Project title:	Cucumbers: the role of environmental and agronomic factors in carbon dioxide toxicity
Project number:	PC 159
Reports:	Final Report (August 2002)
Previous reports:	Annual reports in 2000 and 2001
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Date project commenced:	1 February 1999
Date completion due:	31 March 2002
Keywords:	Photosynthesis, carbon dioxide enrichment, carbon dioxide toxicity, leaf bleaching, RUBISCO, down-regulation, antioxidant activity, senescence, leaf age, nitrogen, shading

The results and conclusions in this report are based on a single series of experiments conducted over a three year period. The conditions under which the experiment was carried out and the results have been reported with detail and accuracy. However, because of the biological nature of the work it must be borne in mind that different circumstances and conditions could produce different results. Therefore, care must be taken with interpretation of the results especially if they are used as the basis for commercial product recommendations.

Authentication

I declare that this work was done under my supervision according to the procedures described herein and that this report represents a true and accurate record of the results obtained.

Signature .....

Prof. W Davies
Report Editor
Lancaster University

Date .....

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Practical Section for Growers

Background

While CO_2 enrichment of cucumbers is routinely used to enhance growth and yield, increasing numbers of growers report that if very high concentrations of CO_2 are employed or if high concentrations occur somewhere in the glasshouse due to faults in the CO_2 delivery system or its improper operation, then leaf bleaching will occur. This damage to the leaves is very unsightly and may ultimately lead to reduced carbon gain, growth and crop yield.

Objectives

This project was designed to try to understand the mechanistic basis of leaf damage due to high CO_2 enrichment and where possible, develop methods to minimise the occurrence of the problem. This might enable growers to use higher concentrations of CO_2 without risk of crop damage and thereby enhance yield and profitability.

Results and Conclusions

Do high concentrations of CO_2 cause damage to cucumber plants and what are the symptoms?

Leaf damage resulting from very high CO_2 concentrations (also known as CO_2 toxicity) which is manifest as leaf bleaching, occurred in this study both in controlled environments and in the glasshouse, when CO_2 concentrations of higher than 1000 vpm were employed but only when these levels of enrichment were supplied during bright, sunny weather (or high radiant loads in the controlled environment chambers). A combination of these factors results in the well-recognised symptoms shown in Plate A.

Other morphological changes to the cucumber plants were observed at 2000 vpm CO_2. These included changes in the xylem diameter and leaf stomatal density and such changes may further stress the shoots and exacerbate the leaf bleaching symptoms observed with CO_2 toxicity.

Plate A A healthy cucumber crop (top photo) and a crop showing clear symptoms of CO_2 toxicity (bottom photo).

The biochemical/physiological basis of damage

Our physiological investigations have shown that these symptoms result from a well-documented physiological condition in the leaves: this is known as oxidative stress. This condition arises when the leaf traps radiant energy from the sun and for a variety of reasons cannot use this energy, which then damages the leaf. The hypothesis here is that very high CO_2 concentrations cause lesions which prevent the leaf from effectively using radiant energy.

Can CO_2 toxicity damage be avoided?

Most importantly, leaf damage was not apparent (even at the highest CO_2 concentrations and the highest radiant loads) when the crop was grown in the glasshouse on the high-wire system. This appears to be because only older leaves are susceptible to damage and with the high wire system of cultivation, these leaves are mostly less exposed to direct, intense sunlight.

The project has also shown that leaf bleaching can be avoided at even the highest CO_2 concentration tested (2000 vpm) if leaves are shaded during the brightest periods in the glasshouse in Spring (from January to April). Mild shading (~35%) at 2000vpm CO_2 prevented the development of leaf bleaching and also severe damage of the photosynthetic systems, as observed in the unshaded treatments.

A small scale controlled environment study demonstrated that doubling N levels in the liquid feed from 144 ppm N to 288 ppm N, prevented the onset of leaf bleaching (leaf damage) under 2000 vpm CO_2 enrichment.

If we avoid leaf bleaching, can we enhance yield?

One glasshouse trial has shown that leaf bleaching can be associated with reductions in fruit production. Preliminary glasshouse trials demonstrated that shading of the crop for an extended period of time avoids the development of leaf bleaching and sustains the rate of photosynthesis but this treatment did not improve fruit yield. Further work is needed here to investigate the possibility that shading only during the brightest periods will allow us to avoid crop damage due to CO_2 levels beyond 1000vpm and enhance yield further.

It has not been possible to test the effects of extra nitrogen fertiliser (288 ppm N) on fruit yield but controlled environment experiments have shown that leaf growth is promoted by this treatment and leaves remain very green and do not show the development of symptoms or biochemistry of oxidative stress under high CO_2 enrichment. Low N (144 ppm N) plants grown under the same conditions showed clear development of symptoms. Further work is needed in this area.

Is there a test that could be used as an early warning of stress?

Our biochemical results suggest that a simple diagnostic test could be developed as an early warning of CO_2 induced oxidative stress and may allow growers to use higher CO_2 concentrations without risk.

Action Points for Growers

Until the findings from this project are evaluated further under semi-commercial conditions, the following guidance is proposed.

How to get the most from CO_2 enrichment without increasing risk of CO_2 toxicity

1. CO_2 enrichment/dosing system

- Calibrate CO_2 detection equipment regularly
- Don't aim for the highest levels of enrichment, particularly when fixed screens are used
- Match boiler output to demand. High boiler output can give very high enrichment peaks at the start of the enrichment period
- Check frequently for leaks in supply mains
- Aim for an even pressure throughout the distribution system
- Check that lay-flat tubes are all inflating
- Be aware of CO_2 dead zones: (Importance of sensor location/ multiple sensors)

2. Sink Strength

- Maintain a balanced fruit load – don't overload the stem
- Maintain a young plant canopy with high wire crops or with a three crop strategy

3. CO_2 enrichment strategy

- Aim for 600 vpm CO_2 from the start of enrichment
- Increase this to 800 vpm [600 + 200] once the crop is established and the light levels improve
- Once the crop is stopped [cordon crops] increase the level to 1000 vpm [800 + 200]
- The above levels are target levels. Your set points may have to be lower to achieve these levels in the growing area

Science Section

Introduction

Previous investigations on the response of *Cucumis sativus* plants to CO_2 enrichment were aimed at understanding the cascade of events that ultimately leads to leaf necrosis. We need to understand the biochemical/physiological basis of leaf necrosis and why, in some cases (e.g. high wire crops, plants grown in shaded conditions), these symptoms can be avoided. As a result of previous work, we strongly believe that the physiological age of the leaf and the incident light environment interact very strongly to produce what we hypothesise is photo-oxidative damage. In the experiments reported here we provide data to support this hypothesis and establish a clearer picture of physiological changes within a leaf subjected to CO_2 enrichment.

As a leaf naturally ages and begins the process of senescence (a genetically regulated process which leads to the death of cells, organs or whole organisms (e.g. Hodges & Forney, 2000)), changes in leaf constitution are taking place, and this could present an additive factor to those changes brought about by elevated CO_2. For example, as a leaf undergoes senescence, decreases in RUBISCO protein and chlorophyll contents are observed (Buchanan-Wollaston, 1997).

Miller *et al* (1997) have advanced a novel explanation of photosynthetic down-regulation. These researchers suggest that in high CO_2 (950ppm CO_2) there has been a temporal shift in the natural ontogenetic development of a leaf, such that senescence-related changes (loss of chlorophyll and RUBISCO activity) occur at an earlier stage of leaf development compared with ambient-grown counterparts. This mechanism for acclimation in high CO_2 environments has also been proposed by Foyer (2001) and it has been suggested that elevated CO_2 causes plants with determinate life histories to develop at a faster rate (Krapp & Stitt, 1999).

Plant senescence is a complex process, involving the co-ordination of many physiological processes and which ever proposed mechanism for photosynthetic down-regulation holds true (either a direct down regulation of photosynthesis enzymes championed by Besford *et al* (1990) or the theory put forward here) the same

reduced photosynthetic performance by a mature leaf grown in elevated CO_2 will eventually be recorded. The decline in P_{max} (maximum rate of photosynthesis) and loss of photosynthetic pigments, especially in an environment with a high radiant load, leaves the plant vulnerable to photo-oxidative stress, caused by the production of free radicals (unstable electrons) within cells. The plant does have a range of defences to fend off an oxidative attack. Foremost among these are carotenoid pigments and superoxide dismutase (SOD).

Carotenoids are probably the most widely distributed class of pigments in nature. As well as having a role in photochemical reactions, they also perform a photoprotective role by quenching singlet oxygen when it is produced and prevent its production by quenching the triplet state of chlorophyll (Young, 1991).

SOD is a key enzyme in the plant's antioxidant system (AOS). Whereas carotenoids are most closely associated with chlorophyll complexes, SOD activity is concentrated in the cytosol (Scandalios, 1993). SOD offers photoprotection by dismutasing the O_2^- radical to hydrogen peroxide (H_2O_2), which is then further processed to water by the action of ascorbate peroxidase or catalase (Hodges & Forney, 2000). An increase in SOD activity has been reported to occur in tomato varieties tolerant to sunscald (which is caused by high light intensities and high temperatures) (Rabinowich & Sklan, 1980). These authors report that plants developing in the shade at low temperatures will have low SOD and will be susceptible to sunscald during sudden exposure to high light/temperature. This could be significant for our research. During the growth of a spring crop the light intensity is generally low in the early stages of growth; but days with a high radiant load become more common at later stages and may override the plant's natural antioxidant defences.

The work presented in this report is split into three parts. Firstly, we report on a glasshouse-scale investigation to elucidate the effect that mild shading has on physiological variables and fruit yield in *Cucumis sativus* grown in two CO_2 regimes, 1000 and 2000vpm. Secondly, we investigate the effect of an elevated nitrogen supply (288ppm, compared with the recommended 144ppm) within the hydroponic feed on photosynthetic performance and the onset of symptoms of CO_2 toxicity in plants

grown at 2000vpm CO_2. Finally an experiment is carried out, whereby the SOD activity of a stress tolerant cucumber cultivar (Enigma) is compared with the industry standard (Sabrina) to assess the possibility of a differential oxidative response to elevated CO_2.

In the first two experiments, gas exchange measurements were undertaken as well as an analysis of RUBISCO content, to determine the extent of photosynthetic downregulation. In order to gain an appreciation of the level of senescence and activity of the AOS, photosynthetic pigments and SOD activity were also quantified.

Materials & Methods

***The Glasshouse crop*:** 2 adjacent modern, 55m^2, glasshouses with CO_2 enrichment and fertigation (Dosatron, France) facilities were made available at Lancaster University for this work. The CO_2 monitoring units (Horiba APBA200E) operated by automatically switching on and off a solenoid valve to regulate CO_2 concentration between set values. Pure CO_2 (BOC) was fed directly into the glasshouses via perforated PTFE tubing laid close to the floor, and allowed to diffuse throughout the crop. Regular measurements of CO_2 concentration were undertaken with a portable analyser (EGM, PP Systems, Hitchin, UK) to determine whether desired concentrations were achieved.

