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The results and conclusions in this report are based on a single series of experiments. 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.

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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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Background and objectives

It is now commonplace in much glasshouse crop production to enrich the aerial environment with CO_2 up to a concentration of 1000 ppm. Many plants respond positively to increasing CO_2 levels as the rate of photosynthesis increases and hence dry matter production and fruit yield. However, a level of CO_2 may be reached (probably species dependent) where the photosynthetic returns are diminished at each stepwise increase in CO_2 concentration.

In addition, many growers of cucumber crops have reported the development of 'phytotoxic plant symptoms' at high concentrations of CO_2 enrichment. The aim of this project is to investigate the response of cucumber plants to high concentrations of CO_2 enrichment, in terms of effects on yield and on plant damage.

Summary of results

Studies conducted under experimental conditions at HRI-Stockbridge House and at Lancaster University over two years have shown that the visual symptoms of CO_2 toxicity in cucumber plants, demonstrated as bleaching of the leaves, result from a combination of:

- High atmospheric CO_2 concentration
- High light intensities
- Leaf age
- And probably mild leaf water deficit

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A glasshouse trial was conducted at HRI-Stockbridge House growing a cucumber crop under a low wire and high wire system. The following CO_2 enrichment regimes were used.

CO_2 set points and crop growth stage

Enrichment Regime	Date (Year 2000)	CO_2 set points (ppm)		Crop growth stage
		Standard	Elevated	
1	Week 1	600 + 200	600 + 200	Planting
2	Week 5	800 + 200	1000 + 200	At the wire
3	Week 8	1000 + 200	2000 + 400	Sub-sub lateral
4	Week 15-17	800 + 200	800 + 200	End of recording

CO_2 toxicity

CO_2 toxicity symptoms developed only in the low wire crop and only under the 2000 ppm CO_2 enrichment regime. No toxicity symptoms were apparent at 1000 ppm in either crop. This experiment indicates that the development of CO_2 toxicity symptoms are a function of high atmospheric CO_2 levels and leaf age.

Crop yield

The glasshouse trial at HRI-Stockbridge House provided no evidence that increasing atmospheric CO_2 concentration to 2000 ppm increased yield above that achieved at 1000 ppm. Most importantly, in the last three weeks of the experiment, yield was significantly reduced in the high CO_2 crop (compared to 1000 ppm). This was the period when atmospheric CO_2 concentration was reduced from 2000 ppm to 1000 ppm. The negative effect of this change in CO_2 concentration on yield is exactly what we would predict if significant down regulation of photosynthesis had occurred over the previous 15 weeks at the higher CO_2 concentration. Down regulation is effectively the reduction by the plant in the amount of photosynthetic apparatus produced in the leaves. When the

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CO_2 concentration is subsequently lowered, the reduced photosynthetic system cannot sustain carbohydrate production and hence yield at the lower CO_2 concentration. Physiological measurements provided clear evidence of photosynthetic down regulation by the crop, even when CO_2 toxicity symptoms were not apparent.

Effect of leaf shading

Controlled environment experiments at Lancaster University showed that mild shading treatments can reduce CO_2 toxicity symptoms as shading helps to lessen down regulation of photosynthesis under high CO_2 concentrations. We predict that if repeated in the glasshouse this effect would increase the yield of cucumbers at higher CO_2 concentrations.

Action points for growers

This hypothesis described above is currently being tested in glasshouse trials and if substantiated, will lead to a clear recommendation to growers to provide mild shading to avoid the exposure of leaves to damagingly high light intensities at higher CO_2 concentrations. This may enable the use of higher CO_2 concentrations to economic advantage in commercial situations.

But at present, the best course of action is as follows:

- Check CO_2 measuring systems for speed and accuracy at the start of the season and check frequently throughout the growing period to ensure that you are achieving the desired level of CO_2 enrichment.
- Enrich to a **maximum of 1000 ppm CO_2** – especially in the early stages of the crop when the ventilators are closed and higher concentrations are easily achieved.
- Restrict input volumes in the early stages of the crop to ensure that you **do not** achieve levels above 1000 ppm CO_2.
- Don't wait for damaged foliage to indicate a problem with CO_2 levels – yield will be reduced before leaf bleaching occurs.

