Effects of sustained elevated CO_2 concentration on two cultivars of barley (*Hordeum vulgare* L.)

THESIS

Submitted in fulfilment of the

requirements for the Degree of

Master of Science

of **Rhodes University**

by

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January 1997

TO

MOM AND DAD

THANKS FOR EVERYTHING

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DEFINITION OF SYMBOLS AND ABBREVIATIONS

Definition of symbols

Symbol	Unit	Definition
A	μmol mol^{-1}	Assimilation of CO_2 per unit leaf area
C_i	μmol mol^{-1}	Intercellular carbon dioxide concentration
E	mmol m^{-2} s^{-1}	Transpiration rate
g_s	mol m^{-2} s^{-1}	Stomatal conductance
PPFD	μmol m^{-2} s^{-1}	Photosynthesis photon flux density
WUE	mmol mol^{-1}	Water use efficiency

Definition of abbreviations

Abbreviation	Definition
ATP	Adenosine triphosphate
CO_2	Carbon dioxide
$[CO_2]$	Carbon dioxide concentration
DAG	Days after germination
DW	Dry weight
LDW	Leaf dry weight
N	Nitrogen
NADPH	Nicotinamide adenine dinucleotide phosphate
NAR	Net assimilation rate
O_2	Oxygen
PAR	Photosynthetically active radiation
P_i	Inorganic phosphate
RGR	Relative growth rate
Rubisco	Ribulose bisphosphate carboxylase oxygenase
RuBP	Ribulose-1,5 bisphosphate
SLS	"Short leaf syndrome"
WSD	Weighted stomatal density

ACKNOWLEDGEMENTS

I would like to express my sincere gratitude to my supervisor Professor C.E.J. Botha for giving me the opportunity of researching toward a higher degree under his excellent supervision in the ecophysiological research unit. Thank you Professor Botha for your constant support and guidance throughout this research; your critical comments and suggestions and your ever-willing assistance during the process of producing this thesis are greatly appreciated.

A special thank you to the Small Grain Centre, Bethlehem, South Africa for donating the seeds without which this research would not have been possible.

I am deeply indebted to Mrs Sarah Radloff for her kind assistance with statistical analysis. Sincere thanks are also due to Mrs Shirley Pinchuck and Mr Robin Cross for assistance with electron microscopy. Mr Bradford Ripley is sincerely thanked for proof-reading parts of this thesis. A special thank you to my friend, Mr Jabulani Mjwara for providing a wealth of references used in the production of this thesis.

Special thanks are due to Ms Gege Kekana for her wonderful assistance and support during the production of this thesis, and to my friend and fellow student, Mr Bernd Sonnenberg for helping in the preparation of this work.

I am very grateful to my family and friends for their unfailing encouragement throughout my studies.

Lastly, the financial support by the Foundation for Research Development (FRD, Pretoria, South Africa) who awarded an M.Sc. bursary is acknowledged. The Botany Department, Rhodes University is thanked for the award of a Graduate Assistant Bursary.

ABSTRACT

The enormous burning of fossil fuel and deforestation have caused an increase in the atmospheric CO_2 concentration ($[CO_2]$) during the last century. This will invariably have profound direct and indirect effects on plant carbon metabolism. The majority of research on the effects of CO_2 enrichment on plants are short-term and are done on other crops, but very little have been done on barley. This project aimed to determine the effects of long-term CO_2 enrichment on photosynthesis, growth and grain yield on barley.

Hordeum vulgare L. cvs Stirling and Schooner plants were grown from seeds in controlled environment chambers at ambient (350) and elevated (600) μmol mol^{-1} $[CO_2]$. Measurements of net assimilation rate (NAR), photosynthetic pigments content and growth parameters were started 7 days after germination (DAG) and continued until senescence. The anatomy of matured fully developed leaves was also monitored.

Elevated $[CO_2]$ resulted in an increase in NAR in the two cultivars from days 7 until 14, after which the stimulation of NAR of CO_2-enriched plants started to decrease. At the onset of senescence, NAR was almost equal in plants grown under both ambient and elevated $[CO_2]$. The response of assimilation as a function of internal $[CO_2]$ (C_i) at the end of the experimental period showed a significant decrease in both the initial

slope of the A/C_i curves and the CO_2-saturated photosynthetic rates in the two cultivars. Stirling showed no significant changes in the content of chlorophyll *a*, chlorophyll *b* or in total carotenoids. However, Schooner showed a stimulation in chlorophyll *a* content at day 7, but decreased at day 28. Chlorophyll *b* and total carotenoids content were not affected by CO_2 enrichment.

While total above-ground biomass was not affected by elevated [CO_2] in the two cultivars, total plant height decreased significantly after 14 days in Stirling whereas no significant change occurred in Schooner throughout the experimental period. Leaf area was not significantly affected by CO_2 enrichment in the two cultivars although the leaves in CO_2-enriched plants were slightly shorter.

Anatomical studies reveal that leaf thickness was significantly increased by CO_2 enrichment in Stirling, but the increase was not significant in Schooner. Both cultivars did not show any significant effect on chloroplast morphology and ultrastructure as a consequence of elevated CO_2 exposure. No signs of starch accumulation were evident in variety Schooner, but Stirling showed some form of starch accumulation, under increased atmospheric [CO_2].

Elevated CO_2 resulted in a significant reduction by more than 50 % in the number of grain yield per plant in both Stirling and Schooner. Results from this study therefore indicate that CO_2 enrichment will not be beneficial in terms of growth and yield in this important crop.

CHAPTER 1: INTRODUCTION

1.1. Atmospheric carbon dioxide concentration is increasing

Historical evidence indicates that atmospheric CO_2 concentration has increased significantly over the past two and half centuries by about 30 % from approximately 280 μmol mol^{-1} to more than 350 μmol mol^{-1} (Fig. 1.1). Between 1750 and 1850 only a small increase in atmospheric CO_2 concentration ($[CO_2]$) occurred from approximately 280 μmol mol^{-1} to 290 μmol mol^{-1}, at a relatively small increase rate of about 0.05-0.1 μmol mol^{-1} CO_2 per year. In contrast, the beginning of the Industrial Revolution, resulted in a dramatic increase of atmospheric $[CO_2]$ from about 290 to approximately 315 μmol mol^{-1} around the 1950s (Newton, 1991; Taylor and Lloyd, 1992). The average rate of increase was approximately 0.35 μmol mol^{-1} per year. The period between 1950 and 1992 showed a more marked increase in the average increase rate of increase in the atmospheric $[CO_2]$ from 0.35 to 0.83 μmol mol^{-1} CO_2 per year. Atmospheric CO_2 increased from 315 to more than 350 μmol mol^{-1} between 1950 and 1992 (Taylor and Lloyd, 1992). Recent literature reveal that CO_2 concentration is currently increasing at the rate of approximately 1.8 μmol mol^{-1} CO_2 per year (Watson *et al.*, 1990; Hendry, 1992).

The increase in atmospheric $[CO_2]$ observed at the beginning of the Industrial Revolution, can be attributed to increases in the burning of fossil fuels which occurred

around that period. Unless radical shifts in fossil fuel emissions occur, it is predicted that atmospheric [CO_2] will be approximately 700 μmol mol^{-1} by the middle of the next century (Vu *et al.*, 1989; Newton, 1991; Coleman *et al.*, 1993; Rogers and Dahlman, 1993; Manderscheid and Weigel, 1995). We will thus have to cope with elevated CO_2 as part and parcel of our day to day lives.

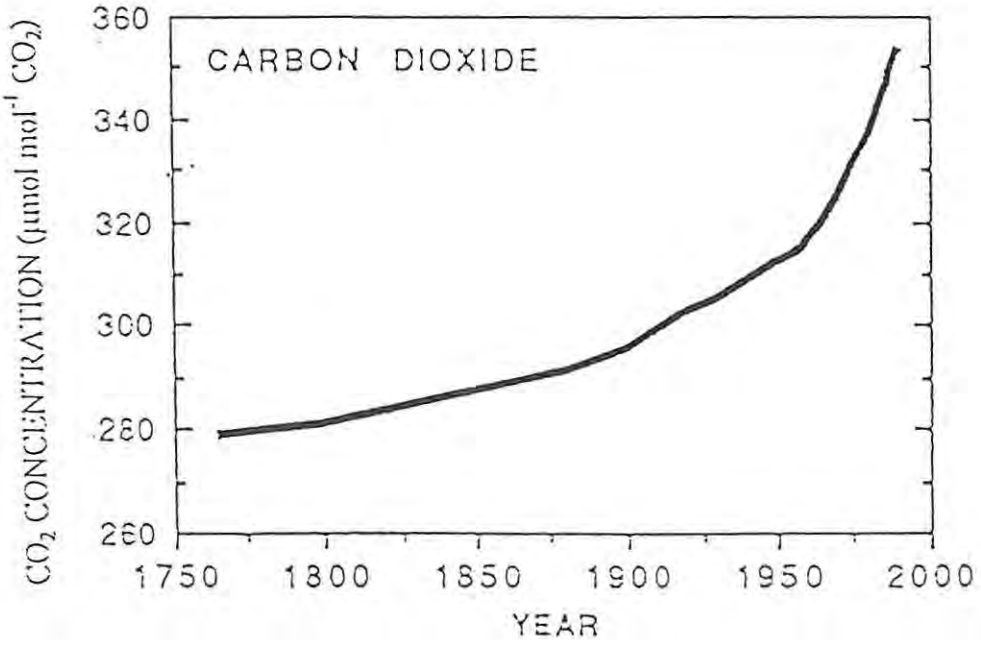

Fig. 1.1. Global increase in atmospheric [CO_2] over the past two decades (Roeckner, 1992)

1.2. Effects of CO_2 enrichment on plant photosynthesis

Atmospheric [CO_2] is amongst the most important of the environmental parameters which affect photosynthetic CO_2 assimilation by plants, and consequently this must affect whole-plant functioning. Photosynthesis in C_3 plants grown in adequate light and other growth conditions require about 800-1000 μmol mol^{-1} for CO_2 saturation. In C_4 plants, however, photosynthesis under adequate light and other non-limiting growth conditions, is almost saturated at the current atmospheric [CO_2]. Increased [CO_2] will therefore significantly increase the rate of photosynthesis in C_3 plants, but affect C_4 plants very little as depicted in Fig. 1.2 (Cure and Acock, 1986; Lawlor and Mitchell, 1991; Wolfe and Erickson, 1993).

As atmospheric [CO_2] increases, more CO_2 will tend to enter the leaves of plants because of the increased CO_2 gradient between the leaf and air, which will result in a higher CO_2:O_2 ratio. Since ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) catalyses both the photosynthetic carbon reduction (PCR) and the photorespiratory carbon oxidation (PCO), increased CO_2:O_2 ratio will favour the (PCR) over the (PCO). Consequently, photorespiration will decrease, thereby increasing photosynthesis (Rowland-Bamford, *et al.*, 1991; Conroy *et al.*, 1994; Woodrow, 1994).

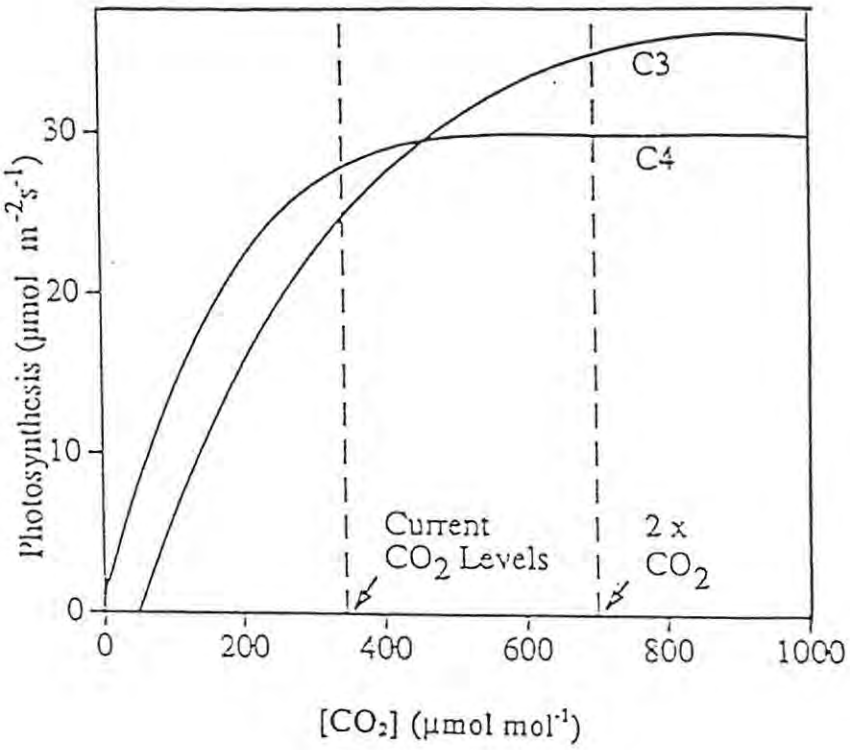

Fig. 1.2. Photosynthesis per unit leaf area in relation to atmospheric [CO_2]. Typical curves for C_3 and C_4 plants. Although the specific photosynthetic values will vary among species in general, C_3 plants will show a greater relative benefit from CO_2 doubling compared to C_4 (From Wolfe and Erickson, 1993).

1.3. Feedback mechanisms and photosynthetic acclimation to CO_2 enrichment

Many workers have reported that the large increases in photosynthesis observed in short-term (periods of hours to a few days) experiments, may not be maintained in the long-term (Drake and Leadley, 1991; Farrar and Williams, 1991; Tissue *et al.*, 1993; Wolfe and Erickson, 1993; Xu *et al.*, 1994a), (see Table 1.1). After an initial short-term enhancement of photosynthesis with CO_2 enrichment, plants may respond to increased CO_2 by reducing the rate of photosynthesis without changing the leaf composition, or photosynthetic capacity. This phenomenon is often referred to as 'photosynthetic down-regulation'. Plants may also respond to long-term CO_2 enrichment by decreasing the photosynthetic components with a concomitant loss in photosynthetic competence and capacity, which is often referred to as 'photosynthetic acclimation' (Delgado *et al.*, 1994).

Although the explanation for this decline of net assimilation rates after long-term elevated [CO_2] exposure is still controversial, several reasons had been suggested. Amongst others, Bowes (1991), Radoglou *et al.* (1992), Long *et al.* (1993) and Xu *et al.* (1994a), reported that the decline in photosynthesis under high CO_2 conditions may be a result of feedback inhibition, by accumulated carbohydrates in the leaves, due to the inability of the carbohydrate sinks to cope with higher initial photosynthesis fluxes. In addition, Cave *et al.* (1981) and Stitt (1991) add that carbohydrate accumulation in leaves disrupts the chloroplast structure and hence function, thereby decreasing photosynthetic rates. Sage *et al.* (1989),

Table 1.1. Photosynthetic responses to CO_2-doubling for several crop species with either C_3 or C_4 photosynthetic pathway. Data are from several experiments and represent the percentage change at 680 μmol mol^{-1} CO_2 compared with controls (300-350 μmol mol^{-1}). Responses of short term and long term (> 1 week) exposure to elevated CO_2 are presented (Adopted from Wolfe and Erickson, 1993).

Photosynthetic pathway	Crop	Percentage change in net photosynthesis after CO_2 doubling	
		Short term	Long term
C_3	Barley	+ 50	+ 14
	Cotton	+ 60	+ 13
	Rice	+ 42	+ 46
	Soybean	+ 78	+ 42
	Tomato	+ 30	+ 9
	Wheat	+ 41	+ 27
C_4	Corn	+ 26	+ 4
	Sorghum	- 3	+ 6

Tissue *et al.* (1993) and Delgado *et al.* (1994), on the other hand, suggested that the decline in photosynthesis after a long-term CO_2 enrichment may be due to the decline in the activity of rubisco. It is however, still unclear whether this decline in rubisco activity is due to the reduction in the quality of rubisco protein, a decrease in the activity of the existing enzyme, alteration in its regulation, or a combination of either of them. In addition a decline in stomatal conductance under elevated CO_2 conditions was suggested as another possible reason leading to the decrease or loss of stimulation in photosynthesis after long term exposure (Bowes, 1991; Kimball an Mauney, 1993; Knapp *et al.*, 1994; Sage, 1994; Thomas *et al.*, 1994; Samarankoon *et al.*, 1995). Cave *et al.* (1981) attributed the decline in photosynthesis under CO_2 enrichment to a concomitant decline in chlorophyll content. However, other (contradictory) reports point out that CO_2 enrichment does not affect chlorophyll content (see Wong, 1979; Ehret and Jolliffe, 1985; Delgado *et al.*, 1994, for example).

1.4. Growth responses to CO_2 enrichment

The response of plant growth to CO_2 enrichment is well documented. In their 1985 review, Acock and Allen pointed out that increasing atmospheric [CO_2] may lead to the so-called 'luxury consumption' of carbon in plants. This may be expressed in plants through increased leaf number, increased leaf area and increased total leaf thickness (Acock and Allen, 1985; Newton, 1991; Bosac *et al.*, 1995). However, contradictory responses have been reported by Scheidegger and Nösberger (1984),

who observed that individual leaf size was not affected by CO_2 enrichment in white clover.

Other common plant responses to elevated [CO_2] are the moderate increases in stem height and stem diameter (Acock and Allen, 1985). Enhanced numbers of branches and tillers have also been widely reported (Acock and Allen, 1985, and references cited; Newton, 1991; Rogers and Dahlman, 1993). All these morphological changes are attributable to CO_2 enrichment and may translate into increased total plant dry weight as reported by Mauney *et al.* (1978); Cure and Acock, (1986); Idso and Idso, (1994); Wheeler *et al.* (1994). However, in their recent publication, Manderscheid and Weigel (1995), reported that the total above ground biomass was not affected by CO_2 enrichment in barley, bean, maize and wheat.

1.5. Elevated CO_2 and yield

A review by Acock and Allen (1985) and references cited therein, reveal that increasing atmospheric [CO_2] from 330 to 660 μmol mol^{-1} resulted in an increase in the yield of about 38 % on the average, in 38 agricultural crops, and 18 other species (see Table 1.2 below). This was subsequently confirmed by Newton (1991) and Conroy *et al.* (1994) in cereals. However, contradictory results by Garbutt and Bazzaz (1984) indicate that the three herbaceous annual species used did not respond to elevated CO_2 by increased yield, but rather showed slight decreases in yield.

Table 1.2. Predicted marketable yield increase for a doubling of [CO_2] from 330 to 660 μmol mol^{-1} (Adopted from Acock and Allen, 1985).

Crop	Number of observation	Percent increase
Barley	1	36
Clover	2	4
Corn	1	16
Cotton	2	104
Rice	1	9
Sorghum	1	79
Soybean	13	17
Tomato	6	13
Wheat	13	38

1.6. Research objectives

Previous sections in this chapter and reviews by Newton (1991); Woodward (1992); Bowes (1993) and Rogers and Dahlman (1993) clearly indicate that responses of plants to elevated CO_2 seem to be largely species-dependent, but also dependent on other growth and environmental conditions, for example, temperature and nitrogen nutrition. The majority of existing CO_2-enrichment studies have dealt with only a few crops in particular broad bean, cotton, soybean and wheat.

In terms of world crops barley is the forth most important (Table 1.3), highlighting its high economic significance. Despite the economic importance, there remains a dearth of knowledge on barley's responses to long-term elevated [CO_2]. In addition, Mauney *et al.* (1978) and Wolfe and Erickson (1993), and references cited by these authors suggest that carbon assimilation rate does not correlate with plant growth or ultimately with yield.

Table 1.3. World rank and photosynthetic type for ten major crops (Adopted from Rogers and Dahlman, 1993).

Crop	World rank (acreage)	Photosynthetic pathway
Alfalfa	-	C_3
Barley	4	C_3
Corn	3	C_4
Cotton	9	C_3
Rice	2	C_3
Sorghum	6	C_4
Soybean	7	C_3
Sweet potato	16	C_3
Wheat	1	C_3
White potato	12	C_3

Given that intraspecific differences on the responses of plants to elevated CO_2 have been previously reported by Wulff and Alexander (1985), the hypothesis upon which the research presented in this thesis is based, was that the two barley cultivars would respond differently to sustained elevated [CO_2].

The aims of these project were firstly to determine the effects of sustained elevated [CO_2] on photosynthesis, growth and yield in barley. Secondly, this study aimed to investigate the potential differences on the responses of the barley cultivars to CO_2 enrichment.

