The effects of oxides of nitrogen and carbon dioxide enrichment on photosynthesis and growth of lettuce (*Lactuca sativa* L.)

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SUMMARY

The response of glasshouse crops to the nitrogen oxide pollutants which may be generated during enrichment with CO_2 has been studied in controlled environments. Lettuce (*Lactuca sativa* L. cv. Ambassador) was grown in air containing either low CO_2 (380 μmol mol^{-1}), high CO_2 (1200 μmol mol^{-1}), or high CO_2 plus oxides of nitrogen (NO_x). Carbon dioxide enrichment increased the rate of emergence and expansion of leaves and the growth of young plants. Addition of NO_x (2 μmol mol^{-1} NO and *c*. 0·5 μmol mol^{-1} NO_2) to CO_2-enriched air significantly reduced the yield, compared with the 'clean', high CO_2 treatment, without producing visible symptoms of toxicity. Fumigation of single plants in high CO_2 with NO_x rapidly inhibited photosynthesis per unit leaf area. This did not appear to be due to a reduction in stomatal conductance. Removal of NO_x from the atmosphere caused a rapid and complete recovery in the rate of photosynthesis. Studies were made of the effects of growing plants for long periods in atmospheres containing high CO_2 and NO_x on the photosynthetic capacity of single leaves when measured in NO_x-free air. The decrease in photosynthetic rate as the fourth leaf aged occurred earlier in plants grown in CO_2-enriched air than in those from the low CO_2 treatment. Leaves which developed in the CO_2-enriched air containing NO_x did not suffer any long-term damage to photosynthetic activity in comparison with those of the 'clean' high CO_2. In mature leaves the principal long-term effect of enrichment (with or without NO_x) was to reduce the rate of photosynthesis in saturating CO_2. In contrast, there was less effect on the rate of photosynthesis in low CO_2. The absence of a long-term effect of NO_x on the photosynthetic capacity suggested that photosynthesis by the lettuce crop will be inhibited only during the transient periods of NO_x accumulation in the glasshouse.

Key words: *Lactuca sativa*, CO_2 enrichment, nitrogen oxides, photosynthesis.

INTRODUCTION

The enrichment of glasshouse atmospheres with CO_2 is a standard practice which enhances photosynthesis by single leaves and canopies (Hand, 1973, 1982) and the harvestable yield of crops (Calvert & Slack, 1975). The stimulation of the rate of net photosynthesis by CO_2 is mainly attributed to the promotion of carboxylation and the inhibition of oxygenation of ribulose bisphosphate (RUBP) and, in addition, to the activation of RUBP carboxylase by CO_2 (see reviews by Keys, 1986; Ogren, Salvucci & Portis, 1986).

The photosynthetic rate of leaves grown in high

CO_2 (double or triple ambient concentrations), when measured in limiting or saturating CO_2, may fall below the rate of those grown at ambient concentrations (Wong, 1979; Caemmerer & Farquhar, 1984; Ehret & Jolliffe, 1985; Peet, Huber & Patterson, 1986; Spencer & Bowes, 1986). The prevalence of photosynthetic adaptation to CO_2 is uncertain since in certain species and conditions it was not observed (Hicklenton & Jolliffe, 1980; Valle *et al.*, 1985). An improved understanding of this phenomenon demands a description of how photosynthetic adaptation to CO_2 is influenced by the conditions during growth and by the stage of plant and leaf development.

In winter, glasshouses may be simultaneously heated and CO_2 enriched by venting the flue gases from the fuel burners directly into the air around the

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crop. The release of SO_2 and unburnt hydrocarbons such as ethyene and propylene can be minimized through using correctly adjusted burners and low sulphur fuels (Hand, 1982). Emissions of nitrogen oxides, formed from the oxidation of atmospheric nitrogen, cannot be avoided even with the use of a 'clean' fuel such as natural gas (c. 92 per cent methane; Hand, 1982). Both NO and NO_2 (collectively known as NO_x), of which the former is often predominant in the glasshouse, can have direct effects on plants. The scale of the problem is not clear, because inhibition of yield due to the nitrogen oxides can be obscured by the stimulatory effect of the CO_2 enrichment. Visible symptoms of injury often do not appear. Controlled experiments in commercial glasshouses by different workers suggest that yield may be both decreased (Law & Mansfield, 1979) and increased (Besford & Hand, 1986) by NO_x. The response of plants to NO_x depends, at least in part, on the nitrogen status of the soil (Anderson & Mansfield, 1979; Rowland, Drew & Wellburn, 1987) and the irradiance (Mortensen, 1986).

Lettuce is an important winter crop grown under glass in the United Kingdom. Although some research in controlled conditions (Mortensen, 1985) suggests that this species is less sensitive to NO_x than is tomato (*Lycopersicon esculentum* Mill.), another major protected crop, winter, lettuce may suffer serious visible damage due to pollutants (Hand, Slack & Hannah, 1986). Further research using controlled fumigation of single plants and crops is needed to resolve the uncertainty over the occurrence of injury due to the accumulation of NO_x in glasshouses.

