



Effects of elevated carbon dioxide and ozone on the growth and yield of spring wheat (*Triticum aestivum* L.)

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Received 25 March 1996; Accepted 22 July 1996

Abstract

Spring wheat cv. Minaret was grown under three carbon dioxide (CO₂) and two ozone (O₃) concentrations from seedling emergence to maturity in open-top chambers. Under elevated CO₂ concentrations, the green leaf area index of the main shoot was increased, largely due to an increase in green leaf area duration. Biomass increased linearly in response to increasing CO₂ (ambient, 550 and 680 ppm). At anthesis, stem and ear dry weights and plant height were increased by up to 174%, 5% and 9 cm, respectively, and biomass at maturity was 23% greater in the 680 ppm treatment as compared to the ambient control. Grain numbers per spikelet and per ear were increased by 0.2 and 5 grains, respectively, and this, coupled with a higher number of ears bearing tillers, increased grain yield by up to 33%.

Exposure to a 7 h daily mean O₃ concentration of 60 ppb induced premature leaf senescence during early vegetative growth (leaves 1–7) under ambient CO₂ concentrations. Damage to the main shoot and possible seedling mortality during the first 3 weeks of exposure altered canopy structure and increased the proportion of tillers 1 and 2 which survived to produce ears at maturity was increased; as a result, grain yield was not significantly affected. In contrast to the older leaves, the flag leaf (leaf 8) sustained no visible O₃ damage, and mean grain yield per ear was not affected. Interactions between elevated CO₂ and O₃ influenced the severity of visible leaf damage (leaves 1–7), with elevated CO₂ apparently protecting against O₃-induced premature senescence during early vegetative growth. The data suggest that the flag leaf of Minaret, a major source of assimilate during grain fill, may be relatively

insensitive to O₃ exposure. Possible mechanisms involved in damage and/or recovery are discussed.

Key words: Carbon dioxide, ozone, spring wheat (cv. Minaret), leaf damage, tiller, yield.

Introduction

Atmospheric carbon dioxide (CO₂) concentrations have increased dramatically since pre-industrial times and are predicted to rise to over 700 ppm by the end of the next century (Watson *et al.*, 1990). In parallel with the recent trend of rising atmospheric CO₂, ozone (O₃) has also increased (IPCC, 1992) and is regarded as one of the most phytotoxic of the air pollutants commonly encountered in developed countries of the Northern Hemisphere (Ashmore and Bell, 1991). In addition to their contribution to the 'greenhouse effect', CO₂ and O₃ have direct impacts on plant physiology and crop production (Krupa and Kickert, 1989; Bowes, 1993).

Although various experimental approaches may be used to investigate the effects of predicted future climate change on plants, previous experiments have largely been confined to studies of the impact of a single atmospheric gas or climate change factor on the growth of a chosen species (Krupa and Kickert, 1989). There have been few studies of the responses of wheat to elevated CO₂ in open-top chambers (OTCs) (Lawlor and Mitchell, 1991), which were originally developed to simulate field conditions. In contrast, OTCs have been used extensively to study the effects of elevated ozone on plant growth and physiology (Krupa *et al.*, 1994) and have also been widely used, particularly in Europe, to examine the responses of spring wheat to elevated O₃ (Skärby *et al.*, 1993).

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Previous studies suggest that elevated atmospheric CO₂ improves the growth and productivity of spring wheat (Weigel *et al.*, 1994) and many other plant species (see Kimball, 1983, for a review) and that, in response to a doubling of CO₂ (700 ppm), these increases are in the order of 30% (Cure and Acock, 1986). This stimulation of growth results primarily from an increase in net photosynthetic rate (Long *et al.*, 1993; Baker and Allen, 1994) and improvement of whole plant water use efficiency resulting from partial stomatal closure (Morison and Gifford, 1984; Baker and Allen, 1994). Although growth responses appear to be non-linear between the current ambient and predicted future CO₂ atmospheric concentrations (Hunt *et al.*, 1991), many studies have simply doubled CO₂ concentration to around 700 ppm (see Idso and Idso, 1994, for references). Quantification of crop growth responses between current ambient CO₂ concentrations and 700 ppm may be important for use in modelling and future crop growth assessments.

In contrast, elevated O₃ has a generally deleterious effect on vegetation, primarily by inducing premature leaf senescence (Krupa and Kickert, 1989), decreasing photosynthesis, altering assimilate partitioning, and suppressing yield (Heagle, 1989; Nouchi *et al.*, 1995; Balaguer *et al.*, 1995). Although effects may vary widely because of differences in exposure regimes or the sensitivity of the species or cultivar involved (Krupa and Kickert, 1989), several recent European studies suggest that the yield of spring wheat may be depressed by elevated atmospheric O₃ concentrations at current ambient levels of CO₂ (Pleijel *et al.*, 1991; Skärby *et al.*, 1993).

Although many previous studies have examined the individual effects of CO₂ and O₃ on plants, information on their interactive effects may be crucial for accurate prediction of crop growth and yield under future climatic conditions involving increased CO₂ and O₃ concentrations. Indeed, existing models already suggest that potential yield losses resulting from increased atmospheric O₃ may be greatly overestimated if O₃ concentration is considered in isolation from other environmental variables (Hertstein *et al.*, 1995). The interactive effects of elevated CO₂ and O₃ on spring wheat have only recently begun to receive experimental attention (Balaguer *et al.*, 1995; McKee *et al.*, 1995). The experiment described here was conducted under field conditions in open-top chambers, with the objective of determining the individual and interactive effects of season-long elevated CO₂ and O₃ exposure on vegetative and reproductive growth, visible foliar injury and yield in spring wheat cv. Minaret.