CHAPTER 2: MATERIALS AND METHODS

2.1. Plant material and growth conditions

Seeds of two cultivars of barley, (*Hordeum vulgare* L. cvs. Stirling and Schooner) donated by the Small Grain Centre, Bethlehem, South Africa, were soaked in water overnight and germinated in petri dishes. Germinated seeds of approximately same size were planted in washed white river sand in 15 cm deep plant pots, as suggested by Ms Careen Du Plessis, Small Grain Centre - Personal communication. One seed was planted per pot to prevent competition and shading. Plants were watered every other day with a complete nutrient solution (Chemicult, Chemicult Products, Pty Ltd, Camps Bay, Cape Town, South Africa); containing 6.5 % N; 27.0 % P; 13.0 % K; 7.0 % Ca; 2.2 % Mg; 7.5 % S; 0.15 % Fe; 0.024 % B; 0.005 % Zn; 0.002 % Cu and 0.001 % Mo as outlined in Mjwara *et al.* (1996). Plants were grown under two different carbon dioxide (CO_2) concentrations in Controlled Environment Cabinets (Conviron, Controlled Environments Ltd. Winnipeg, Canada.). One group, with plants from both cultivars, was grown in an EF 7H Conviron with a CO_2 concentration of 350 ± 10 $\mu mol\ mol^{-1}$ and a 25/20°C day/night temperature. The maximum irradiance of photosynthetically active radiation (PAR, 400-700 nm) obtainable was approximately 500 $\mu mol\ m^{-2}\ s^{-1}$ at the top of the plant canopy. In order to maintain constant vapour pressure deficits in the cabinets, the day/night relative humidity was set at 65/45 %. The other group, also with plants from both cultivars was grown in a second Conviron

(Model S10H) in which the CO_2 concentration could be controlled. The CO_2 concentration was set at 600 ± 10 μmol mol^{-1} , all other environmental factors were the same as in the Conviron EF 7H. The photoperiod in the two cabinets was set at 16 hours. Since two cultivars were used and only two growth cabinets were available for this study, most parts of the experiment were repeated twice.

2.2. Photosynthetic measurements

The rate of photosynthesis was measured using an ADC LCA-2 infrared gas analyser (IRGA, Analytical Development Co. Ltd., Hoddesdon, Herts, UK), set in an open circuit (Fig. 2.1). The first fully expanded healthy leaf of predetermined area was clamped in an ADC PLC (B) Parkinson broad leaf chamber (Analytical Development). An ADC ASUM flowmeter (Analytical Development) was used to maintain flow rate over the leaf at 350 ± 5 ml $min.^{-1}$. Gas exchange measurements were taken in triplicate and recorded using the ADC DL-2 datalogger (Analytical Development).

2.2.1. Light intensity regulation

Irradiance during photosynthetic experiments was provided using a Philips (SON-T) high pressure 400 W sodium lamp, with a maximum irradiance in excess of 2000 μmol m^{-2} s^{-1} photosynthetic photon flux density (PPFD), which was clamped to a stand above the leaf chamber. The required irradiance was maintained by placing fine shade cloth frame(s) between the light source and the leaf chamber.

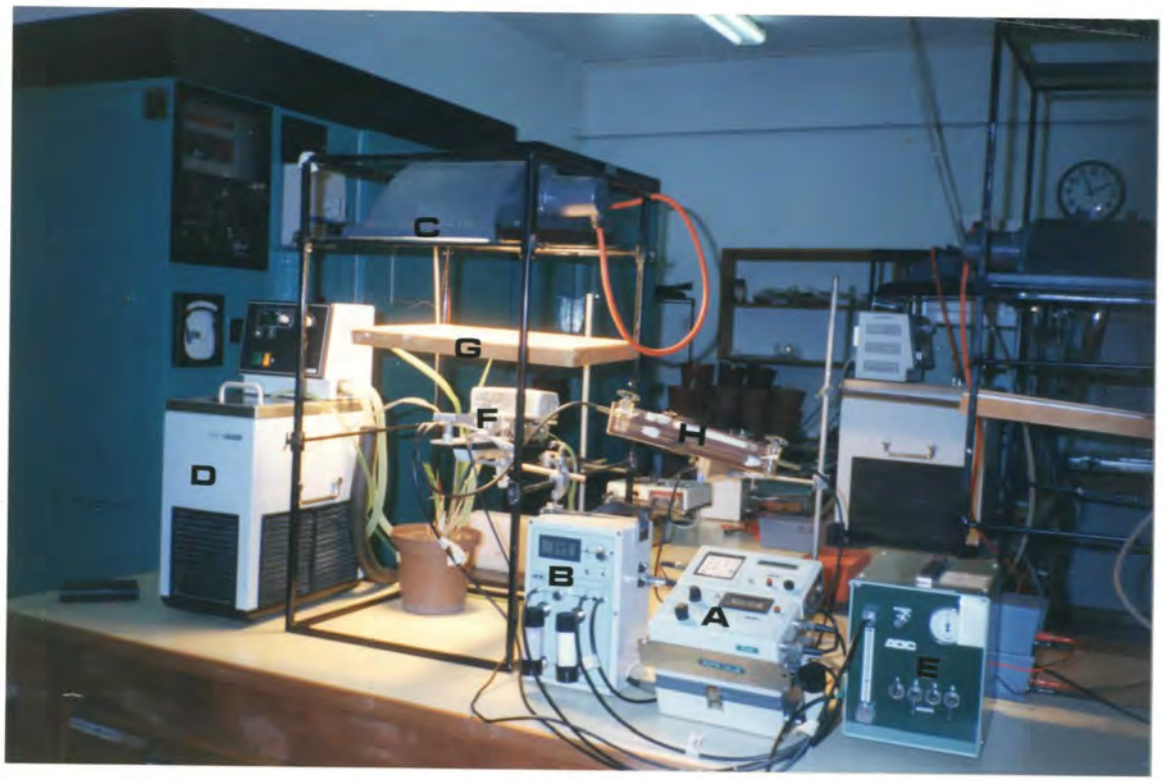

Fig 2.1. Shows the infra-red gas analyser (IRGA) set-up. A - The ADC LCA-2 IRGA coupled to an ADC DL-2 datalogger; B - an ADC ASUM flowmeter; C - a Philips (SON-T) high pressure 400 W Sodium lamp; D - RMS-6 LAUDA refrigeration unit; E - ADC GD-600 gas diluter; F -ADC PLC (B) Parkinson broad leaf chamber; G - shade cloth; H - a wet-and -dry bypass humidifier. Note the Conviron cabinets in the background.

2.2.2. Humidity control

To prevent stomatal closure in response to low humidity during the experiments, a pre-humidifier with a wet-and dry-bypass, connected between the mass flowmeter and the ADC LCA-2 IRGA was used. When gas exchange readings were taken, the humidifier was set on a dry-bypass and allowed to stabilise for a few minutes. Between measurements, the humidifier was set to the humid bypass, and the relative humidity was maintained at approximately 68-70 %.

2.2.3. Temperature control

The temperature of the leaf was regulated by the heat sink coupled to a RMS-6 LAUDA refrigeration unit (Dr R. Wobser GMBH & Co., Königshofen, Germany) connected to the Parkinson broad leaf chamber. All photosynthetic measurements were done at a pre-determined optimum temperature of 25°C. The temperature was carefully monitored and maintained within 0.5°C of the required experimental temperature.

2.2.4. CO_2 concentration control

CO_2 was provided using bottled CO_2 (1000 ppm; 0.3 % O_2; and balanced with N_2, Fedgas, Alrode, Port Elizabeth, South Africa). The required CO_2 concentration was obtained by passing the gas from the gas bottle through two ADC GD-600 gas diluters (Analytical Development) which were connected in line with the ASUM flowmeter.

Readings were taken as CO_2 concentration was varied stepwise from 900 to 0 μmol mol^{-1} , allowing at least fifteen minutes equilibration period at each CO_2 concentration.

2.2.5. Data calculation

Data was downloaded from the datalogger to a computer programme, infra red photosynthetic CO_2 gas analysis calculation (IRCAL, Version 2.0, 1992, Botha, C.E.J. & Brown, B.J.L., Rhodes University, Grahamstown, South Africa). Net assimilation rate and related parameters including stomatal conductance, transpiration rate, water use efficiency, apparent quantum efficiency and intercellular [CO_2] were then calculated using the equations described by von Caemmerer & Farquhar (1981), incorporated into IRCAL (see Appendix 1 for equations)

2.3. Photosynthetic pigments measurements

Samples (0.2 g) from the first fully developed leaves were frozen in liquid N_2 and thereafter homogenised to a pulp using a cold pestle and mortar in 10 ml of ice-cold 80 % acetone. After homogenisation, the slurry was passed through three layers of cheesecloth and the filtrate was centrifuged at 15 000 g for 10 minutes at 4°C using the RC-Sorvall Superspeed Refrigerated Centrifuge (Du Pont Instruments, Newtown, Conn). After decanting the filtrate, the supernatant was measured against 80 % acetone at 663 , 646 and 470 nm using a Philips PU 8670 VIS/NIR spectrophotometer (Pye Unicam Ltd., UK). The concentration of chlorophyll *a* and *b* (C_a and C_b, respectively)

and carotenoids (C_{x+c}) (in μg ml plant extract^{-1}) were calculated using equations 2.1-2.4 as described by Lichtenthaler and Wellburn (1983).

$$C_a = 12.21.A_{663} - 2.81.A_{646} \quad \text{Equation 2.1}$$

$$C_b = 20.13.A_{646} - 5.03.A_{663} \quad \text{Equation 2.2}$$

$$C_{a+b} = C_a + C_b \quad \text{Equation 2.3}$$

$$C_{x+c} = \frac{1000.A_{470} - 3.27.C_a - 104.C_b}{229} \quad \text{Equation 2.4}$$

Since chlorophylls and carotenoids are sensitive to light, the extraction procedures were performed in dim light

2.4. Plant growth measurements

Growth measurements were commenced at 7 days after germination (DAG) and carried out weekly thereafter until the onset of senescence.

2.4.1. Plant height, fresh- and dry weight measurements

Total plant height was measured from the level of the soil to the tip of the legule of the last fully developed leaf, after which plants were harvested. Above-ground biomass (stem and leaves) were separated from below-ground biomass (roots) at the level of the cotyledon. The first fully developed leaf was separated from the shoot for freshweight and leaf area determination. Total above-ground fresh weight was measured as well. The roots were washed free of soil and weighed. Plant materials used for fresh-weight determination were oven-dried at 70°C for 48 hours after which dry weight measurements were done. Relative growth rates were then later calculated

2.4.2. Leaf area and thickness determination

Photogravimetric method was used to determine leaf area. Leaves were placed on an overhead transparency and a photocopier was used to obtain prints of the leaves. The images were cut out and photographed using a Panasonic F10 CCD video camera (Matsushta Communication Industrial Co., Ltd., Japan) connected to a computer; and saved as grey level images. Leaf area was then calculated using a computer programme PC IMAGE Colour VGA 24 for Windows and Screen Machine II, Version 2.1., 1995 (Foster Findlay Associates, Ltd.). The same leaves used for leaf area measurements were also used for fresh- and dry- weight determination. Leaf thickness was measured from pictures of cross-sections obtained from light microscopy using an ultrastructure size calculator (Dunn & Reidman, CA, USA).

2.5. Microscopy

2.5.1. Electron microscopy

2.5.1.1. Transmission electron microscopy

Tissues from the first fully-expanded mature leaves were selected and cut into small pieces approximately 3X5 mm. Materials were fixed in 2.5 % glutaraldehyde in 0.2 M cacodylate buffer (pH 7.2) at 4°C for 12 hrs. The material was then trimmed into smaller pieces of approximately 1X2 mm. Leaf tissues were thoroughly rinsed in the same buffer and post-fixed in 2 % osmium tetroxide. The material was then carried through an ethanol dehydration series followed by two changes in propylene oxide (1 hr total). Leaf tissues were embedded in Araldite Taab 812 resin. Ultrathin sections (silver-gold) were cut using a diamond knife mounted on an RMC MT-7 ultramicrotome (Research & Manufacturing Co. Inc., Tucson, Arizona). The sections were collected on 200 mesh copper grids and double-stained in uranyl acetate and lead citrate in that order. Sections were viewed and photographed using a Joel 100-CX-2 transmission electron microscope (Tokyo, Japan) at an accelerated voltage of 80 kV.

2.5.1.2. Scanning electron microscopy

Plant tissues were cut and fixed in 2.5 % glutaraldehyde as for the transmission electron microscopy preparation. After the final change of the alcohol dehydration series, leaf sections were placed in an amylacetate series allowing amylacetate to

infiltrate for 20 min. in each change. Specimens were then critical point dried for 1-2 hours. Plant materials were then mounted on metal stubs, sputter coated with gold and viewed using a JEOL JEM-840 scanning electron microscope (Tokyo, Japan). SEM images were then saved into an attached computer and used later for stomatal density determination.

2.5.2. Light microscopy

Monitor sections from the blocks prepared for transmission electron microscopy were cut using a glass knife mounted on a RMC MT-7 ultramicrotome. The sections were mounted on clean slides and stained with toluidine blue. The leaf sections were examined using a Zeiss universal microscope (Carl Zeiss (Pty) Ltd., Oberkochen, Germany) and photographed using a Zeiss MC-63 camera system fitted to the microscope.

2.6. Stomatal density determination

SEM images of leaf surfaces were used for stomatal density determination. Images were then recalled from digital storage and the total number of stomata were counted within a 0.25 mm^2 area. Stomata were counted for images of both the adaxial and abaxial surfaces. Leaf surface area was determined using an image measurement software, SigmaScan/Image. Version 1.2, 1993 (Jandel Scientific, CA, USA). Because both the abaxial and the adaxial surfaces were involved in gas exchange

measurements, weighted stomatal density had to be calculated using equation 2.5 below as described by El-Sharkawy *et al* (1985)

$$WSD = \frac{ADSD^2 + ABSD^2}{ADSD + ABSD} \quad \text{Equation 2.5}$$

Where WSD is weighted stomatal density,

ADSD is adaxial stomatal density, and

ABSD is abaxial stomatal density

2.7. Statistical analysis

In most instances, three plants per cultivar per CO_2 treatment were sampled, and each experiment was replicated twice, so unless otherwise stated, each data point represents a mean of six values. All data of photosynthetic responses, photosynthetic pigments, growth responses and yield were subjected to a multifactor ANOVA to test for differences between the means at 5 % level of significance using a statistical software programme, STATGRAPHICS (Manugistics, Inc. & Statistical Graphics Corporation, USA, 1993). One-way ANOVA was used to test significance between days. Scheffe's multiple range test was used to establish where significance occurred.

CHAPTER 3: PHOTOSYNTHESIS, PHOTOSYNTHETIC PIGMENTS AND WATER USE EFFICIENCY IN RELATION TO SUSTAINED ELEVATED CO_2

3.1. Introduction

Increases in the global atmospheric carbon dioxide concentration has prompted a great deal of research on the effects of enhanced [CO_2] on plant morphological, physiological and biochemical processes. Many studies have previously focused on short-term experiments lasting for only a few minutes to hours, but there has been a steady growth in research focused on the effects of sustained CO_2 enrichment in plants - periods of days to weeks to months. Since the initial interaction between plants and atmospheric CO_2 is the diffusion of CO_2 into plant's leaves and its subsequent fixation by photosynthesis, the analysis of the response of plants to the continuously increasing atmospheric [CO_2], has therefore focused mainly on photosynthesis (Gunderson *et al.*, 1993). Reviews by Pearcy and Björkman (1983), Cure and Acock (1986) suggested that photosynthesis increases by between 20 and 200 % with a doubling of [CO_2] depending on the species and other experimental conditions. However, a wealth of experimental data have shown that such initial stimulation of CO_2 assimilation may be reduced or lost following a whole range of primary, secondary and tertiary responses and interactions, including photosynthetic biochemical mechanisms, stomatal

conductance, water use efficiency, changes in tissue chemistry and nutrient use (Gunderson *et al.*, 1993; Mjwara *et al.*, 1996; and references cited therein). This chapter will only focus on the photosynthetic biochemical mechanisms, stomatal conductance and water use efficiency, and how these respond and interact with CO_2 enrichment.

Long *et al.* (1993) and Sage (1994) demonstrated that the A/C_i response curves can be interpreted in terms of the biochemical mechanisms controlling long-term responses of photosynthesis to elevated [CO_2]. According to Sage (1994), (in a typical A/C_i response curve of a C_3 plant), assimilation is limited by the capacity to consume RuBP in CO_2 fixation at low C_i. At intermediate C_i, photosynthesis is usually limited by the capacity of the thylakoid reactions to supply ATP and NADPH for RuBP regeneration. At elevated C_i, assimilation is limited by the capacity of starch and sucrose synthesis to utilise triose phosphate and subsequently regeneration of inorganic phosphate (P_i) for photophosphorylation (Sage, 1994; and references cited therein). In his 1994 review, Sage indicated that the A/C_i response curves of plants grown under CO_2-enriched environments as compared to those grown under ambient [CO_2] may be affected in either of the six general ways:

1. assimilation is reduced at all C_i under CO_2 enrichment. This response means that all photosynthetic components have been reduced as a consequence of growth at elevated [CO_2].

2. CO_2 assimilation is reduced at low and intermediate C_i, but CO_2-saturated A is unaffected. This implies that CO_2 enrichment resulted in the decline of Rubisco and thylakoid-dependent RuBP regeneration capacity.

3. assimilation is reduced at low C_i and increased at high C_i, the response suggesting that whilst Rubisco capacity is reduced, P_i regeneration capacity is increased as a consequence of elevated CO_2 exposure.

4. increasing assimilation at all C_i . This response suggests that all photosynthetic components have been increased as a result of growth under sustained CO_2 conditions.

5. assimilation is increased at high C_i only. This is interpreted to mean that P_i regeneration capacity increased with CO_2 exposure.

6. assimilation remains unaffected at all C_i. This response suggests that elevated [CO_2] has no effect on any photosynthetic components

Given the six possibilities it is therefore relevant that there is a need to focus on the analyses of A/C_i response curves and their interpretation so as to understand the effects of CO_2 enrichment on the biochemical mechanisms of this plant species, in this chapter.

Carbon dioxide influences stomatal opening and hence affects stomatal conductance and water vapour exchange (Prior *et al.*, 1991). It had been demonstrated that elevated [CO_2] decreases stomatal conductance and transpiration rate and increases water use efficiency (Prior *et al.*, 1991; Radoglou *et al.*, 1992; Xu *et al.*, 1994; Samarakoon *et al.*, 1995) in many C_3 plants. Radoglou *et al.* (1992) went further and said that the

magnitude of the response depends on the species and other environmental conditions. Some researchers have attributed decreased stomatal conductance to a decrease in stomatal density (Woodward and Bazzaz, 1988; Ceulemans and Mousseau, 1994; and references cited therein). However, contradictory reports on the effects of increased [CO_2] have also been published, reporting increases and no significant effects of stomatal density under elevated [CO_2] (O'Leary and Knecht, 1981; Drake, 1992; Berryman *et al.*, 1994). Factors leading to the decrease in stomatal conductance in plants grown under elevated CO_2 conditions are still controversial, but many researchers have attributed it with the partial closure of stomata under elevated [CO_2], rather than changes in stomatal density (Grodzinski, 1992; Lawlor, 1993; Hinckley and Braatne, 1994).

3.2. Results

3.2.1. Net assimilation rate

Measured net assimilation rate (NAR) increased continuously from the beginning of the experimental period, reaching its maximum at 14 DAG, and thereafter decreased until the end of the experimental period in both cultivars under ambient and elevated [CO_2]. Elevated CO_2 resulted in a significant increase in net assimilation rate in both cultivars (Fig. 3.1; $P < 0.001$). Similar results were reported by Ingvardsen and

Veierskov (1994) for the same plant species. NAR was stimulated by CO_2 enrichment during the initial growth stages which was followed by a remarkable decline to levels almost similar to those observed in plants grown under ambient [CO_2] in both cultivars. Similar responses had been observed by many workers on other plant species including, beans, rice and wheat (Radoglou *et al.*, 1992; Conroy *et al.*, 1994; Delgado *et al.*, 1994; Mjwara *et al.*, 1996). The greatest increase in NAR as a result of CO_2 enrichment occurred at the beginning of the experimental period with an increase of about 28 % in both cultivars. Schooner exposed to elevated [CO_2] showed slightly lower maximum NAR of approximately 25 μmol m^{-2} s^{-1} whereas Stirling under the same condition had higher rates of about 27 μmol m^{-2} s^{-1} at 14 DAG. At the end of the experimental period NAR in Schooner was almost 40 % lower than that of Stirling.

