The interactive effects of light, temperature and CO_2/O_2 ratios in photosynthesis of *Coix Lachryma-jobi* L.

THESIS

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DEFINITION OF SYMBOLS AND ABBREVIATIONS

DEFINITION OF SYMBOLS

Term	Unit	Definition
A	μmol $m^{-2}s^{-1}$	Assimilation of CO_2 per unit leaf area
A_1	μmol $m^{-2}s^{-1}$	Assimilation in the presence of stomatal limitation
A_{max}	μmol $m^{-2}s^{-1}$	Maximum net assimilation
A_o	μmol $m^{-2}s^{-1}$	Assimilation in the absence of stomatal limitation
APS	μmol $m^{-2}s^{-1}$	Apparent rate of photosynthesis
C_a	μl l^{-1}	CO_2 concentration in the ambient air
CE	μmol $m^{-2}s^{-1}$	Carboxylation efficiency, calculated from the initial slopes of A/C_i
C_i	mol mol^{-1}	CO_2 concentration in the leaf intercellular spaces
E	μmol $m^{-2}s^{-1}$	Rate of transpiration or evapotranspiration
g_b	μmol $m^{-2}s^{-1}$	Boundary layer conductance
g_c	μmol $m^{-2}s^{-1}$	Conductance to diffusion of CO_2
g_s	μmol $m^{-2}s^{-1}$	Stomatal conductance
J_{max}	μmol $m^{-2}s^{-1}$	Light-saturated rate of electron transport
K_i	mmol mol^{-1}	Inhibitor constant at the site of carboxylation
K_m (CO_2)	μmol $m^{-2}s^{-1}$	Michaelis constant for CO_2
K_m (O_2)	μmol $m^{-2}s^{-1}$	Michaelis constant for O_2
L		Latent heat of evaporation
PPFD	μmol $m^{-2}s^{-1}$	Photosynthetic photon flux density
Q	μmol $m^{-2}s^{-1}$	Photon flux of photons
QE	mmol mol^{-1}	Quantum efficiency
R_d	μmol $m^{-2}s^{-1}$	Dark respiration rate
T	°C	Temperature
T_a	°C	Ambient air temperature
T_l		The saturation vapour pressure of the leaf surface
V_{max}	μmol $m^{-2}s^{-1}$	Maximum initial carboxylation velocity
V_{max} (CO_2ase)	μmol $m^{-2}s^{-1}$	Maximum RuBP saturated rate of carboxylation
V_{max} (O_2ase)	μmol $m^{-2}s^{-1}$	Maximum oxygenation rate
WUE	mol mol^{-1}	Water use efficiency
Γ^*	μl l^{-1}	CO_2 compensation point of photosynthesis in the absence on the dark respiration
Γ	μmol $m^{-2}s^{-1}$	Light compensation point
Φ		Quantum yields
ϕ		The ratio of the rate of oxygenation to the ratio of carboxylation reactions
λ		Absolute magnitude of the unit marginal cost of a plant
σ		Stefan-Boltzmann constant

DEFINITION OF ABBREVIATIONS

Abbreviation	Definition
BS	Bundle sheath cells
CO_2	Carbon dioxide
$[CO_2]$	Carbon dioxide concentration
H_2O	Water
KMS	*Kranz* mesophyll cells
ℓ	Stomatal limitation
NAR	Net assimilation rate
O_2	Oxygen
$[O_2]$	Oxygen concentration
PCA	Primary carbon assimilation cells
PCR	Photosynthetic carbon reduction cells
PEP	Phosphoenolpyruvate
PEPC	Phosphoenolpyruvate carboxylase
RH	Relative humidity
RuBP	Ribulose-1,5 bisphosphate
RUBISCO	Ribulose biphosphate carboxylase oxygenase
RPP	Reductive pentose phosphate

ABSTRACT

A portable infra red gas analyzer was used to investigate the interactive effects of light, temperature, and CO_2/O_2 ratios under controlled environmental conditions in an attempt to model gas exchange characteristics of *Coix lachryma-jobi* L. Plotting light response curves as a function of temperature (20, 25 30 and 35°C) revealed no sign of light saturation even at a photosynthetic photon flux density (PPFD) close to 2000 μmol m^{-2} s^{-1}. High net assimilation rates (A) of approximately 24 μmol CO_2 $m^{-2}s^{-1}$ were realized at 30-35°C. Assimilation (A) versus internal CO_2 partial pressure (C_i) curves showed a steep rise with increase in C_i but saturated at approximately 150 (μl l^{-1}) and all the results, either in the absence or presence of O_2, showed a similar response under all temperature regimes.

C. lachryma-jobi exhibited low CO_2 compensation points (Γ^*) between 0 and 10 μl l^{-1} under similar experimental temperatures and either at 0 or 21% O_2. The slopes of double reciprocal plots of $1/A$ versus $1/Ci$, were nearly identical and crossed the y-intercept at almost identical points under all O_2 concentrations. These data indicate first; that there was no apparent O_2 inhibition and second; indicated that the apparent inhibitor constant (K_i) for O_2 at the site of carboxylation did not change with increase in $[O_2]$ from 0 to 21% oxygen. These observations were further confirmed by results obtained from the analysis of apparent carboxylation efficiency (***CE***, as defined as the slope of response of A to increasing CO_2), as no inhibition of A with increase of $[O_2]$ occurred. These characteristics were consistent with typical features of C_4 photosynthesis.

The absence of O_2 inhibition and low Γ^* values indicated that an efficient CO_2 concentrating mechanism which eliminates photorespiration exists in *C. lachryma-jobi*. At the light microscope level, leaf anatomy exhibited typical C_4 structure viz. bundle

sheath with large chloroplasts and this sheath is further surrounded by a radiate Kranz mesophyll cells. Furthermore the anatomical features suggested that *C. lachryma-jobi* was an NADP-ME species. Stomatal conductance (g_s) to assimilation (g_s/A) indicated an increase in A with decrease in g_s, an essential feature of improving water use efficiency (***WUE***), but one which drastically reduces CO_2 diffusion rate. The physical limitation (stomatal limitation, ℓ) to CO_2 diffusion under various $[O_2]$ and temperatures, but constant PPFD, did not exhibit statistically significant change in ℓ values at either 0 or 21% O_2 within each temperature regime, however there was a marked decrease in ℓ as the plant approached its optimum photosynthetic temperature.

CHAPTER 1: INTRODUCTION

The major objective of this study was to investigate the interactive effects of light, temperature and CO_2/O_2 ratios in photosynthetic characteristics of *Coix Lachryma-jobi* L. Most researchers to date, have been able to generate photosynthetic models, using the data obtained from *in vitro* studies of isolated chloroplast and enzyme components (Farquhar, 1979; Farquhar *et al.*, 1980; Gutschik, 1984; Farazdaghi and Edwards, 1988; Sage, 1990). These models have been based mainly on enzyme kinetics, whole chain transport and the energy requirements of RuBP regeneration. These models have been criticized on the basis that they might overestimate photosynthetic responses and sometimes fail to simulate all conditions associated with whole leaf photosynthesis (Tenhunen, *et al.*, 1980; Monson, *et al.*, 1984; Terashima and Saeki, 1985). Nevertheless these models have been used with considerable success in simulating the photosynthetic responses, particularly in C_3 species, for example soybean (Harley, *et al.*, 1985; Harley, *et al.*, 1986).

Early in the 1970's, there was a growing emphasis on combining the internal physiological processes and environmental parameters in photosynthetic studies (Laetsch, 1974, and references quoted therein). Since then, a series of papers on whole leaf photosynthesis have been published (Chartier and Prioul, 1976; Tenhunen *et al.*, 1980; Monson, *et al.*, 1984; Harley, *et al.*, 1985). The aim of these whole leaf photosynthesis models, was to incorporate the three major environmental factors; namely, irradiance, temperature and carbon dioxide, in an attempt to use them in ecophysiological studies. These ecophysiological studies have been well investigated for C_3 species (Terashima and Saeki, 1985; Harley, *et al.*, 1986).

Little work of this nature has been undertaken using C_4 species (Farquhar and von Caemmerer, 1980). Nevertheless, many gas exchange characteristics observed using intact leaves have been predicted using models based on known biochemical and anatomical characteristics (Berry and Farquhar, 1977; Farquhar and von Caemmerer, 1980; Morot-Gaudry *et al.*, 1980).

The CO_2 concentrating mechanism has been implicated as one of the major advantages of C_4 species, because the assimilation would always proceed at high rates even when the internal CO_2 is low in these plants (Hattersley and Watson, 1976; Berry and Farquhar, 1977). The diffusion of CO_2 into the leaves takes place through the stomata, consequently stomata are significant and a major fraction influencing total limitation of photosynthesis (Taylor and Terry, 1984; Düring, 1991; Grantz and Assmann, 1991). Accordingly modelling the photosynthesis of a plant would be neither comprehensive nor complete without the consideration of limitations imposed by stomata.

The present study was undertaken in an attempt to extend available information concerning the interactive effects of light, temperature and CO_2/O_2 ratios in whole leaf photosynthesis of C_4 species. In addition this thesis addresses the limitations imposed by the stomata on photosynthesis *C. lachryma-jobi*, under controlled laboratory conditions.

The results of this thesis are discussed under the following main headings:

1. An anatomical study of the mature leaf blade of *Coix Lachryma-jobi* L.
2. Photosynthetic light and temperature responses
3. A/C_i responses
4. Stomatal limitation studies
5. Water use efficiency

CHAPTER 2: MATERIALS AND METHODS

2.1 Plant Material and Growth Conditions

Coix lachryma-jobi L., is indigenous to the East Indies and belongs to the same tribe as *Zea mays* L., the Maidea in the family Poaceae. This plant has now been introduced to a number of areas which include Africa, America and the Mediterranean region (Schaaffhausen, 1952). It is now growing in virtually all equatorial, tropical and subtropical regions of the World, and often grows spontaneously in damp hot regions. In Natal, particularly in the South Eastern Midlands, it has a large potential in the tourist industry, as *Coix lachryma jobi* produces hard shelled beads which are strung together to form necklaces, rosaries and many other ornaments.

All plant material used for this study was collected in the Amatole Mountains, Hogsback, Eastern Cape approximately 135 km from Grahamstown. Two individual vegetative and flowering plants were pressed, dried and prepared as voucher specimens and were transferred for storage to the Rhodes University Herbarium. Individual vegetative adult specimens were dug up and repotted in plastic pots containing potting soil with a pH of approximately 6.2. The potted plants were placed in the glasshouse. During the first few weeks the plants were watered twice daily and subsequently three times a week. No additional nutrients were supplied. The plants were left to grow under normal temperatures, i.e in the range of 14°C and 28°C for night and day respectively. During the experimental period, some plants were transferred to a controlled environment cabinet (Conviron EF-7H, Controlled Environments LTD, Winnipeg, Canada), which was set at 25°C/18°C for day/night respectively with a 14hr. photoperiod and 60% day/night relative humidity (RH). The light intensity in the conviron was set at approximately 1000 μmol $m^{-2}s^{-1}$ photosynthetic photon flux density (PPFD). Plants were allowed to acclimatize under these conditions for two weeks prior to the measurement of leaf gas exchange.

2.2 Leaf Gas Exchange Measurements

In order to reduce interplant variability, all gas exchange measurements were performed on the fully expanded 4th visible healthy leaf. Attached leaf of predetermined area was clamped in the broad leaf chamber (ADC PLC (B) Parkinson broad leaf chamber). The flow rate of the gas stream over the leaf was kept constant at 350ml per min. using a flow meter (ADC ASUM). The photosynthetic data were logged at 15 minute intervals, after the achievement of steady state conditions, using an ADC DL-2 datalogger LCA-2. The infra red gas analyzer (IRGA) was set up in differential mode, in open circuit. All three components of the IRGA were manufactured by Analytic Development company Co. Ltd., Hoddesdon, Herts., U.K. The IRGA was calibrated weekly using bottled gas (Fedgas Alrode, Port Elizabeth, S.A) with a known CO_2 concentration, for the duration of the experimental period.

2.3 Light Experiments

Actinic light was provided by a Phillips (SON-T) high pressured 400 Watt sodium lamp with maximum light intensity in excess of photosynthetic photon flux density (PPFD) of 2000 μmol $m^{-2}s^{-1}$. For light response experiments, fine shade cloth frames were mounted under the light source, above the leaf chamber. The temperature of the leaf chamber was closely monitored and the desired temperature was regulated using a Lauda RM-3 (Contolabor, Johannesburg) Multitemp water circulator.

In order to prevent stomatal closure in the normally dry airstream, a pre-humidifier, with a wet and dry bypass, was incorporated into the gas flow system and kept on the humid bypass when the leaf was being acclimatized between temperature, light intensity or CO_2 concentration studies. The humidifier was switched on to the dry cycle prior to taking readings from the IRGA. Readings were only taken once the relative humidity (RH) readout had stabilized (usually within 10-20 seconds). The humidification system was incorporated into the system, to prevent unwanted stress effect manifestation. Three readings were taken at each light intensity and desired temperature and each experiment on a same leaf was performed in triplicate.

2.4 **Assimilation (*A*) versus Internal CO_2 Experiments (*A*/C_i).**

The *A*/C_i curves were derived using bottled gas (Fedgas Alrode, Port Elizabeth, S.A) with different CO_2/O_2 concentrations balanced against nitrogen. These gas cylinders contained CO_2 with 0, 4, 8, 16, and 21% O_2 concentrations. *A*/C_i responses at 20-35°C were determined by decreasing the concentration of CO_2 stepwise from the lowest possible level, using an ADC GD-600 gas diluter (Analytic Development Co. Ltd. Hoddesdon, Herts., U.K.), which was connected in line to the ASUM mass flow-meter. Measurements of *A* were taken as CO_2 concentration was decreased in stages from 999 to 0 $\mu l\ l^{-1}$. Three readings were taken for each CO_2 concentration and each experiment was performed in triplicate.

The concept that photosynthesis is measured with a system, rather than a single instrument, is an important one. The system concept emphasizes the fact that there is no discrete photosynthetic sensor. Photosynthesis is always a calculated parameter, determined from measurements of internal CO_2 concentrations, gas flow, leaf area, temperature, humidity, stomatal conductance and transpiration (Field *et al.* 1989). It was therefore essential to use calculations incorporating these parameters, similar to those described by von Caemmerer and Farquhar, (1981), (See Appendix B, for calculations used). However, the calculations in this case were adapted by Botha and Brown (1991) in their software package, infra-red photosynthetic CO_2 gas analysis calculation (IRCAL, Version 2.0), (Copyright by C.E.J. Botha and B.J.L. Brown, Rhodes University, Grahamstown, 1991). Once the data had been computed, it was produced as a hard copy output on a dot-matrix printer, as well as in a comma separated value (CSV) file. The final computations exhibit net assimilation rates (NAR) and also display characteristics which are simultaneously computed, these include leaf temperature, internal CO_2 concentration, stomatal conductance, stomatal resistance, the slope of the supply function, the apparent water use efficiency (*WUE*) and apparent quantum efficiency. All graphs were created using FIGP software package (FIG-P. Software Corp. Durham, N.C., USA).

2.5 **Statistical Analysis**

Steady state CO_2 assimilation rates of the A/C_i experiments were statistically evaluated using analysis of variance (ANOVAR), since it offered a multiple range of tests, where values could be compared across and down the tables. All statistical calculations were based on actual data points, as well as on the means of the maximum assimilation rates attained at ambient and at internal CO_2 of 350 $\mu l\ l^{-1}$ using various gases of varying but known O_2 concentrations. Calculations were computed using a Stat Graphics (Statgraphics, Version 5.0, Statistical Graphics Corp. Maryland, USA).

2.6 **Leaf Anatomy**

Fully expanded mature leaves of a similar morphological age as those used for the leaf gas-exchange measurements were used for anatomical studies. The leaf was cut into segments of approximately $0.5cm^2$ each starting from the base continuing up to the apex of the leaf. Sections were immediately transferred to Formalin Acetic Acid (FAA) to kill and fix the tissues. After fixation, tissues were gradually dehydrated by alcohol involving a graded series of tertiary butyl alcohol (TBA). Finally the TBA was substituted with liquid paraffin and subsequently with paraffin wax. The specimens were left in a warm oven for two days to allow for impregnation of the tissue by the wax, after which, the specimens were then transferred to the moulds and allowed to set. The set wax-blocks were then affixed to wooden microtome blocks, and the sections cut using Wetzler microtome (Lietz, Johannesburg, RSA) at 12μ. Finally, permanent sections were stained in safranine and fast green. The slides were examined and photographed using a Zeiss photomicroscope III (Zeiss Standard Junior 18, fitted with an Mk-63 camera, Carl Zeiss (Pty) Ltd., Johannesburg, RSA).

CHAPTER 3: THE ANATOMY OF THE MATURE LEAF BLADE

3.1 Introduction

In C_4 plants, the biochemistry of photosynthesis is functionally interwoven with highly specialized leaf anatomy characteristic of these species (Hatch,1987). Typically, the chloroplasts of C_4 species are about equally distributed between two quite distinct cell types. These cells have separate metabolic photosynthetic functions first recognised in the beginning of the 70's by Downton, 1970; and Hatch, *et al.*, (1971). These mesophyll and bundle sheath cells are generally arranged in two concentric layers, with a common interface around the vascular bundles. The inner layer is made of large thick-walled, cylindrical cells containing prominent chloroplasts, termed the PCR (photosynthetic carbon reduction), or bundle sheath (BS) cells, whilst the outer radiating layer is composed of relatively elongated thin-walled cells containing less conspicuous chloroplasts, termed the PCA (primary carbon assimilation) cells. This specialized wreathlike arrangement which is always associated with C_4 photosynthesis is termed *Kranz* anatomy (Tregunna, *et al.*, 1970; Smith and Brown, 1973 and Laetsch, 1974).

It is possible to sub-divide the C_4 plants into three subspecies on the basis of enzyme studies, the Phosphoenolpyruvate carboxykinase (PCK), NAD-malic enzyme (NAD-ME) and NADP-malic enzyme (NADP-ME) types, according to the mechanism each employ for carboxylation C_4 acids in the PCR cells. Furthermore these subgroups have been correlated to distinct anatomical and ultrastructural features (Edwards, *et al.*, 1971; Brown and Gracen, 1972; Gutierrez, *et al.*, 1974; Hattersley and Watson, 1976; Hattersley and Browning, 1981 and Fladung and Hesselbach, 1987). In his study of comparative leaf anatomy in the Poaceae, Ellis (1976) classified the South African grasses into either C_3 or C_4 and further sub-divided the C_4 group into the three subspecies. This study indicates that *C. lachryma-jobi* is an NADP-ME type.

3.2 Aims and Objectives

Most of the early studies in structure, physiology and biochemistry of C_4 plants lead independent and parallel lives (Laetsch, 1974). However, it was soon realized that the faster export rates of photosynthetic products from leaves of tropical plants such as maize and sorghum were associated with C_4 photosynthesis, and slower export rates from the leaves of grasses such as rice and wheat with C_3 photosynthesis (Gallaher, *et al.*, 1975). Since then many workers have attempted to correlate physiological functions to structure and it has become more apparent with recent ecophysiological studies that previous separation or omission of these disciplines has resulted in what are now recognised to be incomplete descriptive ecophysiological models. In fact the gradual convergence of these different lines of study with the resultant synergistic effect on all these disciplines has led to an intriguing aspect of the C_4 plant phenomenon (Ku, *et al.*, 1983; Kemp, *et al.*, 1983; Prendergast, *et al.*, 1988; Araus, *et al.*, 1991). The most comprehensive recent classical treatise of integration of structure-function relationships in C_4 plants, was the one published by Hatch, (1987). The research presented here attempts to integrate the leaf structural aspects of *C. lachryma-jobi* to observed physiological functions, as structure function studies are major aspects of ecophysiological study.

