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THE PHOTOSYNTHETIC AND STOMATAL RESPONSE OF *MEDICAGO SATIVA* CV. SARANAC TO FREE-AIR CO_2 ENRICHMENT (F.A.C.E.) AND NITROGEN

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Biosystems and Process Sciences Division

DEPARTMENT OF APPLIED SCIENCE

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by

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ABSTRACT.

Plots of *Medicago sativa* cv. saranac were grown in the field at ambient (355µmol CO_2 mol^{-1} air) or elevated (600µmol CO_2 mol^{-1} air) CO_2 concentrations. High (200kg yr^{-1}) or low (20kg yr^{-1}) nitrogen levels were applied to two isogenic lines, one able and one unable to use nitrogen fixing bacteria. Plants were in the second year of field growth. Exposure to elevated CO_2 was via a Free-Air CO_2 Enrichment System (FACE). Elevated CO_2 increased diurnal assimilation by between 12% and 92%. Analysis of A/C_i responses showed that effective nitrogen fertilisation was more important to rubisCO and RuBP activity than elevated CO_2. No acclimation was consistently observed. Leaves lower down the canopy were found to have lower Vc_{max} and J_{max} values, though age may be the cause of the latter effect. FACE conditions have only a small effect on these responses. There was some evidence found for the down-regulation of photosynthesis in the late afternoon. The FACE conditions had no affect on stomatal density but did increase epidermal cell density.

Keywords:

Medicago sativa, Free-Air CO_2 Enrichment, photosynthesis, acclimation, A/C_i response, stomatal index, canopy effects.

Abbreviations used in text:

A= CO_2 assimilation rate (µmol CO_2 m^{-2} s^{-1}); A_{sat}= light saturated A; C_a= ambient atmospheric CO_2 concentration (µmol CO_2 mol^{-1} air); C_i= intercellular CO_2 concentration (µmol CO_2 mol^{-1} air); **E**= H_2O transpiration rate (mmol H_2O m^{-2} s^{-1}); J_{max}= maximum light saturated rate of electron transport (µmol m^{-2} s^{-1}); **PPFD**= photosynthetic photon flux density (µmol m^{-2} s^{-1}); **rubisCO**= Ribulose- 1,5 bisphophate Carboxylase-Oxygenase; **RuBP**= Ribulose- 1,5 bisphophate; Vc_{max} = maximum rate of carboxylation (µmol m^{-2} s^{-1}).

1: INTRODUCTION.

At present the Earth's atmosphere is receiving an extra 8 Gt of carbon each year, mainly from fossil fuel burning and changes in land use (Idso, 1989; Schneider, 1990). The atmospheric concentration of CO_2 (C_a) is, at present, 350ppm. This is a 30% increase from the pre-industrial level of 280ppm. Much of this increase has occurred in the last 30 years. The rate of this increase is such that it has been estimated that C_a may be double the pre-industrial level by 2050 and may reach 700ppm by the end of the next century (Watson *et.al.* 1990; IPCC business as usual scenario). CO_2 is the substrate for photosynthesis and photosynthesis is the major physiological process by which plants sense and respond directly to changes in C_a (Mott, 1990; Long & Drake, 1992). Understanding the responses of photosynthesis to increased C_a is therefore fundamental to understanding the responses of plants (Long *et.al.* 1993).

In C_3 species, photosynthesis and transpiration are directly affected at elevated C_a (Poorter, 1993). Transpiration is reduced due to decreased stomatal conductance (Morison,1987), whilst photosynthesis is stimulated (Stitt, 1991), this stimulation occurs for two reasons:

1) Most importantly, CO_2 is a competitive inhibitor of the oxygenation of RuBP by rubisCO, so photorespiration is partially suppressed (Webber *et.al.* 1994). Since photorespiration may depress the CO_2 assimilation rate (A, μmol CO_2 mol^{-1} air) by up to 50% in temperate climates, substantial increases in A (Long, 1991) and plant productivity (Cure & Acock,1986), may be expected if C_a continues to rise.

2) The current C_a is insufficient to saturate rubisCO in C_3 species, therefore any increase in C_a will allow this extra capacity to be used to increase carboxylation velocity by increasing substrate binding (Webber *et.al.* 1994).

Poorter (1993), in a review of 89 reports of 156 species, found an average increase of 37% in plant growth with and doubling of growth C_a. However, it has been shown in many studies that in some plants grown under elevated C_a, this stimulation does not last longer than a few weeks or months (Sage *et.al.* 1989; DeLucia *et.al.* 1985; Ehret & Jolliffe, 1985). This down-regulation or 'acclimation' of the photosynthetic response varies between plant species and appears to be most pronounced in plants which have the least ability to produce new or larger sinks for the increased photosynthate produced in elevated C_a (Arp, 1991; Long & Drake, 1992). The most consistent biochemical changes are:

i) increased leaf carbohydrate (Stitt, 1991), either as starch or soluble sugars;

ii) decreased rubisCO activity (Wong, 1979; Yelle *et.al.* 1989; Sage *et.al.* 1989; Rowland-Bamford *et.al.* 1991).

The decline in rubisCO activity has been quantified using leaf gas exchange measurements and the construction of curves of A_{sat} at different C_i. Analysis of the A/C_i response of plant species provides an *in vivo* method of quantitatively separating the relative limitations imposed by stomata, carboxylation efficiency and capacity for regeneration of RuBP, on photosynthesis in leaves (Long & Drake, 1992). The initial slope of the response is determined by the amount of active rubisCO in the leaf, the slope 'plateau' is determined, mostly, by the rate of RuBP regeneration. For a wide range of C_3 species it has been shown that the C_i which they maintain under the current ambient CO_2 concentrations is usually at the point on the A/C_i curve where both rubisCO and RuBP regeneration are co-limiting (Wullschleger, 1993; Stitt, 1991). This is usually considered to represent an optimisation of resources. It would seem plausible to suggest that plants have evolved to make the best use of potentially limiting resources such as nitrogen, CO_2 and

light. An increase in C_a should lead to a reallocation of resources to maintain the co-limiting balance (Long, 1991). This will be especially true if the plant cannot use the excess photosynthate, i.e. in low rooting volumes or, as rubisCO is a major sink for leaf nitrogen, if nitrogen is scarce (Terashima & Evans, 1988). It has been suggested that sink feedback mechanisms such as leaf carbohydrate accumulation (Cave *et.al.* 1981),could lead to reduced rubisCO activity. A number of mechanisms for the link between carbohydrate accumulation and down-regulation of photosynthetic response has been suggested, these include; phosphate limitation (Stitt *et.al.* 1987), direct effects by starch grains (Grub & Mächler, 1990), changes in photosynthetic protein abundances (Sheen, 1990) and direct changes in genetic transcription rates (Webber *et.al.* 1994). All may play a part in the down-regulation of photosynthesis when photosynthate is not utilised.

Changes in stomatal characteristics could be a factor in observed gas exchange responses in plants grown at elevated C_a . Decreases in stomatal density have been demonstrated in plants grown at high C_a (O'Leary & Knecht, 1981; Woodward & Bazzaz, 1988). Other studies have failed to find this relationship (Korner, 1988; Radoglou & Jarvis, 1993; Ferris & Taylor, 1994). No (known) previous study of this type has considered either *Medicago sativa* or field grown legumes. As stomata represent the first interface between plants and their atmospheric environment, any effects, of C_a ,on their relative numbers could have major implications for plant/C_a interactions.

Medicago sativa L. (Lucerne in Europe, Alfalfa in the U.S.) is a perennial species of the Fabaceae family. It is widely used as a forage crop as it is highly productive and nutritious with an especially high protein content. It also forms root nodules, having a symbiotic relationship with the nitrogen-fixing bacteria *Rhizobium melioti*. *M.sativa* which is cut or grazed at intervals remains productive and vigorous (Langer & Hill, 1991). In dry

climates, such as Mediterranean regions, *M.sativa* can out-yield other forage plants. Ascertaining the physiological behaviour of *M.sativa* in elevated C_a is important for a number of reasons:

1) *M.sativa* is a widely used forage crop, elevated C_a could have implications for its cultivation and management i.e. cutting, grazing and fertilisation regimes. These practices may have to be modified in order to optimise any changes in productivity that may occur in response to elevated C_a.

2) As *M.sativa* is a nodulating plant, it could provide useful information about the general responses, of other such species, to increased C_a. A number of studies have shown that elevated C_a can increase both nodule numbers, mass and specific activity (Stulen & Hertog, 1993; Hardy & Havelka, 1976; Masterson & Sherwood, 1978). It has also been suggested that root nodules represent a large carbohydrate sink as large amounts of carbohydrates are consumed in the nodules to provide the energy for N-fixation (Poorter, 1993). Therefore in reducing nitrogen stress and increasing potential sink strength, root nodules may alter the plants response to elevated C_a. In a review of studies Poorter (1993) concluded that, on average, the photosynthetic response of nodulating C_3 species is higher than that of non-nodulating.

3) Bunce (1993) suggests that in a periodically harvested crop such as *M.sativa*, the stimulation due to elevated C_a, might be more persistent. After harvesting, the source: sink balance would be reduced so reducing feedback inhibition. It is therefore important to examine the relationship between defoliation, i.e. cutting or grazing, and continued productivity and how growth in elevated C_a affects this.

There have been a number of studies involving the effects of elevated C_a on Lucerne (see Table 1).

Table 1: Summary of results found in *Medicago sativa,* grown in doubled C_a.

Effect Study	Growth conditions	Photosynthesis	Comments
Morison & Gifford, 1984, 1984a.	phytotron	75%↑ leaf area. 78%↑ dry weight. 450%↑ dry weight of reproductive parts	Luceme showed some of the biggest gains in comparison to 16 other species
Baysdorfer & Bassham, 1985.	Controlled environment	Photosynthesis only enhanced in seedlings or defoliated plants.	Suggests plant is sink limited over much of life cycle
Bunce, 1993.	open-topped chambers in field	Photosynthesis persistently stimulated, especially at higher temperatures Biomass not increased. Increased water use efficiency not consistently observed	Showed large variation, probably due to precipitation.

These studies appear contradictory, i.e. the phytotron produced large biomass gains not found in the field. In the field studies the photosynthetic rate was consistently stimulated but there was no increase in biomass, this response has been found in other perennial species (Korner *et.al.* 1992; Norby *et.al* 1992), and implies compensating changes in resource allocation (Bunce, 1993). The field study also indicates that *M.sativa* will be influenced by a number of other factors, especially precipitation.

However, confidence in these studies is limited by their lack of realism. All involve enclosure systems which have been shown to significantly alter microclimatic conditions (Lawlor & Mitchell, 1991). It has also been shown that rooting conditions can produce acclimatory responses. These conditions include restricted rooting volumes (Arp, 1991) and nitrogen stress due to porous media (Vessey *et.al.* 1990), neither of which may be encountered in the field. In nearly all crops studied in the field, there is no acclimation of photosynthesis (Campbell *et.al.* 1988). The differences between enclosure and field results

demonstrate the need for field-based experiments to determine the effect of elevated C_a on plant processes in a realistic environment.

The Free-Air CO_2 Enrichment (FACE) system developed at the Brookhaven National Laboratory, provides an open-field growth environment with the ability to control ambient C_a conditions over long time periods, without any alteration of microclimate (Hendrey *et.al.* 1993). This study aims to add field derived data to that from the studies previously mentioned. To this end it aims to examine the photosynthetic and stomatal responses of *M. sativa* to elevated C_a growth conditions. It also aims to examine the effects of nitrogen status and leaf canopy position/age on these responses. Leaf surface cell characteristics will also be examined to see if growth C_a has any influence. To do this the report intends to address the following questions:

i) How does field-growth in ambient and elevated C_a affect the photosynthetic response of *M.sativa*? To address this question A/C_i curves for ambient and elevated C_a grown plants were constructed.

ii) How does nitrogen status affect these responses? In both CO_2 environments three treatments were studied, 1) nodulating and 2) non-nodulating growing in low-N, and 3) non-nodulating but fertilised with inorganic N. A/C_i responses were compared between these treatments.

iii) Does acclimation occur or is it merely an artefact of experimental conditions? To look at this a time series of A/C_i responses was produced and responses in terms of C_a and nitrogen compared.

iv) Does cutting produce any changes in A/C_i response? Are changes different depending on the C_a and/or N treatment? The crop was cut twice during the season to allow this to be studied.

iv) Are there any differences in the diurnal response of these plants (ambient/elevated C_a, nitrogen)? Diurnal sequences of A under natural light levels were made to examine this.

vi) How does the position of a leaf within the plant canopy affect A/C_i response? Leaves shaded within canopies such as *M. sativa* show acclimatory changes in the photosynthetic system typical of shade-adapted leaves (Evans, 1993). The photosystem antenna and light-harvesting complex protein content increase, and their stoichiometry change, leading to more efficient light capture and energy transduction (Baker & McKiernan, 1988). Photosynthetic components important to light-saturated capacity decrease, leading to a reduction in A_{sat} (Boardman, 1977). Shade-acclimated leaves in *M. sativa* have been shown to experience decreases in both V_{cmax} and A_{max} (Evans, 1993). How does field growth in elevated C_a affect these changes?

vii) Does C_a or N treatment have any effect on the epidermal cell characteristics of *M.sativa*? Adaxial and abaxial imprints of leaves on acrylic plastic slides were examined and epidermal/stomatal cell numbers and stomatal index values were calculated and compared.

2:MATERIALS AND METHODS.

2.1: Growth Conditions.

2.1.1: Atmospheric-

Medicago sativa cv. Saranac was exposed to elevated C_a in the field using a Free Air Carbon Dioxide Enrichment (FACE) system. This system, designed and built by Brookhaven National Laboratory is able to achieve controlled elevation of C_a within and above the crop under field conditions. The F.A.C.E. apparatus consists of a 14m-diameter toroidal plenum buried under the soil surface. 32 vertical vent pipes are connected to the plenum at 2m intervals. Air, enriched with CO_2, was blown over the plots through tri-directional jets in the vertical pipes at elevations of 0.5 - 1.5 m, depending on crop height. A computer control system used wind speed, direction and CO_2 concentration, measured at the center of each ring, to determine which valves upwind of the plots should be opened to maintain the set-point concentration. The enrichment was maintained from March to November during all daylight hours when the leaf temperature is expected to be greater than 8°C. One-minute integrals of C_a were within 10% of the target concentration (540 to 660 $\mu mol\ mol^{-1}$), for 89 to 94% of the time, in 1993 and 1994 (Nagy *et.al.* 1995). There were three F.A.C.E. rings and three control rings, the layout of these rings is shown in Appendix 1. The control rings do not have any F.A.C.E. apparatus, in 1993 and 1994 normal ambient C_a varied slightly with season and time of day but averaged 355 $\mu mol\ mol^{-1}$ (Nagy *et.al.* 1995). Experimental plots were located in each of the six rings producing 3 replicates per atmospheric treatment. A plan of the plots in the rings is shown in Fig 1.

FIG 1: The layout of the various plots/treatments within a ring.