3.2.2. A/C_i responses

Figs. 3.2 and 3.3 show the responses of assimilation as a function of intercellular [CO_2] (C_i) measured at two different growth stages, 7 DAG and 28 DAG respectively for the two cultivars. At both growth stages the two cultivars responded to increase in C_i by a rapid initial increase in NAR. At 7 DAG photosynthetic saturation was at approximately 400 μmol mol^{-1} C_i. Whilst the initial slope of the A/C_i response curves was not affected by the [CO_2] under which the plants were grown at 7 DAG, the CO_2-saturated photosynthetic rates in both Stirling and Schooner were significantly higher under CO_2 enrichment (Fig. 3.2; $P \leq 0.05$). The A/C_i response curves of the two

cultivars at the end of the experimental period were also similar in both cultivars. In both Stirling and Schooner, the response of assimilation as a function of C_i showed a significant decrease ($P < 0.05$) in both the initial slope of the A/C_i curve and the CO_2-saturated photosynthetic rates (Fig 3.3). The response reported here are very common for a wide range of C_3 plants (Sage, 1994; and references cited therein).

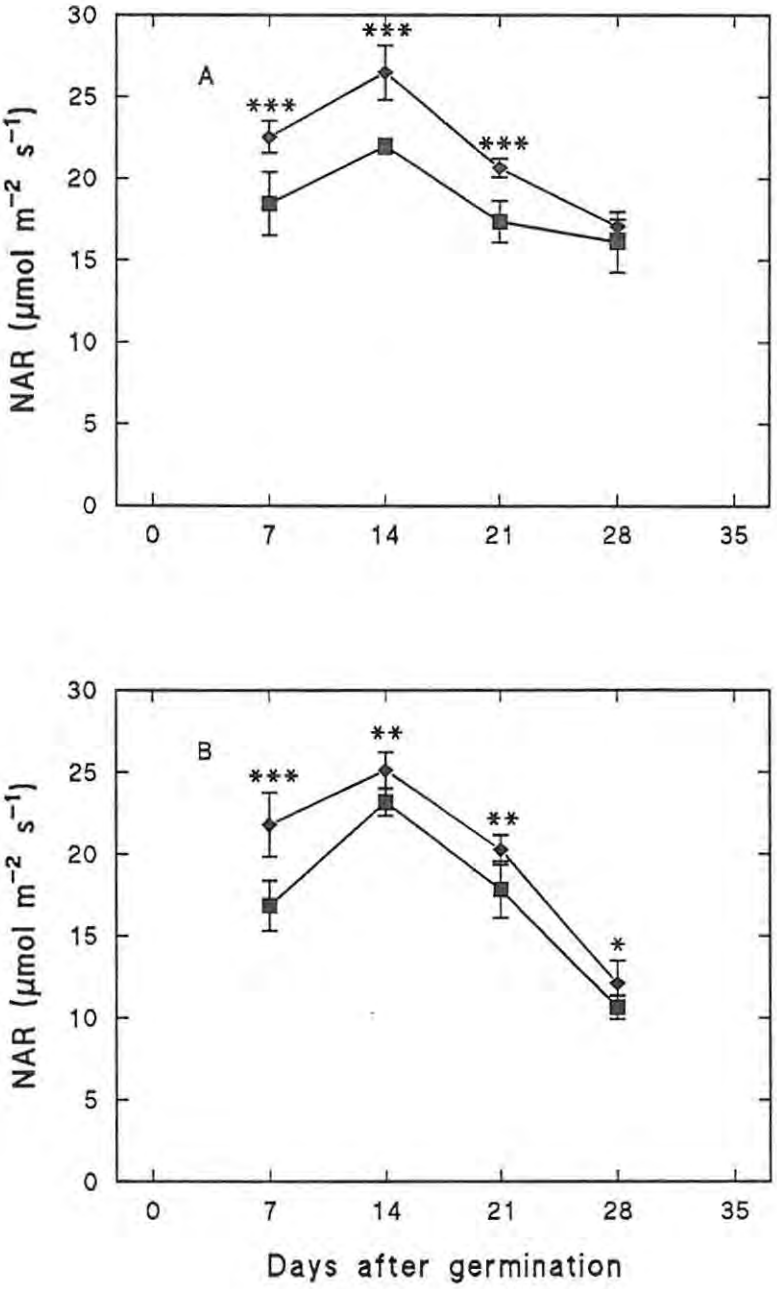

Fig. 3. 1. NAR responses of barley plants grown and measured at 350 (■) and 600 (♦) µmol mol^{-1} [CO_2] and light intensity of 500 µmol m^{-2} s^{-1}. A, Stirling; B, Schooner. n = 6 and error bars denote ± SE. *, **, and *** indicate significant difference at $P \leq 0.05$, $P \leq 0.01$ and $P \leq 0.001$, respectively.

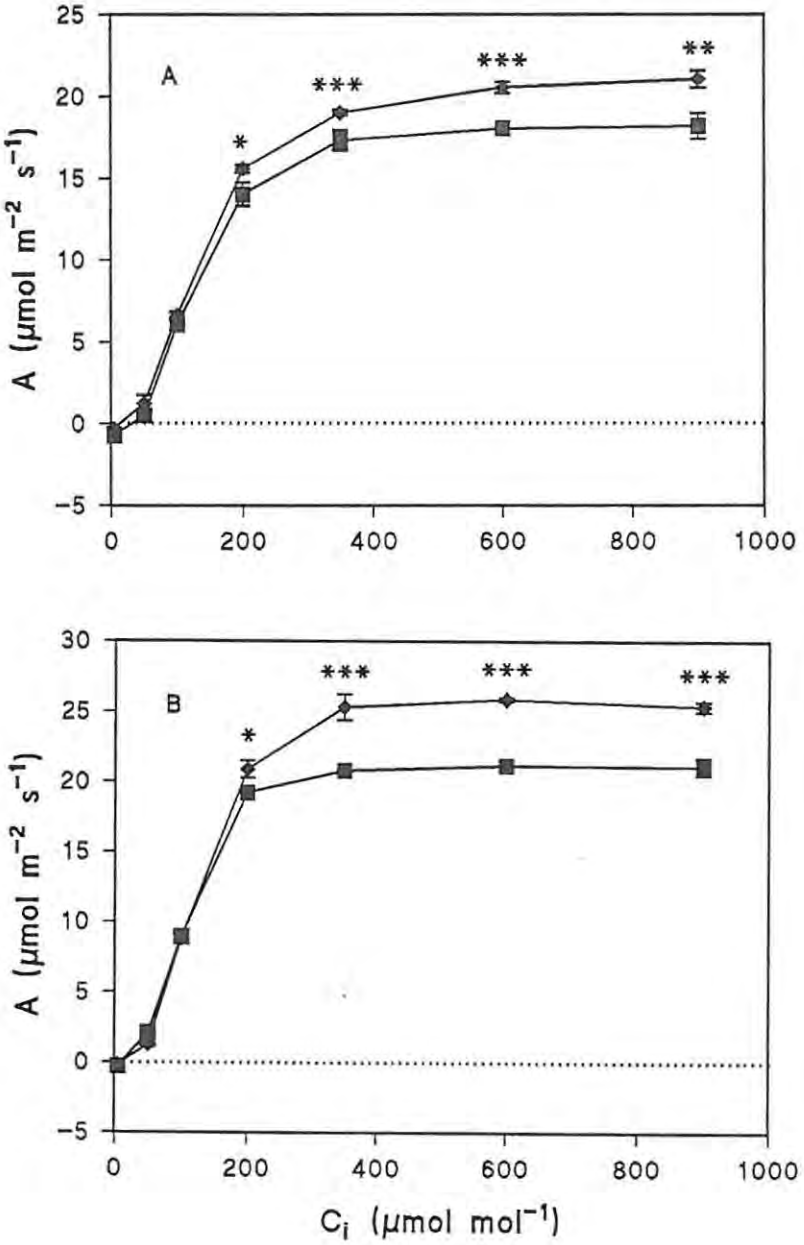

Fig. 3. 2. Responses of assimilation as a function of C_i for barley plants grown at 350 (■) and 600 (♦) µmol mol^{-1} [CO_2]. A, Stirling; B, Schooner. Measurements were done at 25 °C and a light intensity of 500 µmol m^{-2} s^{-1}. Plants were one week old. Error bars denote ± SE, n = 6. *, ($P \leq 0.05$); **, ($P \leq 0.01$); ***, ($P \leq 0.001$).

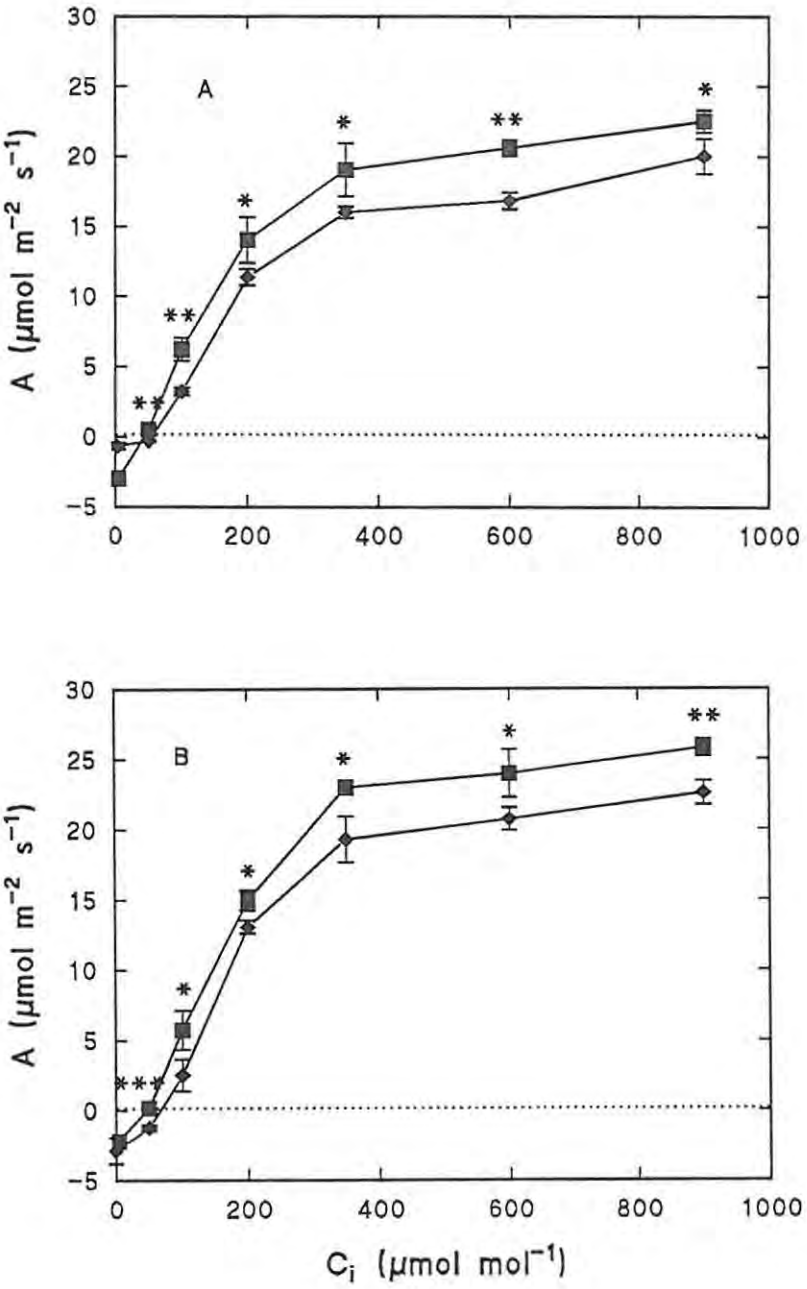

Fig. 3.3. Responses of assimilation as a function of C_i for barley plants grown at 350 (■) and 600 (◆) μmol mol^{-1} [CO_2]. A, Stirling; B, Schooner. Measurements were done at 25 °C and a light intensity of 500 μmol m^{-2} s^{-1}. Plants were four weeks old. Error bars denote ± SE, n = 6. *, ($P \leq 0.05$); **, ($P \leq 0.01$); ***, ($P \leq 0.001$).

3.2.3. Photosynthetic pigments

Elevated [CO_2] did not cause any significant changes in the content of chlorophyll *a* in Stirling (Fig. 3.4. B). Chlorophyll *a* content in Stirling increased continuously under both CO_2 treatments over the experimental period. Schooner on the other hand exhibited a different trend. Chlorophyll *a* content increased with time in Schooner, reaching its maximum of almost 3 μg ml plant extract^{-1} at 21 DAG, and decreased thereafter (Fig 3.4. B). Elevated CO_2 resulted in a significant increase ($P = 0.01$) in chlorophyll *a* content in schooner at 7 DAG, but it is however interesting to note that at 28 DAG CO_2 enrichment resulted in a significant decrease ($P < 0.001$) in chlorophyll *a* content, a result which had been widely reported for various other plant species (Madsen, 1979; Cave *et al.*, 1981; Wulff and Strain, 1981; see Fig. 3.4. B). Chlorophyll *b* content in both cultivars were not significantly affected ($P > 0.05$) by CO_2 enrichment (Fig. 3.5). When comparing chlorophyll *a* and *b*, it is noteworthy that chlorophyll *b* content was always lower than chlorophyll *a* content in both cultivars under both ambient and elevated [CO_2], with the a chlorophyll *a* maximum of approximately 3 μg ml plant extract^{-1}, whereas the maximum of chlorophyll *b* extracted was no greater than 1 μg ml plant extract^{-1}. The total chlorophyll (*a*+*b*) content was not significantly affected ($P > 0.05$) in the two cultivars by CO_2 enrichment (Fig. 3.6). Stirling showed a continuous increase in total chlorophyll content throughout the experiment (Fig. 3.6. A). In contrast, Schooner showed a sharp

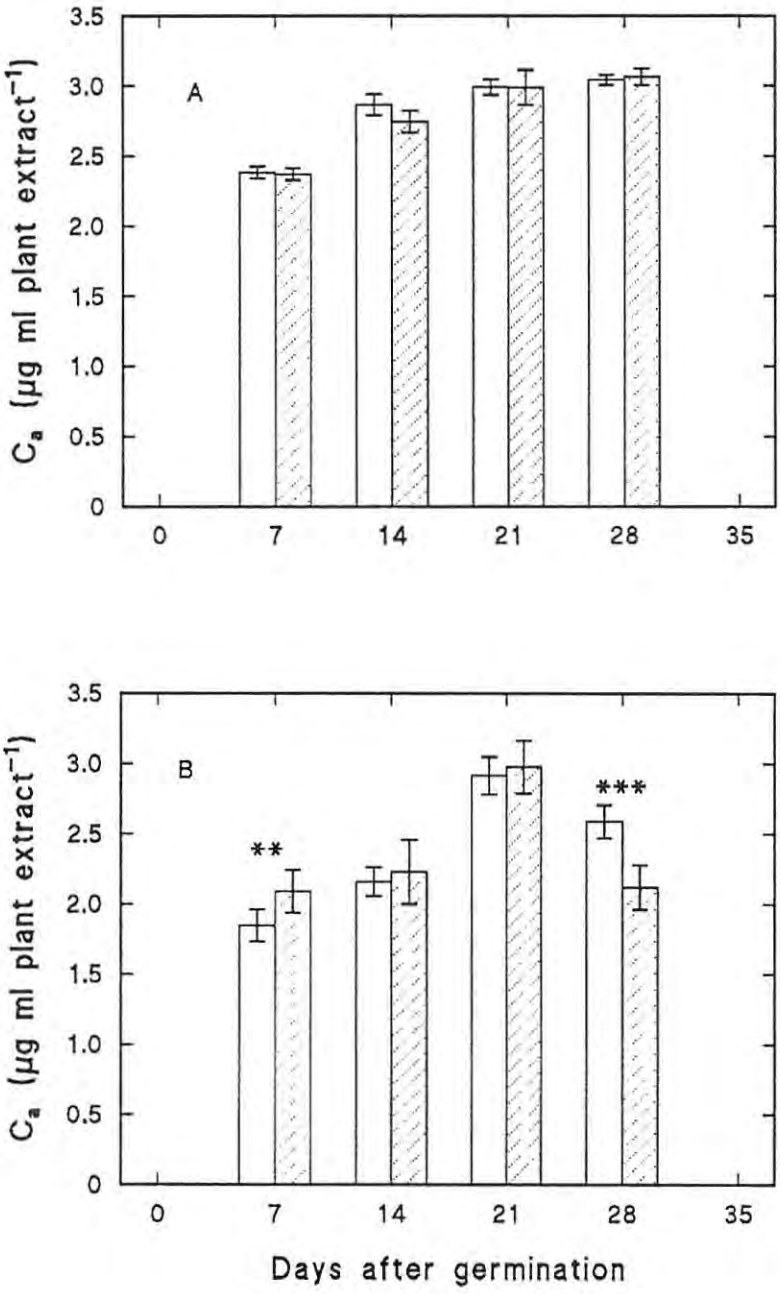

Fig. 3. 4. A time course comparison of chlorophyll *a* content in barley plants grown at a [CO_2] of 350 (open bars) and 600 (hatched bars) µmol mol^{-1}. A, Stirling; B, Schooner. n = 6, error bars denote ± SE. ** and *** indicate significant difference at $P \leq 0.01$ and $P \leq 0.001$, respectively.

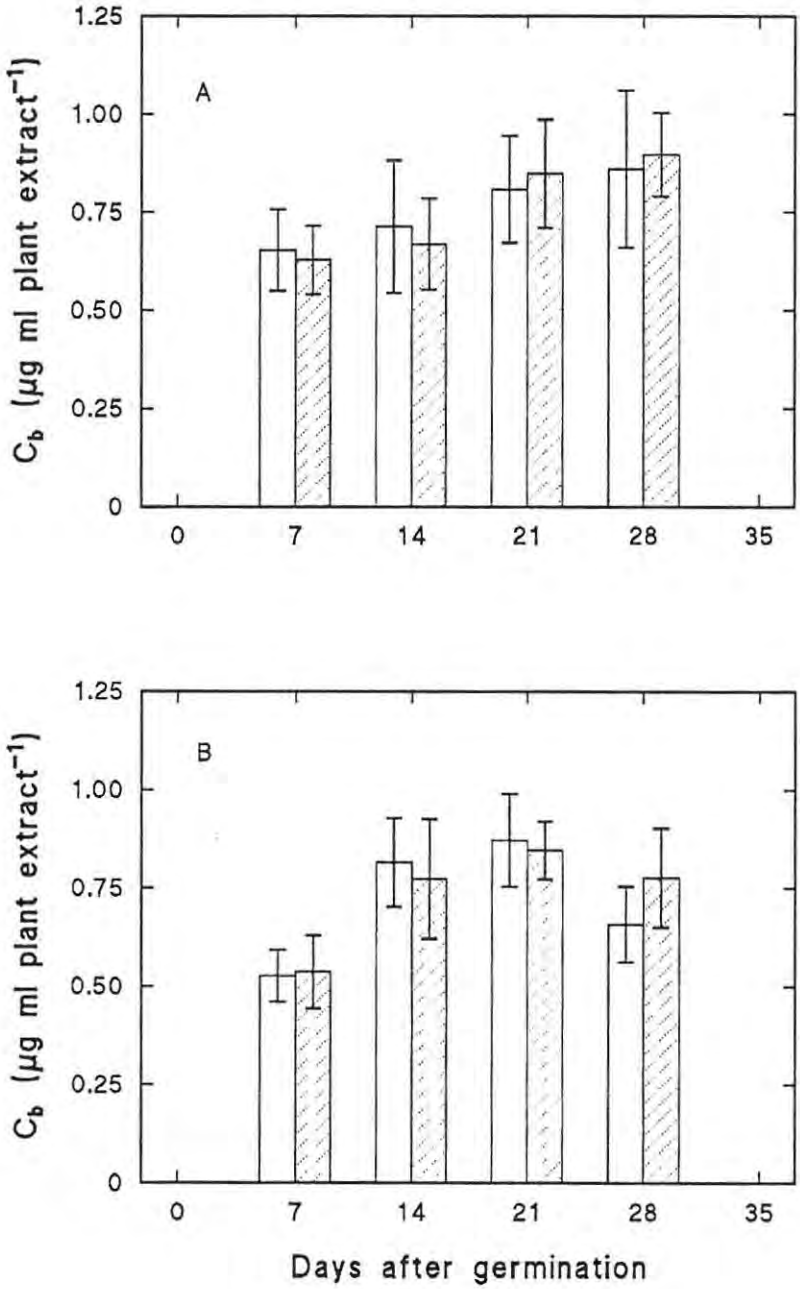

Fig. 3.5. A time course comparison of chlorophyll *b* content in barley plants grown at a [CO_2] of 350 (open bars) and 600 (hatched bars) µmol mol^{-1}. A, Stirling; B, Schooner. n = 6, error bars denote ± SE.

