

# Growth, light interception and yield responses of spring wheat (*Triticum aestivum* L.) grown under elevated CO<sub>2</sub> and O<sub>3</sub> in open-top chambers

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## Abstract

Spring wheat cv. Minaret was grown to maturity under three carbon dioxide (CO<sub>2</sub>) and two ozone (O<sub>3</sub>) concentrations in open-top chambers (OTC). Green leaf area index (LAI) was increased by elevated CO<sub>2</sub> under ambient O<sub>3</sub> conditions as a direct result of increases in tillering, rather than individual leaf areas. Yellow LAI was also greater in the 550 and 680 µmol mol<sup>-1</sup> CO<sub>2</sub> treatments than in the chambered ambient control; individual leaves on the main shoot senesced more rapidly under 550 µmol mol<sup>-1</sup> CO<sub>2</sub>, but senescence was delayed at 680 µmol mol<sup>-1</sup> CO<sub>2</sub>. Fractional light interception (*f*) during the vegetative period was up to 26% greater under 680 µmol mol<sup>-1</sup> CO<sub>2</sub> than in the control treatment, but seasonal accumulated intercepted radiation was only increased by 8%. As a result of greater carbon assimilation during canopy development, plants grown under elevated CO<sub>2</sub> were taller at anthesis and stem and ear biomass were 27 and 16% greater than in control plants. At maturity, yield was 30% greater in the 680 µmol mol<sup>-1</sup> CO<sub>2</sub> treatment, due to a combination of increases in the number of ears per m<sup>-2</sup>, grain number per ear and individual grain weight (IGW).

Exposure to a seasonal mean (7 h d<sup>-1</sup>) of 84 nmol mol<sup>-1</sup> O<sub>3</sub> under ambient CO<sub>2</sub> decreased green LAI and increased yellow LAI, thereby reducing both *f* and accumulated intercepted radiation by ~16%. Individual leaves senesced completely 7–28 days earlier than in control plants. At anthesis, the plants were shorter than controls and exhibited reductions in stem and ear biomass of 15 and 23%. Grain yield at maturity was decreased by 30% due to a combination of reductions in ear number m<sup>-2</sup>, the numbers of grains per spikelet and per ear and IGW. The presence of elevated CO<sub>2</sub> reduced the rate of O<sub>3</sub>-induced leaf senescence and resulted in the maintenance of a higher green LAI during vegetative growth under ambient CO<sub>2</sub> conditions. Grain yields at maturity were nevertheless lower than those obtained in the corresponding elevated CO<sub>2</sub> treatments in the absence of elevated O<sub>3</sub>. Thus, although the presence of elevated CO<sub>2</sub> reduced the damaging impact of ozone on radiation interception and vegetative growth, substantial yield losses were nevertheless induced. These data suggest that spring wheat may be susceptible to O<sub>3</sub>-induced injury during anthesis irrespective of the atmospheric CO<sub>2</sub> concentration. Possible deleterious mechanisms operating through effects on pollen viability, seed set and the duration of grain filling are discussed.

**Keywords:** CO<sub>2</sub>, growth, grain yield, O<sub>3</sub>, *Triticum aestivum*

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## Introduction

Atmospheric carbon dioxide (CO<sub>2</sub>) concentration is expected to increase substantially by the end of the next century (IPCC 1996) and the importance of ozone (O<sub>3</sub>)

as a pollutant is also increasing in Europe, North America and the urban/industrial areas of developing nations (Chameides *et al.* 1994). CO<sub>2</sub> (Bowes, 1993) and O<sub>3</sub> (Krupa & Kickert 1989) are both known to have direct impacts on plant physiology and crop production. Increasing atmospheric CO<sub>2</sub> concentration above current ambient levels can stimulate photosynthesis (Long *et al.* 1993) and

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there is little doubt that crop yields are increased at CO<sub>2</sub> concentrations around 700  $\mu\text{mol mol}^{-1}$  (Idso & Idso 1994). In contrast, O<sub>3</sub> is considered to be one of the most phytotoxic air pollutants, and the greater incidence of chronic O<sub>3</sub> episodes is expected to increase losses in crop yield in many industrially or agriculturally developed areas (Chameides *et al.* 1994). Current ambient concentrations of O<sub>3</sub> in Europe are sufficiently high to depress grain yield in spring wheat (Skärby *et al.* 1993), and comparison of open-top chamber (OTC) experiments in Europe and the USA has shown that European spring cultivars are more sensitive than North American winter cultivars when exposed to a 7–8 h seasonal mean of 60–120  $\text{nmol mol}^{-1}$  O<sub>3</sub> (Miller 1993). OTC experiments have also shown that the grain yield of barley and oats is less sensitive than that of spring wheat (Skärby *et al.* 1993). However, the losses resulting from the current O<sub>3</sub> climate may be modified by the growth stimulation promoted in spring wheat by elevated atmospheric CO<sub>2</sub> (Hertstein *et al.* 1995). Although there have been many studies of the individual effects of CO<sub>2</sub> (Idso & Idso 1994) and O<sub>3</sub> (Krupa *et al.* 1994), relatively few have examined the interactive effects of elevated CO<sub>2</sub> and O<sub>3</sub>, and in most of these have been largely confined to the vegetative phase (Balaguer *et al.* 1995; McKee *et al.* 1995; Rao *et al.* 1995). However, detailed information on the interactive effects on wheat yields of season-long exposure to these greenhouse gases is now beginning to emerge (Fangmeier *et al.* 1996; Mulholland *et al.* 1997).