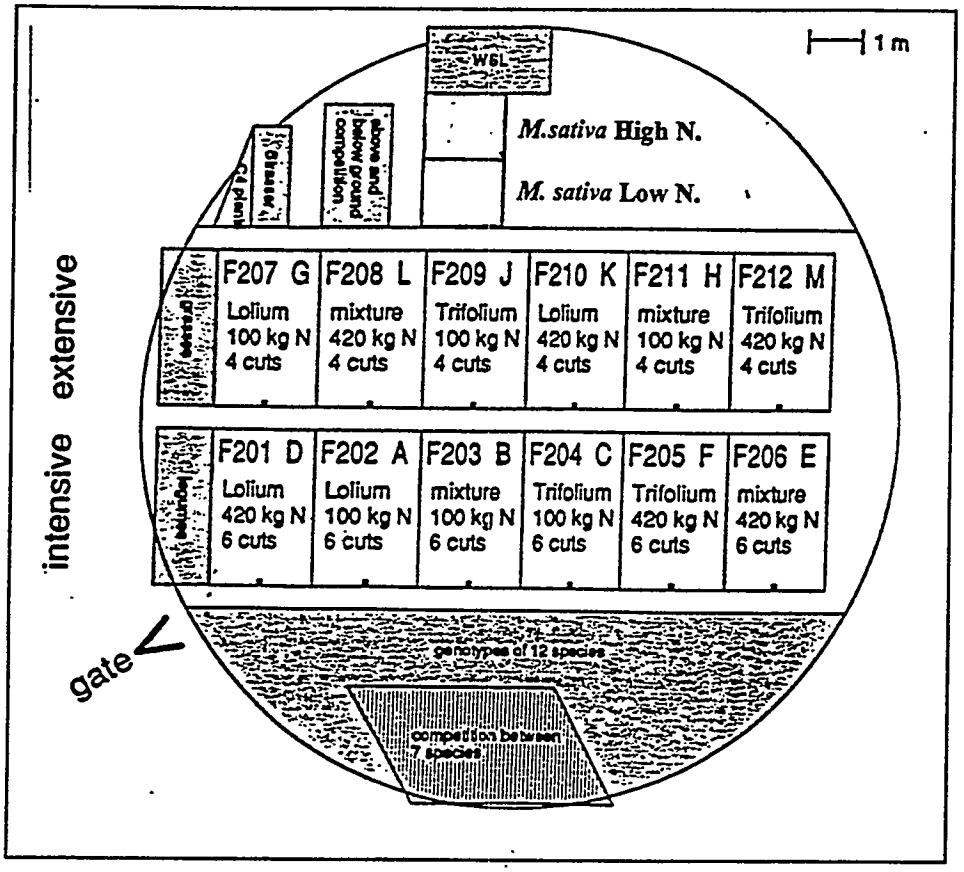
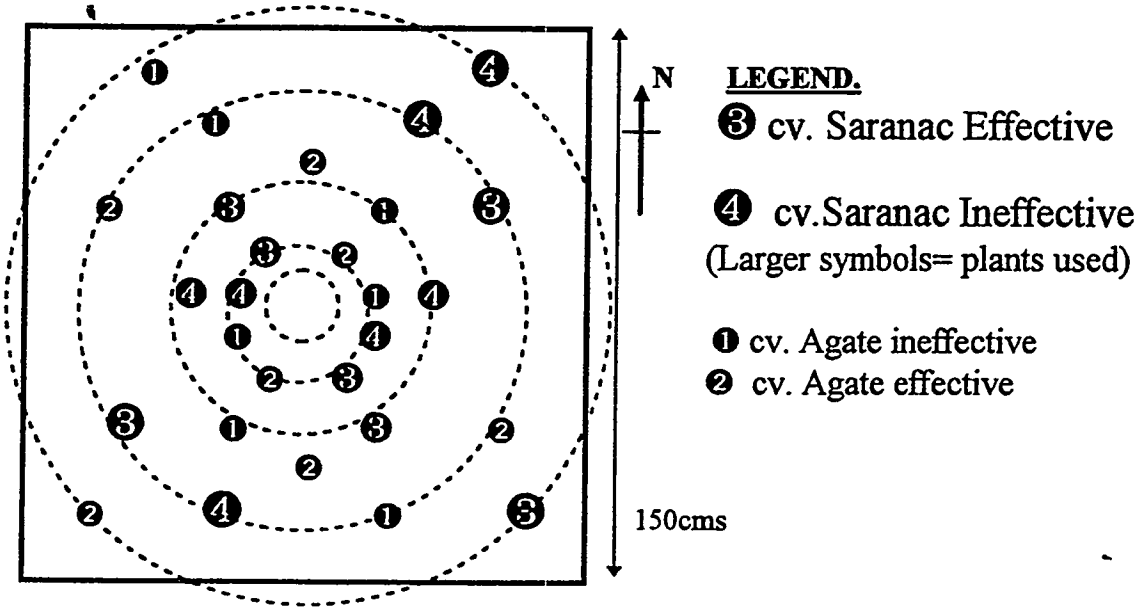


FIG 2: Plot Design for *Medicago sativa.*



2.1.2: Plot design-

Medicago sativa cv.Saranac was grown in plots 145cms by 150cms (see fig. 2). There were two plots per ring, one a low nitrogen treatment, the other high, Table 2 shows these nitrogen treatments.

Table 2 : Nitrogen treatments for *Medicago sativa.* F.A.C.E and Control,1994 & 1995.

	High Nitrogen kg N year $^{-1}$	**Low Nitrogen kg N year $^{-1}$**
1994	580	0
1995	200	20

Within each plot the plants were in density gradient with the highest density at the centre (see fig 2). Each plot had two cultivars and two isogenic lines of each, one line could effectively nodulate with *Rhizobium meliloti* (hereafter referred to as nodulating) whilst the other could not (hereafter referred to as non-nodulating). The plants were seeded in a glasshouse in March 1994 and planted into the field in mid-May (1994). The plants used for this report were the nodulating and non-nodulating cv. Saranac, in the fifth density ring (diameter 106cms, see fig. 2).

2.2: Gas Exchange Measurements.

2.2.1: Equipment-

Leaf gas exchange measurements were made using a CIRAS 1 analyser (version 1.4, PP Systems, Hitchin, Herts, UK). This is an open, combined infra-red gas analysis system. This was attached to a Parkinson Leaf Cuvette (Version 1.1, PP Systems, Hitchin, Herts, UK). The leaf chamber allowed 2.5cm^2 leaf area to be analysed.

Water vapour calibration for the CIRAS was via a water vapour generator (Type WG-600, Analytical Development Co.Ltd. Hoddesdon, Herts, UK). The CIRAS was calibrated for 10.1mb water vapour. CO_2 calibration was at 608 µmol mol^{-1} (27548-Type 30L- Carbagas, Swiss Calibration). The gas exchange parameters A, C_i, gs and E were calculated according to Von Caemmerer and Farquhar (1981) and standardised to 25°C (Harley *et.al.* 1992).

All measurements, unless otherwise stated, were taken using the central leaflet of the first trifoliate leaf to have a visible internode above the petiole. As far as possible, only leaves were all three leaflets were intact were used. Leaf area was calculated by drawing leaf outlines onto acetate sheets and running them though a leaf area analyser (Li-Cor Portable Area Méter, LI-3000A, Li-Cor Inc. Lincoln, Nebraska, USA).

2.2.2: Diurnal Measurements-

2 sets of diurnal measurements were taken (see Table 3 for dates and treatments). 2 leaves per treatment were measured in each of the 6 rings every 2 hours from 06:00 to 20:00. Measurements were made at the growth C_a (CO_2 355 or 600 µmol mol^{-1}), and at ambient light levels. The leaf cuvette was held horizontal. Diurnals were performed on clear, dry days to reduce the effect of clouds on irradiance and to reduce the chances of rain preventing completion. For logistical reasons, the rings were always sampled in the following order; C2, F1, C1, F2, F3, C3 (see Appendix 1).

Table 3: Diurnal Measurements, Dates and Samples.

Diurnal No.	Date	Treatments Used	Leaves per treatment	Days after cut.
1	17/6/95	All FACE & Control. Low nitrogen. Effective & Ineffective	2	25
2	30/6/95	All FACE & Control. Low nitrogen effective & ineffective. High nitrogen ineffective.	2	38

2.2.3: A versus Ci Measurements-

The leaf response in terms of A with varying C_i , was measured by altering the CO_2 concentration in the leaf chamber in steps. For ambient grown plants the concentration sequence was 355, 250, 150, 50, 600, 900, the first 4 concentrations should correspond with the intial slope of the A/C_i response, so indicating Vc_{max} , whilst the final 2 concentrations should correspond to the 'plateau' area of the response determined by J_{max}. For FACE grown plants the sequence of CO_2 concentration was 600, 355, 250, 150, 50, 750, 900, the concentrations upto 600 umol mol-1, should allow determination of Vc_{max} , whilst 750 and 900 allow J_{max} to be calculated. Measurements were taken at +/- 40 µmol mol^{-1} of the target value, except for the growth CO_2 value, which was taken at +/- 15 µmol mol^{-1} .

A stabilised quartz-iodide light source was used to provide uniform, near-saturating photosynthetic photon flux densities (750 µmol m^{-2} s^{-1}). A 12 volt battery power supply allowed constant illumination for upto 8 hours.

A/Ci measurements were taken on 2 leaves per treatment per ring. Treatments sampled for each occasion are shown in table 4. Where possible 3 treatments were used,

however a lack of concurrent, suitable days prevented this in some cases. Measurements were taken between 06:00 and 15:00. The afternoon time limit was used to avoid the possibility of feedback inihibtion of photosynthesis due to carbohydrate accumulation and cytosolic inorganic phosphate limitation (Stitt, 1990).

Vc_{max} and J_{max}, as *in vivo* measures of rubisCO activity and maximum capacity for RuBP regeneration respectively, were calculated by fitting the equations of McMurtrie & Wang (1993).

Table 4: A/Ci Measurements & Cuts, Dates.

A/Ci No.	Dates	Treatments Used	Leaves per Treatment per ring	Days after cut.
1	19/5/95-21/5/95	All FACE & Control. Low nitrogen. Effective & Ineffective	2	
1st CUT	23/5/95			0
2	8/6/95-10/6/95	FACE 1, 2, 3 Control 2 Low nitrogen Effective & Ineffective	2 only 1 for ineff. 1 for both 2 for both	16-18
3	21/6/95-23/6/95	All FACE & Control Low Nitrogen effective & ineffective. High nitrogen ineffective.	2	29-31
4	10/7/95-13/7/95	All FACE & Control Low Nitrogen effective & ineffective. High nitrogen ineffective.	2	48-51
2nd CUT	17/7/95			0

2.2.4: Diurnal A/Ci Measurements-

To determine if the A/Ci response does alter during the course of the day, A/Ci measurements were conducted in the late afternoon (15:00 to 17:00). So that the same leaves were used for the measurements, the leaves used for the pre-15:00 measurements

were tagged for iden tification. The treatments and rings used for this experiment are given in Table 5.

Table 5: Diurnal A/Ci Measurements, Dates & Samples

Diurnal A/Ci No.	Dates	Treatments Used	Leaves per Treatment per ring
1	27/6/95	F1 & C2, effective and ineffective.	2
2	10/7/95-11/7/95	F1,F3 & C2,C3, effective and ineffective.	2

2.2.5: Canopy Position/Leaf Age Effects-

There will be two categories of leaf in the lower canopy, those formed there and older leaves which were once at the top but are now shaded by leaves formed later. Two experiments were performed to see if there was any difference in the A/C_i responses of these two leaf groups compared to the young fully expanded leaves at the top of the canopy.

The first experiment looked at the older leaves which were once at the top of the canopy. The treatments used were effective and ineffective in the low nitrogen plots. Two leaves per plant were tagged after a week of regrowth. Two leaves were tagged to allow for some loss due to herbivory etc. Tagging was with small rings of masking tape, to minimise any possible effects of the leaves. The leaves all had three intact leaflets and a visible internode. 24 days later (24/6/95), after the tagged leaves had been overtopped for some time, one tagged leaf from each plant was measured for its A/C_i response. This work

also coincided with the 3rd set of 'normal' A/Ci curves so that comparisons could be made between old and new leaves.

The second experiment looked at new leaves formed lower in the canopy. The treatments used were effective and ineffective in the low nitrogen plots. These leaves were selected by looking for new growth branches from the main stems and then using leaves growing from the base of the petioles of large leaves on these branches. This experiment coincided with the 4th set of 'normal' AC_i measurements so comparisons with new upper canopy leaves were possible.

2.3: Stomatal Measurements-

Impressions of abaxial and adaxial leaf surfaces were taken using acrylic plastic slides and acetone. Three effective plants per ring, and three leaves per plant were used. The third plant was in the outer-most density ring. Impressions were formed using leaves still attached to the plant and by rolling a metal cylander over the leaf to give an even spread of pressure. Each impression was viewed under a light microscope (x 100) magnification. Three fields of view per leaf were studied (field of view area= 3.141×10^{-4} mm^2). Epidermal and stomatal cell numbers were recorded. The number of cells per field of were added to give a total value for each leaf surface. Stomatal index (S.I.) was calculated as (after Ferris & Taylor, 1994):-

$$S.I. = (S/E+S) \times 100$$

S = number of stomata per unit area

E = number of epidermal cells per unit area.

Fig 3: 1st Diurnal Experiment (17/6/95).

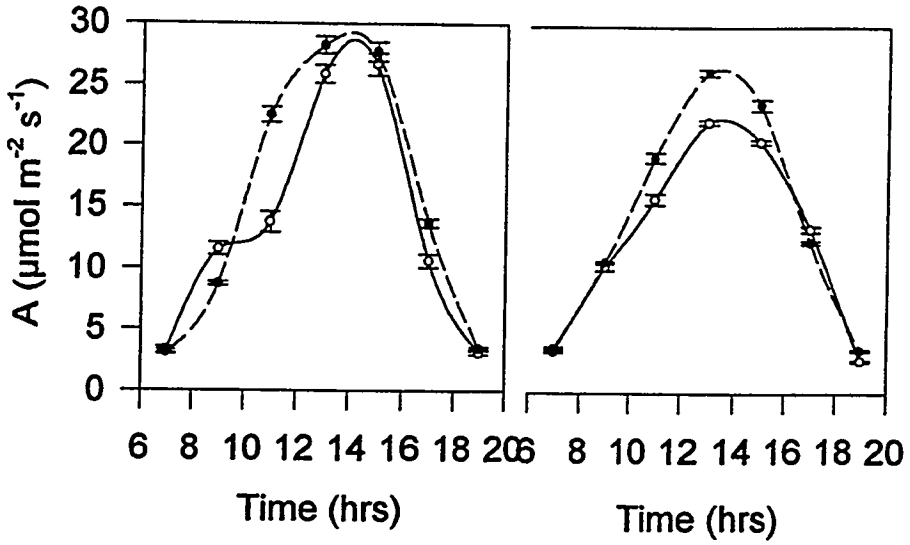


Fig 3a: Diurnal assimilation rate for ineffective *M.sativa* grown in normal ambient (—O—) and FACE (---●---) plots (17/6/95).

Fig 3b: Diurnal assimilation rate for effective *M.sativa* grown in normal ambient (—O—) and FACE (---●---) plots (17/6/95).

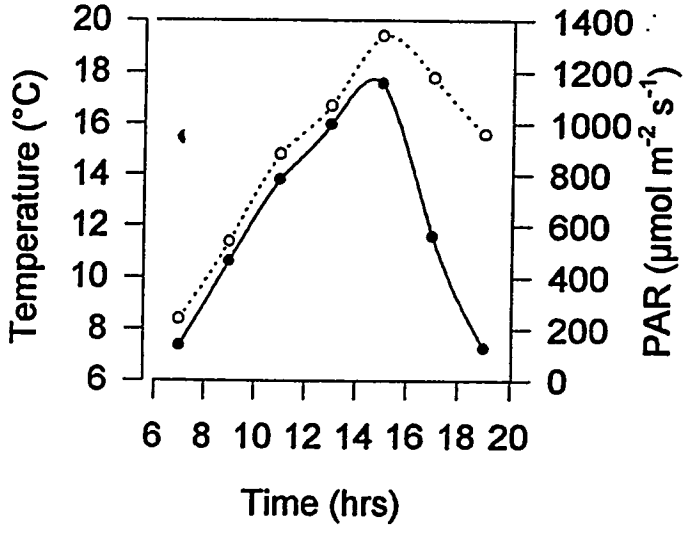


Fig 3c: Diurnal air temperature (°C) (····O····) and PAR (µmol m^{-2} s^{-1}) (—●—) (17/6/95). Mean air temp. 06:00-20:00= 18.37°C. Mean global radiation 06:00- 20:00= 0.376MJ m^{-2}.

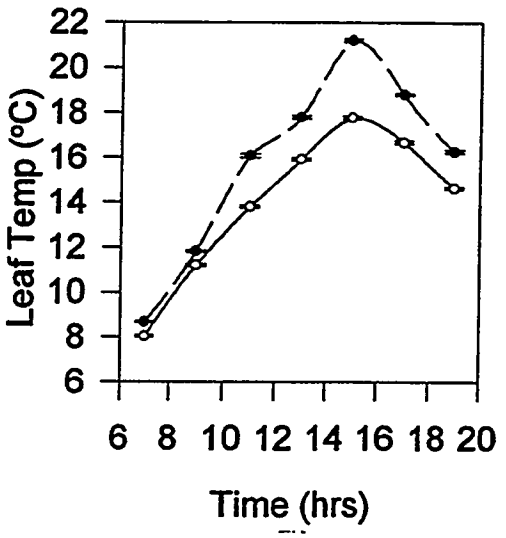


Fig 3d: Diurnal leaf temperature (°C) in normal ambient (—O—) and FACE (---●---)plots (17/6/95). Mean control leaf temp. 06:00-20:00= 14.01°C.Mean FACE leaf temp. 06:00-20:00= 15.81°C

3: Results.