Temperature, humidity and light intensity sensors, connected to a datalogger (Delta-T Devices, UK) facilitated the recording of glasshouse environmental conditions. Shading treatments were achieved by placing 35% shade netting (LBS, Colne, UK) in the roof of one half of each glasshouse in such a way (N-S orientation) that overlap shading did not occur in the unshaded treatments at the brightest period of the day.

80 Seedlings of cultivar Sabrina were obtained from Crystal Heart Growers, and allowed to acclimatise to the glasshouse environment for a week before the imposition of treatments (2000vpm and 1000vpm CO_2 respectively).

Plants were grown in a standard hydroponic rockwool system and manipulated in accordance with industry guidelines for best glasshouse practice for a low wire crop system.

***Fruit Yield*:** Fruits were harvested as they matured and numbers and weights collated on an individual plant basis.

***Leaf Tagging*:** Leaves at node 10 were tagged and subsequently all physiological measurements were taken on this cohort. When the plants reached the wire (1.8m), the youngest leaves on the plant at this time were also tagged.

***Controlled Environment (CEC) Crop*:** Seeds of *Cucumis sativus* (Nunhems, Holland for **Sabrina**, Rijk Zwaan, UK, for **Enigma** cultivars) were germinated on rockwool (Grodan, Fargro, UK) blocks in a humid environment. Supplementary lighting was provided as necessary. When the seedlings had reached the 4^{th} leaf stage they were transferred to the CEC and allowed to acclimatise for 5 days before the treatments were imposed. The environmental conditions and CEC set-up have been described previously (HDC report for project PC 159, 2001). The daily average target for temperature was 20 °C and the photoperiod experienced was 12hr, with the light intensity increasing and decreasing in a step-wise fashion at the start and end of the photoperiod.

***Leaf Tagging*:** The youngest leaves (Leaf 4 or 5) were tagged at the start of the experiment and measured throughout the investigation. Leaves were subsequently tagged in mid- and upper-canopy positions to allow an investigation of leaf age on measured physiological variables.

***Gas Exchange*:** An Infra-red Gas Analysis (IRGA) system (CIRAS-1, PP Systems, Hitchin, UK) was used in the way previously described (HDC report for project PC 159, 2001) to determine gas exchange variables both in the growth environment of the plant measured, and in the other treatment concentration (e.g. For an ambient plant, measurements were taken at 360vpm and 2000vpm CO_2). In each investigation, tagged leaves were measured every 5 or 7 days throughout the experimental period.

***RUBISCO Content*:** The amount of Ribulose-1, 5, Bisphosphate (RUBISCO) in leaf samples was determined semi-quantitatively in a two-step approach. Samples were extracted in buffer and introduced to a mini-gel system (BioRad Mini-Protean III) and subject to SDS-PAGE with relevant RUBISCO standards. The band corresponding to the RUBISCO protein was subsequently macerated and eluted for 12hr. Spectrophotometry was then performed, alongside soluble protein analysis (Bradford's assay, Bradford, 1976) to allow the determination and expression of RUBISCO on a total soluble protein basis. (For complete method, refer to HDC report for project PC 159, 2001)

***Leaf Pigment Analysis*:** Samples were collected for the determination of Chlorophyll and Carotenoid concentration. Three 1cm diameter leaf discs were taken for each sample and stored temporarily under liquid nitrogen. The fresh weight of each group was taken and the leaf discs were homogenised by pestle and mortar in a 1.5ml solution of chloroform/methanol (3:1). After the addition of 1.5ml distilled water, 500µl of supernatant was removed and centrifuged at 13000rpm for 5 minutes. 200µl of the resulting supernatant was then taken and 1.8ml chloroform was added. The absorbance of these solutions at wavelengths of 480, 647.6, and 665.5nm respectively was determined spectrophotometrically (Uvikon 860) and the pigment content was calculated based on the equations of Welburn (1994) (Fig. A), and expressed on a µg pigment FW^{-1} basis.

Fig. A. Equations for leaf pigment determination:

Chlorophyll a (Chla) = $(11.47 * Abs_{665.6}) – (2 * Abs_{647.6})$

Chlorophyll b (Chlb) = $(21.85 * Abs_{647.6}) – (4.53 * Abs_{665.6})$

Total Carotenoids = $(1000 * Abs_{480}) – (1.33 * Chla) – (23.9 * Chlb) / 198$

***Superoxide Dismutase Activity (SOD)*:** This assay for measuring a prominent enzyme of the antioxidant system is based on the methods developed by Seven *et al* (2000) and measures the capacity of SOD, in samples, to inhibit the reduction of nitroblue tetrazolium (NBT) by superoxide. One unit of activity is defined as that amount of enzyme causing half the maximum inhibition of NBT reduction.

Three 1cm leaf discs were taken for each sample and extracted at a concentration of 0.1g FW per 1ml of buffer solution (phosphate buffer, pH 7.5) with a pestle and mortar. Samples were centrifuged for 2 minutes at 12000rpm and the subsequent supernatant was retained and stored at -18°C (if necessary). For the reaction, 96-well microtitre plates were used and samples were replicated within this system in triplicate. 25µl of sample was added to a well, followed by 225µl of buffer solution (90% Phosphate buffer, pH 7.5, 6.6% EDTA, 0.1M, 3.3% Nitroblue tetrazolium

(NBT), 1.5mM). 50µl of Riboflavin was rapidly added and the well was left to stand on a light-box for 20 minutes. Appropriate standards were included with each plate, and the absorbance at 600nm was subsequently determined using a microplate reader (Labsystems, operated by GENESIS software).

Results and Discussion

Part I:

The interactive effects of high irradiance and elevated CO_2 on gas exchange parameters and the onset of leaf bleaching in a glasshouse crop of *Cucumis sativus*.

Introduction

The aim of this investigation was to confirm, on a glasshouse scale, hypotheses produced as a result of experiments conducted in CEC conditions in the year 2000. We established that mild shading can ameliorate the negative effects of high CO_2 on photosynthesis in leaves of *Cucumis sativus* and also prevent the formation of CO_2 induced necrotic regions. We would like to determine whether this theory holds in a system where plants experience similar conditions to those in a commercial glasshouse. Also, by growing a full size crop we will be able to investigate differences in fruit yield in respective treatments.

20 plants were grown to maturity (grown for 6 weeks) in each of the following treatments:

Glasshouse ID	**Treatment**
GH1US	1000vpm CO_2 Unshaded
GH1S	1000vpm CO_2 Shaded (35%)
GH2S	2000vpm CO_2 Unshaded
GH2US	2000vpm CO_2 Shaded (35%)

Gas Exchange: Repeated gas exchange measurements were taken once a week on leaves that had been tagged on day 1 of initiation of treatments (Leaf 10). At the end of the experiment (Day 40), the youngest leaves at the top of the canopy were also measured. Assimilation rates and stomatal conductance of leaves was measured at 360, 1000, and 2000vpm CO_2 respectively.

RUBISCO Content: Samples were taken for analysis, both in mature and immature leaves at day 40, to assess if growth in elevated CO_2 could cause a downregulation in the synthesis of this protein.

Photosynthetic Pigment Analysis: Chlorophyll a, b and total carotenoids were quantified in leaf 10 and in the youngest leaf at the beginning (day 5) and end (day 40) of the experiment. 3 replicates were taken per leaf and per treatment.

SOD activity: Samples were taken for analysis at the end of the experiment. It was postulated that SOD activity would increase before any visual signs of leaf damage and hence could act as an early warning system for CO_2-induced damage.

Results

Symptom Development: Plants in all treatments were visually similar throughout the experimental period, until 5 days from the final sampling day. At this juncture, there were several days of high radiant load, after which plants in the 2000vpm unshaded treatment became chlorotic with large areas of necrosis evident on leaf surfaces. Leaves in the shaded treatment under 2000 vpm CO_2 were healthier looking, but not as green as those in 1000vpm CO_2 treatments.

Gas Exchange: It is clear that even after 5 days, a differential stomatal response is apparent between treatments. The stomatal conductance in leaves grown at 2000vpm CO_2 is significantly lower than that measured at 1000vpm (Fig. 1). This relative closing suggests a potentially stronger non-biochemical limitation for photosynthesis at higher CO_2 levels. Even so, photosynthesis seems relatively unaffected in all treatments. At day 40 the damaging effect of 2000vpm CO_2 is striking and a protective role of shading is evident. Photosynthesis in 2000vpm CO_2, unshaded treatments is severely downregulated, 35% shading achieving partial amelioration of this but only to half the rates of the 1000vpm CO_2 crop.

Pigment analysis mirrors this trend. Mature leaves grown in an atmosphere of 2000vpm CO_2 and receiving no shading had significantly lower chlorophyll and carotenoid concentrations (Fig. 2a), whereas shading in this case was statistically

indistinguishable from other treatments. In younger leaves (Fig. 2b), growth in 2000US caused a significant decrease in total chlorophyll compared with 2000S and the latter treatment had a lower value than the 1000vpm treatments. The ratio (Chl a:b), which is often regarded as an indicator of senescence, shows a sharp decline in the 2000US treatment (Fig. 3). Although a downregulation of photosynthesis was apparent, no significant differences in total RUBISCO content were apparent (Fig. 4)

The status of the antioxidant system, represented as total **SOD activity** (Fig. 5) was affected by the treatments. There is a significant effect of CO_2 and shading, suggesting that plants grown in 2000vpm CO_2 treatments endure a greater oxidative insult than in other treatments.

After scrutinising the photosynthesis data, it is perhaps surprising that, when expressed on an individual plant basis, plants in shaded treatments yielded fewer fruits than those in unshaded compartments (shaded plants had higher rates of carbon gain). With hindsight however, a much clearer effect of treatment on yield would have been obtained had we continued to sample. Because the biology of dead/dying plants was of limited interest, the experiment was terminated at the point that severe leaf damage occurred on the high CO_2/unshaded crop. This damage would undoubtedly have reduced yield had we continued to sample. The levels of CO_2 employed in this experiment made no significant impact on fruit yield (Fig. 6 & 6a).

