhanced CO₂ uptake via C₃ photosynthetic pathway relatively more than by the CAM pathway.

Ziska et al. (1991) reported that neither mean net CO₂ uptake rate nor plant dry weight of pineapple was increased after plants had been exposed to doubled CO₂

for three months. The results from the present study clearly show that an increase in environmental CO₂ significantly enhanced daily CO₂ uptake by pineapple grown in three different temperature regimes. Zhu (chapter 4) also showed that there was a significant increase in dry weight for pineapple grown at elevated CO₂. The increased net CO₂ uptake during the light period (phases II and IV) was consistent with the response of some C₃ species (Ziska et al., 1991), where enhancement is attributed to the fact that carboxylation of RuBP by Rubisco is not saturated at ambient CO₂ levels (Bowes, 1991; Stitt and Schulze, 1994). As air temperature increases, the photorespiration rate will increase (Taiz and Zeiger, 1991). Therefore, greater enhancement in daytime CO₂ uptake at high CO₂ would be expected for plants grown at 35/25°C than at 30/25 and 30/20 °C due to the partial inhibition of photorespiration by elevated CO₂.

The enhanced nocturnal CO₂ uptake at elevated CO₂ for pineapple grown at 35/25 and 30/25 °C was inconsistent with the results obtained for the CAM species *Agave deserti* and *Ferocactus acanthodes* grown at 25/15 °C (Nobel and Hartsock, 1986). For these species daytime CO₂ uptake was enhanced by nearly 30%, while nocturnal uptake was basically unaffected as environmental CO₂ level increased from 350 to 650 μmol mol⁻¹. Nocturnal carbon fixation by the CAM plants *Kalanchoë daigremontiana* and *Agave vilmoriniana* also were relatively unaffected by elevated CO₂ (Holtum et al., 1983; Szarek et al., 1987). However, CO₂ uptake of the CAM species *Kalanchoe pinnata* increased by 30% in the light and 10% in the dark when the CO₂ level was raised from 330 to 640 μmol mol⁻¹ (Winter, 1985). When the CO₂

level was doubled, both day and night CO₂ uptake were enhanced for the CAM species *Opuntia ficus-indica* (Nobel and Israel, 1994) and *Hylocereus undatus* grown at 15/5, 25/15, 35/25 and 45/35 °C (Raveh et al., 1995), with greater effects being observed when plants were grown at higher temperatures. It appears that CO₂ enhancement of carbon fixation at night for CAM plants may be a function of the species or the day/night temperatures, or both.

In this study, the increased nocturnal CO₂ uptake at elevated CO₂ for plants grown at 35/25 and 30/25 °C could be due in part to the enhanced synthesis of PEP in the dark as a result of enhanced soluble sugar content at elevated CO₂ (Cui and Nobel, 1994), as soluble sugars were considered to be the main source of PEP production in pineapple leaves during the phase I (Medina et al, 1991). It may also be possible that elevated CO₂ reduce dark respiration at higher temperatures, thus enhancing CO₂ dark fixation (Amthor, 1991; Nobel et al., 1994). It could be further deduced that for pineapple grown at ambient CO₂, the relatively lower daytime CO₂ uptake at 35/25 °C and smaller nocturnal uptake at 35/25 and 30/25 °C might be due partly to elevated photo and dark respirations stimulated by higher temperatures. Although data on the effect of CO₂ levels on respiration are scarce and no data were found for CAM species. Available reports indicated that respiration of some C₃ species was reduced at higher CO₂ levels, with a greater effect occurring at higher temperatures (Wong, 1990; Bunce, 1990; Amthor, 1991; Eamus, 1991; Hogan et al., 1991; Long, 1991; Drake and Leadley, 1991; Ziska and Teramura, 1992; Ziska and Bunce, 1993). Dark respiration was significantly lower in temperate species of

Triticum aestivum (Gifford et al., 1985), *Glycine. max* and *Lycopersicon esculentum* (Bunce, 1990) and *Tabebuia rosea*, a tropical tree species (Hogan et al., 1991).

In current study, a simultaneous increase in CO₂ level, and daily or night temperatures by 5 °C significantly enhanced total daily CO₂ uptake in spite of reduced CO₂ dark uptake, which was supported by the data of biomass accumulation in the same study. The results of current research indicates that elevated CO₂ could enhance total daily CO₂ uptake by pineapple if the global temperatures could increase concurrently.

The c_i/c_a ratios ranged from 0.6 to 0.8 for C₃ species and 0.2 to 0.4 for C₄ species (Winter, 1985). The c_i averaged 220 $\mu\text{mol mol}^{-1}$ for eight C₃ species and 100 $\mu\text{mol mol}^{-1}$ for four C₄ species at near a ambient CO₂ level of 320 $\mu\text{mol mol}^{-1}$ (Wong et al., 1979). For pineapple grown at about 350 $\mu\text{mol mol}^{-1}$ CO₂ in this study, the c_i/c_a ratio ($\sim 0.55-0.65$) and c_i ($\sim 170-240 \mu\text{mol mol}^{-1}$) in phase I were relatively close to the values observed for C₃ species, which might indicate that the magnitude of nocturnal carbon fixation by PEPCase in pineapple was similar to that in C₃ species via photosynthetic carbon reduction cycle if g_s was the same. While the c_i/c_a ratio ($\sim 0.2-0.3$) and c_i ($\sim 100 \mu\text{mol mol}^{-1}$) in phase IV were basically similar to the values observed for C₄ species, implying that carbon assimilation during phase IV might involve the direct fixation of external CO₂ into carbohydrates by Rubisco and the carboxylation of CO₂ into malate by PEPCase. Data on organic acid accumulation, instantaneous carbon isotopic discrimination and O₂/CO₂ exchange for some CAM species including pineapple (Cote et al., 1989; Griffiths et al., 1990; Winter, 1985;

Borland et al., 1993; Franco, personal communication) indicated that substantial amount of external CO₂ was incorporated into malate during phase IV.

Water use efficiency (WUE) depends on the environmental CO₂ level (c_a), the c_i/c_a ratio and vapor pressure deficit between the leaf intercellular space and ambient air. Therefore, WUE is a comprehensive index that reflects the effects of both environmental and physiological factors on leaf gas exchange. Mean WUE approximated 0.9 and 1.7 mmol CO₂ m⁻² day⁻¹/mol H₂O m⁻² day⁻¹, respectively, for six of the most productive C₃ and C₄ plants (Nobel, 1994), averaged 5.1 and 5.5 mmol CO₂ m⁻² day⁻¹/mol H₂O m⁻² day⁻¹, respectively for the cultivated CAM species *Agave mapisaga* and *Opuntia ficus-indica* (Nobel, 1994). For pineapple in this study, daily mean WUE averaged 7.9, 6.9 and 9.5 mmol CO₂ m⁻² day⁻¹/mol H₂O m⁻² day⁻¹, respectively, for plants grown at 35/25, 30/25 and 30/20°C in ambient CO₂, showing very high WUE, even among CAM species. The higher daily WUE of pineapple was due primarily to high WUE in the dark when most carbon was assimilated by this species. WUE can be increased in three ways: 1. increase in net CO₂ uptake, 2. decrease in water loss via transpiration, and 3. a combination of 1 and 2 (Eamus, 1991; Hogan, 1991). Significantly increased WUE for pineapple grown at high CO₂ in 35/25 and 30/25 °C regimes was due to simultaneous increase in net CO₂ uptake and a decrease in transpiration, while increased WUE at high CO₂ in 30/20 °C regime was attributable primarily to reduced transpiration since CO₂ dark fixation was not enhanced by elevated CO₂ at this temperature. In addition, intensified CAM activity also enhances WUE, thus accounting partly for higher mean daily WUE at 35/25 and

30/20 °C than 30/25 °C for plants grown at low CO₂.

In summary, as was expected from the response of C₃ species, the daytime CO₂ uptake via C₃ pathway in pineapple was significantly increased by elevated CO₂. The nocturnal CO₂ uptake via CAM pathway was also enhanced at elevated CO₂ for plants grown at higher temperatures, a response inconsistent with the observations for C₄ plants and some CAM species. There are only a few mechanisms that could account for the enhanced CO₂ assimilation in the dark period for CAM species, but none of them has been confirmed experimentally. Elevated CO₂ increased daily CO₂ uptake, reduced stomatal conductance and transpiration, thus enhanced WUE. A simultaneous increase in CO₂ level and temperature also enhanced the total daily CO₂ assimilation and WUE in pineapple. Although the enhancement in CO₂ fixation by elevated CO₂ was greater for pineapple grown at 35/25 and 30/25 °C, net CO₂ uptake and WUE were consistently greater for plants grown at 30/20°C in both ambient and twice ambient CO₂. It appears that carbon assimilation and WUE in pineapple would significantly increase in elevated CO₂ and temperature environment predicted to occur in the next century.

Table 3.1 Integrated net CO₂ uptake by the youngest physiologically mature leaves of pineapple grown at two CO₂ levels and three day/night temperatures. Data are means (n=4) ± SE. Meas./grow refers to CO₂ uptake measured at specified levels for plants grown at specified levels.

CO ₂ level	Temperature	Day	Night	24 hours
(meas./grow) (μmol mol ⁻¹)	°C	Net CO ₂ uptake		
mmol CO ₂ m ⁻² period ⁻¹				
Long-term effect				
350/350 ^a	35/25	23.5 ± 3.3	163.2 ± 1.2	186.7 ± 4.1
	30/25	55.1 ± 3.6	120.1 ± 5.8	175.2 ± 9.4
	30/20	57.0 ± 1.0	249.2 ± 9.8	306.2 ± 9.9
700/700 ^a	35/25	107.0 ± 0.9	235.6 ± 4.8	342.6 ± 4.9
	30/25	129.1 ± 9.9	216.6 ± 16.1	345.7 ± 25.7
	30/20	114.4 ± 9.8	237.7 ± 15.6	352.1 ± 6.1
LSD ^d	CO ₂ /T	32.0	78.5	89.8
	T/CO ₂	27.4	35.9	43.6
Short-term effect				
350/700 ^b	35/25	60.0 ± 6.3	198.4 ± 7.8	258.4 ± 10.5
	30/25	67.5 ± 3.7	180.3 ± 5.9	247.8 ± 5.6
	30/20	68.5 ± 4.9	215.4 ± 19.2	283.9 ± 16.7
700/350 ^c	35/25	72.0 ± 3.9	199.7 ± 38.8	271.7 ± 34.9
	30/25	96.9 ± 14.4	199.2 ± 18.2	296.1 ± 3.9
	30/20	95.0 ± 11.0	271.7 ± 29.0	366.7 ± 18.0

^{a, b, c}: Data are means of four, five and two plant leaves, respectively.

^d: LSD: Difference required for significance at 0.05 level of probability.

CO₂/T and T/CO₂ refer to LSD values between CO₂ levels within temperature treatments and between temperature treatments within CO₂ levels.

Table 3.2 Mean ratio of intercellular space CO₂ (c_i) to ambient CO₂ (c_a) concentration (c_i/c_a) and mean c_i in the dark (phase I) and afternoon (phase IV) of the youngest physiologically mature leaves of pineapple grown six months at two CO₂ levels and three day/night temperatures. Data are means ± SE.

CO ₂ levels (μmol mol ⁻¹)		Phase	Day/night temperature (°C)		
			35/25	30/25	30/20
c _i /c _a					
350	I ^a	0.553 ± 0.035	0.458 ± 0.021	0.645 ± 0.030	
	IV ^b	0.293 ± 0.040	0.222 ± 0.040	0.237 ± 0.033	
700	I ^b	0.366 ± 0.011	0.283 ± 0.011	0.518 ± 0.021	
	IV ^c	0.142 ± 0.025	0.107 ± 0.009	0.221 ± 0.028	
c _i (μmol mol ⁻¹)					
350	I ^a	200 ± 13	169 ± 7	237 ± 10	
	IV ^b	105 ± 14	80 ± 14	85 ± 11	
700	I ^b	258 ± 7	200 ± 9	365 ± 16	
	IV ^c	106 ± 17	79 ± 8	177 ± 18	

^a, ^b, ^c: Data are means of five, six and ten 'D' leaves, respectively. Each leaf is from a different plant.

Table 3.3 Mean water use efficiency (WUE) for the youngest physiologically mature leaves of pineapple grown at two CO₂ levels and three day/night temperatures. Data are means (n=4) ± SE. Meas./grow refers to CO₂ uptake measured at specified levels for plants grown at specified levels.

CO ₂ level	Temperature	Day	Night	24 hours
(meas./grow) (μmol mol ⁻¹)	°C	WUE		
Long-term effect				
350/350 ^a	35/25	2.5 ± 0.4	12.1 ± 0.7	7.9 ± 0.1
	30/25	3.9 ± 0.3	10.7 ± 0.2	6.9 ± 0.3
	30/20	3.7 ± 0.2	14.5 ± 0.6	9.5 ± 0.2
700/700 ^a	35/25	6.7 ± 0.1	13.2 ± 0.4	10.1 ± 0.1
	30/25	8.6 ± 0.3	13.2 ± 0.6	11.0 ± 0.4
	30/20	8.4 ± 0.5	17.4 ± 0.5	13.0 ± 0.2
LSD ^d	CO ₂ /T	1.8	4.5	1.7
	T/CO ₂	1.3	2.6	1.1
Short-term effect				
350/700 ^c	35/25	3.2 ± 0.3	7.5 ± 0.2	5.7 ± 0.3
	30/25	4.0 ± 0.1	8.0 ± 0.3	6.3 ± 0.2
	30/20	3.9 ± 0.1	10.6 ± 0.1	7.5 ± 0.2
700/350 ^d	35/25	7.9 ± 1.8	17.4 ± 0.4	13.0 ± 0.9
	30/25	10.0 ± 1.3	24.0 ± 3.2	16.3 ± 1.3
	30/20	13.6 ± 0.1	27.6 ± 2.5	21.9 ± 1.9

^{a, b, c}: Data are means of four, five and two plant leaves, respectively.

^d: LSD: Difference required for significance at 0.05 level of probability.

CO₂/T and T/CO₂ refer to LSD values between CO₂ levels within temperature treatments and between temperature treatments within CO₂ levels.

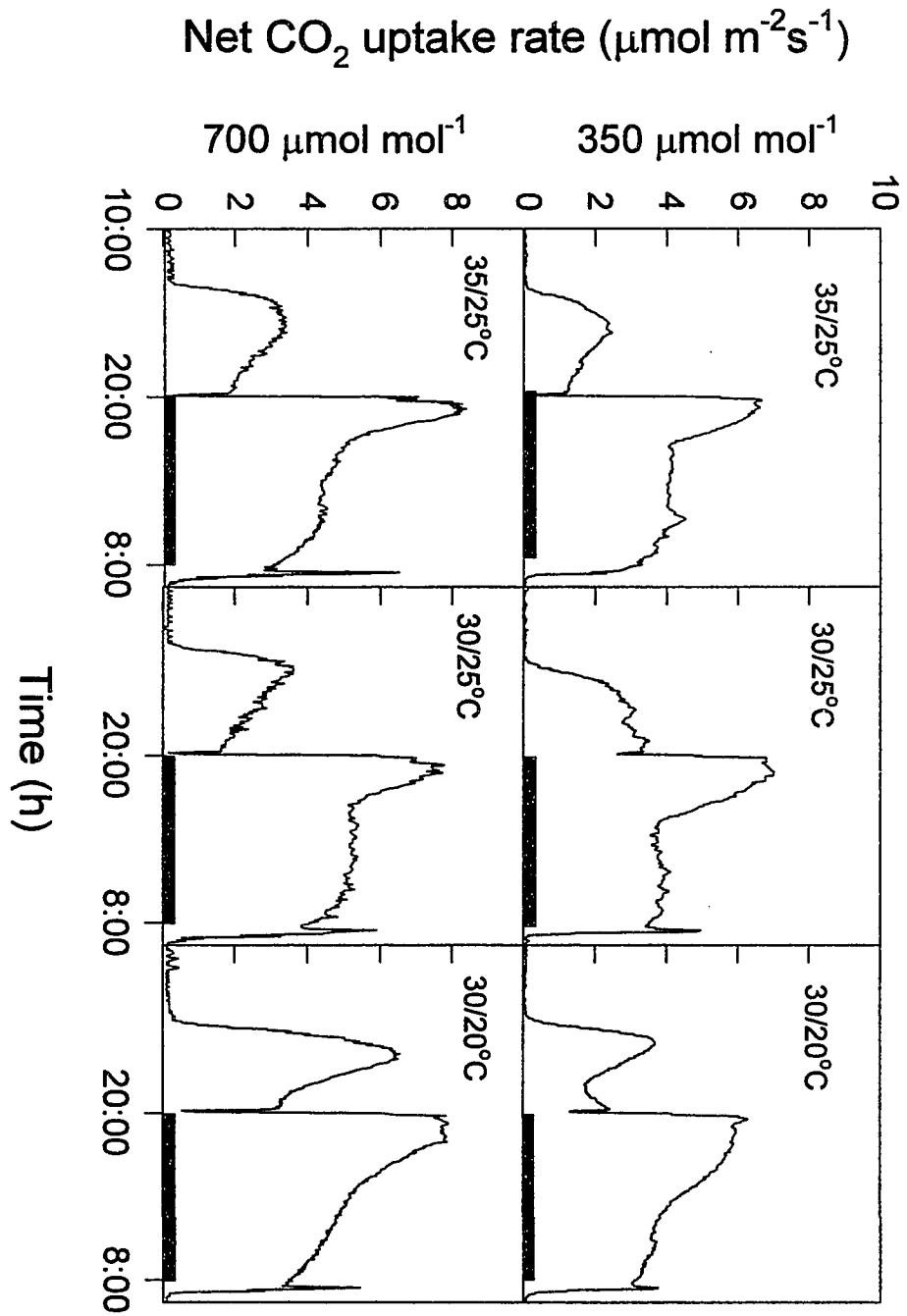


Fig. 3.1 Net CO₂ exchange rates of the youngest physiological mature leaves of pineapple grown at two CO₂ and three day/night temperatures. Dark bars refer to the dark period. Data were collected about three months after the treatments were started.

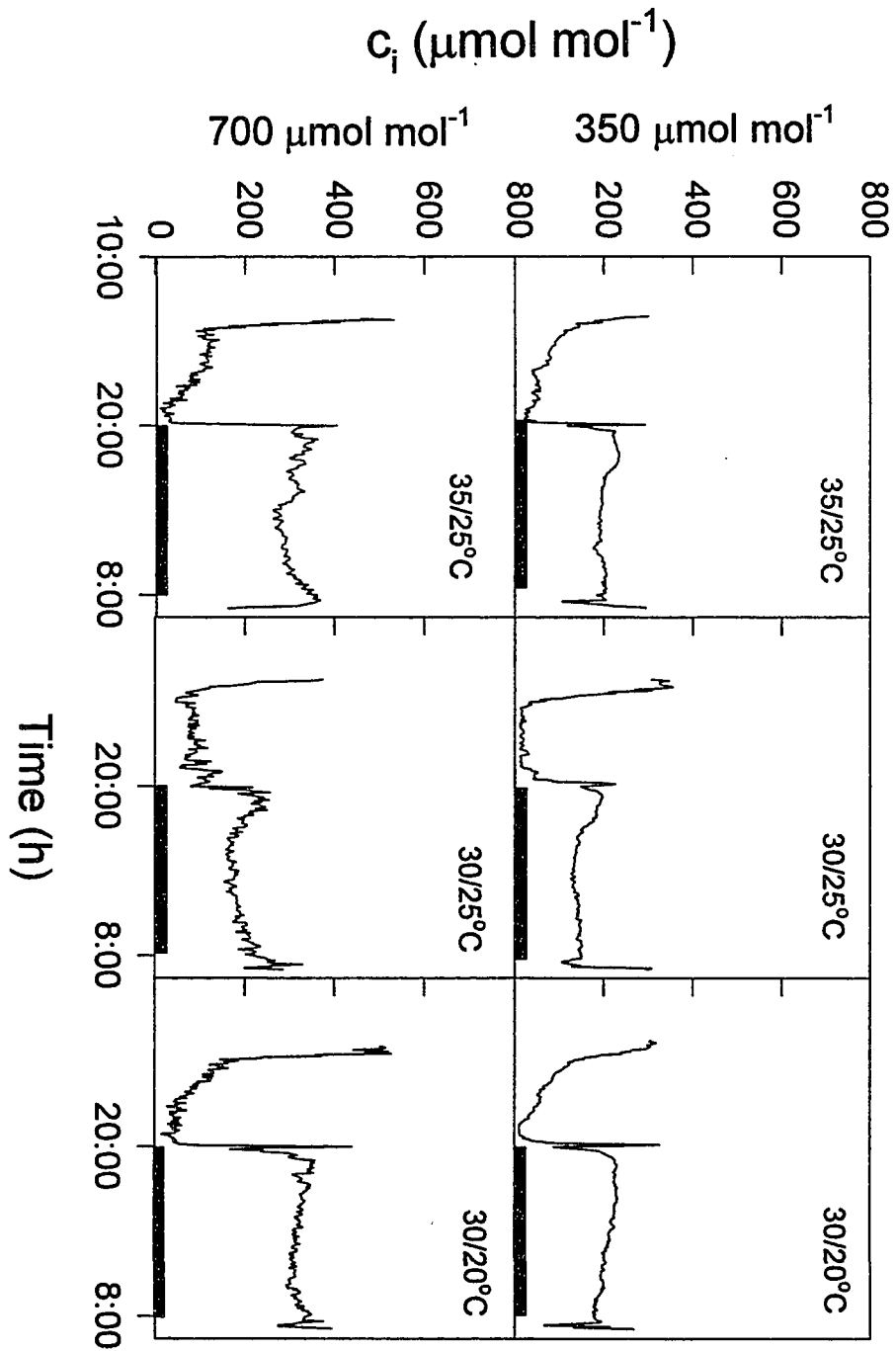


Fig. 3.2. Intercellular space CO_2 levels (c_i) of the youngest physiological mature leaves of pineapple at two CO_2 and three day/night temperatures. Dark bars refer to the dark period. Data were collected about three months after treatments were started.