Materials and methods

Site, preparation, and open-top chambers

The experiment was situated at the University of Nottingham, Sutton Bonington Campus, Nottingham, UK (lat. 52° N, long.

1°15' W). The experimental plot (160 m long and 40 m wide; 6400 m² ground area) was located on a sandy loam of the Astley Hall series, and was cultivated prior to sowing using normal agricultural practices as part of the normal rotation. Minaret seed was sown on 20 March 1995 using a Nordston drill at a rate of 180 kg ha⁻¹ to give a mean population density of 371 plants m⁻². The open-top chambers were of the Heagle design (Heagle *et al.*, 1973), 3.1 m in diameter and 2.4 m in height, fitted with a 45° sloping frustum, and placed on 15.2 m centres (north-south) and 12.2 m centres (east-west) to avoid shading between neighbouring chambers. The chambers were clad in 200 µm PVC covers in three sections comprising a frustum, middle and bottom cover. The bottom cover was double-skinned, the inner wall being perforated with 306 × 2.5 cm diameter holes to act as a plenum which distributed air evenly into the chamber and through the crop from a fan box (Model PSA 402/2, Jones and Attwood, Stourbridge, UK).

Crop management

The sown crop was sprayed with a pre-emergence herbicide 'Flexidor' (Dow Elanco, UK) at a rate of 0.25 l ha⁻¹, and allowed to germinate and emerge under ambient conditions. At the terminal spikelet stage, 75 kg N ha⁻¹ was applied as a compound NPK fertilizer to all plots. The crop was treated for powdery mildew on 26 June 1995, using Bayleton (Bayer, Germany) at a rate of 0.04 l m⁻², and sprayed for aphids with 'Rapid' (ICI, UK) at a rate of 0.01 l m⁻² on 1 July 1995. Powdery mildew was not observed in the elevated O₃ or open field plots, but limited infection occurred in the elevated CO₂ treatments; powdery mildew and aphids were both successfully controlled by the measures applied.

The crop was irrigated on a weekly basis from emergence to the end of the season to return the soil to a water deficit of 20 mm. Evapotranspiration was estimated using the Priestley-Taylor equation (Jamieson, 1982), and the quantities of irrigation applied were adjusted for rainfall during the intervening period.

Gas exposure

Ozone was generated from O₂ by electrical discharge (Model LN103, Ozonia, Switzerland) and added to the elevated O₃ chambers at three times the ambient concentration up to a maximum of 140 ppb, above which irreversible injury might have occurred. CO₂ was supplied from a 25 tonne storage tank with a vaporization facility capable of producing up to 50 kg h⁻¹ at a working pressure of 2 kg cm⁻² (Hydrogas, Middlesex, UK), and was distributed using a manually controlled 12 valve manifold. Gas treatments were applied from seedling emergence to maturity.

Air was sampled at a height of 1.2 m above ground level throughout the season and was drawn from a single replicate of each treatment through 70 m lengths of PTFE tubing, using two Lacy-Hulbert vacuum pumps (Model TR10V; capacity 10 m³ air h⁻¹). A roving line was used daily to cross-check gas concentrations in those chambers which were not continuously monitored. Using a series of eight solenoid valves, air from individual plots could be analysed within a 220 s period, enabling a full cycle of all sampled plots to be completed within 30 min. This cycle, together with data logging and other system functions, was controlled using a Turbo-Basic computer program and intelligent interface (Model Thinklab, 3D Digital Design and Development, London, UK). Each sample was analysed for ozone by ultraviolet absorption (Model 8810, Monitor Labs, San Diego, USA) and for CO₂ by infrared gas

analysis (Model 225 MK3, Analytical Development Company, Hoddesdon, Herts, UK).

Aspirated psychrometers containing two copper constantan thermocouples were used to measure wet and dry bulb temperatures in two replicate plots of each treatment, and incident short wave radiation was measured in the centre of each replicate plot using tube solarimeters.

Experimental design

A factorial design of 18 OTCs containing three CO₂ and two O₃ treatments and an unchambered treatment, randomized in three blocks, was used; each treatment was therefore replicated three times. Elevated CO₂ was maintained for 24 h d⁻¹ and O₃ was applied for 7 h d⁻¹ for 5 d week⁻¹ (0900–1600 GMT) in the elevated O₃ plots. The treatments imposed included unchambered ambient air field plots (AA), ambient air OTC control plots (chAA), elevated CO₂ OTCs at 550 and 680 ppm (c550 and c680) and elevated O₃ OTC treatments (60 ppb ozone) under ambient (oz), 550 (oz550) and 680 ppm CO₂ (oz680).

Growth analysis

Harvests were carried out on six occasions during the growing season, three of which were timed to coincide with Zadok's Growth Stages (ZGS) 31 (stem extension), 65 (anthesis) and 92 (maturity) (Tottman and Broad, 1987). The timing of these growth stages is critical for the modelling aspects of the European Stress Physiology and Climate Experiment (ESPACE), of which this project forms part. Harvests were carried out at the following intervals: harvest 1, 44 d after sowing (DAS); harvest 2, 56 DAS (ZGS 31); harvest 3, 71 DAS; harvest 4, 91 DAS (ZGS 65); harvest 5, 105 DAS; and harvest 6, 134 DAS (ZGS 92). At each harvest, plants were

sampled from two 25 cm row lengths (10 cm spacing between rows) on the east and west sides of each plot. Plant numbers, heights and visual estimates of the green leaf area ratio (GLAR) (green leaf area estimated in 5% steps) were recorded (eqn. 1).

$$GLAR = \frac{\text{fraction of green leaves per stem}}{\text{total leaf number per stem}} \quad (1)$$

The plants were then separated into main shoots and tillers and further subdivided into leaves, stems and ears. The projected areas of the leaves, stems and ears were measured using a leaf area meter (Model LI-3000A, LI-COR, Lincoln, Nebraska, USA), before drying at 80 °C for 48 h and weighing. At final harvest, the ears were dried as before and the grains carefully removed to determine yield, grain numbers per spikelet and per ear, and individual grain weight (IGW).