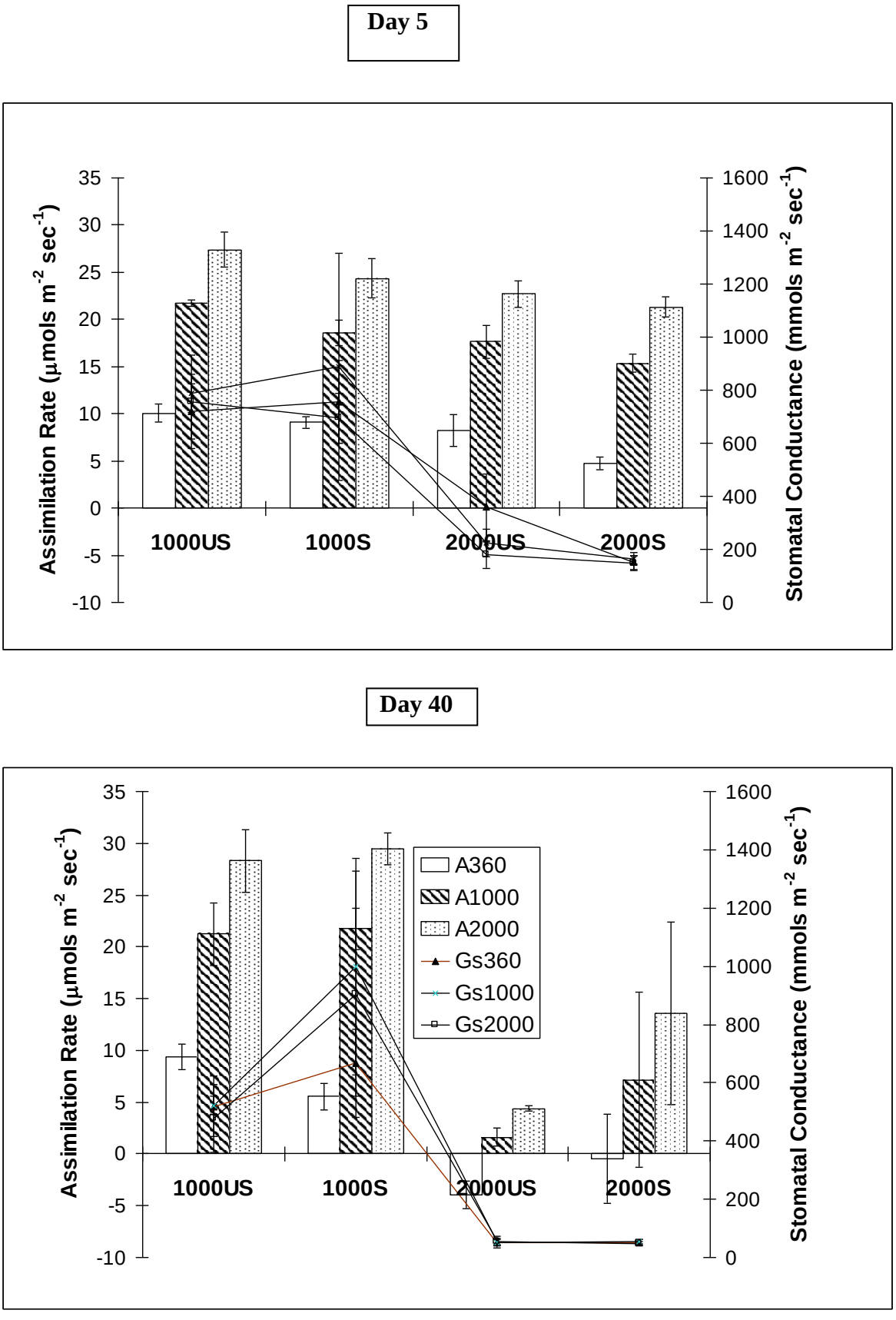


Fig 1. Assimilation Rate (A) & Stomatal conductance (Gs) of leaves, tagged when CO_2 regimes initiated and subsequently measured throughout their development, of *Cucumis sativus* (Sabrina) after 5 and 40 days of exposure to respective treatments (4 reps per treatment. $\pm$ SE). 1000US = 1000 vpm CO_2 & unshaded; 1000S = 1000 vpm CO_2 & shaded; 2000US = 2000 vpm CO_2 & unshaded; 2000S = 2000 vpm CO_2 & shaded.

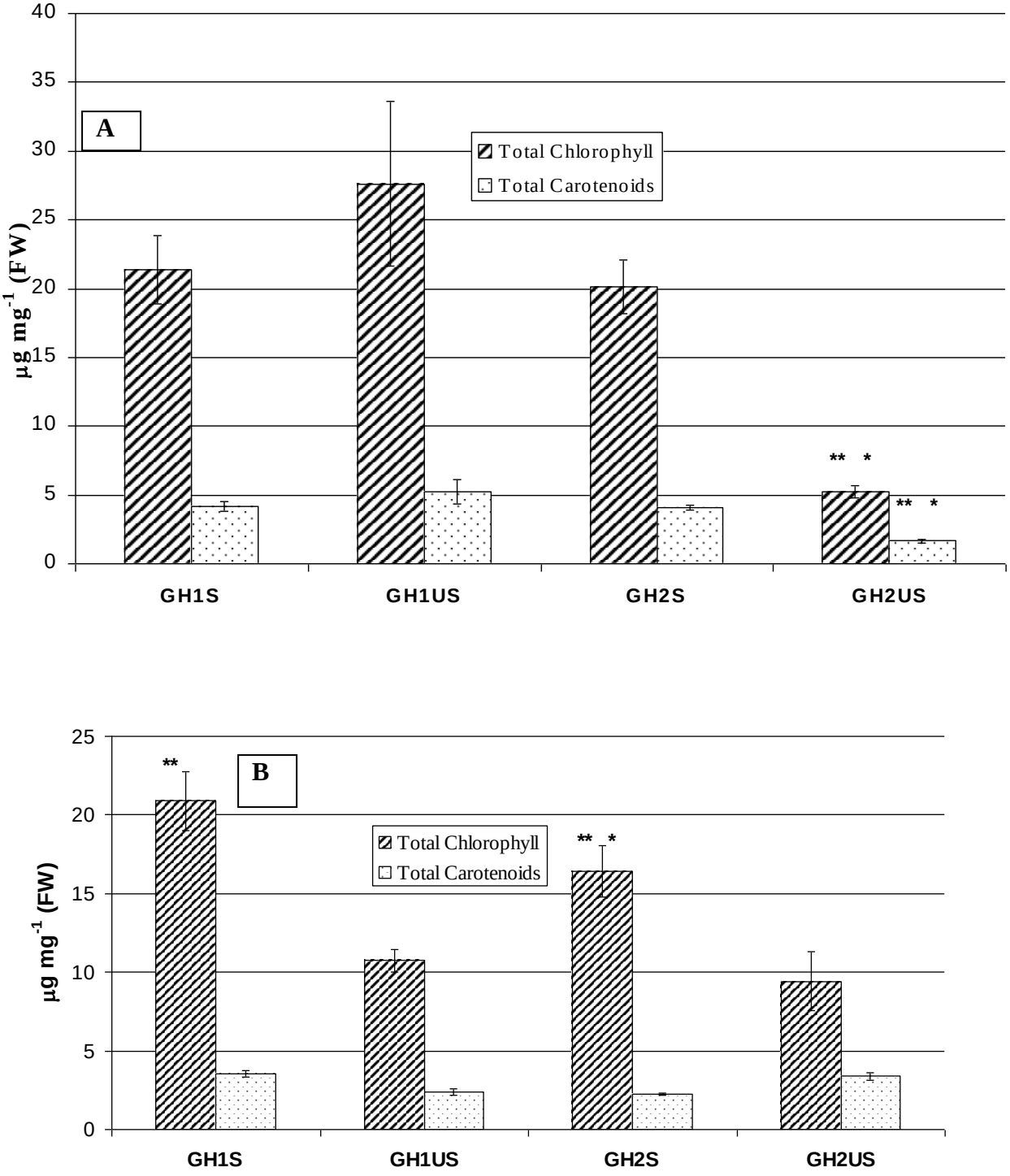


Fig 2. Total Chlorophyll and Carotenoid content, expressed on a fresh weight basis, of leaves of *Cucumis sativus* (Sabrina). Measurements were taken on leaves at node 10 (**A**) and node 26 (**B**)(main stem growth terminated at this point as plants had reached recommended height) after the plants had been exposed to their respective treatments for 40d.(5 reps per treatment (± SE). * and ** denotes significant CO_2 and shade effects between treatments respectively. GH1S = 1000 vpm CO_2 & shaded; GH1US = 1000 vpm CO_2 & unshaded; GH2S = 2000 vpm CO_2 & shaded; GH2US = 2000 vpm CO_2 & unshaded.

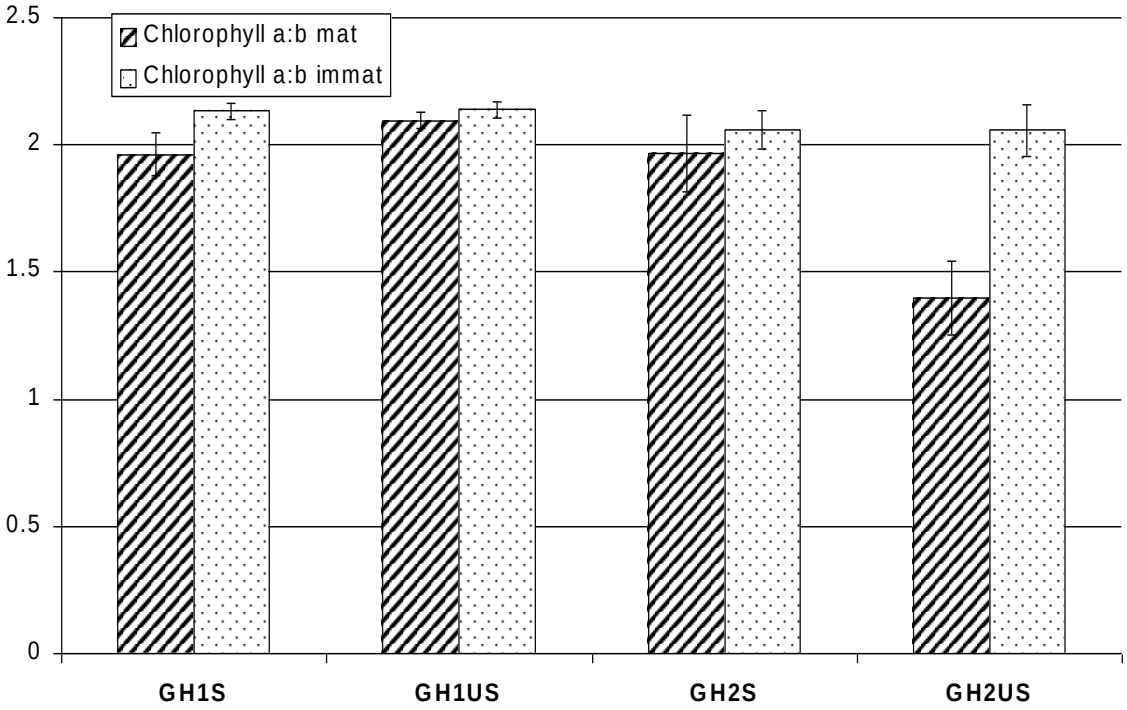


Fig 3. Chlorophyll a to b ratio of mature (Node 10) and immature (Node 26) leaves of *Cucumis sativus* (Sabrina) after 40 days of plant exposure to respective treatments (± SE). GH1S = 1000 vpm CO_2 & shaded; GH1US = 1000 vpm CO_2 & unshaded; GH2S = 2000 vpm CO_2 & shaded; GH2US = 2000 vpm CO_2 & unshaded.

