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**Inter and Intra-Specific Variation in Photosynthetic Acclimation
Response to Long Term Exposure of Elevated Carbon Dioxide**

Matthew Wilkinson
(under the direction of Dr. Stephen Long)

August 1996

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Biosystems and Process Sciences Division

DEPARTMENT OF APPLIED SCIENCE

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Term Exposure of Elevated Carbon Dioxide**

Matthew Wilkinson
(under the direction of Dr. Stephen Long)

**Department of Biology
University of Essex
and University of Writtle College
United Kingdom**

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**Inter and Intra-Specific Variation in Photosynthetic
Acclimation Response to Long Term Exposure of Elevated
Carbon Dioxide**

Matthew Wilkinson

August 1996

**Report of original research submitted in partial fulfillment of the
requirements for the degree of MSc in Crop Production in the Changing
Environment. Department of Biology, University of Essex; and Writtle
College.**

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Abstract

The response of intra and interspecific variation in photosynthetic acclimation to growth at elevated atmospheric CO₂ concentration (600 μmol mol⁻¹) in six important grassland species was investigated. Plants were grown in a background sward of *Lolium perenne* and measurements were made after four years of growth at elevated C_a. Elevated CO₂ was maintained using a FACE (Free-Air Carbon Enrichment) system. Significant intra and interspecific variation in acclimation response was demonstrated.

The response of adaxial and abaxial stomatal conductance to elevated CO₂ was also investigated. The stomatal conductance of both the adaxial and abaxial leaf surfaces was found to be reduced by elevated C_a. Significant asymmetric responses in stomatal conductance was demonstrated in *D.glomerata* and *T.pratense*. Analysis of stomatal indices and densities, indicated that the observed reductions in stomatal conductance, were probably the result of changes in stomatal aperture.

Key-words: Acclimation, Elevated CO₂, FACE (Free-Air Carbon Enrichment), Genotypic variation, Stomatal conductance

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Word Count (inc. References) : 8482

Abbreviations and Symbols

A	Assimilation ($\mu\text{mol m}^{-2}\text{s}^{-1}$)
A_{sat}	Light saturated CO ₂ uptake
ATP	Adenosine Tri-Phosphate
C_a	Atmospheric Carbon Dioxide Concentration ($\mu\text{mol mol}^{-1}$)
CAM	Crassulacean Acid Metabolism
C_i	Leaf internal Carbon Dioxide Concentration($\mu\text{mol mol}^{-1}$)
CO₂	Carbon Dioxide
F.A.C.E	Free-Air Carbon Enrichment
J_{max}	Maximum rate of light saturated electron transport ($\mu\text{mol m}^{-2}\text{s}^{-1}$)
PEPCase	Phosphoenolpyruvate Carboxylase
P_i	Inorganic Phosphate
Rubisco	Ribulose 1,5 biphosphate carboxylase/oxygenase
RubP	Ribulose 1,5 biphosphate
V_{cmax}	Maximum rate of Carboxylation ($\mu\text{mol mol}^{-1}$)
WUE	Water Use Efficiency

1. Introduction

1.1 Climate Change

The rise in atmospheric carbon dioxide concentration (C_a) from a pre-industrial revolution level of $275 \mu\text{mol mol}^{-1}$ to the present day levels of 350 $\mu\text{mol mol}^{-1}$ (IPCC 1996) is currently a well documented and accepted phenomenon. Future predictions of C_a suggest that levels may reach ~ 530 and $700 \mu\text{mol mol}^{-1}$ by 2050 and 2100 respectively. Such relatively rapid increases have been attributed to anthropogenic emissions (primarily the burning of fossil fuels) and through deforestation. In conjunction with this is a predicted rise in mean global temperature of $3-4^\circ\text{C}$ by 2050 (IPCC 1994)

1.2 Crop Responses

One aspect of plant metabolism that has received much attention, in relation to rising C_a is photosynthesis, this is not surprising given that, photosynthesis is the primary way by which plants sense and respond to rising C_a (Mott 1990). Experimentally, short term exposure (hours to days) of C_3 plants to elevated CO_2 has been shown by many workers (Sage 1994) to cause a stimulation of photosynthetic activity, through an increased uptake of CO_2 / unit area of leaf ($A \mu\text{mol m}^{-2} \text{s}^{-1}$). This has been attributed primarily to a suppression of the oxygenation reaction of CO_2 fixation, as CO_2 may act as a competitive inhibitor. Secondly, due to the fact that at present day C_a Rubisco is not fully saturated (Bowes 1991; Stitt 1991; Long *et al* 1993). In addition, when RubP is in excess, the velocity of the carboxylation reaction is increased because Rubisco is not CO_2 -saturated at the current ambient concentration.

However, this stimulation of A has frequently been shown to be only a temporary effect. Long-term exposure of C₃ plants to elevated C_a has been demonstrated to result in a "down regulation" of photosynthetic activity, commonly this is expressed as a reduction in A (Farquhar *et al* 1980). Thus, occurs a reduction in the carbon foraging ability of the C₃ photosynthetic pathway, often to levels less than those obtained by equivalent plants grown at ambient CO₂ (Sage *et al* 1989).

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Photosynthesis is potentially limited by one of three processes; a) the efficiency of carboxylation (consumption of RubP in CO₂ assimilation). b) the capacity of the thylakoid membrane to supply ATP and NADPH for RubP regeneration. c) the capacity for utilisation of triose phosphate in sucrose and starch synthesis and to regenerate inorganic phosphate (P_i) for ATP synthesis in photosynthesis (Arp 1991; Long and Drake 1993; Webber *et al* 1994).

At present C_a it appears that photosynthesis is typically co-limited by Rubisco activity and the capacity of the thylakoid membrane to supply ATP and NADPH for RubP regeneration. Theoretically this may represent an optimisation of resource allocation (especially nitrogen) by the plant (Long and Drake 1992; Long *et al* 1993).

The accumulation of carbohydrate resulting from the short-term stimulation of A is widely believed to cause a disruption of the balance between production and end-product utilisation (Arp 1991). Initially, it was thought that this imbalance may cause direct mechanical damage, to the chloroplast, through the accumulation of soluble carbohydrate (Cave *et al* 1981). However, subsequent research has revealed a further likely mechanism of negative photosynthetic acclimation. The plant's inability to utilise the extra sucrose

produced, may result in a reduction of the flux of inorganic phosphate (P_i) from the cytosol to the chloroplast. Subsequent RubP regeneration may be suppressed, through a limitation in ATP production. The plot of assimilation ($A \mu\text{mol m}^{-2} \text{s}^{-1}$) over internal CO_2 concentration (C_i) or the A/C_i plot (fig 1), is a useful tool for the investigation of CO_2 induced changes of A , since it allows *in vivo* estimation of the limitation imposed by the amount of Rubisco (initial slope), capacity for RubP regeneration.

Fig 1 Hypothetical A/C_i curve (Farquhar *et al* 1980)

The second potential mechanism, which may account for negative photosynthetic acclimation is that of an alteration of the Rubisco status (either amount or activation state) of the leaf. At high C_a values (in the absence of any stomatal restrictions) C_i may increase to a point where A is no longer partially restricted by Rubisco activity, the sole limiting factor to photosynthesis is the capacity of RubP regeneration. The leaf would now contain an excess of Rubisco, given the fact that Rubisco may account for up to 25% of leaf nitrogen, this clearly does not represent an optimal allocation of resources within the leaf / plant.

Thus, given a mechanism, controlled by negative feedback (through an excess of carbohydrate in the sinks) a decline in the amount of Rubisco could be expected. Stitt (1991) referred to the reduction in amount of Rubisco as “adaptive” acclimation as it would allow resources such as nitrogen, to be reinvested in sink production. Indeed evidence exists for long-term exposure to elevated CO_2 being the cause of the repression of specific genes resulting in reduced amounts of photosynthetic proteins, including Rubisco (Stitt 1991).

The efficiency of carboxylation, a representation of the amount / activation state of Rubisco (Long and Drake 1992) may be investigated through a comparison of the initial slope of the A/C_i plot. At low C_i the maximum rate of carboxylation ($V_{c \text{ max}}$) is controlled by Rubisco, as RubP is likely to be in excess. This method allows a comparison of CO_2 effects, through an *in vivo* measure of Rubisco induced changes in carboxylation efficiency.

The accumulation of soluble carbohydrate through an imbalance of excess source and insufficient sink capacity, must therefore be viewed as the primary cause of both short and long term (adaptive) negative photosynthetic acclimation.

1.3 Stomatal Limitations

The above measurement (A/C_i) are taken *in vivo*, where stomatal limitations do not necessarily contribute to the analysis. There is a clear evidence that the stomata of C_3 plants react to an increase in C_a , resulting in partial closure (Mansfield 1985; Mott 1988; Mousseau^s and Saugier 1992). Potentially, short-term stomatal closure would reduce carbon diffusion to the site of carboxylation, the result of which would be a negative effect on A . In the long term, there is less evidence that exposure to elevated C_a may result in a reduction of stomatal

density (Woodward 1987; Beerling and Woodward 1995) again this is likely to have a negative effect of assimilation through a reduction in stomatal conductance. Evidence also exists that exposure of plants to elevated CO_2 may affect stomatal conductance on the adaxial and abaxial on the same leaf, to a different degree. Potentially, this may result in amphistomatous leaves developing a distribution of conductance approaching that of hypostomatous leaves (stomata on abaxial only) . The implications for photosynthesis of asymmetric stomatal response to elevated CO_2 , are clearly important. Therefore, it seems appropriate to examine whether some stomatal component plays a role in long term negative photosynthetic acclimation.

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1.4 Inter and Intraspecific Variation in Acclimation Response

Within those species that display a C_3 type of carbon fixation photosynthetic pathway, there is clearly a wide range of interspecific variation in photosynthetic response, to both short and long-term exposure to elevated C_a (Wullschlegel 1993). Rogers *et al* (1996) attributed interspecific variation to differences in sink size /strength.