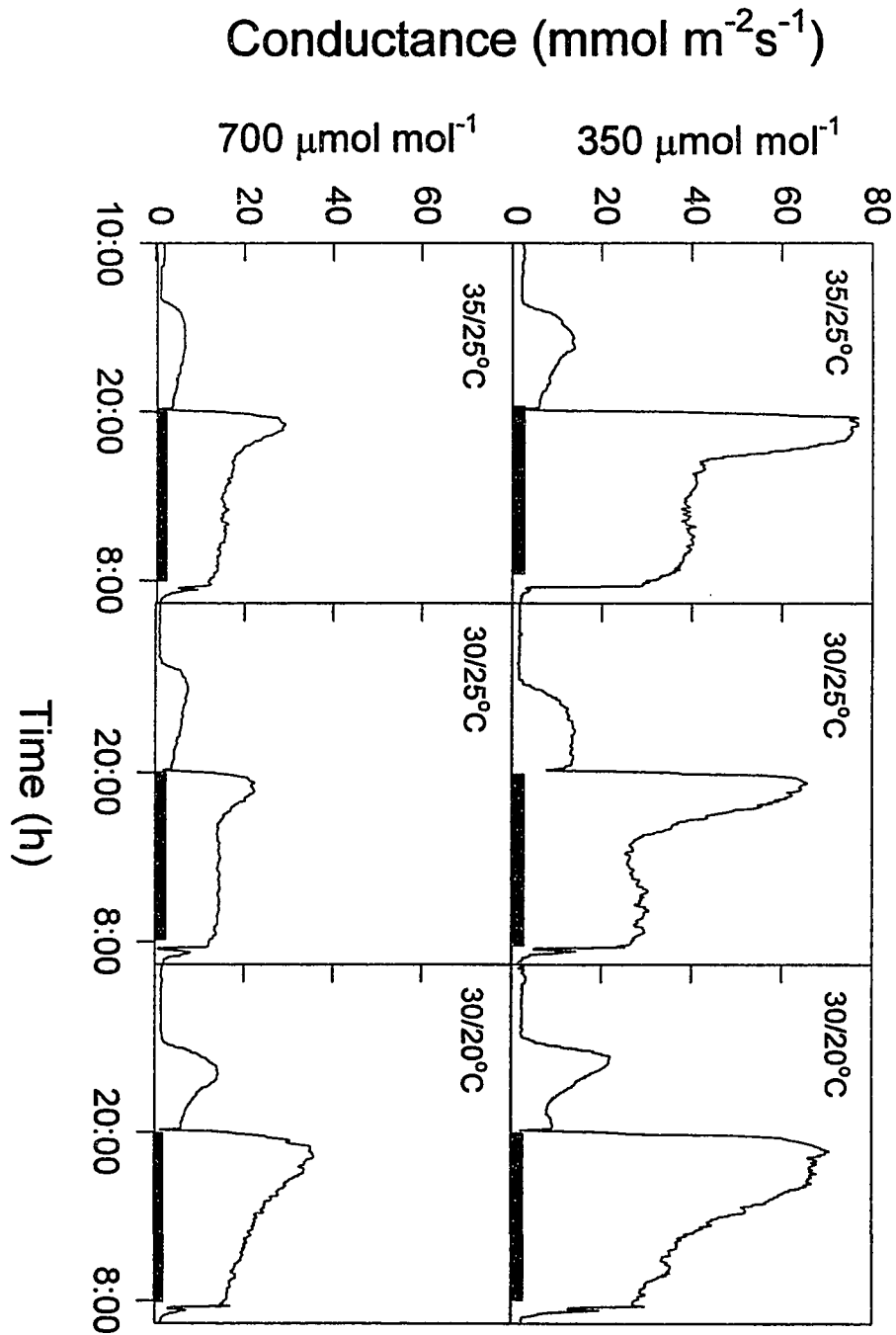


Fig. 3.3 Stomatal conductance (g_s) of the youngest physiological mature leaves of pineapple grown at two CO_2 levels and three day/night temperatures. Dark bars refer to the dark period. Data were collected about three months after treatments were started

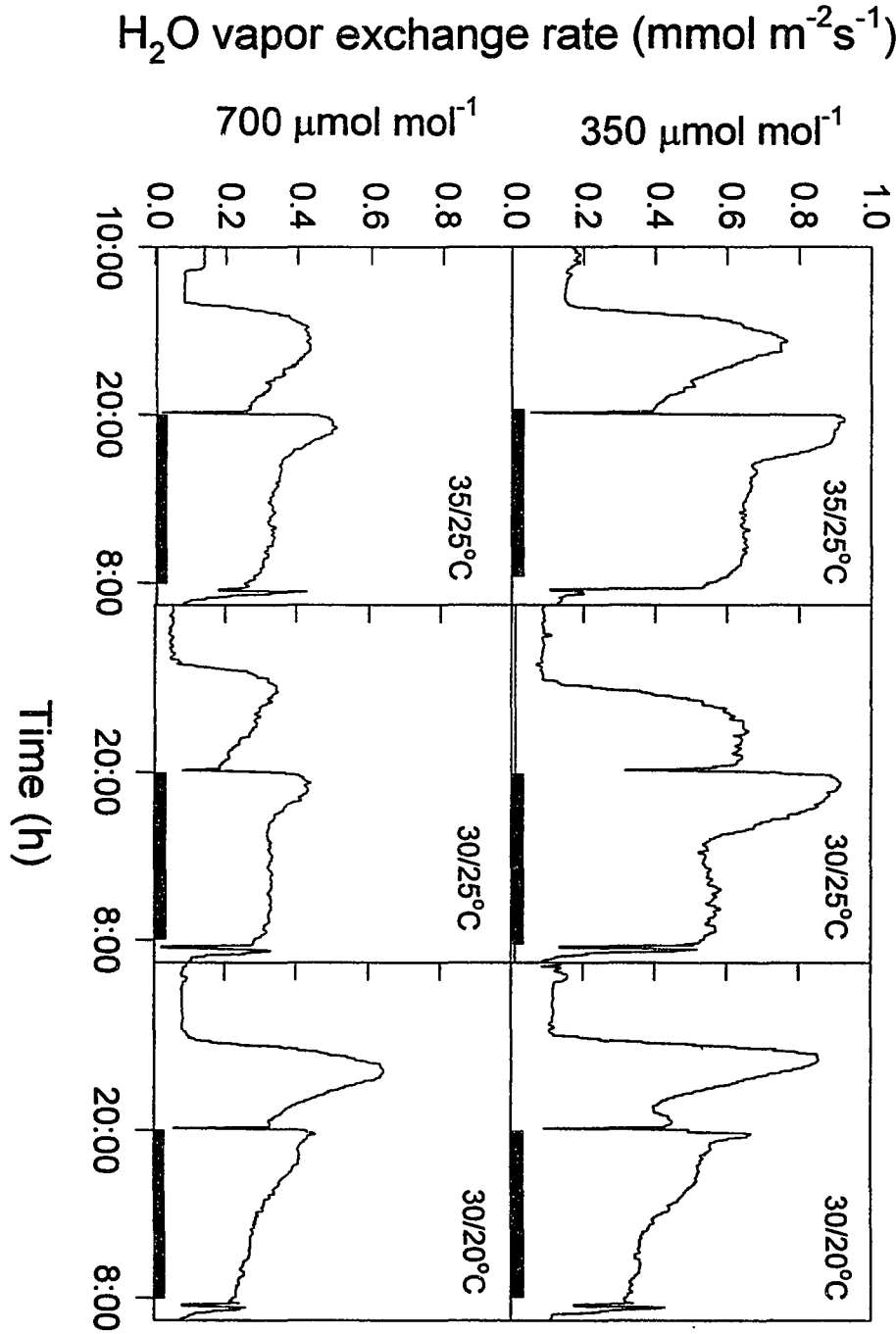


Fig. 3.4. H_2O exchange rates of the youngest physiological mature leaves of pineapple grown at two CO_2 levels and three day/night temperatures. Dark bars refer to the dark period. Data were collected about three months after treatments were started.

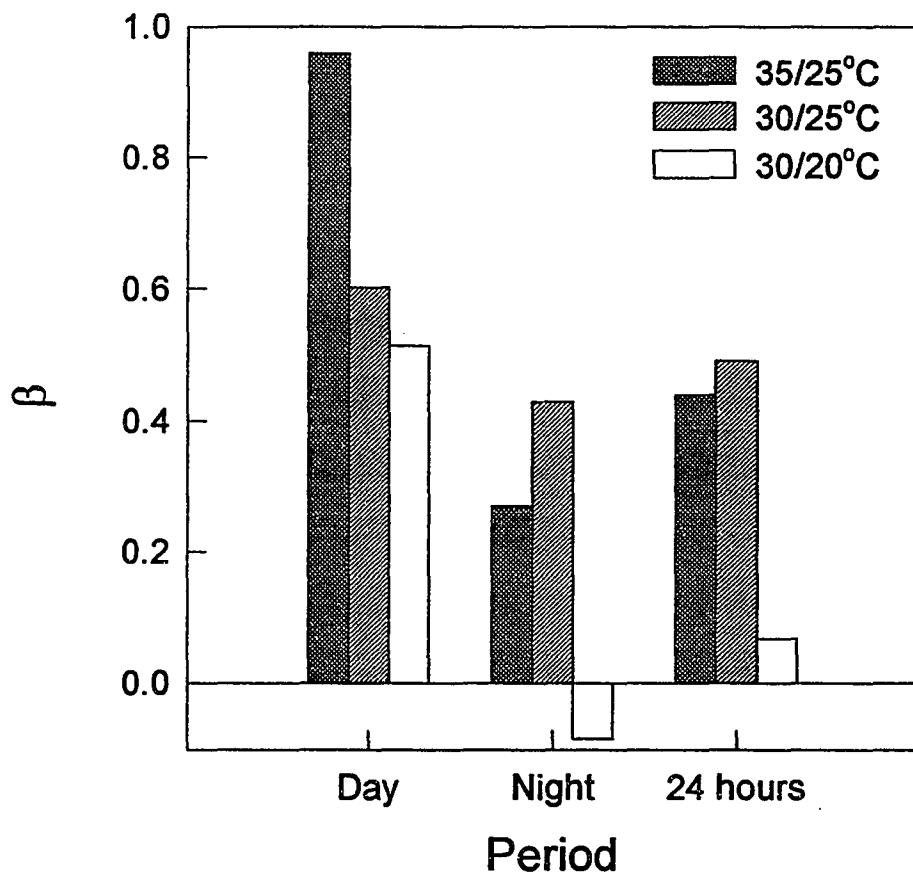


Fig. 3.5 Sensitivity (β) of total CO₂ uptake to an increase in CO₂ levels during the day, night and 24-h periods by the youngest physiological mature leaves of pineapple grown at three day/night temperatures.

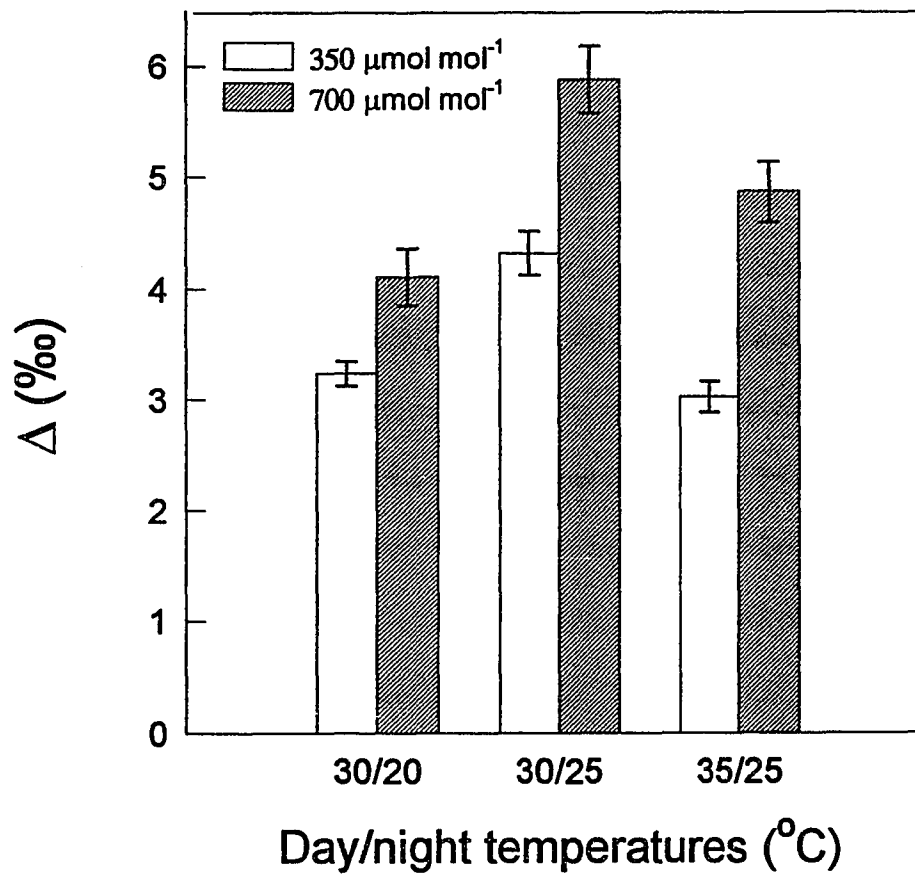


Fig. 3.6 Carbon isotopic discrimination (Δ) in the youngest physiological mature leaves of pineapple grown at two CO_2 levels and three day/night temperatures.

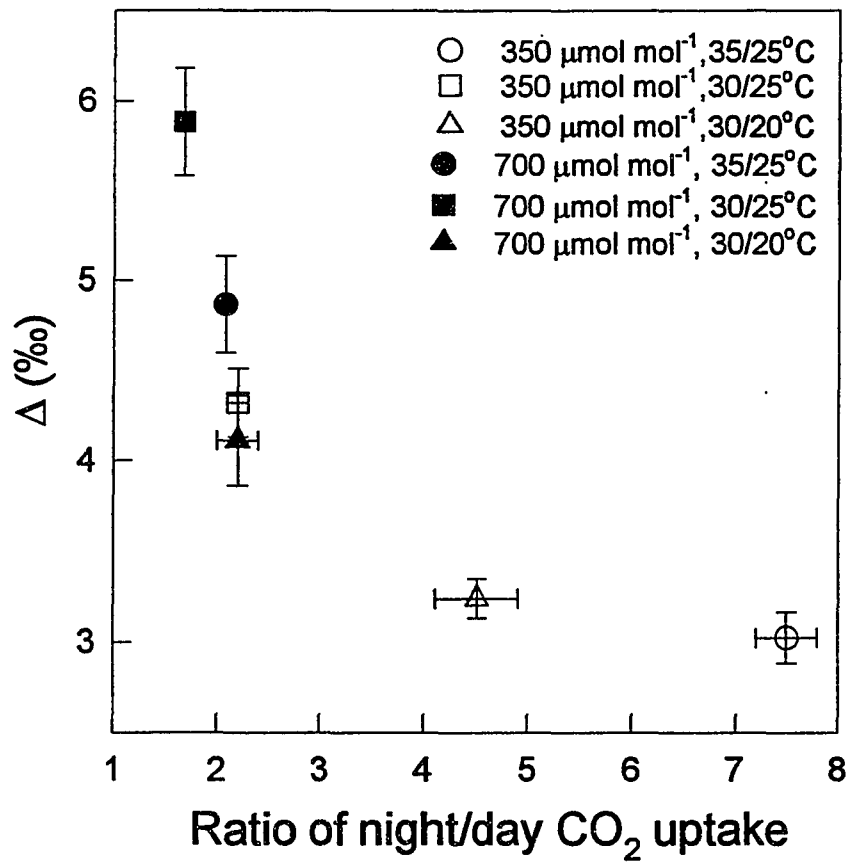


Fig. 3.7 Change in mean carbon isotopic discrimination (Δ) with the mean ratio of night/day CO₂ uptake by the youngest physiological mature leaves of pineapple at two CO₂ levels and three day/night temperatures.

CHAPTER 4

BIOMASS ACCUMULATION AND PHOTOSYNTHETIC CHARACTERISTICS OF PINEAPPLE IN RESPONSES TO ELEVATED CO₂ AND TEMPERATURES

ABSTRACT

The effects of CO₂ and temperatures were studied on some of the photosynthetic characteristics of pineapple (*Ananas comosus* (L.) Merr.), a species having crassulacean acid metabolism (CAM). The primary focus of this study was on biomass accumulation and partitioning, leaf titratable acidity (TA), tissue sap osmolarity, leaf chlorophyll and nitrogen contents. Plants were grown in controlled environment chambers at day/night temperatures of 35/25, 30/25 and 30/20 °C with near ambient (350 μmol mol⁻¹) and twice ambient (700 μmol mol⁻¹) CO₂. For plants grown at ambient CO₂, net assimilation rate (NAR) was 1.46 g m² day⁻¹ at 30/20°C, a value about 22% greater than those at 35/25 and 30/25°C, with little difference between 35/25 and 30/25 °C treatments. Elevated CO₂ increased the NAR of pineapple grown at 35/25, 30/25 and 30/20°C, respectively, by 76, 123 and 68%. Therefore, the growth enhancement by elevated CO₂ was greatest for plants grown at small diurnal temperature differential of 5 °C and warm night temperature. Simultaneous increase in CO₂ levels and day/night temperature from 30/20 to 35/25 °C or night temperature from 30/20 to 30/25 °C enhanced NAR by 45 and 84%, respectively. Increased plant growth at elevated CO₂ partitioned more biomass to stem and root, but less to leaf. Greater leaf area expansion and thickness at elevated CO₂

indicated that leaf growth was also enhanced. At twice ambient CO₂, nocturnal accumulation of organic acids was enhanced, while leaf chlorophyll content was reduced.

INTRODUCTION

Consumption of fossil fuels and deforestation over the past century have resulted in a steady increase in the atmospheric CO₂ concentration (Sarmiento and Bender, 1994; Porter and Carter, 1991). Global climate models predict that atmospheric temperature will rise 1.5 to 4.5°C by the middle of the next century (Bakin, 1993). Increased atmospheric CO₂ levels increase the photosynthesis and dry matter accumulation of plants having the C₃ photosynthetic pathway but have a minimal effect on C₄ plants. To date, the effects of elevated CO₂ and temperature have been intensively studied on at least eight important C₃ crops (wheat, barley, rice, soybean, cotton, alfalfa, potato and sweet potato) and 2 major C₄ species (corn and sorghum) (Acock and Allen, 1985; Cure and Acock, 1986). Available studies indicated that elevated CO₂ significantly increased the growth in some other C₃ crops (Gifford et al., 1985; Idso et al., 1987; Yelle et al., 1989; Peet et al., 1986; Hogan et al., 1991). Only a few studies were found to examine the effects of CO₂ enrichment on some CAM species, including *Kalanchoë daigremontiana* (Holtum et al., 1983), *Agave deserti*, *Ferocactus acanthodes* and *Opuntia ficus-indica* (Nobel and Hartsock, 1986; Cui and Nobel, 1994; Nobel, 1991; Israel and Nobel, 1994). Elevated CO₂ increased the daily carbon fixation and biomass accumulation of the CAM species

(Nobel and Hartsock, 1986; Idso et al., 1986; Cui et al., 1993; Nobel et al., 1994). The enhancement was primarily due to increased CO₂ fixation in the light with little enrichment in the night (Nobel and Hartsock, 1986; Cui et al., 1993). But enhanced fixation in the dark was also found for some CAM species grown at elevated CO₂ (Winter, 1985; Black, 1986; Nobel and Israel, 1994; Raveh et al., 1995). Leaf nitrogen and chlorophyll contents were found to decrease for the CAM species *Opuntia ficus-indica* (Cui and Nobel, 1994; Nobel et al., 1994) and some C₃ plants (Rowland-Bamford et al., 1991; Coleman et al., 1991; Wong, 1990; Ryle and Powell, 1992) after plants were exposed to elevated CO₂ for extended period.

The environmental physiology of pineapple (*Ananas comosus* (L.) Merr.) was recently reviewed by Bartholomew and Malézieux (1994) including the effect of various day/night temperature regimes. No studies were found that examined the combined effects of elevated CO₂ and temperatures on growth and organic acid accumulation of pineapple, the world's most important commercial CAM species. The principal objective of this study was to investigate the biomass accumulation and other physiological responses of pineapple to elevated CO₂ and temperatures.

MATERIALS and METHODS

Plant Materials and Treatments

Fruit top (crowns) from the Smooth Cayenne clone Champaka 153 were obtained from a local pineapple company and were grown in artificial media in pots for three months prior to imposing the treatments. The potting media consisted of a

1:1 (by volume) mixture of Sunshine #4 (a commercial potting mixture) and horticultural Perlite. The plants were watered every five days and fertilized once per two weeks with a dilute nutrient solution containing 0.4% (w/v) urea and 0.6% (w/v) Gaviota Foliar 62 (Brewer Environmental Industries, Honolulu), a commercial soluble fertilizer mix consisting 12% N, 24% P₂O₅, 24% K₂O, 0.04% Mg, 0.10% Fe, 0.013% Cu, 0.01% B, 0.02% Mo, 0.012% Mn, 0.014% Zn and 0.0005% Vitamin B1.

Plants were grown in controlled environment chambers with CO₂ level of 350 ±30 (low) μmol mol⁻¹ CO₂ from November, 1993 to May, 1994 in the first experiment, and 700 ±50 (high) μmol mol⁻¹ CO₂ from August 1994 till February, 1995 in the second experiment because only three chambers were available. In each experiment, day/night temperatures were 35/25, 30/25 and 30/20 °C. The photosynthetic photon flux density in the growth chambers was maintained at about 400 μmol m⁻² s⁻¹ at the mid-plant height during a 12 h photoperiod. The temperature treatments were based on the data obtained at Wahiawa, Hawaii, USA, one of the better pineapple producing areas of the world. The mean monthly temperature at Wahiawa ranges from 20.1 °C in February to 24.3 °C in August (data for 1953-1962). February temperatures are too cool for optimum growth so fruit tops (crowns) are planted through black plastic mulch to take advantage of the increased soil temperature under the mulch (Ekern, 1969). Thus, 30/20 °C was considered to be near the optimum for pineapple growth and was used as the control in the current study.

After three months of the initial establishment, about 22 plants were placed in each of the chambers. The low CO₂ level was maintained by adjusting the rate of air exchange between the growth chambers and the outside. The high CO₂ level was established by bleeding pure CO₂ from a gas cylinder into the growth chambers. The CO₂ concentration was achieved by adjusting the flow rate of the pure CO₂ into the chambers and the rate of air exchange between the growth chambers and the outside. The CO₂ concentration in the chambers was measured frequently with a Li-Cor 6262 gas analyzer (Li-Cor, Inc., Lincoln, NB) until the desired set point was established. At six months after planting, all plants were repotted into ~ 8 L pots for the remainder of the experiment to minimize the effects of limited rooting volume on photosynthesis and growth.

Physiological Measurements and Data Sampling

Biomass Data

Fresh and dry weights of leaves, stems and roots were measured on three to four plants per treatment about every six weeks for a period of at least 24 weeks. Dry weight was determined after oven-drying at 70 °C for two weeks. Green leaf area was measured using a Li-Cor Li-3100 leaf area meter (Li-Cor, Inc, Lincoln, Nebraska, USA).

Leaf Tissue Titratable Acidity and Osmolarity

Leaf titratable acidity (TA) and osmolarity were measured on four 'D' leaves per treatment collected at end of dark period (predawn) and end of the photoperiod

(afternoon). The leaf was split longitudinally and one-half was removed at dawn and frozen at -75°C to disrupt the cell membranes. The second segment of the leaf was harvested in the afternoon and similarly frozen. To determine TA, two 1.3 cm diameter leaf discs were collected from the middle one-third of each frozen leaf segment, ground in a mortar with ~ 20 ml of distilled water, and the total volume was titrated to pH 7.2 using 0.01 N NaOH. Leaf sap was extracted from frozen-and-thawed tissues and the osmolarity of the leaf sap was determined using a 5500 vapor pressure osmometer (Model 5500S, Wescor Inc. Logan, Utah, USA).

Chlorophyll and Nitrogen Contents

Leaf chlorophyll content was determined using the techniques described by Coombs et al. (1985) and Ranjith et al., (1995). The leaves were frozen at -75°C and ground to a powder in liquid nitrogen. Chlorophyll was extracted by grinding 200 mg of frozen leaf sample with 80 % (V/V) acetone, and adjusting the final volume to 20 ml by adding 80 % acetone. The mixture was incubated overnight at room temperature (22°C) and centrifuged at 12000 g for 1 minute. The absorbance of the leaf supernatant was read at 647 and 664 nm wavelengths with a spectrophotometer (Spectronic 21, Bausch & Lomb, New York), and chlorophyll content was calculated according to Coombs et al. (1985).

Leaf total nitrogen (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, University of Hawaii, Manoa. The data were

reported as the percentage N in the leaf on a dry weight basis (W/W %).

Carboxylating Enzyme Activities

Attempts to assay the activities of phosphoenolpyruvate carboxylase (PEPCase) and ribulosebiphosphate carboxylase/oxygenase (Rubisco) were according to the methods described by Ranjith et al. (1995) for sugarcane. The 'D' leaf tissues were freshly harvested and ground to a powder in liquid nitrogen. A 1 g sample of powder was added to 4 ml of extraction solution containing 100 mM Bicine (pH 8.1), 1% (W/V) PEG 6000, 7.5 mM DTT and 1 mM EDTA Na₂. Then, tissue extracts were centrifuged at 4°C for 12 minutes at 15,000 g. For the determination of Rubisco activity, 1 ml of tissue extract supernatant was mixed with 1 ml of activation solution consisting of 100 mM Bicine (pH 8.1), 20 mM MgCl₂ and 20 mM NaHCO₃, and the mixture was incubated for 20 minutes to activate Rubisco. The reaction of Rubisco was initiated by adding 200 µl of activated enzyme extract to 800 µl of Rubisco assay mixture consisting of 100 mM Bicine (pH 8.1), 5 mM DTT, 0.1 mM EDTA Na₂, 0.4 mM RuBP, 20 mM MgCl₂, 10 mM NaHCO₃ and 0.5 µCi NaH¹⁴CO₃. The reaction of PEPCase was initiated by adding 200 µl of enzyme extract to 800 µl of PEPCase assay mixture consisting of 100 mM Bicine (pH 8.1), 5 mM DTT, 0.1 mM EDTA Na₂, 5 mM PEP, 5 mM Na glutamate, 10 mM MgCl₂, 10 mM NaHCO₃ and 0.5 µCi NaH¹⁴CO₃. The reaction was terminated after 2 minutes at 22°C by adding 70 µl of 1 M HCl, and the reaction vials were kept in an oven at about 50°C for 3 hours to remove unfixed ¹⁴CO₂. A 5 ml aliquot of scintillation fluid was added to the solution

in the vial and the radioactivity was determined by a scintillation counter (Beckman LS 1801, Beckman Instruments, Inc. California, USA).

Because of difficulties in obtaining reasonable levels of activities, the method was slightly modified several times to increase the ^{14}C specific activities. The first modification was to raise the Bicine pH from 8.1 to 8.8 to minimize the pH change due to the acidity of the pineapple leaf. The second modification was to increase the amount of $\text{NaH}^{14}\text{CO}_3$ and reduce the amount of NaHCO_3 in order to raise the ^{14}C concentration in the assay mixture. This procedure enabled the enzymes to combine more radioactive carbon into carbohydrates during the reaction. The last modification, based on the method described by Black (Personal communication, 1995), was to add $10\ \mu\text{M}$ Leupetin and 2% (W/V) PVPP to the extraction buffer to inhibit the leaf tissue protease during enzyme extraction. The activities of both pineapple and sugarcane were measured to confirm that the technique worked appropriately.

Growth Analysis

Dry matter partitioning was assessed by calculating stem weight, root weight and leaf weight to total plant dry weight ratios, which were then expressed as the percentage of dry weight in stem (SWR), root (RWR) and leaf (LWR) relative to the total plant dry weight. The mean relative growth rate (RGR) and net assimilation rate (NAR) over the entire growing period (Chiariello et al., 1989) were calculated as:

$$RGR = \frac{1}{\bar{W}} \frac{dW}{dt} = \frac{\ln W_2 - \ln W_1}{t_2 - t_1} \quad (4.1)$$

$$NAR = \frac{1}{A} \frac{dW}{dt} = \frac{2(W_2 - W_1)}{(A_2 + A_1)(t_2 - t_1)} \quad (4.2)$$

where W is total dry weight, A is green leaf area, t is time, and the subscripts 1 and 2 refer to samples collected at beginning (1) and end (2) of treatments.