3.1: Diurnal.

Both diurnal experiments showed enhanced A rates in F.A.C.E. grown plants (see Figs 3a, 3b and 4a, 4b, 4c). In the second experiment this enhancement was especially evident in the latter part of the day. In the first experiment the enhancement was greatest in the middle of the day for the effective plants.Table 6 shows the important A values for both diurnal experiments.

Table 6: Diurnal experiments1 and 2. Photosynthetic assimilation (A), summary data.

Diurnal Number.	Control/ F.A.C.E.	Nitrogen.	Average bi-hourly A.	Sum of diurnal bi-hourly A (A_{tot}).	Enhancement of A_{tot} in FACE rings. (%).
1	Control	effective	12.65	88.53	0
17/6/95		ineffective	13.57	95.01	0
	F.A.C.E.	effectiye	14.2	99.43	12.3
		ineffective	15.38	107.64	13
2	Control	effective	13.94	97.6	0
30/6/95		ineffective	12.8	89.58	0
		High N. ineffective	15.75	110.28	0
	F.A.C.E.	effective	22.23	155.59	59.4
		ineffective	24.60	172.2	92.2
		High N. ineffective	21.65	192.2	37.4

In both experiments it can be seen that F.A.C.E. produces an enhancement in A in all plant treatments. In the first experiment this enhancement is relatively small and is statistically insignificant (see Table 7), however in the second experiment the enhancement of A due to F.A.C.E. is much greater and highly significant ($P< 0.01$).

Nitrogen treatment did not give produce statistically significant differences in A or the effect of growth C_a. However in both experiments it is the ineffective low nitrogen plants which are most enhanced by elevated C_a. The differences, in A, between nitrogen and

Fig 4: 2nd Diurnal Experiment (30/6/95.)

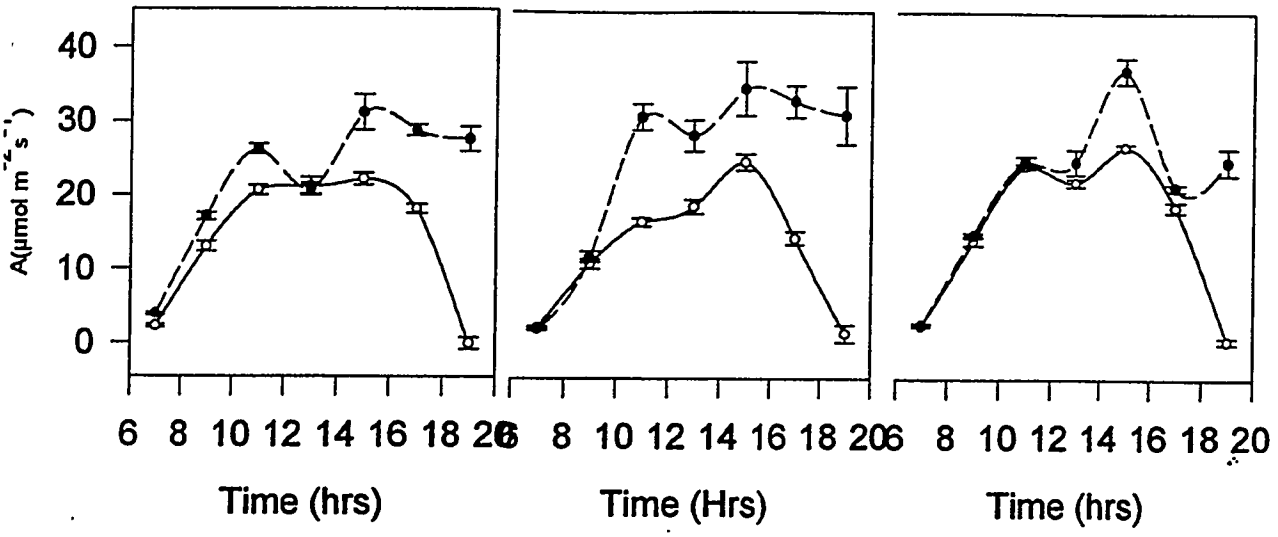


Fig 4a: Diurnal assimilation rate for ineffective *M.sativa* grown in normal ambient (—O—) and FACE (---●---) plots (30/6/95).

Fig 4b: Diurnal assimilation rate for effective *M.sativa* grown in normal ambient (—O—) and FACE (---●---) plots (30/6/95).

Fig 4c: Diurnal assimilation rate for ineffective high nitrogen *M.sativa* grown in normal ambient (—O—) and FACE (---●---) plots (30/6/95).

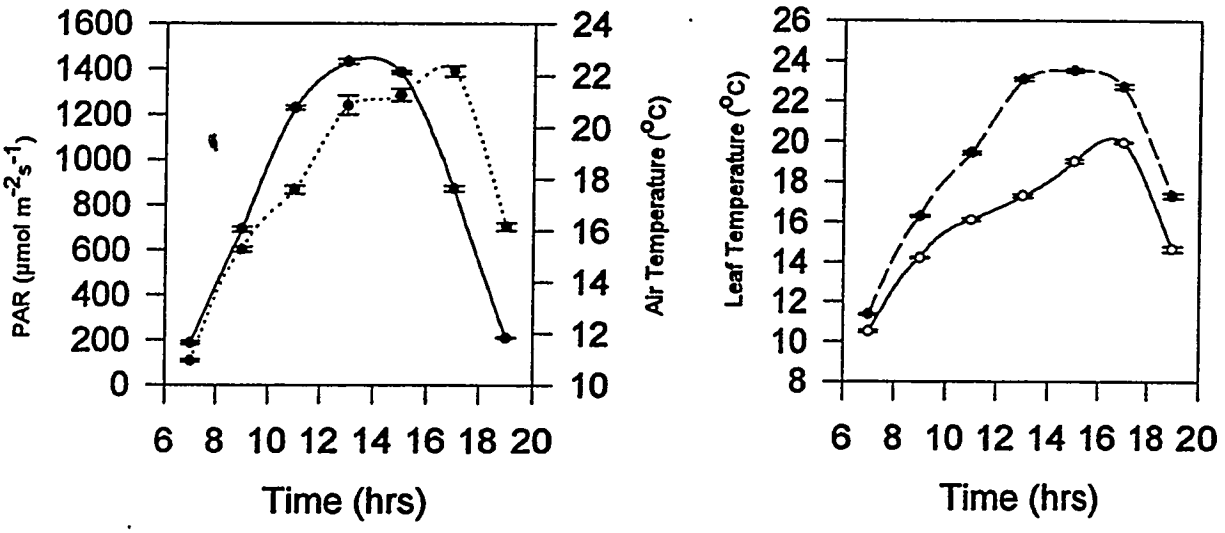


Fig 4d: Diurnal air temperature (°C) (····O····) and PAR(μmolm^{-2} s^{-1}) (—●—) (30/6/95). Mean air temp. 06:00-20:00= 23.12°C. Mean global radiation 06:00-20:00= 0.505MJ m^{-2}.

Fig 4e: Diurnal leaf temperature (°C) in normal ambient (—O—) and FACE (---●---) plots (30/6/95). Mean control leaf temp. 06:00-20:00= 15.97°C.Mean FACE leaf temp. 06:00-20:00= 19.11°C

growth C_a treatments are much greater in the second experiment which was conducted 13 days later.

Table 7: Results of two-way ANOVA examining the effects of C_a and nitrogen conditions on diurnal A in *Medicago sativa.*

Diurnal No.	Source of variation.	Degrees of freedom.	P. value	significance. * = 0.05 **= 0.01
1	CO_2	1	0.64	
	nitrogen	1	0.77	
	interaction	1	0.97	
	within	24		
2	CO_2	1	0.0091	**
	nitrogen	2	0.983	
	interaction	2	0.745	
	within	36		

The diurnal experiments also showed that F.A.C.E. conditions increase leaf temperatures (see Figs 3d, 4e). This increase due to F.A.C.E. growth conditions was, on average, 1.79°C and 3.56°C in the first and second diurnal experiments respectively. The avarage control leaf temperature was 14.01°C in the first experiment and 15.97°C in the second experiment. Table 8 shows the results of paired T-test on the leaf temperature data. The F.A.C.E. conditions produce highly significant increases in leaf temperatures.

Table 8: Results of 2 tailed paired T-test analysis examining the effect of growth C_a on the diurnal leaf temperature in *Medicago sativa.*

Diurnal No.	degrees of freedom	t-stat	P-value	t-crit
1	6	4.859	0.0028**	2.447
2	6	4.703	0.0033**	2.447

Figs 5 & 6: Vc_{max} and J_{max} Results from *Medicago sativa.*

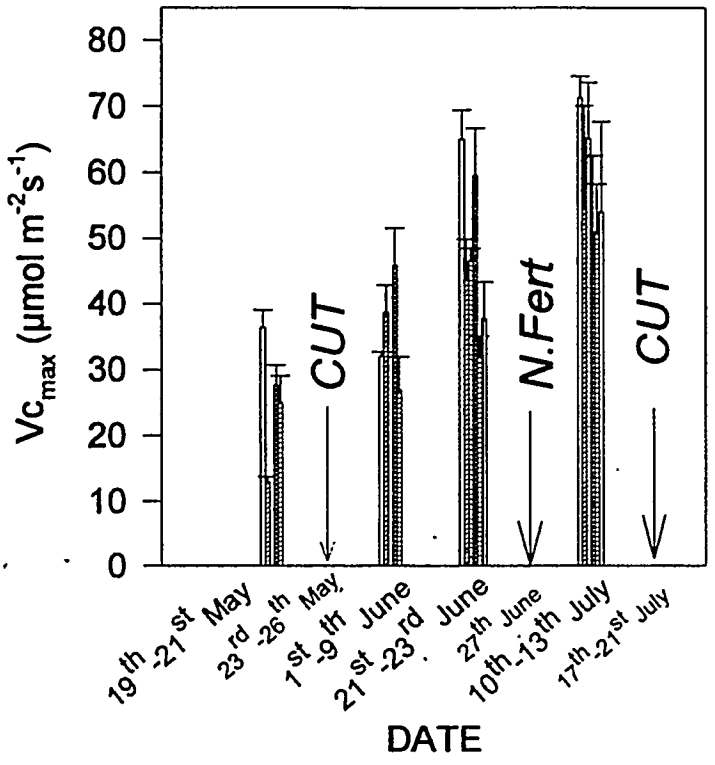
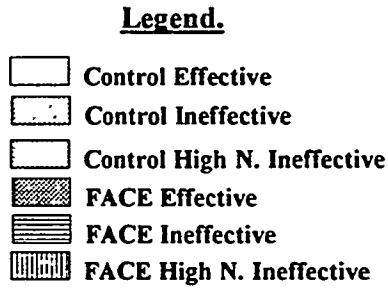


Fig 5: Mean maximum rate of carboxylation of rubisCO (µmol m^{-2} s^{-1}) ($V_c$max) values for ineffective, effective and ineffective high nitrogen grown *Medicago sativa* plants grown in normal ambient CO_2 and F.A.C.E. conditions.



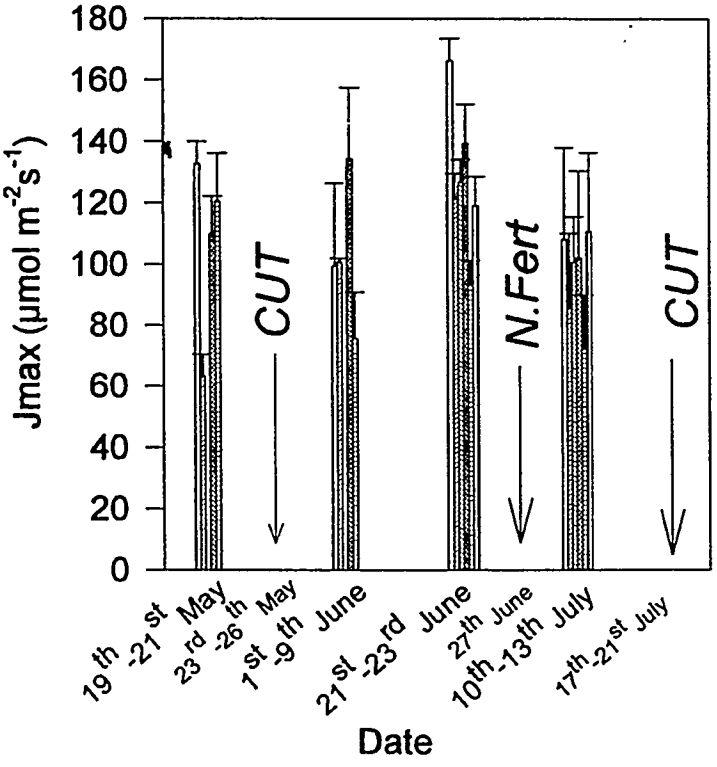


Fig 6: Mean maximum light saturated rate of electron transport (µmol m^{-2} s^{-1}) (Jmax)for ineffective, effective and ineffective high nitrogen grown *Medicago sativa* plants grown in normal ambient CO_2 and F.A.C.E. conditions.

3.2: A versus C_i.

Table 9 shows the Vc_{max} and J_{max} results of the four A vs. C_i experiments (see also Figs 5 and 6).

Table 9: Mean Vc_{max} and J_{max} results for each nitrogen and CO_2 treatment for all four A vs. C_i experiments.

	Vc_{max}			J_{max}		
Experiment / CO_2	Ineff. low N.	Effective low N.	Ineff. High N.	Ineff. low N.	Effective low N.	Ineff. High N.
1 Control	12.91	36.40	—	63.39	132.72	—
1F.A.C.E.	25.13	27.63	—	120.53	110.05	—
2 Control	38.67*	32.03*	—	100.72*	99.69*	—
2F.A.C.E.	27.75	45.77	—	78.66	134.38	—
3 Control	43.67	65.02	46.57	121.35	166.32	126.90
3 F.A.C.E.	31.83	59.52	37.72	93.71	139.31	119.06
4 Control.	54.62	71.36	57.06	95.75	107.99	110.97
4F.A.C.E.	33.25	62.53	56.36	98.42	119.28	132.60

* Results based on only 2 measurements (others based on 6)

The results from each experiment were statistically analysed using ANOVA procedures. These results are shown in Table 10.

Table 10: Results of two-factor ANOVA tests on Vc_{max} and J_{max} results from four experiments. Tests examine the effects of growth C_a and nitrogen treatment and any interaction of these factors, on Vc_{max} and J_{max}.

		Vcmax				Jmax			
Experiment	Source of Variation	df	F-value	P-value	Significance *=<0.05 **=<0.01	df	F-value	P-value	Significance *=<0.05 **=<0.01
1	CO2	1	0.36	0.55		1	2.39	0.14	
	Nitrogen	1	20.28	0.0002	**	1	6.95	0.016	*
	Interaction	1	13.23	0.0016	**	1	12.78	0.002	**
2		possible Not to use ANOVA as unequal data sets							
3	CO2	1	4.422	0.044	*	1	7.98	0.008	**
	Nitrogen	2	13.22	<0.001	**	2	13.01	<0.001	**
	Interaction	2	0.19	0.82		2	0.78	0.47	
4	CO2	1	0.35	0.30		1	0.52	0.48	
	Nitrogen	2	0.91	0.17		2	0.78	0.47	
	Interaction	2	0.49	0.68		2	0.11	0.89	

Fig 7 : 1st Diurnal A/C_i Vc_{max} and J_{max}.