This paper describes the effects of CO_2 enrichment in the presence and absence of NO_x on the growth of lettuce. Two types of study were made of the effect of elevated levels of CO_2 and NO_x on the gas exchange of this species: (1) the immediate response to a transient fumigation with NO_x on the rate of photosynthesis and transpiration; (2) the long-term effect of growth in atmospheres of either low or high CO_2, or high CO_2 plus NO_x, on the photosynthetic capacity of leaves when measured in NO_x-free air.

MATERIALS AND METHODS
Growth conditions

Lettuce (*Lactuca sativa* L. cv. Ambassador) seeds were germinated in a peat-based compost, grown in a glasshouse in ambient air until the third leaf had appeared and was approximately 10 mm long (c. day 14), and then transferred to the controlled environment cabinets described by Whitmore (1985). Plants were watered frequently with a horticultural nutrient solution containing all essential elements. In the cabinets the plants received a constant irradiance of $250\ \mu mol\ m^{-2}\ s^{-1}$ of photosynthetically active radi-

ation during an eight-hour day. The temperature was dependent upon that of the air-conditioned room and changed slightly through the year; values in the cabinets ranged from 22 to 24 °C (day) and from 14 to 17 °C (night). The plants grew well although these temperatures are higher than the normal for winter lettuce. The water vapour pressure deficit was approximately 1·0 kPa (day) and 0·6 kPa (night), acceptable for glasshouse crops (Grange & Hand, 1987). Cool air was drawn from the temperature-controlled room through a charcoal filter and mixed in an antechamber with pure CO_2 or 1 per cent NO in N_2. The air was then pushed from the antechamber across the cabinet so that it passed horizontally and turbulently through the plant canopy. Part of this air was recycled and the remainder was exhausted to the atmosphere. The rate of ventilation was approximately 0·8 changes per minute or $3·0\ m^3\ m^{-2}$ floor area.

The concentrations of CO_2 were measured using an infrared gas analyser (model 225 mark II, Analytical Development Company, UK) and oxides of nitrogen were determined using a chemiluminescent Analyser (model NA530R; Meloy, USA). Periodic full calibration of analysers was performed using a range of CO_2 concentrations and NO or NO_2 permeation devices. Single concentrations of CO_2 in air, NO in N_2, and NO_2 in air (British Oxygen Company, London) were employed for routine calibration. The concentration of CO_2 in the cabinets was either a little above ambient (c. 380 $\mu mol\ mol^{-1}$) or enriched about three-fold (c. 1200 $\mu mol\ mol^{-1}$). Fumigation with NO was inevitably accompanied by the primary oxidation product NO_2. The concentrations of NO (with approximate NO_2 concentrations) used in experiments were either 0·5 (0·15) $\mu mol\ mol^{-1}$ or 2·0 (0·5) $\mu mol\ mol^{-1}$. The NO concentration in the atmosphere of the unpolluted, control, cabinets varied because it is poorly absorbed by the charcoal filters, but the concentrations were much lower than those of the experimental treatments. Outdoor, atmospheric levels of NO at the Lancaster site are usually less than 0·02 $\mu mol\ mol^{-1}$.

Growth experiments

In two separate experiments, plants were grown from 14 days until 30 and 36 days after sowing in atmospheres containing different amounts of CO_2 and NO_x, which were applied continuously, and were then harvested for measurements of the mass of shoots and roots and the numbers of leaves. Data were analysed by a one-way analysis of variance.

Gas exchange measurements

The apparatus was a conventional 'open' system with a continual flow of air through a glass chamber. The rates of CO_2 exchange and transpiration were

determined from the change in the mole fractions of CO$_2$ and H$_2$O in the air stream before and after the chamber. Two types of chamber were used, one for measurements of the shoot and another for single leaves. The shoot chamber consisted of a double-skinned glass cylinder of internal diameter 15 cm and height 20 cm. This was open at one end and was placed over the shoot the stem of which was sealed with a neoprene gasket into a base plate of acrylic plastic. The chamber and the base plate were joined together using a similar gasket and vacuum grease. A flow of between 2·1 and 2·8 mmol s^{-1} of air passed through the plant chamber via an inlet and outlet port in the base and was mixed by a small fan (Micronel, Switzerland). An estimate of the boundary layer conductance, made using a filter paper replica of a lettuce plant, was 0·65 mol m^{-2} s^{-1} at a flow rate of 2·1 mmol s^{-1}. The chamber temperature was regulated and maintained at 20 °C (± 0.5 °C) by chilled water circulating between the inner and outer skins of the glass cylinder.

Measurements on the distal portions of single leaves were made using a small clip-on glass cuvette which enclosed a leaf area of 11·34 cm^2. On both sides of the leaf air entered the cuvette through a single port and left through two. A high flow rate (0·69 mmol s^{-1}) encouraged turbulence and increased the conductance of the boundary layer (G_{wv}) which, measured using a filter paper disc, was 0·31 mol m^{-2} s^{-1} at this flow rate. Water jackets, on both sides of the cuvette, regulated the internal temperature.

Both types of chamber were illuminated by a 400 Watt metal halide lamp (Thorne, UK) and photosynthetically active radiation was measured at plant height using a quantum sensor (Crump, UK). Temperatures in the cuvette were measured with soldered copper–copper/nickel thermocouples which were either attached to the abaxial surface for leaf measurements or below the leaves for air measurements.