Within the literature it appears that very little work, still as yet, has addressed intraspecific variation in photosynthetic response to elevated C_a this would agree with the work of Wulff & Alexander (1985) and Ziska & Teramura (1992). In many ways this is surprising and perhaps worrying for two reasons; it is evident that C_a levels are rising at an unprecedented rate (IPCC 1996). Secondly, it has been known that genotypic variation within populations in response to elevated C_a does exist, both between naturally occurring ecotypes and

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within existing cultivars of agronomically important species (Eagles 1967; Nösberger *et al* 1983).

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Intraspecific variation in response to elevation of C_a , within natural ecosystems could act to favour those genotypes that show improved fitness under the new environmental conditions (Ziska and Teramura 1992). Thus, potentially resulting in significant population changes with dynamic ecosystems. Rapid evolution of tolerance causing evolutionary shifts in populations, within a few generations, has already occurred, i.e. where species have been exposed to strong selection under pollution (Bradshaw and McNeilly 1991).

Within agricultural systems evidently more research is needed, studying the responses of different genotypes/cultivars to elevation of C_a (Heichel & Musgrave 1996; Hesketh & Hellmers 1973). Since photosynthesis is the major physiological process by which plants sense and by which their production can respond directly to, rising atmospheric CO_2 concentrations (Mott 1990; Long & Drake 1992). Clearly intraspecific variation in photosynthetic response to rising C_a is an important area of research, since many interspecific studies have clearly demonstrated acclimation of photosynthesis (positive and negative) after long term exposure to elevated CO_2 .

Overall it appears that intraspecific variation in response to elevated C_a represents a large gap in the understanding of plant physiology, and in terms of photosynthesis it is largely and unanswered question.

1.5 F.A.C.E. (Free Air Carbon Enrichment)

The investigation of photosynthetic adjustment in plant material grown within a F.A.C.E. (Free Air Carbon Enrichment) system as developed by Brookhaven National Laboratory, allows field scale measurements to be taken in the absence of any “chamber effects”. Field CO₂ fumigation, minimises alteration of microclimatic factors, such as wind speed, temperature, relative humidity which could potentially affect photosynthesis. FACE technology also allows the investigation of plant material in the absence of artificially induced, sink limitations, e.g. pot size, (Arp 1991) which in the past have been shown to be a partial causal agent of negative photosynthetic adjustment (Hendry 1994)..FACE technology also allows fumigation of large areas, thus making it suitable for experiments such as this species x genotype x CO₂ experiment, which was established in 1993.

1.6 Aim of Study

This study addresses the following questions that, as yet are clearly lacking in the existing literature;

- 1) What is the size of intraspecific variation in acclimation response, after prolonged exposure to elevated CO₂? This will be investigated using several important lowland grassland species.
- 2) Does interspecific variation exceed intraspecific variation in acclimation response, within these species?

In term of stomatal response to elevation of field C_a, using a combination of porometry and stomatal impressions techniques, the project addresses the following questions;

- 3) How does long-term exposure to elevated C_a effect the ratio of upper to lower axial, stomatal conductance?
- 4) To what extent does interspecific variation stomatal conductance sensitivity occur in response to CO_2 ?

2. Materials and Methods

2.1 The Site

The ETHZ research station located at Eshicon, includes the Free-Air Carbon Enrichment (F.A.C.E.) experiment, as developed by Brookhaven National Laboratory (Hendrey *et al*, 1993). This system comprises of a subterranean 14m diameter toroidal plenum, above ground 32 vertical vent pipes emerge at regular intervals from the plenum.

Within the FACE rings, a central computer (I.B.M. PC), processing data of wind speed, wind direction and air temperature allows the maintenance of elevated C_a , these rings have a target concentration of $600\mu\text{mol mol}^{-1}$, by releasing CO_2 according to the wind speed and direction. There are six rings, paired within three blocks, three control rings at ambient C_a ($355\mu\text{mol mol}^{-1}$) and three FACE rings at elevated C_a ($600\mu\text{mol mol}^{-1}$).

Fumigation of the FACE rings commenced in 1993, and has continued throughout each growing season. This year fumigation commenced on April 5th. In 1995, measured over one minute intervals, elevated C_a was maintained within 10% of the target CO_2 concentration ($540\text{--}660\mu\text{mol mol}^{-1}$) for 89-94% of the time and within 20% of the target concentration for 98% of the time (Bluhm 1996, personal communication).

The species x genotype competition experiment was established in 1993 and is therefore presently in it's forth year of growth at elevated CO_2 . Figure 2.1 details the layout of the genotype plots in one of the six rings. Twelve species each comprising of twelve genotypes (Table 2.1) were established into a background sward of *L.perenne* L. (cv Bastion). The plots have been managed

extensively, i.e. they have been cut 3-4 times per year, to a sward height of 5 cm and 100 kgN ha⁻¹yr⁻¹ has been applied. The soil may be characterised as a clay loam (25% clay, 35% silt and 37% sand) (Jongen *et al* 1995), with a pH of 6.8.

Figure 2.1 Layout of one ring.

Table 2.1 Species Present in Genotype Plots

Grass Species

A.elatius
D.glomerata
F.pratense
H.lanatus
L.multiflorum
L.perenne

Di-cot Species

R.acetosa
R.frisianus
R.obtusifolius
T.flavescens
T.pratense
T.repens

↑ species in plot

2.2 Materials

Data collected consisted of leaf gas analysis measurements, porometer measurements and stomatal densities/indices. Leaf gas analysis, obtained non-destructively was used as a means of indicating possible photosynthetic acclimation. For those genotypes, within each species where the largest

intraspecific variation in photosynthetic response was displayed, the variables of A and C_i were derived, based on the equations of Von Caemmerer and Farquar (1981) and Harley (1992) to standardise to 25°C (Long and Fischer 1996, unpublished). Photosynthetic activity was measured using an Open Combined Infra-Red Gas Analysis System (CIRAS 1 model 1.4, PP Systems, Hitchin, Herts UK) in conjunction with a Parkinson Leaf Curvette (model 1.1, PP Systems, Hitchin, Herts UK). In association with both of the above, a palm top PC (Hewlett Packard 1000CX) was used to control the system. Regular calibration of the CIRAS was made for CO_2 at $600 \mu\text{mol mol}^{-1}$ against a gravimetric CO_2 concentration (Carbagas, Zurich). The water vapour measurements were calibrated at 10mb generated by a water vapour generator (Type WE 600, Analytical Development Co, Hoddesden UK).

Leaf gas analysis measurements always comprised of the adaxial of the leaf being displayed in the upper leaf chamber. For each species, sampled leaves were always at comparable growth stages, measurements always being taken at a similar position along the leaf. For grass species this was always taken as the youngest fully expanded leaf, i.e. the upper most leaf bearing a ligule. Leaf area (where it was not sufficiently large to completely fill the leaf chamber) was calculated by multiplying the width of the leaf by either, the diameter of the chamber where a small, circular leaf chamber was used, or the length where a large rectangular chamber was used.

For construction of A/C_i curves saturating photon flux densities were required. A quartz iodide lamp was attached to the upper surface of the leaf

chamber, using a diffuse filter a constant light saturating photon flux density of $800 \mu\text{mol m}^{-2} \text{s}^{-1}$ PAR was achieved.

Stomatal conductance for ab/adaxial ^{Subo} was measured independently using a transit-time porometer (AP4 Porometer, Delta-T, Cambridge, UK). The term "stomatal conductance" is used to describe total leaf conductance, where it was assumed that direct diffusion through the leaf epidermis was minimal. Regular calibration, against the manufactures calibration plate which corrected theoretical conductance values of the plate, to those of the prevailing conditions during measurement, were carried out as required (i.e. following a change in atmospheric temperature of $\geq 5^\circ\text{C}$ and/or a change in relative humidity (R.H.) of $\geq 10\%$). Atmospheric pressure was measured with a miniature barometer (Type Alti-Thermo, Casio) so that all measurements could be corrected to a standard pressure.

Measurements ^{Subo} were made in the geometric centre of each leaf as described in Pearson *et al* (1995). In order to avoid the measurement of one surface having a detrimental effect on the other, the measurement cup was moved along the leaf 1-1.5cm after each measurement had been taken.

Impressions of leaf epidermis from mature, recently expanded leaves on either side of the mid rib were made onto acetate sheets. The impressions were taken from the same portion of the leaves as used in the porometer measurements. Stomatal and epidermal cell densities (number per area of leaf surface) were used for calculating stomatal index;

Stomatal index = [stomatal density/(stomatal density + epidermal cell density) x 100]

Guard cells were not counted as epidermal cells. Counts were made using a microscope (Ortholux II, Leitz, Wetzlar, Germany) giving a field area of 0.1128mm^2 at x 312.5 magnification, the size of the field was determined using a calibrated eye-piece graticule. A total of twelve fields were counted per leaf surface, per CO_2 treatment for each block, giving a final replication of thirty six counts per leaf surface, per treatment

2.3 Methods

2.3.1 Genotype Acclimation Sampling

Measurements of photosynthesis and transpiration were performed on six genotypes from each species, as means of rapidly determining potential intraspecific acclimation. Genotypes in each of the six rings (control and FACE) were measured at a C_a of $360\text{ }\mu\text{mol mol}^{-1}$. Two leaves per plant were sampled, on the leaf and in the position as described above. No artificial lighting was imposed on the leaf, measurements always being taken in ambient sunlight. Measurements commenced at 10.00hrs and were always completed by 15.00hrs in order to avoid the complication of potential feedback inhibition of photosynthesis (Delucia *et al* 1985; Arp 1991; Grulke *et al* 1993; Webber *et al* 1994) in the latter part of the afternoon. Rings were always sampled in the following order, C2, F1, C1, F2, F3, C3. See appendix 1 for ring locations.