Statistical analysis

The data were analysed as a seven treatment randomized block by ANOVA using GENSTAT. As the seven treatments comprised a factorial three CO₂ × two O₃ treatment structure plus an unchambered ambient plot, the treatment sum of squares was partitioned into a chambered versus unchambered contrast and then into the three CO₂ × two O₃ factorial within the chambered treatments. The CO₂ effects were partitioned into linear and quadratic components to test for dose response, and significant linear trends are prefixed by 'Linear'. As preliminary analysis of the growth data revealed that the unexplained residual error increased between successive harvests, the data were log transformed for statistical analysis.

Results

Microclimatic conditions in OTCs and gas exposure levels

Table 1 summarizes the mean values for microclimatic variables measured within the OTC and AA plots in 1995. Compared to the open field, environmental conditions within the OTCs were characterized by slightly higher daily mean and maximum temperatures, decreased short wave radiation receipts and lower saturated vapour pressure deficits (SVPD). Seasonal total radiation within the OTCs was 13.9% lower than the ambient value.

The gas concentrations achieved in the various treatments are summarized in Table 2. CO₂ concentrations in the elevated treatments were within 3% of the target levels (Fig. 1A; Table 2), while the elevated ozone treatment

Table 1. Incident short wave radiation, air temperature and saturation vapour pressure deficit (SVPD) in the ambient plots (AA) and OTCs during the 1995 experimental period

Data for OTCs are means for all six treatments ± 1 standard error.

Environmental variable	OTCs	AA
Solar radiation (MJ m ⁻²)		
Daily mean	14.1 ± 0.21	16.3
Integrated*	1661 ± 14.6	1929
Air temperature (°C)		
Daily mean	14.1 ± 0.015	13.2
Daily mean maximum	20.6 ± 0.044	18.3
SVPD (kPa)		
Daily mean	0.80 ± 0.014	0.82

* Seasonal total between sowing and final harvest.

Table 2. Seasonal mean gas concentrations for O₃ (7 h means and 7 h maximum) and CO₂ (24 h mean) in 1995

Ozone was applied for 7 h d⁻¹ and 5 d week⁻¹; however, means were calculated over all days, in order to express the data as a seasonal mean. The accumulated O₃ exposure over a threshold concentration of 40 ppb (AOT (40)), was calculated as the number of ppb h of ozone between astronomic sunrise and sunset, from 20 April to 1 August 1995

Gas variable	Treatment						
	AA	chAA	c550	c680	oz	oz550	oz680
O ₃ 7 h mean (ppb)	29	26	27	27	60	59	61
O ₃ 7 h max (ppb)	69	62	63	62	131	123	130
AOT (40) (ppb h)	2158	1357	1549	1589	18671	17862	19496
CO ₂ 24 h mean (ppm)	379	379	550	683	380	554	700