Outside air was compressed and pushed through filters of potassium permanganate ('Purafil', Jones and Attwood, UK) and then activated charcoal to reduce the concentrations of ambient pollutants including NO. Atmospheric carbon dioxide was also removed, except when high concentrations were used in the chambers, by passing through soda lime. The dewpoint was set by bubbling the air through a dreschel bottle at approximately 12 °C; this provided a water-vapour pressure deficit in the chamber, at 20 °C, of approximately 1·0 kPa. Concentrated CO$_2$ (1 or 3 per cent in air) or NO (0·1 per cent in N$_2$) were added at this stage to the conditioned air and were sufficiently mixed in a 1·0 litre glass vessel. A second pump passed samples of this air, under positive pressure, to reference and analysis streams, the latter passing to the plant chamber. Excess conditioned air was flushed to the atmosphere.

The rate of transpiration was estimated from the difference in the dew point temperatures of the analysis line determined before and after the chamber; these were measured using a dewpoint hygrometer (model 911, EG & G, USA). The CO$_2$ differences between the reference and the analysis lines from the cuvette were compared using the infrared gas analyser after the water-vapour contents of these air streams had been equalized by passing through glass flasks at *c*. 12 °C.

Experimental procedures

The photosynthetic response at 20 °C to step-wise increases in irradiance was measured from darkness to light saturation, allowing equilibration of gas exchange at each level. The response to changing CO$_2$ in the cuvette was started at approximately 345 μmol mol^{-1} and raised over 4–5 steps to around 800 μmol mol^{-1}, then returned to ambient followed by step-down changes to the compensation point. The principal aims were to examine (*a*) the initial slope of the response to CO$_2$, and (*b*) the CO$_2$ saturated rate of photosynthesis. These should be measured without light limitation (Caemmerer & Farquhar, 1981; Evans, 1986). However, in preliminary work, the rate of photosynthesis in lettuce declined if held at saturating irradiance for more than about 15 minutes. Measurements were, therefore, made at the growth irradiance. The IRGA calibration, differential, and the background CO$_2$ level were determined using one analyser (ADC, model 225, Mark II) adopting the protocol described by Jarvis & Sandford (1985, see their Table 3.6). Calculation of the rates of net photosynthesis and transpiration, the derived conductances to H$_2$O and CO$_2$, and the estimates of the intercellular CO$_2$ concentration, were made using the principles and the equations described by Long & Hällgren (1985). The value 1·6 was used for the ratio of the diffusivity of CO$_2$ and H$_2$O in air.

The large chamber was used for measurements of the whole shoot; rates were determined in clean air and then after the addition of 2 μmol NO mol^{-1} (*c*. 0·46 μmol NO$_2$ mol^{-1}). Other experiments used the small cuvette to measure the photosynthetic capacity of leaves in NO$_x$-free air which had been grown in either clean air, or air enriched with CO$_2$ with or without NO$_x$. The fourth leaf above the cotyledons was used; this had developed without shading from leaves of the same or other plants. In some cases following the gas exchange analysis the area of the fourth leaf was measured in order to study the expansion the laminae grown in different atmospheres. Area was determined by tracing the outline of the leaf on to paper of known density which was then weighed.

Table 1. *Effect of CO_2 enrichment, alone or in the presence of oxides of nitrogen, given after the third leaf, 14-day stage, on the growth of* Lactuca sativa

	Treatment				
CO_2... NO...	Ambient Zero	High Zero	High 0·5	High 2·0	L.S.D. ($P < 0.05$)
(a) 30 days old; two concentrations of NO_x					
Shoot fresh mass (g)	9·22	13·94	12·44	10·64	1·4
Plant dry mass (g)	0·543	0·791	0·703	0·654	0·078
log_{10} (shoot/root) (dry mass basis)	0·756	0·885	0·842	0·840	0·090
Leaf number	14·0	15·9	15·25	14·38	0·73
(b) 36 days old; a single concentration of NO_x					
Shoot fresh mass	35·5	53·2	—	35·4	7·1
Plant dry mass	1·99	2·72	—	1·86	0·28

Gas concentrations in $\mu mol\ mol^{-1}$, ambient and high CO_2 were c. 380 and 1200 respectively. NO was accompanied by some NO_2 (see Methods section). Sample sizes, $n = 8$ (*a*), $n = 6$ (*b*). Analysis of variance indicated a significant mean square ratio ($P < 0.05$) in each case. L.S.D. is the least significant difference ($P < 0.05$).

Statistical analysis of the initial gradients of the CO_2 response curves

A linear regression of the initial points from each individual CO_2 response curve was performed to establish at which concentration of CO_2 the response became non-linear. The points in the linear section were then included in an analysis of covariance to compare the initial slopes between plants and between treatments. The analysis was performed with the GLIM™ package, which is used to fit generalized models to unbalanced data and included an analysis of covariance. (GLIM is a trademark of the Royal Statistical Society.)

RESULTS
Growth responses to CO_2 and NO_x

The three-fold CO_2 enrichment produced a large increase of 37–51 per cent in the yield of 30- and 36-day-old lettuce in both the fresh weight of the shoot and the dry weight of the whole plant compared with the plants raised in low CO_2 (Table 1 *a,b*). More of the dry mass was in the tops compared with the roots. Enrichment with CO_2 raised the rate of leaf emergence and the rate of growth of individual leaves (Table 1 and Fig. 1) but the effect on the final area of the fourth leaf was slight (Fig. 1).