Although there is good evidence that exposure to O<sub>3</sub> may reduce yield in spring wheat (Skärby *et al.* 1993), few field experiments have attempted to elucidate the mechanisms involved (Unsworth *et al.* 1984). The quantity of radiation intercepted by vegetative canopies is known to be a major determinant of growth and yield, and biomass production is closely related to accumulated seasonal radiation interception in wheat (Gallagher & Biscoe 1978) and other crop species. Accelerated leaf senescence induced by exposure to O<sub>3</sub> (Krupa & Manning 1988) may therefore be a major factor in reducing light interception, assimilate production and hence grain yield. This paper describes results obtained during the second year of an OTC study to examine the impact of elevated atmospheric CO<sub>2</sub> and/or O<sub>3</sub> on spring wheat. In the preceding year, biomass and grain yield at maturity were increased by season-long exposure to elevated CO<sub>2</sub>, but were not significantly affected by the ozone treatments applied (Mulholland *et al.* 1997). In the present study, a higher O<sub>3</sub> concentration was applied (84 vs. 60  $\text{nmol mol}^{-1}$  O<sub>3</sub>) in order to: (i) establish the individual and interactive effects on growth, canopy development, radiation interception and yield of season-long exposure of spring wheat cv. Minaret to three levels of CO<sub>2</sub> and two

levels of ozone in open-top chambers and (ii) to examine the mechanisms responsible for the observed responses.

## Materials and methods

### Experimental design

A factorial design of 18 OTCs containing three CO<sub>2</sub> and two O<sub>3</sub> treatments plus an unchambered ambient air treatment, randomized in three blocks, was used; each treatment was replicated three times. Elevated atmospheric CO<sub>2</sub> was maintained for 24 h d<sup>-1</sup> and O<sub>3</sub> was applied for 7 h d<sup>-1</sup> for 5 d weeks<sup>-1</sup> (0900–1600 GMT) in the elevated O<sub>3</sub> plots. The treatments imposed included unchambered ambient air field plots (AA), ambient air OTC control plots (chAA), elevated CO<sub>2</sub> OTCs at 550 and 680  $\mu\text{mol mol}^{-1}$  (c550 and c680) and elevated O<sub>3</sub> OTC treatments (84  $\text{nmol mol}^{-1}$  ozone) under ambient (oz), 550 (oz550) and 680  $\mu\text{mol mol}^{-1}$  CO<sub>2</sub> (oz680).

### Site preparation, open-top chambers and gas exposure

The experiment was situated at the University of Nottingham, Sutton Bonington Campus, Nottingham, UK (lat. 52°N, long. 1°15' W). The experimental area (160 × 40 m; 6400 m<sup>2</sup> ground area) was located on a sandy loam of the Astley Hall series, and was cultivated prior to sowing using standard agricultural practices as part of the normal crop rotation. Seed of cv. Minaret was sown on 20 March 1996 using a Nordston drill at a rate of 173 kg ha<sup>-1</sup> to give a mean population of 347 plants m<sup>-2</sup>. Ozone was generated from oxygen (O<sub>2</sub>) by electrical discharge (Model LN103, Ozonia, Switzerland) and supplied to the elevated O<sub>3</sub> treatment chambers to provide a concentration five times the ambient up to a maximum of 170  $\text{nmol mol}^{-1}$ , above which acute injury might have been induced. CO<sub>2</sub> was supplied from a 25 t storage tank with a vaporization facility capable of producing up to 50 kg h<sup>-1</sup> at a working pressure of 2 kg cm<sup>-2</sup> (Hydrogas, Middlesex, UK), and was distributed using a manually controlled 12 valve manifold. Gas treatments were applied from seedling emergence to maturity. Gas concentrations were measured in eight individual plots which included one replicate of each treatment plus a roving line which was used daily to crosscheck concentrations in those chambers which were not continuously monitored. A series of eight pc-controlled solenoid valves were used to sample air from individual plots within a 220-s period, enabling a full cycle of all measured plots to be completed within a 30-min period. A 2-m long perforated sampling tube located horizontally 1.2 m above ground level was used to ensure that representative air samples were obtained. Full details of OTC design and gas analysis are provided by Mulholland *et al.* (1997).

### Crop management

The sown crop was sprayed with the pre-emergence herbicide 'Flexidor' (Dow Elanco, UK) at a rate of 0.25 L ha<sup>-1</sup> and allowed to germinate and emerge under ambient conditions; irrigation was applied on a weekly basis throughout the growing season to maintain a soil water deficit of 20 mm (Mulholland *et al.* 1997). At the terminal spikelet developmental stage, 75 kg N ha<sup>-1</sup> was applied as a compound NPK fertilizer to all plots. The crop was treated for powdery mildew on 30 June 1996, using Bayleton (Bayer, Germany) at a rate of 0.04 L m<sup>-2</sup>, and sprayed for aphids with 'Rapid' (ICI, UK) at a rate of 0.01 L m<sup>-2</sup> on 1 July 1996. Powdery mildew was not observed in the elevated O<sub>3</sub> or open field plots, but limited infection occurred in the elevated CO<sub>2</sub> treatments; powdery mildew and aphids were both successfully controlled by the measures applied.