3.3 Results

3.3.1 *Leaf Morphology*

C. lachryma-jobi leaves are generally linear, long and lanceolate (Hickey, 1973), and widest at the midpoint, and tapering towards their bases and tips. The leaves are 70-100 long and 1.5-3.0 cm in width. The leaf consists of three parts; basal sheath, which represents about 10% of the total leaf length, ligule, a short membranous structure at the point of attachment of the leaf and the stem, and finally, the blade. A prominent midrib with a scabrid margin projects from the lower surface, beginning about half way from the tip. Healthy leaves are glabrous (lack hairs) and are dark green in colour.

3.3.2 *Description of Leaf Blade Anatomy*

In transverse section, the blade exhibit characteristic Kranz anatomy, with mesophyll radially arranged around a chlorenchymatous bundle sheath (BS) (Fig. 1). The BS cell's chloroplasts are centrifugally arranged, a feature associated with NADP-ME type species (Ellis, 1976). PCA cells of adjacent vascular bundles are separated by relatively colourless cells. This interveinal distance between two PCA cells was also typical of C_4 photosynthesis since the maximum lateral cell count (Hattersley and Watson, 1975 and Kawamitsu, *et al.*, 1985) was less than four. Large substomatal cavities occur beneath the adaxial epidermis and such cavities open directly to the PCA cell surface (Fig. 1-4). The adaxial epidermis is interspersed with large colourless cells called bulliform cells (BC). In any given section, excluding the midrib, there are three types or orders of vascular bundles (VB), and these orders of VB were described fully elsewhere for *Themeda triandra*, (Botha, *et al.*, 1982), namely; large (first-order), intermediate (second-order) and small bundles.

Generally, small bundles alternate with intermediate bundles and large bundles are flanked by small ones (Fig. 1). Some longitudinal bundles are interconnected by small transverse bundles. By definition, a vascular bundle is a strand-like part of the vascular system, consisting solely of xylem and phloem (Esau, 1977; Colbert and Evert, 1982). For convenience, in the following detailed description of the different vascular bundles, some associated structures such as bundle sheaths, hypodermal sclerenchyma and neighbouring mesophyll cells will also be considered, as these form the specialized boundary to the vascular bundles.

3.3.3 *Large and median bundle*

The large bundles are characterized by the presence of large metaxylem vessels (V) on either side of the protoxylem (PX) or protoxylem lacuna (Fig. 1 and 2). Though not clearly visible in these light micrographs the metaphloem consists of both thick and thin walled sieve-tubes, and vascular parenchyma cells including companion cells. The inner mestome sheath with thickened walls is not well defined and does not completely encase the vascular tissue. It is only associated

with the xylem (Fig. 1 and 2). Generally, large bundles in *C. lachryma-jobi* are more or less completely surrounded by a chlorenchymatous BS layer, which is interrupted on the adaxial and abaxial surfaces by hypodermal sclerenchyma girders.

However the midrib is subtended by chlorenchymatous BS cells. The hypodermal sclerenchyma girders are only present on the abaxial surface of vascular tissue (Fig. 2). Except in the midrib, large bundles essentially extend from epidermis to epidermis (Fig. 2).

3.3.4 ***Intermediate Bundles***

Intermediate bundles are completely surrounded by BS cells and are associated with hypodermal sclerenchyma strands only abaxially (Fig. 3). Unlike the large bundles, intermediate bundles lack sclerenchyma girders and in addition they lack large metaxylem vessels and protoxylem. The phloem consists of sieve tubes, parenchyma and companion cells. The chlorenchyma BS cells are always bordered by PCA cells. The intermediate bundles together with their BS cells and associated Kranz mesophyll, essentially extend nearly all the way from adaxial to abaxial epidermis (Fig. 3).

3.3.5 ***Small Bundles***

Small bundles are completely surrounded by a chlorenchymatous BS layer which is in direct contact with the PCA or Kranz mesophyll cells (Fig. 4). These bundles consist entirely of metaxylem and metaphloem. Unlike the large and intermediate bundles, the small bundles lack both hypodermal sclerenchymatous girders or strands.

In addition, small bundles occupy largely the lower half (abaxial) of the leaf cross section and lie closer to the abaxial epidermis and topped by large bulliform cells within the adaxial epidermis (Fig. 4). In conclusion, *C. lachryma-jobi* appears to be an NADP-ME species based on its leaf anatomy.

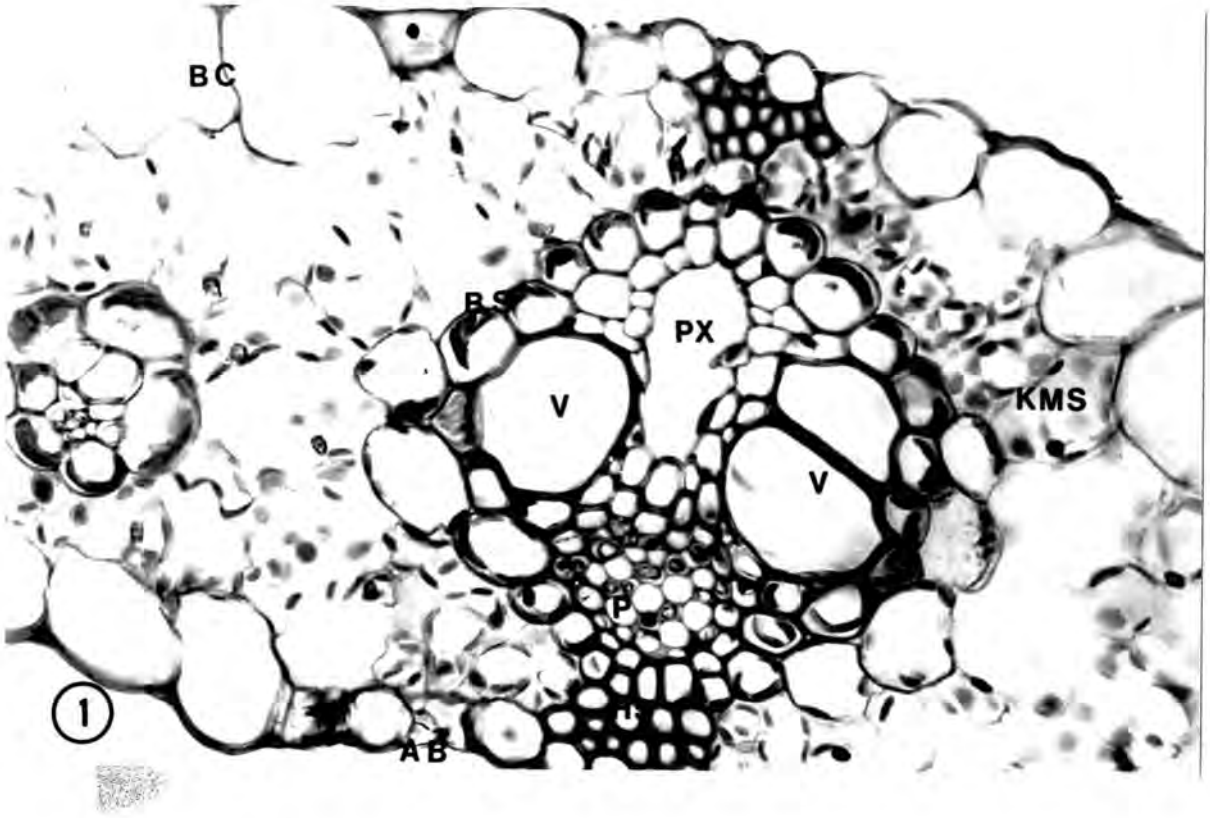

Fig. 1 Light micrograph of cross section of *C. lachryma-jobi* leaf showing a large vascular bundle flanked on the left by a small bundle. The chlorenchymatous bundle sheath (***BS***) is interrupted on both the abaxial and adaxial surfaces by hypodermal sclerenchyma girders (***HS***). ***AB*** abaxial epidermis; ***BC*** bulliform cells; ***KMS*** Kranz mesophyll or ***PCA*** cells; *V* mature metaxylem vessel; *P* phloem tissue consisting sieve tubes, parenchyma and companion cells. ×2000

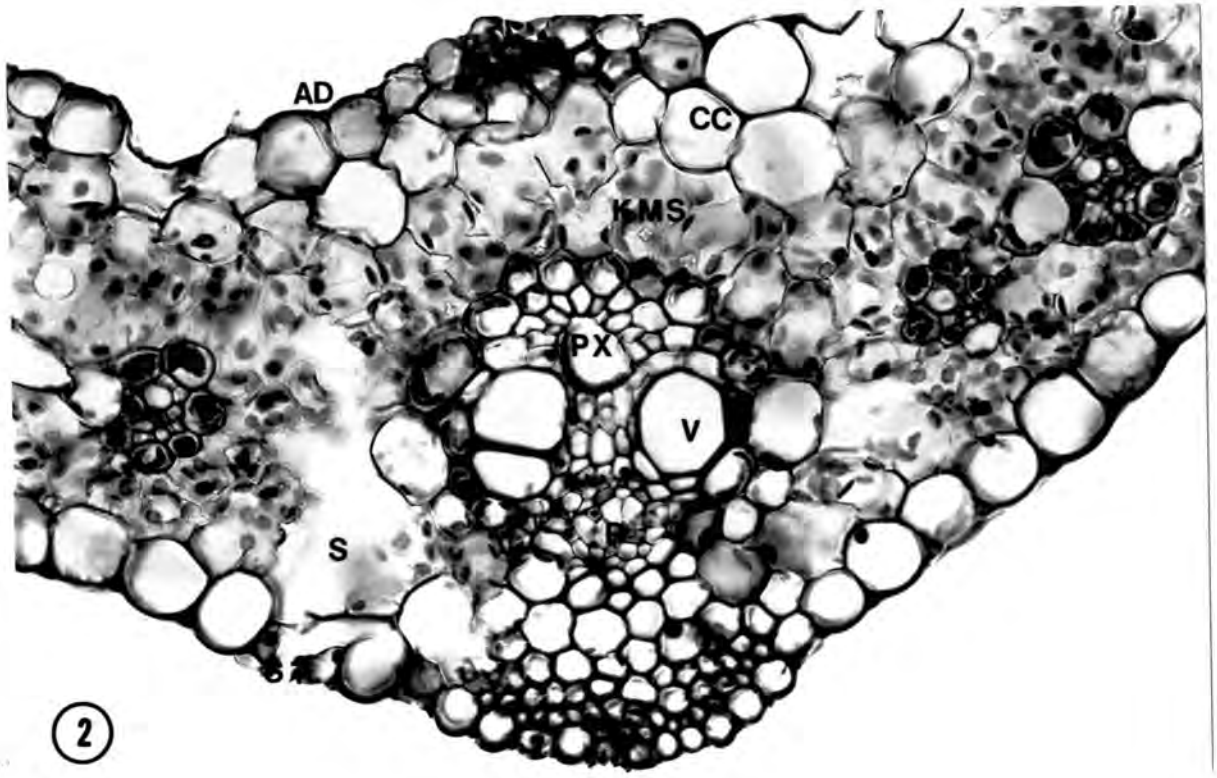

Fig. 2 Light micrograph of cross section of *C. lachryma-jobi* leaf showing the median bundle, which is flanked on both sides by small bundles. In the midrib, large colourless cells (*CC*) intervene between the *VB* and the adaxial epidermis (*AD*). In this bundle the *HS* cells are present on the abaxial surface only. *S* substomatal cavity; *KMS* Kranz mesophyll cells; *V* mature metaxylem vessel; *P* phloem tissue consisting sieve tubes, parenchyma and companion cells. ×1890

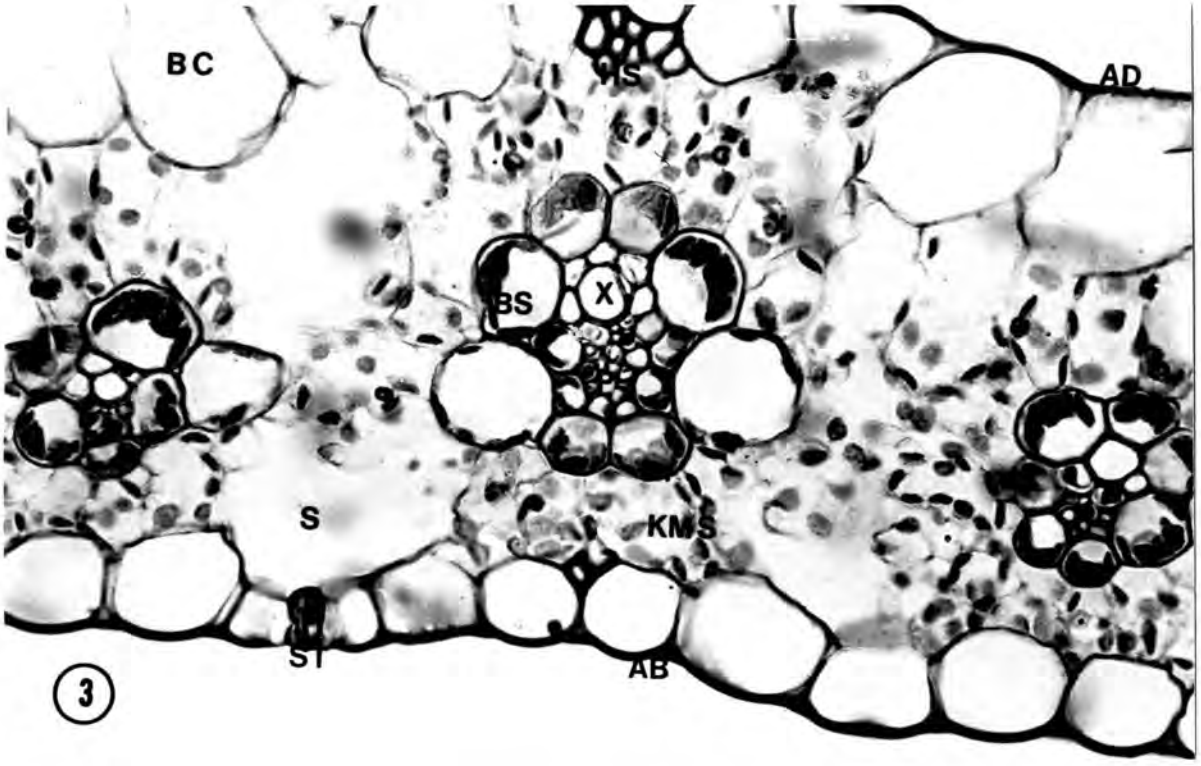

Fig. 3 Light micrograph of cross section of *C. lachryma-jobi* leaf showing the intermediate bundle, which is also flanked by small bundles on both sides. In this bundle the ***HS*** cells are present on the adaxial surface but do not interrupt the ***BS*** cells. ***KMS*** Kranz mesophyll cells; ***S*** substomatal cavity; ***X*** xylem cells; ***P*** phloem tissue consisting sieve tubes, parenchyma and companion cells. ×2020

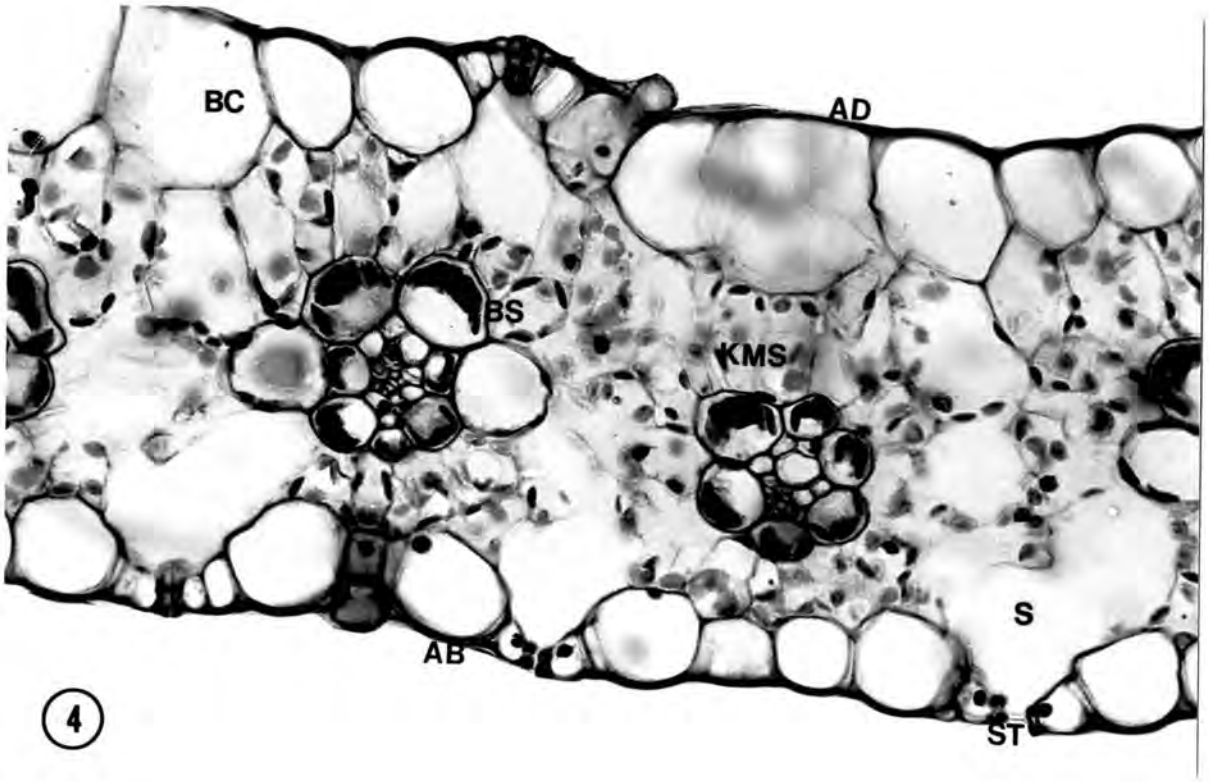

Fig. 4 Light micrograph of cross section of *C. lachryma-jobi* leaf showing the small bundles. ***BC*** cells are very pronounced and are present on both surfaces of the epidermis. In these bundles, ***HS*** cells are not present. ***KMS*** Kranz mesophyll cells; ***ST***; stoma and beneath it large substomatal cavity present (***S***); ***VB*** vascular bundle, consisting of protoxylem and protophloem i.e sieve tubes, parenchyma and companion cells. ×1900

CHAPTER 4: LIGHT AND TEMPERATURE RESPONSE

4.1 Introduction

Photosynthesis is strongly affected by light quality and intensity as well as temperature. In most plants, changes in photosynthetic rate in response to temperature are reversible over the range from 10 to 40°C, but exposure below or above this range may cause irreversible damage (Berry and Björkman 1980). Unfortunately attempts to compare the results obtained by different investigators on different species under different environments suffer from the problems that the temperature dependence of photosynthesis, even for a single leaf, is strongly influenced by other environmental factors e.g light, internal CO_2 and leaf water content or humidity (Welschmeyer and Lorenzen, 1981; Buryzynski and Lechowiski, 1983; Ludlow, 1987).

Temperature and light effects only will be discussed in this section. The temperature dependence of photosynthesis becomes increasingly pronounced when either light intensity, or intercellular CO_2 level is altered. A close association exists between these factors and stomatal conductance which is in turn, influenced by temperature (Meidner, 1986). It is only recently that there has been a growing awareness among researchers of the need to simultaneously assess the effect of these interacting factors in photosynthesis and not merely to describe one factor in isolation from other factors.