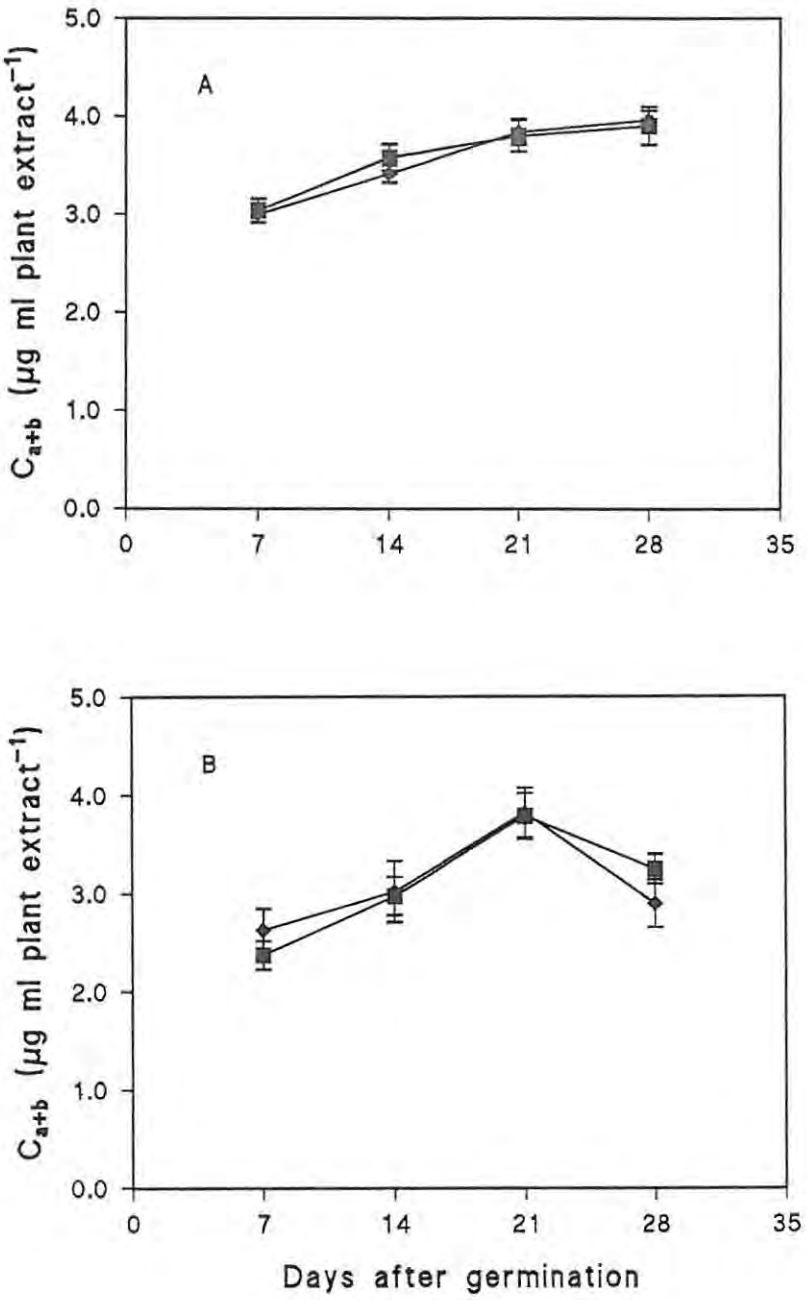

Fig. 3.6. A time course comparison of total chlorophyll content (C_{a+b}) in barley plants grown at a [CO_2] of 350 (■) and 600 (♦) µmol mol^{-1}. A, Stirling; B, Schooner. n = 6, error bars denote ± SE.

increase in the total chlorophyll content up to 21 DAG after which it decreased (Fig. 3.6.B). Fig. 3.7 shows the effects of CO_2 enrichment on chlorophyll *a/b* ratio in Stirling (A) and Schooner (B). CO_2 enrichment did not affect chlorophyll *a/b* ratios in the two cultivars. This result is consistent with the findings by Nie *et al.* (1995) for wheat. The differences in chlorophyll *a/b* ratios between days were also not significant at the 5 % level of significance ($P > 0.05$) for both Stirling and Schooner. Total carotenoid content remained constant at about 0.5 μg ml plant extract^{-1} under ambient and elevated carbon dioxide concentration throughout the experimental period in both the cultivars (Fig. 3.8).

3.2.4. Stomatal conductance and stomatal density

Fig. 3.9 shows the responses of stomatal conductance (g_s) to CO_2 enrichment in Stirling (A) and Schooner (B). In both cultivars plants grown under ambient [CO_2] showed an increase in g_s over time up to 14 DAG, after which there was a linear decline in g_s. On the contrary, plants grown in CO_2-enriched environments showed a linear decrease in g_s with time throughout the experimental period. CO_2 enrichment did not affect g_s at the beginning of the experiment in the two cultivars. However, from 14 DAG until the end of the experimental period, CO_2 enrichment resulted in a significant decrease ($P < 0.05$) in g_s in the two cultivars. Similar response had been previously demonstrated in a number of species (Jarvis, 1989; Nederhoff, 1992; Berryman *et al.*, 1994; Guehl *et al.*, 1994; Xu *et al.*, 1994b).

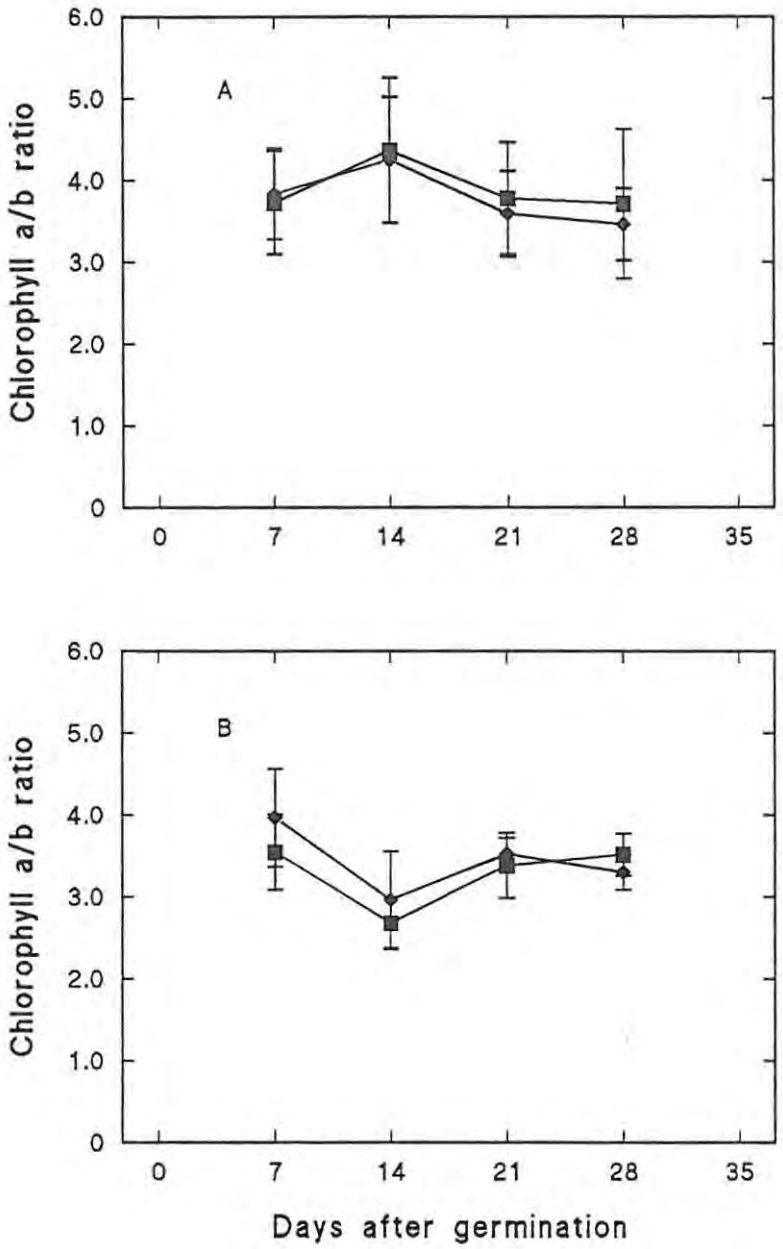

Fig 3.7. Chlorophyll *a/b* ratio of barley plants grown at a carbon dioxide concentration of 350 (■) and 600 (♦) μmol mol^{-1}. A, Stirling; B, Schooner. n = 6, error bars denote ± SE.

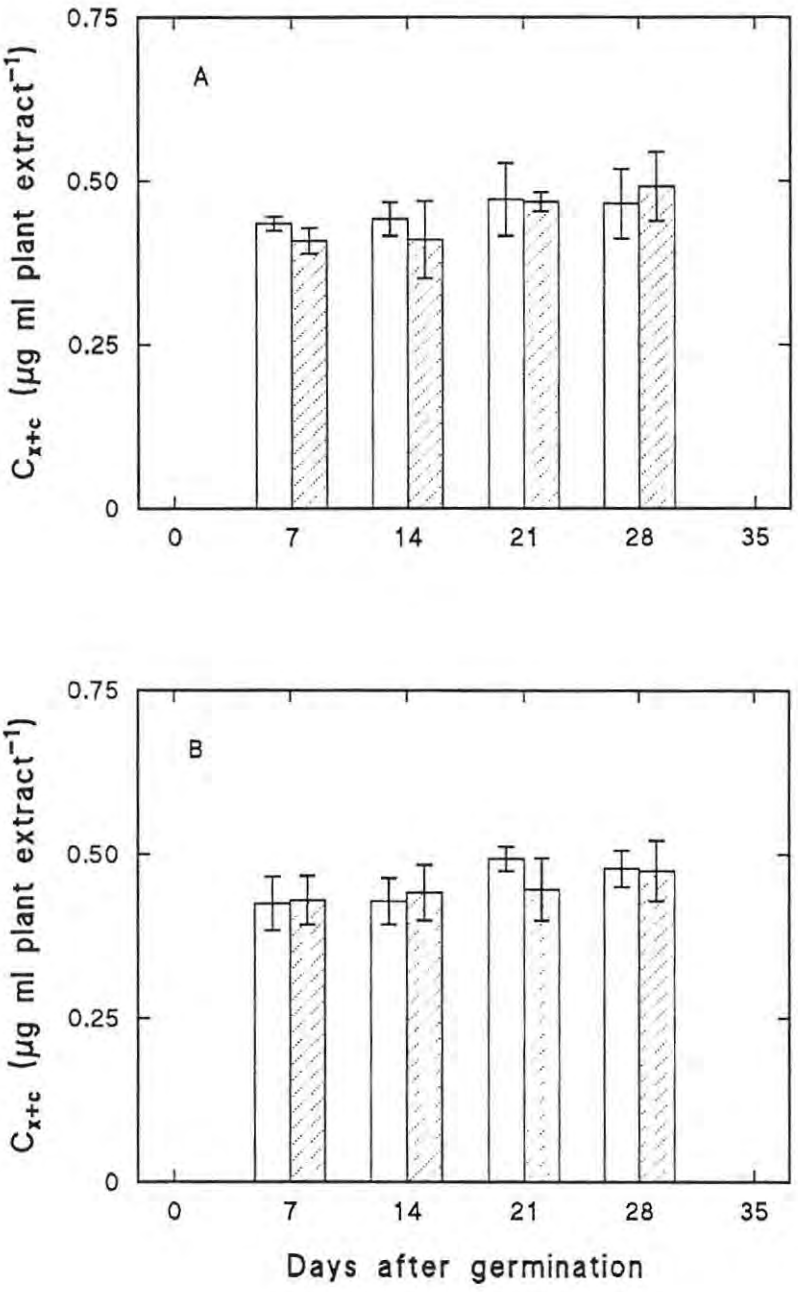

Fig 3.8. Total carotenoids content of barley plants grown at 350 (open bars) and 600 (hatched bars) µmol mol^{-1} [CO_2]. A, Stirling; B, Schooner. Error bars denote ± SE, n = 6.

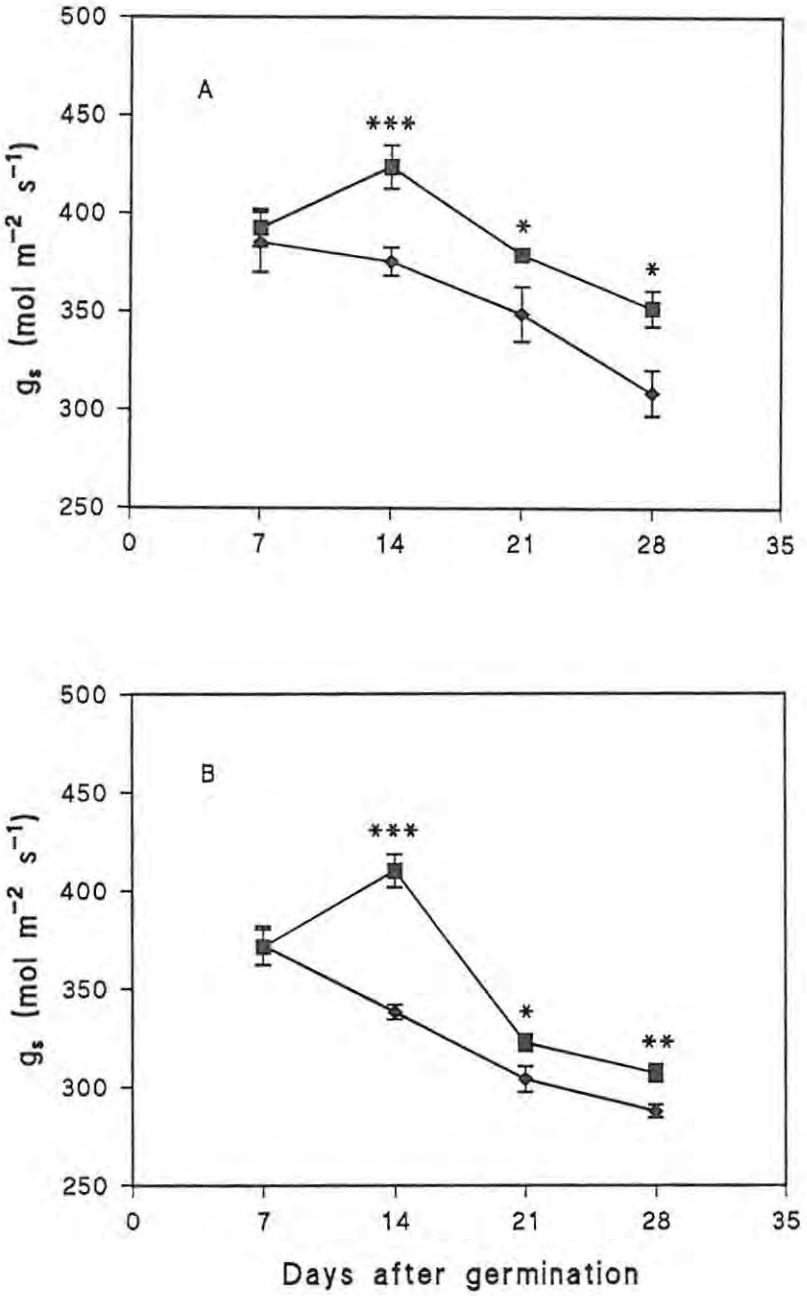

Fig. 3.9. Stomatal conductance of barley plants grown at a [CO_2] of 350 (■) and 600 (♦) μmol mol^{-1}. A, Stirling; B, Schooner. n = 6, error bars denote ± SE. *, ** and *** indicate significant difference at $P \leq 0.05$, $P \leq 0.01$ and $P \leq 0.001$, respectively.

Responses of stomatal density to CO_2 enrichment at the two growth stages (7 DAG and 28 DAG) of barley are presented in Table 3.1. At both 7 DAG and 28 DAG adaxial stomatal density was not significantly affected ($P > 0.05$) by CO_2 enrichment, the result which is in agreement with the findings reported by O'Leary and Knecht (1981) for beans and Xu *et al.* (1994b) for soybean. Stirling showed an increase in abaxial stomatal density at both the growth stages under elevated CO_2 conditions, and consequently weighted stomatal density was significantly increased as well at the two growth stages. However, Schooner showed a different response. At the beginning of the experiment (7 DAG) exposure to elevated [CO_2] lead to a significant increase in the abaxial stomatal density ($P = 0.031$), but weighted stomatal density was not significantly affected ($P > 0.05$). Schooner showed significant effect of CO_2 enrichment on abaxial stomatal density at 28 DAG, but a significant increase in weighted stomatal density ($P = 0.035$).

3.2.5. Transpiration rate and water use efficiency

Transpiration rate (E) increased from 7 DAG until 14 DAG after which it started to decline in Stirling under both ambient and elevated [CO_2] (Fig. 3.10. A). A similar trend was observed for Schooner exposed to ambient [CO_2], but E for Schooner exposed to elevated [CO_2] decreased linearly with time throughout the experiment (Fig. 3.10. B). CO_2 enrichment caused a significant decrease ($P < 0.05$) in E for both Stirling and Schooner . The transpiration rate for Schooner showed great sensitivity to elevated CO_2 exposure, and decreased by values above 20 % whereas E in Schooner only decreased by values not greater than 10 % in response to CO_2 enrichment. As would be expected for a C_3 plant, CO_2 enrichment increased water use efficiency (WUE) in both Stirling and Schooner (Fig. 3.11; $P < 0.05$). This result is consistent

with a number of reports on a wide range of species exposed to elevated CO_2 (Cure and Acocks, 1986; Bazzaz, 1990; Grodzinski, 1992; Lawlor, 1993). For both cultivars under both ambient and elevated [CO_2] there was a gentle increase in WUE over time.

Table 3.1 Responses of stomatal density to CO_2 enrichment. Results are means of four replicates. (^{ns}, not significant, $P > 0.05$; *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$).