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Science Section

Part 1 The Effect of Leaf Age on the Susceptibility of *Cucumis sativus* to CO_2-induced Leaf Necrosis. A glasshouse trial conducted at HRI Stockbridge House

Introduction

It is generally believed that since the concentration of CO_2 is a limiting factor in photosynthesis of C_3 plants, an increase in the atmospheric concentration of this gas will increase the rate of photosynthesis and hence dry matter production and fruit yield. It is now commonplace in much glasshouse crop production to enrich the aerial environment with CO_2 up to a concentration of 1000ppm. This practice may be restricted to a spring cropping since minimal ventilation is required at this time of year. Many plants respond positively to increasing CO_2 levels but a level is reached (probably species dependent) where the photosynthetic returns are diminished at each stepwise increase in CO_2 concentration. For example, Heij & Van Uffelen (1984) calculated, after growing cucumber crops (cv Corona) at six different CO_2 regimes, that the number of days to achieve a production level of 16 and 32 kg m^{-2} respectively was virtually identical for growth concentrations of 1500 and 2800 ppm. Nevertheless, cucumber production during the first eleven weeks of the experiment was 27% higher in a 2870ppm atmosphere and 15% in 1500 compared with ambient. It was believed that the crop grown at the higher concentration suffered leaf damage such that the increase in production tailed off with time.

From previous experiments, both at HRI Stockbridge House in a commercial crop and in controlled environment conditions (CEC) at Lancaster University, we have found that downregulation of photosynthesis, a phenomenon rigorously investigated by climate change physiologists, occurs before and also in the absence of leaf damage. We believe leaf age to be an important component of any downregulation or damage to the photosynthetic system since as a leaf senesces it becomes less photosythetically active. In this investigation we aim to assess how leaf age affects photosynthetic performance of cucumber plants grown with two CO_2 enrichment growth strategies (1000ppm CO_2 and

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2000ppm). Based on earlier work, measurements will be taken to determine the amount of RUBISCO in the leaves (RUBISCO is the primary carboxylating enzyme for C3 photosynthesis) and to determine possible morphological differences that could relate to leaf damage. The influence of modified photosynthesis on crop yield will also be assessed.

Materials and Methods

Investigation protocol. We have demonstrated during 1999 that we can induce classic CO_2 damage in cucumber plants grown at Stockbridge House using a traditional low wire system when these plants are exposed to 2000ppm CO_2. However with this traditional cropping system the plant architecture and fruit load changes during the season thus complicating measurement procedures relating to the development of CO_2 toxicity symptoms.

By growing a high wire crop in Year 2000 we were be able to keep the same plant architecture: that is a plant stem with a set number of leaves and developing fruit. This made it possible to tag leaves in set positions and follow their physiological development over time. Any changes in nutrient uptake by the plant (particularly N), changes in stomatal density, rates of photosynthetic activity and the RUBISCO content of leaves as a result of CO_2 enrichment strategy could be detected and potentially linked to the onset of down regulation and photo-oxidative damage to the photosynthetic system of the leaf.

Our view is that CO_2 damage to cucumber leaves may be particularly acute in older leaves. However the nature of a high wire cropping system means that as you layer the plant you remove older, and probably damaged leaves where photosynthesis is downregulated. We hypothesise, therefore, that we will not see damage at supra-optimal CO_2 levels (2000 ppm) in a high wire cucumber crop. Hence, we included a low wire crop as a control in the same glasshouse. We used the developmental stages of the plant architecture in the low wire crop to change the CO_2 regimes as we did in 1999 (HDC Report, 2000).

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The crop. A cucumber crop cv Sabrina was sown on 13 December 1999 and planted on 6 January 2000 into two adjacent, modern, well-sealed, 200m^2, 4.2 m high Venlo glasshouses. During propagation the plants were exposed to 1000 ppm CO_2 and supplementary lighting was provided using 400 W high-pressure sodium lamps.

Within each glasshouse two crop canopies were produced on the V-system, namely a high wire crop trained to a 3.6 m wire and a traditional plant canopy where the main stem was stopped when it reached the 2.2 m wire and two lateral branches were allowed to develop. In each case the plant density was 1.4 m^{-2}. A side shoot was taken from every plant within the high wire canopy on 20 January to increase the head density to 2.8 m^{-2}.