2.3.2 A/C_i Measurements

Using data obtained from the above set of measurements, for those two genotypes that displayed the strongest contrasting evidence for acclimation (either positive or negative), plots of light saturated CO_2 uptake, per unit area

(A_{sat}) to intercellular CO_2 concentration (C_i) were made (A/C_i). For each species, A/C_i curves were constructed for two leaves, in the following sequence of seven C_a values ($\mu\text{mol mol}^{-1}$);

Control plots: 360, 200, 150, 100, 50, 600, 900

F.A.C.E. plots: 600, 200, 150, 100, 50, 360, 900

This sequence of C_a values was required to minimise deactivation of the Rubisco enzyme. Measurements were always taken at C_a concentrations within + or - $15 \mu\text{mol mol}^{-1}$ of the above values. The leaf was illuminated with a stabilised quartz halide source at $\text{Ca. } \mu 800 \text{ mol mol}^{-1}$ to ensure a constant near saturating irradiance. Measurements commenced at 10.00hrs and were always completed by 15.00hrs.

For each genotype, values of $V_{c \text{ max}}$ (maximum rate of carboxylation, $\mu\text{mol mol}^{-1}$) were calculated to provide an *in vivo* estimate of Rubisco activity. Values of J_{max} were also fitted, thus allowing a genotypic comparison of the maximum rate of light saturated electron transport ($\mu\text{mol m}^{-2} \text{ s}^{-1}$) or the maximum rate for RubP regeneration.

Data was analysed for significant differences in inter and intraspecific acclimation of photosynthetic capacity in relation to the CO_2 treatment using analysis of variance techniques.

2.3.3 Stomatal Conductance Measurements

In order to assess the effect of prolonged exposure to elevated C_a , on the interspecific variation in stomatal behaviour, stomatal conductance measurements were taken. Investigations always commenced at 10.00hrs and were always completed by 15.00hrs. For the three grass species investigated, six leaves per plant were measured on both ^{Surf}axial. For the three broadleaf species, three leaves per plant were measured, in each half of both axial. There was therefore a minimum replication of twenty four measurements, per axial, per CO_2 treatment, for each species sampled.

2.3.4 Data Analysis

Data was analysed using one and two way, analysis of variance techniques. The level of significance, $P < 0.05$ was used.

3. Results

3.1 Genotype Acclimation Sampling Results

Measurements of photosynthesis and transpiration, on six genotypes from each species (three on *A.elatius*) demonstrated the existence of considerable intraspecific variation in photosynthetic response (A) to elevation of C_a (fig 3.1, fig 3.2 & fig 3.3).

When measured at C_a 360 $\mu\text{mol mol}^{-1}$ all genotypes grown at elevated C_a displayed a reduction in A, with respect to those grown at ambient C_a . The only exception to this trend was one genotype of *T.pratense* and all three genotypes of *A.elatius*.

Table 3.1 displays those genotypes within each species that exhibited the greatest intraspecific change in assimilation rate, between genotypes grown at ambient C_a in comparison to genotypes grown at elevated C_a . These genotypes were subsequently used for further investigation of V_c and J_{max} .

Table 3.1 Genotypes Displaying Greatest Change in Assimilation, Ambient Compared to Elevated CO_2 Grown Plants

Species	<i>H.lanatus</i>	<i>A.elatius</i>	<i>D.glomerata</i>	<i>T.pratense</i>	<i>R.frisianus</i>	<i>R.obtusifolius</i>
Genotype	554 504	202 257	359 308	1102 1151	955 910	1053 1051

3.2 A/C_i Results

3.2.1 Interspecific Variation in Grass Species Results

Among the three grass species, significant interspecific variation ($f=7.32$; $p<0.05$) in $V_{c \text{ max}}$, an indication of Rubisco activity and carboxylation, was demonstrated (fig 3.4a & table 3.2). Whilst significant up-regulation of $V_{c \text{ max}}$

was demonstrated within *D.glomerata*, after prolonged exposure to elevated C_a , this was not demonstrated in either *H.lanatus* or *A.elatius*.

Table 3.2 Mean Separation of $V_{c \max}$, Grass Species. Showing Ambient Vs Elevated CO_2 Grown Plants

		Ambient CO_2 Grown Plants		
Species		<i>D.glomerata</i>	<i>H.lanatus</i>	<i>A.elatius</i>
Elevated				
CO_2	<i>D.glomerata</i>	34.00	52.07	40.01
Grown	<i>H.lanatus</i>	-35.99	4.12	7.94
Plants	<i>A.elatius</i>	-34.49	3.05	9.01
LSD = 27.38				

- Figures in bold represent those means that are significantly different ($p < 0.05$)
- LSD = Least Significant Difference

A significant reduction in $V_{c \max}$ was demonstrated between both *H.lanatus* and *A.elatius* after exposure to elevated C_a when compared to *D.glomerata* grown at ambient C_a . Table 3.2 also demonstrates a significant up-regulation of $V_{c \max}$ within elevated C_a grown *D.glomerata*, as compared to ambient C_a grown *H.lanatus* and *A.elatius*.

Within the grass species, significant interspecific variation in $J_{\max}$ ($f=5.98$; $p < 0.05$) was demonstrated after prolonged exposure to elevated C_a (Fig 3.4b & table 3.3). A significant reduction in $J_{\max}$ was present within *D.glomerata* at the high CO_2 treatment. In addition a significant reduction in $J_{\max}$ occurred between ambient grown C_a *D.glomerata* and elevated C_a grown *H.lanatus* and *A.elatius*.

3.2.2 Interspecific Variation in Broad-leaved Species Results

Within the broad-leaved species, significant interspecific variation in $V_{c \max}$ was demonstrated ($f=3.86$; $p < 0.05$). A significant reduction in $V_{c \max}$ was

FIG 3.1 Genotype acclimation trials for a) *H. lanatus* and b) *A. elatius*. Measurements made at C_a 360 $\mu\text{mol mol}^{-1}$

FIG 3.2 Genotype acclimation trials for a) *D.glomerata* & b) *T.pratense*. Measurements made at C_a 360 $\mu\text{mol mol}^{-1}$

FIG 3.3 Genotype acclimation trials for a) *R. frisianus* & b) *R. obtusifolius*. Measurements made at C_a 360 $\mu\text{mol mol}^{-1}$

demonstrated within *R.frisianus* exposed to elevated C_a , as compared to the equivalent plants grown at ambient C_a .

Table 3.3 Mean Separation of J_{max} , Grass Species. Showing Ambient Vs Elevated CO_2 Grown Plants

		Ambient CO_2 Grown Plants		
Species		<i>D.glomerata</i>	<i>H.lanatus</i>	<i>A.elatius</i>
Elevated CO_2 Grown Plants	<i>D.glomerata</i>	-138.59	73.9	-45.31
	<i>H.lanatus</i>	-189.15	11.67	-5.25
	<i>A.elatius</i>	-208.44	2.02	-24.54

LSD = 101.26

In addition, significant differences also occurred between *R.frisianus* exposed to elevated C_a and both *T.pratense* and *R.obtusifolius* grown at ambient C_a (fig 3.5a & table 3.4)

Table 3.4 Mean Separation of $V_{c\ max}$, Broad-leaved Species. Showing Ambient Vs Elevated CO_2 Grown Plants

		Ambient CO_2 Grown Plants		
Species		<i>T.pratense</i>	<i>R.frisianus</i>	<i>R.obtusifolius</i>
Elevated CO_2 Grown Plants	<i>T.pratense</i>	17.63	5.	2.96
	<i>R.frisianus</i>	-36.63	-0.86	-3.67
	<i>R.obtusifolius</i>	7.04	14.07	16.88

LSD=22.0

The pattern of J_{max} response, within these species, was identical to that of $V_{c\ max}$ response (fig 3.5b) despite the magnitude of the response being different. Field elevation of C_a was found to have no significant effect on J_{max} at the species level ($f=2.75$; $p<0.05$)

FIG 3.4 Interspecific Variation in a) $V_{c \text{ max}}$ & b) J_{max} for all Grass Species

FIG 3.5 Inter-specific Variation in a) $V_{c \max}$ & b) $J_{\max}$ for all broadleaved species

3.3 Intraspecific Variation in Grass Species Results

Analysis of the mean separation of $V_{c\max}$ (fig 3.6a,b; fig 3.7a & table 3.5) demonstrates a lack of significant evidence for intraspecific variation in acclimation response after prolonged exposure to elevated C_a ($f=0$; $p<0.05$). This was despite a wide range of response at elevated C_a , from a stimulation (*A.elatius*) to a reduction (*H.lanatus*) being demonstrated. However contrary to this, significant intraspecific variation ($f=9.62$; $p<0.05$) in $J_{\max}$ was located within *D.glomerata* under identical conditions (table 3.6) both genotypes of *D.glomerata* (359 & 308) being significantly reduction.

3.4 Intraspecific Variation in Broad-leaved Species Results

Analysis of the mean separation of $V_{c\max}$ within the broad-leaved species, demonstrated significant evidence ($f=2.65$; $p<0.05$) for the existence of intraspecific response in acclimation. This was demonstrated within the three species investigated (table 3.7). Whilst genotypes of *R.obtusifolius* and *R.frisianus* showed a reduction in $V_{c\max}$ at elevated C_a , one genotype of *T.pratense* demonstrated an up-regulation. These results are consistent with the findings of $J_{\max}$, (fig 3.7b, 3.8a, 3.8b & table 3.8) where significant intraspecific variation in acclimation response was demonstrated.