The addition of NO_x to the CO_2-enriched atmosphere progressively reduced yield as the concentrations of NO was increased to 0·5 and to 2·0 $\mu mol\ mol^{-1}$ (Table 1). With greater NO_x the benefits to growth of the 'clean' CO_2 enrichment were greatly reduced. Fumigation with NO_x never caused large areas of necrosis on the leaves of lettuce but such signs of toxicity were visible in tomato plants placed for a few days in the same chambers.
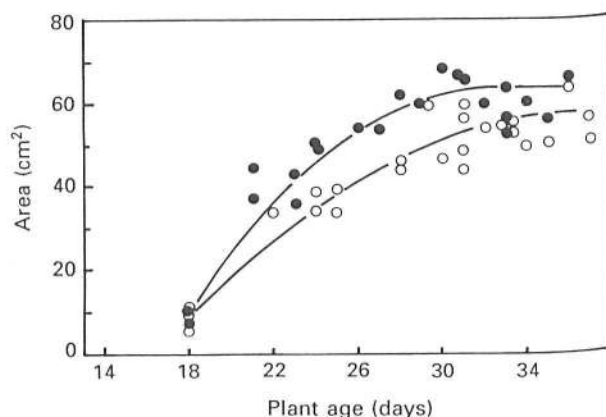
Figure 1. Expansion of the fourth leaf of *Lactuca sativa* in plants grown after day 14, when the third leaf had emerged, in low (O, 380 $\mu mol\ mol^{-1}$) or high (●, 1200 $\mu mol\ mol^{-1}$) CO_2.

### Short term effects of NO fumigation on photosynthesis

After a steady rate of photosynthesis had been reached, the addition of NO_x to the cuvette reduced the assimilation by the shoot within several minutes to a new, stable, level (Fig. 2 and Table 2). Subsequent removal of the NO_x was followed by an equally fast recovery of photosynthetic activity to close to the initial rates. These effects of NO_x on photosynthesis were demonstrated in lettuce of different ages. In some cases the changes in photosynthesis were concomitant with those in the stomatal conductance to water vapour as shown in the example (Fig. 2); in others the stomatal aperture was not affected by the pollutant. There was no reduction in the estimated concentration of CO_2 in the intercellular spaces during the monitoring period (data not shown). The addition of NO_x in the absence of a plant produced no measureable gradient in CO_2 across the chamber.

Table 2. *Effect of a transient fumigation with NO on photosynthesis and conductance to water vapour by young* Lactuca sativa

Plant no.	Photosynthesis (μmol CO$_2$ m^{-2} s^{-1})			Conductance (μmol H$_2$O m^{-2} s^{-1})		
	Before	During	After	Before	During	After
1	5·95	4·64 (78)	5·49 (92)	69·1	51·1 (74)	45·4 (66)
2	7·31	6·30 (86)	7·56 (103)	114·8	100·2 (87)	108·0 (94)
3	7·91	7·35 (93)	7·85 (99)	107·2	118·9 (111)	121·4 (113)
4	5·33	5·00 (94)	5·60 (105)	76·9	73·0 (95)	71·4 (93)
5	5·01	4·46 (89)	4·99 (99)	114·3	108·9 (95)	103·2 (90)

Measurements made at 20 °C, 250 μmol m^{-2} s^{-1} P.A.R., in high CO$_2$ (950–1000 μmol mol^{-1}) on whole shoots before, during and after a one-hour fumigation with 2·0 μmol mol^{-1} NO. Values represent steady-state rates; those in parentheses are the rates as a percentage of the initial value.
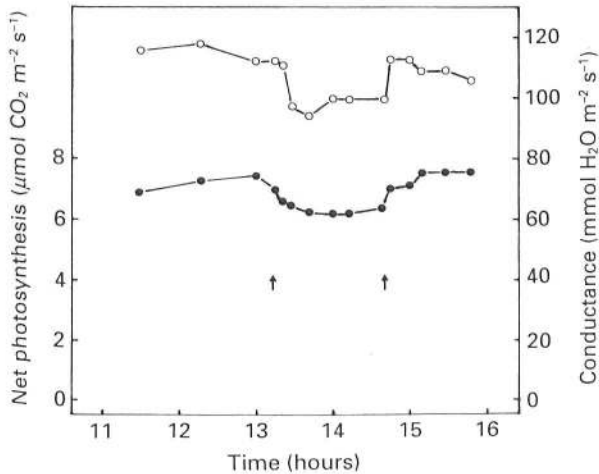
Figure 2. The effect of a transient treatment with NO (2 μmol mol^{-1}) on the photosynthesis (●) and the leaf conductance to water vapour (○) of a whole lettuce shoot aged 29 days. Measured in high CO$_2$ (950 μmol mol^{-1}) and at a photon fluence of 250 μmol m^{-2} s^{-1}. The time between the arrows indicates the period of fumigation.