Mean leaf expansion rate (LER) over the entire growing period was calculated as:

$$LER = \frac{dA}{dt} = \frac{A_2 - A_1}{t_2 - t_1} \quad (4.3)$$

Specific leaf weight (SLW), a measure of leaf thickness, was calculated as the ratio of leaf dry weight to green leaf area (Chiariello et al., 1989). Dry matter content of leaf (or stem) was also calculated as the percentage of leaf (or stem) dry weight relative to fresh weight of leaf (or stem).

RESULTS

Growth Responses

Average total dry weight per plant (W_t) was consistently higher at 30/20 °C than at 35/25 and 30/25 °C in low CO₂ (Fig. 4.1). While in high CO₂, the differences in W_t between temperature treatments were much smaller and more variable (Fig. 4.1); final W_t after six months of growth was about 30 and 20 g greater at 30/25 and 30/20 °C, respectively, than at 35/25 °C. The initial W_t of plants grown at high CO₂ were 15 g smaller than that of plants grown in low CO₂, yet the final W_t after six months was 32, 62 and 40 g greater in high CO₂, respectively, at 35/25, 30/25 and

30/20 °C (Fig. 4.1). Therefore, biomass accumulation of pineapple during six-month growth period was greater for plants grown in high CO₂ at all temperatures.

Average leaf surface area per plant (A_L) was somewhat variable and differences between temperature treatments were small (Fig. 4.1). The final A_L after six-month of growth was 860 and 760 cm² greater at 35/25 and 30/25 °C than at 30/20 °C in low CO₂, and 580 and 265 cm² greater at 35/25 and 30/20 °C than at 30/25 °C in high CO₂. Therefore, the final A_L was always greatest for plants grown in the 35/25 °C regimes at both ambient and twice ambient CO₂. The initial A_L was 913 cm² greater for plants grown at low than high CO₂, and the final A_L after six months was 530 and 1000 cm² greater at 35/25 and 30/25 °C in low than high CO₂, and comparable between Low and high CO₂ at 30/20 °C.

In low CO₂, net assimilation rate (NAR) was greater at 30/20 °C than at other two temperatures, while NAR values at 35/25 and 30/25 °C were not different (Table 4.1). In high CO₂, NAR was greatest at 30/25 °C and smallest at 35/25 °C (Table 4.1). NAR was 0.76, 1.23 and 0.69 time greater for plants grown at high CO₂ than the values in low CO₂, respectively, at 35/25, 30/25 and 30/20 °C. Relative growth rate (RGR) was also highest at 30/20°C, and about 6% lower at 35/25 and 30/25°C for plants grown at low CO₂, and greatest at 30/25°C and smallest at 35/25°C for plants at high CO₂ (Table 4.1). Elevated CO₂ enhanced RGR by 57% at 35/25 °C, 70% at 30/25° and 56% at 30/20°C (Table 4.2). Simultaneous increases in CO₂ level and temperatures from 30/20 to 35/25 and 30/25 °C enhanced NAR by 45 and 84%, and RGR by 48 and 60%, respectively.

Leaf area expansion rate (LER) was greatest at 35/25 °C and smallest at 30/20 °C for plants grown at low CO₂ (Table 4.1). At high CO₂, LER was still greatest at 35/25 °C but smallest at 30/25 °C, with relatively smaller temperature effect (Table 4.1). LER was 13, 4 and 28% greater at high than at low CO₂, respectively, at 35/25, 30/25 and 30/20 °C. Simultaneous increases in CO₂ level and temperatures from 30/20 to 35/25 or 30/25 °C increased LER by 35 or 21%, respectively.

Mean dry matter contents (DMC) of leaf and stem were consistently higher during six-month growth period for plants grown at high CO₂ (Table 4.2). Although the CO₂ effect on DMC was not consistent along plant age, the average increase in DMC during six months was about 8-10% in leaf and 14-16% in stem at 35/25 and 30/20 °C, and 16% in leaf and 26% in stem at 30/25 °C. There was no consistent effects of plant age and temperature treatments (Table 4.2).

At low CO₂, day/night temperature had no consistent effect on stem weight ratio (SWR), while at high CO₂, SWR was greatest at 30/25 °C and lowest at 35/25 °C (Table 4.3). Root weight ratio (RWR) was greater at 30/20 °C and smaller at 35/25 °C in low CO₂. RWR in high CO₂ was greater at 30/25°C and smaller at 35/25 °C (Table 4.3). Leaf weight ratio (LWR) at low CO₂ was greater at 35/25 and 30/25 °C than at 30/20 °C. In high CO₂, the LWR in the 35/25°C regime was comparable with that in low CO₂, while LWRs in other two temperature regimes were lower than those in low CO₂ (Table 4.3). Based on data from the final harvest, elevated CO₂ enhanced dry matter partitioning to stem (SWR) by 8 and 23%, respectively, at 35/25 and 30/25 °C, with 1.4% reduction at 30/20 °C; elevated CO₂ also enhanced dry

matter partitioning to root (RWR) by 37 and 24%, respectively, at 30/25 and 30/20 °C; partitioning to leaf (LWR) was generally reduced at high CO₂ by 5 and 2% at 30/25 and 30/20 °C with only 1% increase at 35/25°C; simultaneous increases in CO₂ level and day/night temperature from 30/20 to 35/25 decreased SWR and RWR by 10 and 23%, respectively, while increased LWR by 3%; simultaneous increases in CO₂ level and night temperature from 30/20 to 30/25 increased SWR and RWR by 16 and 20%, respectively, but decreased LWR by 3%.

Specific leaf weight (SLW), which is positively correlated with leaf thickness (Chiariello et al., 1989), generally increased with plant age, with the greatest response at 30/20 °C (Fig. 4.2). The effect temperature on SLW was relatively smaller than that of CO₂, and also increased with plant age (Fig. 4.2). Elevated CO₂ significantly increased SLW for plants grown in all temperature regimes, with the greatest effect at 30/25 °C, and comparable response at 35/25 and 30/20°C (Fig. 4.2). The similar curve shape within a temperature regime suggested that the relation between SLW and plant age was temperature specific rather than CO₂ dependent (Fig. 4.2).

Physiological Responses

As expected for a species with CAM, the predawn titratable acidity (TA) in pineapple leaves was substantially higher than afternoon values and increased with plant age (Fig. 4.3). At low CO₂, predawn TA was highest at 30/20 °C and lowest at 30/25 °C throughout the six-month period (Fig. 4.3). At high CO₂, predawn TA

values were greater than those at low CO₂, with the greatest effect occurring to plants grown at 30/25 °C; the response to temperature was similar during the early part of growth period, but the TAs at 35/25 °C dropped below the values at 30/25 °C during the late part of the growth period (Fig. 4.3). The effect of CO₂ enrichment on predawn TA levels decreased during the late part of growth period. The afternoon TA values were unaffected by CO₂, temperature and plant age (Fig. 4.3).

Predawn osmolarity at high CO₂ was 35 to 40 mmol kg⁻¹ greater than at low CO₂ in the 35/25 and 30/25 °C regimes, but about 18 mmol kg⁻¹ smaller than at low CO₂ in the 30/20 °C regime (Fig. 4.4a). Afternoon osmolarity was 50 to 55 mmol kg⁻¹ greater at high than at low CO₂ in all temperature regimes (Fig. 4.4b), and was approximately 120 to 190 mmol kg⁻¹ lower than predawn values, depending on the CO₂ levels and temperatures (Fig. 4.4). Predawn osmolarity and TA were linearly correlated at both low ($r^2=0.706$, $p<0.05$) and high CO₂ ($r^2=0.645$, $p<0.05$), while afternoon osmolarity and TA values were not statistically correlated (data not shown).

Mean nitrogen (N) content of 'D' leaves at the final harvest was 1.79, 1.80 and 1.62%, respectively at 35/25, 30/25 and 30/20 °C for plants grown in low CO₂, and 1.53, 1.63 and 1.62%, respectively at 35/25, 30/25 and 30/20 °C for plants grown in high CO₂. Therefore, leaf N content was reduced at elevated CO₂ in the 35/25 and 30/25 °C regimes, but the differences was only significant at 35/25 °C ($p<0.05$). Leaf Chlorophyll content was consistently highest at 30/25 °C and lowest at 35/25 °C at both low and high CO₂ during six months of growth (Fig. 4.5). Elevated CO₂ appeared to reduce the effect of temperature on the chlorophyll content.

Mean leaf chlorophyll content at the final harvest was significantly lower ($p < 0.01$) at high CO₂ by 31, 26 and 27%, respectively, at 35/25, 30/25 and 30/20 °C (data not shown).

Attempts to measure the Rubisco and PEPCase activities using the original and modified methods were unsuccessful. The radioactivity counts were about 3000 to 4000 for sugarcane leaves, and approximately 300 to 500 for pineapple leaves, and 40 to 50 for background levels (data not shown). The reasons for the low activities of pineapple leaves is not known. No differences due to treatments could be detected.

DISCUSSION

When grown at ambient CO₂, biomass accumulation of pineapple was greater for plants at 30/20 °C due to a higher NAR, as a result of greater fixation of external CO₂ in the light (chapter 3), and organic acid accumulation in the dark. Although initial dry weights of were smaller for plants grown at high CO₂, greater dry weight at the final harvesting indicated that biomass accumulation was significantly enhanced by elevated CO₂ at all three temperatures. As CO₂ was doubled, more dry matter was partitioned to stem and root, thereby leading to relatively lower LWR. Increased RWR for pineapple grown at elevated CO₂ was consistent with the observation for the CAM species *Opuntia ficus-indica*, which showed that root growth was enhanced at elevated CO₂ (Cui and Nobel, 1994). Leaf growth was also enhanced at elevated CO₂ as evidenced by greater LER and SLW. It appears that higher temperature of 35/25 °C increased leaf surface area at both CO₂ levels, while relatively lower temperature

of 30/20 °C promoted the effect of CO₂ enhancement on leaf area. Increased LER at high CO₂ indicated that leaf area could be greater for plants grown at elevated CO₂ if the initial A_L was the same for plants at both ambient and twice ambient CO₂. All these growth enhancements on pineapple were attributable primarily to increased NAR induced by elevated CO₂. This effect was greater for pineapple grown at relatively higher temperatures, which was consistent with the theoretical prediction on the photosynthetic response to CO₂ and temperature in C₃ species (McMurtrie and Wang, 1993; Kirschbaum, 1994). Data in carbon isotope discrimination and carbon assimilation indicated that plants grown at 35/25 and 30/25 °C responded relatively more to doubled CO₂ than did plants at 30/20 °C (chapter 3). This greater effect of CO₂ enrichment for plants grown at higher temperatures might be due partially to reduced photo and dark respirations at elevated CO₂ as indicated earlier (chapter 3).

The available data shows that stem dry matter (DMC) and starch contents of pineapple were positively correlated (Bartholomew and Paull, 1986). Thus, the increased stem DMC observed at high CO₂ might have resulted from an increased starch content in the stem. Although no data are available on the relationship between leaf DMC and leaf starch content for pineapple, other studies indicate that increased leaf thickness at elevated CO₂ could be due partially to the greater accumulation of carbohydrates such as starch (Arp, 1991; Rowland-Bamford et al., 1990). The largest increase in DMC at elevated CO₂ for plants grown at 30/25 °C may partly account for the greatest enhancement in plant dry weight in this temperature regime. It was evident that the responses in dry matter accumulation, biomass partitioning and leaf

thickness to elevated CO₂ was greatest for plants grown at smaller diurnal temperature range and warm night temperature (30/25 °C), which was due to the greater enhancement in both C₃ and CAM-type activities in that temperature regime. Data in this study indicated that simultaneous increase in CO₂ level and day/night temperature or night temperature by 5 °C was expected to enhance both growth rate and leaf area in pineapple.

Increased predawn TA levels with plant age suggest that CAM activity of pineapple intensified with the age during the six months of growth. For plants grown at ambient CO₂, the temperature response of predawn TA levels was completely consistent with the nocturnal CO₂ fixation reported previously and the dry matter accumulation at the three temperatures. At twice ambient CO₂, the predawn TA level was not consistent with the CO₂ fixation and biomass accumulation. These results may suggest that at ambient CO₂, accumulation of organic acids accounted for the major proportion of total carbon fixed, while at doubled CO₂, the fixation of external CO₂ via C₃ photosynthetic pathway contributed relatively more to biomass accumulation than was the case at ambient CO₂. Greater TA levels for plants grown at high than at low CO₂ indicated that accumulation of organic acids in the dark was enhanced by elevated CO₂. The greatest CO₂ enrichment in TA levels at 30/25 °C suggested that elevated CO₂ favored the nocturnal synthesis of organic acids for plants grown at smaller diurnal temperature range of 5 °C, a result fairly consistent with the growth data where the greatest enhancement in NAR and RGR at elevated CO₂ also occurred in this temperature regime. Decreased CO₂ enrichment in TA levels at 35/25 and

30/20 °C during the late part of growth period may reflect reduced CAM activity due possibly to an inadequate nutrient supply or limitation in rooting volume for plants grown at elevated CO₂.

Studies on cultivated and wild *Ananas comosus* species by Sideris et al. (1948) and Medina et al. (1993) indicated that the concentration of organic acids in leaf tissue neared the maximum, while soluble sugars approached a minimum at the end of dark period. Therefore, the predawn osmolarity level, which reflects the concentration of solutes in leaf sap, was primarily determined by the predawn TA levels, thus explaining the good correlation between predawn TA and osmolarity. Greater afternoon osmolarity at high CO₂ may be attributable to increased synthesis of organic acids in the dark, which may enhance decarboxylation of these acids into soluble photosynthates in the light, or direct fixation of external CO₂ via C₃ pathway, or both.

Reduced chlorophyll content for pineapple grown at high CO₂ was consistent with the observation of Cui and Nobel (1994), which showed that Leaf chlorophyll and nitrogen contents concomitantly decreased in the CAM species *Opuntia ficus-indica* after plants had been exposed to elevated CO₂ for three months. Leaf nitrogen and chlorophyll contents generally are closely correlated, and decrease in leaf nitrogen level may result in reduced chlorophyll content (Stitt, 1991; Bowes, 1991). But there was no consistent relationship between them in this study. Leaf nitrogen content was also reduced in a higher CO₂ environment for the C₃ species *Oryza sativa* (Rowland-Bamford, 1991), *Gossypium hirsutum* (Wong, 1990), *Abutilon theophrasti*,

Sinapis alba and C₄ species *Amaranthus retroflexus* (Coleman et al., 1991) and loblolly pine (*Pinus taeda*) (Tissue et al., 1993). The decrease in chlorophyll and nitrogen contents may be an adaptive response of plants to elevated CO₂ during a long-term exposure.

In summary, at ambient CO₂, the greatest plant dry weight and titratable acid levels at 30/20 °C confirmed that 30/20 °C is the optimal temperature regime for pineapple growth among the three temperatures. Elevated CO₂ increased biomass accumulation of pineapple grown at three day/night temperatures due in part to increased fixation of external CO₂ into organic acids in the dark. This conclusion is further supported by the results of net CO₂ fixation. CO₂ enrichment had a greater effect for plants grown at temperatures of 30/25 and 35/25 °C. Simultaneous increase in CO₂ levels and day/night temperature or night temperature by 5 °C enhanced the growth rate and leaf area in pineapple. This suggests that the pineapple growth could be expected to increase in the environment having elevated atmospheric CO₂ and temperatures. If the day/night temperature differential decreases and night temperature increases as predicted, the growth enhancement would be greatest based on the results of this study. The increased leaf surface area at twice ambient CO₂ could provide more assimilatory apparatus, thereby enhancing photosynthesis. Reduced leaf nitrogen and chlorophyll contents might be due to the physiological responses to elevated CO₂, but nutrient management and rooting space could be other important concerns, which need further studies.

Table 4.1 Mean values of relative growth rate (RGR), net assimilation rate (NAR) and leaf expansion rate (LER) of pineapple grown six months at two CO₂ levels and three day/night temperatures.

Growth parameters	CO ₂ levels (μmol mol ⁻¹)	Day/night temperature (°C)		
		35/25	30/25	30/20
NAR (g m ⁻² day ⁻¹) ^a	350	1.20	1.21	1.46
	700	2.11	2.69	2.46
RGR (g kg ⁻¹ day ⁻¹) ^a	350	6.70	6.68	7.10
	700	10.54	11.36	11.09
LER (cm ² day ⁻¹) ^a	350	27.68	27.17	23.17
	700	31.35	28.13	29.60

^a: Values are calculated from the initial biomass data and the data for the final harvest made after six months of growth.

Table 4.2 Mean leaf and stem dry matter contents (DMC) (n=3-4) of pineapple plants grown six months at two CO₂ levels and three day/night temperatures

Parameters	CO ₂ levels ($\mu\text{mol mol}^{-1}$)	Days*	Day/night temperature (°C)		
			35/25	30/25	30/20
Leaf DMC (%)	350	45	11.7	10.7	11.0
		98	12.3	11.6	11.6
		142	12.5	13.0	12.3
		190	11.4	12.1	12.9
	700	40	12.4	12.9	12.4
		90	13.7	14.8	12.8
		132	12.7	13.4	12.9
		180	13.9	13.7	13.5
Stem DMC (%)	350	45	10.1	10.8	11.0
		98	11.2	10.7	11.5
		142	11.0	11.2	13.1
		190	11.1	10.9	12.8
	700	40	13.3	12.9	13.0
		90	13.2	15.2	13.7
		132	12.1	12.3	14.3
		180	11.7	14.0	14.0

* Days after plants were exposed to treatments in growth chambers.

Table 4.3 Stem (SWR), root (RWR) and leaf weight ratio (LWR) (n=3-4) of pineapple grown six months at CO₂ levels and three day/night temperatures. Data are expressed as the percentage of stem, root and leaf dry weights relative to total plant dry weight.

Growth parameters (°C)	CO ₂ levels (μmol mol ⁻¹)	Days ^a	Day/night temperature	
			35/25	30/25
SWR (g g ⁻¹)	350	98	7.3	6.66.4
		142	7.1	7.37.4
		190	6.1	6.97.3
	700	90	6.8	8.58.5
		132	6.9	8.37.4
		180	6.6	8.57.2
RWR (g g ⁻¹)	350	98	5.6	6.39.1
		142	5.4	6.78.5
		190	7.0	6.57.4
	700	90	5.4	8.27.2
		132	6.7	8.66.9
		180	5.7	8.99.2
LWR (g g ⁻¹)	350	98	87.2	87.184.4
		142	87.5	86.184.1
		190	86.9	86.685.4
	700	90	87.7	83.684.3
		132	86.4	83.185.7
		180	87.8	82.583.6

^a Days after plants were exposed to treatments in growth chambers.

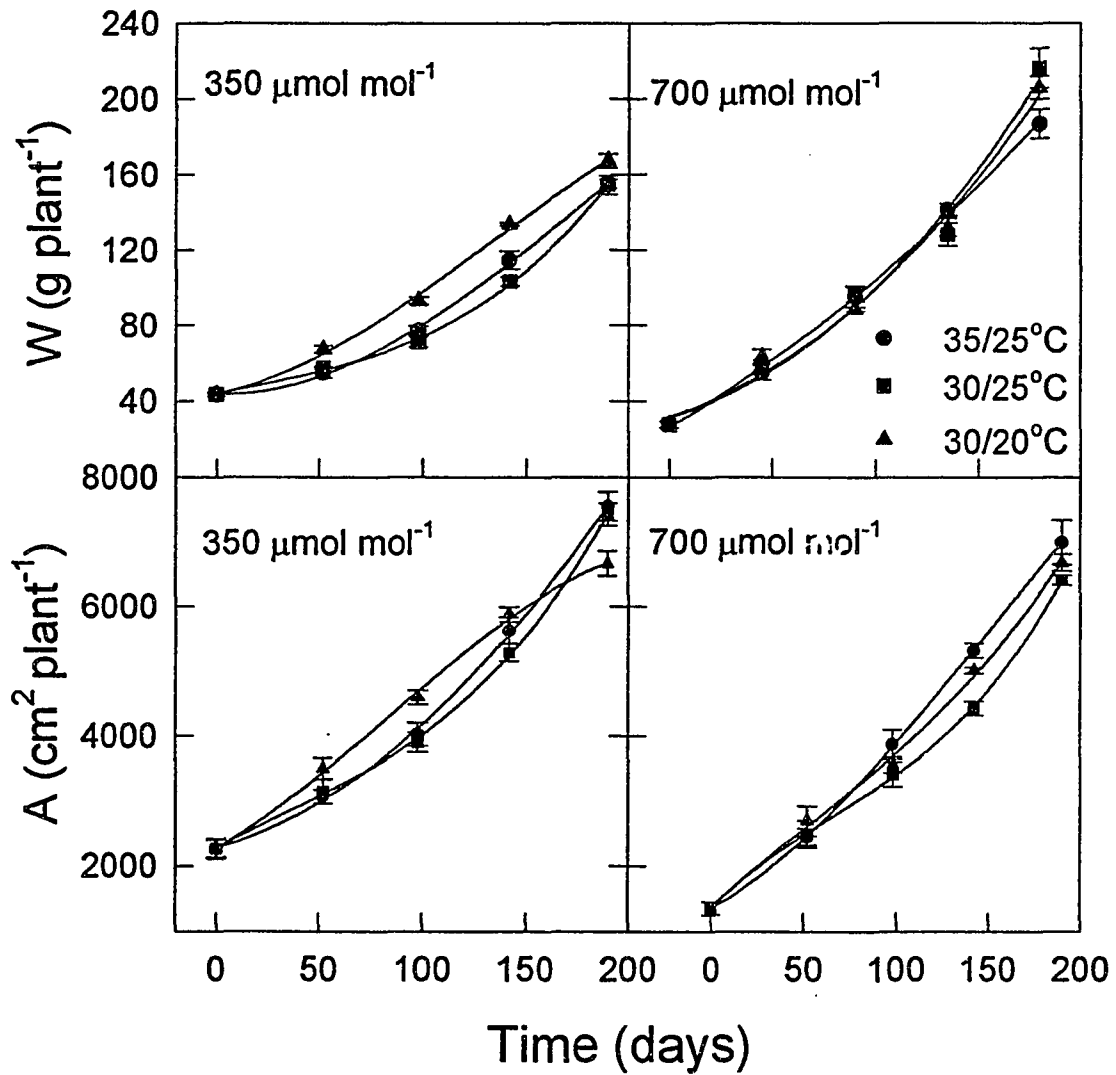


Fig. 4.1 Plant dry weight (W) and leaf area (A) of pineapple grown for six months at two CO₂ levels and three day/night temperatures. Data are means of 3 to 4 plants per treatment.

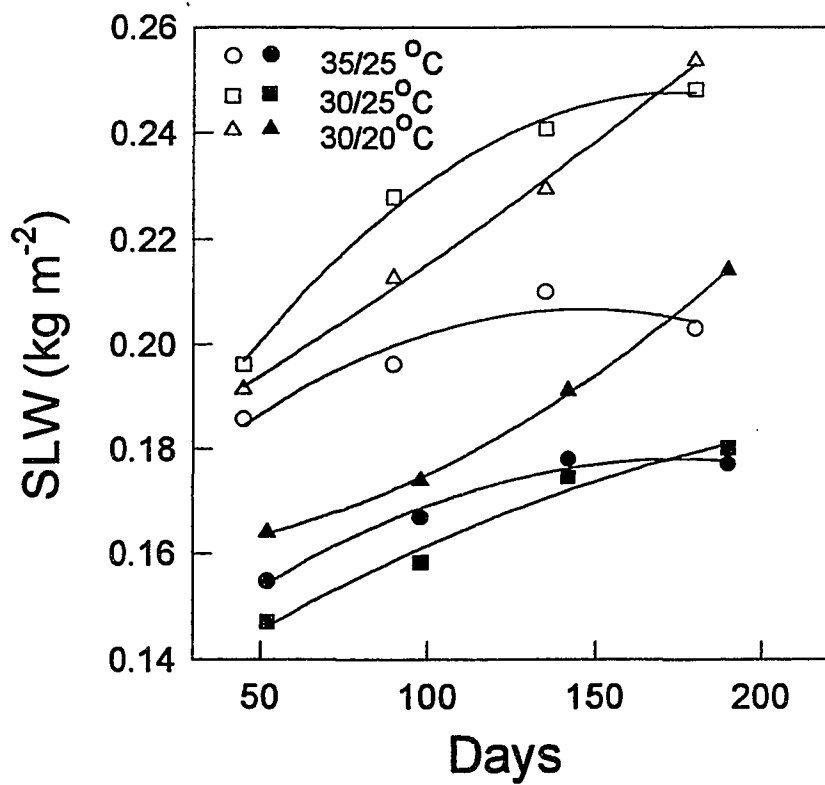


Fig. 4.2 Specific leaf weight (SLW) of pineapple grown six months at near ambient ($350 \mu\text{mol mol}^{-1}$, closed symbols) and elevated ($700 \mu\text{mol mol}^{-1}$, open symbols) CO₂ levels and three day/night temperatures.