4.2 Effect of Temperature and Light on C_4 Enzymes

Björkman and Holmgren (1963) in their studies of the effect of light and temperature on plants grown in two contrasting environments, namely shaded and exposed habitats, found that A_{max} was generally higher in the plants from exposed habitat compared to those grown in shaded habitat. These responses were linked to enzymatic changes, where ribulose bisphosphate carboxylase (RUBP) activity, was 0.25 μmol CO_2 mg^{-1} protein min^{-1} for exposed habitat plants grown in strong light and reduced to 0.15 for plants grown in weak light. These observations suggested a strong correlation in enzyme activation by light. Usuda (1985), in his studies of changes of levels of C_4 and reductive pentose phosphate enzymes during induction of

photosynthesis in maize, found that light seems to play a major role in activation of RuBP, Phosphoenolpyruvate carboxylase (PEPC) and other enzymes associated with C_3 and C_4 photosynthesis. Further conclusive evidence of this light activation has been provided by (Kobza and Seemann, 1988; Leegood and von Caemmerer, 1989 and Tanaka, *et al.*, 1990). However, the degree of light activation of enzymes has been observed and does not seem to follow a fixed pattern (von Caemmerer and Farquhar, 1981; Evans, 1987), but the degree to which the major proteins limit the rate of photosynthesis may vary with light condition to which the plant is adapted (Woodrow and Mott, 1988).

It is generally believed that both the reductive pentose phosphate (RPP) and C_4 enzymes are activated by light, but there is also a strong evidence that the function of these enzymes also depend largely on temperature. Studies on RuBP activity have shown that this enzyme functions optimally at temperatures between 18 and 25°C but beyond this range it has been noted that it tends to be oxygenated rather than carboxylated, leading to a process called photorespiration (Zelitch, 1971; Ogren, 1984; MacRae and Ferguson, 1985). Farquhar *et al.*, (1980), derived a kinetic expression which relates the ratio ϕ of the rate of oxygenation reaction (VO_2ase) to the rate of carboxylase reaction (VCO_2ase) in the following equation:

$$\phi = \frac{VO_2ase}{VCO_2ase} = \frac{V_{max}O_2ase}{V_{max}CO_2ase} \times \frac{K_m(CO_2)}{K_m(O_2)} \times \frac{[O_2]}{[CO_2]}$$

..... Equation 1

Where, K_M (CO_2) and K_M (O_2) are the Michaelis constants for CO_2 and (O_2) respectively and finally [CO_2] and [O_2] are the relative concentrations of CO_2 and O_2 at the site of carboxylation of the enzyme. At a given condition there would be ϕ oxygenation of RuBP for each carboxylation. The equation briefly summarizes the factors which may limit the rate of carboxylation *in vivo*. Firstly, the relative partial pressures of CO_2 and O_2 determine the partitioning between carboxylation and oxygenation. Secondly, the

amount of activated enzyme present determines the maximum velocity (V_{max}), of the enzyme for O_2ase relative to carboxylase.

There is sufficient evidence to suggest that the rate of oxygenation increases with temperature (Ku and Edwards, 1977a; 1978). Brooks and Farquhar (1985) argue that these effects can be accounted for by temperature-dependent solubilities of CO_2 and O_2 in water. The solubility of CO_2 to O_2 has been observed to decrease with temperature whether as gaseous partial pressure or as concentrations of dissolved gases. It is therefore apparent that there are more chances of oxygenation of this bi-functional enzyme RuBP at high temperatures because of reduced affinity of CO_2 and less CO_2 to O_2 ratios under these conditions. In contrast to C_3 species, C_4 species exhibit higher photosynthetic rates at high temperature. It has been shown as early as mid sixties (Hatch and Slack, 1966) that these plants use the enzyme PEP carboxylase for the primary fixation of CO_2, and the fixed carbon travels initially through various 4-carbon dicarboxylic acids; namely, oxaloacetate, malate or aspartate, but the CO_2 is eventually released in the BS cells and refixed by Rubisco.

PEP-carboxylase provides the necessary base with sufficiently high assimilatory capacity (combination of V_{max} and affinity for inorganic carbon) to ensure rapid incorporation of carbon dioxide under relatively low internal CO_2 concentration (Jordan and Ogren, 1984; Hatch, 1987). In addition the high CO_2 concentration developed in the bundle sheath cells of C_4 species serves to abolish O_2 inhibition of RuBP oxygenase activity, and associated photorespiration. Consequently the quantum yield of C_4 species remains more or less constant over the range of 20-40°C (Berry and Björkman, 1980; Pearcy and Ehleringer, 1984; Ehleringer and Werk, 1986).

4.3 Light Requirements as Affected by Temperature and Light

In photosynthesis, light drives a current of electrons through the electron-transport of chloroplast thylakoid membranes in the same way as ubiquinone in the respiratory electron-transport chain (Bidwell, 1979). As electrons are being transferred from one form of cytochrome

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such as plastiquinone to another in the presence of suitable enzyme, a molecule of ATP is generated from ADP and Pi for each electron pair that passes down this electron-transport chain (Osmond, *et al.*, 1982; Heber *et al.*, 1986). ATP and NADPH represent the net gain from the light reactions. Generally the energy costs of C_4 photosynthesis (5 ATP and 2 NADPH, mol^{-1} CO_2 fixed) are greater than those of RPP cycle in the absence of photorespiration (3 ATP and 2 NADPH, mol^{-1} CO_2 fixed). However the real cost with oxygenase reaction and accompanying photorespiration is much higher and it is believed to be 5 ATP and 3 NADPH (Osmond, *et al.*, 1982; Ziegler-Jöns and Selinger, 1987). These energy costs are reflected in quantum yields of leaf photosynthesis measured under light-limiting conditions.

Quantum yield (Φ) is the number of moles of CO_2 fixed (A) per μmol m^{-2} s^{-1} or Einstein (mol of photon) absorbed. Quantum yield may be calculated in the following equation:

$$\Phi = \frac{A}{Q}$$

..... Equation 2

Ehleringer and Björkman (1977) showed that Φ, determined at light saturation and under normal ambient air, was temperature dependent in C_3 species and that the effect of temperature on the quantum yield is probably related to a stimulation of oxygenation of RuBP as temperature increases. As a result the Φ of C_3 photosynthesis declined by about 35% over the range of 20-40°C (Ehleringer and Werk, 1986). In contrast the Φ of C_4 photosynthesis are more or less constant over the similar temperature range, as the transfer of CO_2 from PEP carboxylase means that RUBP carboxylase operates at an optimal CO_2 concentration, which in turn, inhibits the oxygenase reaction of RuBP (Ehleringer and Pearcy, 1983). Consequently the photosynthetic efficiency in terms of chemical energy requirements, and also general CO_2 assimilation, increases on average. However, the advantage is negated at temperatures below 20°C (Ludlow *et al.*, 1985; Fitter and Hay, 1987). This temperature-dependence may also explain the general distribution of C_4 plants, which normally frequent hot dry sunny environments and

are largely confined to low latitudes (Berry and Björkman, 1980; Vogel, *et al.*, 1986).

4.4 Aims and Objectives

Models on the relationships between photosynthesis and environmental factors, such as curves fitted to measured relationships between photosynthesis and light and temperature, are valuable for practical purposes. Light and temperature, undergo significant, often rapid changes under natural conditions. The influence of these factors on the photosynthetic process is interconnected and therefore, they must be taken into consideration jointly when planning experiments. Light response curves obtained under laboratory conditions can be used subsequently to compare other experimentally-applied parameter changes in conditions imposed on the plant. Such responses may be used to estimate the apparent internal biochemical activity without actually isolating enzymes, thus contributing immensely to the concept of whole leaf photosynthesis. The aim of this section of the experimental investigation was to determine the response of *C. lachryma-jobi* to light and temperature under laboratory conditions.

4.5 Results

4.5.1 *Light and Temperature Experiments*

Light curves of net assimilation of *C. lachryma-jobi* against increasing photosynthetic photon flux density, under different thermal conditions are presented in Fig. 5.

Assimilation at all temperatures (20, 25, 30 and 35°C) show a curvilinear progression, consisting of two phases, an initial linear phase of steep rise in *A* with light below 400 μmol $m^{-2}s^{-1}$ PPFD and followed by a progressive decrease in the slope with increase in light, most notably above PPFD of 1200 μmol $m^{-2}s^{-1}$.

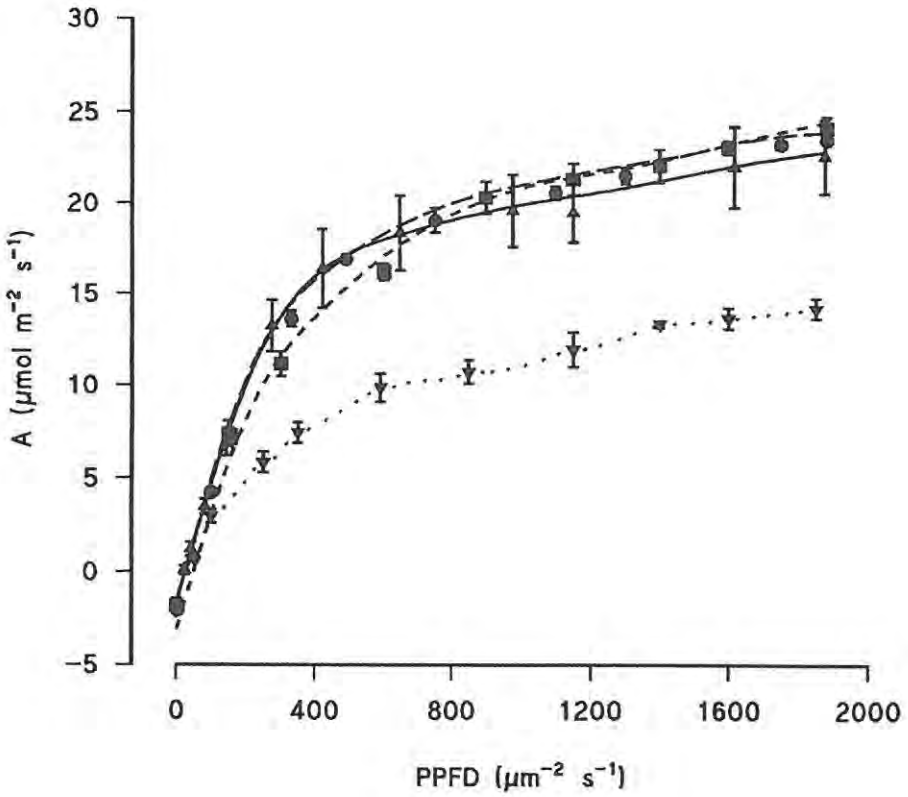

Fig. 5. Illustrates net assimilation rates (*A*, μmol $m^{-2}s^{-1}$) in *Coix* as a function of incident light intensity (PPFD, μmol $m^{-2}s^{-1}$) at different temperatures in ambient air. Both characters of the light curves of *Coix* and absolute values of *A* under conditions of thermal optimum were typical of the C_4 species. When the thermal optimum was exceeded, i.e above 30°C no further increase in *A* was observed. (▼20, ▲25, ■30 and ●35°C)

At low PPFD (i.e below 400 μmol $m^{-2}s^{-1}$) temperature has little effect in net assimilation rates, but with the increase in PPFD there was a marked separation of the curves with the response at 30°C attaining a slightly higher NAR than the remaining temperature regimes. No evidence of light saturation is apparent between 20 and 35°C, at light intensities near 2000 μmol $m^{-2}s^{-1}$ PPFD. Comparison of the curves shows that the photosynthetic performance of *C. lachryma-jobi* was reduced drastically at low temperature irrespective of increased light intensity (Fig. 5). Maximum assimilation (A_{max}) was attained between 30 and 35°C at approximately 24 μmol CO_2 $m^{-2}.s^{-1}$, however the A_{max} was reduced at 20°C to approximately 15.00 μmol CO_2 $m^{-2}.s^{-1}$. These results are typical features of C_4 species and are in accordance with other research reports of similar work on C_4 photosynthesis (Buryznski and Lechowski, 1983; Grammatikopoulos and Manetas, 1990).

All curves converged at almost the same point at PPFD of 0 μmol $m^{-2}s^{-1}$, below the x-Axis, thus suggesting that *C. lachryma-jobi* also exhibit dark respiration (R_d). However there was a slight variation in light compensation points (Γ), i.e the points at which photosynthesis equals respiration and no net gas exchange occurs.

4.5.2 *Light Requirement and Apparent Quantum Efficiency*

Apparent quantum efficiency (Table 1) was computed simultaneously with net assimilation and other parameters associated with photosynthesis.

Table 1. Illustrates quantum efficiency (**QE**) [essentially quantum yield (Φ, from equation 2) but not corrected for leaf absorptance (Botha and Russell, 1988)], determined at rate-limiting light intensities (2000 μmol $m^{-2}s^{-1}$ PPFD) under all temperature regimes.

Temperature °C	Maximum Net Assimilation A_{max} (μmol m^{-2} sec^{-1})	Quantum Efficiency QE (moles CO_2 mole-$quanta^{-1}$)
20	15.00	0.0476
25	21.45	0.0482
30	24.00	0.0478
35	23.89	0.0484

There was no significant differences in the QE values with increase in temperature, but a steady increase in net assimilation, under similar conditions, was observed. Although, the values obtained under these light and temperature conditions, were similar to those reported in similar studies by Ehleringer and Björkman (1977) the trend was found to be essentially the same as that of *Atriplex rosea*, also a C_4, despite the fact that the QE of *A. rosea* was marginally higher than those of *C. lachryma-jobi*.

In conclusion, the character of the light response curves and apparent quantum efficiency, observed under controlled laboratory conditions, suggests that *C. lachryma-jobi* is photosynthetically C_4 and that the data indicates that it is an NADP-ME subtype.

CHAPTER 5: ASSIMILATION VERSUS INTERNAL CO_2 CONCENTRATION

5.1 Introduction

Carbohydrate formation in all higher plants, regardless of photosynthetic mode, depends upon the carboxylation of RuBP. However this enzyme exhibits different kinetic properties depending upon its degree of activation (Lorimer and Andrews, 1973). When fully activated this unstable enzyme could either be carboxylated and enters the RPP cycle or could be oxygenated resulting in the formation of phosphoglycolate which becomes the primary substrate for photorespiration or C_2 cycle. It is generally believed that net photosynthesis could be increased by approximately 33% when a low oxygen 1-2% gas stream is substituted for normal air (Kriedmann and Downton, 1981; Brown, *et al.*, 1986). Consequently the degree of O_2 inhibition of photosynthesis mainly depends upon CO_2 concentration, temperature and leaf water status (Brown and Morgan, 1980).

5.2 Effects of CO_2/O_2 Ratios in C_4 Photosynthesis

Plants which use the classical calvin cycle or C_3 pathway of photosynthesis rely upon RuBP carboxylase to catalyse the primary step in CO_2 fixation. In C_4 photosynthesis however, the initial fixation is catalyzed by PEP, an enzyme with higher affinity for CO_2 (Hatch, 1976). Using this enzyme, C_4 plants effectively concentrate CO_2 at the site of carboxylation, thus enabling these plants to efficiently remove CO_2 from the atmosphere, particularly when CO_2 levels are limiting (Tolbert and Zelitch, 1983; Newton, 1991). Hatch (1976) stated that the substomatal CO_2 concentration of C_3 species is approximately 6 μM whilst the level in C_4 plants is an order of magnitude higher.

The CO_2 pump through the C_4-dicarboxylic acid pathway is thought to increase the CO_2/O_2 ratio in bundle sheath (BS) cells thus preventing oxygen inhibition of photosynthesis (Zelitch, 1971;

Hatch and Osmond, 1976). Inhibition of photosynthesis by atmospheric O_2 has not been observed in C_4 plants (Morgan and Brown, 1979). However, as early as mid 60's, Forrester, *et al.* (1966) reported a slight inhibition of photosynthesis by O_2 in C_4 photosynthesis. This was later confirmed by other researchers who conducted similar experiments (Canvin, *et al.*, 1980; Berger and Fock, 1983). Krall and Edwards (1990) indicated that despite the high concentration of CO_2 in the BS, a limited but undetermined amount of O_2 is presumably utilised by C_4 plants through RuBP, since evidence exists for function of the C_2 pathway in these plants. Therefore the basis for O_2 inhibition of photosynthesis in the C_4 plants is still unresolved.

5.3 Carboxylation efficiency in C_4 photosynthesis

Lineweaver-Burk plots (Clark and Switzer, 1977) have been employed in the investigation of apparent oxygen effects. These plots of $1/A$ versus $1/C_i$ yield a straight line with an intercept of $1/V_{max}$ and a slope of K_M/V_{max}; [where K_M is the Michaelis constant and V_{max}, the maximum initial carboxylation velocity]. Although double reciprocal plots are used for the most part in isolated enzymes (Seemann and Berry, 1982; Laing, *et al.*, 1984), they have also been successfully employed by a number of researchers on whole leaf photosynthesis (Ku and Edwards, 1977a; Monson *et al.*, 1984). Furthermore, carboxylation efficiency on whole leaf photosynthesis which was proposed by Ku and Edwards (1977a) and later Monson, *et al.* (1984) at different CO_2/O_2 concentrations and at different temperatures has been determined and employed to supplement the *Lineweaver-Burk plots* application.

Carboxylation efficiency (*CE*) is defined as the initial slope of response of photosynthesis to increasing CO_2 as illustrated in the following equation:

$$CE = \frac{APS}{CO_2 - \Gamma^*}$$

.....Equation 3

where ***APS*** is the apparent rate of photosynthesis, CO_2 is the internal carbon dioxide concentration, and Γ^* is the CO_2 compensation point. Though this method is, as stated earlier, preferably used in studies on isolated enzymes, it is applicable to studies of whole leaf photosynthesis, and the resultant plot takes into consideration all limiting factors that affect assimilation, in contrast to its use with isolated enzyme systems where conditions in many cases are far from the natural leaf environment (Ku and Edwards, 1977a; Monson, *et al.*, 1984). Furthermore ***CE***, (based on soluble CO_2 in the leaf), closely approximates carbon assimilation efficiency per unit of CO_2 available at the site of carboxylation of photosynthetic cells and thus allows reliable comparisons of ***CE*** with varying temperature and at different CO_2/O_2 concentrations (Ku and Edwards, 1977a; Moore, *et al.*, 1985).

5.4 Aims and Objectives

As was briefly stated in the introduction, the main objective was to develop an analytical model of whole leaf photosynthesis for comparing the responses of *C. lachryma-jobi* to different light intensity, temperature and CO_2/O_2 concentrations. The results on light and temperature have been dealt with in chapter four. This section of the thesis focuses on assimilation in *C. lachryma-jobi* exposed to different CO_2/O_2 concentrations and at varying temperatures.

5.5 Results

5.5.1 *A/C$_i$ Experiments*

The effects of varying C_i on net assimilation rate investigated at PPFD of 1200 μmol $m^{-2}s^{-1}$ and at different temperatures are presented in Figs. 6-9.

The slope of the plotted A/C_i, at varying temperatures and $[O_2]$, exhibit a rapid initial increase in A in response to availability of substrate, C_i which is caused by increase in ambient CO_2. Carbon assimilation responses at each temperature regime saturated between C_i of 150-200 $\mu l\ l^{-1}$ but the curves increased steadily in response to CO_2 close to 600 $\mu l\ l^{-1}$. The curves were nearly identical within each temperature regime under either 0, 4, 8, 16, and 21% O_2 at all the experimental C_i points in the range of 0-600 $\mu l\ l^{-1}$ (Figs. 6-9). This trend is best illustrated in Table 2 below, where A_{max} values under all O_2 concentration and at all temperatures are illustrated.

Table 2. Maximum net assimilation response at varying $[O_2]$ but constant PPFD (1200 μmol m^{-2} s^{-1}) under all temperature regimes.