### Meteorological measurements

Aspirated psychrometers containing two copper constantan thermocouples were used to measure wet and dry bulb temperatures 1.2 m above the ground level in two replicate plots of each treatment. Incident, reflected and transmitted shortwave radiation was measured in the centre of each replicate plot using 1-m long tube solarimeters (constructed after Green & Deuchar 1985). Data were logged for all instruments at 15 s intervals using Campbell 21X dataloggers (Campbell Scientific, Shepshed, UK), from which hourly means were calculated and stored. Intercepted radiation was calculated by subtracting transmitted and reflected radiation from the incident value; the radiation conversion coefficient ( $\epsilon_s$ ; g MJ<sup>-1</sup>) between 53 and 141 days after sowing (DAS) (maturity) was calculated as described in Unsworth *et al.* (1984).

### Leaf production, senescence and growth analysis

At emergence, five randomly selected plants within each experimental plot were tagged; leaf counts were then made at weekly intervals throughout the season to determine the time courses of leaf production. The same leaves were used for visual estimates of the fractional green leaf area (FGLA) (estimated in 0.05 steps where 1 = totally green leaves) to provide a measure of leaf senescence for individual leaves.

Destructive harvests were carried out at the following times: harvest 1, 62 DAS (terminal spikelet as defined by Tottman & Broad 1987); harvest 2, 83 DAS; harvest 3, 97 DAS (anthesis; defined as occurring when 50% of the plants had anthers exerted from the florets); harvest 4, 141 DAS (maturity; defined as when the caryopsis was

hard (not dented by thumbnail)). All plants from three randomly selected 25 cm row lengths at harvests 1–3 and from six 25 cm row lengths at harvest 4 (10 cm spacing between rows), and their numbers and heights were recorded. The plants were then separated into main shoots and tillers and further subdivided into leaves (green and yellow), stems and ears. The projected areas of the leaves, stems and ears were measured using a leaf area meter (Model LI-3000 A, LI-COR, Lincoln, Nebraska, USA) before drying at 80 °C for 48 h and weighing. At final harvest, the ears were dried as described above before carefully removing the seed 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 CO<sub>2</sub> × two O<sub>3</sub> treatment structure plus an unchambered ambient plot, the treatment sum of squares was partitioned into a chambered vs. unchambered contrast and then into the three CO<sub>2</sub> × two O<sub>3</sub> factorial within the chambered treatments. Split plot analyses were used to compare results from different harvests, with the treatments being considered as seven levels of the main plot factor and the harvests as levels of the subplot factor. Thus both between-chamber and within-chamber errors are presented. The CO<sub>2</sub> effects were partitioned into linear and quadratic components to test for dose response; significant linear trends are prefixed by 'Linear'.

## Results

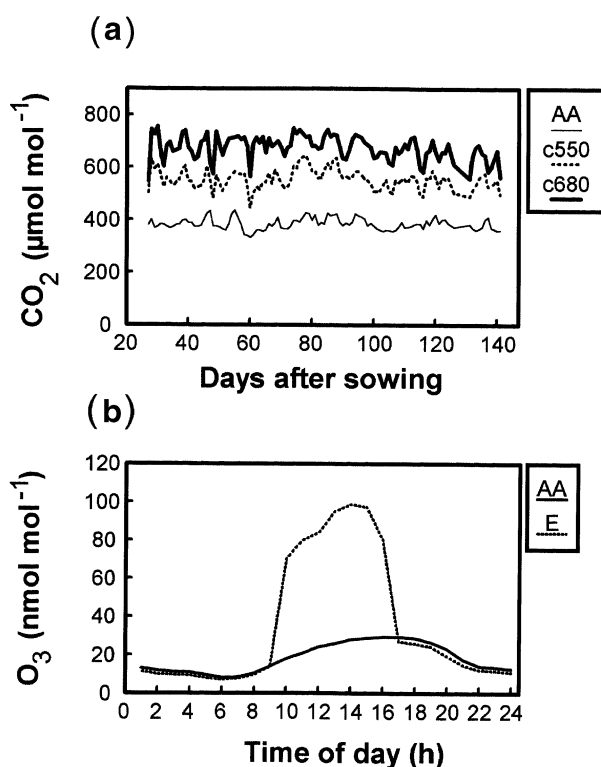
### Microclimatic conditions and gas exposure levels

Compared to the open field, environmental conditions within the OTCs were characterized by slightly higher daily mean (11.8 vs. 13.0 ± 0.022 °C) and maximum temperatures (16.9 vs. 19.2 ± 0.15 °C) and slightly lower saturation vapour pressure deficits (svpd; 0.64 vs. 0.78 ± 0.016 kPa) for ambient and chambered plots, respectively. Cumulative short wave radiation receipts between crop emergence and maturity (22–141 DAS) were 1493.5 and 1214.8 ± 11 MJ m<sup>-2</sup> for ambient and chambered plots, respectively, representing a reduction of 19% in incident radiation resulting from the OTC structure and covers.