CO_2 regimes. Pure CO_2 was used for all enrichment. Immediately following planting a CO_2 regime of 600 ppm rising by 200 ppm on lighting was set in the environmental computer. CO_2 strategies in each compartment were changed according to crop growth stage of the low wire crop (Table 1).

Table 1. CO_2 set points and crop growth stage.

Regime	Date (Year 2000)	CO_2 set points (ppm)		Crop growth stage
		Standard	Elevated	
1	Week 1	600 + 200	600 + 200	Planting
2	Week 5	800 + 200	1000 + 200	At the wire
3	Week 8	1000 + 200	2000 + 400	Sub-sub lateral
4	Week 15-17	800 + 200	800 + 200	End of recording

Measurement protocol. At the initiation of each regime, the second youngest leaf on each plant was tagged so the subsequent development could be monitored. Then, measurements were taken of the tagged leaves and of leaves that had developed during the time frame. Since regime 3 was of a lengthier duration to the other two, a second cohort of leaves was tagged, which meant towards the end of the measurement period, 3 leaves were measured. Due to time limitation this brought the replication down from 3

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replicates per treatment per leaf age to two replicates. At the same time as photosynthesis measurements were carried out, samples were also taken for RUBISCO analysis. These were always taken soon after 9 am on the last measurement day. During growth in regime 1 it was determined whether or not the time of day (morning versus afternoon) affected the photosynthetic rates.

Gas Exchange Measurements. A portable gas exchange unit (Ciras-1, PP Systems, Hitchin, UK) in combination with a cuvette, which completely seals over a section of leaf, allows the input of a flow of air of specified gas composition. The assimilation rate of the plant is established by measuring the difference in [CO_2] in the air before and after it has passed through the leaf cuvette. Ciras-1 automatically calculates intercellular CO_2 concentration (C_i). By comparing photosynthetic rates at different intercellular CO_2 concentrations, we can factor out the effect of the stomata and directly assess the influence of the environment on the metabolic activity of the plant.

RUBISCO. The amount of RUBISCO protein present in leaf extracts was determined by the spectrophotometric and densitometric analysis of the amount of dye bound to the two sub-units after separation by sodium dodecyl-sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and staining with Coomassie Brilliant Blue R-250 (Servaites, 1984). Initially however, a subset of samples was analysed both by this method and also by Western analysis, using a LKB 2117 Multiphor II Electrophoresis Unit, where primary (RUBISCO (RI for SH)) and secondary (Anti-rabbit IgG) antibodies fixed the protein, and Sigma fast tablets developed the colour, onto nitrocellulose paper. This comparison was implemented in order to determine whether a qualitative difference could be established before a semi-quantitative method was utilised.

RUBISCO samples were taken on each site visit (every 5 days) to HRI Stockbridge House. Leaf discs were taken, (3 replicates per treatment and leaf position), temporarily stored on ice and subsequently transferred to liquid nitrogen for storage. Samples were ground to a fine powder in a liquid nitrogen-chilled mortar and extracted with buffer (4%

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(w/v) SDS, 10% (v/v) 2-mercaptoethanol, 10% (v/v) glycerol, 50 mM Tris-HCl, 1M NaOH as pH adjuster and boiled for 2 mins, pH 6.8).

In order to determine protein loadings for the gels, extracted samples were diluted as necessary and added to diluted dye (Coomassie Brilliant Blue G-250) reagent (Bio-Rad) and the absorbance at 595 nm (A_{595}) was determined for the subsequent solutions and compared to BSA (Bovine serum albumin) standards. Leaf extract (0.1g ml^{-1}) was added to an equal volume of loading buffer (5g glycerol, 5ml β-mercaptoethanol, 2.3g SDS, 12.5ml stacking buffer) and boiled for 2-5 mins. Standards from purified clover RUBISCO were also prepared in the same way and 10µl of sample was loaded per gel well. Using a Bio-Rad Mini Protean II system, samples were subjected to SDS-PAGE. Gels were destained in methanol/acetic acid/glycerol/water (21:2:3:74, v/v/v/v) until the gel background became clear. In order to quantify the amount of RUBISCO protein present in samples, two methods of analysis, spectrophotometry and densitometry, were trialled.