Temperature °C	A_0	A_4	A_8	A_{16}	A_{21}
20	18.40	17.60	18.55	19.00	18.15
25	22.98	20.90	23.98	21.71	23.71
30	23.75	24.03	23.66	24.00	23.52
35	24.11	22.78	23.99	24.68	25.05

A_0 - CO_2 0% O_2 Balanced with Nitrogen.
A_4 - CO_2 4% O_2 Balanced with Nitrogen.
A_8 - CO_2 8% O_2 Balanced with Nitrogen.
A_{16} - CO_2 16% O_2 Balanced with Nitrogen.
A_{21} - CO_2 21% O_2 Balanced with Nitrogen.

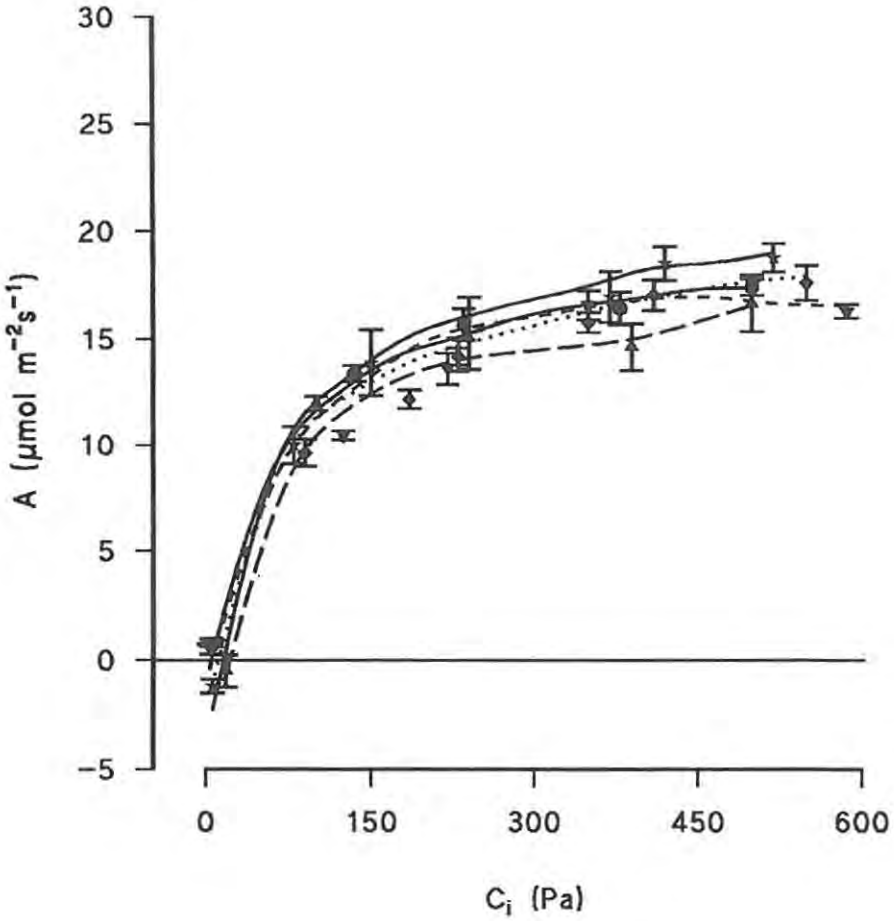

Fig. 6. Shows the response of CO_2 assimilation to intercellular CO_2 at 0, 4, 8, 16, and 21% O_2 (N-balanced CO_2). Measurements were made at PPFD of 1200 μmol $m^{-2}s^{-1}$ at 20°C. (●0, ▲4, ▼8, ♦16, ★21% O_2)

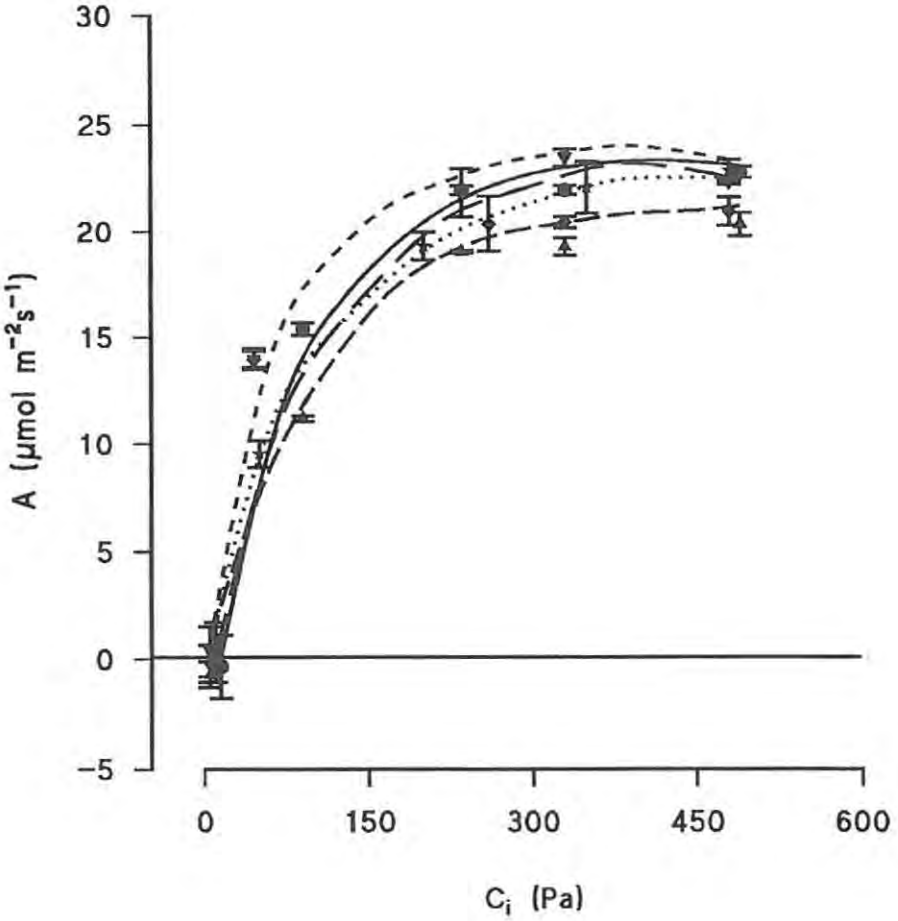

Fig. 7. Shows the response of CO_2 assimilation to intercellular CO_2 at 0, 4, 8, 16, and 21% O_2 (N-balanced CO_2). Measurements were made at PPFD of 1200 μmol $m^{-2}s^{-1}$ at 20°C. (●0, ▲4, ▼8, ♦16, ★21% O_2)

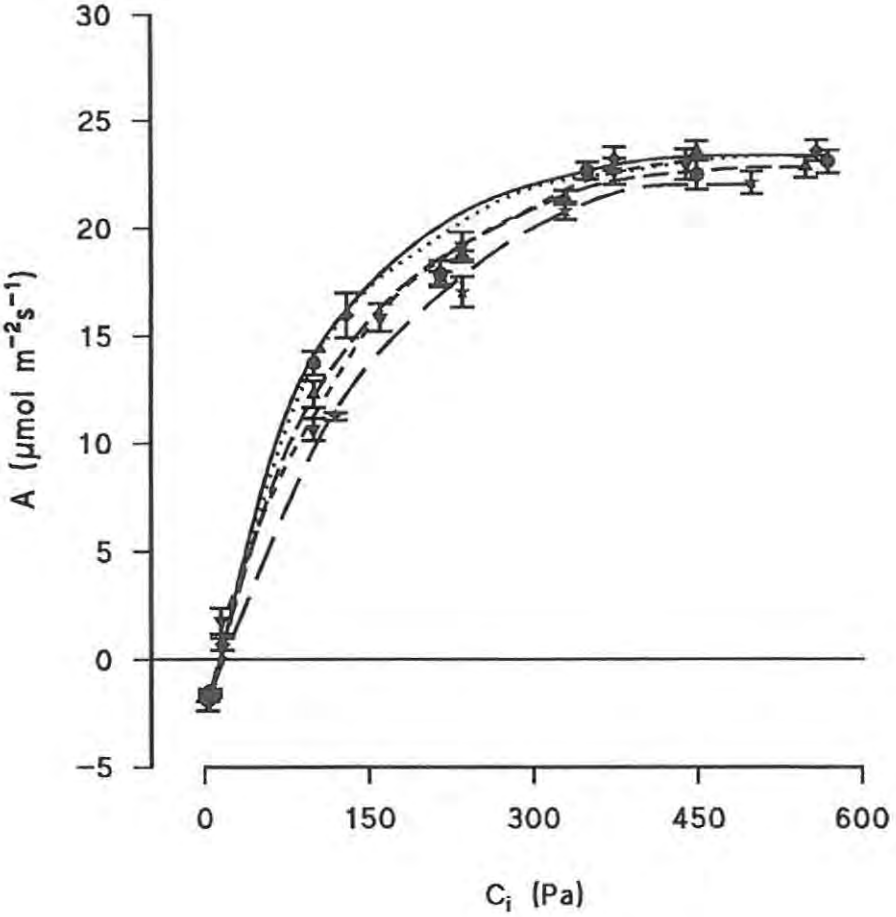

Fig. 8. Shows the response of CO_2 assimilation to intercellular CO_2 at 0, 4, 8, 16, and 21% O_2 (N-balanced CO_2). Measurements were made at PPFD of 1200 μmol $m^{-2}s^{-1}$ and at 30°C. (●0, ▲4, ▼8, ◆16, ★21% O_2)

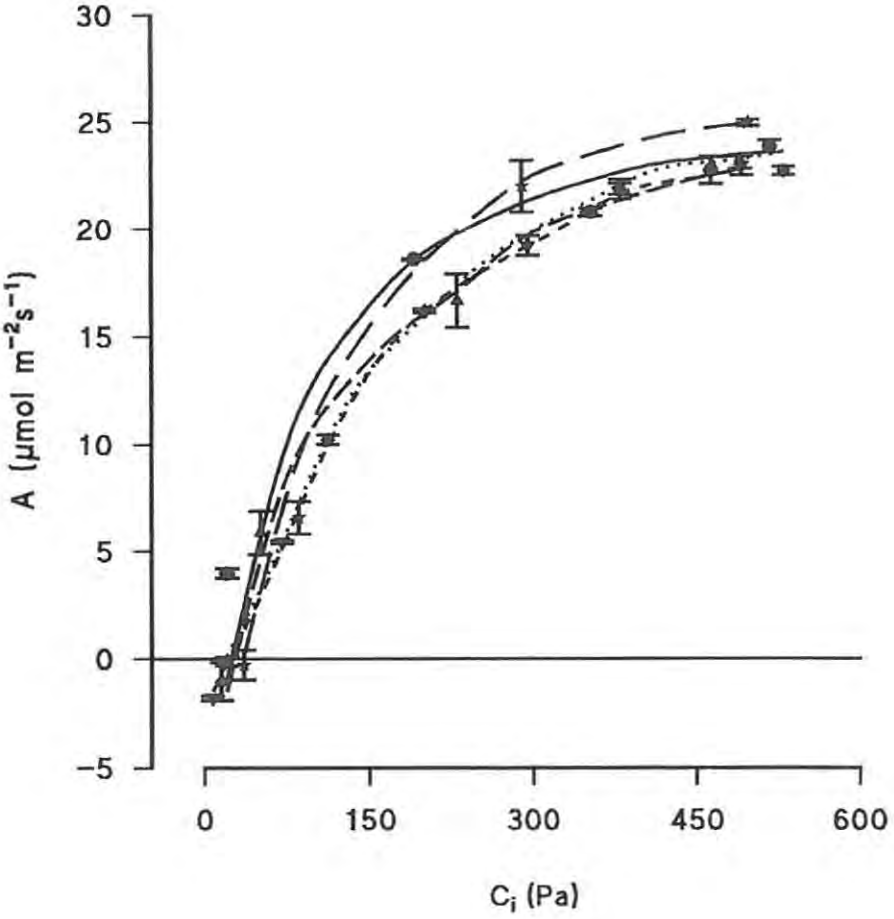

Fig. 9. Shows the response of CO_2 assimilation to intercellular CO_2 at 0, 4, 8, 16, and 21% O_2 (N-balanced CO_2). Measurements were made at PPFD of 1200 μmol $m^{-2}s^{-1}$ and at 35°C. (●0, ▲4, ▼8, ◆16, ★21% O_2)

Statistical analysis using analysis of variance did not indicate any significant difference in the values in Table 2, within each temperature regime, except those where 4% O_2 was used. Irrespective of experimental replication, results at this O_2 concentration were not consistent. The CO_2 compensation point (Γ^*) is generally considered to be indicative of photorespiratory activity. Γ^* did not change with increase in $[O_2]$ i.e either at 0 or 21% O_2, at all temperatures, and under all O_2 concentration was between 0 and 12 $\mu l\ l^{-1}$ (Table 3). The oxygen insensitivity observed at either 0 or 21% O_2 concentration are consistent with typical previously reported results to C_4 species (Bidwell, 1979; Taylor and Terry; 1984; Bunce, 1990).

5.5.2 *Analysis of A/C_i Curves*

The data from Figs. 6-9, was used to plot double reciprocal plots or plots of $1/A$ versus $1/C_i$, in an attempt to investigate apparent O_2 inhibition. Though it was not possible to calculate the actual $K_i(O_2)$ and $K_m(CO_2)$ from the data generated by the double reciprocal plots, the type of inhibition and also the apparent response of A to varying $[O_2]$ are obvious from these plots.

Double reciprocal plots of apparent rate of photosynthesis under different $[O_2]$ and at 20, 25, 30 and 35°C respectively, indicate a competitive inhibition of assimilation by O_2 at all temperatures. The curves at all temperature regimes were almost identical at either 0 or 21% O_2, and cross the y-intercept at nearly the same point which indicates that the data presented in figures 6-9 (A/C_i plots) indicate no apparent O_2 inhibition in *C. lachryma-jobi*. The competitive assimilation inhibition of CO_2 fixation by O_2 may be determined by calculating ***CE*** (equation 3) using the data in (Figs. 6-9 and 10-13). The resultant ***CE*** values obtained at all temperatures and under all $[O_2]$ are presented in Table 3.

Analysis of variance, within each temperature regime and at either 0 or 21% O_2 showed no significant difference in ***CE***. The results presented in Table 3 do not support photosynthetic O_2 sensitivity in *C. lachryma-jobi* and further support the trends observed in the A/C_i plots (Figs. 6-9) and Lineweaver-Burk plots (Figs. 10-13). Thus the observed trend in *C. lachryma-jobi*

photosynthesis compares favourably with results obtained by Monson, *et al.* (1984) in their comparative study of ***CE*** on whole leaf photosynthesis in C_3 and C_4 species.

Table 3. Illustrates carboxylation efficiency (***CE***, calculated from the initial slopes of the ***A*** versus C_i relationships, μmol $m^{-2}s^{-1}$ and photosynthetic CO_2 compensation points Γ^*, μl l^{-1}) at all CO_2/O_2 concentrations, under all temperature regimes and at 1200 μmol $m^{-2}s^{-1}$ PPFD. Only the highest and the lowest values are presented. Similarly, values from steady state gas exchange of A/C_i relationship from the data of (Figs. 6-9), were used but interpolated at 150 μl l^{-1} i.e before the point of inflexion.

Temperature °C	CO_2 Compensation (Γ^*, μl l^{-1}) 0-21% [O_2]	Carboxylation Efficiency (***CE***) μmol m^{-2} s^{-1}, 0-21% [O_2]
20	4.0 - 10.0	0.123 - 0.128
25	3.5 - 8.5	0.125 - 0.130
30	4.5 - 12.0	0.127 - 0.129
35	4.5 - 10.0	0.126 - 0.131

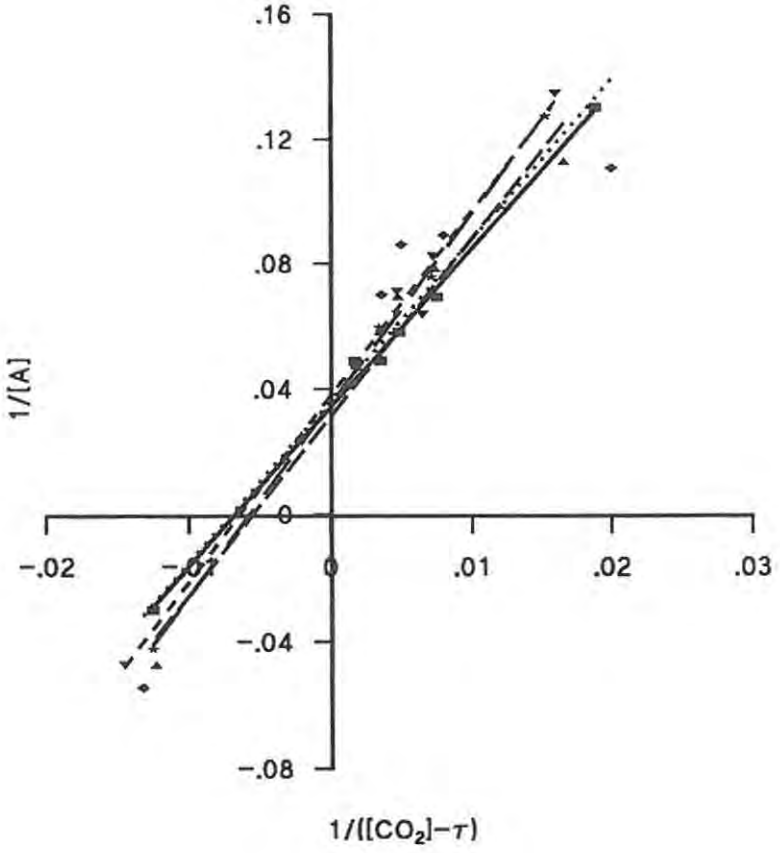

Fig 10. Double reciprocal plots of apparent rate of photosynthesis versus internal CO_2 at various O_2 and at 20°C. For investigation of apparent O_2 concentrations inhibition, values from steady state gas exchange of A/C_i relationship from the data of Fig. 6 was used. (●0, ▲4, ▼8, ◆16, ★21% O_2)

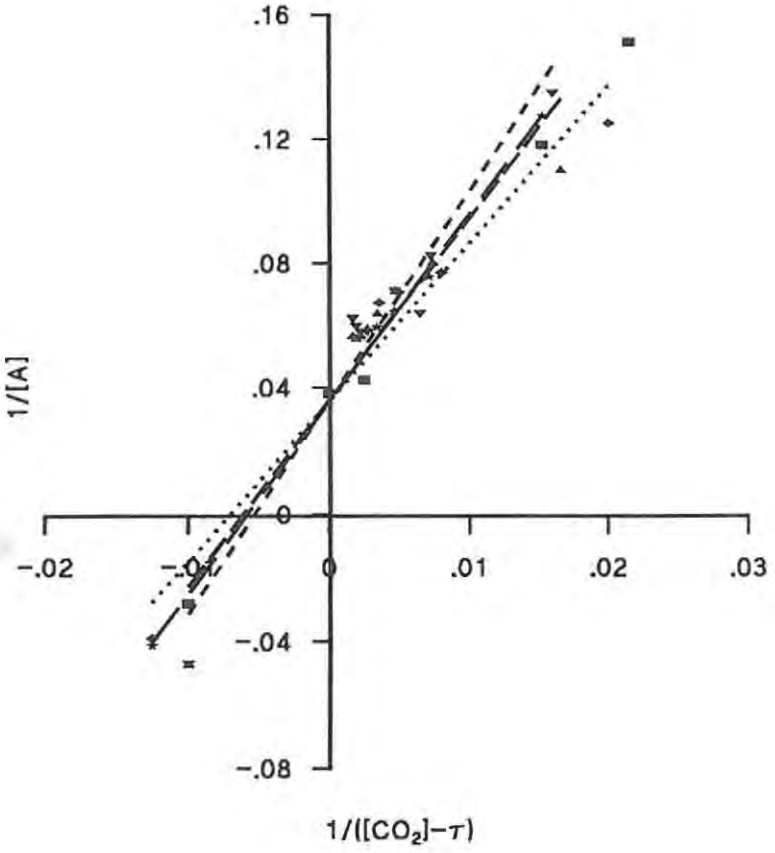

Fig 11. Double reciprocal plots of apparent rate of photosynthesis versus internal CO_2 at various O_2 concentrations and at 25°C. For investigation of apparent O_2 inhibition, values from steady state gas exchange of A/C_i relationship from the data of Fig. 7, was used. (●0, ▲4, ▼8, ◆16, ★21% O_2)