The gas concentrations achieved in the various treatments are summarized in Table 1. Although some day-to-day variation in CO<sub>2</sub> concentrations within the OTCs was observed depending on windspeed and direction, the seasonal mean CO<sub>2</sub> concentrations were within 3%

**Table 1** Seasonal mean concentrations of O<sub>3</sub> (7 h mean) and CO<sub>2</sub> (24 h mean) in 1996. Ozone was applied for 7 h d<sup>-1</sup> and 5 d week<sup>-1</sup>; but mean values were calculated over all days in order to express the data as a seasonal average. The accumulated O<sub>3</sub> exposure over a threshold concentration of 40 nmol mol<sup>-1</sup> (AOT (40)) was calculated as the number of nmol mol<sup>-1</sup> h of ozone between astronomical sunrise and sunset between 16 April and 8 August 1996

Variable	Treatment						
	AA	chAA	c550	c680	oz	oz550	oz680
O <sub>3</sub> 7 h mean (nmol mol <sup>-1</sup> )	29	26	27	27	84	84	84
AOT (40) (nmol mol <sup>-1</sup> h)	2545	1887	1879	1931	35498	35695	35709
CO <sub>2</sub> 24 h mean (μmol mol <sup>-1</sup> )	385	384	541	668	386	566	682

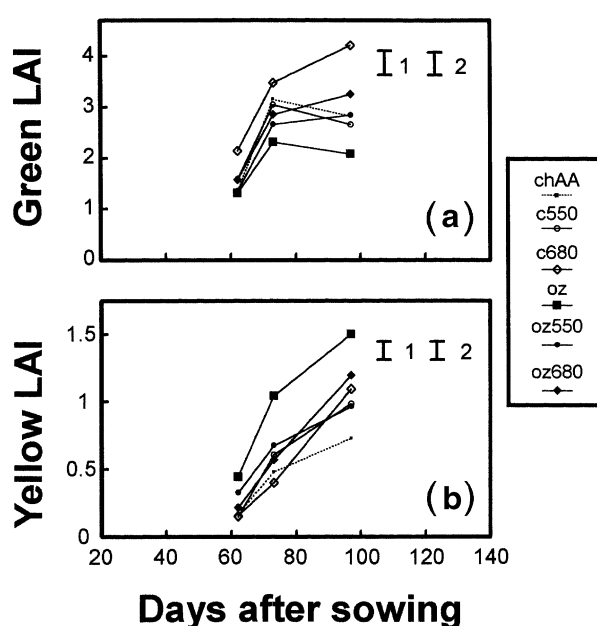


**Fig. 1** Daily mean (a) CO<sub>2</sub> concentrations (24 h d<sup>-1</sup>) for the ambient air (AA), 550 (c550) and 680 μmol mol<sup>-1</sup> (c680) OTC treatments, and (b) mean diurnal time courses of O<sub>3</sub> concentration for the ambient air (AA) and elevated O<sub>3</sub> (E) OTC treatments in 1996

of the target levels (Fig. 1a and Table 1), while the elevated ozone treatment provided a seasonal mean 7 h d<sup>-1</sup> exposure averaged over all days of 84 nmol mol<sup>-1</sup> as compared to 26–29 nmol mol<sup>-1</sup> in the ambient O<sub>3</sub> treatment (Fig. 1b and Table 1).

#### Light use and canopy development

**CO<sub>2</sub> effects.** Green leaf area index (LAI) was increased by elevated CO<sub>2</sub> by up to 49% relative to the chAA control at anthesis (97 DAS) in the c680 treatment (Lin;



**Fig. 2** Effect of season-long exposure to elevated CO<sub>2</sub> and O<sub>3</sub> on (a) green leaf area index (LAI) and (b) yellow LAI for harvests 1–3. Note the differing scales on the y axes. Vertical bars denote single standard errors of the difference (SEDs) for comparing (1) individual treatments at each harvest and (2) all treatments and harvests, with 12 and 42 degrees of freedom (d.f.), respectively

$P < 0.05$ ; Fig. 2a). Although the area of individual leaves was unaffected by CO<sub>2</sub> (data not shown), their green leaf area duration was increased in the c680 treatment ( $P < 0.001$ ; Fig. 3a–d); this effect, coupled with a larger number of tillers produced ( $P < 0.05$ ; cf. Table 3), was responsible for the observed increases in green LAI. Although the onset of visible leaf senescence was delayed in the c680 treatment, individual leaves began to senesce slightly earlier in the c550 treatment than in the chAA control (Fig. 3a – d; nonsignificant); this effect was detectable at harvests 2 and 3 by a slight reduction in green LAI (Fig. 2a; nonsignificant). Concurrent increases were also observed in yellow LAI in the c550 and c680 treatments (Lin;  $P < 0.05$ ; Fig. 2b).