The spectrophotometric assay measures the quantity of dye bound to the RUBISCO protein, but first, elution of samples is necessary. Elution of dye from the gel bands was performed firstly by cutting out the band of interest, followed by maceration with pestle and mortar and finally extraction of the dye by incubation in 1% SDS (w/v) for 12h at 25 °C. The absorbance value of the resulting solution was measured at 600nm using a microtitre plate reader (Labsystems, operated by GENESIS software) and leaf samples were compared with RUBISCO standards.

For densitometric analysis, Coomassie gels were placed into a laser densitometer (LKB 2202 Ultrascan laser densitometer, Bromma, Sweden) and the system was set up such that only the RUBISCO bands were scanned. A recording integrator was attached so the area under the absorbance peaks could be determined. Again, samples were compared with RUBISCO standards.

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Xylem Vessel and Stomatal Analysis. At the end of each growth regime, leaves were collected (5 replicates per treatment and leaf). Sections of petiole were taken and stored temporarily in 10% ethanol. Using a light microscope, xylem vessel diameter and number of vessels per petiole were determined.

In order to take stomatal measurements, a mixture of approximately 10:1 Xantopren VL plus and Activator (Optosil) (Heraeus, Germany) was smoothed over both leaf surfaces. After this dried it was removed. It then acted as a leaf template from which nail varnish peels could be taken and viewed under a light microscope. Stomatal indices for both the abaxial and adaxial surface were determined.

Symptom Development. During the development of the crops, visual appearance was monitored daily and photographs were taken when toxicity symptoms appeared.

Crop Yield. Cucumbers were picked at maturity and both numbers harvested and weight harvested were recorded.

Results and Discussion

Symptom Development. Dramatic symptoms of CO_2 toxicity were recorded at 2000 ppm approximately 3 weeks after the period during the spring when light intensities first reached high levels (March 2000 – week 11). Interestingly, these symptoms only developed in the low wire crop.

A/C_i Analysis. The same CO_2 enrichment regimes were initially applied to both compartments. A difference in photosynthetic rate was not expected between leaves from the two compartments (Fig. 1a). Due to diurnal rhythms, photosynthetic variables may differ between morning and afternoon and this factor may confound later measurements. However, in cucumber, there was no clear difference in photosynthetic rates in the morning and the afternoon (Figs. 1b and c). A significant down-regulation, certainly of RuBP regeneration was evident after leaves had been exposed to the two different treatments in Regime 2 for 20 days (data not shown). The fact that the A/C_i curve for

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ambient grown plants had also shown down-regulation suggests the apparent downregulation at elevated CO_2 is due to a combination of leaf age and supra-optimal levels of CO_2. However, this effect was not apparent in the 2nd youngest leaves measured at the initiation of Regime 3 (Fig. 1d). These leaves were then followed for 20 days and it is apparent there was a dramatic reduction in both carboxylation efficiency and RuBP regeneration. The downregulation in the standard crop was not so severe (Figs. 1e & f). It is interesting to note that similar P(max) values were achieved in new (2nd youngest) leaves, even after 45 days of crop exposure to treatments, which suggests that any alterations to leaf photosynthetic properties occur as an acclimatory response to the environment rather than by signalling from lower leaves to the developing apex (Figs. 1g and h).

RUBISCO Content. No clear differences exist between treatments in regimes 1 and 2 (Fig. 2) although it is tempting to suggest a small decline from regime 1-2 in both treatments, consistent with the observed down-regulation of photosynthesis previously mentioned. However, the apparent increase in levels of RUBISCO in the older leaves at the end of regime 3 is harder to explain, especially since the qualitative analysis suggested that there would be less (RUBISCO bands seemed less dense at the end of regime 3 samples, Plate 2). It may have been the case that there was insufficient replication (2 replicates per data point.) to detect significant differences and in future work this should be taken into consideration.

Water Relations. Measurements of xylem vessel diameter and number in petioles of newly formed (2nd youngest) leaves during regime 1 and at the end of regime 3 (Graphs j & k) showed an apparent decrease in xylem vessel diameter which may be the result of continued growth in elevated CO_2. The effect of this change may be offset by a concurrent increase in the number of vessels. Pressure bomb measurements of hydraulic conductivity of petioles were undertaken but yielded no clear information.