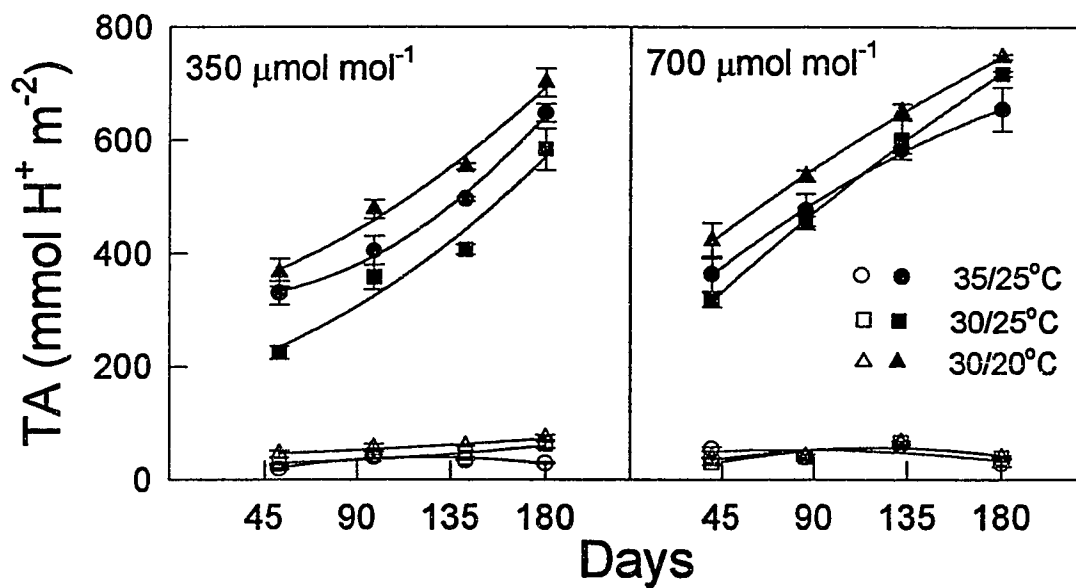


Fig. 4.3 Effects of CO₂ levels on the titratable acidity (TA) of the youngest physiologically mature leaves of pineapple grown at three day/night temperatures. Close symbols: am data, open symbols: pm data.

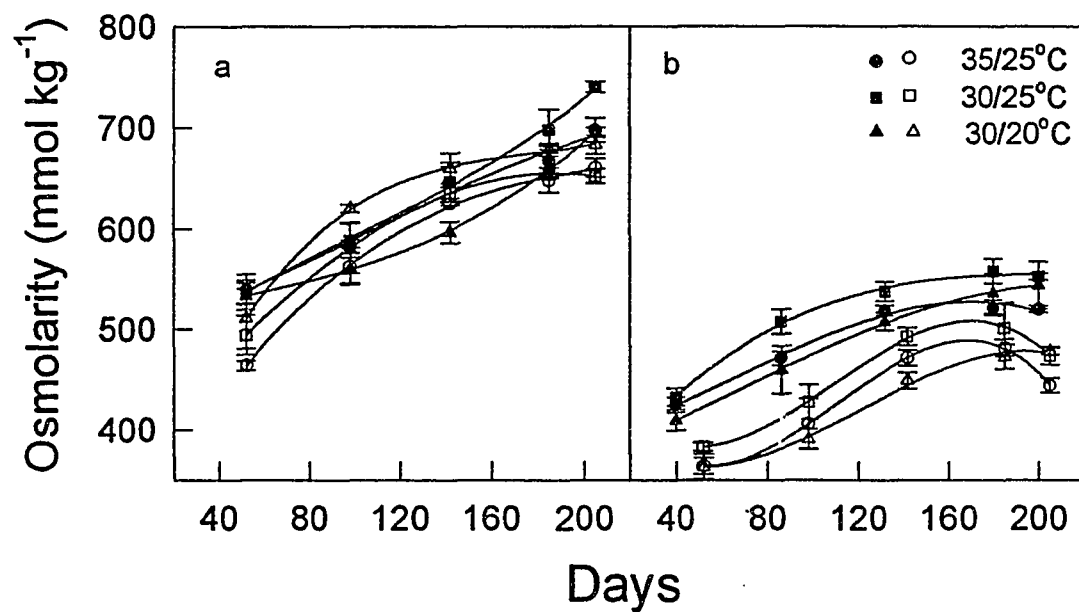


Fig. 4.4 Leaf sap osmolarity of the youngest physiologically mature leaf of pineapple grown six months at two CO₂ levels and three temperatures. Close symbols: 700 μmol mol⁻¹; open symbols: 350 μmol mol⁻¹. Predawn data: a; afternoon data: b.

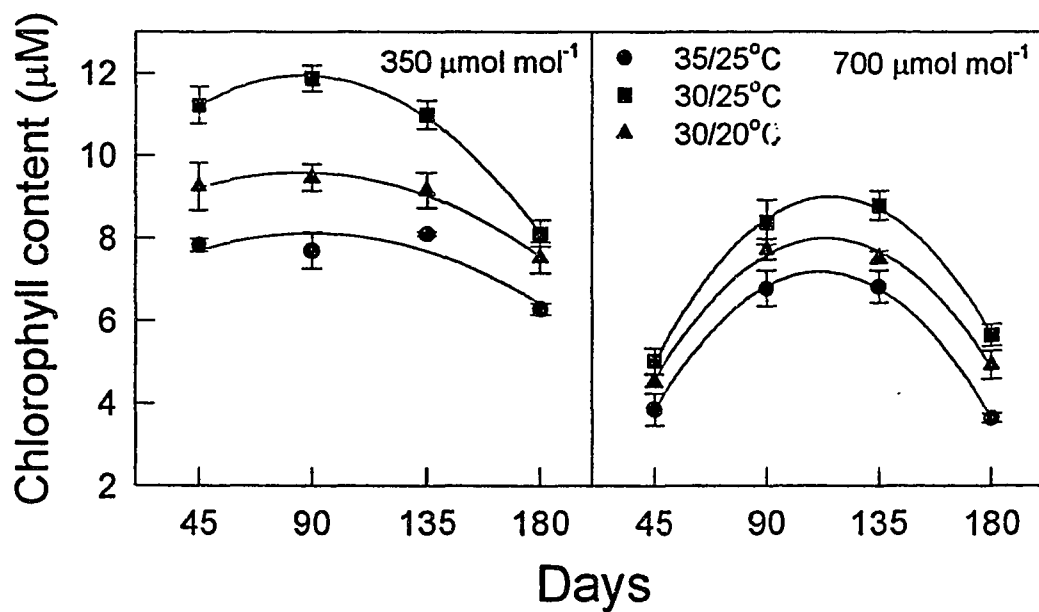


Fig. 4.5 Chlorophyll content (n=3 to 4) of the youngest physiologically mature leaves of pineapple during six months of growth at near ambient (350 µmol mol⁻¹) and elevated (700 µmol mol⁻¹) CO₂ and three day/night temperatures.

CHAPTER 5

WATER RELATIONS, GAS EXCHANGE AND ACIDIFICATION DURING DROUGHT FOR PINEAPPLE GROWN AT AMBIENT AND ELEVATED CO₂ AND THREE TEMPERATURES

ABSTRACT

The effect of water deficit on leaf water potential components, gas exchange and titratable acidity of pineapple (*Ananas Comosus* (L.) Merr.), a species having crassulacean acid metabolism (CAM), was studied under near ambient ($350 \mu\text{mol mol}^{-1}$) and elevated ($700 \mu\text{mol mol}^{-1}$) CO₂ levels and day/night temperatures of 35/25, 30/25 and 30/20 °C. Water was withheld from nine-month-old plants for two months. Leaf relative water content (RWC), titratable acidity (TA), total leaf water potential and osmotic potential, and chlorophyll fluorescence (F_v/F_m) were measured every 10 days during a two-month period. CO₂ and H₂O vapor exchange rates were measured every 5 days for 30 days, after which the diurnal gas exchange rate was negligible. The decrease in leaf water content during the drought was slower for plants grown at 30/25 and 30/20 °C and at high CO₂. After withholding watering for 10 days, total net CO₂ uptake in the light decreased from 27-37 to 13-15 mmol m⁻² at 35/25 and 30/25 °C, and from 57 to 30 at 30/20 °C for plants grown at low CO₂, but from 78-80 to 12-14 mmol m⁻² at 35/25 and 30/25 °C, and from 73 to 29 mmol m⁻² at 30/20 °C for plants grown at high CO₂, while the decrease in total CO₂ uptake in the dark was relatively closer. Therefore, the total daily CO₂ uptake decreased more rapidly for

plants grown at high than low CO₂. Stomatal conductance and H₂O vapor exchange were much lower at high CO₂, thus, leaf water content and water potential were higher during the drought. As a result of reduced nocturnal CO₂ uptake, predawn TA levels dropped to less than 20% of the original values after 40 days of drought for plants grown at 35/25 and 30/25 °C, while plants grown at 30/20 °C still maintained about 33-36% of the original level at the end of drought period. Leaf osmotic and water potentials initially were higher at low than at high CO₂ but the values decreased more rapidly over time at low CO₂ than they did at high CO₂. F_v/F_m values increased early in drought period, and then consistently decreased as water deficit continued.

INTRODUCTION

Species with crassulacean acid metabolism (CAM) have very low consumptive water loss relative to mesophytic plants due to such morphological, anatomical and physiological characteristics as inverted stomatal rhythm, low conductance in the light and small leaf surface to volume ratios (Osmond, 1989; Nobel, 1994). Pineapple, for example, sustains transpiration rates approximately one-tenth to one-twenty-fifth of those for mesophytic species (Ekern, 1965; Joshi et al., 1965; Neales et al., 1968; Bartholomew and Malézieux, 1994). These attributes permit CAM species to tolerate long periods of drought. Severe drought, however, still depresses endogenous physiological activities in CAM plants (Guralnick and Ting, 1987; Goldstein et al, 1991; Bastide et al., 1993).

A large amount of world agricultural land is located in areas having hot, arid

climates where crop production is largely limited by water availability (Parry, 1990). Soil water status is one of three important environmental factors in predicting the productivity of the CAM species *Agave deserti*, *Ferocactus acanthodes* and *Opuntia ficus-indica* (Nobel and Hartsock, 1986; Nobel, 1991). A soil water deficit can significantly alter the diurnal gas exchange pattern of CAM plants (Kluge and Ting, 1978). After prolonged drought, stem succulents have negligible CO₂ or H₂O vapor exchange and greatly damped nocturnal accumulation of organic acids due to the internal re-fixation of respiratory CO₂ (Szarek et al., 1973), which is termed CAM-idling (Ting, 1985).

Pineapple exhibits the typical features of CAM plants, showing substantial C₃-type CO₂ uptake in the light when well watered, but when plants are under water stress, most CO₂ is fixed at night (Bartholomew and Kadzimin, 1977; Bartholomew and Malézieux, 1994). Quite a few studies have been conducted on the impact of water availability on tissue water relations, stomatal responses, net CO₂ uptake and acidification of CAM species (Goldstein et al., 1991; Kluge and Ting, 1978; Smith et al., 1987; Guralnick and Ting, 1987; Bastide et al., 1993). However, no studies were found where the physiological responses of CAM species to water deficit were examined for plants grown at different CO₂ levels and temperatures. The interactive effects of CO₂ and temperature on the gas exchange characteristics of CAM species are expected to be quite different when soil water availability is limited. For instance, with watering twice weekly, the dry weight of the CAM plant *Agave vilmoriniana* was not significantly increased when the environmental CO₂ level was raised from 350 to

675 $\mu\text{mol mol}^{-1}$; however, when the irrigation was slightly greater than that in natural conditions (dry treatment), biomass at 675 $\mu\text{mol mol}^{-1}$ increased substantially relative to that obtained at 350 $\mu\text{mol mol}^{-1}$ (Idso et al., 1986). The hypothesis to be tested in this study was that better water status will be maintained at elevated than ambient CO_2 when pineapple is grown under prolonged drought. The objective of this study was to examine the responses of leaf water relations, diurnal gas exchange and nocturnal titratable acidity of pineapple, a species with Crassulacean acid metabolism, to water deficit when growing at near ambient and elevated CO_2 levels and in three day/night temperature regimes.

MATERIALS and METHODS

Plant Materials and Environmental Conditions

Pineapple crowns from the Smooth Cayenne clone champaka 153, obtained from a local pineapple company, were initially grown in pots outdoors for three months and then in controlled environment chambers for six months to fully adapt plants to the CO_2 and temperature treatments. Then, a water deficit was imposed on a subset of the plants by completely withholding irrigation for up to 70 days, while the control plants were watered every five days to keep the potting media moist. The photosynthetic photon flux density in the growth chambers was maintained at about 400 $\mu\text{mol m}^{-2} \text{s}^{-1}$ at the mid-plant height during the 12 h photoperiod. Air humidity in the chambers was not controlled. The treatments consisted of near ambient (355 ± 20 $\mu\text{mol mol}^{-1}$) and elevated (710 ± 50 $\mu\text{mol mol}^{-1}$) CO_2 concentrations, and day/night

temperatures of 35/25, 30/25, and 30/20°C. The plants were grown in 8-liter pots in a 1:1 (by volume) mixture of sunshine #4 (a commercial potting media) and horticultural perlite. Because the number of growth chambers was limited, two experiments were performed. The first experiment was begun in May and ended in July, 1994, and the plants were grown at low CO₂. The second experiment was begun in Jan. and ended in March, 1995, and the plants were exposed to high CO₂. The elevated CO₂ level in the second experiment was achieved by bleeding pure CO₂ into the growth chambers, and the CO₂ level was maintained by adjusting the flow rate from a cylinder containing pure CO₂ and the air exchange rate between the growth chambers and the outside. The CO₂ concentration in the chambers was measured frequently until the desired set point was established.

Physiological Measurements

Gas Exchange

All measurements were made on the youngest fully expanded and the tallest leaf on the plant from the ground level, termed the 'D' leaf in the pineapple literature and the tissues of choice for indexing growth, nutrient and water status of plants (Bartholomew and Kadzimin, 1977). Diurnal CO₂ uptake and H₂O vapor exchange were measured using an open gas exchange system consisting of a 1.57-liter leaf cuvette with a recirculating fan and water jacket connected to a refrigerated water bath to control the leaf temperature, a CO₂/H₂O infra red (IR) gas analyzer (Li-6262, Li-Cor, Inc., Lincoln, Nebraska, USA), and a dew point generator (Li-610, Li-Cor,

Inc.). A datalogger (21X, Campbell Scientific Inc., Logan, Utah, USA) was used to record data and control the operation of the gas exchange system via solid state relays and solenoid valves. The diurnal cycle of gas exchange was monitored with a 5-minute averaging interval over 24-h periods and the measurements were made at approximately 5, 10, 15, 20 and 30 days after withholding irrigation. The CO_2 sources for gas exchange measurements were the ambient air pumped from outside the building in the first experiment, and ambient air plus pure CO_2 from a cylinder containing pure CO_2 to average $710 \pm 50 \mu\text{mol mol}^{-1} \text{CO}_2$ in the second experiment. Net CO_2 and H_2O exchange rates, stomatal conductance (g_s) were calculated according to the procedures of Long and Hallgren (1985) and Christopher et al. (1989).

Titrateable Acidity, Osmotic and Water Potentials

Leaf titrateable acidity (TA) and osmolarity were measured on one 'D' leaf from each of four plants per chamber collected at the end of the dark period (predawn) and 15 minutes before the start of the next dark period (afternoon). The 'D' leaf for determining TA and osmolarity was split longitudinally and one-half was removed at predawn and frozen at -75°C to disrupt the cell membranes. The second segment of the leaf was harvested in the late afternoon and similarly frozen. To determine TA, two 1.3 cm diameter leaf discs were collected from the middle one-third of each frozen leaf segment, ground in a mortar with 20 ml of distilled water, and the total volume was titrated to pH 7.2 using 0.01 N NaOH. Tissue osmolarity was measured on leaf sap extracted from freeze-thawed tissues with a vapor pressure osmometer (5500S, Wescor Inc. Logan, Utah, USA), and then converted to osmotic

potential (ψ_x) according to the Van't Hoof relation (Nobel, 1991). A fresh leaf disc for determining water potential (ψ_w) was collected from the middle-third of the leaf. The ψ_w values were measured using a thermocouple psychrometer (Decagon Devices, Pullman, Washington). The instrument was calibrated against standard calibration solutions, and the values were converted to ψ_w using the Van't Hoof relation (Nobel, 1991).

Relative Water Content (RWC)

Leaf RWC was measured on leaf discs at the beginning (morning) and end (afternoon) of the photoperiod at 10-day intervals over the 70-day drought period. RWC was calculated by the equation:

$$RWC = \frac{\text{fresh weight} - \text{dry weight}}{\text{turgid weight} - \text{dry weight}} \times 100 \quad (5.1)$$

Chlorophyll Fluorescence

Chlorophyll fluorescence was measured using a portable SF-20 plant productivity fluorometer (Richard Brancker Research Ltd. Ottawa, Ontario, Canada). This technique permits fast in-situ measurement of initial fluorescence yield (F_0) and maximum fluorescence yield (F_m) (dark adapted). Variable fluorescence (F_v) was calculated as $F_m - F_0$, and the photochemical efficiency was estimated as F_v/F_m (Jones, 1992). F_0 and F_m values were measured on dark-acclimated (30 minutes) 'D' leaves about 15 minutes before the onset of morning illumination (phase II) and 4 hours after midday (phase IV) when the C_3 -type photosynthetic activity was expected to be high. Since only a few measurements of F_v/F_m were made for plants grown at low

CO₂, only data collected every 10 days for 70 days for plants grown at high CO₂ are presented.

RESULTS

Water Relations

Both predawn and afternoon relative water content (RWC) decreased continuously over the 70-day period, and the rate of decrease was more rapid at temperatures of 35/25 and 30/25 °C than at 30/20 °C (Fig. 5.1). At the onset of the water deficit, leaf RWC was somewhat higher for plants grown at low CO₂ compared to plants grown at high CO₂ (Fig. 5.1). By the end of the drought, predawn RWC was comparable between low and high CO₂ for plants grown at 35/25 and 30/25 °C, and 6.5% lower at high CO₂ for plants grown at 30/20 °C

The effect of CO₂ levels on the RWC was not consistent among temperature regimes. Decrease in RWC was comparable at both low and high CO₂ for plants grown at 30/20 °C, while RWC decreased more rapidly at low than at high CO₂ for plants grown at 35/25 and 30/25 °C (Fig. 5.1). The difference in RWC between temperature treatments was about 3-fold greater for plants grown at low CO₂ than at high CO₂ near the end of drought period (Fig. 5.1). Afternoon RWC similar to predawn values. Therefore, for plants grown at elevated temperatures, leaf tissues of pineapple conserved more water at high CO₂ than at low CO₂ after a 70-day drought.

Predawn water potential (ψ_w) at low CO₂ dropped markedly during the two-month drought, with the greatest decline at 35/25 and 30/25 °C (Fig. 5.2). At high

CO₂, predawn ψ_w was more negative at the onset of the water deficit and steadily increased, followed by a slight decrease after 40 days of drought (Fig. 5.2). Changes in the predawn osmotic potentials (ψ_s) was smaller than but similar to the changes in ψ_w for plants grown at both low and high CO₂ during the drought period, with the exception of the treatment at 30/20 °C in high CO₂ which remained relatively constant during the drought period (Fig. 5.2). Predawn turgor pressure (ψ_p) decreased steadily for plants grown at low CO₂, but at high CO₂ the ψ_p values initially increased and then remained relatively constant at 35/25 and 30/25 °C or decreased at 30/20 °C for rest of drought period (Fig. 5.2).

Gas Exchange, Acidification and Chl Fluorescence

Measurement of gas exchange and stomatal conductance started five days after the onset of drought, before which net CO₂ uptake remained relatively constant (Zhu, unpublished data). After withholding watering for 10 days, the CO₂ exchange rate dropped substantially (Fig. 5.3). The greatest decrease occurred early in the light (phase II) or more in the late afternoon (phase IV) (Fig. 5.3). After 10 days without watering, CO₂ exchange rates were reduced relatively more at high than at low CO₂, especially in the 30/25 and 35/25 treatments (Fig. 5.3). In addition to the damped amplitude, at or after 15 days of drought, nocturnal CO₂ uptake underwent a phase shift, reaching the peak uptake rate at the end of the dark period rather than at the beginning (Fig. 5.3).

The diurnal stomatal conductance (g_s) exhibited a pattern similar to that for

CO₂ uptake (Fig. 5.4). g_s decreased steadily with time after drought started, with greater reduction for plants grown at high CO₂ and elevated temperatures (35/25 and 30/25 °C) (Fig. 5.4). After 15 days of drought, stomata opened later in the night, and the maximum g_s shifted towards the end of dark period, especially at high CO₂ (Fig. 5.4). H₂O vapor exchange rates were consistent with data for g_s (Fig. 5.5). The rate of decrease in H₂O vapor exchange with increasing water deficit was also greater for plants grown at high CO₂ and at elevated temperatures (Fig. 5.5). After about 15 days of water stress, H₂O vapor exchange rates at high CO₂ increased in a stepwise fashion during the later part of phase I (Fig. 5.5).

Total net CO₂ uptake over the light and dark periods decreased substantially during the 30-day period after the onset of drought for plants grown at both low and high CO₂, with the greatest reduction in the light period (Table 5.1). At low CO₂, the decrease in CO₂ uptake in the light was comparable among the three temperature regimes, while the decrease in nocturnal uptake was much greater at 35/25 and 30/25 °C (Table 5.1). At high CO₂, CO₂ uptake in both the light and dark decreased more rapidly at 35/25 and 30/25 °C than at 30/20 °C (Table 5.1). The decrease in CO₂ uptake was much greater for plants grown at high CO₂ during the early drought period. The decrease in total H₂O vapor exchange was basically analogous to that of net CO₂ uptake, with a greater decrease occurring at high CO₂ and at the higher temperatures of 35/25 and 30/25 °C (Table 5.1).

Predawn titratable acidity (TA) was greatly reduced by water deficit, and gradually approached the afternoon values as drought continued for plants grown at

35/25 and 30/25 °C, while afternoon TA values were unaffected by CO₂ levels or temperature treatments (Fig. 5.6). Predawn TA levels at 30/20 °C remained much higher than the afternoon values, even at the end of drought period (Fig. 5.6). Initial TA values at the low and high CO₂ levels were comparable for plants grown at 35/25 °C, and greater in high CO₂ at 30/25 and 30/20 °C. After 10 days of withholding water that there were no consistent effects of CO₂ levels on leaf TA values (Fig. 5.6).

For well watered plants, both predawn and afternoon F_v/F_m values remained relatively constant over the 70-day drought (Fig. 5.7). For water-stressed plants, predawn F_v/F_m increased slightly at the onset of water deficit, followed by a steady decrease until the end of the drought, while the afternoon values increased for nearly 30 days, and then decreased thereafter (Fig. 5.11). On most days, the highest values were obtained from plants grown at 30/20 °C. Similar changes in F_v/F_m to drought was also observed for plants grown at low CO₂, but detailed data for the entire drought period were not collected. For plants under water stress, the greatest F_v/F_m values were consistently obtained at 30/20 °C, while the lowest values were measured at 30/25 °C during most of the drought period and shifted to 35/25 °C regime near the end of the drought (Fig. 5.7).

DISCUSSION

Although pineapple is a drought avoidant plant (Bartholomew and Malézieux, 1994), withholding watering for two months significantly reduced the leaf RWC. The

day/night temperature regime had a greater effect on leaf RWC than did CO₂ levels. Due to the fact that stomatal conductance in the light decreased rapidly early in the drought, the leaf gas exchange primarily occurred in the dark. Thus, night temperature played a major role in controlling CO₂ and H₂O vapor exchange, leaf water content and water potential components during the drought. Leaf RWC did decrease more slowly at high than at low CO₂ during the drought, and the diurnal fluctuations were smaller between temperature treatments for plants grown at high CO₂. This was the consequence of decreased leaf transpiration due to reduced stomatal conductance at high CO₂. The slight increase in leaf RWC for plants grown at high CO₂ and 30/20 °C during the initial phase of the drought resulted possibly from the reduced transpiration and stomatal conductance induced by both high CO₂ and water deficit, while water absorption by the root system continued when the soil was still moist.

At onset of drought, the more negative water potential components for plants grown at high than at low CO₂ might be due to the lower initial leaf RWC and the greater leaf sap osmolarity as a result of greater accumulation of organic acids in those plants when soil was moist. There was no obvious explanation for the lower RWC occurred early in the drought for plants grown at high CO₂. It was unclear why water potential components increased for plants grown at high CO₂ during the early half of drought period.