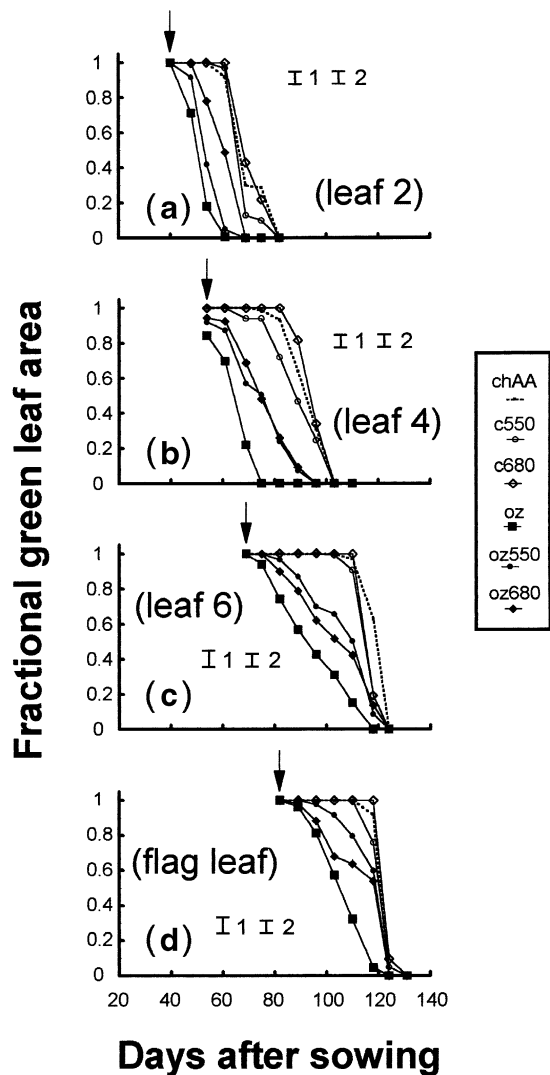


Fig. 3 Effect of season-long exposure to elevated CO<sub>2</sub> and O<sub>3</sub> on the fractional green leaf area (FGLA) of (a) leaf 2, (b) leaf 4, (c) leaf 6, and (d) leaf 8 (flag). Arrows mark the timing of leaf full expansion and vertical bars indicate SEDs for comparing (1) individual treatments at each harvest and (2) all treatments and harvests with (a) 12 and 98, (b) 12 and 126, (c) 12 and 126, and (d) 12 and 112 d.f. for leaves 2, 4, 6 and 8, respectively

The substantial increase in green LAI in the c680 treatment between 53 DAS and anthesis (89 DAS) was accompanied by a 26% increase in fractional light interception of radiation ( $f$ ) relative to the chAA control (Fig. 4a). However, total seasonal interception of radiation was only 0.3 and 8% higher in the c550 and c680 treatments than in the chAA control (Fig. 4b). The conversion coefficient for intercepted radiation ( $\epsilon_s$ ) between 53 and 141 DAS was 3 and 15% higher in the c550 (3.4 g MJ<sup>-1</sup>;  $r = 0.98$ ;  $P < 0.001$ ) and c680 (3.9 g MJ<sup>-1</sup>;  $r = 0.99$ ;  $P < 0.001$ ) treatments than in the chAA control (3.3 g MJ<sup>-1</sup>;  $r = 0.96$ ;  $P < 0.001$ ).

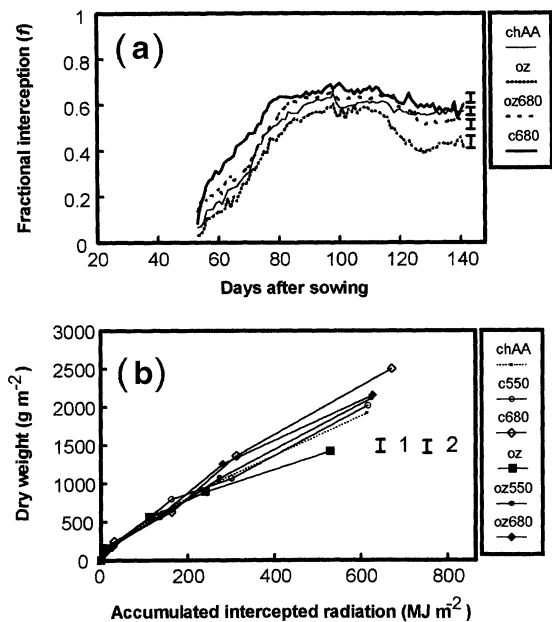


Fig. 4 Effect of season-long exposure to elevated CO<sub>2</sub> and O<sub>3</sub> on (a) fractional light interception and (b) the relationship between total dry weight and accumulated intercepted radiation. Vertical bars in (a) indicate standard errors, with 87 d.f. and (b) SEDs for comparing (1) individual treatments for each harvest and (2) all treatments and harvests with 12 and 56 d.f.

**O<sub>3</sub> effects under ambient atmospheric CO<sub>2</sub> concentrations.** Significant canopy damage was observed in the oz treatment, whereby green LAI was decreased by up to 27% at harvests 2 and 3, and yellow LAI was more than doubled relative to the chAA control (Fig. 2a,b;  $P < 0.001$ ). This was reflected by a marked decrease in the FGLA of individual leaves, which exhibited visible damage at or soon after (6–7 days) achieving full expansion (Fig. 3a–d;  $P < 0.001$ ); as a result, leaves in the oz treatment senesced completely 7–28 days earlier than in the chAA control. The extensive damage caused to all leaves decreased mean fractional light interception between 53 and 89 (anthesis) DAS by 17% (Fig. 4a) and cumulative seasonal interception by 16% relative to the chAA control (Fig. 4b). The mean  $\epsilon_s$ -value for this period was decreased by 12% from 3.3 in the chAA control to 2.9 g MJ<sup>-1</sup> in the oz treatment ( $r = 0.97$ ;  $P < 0.001$ ).