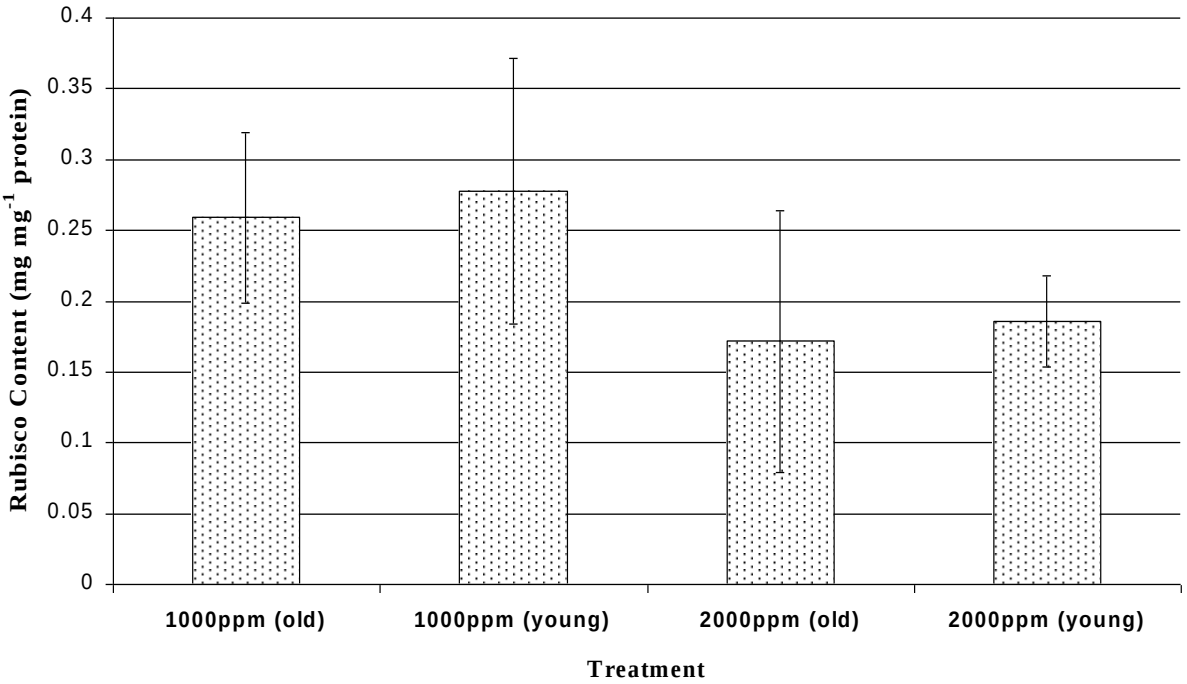


Fig. 4. SDS-PAGE Rubisco measurements of old (Node 10) and young (Node 26) leaves of *Cucumis sativus* (Sabrina) after 40d of treatment exposure (3 reps ± SE).No significant differences (2-Way ANOVA).

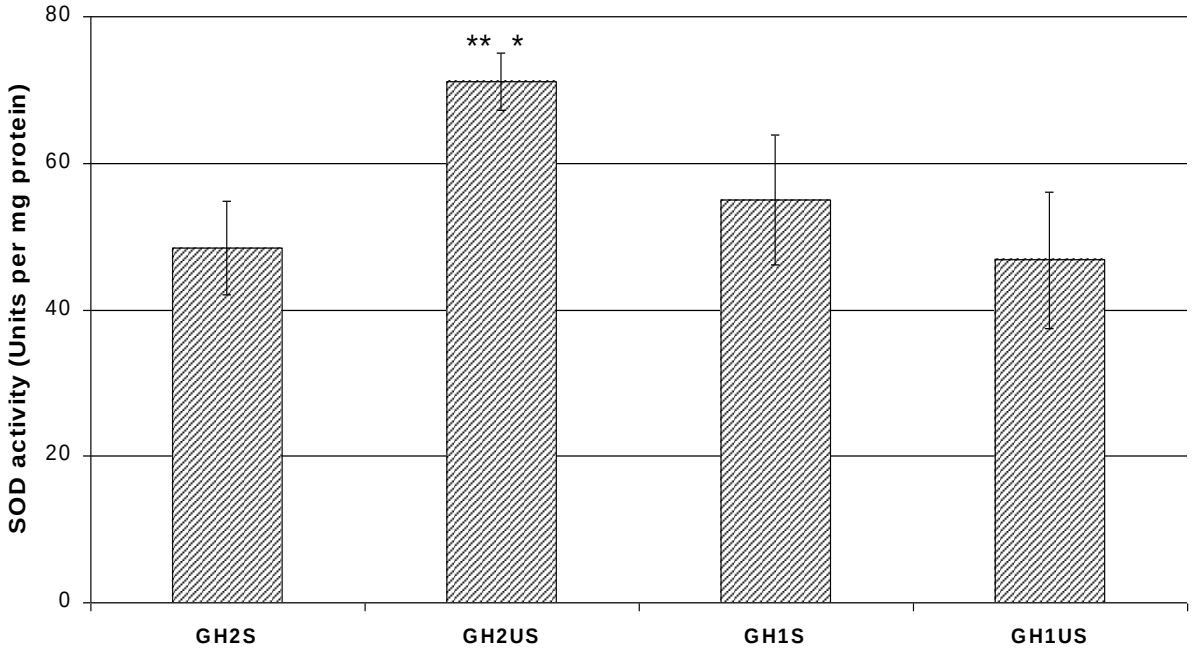


Fig. 5. Superoxide Dismutase (SOD) activity of mature (Node 10) leaves of *Cucumis sativus* (Sabrina) measured after 40d exposure to respective treatments (3 reps, ± SE). * and ** denote significant CO_2 and shade effects respectively. GH1S = 1000 vpm CO_2 & shaded; GH1US = 1000 vpm CO_2 & unshaded; GH2S = 2000 vpm CO_2 & shaded; GH2US = 2000 vpm CO_2 & unshaded.

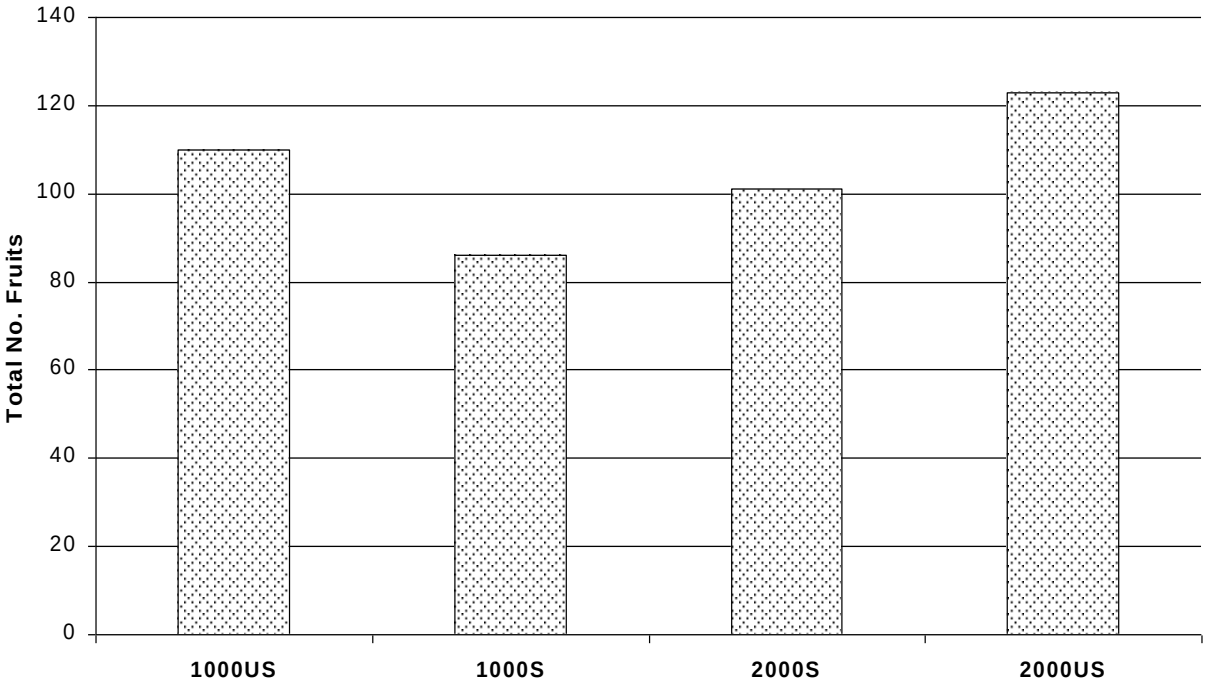


Fig. 6. Total numbers of commercial grade fruits harvested from *Cucumis sativus* (Sabrina) throughout the crops exposure to respective treatments. All mature fruits above the 7th node included.