CO₂ uptake in pineapple was more sensitive to water deficit in the light than in the dark, a response common to CAM species (Kluge and Ting, 1978; Winter, 1985;

Winter et al., 1992; Cote et al., 1992). Elevated CO₂ enhanced CO₂ uptake of pineapple relatively more in the light than in the dark (Zhu, 1996), thus accounting for the greater decrease in CO₂ uptake for plants grown at high CO₂ as the drought continued. Maximum stomatal opening of pineapple as indicated by g_s occurred later in the dark as water deficit became severe, and similar results have been obtained for some CAM species (Nobel, 1994). As a result, the maximum nocturnal CO₂ and H₂O exchange rates shifted towards the end of dark period, which in turn lowered the nocturnal gas exchange and predawn TA values. This effect on leaf gas exchange was particularly evident for plants grown at elevated CO₂ and 25 °C night temperature. During the drought, stomates became fully closed in the light and opened later in the dark. Therefore, during the drought period, nocturnal temperature was the key factor in controlling leaf gas exchange, by its direct effect on stomatal aperture, and indirectly by reducing tissue water content. This explains the greater reduction in daily CO₂ uptake at nighttime temperature of 25 °C than occurred at 20 °C.

With adequate water supply, nocturnal CO₂ uptake and acidification of pineapple were enhanced by elevated CO₂ (chapter 3, 4). Similar results were obtained early in the drought, but as water became depleted and stress more severe, the enhancement due to elevated CO₂ disappeared. The combination of water deficit and elevated CO₂ resulted in a rapid closure of the stomata. This combined effect could reduce the carbon gain contributed by elevated CO₂, as occurred with well-watered plants, decrease the nocturnal CO₂ uptake, and thus sacrifice CO₂ assimilation to conserve tissue water. Greatly but continued damped diurnal fluctuations in TA

levels as the drought proceeded indicated the occurrence of CAM-idling in pineapple leaf tissue. CAM-idling is characterized by continued but very low diurnal oscillations in organic acids due to almost solely to the re-fixation of respiratory CO₂ with no apparent fixation of external CO₂ (Szarek et al., 1973; Ting, 1985). This CAM-idling was particularly pronounced for plants grown at 35/25 and 30/25 °C after about 40 days of drought, but was not observed for plants grown at 30/20 °C, even after two-months of water deficit. In the latter treatment, leaves continued to assimilate external CO₂ in the dark with about 33-36% of the original rates. The above data indicated that in a warm dark-period environment, soil water deficit might impose a relatively but not an absolutely greater limitation on leaf gas exchange and acidification for pineapple grown at high CO₂ than does the current environment.

Photochemical efficiency (F_v/F_m) describes the proportion of absorbed light energy used by photochemical processes, e.g. generation of ATP and NADPH (Jones, 1992). Increased F_v/F_m values in pineapple early in the drought period indicated that C₃-type photosynthetic activity was enhanced by mild water stress. F_v/F_m values also increased during the early stages of water deficit for *Clusia uvitana*, a facultative CAM species (Winter et al., 1992). Greater F_v/F_m values might indicate that rate of decarboxylation of organic acids was increased, resulting in a release of a large amount of CO₂ internally (Winter et al., 1992), and therefore enhancing the production of carbohydrates via the C₃ pathway. Elevated CO₂ assimilation in the dark was also observed during the initial phase of drought for pineapple grown at 30/25 and 30/20°C in this study. Similar results were also reported for pineapple by others

(Cote et al., 1992; Zhu, unpublished data). Nocturnal CO₂ uptake was enhanced up to nine days after withholding watering from the facultative CAM species *Clusia uvitana* (Winter et al., 1992). Idso et al. (1986) reported that the growth rates of the CAM plant *Agave vilmoriniana* were significantly increased at elevated CO₂ when plants received slightly more water than was available in natural conditions, while the well-watered plants (irrigated twice per week) lacked such a response. Higher nocturnal acidification would result in greater decarboxylation of the organic acids in the light and thus raise the c_i value, which might account for the increased F_v/F_m ratio during the early period of drought for pineapple under water stress. These evidences might indicate that mild water stress could either temporarily enhance the assimilatory capacity in pineapple, such as the increase in PEPcase activity (Cote et al., 1992), or imply that the irrigation practice used in this study provided more water than was required. After extended drought, the stronger quenching in F_v/F_m values for plants grown at higher temperatures may suggest greatly reduced c_i levels due presumably to substantially damped nocturnal accumulation of organic acids.

In conclusion, although pineapple can survive in habitats where water is scarce, water deficit will inevitably impose limitations on its physiological activities. The decrease in CO₂ fixation due to water deficit was more rapid for plants grown at elevated CO₂ and temperatures than was true at ambient levels. Thus, in an environment where elevated CO₂ and temperatures are predicted to occur in the next century, CO₂ fixation of pineapple would be reduced more rapidly than would occur in the current environment if a soil water deficit occurred. However, greater tissue

water content and leaf turgor pressure would help sustain some of physiological activities, such as CAM-idling, for a longer period during the prolonged water deficit.

Table 5.1 Responses of integrated net CO₂ uptake and H₂O vapor exchange to water deficit during the indicated periods for the youngest physiologically mature leaves of pineapple grown at two CO₂ levels and three day/night temperatures.

CO ₂ levels ($\mu\text{mol mol}^{-1}$)		Day/night temperature ($^{\circ}\text{C}$)					
Time ^a		35/25		30/25		30/20	
		Day	Night	Day	Night	Day	Night
Net CO ₂ uptake ($\text{mmol m}^{-2} \text{ period}^{-1}$)							
350	05	27.0	208.1	37.0	227.0	57.3	360.2
	10	14.7	169.0	13.3	194.4	29.5	353.3
	15	2.3	86.9	3.2	84.7	4.7	258.1
	20	1.7	56.4	1.9	34.5	2.3	172.3
	30	0.2	10.1	0.6	16.6	1.2	129.4
700	05	78.1	287.0	80.2	224.4	73.0	280.4
	10	11.8	136.8	14.1	141.1	28.8	260.2
	15	1.5	82.2	2.9	74.5	7.2	184.1
	20	1.2	38.4	2.2	57.4	2.1	111.2
	30	0.6	9.0	1.8	42.6	1.9	91.0
H ₂ O vapor exchange ($\text{mol m}^{-2} \text{ period}^{-1}$)							
350	05	11.4	17.1	8.2	18.7	13.2	19.5
	10	9.4	15.5	5.6	15.2	6.9	22.5
	15	3.0	9.1	3.0	7.5	3.7	18.8
	20	1.1	5.4	1.8	5.5	2.8	10.7
	30	0.4	2.3	0.4	5.4	0.4	7.3
700	05	10.1	17.4	8.9	15.2	7.6	12.9
	10	9.2	11.7	3.4	10.0	4.9	12.9
	20	0.2	7.0	0.9	5.4	0.6	8.3
	30	0.2	2.8	0.4	3.7	0.4	4.6
	15	0.1	0.4	0.3	3.3	0.2	2.9

^a: Days after withholding irrigation.

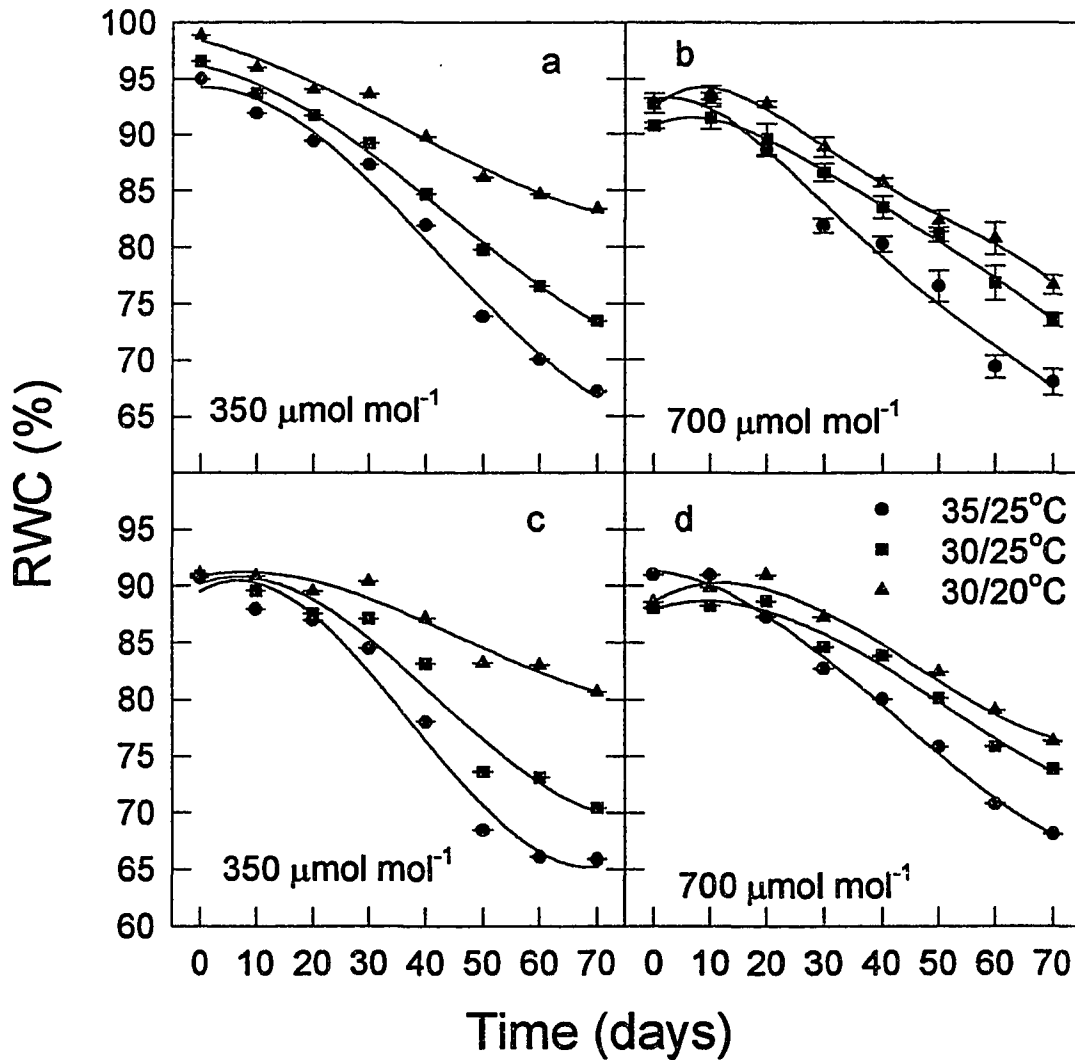


Fig. 5.1 Relative water content (RWC) the youngest physiological mature leaves of pineapple grown at two CO₂ levels and three day/night temperatures during a 70-day drought period. a,b; predawn; c,d; afternoon.

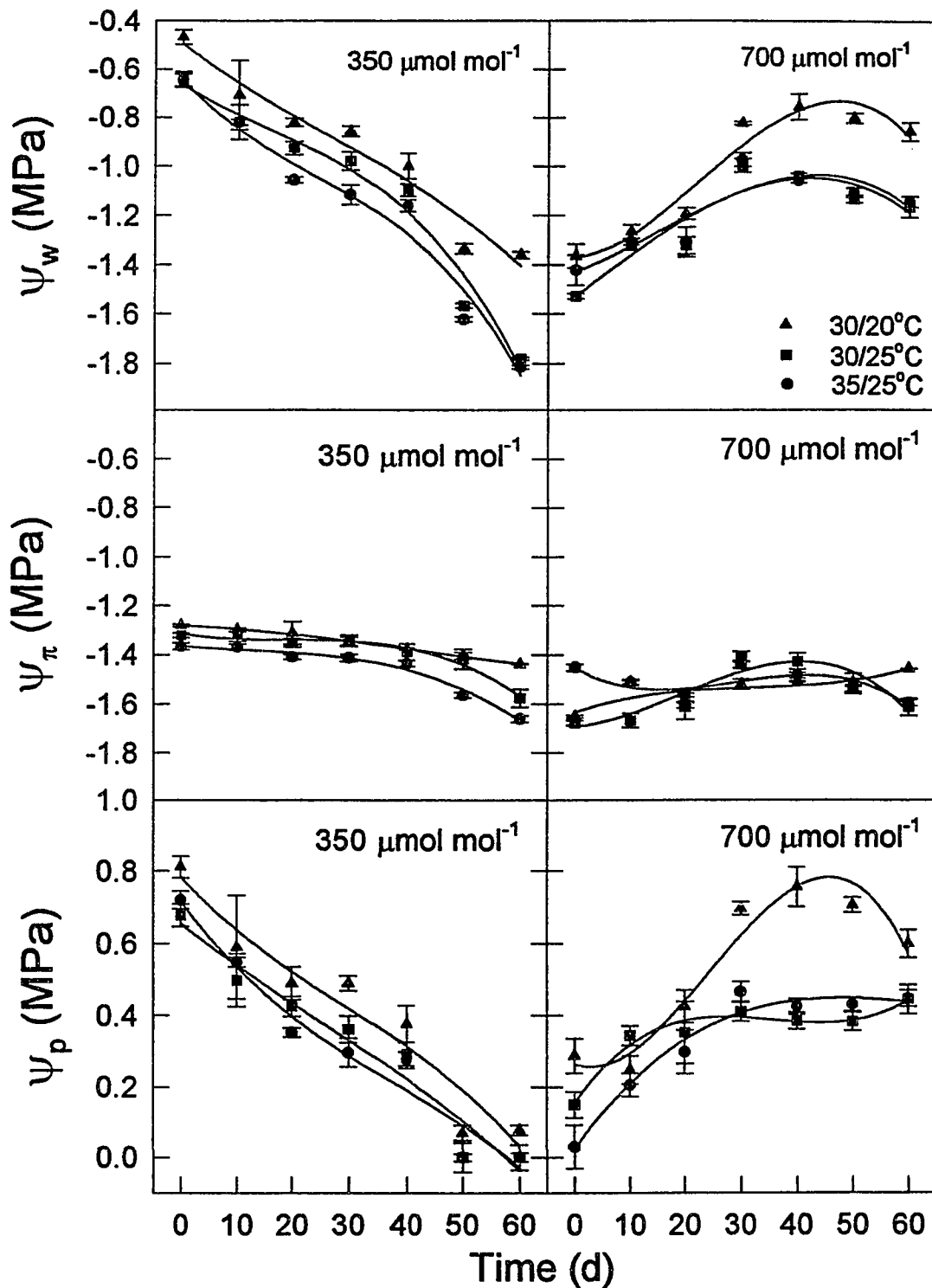


Fig. 5.2 Predawn water (ψ_w), osmotic (ψ_π) and turgor (ψ_p) potentials of the youngest physiologically mature leaves of pineapple grown at two CO_2 and three day/night temperatures during a 60-day drought period.

CO₂ exchange rate ($\mu\text{mol m}^{-2}\text{s}^{-1}$)

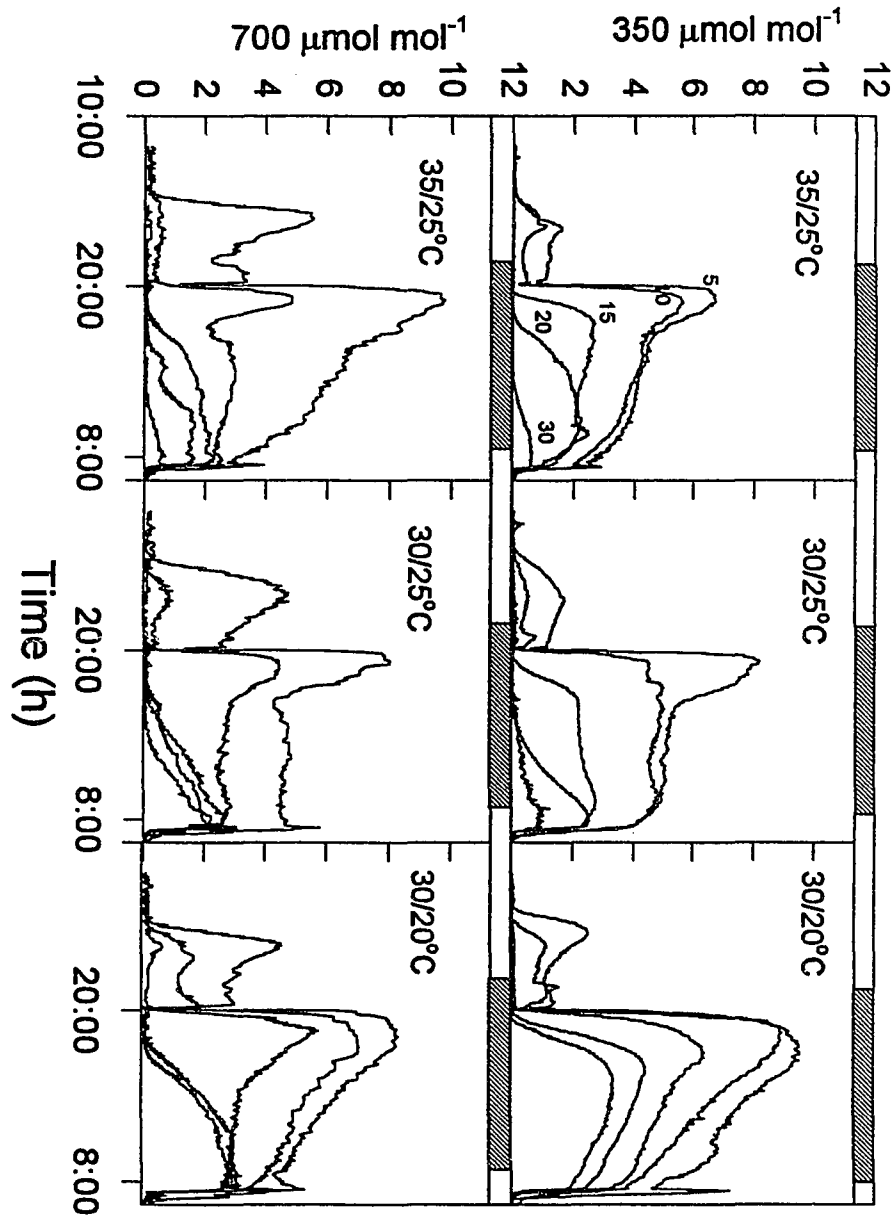


Fig. 5.3 CO₂ exchange rates of the youngest physiological mature leaves of pineapple grown at two CO₂ levels and three temperatures measured on 5, 10, 15, 20 and 30 days after withholding irrigation. Shaded bars indicate the dark period.

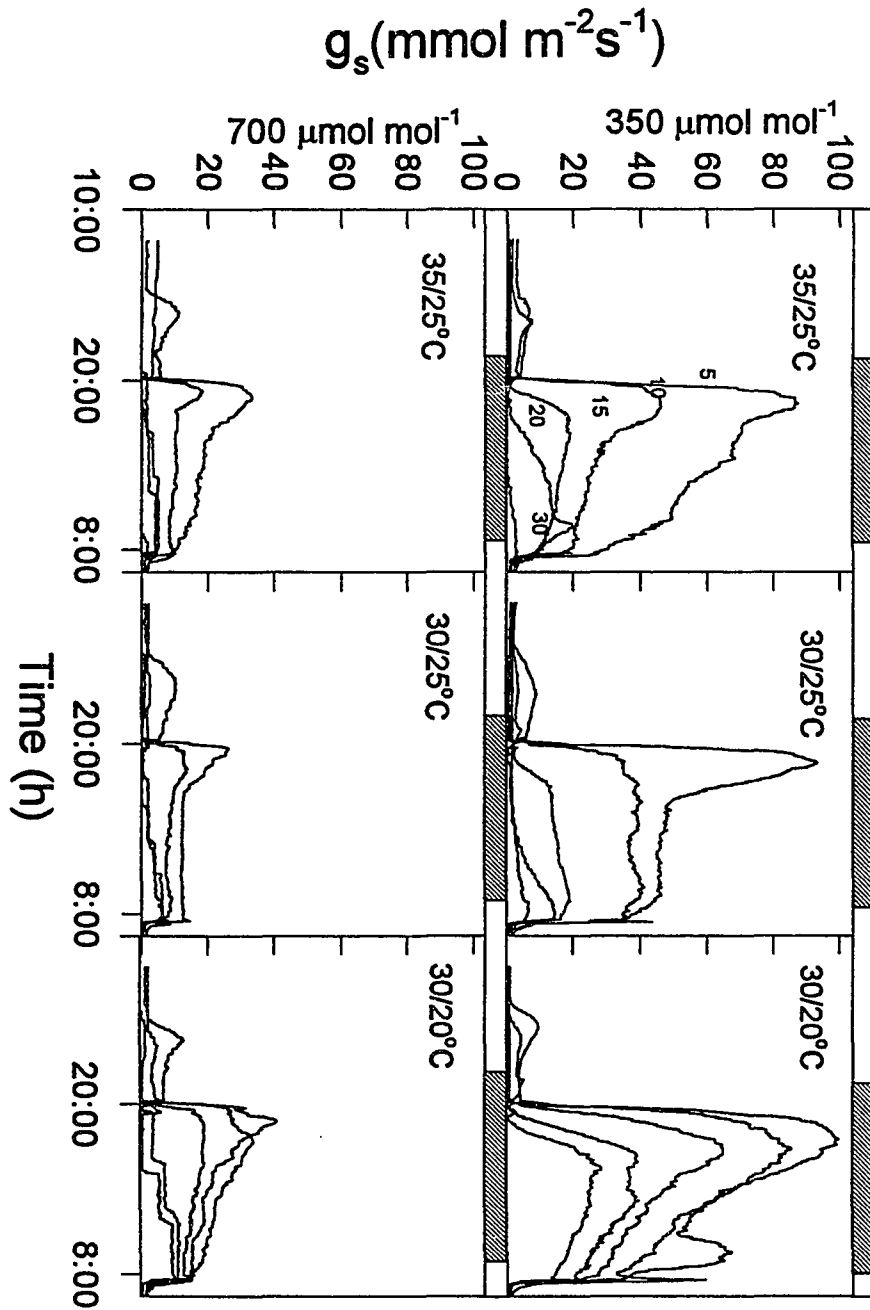


Fig. 5.4 Stomatal conductance (g_s) of the youngest physiological mature leaves of pineapple grown at two CO_2 levels and three temperatures measured on 5, 10, 15, 20 and 30 days after withholding irrigation. Shaded bars indicate the dark period.

H₂O vapor exchange rate (mmol m⁻²s⁻¹)

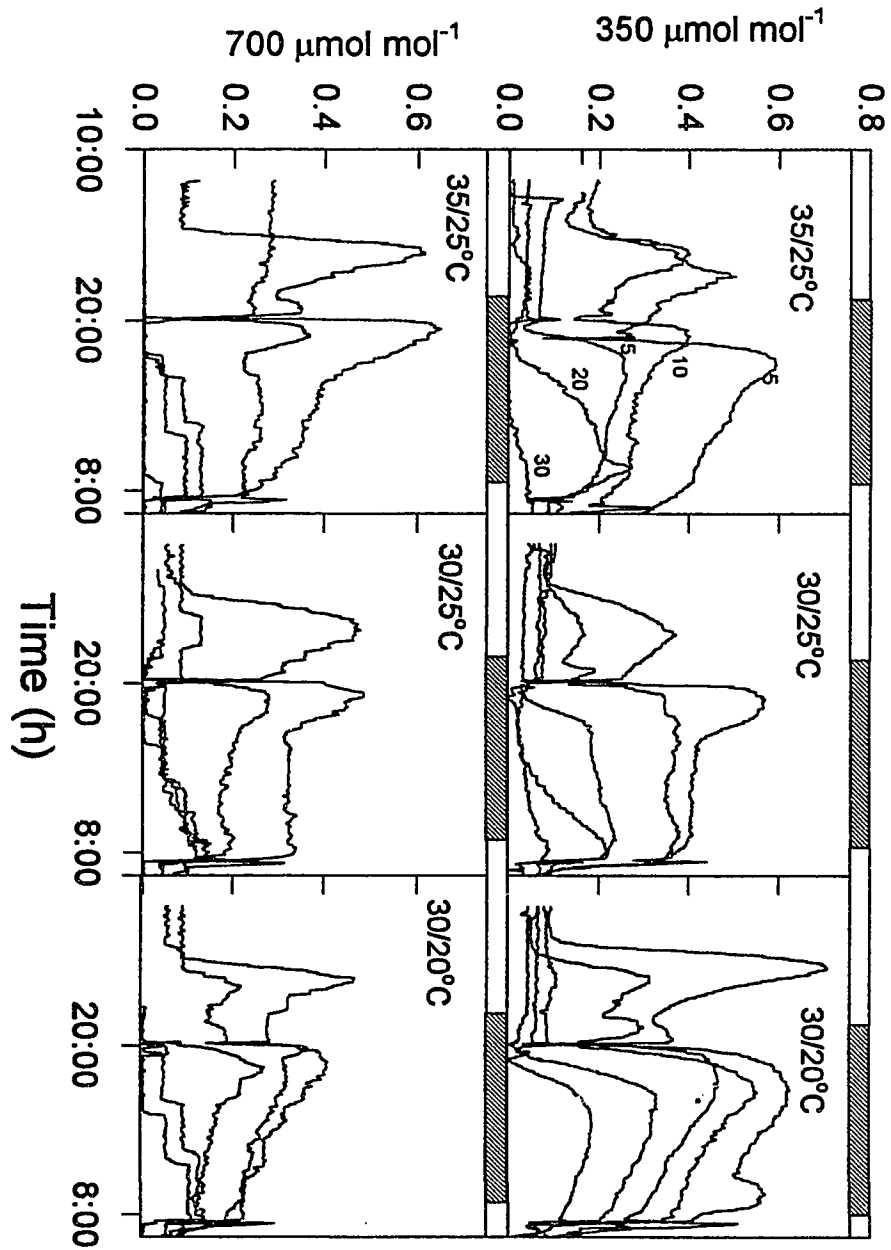


Fig. 5.5 H₂O vapor exchange rates of the youngest physiological mature leaves of pineapple grown at two CO₂ levels and three temperatures measured on 5, 10, 15, 20 and 30 days after withholding irrigation. Shaded bars indicate the dark period.