**CO<sub>2</sub> × O<sub>3</sub> interactions.** The influence of O<sub>3</sub> in increasing yellow LAI was reduced by the presence of elevated CO<sub>2</sub> in the oz550 and oz680 treatments ( $P < 0.001$ ; Fig. 2b). These effects were substantiated by the observation that FGLA was greater in the oz550 and oz680 treatments than in oz for leaves 2, 4, 6 and the flag leaf (Fig. 3a–d); senescence, as indicated by the lower FGLA values, was nevertheless greater than in the corresponding c550

**Table 2** Effects of CO<sub>2</sub> and O<sub>3</sub> on growth characteristics 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 nonsignificant

Variable	Treatment							ANOVA					
	AA	chAA	c550	c680	oz	oz550	oz680	ch	CO <sub>2</sub>	O <sub>3</sub>	CO <sub>2</sub> ×O <sub>3</sub>	SED	d.f.
Stem DW (g m <sup>-2</sup> )	653.9	619.0	638.0	847.0	526.0	671.0	772.0	NS	<i>P</i> < 0.001	NS	NS	48.7	12
Flag leaf SLA <sup>a</sup> (cm <sup>2</sup> g <sup>-1</sup> )	197.1	183.5	204.0	202.2	209.4	193.8	195.7	NS	NS	NS	NS	11.4	12
Ear DW <sup>b</sup> (g m <sup>-2</sup> )	168.8	246.2	256.9	294.0	188.7	233.1	273.4	<i>P</i> < 0.001	<i>P</i> < 0.001	<i>P</i> < 0.05	NS	20.9	12
Plant height (cm)	88.9	94.2	95.3	102.4	90.3	97.1	99.3	<i>P</i> < 0.05	<i>P</i> < 0.001	<i>P</i> < 0.05	NS	2.4	12

<sup>a</sup>, specific leaf area of the main shoot flag leaf and <sup>b</sup>, dry weight.

**Table 3** Effects of CO<sub>2</sub> and O<sub>3</sub> on 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 nonsignificant

Variable	Treatment							ANOVA					
	AA	chAA	c550	c680	oz	oz550	oz680	ch	CO <sub>2</sub>	O <sub>3</sub>	CO <sub>2</sub> ×O <sub>3</sub>	SED	d.f.
Stand ears m <sup>-2</sup>	733	635	697	750	582	726	733	NS	<i>P</i> < 0.05	NS	NS	69.80	12
MS <sup>a</sup> Grains per ear	44.6	46.0	49.9	53.5	43.2	49.1	48.6	<i>P</i> < 0.1	<i>P</i> < 0.01	<i>P</i> < 0.05	NS	2.30	12
MS Grains per spikelet	2.1	2.2	2.4	2.5	2.0	2.3	2.3	NS	<i>P</i> < 0.01	<i>P</i> < 0.05	NS	0.11	12
MS IGW <sup>b</sup> (mg)	37.5	38.2	39.1	40.9	33.8	38.9	38.5	NS	<i>P</i> < 0.01	<i>P</i> < 0.05	NS	1.59	12
Stand grain DW(g m <sup>-2</sup> )	922	918	995	1227	644	969	1007	NS	<i>P</i> < 0.01	<i>P</i> < 0.01	NS	128.40	12
Stand HI <sup>c</sup>	0.41	0.48	0.49	0.49	0.45	0.46	0.47	<i>P</i> < 0.001	NS	<i>P</i> < 0.01	NS	0.01	12

<sup>a</sup>, Main shoot; <sup>b</sup>, individual grain weight and <sup>c</sup>, harvest index.

and c680 treatments (*P* < 0.05). This conservation of green leaf area by elevated CO<sub>2</sub> probably contributed to the observed 2% increase in *f* and 9% increase in *ε<sub>s</sub>* (3.6 g MJ<sup>-1</sup>; *r* = 0.96; *P* < 0.001) in the oz680 treatment as compared to the chAA control.

### Biomass production and grain yield

**Effects of elevated CO<sub>2</sub>.** Stem dry weight (DW) and plant height at anthesis were increased by 27% and 8 cm, respectively, in the elevated CO<sub>2</sub> c680 treatment relative to the chAA control (Lin; *P* < 0.001; Table 2). Ear dry weight was also increased by 16% (Lin; *P* < 0.001), but specific leaf area for the main shoot flag leaf was not significantly affected (Table 2). Total above-ground biomass at maturity was increased by 5 and 30% in the c550 and c680 treatments, respectively (Lin; *P* < 0.001; Fig. 4b). The number of ears m<sup>-2</sup> was increased by elevated CO<sub>2</sub> (*P* < 0.05; Table 3), contributing to the 8 and 34% higher grain yields in the c550 and c680 treatments relative to the chAA control (Lin; *P* < 0.01; Table 3;

Fig. 5); harvest index (HI) was unaffected by CO<sub>2</sub> treatment (Table 3). Several other parameters also contributed to the significantly greater grain yields obtained under elevated CO<sub>2</sub>; thus the number of grains per ear and per spikelet and IGW were up to 7.5, 0.3 and 2.7 mg greater in the c680 treatment than in the chAA control (Lin; *P* < 0.001; Table 3).