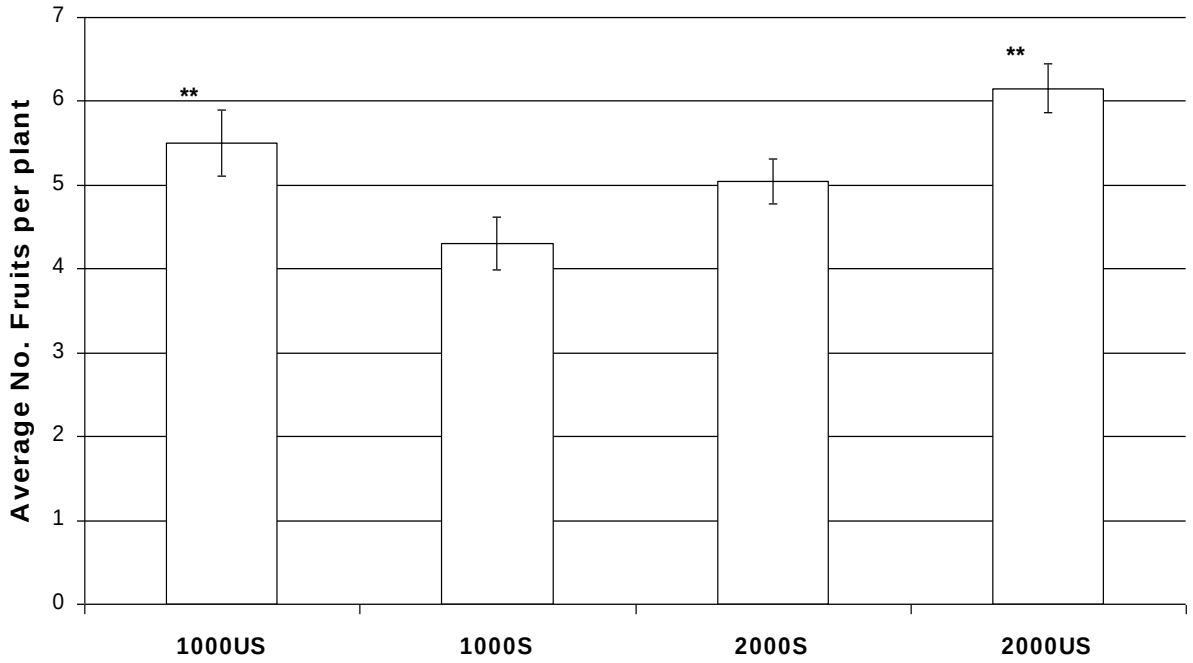


Fig 6a. Average number of fruits harvested per *Cucumis sativus* plant. ** denotes a significant effect of shading. Data represented as averages of 20 independent samples (± SE). 1000US = 1000 vpm CO_2 & unshaded; 1000S = 1000 vpm CO_2 & shaded; 2000US = 2000 vpm CO_2 & unshaded; 2000S = 2000 vpm CO_2 & shaded.

Part II:

The effect of nitrogen concentration and CO_2 enrichment on photosynthetic performance and superoxide dismutase (SOD) profiles in *Cucumis sativus*.

Introduction

We report here the effects on growth and CO_2 toxicity symptoms of an elevated concentration of nitrogen in the hydroponic feed. We hypothesise that a system that contains twice the standard nitrogen concentration (288ppm) will have a larger resource pool to maintain levels of photosynthetic components when grown in an atmosphere of 2000vpm CO_2 compared with a hydroponic feed containing standard N (144ppm) (remaining nutritional components within the feed were unchanged from that recommended by HDC projects PC 111 and PC 124).

4 treatment cabinets were modified to allow the required nutrient deliveries and 6 plants were introduced for each treatment and a separate growth cabinet was assigned for each. (Fig. b)

Fig. b Treatment protocol

Cabinet No.	Treatment	Treatment Code
1	360vpm CO_2, 144ppm N (as nitrate)	AN
2	360vpm CO_2, 288ppm N	A2N
3	2000vpm CO_2, 288ppm N	22N
4	2000vpm CO_2, 144ppm N	2N

Growth Non-destructive leaf area measurements were made on newly expanding leaves at day 5 and subsequent determinations were made every 2 days during the experiment until the leaves had reached full expansion.

Gas Exchange, Photosynthetic pigments and SOD activity Measurements for these variables were taken every 5 days throughout the duration of the experiment (30d). Initially, only leaf 4 was sampled, but towards the end of the investigation leaves from mid (leaf 10) and upper (leaf 14) canopy positions were also measured. When the

plants reached the top of the cabinet, the growing points were removed. Leaf 4 therefore became the youngest leaf developing in this system.

Results

Growth & Symptom development: In the 2000vpm CO_2, 288 ppm N treatment, leaf area development over time (Fig. 7) was significantly enhanced compared to leaf area development in other treatments, 7 DAE (days after exposure). Symptoms of leaf necrosis were becoming apparent at the end of the experiment in the 2000vpm CO_2, 144 ppm N treatment, but not in the higher nitrogen treatment, where the leaves remained dark green. Generally, leaves in the higher nitrogen treatments, especially ones in lower canopy positions, were of a darker green colouration than their 'standard' nitrogen counterparts.

Assimilation rates of leaf no. 4 at day 30 do show signs of downregulation (Fig. 9); although there appears to be little differences between the two 2000vpm CO_2 treatments. A significant decrease in **RUBISCO content** with time is apparent in the 2000vpm CO_2, 144 ppm N treatment, supporting the notion of a biochemical down regulation event (Fig. 8).

The pigment dynamics are not as clear as the previous experiment, but the results do seem to be strongly dependent on leaf age (Fig. 10). There is a significant CO_2 effect ($P<0.05$), with chlorophyll and carotenoid values lower in 2000vpm treatments than in ambient conditions and with the 288 ppm N plants having slighter lower values than the standard N plants. In leaf 10, the nitrogen level appeared to be a more significant factor than the CO_2 level in influencing pigments. The 2000vpm CO_2, 288 ppm N treatment had significantly higher levels of chlorophyll than the 2000vpm CO_2, 144 ppm N treatment and within the standard nitrogen concentration; plants grown in 2000vpm CO_2 seemed to have lower pigment levels than ambient-grown plants, though not significantly so ($P=0.059$). The youngest leaves sampled showed no clear differences.

SOD activity was also dependent on leaf age. In both leaves no. 4 and 10, elevated CO_2 plants had higher SOD activity than ambient-grown plants. Standard nitrogen-raised plants within 2000vpm CO_2 expressed higher SOD activity than their elevated CO_2 counterparts; the youngest leaves showed no significant deviation in SOD activity between treatments (Fig. 11).

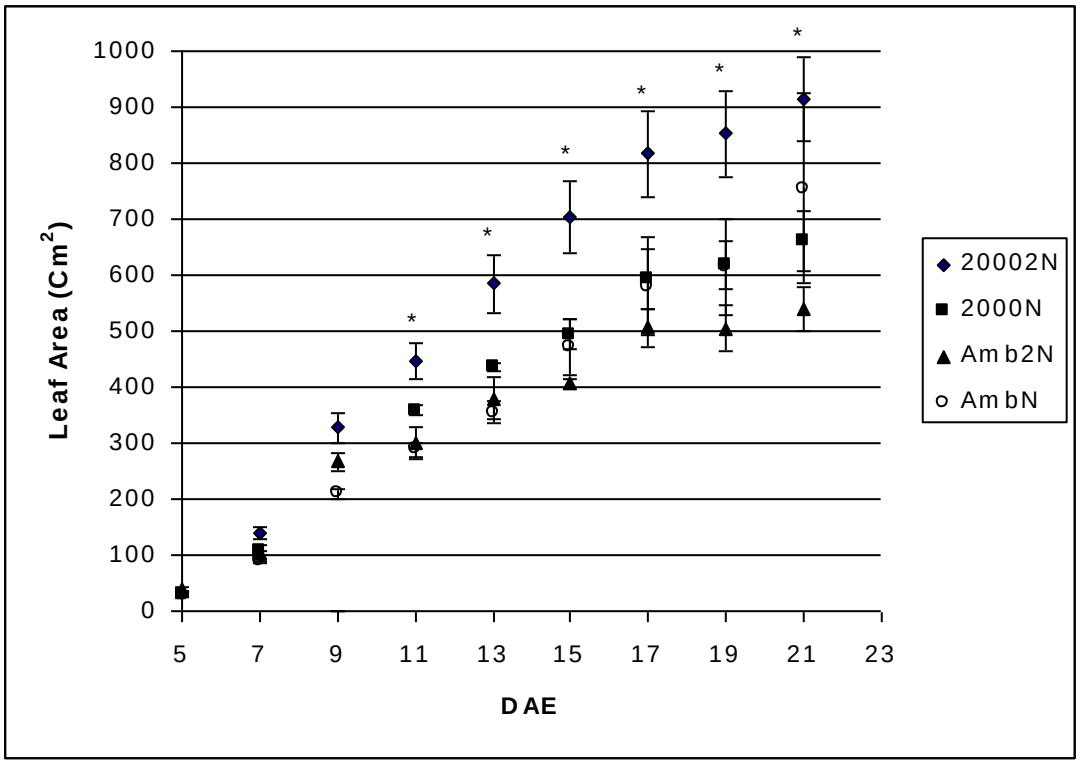


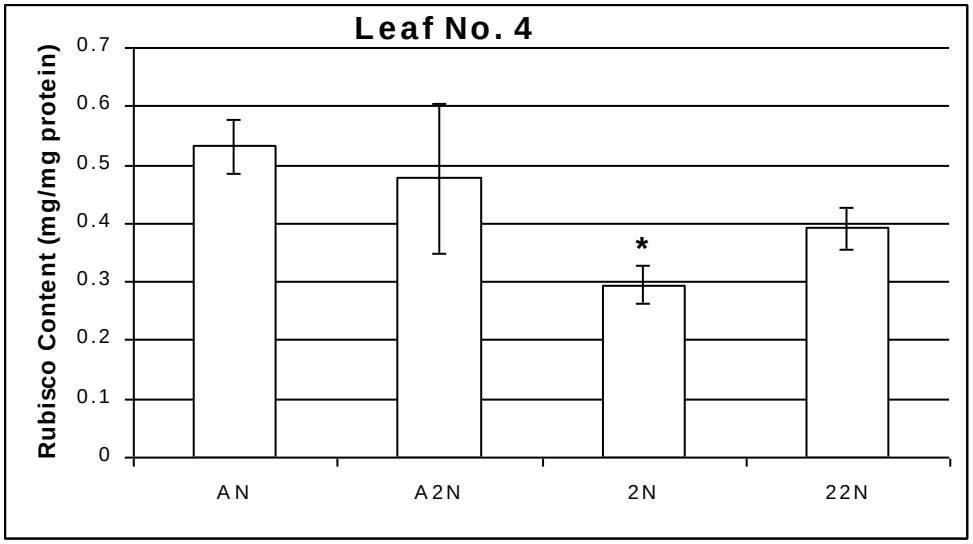
Fig. 7. Leaf area determinations for *Cucumis sativus* (Sabrina) plants grown in ambient or 2000 vpm CO_2 and supplied with a hydroponic feed containing either standard (N, 144ppm) or double (2N, 288ppm) Nitrogen (as Nitrate) concentrations (4 reps, $\pm$ SE).* denotes $P<0.001$ (Repeated Measures 2-way ANOVA).