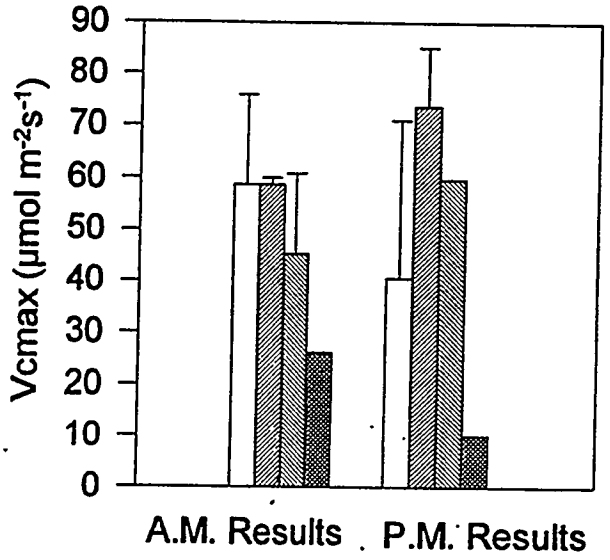


Fig 7a: Mean A.M. and P.M. maximum rate of carboxylation of rubisCO (µmol m^{-2} s^{-1}) ($V_c$max) values for ineffective, and effective grown *Medicago sativa* plants grown in normal ambient CO_2 and F.A.C.E. conditions.

Legend
- Control Effective
- Control Ineffective
- F.A.C.E. Effective
- F.A.C.E. Ineffective

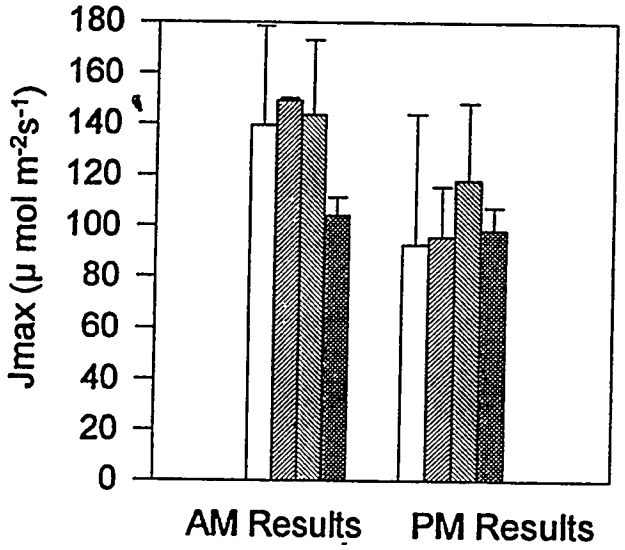


Fig 7b: Mean A.M. and P.M. maximum light saturated rate of electron transport (µmol m^{-2} s^{-1}) (Jmax) values for ineffective, and effective grown *Medicago sativa* plants grown in normal ambient CO_2 and F.A.C.E. conditions.

Vc_{max} values (and by inference rubisCO activities) show an increasing trend over the course of the fieldwork (see Fig 5), this pattern is not shown by J_{max} values. In the first and third experiments there were highly significant nitrogen effects on both Vc_{max} and J_{max} values. In the first experiment the nitrogen and CO_2 treatments interacted to a highly significant degree, but CO_2 treatment alone was not a significant factor in determining Vc_{max} or J_{max} values. In the third experiment the CO_2 treatment was significant for Vc_{max} values and highly significant for J_{max} values. In the fourth experiment, different nitrogen and CO_2 treatments produced no significant changes in Vc_{max} or J_{max}.

The effectively nodulating nitrogen usually produced the highest Vc_{max} values in both C_a treatments. The only exception to this was in the control CO_2 results from the second experiment, however it should be stressed that these results are from a reduced data set so cannot really be compared to the other 'complete' data sets.

The fertilization of the high nitrogen plot on the 27th June was followed by an increase in the Vc_{max} values of the high nitrogen ineffectively nodulating plants. This increase was however, not significant (P= 0.0675, F= 3.85).

3.3: Diurnal A/C_i.

The results to the first diurnal A/C_i are shown in Figs 7a and 7b. The results of the statistical analyses is given in Table 11. These analyses show no significant differences in morning and late-afternoon Vc_{max} and J_{max} values, if the effects of nitrogen treatment are not included in the analyses. Analyses of the ineffective and effective nitrogen treatments was not done for the first diurnal A/C_i experiment as there were so few replications.

Fig 8: 2nd Diurnal A/C_i Vc_{max} and J_{max}.

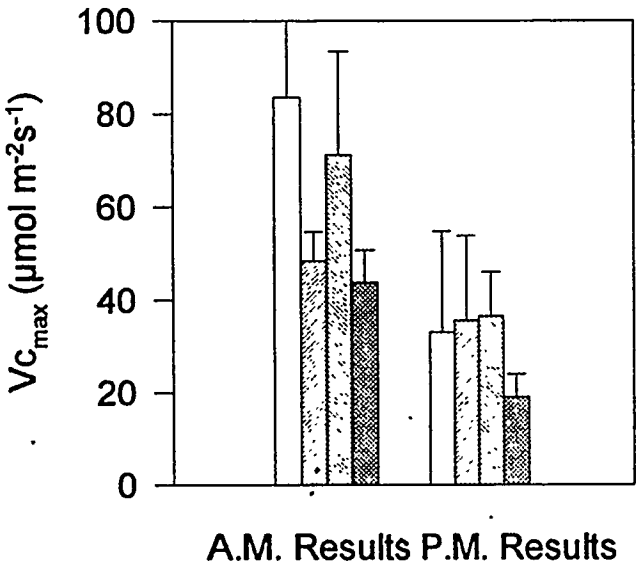
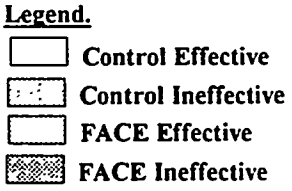


Fig 8a: Mean A.M. and P.M. maximum rate of carboxylation of rubisCO (µmol m^{-2} s^{-1}) ($V_c$max) values for ineffective, and effective grown *Medicago sativa* plants grown in normal ambient CO_2 and F.A.C.E. conditions.



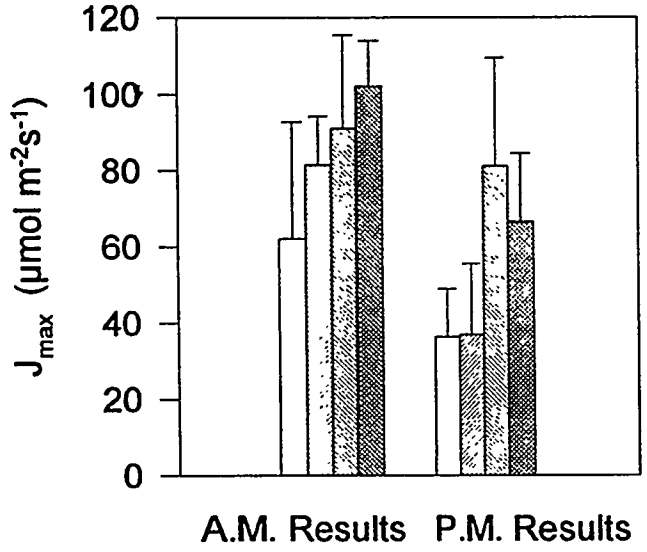


Fig 8b: Mean A.M. and P.M. maximum light saturated rate of electron transport (µmol m^{-2} s^{-1}) (Jmax) values for ineffective, and effective grown *Medicago sativa* plants grown in normal ambient CO_2 and F.A.C.E. conditions.

Table 11: Results of ANOVA, on the Vc_{max} and J_{max} values, for the first diurnal A/C_i experiment, examining the effects of time of day and CO_2 treatment.

	Vcmax				Jmax			
Source of Variation	df	F-value	P-value	Significance * =<0.05 **=<0.01	df	F-value	P-value	Significance * =<0.05 **=<0.01
CO_2	1	3.43	0.089		1	0.034	0.856	
Time of day	1	0.01	0.922		1	3.489	0.863	
Interaction	1	0.001	0.977		1	0.955	0.348	
Within	12				12			

The results to the second diurnal A/C_i are shown in Figs 8a and 8b. The results of the statistical analyses are given in Table 12.

Table 12: Results of ANOVA, on the Vc_{max} and J_{max} values, for the second diurnal A/C_i experiment, examining the effects of time of day and CO_2 treatment. Values were tested both within the same nitrogen treatments and ignoring nitrogen treatments.

		Vcmax				Jmax			
Nitrogen Treatment	Source of Variation	df	F-value	P-value	Significance * =<0.05 **=<0.01	df	F-value	P-value	Significance * =<0.05 **=<0.01
Effective & Ineffective tested together.	CO_2	1	0.86	0.36		1	1.01	0.32	
	Time of day	1	6.92	0.016	*	1	5.59	0.02	*
	Interaction	1	0.03	0.87		1	0.02	0.89	
	Within	20				28			
Effective Low N.	CO_2	1	0.27	0.61		1	0.613	0.44	
	Time of day	1	4.26	0.073		1	3.46	0.09	
	Interaction	1	0.016	0.90		1	0.002	0.96	
	Within	8				12			
Ineffective Low N.	CO_2	1	1.01	0.34		1	0.35	0.56	
	Time of day	1	3.23	0.11		1	1.83	0.20	
	Interaction	1	0.32	0.58		1	0.03	0.86	
	Within	8				12			

These results show that a significant (<0.05) diurnal reduction occurs for both Vc_{max} ($p= 0.016$) and J_{max} ($p= 0.02$), but only when the data are not split into nitrogen treatments. There is no significant CO_2 effect on these values and, no interaction, whether the values are considered within or ignoring nitrogen treatment.

Fig 9 : 1st Lower Canopy Results. Vc_{max} & J_{max}.

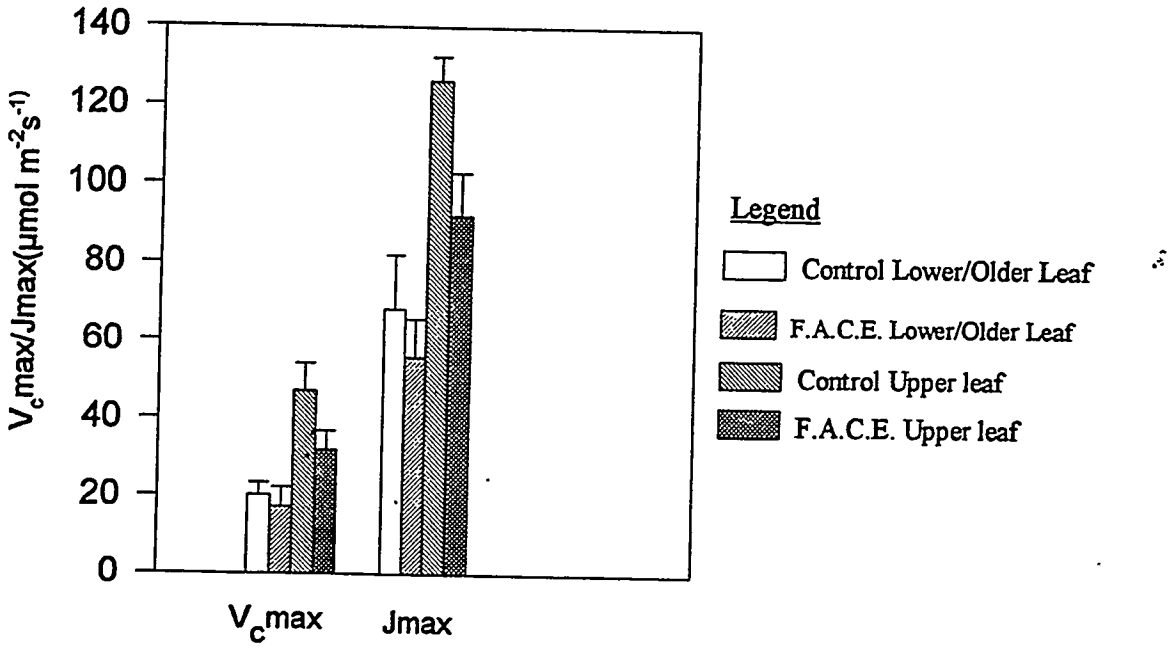


Fig 9: Mean maximum rate of carboxylation of rubisCO (µmol m^{-2} s^{-1}) ($V_c$max) and maximum light saturated rate of electron transport (µmol m^{-2} s^{-1}) (Jmax) values of young upper and old over- topped lower canopy leaves of ineffective low nitrogen grown *Medicago sativa* grown in normal ambient CO_2 and F.A.C.E.

3.4: Canopy Experiments.

3.4.1: Older Canopy Leaves-

The Vc_{max} and J_{max}, results for the first canopy experiment, are shown in Fig 9. The results of two-factor ANOVA analyses of Vc_{max} and J_{max} are shown in Table 13.

Table 13: Results of two-factor ANOVA's between Vc_{max} and J_{max} for young upper canopy and over-topped lower canopy leaves. All leaves ineffective low nitrogen.

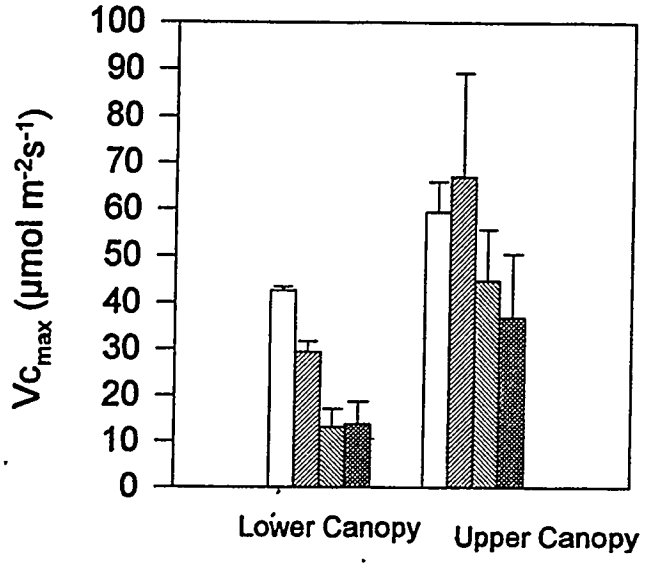
	Vcmax				Jmax			
Source of Variation	df	F-value	P-value	Significance * =<0.05 **=<0.01	df	F-value	P-value	Significance * =<0.05 **=<0.01
CO_2	1	2.913	0.113		1	4.90	0.046	*
Leaf Position/Age	1	15.45	0.002	**	1	20.03	0.0007	**
Interaction	1	1.27	0.281		1	1.07	0.320	
Within	12				12			

As can be seen there are highly significant effects by leaf position/age on both Vc_{max} and J_{max}. The CO_2 treatment also has a significant effect on J_{max}. There are no interactive effects, of CO_2 and leaf position/age, on either Vc_{max} or J_{max}.

3.4.2: Young Canopy Leaves-

The Vcmax and Jmax results of the second lower canopy experiment are shown in Fig 10a and 10b. The results of the ANOVA analyses are shown in Table 14. As can be seen on Fig10a, there appears to be a reduction in Vc_{max} values in young lower canopy leaves and in the F.A.C.E. grown leaves. This is shown in the highly significant CO_2 and

Fig 10 : 2nd Lower Canopy Results. Vc_{max} & J_{max}.



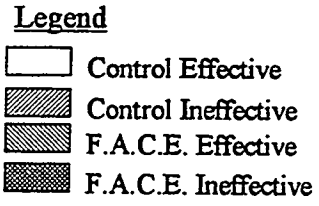


Fig 10a: Mean maximum rate of carboxylation of rubisCO (µmol m^{-2} s^{-1}) ($V_c$max) values of young upper and young lower canopy leaves of ineffective low nitrogen grown *Medicago sativa* grown innormal ambient CO_2 and F.A.C.E.

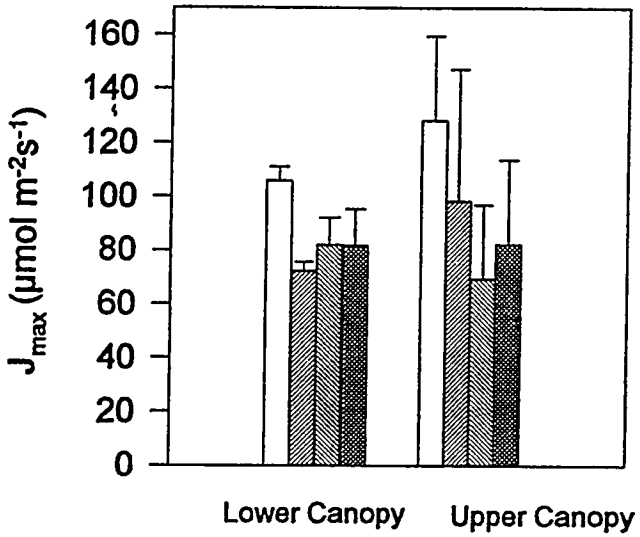


Fig 10b: Mean maximum light saturated rate of electron transport (µmol m^{-2} s^{-1}) (Jmax) values of young upper and young lower canopy leaves of ineffective low nitrogen grown *Medicago sativa* grown in normal ambient CO_2 and F.A.C.E.