3.5 Stomatal Conductance Results

Within grass species, stomatal conductance was consistently reduced, on both axial at the high CO₂ treatment (fig 4.1). Table 3.9 illustrates the significant reduction demonstrated on both axial, by *D.glomerata* and *H.lanatus*. In addition CO₂ was shown to have a significantly negative effect on the adaxial stomatal

Grass Species Inter and Intra-specific Variation in 3.5) $V_{c \max}$ & 3.6) $J_{\max}$

Table 3.5 Mean Separation of $V_{c \max}$ ($\mu\text{mol mol}^{-1}$) in All Grass Species, Ambient Vs Elevated CO_2 Grown Plants

		Ambient CO_2 Grown Plants					
Species		<i>D.glomerata</i>		<i>H.lanatus</i>		<i>A.elatius</i>	
Genotype		359	308	554	504	202	257
Elevated CO_2	359	22.57	-6.49	79.85	71.00	75.00	56.56
	308	-24.27	-6.5	-33.0	24.3	28.47	9.71
Grown Plants	554	55.9	25.00	-1.38	-7.32	1.11	21.91
	504	7.98	10.93	-16.00	-6.87	11.04	7.72
	202	35.23	14.01	-12.8	3.79	7.96	-10.8
	257	4.68	24.20	51.71	6.39	-2.22	-20.98

LSD = 27.38

Table 3.6 Mean Separation of $J_{\max}$ ($\mu\text{mol mol}^{-1}$) in All Grass Species, Ambient Vs Elevated CO_2 Grown Plants

		Ambient CO_2 Grown Plants					
Species		<i>T.pratense</i>		<i>R.frisianus</i>		<i>R.obtusifolius</i>	
Genotype		359	308	554	504	202	257
Elevated CO_2	359	-132.08	-81.41	-130.39	-81.1	65.32	-216.49
	308	-195.76	-145.09	-66.71	-17.42	-1.64	-25.31
Grown Plants	554	266.28	215.61	3.81	-53.1	68.88	45.21
	504	162.67	112.0	99.8	-50.51	-34.73	-58.4
	202	249.47	198.8	-13	36.29	-52.07	-28.4
	257	218.07	167.4	-44.4	4.89	-20.67	3

LSD = 101.26

- 1) Figures in bold represent those means that are significantly different ($p < 0.05$)
- 2) Figures in shaded boxes represent Intra-Specific Variation
- 3) LSD = Least Significant Difference ($P < 0.05$)

Broad Leafed Inter and Intra-Specific Variation in 3.7) $V_{c \max}$ and 3.8) $J_{\max}$

Table 3.7 Mean Separation of $V_{c \max}$ ($\mu\text{mol mol}^{-1}$) in All Broad leaf Species, Ambient Vs Elevated CO_2 Grown Plants

		Ambient CO_2 Grown Plants					
Species		<i>T.pratense</i>		<i>R.frisianus</i>		<i>R.obtusifolius</i>	
Genotype		1102	1151	955	910	1053	1051
Elevated CO_2	1102	22.54	10.8	-2.39	-7.59	-2.34	-15.32
	1151	10.8	-12.7	9.35	4.14	9.41	-3.57
Grown Plants	955	-81.54	26.46	-48.53	-43.32	48.58	35.6
	910	-23.32	-25.24	-3.17	-8.38	-3.12	-16.1
	1053	-5.12	-7.04	15.03	9.82	-15.08	-2.1
	1051	11.45	9.53	31.6	26.39	-31.65	-18.67

LSD = 22.34

Table 3.8 Mean Separation of $J_{\max}$ ($\mu\text{mol mol}^{-1}$) in All Broad leaf Species, Ambient Vs Elevated CO_2 Grown Plants

		Ambient CO_2 Grown Plants					
Species		<i>T.pratense</i>		<i>R.frisianus</i>		<i>R.obtusifolius</i>	
Genotype		1102	1151	955	910	1053	1051
Elevated CO_2	1102	0.19	70.07	-76.37	-46.2	19.3	-16.97
	1151	-21.44	48.44	-54.74	-24.57	40.93	4.66
Grown Plants	955	84.75	14.87	-8.57	-38.74	104.24	67.97
	910	-51.72	-121.6	-127.9	-97.73	-32.23	-68.5
	1053	22.42	-47.47	-53.77	-23.6	-41.9	-5.63
	1051	64.28	-5.6	-11.9	18.27	-83.77	-47.5

LSD = 49.02

- 1) Figures in bold represent those means that are significantly different ($p < 0.05$)
- 2) Figures in shaded boxes represent Intra-Specific Variation.
- 3) LSD = Least Significant Difference ($p < 0.05$)

FIG 3.6 Intraspecific Variation in $V_{c \max}$ and $J_{\max}$ for a) *H. lanatus* (genotypes 554 & 308) & *A. elatius* (genotypes 202 & 257)

FIG 3.7 Intraspecific variation in $V_{c \max}$ and $J_{\max}$ for a) *D.glomerata* (genotypes 359 & 308) & b) *T.pratense* (genotypes 1102 & 1151)

a) *D.glomerata*

b) *T.pratense*

FIG 3.8 Intraspecific variation in $V_{c \max}$ & $J_{\max}$ for a) *R.obtusifolius* (genotypes 1053 & 1151) & b) *R.frisianus* (genotypes 910 & 955)

a) *R.obtusifolius*

b) *R.frisianus*

**FIG 4.1 Stomatal Conductance of ad & abaxial for
a) grass species & b) broad leaved species**

conductance of *T.pratense* ($f=5.75$; $p,0.05$) and on abaxial stomatal conductance of *R.frisianus* ($f=12.44$; $p<0.05$)

Table 3.9 Differences Between Control and FACE Stomatal Conductance Means ($\text{mmol m}^{-2} \text{s}^{-1}$). Showing both Ad and Abaxial

	D.glomerata	H.lanatus	A.elatius	T.pratense	R.frisianus	R.obtusifolius
Adaxial	-122.67	-99.26	-26.01	-68.26	+8.37	-49.36
Abaxial	-56.68	-79.07	-4.23	+22.01	-38.13	-167.61

- Figures in bold represent significant difference between control means and FACE means. ($p<0.05$)
- Negative figures represent a reduction in stomatal conductance at elevated CO_2

3.6 Stomatal Index and Density Results

There was no significant effect of CO_2 on the stomatal index of either the ab or adaxial within any of the grass species. Despite this, greater variation in stomatal index existed on the abaxial (table 3.10 & fig4.3b). However, a significant difference between species ($f=5.25$; $p<0.05$) was demonstrated.

Table 3.10 Difference Between Control and FACE Stomatal Index Means for Grass Species only. Showing both Ad and Abaxial

	D.glomerata	H.lanatus	A.elatius
Adaxial	+0.8	+1.5	-0.35
Abaxial	-4.5	-4.35	+4.5

- No significant CO_2 effect was displayed ($f=1.75$, $p<0.05$)
- Negative figures represent a reduction in stomatal index at elevated CO_2 .

The results of stomatal density (fig 4.4) were consistent with the findings of stomatal index. Whilst neither axial demonstrated a significant CO_2 effect, the abaxial appeared to be more sensitive to CO_2 , displaying a greater degree of variability (fig 4.4b).

**FIG 4.2 Stomatal Conductance Upper/ Lower Surface
for a) grass species & b) broad leaved species**

FIG 4.3 Stomatal Indices for Grass Species a) Adaxial & b) Abaxial

FIG 4.4 Stomatal Density for all Grass Species a) Adaxial & b) Abaxial

4. Discussion

In this study, significant ($p < 0.05$) inter and intraspecific variation in acclimation response was demonstrated, both within $V_{c \max}$ and $J_{\max}$, after long term exposure to elevated CO_2 . The location of intraspecific variation, in response to elevated CO_2 , is consistent with the work of Wulff and Alexander (1985) and Bazzaz (1995) however, in neither of these studies were plants grown in competition (in contrast to this study). Previously where plants have been grown in competition (and at FACE) significant intraspecific variation in harvestable biomass was not located (Luscher *et al* 1996).

In this study, significant interspecific differences occurred in 48% of cases, whilst intraspecific differences occurred in 27% of cases. The literature is vague in recording the relative sizes of inter and intraspecific variation in photosynthetic acclimation responses, as such this is clearly an area for future research projects.

The down regulation of $V_{c \max}$, observed as a decrease in the initial slope of the A/C_i plot, is indicative of a reduction in the amount (or activity) of Rubisco (Long *et al* 1993). Within the literature several mechanisms accounting for the reduction in $V_{c \max}$ are proposed, a redistribution of limiting resources (especially Nitrogen) may occur since at elevated C_a , the leaf may be in possession of excess Rubisco (Reinling 1994; Webber *et al* 1994). In this study a reduction in $V_{c \max}$ was demonstrated both at the species and genotype level, this is consistent with the work of Sage *et al* (1989) who demonstrated a reduction in $V_{c \max}$ in *C.album*, *P.vulgaris*, *B.oleracea* and *S.melongena* after exposure to elevated C_a . Many workers have attributed a low level of plant nutrition availability to a causal effect of negative photosynthetic adjustment, it is likely

that nitrogen is especially important (Sage *et al* 1989; Webber *et al* 1991; Long *et al* 1992; Rodgers *et al* 1996). Given the fact that in this study only one level of nitrogen was used ($100\text{Kg N ha}^{-1}\text{yr}^{-1}$) it is unclear as to what extent nitrogen had on negative photosynthetic adjustment. However, evidence supporting plant nutrition limitation leading to negative acclimation (Sage *et al* 1989; Webber *et al* 1991; Long *et al* 1992) was highlighted. The only broad-leaved species to display an up-regulation of both $V_{c\text{ max}}$ and J_{max} at elevated CO_2 was *T.pratense*. Both genotypes of the other two broad-leaved species showed a reduction in $V_{c\text{ max}}$ and J_{max} at elevated CO_2 .

The symbiotic relationship that forms between the *rhizobium* bacteria and the plant *T.pratense*, may potentially place the legumes at a competitive advantage in a future CO_2 enriched atmosphere. This up-regulation of the photosynthetic mechanism, supports the sink limitation theory of negative acclimation (Delucia *et al* 1985; Sasek *et al* 1985; Sage *et al* 1989; Arp 1991; Bunce 1992; Mousseau and Saugier 1992; Grulke *et al* 1993). The symbiotic relationship may cause a large carbon sink, it is unlikely therefore, that a disruption between sucrose production and utilisation would occur. In addition to this, the mycorrhizal infection common on this type of plant (legume) would act to further increase to the sink demand. Alternatively (or in addition) given that *T.pratense* is less likely to be nitrogen limited than the surrounding plants that it is in competition with, a physical limitation to sink size through a lack of available nitrogen is less likely than in non legumes. An up-regulation of both $V_{c\text{ max}}$ and J_{max} in *T.pratense*, is consistent with previous work (Nijs *et al* 1989; Luscher *et al* 1996). Luscher *et al* (1996) demonstrated an increase in harvestable biomass of 181% in legume species (*T.pratense* and *T.repens*) when grown at

elevated CO₂, as compared to an increase of 30% in non legume species. However, Luscher *et al* (1996) found no evidence for intra-specific variability in response to elevated CO₂. One limitation of this study, is that only one legume species was investigated, without doubt it would have been extremely useful to have data on another legume species e.g. *T.repens*. Such an examination would have facilitated a further strengthening of this discussion.