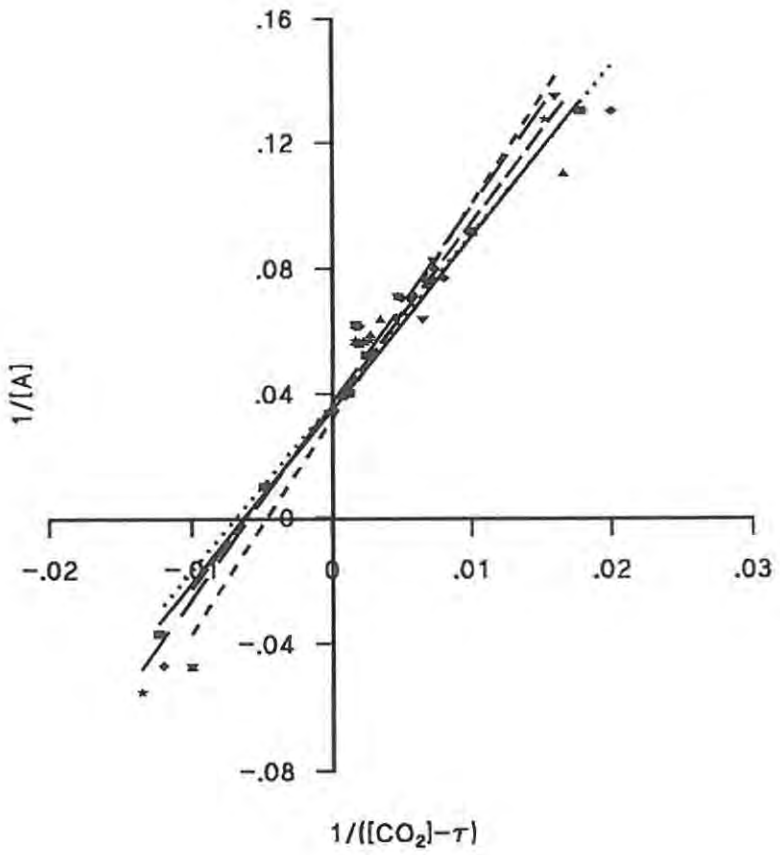

Fig 12. Double reciprocal plots of apparent rate of photosynthesis versus internal CO_2 at various O_2 concentrations and at 30°C. For investigation of apparent O_2 inhibition, values from steady state gas exchange of A/C_i relationship from the data of Fig. 8, was used. (●0, ▲4, ▼8, ◆16, ★21% O_2)

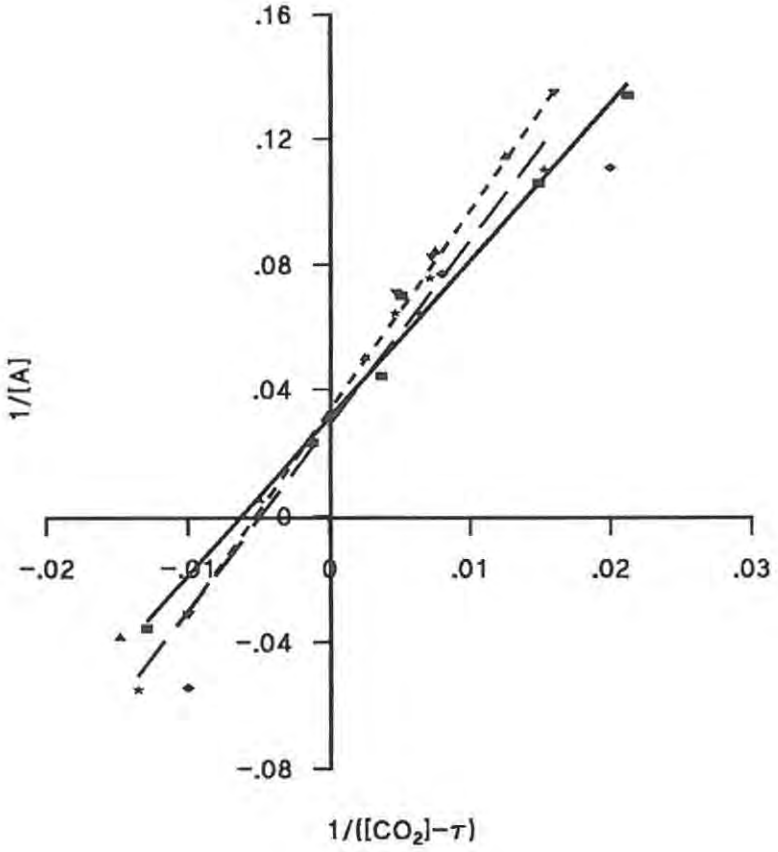

Fig. 13. Double reciprocal plots of apparent rate of photosynthesis versus internal CO_2 at various O_2 concentrations and at 35°C. For investigation of apparent O_2 inhibition, values from steady state gas exchange of A/C_i relationship from the data of Fig. 9, was used. (●0, ▲4, ▼8, ◆16, ★21% O_2)

CHAPTER 6: STOMATAL LIMITATION (ℓ) STUDIES

6.1 Introduction

The regulation of stomatal aperture to restrict damage to the tissues by dehydration is of major importance to plants. Stomatal closure is indeed one of the first lines of defence against desiccation (Kramer, 1980) since it is a quicker and more flexible process than alternatives such as changes in life cycle, root growth or leaf area, which are better suited for long-term adaptation (Schulze and Hall, 1982; Chaves, 1991). However, when stomata close, they protect the plant against water loss and simultaneously restrict carbon assimilation by the plant.

Stomata thus serve the conflicting roles of permitting CO_2 to diffuse into the leaf to support photosynthesis, and the simultaneous restriction of loss of water via transpiration from the leaf. To maximise one role must necessarily lead to total failure of the other. It can be argued that the regulatory system must therefore strike an appropriate compromise between permitting photosynthesis and restricting water loss (Schwartz and Zeiger, 1984; Zeiger, *et al.*, 1985; Field, *et al.*, 1989). In addition, carbon assimilation is regulated by the responsiveness or sensitivity to the changes in specific enzyme concentrations and their kinetic properties (Jones, 1985; Seemann, *et al.*, 1988). These factors limiting the rate of assimilation under various conditions could be ascribed to physical and also to internal physiological and biochemical parameters.

6.2 Separation of Stomatal and Mesophyll Limitations

A/C_i plots illustrate the response of assimilation rate to elevated CO_2 concentration in the absence of stomatal limitations as the internal CO_2 concentration is raised above that of normal atmospheric CO_2 at the site of decarboxylation in the mesophyll cells. The response has had two applications: First, as an alternative method of separating stomatal from mesophyll limitation; and second, in separating *in vivo* carboxylation from electron transport limitations within the mesophyll cells (Farquhar and Sharkey, 1982; Long and Hällgren, 1985).

Farquhar and Sharkey (1982) separated the limiting factors into internal and external factors. These authors referred to the former as mesophyll limitations, which were mainly biochemical in nature, in which CO_2 and O_2 concentration at the site of carboxylation, enzyme amount, rate of reaction and regeneration were implicated. The external limitations were mainly physical and were defined as the limitation to the rate of CO_2 fixation, imposed by stomata. These result in a relative difference between any particular steady state rate and that rate which would occur if the intercellular CO_2 partial pressure were raised to that of the ambient CO_2. Under these conditions stomatal conductance is taken as being infinite. Since then, many researchers have actually reported enhanced photosynthetic rates when internal CO_2 has been raised to a level above that of ambient or atmospheric CO_2, thus supporting the findings of Farquhar and Sharkey (Taylor and Terry, 1984; Santrucek and Slavik, 1990; Woodrow, *et al.*, 1990; Grantz and Assmann, 1991).

Farquhar and Sharkey (1982) proposed a simple equation for calculating the apparent limitation (ℓ) from the response of A/C_i, where the actual measured assimilation rate, A_1 measured under normal atmospheric CO_2 concentration, is subtracted from the rate which would occur should there be no stomatal limitations (A_o, i.e the value of A interpolated from response curve of A/C_i at 350 $\mu l\ l^{-1}$), in the following manner:

$$\ell = \frac{A_o - A_1}{A_o}$$

.....Equation 4

ℓ = relative measure of stomatal limitation

The major advantage afforded by the use of this equation its measured by simply raising the ambient CO_2 partial pressure to a point where the internal and normal ambient levels of CO_2 are equal.

6.3 Aims and Objectives

Of fundamental importance is to examine the rate of CO_2 assimilation and its response to change in the internal CO_2 concentration, coupled with changes in transpiration, as both processes are intricately linked sharing as they do the same gas exchange pathway in and out of the leaf. The objective of this section of the thesis was thus to examine the integration of stomatal conductance in relation to the magnitude of stomatal limitation to diffusion of CO_2 into the leaf, and also to examine the resultant water use efficiency of the plant under differing $[CO_2]$ and $[O_2]$. In addition, experiments were conducted to examine the effect of varying O_2 concentration on stomatal limitation, as it was felt that this could contribute to the overall development of the analytical model of whole leaf photosynthesis in *C. lachryma-jobi*.

6.4 Results

Calculation of stomatal limitation (ℓ) (equation 4) requires the two values, A_I (Table 4), which is the assimilation in the presence of stomatal limitation and is normally the value interpolated from the light response curve at optimum light intensity [generally taken at or as near to the point of inflexion as possible] at ambient CO_2 concentration (normally taken as 350 $\mu l\ l^{-1}$); and A_o, which is the assimilation achieved at elevated CO_2 concentration. In order to determine if the measured A_o values reflected stomatal limitation due to oxygen effects or whether they were a result of changes in the internal biochemical activity, a series of experiments was undertaken to determine the effect of varying oxygen concentration on assimilation with CO_2 at near ambient (350 $\mu l\ l^{-1}$) levels (Table 4).

Statistical analysis did not show any significant difference between the previously interpolated A_I values under ambient $[CO_2]$ and the A_I values attained using bottled gases of varying $[O_2]$, for each temperature regime. Both values, however, increased with increase in temperature. The increase between 20 and 25°C was very marked compared with the other temperatures which exhibited a slow but steady increase with increase in temperature (Table 4).

Table 4. Illustrates the A_1 values interpolated from A versus light intensity curves and A_1 values obtained using bottled gases at 350 µl l^{-1}, with varying oxygen concentration at light intensity of 1200 μmol $m^{-2}s^{-1}$ PPFD.

Temperature °C	A_1	Assimilation in absence of ℓ				
		A_0	A_4	A_8	A_{16}	A_{21}
20	12.49	12.61	13.00	11.83	12.09	11.74
25	19.47	19.50	19.90	19.92	19.85	19.75
30	20.97	19.87	20.73	21.40	22.31	21.11
35	20.20	20.50	20.63	20.67	21.10	21.20

The results of assimilation versus internal CO_2 response experiments conducted at various O_2 concentrations, at 350 µl l^{-1} and 20, 25, 30, and 35°C are presented in Table 5. These results show no significant change in A_o values for each temperature regime. However the A_o values increased with temperature. At 20°C, the A_o values ranged from 15.35 to 17.52 μmol $m^{-2}s^{-1}$, while at 35°C the A_o values ranged between 21.45 to 23.85 μmol $m^{-2}s^{-1}$ (Table 5). The average difference between these two temperature regimes was approximately 6.2 μmol $m^{-2}s^{-1}$.

Table 5. Illustrating calculated A_o values obtained at 350 $\mu l\ l^{-1}$ for all CO_2/O_2 concentrations and at light intensity of 1200 μmol $m^{-2}s^{-1}$ PPFD.

Temperature °C	A_o	A_4	A_8	A_{16}	A_{21}
20	17.30	15.35	16.45	16.85	17.52
25	23.67	20.32	23.85	21.00	24.45
30	21.56	22.62	23.25	22.69	23.64
35	22.40	21.70	21.45	21.85	23.85

Analysis of variance did not show any significant difference when results within each temperature regime were compared (Table 5). However, a marked difference in apparent stomatal limitations was observed as the plant approached optimum photosynthetic temperature between 30 and 35°C, irrespective of $[O_2]$. The individual A/C_i curves (Appendix A) at 0 and 21% $[O_2]$ show this response clearly but this trend is illustrated when these responses are combined as shown in Figs. 14-15 and 16-17; at 20-30 and 25-35°C respectively. The large differences observed between the curves obtained at ambient (A_1) and elevated CO_2 concentration A_o, indicate limitations imposed by stomata (ℓ). The remaining results observed at other $[O_2]$ (not presented in Figures) are summarized in Table 6.

Table 6. Shows ℓ values at varying CO_2/O_2 concentrations at different temperatures, calculated using equation (4) and the data presented in Table 4 and 5.

Temperature °C	ℓA_o	ℓA_4	ℓA_8	ℓA_{16}	ℓA_{21}
20	0.278	0.186	0.241	0.259	0.287
25	0.177	0.042	0.183	0.073	0.201
30	0.027	0.072	0.081	0.075	0.112
35	0.091	0.070	0.060	0.750	0.153

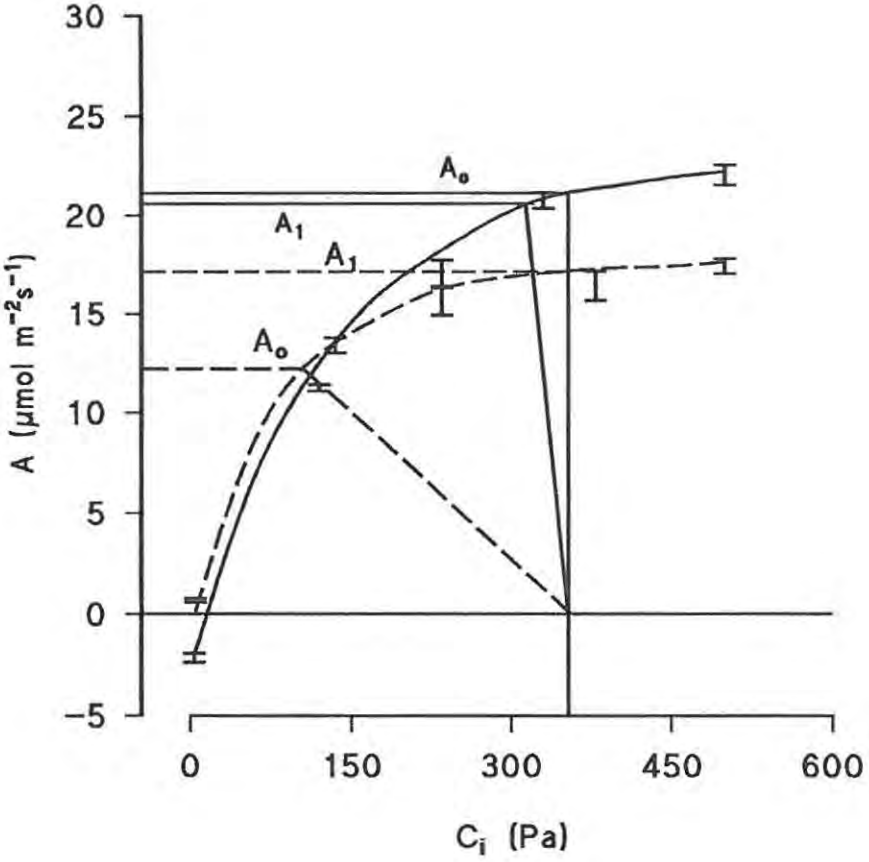

Fig. 14. Shows combined A/C_i curves at light intensity of 1200 μmol $m^{-2}s^{-1}$ PPFD and at 0% [O_2]. Stomatal limitations (ℓ) vary with temperature and appear not to be affected by O_2. Solid lines represent response at 30°C and dashed lines represent 20°C.

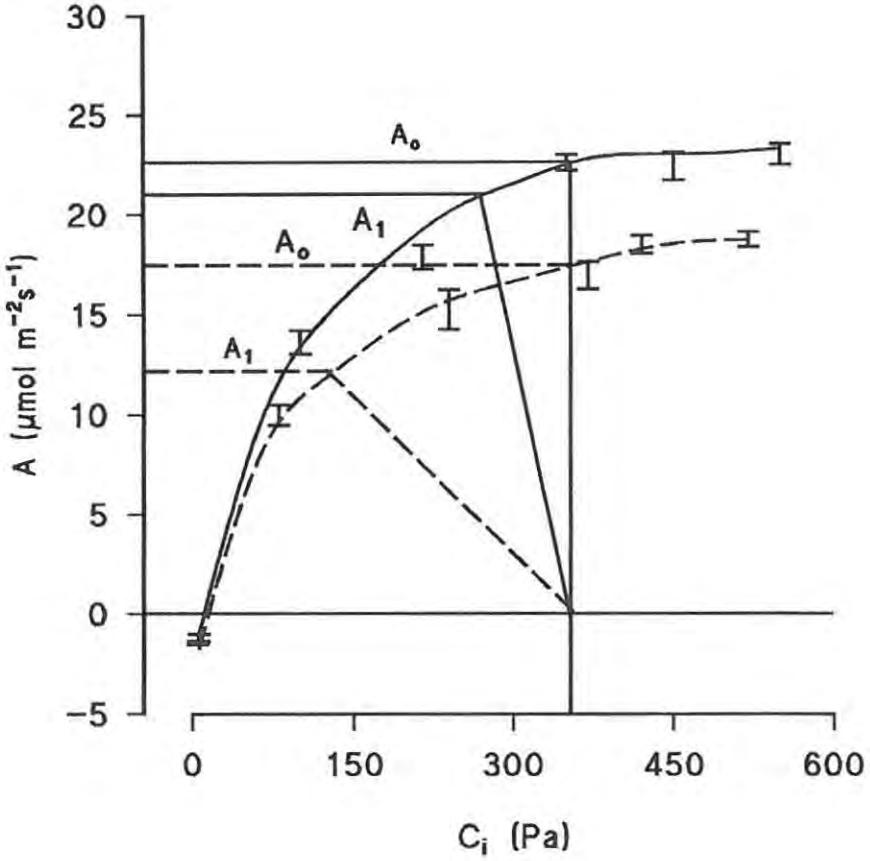

Fig. 15. Shows combined A/C_i curves at light intensity of 1200 μmol $m^{-2}s^{-1}$ PPFD and at 21% $[O_2]$. Stomatal limitations (ℓ) vary with temperature and appear not to be affected by O_2. Solid lines represent response at 30°C and dashed lines represent 20°C.