Table 14: Results of two-factor ANOVA's between Vc_{max} and J_{max} for young upper canopy and young lower canopy leaves.

Nitrogen Treatment	Source of Variation	Vcmax				Jmax			
		df	F-value	P-value	Significance * =<0.05 **=<0.01	df	F-value	P-value	Significance * =<0.05 **=<0.01
Effective and Ineffective considered together.	CO_2	1	8.26	0.009	**	1	1.26	0.27	
	Leaf Position	1	11.64	0.003	**	1	0.14	0.71	
	Interaction	1	0.14	0.71		1	0.51	0.48	
	Within	20				20			
Effective	CO_2	1	3.95	0.082		1	1.274	0.29	
	Leaf Position	1	4.60	0.064		1	0.083	0.78	
	Interaction	1	0.0008	0.98		1	0.040	0.85	
	Within	8				8			
Ineffective	CO_2	1	2.96	0.123		1	0.140	0.72	
	Leaf Position	1	5.20	0.051		1	0.519	0.49	
	Interaction	1	0.30	0.598		1	0.495	0.50	
	Within	8				8			

leaf position effects when the nitrogen treatments are analysed together. There is no interaction between CO_2 and leaf position. There are no significant effects of CO_2 or leaf position on J_{max} values. However if the two nitrogen treatments are considered separately there are no significant differences found between the Vc_{max} or J_{max} values of upper/lower or F.A.C.E./normal CO_2 grown leaves.

3.5: Stomatal Measurements.

Fig 11 shows the adaxial and abaxial leaf cell numbers and the stomatal index values calculated. Statistical analyses (see Table 15) show a highly significant increase in the number of epidermal cells on the surface of F.A.C.E. grown leaves. There is also a significant reduction in the number of abaxial epidermal cells compared to adaxial epidermal cells. Stomatal cell numbers and Stomatal Index showed no significant response to F.A.C.E. conditions. There is a highly significant increase in the number of stomatal cells

Fig 11: Leaf Surface Cell Characteristics.

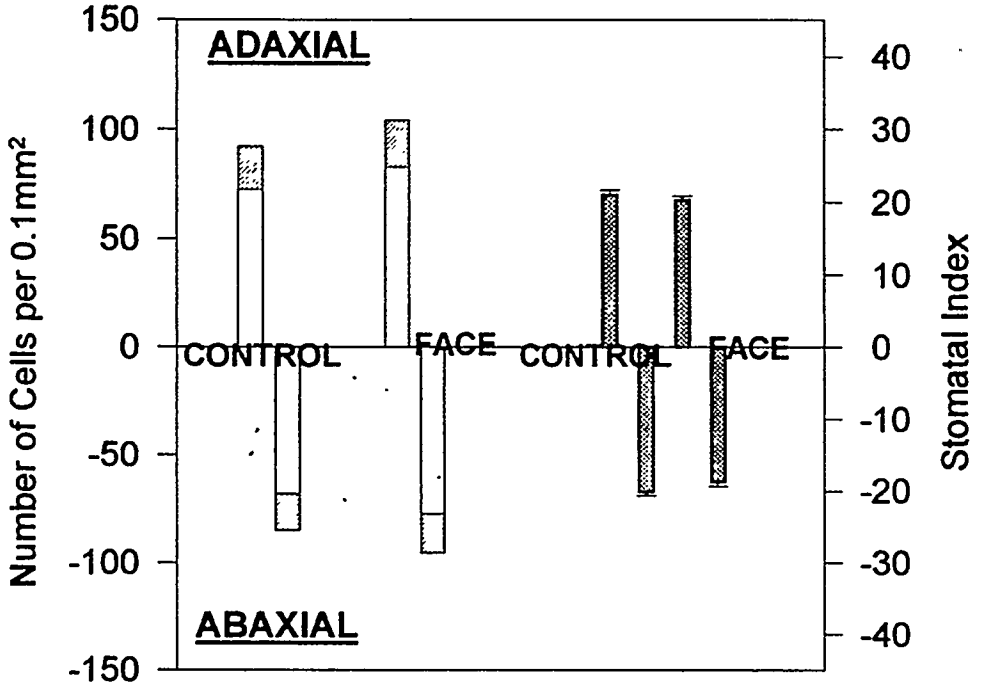
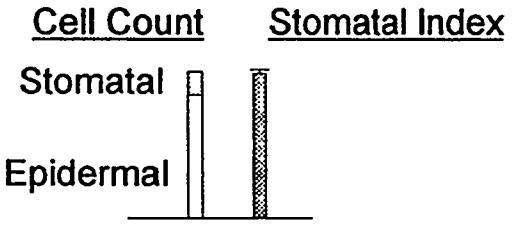


Fig 11: Epidermal/stomatal cell density and stomatal index from adaxial and abaxial leaf surfaces from *Medicago sativa* grown in normal ambient CO_2 and F.A.C.E.



on the adaxial leaf surface compared to the abaxial. Stomatal index is significantly greater on the adaxial surface.

Table 15: Results of two-factor ANOVA's between cell numbers examining the effects of CO_2 and leaf surface. All leaves effective low nitrogen.

		Epidermal Cell Number			Stomatal Cell Number			Stomatal Index		
Source of Variation	df	F-val.	P-val.	Signif.	F-val.	P-val.	Signif.	F-val.	P-val.	Signif.
CO_2	1	19.07	< 0.00001	**	3.44	0.066		3.56	0.062	
Adaxial/ Abaxial	1	5.37	0.022	*	12.3	0.0007	**	5.53	0.020	*
Interaction	1	0.021	0.884		0.49	0.48		.40	0.528	
Within	104									

* = < 0.05

** = < 0.01

4: Discussion.

4.1: Diurnal Experiments.

The results from the diurnal measurements of photosynthesis in *M. sativa* show that under increased C_a there is a diurnal enhancement of A, this enhancement is much greater and highly significant in the second experiment (see Table 6). The enhancement is highly significant (see Table 7) in terms of CO_2 but not nitrogen treatment. In the first experiment the enhancement is a smooth line following the normal diurnal pattern (see Fig 3a &b), in the effective plants enhancement is restricted to the middle of the day (10:00-16:00) (see Fig 3b). In the second experiment the enhancement is more pronounced in the latter part of the measurement period and in all three treatments is interrupted by an early afternoon reduction in A which is most pronounced in the F.A.C.E. plants (see Figs 4 a, b & c). Another highly significant effect of the F.A.C.E. treatment is the increase in leaf temperatures over the whole diurnal measurement period (see Figs 3d & 4e). The partial closing of stomates in elevated C_a has been shown to reduce the effectiveness of evaporative cooling. The findings of this study broadly agree with the suggestion that doubling of C_a could lead to increases in leaf temperatures of 1-2.5°C (Morison & Gifford, 1984), though in the second experiment, F.A.C.E. grown leaf temperatures were up to 5.78°C greater than normal ambient C_a grown leaves. The increase in diurnal assimilation rates under F.A.C.E conditions is likely to be the result of a combination of a number of factors:-

1)Elevated C_a conditions directly increase photosynthesis rates due to reduced photorespiration and the saturation of the rubsiCO in the leaves (Webber *et.al.* 1994).
2)The increased leaf temperatures of F.A.C.E. grown plants also may lead to an increase in A (Bunce, 1993). The much greater A enhancements in F.A.C.E. plants in second experiment are likely to be due to the greater differences in leaf temperatures between normal ambient C_a and F.A.C.E. grown plants and the higher PPFD levels.This study supports the assertion of Long & Drake (1992) that the interactive effects of elevated C_a and increased temperature mean that photosynthetic rates will continue to rise.

The stomatal conductances (gs) for the diurnal experiments are not included in this report as they were highly variable, showed no diurnal pattern and were probably incorrect due to machine problems. However the second diurnal experiment shows a distinct reduction in assimilation rate of the F.A.C.E. grown plants in the middle of the day. This reduction is almost certainly due to stomatal closure reducing the CO_2 supply to the chloroplasts. It seems likely that the F.A.C.E. grown plants have lower gs and so any further reduction due to high temperatures will more quickly, when compared to normal ambient C_a grown plants, lead to a reduction in gas fluxes between the atmosphere and intercellular leaf spaces.

The lack of significant differences in A between the different nitrogen treatments is somewhat unexpected as low nitrogen availability has been shown to reduce total concentrations of rubisCO (Long & Drake, 1992). Also N fertilization has been shown to

enhance growth responses to elevated C_a to a proportionally greater degree than responses at ambient C_a (Wong, 1979; Cure *et.al.* 1988) . In *M.sativa* the presence of active N-fixing bacteria in root nodules does not lead to any significant increase in diurnal A rates or to any difference in the enhancement of A caused by elevated C_a. In both experiments it is the ineffectively nodulating low nitrogen plants, that have the greatest nitrogen deficiency, which experience the greatest enhancement of A under F.A.C.E. conditions. It has been suggested that N-fixing species should profit more from elevated C_a conditions because their nodules represent a large sink, so sink-limitation should not occur (Poorter 1993). In this study the plants have been growing in the field since May 1994, after cutting down to the crown the plants regrow using the same root systems, which survive underground. This may mean that the chances of sink limitation are reduced as the root systems are, compared to the above-ground part of the plant, very well developed. In these terms it is less likely that sink limitation will be a problem until the above-ground parts of the plant get close to their maximum biomass.

4.2: A versus C_i Experiments.

The results from the A vs C_i experiments do not show any clear patterns over the course of the field season, though there is a general trend for Vc_{max} to increase. The most important factor determining Vc_{max} values is nitrogen treatment. Except in the poorly replicated second A/C_i experiment, the effectively nodulating plants have consistently higher Vc_{max} values and therefore consistently higher rubisCO activities. This supports the idea that growth in low nitrogen results in decreased concentrations of total rubisCO and so

lower Vc_{max} values (Stitt,1990). One interesting point concerning nitrogen is illustrated by the third and fourth A/C_i experiments. Before the nitrogen fertilization on the 27/6/95, the Vc_{max} values for the ineffectively nodulating high and low nitrogen plants are quite similar. In this third experiment the effectively nodultaing plants have significantly greater values. In the final A/C_i experiment, after the fertilization the ineffective high nitrogen plants have increased Vc_{max} values. This increase in Vc_{max} values after the fertilization is not statistically significant ($P= 0.0675$, $F = 3.85$), but with a greater number of replications a significant result could have possibly been obtained. This increase also supports the link between N and rubisCO activity. In the final A/C_i experiment there are no significant differences in Vc_{max} values between the different nitrogen treatments.

Significant effects of F.A.C.E. growth conditions on Vc_{max} values are only seen in the third A/C_i experiment ($P= 0.044$, $F= 4.422$). In this experiment he F.A.C.E grown plants have consistently lower Vc_{max} values compared to the normal ambient C_a grown plants. This can not be called a consistent acclimation response as it is not repeated in the final A/C_i experiment. The highly significant ($P=0.0016$, $F= 13.23$) interaction between nitrogen treatment and C_a, and Vc_{max} values in the first experiment is due to the very small value of Vc_{max} recorded for the control ineffective plants, it seems possible that this value is caused by a small number of anomalous results, so caution should be exercised in interpreting this particular result.

There are no clear trends in the J_{max} values other than all treatments have their maximum values in the third experiment and the effective plants usually have the higher

values, this lends some support to the connection between low nitrogen status and reduced RuBP regeneration (Stitt, 1990). The exception to this is the J_{max} values for the control plants in the second experiment, but again it must be borne in mind the this involves a small number of replications. The J_{max} values have the same pattern of significances as the Vc_{max} values, i.e. significant nitrogen and interaction effects in the first experiment and significant CO_2 and nitrogen in the third, but no significant effects of treatment in final experiment. In the third experiment the effective low nitrogen plants have the greatest mean J_{max} values and all the normal ambient grown plants have higher mean J_{max} values than the F.A.C.E. grown counterpart. Again this apparent 'down-regulation' cannot be termed acclimation as it is not found in the final experiment.

The main findings of the A/C_i experiment are therefore :-

1) The major factor influencing Vc_{max} and J_{max} values is nitrogen treatment. Effective nodulation usually produces the highest Vc_{max} and J_{max} results. This supports the findings of studies with a number of plants including cotton, wheat, and spinach, which also found that in low nitrogen conditions rubisCO activity and RuBP regeneration are reduced (Stitt,1990).

2) The responses of Vc_{max} and J_{max}, to F.A.C.E. conditions, are much more variable. In the second two experiments the normal ambient C_a grown plants had the higher mean Vc_{max} values. J_{max} values were higher than in the normal ambient C_a grown plants than in the F.A.C.E. in the third experiment and vice versa in the final experiment. This variablity may reflect a number of factors, air and leaf temperature effects have already been discussed,

though the equations used are supposed to account for this. It is also possible that plot and ring effects such as shading, slug damage, soil differences etc. could also cause 'experimental noise' which could mask, what may already be relatively small, differences. It should always be remembered that this is a field experiment which can lead to greater variability in growth conditions.

4.3: Diurnal A/C_i Experiments.

The diurnal A/C_i experiments are somewhat inconclusive. The lack of significant results in the first experiment would suggest that diurnal feedback limitation was not occurring in *M.sativa.* However the second experiment had more replication and when the effective and ineffective results were considered together, a significant reduction in both Vc_{max} and J_{max} was observed over the course of the day. The need to combine the data sets together suggests that this experiment may produce more compelling results if a higher number of replicates were used, however this was not possible due to time constraints. There were no significant differences between normal ambient C_a grown and F.A.C.E. grown plants suggesting that source/sink imbalances are not being induced by the elevated C_a conditions. The results suggest that feedback limitation may occur in *M.sativa* during the late afternoon when daily temperatures and PPFD densities, and therefore daily A, are close to their maximum annual values. It should also be remembered that the second experiment was conducted at the end of a period of growth, when the source:sink ratio would be reaching its maximum.This also suggests a sink feedback effect. Further work

could confirm this result and determine whether the high source:sink ratio i.e. root nodule activity is an important factor in diurnal effects on A/C_i responses.

4.4: Canopy Experiments.

The canopy experiments showed significant effects both of leaf position and F.A.C.E. exposure. In the first experiment, reduction of Vc_{max} values in the lower over-topped leaves compared to the young upper leaves was highly significant (P= 0.002, F=15.45). Whilst CO_2 treatment was not statistically significant, it can be seen on Fig. 9 that the normal ambient C_a grown plants have the higher mean Vc_{max} results. The J_{max} values do show significant reductions in F.A.C.E. grown plants (P= 0.046, F= 4.9) and highly significant reductions in lower canopy, over-topped leaves (P= 0.0007, F= 20.03). These Vc_{max} results indicate that there is a reduction of rubisCO activity lower down the canopy, which is dependent on leaf position/age. This agrees with other studies which have shown that the photosynthetic capacity of *M.sativa* leaves deep within the canopy is only one-fifth of that of leaves at the top of the canopy (Evans 1993). This decline is due to two factors, firstly leaves remobilise and export their nitrogen as irradiance is reduced by shading and secondly the photosynthetic system adapts to the lower irradiances by reducing the electron transport capacity per unit of chlorophyll and altering the nitrogen partitioning within the leaf (Evans,1993). Both these changes are shown in the first canopy experiment with the reduction in electron transport (J_{max}) being less under F.A.C.E. conditions (see Fig 9), there is however, no significant interaction between CO_2 and leaf position. This result

seems to support the assertion of Long & Drake (1992) that elevated C_a conditions would be expected to increase the capacity for RuBP regeneration, if leaf resources are optimized.