Given that the genotypes x species plots were in their fourth year of growth at elevated C_a, the results obtained provide a firm basis for determining the extent to which inter and intra-specific variation in photosynthetic acclimation response exists. The results obtained by this study suggest that long-term exposure to elevated C_a will cause species within a community and genotypes within a species to respond differently. It is widely regarded within the available literature (despite there being a radical lack of evidence) that if genotypes within a given population differ in their response to rising levels of CO₂, selection would act to favour those genotypes that poses the greatest ability to effectively utilise the CO₂ (Zisca and Teramura 1992). However, much further work is needed to determine how the utilisation of extra CO₂, expresses itself as an evolutionary advantage, it is clearly not sufficient to assume a competitive advantage, would be gained.

In this study, stomatal conductance of both ab and adaxial were found to decrease across a range of species at elevated CO₂. This is as would be expected, since a reduction in stomatal conductance at elevated CO₂ has been widely demonstrated (Cure and Acock 1986). Morison (1987) noted that at twice ambient C_a a typical reduction in stomatal conductance in the region of 40%

could be expected. It is probable that the greater degree of stomatal closure at elevated CO_2 partially accounted for the reduction in assimilation, as demonstrated in the genotype acclimation sampling.

Of main concern to this study was the finding of asymmetric responses between ab and adaxial to elevated C_a . Obvious conflicting differences were demonstrated in *D.glomerata* and *T.pratense*. In *D.glomerata* this study demonstrated a reduction in the importance of the abaxial at elevated CO_2 , whereas in *T. pratense* the abaxial clearly becomes more important. Pearson *et al* (1995) found a greater reduction in conductance for the adaxial, than for the abaxial in *R.obtusifolius*, consistent with the findings of *T.pratense* in this study. Possibly suggesting a functional distinction between grass species (mono-cots) and broad-leaved species (di-cots)

In this study, measurements of stomatal index and density, of grass species have shown there was no significant effect of CO_2 on either of these parameters. These findings are consistent with those of Radoglou and Jarvis (1990) and Rylà and Stanley (1992) who also demonstrated that elevated C_a had no effect on either stomatal index or density. A lack of significant differences in either of these parameters indicates that there were no direct effects of CO_2 enhancement on the initiation of the number of stomata. Initiation that occurs during ontogenesis, in addition there was no CO_2 induced effect on epidermal cell expansion at a later stage (Radoglou and Jarvis 1990). However, the results of this study, although not significant, did demonstrate a slight reduction in stomatal index and density on the adaxial of both *D.glomerata* and *H.lanatus* at elevated CO_2 . Woodward and Bazzaz (1988) noted that at elevated C_a there was a

tendency for a “slight” reduction in stomatal density, in a range of tree and shrub species.

Regarding the mechanisms surrounding shifts in stomatal densities at elevated C_a , Beerling and Woodward (1994) using variegated leaves demonstrated that such responses (as shown above) are more likely to be related to leaf structure. It was previously thought that stomatal density was closely linked to the carboxylation capacity of the mesophyll cells (Friend and Woodward 1990). Further to this, Boetsch *et al* (1996) commented that the mechanisms of stomatal partitioning (during leaf development) were largely unknown, but that environmental conditions reportedly influenced their frequency.

Contrary to what was demonstrated in this study, Woodward (1987) demonstrated a clear (40%) decrease in stomatal density in the leaves of eight temperate arboreal species, collected over the last two hundred years. This startling reduction in stomatal density was accounted for by the corresponding increase in C_a . A further contradiction to the findings of this study, were demonstrated by Beerling and Woodward (1995), this work commented that a reduction in stomatal density (level of significance not specified) with increasing C_a had been documented for 109 vascular plant species. It therefore seems likely that different functional groups of plants will react in different ways, regarding stomatal development under elevated CO_2 . These data suggest that the demonstrated reductions in stomatal conductance, were almost certainly as a result of a reduction in stomatal aperture (pore- length/width) rather than a reduction in stomatal frequency. Pearson *et al* (1995) noted that it was unlikely that a change in pore depth would occur, although in the absence of any

experimental data, this is a possible alternative hypothesis. The slight reduction in the stomatal index of *D.glomerata* and *H.lanatus* is unlikely to be sufficient enough to have had an effect on stomatal conductance.

The asymmetrical partitioning of gas exchange between the leaf surfaces, may have important implications for not only photosynthesis, but also for photosynthetic adjustment at elevated CO₂. A possible effect of this could be that due to a stomatal induced reduction in C_i the photosynthetic apparatus only acclimates on one axial.

From this study it appears that the reduction in stomatal conductance at elevated CO₂ in grass species is greater on the abaxial, it is likely therefore that negative photosynthetic acclimation may be more pronounced on the adaxial. In broad-leaved species, it is possible that this effect may be reversed. In this study, it was not possible to record partitioned photosynthetic gas exchange studies, between each axial, this undoubtedly would have been of considerable use. Considerable asymmetric responses in transpiration gas exchange, have been demonstrated, in this study and in other work (Pearson *et al* 1995). It is likely that such reduction in stomatal conductance, does have important implications for intra-plant photosynthesis and acclimation, in response to long-term exposure of elevated C_a this is clearly an area for future research projects.

5. Conclusion

This project has clearly demonstrated the presence of intra and considerable interspecific variation in acclimation response to long-term exposure of elevated C_a. As such, the results obtained provide a firm basis for determining the extent to which inter and intra-specific variation in

photosynthetic acclimation response exists. Such information is invaluable for long-term predictions on the development of the botanical composition of dynamic plant communities. This work is especially important given that agricultural and land-use policy, over the next fifty years is likely to dictate a return to extensively managed species rich grasslands. This work has demonstrated that species within a community are likely to be affected to a greater extent in their photosynthetic adjustment to elevated CO₂, than genotypes within a species. However, the fact that genotypic variation does exist, will have important implications for the evolutionary adaptations of plant populations.

In terms of ecosystem dynamics, it is possible that legume species (with an ability to “fix” atmospheric nitrogen) will increase their domination of species rich grassland communities.

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Appendix 1

Genotype Plots

Plate 1) Pre-cut

Plate 2) Post Cut

Plate 2. Layout of The Eschikon FACE Rings

1. Introduction

The rise in atmospheric carbon dioxide concentration (C_a) from a pre-industrial revolution concentration of $275 \mu\text{mol mol}^{-1}$ to the present day level of $\sim 350 \mu\text{mol mol}^{-1}$ (IPCC 1996) is currently a well documented and accepted phenomena. Driven by deforestation and more importantly anthropogenic emissions (primarily the burning of fossil fuel) projections suggest that C_a will reach 530 and $700 \mu\text{mol mol}^{-1}$ by 2050 and 2100 respectively. The draconian measures required to significantly reduce emissions are unlikely (IPCC 1996). In conjunction with this is a predicted rise in mean global temperature of 3°C to 4°C by 2050 (Watson *et al* 1990). This is likely to have significant effects on plant life.

Elevation of C_a will stimulate the carboxylation reaction of carbon dioxide fixation, catalysed by Rubisco (ribulose 1,5 bisphosphate carboxylate/oxygenase). Potentially this will allow higher rates of photosynthesis providing more carbohydrate for plant growth.

However in many cases, C_3 plants exposed to enhanced C_a at concentrations similar to those predicted for the middle of the next century, often show an erosion over time of this short term photosynthetic stimulation. Photosynthetic capacity when measured at ambient C_a is characteristically decreased (Cure & Acock 1986; Sage *et al* 1989) often to levels of CO_2 assimilation (A) less than equivalent plants only exposed to ambient CO_2 concentrations. The term “acclimation” has been used to describe this reduction in photosynthetic capacity (Long 1991).

Therefore it is evident that the complex interactions of these two parameters are likely to have significant effects on photosynthesis, growth and reproduction of plants within natural and semi-natural ecosystems (Kimball 1983; Cure & Acock 1986).

As yet genotypic variation has received very little attention in relation to plant responses at elevated CO_2 (Wulff & Alexander 1985; Zisca & Teramura 1992). If genotypic variation in photosynthetic response to long term exposure of elevated CO_2 does exist within a species, selection is likely to favour those genotypes that possess the greatest ability to effectively utilise the CO_2 . Potentially this could result in shifts in populations within dynamic ecosystems. The potential impacts of intraspecific variation in acclimation response within agriculture C_3 crops are considerable.

2. Acclimation

2.1 Short Term Stimulation by CO_2

A common response of C_3 plants grown at ambient C_a , when placed in an environment that is rich in CO_2 , is a short term stimulation in photosynthesis. Within the literature this is a widely reported and generally accepted phenomena (Long 1991).

Initially the rate of carboxylation is stimulated in favour of oxygenation, primarily for two reasons; Rubisco has a relatively low affinity for CO_2 and, at present atmospheric CO_2 concentrations (C_a), Rubisco is substrate limited. Secondly increasing the internal CO_2 concentration (C_i) increases its competitive ability relative to oxygen, in relation to access to Rubisco active sites. Thus, oxygenation and hence subsequent photorespiration is suppressed (Lorimer 1987;

Bowes 1991; Stitt 1991; Long *et al* 1993). Therefore on initial transfer to elevated CO₂ the uptake of CO₂/unit area leaf ($A-\mu\text{mol m}^{-2} \text{s}^{-1}$) is increased. Kimball (1983) suggested that a doubling of C_a (in the absence of any photosynthetic down regulation) could be expected to produce a general yield (biomass) increase of $33\% \pm 6\%$, across a wide range of C_3 plants.