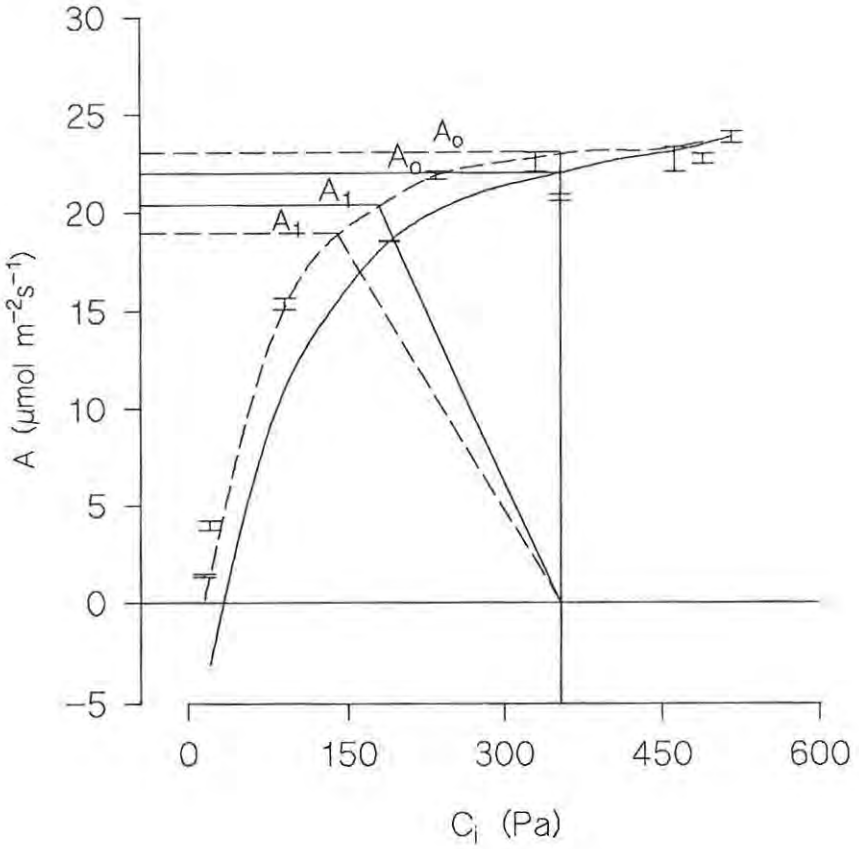

Fig. 16. Shows combined A/C_i curves at light intensity of 1200 μmol $m^{-2}s^{-1}$ PPFD and at 0% [O_2]. Stomatal limitations (ℓ) vary with temperature and appear not to be affected by O_2. Dashed lines represent response at 25°C and Solid lines represent 35°C.

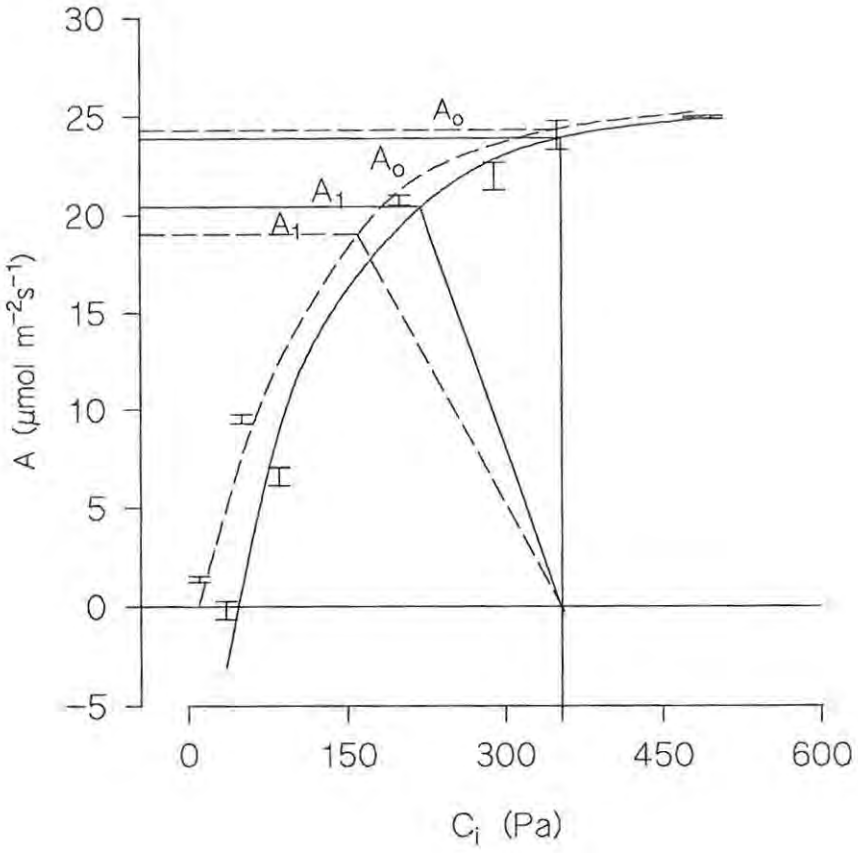

Fig. 17. Shows combined A/C_i curves at light intensity of 1200 μmol $m^{-2}s^{-1}$ PPFD and at 21% [O_2]. Stomatal limitations (ℓ) vary with temperature and appear not to be affected by O_2. Dashed lines represent response at 25°C and Solid lines represent 35°C.

Under these steady state conditions, ℓ values between 30 and 35°C were reduced by approximately 75% as compared to those at 20 and 25°C (Figs. 14-17 and Table 6). The trend observed in stomatal limitations of *C. lachryma-jobi* was consistent with that obtained by Grantz and Assmann (1991) in their comparative study of stomatal limitation in sugarcane and soybean.

In conclusion the A_I values obtained under ambient air and used in the calculation of ℓ were very close to the results obtained using bottled gases at 350 $\mu l\ l^{-1}$ with varying oxygen concentration, thus suggesting that the calculated ℓ values are real and do not result from an apparent oxygen effect.

CHAPTER 7: WATER USE EFFICIENCY (*WUE*)

7.1 Introduction

As pointed out in the previous chapter (Chapter 6), a close association exists between carbon assimilation and water loss, because water leaving the leaf and carbon dioxide entering must follow identical paths (Jarvis, 1976). This is one of the major dilemmas facing terrestrial plants. It is essential for a plant to regulate water loss while allowing sufficient CO_2 to diffuse through the stomata into the mesophyll cells where photosynthesis takes place (Tyree and Yianoulis, 1980). To reconcile this dilemma plants have evolved an epidermis with cuticle relatively impermeable to both CO_2 and H_2O, and turgor operated valves (stomata) (Raschke, 1975; Kramer, 1980). The presence of an epidermis thus not only reduces the rates of CO_2 and water vapour exchange, but it also provides the means of controlling CO_2 assimilation and transpiration through the size of the stomatal pores (Ludlow, 1980).

7.2 Water Use Efficiency and Assimilation

Until very recently, CO_2 assimilation and transpiration have always been treated separately (Schulze and Hall, 1982), rapid advances in new infra red gas analysis (IRGA) techniques have made it possible to measure the CO_2 and H_2O exchange simultaneously (Richards, *et al.*, 1986; Field, *et al.*, 1989). The need to incorporate water use efficiency studies in this research stems from the fact that CO_2 and H_2O are inseparable and involve stomatal functioning which is dealt with in some detail in this thesis (Chapter 6). Cowan (1977) used the term ***WUE*** (mol mol^{-1}) describing the relationship of net assimilation (*A*) to water evaporated or transpired (*E*) in the following equation:

$$WUE = \frac{A}{E}$$

.....Equation 5

This relationship, expressed as mol mol^{-1} is very dependent on the aperture of the stomata and hence on the stomatal conductance (g_s). If the stomata were widely opened, A and E would generally be greater than if they were less open. Cowan (1982) concluded that this relationship is an implicit parameter, as it represents all possible compromises that may be made between taking up CO_2 and losing H_2O. Cowan (1982) and later Williams (1983) recast the problem by proposing a daily quota or cost of carbon gained by the leaf per minimum water lost. They predicted that, in order to lose the least possible water for a given amount of carbon gained, a plant's stomata should be controlled so that the following relationship is achieved:

$$\left(\frac{\partial E}{\partial A}\right)_{s,t} = \lambda$$

.....Equation 6

where λ is constant over the day, s is a coordinate representing a specific leaf surface area and t is time. Cowan (1982) and later Williams (1983) suggest that the λ of any particular leaf area and time must at least be constant, or the integral be minimized so that the relationship of $(\partial E/\partial A)$ is always greater than 0, as expressed by the following equation:

$$\left(\frac{\partial^2 E}{\partial A^2}\right)_{s,t} > 0$$

..... Equation 7

If A is regarded as a benefit to the plant and E as a cost, then the slope of $\partial E/\partial A$ plots would be one representing the marginal energy cost to the plant over time (t). The $\partial E/\partial A$ ratio,

should therefore remain fairly constant or be greater than 0 for the survival of the plant irrespective of irradiance (intensity and quality), leaf water potential, leaf temperature, internal CO_2, relative humidity and wind speed (Cowan, 1982). However, the optimal variation in the rate of transpiration and assimilation rate, essentially ***WUE***, do not only depend on the magnitude of an undetermined parameter, λ, but also on characteristics of the physical environment (such as irradiance, internal CO_2 and relative humidity) and leaf internal metabolism (Dwelle, *et al.*, 1983; Vos and Ayarzún, 1987; Assmann and Grantz, 1991). Thus a model integrating the physical and internal biochemical components was necessary.

7.3 An Integrated Model for *WUE*

Water use efficiency described above is easy to interpret but for a given set of environmental parameters, the relationship and applicability of these calculations (Equations 5, 6 and 7) changes altogether as has already been pointed out above. Work by Farquhar, *et al.* (1980); Cowan (1982) and later Collatz, *et al.* (1991) led to the concept that stomatal responses to changes in environment can be separated into components that are dependent on photosynthesis and others that are independent of photosynthesis. For example, a change in light (which primarily affects the kinetics of photosynthesis) with all other factors held constant, causes a change in g_s to maintain a constant proportionality of g_s to A. On the other hand, a change that affects only the kinetics of water vapour diffusion (e.g. a change in diffusion gradients brought about by temperature) generally causes g_s/A ratio to change. It should be clear that ***WUE*** must be affected, as both light and temperature affect g_s. Consequently a model explaining other environmental and biochemical parameters that directly or indirectly affect ***WUE***, was of prime importance. Farquhar and Sharkey (1982) proposed a mathematical equation that incorporated the implicit equations 5, 6 and 7 in the attempt to broaden their practicability, in the following manner:

$$A=g_c(C_a-C_i)-\left(\frac{C_i+C_a}{2}\right)E$$

..... Equation 8

Where g_c is the conductance to diffusion of CO_2, C_a and C_i are partial pressures of CO_2 in the air and inside the leaf respectively, and E is the transpiration rate. However this equation still does not incorporate all the variables that affect A/E relationship. Hence Cowan (1982) added other parameters and rearranged the above equation to the following:

$$T_l=T_a+\frac{\varnothing_0-LE}{C_p g_b+8\sigma T_a^3}$$

..... Equation 9

Where T_l is the saturation water vapour pressure of the leaf surface, T_a is the ambient air temperature, ϕ_0 is the radiation absorbed per unit area of the leaf minus the flux of radiation emitted per unit area of the leaf. L is the latent heat of evaporation, g_b is the boundary layer conductance and σ is the Stefan-Boltzmann constant. Considering the above equation it is apparent that the relationship between A and E is not a simple one, but involves a number of complex, possibly interactive factors.

Cowan's (1982) model is the one that is widely used either in the form presented above (Equation 9) or slightly modified (Appendix B) (Farquhar and Sharkey, 1982 and Field, *et al.*, 1989) as it attempts to relate empirical measurements of net CO_2 assimilation in leaves to the biochemistry of photosynthesis and to transpiration (Atkinson, *et al.*,1986). The variables which primarily determine the net rate of fixation in a given leaf are C_i, temperature and irradiance

and the relationship between these factors must be considered, else drastic simplifications to the system are made (Passioura, 1988). In such instances, as Field, *et al.* (1989) and later Ishikawa, *et al.* (1990) stated that, that would lead to simplified equations which would result in incorrect estimation of ***WUE*** and thus limit their application.

7.4 **Aims and Objectives**

From the discussion above, it is clear that the measurements of CO_2 and H_2O exchange are synergistic due to the interdependence of the two processes. Thus, the diffusion limitation to photosynthesis must be viewed as the product of compromise between the responses that tend to increase photosynthesis and those that tend to decrease water loss (Field *et al.* 1989). Cowan and Farquhar (1977) defines this phenomenon as optimization theory. The present attempt to model *C. lachryma-jobi* photosynthesis must centre around the crucial mechanism of stomatal functioning, as stomata are the only apertures that determine the internal CO_2 inside the mesophyll cells and inevitably control water diffusion to or from the leaf. Both factors relate to photosynthetic efficiency, which can be used as an index of the adaptability and survival of a plant in a particular habitat, which justifies an examination of the variance of $\partial E/\partial A$ relationship to humidity, irradiance and temperature change under laboratory conditions. It was felt that the examination of ***WUE*** results (although may be affected by other factors, such as preconditioning time in growth cabinets) would yield information which could be used to model ***WUE*** and further explore $\partial E/\partial A$ in *C. lachryma-jobi.*

7.5 **Results**

The results of the experiments to determine water use efficiency of *C. lachryma-jobi* under ambient air (CO_2 concentration approximately 350 $\mu l\ l^{-1}$) and between 20 and 35°C are presented in Fig. 18.

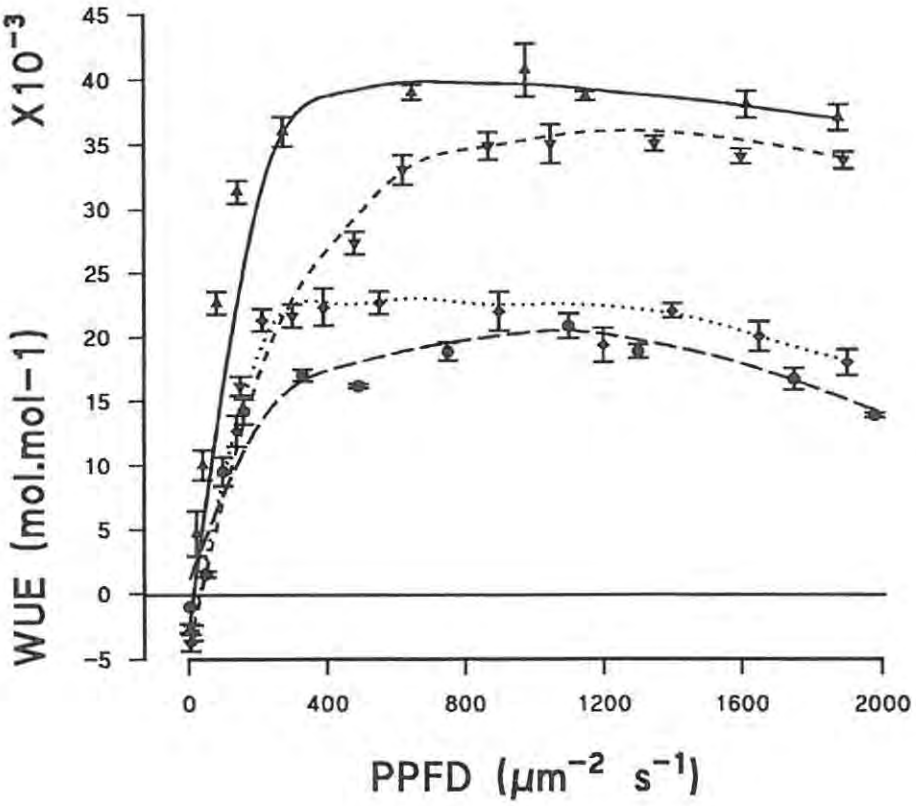

Fig. 18. Shows the response curves of ***WUE*** (mol mol^{-1}), to increasing PPFD under four temperature regimes. Measurements were made at 70% relative humidity and at ambient CO_2 concentration (350 µl l^{-1}). (▾20, ▴25, ◆30, ●35°C)

As illustrated in Fig. 18, the ***WUE*** increased linearly with increasing PPFD up to a light intensity of approximately 400 μmol $m^{-2}s^{-1}$ PPFD, at all temperatures. Above this light intensity, a progressive decrease in ***WUE*** was evident at all temperatures. This trend was very apparent when ***WUE*** values were plotted against temperature at high light intensity (2000 μmol $m^{-2}s^{-1}$ PPFD) (Fig. 19).

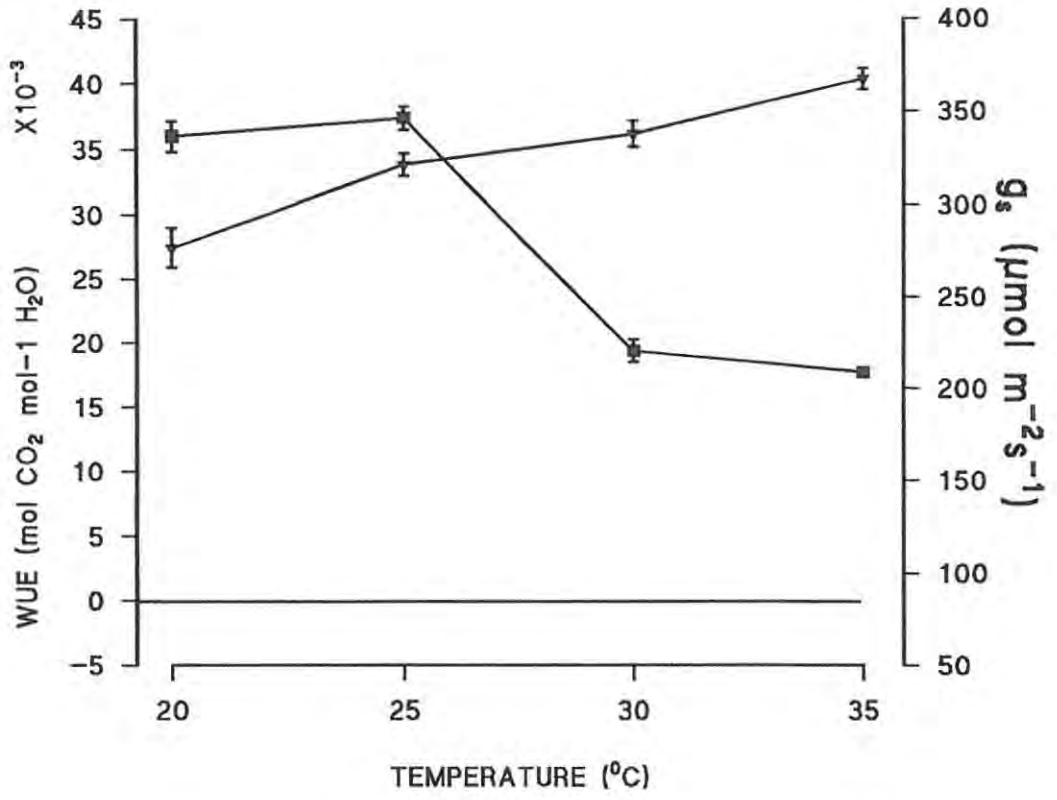

Fig. 19. Illustrates the calculated ***WUE*** (mol mol^{-1}) and g_s (μmol $m^{-2}s^{-1}$) in relation to increasing temperature at light intensity of 2000 μmol $m^{-2}s^{-1}$ PPFD under ambient CO_2 (350 $\mu l\ l^{-1}$). (■***WUE***; ▼g_s)

C. lachryma-jobi had high ***WUE*** at 25°C (approximately 38 x 10^{-3} mol CO_2 mol^{-1} of H_2O), but thereafter decreased with further increase in temperature (Fig. 19). This trend was essentially the same as that of *Ischaemum anthephoroides*, also a C_4 plant (Ishikawa, *et al.*, 1990). Although *C. lachryma-jobi*'s ***WUE*** at 25°C is highest compared to all other temperature regimes, assimilation rate (*A*) is significantly less (19.52 μmol $m^{-2}s^{-1}$) compared to *A* attained between 30 and 35°C (23.00 μmol $m^{-2}s^{-1}$) (Table 7). Despite a marked decrease in ***WUE*** with increase in temperature, *C. lachryma-jobi* showed a significant increase in stomatal conductance under

similar experimental conditions.