	Stirling		Schooner	
	Ambient $[CO_2]$	Elevated $[CO_2]$	Ambient $[CO_2]$	Elevated $[CO_2]$
Adaxial stomatal density (number mm^{-2}) at 7 DAG	42 ± 4	47 ± 3 ^{ns}	57 ± 5	55 ± 4 ^{ns}
Abaxial stomatal density (number mm^{-2}) at 7 DAG	33 ± 4	45 ± 6 **	43 ± 2	52 ± 5 *
Weighted stomatal density (number mm^{-2}) at 7 DAG	39 ± 5	46 ± 4 *	51 ± 5	54 ± 3 ^{ns}
Adaxial stomatal density (number mm^{-2}) at 28 DAG	39 ± 4	52 ± 4 ^{ns}	45 ± 3	54 ± 3 ^{ns}
Abaxial stomatal density (number mm^{-2}) at 28 DAG	36 ± 3	53 ± 6 ***	38 ± 3	45 ± 5 ^{ns}
Weighted stomatal density (number mm^{-2}) at 28 DAG	38 ± 4	53 ± 5 ***	43 ± 4	50 ± 4 *

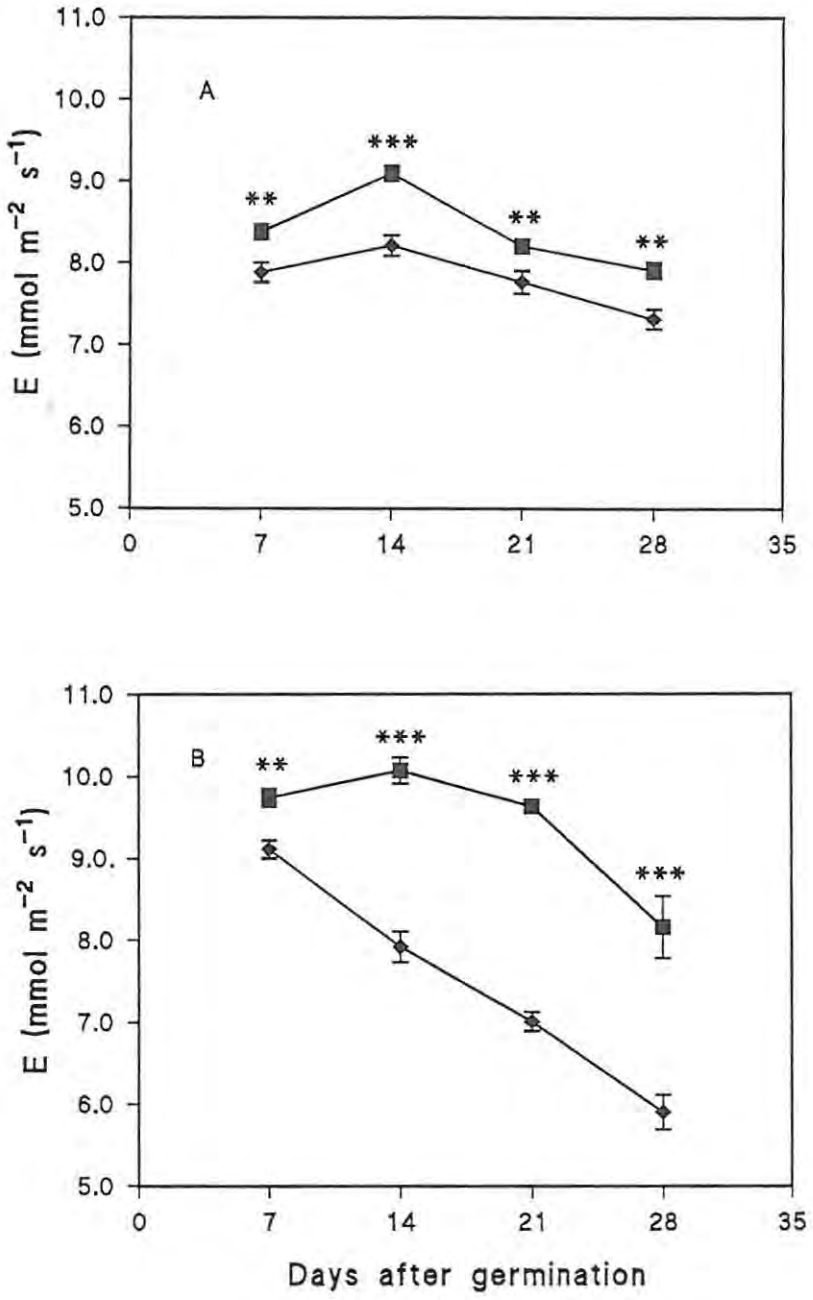

Fig. 3.10. Transpiration rate responses of plants grown at a [CO_2] of 350 (■) and 600 (♦) μmol mol^{-1}. A, Stirling; B, Schooner. Error bars denote ± SE, n = 6. **, ($P \leq 0.01$); ***, ($P \leq 0.001$)

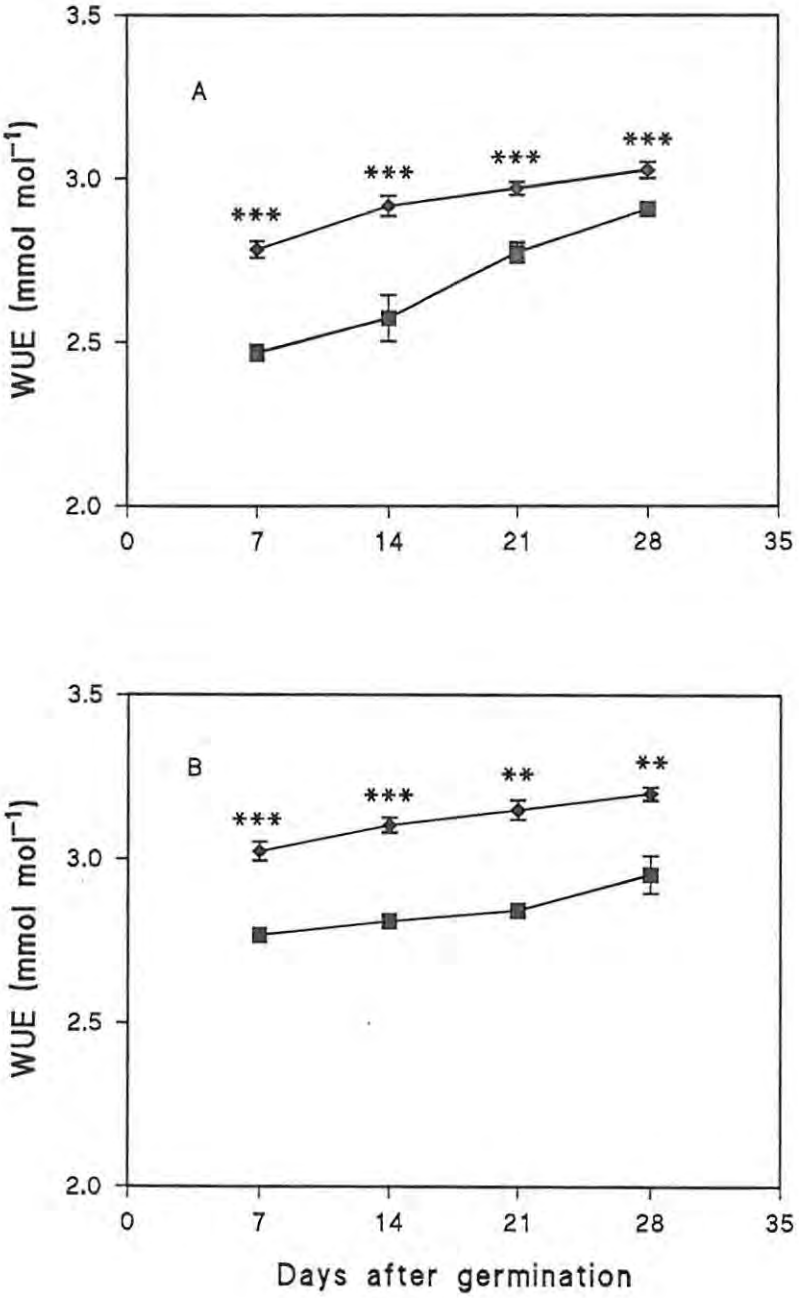

Fig. 3.11. Responses of water use efficiency of barley plants grown at a [CO_2] of 350 (■) and 600 (♦) μmol mol^{-1}. A, Stirling; B, Schooner. Error bars denote ± SE, n = 6. **, ($P \leq 0.01$); ***, ($P \leq 0.001$).

CHAPTER 4: LEAF ANATOMY OF BARLEY GROWN UNDER CONTINUOUS AMBIENT AND ELEVATED [CO_2]

4.1. Introduction

The anatomy of leaves of plants grown under sustained elevated CO_2 concentration is still under-researched. Since the structure and function of all living organisms are directly related, it is of immediate concern that the changes in leaf anatomy should be monitored in order to gain a better understanding of the changes in the major physiological and biochemical functions in plants exposed to enriched CO_2 environments.

Growth of plants under enriched CO_2 conditions was seen to increase the total leaf thickness in plants (Thomas and Harvey, 1983; Vu *et al.*, 1989). Such increases in leaf thickness were suggested to be due to an increased mesophyll thickness. Vu *et al.* (1989) reported that elevated CO_2 concentration resulted in an increase in the number of cells in the mesophyll and less intercellular spaces, and this may be the direct cause of increase in leaf thickness. Thomas and Harvey (1983) also observed that the effects of CO_2 enrichment on mesophyll thickness was variable and species-dependent. Soybean and sweet gum showed an appreciable increase in mesophyll thickness, whereas corn and pine species showed no significant change in mesophyll thickness under elevated CO_2 concentration. In all four species observed by Thomas and Harvey

(1983), CO_2 enrichment did not cause any alteration in the thickness of the epidermal layer.

CO_2 enrichment affects plant chloroplasts differently in different species. Kutík *et al.* (1995) and Thomas and Harvey (1983) reported that CO_2 enrichment lead to an increase in the number of chloroplasts in the mesophyll cells, but no effect on the chloroplast numbers as a consequence of CO_2 enrichment was observed by Robertson and Leech (1995). Chloroplast cross-sectional area was not affected by CO_2 enrichment (Kutík *et al.*, 1995). One well-documented aspect on the effects of CO_2 enrichment on chloroplasts ultrastructure, is that increased [CO_2] results in an increase in the numbers and size of starch grains inside the chloroplast (Cave *et al.*, 1981; Wulff and Strain, 1981; Thomas and Harvey, 1983; Ehret and Jolliffe, 1985; Vu *et al.*, 1989; Kutík *et al.*, 1995). Robertson and Leech (1995) on the contrary, observed a marked reduction in starch accumulation as a result of growth in elevated CO_2 in young wheat leaves. Increased starch grains had been reported to result in the concomitant reduction of total thylakoids (Yelle *et al.*, 1989; Kutík *et al.*, 1995). On the other hand, Vu *et al.* (1989) reported that dispite the increase in starch content, elevated CO_2 did not result in any other alteration of chloroplast ultrastructure in soybean. This result confirms that the effects of CO_2 enrichment on leaf anatomy is also species-dependent. Foliar deformation, including chlorosis, necrosis and leaf rolling had been observed in plants grown under elevated CO_2 concentrations and had

been correlated with higher leaf starch levels (Cave *et al.*, 1981; Ehret and Jolliffe, 1985 and references cited therein; Tripp *et al.*, 1991).

Given the variation in results reported here, it was felt that an examination of the leaf morphology and anatomy of these varieties was necessary. This chapter compares the leaf anatomy and chloroplast ultrastructure in the two cultivars of barley grown under ambient and elevated carbon dioxide concentrations.

4.2. Results

4.2.1. External observation

Visual inspection revealed foliar injury to plants grown under CO_2 enrichment. Injury symptoms were first observed as regions of interveinal chlorosis and at the leaf tips, after which it progressed to complete chlorosis in elevated CO_2-grown plants. Chlorosis appeared first in the lower leaves and progressed to the upper leaves over the growing season until the entire plant appeared stressed. The affected leaves then became necrotic. Similar results were observed by Ehret and Jolliffe (1985) for bean plants and also by Tripp *et al.* (1991) for tomato plants. It should be noted that Schooner exposed to elevated [CO_2] showed earlier sings of leaf injury by about four days as compared to Stirling.

4.2.2. Leaf thickness

Figs. 4.1 and 4.2 show cross-sections of mature leaves of Stirling and Schooner respectively, grown under ambient and elevated [CO_2]. The most visible result from Fig. 4.1 is the enormous reduction of intercellular spaces in elevated CO_2-grown plants. This may be attributed to an increase in the number of mesophyll cells resulting in the cells so closely packed within the tissue. CO_2 enrichment resulted in a significant increase in total leaf thickness in Stirling (Table 4.1, $P = 0.01$). The increase in the total leaf thickness in Stirling as a consequence of CO_2 enrichment was 21 % from 230 ± 29 to 290 ± 26 μm. Increased total leaf thickness when plants were exposed to elevated [CO_2] have also been observed by Thomas and Harvey (1983) for corn, pine, soybean and sweet gum species. Similarly the mesophyll tissue of plant exposed to CO_2-enriched air increased by 17 %. The epidermal thickness was also increased CO_2 enrichment. Adaxial epidermis thickness increased by 33 % whereas there was a 29 % increase in the thickness of the abaxial epidermis in Stirling grown under elevated [CO_2].

Schooner showed a different response to CO_2 enrichment as compared to Stirling in relation to leaf thickness. Although the increase in total leaf thickness in Schooner grown under increased CO_2 conditions of 9 % was observed, the change was not statistically significant at a 5 % significance level (Table 4.1, $P > 0.05$). Similarly, there was no significant change in the mesophyll thickness under CO_2 enrichment, the increase was only 2 %. However, the thickness of adaxial epidermis significantly

increased by 38 % ($P \leq 0.01$) as a result of elevated CO_2 exposure. Although the thickness of abaxial epidermis increased by 12 % under CO_2-enriched air, such increases were not significant.

When comparing Stirling and Schooner, Schooner developed slightly thinner leaves with an average total leaf thickness of 224 ± 27 μm compared to 230 ± 29 μm in Stirling, under ambient [CO_2]. Plants grown under CO_2 enrichment conditions also showed similar results, with the leaf thickness of Schooner being 15 % less than that of Stirling which is reflected by the thinner mesophyll in Schooner under the same CO_2 treatment. There was no appreciable difference between the thickness of adaxial epidermis of the two cultivars. However, it is worth noting that Schooner showed slightly thicker abaxial epidermis than Stirling.

Table 4.1. Effect of CO_2 enrichment on total leaf thickness and on epidermal and mesophyll layers on two cultivars of barley. n = 9. ^{ns}, not significant ($P > 0.05$); * and **, represent significance at $P \leq 0.05$ and $P \leq 0.01$ respectively.

Cultivar	$[CO_2]$ ($\mu mol\ mol^{-1}$)	Thickness (μm)			
		Total	Mesophyll	Epidermis	
				Adaxial	Abaxial
Stirling	350	230 ± 29	174 ± 16	27 ± 8	29 ± 9
	600	290 ± 26**	209 ± 25*	40 ± 9*	41 ± 12*
Schooner	350	224 ± 27	162 ± 19	24 ± 4	38 ± 12
	600	247 ± 28^{ns}	165 ± 24^{ns}	39 ± 12**	43 ± 9^{ns}

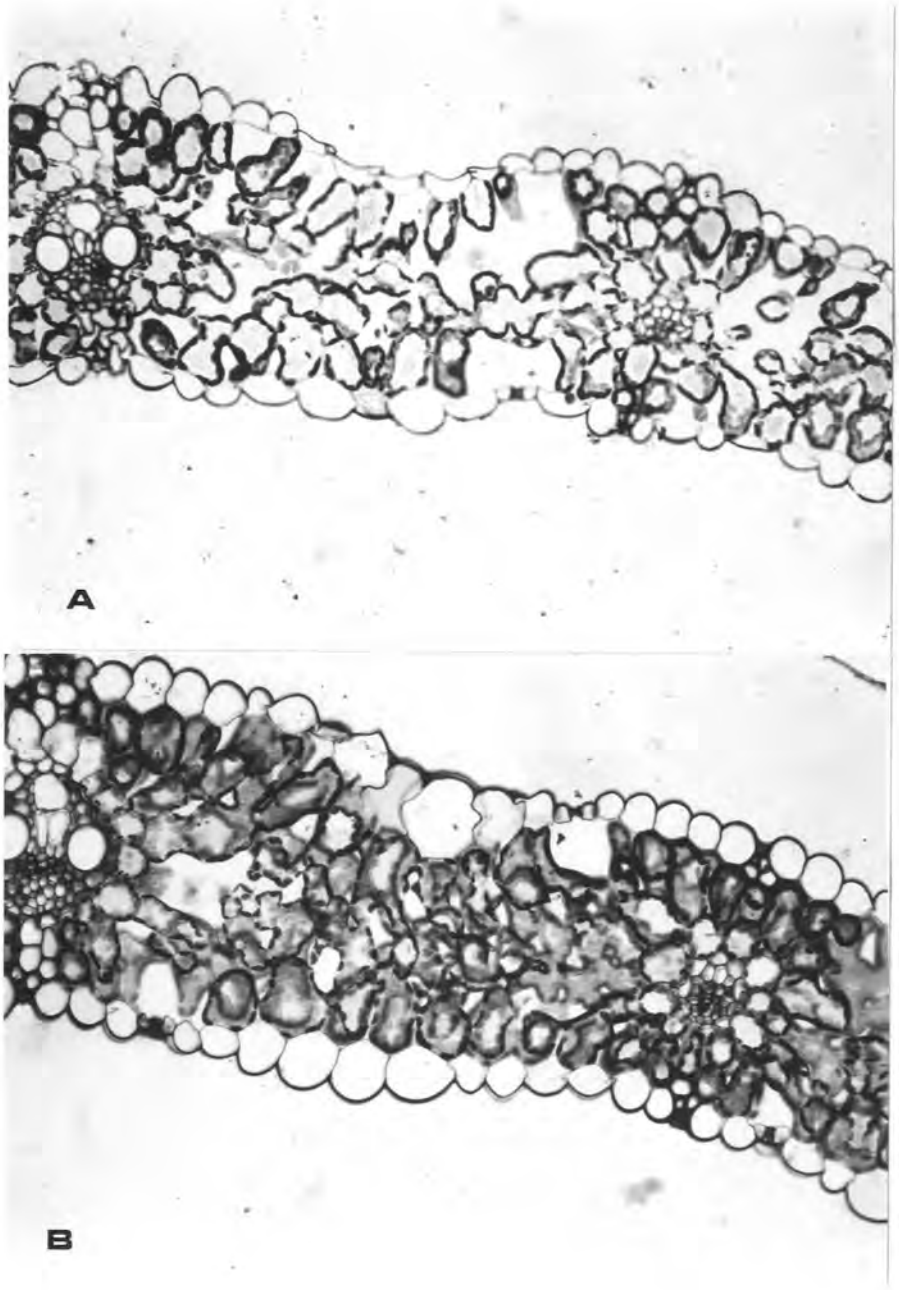

Fig. 4.1. Cross-sections of mature first fully developed leaves of barley cv. Stirling grown under ambient (A) or elevated (B) [CO_2]. Plants were 14 DAG old.

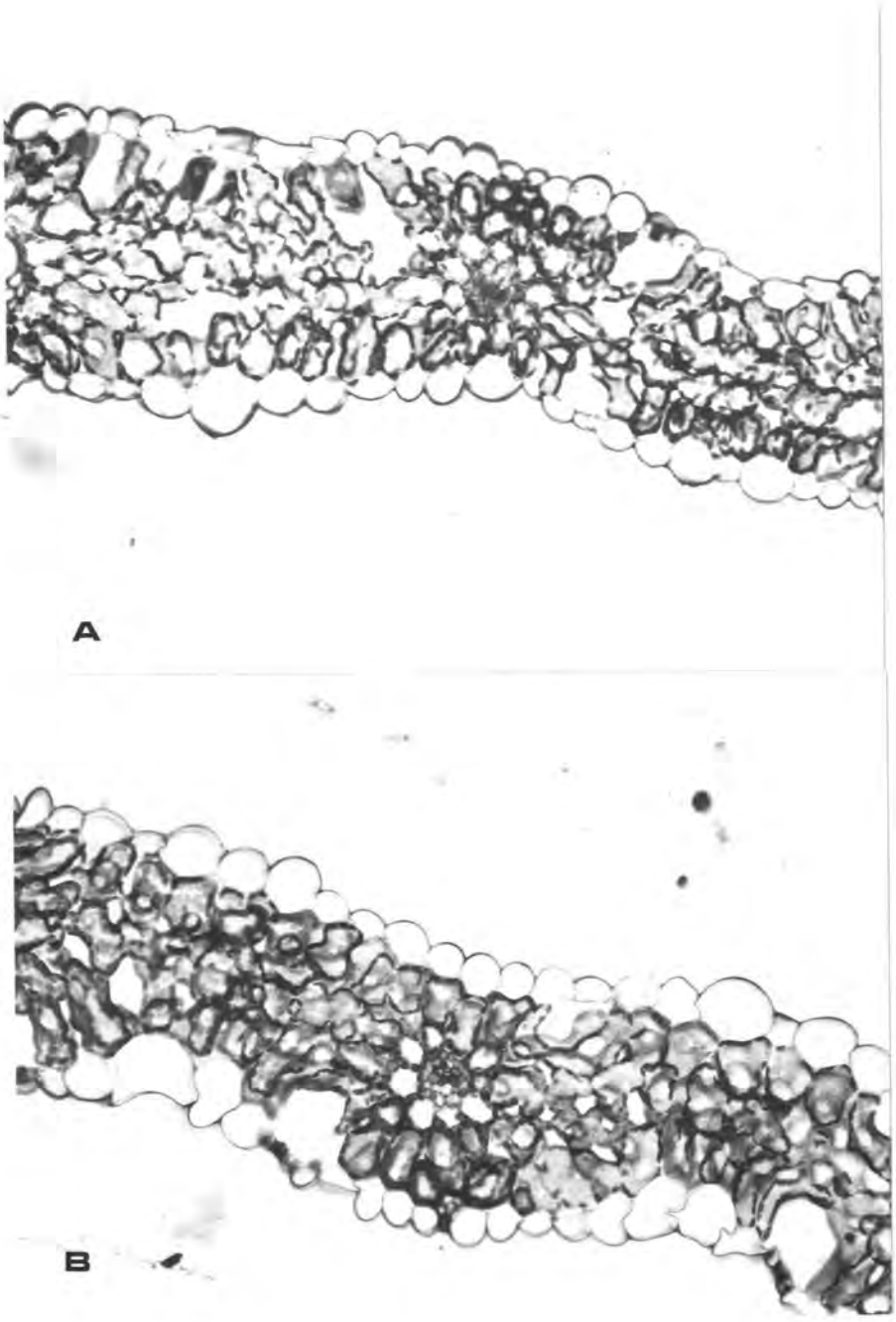

Fig. 4.2. Cross-sections of mature first fully developed leaves of barley cv. Schooner grown under ambient (A) or elevated (B) [CO_2]. Plants were 14 DAG old.