**Effects of elevated O<sub>3</sub> under ambient CO<sub>2</sub> concentrations.** Exposure to O<sub>3</sub> reduced plant height at anthesis by 3.9 cm (*P* < 0.05) and stem biomass was also 15% lower although this difference was not significant. The specific leaf area of the flag leaf was unaffected by O<sub>3</sub>, but ear DW was reduced by 23% (*P* < 0.05; Table 2). Above-ground biomass and grain yield at maturity were reduced by 35 and 30%, respectively (*P* < 0.01; Fig. 4b; Table 3; Fig. 5), and harvest index was reduced to 0.45 as compared to 0.48 in the chAA control (*P* < 0.01; Table 3). Several factors contributed to these effects on grain yield, including a small but nonsignificant decrease in ear number m<sup>-2</sup> (nonsignificant), significant reductions

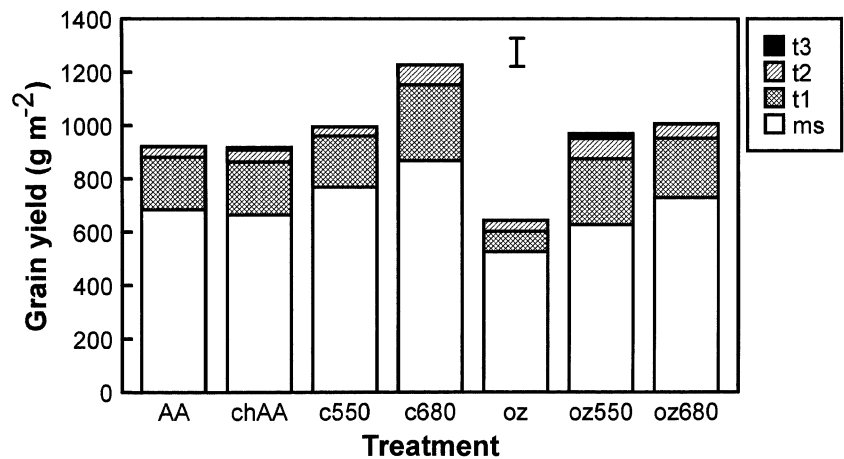


Fig. 5 Effect of elevated CO<sub>2</sub> and O<sub>3</sub> on the contributions of the main shoot (ms) and tillers (t) 1, 2 and 3 to total grain yield. Vertical bar denotes one SED with 12 d.f.

of 2.8 and 0.2 in the numbers of grains per ear and per spikelet ( $P < 0.05$ ) and a decrease in IGW of 4.4 mg ( $P < 0.01$ ; Table 3).

**CO<sub>2</sub> × O<sub>3</sub> interactions.** Although grain yield at final harvest was 3 and 18% lower in the oz550 and oz680 treatments and the numbers of grains per ear and spikelet and IGW were also consistently lower than in the corresponding ambient O<sub>3</sub> c550 and c680 treatments, none of these differences were statistically significant (Table 3). The consistency of the data provide evidence that grain yield was reduced by O<sub>3</sub> irrespective of CO<sub>2</sub> concentration. Thus, grain yield apparently exhibited a nonlinear relationship to atmospheric CO<sub>2</sub> concentration under elevated O<sub>3</sub> conditions, with reductions being particularly marked in the oz680 treatment, whereas a linear relationship was obtained under ambient O<sub>3</sub> concentrations.

#### Chambered vs. unchambered plots

Plants grown in the unchambered field plots (AA) were shorter ( $P < 0.05$ ; Table 2) and had a significantly lower ear DW than those in the chAA treatment at anthesis ( $P < 0.001$ ) but grain DW at maturity was comparable even though HI was slightly lower ( $P < 0.001$ ; Table 3). The numbers of grains per ear and per spikelet and IGW were not significantly affected (Table 3).

#### Discussion

Season-long elevation of atmospheric CO<sub>2</sub> produced linear increases in shoot biomass and grain yield of up to 30 and 34%, respectively, in the 680 µmol mol<sup>-1</sup> treatment under current ambient O<sub>3</sub> conditions, in agreement with previous results for cv. Minaret (Mulholland *et al.* 1997). The increase in yield under CO<sub>2</sub> enrichment resulted primarily from an increased number of ears m<sup>-2</sup> (Table 3), as has been observed in other studies (Goudriaan & De

Ruiter 1983; Hocking & Meyer 1991). The increased proportion of plants producing 1–2 ear bearing tillers in the c550 and c680 treatments (Table 3), may reflect an increase in the initiation and development of new sinks resulting from changes in meristematic activity brought about by increased assimilate availability during vegetative growth (Lawlor & Mitchell 1991; Stitt 1991). The main shoots of plants grown under elevated CO<sub>2</sub> out-yielded those of chAA control plants, primarily because of linear increases in the numbers of grains per ear and per spikelet and IGW between the chAA control and c680 treatments. The linear increase in IGW contrasts with some previous studies in which grain weight decreased under elevated CO<sub>2</sub> (Apel 1976; Weigel *et al.* 1994), but supports numerous other reports (Gifford 1979; Chaudhuri *et al.* 1990a). The greater stem and ear biomass observed at anthesis under elevated CO<sub>2</sub> may have increased assimilate availability prior to and during grain filling, thereby providing a possible explanation for the greater grain number per ear and IGW recorded. HI remained constant at 0.49 under elevated CO<sub>2</sub>, reflecting the proportionate increase in grain weight and stem and leaf biomass which has been reported previously (Gifford 1979), but contrasting with the decreases described by Weigel *et al.* (1994). The linear increase in grain yield between the ambient and 680 µmol mol<sup>-1</sup> CO<sub>2</sub> treatments contrasts with the nonlinear responses observed in a study of two other spring wheat cultivars (cvs. Star and Turbo), in which the greatest promotion of yield was obtained at 550 µmol mol<sup>-1</sup> CO<sub>2</sub> (Weigel *et al.* 1994). These contrasting responses suggest that the impact of future CO<sub>2</sub> concentrations on spring wheat may vary greatly depending on the cultivar involved.