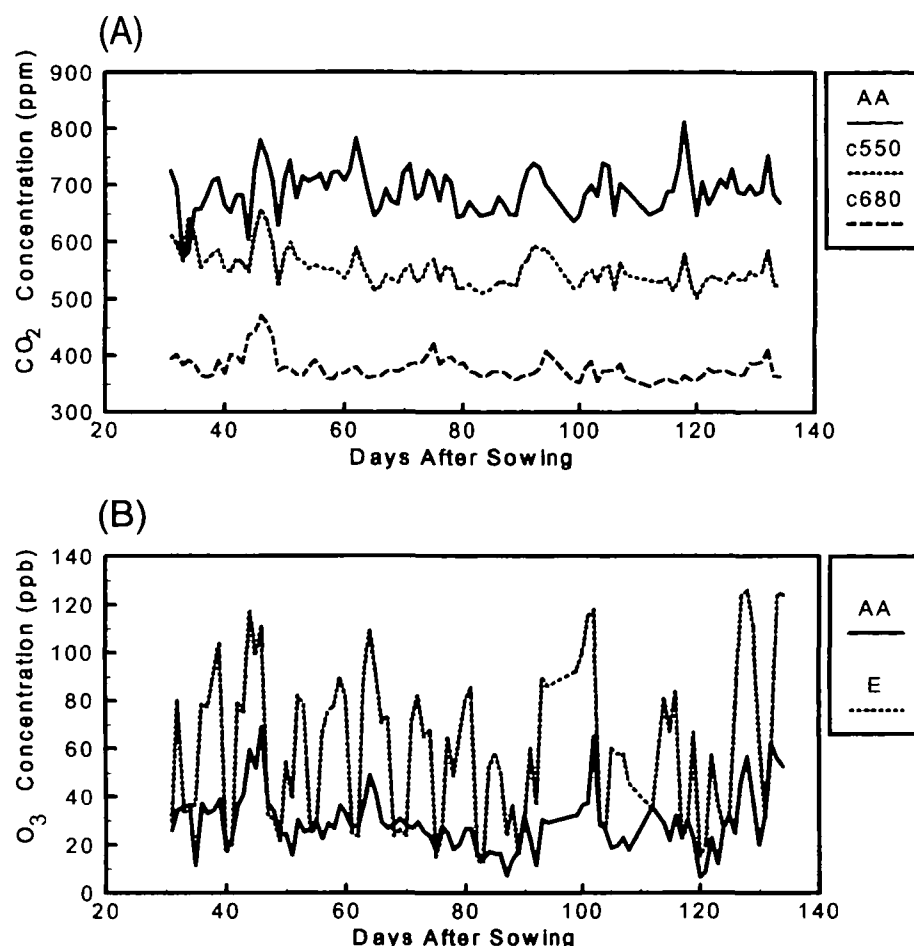


Fig. 1. Daily mean (A) CO₂ concentrations (24 h d⁻¹) for the ambient air (AA), 550 ppm CO₂ (c550) and 680 ppm CO₂ (c680) OTC treatments, and (B) O₃ concentrations for the ambient air (7 h d⁻¹; AA) and elevated O₃ OTC (7 h d⁻¹; E) treatments in 1995.

gave a seasonal mean 7 h d⁻¹ exposure averaged over all days of 60 ppb (Fig. 1B; Table 2).

Effects of season-long exposure to elevated CO₂

Minaret exhibited a linear increase in shoot dry weight (DW) in response to increasing CO₂ concentration and dry matter accumulation at maturity was 7% and 23% greater at 550 and 680 ppm than under ambient conditions (Linear; $P < 0.01$; Fig. 2). For most growth variables no statistically significant interaction was observed between the elevated CO₂ and O₃ treatments (i.e. the response to 550 or 680 ppm CO₂ was unaffected by the presence of O₃); the combined means for the c550 + oz550 and c680 + oz680 treatments are therefore discussed unless otherwise indicated. Table 3 summarizes results for the main growth variables measured at anthesis for the main shoot. CO₂-enriched plants exhibited increases in stem and ear DW of up to 174% and 5%, respectively, as compared to chAA control plants (Linear; $P < 0.001$). The greater stem biomass under elevated CO₂ was partly

attributable to an increase in plant height of up to 9 cm (Linear; $P < 0.01$). The green leaf area index (*LAI*) of the main shoot was greater throughout the season under elevated CO₂ (Linear; $P < 0.01$; Fig. 3A), but overall canopy *LAI* was unaffected (data not shown). The specific leaf area (*SLA*) of the flag leaf was also unaffected which, when combined with the canopy *LAI* data, demonstrates that the increased vegetative biomass under elevated CO₂ originated mainly from the greater stem DW. Ear DW was significantly increased at anthesis (Linear; $P < 0.05$; Table 3) and analysis of *GLAR* revealed that elevated CO₂ increased the longevity of green leaf area, as shown by the data obtained at anthesis (Linear; $P < 0.001$; Table 3).

At final harvest, total grain dry weight was increased by up to 10% and 33% in the 550 and 680 ppm CO₂ treatments, respectively, relative to the chAA control (Linear; $P < 0.01$; Table 4; Fig. 4). No beneficial effect of elevated CO₂ was observed on either the number of tillers produced, or the number surviving until maturity (1–2

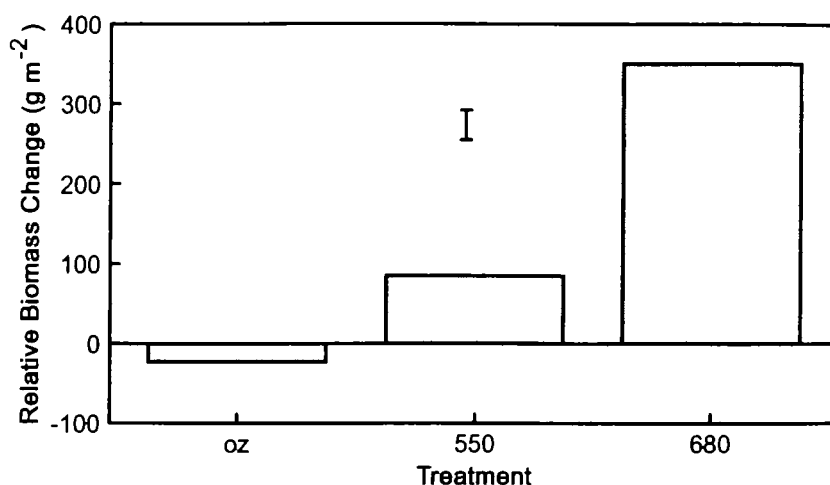


Fig. 2. Above-ground biomass change (g m^{-2}) at maturity relative to the chAA control for the elevated O_3 (oz) and CO_2 treatments (ambient and elevated O_3 treatments averaged for 550 and 680 ppm CO_2). Vertical bar denotes one standard error of the difference (SED) with 12 degrees of freedom (d.f.).

Table 3. Effects of CO_2 and O_3 on the principal growth characteristics of the main shoot at anthesis

Data are expressed as means and an ANOVA summary is presented; ch represents the comparison between chambered and unchambered plots, P values indicate the level of significance and n.s. denotes non-significant

Growth variable	Treatment							ANOVA					
	AA	chAA	c550	c680	oz	oz550	oz680	ch	CO_2	O_3	$\text{CO}_2 \times \text{O}_3$	SED	d.f.
Stem DW (g m^{-2})	153.0	200.5	537.0	548.4	125.5	541.9	539.7	$P < 0.05$	$P < 0.001$	$P < 0.05$	n.s.	18.6	12
Flag leaf DW (g m^{-2})	36.5	39.0	43.2	37.7	37.2	35.2	38.1	n.s.	n.s.	n.s.	n.s.	2.1	12
Flag leaf SLA^a ($\text{cm}^2 \text{g}^{-1}$)	196	198	211	197	212	216	235	n.s.	n.s.	n.s.	n.s.	16.1	12
Ear DW (g m^{-2})	87.8	114.4	119.0	120.2	96.9	109.1	114.1	$P < 0.05$	$P < 0.05$	$P < 0.05$	n.s.	4.1	12
Plant height (cm)	89.1	97.5	106.0	106.7	95.6	104.6	107.0	$P < 0.001$	$P < 0.01$	$P < 0.01$	n.s.	4.0	12
GLAR^b	0.42	0.46	0.49	0.50	0.36	0.42	0.43	$P < 0.05$	$P < 0.001$	$P < 0.001$	$P < 0.001$	0.01	12

^a Specific leaf area, ^b Green leaf area ratio.