The relationship between *WUE* and g_s is also illustrated in Fig. 19, where g_s increased linearly to increasing temperature. This trend was consistent with findings of Morison (1987) in his study of stomatal conductance and water use efficiency under high CO_2 concentrations. Similarly, $\partial E/\partial A$ relationship increased markedly with increase in temperature (20°C= 0.028; 35°C=0.055) indicating that the marginal energy cost constant (λ, equation 6 and 7), was improving with increase in temperature or was getting away from 0 (Table 7). The efficiency noted in marginal energy cost was manifested in net assimilation rates which steadily increased across the range of experimental temperatures.

Table 7. Illustrates the relationships among gas exchange parameters, i.e temperature, stomatal conductance (g_s μmol $m^{-2}s^{-1}$); $\partial E/\partial A$; *WUE* (mol CO_2 mol^{-1} H_2O) and net assimilation rate (*A* μmol $m^{-2}s^{-1}$) at 70% RH and at light intensity of 2000 μmol $m^{-2}s^{-1}$ PPFD under ambient CO_2 concentration (350 μl l^{-1}).

Temperature °C	g_s μmol m^{-2} s^{-1}	$\partial E/\partial A$	*WUE* mol CO_2 mol^{-1} H_2O	A μmol m^{-2} s^{-1}
20	283.20	0.028	35	13.42
25	324.00	0.027	37	19.52
30	340.00	0.050	19	23.31
35	375.10	0.055	18	21.40

In conclusion it is apparent from Table 7, that *C. lachryma-jobi*, though showing a decreased, *WUE* between 30 and 35°C (18 x 10^{-3} mol CO_2 mol^{-1} H_2O), it is markedly efficient in terms of *A* (23 μmol $m^{-2}s^{-1}$) and also in terms of marginal energy cost (0.055) compared to the *A* value obtained at 25°C, where the plant attained the highest *WUE* (35 mol CO_2 mol^{-1} H_2O) but low *A* (19.50 μmol $m^{-2}s^{-1}$).

CHAPTER 8: DISCUSSION

The interactive effects of light, temperature and CO_2/O_2 ratios in whole leaf photosynthesis of *Coix lachryma-jobi* L., were investigated under controlled laboratory conditions. Furthermore the effect of varying $[O_2]$ on stomatal limitation was investigated. The results will be discussed in sections though not strictly following the pattern that was adopted in the preceding chapters.

8.1 Light and Temperature

The light response curves of *C. lachryma-jobi* conform to a generalized hyperbola and consists of two phases; an initial linear phase, with a steep rise in A when plotted against light intensity, and a progressive decrease in slope with a further increase in light intensity. The curves did not show any signs of light saturation at the range of temperatures examined (Fig. 5). There were no significant differences in $\mathbf{R_d}$ points but A_{max} in the range 30 and 35°C was higher (approximately 24 μmol $m^{-2}s^{-1}$) and the lowest value (approximately 15μmol $m^{-2}s^{-1}$) realized at 20°C. Though A_{max} at 30 and 35°C is not as high as the values for other C_4 species (Black, 1973), the photosynthetic optimum observed was similar to other reports (Berry and Björkman, 1980 and Botha and Russell, 1988). The effect of varying light intensity is thought to result in changes in amounts and of PEPC and in terms of feedback interaction between the C_3 and C_4 cycles (Fig. 20), in leaves of C_4 plants during steady-state photosynthesis (Usuda, 1985; Leegood, *et al.*, 1989; Doncaster, *et al.*, 1989). C_4 cycle is obligatory coupled with calvin cycle.

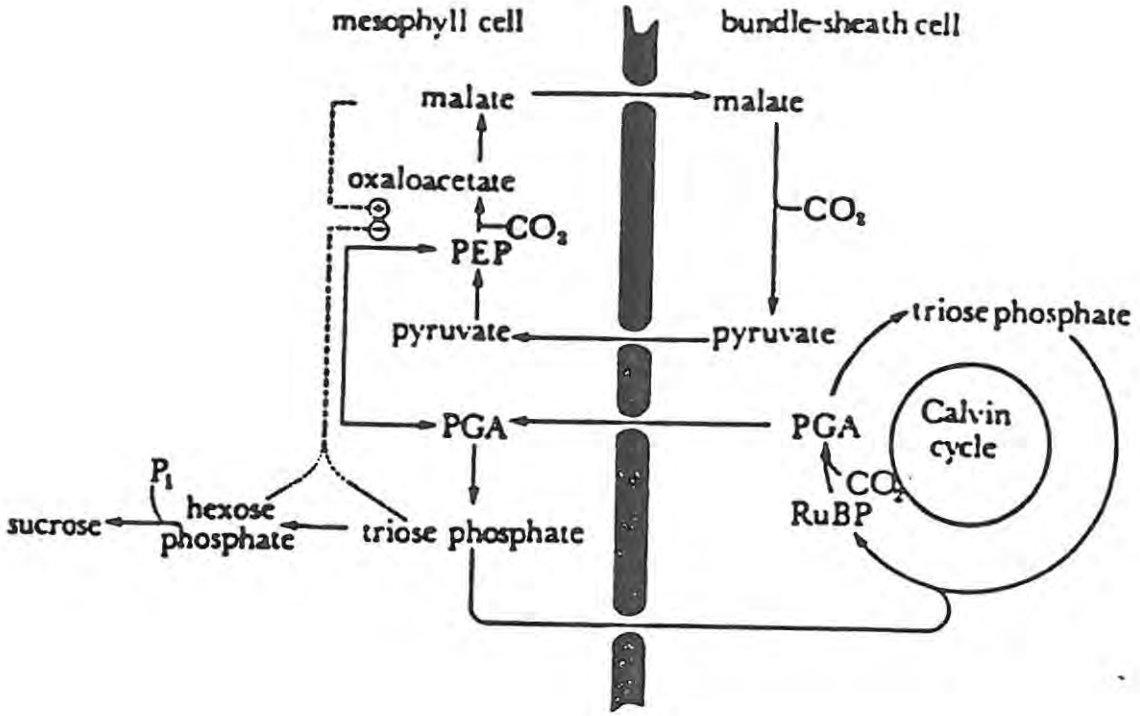

Fig. 20. Scheme for photosynthetic carbon assimilation in an NADP-Malic enzyme type C_4 such as maize (*C. lachryma-jobi* belongs to the same group). Adapted from (Leegood, *et al.*, 1989).

The interdependence mainly exists in the regeneration of PEPC from PGA which is a resultant product after decarboxylation of malate in the BS cells (Leegood, *et al.*, 1989). Leegood, *et al.* (1989) in their study of C_4 intermediates and their enzymes, found that triose phosphate and PGA ratios were drastically reduced at low light intensity. Recently evidence has been accumulating that different forms of PEP exist at low and high light intensities. But it is not yet clear whether the two kinds of PEP suggested have any significance in the carboxylation efficiency of C_4 species or whether they constitute an alternative measure of adjusting to low light intensity (Hatch, 1987; Doncaster, *et al.*, 1989). The low *A* observed in *C. lachryma-jobi* under low light intensities could, in part, be attributed to this.

As was pointed out in Chapter 4, light and temperature are inseparable as parameters affecting the photosynthetic machinery. Many researchers have indicated that at low light, temperature

seems to play no role in photosynthesis (Berry and Björkman, 1980; Meidner, 1986). This could explain the *C. lachryma jobi* $\mathbf{R_d}$ values which almost converged at an identical point below the light compensation point (Γ) (Fig. 5). However as light increases, the role of temperature becomes apparent (Fig. 5). Rubisco has been found to be an unstable enzyme that will tend to be oxygenated rather than carboxylated particularly at high light intensities and elevated temperatures (Brooks and Farquhar, 1985; Woodrow and Mott, 1988), thus lowering *A*. This is not in accordance with the results obtained in (Fig. 5), where curves of net assimilation increased with temperature. Unlike RuBP, PEPC has been observed to function more efficiently at high light intensity and elevated temperature (Buryzynski and Lechowisk, 1983; Woodrow and Mott, 1988), which could explain the higher photosynthetic performance in *C. lachryma jobi* at high light intensities and temperatures. These factors could in part explain the relative constant apparent **Φ** or **QE** observed in *C. lachryma-jobi* at all temperatures (Table 1).

The values presented in Table 1 essentially indicate high **QE**, which is a typical feature of C_4 species (Ehleringer and Björkman, (1977). These results suggests that *C. lachryma-jobi* may have a limited ability to acclimatize to low light and temperature. In fact, according to Ehleringer and Pearcy (1983) and later Fitter and Hay (1987) the C_4 advantage in terms of the efficiency in chemical energy requirements, and also in general CO_2 assimilation is on averaged increased, but under low light intensities and temperatures the C_4 advantage disappears completely.

8.2 Assimilation versus Internal CO_2 (A/C_i)

The response curves of A/C_i (Figs. 6-9) exhibited saturation below approximately 150 μl l^{-1} but did not show any signs of O_2 inhibition either at 0 or 21% O_2 within each temperature regime. The A_{max} values of these curves varied with change in temperature (Table 2.). Forrester and co-workers (1966) reported O_2 inhibition in C_4 species. Subsequently there has been a lot of controversy with regard to the response of C_4 photosynthesis to $[O_2]$ (Barger and Fock, 1983; Edwards and Krall, 1990). The results obtained using *C. lachryma-jobi*, exposed to increased $[O_2]$ in this study are in accordance with results obtained previously by Morgan and Brown (1979);

Monson *et al.* (1984); Furbank, *et al.* (1989). The O_2 insensitivity was further supported by double reciprocal plots of A/C_i curves, where similar slopes crossed the y-axis at almost identical points either at 0 or 21% O_2 (Figs. 10-13). This suggests that the inhibitor constant K_i (O_2) and substrate K_m (CO_2) under all CO_2/O_2 concentration may be the same at the active site of Rubisco (Ku and Edwards, 1977a).

Furthermore, the *CE* values, determined from the initial slopes of *A/Ci* curves, did not exhibit any statistically significant differences under similar conditions (Table 3.). The present results are then consistent with other research reports on similar work on whole leaf photosynthesis (Monson, *et al.*, 1984; Grantz and Assmann, 1991). The response can thus be attributed to the basic function of the specialized reactions of the C_4 photosynthesis which is to concentrate CO_2 in the BS cells, the site of RuBP (Kriedemann and Downton, 1981). Elevated CO_2:O_2 ratios would serve to suppress the oxygenase reaction and thus inhibition of Rubisco by increased O_2 is decreased (Newton, 1991).

8.3 CO_2 Concentrating Mechanism and C_4 Anatomy

Anatomical and ultrastructural features also contribute positively to the functional physiological and biochemical activities of the C_4 species (Laetsch, 1974; Hattersley and Browning, 1981). *C. lachryma-jobi* has a typical Kranz anatomy (Figs. 1-4). In addition, the small intervainal distances observed in *C. lachryma-jobi* and other C_4 plants and also the close proximity of the photosynthetic tissues (e.g BS cells) to the vascular bundles, (Hattersley and Watson, 1976; Colbert and Evert, 1982), add to the overall efficiency of C_4 photosynthesis. Evert, *et al.* (1977) noted numerous plasmodesmatal connections between the Kranz (PCA) and bundle sheath (PCR) cells in their study of the distribution of plasmodesmata in BS and PCA cells of *Zea mays*. High plasmodesmatal frequencies have been recorded at this cell interface in C_4 grasses (Botha and Evert, 1988; Botha, 1992; Botha and van Bel, 1992), which are thought to facilitate rapid transport of photosynthates to the sink and may also contribute in the maintenance of high CO_2 concentration particularly at the site of carboxylation, thus promoting higher net

assimilation rates (NAR).

In addition the C_4 species can continue photosynthesizing with their stomatal apertures almost closed (Hatch, 1987; Furbank, *et al.*, 1989). Thus stomatal aperture is closely coupled with the maintenance of leaf water status (Schulze and Hall, 1982; Chapin, *et al.*, 1987; Chaves, 1991).

8.4 **Stomatal Limitation and Water Use Efficiency**

The apparent A_o values presented in Table 4 obtained from *A/Ci* curves show no statistically significant differences with varying $[O_2]$ at the experimental temperatures. However there was a marked decrease in apparent stomatal limitation (ℓ) values as the plant approached its optimum photosynthetic temperature at 35°C, (Figs. 14 to 17) at 0 and 21% O_2 and between 20 and 35°C. The remaining data including other values obtained at other $[O_2]$ levels and at all temperatures are summarized in Table 5 (Appendix A, for individual responses at varying $[O_2]$ and temperatures). The decreased ℓ values noted at 35°C, the optimum temperature, was consistent with other temperature optima of other research reports of similar work (Eaumus and Fowler, 1990 and Grantz and Assmann, 1991).

Even though stomata may impose limitations particularly at low temperatures, other factors associated with the C_4 mechanism do contribute, such as limitations imposed by PEPC at low temperatures and effect resulting from differential solubilities of CO_2 to O_2 at the site of carboxylation (Vavasseur, *et al.*, 1990; Woodrow, *et al.*, 1990). A/C_i curves have been used extensively to demonstrate the limitations imposed by stomata on photosynthesis (Farquhar and Sharkey, 1982; Harley *et al.*, 1986; Day, *et al.*, 1991). The term 'apparent stomatal limitation (ℓ)' was used in this thesis, in that the limitation proposed by Farquhar and Sharkey (1982) fails to separate both direct and interactions between the stomatal conductance (g_s) to water and the conductance of CO_2 (Woodrow, *et al.*, (1990). Consequently in a more realistic situation as g_s increases, the rate of photosynthesis may initially increase then decrease as water and associated heat loss becomes a limiting influence.

This phenomenon is illustrated by the data presented in Fig. 18, where the calculated ***WUE*** in *C. lachryma-jobi* becomes reduced particularly at higher temperatures compared with that at low temperatures. As stated by Kramer (1980) and later by Schulze and Hall (1982) plants must increase transpiration at higher temperatures in the bid to protect themselves against excessive heat. This effect is shown in Fig. 19, but inherent to this strategy is a lowering of ***WUE***. In terms of optimization theory proposed by Cowan (1977) and in terms of marginal energy cost to the plant as defined by the function of $\partial E/\partial A$ (Cowan, 1977; Williams, 1983), *C. lachryma-jobi* exhibits increased photosynthetic efficiency, though it loses more water per mol of CO_2 fixed, which is further substantiated by the results illustrated in Table 7 and Fig. 19. The results presented in this thesis suggest that *C. lachryma-jobi* is still capable of efficient photosynthesis even at low ***WUE*** and in this respect, characteristically C_4.

8.5 **Conclusion**

The gas exchange characteristics of *C. lachryma-jobi* presented here do not represent the entire spectrum of parameters associated with whole leaf photosynthesis. The emphasis within this thesis was to demonstrate the interaction between the major parameters affecting net assimilation, under controlled conditions. More quantitative work under natural environment in the field is needed in order to provide a more comprehensive ecophysiological model for this species. Furthermore, the investigation of the effect of elevated CO_2 was done on *C. lachryma-jobi* leaves, only on the short-term periods (approximately 6 hours exposure to elevated $[CO_2]$, per day), therefore more work is justified to study the long-term effects of this phenomenon. To date, there is still insufficient data documented on the imbalance brought about by a CO_2 increase at the level of plant water economy or on other related factors. These factors are first; Rubisco and consequently enzyme availability, rate of carboxylation and the subsequent regeneration of Rubisco; and second; stomatal conductance - specifically stomatal limitation and water use efficiency.

Recent evidence suggests that enhanced CO_2 levels lead to a change in source to sink balance

(Hoddinott and Jolliffe, 1987; Stitt, 1991). This is also an area that needs considerable attention as this will indirectly affect the movement of photosynthates from source (leaves) to sink, thus creating an overflow of products such as carbohydrates (e.g sucrose) at the source. Accordingly, such a situation will adversely affect net assimilation rates. Some other workers have noted new morphological and anatomical structures developing in plants exposed to elevated CO_2, thought to be the initiation of new sinks to cope with sudden increase in levels of photosynthates within the leaf (Long, 1991; Neuhaus and Stitt, 1990; Stitt, 1991). Therefore studies of the structure-function relationships will also provide necessary information that we feel would help in the development of an ecophysiological model for whole leaf photosynthesis. Thus the open question as to the effects of elevated CO_2 in plants still remains.

The whole leaf photosynthetic models will undoubtedly enhance our understanding of the underlying processes involved in photosynthesis and their interactions which control net assimilation, particularly in C_4 species (the group that has received little attention to date). These models will be invaluable in improving agricultural and biotechnological techniques related to crop production and would also identify targets of possible manipulation which would ensure continued overall plant performance as measured by growth, yield and reproduction, particularly as so many agriculturally important plants belong to this group of C_4 species.

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APPENDIX A

Figures showing changes in stomatal limitation at various [O_2] and temperatures

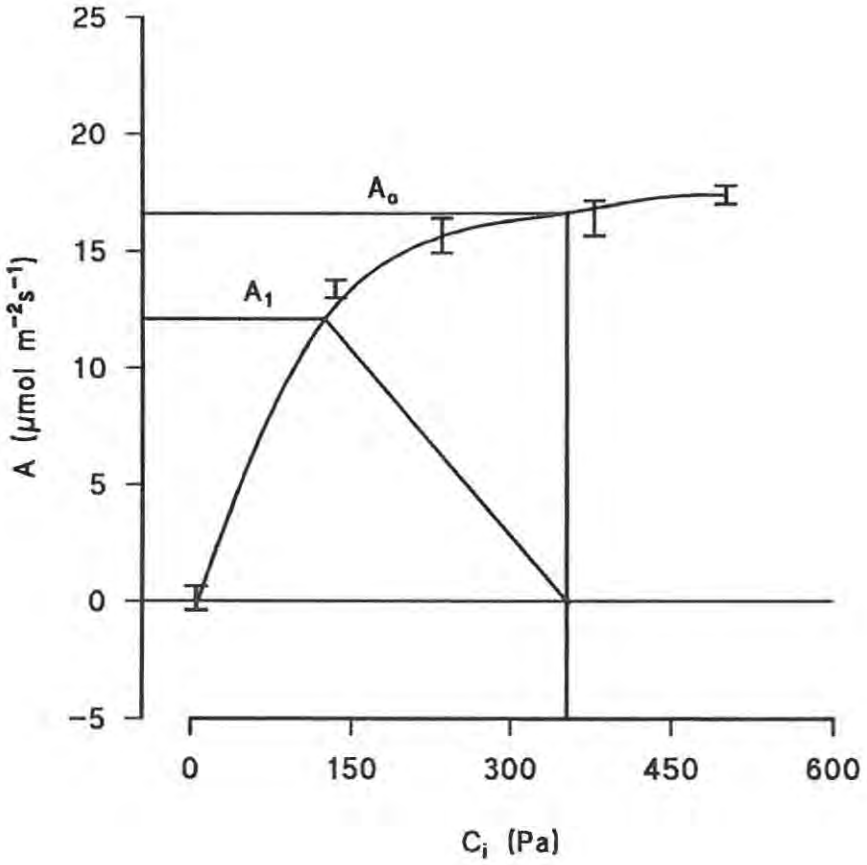

Fig. 21 A/C_i curve showing changes in stomatal limitation at 20°C in the absence of oxygen.