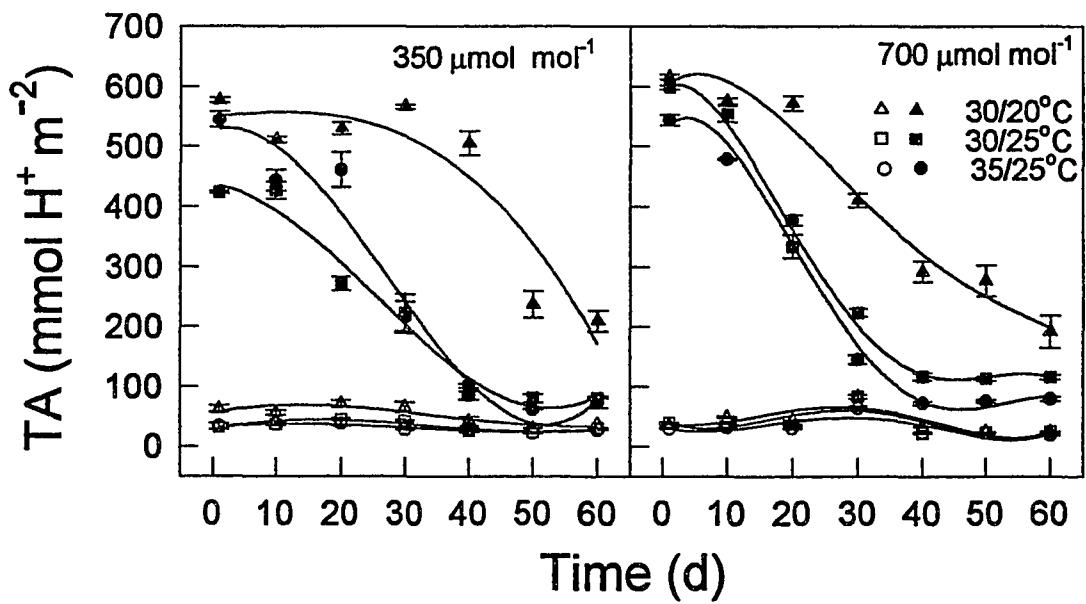


Fig. 5.6 Titratable acidity (TA) during a 60-day drought period for the youngest physiologically mature leaves of pineapple grown at two CO₂ and three day/night temperatures. Close symbols; predawn TA; open symbols: pm TA.

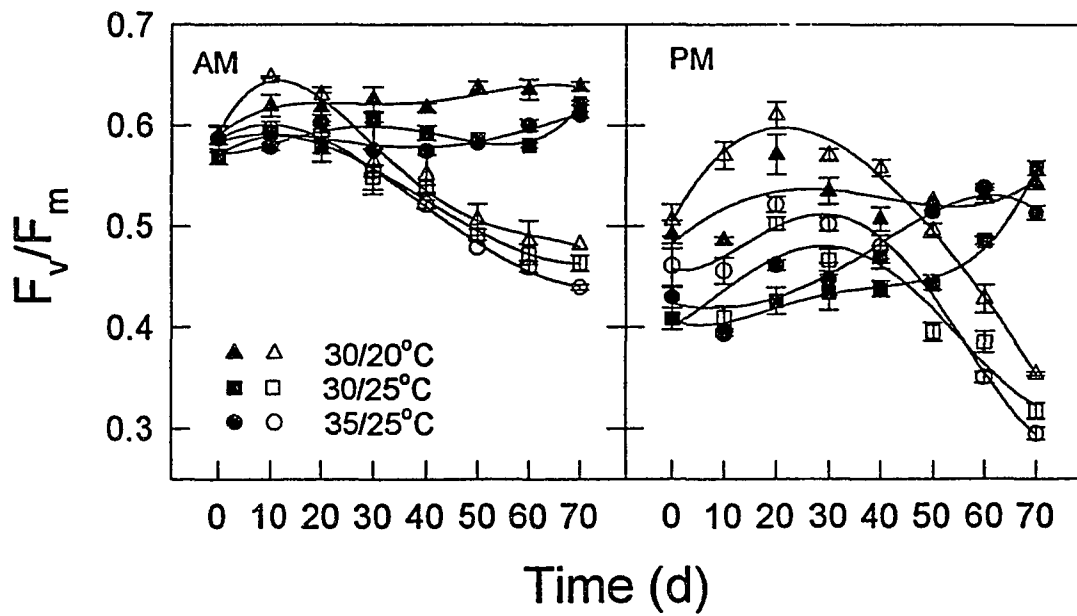


Fig. 5.7 Chlorophyll fluorescence (F_v/F_m) during a 70-day drought period for the youngest physiologically mature leaves of pineapple grown at $700 \mu\text{mol mol}^{-1} \text{CO}_2$ and three temperatures. Open symbols; droughted; close symbols: watered.

CHAPTER 6

PHYSIOLOGICAL RESPONSES TO AMBIENT AND ELEVATED CO₂ IN PINEAPPLE GROWN IN OPEN-TOP CHAMBERS

ABSTRACT

Pineapple (*Ananas comosus* (L.) Merr.), a species having crassulacean acid metabolism (CAM), was grown in open-top chambers for four months at near ambient ($\sim 330 \mu\text{mol mol}^{-1}$) and elevated ($\sim 730 \mu\text{mol mol}^{-1}$) CO₂ levels to study the effect of elevated CO₂ on growth and physiological responses. The mean day/night air temperature in the chambers was about 40/24°C. The non-photochemical quenching coefficient (q_N) of leaves was about 45% lower in the early morning for plants grown at high CO₂. Both photochemical quenching (q_P) and q_N in the afternoon were comparable between low and high CO₂ levels. Thus, the chlorophyll fluorescence kinetics indicated that the fixation of external CO₂ via the C₃ pathway was enhanced in the morning, but not in the afternoon, possibly due to the high leaf temperature. The diurnal differential in titratable acidity of plants at high CO₂ reached 347 mmol H⁺ m⁻², which was up to 42% greater than that obtained from plants at low CO₂. Carbon isotopic discrimination (Δ) of plants was 3.75‰ at low CO₂ and 3.17‰ at high CO₂, indicating that CO₂ uptake via the CAM pathway was enhanced more by elevated CO₂ than was uptake via the C₃ pathway. Average plant dry weight was 180 (g plant⁻¹) at high CO₂ and 146 (g plant⁻¹) at low CO₂, with more biomass partitioned to stem and root but less to leaf for plants grown at high CO₂. Leaf thickness was 11% greater at high than at low CO₂. There was no effect of CO₂ levels on leaf

nitrogen or chlorophyll contents after a four-month exposure to elevated CO₂.

INTRODUCTION

Atmospheric CO₂ levels have steadily increased from 275 to 350 μL L⁻¹ since the beginning of the industrial revolution (Sarmiento and Bender, 1994). The CO₂ level has been predicted to double by the middle of the next century (Cure and Acock, 1986). Biomass accumulation by plants that fix CO₂ via the C₃ photosynthetic carbon reduction cycle (C₃ plants) was substantially increased in elevated CO₂ but had little effect on C₄ species (Cure and Acock, 1986). As might be expected from the responses of C₄ species, elevated CO₂ had little effect on nocturnal CO₂ assimilation of some CAM species, but did enhance carbon assimilation of these species in the light (Nobel and Hartsock, 1986; Szarek et al., 1987). However, other studies with CAM plants showed that CO₂ uptake also increased in the dark at elevated CO₂ (Winter, 1985; Black, 1986; Nobel and Israel, 1994; Raveh et al., 1995). A few studies indicated that nitrogen (N) content in leaf tissue of some C₃ plants decreased after prolonged exposure to elevated CO₂ (Rowland-Bamford et al., 1991; Coleman et al., 1991; Wong, 1990; Ryle and Powell, 1992). The reduction in N content was partially responsible for the decreases in tissue chlorophyll content and activity of ribulosebisphosphate carboxylase/oxygenase (Rubisco) (Sage et al., 1987).

Pineapple (*Ananas comosus* (L.) Merr.), a species having Crassulacean acid metabolism (CAM), exhibits the four phases of CO₂ fixation typical of CAM species (Osmond, 1978). Phase I occurs in the dark when CO₂ is assimilated by the enzyme

phosphoenolpyruvate (PEP) carboxylase (PEPCase) and stored as malic acid in cell vacuoles. Phase II occurs at the beginning of the light period during the transition from the carboxylation of PEP by PEPCase to carboxylation of ribulosebiphosphate (RuBP) by ribulosebiphosphate carboxylase/oxygenase (Rubisco). During phase III, the stomates are closed, malic acid is decarboxylated and the CO₂ thus released is assimilated by Rubisco into carbohydrates via the C₃ cycle. Phase IV occurs later in the light period when external CO₂ is directly fixed via the C₃ pathway.

Chlorophyll (Chl) fluorescence induction kinetics (Kautsky effect) is a powerful method for studying the responses of photosynthetic characteristics to various environmental factors (Krause and Weis, 1984; Krause, 1991). Fluorescence signals permit the estimation of the photochemical (q_p) and non-photochemical (q_N) fluorescence quenching coefficients, and the photochemical efficiency of photosystem II (PSII) (F_v/F_m) (Jones, 1992). The coefficient q_p is a measure of the proportion of excitation energy captured by open centers of PSII (Krause and Weis, 1984, 1988; Krause, 1991; Jones, 1992). The coefficient q_N estimates the proportion of energy that does not contribute to biochemical processes in photosynthesis, but is lost through such process as the thermal dissipation of excitation energy (Hormann et al., 1994; Raskin et al., 1993). The ratio of variable (F_v) to maximum (F_m) fluorescence (F_v/F_m) is an indicator of the proportion of absorbed light energy used in photochemical processes and measures the efficiency of the open centers to transport electrons (Jones, 1992). Chl fluorescence kinetics thus can help to interpret the results of the effects of environmental factors on plant photosynthesis (Guralnick et al., 1992).

Stable carbon isotopic discrimination (Δ) is an effective technique for determining the relative contribution of the day (C_3) and night (CAM) CO_2 fixation pathways to total carbon assimilation in CAM species (Ehleringer and Osmond, 1989; Griffiths, 1988; Griffiths, 1992). Smaller Δ values indicate enrichment in ^{13}C relative to ^{12}C in leaf tissues, reflecting higher CAM activity, while higher Δ values indicate relatively greater C_3 -type activity (Ehleringer and Osmond, 1989; Griffiths, 1992). Therefore, Δ value provides an effective tool for characterizing the integrated effect of both C_3 and CAM activities in carbon assimilation of pineapple. This study was designed to investigate the growth and photosynthetic responses of pineapple (*Ananas comosus* (L.) Merr.), a species with CAM, to near ambient and elevated CO_2 in open-top chambers.

MATERIALS and METHODS

Plant Materials and Chamber Conditions

Fruit tops (crowns) of smooth cayenne pineapple obtained from a local pineapple company were grown in pots in a 1:1 (by volume) mixture of black cinder and horticultural vermiculite for four months from Jan. 1 to April 30, 1995 prior to imposing the CO_2 treatments. Then the plants were exposed to near ambient ($330 \pm 20 \mu\text{mol mol}^{-1}$) and elevated ($730 \pm 35 \mu\text{mol mol}^{-1}$) CO_2 levels in open-top chambers for four months from May 1 to Aug. 31, 1995 in a greenhouse at the University of Hawaii, Manoa campus. The plants were grown in ~4-liter pots for five months and then transferred to ~8-liter pots for the remainder (three months) of

the experiment. Transplanting was done to minimize the effect of limited rooting volume on plant growth. There were six plants in each chamber. Plants were irrigated twice weekly with a dilute nutrient solution specifically developed for pineapple (Bartholomew, personal communication), consisting of 0.5mM KH_2PO_4 , 0.82 mM $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.5mM K_2SO_4 , 2.5mM NH_4NO_3 , 0.5mM CaCl_2 (anhydrous), 50 μM H_3BO_3 , 9 μM $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$, 1 μM $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 0.3 μM $\text{CaSO}_4 \cdot 5\text{H}_2\text{O}$, 0.1 μM $\text{H}_2\text{MoO}_4 \cdot \text{H}_2\text{O}$, and 0.1 μM Fe_3BO . The pots were completely flushed with water every month to prevent the accumulation of nutrients in the potting media. This practice provided plants with an optimum nutrient supply and minimized any nutrient toxicity that might occur during the experimental period.

The open-top chambers, which were 1.5 m long, 1.3 m wide and 1.4 m tall, consisted of a frame covered with transparent mylar film. A table fan (~20 cm in diameter) was used in each chamber to circulate the air. Elevated CO_2 was achieved by bleeding pure CO_2 into the open-top chamber and the CO_2 concentration was maintained by adjusting the flow rate from the cylinder containing pure CO_2 . The CO_2 concentration in the chambers was measured with an IR gas analyzer (Li-6262, Li-Cor, Inc, Lincoln, Nebraska, USA) until the desired set point was established. Daily air and leaf temperature were measured periodically using fine-wire thermocouples and a datalogger (21X, Campbell Scientific Inc., Logan, Utah, USA). Leaf temperature was measured by inserting the thermocouples into the tissue of the youngest fully expanded and tallest leaf on the plant from the ground level, termed the 'D' leaf in the pineapple industry (Bartholomew and Kadzimin, 1977). The 'D'

leaf has been used to index plant nutrient levels and evaluate the effects of environmental factors on plant growth and physiology (Bartholomew and Kadzimin, 1977). Air temperature was monitored at the mid-plant height with thermocouples shaded with aluminum foil. Photosynthetic photon flux density, measured by a Li-Cor quantum sensor (Li-Cor, Inc., Lincoln, Nebraska, USA), was maintained at about 500 $\mu\text{mol m}^{-2} \text{s}^{-1}$ at midday at the mid-plant height on sunny days using shade cloth, which also prevented the air temperature inside the chambers from greatly exceeding 40°C.

Physiological Measurements and Growth Analysis

Carbon Isotopic Discrimination

Relative abundance of ^{13}C and ^{12}C in leaf tissue was determined on a subsample of the 'D' leaf. The leaf tissues were oven dried at 70°C and finely ground. The natural abundance $^{13}\text{C}/^{12}\text{C}$ ratios of the leaf tissue was measured on a SIRA series II isotope ratio mass spectrometer (VG Isotech, Middlewich, UK) at the Duke University Phytotron. The natural abundance $^{13}\text{C}/^{12}\text{C}$ ratios of source air in the open-top chambers was measured by collecting air samples in 250 ml pyrex gas collecting tubes. The carbon isotope composition of the air samples was measured on a mass spectrometer (Finnigan MAT 252, Finnigan Corp., Germany) in the Isotope Laboratory of the Institute of Geophysics, University of Hawaii. The data on stable carbon isotope composition ($\delta^{13}\text{C}$) were used to calculate carbon isotope discrimination (Δ) by the equation:

$$\Delta = \frac{\delta_a - \delta_p}{1 + \delta_p} \quad (6.1)$$

where δ_a is the isotopic composition of air, and δ_p is that of the leaves.

Chlorophyll Fluorescence Kinetics

Fluorescence induction kinetics (The Kautsky effect) was measured using a PAM 101 chlorophyll fluorometer (Walz, Effeltrich, Germany). The measurement was made at about 8:00 am (phase II) and 5:00 pm (phase IV) when C₃-type photosynthesis was expected to be high based on an earlier study of leaf gas exchange of pineapple in the greenhouse (Zhu, unpublished data). Fluorescence data were collected every two weeks after plants had been exposed to CO₂ treatments for one month. The plants were dark acclimated for about 30 minutes and the fluorescence intensity of the 'D' leaves was detected following the procedure described by Jones (1992) and Guralnick et al. (1992). Leaves were illuminated with modulated light (PPFD \approx 2-4 $\mu\text{mol m}^2 \text{s}^{-1}$) to induce the initial fluorescence yield (F_0), followed by a saturating pulse (PPFD \approx 2200 $\mu\text{mol m}^2 \text{s}^{-1}$) to create maximum fluorescence (F_m , dark adapted) and peak value of fluorescence ($F_m(t)$, light adapted), and further by actinic light (PPFD \approx 350 $\mu\text{mol m}^2 \text{s}^{-1}$) to drive photosynthesis and thus increase fluorescence yield. The resulting fluorescence induction curve was recorded on a strip chart recorder. Variable fluorescence (F_v) was calculated as $F_m - F_0$ and photochemical efficiency of PSII was estimated as F_v/F_m (Jones, 1992). The photochemical (q_p) and non-photochemical (q_N) were calculated using the equations described by Jones (1992) and Guralnick et al. (1992).

Titrateable 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 segment of the leaf was harvested in the late afternoon and similarly frozen. To determine TA, two 1.3 cm diameter leaf discs were collected from the middle one-third of each frozen leaf segment, ground in a mortar with ~20 ml of distilled water, and the total volume was 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 Inc. Logan, Utah, USA).

Chlorophyll and Nitrogen Contents

Leaf chlorophyll content was determined using the techniques described by Ranjith et al., (1995). The leaves were frozen at -75°C and ground to a powder in liquid nitrogen. Chlorophyll was extracted by grinding 200 mg of frozen leaf sample with 80 % (V/V) acetone, and adjusting the final volume to 20 ml by adding 80 % acetone. The mixture was incubated overnight at room temperature (22°C) and centrifuged at 12000 g for 1 minute. The absorbance of the leaf supernatant was read at 647 and 664 nm wavelengths with a spectrophotometer (Spectronic 21, Bausch & Lomb, New York), and chlorophyll content was calculated according to Coombs et al. (1985).

Leaf total nitrogen (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, University of Hawaii, Manoa. The data were reported as the percentage N in the leaf on a dry weight basis (W/W %).

Growth Analysis

Fresh and dry weights of leaves, stems and roots were measured on comparable planting material at the beginning of the experiment to establish the initial weights, and at the end of the experiment to obtain the final growth data. Dry weight was determined after oven-drying at 70 °C for two weeks. Green leaf area was measured using a Li-3100 leaf area meter (Li-Cor, Inc, Lincoln, Nebraska, USA). Stem weight ratio (SWR), root weight ratio (RWR), leaf weight ratio (LWR), relative growth rate (RGR) and net assimilation rate (NAR) were calculated as described by Chiariello et al. (1989). Specific leaf weight (SLW), a parameter positively related to leaf thickness (Chiariello et al., 1989), was determined as the ratio of leaf dry weight to green leaf area. Tissue dry matter contents (1- water content) were calculated from fresh and dry weight measurements.

RESULTS and DISCUSSION

The average daytime (7:30 am to 6:30 pm) air and leaf temperatures were 39.6 and 41.5 °C, and night (6:30 pm - 7:30 am) air and leaf temperatures were 24.2 and 24.1 °C during the experimental period. The high day temperature that prevailed during the study resulted from the fact that it was carried out during midsummer and

the absence of good temperature control in the greenhouse. The night temperatures were typical of those occurring at night during the summer in Honolulu. The q_p value from plants grown at high CO_2 was slightly higher than the values for plants grown at low CO_2 in both phase II and phase IV but the differences were not significant (Table 6.1). The q_N value for plants grown at high CO_2 was 45% lower than for plants grown at low CO_2 in phase II, but the values in phase IV were similar (Table 6.1). Greater CO_2 fixation results in a decrease in q_N and increase in q_p as result of a reduced pH gradient across the thylakoid membranes due to faster consumption of ATP and NADPH (Guralnick et al., 1992). The q_N and q_p data for pineapple suggest that photosynthetic CO_2 uptake via C_3 pathway was significantly greater in the phase II for plants grown at high CO_2 , but not in phase IV. With near optimum day temperatures, photosynthetic CO_2 uptake via the C_3 pathway in pineapple occurred mainly in phase IV, while uptake in phase II was very minor because of its short duration (Bartholomew, 1982; Cote et al., 1991). Thus, in this study it was assumed that CO_2 uptake via the C_3 pathway during phase IV was largely suppressed by the 40°C temperature. The F_v/F_m values at high CO_2 were slightly but not significantly greater than those at low CO_2 in the both phase II and IV (Table 6.1), indicating that the photochemical efficiency of pineapple leaves was not significantly enhanced by elevated CO_2 under the conditions of this study.

Plants grown at low CO_2 had a slightly but significantly greater carbon isotopic discrimination value (Δ) in 'D' leaf tissues than did plants grown at high CO_2 (Fig. 6.1). This response was in contrast to the results obtained in an earlier growth-

chamber study with lower day/night temperatures, where the Δ values were significantly greater at 700 than at 350 $\mu\text{mol mol}^{-1}$ CO_2 (chapter 3). The Δ values from this study indicated that in a 40/24°C temperature regime, the relative contribution of the CAM pathway to total CO_2 uptake was greater for pineapple grown at high than at low CO_2 .

Predawn (nocturnally accumulated) and afternoon TA values were significantly greater at high than at low CO_2 (Table 6.2), but the differences in the afternoon values were small (Table 6.2). The diurnal difference in TA (ΔTA) between predawn and afternoon, a measure of net dark CO_2 fixation (Griffiths, 1988), was also significantly greater for plants grown at high CO_2 (Table 6.2). Therefore, the TA data clearly indicated that phase-I CO_2 uptake via the CAM pathway was considerably enhanced by elevated CO_2 . Both carbon isotopic discrimination and TA data showed that the integrated effect of CO_2 enrichment over 24 hours was greater in the dark than in the light. This result was not consistent with other studies on the CAM species (Szarek et al., 1987; Nobel and Hartsock, 1986). As noted previously, nocturnal carbon assimilation via PEPCase was reported to be insensitive to CO_2 enrichment causing researchers to assume that carboxylation by PEPCase saturate at ambient CO_2 levels (Winter, 1985; Bowes, 1991). Photosynthetic rates of C_4 plants, which also utilize PEPCase, saturate at an intercellular space CO_2 concentration (c_i) of about 200 $\mu\text{mol mol}^{-1}$ (Taiz and Zeiger, 1991). The mean c_i values during the dark period for pineapple grown at 35/25°C, a temperature regime relatively close to that inside the open-top chambers in this study, averaged 200 and 260 $\mu\text{mol mol}^{-1}$, respectively at

350 and 700 $\mu\text{mol mol}^{-1}$ CO_2 levels (chapter 3). If it can be assumed that carboxylation via PEPCase in pineapple saturates at about 200 $\mu\text{mol mol}^{-1}$ CO_2 , the enhanced nocturnal CO_2 uptake of pineapple by elevated CO_2 could not be interpreted in terms of PEPCase activity. The greater carbon assimilation and accumulation of organic acids during the dark period for plants grown at high CO_2 might result from some other physiological responses to elevated CO_2 , such as reduced dark respiration at high CO_2 (Amthor, 1991; Nobel and Israel, 1994) or enhanced synthesis of PEP in the dark as a result of increased soluble sugar content (Cui and Nobel, 1994) which was considered to be the main source of PEP synthesis in pineapple during the dark period (Medina et al., 1991). But none of these effects has been confirmed experimentally.

Both predawn and afternoon osmolarity were significantly higher at high than at low CO_2 , with a greater effect on the predawn values (Table 6.2). The greater predawn osmolarity at high CO_2 is attributed primarily to the higher nocturnal organic acid levels (Medina et al., 1991; 1993). The elevated osmolarity in the afternoon at high CO_2 may result from greater accumulation of assimilates, such as soluble sugars, in leaf tissues (Medina et al., 1993; Cui and Nobel, 1994), since soluble sugars in pineapple leaves generally approached a maximum towards the end of the light period (Sideris et al., 1948; Medina et al., 1993).

Biomass accumulation over the four-month of growing period was significantly greater at high than at low CO_2 (Table 6.3). The higher relative growth rate (RGR) at elevated CO_2 was due primarily to a greater net assimilation rate (NAR) because leaf

area was slightly but not significantly greater (Table 6.3). Enhanced plant growth at elevated CO₂ was also observed for the CAM species *Agave deserti* and *Ferocactus acanthodes* (Nobel and Hartsock, 1986), *Agave vilmoriniana* (Idso et al., 1986) and *Opuntia ficus-indica* (Cui and Nobel, 1994). At high CO₂, 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 (SWR) and root (RWR), and the smaller portion partitioned to leaf (LWR) (Table 3). Leaf thickness (SLW) was significantly greater at high CO₂ (Table 3). The CAM species *Opuntia ficus-indica* also had greater cladode thickness when plants were grown at high CO₂ (Nobel et al., 1994; Nobel and Israel, 1994). Pineapple stem dry matter content (DMC) was significantly greater for plants grown at high CO₂, but there was no effect on the leaf DMC (Table 6.3). Pineapple stem DMC and stem starch content are positively correlated (Bartholomew and Paull, 1986). Thus, greater stem DMC may indicate increased stem starch content at high CO₂.