AmbN = 360vpm CO_2, 144ppm N

Amb2N = 360vpm CO_2, 288ppm N

2000N = 2000vpm CO_2, 144ppm N

20002N = 2000vpm CO_2, 288ppm N



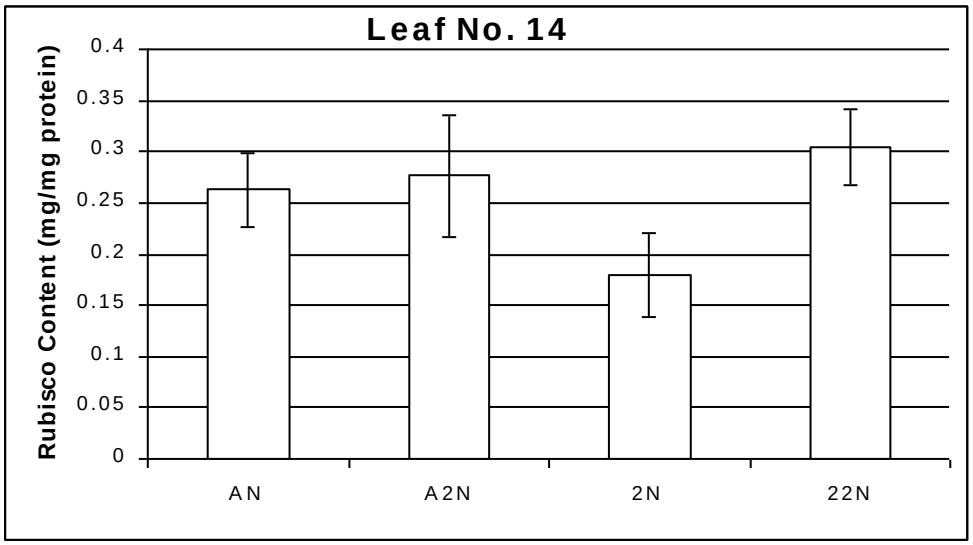


Fig 8. SDS PAGE Rubisco measurements taken from mature (leaf no.4) and immature (leaf no.14) leaves of *Cucumis sativus* (Sabrina) exposed to respective treatments for 30 days (4 reps $\pm$ SE). * denotes a significant CO_2 effect. AN = 360vpm CO_2, 144ppm N; A2N = 360vpm CO_2, 288ppm N; 2N = 2000vpm CO_2, 144ppm N; 22N = 2000vpm CO_2, 288ppm N

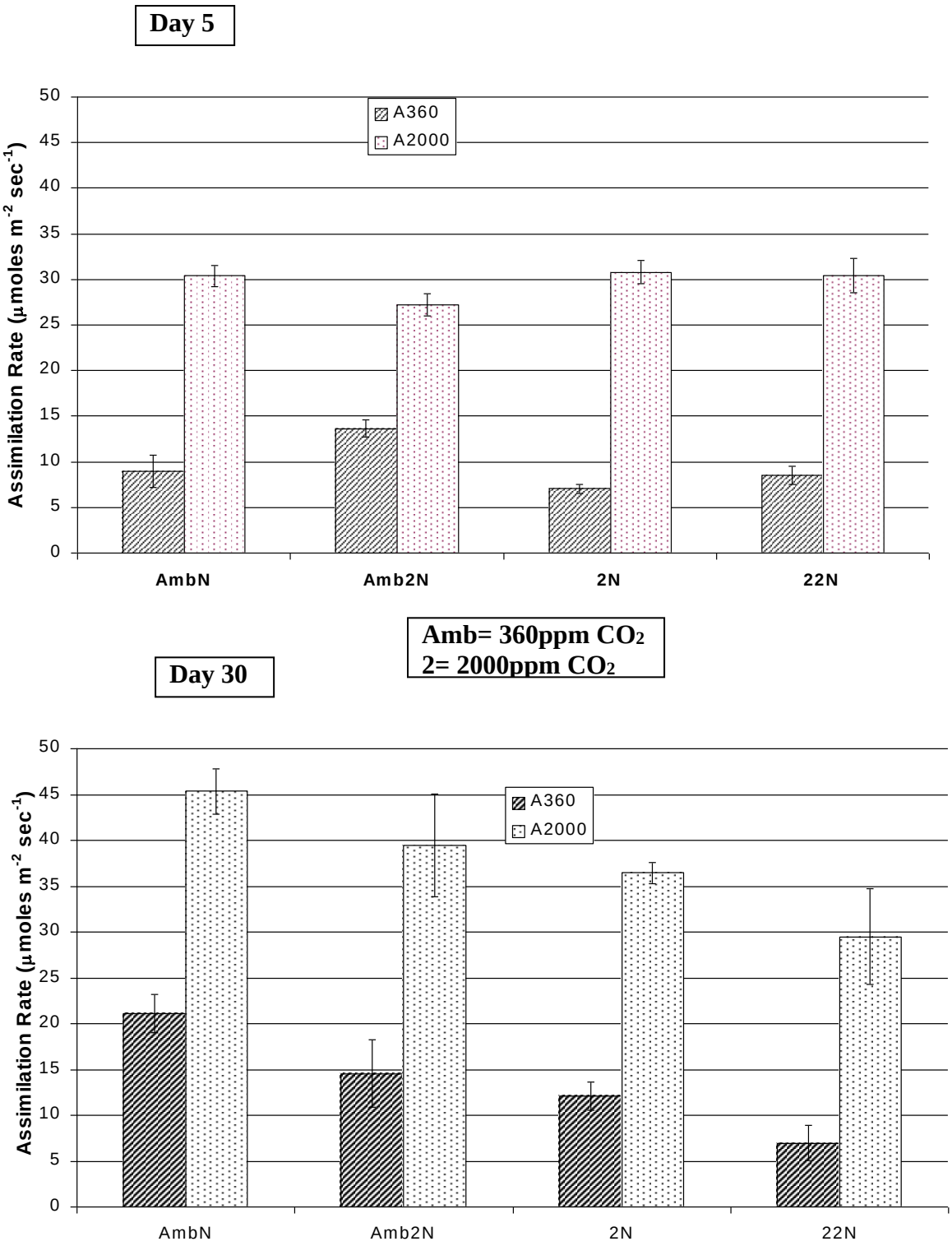


Fig 9. Assimilation Rate (A) of leaves (Leaf no. 4) tagged when CO_2 regimes initiated and subsequently measured throughout their development, of *Cucumis sativus* (Sabrina) after 5 and 30 days of exposure to respective treatments (4 reps per treatment. $\pm$ SE). AmbN = 360vpm CO_2, 144ppm N; Amb2N = 360vpm CO_2, 288ppm N; 2N = 2000vpm CO_2, 144ppm N; 22N = 2000vpm CO_2, 288ppm N.

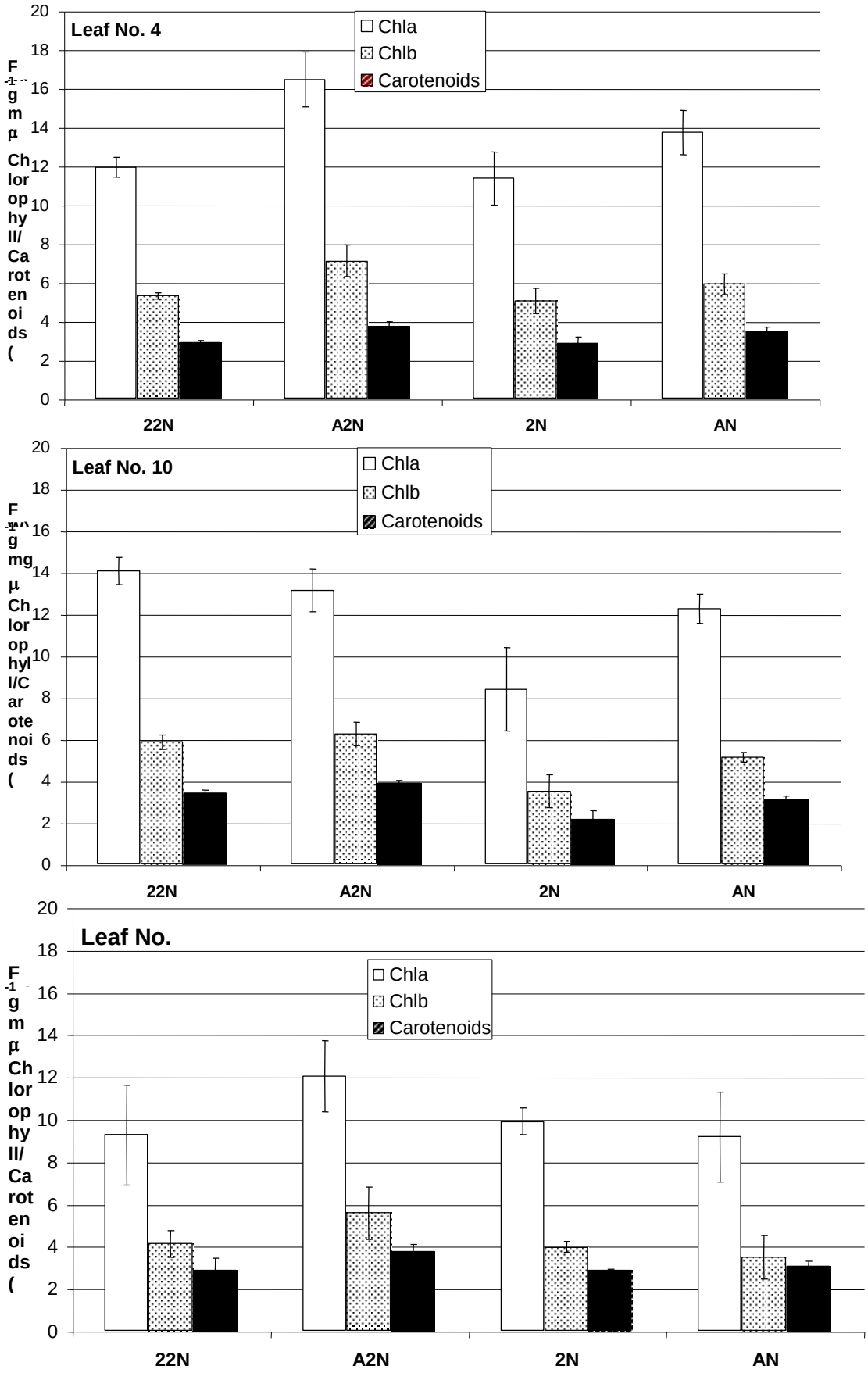


Fig 10. Total Chlorophyll and Carotenoid content, expressed on a fresh weight basis, of leaves of *Cucumis sativus* (Sabrina). Measurements were taken on leaves at node 4,10 and 14 after the plants had been exposed to their respective treatments for 30d. (5 reps per treatment (± SE)). AN = 360vpm CO_2, 144ppm N; A2N = 360vpm CO_2, 288ppm N; 2N = 2000vpm CO_2, 144ppm N; 22N = 2000vpm CO_2, 288ppm N