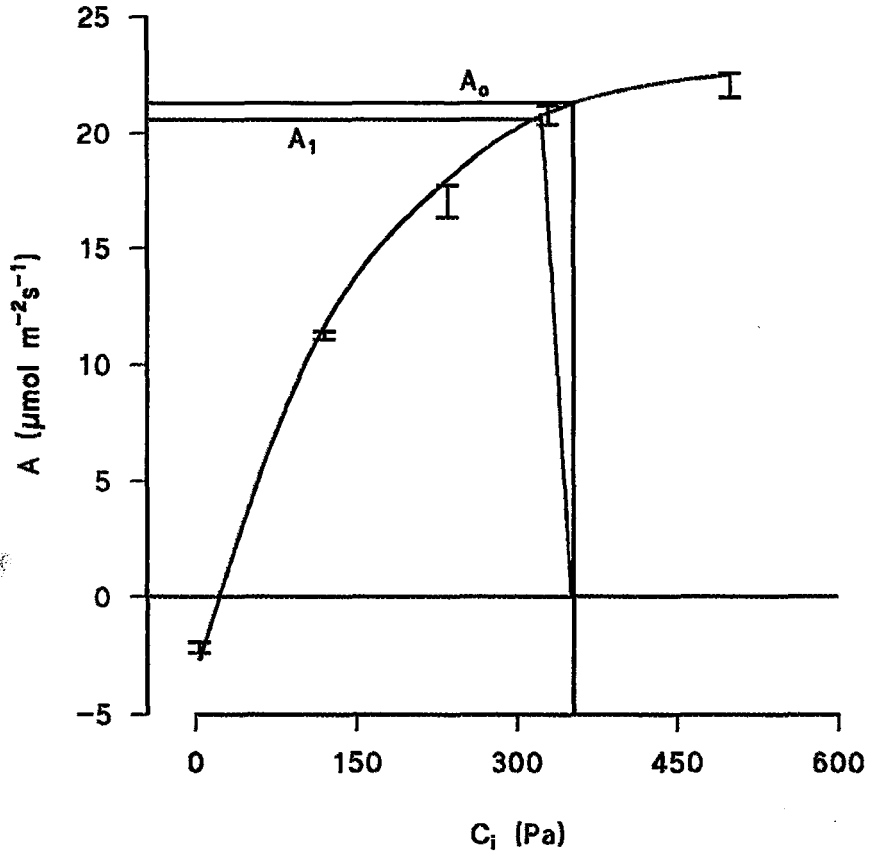

Fig. 22. A/C_i curve showing changes in stomatal limitation at 30°C in the absence of oxygen.

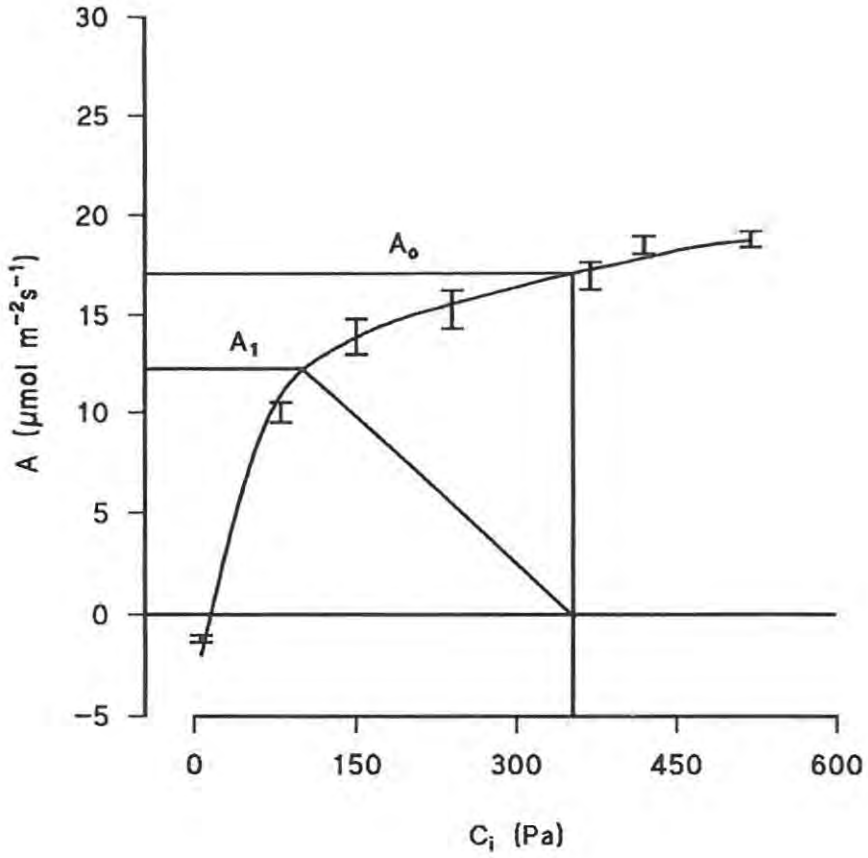

Fig. 23. A/C_i curve showing changes in stomatal limitation at 20°C and 21% [O_2].

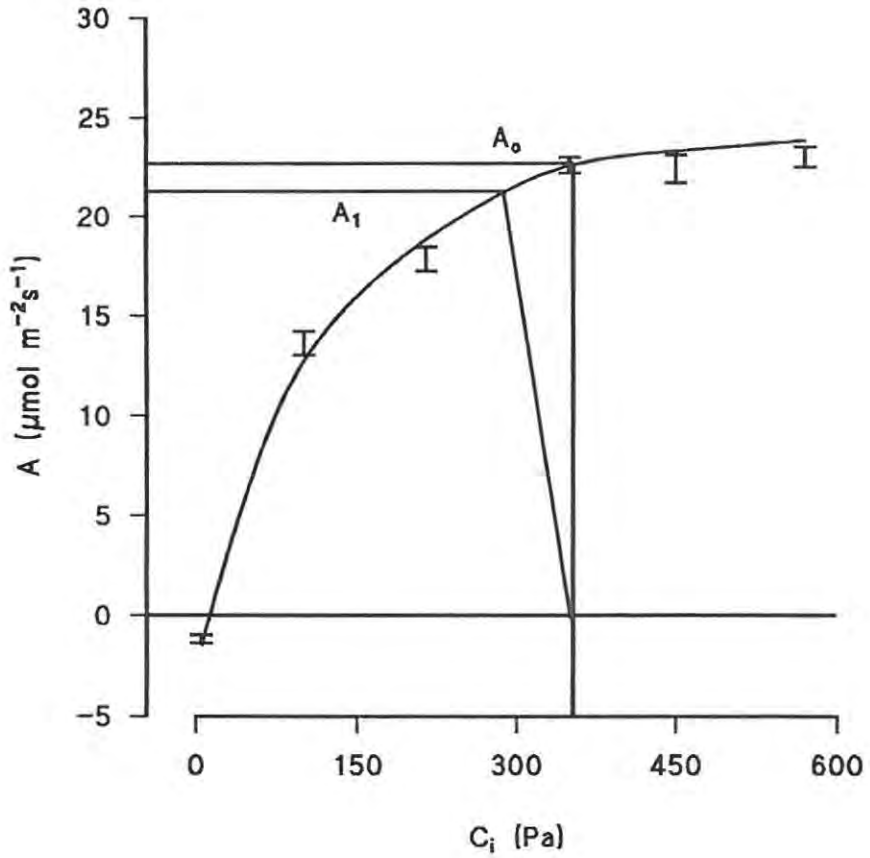

Fig. 24. A/C_i curve showing changes in stomatal limitation at 30°C and 21% $[O_2]$.

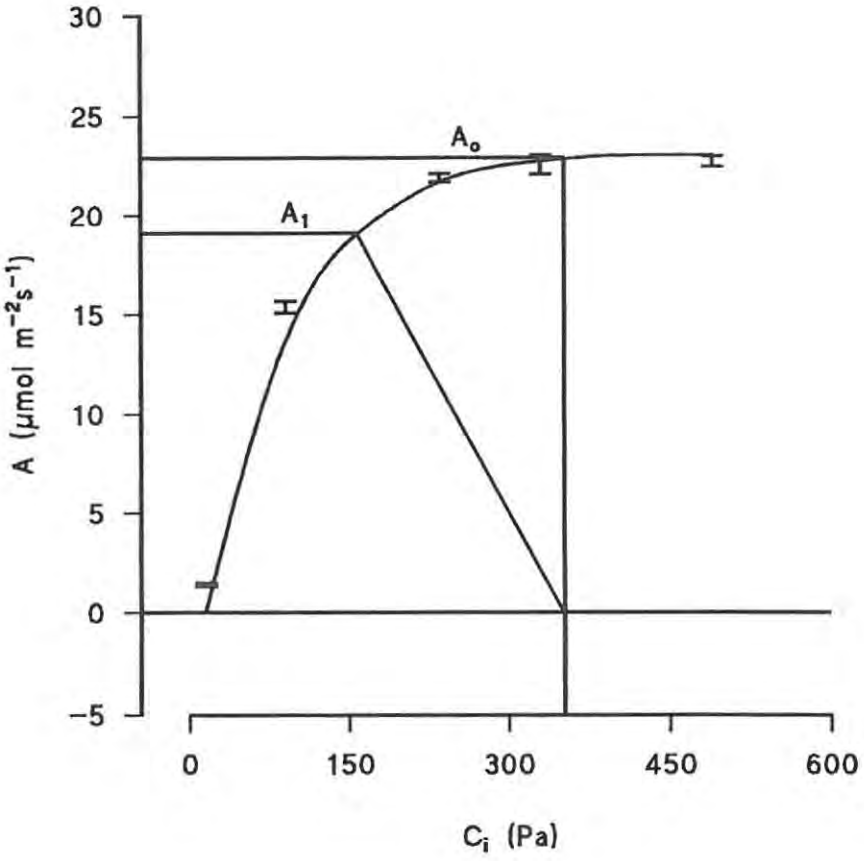

Fig. 25. *A*/C_i curve showing changes in stomatal limitation at 25°C in the absence of oxygen.

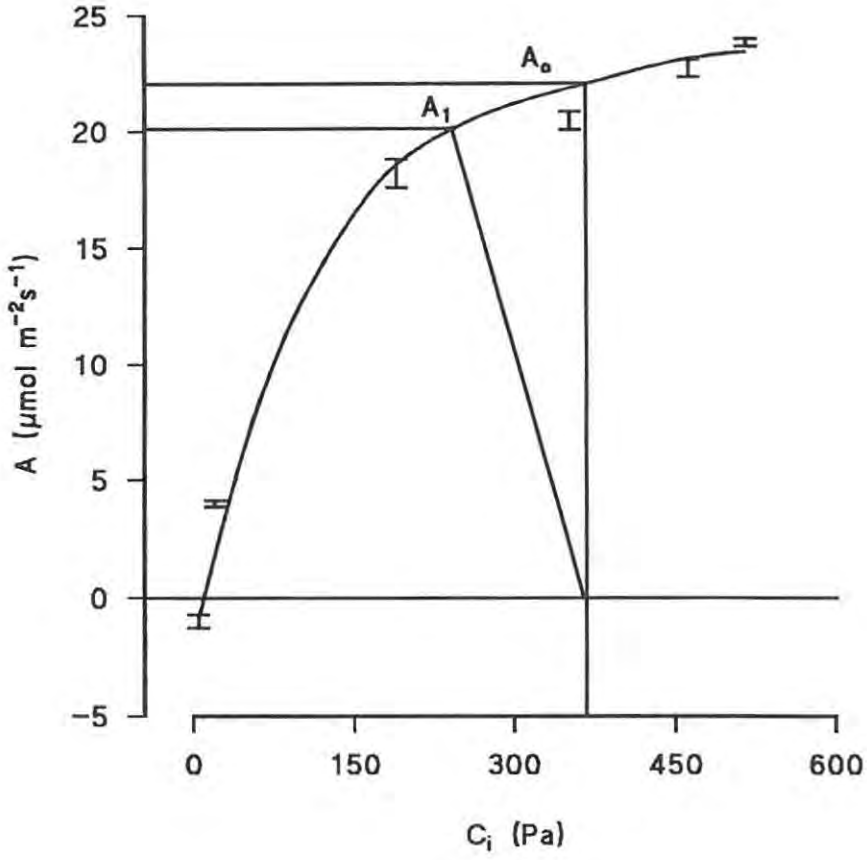

Fig. 26. ***A/C**_i* curve showing changes in stomatal limitation at 35°C in absence of oxygen.

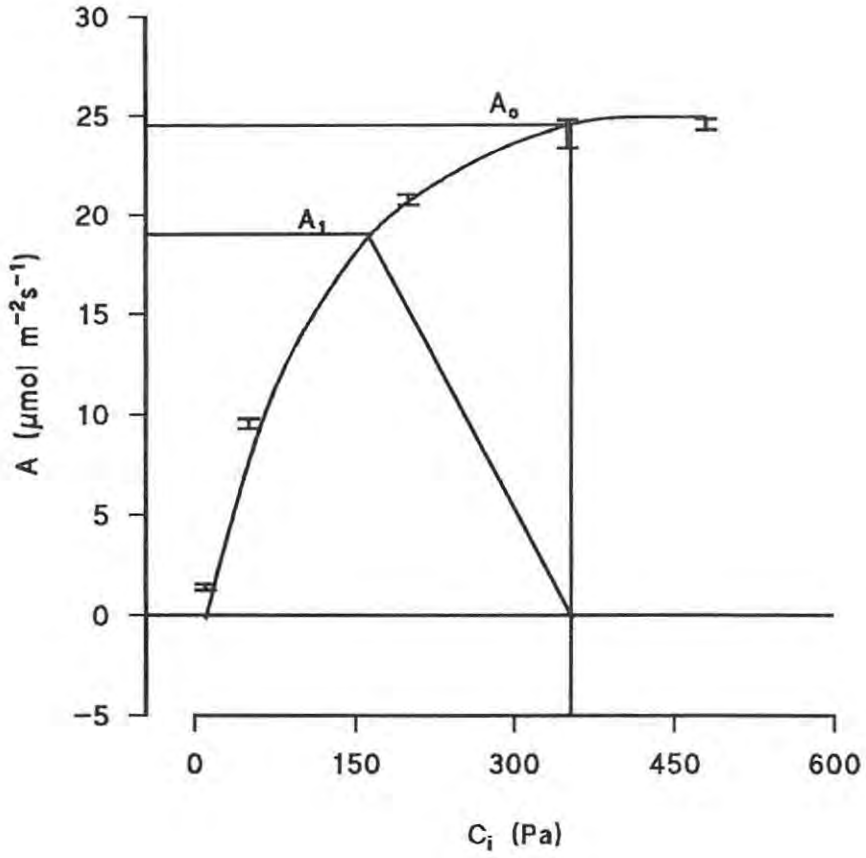

Fig. 27. ***A/C***$_i$ curve showing changes in stomatal limitation at 25°C and 21% [O_2].

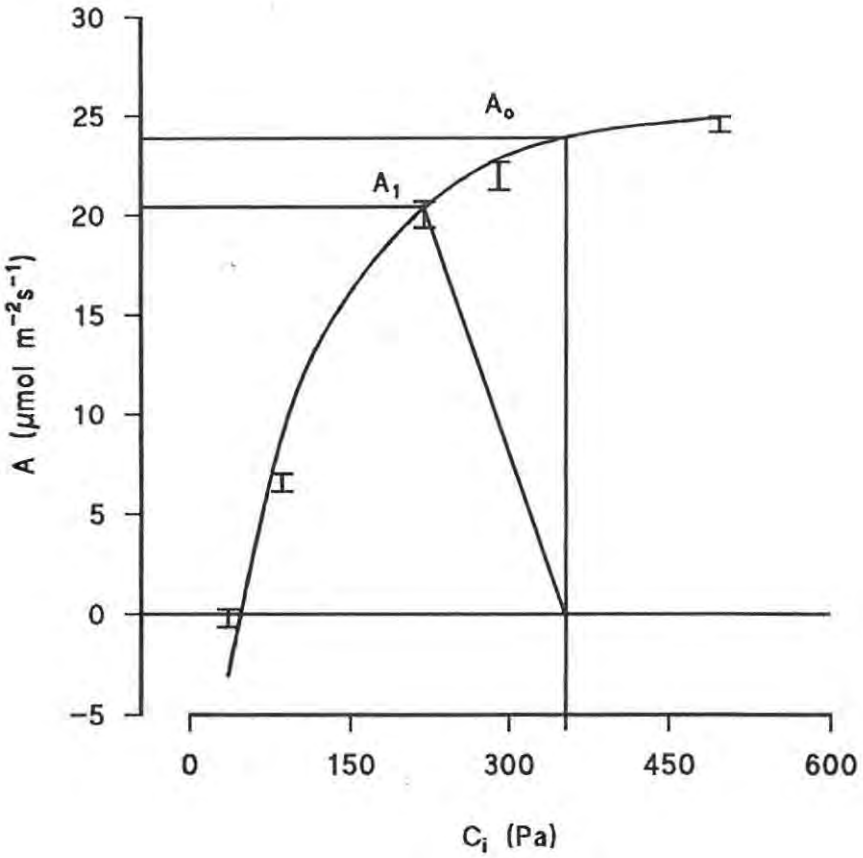

Fig. 28. ***A/C***$_i$ curve showing changes in stomatal limitation at 35°C and 21% [O_2].

APPENDIX B

Parameter calculations and equations

The basic equations (Farquhar, *et al.*, 1980; Von Caemmerer and Farquhar, 1981; Ziegler-Jöns and Selinger, 1987; Field, *et al.*, 1989) which were also incorporated in the IRCAL programme (materials and methods), are presented here without details of the theory. Photosynthetic CO_2 assimilation (A, μmol $m^{-2}s^{-1}$) was calculated from the depletion of CO_2 in the gas stream. According to the above mentioned authors, A depends on the velocity of carboxylation, V_{cmax}, **RUBISCO** which is an unstable enzyme capable of carboxylation or oxygenation, hence K_c and K_o which are the Michaelis-Menten constant for CO_2 and O_2 are used. Thus the net rate of CO_2 assimilation in the absence of day respiration (R_d) is:

$$A = V_{cmax}\left(\frac{C-\Gamma^*}{C+K_c\left(\frac{1+0}{K_o}\right)}\right)-R_d$$

.....Equation 9

Farquhar, *et al.* (1980) established the dependence of A to intercellular CO_2 using equation 9. The resultant equation (Equation 10) is directly related to the equation proposed by Ku, *et al.* (1977a), (Equation 3) which estimated carboxylation efficiency (*CE*) from the initial slopes of A versus C_i.

The dependence of A on the intercellular CO_2 is then:

$$\frac{dA}{dC} = V_{cmax}\left(\frac{\Gamma^* + K_c\left(\frac{1+0}{K_o}\right)}{\left[C + K_c\left(\frac{1+0}{K_o}\right)\right]^2}\right)$$

..... Equation 10

Carbon dioxide compensation point (Γ^*, $\mu l\ l^{-1}$) has been used extensively in this thesis and its calculation is based on the following equation:

$$\Gamma^{*}=\frac{\Gamma+K_c\left(\frac{1+0}{K_o}\right)\frac{R_d}{V_{cmax}}}{1-\frac{R_d}{V_{cmax}}}$$

..... Equation 11

Farquhar and Sharkey (1982) and later Field, *et al.* (1989) pointed out that the power of photosynthetic measurement is greatly increased by simultaneous measurement of transpiration (***E***, μmol $m^{-2}s^{-1}$E) as was illustrated in equation 8. Once ***E*** has been calculated, it is possible to calculate leaf conductance to water vapour, which is the critical parameter for the determination of internal CO_2 concentration (C_i). By rearranging equation 8 as given in Chapter 7 (page 45), i.e.

$$A=g_c(C_a-C_i)-\left(\frac{C_l+C_a}{2}\right)E$$

the resultant equation (12, below) is incorporated in the IRCAL software packaged that was used adequately estimates C_i as outlined by Field, *et al.* (1989).

$$C_i = \frac{\left(g_{tc} - \frac{E}{2} c_a\right) - A_n}{\left(g_{tc} + \frac{E}{2}\right)}$$

..... Equation 12

where g_{tc} is the total conductance to CO_2 (μmol $m^{-2}s^{-1}$), C_a is the mole fraction of CO_2 in the ambient air (mol mol^{-1}) and A_n is net assimilation rate (μmol $m^{-2}s^{-1}$).