4.2.3. Chloroplast morphology

The results on the effects of CO_2 enrichment to chloroplast sizes are presented in Table 4.2. Elevated CO_2 did not elicit significant changes in chloroplast sizes in both Stirling and Schooner. There was a large increase of about 29 % in chloroplast length in Stirling (also see Fig. 4.3), but statistical analysis reveal that such changes were not significant at the 5 % level of significance ($P = 0.31$). Chloroplast breadth increased by about 29 % but the increase was not statistically significant ($P = 0.29$) in Stirling. The increase in chloroplast length due to CO_2 enrichment was relatively smaller in Schooner, only about 8 %, from 4.7 ± 0.4 μm under ambient [CO_2] to 5.1 ± 0.6 μm under CO_2 enrichment (also see Fig. 4.4). The increase in chloroplast breadth in Schooner was increased by about 24 % when plant were grown in CO_2-enriched air. This is similar to the observation of Kutík *et al.* (1995) in sugar beet.

Table 4.2. Chloroplast length and breadth for two cultivars of barley grown under ambient and elevated [CO_2]. n = 9. ^{ns}, not significant ($P > 0.05$).

Cultivar	[CO_2] (μmol mol^{-1})	Chloroplast length (μm)	Chloroplast breadth (μm)
Stirling	350	4.4 ± 0.6	1.9 ± 0.3
	600	6.2 ± 0.9^{ns}	2.4 ± 0.3^{ns}
Schooner	350	4.7 ± 0.4	1.9 ± 0.3
	600	5.1 ± 0.6^{ns}	2.5 ± 0.3^{ns}

4.2.3. Chloroplast ultrastructure

Electron micrographs of mesophyll cells chloroplasts of mature first fully developed leaves of plants grown under ambient and elevated CO_2 concentrations are shown in Figs. 4.3 and 4.4. In most of the cells, chloroplasts had no starch (Figs. 4.3 and 4.4). Robertson and Leech (1995) observed a similar result, in *Triticum aestivum* cv Hereward. Stirling grown under elevated CO_2 showed some starch grains (Fig. 4.3). The accumulation of starch as a consequence of CO_2 enrichment have been previously reported by many workers (Cave *et al.*, 1981; Wulff and Strain, 1982; Ehret and Jolliffe, 1985; Kutík *et al.*, 1995). Despite the appearance of starch grains in Stirling grown under CO_2-enriched air, no other alterations in the chloroplast ultrastructure

was evident (Fig. 4.3). This result is in harmony with the findings by Vu *et al.* (1989), that CO_2 enrichment only resulted in the accumulation of starch in soybean, but did not cause any other ultrastructural changes. Schooner did not show any appreciable changes in the chloroplast with CO_2 enrichment (Fig. 4.4).

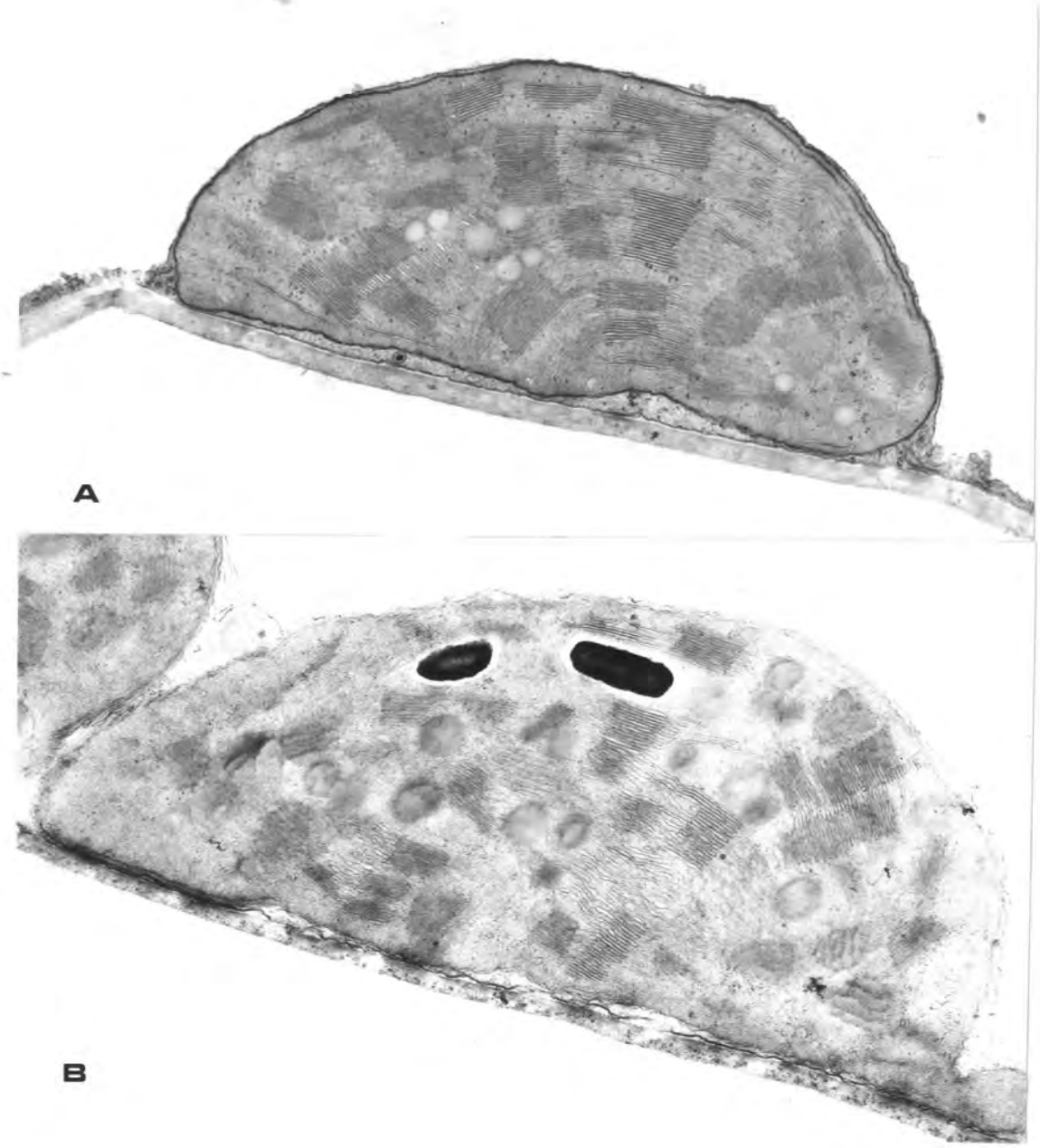

Fig. 4.3. Electron micrographs of mesophyll cell chloroplasts of barley cv. Stirling for plants grown under ambient (A) or elevated (B) [CO_2].

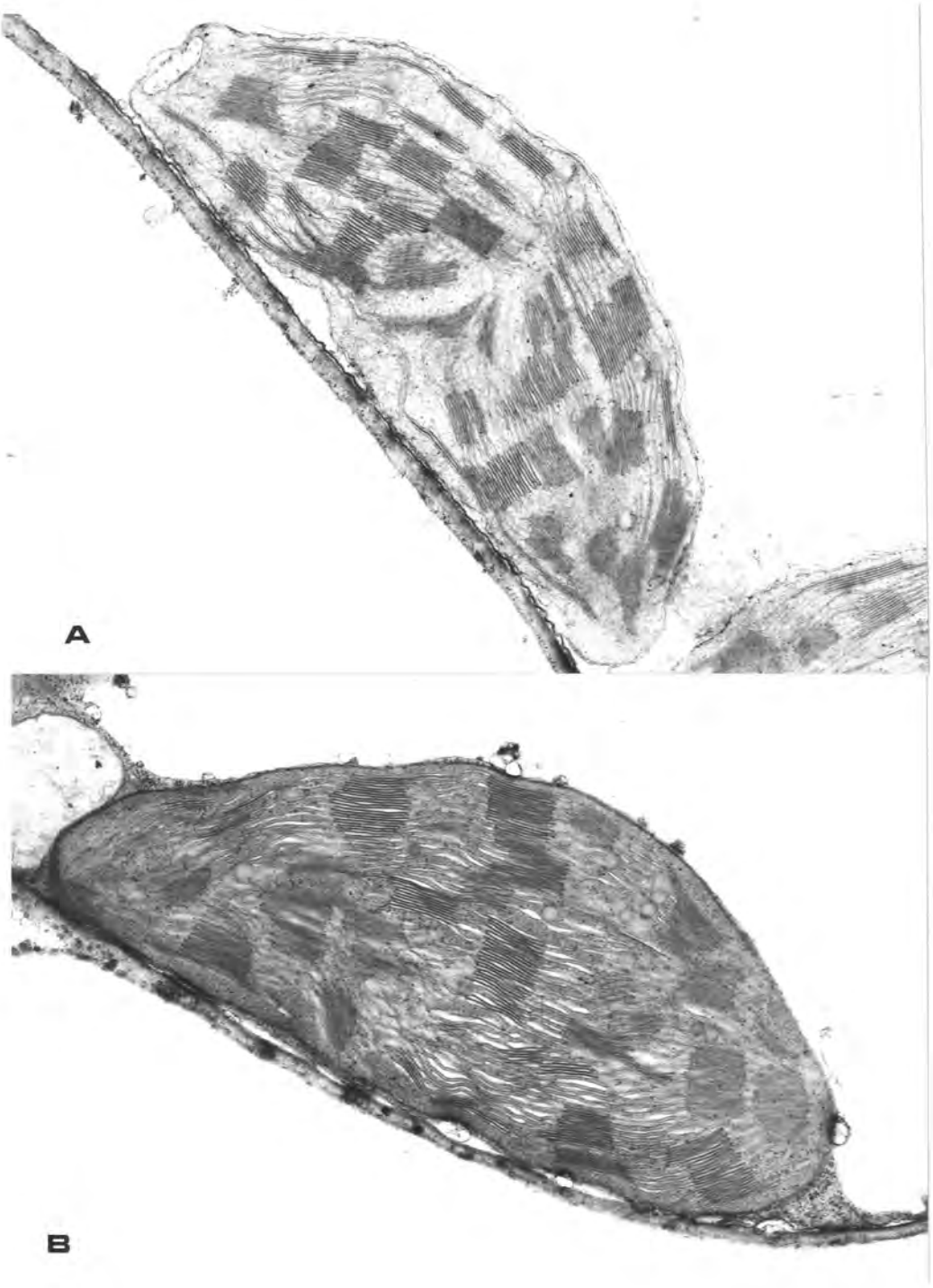

Fig.4.4. Electron micrographs of mesophyll cell chloroplasts for barley cv. Schooner grown under ambient (A) or elevated (B) [CO_2]. Note the absence of starch grains under elevated CO_2

CHAPTER 5: RESPONSES OF GROWTH AND GRAIN YIELD TO CO_2 ENRICHMENT

5.1. Introduction

While many workers have attempted to assess the effects of increasing [CO_2] on plant growth and yield, there is still no consensus as to the quantitative effects of increased CO_2 concentration on both growth and yield. In many plant species, elevated CO_2 concentration have been found to stimulate leaf growth and total above-ground biomass (Wulff and Strain, 1981; Poorter, 1993; Retuerto and Woodward, 1993; Ingvardsen and Veierskov, 1994). The increased growth on a wide variety of plants maintained under CO_2 enrichment may be in part attributed to higher rates of leaf photosynthesis brought about by the increased availability of CO_2 at the site of carboxylation (Madore and Grodzinski, 1985). Consequently, the enrichment of plant environments with above-ambient levels of carbon dioxide has been a widely used horticultural practice for increasing greenhouse plant productivity (Wolfe and Erickson, 1993).

5.1.1. Effects of CO_2 enrichment on above-ground biomass

Various contradictory responses have also been reported on the effects of CO_2 on above-ground biomass. Mousseau and Enoch (1989) reported that exposure of plants to CO_2-enriched conditions resulted in a 25 % reduction in total plant leaf area and consequently a decrease in total shoot weight. Various other reports reveal that CO_2 enrichment has no effect on leaf growth and total above-ground biomass production (Ehret and Jolliffe, 1985; Oberbauer *et al.*, 1986; Barnes and Pfirrmann, 1992; Drake, 1992; Stulen and den Hertog, 1993).

5.1.2. Responses of below-ground biomass

Although a great deal of research had been done on the effects of CO_2 enrichment on plants, there is still a paucity of information on the responses of below-ground processes to rising CO_2 concentration (Rogers *et al.*, 1992). The vital role of roots as an interface between the lithosphere and the biosphere is of pivotal significance in moving towards a fuller understanding of plant reaction to elevated [CO_2]. Continued research in this area is therefore warranted. Reports related to the effects of CO_2 enrichment on below-ground biomass are still controversial. For example, below-ground biomass had been reported to respond to CO_2 enrichment by either an increase, a decrease or to exert no effect (Cure and Acock, 1986; Rogers *et al.*, 1994; Poorter, 1993; Stulen and den Hertog, 1993).

5.1.4. Root/shoot ratios

No general pattern emerges when data on the effects of CO_2 enrichment on the distribution of dry matter between above- and below-ground biomass is expressed as root to shoot (R/S) ratios. Root to shoot ratios have been reported to either increase, decrease or not affected by CO_2 enrichment (Oberbauer *et al.*, 1986; Wong, 1990; El Kohen *et al.*, 1993; Stulen and den Hertog, 1993). This clearly reveals that the responses of plant dry matter partitioning to CO_2 enrichment are highly species-dependent. It is however, striking that plants which were reported as unaffected by CO_2 enrichment in one report were sometimes found to be very responsive in others. This suggests that dry matter partitioning in plant organs is not only influenced by CO_2 enrichment, but also by strong interaction of other environmental variables. A large variation in responses of R/S ratios of plants exposed to CO_2 enriched environments had been associated with plant nutrition. Many experiments performed under well-fertilised and well-watered conditions showed that dry matter partitioning was not influenced by CO_2 enrichment, whereas experiments in which nutrients limitation was imposed showed either no change in dry matter partitioning, or alternatively a greater investment in below-ground dry matter, and therefore higher R/S ratios under increased [CO_2] (Sionit *et al.*, 1981; Norby *et al.*, 1986; Stulen and den Hertog, 1993). Water limitation was showed to result in higher investment of dry matter in root, the result which is believed to allow roots to probe deeper layers of moist soils to ameliorate water and nutrient stress (Stulen and den Hertog, 1993).

5.1.4. Effects on yield

A common response on the effects of CO_2 enrichment on the plant's economic yield is that CO_2 enrichment stimulates higher plant yield (Kimball, 1983; Miglieta *et al.*, 1993; Wheeler *et al.*, 1994). However, reports that CO_2 enrichment has no effect on yield also exist (Nederhoff *et al.*, 1992). Garbutt and Bazzaz (1984) also reported slight decrease in seed number as a consequence of CO_2 enrichment in *Datura stramonium*. Reports on the effects of CO_2 enrichment on individual seed sizes are still controversial. Whilst Sionit *et al.* (1981) and Miglieta *et al.* (1993) observed increases in seed sizes under CO_2 enrichment, reports that elevated [CO_2] exposure result in seed decreases in seed sizes also exist (Wulff and Alexander, 1985).

This chapter will explore the effects of increased [CO_2] on dry matter partitioning and grain yield in the two cultivars of barley so that more sensible predictions could be made on the effects of the future CO_2-enriched environments on these two cultivars of barley.

5.2. Results

5.2.1. Leaf growth

Fig. 5.1. shows the responses of leaf length in barley to CO_2 enrichment. Elevated [CO_2] did not elicit significant changes in leaf length in both Stirling and Schooner (*P*

> 0.05), except for one instance at 28 DAG, where leaves of plants grown under CO_2 enrichment were significantly shorter than their ambient CO_2-grown counterparts. At 28 DAG, leaf length of Stirling and Schooner grown under elevated CO_2 was 19 % and 24 % less, respectively, than that of ambient CO_2-grown plants. Shorter leaves in plants grown under elevated [CO_2] as compared to plants grown under ambient [CO_2] had been previously observed by Nederhoff *et al.* (1992). Leaf length remained constant throughout the whole experiment at approximately 18 ± 5 cm and 16 ± 3 cm for Stirling grown under ambient and elevated [CO_2] respectively. Schooner showed a similar trend, but with slightly shorter leaves of 17 ± 4 cm and 14 ± 4 cm for plants grown under ambient and elevated [CO_2] respectively.

5.2.2. Leaf area

The leaf area of the first fully developed leaves were negatively affected by CO_2 enrichment. In both Stirling and Schooner, from 14 DAG leaf areas of plants grown under CO_2 enrichment were significantly lower than those for ambient CO_2-grown plants (Fig. 5.2; $P \leq 0.05$). These results are in agreement with those of Mousseau and Enoch (1989) and Nederhoff *et al.* (1992). Whilst leaf area in Stirling increased slightly over time under the two CO_2 treatments, Schooner showed a different response, with leaf areas of plants grown under CO_2-enriched air remaining constant at approximately 7 cm^2. Leaf area in Schooner grown under ambient [CO_2] increased

from the beginning of the experimental period until 14 DAG after which leaf area remained constant until the end of the experiment (Fig. 5.2. B).

Leaf dry weight (LDW) was similarly not significantly affected by CO_2 enrichment in the two cultivars (Fig. 5.3; $P > 0.05$). Lack of response in LDW in plants exposed to elevated [CO_2] have been previously reported by Morison and Gifford (1984) for cotton and Oberbauer *et al.* (1986) for three Alaskan tundra plant species. LDW increased over time in the two cultivars under both [CO_2]. Stirling showed slightly higher LDW values with a mean of 31 ± 7 mg at the end of the experimental period, whereas Schooner had a mean LDW value of 25 ± 5 mg.