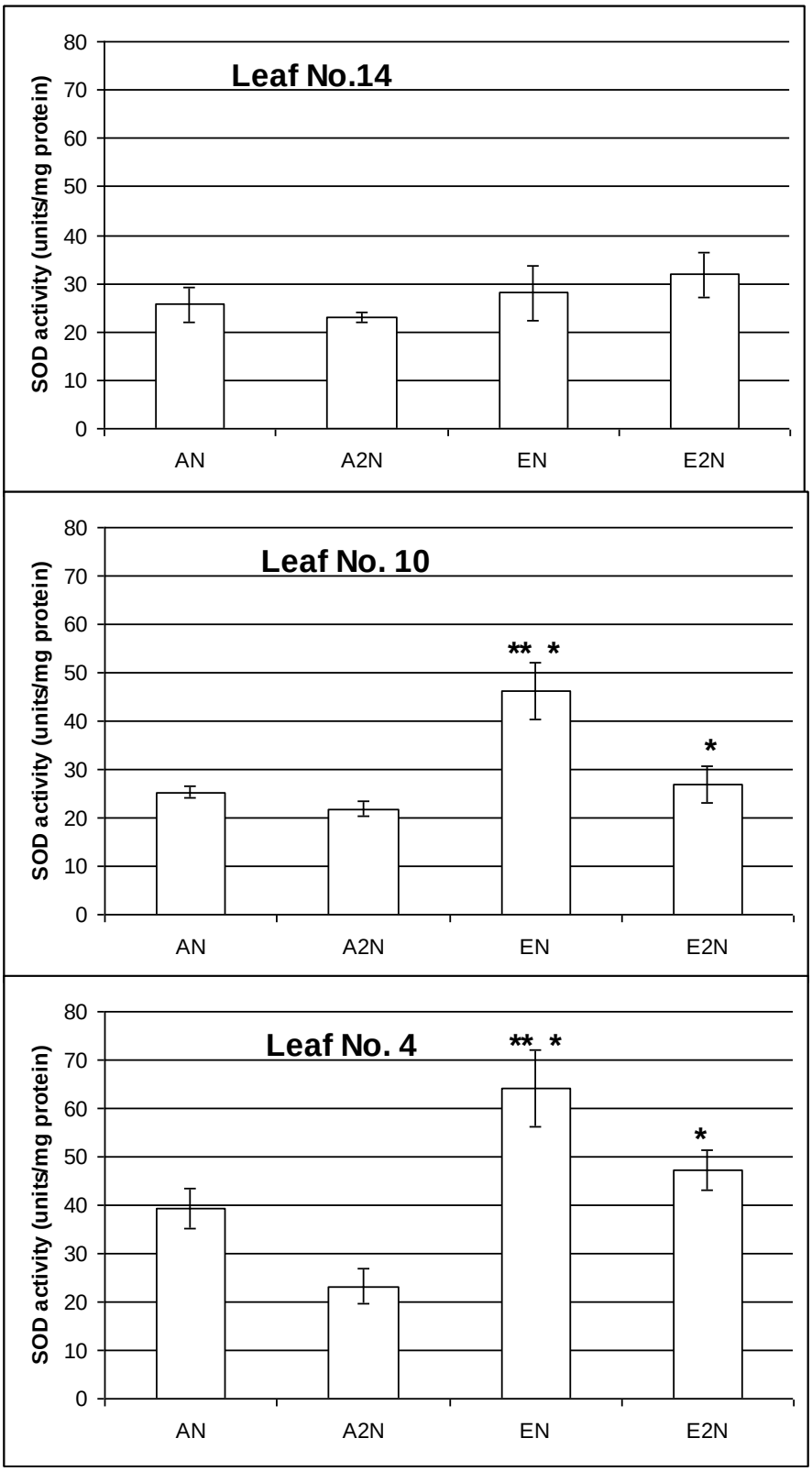


Fig. 11. Changes in superoxide dismutase (SOD) activity as a function of leaf age, nitrogen, and CO_2 concentration. Measurements were taken after individuals of *Cucumis sativus* (Sabrina) had been exposed to respective treatments for 30d (4 reps, $\pm$ SE). * and ** denote significant CO_2 and nitrogen effects respectively.
AN = 360vpm CO_2, 144ppm N; A2N = 360vpm CO_2, 288ppm N; EN = 2000vpm CO_2, 144ppm N; E2N = 2000vpm CO_2, 288ppm N

Part III:

Genotypic responses in SOD activity of *Cucumis sativus* cultivars grown in elevated CO_2.

Introduction

Results from previous experimentation have shown an increase in SOD activity, before any visual symptoms of CO_2-induced toxicity damage are apparent. In this final experiment we aim to assess any changes in genotypic response in SOD activity to elevated CO_2. Seeds of the cultivar Enigma which is tolerant to powdery mildew, were grown alongside the commercial standard, Sabrina, in both ambient (360vpm CO_2) and elevated (2000vpm CO_2) condition. We hypothesise that because Enigma is tolerant to fungal infection, and that part of this tolerance is related to a response to oxidative stress, this cultivar may show stronger oxidative stress defences than Sabrina.

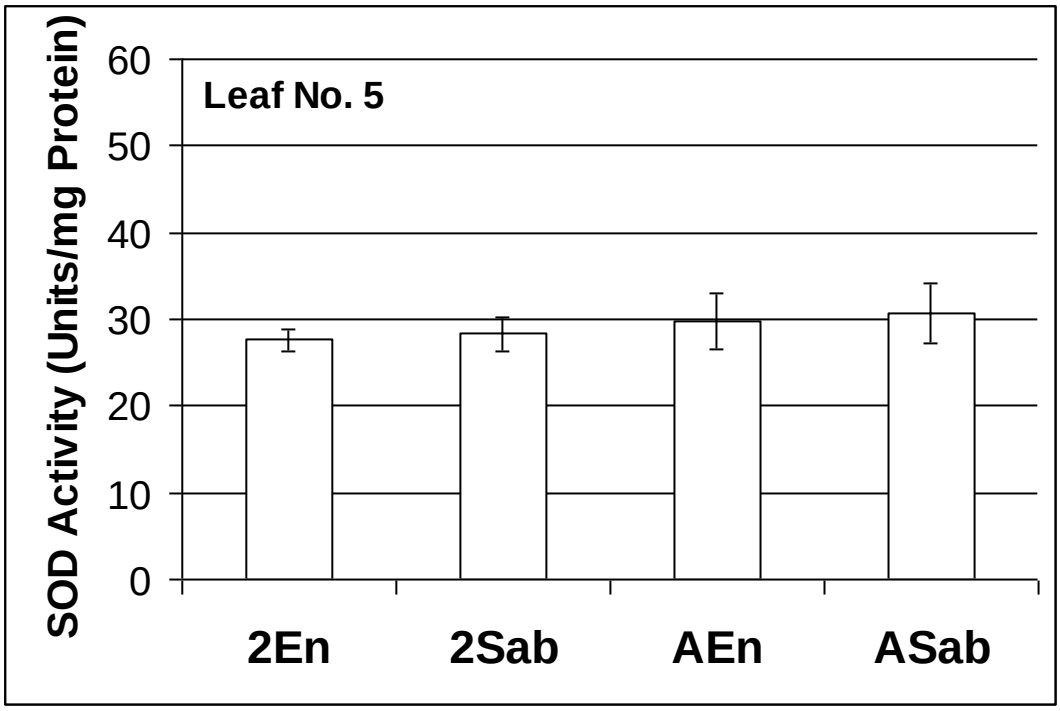
Samples were taken every 5 days for SOD activity. As in Part II, leaves throughout the canopy were sampled as the growing period (30d) progressed.

Results

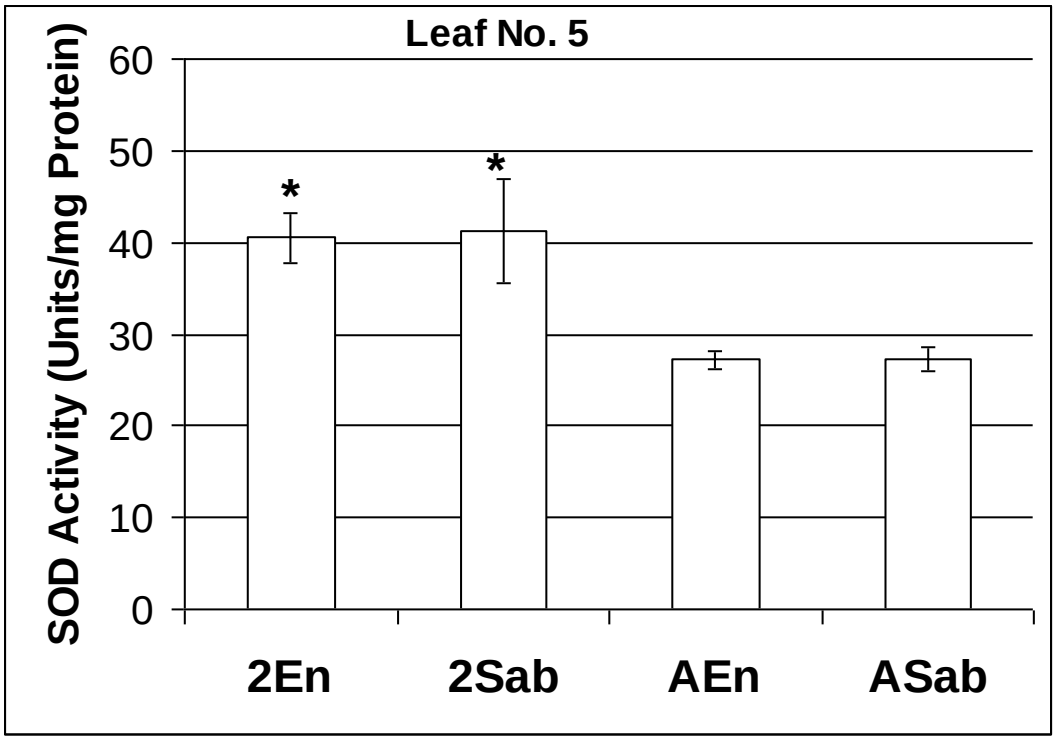
Symptom Development At day 34, symptoms of CO_2 damage were observed in both cultivars of *Cucumis sativus* grown at 2000vpm CO_2.

The SOD data reveals that no significant differences with respect to antioxidant status exist between the two cultivars tested. The data do confirm the oxidative stress response described elsewhere in this report (Part II). SOD activity was significantly higher in leaf 5 at day 15 in the 2000vpm CO_2 treatment and in leaf 8 at day 30 (Fig. 12). It is apparent that leaf age and exposure time to elevated CO_2 are fundamental in influencing the extent of oxidative stress in leaves of *Cucumis sativus*. These data tie in with previous observations where leaf damage occurs in mid-canopy positions (i.e. maturing/ mature leaves) after 2-3 weeks exposure to 2000vpm CO_2 (HDC Report 2001, PC 159).