Table 4. Effects of CO_2 and O_3 on principal grain yield characteristics at maturity

Data are expressed as means and an ANOVA summary is presented; ch represents the comparison between chambered and unchambered plots, P values indicate the level of significance and n.s. denotes non-significant.

Growth variable	Treatment							ANOVA					
	AA	chAA	c550	c680	oz	oz550	oz680	ch	CO_2	O_3	$\text{CO}_2 \times \text{O}_3$	SED	d.f.
Stand ears m^{-2}	520	466	500	520	546	519	566	n.s.	n.s.	n.s.	n.s.	68.9	12
MS ^a grains per ear	37.5	35.3	39.6	38.3	39.7	41.0	40.2	n.s.	$P < 0.05$	$P < 0.05$	n.s.	1.96	12
MS grains per spikelet	1.9	1.8	2.0	2.0	2.0	2.0	2.0	n.s.	$P < 0.05$	$P < 0.05$	n.s.	0.09	12
MS IGW ^b (mg)	39.4	39.2	40.5	37.4	40.3	41.3	40.8	n.s.	n.s.	n.s.	n.s.	2.45	12
Stand grain DW ^c (g m^{-2})	565.2	627.4	688.1	789.1	613.1	679.2	834.4	n.s.	$P < 0.01$	$P < 0.01$	n.s.	81.6	12
HI ^d	41.1	46.1	47	47.1	45.5	46.5	46.5	$P < 0.05$	n.s.	n.s.	n.s.	2.4	12

^a Main shoot; ^b Individual grain weight; ^c Grain dry weight; ^d Harvest index.

per plant). However, there was a small but non-significant increase in the number of ear-bearing tillers 1 and 2 under elevated CO_2 which resulted in an increased contribution by the tillers to grain yield (Fig. 4). Analysis of the yield components for the main shoot revealed that the number of grains per ear increased from 35 (chAA) to 41 (oz550) under elevated CO_2 (Linear; $P < 0.05$; Table 4), reflecting an increase from 1.8 (chAA) to 2.0 grains per spikelet

under elevated CO_2 (Linear; $P < 0.05$; Table 4). The harvest index for the entire stand was unaffected (Table 4).

Elevated O_3 effects under ambient CO_2 concentrations

Exposure to O_3 reduced the LAI of the main shoot in the oz treatment ($P < 0.01$; Fig. 3A), although canopy LAI was unaffected (data not shown). This reduction in the