The lack of a significant effect of high CO₂ on the leaf nitrogen and chlorophyll contents in this experiment (Table 6.4) was in contrast to the results obtained earlier (chapter 4). The different responses in the two studies was attributed to improved nutrient management in this study as a result of the use of a specially prepared nutrient solution and more frequent application of nutrients to the plants. Leaf chlorophyll and nitrogen contents decreased for the CAM species *Opuntia ficus-indica* after plants had been grown at elevated CO₂ for about three months (Cui and Nobel, 1994). Leaf nitrogen content also declined as CO₂ increased for the C₃ plants

Oryza sativa (Rowland-Bamford et al, 1991), *Gossypium hirsutum* (Wong, 1990), *Abutilon theophrasti*, *Sinapis alba* and loblolly pine (*Pinus taeda*) (Tissue et al., 1993), and the C₄ species *Amaranthus retroflexus* (Coleman et al., 1991). Most of those reports clearly stated that the nutrients applied were sufficient to prevent nitrogen deficiency. However, the results from this study indicated that with careful nutrient management and adequate rooting volume, long-term exposure to high CO₂ did not reduce leaf nitrogen or chlorophyll contents if complete nutrients were applied twice a week. Further investigation may be needed to understand the fundamental cause of the decrease in tissue nitrogen and chlorophyll contents in response to elevated CO₂.

The results of this study indicated the growth of pineapple was enhanced after four-month exposure to elevated CO₂, even in a high day temperature. Further, the Δ and TA data demonstrated unequivocally that this enhancement was mainly the result of greater nocturnal CO₂ fixation.

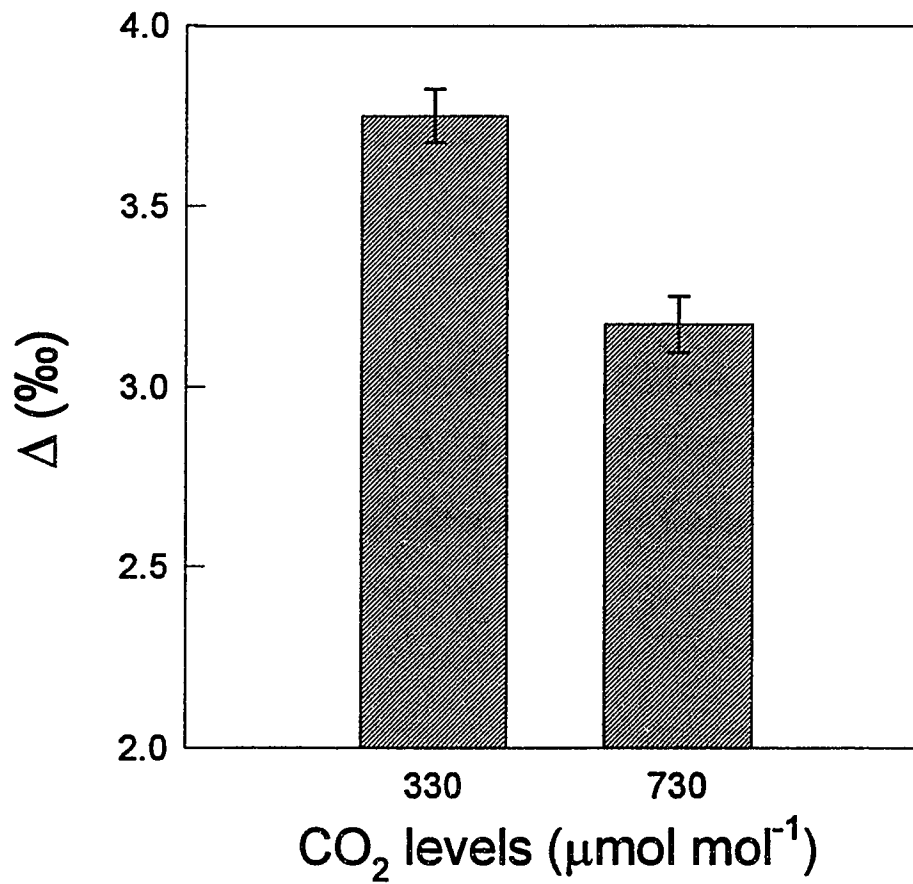


Fig. 6.1 Carbon isotopic discrimination (Δ) of the youngest physiologically mature leaves of pineapple grown at 330 and 730 $\mu\text{mol mol}^{-1}$ CO_2 levels in open-top chambers.

Table 6.1 Chlorophyll fluorescence kinetics coefficients (n=6) of the youngest physiologically mature leaves of pineapple grown at ambient (330 $\mu\text{mol mol}^{-1}$) and elevated (730 $\mu\text{mol mol}^{-1}$) CO_2 in open-top chambers

Parameters	CO ₂ level ($\mu\text{mol mol}^{-1}$)		T test
	330	730	
q _P (morning)	0.727	0.841	NS
q _P (afternoon)	0.809	0.897	NS
q _N (morning)	0.681	0.377	*
q _N (afternoon)	0.643	0.651	NS
F _v /F _m (morning)	0.831	0.864	NS
F _v /F _m (afternoon)	0.790	0.833	NS

*, ** and NS; Statistically significant at 0.05 (*) and 0.01 (**) levels of probability, and non-significant at 0.05 (NS).

Table 6.2. Titratable acidity and osmolarity of the youngest physiologically mature leaves (n=6) of pineapple grown at near ambient (330 $\mu\text{mol mol}^{-1}$) and elevated (730 $\mu\text{mol mol}^{-1}$) CO_2 in open top chambers. TA is titratable acidity and $\Delta(\text{TA})$ is diurnal TA differential.

Parameters	CO ₂ level) ($\mu\text{mol mol}^{-1}$)		T test
	330	730	
TA ($\text{mmol H}^+ \text{ m}^{-2}$)			
06/30/95 (predawn)	225.5	293.2	**
08/31/95 (predawn)	278.2	384.3	**
TA ($\text{mmol H}^+ \text{ m}^{-2}$)			
06/30/95 (afternoon)	31.6	32.9	*
08/31/95 (afternoon)	35.4	37.4	*
$\Delta(\text{TA})$ ($\text{mmol H}^+ \text{ m}^{-2}$)			
06/30/95	194.0	260.3	**
08/31/95	242.8	347.0	**
Osmolarity (mmol kg^{-1})			
08/31/95 (predawn)	418.4	504.5	**
Osmolarity (mmol kg^{-1})			
08/31/95 (afternoon)	441.2	484.0	*

*, **; Defined in table 6.1

Table 6.3. Growth responses to CO₂ levels for pineapple grown at near ambient (330 μmol mol⁻¹) and elevated (730 μmol mol⁻¹) CO₂ in open top chambers. W_t and A_L are plant dry weight and leaf area. Data were mean of six plants per treatment.

Parameters	CO ₂ level (μmol mol ⁻¹)		T test
	330	730	
W _t (g plant ⁻¹)	146.0	180.1	**
A _L (cm ² plant ⁻¹)	6404	6746	NS
NAR (g m ⁻² d ⁻¹)	1.64	2.23	
RGR (g kg ⁻¹ d ⁻¹)	7.08	8.83	
SWR (%) ^a	7.2	9.8	*
RWR (%) ^a	8.7	11.6	**
LWR (%) ^a	84.1	78.7	**
SLW (g cm ⁻²)	0.019	0.021	**
DMC (stem) (%)	11.20	13.12	**
DMC (leaf) (%)	11.35	11.21	NS

^a Data are expressed as percentage of stem, root and leaf dry weights relative to total plant dry weight.

*, **, NS; defined in table 6.1

Table 6.4. Chlorophyll and nitrogen contents in the youngest physiologically mature leaves (n=6) of pineapple grown at near ambient (330 μmol mol⁻¹) and elevated (730 μmol mol⁻¹) in open top chambers

Parameters	CO ₂ level (μmol mol ⁻¹)		T test
	330	730	
Total N (%)	1.28	1.20	NS
Chlorophyll content (μM)	3.83	4.03	NS

NS; defined in table 6.1

CHAPTER 7

SUMMARY

In the current project, studies have been conducted to examine the responses of pineapple to CO₂, temperature and water deficit. The primary focus of this study was to examine the effects of CO₂ and temperatures on leaf gas exchange, titratable acidity, carbon isotopic composition, biomass accumulation and partitioning, as well as some of the relevant physiological characteristics. Besides, the leaf water relation, gas exchange and accumulation of organic acids during the drought were studied for plants grown at two CO₂ levels and three temperatures.

Greater day/night temperature differential of 10 °C intensified CAM activity of pineapple, while smaller diurnal temperature range of 5 °C and warm night temperature of 25°C enhanced the relative contribution of daytime CO₂ fixation via C₃ pathway to total carbon assimilation. Elevated CO₂ significantly increased total daily CO₂ uptake and reduced stomatal conductance, thus enhanced water use efficiency, with greatest effect during the light period. Nocturnal CO₂ fixation and acidification also increased, which was in contrast to data obtained for some CAM species. Carbon isotopic discrimination data of leaf tissues indicated that elevated CO₂ increased the relative contribution of carbon fixation via C₃-type photosynthetic pathway, with the greater effect on plants grown in higher temperatures. Elevated CO₂ largely increased the CO₂ fixation for plants grown at 35/25 and 30/25 °C, while the enhancement was relatively smaller at 30/20 °C. Therefore, there was CO₂ by temperature interaction

on plant net CO₂ uptake.

Elevated CO₂ increased biomass accumulation of pineapple as a result of enhanced NAR. This effect was greater for plants grown at higher temperatures, which was in good agreement with the data of CO₂ assimilation obtained from pineapple in this study and some C₃ species in other studies. At elevated CO₂ more biomass was partitioned to stem and root, but less to leaf. However, leaf growth was also promoted as indicated by greater leaf thickness and surface area expansion. These responses were greatest for plants grown at smaller daily temperature range of 5 °C and warm night temperature of 25 °C. Leaf and stem dry matter contents were greater at elevated CO₂, which may be due to increased starch content. Simultaneous increase CO₂ levels and day/night temperature or night temperature by 5 °C also enhanced the growth rates of pineapple.

In elevated CO₂, nocturnal acidification was enhanced, a result being in contrast with the studies on some CAM species. This response was also greatest for plants grown at 30/25 °C. Afternoon osmolarity was greater for plants grown at elevated CO₂, which was due presumably to increased decarboxylation of organic acids or the fixation of external CO₂ into carbohydrates. Decreased chlorophyll and nitrogen contents may relate with physiological acclimation to increased CO₂, insufficient nutrient application or limitation of rooting volume.

Prolonged drought significantly reduced leaf water content, water and osmotic potentials. The effect was greater for plants grown at higher night temperature of 25°C. Total daily CO₂ fixation and stomatal conductance also reduced during the

drought, with the greatest effect in the light and at elevated CO₂. Reduced CO₂ dark fixation resulted in a decrease in accumulation of organic acids in the dark, with a result of CAM-idling occurring to plants grown at night temperature of 25°C after drought became severe. Elevated CO₂ reduced the water loss from leaf tissue as a consequence of decreased stomatal opening. This response only occurred to plants grown at night temperature of 25 °C. Leaf potential components at elevated CO₂ were sustained at relatively higher levels during the late part of drought, which may help maintain some of physiological activities of pineapple at relatively higher level that might not be possible at lower potentials. Decrease in leaf water content was relatively lower for plants grown at night temperature of 20 °C, thus the nocturnal CO₂ fixation and accumulation of organic acids were maintained at relatively greater rates during the drought period.

The study of pineapple grown in open-top chambers indicated that plant dry weight was greater for plants grown at elevated CO₂ due to enhanced NAR. This growth enhancement resulted primarily from greater accumulation of organic acids in the dark at elevated CO₂. Data of chlorophyll fluorescence indicated that the fixation of external CO₂ via C₃ photosynthetic pathway was largely suppressed presumably by a ~40 °C temperature. Therefore, nocturnal CO₂ fixation was expected to play more important role in daily carbon fixation than was for plants grown at more favorite temperatures. Leaf nitrogen and chlorophyll contents did not decrease after four months of exposure to elevated CO₂ as a result of improved nutrient management.

In general, at ambient CO₂, pineapple grown at 30/20 °C had the greatest

carbon fixation, organic acid synthesis and biomass accumulation, while plants grown at 30/25 °C had the smallest growth rate. In elevated CO₂ and temperatures, the effect of CO₂ enhancement was greater for pineapple grown at relatively higher temperature of 35/25 and 30/25 °C. If drought would occur at elevated CO₂ and temperature environment, the decrease in CO₂ fixation would be more rapid than it would be at current atmospheric conditions, but leaf water content and water potential components would be maintained at relatively greater levels than could for plants grown at ambient CO₂.

APPENDIX A

Integrated net CO₂ uptake over day, night and 24-h periods by the youngest physiologically mature leaves of pineapple grown at near ambient and elevated CO₂ levels and three day/night temperatures. Data are presented as sequence of observations.

Temperature (°C)	Observations	Day	Night	24 hours
		Net CO ₂ uptake		
		mmol CO ₂ m ⁻² period ⁻¹		
		Grown and measured at 350 μmol mol ⁻¹		
35/25	1	33.5	226.6	260.0
	2	72.8	233.8	306.6
	3	17.4	183.2	200.6
	4	34.9	159.1	193.9
	5	21.1	153.6	174.7
	6	20.7	158.6	179.3
	7	13.9	153.1	167.0
	8	15.0	168.0	182.9
30/25	1	78.1	147.7	225.8
	2	60.9	155.4	216.3
	3	55.0	104.1	159.2
	4	45.1	109.2	154.3
	5	60.5	136.3	196.9
	6	59.9	130.8	190.5
	7	59.0	135.9	194.8
	8	53.4	125.6	179.1
30/20	1	48.9	187.6	236.6
	2	70.1	250.1	320.2
	3	69.0	246.5	315.5
	4	54.0	285.8	339.8
	5	54.0	205.5	259.5
	6	50.1	259.1	309.2
	7	56.2	162.3	198.5
	8	54.4	223.5	277.8

Grown and measured at 700 $\mu\text{mol mol}^{-1}$

35/25	1	72.6	195.0	267.6
	2	39.8	209.0	248.9
	3	80.6	306.9	387.5
	4	88.5	206.2	294.7
	5	113.7	258.8	372.5
	6	115.0	236.9	351.8
	7	110.8	240.5	351.3
	8	70.0	182.3	252.3
	9	79.3	210.8	290.2
	10	98.9	182.7	281.5
	11	78.1	287.0	365.1
30/25	1	134.9	225.6	360.5
	2	87.4	211.9	299.3
	3	90.4	155.8	246.2
	4	129.9	158.8	288.7
	5	145.9	276.9	422.7
	6	140.8	232.8	373.6
	7	99.6	198.0	297.6
	8	108.0	244.3	352.2
	9	101.3	223.9	325.2
	10	83.9	193.8	277.7
	11	80.3	221.2	301.5
30/20	1	106.5	222.3	328.8
	2	167.7	346.4	514.2
	3	115.1	200.6	315.6
	4	107.3	233.3	340.6
	5	96.3	276.8	373.1
	6	108.8	239.9	348.8
	7	145.1	200.6	345.7
	8	99.0	218.6	317.6
	9	93.3	197.2	290.5
	10	134.3	197.7	332.0
	11	93.0	270.4	363.4

		Grown at 700 and measured at 350 μmol		
		mol ⁻¹		
35/25	1	47.5	215.9	263.5
	2	50.5	196.6	247.1
	3	77.8	220.6	298.4
	4	47.8	178.7	226.5
	5	76.3	180.4	256.6
30/25	1	59.4	179.9	239.3
	2	67.1	173.6	240.6
	3	68.1	165.2	233.3
	4	82.4	178.1	260.5
	5	60.3	204.8	265.1
30/20	1	54.2	300.6	354.8
	2	82.3	200.7	283.0
	3	57.3	192.7	250.0
	4	74.3	184.3	258.6
	5	74.3	198.8	273.1

		Grown at 350 and measured at 700 μmol		
		mol ⁻¹		
35/25	1	77.6	144.8	222.4
	2	66.4	254.6	321.0
30/25	1	117.2	173.4	290.6
	2	76.6	225.0	301.5
30/20	1	110.5	230.7	341.3
	2	79.4	312.7	392.1

REFERENCES

- Acock, B. 1990. Effects of carbon dioxide on photosynthesis, plant growth, and other processes. pp. 45-59 In Kimball, B.A., Rosenberg, N.J. and Allen, L.H. (eds.) *Impact of Carbon Dioxide and Trace Gases, and Climate Change on Global Agriculture*, ASA special pub. No. 53.
- Acock, B and L.H. Allen, Jr. 1985. Crop responses to elevated carbon dioxide concentrations. In Strain, B.R. and Cure, J.D. (eds.) *Direct effects of increasing carbon dioxide on vegetation*. DOE/ER-0238. U.S. Dept. of Energy, Washington, DC. pp 54-97. Anonymous. 1990. *Production*. FAO Yearbook, FAO, Rome. 167 pages.
- Amthor, J.S. 1991. Respiration in a future, higher-CO₂ world. *Plant, Cell and Environment*. 14: 13-20.
- Arp, W.J. Effects of source-sink relations on photosynthetic acclimation to elevated CO₂. *Plant, Cell and Environment*, 14: 869-87.
- Baker, N.R. and P. Horton. 1987. Chlorophyll fluorescence quenching during photoinhibition. pp 145-168. In Kyle, D.J., Osmond, C.B. and Arntzen, C.J. (eds.) *Photoinhibition*, Elsevier Science Publishers, New York.
- Bartholomew, D.P. 1982. Environmental control of carbon assimilation and dry matter production by pineapple. pp 278-294 In Ting, I.P. and Gibbs, M. (eds.) *Crassulacean Acid Metabolism*, Am. Soc. Plant Physiol. Rockville.
- Bartholomew, D.P and S.B. Kadzimin. 1977. Pineapple. pp 113-156. In Alvim, P. de T., and Kozlowski, T. T. (eds.) *Ecophysiology of Tropical Crops*, Academic Press, New York.
- Bartholomew D.P. and E. Malézieux. 1994. Pineapple. pp 243-291. In Schaffer, B. and P.C. Anderson (eds.) *Handbook on Environmental Physiology of Fruit Crops*, CRC Press, Inc., Boca Raton.
- Bartholomew D.P. and R. Paull. 1986. Pineapple. pp 371-388 In Monselise, S.P. (eds.) *CRC Handbook of Fruit Sets and Development*, CRC Press, Inc., Boca Raton.
- Baskin Y. 1993. Ecologists put some life into models of a changing world. *Science*. 259: 1694-1696.

- Bastide, B., D. Sipes, J. Hann and I.P. Ting. 1993. Effect of severe water stress on aspects of Crassulacean acid metabolism in *Xerosicyos*. *Plant Physiol.* 103: 1089-1096.
- Beadle, C.L. 1985. Plant Growth Analysis. Techniques in bioproductivity and photosynthesis. pp. 20-25. In J. Coombs, D.O Hall, S.P. Long and J.M.O. Scurlock (eds.). Second edition.
- Beadle, C.L., M.M. Ludlow and J.L. Honeysett. Water Relations. 1985. pp 50-61. In Coombs, J., D.O. Hall, S.P. Long and J.M.O. Scurlock (eds.) Techniques in Bioproductivity and Photosynthesis. Second edition. Pergamon Press Ltd.
- Bender, M.M., I. Rouhani, H.M. Viner and C.C. Black. 1973. $^{13}\text{C}/^{12}\text{C}$ ratio changes in Crassulacean Acid Metabolism plants. *Plant Physiol.*, 42: 427-430.
- Besford, R.T., L.J. Ludwing, and A.C. Withers. 1990. The greenhouse effect: Acclimation of tomato plants growing in high CO_2 , photosynthesis and ribulose-1, 5-bisphosphate carboxylase protein. *J. exp. Bot.* 41:925-931.
- Black, C.C., Jr. 1986. Effects of CO_2 concentration on photosynthesis and respiration of C_4 and CAM plants. pp 29-40. In Enoch, H.Z and B.A. Kimball (eds.) Physiology, Yield, and Economics. Vol. II. Carbon dioxide enrichment of greenhouse crops, CRC Press, Inc., Boca Raton, Florida.
- Black, C.C. 1986. Effects of CO_2 concentration on photosynthesis and respiration of C_4 and CAM plants. pp 29-40. In Enoch, H.Z. and Kimball, B.A. (eds.) Carbon dioxide enrichment of greenhouse crops. Vol.II physiology, yield and economics. CRC Press, Inc. Boca Raton, Fl.
- Black, C.C., N.W. Carnal, and W.H. Kenyon. 1982. Compartmentation and the regulation of CAM. In Ting, I.P. and M. Gibbs (eds.) Crassulacean Acid Metabolism. Am. Soc. Plant Physiol., Rockville.
- Borland, A.M. and H. Griffiths. 1989. The regulation of citric acid accumulation and carbon recycling during CAM in *Ananas comosus*. *J. Expt. Bot.* 40:53.
- Borland, A.M., H. Grffiths, M.S.J. Broadmeadow, M.C. Fordham and C. Maxwell. 1993. Short-term change in carbon-isotope discrimination in the C_3 -CAM intermediate *Clusia minor* L. growing in Trinidad. *Oecologia.* 95: 444-453.
- Bowes, G. 1991. Growth at elevated CO_2 : photosynthetic responses mediated through Rubisco. *Plant, Cell and Environment.* 14: 795-806.
- Boyer, J.S. 1969. Physiological limitations on crop production under temperature and

- moisture stress. Nat. Acad. Sci., Washington, D.C.
- Bunce, J.A. 1990. Short- and long-term inhibition of respiratory carbon dioxide efflux by elevated carbon dioxide. *Annals of Botany*, 65: 637-642.
- Carnal, N.W and C.C. Black. 1990. Soluble sugars as the carbohydrate reserve for CAM in pineapple leaves: implication for the role of pyrophosphate-6-fructokinase in glycolysis. *Plant Physiol.*, 90: 91-100.
- Chiariello N.R, H.A. Mooney and K. Williams. 1989. Growth, carbon allocation and cost of plant tissues. pp. 327-365. In Percy, R.W., J.R. Ehleringer, H.A. Mooney and P.W. Rundel (eds.) *Plant Physiological Ecology: Field Methods and Instrumentation*, Chapman and Hall. London.
- Christopher, B.F, T. Ball and J.A. Berry. 1989. Photosynthesis: principles and field techniques. pp 209-253. In Percy, R.W., J.R. Ehleringer, H.A. Mooney and P.W. Rundel (eds.) *Plant Physiological Ecology: Field Methods and Instrumentation*, Chapman and Hall. London.
- Choudhury, B.J. and J.L. Monteith. 1986. Implications of stomatal responses to saturation deficit for the heat balance of vegetation. *Agric. For. Meteorol.*, 36: 215-225.
- Cockburn, W. 1979. Relationships between stomatal behavior and internal carbon dioxide concentration in Crassulacean acid metabolism plants. *Plant Physiol.* 63:1029-1032.
- Coleman, J.S , L. Rochefort, F.A. Bazzaz and F.I. Woodward. 1991. Atmospheric CO₂, plant nitrogen status and the susceptibility of plants to an acute increase in temperature. *Plant, Cell and Environment*. 14: 667-674.
- Connelly, P.R. 1969. The relative response of the pineapple plant, *Ananas comosus* (L.) Merr., under varying nitrogen rates and carriers under different levels of light intensity. Master thesis. University of Hawaii, Manoa.
- Coombs, J., G. Hind, R.C. Leegood, L.L. Tieszen and A. Vonshak. 1985. Analytical techniques. *Techniques in bioproductivity and photosynthesis*. pp 219-228. In J. Coombs, D.O Hall, S.P. Long and J.M.O. Scurlock (eds.). Second edition.
- Cortazar, V.G.D. and P.S. Nobel. 1990. Worldwide environmental productivity indices and yield predictions for a CAM plant, *Opuntia ficus-indica*, including effects of doubled CO₂ levels. *Agric. Forest Met.* 49:261-279.