2.2 Mechanisms of Short Term Stimulation

Fig1 Simulated response of light saturated leaves CO₂ uptake (A), with intercellular CO₂ concentration (C_i) (Farquhar *et al* 1980)

Fig1 clearly demonstrates the two initial responses in the fate of a typical C_3 leaf exposed to current C_a for its development stages. The model (Farquhar *et al* 1980) suggest that depending on C_i photosynthetic rate (A) is initially determined by the efficiency of carboxylation (i.e. amount of active Rubisco). The maximum rate of carboxylation ($V_{c \text{ max}}$) is given by $\delta A / \delta C_i$ and therefore provides an *in vivo* measure of the activity of Rubisco ($\text{mol m}^{-2} \text{s}^{-1}$) (Long *et al*

1993; Bowes 1991). Figure 1 also clearly demonstrates a change to a slower rise, in this region A is limited by the supply of RubP (Ribulose 1,5 bisphosphate) regeneration for carboxylation (dotted line), two factors are accepted to limit A in this region of the curve; 1) W_j is the potential rate of non cyclic electron transport, (capacity of the thylakoid membranes to supply ATP and NADPH). 2) W_p Represents the capacity of starch and sucrose to utilise triose phosphate and subsequently regenerate inorganic orthophosphate (P_i) (Sage *et al* 1994). Sharkey (1985) noted that this may be particularly important during carbohydrate synthesis.

Thus clearly the limitations of photosynthesis and mechanisms of acclimation may be evaluated in terms of Rubisco and RubP regeneration (Bowes 1993).

2.3 Acclimation of the Photosynthetic Apparatus to Long Term Exposure of Elevated CO_2

Long term (weeks to years) exposure of C_3 (Farquhar *et al* 1980) plants to elevated C_a has been shown by many workers to result in a “down regulation” of photosynthetic activity. Sage *et al* (1989) demonstrated that in some species under certain conditions, down regulation occurred to such an extent that the stimulation of A through the inhibition of photorespiration was completely lost. Therefore, after long term exposure to elevated C_a , A may be less than that, attained by equivalent plants grown at ambient CO_2 concentration.

The term “acclimation” has been used by several authors as an adjective to describe the observation of “down regulation,” (Long 1991; Bunce 1993; Grulke *et al* 1993). However, perhaps more correctly acclimation should be

referred to as, “negative photosynthetic adjustment,” since down regulation is not a universal feature of C_3 plants exposed to elevated C_a (Long *et al* 1993) (as will be demonstrated). This would appear in direct contrast to Reining (1994) who stated that down regulation was often assumed to be the typical reaction of C_3 plants when exposed to long term elevation of C_a . As such the term positive or negative acclimation should be used more correctly, to mean either stimulation or negative adjustment of the photosynthetic apparatus.

2.4 Mechanisms of Negative Down Regulation

Referring back to figure 1, analysis of the A/C_i response curve suggests that at present C_a , Rubisco activity and the capacity for RubP regeneration are co-limiting photosynthesis. Several authors (Long & Drake 1992; Long *et al* 1993) have suggested that this represents an optimisation of resources between Rubisco and the apparatus involved in RubP regeneration. It is suggested that nitrogen (N) is especially important, due to the large investment that the plant makes in N allocation (Rubisco may account for up to 25% of leaf N).

Thus when a plant is exposed to elevated C_a , (in the absence of any stomatal change) C_i could be increased to a point where A is solely limited by the capacity for RubP regeneration (Long personal communication 1995). As a result the leaf now experiences a situation where it is in excess of Rubisco, this clearly does not represent an optimal allocation of resources.

Thus given a control mechanism which allows plants to optimise their Rubisco concentration, a decline in amount of Rubisco would be expected (Webber *et al* 1994). Acock & Pasternak (1986) suggested that acclimation (negative) involves a redistribution of resources towards that most limiting

process, resulting in optimal survival, reproduction and growth under the prevailing environmental conditions.

The literature cites several mechanisms accounting for negative acclimation (Reining 1994). 1) A high C_a has been shown to lead to partial stomatal, potentially this would reduce carbon transport to the site of carboxylation (Mousseau & Saugier 1992). However, in order to eliminate the effect of stomatal conductance, A is generally measured against C_i . Thus A/C_i curves gained in this manner indicate that the limitations to A , after long term exposure to elevated CO_2 cannot be accounted for only by a limitation of CO_2 influx through the stomata (Delucia *et al* 1985).

As such, the literature cites further hypothesis. 2) The greater rate of assimilate production at elevated CO_2 (fig1) eventually leads to an excess of carbohydrate production, at rates greater than requirement. This potentially could lead to feed back inhibition of photosynthesis (Delucia *et al* 1985; Arp 1991; Grulke *et al* 1993; Webber *et al* 1994).

3) The third proposed mechanism accounting for negative acclimation is the frequently found reduction in activity (or amount) of Rubisco, thus potentially reducing the rate of carboxylation. The latter two of these hypotheses will be reviewed in the subsequent section.

2.4.1 Feedback Inhibition

The reduction in photosynthetic capacity has been attributed to end product inhibition, caused by an insufficient demand for carbohydrate, resulting in a disruption of the balance between production and utilisation (Arp 1991). Several workers have demonstrated an increase in accumulation of starch and sucrose in plants exposed to elevated C_a . Many workers have also demonstrated

support for this hypothesis of this negative inhibition of photosynthesis through sink limitation (Delucia *et al* 1985; Sasek *et al* 1985; Sage *et al* 1989; Bunce 1992; Moussea & Saugier 1992; Grulke *et al* 1993). At the extreme, it was thought that this imbalance may cause chloroplastic accumulation of soluble carbohydrate, in the form of starch, giving rise to mechanical damage (Cave *et al* 1981), or some other structural change (Crionit *et al* 1984). However these events now seem to be rare. Subsequent research, has revealed it to be much more likely that the plant's inability to utilise the extra sucrose produced, results in a reduction of flux of inorganic phosphate (P_i) from the cytosol to the chloroplast. Subsequent ATP and NADPH production is likely to be inhibited, therefore suppressing the rate of RubP regeneration (Arp 1991; Long & Drake 1992; Webber *et al* 1994).

A comprehensive understanding of negative acclimation through sink limitation has come from plants grown in pots where root growth (naturally a large sink) is limited. Arp (1991) demonstrated a strong correlation between pot size and photosynthetic capacity. It is generally accepted within the literature, that in the absence of restricted rooting volume (i.e. in field grown plants) plants grown at elevated C_a will have significantly stimulated root growth (Jongen *et al* 1995). Therefore it is reasonable to predict that pot grown plants exposed to elevated C_a will have altered root/shoot ratios, this has been shown to occur experimentally (Arp 1991).

Within the literature exists evidence for other forms of sink limitation resulting in negative photosynthetic acclimation of plants grown at elevated CO_2 (Clough *et al* 1981; Peet 1984; Arp & Drake 1991).

2.4.2 Importance of Nitrogen

Many workers have attributed low plant nutrient availability to a causal effect of negative photosynthetic adjustment (especially N) (Wong 1979; Sage *et al* 1989; Webber *et al* 1991; Long *et al* 1992; Rodgers *et al* 1996). Low N availability has been shown to reduce photosynthetic rate in both ambient grown and elevated grown plants, but suppression tends to be largest in elevated grown plants (Long *et al* 1992). Thus suggesting that low N availability reduces potential sink size and strength, possibly causing negative inhibition. In addition it is likely that under N limiting conditions, negative acclimation may occur as a result of the re-allocation of N from Rubisco to a more limiting process (Arp 1991).

2.4.3 Rubisco Limitations

The third of the limitations cited separately by Reinning (1994) as being a hypothesis accounting for negative acclimation of C₃ plants at elevated CO₂ is that of a reduction in the amount (or activity) of Rubisco. This is commonly observed through a decrease in the initial slope of the A/C_i plot (Long *et al* 1993).

Stitt (1991) referred to this hypothesis as “adaptive” regulation, as a reduction in amount of Rubisco, would allow resources such as amino acids to be mobilised from leaves to be re-invested in sinks. The literature provides convincing evidence for the existence of mechanisms, by which the accumulation of soluble carbohydrate could lead to the repression of specific genes, leading to decreased levels of Rubisco (and other Calvin cycle enzymes). It is likely that the observed decrease in photosynthetic proteins could be triggered by either;

- A) Changes in metabolites or P_i , occurring as a result of carbohydrate accumulation.
- B) Direct interaction of carbohydrate with a receptor.
- C) Physical changes within the leaf. (Webber *et al* 1994)

However some workers have demonstrated a reduction in activity of Rubisco in the absence of a reduction in amount (Von Caemmer & Farquhar 1984; Sage *et al* 1989). This is likely to indicate that Rubisco is being activated in response to a restriction elsewhere in the photosynthetic apparatus (Stitt 1991).

The accumulation of soluble carbohydrate produced by an imbalance between excess source and insufficient sink, must therefore be viewed as the primary cause of both short and long-term negative photosynthetic acclimation. In the short term the literature suggests that reductions in ATP and NADPH synthesis limit the rate of RubP regeneration. In the longer term “adaptive” acclimation, is likely to cause the repression of specific genes resulting in a reduced amount of photosynthetic proteins (including Rubisco) (Stitt 1991).

3. Inter/Intra Specific Variation in Response to Elevated C_a

3.1 Inter C_3 , C_4 and CAM Differences

Within the literature, there is a great deal of evidence suggesting that interspecific differences between many plant species, in response to elevation of C_a , is large. Such interspecific differences affect many plant physiological processes, (both photosynthetic and non photosynthetic). Perhaps the most obvious differences are the associated with between the species that differ in their photosynthetic carbon fixation pathway (Poorter 1993). A general assumption by many authors (Percy & Bjorkman 1983; Newton 1991) is that those plants with

a C_4 type pathway, through their ability to concentrate CO_2 at the site of Rubisco should not show a response in elevation of C_a .