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Crop Yield. Fig. 4a shows the influence of harvest date and CO_2 treatment on the numbers and weight of cucumbers harvested during the experiment. These data are made more accessible by plotting the relative yields in high CO_2 compared to the yield in a lower CO_2 concentration (Fig. 4b). Interestingly, there was a small reduction in yield in high CO_2. This became partcularly apparent at the end of the experiment during regime 4.

Conclusions

The possibility that increasing leaf age can exacerbate the negative effects of elevated CO_2 is supported by previous investigations. Frydrych (1976), investigating photosynthetic characteristics of cucumber at 320 and 1500 ppm CO_2, also found a decrease in photosynthetic rate (measured as dry weight increases) from youngest leaf to cotyledon, but the decline in both regimes was similar.

Using tomato plants grown in a range of concentrations (350, 700, 1050, 1400) for 31d, Van Oosten & Besford (1995) showed that the initial stimulation of assimilation rate (A) by high levels of CO_2 is lost after full leaf expansion is achieved. In fact, when measured at their growth concentration, assimilation rate of plants exposed to the highest concentrations was found to be negatively correlated with the CO_2 levels in which they were grown. This supports the results found in this investigation. Both treatments showed a down regulation of photosynthesis as the leaf matured, the effect in the 2000ppm CO_2 regime being more severe than that in the 'standard' regime.

The degree to which the low wire crop in the 2000ppm CO_2 treatment was damaged compared to the high wire crop was startling. This served as convincing evidence that a mature, down regulated leaf that intercepts high radiant loads is more susceptible to leaf bleaching than an immature leaf in a similar canopy position. By layering the crop, and thus maintaining a young upper canopy, the deleterious effects of supra-optimal levels of CO_2 appear to have been avoided, even though the high wire crop showed signs of significant down regulation. Part of the layering process involves removing older leaves, possibly before they are drastically down regulated. It is also interesting to note that main stem fruits tend to be of a better quality than those harvested from a low wire crop. It

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should be appreciated however, that maintaining the high wire crop involves an increased labour input and hence increased running costs. Nevertheless, it has been shown that options exist that will reduce crop damage if glasshouse managers choose to grow their crop under these CO_2 conditions. Whether or not high wire 2000ppm CO_2-grown crops return a higher yield than a low wire crop under these conditions remains to be firmly established. Our yield data are from a relatively restricted number of plants but it does appear to be clear that increases of CO_2 concentration will not increase yield above values shown by plants grown at 1000 ppm. One very interesting observation is the very significant reduction in yield in plants in regime 4 which have previously been exposed to 2000 ppm. Following a reduction in CO_2 supplementation yield is reduced. This is exactly what would be predicted if downregulation of photosynthesis is occurring. This combined with lowered CO_2 supplementation will result in reduced carbohydrate production and yield.

Future work in this area will look more closely at the interactions between CO_2 enrichment and radiant load, since these two factors seem to be the most important interaction that will cause photodamage to the plants' photosynthetic apparatus at 2000ppm CO_2.

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Part 2. The Interactive Effects of High PPFD and Elevated CO_2 on Gas Exchange Parameters and the Onset of Leaf Bleaching in *Cucumis sativus*. A controlled environment study conducted at Lancaster University.

Introduction

Following work in commercial glasshouses at HRI Stockbridge House, in which a *Cucumis sativus* crop (Cv Sabrina) was grown in two enrichment regimes, 1000ppm and 2000 ppm, it was discovered that mature leaves in the uppermost part of the canopy were more susceptible to CO_2-induced leaf bleaching in the 2000ppm treatment.

The accumulation of starch in the chloroplast has been suggested as a mechanism for this toxic damage in Basil (Wallick, 1994), Bush bean (Ehret & Jolliffe, 1985), tomato (Van Berkel, 1984) and cucumber. Madsen (1974), cited in Tripp et al (1991) also propose starch accumulation as the main factor in leaf necrosis under CO_2 enrichment. However, Tripp et al (1991) found, over an entire growing season, that there was no significant relationship between foliar starch concentration and deformation severity. However, it is known that certain species have adapted different strategies for processing photosynthates, therefore starch accumulation may be a more important factor in some species than others. Cucumber plants are known as starch accumulators and have high levels of acid invertase activity which means they have a higher starch storing capacity. Tomato is apparently incapable of storing starch to such an extent (Goldscmidt & Huber, 1992). Also, in cucumber, sucrose is rapidly converted to stachyose and is exported in this form, which may explain why Pmax is sustained in this crop (prevention of feedback inhibition of photosynthesis) for a longer period compared with a crop like tomato.