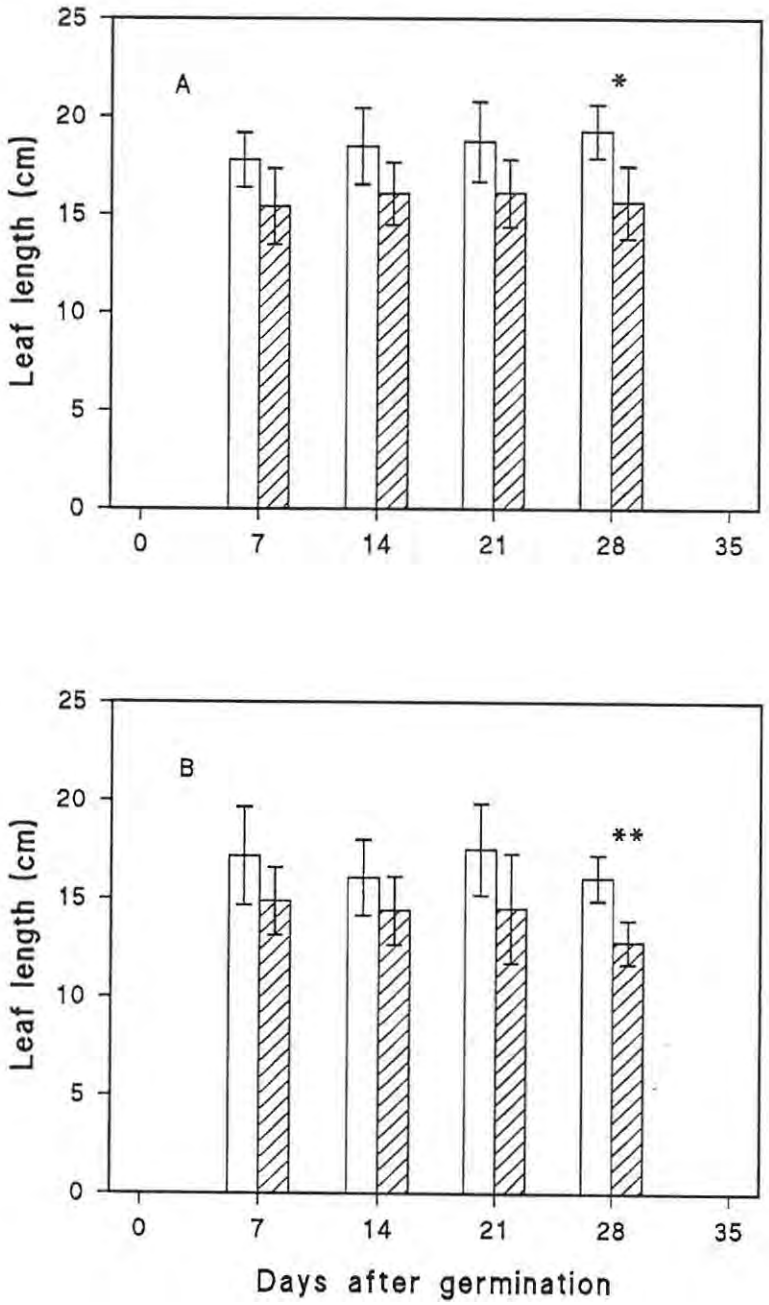

Fig. 5.1. Shows responses of leaf length in barley grown at 350 (open bars) or 600 (hatched bars) μmol mol $^{-1}$. A, Stirling; B, Schooner. *, ($P \leq 0.05$); **, ($P \leq 0.01$).

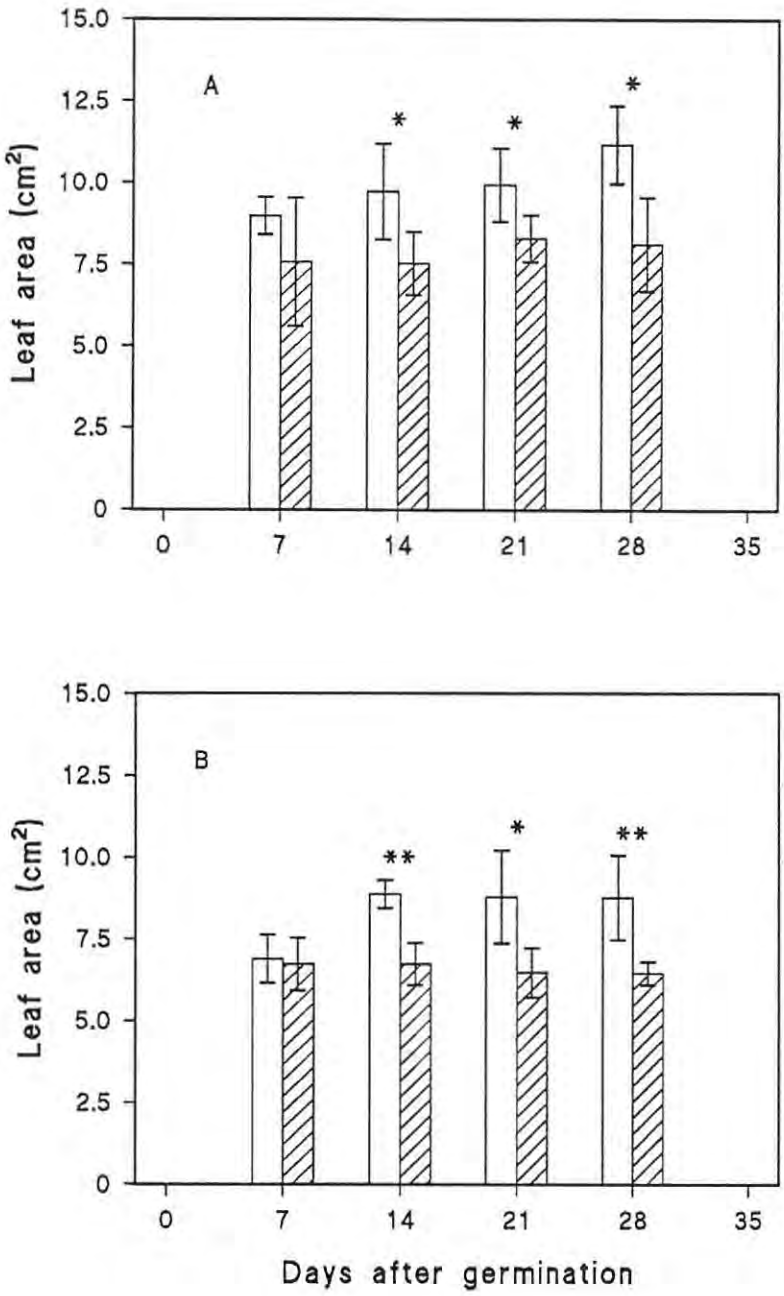

Fig. 5.2. The response of leaf area in barley plants grown at 350 (■) or 600 (♦) μmol mol^{-1}. A, Stirling; B, Schooner. *, ($P \leq 0.05$); **, ($P \leq 0.01$).

5.2.2. Total above- and below-ground biomass

Elevated CO_2 did not induce any significant change in the number of tillers in either Stirling or Schooner (Table 5.1). Similar results were observed for *Castanea sativa* Mill. by Mousseau and Enoch (1989). Responses of total plant height to carbon dioxide concentration are depicted in Fig. 5.4. Under elevated CO_2 conditions, barley showed a steep increase of plant height from the commencement of the experiment until 21 DAG, after which no appreciable increase in plant height was observed. Total plant height was not significantly affected by CO_2 enrichment in Schooner (Fig. 5.4. B; $P > 0.05$). In contrast, Stirling exhibited a different response. Plant height was stimulated by CO_2 enrichment during the initial stages of development, but was significantly less from 21 DAG due to elevated CO_2 exposure (Fig. 5.4. A; $P \leq 0.05$). Ceulemans and Mousseau, (1994) observed similar responses in poplar plants where plants grown under elevated [CO_2] were shorter than those grown under ambient CO_2 concentration.

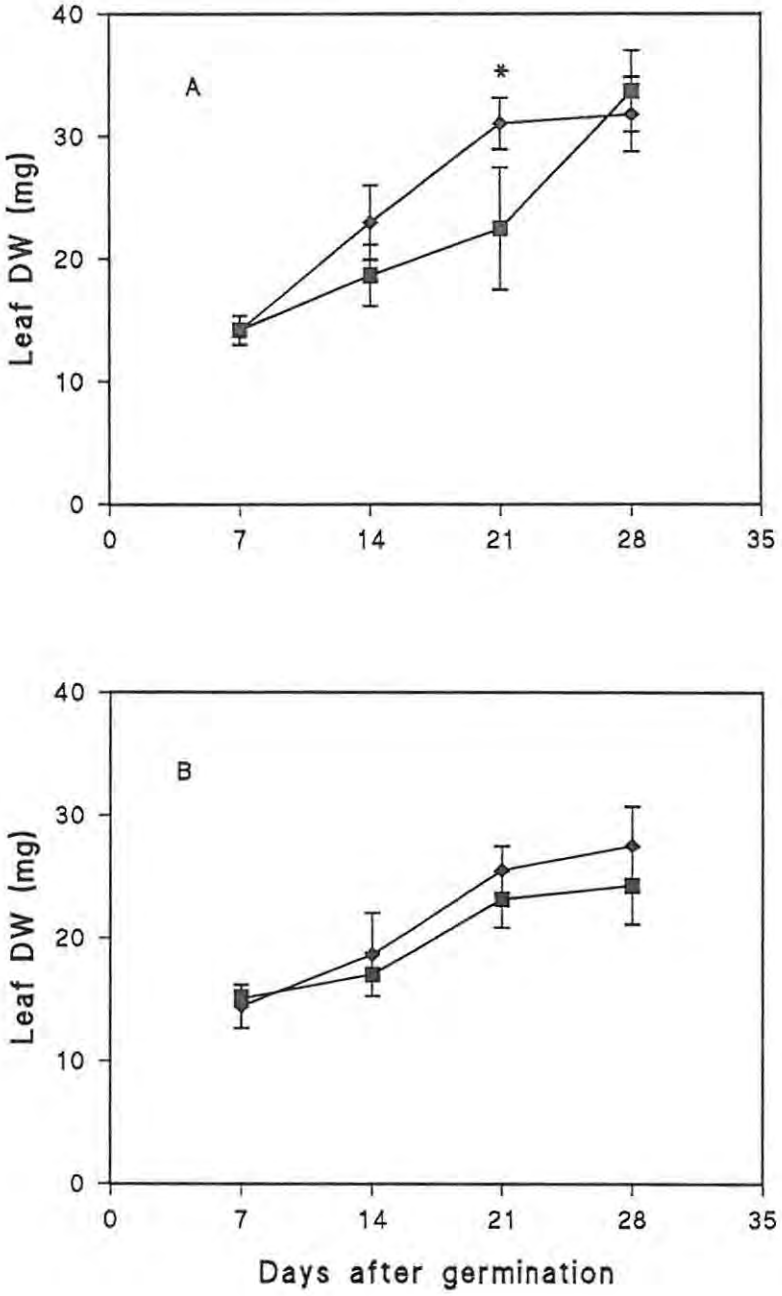

Fig 5.3. Shows leaf dry weight responses in barley plants grown at 350 (■) or 600 (♦) μmol mol^{-1}. A, Stirling; B, Schooner. *, ($P \leq 0.05$).

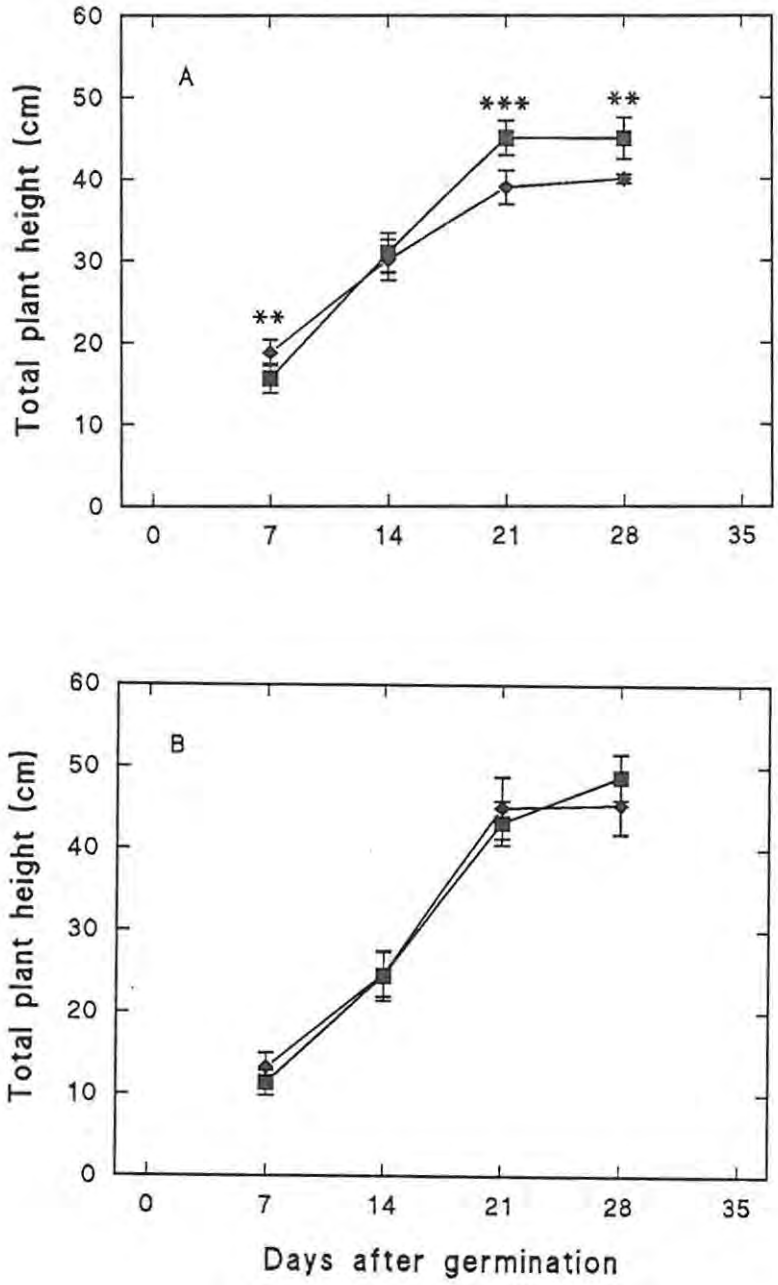

Fig. 5.4. Total plant height in barley plants grown at 350 (■) or 600 (◆) μmol mol^{-1}. A, Stirling; B, Schooner. **, ($P \leq 0.01$); ***, ($P \leq 0.001$).

Table 5.1. Effects of CO_2 enrichment on the number of tillers in barley. Results are means of nine replicates. (^{ns}, not significant, $P > 0.05$).

Cultivar	$[CO_2]$ ($\mu mol\ mol^{-1}$)	No. of tillers
Stirling	350	8 ± 1.37
	600	8 ± 1.98^{ns}
Schooner	350	8 ± 1.93
	600	8 ± 1.07^{ns}

The effects of elevated $[CO_2]$ on relative growth rate (RGR, the dry weight increase per unit of dry weight present expressed per unit time) were insignificant throughout the growth period in both cultivars (Table 5.2; $P > 0.05$). Similar result had been previously reported by Rogers *et al.* (1986) for soybean and recently by Mjwara *et al.* (1996) for common broad beans. Similarly, total above-ground dry weight was not significantly by CO_2 enrichment in either Stirling or Schooner (Fig. 5.5; $P > 0.05$). The se results are in agreement with those of El Kohen *et al.* (1993) and Ferris and Taylor (1993).

Table 5.2. Mean relative growth rates (RGR) at 14, 21 and 28 DAG, of two cultivars of barley grown under ambient and elevated [CO_2]. Data are means of four replicates. (^{ns}, not significant, $P > 0.05$).

Cultivar	DAG	[CO_2] (μmol mol^{-1})	RGR
Stirling	7 - 14	350	0.102
		600	0.105^{ns}
	14 - 21	350	0.081
		600	0.097^{ns}
	21 - 28	350	0.031
		600	0.023^{ns}
Schooner	7 - 14	350	0.098
		600	0.085^{ns}
	14 - 21	350	0.080
		600	0.079^{ns}
	21 - 28	350	0.041
		600	0.050^{ns}

Root DW was not significantly affected by CO_2 enrichment, except at 14 DAG in Schooner where CO_2 enrichment resulted in an increase in root DW (Fig. 5.6; $P >$ 0.05). Similar responses were observed by Ferris and Taylor (1994) and Bosac *et al.* (1994) but in different plant species. It is interesting to note that root development in the two cultivars was different. Root DW in Stirling increased linearly over time under both [CO_2]. However, root DW in Schooner increased over time until 21 DAG after which it remained constant.

Root to shoot ratios (R/S) remained unchanged due to CO_2 enrichment in Stirling, but Schooner showed increased root to shoot ratios under increased [CO_2] (Table 5.3). Root to shoot ratios decreased over time in both cultivars grown under either ambient or elevated [CO_2], for example R/S ratios in Stirling grown under ambient [CO_2] decreased from 0.475 at 7 DAG to 0.208 at 28 DAG. Different responses of root to shoot ratios to CO_2 enrichment, ranging from no response, to decreases to increases in root to shoot ratios in different plant species were also been reported by Morison and Gifford (1984); Oberbauer *et al.* (1986) and Stulen and den Hertog (1993).

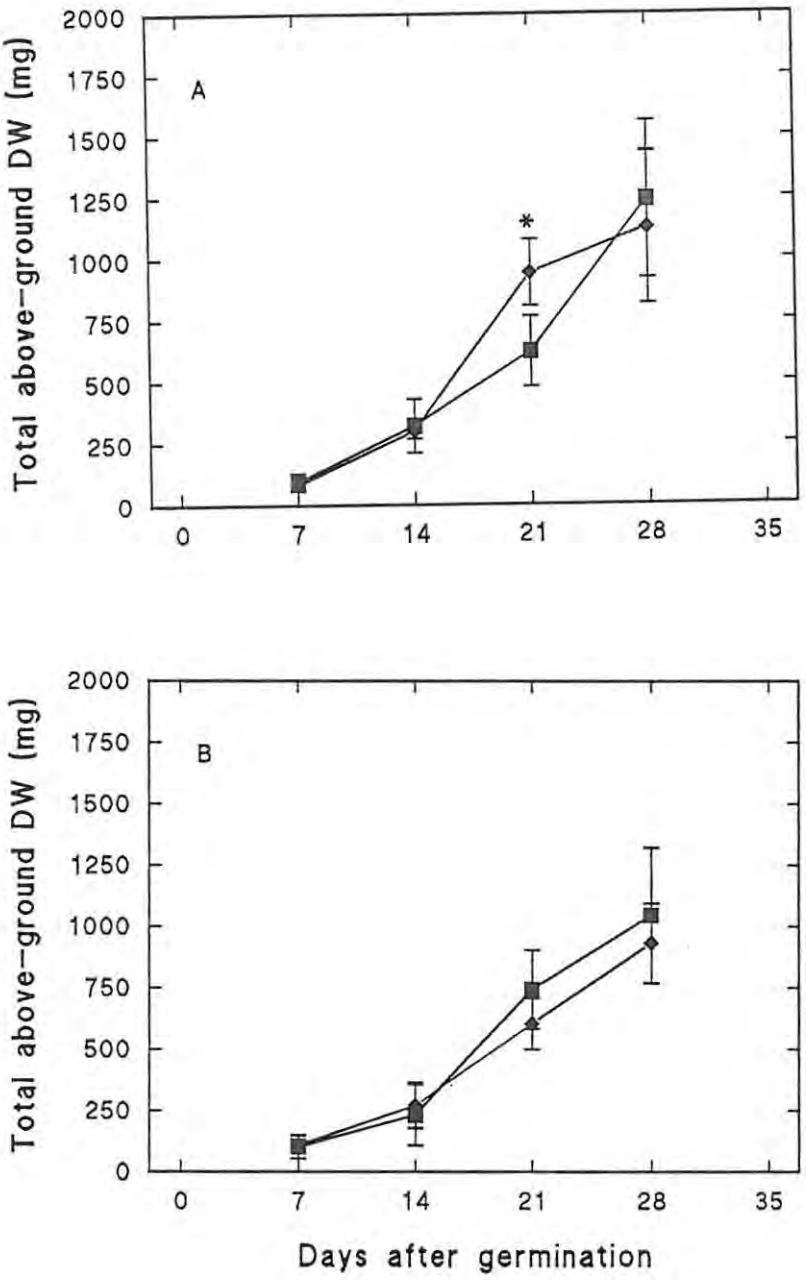

Fig. 5.5. Total above-ground dry weight in barley plants grown at 350 (■) or 600 (◆) μmol mol^{-1}. A, Stirling; B, Schooner. *, ($P \leq 0.05$).