Long-term effects of growth in atmospheres of varying CO$_2$ and NO$_x$ on the photosynthetic potential of single leaves

Measurements at varying irradiance in ambient CO$_2$. The photosynthetic rate of the fourth leaf, measured at increasing irradiance, changed with the stage of leaf development. A comparison was made between plants grown in high or low CO$_2$ (without NO$_x$). Activity was highest in the younger leaves and decreased with advancing age (Figs 3 and 4), the decline being apparent at an earlier stage in the leaves grown in the CO$_2$-enriched air. For instance, the light-saturated rate of photosynthesis fell to one-third of the maximum after 31 days (17-day old leaves) in high CO$_2$ plants and after 37 days (23-day-old leaves) in low CO$_2$ grown plants. The leaves of both of these treatments had fully expanded by this stage but neither showed obvious signs of senescence such as chlorophyll degradation.

The changes in photosynthesis and leaf conductance, with respect to age and the growth environ-

ment, were similar (Fig. 4a,b). However, the lower conductance for CO$_2$ in the mature, enriched, leaves was not likely to have caused the changes in photosynthesis because the estimated C$_i$ was similar or a little higher than in the comparable leaves of the ambient treatment (Fig. 4c).

Measurements at the growth irradiance in changing CO$_2$. When the initial response of photosynthesis to CO$_2$ was measured at different levels of irradiance it was found that 250 μmol m^{-2} s^{-1} was sufficient to attain the maximum gradient (e.g. Fig. 5). Therefore the photosynthetic activity, measured at very low CO$_2$ and at the growth irradiance, should not have been limited by the rate of regeneration of RUBP (Caemmerer & Farquhar, 1981; Weber *et al.*, 1987).

Since the rate of photosynthesis, measured in saturating light and ambient CO$_2$ (c. 345 μmol m^{-2}
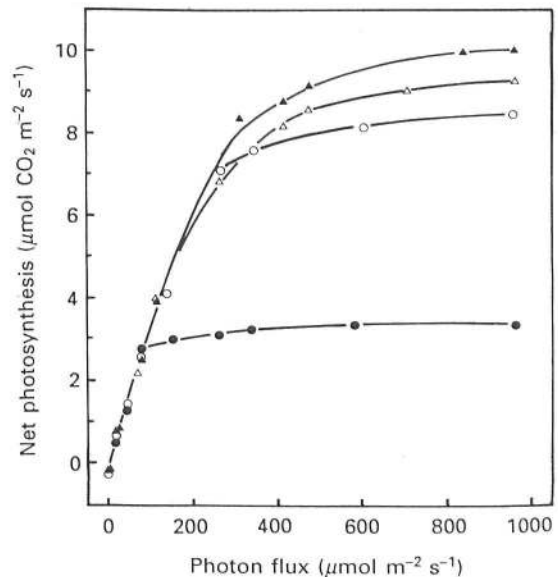
Figure 3. The response of photosynthesis to increasing photon flux in young (plant age 21–22 days; $\triangle$, $\blacktriangle$) and mature leaves (36 days; ○, ●) of lettuce plants grown in low CO$_2$ (open symbols) or high CO$_2$ (closed symbols). Measured in c. 345 μmol CO$_2$ mol^{-1}.
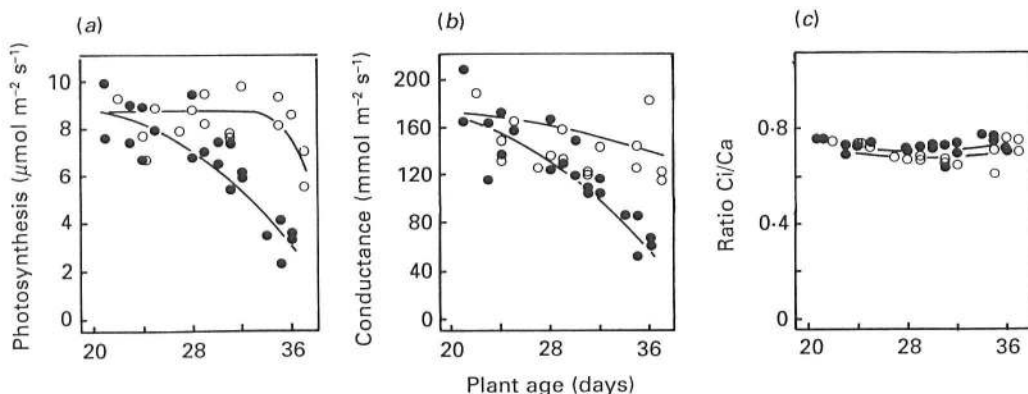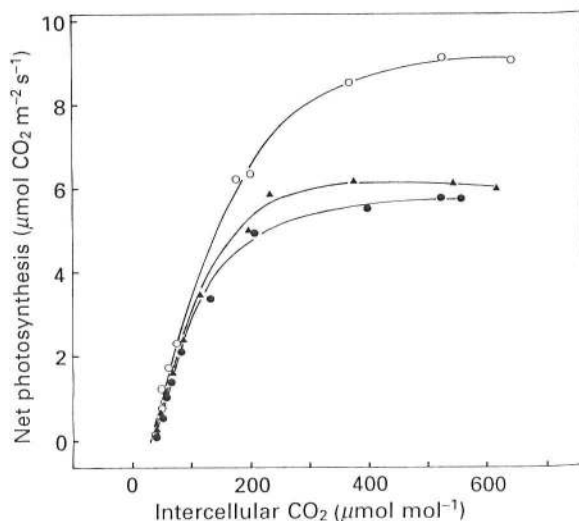
Figure 4. The light-saturated rate of photosynthesis (*a*), conductance to water vapour (*b*), and ratio of the CO_2 mole fraction in the intercellular spaces and the cuvette air (*c*) measured during ageing of the fourth leaf of *Lactuca sativa* grown in low (○) or high (●) CO_2 and measured in low CO_2 (*c.* 345 μmol mol^{-1}).
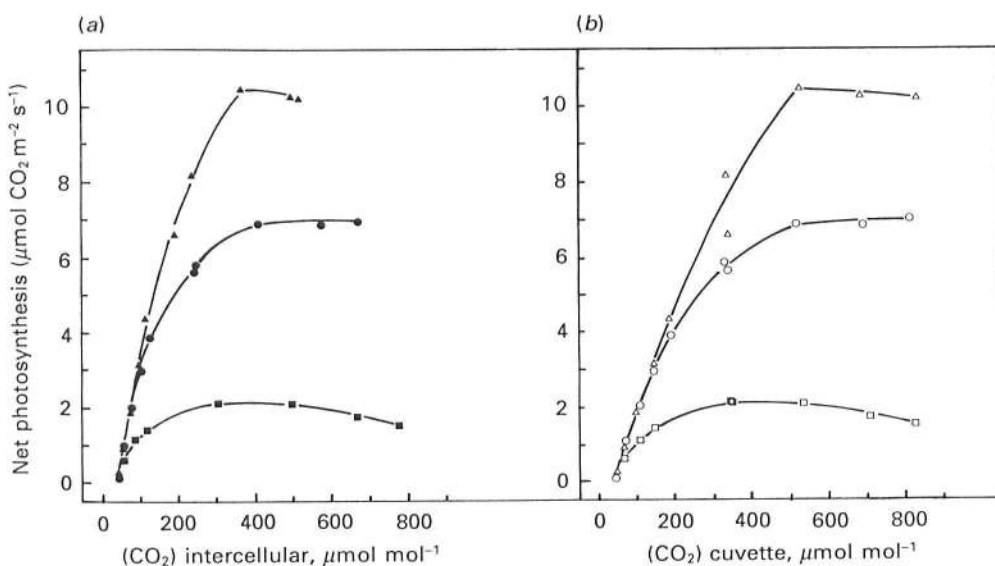