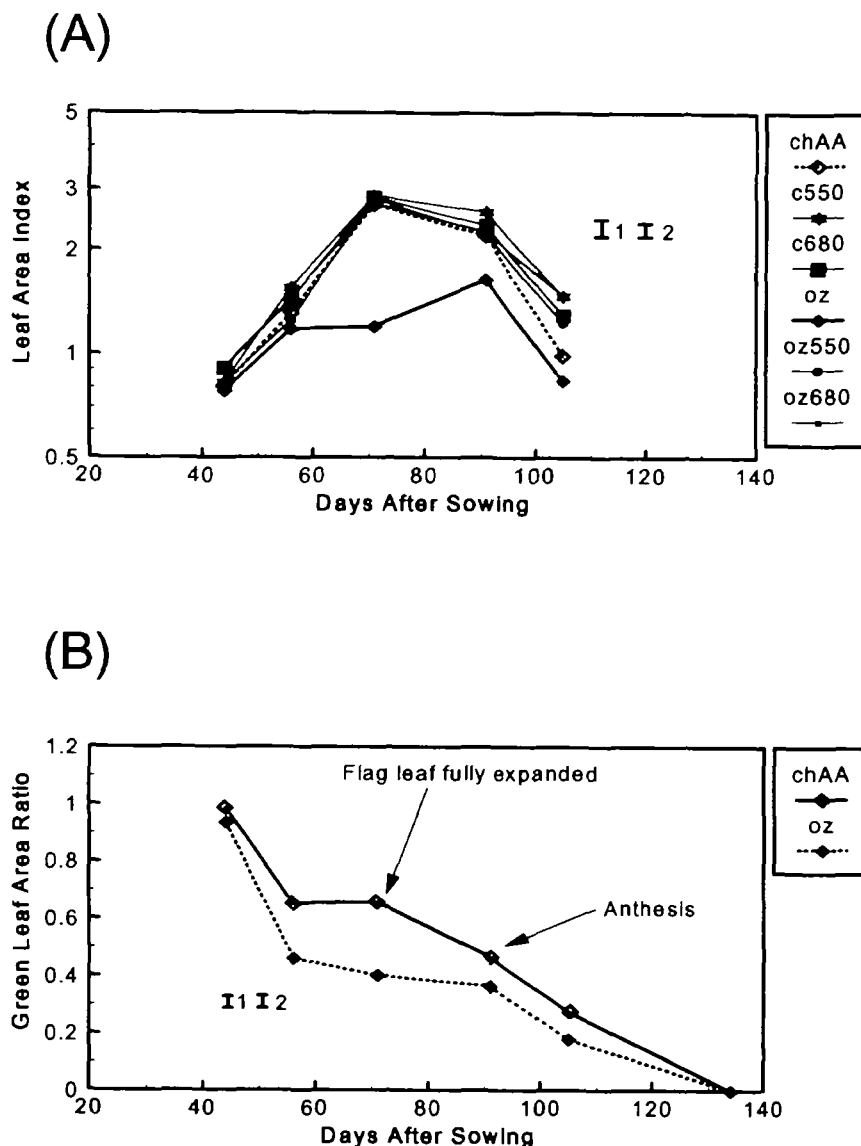


Fig. 3. Effect of elevated CO₂ and O₃ on (A) the green leaf area index (note log scale on y axis) of the main shoot at harvests 1–5 and (B) the effect of elevated O₃ on the green leaf area ratio (*GLAR*) of the main shoot for the oz and chAA control treatments at harvests 1–6. Vertical bars denote single SEDs for (1) comparing individual treatments at each harvest and (2) comparing all treatments and harvests, with (A) 12 and 126 d.f. and (B) 12 and 155 d.f., respectively.

leaf area of the main shoot was reflected by a decrease in *GLAR* ($P < 0.001$; Fig. 3B), suggesting that exposure to ozone promoted premature leaf senescence.

At anthesis, main stem dry weight was significantly reduced in the oz treatment ($P < 0.05$; Table 3), possibly due in part to reductions in plant height (non-significant). Ear dry weight was also decreased in oz relative to chAA ($P < 0.05$; Table 3) and premature leaf senescence was observed during early vegetative growth (up to leaves 6–7). In contrast, the DW and *SLA* of the flag leaf (in general leaf 8 on the main shoot) were not significantly affected by ozone (Table 3). These observations are supported by the evidence that *GLAR* in the oz treatment stabilized around the time when the flag leaf reached full

expansion (Fig. 3B) and subsequent senescence occurred at a rate similar to the chAA control from 91 DAS onwards. However, despite the occurrence of considerable leaf damage during early vegetative growth in oz, grain yield was not significantly affected at final harvest (Table 4; Fig. 4). Detailed examination of tiller survival throughout the season revealed that the proportion of tillers which survived to produce ears was significantly greater in oz than in the chAA treatment (54% versus 26%; $P < 0.01$; Fig. 5), and that the absolute number of ear-bearing tillers was also increased ($P < 0.1$; data not shown). It is clear from Fig. 4 that the main shoots of chAA plants out-yielded those in the oz treatment by 14% when expressed on a land area basis, largely due to

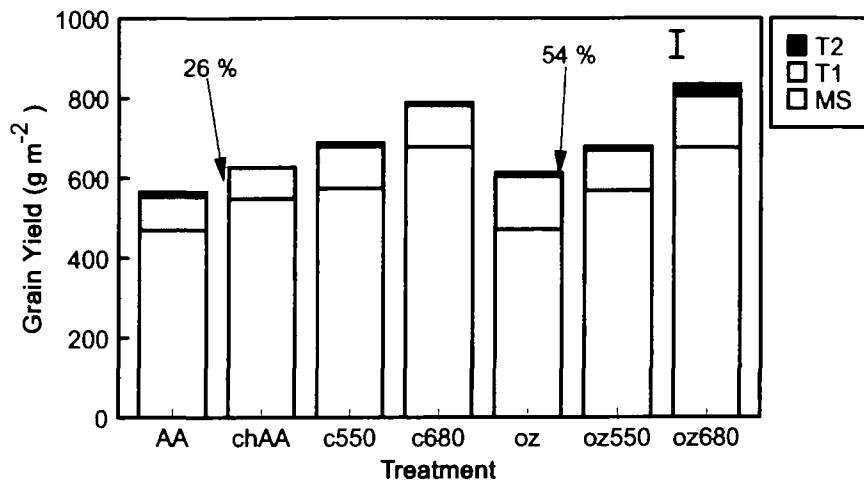


Fig. 4. Effect of elevated CO_2 and O_3 on the grain yield contributions of the main shoot and tillers 1 and 2. 26% and 54% represent the percentage tiller survival values for the chambered ambient control (chAA) and the oz elevated O_3 treatment, respectively. Vertical bar denotes one SED with 12 d.f.

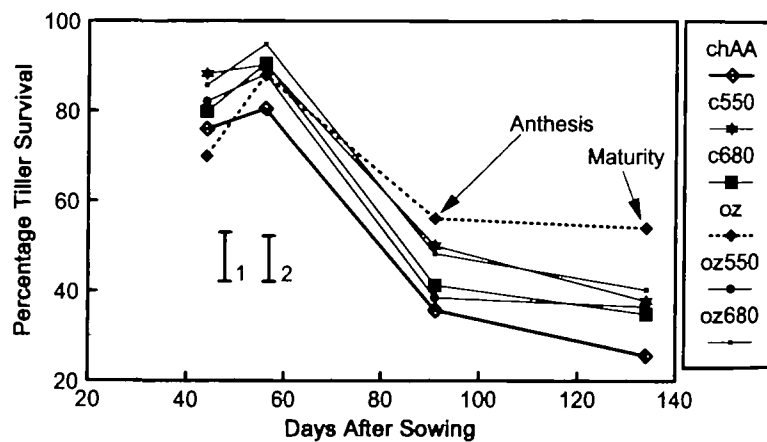


Fig. 5. Effect of elevated CO_2 and O_3 on seasonal time-courses of percentage tiller survival for harvests 1, 2, 4, and 6. Vertical bars denote single SEDs for (1) comparing individual treatments at each harvest and (2) comparing all treatments and harvests, with 12 and 99 d.f., respectively.