- Cote, F.X. 1988. Photosynthèse et photorespiration d'une plante à métabolisme crassulacéen: *Ananas comosus* (L.) Merr. Etude des échanges gazeux. Thèse de doctorat en Sciences. Université de Toulouse, Toulouse, France.
- Cote, F.X., M. Folliot, and M. Andre. 1992. Photosynthetic crassulacean acid metabolism in pineapple: Diel rhythm of CO₂ fixation, water use, and effect of water stress. pp 113-129. In Bartholomew, D.P. and K.G. Rohrbach (eds.) First International Pineapple Symposium. Acta Horticulture. 344.
- Cote, F.X., M. Andre, M. Folliot, D. Massimino, and A. Daguene. 1989. CO₂ and O₂ exchanges in the CAM plant *Ananas comosus* (L.) Merr. Plant Physiol. 89: 61-68.
- Crews, C.E., H.M. Vines, and C.C. Black. 1975. Post-illumination burst of carbon dioxide in Crassulacean acid metabolism plants. Plant Physiol. 55:652-657.
- Cui, M., P.M. Miller and P.S. Nobel. 1993. CO₂ exchange and growth of the Crassulacean acid metabolism plant *Opuntia ficus-indica* under elevated CO₂ in open-top chambers. Plant Physiol. 103: 519-524.
- Cui, M. and P.S. Nobel. 1994. Gas exchange and growth responses to elevated CO₂ and light levels in the CAM species *Opuntia ficus-indica*. Plant, Cell and Environment. 17: 935-944.
- Cure, J.D. and B. Acock. 1986. Crop responses to carbon dioxide doubling: A literature survey. Agric. Forest Met. 38:127-145.
- Drake, B.D. and P.W. Leadley. 1991. Canopy photosynthesis of crops and native plant communities exposed to long-term elevated CO₂. Plant, Cell and Environment. 14: 853-860.
- Eamus, D. 1991. The interaction of rising CO₂ and temperature with water use efficiency. Plant, Cell and Environment. 14: 843-852.
- Ehleringer, J.R. 1989. Temperature and energy budgets. pp 117-134. In Pearcy, R.W., J.R. Ehleringer, H.A. Mooney and P.W. Rundel (eds.) Plant Physiological Ecology: Field Methods and Instrumentation, Chapman and Hall. London.
- Ehleringer, J.R. and C.B. Osmond. 1989. Stable isotopes. pp 281-290. In Pearcy, R.W., J.R. Ehleringer, H.A. Mooney and P.W. Rundel (eds.) Plant Physiological Ecology: Field Methods and Instrumentation, Chapman and Hall. London.

- Ekern, P.C. 1965. Evapotranspiration of pineapple in Hawaii. *Plant Physiol.* 40:736-739.
- Farquhar, G.D., O'Leary, M.H., Berry, J.A. 1982. On the relationship between carbon isotope discrimination and intercellular carbon dioxide concentration in leaves. *Aust. J. plant Physiol.* 9:121-137.
- Farquhar, G.D., Sharkey, T.D. 1982. Stomatal conductance and photosynthesis. *Ann. rev. Plant Physiol.* 33: 317-345.
- Fleisch, H. and D.P. Bartholomew, 1987. Development of a heat unit model of pineapple ('Smooth Cayenne') fruit growth from field data. *Fruits* 42:709-715.
- Gifford, R.M., H. Lambers and J.I.L. Morison (1985) Respiration of crop species under CO₂ enrichment. *Physiologia Plantarum.* 63: 351-256.
- Goldstein, G., J.K.E. Ortega, A. Nerd and P.S. Nobel. 1991. Diel patterns of water potential components for the Crassulacean acid metabolism plant *Opuntia ficus-indica* when well-watered or droughted. *Plant Physiol.* 95: 274-280.
- Griffiths, H. 1988. Crassulacean Acid Metabolism: a Re-appraisal of physiological plasticity in form and function. *Advances in Botanical Research.* 15: 43-92.
- Griffiths, H. 1992. Carbon isotope discrimination and the integration of carbon assimilation pathway in terrestrial CAM plants. *Plant, Cell and Environment.* 15: 1051-1062.
- Griffiths, H., M.S.J. Broadmeadow, A.M. Borland and C.S. Hetherington. 1990. Short-term changes in carbon-isotope discrimination identify transitions between C₃ and C₄ carboxylation during Crassulacean acid metabolism. *Planta*, 181: 604-610.
- Guralnick, L.J. and I.P. Ting. 1988. Seasonal patterns of water relations and enzyme activity of the facultative CAM plant *Portulacaria afra* (L.) Jacq. *Plant Cell Environ.* 11: 811-818.
- Guralnick, L.J., P.A. Rorabaugh and Z. Hanscom. 1984. Seasonal shifts of photosynthesis in *Portulacaria afra* (L.) Jacq. *Plant Physiol.* 76: 643-646.
- Guralnick, L.J. and Ting, I.P. 1987. Physiological changes in *Portulacaria afra* (L.) Jacq. during a summer drought and rewatering. *Plant Physiol.* 70: 85-91.
- Guralnick, L.J., R.L. Heath, G. Goldstein and I.P. Ting. 1992. Fluorescence

- quenching in the varied photosynthetic modes of *Portulacaria afra* (L.) Jacq. *Plant Physiol.* 99: 1309-1313.
- Hogan, K.P, Smith, A. P., Ziska, L. H. 1991. Potential effects of elevated CO₂ and changes in temperature on tropical plants. *Plant, Cell and Environment.* 14: 763-778.
- Holloway W.D, M.E. Argall, W.T. Jealous, J.A. Lee and J.H. Bradbury. 1989. Organic acids and calcium oxalate in tropical root crops. *J. Agric, Food chem.* 37: 337-341
- Holtum, J.A., M.H. O'Leary, and C.B. Osmond. 1983. Effect of varying CO₂ partial pressure on photosynthesis and on carbon isotope composition of carbon-4 of malate from the Crassulacean acid metabolism plant *Kalanchoe daigremontiana* Hamet Perr. *Plant Physiol.* 71: 602-609.
- Hormann, H., C. Neubauer and U. Schreiber. 1994. On the relationship between chlorophyll fluorescence quenching and the quantum yield of electron transport in isolated thylakoids. *Photosynthesis research.* 40: 93-106.
- Huerta, A.J. and I.P. Ting. 1988. Effects of various levels of CO₂ on the induction of Crassulacean acid metabolism in *Portulacaria afra* (L.) Jacq. *Plant Physiol.* 88:183-188.
- Idso, S.B. 1990. Interactive effects of carbon dioxide and climate variables on plant growth. pp. 61-68. In Kimball, B.A., Rosenberg, N.J. and Allen, L.H. (eds.) *Impact of Carbon dioxide and trace gases, and climate change on global agriculture*, ASA Special Pub. No. 53.
- Idso, S.B. 1992. U.S. temperature/precipitation relationships: Implications for future 'greenhouse' climates. *Agric. Forest Met.* 58:143-147.
- Idso, S.B., B.A. Kimball, M.G. Anderson and S.R. Szarek. 1986. Growth response of a succulent plant, *Agave vilmoriniana*, to elevated CO₂. *Plant Physiol.* 80: 796-797.
- Idso, S.B., B.A. Kimball, M.G. Anderson and J.R. Mauney. 1987. Effects of atmospheric CO₂ enrichment on plant growth: the interactive role of air temperature. *Agriculture, Ecosystem and Environment*, 20: 1-10.
- Jarvis, P.G and K.G. McNaughton. 1986. Stomatal control of transpiration. *Advances in Ecological Research.* 15: 1-49.
- Jones, H.G. 1992. *Plants and Microclimate: A quantitative approach to environmental*

- plant physiology. Cambridge University Press. Second edition, 429 pages.
- Joshi, M.C., J.S. Boyer and P.J. Kramer. 1965. Growth, carbon dioxide change, transpiration, and transpiration ratio of pineapple. *Bot Gaz. (Chicago)* 126: 174-179.
- Kaplan, A., J. Gale, and A. Poljakoff-Mayber. 1976. Resolution of net dark fixation of carbon dioxide into its respiration and gross fixation components in *Bryophyllum daigremontianum*. *J. Exp. Bot.* 97:220-230.
- Kenyon, W.H., R.F. Severson and C.C. Black. 1985. Maintenance carbon cycle in Crassulacean acid metabolism plant leaves. *Plant Physiol.* 77:183-189.
- Kimball, B.A. 1985. Adaptation of vegetation and management practices to a higher carbon dioxide world. pp 187-202. In Strain, B.R. and Cure, J.D. (eds.) Direct effects of increasing carbon dioxide on vegetation. DOE/ER-0238. U.S. dept. of Energy, Washington, DC.
- Kimball, B.A. and S.B. Idso. 1983. Increasing atmospheric CO₂: effects on crop yield, water use and climate. *Agricultural Water Management.* 7: 55-72.
- Kirschbaum, M.U.F. 1994. The sensitivity of C₃ photosynthesis to increasing CO₂ concentration: a theoretical analysis of its dependence on temperature and background CO₂ concentration. *Plant, Cell and Environment.* 17: 747-754.
- Kluge, M., A. Fischer and I.C. Buchanan-Bollig. 1982. Metabolic control of CAM. In Ting, I.P. and M. Gibbs (eds.) *Crassulacean Acid Metabolism*. Waverly Press. 31-50.
- Kluge, M and I.P. Ting. 1979. *Crassulacean Acid Metabolism: Analysis of an ecological adaptation*. Springer Verlag. New York.
- Krause, G.H. 1991. Chlorophyll Fluorescence and photosynthesis: the basis. *Annu. Rev. Plant Physio. Plant Mol. Biol.* 1991. 42: 313-349.
- Krauss, B.H. 1949. Anatomy of the vegetative organs of the pineapple, *Ananas comosus* (L.) Merr. II. The leaf, *Bot. Gaz.*, 110, 333.
- Krause, G.H and E. Weis. 1984. Chlorophyll fluorescence as a tool in plant physiology. II Interpretation of fluorescence signals. *photosynthesis Research.* 5: 139-157.
- Krause, G.H and E. Weis. 1988. The photosynthetic apparatus and chlorophyll

- fluorescence. An introduction. pp 3-11. In Lichtenthaler, H.K. (eds.) Application of Chlorophyll Fluorescence. By Kluwer Academic Publisher.
- Little, T.M. and F.J. Hills, 1977. Agricultural experimentation: Design and analysis. John Wiley & Sons, Inc.
- Long, S.P. 1991. Modification of the responses of photosynthetic productivity to rising temperature by atmospheric CO₂ concentration: Has its importance been underestimated? *Plant, Cell, and Environment*. 14: 729-739.
- Long, S.P. and Hallgren. 1985. Measurement of CO₂ assimilation by plants in the field and the laboratory. pp 62-94. In Coombs, J., D.O. Hall, S.P. Long and J.M.O. Scurlock (eds.) *Techniques in Bioproductivity and Photosynthesis*. Second edition. Pergamon Press Ltd.
- Lüttge, U. 1987. Carbon dioxide and water demand: Crassulacean acid metabolism (CAM), versatile ecological adaptation exemplifying the need for integration in ecophysiological work. *Tansley review No. 10. New Phytol.* 106: 593-629.
- Mansfield, T.A., A.M. Hetherington and C.J. Atkinson. 1990. Some current aspect of stomatal physiology. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 41: 55-75.
- Marzola, D.L. and D.P. Bartholomew. 1979. Photosynthetic pathway and biomass energy production. *Science* 205: 555-559.
- Mcmurtrie, R.E and Y.-P. Wang. 1993. Mathematical models of the photosynthetic response of tree stands to rising CO₂ concentrations and temperatures. *Plant, Cell and Environment*. 16: 1-13.
- Medina, E., M. Popp, E. Olivares, H.P. Janett and U. Lüttge. 1993. Daily fluctuations of titratable acidity, content of organic acids (malate and citrate) and soluble sugars of varieties and wild relatives of *Ananas comosus* L. growing under natural tropical conditions. *Plant, Cell, and Environment*. 16:55-63.
- Medina, E., M. Popp, U. Lüttge and E. Ball. 1991. Gas exchange and acid accumulation in high and low irradiance grown pineapple cultivars. *Photosynthetica*. 25(4): 489-498.
- Monteith, J.L. 1975. *Vegetation and the atmosphere. Volume 1: Principles.* Academic Press. London. Chap. 4.
- Moradshahi, A., H.M. Vines, and C.C. Black. 1977. CO₂ exchange and acidity levels in detached pineapple, *Ananas comosus* (L.) Merr., leaves during the

- day at various temperatures, O₂ and CO₂ concentrations. *Plant Physiol.* 59:274-278.
- Mott, K.A. 1990. Sensing of atmospheric CO₂ by plants. *Plant, Cell and Environment.* 13: 731-737.
- Neales, T.F., A.A. Patterson and V.J. Hartney. 1968. Physiological adaptation to drought in the carbon assimilation and water loss of xerophytes. *Nature*, 219: 469-472.
- Neales, T.F., P.J.M. Sale, and C.P. Meyer. 1980. Carbon dioxide assimilation by pineapple plants, *Ananas comosus* (L.) Merr. II: Effects of variation of the day/night temperature regime. *Aust. J. Plant Physiol.* 7:375-385.
- Neales, T.F., A.A. Patterson and V.J. Hartney. 1968. Physiological adaptation to drought in the carbon assimilation and water loss of xerophytes. *Nature*, 219: 469-472.
- Neild, R.E., and Boshell, F. 1976. An agroclimatic procedure and survey of the pineapple production potential of Colombia. *Agric. Met.* 17: 81-92.
- Nobel, P.S. 1991. *Physicochemical and environmental plant physiology.* Academic Press, San Diego.
- Nobel P.S. 1994. *Remarkable Agaves and Cacti.* Oxford University Press, Inc. 166 pages.
- Nobel, P.S. 1991. Environmental productivity indices and productivity for *Opuntia ficus-indica* under current and elevated atmospheric CO₂ levels. *Plant, Cell and Environment*, 14: 637-646.
- Nobel, P.S and V.G. de Cortázar. 1991. Growth and predicted productivity of *Opuntia ficus-indica* for current and elevated carbon dioxide. *Agron. J.* 83:224-230.
- Nobel, P.S., M. Cui and A.A. Israel. 1994. Light, chlorophyll, carboxylase activity and CO₂ fixation at various depths in the chlorenchyma of *Opuntia ficus-indica* (L.) Miller under current and elevated CO₂. *New Phytol.* 128: 315-322.
- Nobel, P.S. and T.L. Hartsock. 1986. Environmental influences on the productivity of three desert succulents in the south-western United States. *Plant, Cell and Environment.* 9: 741-749.
- Nobel. P.S. and T.L Hartsock. 1986. Short-term and long-term responses of

- Crassulacean acid metabolism plants to elevated CO₂. *Plant Physiol.* 82:604-606.
- North, G.B., T.L. Moore and P.S. Nobel. 1995. Cladode development for *Opuntia ficus-indica* (Cactaceae) under current and doubled CO₂ concentrations. *American Journal of Botany.* 82(2): 159-166.
- Nose, A., M. Shiroma, K. Miyazato, and S. Murayama. 1977. Studies on matter production in pineapple plants. I. Effects of light intensity in light period on the CO₂ exchange and CO₂ balance of pineapple plants. *Japan. J. Crop Sci.* 47:580-587.
- Nose, A., K. Miyazato, and S. Murayama. 1981. Studies on matter production in pineapple plants. II. Effects of soil moisture on the gas exchange of pineapple plants. *Japan. J. Crop Sci.*, 50 (4): 525-535.
- Osmond, C.B. 1978. Crassulacean Acid Metabolism: A curiosity in context. *Ann. Rev. Plant Physiol.* 29: 379-414.
- Osmond, C.B., Allaway, W.G., Sutton, B.G., Troughton, J.H., Queiroz, O., Luttage, U., Winter, K. 1973. Carbon isotope discrimination in photosynthesis of CAM plants. *Nature (London)* 246: 41-42.
- Osmond, C.B., W.W. Adams III and S.D. Smith. 1989. Crassulacean acid metabolism. pp 255-269. In Percy, R.W., J.R. Ehleringer, H.A. Mooney and P.W. Rundel (eds.) *Plant Physiological Ecology: Field Methods and Instrumentation.* Chapman and Hall.
- Osmond, C.B. and O. Bjorkman. 1975. Pathway of CO₂ fixation in the CAM plant *Kalanchoe daigremontiana*. (Part 2) Effects of O₂ and CO₂ concentration on light and dark CO₂ fixation. *Aust. J. Plant physiol.*, 2: 155-162.
- Osmond, C.B., W.W. Adams III and S.D. Smith. 1989. Crassulacean acid metabolism. pp 255-269. In Percy, R.W., J.R. Ehleringer, H.A. Mooney and P.W. Rundel (eds.) *Plant Physiological Ecology: Field Methods and Instrumentation.* Chapman and Hall.
- Parry, M. 1990. *Climate change and world agriculture.* Earthscan Publications Ltd, London.
- Peet, M.M., S.C. Huber, and D.T Patterson. 1986. Acclimation to high CO₂ in monoecious cucumbers. II. Carbon exchange rates, enzyme activities, and starch and nutrient concentrations. *Plant physiol.* 80: 63-67.

- Porter, J.H., M.L. Parry and T.R. Carter. 1991. The potential effects of climatic change on agricultural insect pests. *Agric. Forest Met.* 57: 221-240.
- Ranjith, S.A., F.C. Meinzer, M.H. Perry and M. Thom. 1995. Partitioning of carboxylase activity in nitrogen-stressed sugarcane and its relationship to bundle sheath leakiness to CO₂, photosynthesis and carbon isotope discrimination. *Aust. J. Plant Physiol.*, 22: 903-911.
- Raskin, V.I and J.B. Marder. 1993. How plants limit the photodestructive potential of chlorophyll. pp 156-159. In Yamamoto, H and C.M. Smith (eds.) *Photosynthetic responses to the environment.*
- Raveh, E., M. Gersani and P.S. Nobel. 1995. CO₂ uptake and fluorescence responses for a shade-tolerant cactus *Hylocereus undatus* under current and doubled CO₂ concentrations. *Physiologia plantarum.* 93: 505-511.
- Reibach, P.H. and C.R. Benedict. 1977. Fractionation of stable carbon isotopes by phosphoenolpyruvate carboxylase from C₄ plants. *Plant Physiol.*, 59: 564-568.
- Rosenberg, N.J. 1989. Evapotranspiration in a greenhouse-warmed world: A review and a simulation. *Agric. Forest Met.* 47:303-320.
- Sale, P.J.M. and T.F. Neales. 1980. Carbon dioxide assimilation by pineapple plants, *Ananas comosus* (L.) Merr. I: Effects of daily irradiance. *Aust. J. Plant Physiol.* 7:363-373.
- Rowland-Bamford, A.J. Allen, L.H., Jr, Baker, J.T. and Boote, K.J. 1990. Carbon dioxide effects on carbohydrate status and partitioning in rice. *Journal of Experimental Botany*, 41: 1601-1608.
- Rowland-Bamford, A.J., J.T. Baker, L.H. Allen Jr and G. Bowes. 1991. Acclimation of rice to changing atmospheric carbon dioxide concentration. *Plant, Cell and Environment.* 14: 577-583.
- Ryle, G.J. and C.E. Powell. 1992. The influence of elevated CO₂ and temperature on biomass production of continuously defoliated white clover. *Plant, Cell and Environment.* 15: 593-599.
- Sage, R.F., R.W. Pearcy, and J.R. Seemann. 1987. The nitrogen use efficiency of C₃ and C₄ plants. III. Leaf nitrogen effects on the activity of carboxylating enzymes in *Chenopodium album* (L.) and *Amaranthus retroflexus* (L.). *Plant Physiology.* 85: 355-359.
- Sanford, W.G. 1962. Pineapple crop log-concept and development. *Better Crops plant Food.* 46: 32-43.

- Sarmiento, J.L and M. Bender. 1994. Carbon biogeochemistry and climate change. *Photosynthesis Research*. 39: 209-234.
- Schreiber, U. and W. Bilger. 1987. Rapid assessment of stress effects on plant leaves by chlorophyll fluorescence measurements. pp 27-53. In Tenhunen, J.D., Catarino, F.M., Lange, O.L. and Oechel, W.L. (eds.), *Plant Response to Stress*. Springer-Verlag, Berlin.
- Schulze, E.-D. 1986. Carbon dioxide and water vapor exchange in response to drought in the atmosphere and in the soil. *Ann. Rev. Plant Physiol.* 37: 247-274.
- Sideris, C.P., Young, H.Y., and Chun, H.H.Q. 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.
- Stitt, M. 1991. Rising CO₂ levels and their potential significance for carbon flow in photosynthetic cells. 14: 741-762.
- Stitt, M. and D. Schulze. 1994. Does Rubisco control the rate of photosynthesis and plant growth? An exercise in molecular ecophysiology. *Plant, Cell and Environment*. 17: 465-487.
- Szarek, R.S, P.A. Holthe, and I.P. Ting. 1987. Minor physiological response to elevated CO₂ by the CAM plant *Agave vilmoriniana*. *Plant Physiol.* 83:934-940.
- Smith, B.N. and S. Epstein. 1971. Biogeochemistry of the stable isotopes of hydrogen and carbon in salt marsh biota. *Plant Physiol.*, 46: 738-742.
- Smith, J.A.C., P.J. Schulte and P.S. Nobel. 1987. Water flow and water storage in *Agave deserti*: osmotic implications of Crassulacean acid metabolism. *Plant, Cell and Environment*. 10: 639-648.
- Stitt, M. 1991. Rising CO₂ levels and their potential significance for carbon flow in photosynthetic cells. *Plant, Cell and Environment*, 14: 741-762.
- Strain, B.R. 1985. Background on the response of vegetation to atmospheric carbon dioxide enrichment. pp 3-10. In Strain, B.R. and Cure, J.D. (eds.) *Direct effects of increasing carbon dioxide on vegetation*. DOE/ER-0238. U.S. Dept. of Energy, Washington, DC.
- Strain, B.R. and J.D. Cure. 1985. Executive summary. In Strain, B.R. and Cure,

- J.D. (eds.) Direct effects of increasing carbon dioxide on vegetation. DOE/ER-0238. U.S. Dept. of Energy, Washington, DC.
- Strain, B.R. and J.D. Cure. 1985. Status of knowledge and recommendation for future work. pp 207-213. In Strain, B.R. and Cure, J.D. (eds.) Direct effects of increasing carbon dioxide on vegetation. DOE/ER-0238. U.S. Dept. of Energy, Washington, DC.
- Szarek, R.S, P.A. Holthe, and I.P. Ting. 1987. Minor physiological response to elevated CO₂ by the CAM plant *Agave vilmoriniana*. *Plant Physiol.* 83:934-940.
- Szarek, S.R., H.P. Johnson and I.P. Ting. 1973. Drought adaptation in *Opuntia basilaris*. Significance of recycling carbon through crassulacean acid metabolism. *Plant Physiol.* 52: 539-541.
- Taylor, K.E. and M.C. MacCracken. 1990. Projected effects of increasing concentration of carbon dioxide and trace gases on climate. pp 1-15. In Kimball, B.A., Rosenberg N.J. and Allen, L.H. (eds.) *Impact of Carbon Dioxide and Trace Gases, and Climate Change on Global Agriculture*, ASA Special Pub. No. 53.
- Taiz, L and E. Zeiger. 1991. *Plant Physiology*. The Benjamin/Cummings Publishing Company, Inc. 566 pages.
- Ting, I.P. 1985. Crassulacean Acid Metabolism. *Ann. Rev. plant physiol.* 36:595-622.
- Ting, I.P. 1993. CO₂ and Crassulacean acid metabolism plants: A review. In Tolbert, N.E. and J. Preiss (eds.) *Photosynthetic Carbon Metabolism and Regulation of Atmospheric CO₂ and O₂*. (Expected publication by Oxford Press)
- Tissue, D.T., R.B. Thomas and B.R. Strain. 1993. Long-term effects of elevated CO₂ and nutrients on photosynthesis and rubisco in loblolly pine seedlings. *Plant, Cell and Environment.* 16: 859-865.
- White, R.M. 1990. The great climate debate. *Sci. Am.* 263: 36.
- Winter, K. 1982. Regulation of PEP carboxylase in CAM plants. pp 153-169. In Ting, I.P. and M. Gibbs (eds.) *Crassulacean Acid Metabolism*. Waverly Press.
- Winter, K. 1979. Effect of Different CO₂ regimes on the induction of Crassulacean acid metabolism in *Mesembryanthemum crystallinum* L. *Aust. J. Plant*

physiol., 6: 589-595.

- Winter, K. 1985. Crassulacean acid metabolism. pp 329-387. In Barber, J and N.R. Baker (eds.) Photosynthetic mechanisms and the environment. Elsevier, Amsterdam.
- Winter, K., G. Zotz, B. Baur and K-J. Dietz. 1992. Light and dark CO₂ fixation in *Clusia uvitana* and the effects of plant water status and CO₂ availability. *Oecologia*. 91: 47-51.
- Wong, S.C. 1990. Elevated atmospheric partial pressure of CO₂ and plant growth. II. Non-structural carbohydrate content in cotton plants and its effect on growth. *Photosynthesis research*. 23: 171-180.
- Wong, S.C., I.R. Cowan and G.D. Farquhar. 1979. Stomatal conductance correlates with photosynthetic capacity. *Nature*. 282:424-426.
- Yelle, S., Beeson, R.C. Jr, Trudel, M.J. and Gosselin, A. 1989. Acclimation of two tomato species to high atmospheric CO₂. I. Sugar and starch concentrations. *Plant Physiol*, 90, 1465-1472.
- Ziska, L.H., K.O. Hogan, A.P. Smith and B.G. Drake. 1991. Growth and photosynthetic response of nine tropical species with long-term exposure to elevated carbon dioxide. *Oecologia*, 86: 383-389.
- Ziska, L.H. and A.H. Teramura. 1992. CO₂ enhancement of growth and photosynthesis in rice (*Oryza sativa*). Modification by increased Ultraviolet-B radiation. *Plant physiology*, 99: 473-481.
- Ziska, L.H. and J.A. Bunce. 1993. Inhibition of whole plant respiration by elevated CO₂ as modified by growth temperature. *Physiologia Plantarum*, 87: 459-466.