Figure 5. The response of photosynthesis of leaves of lettuce to the CO_2 mole fraction estimated in the intercellular spaces (*a*) and in the cuvette air (*b*) determined at three irradiances: 156 (squares), 250 (circles), or 560 (triangles) μmol photons m^{-2} s^{-1}.

s^{-1}), was strongly influenced by the age of the fourth leaf (Fig. 4) the photosynthetic CO_2 response was also determined at various stages of development. Examples of the relationship between assimilation and C_i in mature leaves grown in the different environments are shown (Fig. 6). The assimilation rate was frequently maximal at a C_i of 500 μmol mol^{-1} although in some instances the activity was still not completely saturated when C_i was raised to *c.* 700 μmol mol^{-1}.

The maximum rate of photosynthesis (or, in the absence of saturation, the highest obtained below a C_i of 700 μmol mol^{-1}) was compared between leaves grown in low CO_2, high CO_2, or high CO_2 plus NO_x. In comparison with those grown in low CO_2, high CO_2 caused a fall in rate of photosynthesis in the mature leaves. When NO_x had accompanied high CO_2 in the growth atmosphere there was no additional effect on the rate of photosynthesis (Figs

Figure 6. The photosynthetic CO_2 response of mature leaves of *Lactuca sativa* (age 33–34 days) grown in either low CO_2 (○), high CO_2 (●), or high CO_2 plus NO_x (▲). Measured at an irradiance of 250 μmol m^{-2} s^{-1}.
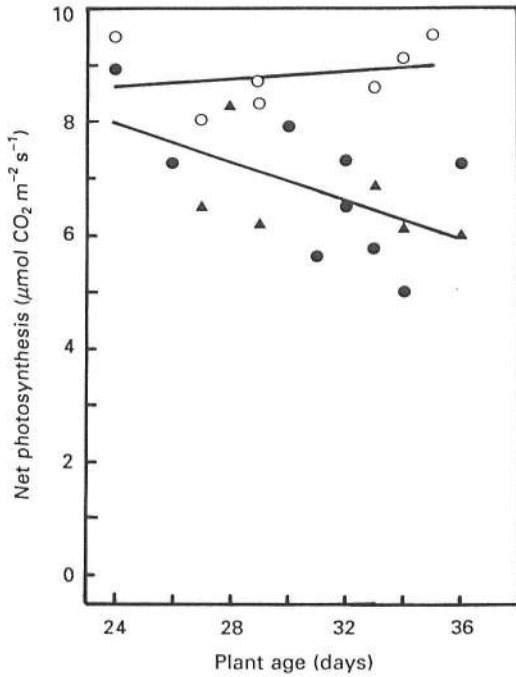
Figure 7. The change in the CO_2-saturated rate of photosynthesis during ageing of the fourth leaf of *Lactuca sativa* grown in either low CO_2 (○), high CO_2 (●), or high CO_2 plus NO_x (▲). Measured in high CO_2 (C_A of *c*. 800 μmol mol^{-1}) and an irradiance of 250 μmol m^{-2} s^{-1}. The regression for the low CO_2 data was $y = 7 \cdot 89 + 0 \cdot 0318x$, while for the combined data of the CO_2 enriched treatments it was $y = 11 \cdot 98 - 0 \cdot 167x$.
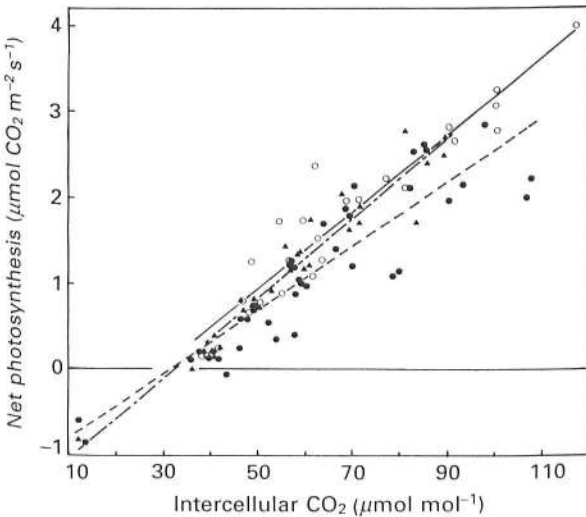
Figure 8. The initial gradient of the photosynthetic response to CO_2 in leaves grown in either low CO_2 (○, single line, $y = 0 \cdot 0446x - 1 \cdot 294$), high CO_2 (●, dashed line, $y = 0 \cdot 0375x - 1 \cdot 212$) or high CO_2 plus NO_x (▲, dotted and dashed line, $y = 0 \cdot 0466 - 1 \cdot 517$). Measurements made at an irradiance of 250 μmol m^{-2} s^{-1} which was saturating for photosynthesis at this range of CO_2

6 and 7). The CO_2-saturated rate measured in leaves grown in low CO_2 showed no significant change with age (correlation coefficient, $r = 0 \cdot 223$, $P = 0 \cdot 636$). But those grown in a high CO_2 atmosphere (pooling

data of plants enriched in the presence and absence of NO_x) declined over this period ($r = -0 \cdot 568$, $P = 0 \cdot 03$).

Study of the initial gradients of the photosynthetic CO_2 response suggest that enrichment, with or without NO_x, had less effect on this characteristic than on the saturated rate (Figs. 6 and 8). The linear regressions for the individual leaves did not appear to depend on age and in Fig. 8 the regressions for the combined data within a treatment are shown. Although the data suggest a lower slope for plants grown in high CO_2, a statistical comparison between treatments (6 to 9 replicates) using an analysis of covariance found no significant difference between the initial gradients ($F = 1 \cdot 517$; $0 \cdot 1 < P < 0 \cdot 25$).

DISCUSSION

Effects of 'clean' CO_2 enrichment

The benefit of CO_2 enrichment to the growth of lettuce in the absence of oxides of nitrogen can be attributed to an increased rate of photosynthesis per unit of leaf area (Figs 5 and 6). Other direct effects of CO_2 on growth which do not act via photosynthesis, such as alteration of the yield threshold for cell wall extension, require investigation.

In the longer term the exposure to high CO_2 altered the response of photosynthesis to light and CO_2 in the fourth leaf. Several observations of changes in leaf photosynthetic activity in response to CO_2 enrichment have been reported, but rarely has it been appreciated that such adaptation in single leaves should, perhaps, be viewed in terms of leaf ontogeny. Both in lettuce (this paper) and tomato (Besford, Withers & Ludwig, 1984) growth in high CO_2 did not appreciably alter the photosynthetic rate, measured in high or low CO_2, in expanding leaves; but it did hasten the decline in activity as the leaves aged. A similar effect of growth in high CO_2 on the rate of photosynthesis in leaves of different ages in *Glycine max* L. was recorded by Hofstra & Hesketh (1975).

Since CO_2 enrichment hastened the decline of photosynthetic activity yet promoted both the rates of emergence and initial expansion of the fourth leaf this treatment appears to have increased the rate of leaf turnover. Confirmation of this demands a closer examination of the rates of foliar emergence and senescence.

Despite adaptation to high CO_2, measurements of the rate of assimilation in the CO_2 and light environments in which the plants were grown underline the benefits of enrichment to photosynthesis. A. C. Withers and L. J. Ludwig (unpublished data) found that the rate of photosynthesis in the fifth leaf of tomato grown and measured at 1000 μmol mol^{-1} exceeded that of those grown and assayed at 300 μmol mol^{-1} at all stages of foliar development. This was also true of the young leaves of lettuce

though in the mature stage the advantage of enrichment was lost.