The glasshouse work at Stockbridge House showed that CO_2 enrichment will cause downregulation of photosynthesis and since it was only the mature upper canopy leaves that showed signs of leaf bleaching, we believe that a combination of intermittent periods of high radiant load and downregulated rates of photosynthesis caused these symptoms. The mechanism for this can be postulated as an excess of electrons from the light reaction centres unable to be dissipated by the Photosynthetic Carbon Reduction cycle. These will

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form free radicals which could potentially cause photo-oxidative damage within the plant cell and hence the classic symptoms of CO_2 toxicity. Research by Sicher (1997) focussed upon chlorosis in primary leaves of barley in ambient and twice ambient CO_2 for 17 days. It was found that the extent of chlorosis was higher in the elevated treatment and that increasing the light intensity from 800 to 1100 μmoles m^{-2} sec^{-1} further increased the extent of chlorosis, as measured by chlorophyll content.

In this investigation we aim to establish the effect of a reduced radiant load on P(max) and also on the appearance of leaf bleaching. Plants were grown in ambient (360ppm) and elevated (2000ppm) CO_2 and were either shaded or unshaded. Levels of the photosynthetic carboxylating enzyme, RUBISCO, were determined to assess its role in the downregulation of photosynthesis. Water relations measurements were also taken to ascertain if this factor could contribute another stress, leading to leaf bleaching. The hypothesis under investigation is: High PPFD interacts with mild water deficit at elevated CO_2 to cause observed CO_2 toxicity symptoms.

Materials and methods

Crop Propagation and growth. *Cucumis sativus*, cv Sabrina, seeds were sown onto rockwool blocks (Grodan, Fargro Ltd., UK) germinated, and propagated in ambient conditions until the 4^{th} leaf stage. At this point, plants were transferred to the CEC and acclimated to this environment for a week. The crop was grown in rockwool fed with a hydroponic nutrient solution using a standard commercial feed recipe supplied by HRI Stockbridge House. Pure CO_2 was fed directly into the cabinet inflow ducting of the two treatment cabinets at the rate of 2.5 l min^{-1}, which gave a consistent growth concentration inside the cabinet of 2000ppm CO_2, measured regularly with a portable CO_2 analyser (EGM-1, PP Systems, UK). Two contol cabinets were maintained at ambient CO2 concentrations. The growth period for the crop was 30 days by which time approximately 23 leaves had emerged on each plant and a significant number of fruits had been harvested.

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The experimental facility consisted of four 105 cm x 180 cm controlled environment cabinets (CEC). A central cooling unit was used to maintain the temperature within the desired set points. An air conditioning unit (ACU) provided, via internal ducting, continual inflow of a steady stream of air, the velocity of which could be controlled by baffles in the ducting. . The humidity was controlled using Vapac humidifiers, controlled via a Quick Basic computer programme. The same computer programme was also used to control the lights and to provide graphical output of the actual environmental conditions achieved. Thermostatted fan heaters were installed in each cabinet to achieve better thermal control when the lights were off in the evening. In addition to previous experiments in the CEC, a solenoid and timer were installed on the CO_2 line and also a timer and relay switch incorporated in the ACU. This enabled CO_2 enrichment on a 12hr cycle and, with the ACU off at night resulting in still air in the cabinets, resulted in less temperature fluctuation in the evening. The photoperiod for this set of investigations was 12hr, with the light intensity increasing and decreasing in a step-wise fashion at the start and end of the photoperiod. Sufficient shading in the cabinets was achieved by a combination of wire gauze placed over the sodium bulbs in the lighting array and also by covering the top of the chamber with muslin. The decrease in light intensity is shown (Fig. 5).