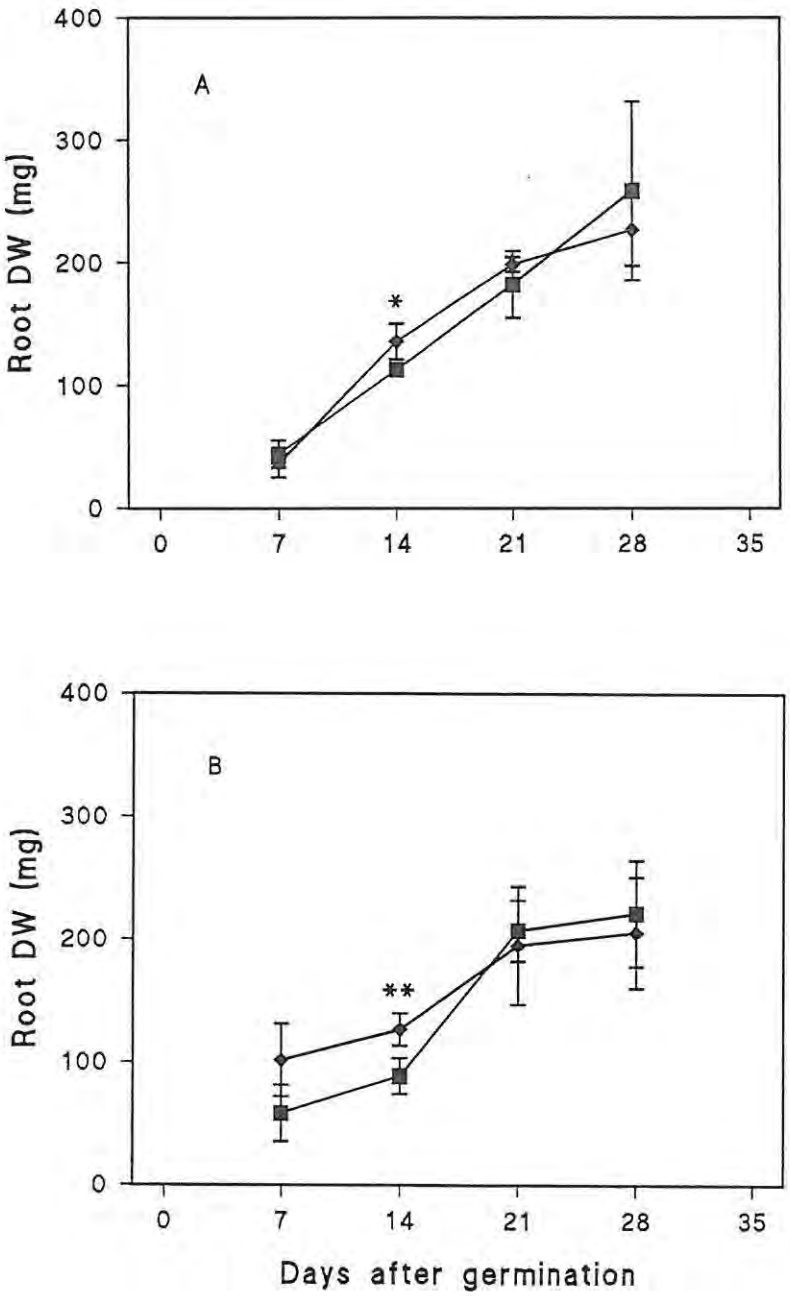

Fig. 5.6. Shows total root dry weight in barley plants grown at 350 (■) or 600 (♦) μmol mol^{-1}. A, Stirling; B, Schooner. *, ($P \leq 0.05$); **, ($P \leq 0.01$).

Table 5.3. Root to shoot (R/S) ratios of two cultivars of barley grown under ambient (R/S 350) and elevated (R/S 600) [CO_2] at 7, 14, 21 and 28 DAG. Data are means of four replicates. (^{ns}, not significant, $P > 0.05$; * and ***, significant at $P \leq 0.05$ and $P \leq 0.001$ respectively).

Cultivar	DAG	R/S 350	R/S 600
Stirling	7	0.475	0.456^{ns}
	14	0.451	0.436^{ns}
	21	0.209	0.210^{ns}
	28	0.208	0.201^{ns}
Schooner	7	0.574	0.933***
	14	0.382	0.468*
	21	0.279	0.323*
	28	0.164	0.169^{ns}

5.2.3. Grain yield

Stirling showed a significant reduction in of about 50 % in the number of grains per head under elevated [CO_2] (Table 5.4; $P < 0.001$). Garbutt and Bazzaz (1984) have also reported slight decreases in seed number, but in a different plant species, *Datura stramonium*. The decrease in the number of grains per head resulted in a proportional 79 % reduction in the number of grains produced per plant, and similarly about 79 % reduction in the total grain weight per plant. The total dry weight per individual grain was, however not affected by CO_2 enrichment, it remained at about 49 ± 4 mg. Schooner also exhibited similar trends, but with smaller percentage reductions in the number of grains produced per head, the total number of grains per plant and the total grain weight per plant (Table 5.4).

Table 5.4. Influence of elevated [CO_2] on yield components of barley. Results are means of nine replicates. (***, P ≤ 0.001; ^{ns}, not significant, $P > 0.05$).

Cultivar	[CO_2]	No. of grains per head	Total DW per grain (mg)	No. of grain per plant	Total grain DW per plant
Stirling	350	8 ± 1.21	49 ± 5	42 ± 4.21	2.10 ± 0.59
	600	4 ± 0.54***	49 ± 4^{ns}	10 ± 2.44***	0.44 ± 0.04***
Schooner	350	7 ± 1.09	46 ± 3	39 ± 3.92	1.97 ± 0.48
	600	4 ± 0.41***	49 ± 5^{ns}	16 ± 1.91***	0.72 ± 0.003***

CHAPTER 6: DISCUSSION

6.1. Elevated CO_2 concentration and the effects on photosynthesis

In chapter 3, the results of experiments in which barley was exposed to elevated CO_2 showed an initial stimulation of photosynthetic rates (Fig. 3.1). This is in agreement with the findings presented by Ingvardsen and Veierskov (1994) in the same species. Similar responses have been reported by other workers using various species including beans, rice and wheat (Radoglou *et al.*, 1992; Conroy *et al.*, 1994; Delgado *et al.*, 1994).

However, the initial positive stimulation of photosynthesis decreased to values almost the same as those of ambient air-grown barley plants with time, in the experiment reported here. Again this response of plants to CO_2 enrichment is well-documented for a wide range of species (see Mjwara *et al.*, 1996 and references cited). Many reasons for the decrease or even total loss of stimulation of photosynthesis due to sustained CO_2 enrichment have been suggested.

Some researchers have ascribed the decline or complete loss of stimulation of NAR to CO_2 enrichment, or to a decrease in stomatal conductance (g_s) which in turn lowers the rate of diffusion of CO_2 into the leaf (Jarvis, 1989; Grodzinski, 1992; Lawlor, 1993; Berryman *et al.*, 1994). In this study, CO_2 enrichment resulted in a significant decrease

in g_s in the two barley cultivars. Whilst Grodzinski (1992) and Lawlor (1993) have attributed the decline in g_s to the partial closure of stomata, decline in g_s has sometimes also been attributed to the reduction in stomatal density in plants grown under elevated [CO_2] (Woodward and Bazzaz, 1988; Cuelemans and Mousseau, 1994). The decline in stomatal conductance demonstrated in this study, was not due to the reduction in stomatal density, since weighted stomatal density was significantly increased in Stirling, whereas no significant change was observed on weighted stomatal density in Schooner (Table 3.1). It follows that the decline in g_s in barley plants grown under elevated CO_2 conditions must be due, at least in part, to the partial closure of stomata under the reported experimental conditions.

The decline in stomatal conductance in plants exposed to CO_2-enriched air was associated with a concomitant decline in transpiration rate (E, Fig. 3.10). This has been observed previously by other workers on various species (Jones *et al.*, 1985; Cure and Acock, 1986; Rogers and Dahlman, 1993; Polley *et al.*, 1994). On the contrary, in their study with wheat, André and Du Cloux (1993) observed that E was not significantly affected by CO_2 enrichment. The decline in E under elevated [CO_2] contributed to an increase in water use efficiency (WUE, the ratio of carbon fixed to water transpired) as shown in Fig. 3.11. Positive stimulation of WUE has long been recognised as a characteristic change in plants exposed to high [CO_2] (Radoglou *et al.*, 1992; Xu *et al.*, 1994; Samarakoon *et al.*, 1995). Enhanced WUE results suggest that CO_2 enrichment will be beneficial to plants growing in arid and semi-arid conditions,

in that it will decrease the water requirements for plants. However, studies by Rogers and Dahlman (1993) and Wolfe and Erickson (1993) pointed out that the potential water savings benefit associated with partial closure of stomata may be counteracted entirely by larger plant sizes, transpiring greater volumes of water, resulting in no change in the plant's water use under CO_2 enrichment.

As was indicated in chapter 3, assimilation (A), in terrestrial C_3 plants under photosynthetic light saturating conditions, is limited by Rubisco capacity at low C_i, thylakoid-dependent RuBP regeneration is limiting at intermediate C_i,and P_i regeneration becomes limiting at elevated C_i. Analysis of A/C_i response curves of this study (Figs. 3.2 and 3.3) suggest therefore, that the initial photosynthetic stimulation under CO_2 enrichment, at the beginning of the experiment was due to the stimulation of thylakoid-dependent RuBP regeneration and also due in part to an increase in P_i regeneration capacity. At the end of the experimental period (28 DAG), the reduction in the initial slope of the A/C_i response curves in high CO_2-grown plants indicate that Rubisco capacity was lower than that of the control plants. The decrease in Rubisco capacity may have been caused by the decrease in its activity, or rather in the activity of the catalytically-competent active sites as was envisaged by Sharkey (1985). It is possible that the concentration of the Rubisco in the experiment reported here also decreased as was the case in other reports (see Besford *et al.*, 1989 and literature cited). Sage *et al.*(1989) and Tuba *et al.* (1994) have also reported similar reductions in the initial slope of A/C_i response curves in *Chenopodium album* and *Triticum aestivum*

L. respectively. In addition, it is evident from the A/C_i response curves that thylakoid-dependent RuBP regeneration may also have caused limitation on A in this study. Furthermore, the suppression of A at elevated C_i suggest that P_i regeneration may be a contributing and a limiting factor in barley grown under CO_2 enrichment. At 28 DAG and under elevated CO_2, a decrease in photosynthetic stimulation coincided with a significant decrease in chlorophyll *a* content in variety Schooner. This data corresponds with earlier reports for various species (see Madsen, 1976; Cave *et al.*, 1981; Wulff and Strain, 1981 and literature cited). Clearly, the decrease in NAR stimulation at the end of the experimental period in Schooner may be attributed to decreased chlorophyll *a* content. The stimulation of NAR at 7 DAG in Schooner appears to be correlated to increased chlorophyll *a* content (Fig. 3.4.B). In Stirling however, (Fig. 3.4.A) no significant stimulatory effects of elevated CO_2 on either chlorophyll *a* and *b* content were observed, which agrees with previous work on cotton, beans and wheat by Wong (1979), Ehret and Jolliffe (1985) and Delgado *et al.* (1994) respectively. Increased NAR in Stirling under CO_2 enrichment (Fig. 3.1. A) was therefore not associated with neither chlorophyll *a* nor *b* content. Consequently, chlorophyll results reflect intra-specific differences on the responses of plants to CO_2 enrichment as was reported by Wulff and Alexander (1985). The lack of response of total carotenoids content in barley to CO_2 enrichment suggests that there is no correlation between NAR and total carotenoids content in these two cultivars of barley.

6.2. Effects of sustained elevated CO_2 on leaf anatomy

Enlarged starch grains in plants grown under CO_2 enrichment have previously been proposed as the main cause of chloroplast disruption and the subsequent deformation of the membranous systems of the thylakoids (Cave *et al.*, 1981). In addition, Ehret and Jolliffe (1985) suggested that the loss of photosynthetic stimulation in plants grown in sustained CO_2-enriched conditions is due to chloroplast disruption. Unlike most species reported in the literature, barley cv. Schooner, did not show starch accumulation in leaves which could be attributed to CO_2 enrichment (Fig. 4.4). Although mesophyll and bundle sheath cells in Stirling (Fig.4.3) demonstrated appearance of some starch grains under elevated CO_2 conditions, the overall chloroplast ultrastructure did not appear to be adversely affected. It follows then that the loss of stimulation in NAR in barley grown under CO_2 enrichment cannot be attributed to negative feedback inhibition caused by starch accumulation. Thus the results reported here suggest that starch grain number and size has no correlation with NAR in barley, which substantiates the previous argument by Gucci *et al.* (1991).

6.3. Elevated [CO_2] and its effects on plant growth

As a result of the increase in photosynthesis and a probable concomitant decrease in water loss, an increase in growth is to be expected in plants grown under CO_2 enrichment (Poorter, 1993). However, barley did not show any significant change in total above-ground dry weight under elevated [CO_2] in the present study (Fig. 5.5).

These results are in contrast with many previously-published observations, including those of Thompson and Woodward (1994) and Weigel *et al.* (1994), who reported (small) increases in total above-ground dry weight as a result of CO_2 enrichment for the same species. On the other hand, the present results are in close harmony with those of Smith *et al.* (1987) and Radoglou and Jarvis (1993) who observed no significant changes in above-ground dry weight under elevated CO_2 conditions in *Agropyron smithii* and a plant with long cotyledons, *Vivia faba*, respectively.

The lack of change in total above-ground dry weight may be correlated with the lack of stimulation of total plant height in Schooner. In contrast, Stirling responded to CO_2 enrichment by the early cessation of stem elongation, which was also observed by Mousseau and Enoch (1989) for sweet chestnut seedlings. Reasons for such early cessation of stem elongation are still unclear.

Elevated CO_2 resulted in slightly shorter leaves, but the difference was not statistically significant in the present experiments. Similar results have been observed for tomato plants by Nederhoff *et al.* (1992). The common result however, is that CO_2 enrichment stimulates leaf expansion (Wong, 1990; Ferris and Taylor, 1993; Bosac *et al.*, 1995). Nederhoff *et al.* (1992) have called this response a "Short Leaves Syndrome" (SLS - a response whereby plants exposed to CO_2 enrichment have shorter leaves compared to ambient CO_2-grown plant). Nederhoff *et al.* (1992) have attributed SLS to a nutrient

deficiency, particularly of calcium, where deficiency symptoms in plants have been described to include yellow margins in young leaves and scorched leaf tips.

Plants grown in CO_2-enriched air in the present study showed some form of yellowing of margins in leaves and also a degree of scorching of leaf tips. Thus, SLS as observed in barley grown under CO_2 enrichment conditions in this study, could at least in part be due to calcium deficiency. However this effect is suprising as the plants were watered with a full-strength nutrient solution every second day. Clearly, further research on the interactive effects of CO_2 enrichment and calcium nutrition on these two barley cultivars is therefore warranted.

It is generally assumed that in most plants a large proportion of the extra dry matter produced by CO_2 enrichment is allocated to the roots, and therefore that plants grown under elevated [CO_2] would have larger root systems (Bazzaz, 1990; Enoch, 1990). However, in the present study barley did not show any significant effect of CO_2 enrichment on root mass. Similar results were observed by Ferris and Taylor (1993) for *Anthyllis vulneraria* L. and *Plantago media*. When growth responses for this study were expressed as root to shoot (R/S) ratios, the two barley cultivars responded differently. There was a significant increase in R/S ratios in Schooner exposed to elevated [CO_2], but Stirling did not show any significant change in R/S ratios for plants grown under ambient and elevated [CO_2]. In their 1993 publication, Stulen and den Hertog associated increases in R/S ratios in plants to nutrient limitations and

decreases in R/S ratios to self-shading due to enhanced leaf sizes. It is generally accepted that in plants grown under non-limiting nutrient conditions, R/S ratios would not be altered by CO_2 enrichment (Stulen and den Hertog, 1993). The increases in R/S ratios in Schooner grown under CO_2 enrichment in this study may therefore be due to nutrient limitations. Since equal amounts of nutrients were supplied to plants grown under ambient and elevated [CO_2], it is suggested that nutrient limitations in CO_2-enriched plants could be due to increased nutrient requirements in these plants as a consequence of CO_2 enrichment.

6.4. CO_2 enrichment and plant yield

Unlike most species that have been studied, barley is unusual in that CO_2 enrichment did not seem to enhance grain yield (Table 5.4). The number of flowering heads remained the same for plants grown under either ambient or elevated [CO_2]. The decrease in grain yield was due to enhanced abortion of high numbers of flowers under CO_2 enrichment. Whilst the reason for the abortion of flowers is not yet fully understood, it is thought that the potential nutrient stress discussed in the previous sections could exert an effect here. Sionit (1983) observed that soybean plants grown under low nutrient supply had lower yields as compared to plants grown with high nutrient level. This author argues that a low supply of nitrogen (N) in the root zone during seed production leads to withdrawal of large amounts of N from the vegetative portion, mainly leaves. The result thereof will be that the leaves will senesce earlier,

thereby shortening the duration of seed development. Consequently a large number of flowers will abort, and seed yield will be lowered. In the present study, leaf senescence in CO_2-enriched plants indeed started about four days earlier and this may have contributed to the shortening of the duration of seed development and hence the decline in seed numbers. However, further research on the effects of N nutrition in relation to grain yield in these two barley cultivars needs to be done if sensible conclusions on the yield component of the two barley cultivars grown under CO_2 enrichment are to be made.

6.5. Conclusion

The results presented in this thesis demonstrate that the effects of short term exposure of plants to elevated [CO_2] resulted in improved photosynthetic rates. However, sustained CO_2 enrichment caused some deleterious effects on photosynthesis and yield. Although variety Stirling showed slightly higher photosynthetic rates, greater stomatal conductance, and larger leaves and chloroplasts than Schooner grown under the same CO_2 treatments, no overall significant differences were observed on the responses of the two cultivars to sustained CO_2 enrichment. The results of this study therefore do not support the hypothesis upon which this thesis was based, that the two barley cultivars used would respond differently to CO_2 enrichment.

This study has highlighted several contributing factors that need further clarification.

1) The relationship between calcium and nitrogen concentration and sustained elevated [CO_2] on the availability of these nutrients to the plant.

2). The shift in senescence under elevated [CO_2] with respect to flower abortion and yield.

3). The lack of starch accumulation in variety Schooner under sustained elevated carbon dioxide concentration

4). The relationship(s) between pool size of carotenoids with respect to photosynthesis and yield bears further detailed examination.

5). The effects of sustained elevated CO_2 on the manifestation of the "Short Leaf Syndrome" needs further study.

6). Last, but perhaps the most significant is the decline in absolute terms of seed yield under sustained elevated CO_2.

All these factors suggest that CO_2 enrichment may have deleterious effects on barley growth and yield, which may necessitate increased planting, increased or changed fertilisation and nutrient-feeding regimes and for screening the current varieties, for CO_2 insensitive plants from which to breed barley which will sustain present day yields, under the future elevated CO_2 environment.

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APPENDIX 1

Parameters calculations and equations

(Adopted from Mjwara, 1991)

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 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 used Γ* is the CO^2 compensation point of photosynthesis in the absence of dark respiration and C_i is the internal carbon dioxide concentration. Thus the net rate of CO_2 assimilation in the absence of day respiration R_d is: V_{cmax}, Rubisco which is an unstable enzyme capable of carboxylationor 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:

$$A = V_{c\max}\left[\frac{C_i - \Gamma^*}{C_i + K_c\left(\frac{1+0}{K_o}\right)}\right] - R_d \qquad \text{Equation 7.1}$$

Farquhar *et al.*, (1980) established the dependence of A to intercellular CO_2 using equation 7.1. The resultant equation (Equation 7.3) is directly related to the equation proposed by Ku and Edwards (1977):

$$CE = \frac{APS}{CO_2 - \Gamma^*} \qquad \text{Equation 7.2}$$

which estimated carboxylation efficiency (*CE*) from the initial slope of *A* versus C_i, where *APS* is the apparent rate of photosynthesis.

The dependence of *A* on the intercellular CO_2 is then:

$$\frac{dA}{dC} = V_{c\max}\left[\frac{\Gamma^* + K_c\left(\frac{1+0}{K_o}\right)}{\left[C + K_c\left(\frac{1+0}{K_o}\right)\right]^2}\right] \qquad \text{Equation 7.3}$$

Carbon dioxide compensation point (Γ*, μmol m^{-2} s^{-1}) has bee used for the calculation of *ACE* and its calculation is based on the following equation:

$$\Gamma^* = \frac{\Gamma + K_c\left(\frac{1+0}{K_o}\right)\frac{R_d}{V_{c\max}}}{1-\frac{R_d}{V_{c\max}}} \qquad \text{Equation 7.4}$$

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*, mmol m^{-2} s^{-1}) as illustrated in equation 7.5. 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 7.5

$$A = g_c(C_a - C_i) - \left(\frac{C_i + C_a}{2}\right)E \qquad \text{Equation 7.5}$$

The resultant equation (7.6, below) is incorporated in the IRCAL software package 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 7.6

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