In the second canopy experiment the effect of leaf age was removed, so any significant results would be due to canopy effects only. In this experiment the Vc_{max} values were significantly reduced in the lower young lower canopy leaves compared to the young upper canopy leaves (P= 0.003, F=11.64). The F.A.C.E. treatment also led to a highly significant reduction in Vc_{max} values (P= 0.009, F=8.26) but there was no interaction between the two factors. In this experiment both effective and ineffective plants were used, however the significant results were only found if the nitrogen treatment was ignored and the data sets combined. This is a similar problem to the second diurnal A/C_i experiment and again points to a problem concerning replication. J_{max} values showed no significant change with either leaf position within the canopy or with CO_2 treatment. The lack of significant response to leaf position or CO_2 treatment in the J_{max} values suggests that when compared to older lower canopy leaves, young lower canopy leaves show little loss in RuBP regeneration capacity due to their position. In *M.sativa* loss of RuBP regeneration capacity is an effect primarily of leaf aging.

4.5: Stomatal Measurements.

The results from the stomatal measurements showed that there were significant differences between the adaxial and abaxial leaf surface, with the adaxial surface having significantly greater densities of epidermal cells (P= 0.022, F= 5.37), stomatal cells (P=

0.0007, F= 12.3) and stomatal index (P= 0.02, F= 5.53). The only effect of the F.A.C.E. conditions was to produce a highly significant (P= <0.00001, F= 19.07) increase in the epidermal cell density. One explaination for this may be the increased speed of development caused by elevated C_a growth conditions (Baysdorfer & Bassham, 1985).This increase in development speed could be a direct effect of elevated C_a or an indirect effect of higher leaf temperatures. If development is accelarated, cell division will increase but water uptake may not, this could lead to more, smaller epidermal cells so higher densities. Stomatal denisities and indexes were statistically unaffected by the CO_2 treatment, however there was a tendency (significant at P= 0.1) to lower stomatal density and index in the F.A.C.E. grown plants. This study lends support to the idea that elevated C_a has little effect on stomatal indexes (Körner, 1988).

4.6: Conclusions.

The results of this study suggest that *Medicago sativa* cv. Saranac, grown in the field, does not show any consistent or long-term acclimation response to elevated C_a conditions. This adds support to the findings of Campbell *et.al.* (1988).This most significant variable in terms of A/C_i response is the nitrogen available for the plant. This can be in the form of N-fixing bacteria or inorganic fertilizer, but the results of this study suggest that N-fixing bacteria allow continous benefits, in terms of rubisCO activity and RuBP regeneration, whilst the effects of inorganic N-fertilizers may decrease over time. Elevated C_a conditions produced definate increases in A on a diurnal basis, this is to be expected in view of the lack of photosynthetic acclimation, this agrees with the work of

Bunce (1993). Elevated C_a also lead to significant increase in leaf temperature, probably due to stomatal closure. There was no evidence that reduced stomatal numbers could cause this reduction in evaporative cooling. The increase in epidermal cell numbers, under F.A.C.E. conditions could be due to accelerated development.

The study also shows that *M.sativa* may suffer from a diurnal downregulation in both rubisCO activity and RuBP regeneration capacity over the course of the day. This down regulation probably only occurs when photosynthetic rates and/or source : sink ratios are at their peak. The C_a conditions had no effect on the diurnal A/C_i response. The position of a leaf within the *M.sativa* canopy also plays an important role in the photosynthetic response with older over-topped leaves showing reduced rubisCO activity and RuBP regeneration. RuBP regeneration is less pronounced in F.A.C.E. grown plants. In young lower canopy leaves both position and elevated C_a produced a loss a rubisCO activity. These results imply that the loss of rubisCO activity is linked to low irradiance levels whilst reduction in RuBP regneration may be age-dependant.

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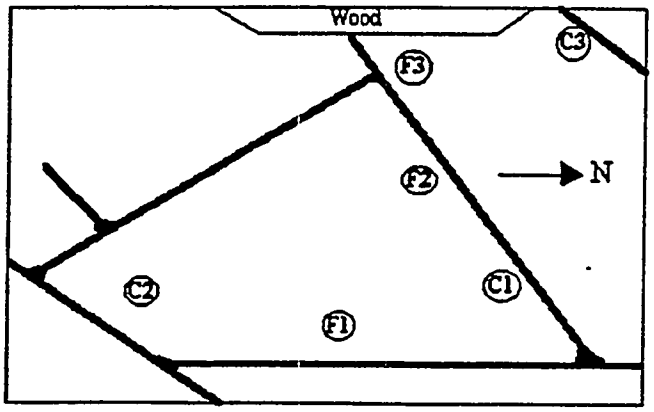
Appendix 1. Site map of the FACE facility. C = control rings; F= F.A.C.E. rings.



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Neil P. Bridson
MSc Crop Production in the Changing Environment.

Literature Review.

The Direct Effects of Increased Atmospheric Carbon Dioxide Concentration on Plant Photosynthesis:
a review of experimental results and predictions.

TABLE 1: Milestones in CO_2 enrichment.

YEAR	OBSERVATIONS	OBSERVER
1648	Major increase in mass of a willow came from from atmosphere.	Van Helmont
1804	First observations of CO_2 enhancement of plant growth.	de Sassure
1902	Negative effects of CO_2 enhancement on plant growth.	Brown & Escombe
1902-1894	Positive effects of CO_2 enhancement on plant growth (Europe)	Demoussy
1918	Positive effects of CO_2 enhancement on plant growth (U.S.A.).	Cummings & Jones
1931	6000 nurseries reported using CO_2 in Germany	Reinau
1959	Basic studies on CO_2 and light responses in plants.	Gaastra
1961	Dutch growers add CO_2 for improving yields of 4000 acres of lettuce.	Anon.
1962	Response of cucumber reported and the complimentary effects of CO_2 and light.	Hopen & Ries: Daunicht
1962-1966	Responses of flower crops reported.	Goldsberry & Holley
1964	Comprehensive studies on tomato and cucumber	Wittwer & Robb
1976	Positive effects noted for the growth of tree seedlings	Hannover *et.al.*

1:INTRODUCTION.

At present the Earth's atmosphere is receiving an extra 8 Gt of carbon each year, mainly from fossil fuel burning and changes in land use (Idso, 1989; Schneider, 1990). Measurements begun in the 1950's have shown an increase in atmospheric CO_2 concentration from 315ppm to a present value of about 350ppm (Keeling *et.al.* 1989). The rise in CO_2 is perhaps the one global alteration that can be anticipated with certainty (Rogers & Dahlman, 1993). The rate of increase is such that it has been estimated that CO_2 concentration may be double the pre-industrial level of 280ppm by the year 2050 and may reach 700ppm by the end of the next century (Houghton *et.al.*, 1990; IPCC buisness as usual scenario).

The effects of this increase may have a number of important and far-reaching consequences for many global systems, including the biosphere and climate. Global warming due to the increase of CO_2 and other 'greenhouse gases' (methane, carbon monoxide, nitrous oxide and CFC's), has been predicted to increase the mean global temperature by 1.5°C to 4.5°C (IPCC, 1990). This is because these gases can trap longwave radiation emitted from the Earth and so, theoretically, produce a warming effect as their concentrations rise. The rise in temperature may change climate patterns including precipitation and mean regional temperatures, both spatially and temporaly. At present, the exact nature of these changes are still debated by various scientific groups.

Rising CO_2 could have many consequences for the biological components of the Earth and especially on the photosynthesising organisms of these ecosystems. CO_2 is the essential substrate for photosynthesis and photosynthesis is the major physiological process by which plants sense and respond directly to changes in CO_2 (Mott, 1990; Long & Drake, 1992). Understanding the response of photosynthesis to increased concentrations of CO_2 is therefore fundamental to understanding the responses of plants (Long, *et.al.* 1993). Given that on a world scale about 88% of the calorie requirements and 90% of the protiens, for the human population, come directly from plant sources, (Langer & Hill, 1991), the responses of agricultural plants will have crucial consequences for the world food supply.

The aim of this literature review is to present a brief overview of the major direct effects of increased CO_2 concentrations on plants, followed by a more detailed discussion on the direct effects on photosynthetic processes. Also considered will be interactions with other environmental factors such as temperature, nitrogen supply and water supply.

2: COMMONLY OBSERVED DIRECT EFFECTS OF GROWTH IN ELEVATED CO_2.

The first recorded observation of the effect of CO_2 on plant growth is attributed to de Sassure in 1804, who noted that pea plants grew faster when exposed to an atmosphere enriched with CO_2 (Kimball, *et.al.* 1993). Table 1 (adapted from Wittwer, in Kimball *et.al.* 1993) gives a brief outline of pre-1980's research.

In C_3 species, studied at elevated CO_2, two physiological processes are directly affected: photosynthesis and transpiration (Poorter, 1993). Net photosynthesis is raised partly due to a decrease in photorespiration and partly due to an increase in substrate supply. Transpiration is reduced due to a lower stomatal conductance.

2.1: Increased Photosynthetic Carbon Assimilation-

When plants from many species are placed into high CO_2 (i.e. 650-700 μmol mol^{-1}) growth conditions, the rate of photosynthesis increases. Typical C_3 plants respond in an almost linear manner, whilst C_4 plants respond more rapidly but only to about 360 μmol mol^{-1} CO_2, after which their response levels off (Kimball *et.al.* 1993). This stimulation occurs for two reasons:

(i) Because CO_2 is a competitive inhibitor of the oxygenation of RuBP (ribulose 1,5 bisphophate) by the primary carboxylase rubisCO (RuBP carboxylase/oxygenase), photorespiration is partially suppressed (Webber *et.al.* 1994). This means that less active sites of the rubisCO enzyme are concerned with the 'wasteful' oxygenation of RuBP thus increasing carboxylation efficiency. This will increase the rate of photosynthetic CO_2 uptake regardless of any limitation by either substrate (RuBP) or enzyme concentration, even when light is limiting (Long and Drake, 1992). A doubling of C_a from 350 to 700 μmol mol^{-1} CO_2 should increase the rate of carboxylation by 67% (Stitt, 1991), this assumes that the ratio of internal to external leaf CO_2 concentration (C_i : C_a) is maintained at the present value of 0.7. and also that the ratio of carboxylation to oxygenation of 1.94 is also constant.

(ii) The current atmospheric CO_2 concentration (C_a) is insufficient to saturate rubisCO in C_3 species, therefore any increase in C_a will allow this extra capacity to be used to increase carboxylation velocity by increased substrate binding (Webber *et.al.* 1994). This second effect an only operate when RuBP is in excess.

C_4 plant species should respond less to increased C_a as they actively concentrate CO_2 at the site of rubisco, so inhibiting oxygenation of RuBP via morphological adaptation. It may be expected therefore that yields of C_3 wheat and sugarbeet will increase relatively more than those of C_4 corn and sugarcane (Kimball *et.al.* 1993). It has also been suggested that these differences may lead to changes in the competitiveness of species, i.e. C_3 weeds of C_4 crops may become more economically damaging and vice versa for C_4 weeds of C_3 crops (Patterson & Flint, 1990).

2.2: Decreased Stomatal Conductance-

Morison (1987) analyzed data from the literature and showed that a doubling of CO_2 concentration to 660 μmol mol^{-1}, reduced stomatal conductance to 60% of that at 330 μmol mol^{-1}. There was no difference found between C_3 and C_4 plants.

The partial closing of stomates has several consequences. Transpirational cooling of leaves will be reduced, leading to an increase in leaf temperatures in elevated CO_2. A rise of 1°C has been reported by Idso *et.al.* (1987), for cotton leaves. Morison & Gifford (1984) used energy balance equations to calculate an increase of 1-2.5°C for double CO_2 grown plants, they measured an average rise of 2.1°C in the 16 species studied. The potential effects of this temperature rise are discussed in section 6.4.

Another consequence of the partial stomatal closure could be the significant reduction in evapotranspiration (ET) from plants. In over 450 studies reviewd by Kimball (1983), average crop transpiration in elevated CO_2 decreased by 34%. Morison & Gifford (1984) showed that, on average, the daily transpiration rate of 16 agricultural/horticultural species, decreased to 79%, though the overall water use was similar to ambient CO_2 grown due to the increase in leaf area.

2.3:Effects of Increased CO_2 at the Whole Plant Scale-

It is usually found that elevated CO_2 grown plants have higher dry yield. Reviews by Kimball (1983) and Cure & Acock (1986) of experiments done under a wide range of conditions show that a doubling of growth CO_2 concentrations increases the productivity of a large number of C_3 crop species, on average by 33%. In C_4 species the same conditions produced only a 10% increase. However, Kimball (1983) used only data on flower, fruit and grain yields, important from an agricultural point of view but perhaps not the best indicators of plant growth. Poorter (1993) in a review of 89 reports of 156 species found a large variation in response, even within the same species. Intraspecific differences in response to elevated CO_2 may be genetic, or experimental in origin. Overall, Poorter (1993) found the average response, of plant growth, to a doubling of CO_2 concentration to be 37%.

When comparing C_3 and C_4 plants Poorter (1993) found a relatively small difference in response, 41% and 22% increase respectively in relative growth rates. It is suggested that the C_4 species show a response for a number of reasons. The decreased stomatal conductance at high CO_2, also found in C_4 species may relieve water stress in some of the experiments, thus increasing growth, however this is unlikely for all cases. The CO_2 response curves of photosynthesis of various C_4 species indicate that photosynthesis is not saturated at an ambient CO_2 concentration as high as 600 $\mu mol\ mol^{-1}$. It seems that in some species the C_4 pathway is not as tightly controlled as usually suggested and therefore allows for some response to CO_2.

Increases in leaf thicknesses have been demonstrated in tomato leaves with elevated CO_2 (Ho, 1977). This may be partially due to the accumulation of starch (Arp,1991). Acock & Pasternak (1986) have also suggested that increasing numbers of pallisade cells may increase leaf thickness. This may be beneficial in a high CO_2 environment, but in ambient CO_2 the extended diffusion path for gases may make it unprofitable.

3:LONG-TERM PLANT RESPONSES.

Plants have evolved mechanisms for adapting to changing light (Björkman, 1981) and temperature (Berry & Björkman, 1980). These mechanisms allow plants to maximise their survival and reproductive rates under the prevailing conditions, i.e. by reallocating resources to produce morphological changes. Light and temperature may change relatively quickly compared to atmospheric CO_2 concentrations. However, because changes in stomatal aperture can alter the CO_2 concentration in the intercellular spaces (C_i), of plant leaves, it is conceivable that plants have evolved a mechanism for adapting to variable CO_2 concentrations (Mott, 1990).

3.1: Acclimation-

Acclimation (or acclimatisation) is the term given to the effect of prolonged growth in elevated CO_2 on the development and maintenance of the photosynthetic apparatus, which in turn determines photosynthetic capacity (Long *et.al.* 1993). In many studies, plants grown under elevated CO_2 conditions do not show a continued positive acclimation effect. The stimulation of photosynthetic carbon assimilation and growth measured after plants have grown in elevated CO_2 is often lower than when measured at the start of the CO_2 treatment (DeLucia *et.al.*, 1985; Ehret & Jolliffe, 1985; Sage *et.al.*, 1989). In some studies, the acclimation is so great that there is no difference between the photosynthetic rate of leaves

TABLE 2: Stimulation of photosynthsis in a range of crop species on exposure to elevated CO_2 (700-1000 µmol mol^{-1}). (After Stitt, 1991)

Species	**Photosnthesis rate as a %age of that in normal CO_2**	**Reference**
Soybean	295	Campbell *et.al.* 1988
	200	Clough *et.al.* 1981
	161	Havelka *et.al.* 1984
	153	Cure *et.al.* 1989
	100 *	Clough *et.al.* 1981
Potato	202	Sage *et.al.* 1989
Cotton	150 (High N)	Wong 1979
	78 (Low N)	Wong 1979
Bean	102	von Caemmerer & Farquhar 1984
	104	Sage *et.al.* 1989
Tomato	120	Ho 1977
	102	Yelle *et.al.* 1989
Water hyacinth	99	Spencer & Bowes 1986
Tobacco	78	Raper & Peedin 1978

* with sinks removed

grown and measured at elevated CO_2 and those grown and measured at ambient CO_2 (DeLucia *et.al.* 1985). Cure & Acock (1986) in their literature survey estimate that the stimulation due to elevated CO_2 decreases from 52% to 29%. Table 2 (adapted from Stitt, 1991), shows some of the photosynthetic responses of a range of plant species, to elevated CO_2,after several weeks.