Gas Exchange Measurements. It was decided that the A/C_i approach that had been used in previous experiments was too time-consuming and did not allow satisfactory replication of samples. The CO_2 regimes that were applied in this experiment were such that when simple gas exchange measurements were taken we would have information on both the carboxylation limited and RuBP limited parts of the A/C_i curve. Also we will have an assessment of how each plant performs in its growth atmosphere and in the growth concentration of the other treatment. Measurements were taken once a week for the 30 day duration of the experiment. On the final day, a comparison was also made between the tagged leaves (5^{th} leaf) and the youngest leaves at this time (~ leaf 16) to assess the extent that leaf age affected the downregulation of photosynthesis.

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RUBISCO Determination. The method previously described was used to quantify the level of this protein. At least 3 replicate samples were taken per treatment from the second youngest leaf of each plant. At the end of the investigation, samples were also taken from the tagged 5th leaf, from which gas exchange measurements had been taken throughout the experiment.

Xylem Vessel Number and Diameter. As in part one, sections of petiole were taken and viewed under a light microscope. In this experiment samples were taken at time-points of 15 days. These measurements were taken on the first experimental crop, where a red spider mite infestation became quite severe in the unshaded, 2000 ppm CO_2 cabinet. However, it was the belief that this type of damage would not affect xylem differentiation In the repeat experiment carried out in November, focus was turned to taking pressure bomb measurements to obtain an assessment of the hydraulic conductivity of the petioles.

Stomatal Measurements. Stomatal density measurements were taken from the 3rd youngest leaves in each treatment plant canopy as described previously.

Water Flux Measurements. A Scholander Pressure Bomb (Soil Moisture Equipment Corporation, CA, U.S.A.) was employed to determine rate of water movement in the xylem vessels. A vial of water was placed, on a stand, in the pressure chamber and the petiole was sealed in such a way that one end was immersed in the water. Applying an external pressure of 0.3 MPa (using N gas) forced water through the xylem. This was collected in a pre-weighed vial containing absorbent tissue and after a set period the vial was re-weighed to determine the flux per unit time. The procedure was repeated with leaves which had grown at both ambient and elevated CO_2 concentrations.

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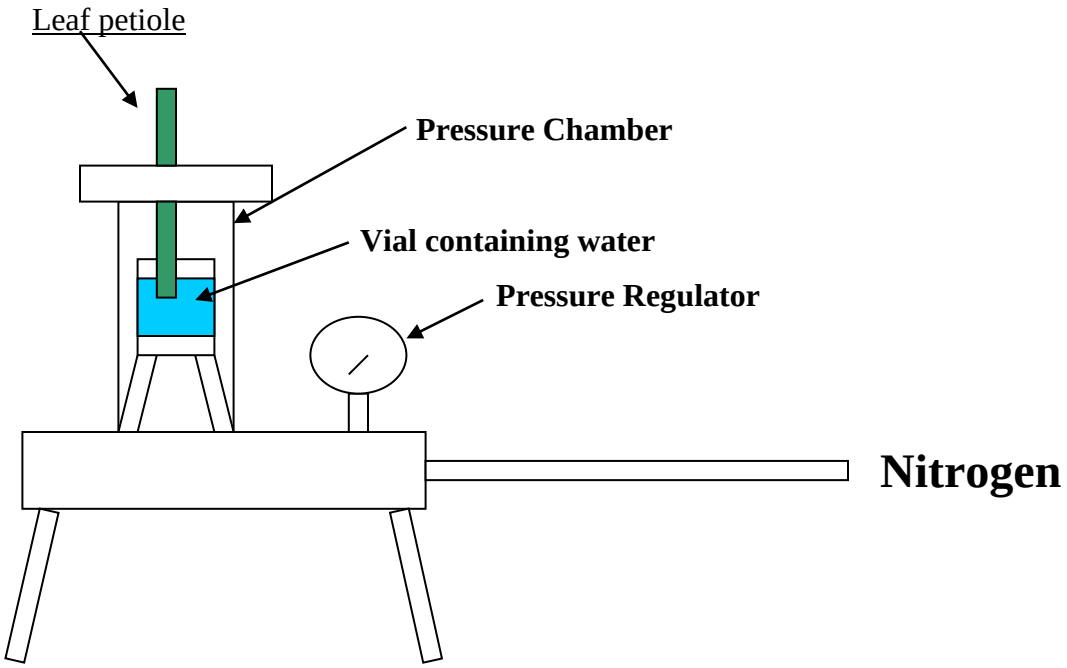

Diagrammatic representation of a Scholander Pressure Bomb.