Interpretation of the photosynthetic CO_2 response curves according to the photosynthesis model of Farquhar, Caemmerer & Barry (1980) offers some cluse to the cause of changes in photosynthesis induced by CO_2 enrichment. Growth in high in comparison to low CO_2 principally reduced the capacity for RUBP regeneration, as indicated by the CO_2-saturated rate, and this was pronounced as the leaves matured. There was no significant effect on the RUBP-saturated carboxylase activity, as indicated by the initial slope. Besford et al. (1984) found that the earlier fall in the assimilation rate (measured at 300 $\mu mol\ mol^{-1}$) during the life of the fifth leaf of CO_2-enriched tomato plants was correlated with the decreased in vitro activity of the CO_2 saturated RUBP carboxylase and this reflected the absolute amount of RUBP carboxylase protein. Comparable observations, in plants grown in high CO_2, of the reduction in both the in vitro activity of the carboxylase and the rate of photosynthesis, measured in limiting CO_2, were made in a range of species by Caemmerer & Farquhar (1984), Wong (1979), Peet et al. (1986), and Spencer & Bowes (1986).

Although several reports describe reductions in the photosynthetic capacity of leaves grown in high CO_2 there are some conflicting observations. Valle et al. (1985) found that leaves of soybean grown at a CO_2 concentration of 600–660 $\mu mol\ mol^{-1}$ had higher rates of CO_2-saturated photosynthesis than those grown in 300–330 $\mu mol\ mol^{-1}$. In Phaseolus vulgaris the photosynthetic changes induced by growth in high CO_2 (1400 $\mu mol\ mol$) were noted only in young and not in older leaves (Ehret & Jollife, 1985). This contrasts with the results with lettuce and those of Besford et al. (1984). Possibly in different species and environments the 'sinks' for carbon and the accumulation in leaves of starch play varying regulatory roles in the photosynthetic adjustment to an altered CO_2 environment (Clough, Peet & Kramer, 1980; Huber, Rogers & Israel, 1984).

Effects of CO_2 enrichment in the presence of NO_x

When CO_2 enrichment is achieved by the combustion of hydrocarbons the accompanying nitrogen oxide may reduce the benefits of the additional CO_2. This was clear both from the measurements of the yields and the photosynthetic rates of whole lettuce when fumigated with NO_x. The rapid effects of NO_x were distinct from those expressed in the long term. Fumigation immediately reduced the rate of photosynthesis and this change was quickly reversible. Very similar observations were made by Hill & Bennett (1970), using Avena fatua L. and Medicago sativa L., but they used low, ambient concentrations of CO_2 which did not stimulate the glasshouse environment. Because NO is spontaneously oxidized

to NO_2, the treatment of plants in aerobic conditions with pure NO cannot be achieved, and the effects described here cannot, with certainty, be ascribed to the monoxide even though this was the dominant component. The mechanism of the inhibition is not understood, but it involves a direct effect on photosynthetic machinery and is not a result of stomatal closure. Since NO_x in the glasshouse can rise quickly to high concentrations (Law & Mansfield, 1982) the rapid effect of these pollutants on photosynthesis should be viewed with concern.

Although there were large reductions in the yield of lettuce plants after continuous NO_x fumigation the photosynthetic CO_2 response indicated that there was, surprisingly, no deleterious effect on the development of the photosynthetic capacity in the fourth leaf. Experiments of Besford & Hand (1986) on a lettuce crop showed that plants grown in CO_2-enriched air contaminated with NO_x had a greater in-vitro activity of RUBP carboxylase compared with those cultivated in 'clean' CO_2-enriched air. This stimulation in enzyme activity was associated with a small increase in the yield of mature lettuce. In their glasshouse study NO_x emissions fluctuated in phase with the periods of fuel burning (Hand et al., 1986) reaching lower minimum concentrations than in our experiments; this may explain the different results. Other features of the growth cabinets and winter glasshouse conditions, such as irradiance (Mortensen, 1986) and temperature are likely to influence the effect of the pollutant on plant growth.

Glasshouse crops may differ in tolerance to oxides of nitrogen (Saxe, 1986; Mortensen, 1985). Using leaves of tomato (cv. Moneymaker), Capron & Mansfield (1976) found a 28 per cent inhibition of the rate of net photosynthesis after an addition of just 0·5 $\mu mol\ mol^{-1}$ NO. This differs considerably from the average inhibition of 12 per cent found here in lettuce after the addition of 2 $\mu mol\ mol^{-1}$. In another study using a range of concentrations, Mortensen (1986 and pers. comm.) also found that high concentrations (up to 2 $\mu mol\ mol^{-1}$) of NO caused a comparatively small inhibition of the rate of photosynthesis in both lettuce and tomato (cv. Rianto).

The extent of the problem for growers is uncertain because continuous fumigation with NO_x does not accurately mimic the glasshouse environment in which the concentrations of NO_x as well as CO_2 fluctuate on an hourly and weekly basis. Nevertheless, there are strong grounds for expecting that NO_x in the commercial glasshouse does affect the rate of plant growth.

It is worthy of note that reductions in the yield of young lettuce occurred in the absence of visible injury which have been observed in commercial lettuce crops at the Institute of Horticultural Research, Littlehampton, UK (D. W. Hand, pers. comm.). Therefore, assuming that the threshold

concentration for an effect on growth is lower than that for causing visible damage, the suppression of yield of commercial lettuce due to the accumulation of NO_x is likely. The absence of a long-term effect of NO_x on the photosynthetic capacity indicates that the inhibition of photosynthesis in the presence of the pollutant is reversible. Therefore, photosynthesis by the lettuce crop may be disrupted only during the transient periods of NO_x accumulation in the glasshouse.

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