As can be seen the down regulation of the photosynthetic response varies between plant species and appears to be most pronounced in plants which have the least ability to produce new or larger sinks for the increased photosynthate produced in elevated CO_2 (Arp, 1991). The down-regulation of photosynthesis during acclimation has often been attributed to end-product inhibition (Clough *et.al.* 1981; Arp, 1991).

3.2:Negative Acclimation to Elevated CO_2-

Decreases in photosynthetic assimilation with prolonged exposure to elevated CO_2 have been commonly associated with a number of physiological and morphological changes (Arp,1991).

3.2.1: Increased leaf carbohydrate-

Leaf carbohyradate levels has been seen to increase in almost all studies of plant growth in enhanced CO_2 (Stitt, 1991). In cotton (Wong, 1990), soybean (Mauney *et.al.* 1979), and *trifolium* (Cave *et.al.* 1981), the accumulation is as starch. In wheat (Havelka *et.al.* 1984) soluble sugars accumulate, these differences reflect the normal storage strategy of these species (Stitt, 1991). Elevated CO_2 has also been shown to change the pattern of starch deposition. In tomato, basal leaf starch content increases in elevated CO_2 growth (Yelle *et.al.* 1989).

Long & Drake (1992) surveyed different studies of acclimation of photosynthesis to increases of growth CO_2 (x 1.5-2.5). In the 28 studies in which leaf carbohydrate levels where examined, all showed some increase. Sucrose concentrations where on average 52% greater, the increase in starch was 160% on average. Starch responds more than sucrose (Farrar & Williams, 1991), Sharkey *et.al.* (1989) reported that the sucrose:starch ratio of soybean leaves shows a marked reduction with increased CO_2.

In general the greater the increase in leaf carbohydrate concentration the greater the decrease in photosynthetic capacity. Both the increase in leaf carbohydrate concentration and the decrease in photosynthetic capacity in elevated CO_2 appear to be related to sink capacity, the increase in carbohydrates was least pronounced in plants grown in large rooting volumes (Webber *et.al.* 1994). Stitt (1991) suggests that the inability of a plant to use extra photosynthate leading to the build up of excess carbohydrates in leaves could produce reduce photosynthesis via feedback mechanisms (Herold 1980), . It is unlikely that direct inhibition of photosynthesis rates by starch occurs (Farrar & Williams, 1991). Gynoecious cucumber (with many seeds set providing many sinks) respond positively to increased CO_2 throughout their growth cycle (Kimball, 1983). Monoecious cucumber varieties, with less seeds show dramatic accumulation of carbohydrate and inhibition of photosynthesis in the older leaves (Peet *et.al.* 1986).

3.2.2: Decreased rubisco activity-

A common, but not universal acclimation response is the reduction of rubisCO activity soybean studies has been the exception to this (Campbell *et.al* 1988) Table 3 summarises some

TABLE 3: Decrease in rubisCO in a range of species in elevated CO_2.

Species	%age reduction in rubisCO	Reference
Cotton (low N)	60	Wong 1979
Tomato	50	Yelle *et.al.* 1989
Bean	26-12	von Caemmerer & Farquhar 1984 Sage *et.al.* 1989
Potato	4	Sage *et.al.* 1988
Soybean	0	Havelka *et.al.* 1984 Campbell *et.al.* 1988

of the data.. In rice, the activity of rubisCO per unit area and the amount were found to decline linearly with the growth CO_2 concentration (Rowland-Bamford *et.al.* 1991). RubisCo is the largest single investment of nitrogen in leaves, usually amounting to 30-50% of total leaf protein. Terashima & Evans (1988), found that plant grown in low nitrogen allocate relatively less nitrogen to rubisCO and more to thylakoid proteins. Wong (1979) showed cotton grown in low nitrogen conditions experienced a 60% decline in rubisco activity. Other crop species have shown much lower reductions after acclimation, Havelka *et.al.* (1984) and Campbell *et.al.* (1988) reported no loss in soybean rubisCO activity.

3.2.3: A/C_i Responses-

The decline in rubisco activity has been quantified using leaf gas exchange measurements and the construction of curves of net leaf rate of CO_2 uptake at saturating light levels (A_{sat}) at different C_i concentrations. Analysis of the A/C_i responses of plant species provides an *in vivo* method of quantitatively separating the relative limitations imposed by stomata, carboxylation efficiency and capacity for regeneration of RuBP on photosynthesis in acclimated and non-acclimated leaves (Long & Drake, 1992).

The response of CO_2 uptake to C_i shows two distinct phases predicted by the mathematical models of Farquhar *et.al.* (1980) see FIG 1. There is an initial linear phase, the angle of which is determined by rubisCO activity, here the RuBP is saturated. This is followed by an inflection after which a shallower slope which indicates the C_i concentrations where A_{sat} is limited by the rate of RuBP regeneration (Long & Drake, 1992). This regeneration limitation can be due to inadequate activity of electron transport or Calvin cycle enzymes or by limitation by end-product synthesis (Stitt, 1991). For a wide range of C_3 species it has been shown that the C_i which they maintain under ambient CO_2 concentrations is usually at the point of inflection, where both rubisCO and RuBP regeneration are co-limiting (von Caemmerer & Farquhar, 1981; Long, 1985; Stitt, 1991, reviewed and updated by Wullschleger, 1993). This balance of enzymes may reflect an optimisation of the distribution of resources within the chloroplast so that neither active rubisCO nor the apparatus for regeneration of RuBP are in excess. The production of these sets of apparatus requires large investments in both energy and materials, so strong selective pressures for adjustment of carboxylase levels are likely.

Long (1991) observed that if leaves where transferred to a doubled CO_2 atmosphere, the increase in carboxylation rate and efficiency would mean that even if a reduction in rubisco activity, of more than 30% occurred it would still only impose the same limitation on photosynthetic assimilation as it does in the present atmospheric CO_2 concentration. It seems likely then that an increase CO_2 concentration experienced by a plant, should lead to a reallocation of resources. This will be especially true if the plant cannot use the excess photosynthate i.e. if rooting volume is low or nitrogen is scarce.

Webber *et.al.*(1994) have suggested three possible methods, depending on the limiting factors, by which plants could optimise their photosynthetic apparatus. The three acclimation responses will each have different effects on the A/C_i curves produced by the plants, (see FIG 1).

I) If a plant cannot use the excess photosynthate produced by elevated CO_2 concentrations, then it will be 'sink-limited'. In this case in would be expected to maintain its photosynthetic rate at a constant level, regardless of the CO_2 concentration it growns in. The plant may be

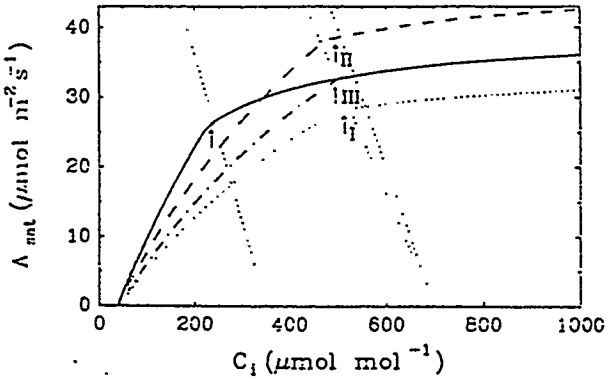


Fig. 1. The simulated response of light-saturated rates of leaf CO_2 uptake (A) with intercellular CO_2 concentration (c_i) calculated from the equations of Farquhar et al. (1980) and Evans and Farquhar (1991) and parameters provided in Long and Drake (1992). The solid line indicates the curve based on the parameters typical of a C_3 leaf developed at current atmospheric CO_2 concentration. Arrows indicate the operating points, i.e., the c_i obtained when $c_a = 350$ μmol mol^{-1} for the solid line and $c_a = 700$ μmol mol^{-1} for the broken lines. The broken lines illustrate the functional consequences of three patterns of acclimation. (I) A reduction in both Rubisco activity and capacity for regeneration of RuBP such that A for leaves grown and measured in 700 μmol mol^{-1} equals that of leaves grown and measured at the current atmospheric CO_2 concentration. (II) A reduction in Rubisco facilitating an increase in capacity for regeneration of RuBP, i.e., a re-optimization of investment reflecting the increased CO_2 concentration. (III) A reduction in Rubisco, reflecting the decreased limitation imposed by this enzyme at elevated CO_2 concentration, but with no change in capacity for regeneration of RuBP.

able reallocate resources away from rubisco, chloroplast membrane proteins and other Calvin cycle enzymes to other parts of the plant.
II) If the nitrogen supply is strictly limiting to growth then the decreased requirement for rubisCO means that plant nitrogen could be reinvested into RuBP regeneration apparatus so allowing the plant to increase its photosynthetic carbon assimilation rate.
III) Hypothesis II assumes that there is no change in the partioning of the nitrogen within the plant organs. But the nitrogen released by rubisCO reductions could be used in other plant organs so increasing plant biomass, sink size, root system etc. thus making the plant more competitive.

The are a number of observations which support this idea. Machler *et.al.* (1988) found that wheat seedlings grown in low nitrogen had reduced glycerate-3-phosphate to triose phosphate ratios, increased ATP/ADP ratios and increased activation of rubisCO, these changes would be expected if photosynthesis was increasingly limited by rubisCO. A/C_i studies of cotton, bean, wheat, spinach and Chenopodium album in low nitrogen show that the initial slope decreases and the curvilinear region occurring at the same C_i indicating that rubisCO and RuBP regeneration are both reducued (Stitt, 1990). Some A/C_i responses indicate an increased capacity for regeneration of RuBP following acclimation, but as yet there is very little evidence that reduced allocation of nitrogen to rubisCO is matched by any increased allocation to other enzymes of the Calvin cycle or thylakoid proteins (Long & Drake 1992). There are a number of common morphogical changes which could indicate a reallocation of resources to sinks, these include, increased tillering in cereals (du Cloux *et.al.* 1987), increased mainshoot development in trees (Oberbauer *et.al.* 1985), induction of flowering (Morison and Gifford, 1984a), increased root nodule activiy in legumes (Idso, 1989). Some possible mechanism by which plants could alter their leaf protein levels in response to changes in their CO_2 environment are discussed later.

4: ACCLIMATION AS A RESPONSE TO SOURCE -SINK IMBALANCE.

4.1: Manipulation of source-sink balance-

A number of studies have artificially changed the balance of sources to sinks to observe any changes this produces. Clough *et.al.* (1981) trimmed the pods of soybean plants and exposed trimmed and untrimmed plants to elevated and ambient CO_2. The rate of photosynthesis was found to decrease faster and further with elevated CO_2 and trimmed (low sink) plants. Peet (1984), decreased the source of photosynthates in soybean by trimming the leaves, this was found to alleviate the negative acclimation in elevated CO_2 compared to untrimmed leaves. Ehret & Joliffe (1985), shaded bean plants in elevated CO_2 and found no negative acclimation.

4.2: Response Patterns Within C_3 Plant Species-

If the hypothesis that source/sink balance is central to elevated CO_2 responses, it would be expected the species with strong sinks, or the ability to increase sink size, to be more responsive. Indeterminate plants with the ability to produce more sinks, should be better able to use the excess photosynthate produced under elevated CO_2. Mauney *et.al.* (1978) ascribed

TABLE 4: Environmental Differences between controlled environments and the field.
(after Lawlor & Mitchell, 1991)

Environmental factor	**Controlled environment**	**Field**
Light	1) Often low intensity 2)Constant 3)Spectral differences from daylight.	1) Very high intensity in sunlight 2) Highly variable
Temperature	1)Often high 2) Constant during day and night	1) Low in winter or at night 2) Very variable through season and from day to day
Light x temperature	Poorly coupled	Strongly coupled
Water	1) Often high humidity 2) Low wind speed 3) Regular application in small amounts	1) Very variable humidity 2)Wind speed variable, can be very high 3) Application very erratic
Nutrition	Regular application in small amounts	Few applications in larger amount
Rooting volume	Very small	Large

the larger growth stimulation of cotton and maize compared to *Helianthus annuus*, to the indeterminate nature of their growth.

Crop plants have been selected and bred for vigorous growth and strong sinks. It may be expected therefore that they will respond better than wild plants. Poorter (1993) found that, on average, C_3 crop plants do show a significantly larger response to CO_2 enrichment than wild species. In a long-term study (one growing season), on winter wheat, Mitchell *et.al.* (1993) found that photosynthesis rate remained high in elevated CO_2. This might be expected as cereals have large and expandable sinks, they can increase tillering in the vegetative phase and the ear is a large sink in the reproductive phase. In most studies of cereals, in elevated CO_2, photosynthesis rates remain stimulated, as shown by gas exchange measurements (Petterson & McDonald, 1994).

4.3 Nitrogen status as a factor in sink/source balance-

If low nitrogen levels reduce the ability of a plant to form larger sinks, responses to high CO_2 could be limited. Wong (1979) reported that for elevated CO_2 grown cotton, photosynthesis rates were more suppressed in low nitrogen growth conditions that in non-nitrogen limiting conditions. However in another study, Wong (1990) reported no loss of photosynthetic rate. Oberbauer *et.al.* (1986), in a three-month study of *Ledlum*, in elevated CO_2, found low nitrogen supply caused a larger down regulation of photosynthesis rate than high nitrogen.

Poorter (1993) suggests that nitrogen fixing species should profit more from CO_2 elevation than other species because their nodules represent a large sink. In his review of studies he concludes that, on average the response of C_3 species capable of symbiosis with nitrogen fixing organism (both herbacious and woody plants) is higher than that of other species. Increased root nodule activity has been reported by Idso (1989). Enhanced CO_2 could have complex indirect effects on growth and photosynthesis rate in nodulating plants (Stitt 1991). Increased nodule development, due to increased photosynthate supply, will supply increased amount of organic nitrogen to the plant. This could be one of the reasons for the sometimes spectacular growth produced by nodulating species in elevated CO_2. Morison & Gifford (1984) found doubling of CO_2 concentrations to increase a number of growth parameters in a range of species with the highest increases in the nodulating species alfalfa (*Medicago sativa*). This species had showed an increase in leaf area of 75% (the largest gain), an increase in dry weight gain of 78%, also one of the highest increases and a 450% increase in the dry weight of reproductive parts (Morison & Gifford 1984a).

4.4: Rooting Volume as a Sink Limitation-

The bulk of CO_2 experiments have been done in controlled environments, Table 4, (adapted from Lawlor & Mitchell, 1991) shows some of the major differences between controlled environments and field conditions. If the source/sink balance, of plants under study, is of critical importance to the response to elevated CO_2, then the light and temperature conditions and potential rooting volumes will have significant influences on this response.

Robbins & Phar (1988) reported that plants grown in small pots accumulated more starch per unit area, had lower carbon assimilation rates and reduced assimilate export rates when compared to those grown in large pots. Arp (1991) examined whether pot grown plants exhibited a greater acclimatory response to elevated CO_2 concentrations than field grown plants. A strong correlation was found, in a review of a wide range of studies, between the

photosynthetic capacity of the plants at identical intercellular CO_2 (C_i) concentrations, and the volume of the pots they were grown in. In all the experiments with a potting volume of less than 3.5 dm^3 growth in elevated CO_2 reduced the photosynthetic capacity at both high and low C_i. In field grown plants or those in the largest containers, the photosynthetic capacity was either the same or increased. It was also found that pot size exterted control over the root:shoot ratio. When the pot size was large, the root:shoot of plants grown in elevated CO_2 generally increased relative to the ratio of ambient CO_2 grown plants. This would support the hypothesis that plants maintain a functional balance between materials essential for growth by allocating resources to the organs nearest to the source of the most limiting material (Acock & Pasternak, 1986, Webber *et.al.* 1994).

The type of root system may also play a role in the sink limitation. In sugarbeet (Wyse, 1980) and radish (Sionit *et.al.* 1982), potsize has been shown to be less important in determining the acclimation response. Both these species use the root as a storage organ for carbohydrate. This large root sink may make end product inhibition less likely.

If sink limitation causes the negative acclimation response, it would be expected that reductions in rubisco activity would also correlate with pot size. From the limited amount of data available it would seem that this is found. Herold & McNeill (1979) observed that pot bound tobacco plants had decreased rubisco activity, von Caemmerer & Farquhar (1984) and Wong (1979) found a decrease in the rubisCO activity of plants grown in 5dm^3 pots, while Chen & Sung (1990), described an increase in the activity of peanut plants grown in 94.5 dm^3 containers.