Results and Discussion

Photosynthesis. In an initial experiment, preliminary results show that in nearly all cases the photosynthesis rates at ambient CO_2 concentration and at elevated CO_2 concentration (A_{360} and A_{2000} values) were higher for plants grown at ambient CO_2 compared to plants grown at 2000ppm (Fig. 6). This suggests that both carboxylation rates and maximum photosynthesis (pmax) in ambient-grown plants were maintained at a higher level than in the enriched plants. The shading in the elevated CO_2 treatment seemed to protect the plants from the apparent damage to the photosynthetic system, seen in full light. Shaded leaves remained dark green with very little evidence of bleaching. We have drawn no firm conclusions from these experiments, however, as a spider mite infestation damaged leaves and makes CO_2 toxicity symptoms difficult to assess.

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In a second experiment, initial photosynthesis measurements were taken on plants at their own growth concentration (Fig.7) and as predicted, certainly in the short term, both elevated treatments had higher photosynthetic rates than those seen in ambient conditions. The protective role of shading is more striking in this experiment. A steady downregulation over time in both elevated treatments is apparent, more so in the unshaded compared with the shaded treatment (Fig. 7a-d).After 4 weeks (Fig 7d) the A_{360} value of the shaded 2000ppm treatment is similar to that of ambient shaded and the pmax value is not significantly different from that of the plants in the ambient treatment, even as the leaf ages, suggesting little downregulation or damage has occurred. A pattern of increasing downregulation and/or damage of photosynthesis with increasing leaf age is apparent in the 2000ppm full light (unshaded) treatment. After 4 weeks the leaves of much of the canopy were light green with regions of yellow and white in places, indicating photodamage. The shaded leaves in this CO_2 regime remaining dark green and healthy looking.

The youngest leaves in each cabinet were also measured at the end of the experiment (30d) (Fig 8). Both ambient treatments showed similar photosynthesis profiles to initial measurements earlier in the experiment. The elevated, shaded plants showed similar rates to plants grown in ambient CO_2. The elevated, unshaded plants had lower A_{360} and A_{2000} values suggesting these newly emerging leaves had a lower photosynthetic output than their counterparts at the beginning of the experiment.

Water Relations and Stomatal Indices. Although in this experiment there appeared to be no difference in xylem vessel number between treatments after 30d exposure (Fig 9), both shaded treatments resulted in a larger xylem vessel diameter compared with full light treatments. The pressure bomb data revealed widely varying results such that the possible restriction of hydraulic conductance at high CO_2 could not be defined in this experiment. Leaves grown in elevated CO_2 had a higher stomatal density in both light regimes and on both the abaxial and adaxial surfaces compared with that of ambient grown plants.

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Conclusion

The most significant result from this experiment is the role a decreased light intensity plays in preventing the onset of CO_2 toxicity symptoms. In addition, the shade treatment reduced the extent to which down regulation of photosynthesis occurred. Tripp *et al* (1991) also found that the most severe foliar deformation occurred in tomato crops under high solar radiation in the summer. Sicher (1997) concluded that light quality played an equally important role with light quantity in inducing leaf chlorosis in barley. This is an important consideration to take into account when undertaking controlled environment work, especially if the desire is to simulate natural conditions. The lack of a clear result from the pressure bomb work suggests that tyloses (blockage in the xylem, causing a mild water stress to be imposed on the leaf) do not seem to have a pivotal role to play in symptom development during CO_2 enrichment. The morphological changes observed throughout this experiment do not seem to play a significant part in photosynthetic down regulation or the onset of leaf chlorosis.

Trials will be conducted to assess whether the ameliorating effect of shade on CO_2 toxicity can be demonstrated in a glasshouse crop. Yield will be monitored to assess the merits of growing cucumber plants under this enrichment regime. In relation to symptom development, work will focus upon analysing levels of key antioxidant enzymes, specifically Glutathione reductase, to investigate how the antioxidant pool may respond during growth in CO_2 enrichment conditions. Changes in the levels of these enzymes in leaves may provide indications that the toxicity symptoms are related to photo-oxidative stress.

Technology Transfer

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