the lower number of main shoots in the latter treatment. However, the increased contributions of tillers 1 and 2 (Fig. 4) resulted in total grain yield being similar to the chAA control (-2.5%). It was difficult to determine whether the lower plant population in oz resulted from increased seedling mortality, since the plants were exposed from emergence and the first counts of seedling density were made at harvest one, three weeks after emergence. However, the measurements made at each harvest (1–6) showed that mean plant density in oz was consistently slightly lower (358 m^{-2} ; non-significant) than in the chAA control (371 m^{-2}), suggesting that some seedling mortality may have occurred during the first 3 weeks of O_3 exposure. Examination of grain yield and its components for the main shoot showed that, as in the elevated CO_2 treatments, the numbers of grains per spikelet and per ear were increased in the oz treatment relative to the chAA control ($P < 0.05$; Table 4).

Elevated $\text{CO}_2 \times \text{O}_3$ interactions

O_3 had a particularly damaging effect on *LAI* and *GLAR* during early vegetative growth under ambient CO_2 conditions in the oz treatment (Fig. 3A, B). However, both variables were restored almost to the control levels in the elevated CO_2 treatments (oz550 and oz680), suggesting that elevated CO_2 protected the leaves against O_3 damage ($P < 0.001$). Tillering was also affected during the post-anthesis period, since the numbers of ear-bearing tiller 1 shoots present at maturity in the oz550 and oz680 treatments were significantly lower than in the ambient CO_2 oz treatment ($P < 0.05$; data not shown), but were similar to those in the corresponding elevated CO_2 , ambient O_3 treatments (c550 and c680). Greater seedling survival may have been a contributory factor to the lower numbers of ear-bearing tiller 1 shoots in the oz550 and oz680 treatments, in which mean plant densities at maturity

were 376 and 383 m⁻², respectively, as compared to 343 m⁻² in the oz treatment.

Chambered versus unchambered plots

Plants in the unchambered ambient air treatment (AA) had a lower stem biomass at anthesis ($P < 0.05$), partly due to a reduction in plant height ($P < 0.001$; Table 4). Flag leaf characteristics, grain numbers per ear and per spikelet, and individual grain weight in the main shoot were all unaffected (Tables 3, 4), although ear DW was lower in AA than in the OTC treatments ($P < 0.05$; Table 3). Total grain yield was also lower in AA than in the OTC treatments (not significant), possibly contributing to the significantly lower harvest index in the former treatment ($P < 0.05$; Table 4).

Discussion

Season-long exposure of Minaret to elevated atmospheric CO₂ concentrations of 550 and 680 ppm produced a linear increase in biomass accumulation. Stem biomass and plant height were greatly increased by elevated CO₂, in agreement with previous studies (Weigel *et al.*, 1994; Fangmeier *et al.*, 1996), but ear biomass, although significantly increased at anthesis, did not exhibit the dramatic increases reported for Minaret by Fangmeier *et al.* (1996). Main shoot *LAI* was increased by elevated CO₂, primarily due to an increase in green leaf area duration rather than increases in leaf area *per se*. The uniformity of leaf area and number up to the flag leaf irrespective of CO₂ treatment is consistent with the findings of Fangmeier *et al.* (1996), but contrasts with other studies in which decreases in leaf area (Hocking and Meyer, 1991; Weigel *et al.*, 1994) and number were found as CO₂ concentration increased (Weigel *et al.*, 1994). These data show that flag leaf area was unaffected by any of the treatments imposed, whereas Fangmeier *et al.* (1996) reported large increases in flag leaf area and weight for Minaret under CO₂ enrichment.

The increased yield in the elevated CO₂ treatments resulted from changes in a number of growth variables, a key factor being an increase in the number of ear-bearing tillers, which agrees with many other studies of wheat (Gifford, 1977, 1979; Weigel *et al.*, 1994). Other yield components varied in their response; for example, individual grain weight was unaffected (Table 4), but grain number per ear was increased, exhibiting a non-linear response to CO₂ enrichment and reaching a maximum of 41 grains per ear at 550 ppm CO₂ (oz550) as compared to 35 in the chAA control. The concurrent increase in the number of grains per spikelet from 1.8 in chAA to 2.0 in the elevated CO₂ treatments substantiates previous observations (Havelka *et al.*, 1984; Weigel *et al.*, 1994). Elevated CO₂ may increase photosynthesis (Idso

and Idso, 1994), thereby increasing the carbohydrate pools of leaves and stems (Smart *et al.*, 1994), and has its greatest effect during vegetative and reproductive development (Lawlor and Mitchell, 1991), when the additional carbohydrates produced in CO₂-enriched plants may well determine the developmental pattern of the meristematic tissues. For example, an abundance of assimilate may allow young tillers and grain primordia to survive (Lawlor and Mitchell, 1991), thereby providing an explanation for the increased tiller survival and grain number per ear observed in the present study. However, the relative insensitivity of grain size to improved assimilate availability in the stem and ear tissue during grain filling suggests that there is a genetically determined upper limit which is unaffected in CO₂-enriched plants.

The relationship between grain yield and increasing atmospheric CO₂ concentration between the chambered ambient and 680 ppm treatments was strongly linear, in contrast to the non-linear responses observed in two other spring wheat cultivars (cvs Star and Turbo) which exhibited maximal increases at 550 ppm (Weigel *et al.*, 1994). Harvest index was stable, in agreement with previous work (Goudriaan and de Ruiter, 1983; Sionit *et al.*, 1980), indicating that stem, leaf and ear biomass increased proportionately in response to elevated CO₂.