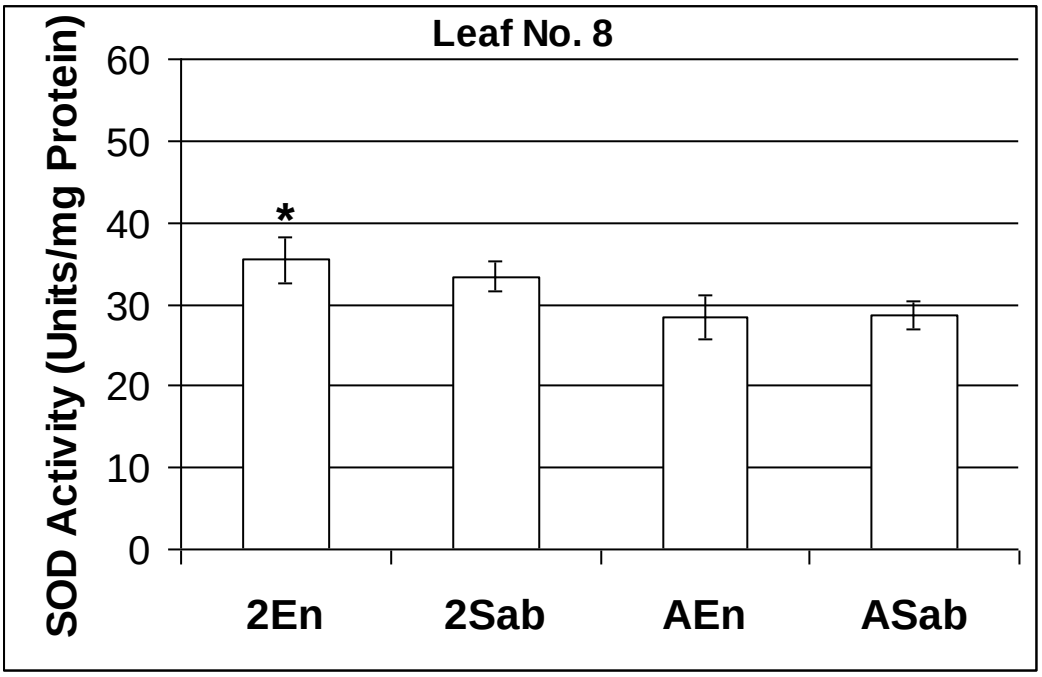
Day 5



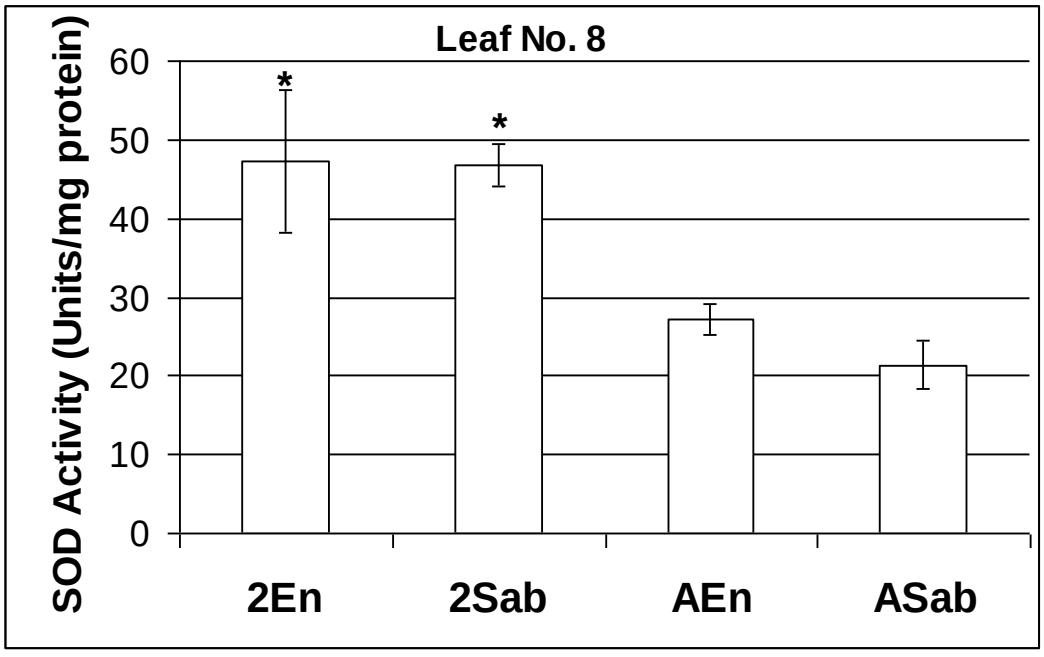
Day 15



Day 15



Day 30



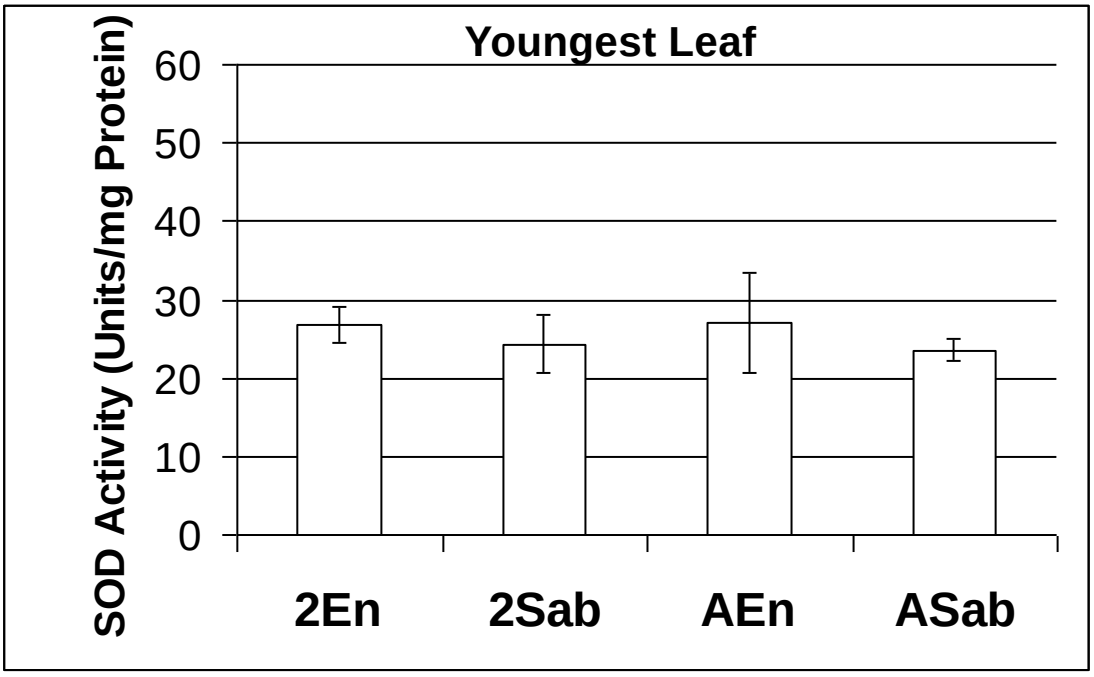


Fig 12. The effects of leaf age, treatment exposure and CO_2 concentration on Superoxide Dismutase (SOD) activity in two cultivars (Sabrina and Enigma) of *Cucumis sativus*. Data represent means of samples from 5 plants (±SE). * denotes a significant CO_2 effect ($P<0.05$, 2-Way ANOVA).
2En = 2000 vpm CO_2 & cultivar Enigma; 2Sab = 2000 vpm CO_2 & cultivar Sabrina; AEn = Ambient & cultivar Enigma; ASab = Ambient & cultivar Sabrina

Conclusions

Data in this report (Part I) have confirmed hypotheses from previous investigations. The protective value of shading has been validated on a glasshouse crop even though an increase in yield did not result from this treatment. A continuation of this experiment would have shown a clear beneficial effect of shading, however, unshaded plants effectively died as a result of the severity of the treatment. Shaded plants remained green and continued to produce fruit. Unfortunately, as the biology of dying plants was no longer of interest, yield assessment was curtailed when the high CO_2 un-shaded plants died. While downregulation of photosynthesis was clear in high CO_2 in all experiments, whether or not direct or indirect (senescence-related changes) downregulation occurs remains to be firmly established. A study of whole-leaf nitrogen allocation would show whether or not there is a favoured allocation of nitrogen away from the RUBISCO protein complex. The analyses undertaken in the reported experiments have expressed RUBISCO as a product of total soluble protein. This does not take into account possible changes in protein levels in membranes and cell walls, insoluble protein components of plant tissue. Nevertheless, even without this information, we can still quantify the physiological phenomena observed.

The decreases in chlorophyll content at high CO_2 observed both in the glasshouse experiment and the nitrogen enrichment experiment (Part I and II) can either be due to accelerated senescence or to changes associated with leaf damage. The lower stomatal conductances observed in Part I could also be involved in symptom development as the leaf will not be able to maintain evaporative cooling and leaf temperatures could increase to a level that will facilitate chlorophyll breakdown.

The SOD activity dynamics reported strongly support the hypothesis of oxidative stress in 2000vpm CO_2, high radiant load, environments, and offers an early indication that leaves will become bleached if the environmental situation persists. The findings from this project emphasise the importance of maintaining a young canopy if very high CO_2 concentrations are to be used. The glasshouse experiment in which a high wire strategy was employed elegantly illustrated this point. These plants did not show any signs of leaf bleaching even though down regulation of photosynthesis did occur. A rigorous yield analysis of this growth system has not been

conducted, although it is well known that fruits grown in this manner are of a higher quality than those grown in a low-wire system, although labour costs may be greater with the high wire system.

If growth of a high wire crop is not possible then CO_2 toxicity symptoms may be avoided by growing the crop with a high nitrogen supply or by shading the leaves during periods of high radiant load. In this way, yields may be increasd to the maximum by employing higher CO_2 concentrations. This may be done safely by employing the simple biochemical tests suggested here as an early warning of toxicity problems (SOD and carotenoid determinations).

Future work

1) To maximise benefits from the use of higher CO_2 concentrations, further test the effects on crop yield of shading during periods of high radiant load.
2) To maximise benefits from the use of higher CO_2 concentrations, further test the effects on crop yield of growing the crop with high nitrogen availability.
3) To maximise benefits from the use of higher CO_2 concentrations, further test the effects on crop yield of developing a biochemical test to provide early warning of potential CO_2 toxicity.
4) To maximise benefits from the use of higher CO_2 concentrations, further test the effects on crop yield of varying the CO_2 concentration throughout the day. E.g. high concentrations early in the day may be beneficial for carbon gain with a lower risk of bleaching during this time. Variation of CO_2 concentrations throughout the day may also reduce the risk of photosynthetic down regulation.

Technology Transfer in 2001-2002

- International Symposium on Growing Media and Hydroponics, Malmo, Sweden, September 2001: Poster presentation.
- Internal Departmental Seminar, Lancaster University, February 2002: Verbal presentation.
- SEB Conference, Swansea, April 2002: Poster presentation.

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