Arp (1991) also found that pot grown plants possessed reduced amounts and/or changes in proportions of chlorophylls. The chlorophyll content of wheat or soybean grown in the field or large containers were not affected by elevated CO_2 (Havelka *et.al.* 1984, 1984a). The chlorophyll levels of a range of species, have been reported as being reduced when grown in pots at elevated CO_2 (Herold & McNeill, 1979; Cave *et.al.* 1981; Chen & Sung, 1990; Oberbauer *et.al.* 1985). The ratio of chlorophyll *a* over chlorophyll *b* has also been found to decrease in plant grown in elevated CO_2. This change in the ratio of *Chla:Chlb* is also found in shade adapted plants (Björkman, 1981). Arp (1991) suggests that in elevated CO_2, this may reflect an adaptation to internal shading caused by an increase in the number of palisade cells.

The obvious links between negative acclimation to elevated CO_2 and source-sink imbalances led Arp (1991) to reach the following conclusions:

> *"While elevated CO_2 may contribute to the reduction of photosynthetic capacity, this is not an acclimation response to elevated CO_2, but to an imbalance between source and sink....CO_2 enrichment does not lead to end product inhibition when sink demand is high, while restricting root growth can induce end product inhibition at normal ambient CO_2".*

5:POSSIBLE MECHANISMS FOR SINK REGULATION OF PHOTOSYNTHESIS.

The occurence of reduced photosynthetic rates and increased leaf carbohydrate levels have led some workers to link the two events (Long & Drake 1992; Stitt 1991; Webber *et.al.* 1994). In some studies photosynthetic rates changes within hours of carbohydrate

accumulation (Herold 1980; Rufty & Huber 1983) and Mayoral *et.al.* (1985) suggested that this could be caused by stomatal closure. In other studies changes in photosynthesis and carbohydrate occur over a period of days. Stitt (1991) has suggested that there may be three possible mechanisms involved in the sink regulation of photosynthesis, each operating at different time scales.

5.1: Starch Grain Accumulation-

It has been suggested that the accumulation of starch into large grains within the chloroplast, could directly affect the structure and function of these organs. Cave *et.al.* (1981) reported an apparent disruption of chloroplasts in *Trifolium subterraneum.* Grub & Mächler (1990) found that the *in vivo* catalytic efficiency of rubisCO was decreased when starch accumulated. The main problem with this hypothesis is that the evidence consists of correlations and this cannot prove that a causal relationship exists (Stitt 1991), it seems most unlikely that direct inhibition of photosynthesis occurs via starch accumulation (Yelle *et.al.* 1989).

5.2: Phosphate (Pi) Limitation-

It has been shown that regulatory mechanisms via fructose-6-phophate (fru6P), fructose-1,6-bisphosphatase (F1,6Pase) and sucrose phosphate synthase (SPS), increase the rate of sucrose synthesis in response to increased rates of photosynthesis and inhibit synthesis when sucrose accumulates in the leaf (Stitt *et.al.* 1987). Accumulation of sucrose is a consistent feature of plants grown in elevated CO_2. This accumulation feeds back on the Calvin cycle as part of the sucrose/starch metabolism. This can lead to an accumulation of phosphorylated intermediates within the cytosol (Herold, 1980). The phosphorylated intermediates keep hold of inorganic phosphate required for the regeneration of RuBP. The idea of Pi limitation limiting photosynthesis explains some commonly reported observations (Long & Drake, 1992). A decrease in Pi return would lead to inhibition of translocation of triose phophates from the chloroplast, this will promote starch synthesis within the chloroplast, which is consistent with many studies. Decreased available Pi could also decrease the chloroplast ATP/ADP ratio, which in turn would inhibit rubisCO activase, so leading to the decreased rubisCO activities commonly observed.

However the Pi limitation hypothesis is not well supported by experimental data (Stitt, 1991). The small amount of data from studies of metatbolism during sink inhibition of photosynthesis is inconsistent with a direct limitation by low Pi (Stitt, 1991). Pi limitation was not detected by Sage *et.al.* (1989), in 4 acclimated crops. Also plants grown in normal nutrient media contain large amount of Pi in their vacuoles (Bligny *et.al.* 1990). It seems that Pi limitation may play a role in the down-regulation of photosynthesis during sink limited acclimation but there are probably other mechaisms involved.

5.3: Adaptative Mechanisms-

This may involves changes in the amounts of key proteins required for photosynthesis. Changes of this type can be viewed as an adaptation to a sink-source imbalance because they would allow resources such as amino acids to be remobilized from the leaves and reinvested in sinks. As discussed above, there are sound resource use efficiency reasons why plants would

want to change photosynthetic protein concentrations with changing CO_2 concentrations (Long & Drake, 1992).

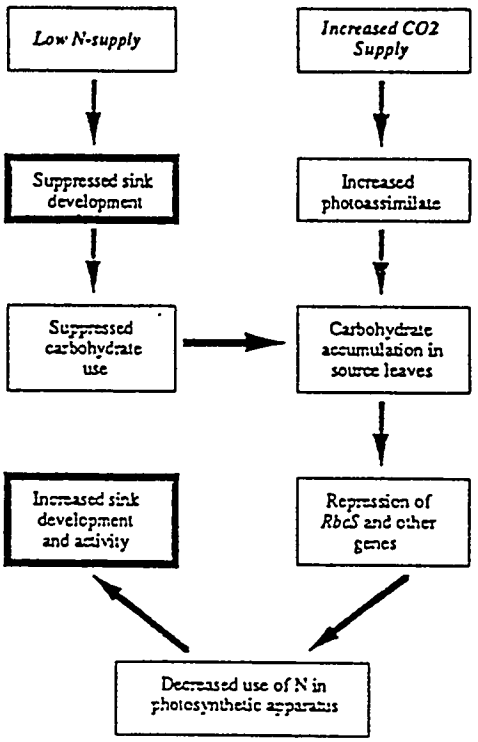
Recent studies with transgenic tobacco plants, expressing yeast invertase in their cell wall, provide strong evidence that accumulating carbohydrate can lead to a decrease of rubisCO and other Calvin cycle enzymes (von Schaewen *et.al.* 1990). Sheen (1990) reported that the expression of reporter genes, linked to several photosynthetic promoters, is inhibited by sucrose, glucose and fructose in a maize protoplast. Carbohydrates produced by secondary metabolism e.g. sorbitol and mannitol, had no effect (Sheen *et.al.* 1992). Leaf age has also been found to affect the reduction of rubisCO gene activity. This may reflect the change from sink to source during leaf development. If carbohydrate levels reduce expression then older leaves, as sources, will accumulates more carbohydrate so will be inhibited first (Stitt 1991).

There is also evidence that CO_2 may directly affect gene expression . Winder *et.al.* (1992) worked with a green algae and reported that decreasing CO_2 levels, decreased the expression of rubisCO genes, both in the chloroplast (*rbcL*) and cell nucleus (*rbcS*). The direction of the change does not lend support the inhibitory effects of elevated CO_2, but the fact that there is a change suggests CO_2 can influence gene expression. The relevance of results from a green algae, to plants is, however, uncertain (Long *et.al.* 1993). Webber *et.al.* (1994) report that in controlled environment studies, a doubling of CO_2 concentration produced an 8% reduction in the levels of *rbcL* and *rbcS* transcripts and a corresponding 8% decrease in total rubisCO, in wheat. When nitrogen was limiting, rubisCO protein levels are 50% reduced in doubled CO_2, with a similar concomitant decline in the steady-state accumulation of *rbcS* and *rbcL*. In wheat growing in field FACE (Free-atmospheric carbon dioxide enrichment) experiments, with no nitrogen limitation, mRNA levels for *rbcS* and *rbcL* decline in relation to leaf age, at elevated CO_2. Fig 2 (from Webber *et.al.* 1994) shows how elevated CO_2 and nitrogen supply may lead to changes in photosynthetic gene expression.

5.3: Times Scales of Acclimation Responses-

Acclimation may be expected to result from a number of processes operating on different time scales. Direct inhibition of genetic expression of rubisCO proteins could occur quickly in plants moved to high CO_2 environments, and would represent a long-term acclimation response (Stitt 1991). Feedback limitation, perhaps caused by phosphate limitation could also occur until the plant had time or resources to produce new sinks.The development of new sinks in perennial species may require years (Arp & Drake, 1992), in these species carbohyrdrate reserves may be carried over to the next season. Long, *et.al.* 1993 have stated that it is possible that loss of photosynthetic capacity in elevated CO_2 conditions, observed over the first weeks or months of growth, may be a temporary phenomenon resulting from insufficient nitrogen uptake or sink size.

To date most of the experiments conducted, that have shown decreased rubisCO and photosynthetic down-regulation, have been with annual crops or perennial species grown for less than one year, (Long & Drake, 1992), usually in controlled environment chambers (Lawlor & Mitchell,1991). Long-lived perennials form the dominant primary producers of most natural terrestrial ecosystems (Webber et.al 1994). Until a large number of experiments are conducted in the field under natural conditions and on a long-term basis, it will not be possible to make predictions about global biological responses to elevated CO_2.



2. Scheme overviewing how elevated c_a and N-supply lead to changes in photosynthetic gene expression.

6: FIELD EXPERIMENTS AND PLANT RESPONSES.

There have been relatively few field based studies concerned with elevated CO_2 responses. The few available results are very variable as would be expected under field conditions. Those that have been conducted have used two methods:

6.1: Open Top Chambers (OTC's)-

OTC's are small greenhouses, they allow plants to grow in the normal soil, so reduce pot size effects, at a controllable CO_2 concentration. They have been used to study a number of species. They do however produce a number of changes to the plants growth conditions; temperatures may be 1-3°C higher than outside, radiation levels may be attenuated, plants are protected from the wind pests and diseases and the air mixing profile, up through the plants may be inverted compared to the normal situation. All these changes may affect growth even before CO_2 concentrations are elevated. Such affects have been recorded, OTC grown sweet potato show substantial decreases in dry mass (Biswas & Hileman 1985). Rogers *et.al.* (1986, 1983) found increases in dry mass yields from soybean and maize plants grown in OTC's.

6.2: F.A.C.E. (Free-atmosphere carbon dioxide enrichment) Technology-

This is a relatively new development. A toroidal plenum (PVC ring) connects to vertical vent pipes (VVP's) and emit CO_2 enriched air over plants growing in the field (Hendrey *et.al.* 1993). The concentration of the CO_2 is kept within certain limits by keeping track of wind speed and direction and altering the pattern of emission accordingly. At present this system is the best way of studying plant responses to elevated CO_2.

6.3: Observed Field Responses-

In nearly all C_3 crops studied in the field, there is no down regulation of photosyntheis (Kimball *et.al.* 1986; Campbell *et.al.* 1988; Arp,1991), except in some cases near the end of the growing season. This has been attributed to decrease growth due to aging, decreased temperature or sink saturation (Kimbal *et.al.* 1986; Radin *et.al.* 1987). In controlled environment measurements on cotton, a marked decline in photosynthetic capacity was observed, but continuous elevation of CO_2 around cotton in a field failed to induce this loss (Radin *et.al.* 1987). Lawlor & Mitchell (1991) compared the effects of CO_2 enrichment on crop productivity under field conditions, with those given in the Cure & Acock (1986) review. Some of these comparisons are given in Table 5.

Two studies have grown perennial plants in OTC's at double ambient CO_2 concentrations, for over five years. The C_3 sedge *Scirpus olneyi* has been found to acclimate over several years, this is attributed to changes in sink sizes, root systems and tissue C/N ratio. During this time photosynthetic capacity has increased by 31% (Arp & Drake, 1991). Sour orange trees (*Citrus aurantium* L.) have been grown from seedlings for 5 years. In both these species, Nie & Long (1992, in Webber *et.al.* 1994), have found a large number of soluble leaf proteins have decreased in concentration. The range of reductions is from less than 10% to more than 50%. These results demonstrate that, in the longer term, acclimation of photosynthesis involves considerably more than a loss of rubisCO protein and activation (Webber *et.al.* 1994).

TABLE 5: Crop productivity under field conditions with elevated CO_2 (After Lawlor & Mitchell, 1991).

Crop/parameter	Cure & Acock (1986) result	Field result	Reference
Cotton			
biomass	+84%	+82%	Idso *et.al.* 1987
seed yield	+209%	+87%	(OTC, 3 years, x2CO_2)
Soybean			
biomass	+39%	+22% & +78%	Rogers *et.al.* 1986 & 1983.
seed yield	+29%	+7% & +55%	(x2 CO_2)
Sweet potato			
yield	+83%	+45%	Biswas & Hileman, 1985
Rice			
biomass	+27%	+27%	Baker, *et.al.*
grain yield	+15%	+47%	1990. (OTC)
Wheat			
dry matter	+31%	+20%	Havelka *et.al.*
grain yield	+35%	+17%	1984a(>x3CO_2)
Maize			
dry matter	+9%	+49%	Rogers *et.al.*
yield	+29%	+55%	1983.

Early FACE, results with cotton have shown a broad agreement with glasshouse and OTC studies (Mauney *et.al.* 1994), some of these results are shown in Table 6. Some of the conclusions from this early work are that shifts in the carbon partioning by plants must be taken into account in carbon balances of the Earth, elevated CO_2 has the potential to increase biomass productivty, in crop systems new cultivars may be able to take advantage of this increase.

6.4: Temperature / Carbon Dioxide Interactions-

Whilst not strictly a direct effect of elevated CO_2, temperature effects on acclimation will need to be investigated. Temperature has been shown to have a great effect on CO_2 enrichment responses. Interactive effects between temperature and CO_2 are little understood but could be very important for a number of reasons. In the future increased plant temperatures are more likely to be experienced as leaf temperature is observed to increase due to reduced stomatal conductance (Morison & Gifford 1984), global warming due to enhanced greenhouse effect has the potential to increase temperatures globally and temperature fluctuations and extremes are more likely in the field than in controlled environments.

Idso *et.al.* (1987) showed that for carrot, radish and cotton, growth stimulation with a doubling of CO_2 was 100% at 32°C but nil at 19°C. With increasing temperature, photosynthetic efficiency decreases as photorespiration increases. This is because increased temperature favours oxygenation by rubisCO due to solubilty and specificity effects (Long & Drake, 1992). However increasing CO_2 concentrations also alter the response of the photosynthetic apparatus to temperature, theoretically increasing the optimum temperature of carbon assimilation, increasing the maximum light saturated assimilation rate (A_{sat}) and increasing the upper temperature at which a positive A_{sat} is maintained (Long & Drake, 1992). These effects could mean that photosynthetic rates continue to be stimulated in an elevated CO_2 environment with increasing temperature.

7: CONCLUSIONS.

It has been realised that initial conclusions about the negative acclimation of crop species to elevated CO_2, may be more of an artifact of sink limitation, of one form or another, than a genuine and likely, natural response to change. The studies of sink feedback mechanisms have provided insights into this important area of plant physiology. In the field it is likely that elevated CO_2 will produce numerous changes to plants, these changes may or may not be sink limited. The changes may occur over many years, giving plants in natural ecosystems a chance to adapt, and giving crop breeders a chance to make the best use of the future atmospheric conditions. To do this it is essential that realistic, field-based, crop responses to atmospheric change are analysed and understood. The real challenge will be to synthesise this new body of information into a practical form, which will allow future changes in species composition and interaction to be assessed. Predicting how species of plants will interact with each other, in the future, may be more difficult than predicting how they interact with the atmosphere in the present.

TABLE 6: Early results from FACE-grown cotton.
(After Mauney *et.al.* 1994, Hendrey *et.al.* 1993)

	Effect of FACE Treatment (% age) Dry Plots	**Effect of FACE Treatment (%age) Wet Plots**
Biomass 1990 1991	+17* +35	+34 +37
Above-ground dry weight	+50	+35
Boll counts	+37	+28
soil respiration	+95	+20
fungal count		+90
	FACE compared to non-FACE.	
Leaf level photsynthesis	+20 to + 40%	
Leaf transpiration	-30 to -33%	
Taproot dry weight	+71	
Lateral root dry weight	+104	

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