Although exposure to O₃ under ambient CO₂ conditions induced substantial foliar damage during early vegetative growth (Fig. 3A, B), there was little effect on grain yield. Leaf damage occurred almost exclusively to the main shoot and altered canopy structure relative to the chAA control plants. The consequent production of a more 'open' canopy due to premature senescence of leaves on the main shoot and the possible mortality of some seedlings in the oz treatment may have promoted tiller growth (Fig. 5), thereby resulting in a similar total *LAI* to that of control plants (data not shown). Thus the elevated O₃ treatment, oz, possessed a greater number of ear-bearing tillers 1 and 2 at final harvest than the chAA control, with the result that grain yield was not significantly reduced. Grain numbers per ear and per spikelet were higher in oz, to an extent similar to that observed in the elevated CO₂ treatments, in agreement with the results of Fangmeier *et al.* (1996). The absence of effects on flag leaf weight, *GLAR* and *SLA* in oz may have been crucial to the ability of plants in this treatment to produce grain yields similar to the chAA control. Visible foliar injury by O₃ was suppressed in the elevated CO₂ treatments, possibly because reductions in stomatal conductance decreased the flux of O₃ into the leaves (Allen, 1990) and/or the production of antioxidants was increased (Rao *et al.*, 1995). The reduced foliar damage to the main shoot under conditions of elevated CO₂ and O₃ was reflected by the observation that tiller 1 numbers at maturity were lower than in the corresponding oz treatment.

Yield in spring wheat has generally been shown to be decreased by prolonged exposure (7–8 h d⁻¹) to O₃ concentrations of around 60 ppb (Miller, 1993; Skärby *et al.*, 1993). However, in the present study, Minaret proved to be relatively insensitive to a 7 h d⁻¹ seasonal mean of 60 ppb O₃, and exhibited a non-significant decrease in grain yield (–2.5%). Although the mechanisms underlying the effects of ozone on grain growth are not clearly understood, it is known that the sink capacity of wheat grain is determined by the number of endosperm cells present (Brocklehurst, 1977), and that the meristematic activity of the endosperm continues for *c.* 2 weeks after anthesis (Brocklehurst, 1977). The ozone climate over the immediate post-anthesis period and during grain filling may, therefore, be important in affecting the sink capacity of the grain and hence final grain yield. As the O₃ concentration applied was proportionately increased above ambient in the present study, the effects on grain set, filling and yield might well vary from year to year depending on the prevailing O₃ climate, especially in a relatively insensitive cultivar such as Minaret. The period between flag leaf emergence (66 DAS), full expansion (73 DAS) and anthesis (91 DAS) was characterized by frequent relatively low daily mean concentrations (Fig. 1B). The daily 7 h mean for the elevated O₃ treatment during this period was 46 ppb, well below the 60 ppb seasonal average. However, during the 2 week period following anthesis, when meristematic activity in the endosperm is at its most intense (Brocklehurst, 1977), the corresponding daily mean O₃ concentration was 74 ppb, and from then until maturity (134 DAS) was 60 ppb. The flag leaves therefore developed and functioned under relatively low O₃ concentrations up to anthesis, after which concentrations were higher, and by approximately two weeks after anthesis had begun to senesce in all treatments (Fig. 3B). These observations suggest that the flag leaf of Minaret may be less sensitive to O₃ than the earlier leaves since, although it developed under relatively low O₃ concentrations, there was little evidence that the higher post-anthesis levels promoted visible damage or premature senescence. Grain yield might have been indirectly reduced by lowered assimilate production and/or partitioning if the flag leaf had been damaged prior to anthesis in the elevated O₃ treatment. However, flag leaf characteristics were not detectably affected, suggesting that grain filling may have proceeded unimpaired, contributing to the observed recovery of O₃-treated (oz) plants from the injury sustained during earlier vegetative growth (Fig. 3A, B). Certainly, there is evidence that injury to the older leaves may be compensated for by increased photosynthetic rates in young or expanding leaves (Heath, 1994), in this case the flag leaf. This may have been an important aspect of the observed recovery from O₃-induced damage since kernel growth rate depends on a combination of current photosynthesis by the flag

leaf and translocation of previously fixed carbon to the sink tissues (grains) for storage (Bruckner and Froberg, 1987); increased photosynthetic activity of the flag leaves may provide a partial explanation for the observed increase in grain number in ozone-treated plants. Ozone exposure regimes around anthesis may therefore be of particular importance in affecting grain yield, an hypothesis supported by the observation that a relatively short exposure to *c.* 120 ppb for 4 h d⁻¹ for 5 d at anthesis induced significant detrimental effects on harvest index and grain yield in wheat (Mulchi *et al.*, 1986).

Conclusions

Minaret exhibited a positive linear growth response to elevated atmospheric CO₂ and was able to compensate for O₃-induced damage sustained by the main shoots under ambient CO₂ conditions, primarily through increased tiller survival. Plants grown under elevated CO₂ were protected against O₃-induced injury. Further studies of the photosynthetic responses of flag leaves before and after full expansion are required to elucidate possible mechanisms of damage and recovery during grain filling. The results suggest that the flag leaves of Minaret may be relatively insensitive to O₃ at the exposure levels applied in view of the substantial visible injury caused to the earlier leaves 1–7.

Acknowledgements

We thank Vanessa Mitchinson, Heather France, Emma Roberts, John Travers, and Paul Nichols for their invaluable technical assistance. This work was funded by CEC-Contract No. EV5V-CT93-0301; ESPACE-Wheat.

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