Much evidence consistent with this hypothesis is available, highlighting the differential growth stimulation between C_3 and C_4 type plants. Hocking and Meyer (1991) investigated wheat (*Triticum aestivum*) and maize (*Zea mays*) grown at ambient ($\sim 355 \mu\text{mol mol}^{-1}$) and at elevated CO_2 ($1500 \mu\text{mol mol}^{-1}$). *T.aestivum* showed a significant increase in dry matter, *Z. mays* showed no CO_2 stimulation in term of extra dry matter production. Consistent with this report, Hunt *et al* (1991) found no stimulation of shoot dry matter again in *Z. mays* when grown at CO_2 concentrations of 540 and $700 \mu\text{mol mol}^{-1}$, whilst in the same trials demonstrating a stimulation of growth in 20 out of 26, C_3 species.

However it should be noted that several pieces of later work have suggested evidence in contrast to the above, generally accepted norm. Poorter (1993) investigated the interspecific variation of 156 different plant species in response to elevated C_a . Stimulation of growth (vegetative dry matter) was larger for C_3 than C_4 species, however a significant increase in dry weight was demonstrated across a wide range of C_4 species (41% for C_3 vs. 22% for C_4). Even under low nitrogen (N) conditions, where it would be expected that C_3 negative acclimation due to limited sink strength would be more prominent (Bazzaz 1990; Sage 1994; Arp 1991; Webber *et al* 1994) Wong & Osmond (1991) demonstrated a stimulation of growth in *Echinochloa frumentacea* (C_4) when grown at elevated as opposed to ambient C_a . This work concluded that under low N conditions there was a possible partial failure of the CO_2 concentrating mechanism in C_4 carbon fixation. Elevation of C_a has also been

demonstrated to significantly increase plant biomass of *Bouteloua gracilis* (C₄) again under low N conditions (Hunt *et al* 1996).

The improved growth of C₄ plants under elevated C_a as demonstrated above may partly be attributed to CO₂ induced stomatal closure and an improved water use efficiency (WUE) (Allen 1990). However, Hunt *et al* (1996) concluded that part of the C₄ growth stimulation could be attributed to a stimulation of photosynthetic activity.

Physiological data regarding CAM (Crassulacean Acid Metabolism) species exposed to elevated CO₂ is scarce. As CAM species utilise PEPCase (Phosphoenolpyruvate Carboxylase) for most of their initial fixation of C_a and as PEPCase can be saturated at current C_a (Ting 1994) little influence of elevated CO₂ may be expected for CAM species. For the six CAM species cited by Poorter (1993) the response was generally even smaller (<15%) than the average C₄ species. However again it is clear that substantial interspecific variation must exist. Nobel and Israel (1994) noted that the widely cultivated and highly productive prickly-pear cactus (*Opuntia ficus-indica*) demonstrated a substantial enhancement in net CO₂ uptake when placed in elevated CO₂. Substantial stimulation of CO₂ uptake was also demonstrated in *Agave salmiana* when placed in elevated C_a. The authors accounted for this through a higher inorganic carbon level in the carboxylating enzyme, a greater substrate affinity for PEPCase and a greater percentage of Rubisco in the activated state (Nobel *et al* 1996).

3.2 Inter C₃ Variation in Response to Elevated C_a

Interspecific variation in response to elevated C_a, within the species that demonstrate C₃ carbon fixation, across a wide range of plant physiological processes has been demonstrated to be large. Existing evidence demonstrates a wide range of responses in term of growth stimulation, reproductive characteristics, dry matter partitioning and photosynthesis.

Even in the absence of positive or negative stimulation, the C₃ photosynthetic mechanism clearly displays a wide range of interspecific variation. Wullschleger (1993) examined this interspecific variation in terms of maximum rate of carboxylation ($V_{c \max}$) and the maximum rate of electron transport ($J_{\max}$). Basing the study on 164 previously published A/C_i curves for 109 C₃ species, values for $V_{c \max}$ ranged from $6 \mu\text{mol m}^{-2} \text{s}^{-1}$ in *Picea abies*, to $194 \mu\text{mol m}^{-2} \text{s}^{-1}$ in *Beta vulgaris*. The average value for $V_{c \max}$ in this study was found to be $64 \mu\text{mol m}^{-2} \text{s}^{-1}$. Values for $J_{\max}$ ranged from $17 \mu\text{mol m}^{-2} \text{s}^{-1}$ again in *Picea abies*, to $372 \mu\text{mol m}^{-2} \text{s}^{-1}$ in *Malvastrum rotundifolium* (desert species), an average value of $134 \mu\text{mol m}^{-2} \text{s}^{-1}$ was demonstrated. Despite at first glances a wide range of values, when the author fitted the data in to the plant groups, herbaceous annuals and woody perennials, the data spread was greatly reduced. (Although studies such as this where many separate experiments are compared together, being a useful tool, indicating possible spread interspecific variation, they cannot be taken as absolute values as different values were not obtained under exactly the same conditions.) Indeed, Poorter (1993) posed the question, whether or not it would be possible to discern groups of C₃ species which differ in their response to an elevation of atmospheric C_a?

3.3 Sink Size/Strength and Inter C₃ Variation

One hypothesis accounting for interspecific variation in photosynthetic response relies on different plant species and their ability to generate sinks (Rogers *et al* 1996). In species where sink strength is limited either genetically or where it is imposed (e.g. cutting of forage species) down regulation may be realistically predicted. (For mechanisms see section 2.4)

Rogers *et al* (1996) commented that the difference in CO₂ response between *T. aestivum* and those species that may have sink limitations, (in the absence of nutrient limitations) may lie in a greater capacity of grasses to form sinks in the form of tillers. Maunney *et al* (1978) ascribed the larger growth stimulation of *Gossypium hirsutum* and *Glycine max* as compared to the relatively small stimulation under elevated CO₂ in *Helianthus annuus*, to the indeterminate nature of growth of the first two species.

Poorter (1993) suggested that further to this idea of sink strength being an important factor, governing interspecific variation it should follow that those plants that have been selected as crop plants with vigorous growth, should have strong sinks. This study then went on to show that the average “crop plant” showed a significantly larger response, to CO₂ enrichment when compared to the wild form of the same species, the difference in yield ratio being comparable to that between C₃ and C₄ species.

However it is clearly not sufficient to determine negative acclimation groups on a rough definition of “crop plants”. Sage *et al* (1989) examined the interspecific of acclimation in five cultivated species; *Phaseolus vulgaris* L, cv Kinden, *Solanum tuberosum*, *Chenopodium album*, *Brassica oleracea* and *Solanum melongena*. All plants were exposed to either 300 $\mu\text{mol mol}^{-1}$ or 900-

1000 $\mu\text{mol mol}^{-1}$ CO_2 from April to July. In all species Rubisco activation state was reduced after prolonged exposure, however only in two species was the actual content of Rubisco/unit leaf area reduced (*C.album* and *B.oleracea*). A/C_i plots indicated a reduced rate of A at elevated C_a in all species apart from *S.tuberosum*, where A was similar to control grown plants up to a C_a of 450 $\mu\text{mol mol}^{-1}$ positive acclimation was observed above this point.

However it should be noted that this experiment maintained an elevated C_a of 900-1000 $\mu\text{mol mol}^{-1}$, this is clearly a large range and may have been the source of large error. Hendry *et al* (1993) commented that FACE (Free Air CO_2 Enrichment) technology allowed field elevation of C_a at a set target $\pm 10\%$ based on 1 minute averages, over 90% of the time, thus giving much more accuracy of control of C_a .

It is clear that even from this study (Sage *et al* 1989) of only five C_3 crop species that interspecific variation in acclimation response is large. No two species exhibited identical quantitative responses to elevated C_a , yet all exhibited reduced Rubisco activity.

3.4 Intraspecific Variation in the Response to Elevated CO_2

Within the literature it appears that very little work has addressed intraspecific variation in plant responses to elevated CO_2 (Wulff & Alexander 1985; Ziska & Teramura 1992). This is surprising and perhaps alarming for several reasons; it is evident that C_a levels are rising at an unprecedented rate (IPCC 1996). Secondly, it has been known that genotypic variation within populations in response to elevated C_a does exist, both between naturally

occurring ecotypes and within existing cultivars of agronomically important species (Eagles 1967; Nosberger *et al* 1983).

Intraspecific variation in response to elevation of C_a , within natural ecosystems could act to favour those genotypes that show improved fitness under the new environmental conditions (Ziska & Teramura 1992). Thus, potentially resulting in significant population changes with dynamic ecosystems. Rapid evolution of tolerance causing evolutionary shifts in populations, within a few generations, has already occurred, e.g. where species have been exposed to strong selection under pollution (Bradshaw & McNeilly 1991).

Within agricultural systems it is evident that more research is needed, studying the responses of different genotypes/cultivars to elevation of C_a (Hesketh & Hellmers 1973; Heichel & Musgrave 1996). This is necessary if future breeding programmes are to select for desired characteristics (such as limitation of negative stimulation). This work would allow crops to optimise in their “resource capture” under future climatic conditions (Morrison 1996).

Since photosynthesis is the major physiological process by which plants sense and by which their production can respond directly to, rising atmospheric CO_2 concentrations (Mott 1990; Long & Drake 1992). Clearly intraspecific variation in photosynthetic response to rising C_a is an important area of research, since many interspecific studies have clearly demonstrated acclimation of photosynthesis (positive and negative) after long term exposure. Within the literature it is not clear at all, as to what extent intraspecific variation in acclimation exists.

Overall it appears that intraspecific variation in response to elevated C_a represents a large gap in the understanding of plant physiology, and in terms of photosynthesis it is largely and unanswered question.

3.5 Evidence for Intraspecific Variation in Response to elevated CO_2

Zisca & Termura (1992) attempted to investigate intraspecific variation in the response of *Oryza sativa* (rice) to elevated CO_2 . They grew cultivars at ambient ($350\mu\text{mol mol}^{-1}$) and elevated CO_2 ($630\mu\text{mol mol}^{-1}$). Elevation of C_a caused an enhancement of photosynthetic activity, resulting in a 50% stimulation of A in both cultivars. Photosynthetic enhancement was still evident after three months.

However, it must be noted that this work attempted to investigate intraspecific variation by using only two cultivars of the same species. It is extremely dubious, that the use of such a limited number of cultivars is sufficient to determine the presence of intraspecific variation. The work also only exposed cultivars to elevated C_a for three months, whether this was sufficient exposure time for intraspecific differences in acclimation to be detected is unclear. The literature is vague in terms of time of response for acclimation to be detected, it seems common to refer to acclimation after “long term exposure” to elevated CO_2 meaning weeks to years (Rowes 1991; Webber *et al* 1994; Amthor 1995; Jacob *et al* 1995).

Work with more genotypes of *Plantago lanceolata* (4 genotypes) revealed significant intraspecific differences in response to elevated C_a ; seed weight of the F_1 generation, germination of these seeds, germination rate and leaf area at harvest all showed significant differences. This work suggested that within

P.lanceolata the probability of genetic variation is high (Wulff & Alexander 1985).

Although no leaf gas exchange measurements were taken during this study (which would have been useful), the intraspecific differences in leaf area growth rate, between control and elevated plots, although not significantly different did show a wide degree of spread ($0.008\text{--}0.26\text{ cm}^2\text{ day}^{-1}$). Thus suggesting that intraspecific differences in photosynthetic response may exist.

Perhaps the most in-depth study of inter Vs intraspecific variation in response to elevation of C_a to date, is that of Farnsworth & Bazzaz (1995). This study aimed to examine variation in growth (and reproductive characteristics) of plants exhibiting a wide range of floral morphologies, within the genera, *Polygonum*, *Ipomoea* and *Cassia*. Within the three *Cassia* genotypes studied, intraspecific variability in terms of vegetative growth in response to elevated C_a was demonstrated. *C. obtusifoli* and *C.fasciculata* responded positively to CO_2 enrichment in terms of early biomass accumulation. *C.nictitans* however exhibited lower rates of branching, height growth and leaf production under elevated CO_2 . The work concluded that intraspecific differences were demonstrated in physiological sensitivity, vegetative architecture, reproductive morphology and dry matter allocation patterns.

A major point that must be noted is that the rise in atmospheric CO_2 concentration is occurring gradually, not as a single doubling effect, as in a majority of above experiments. Consequently the pattern of plant responses across a broad range of CO_2 concentrations must be examined, in order to fully investigate intraspecific variation (Ackerly & Bazzaz 1995).

A further criticism of the above experiments, is that plants within natural (and agricultural) ecosystems, rarely exist by themselves. Thus the effects of competition on intraspecific variation to elevated CO₂ must also be investigated, competition may have important effects on factors such as shading (Wong & Osmond 1991; Mitchell *et al* 1996), water supply (Bruce *et al* 1995; Hunt *et al* 1996) and plant nutrition (Hocking & Meyer 1991; Wong & Osmond 1991).

4. Conclusion

Despite the fact that there is unprecedented evidence of an increase in atmospheric CO₂ concentration, and that this is likely to have highly significant effects on many C₃ plant systems, within the literature there is still uncertainty as to the precise mechanisms controlling photosynthesis under elevated CO₂. These uncertainties will have to form the basis of future research programmes, if the impacts of climate change and especially rising CO₂ on plants are to be fully understood.

The data clearly needs enlarging as to the precise control mechanisms of both short term (hours-days) and long term “adaptive” negative acclimation.

The need for field experiments is paramount in order to avoid the constraints of controlled chamber experiments and the subsequent alteration of the organelle, cell, leaf, plant and canopy microclimate. This requirement for ecologically and agriculturally realistic conditions, where species are grown in competition with each other rather than in pure stands will further advance the understanding of negative acclimation mechanisms.

It is also important that field studies are linked to mechanistic models, allowing specific predictions of plant/climate change interactions to be tested, in

order to facilitate the identification of those areas of study that require more attention.

The understanding of intraspecific variation in response to elevation of CO_2 is evidently only in its embryonic form and as such is under represented within the literature. This is clearly an area for future work if the impacts of increasing C_a on dynamic ecosystems and agricultural plants are to be fully understood. Especially important and as yet largely unanswered, is the question of intraspecific variation in photosynthetic acclimation response to elevated CO_2 . Within those species that intraspecific variation is large this may result in population shifts.

In terms of agricultural systems, the identification of those genotypes/cultivars that display desired characteristics under elevated CO_2 is important, as yet this has not been adequately analysed, it needs to be addressed if crops are to optimise in their resource capture under future climatic conditions.

Appendix 3

Project Plan

By Matthew Wilkinson

A Comparison of Intra and Interspecific Variation of Photosynthetic Acclimation After Four Years Growth at Elevated CO₂.

1. Introduction

The predicted rise in atmospheric Carbon Dioxide concentration (C_a) due to anthropogenic emissions is currently a well documented phenomena. Current trends indicated by Global Climate Models (GCM) predict a future doubling of C_a from $350 \mu\text{mol mol}^{-1}$ in 1990 to 700 mol mol^{-1} by 2100 (Edwards *et al*,1984).

Many workers have demonstrated an initial, short term stimulation of photosynthesis in C_3 plants when exposed to elevated C_a (Strain and Cure, 1985). In many instances however, this initial stimulation of photosynthetic activity erodes with time, often resulting in similar or even lower rates of photosynthesis compared with equivalent plants grown in ambient CO_2 . The term acclimation has been used to describe this effect on photosynthetic development and maintenance (Long *et al.*,1993). As such further understanding of how the photosynthetic capacity of important grassland species will acclimate is vital.

Since photosynthesis is the primary physiological process by which plants sense and respond to rising C_a (Mott, 1990). The question of how different species will respond is clearly an important one, with important long term

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implications for species fitness. The intraspecific genetic spread within a species in terms of acclimation response will play a dominant role in the species survival under future CO₂ concentrations. Within natural ecosystems those species with a low intraspecific variability to acclimation may be out competed by those species with a large spread.

2. Aims and Hypothesis

A majority of the work investigating acclimation of the photosynthesis apparatus in C₃ species to elevated Ca has centred around inter-specific variation. The basis for this project, is to primarily investigate whether inter-specific variation exceeds intraspecific variation in several important grassland species. As yet, this is largely an unanswered question for photosynthesis. The project will therefore aim to address the following areas of study:

1) The study of inter and intraspecific variation of acclimation response after 4 years exposure to elevated CO₂ in genotypes of the following species;

Grasses	Dicots
<i>Lolium multiflorum</i>	<i>Ranunculus frisianus</i>
<i>Dactylis glomerata</i>	<i>Rumex acetosa</i>
<i>Triesetum flavescens</i>	<i>Rumex obtusifolius</i>
<i>Holoccus lanatus</i>	<i>Trifolium pratense</i>
<i>Arrhenatherum elatius</i>	<i>Trifolium repens</i>
<i>Festuca pratensis</i>	

The above plants were sown into a background sward of *Lolium perenne* in 1993, at 35cm gaps; for each species there are about 10-14 genotypes. The acclimation studies are, therefore, in competition with *Lolium perenne*.

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The null hypothesis states that, there is no difference between inter- and intra-specific variation in acclimation to 4 years growth at elevated Ca.

3. Methods

The primary method involved in the above investigation will be analysis of the acclimation index which will be calculated for as many species as possible (dependant on time allowance) using the following equation:-

Acclimation Index

A measured,growth

Index = $\frac{A_{600,600} - A_{360,360}}{A_{600,600} - A_{360,360}}$ enriched Ca

$A_{600,600} - A_{360,360}$ ambient Ca

0 = complete acclimation

1 = no acclimation

>1 = positive acclimation

(Long 1990 Personal-Communication)

Initially a trial will be run to assess how long measurements are likely to take; on the basis of this the number of species that can be realistically investigated will be calculated. Measurements will be replicated three times for representative leaves on each genotype, within each of the three sets of the paired FACE and corresponding ambient CO₂ rings. For those cultivars which demonstrate large intraspecific variation, further investigation (A/C_i response curves) will be carried out to provide more information on photosynthetic acclimation.

A/C_i Response Curves

Using representative leaves for each treatment, in each pair of rings, the response of A (Assimilation) over the range of Ca 50-900 $\mu\text{mol mol}^{-1}$ will be assessed. Curves will be fitted using the maximum likelihood method (Farquhar *et al.*, 1980), providing estimates of the maximum velocity of Rubisco carboxylation ($v_{c \text{ max}}$) and maximum capacity for coupled electron transport (J_{max}).

Further Measurements

In addition to the gas exchange measurements, measurements of plant growth may be made depending on time allowance. Individual leaves within each cultivar will be tagged and the number of leaves formed, and their longevity, assessed. Growth in height (through non-destructive ruler measurements) and growth of leaf area (calculated from length\width measurements) will be made. Final leaf area\ dry weight\ organ partitioning will be assessed by destructive harvesting (as this is their final year of growth at elevated Ca).

4. Design of the Experiment

The FACE system at ETHZ Eshikon, Switzerland comprises of 6 rings in total. Three of the rings are exposed to elevated CO₂ (@ 600 $\mu\text{mol mol}^{-1}$) and three exposed to ambient CO₂ (@ 355 $\mu\text{mol mol}^{-1}$). Such a design allows collected data to be analysed using ANOVA techniques. The statistical significance of :-Genotype x Species x Treatment x Block, will eventually be tested.

5. Time Table

26Apr-7May: Familiarisation with open gas analysis system, Essex

8May: Depart for Eshikon, Switzerland

May: Acclimation index measurements to be commenced. Literature review on going.

June: All measurements continued. A/C_i measurements as necessary.

Early July: All measurements continued

Late July: Measurements brought to an end. Destructive harvesting as required.

31 July: Return to U.K.

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