The response of yield and its components to atmospheric CO<sub>2</sub> concentration was nonlinear in the presence of elevated O<sub>3</sub>, with maximum grain number and IGW being obtained in the intermediate oz550 treatment. Although the observed nonlinear response for individual



yield components substantiates our previous findings (Mulholland *et al.* 1997), the nonlinearity of total grain yield in the elevated O<sub>3</sub> oz, oz550 and oz680 treatments (Fig. 5) contrasts with the previous data set, in which season-long exposure to 60 nmol mol<sup>-1</sup> O<sub>3</sub> (7 h d<sup>-1</sup>) had little effect. The 30% reduction in grain yield induced by exposure to a seasonal mean (7 h d<sup>-1</sup>) of 84 nmol mol<sup>-1</sup> O<sub>3</sub> under ambient CO<sub>2</sub> in the present experiment is in agreement with previous studies (Skärby *et al.* 1993), suggesting the existence of a threshold O<sub>3</sub> concentration between 60 (Mulholland *et al.* 1997) and 84 nmol mol<sup>-1</sup>, above which grain yield is significantly reduced in Minaret. The observed O<sub>3</sub> induced yield losses were largely attributable to a reduction in ear number m<sup>-2</sup>, caused by greater tiller mortality during vegetative growth (data not shown), coupled with significant decreases in the number of grains per ear, IGW and harvest index ( $P < 0.05$ ; Table 3); these effects are consistent with previous studies of spring wheat (Fuhrer *et al.* 1989; Pleijel *et al.* 1991).

Canopy development was markedly influenced by both CO<sub>2</sub> and O<sub>3</sub>. Green LAI was increased at 680 µmol mol<sup>-1</sup> CO<sub>2</sub> due to a combination of increased tillering and delayed senescence of individual leaves (Fig. 3a–d). Yellow LAI was nevertheless increased in both elevated CO<sub>2</sub> treatments because the larger canopy contained a greater absolute quantity of senescent tissue, including tillers. The accelerated yellowing of individual leaves in the c550 treatment was not statistically significant, but agreed with previous observations involving wheat (Weigel *et al.* 1994) and native UK grass species (Baxter *et al.* 1994). This effect was probably not attributable to accelerated leaf development rates since it was not apparent at 680 µmol mol<sup>-1</sup> CO<sub>2</sub> (c680), in which senescence was in fact delayed. Yellow LAI was increased and green LAI markedly reduced by exposure to O<sub>3</sub> under ambient CO<sub>2</sub> concentrations (Fig. 2a,b), a common observation in plants exposed to elevated O<sub>3</sub> (Krupa & Manning 1988; Rao *et al.* 1995). As a consequence, all leaves senesced completely 7–28 days earlier in the elevated O<sub>3</sub> treatment than in the corresponding ambient O<sub>3</sub> treatments. Importantly therefore, and in contrast to our previous work (Mulholland *et al.* 1997), the flag leaf senesced sooner and significantly more rapidly in the O<sub>3</sub>-treated plants than in the chAA control, as has been reported previously for wheat (Sandelius *et al.* 1995). This may have had a deleterious effect on assimilate production and partitioning from the flag leaf to support grain growth (Bruckner & Froberg 1987; Schynder 1993). The lower individual seed weight in O<sub>3</sub>-treated plants may reflect a combination of a decrease in the duration of grain filling (Slaughter *et al.* 1992) and reductions in the availability of assimilate and/or its diversion to support leaf repair or adaptation (Heath 1994).

The contrasting impact on biomass production and grain yield of elevated CO<sub>2</sub> and O<sub>3</sub> when applied individually or in combination may reflect their differing effects on canopy expansion and hence radiation interception and assimilate production (Gallagher & Biscoe 1978). Although above-ground biomass at maturity was increased by up to 34% in the c680 treatment, the seasonal values for accumulated intercepted radiation and the conversion coefficient ( $\epsilon_s$ ) were only 9 and 15% greater than in the chAA control; however, mean fractional interception in the c680 treatment between 53 DAS and anthesis (89 DAS) was 26% higher as compared to the control (Fig. 4a), which more closely matches the increase in biomass observed at maturity. The relatively small improvement observed in  $\epsilon_s$  under elevated CO<sub>2</sub> might be explained if a greater proportion of the assimilates produced were partitioned to the roots (Arp 1991), which were not measured in the present study; indeed, there is evidence that root growth in wheat may be promoted by elevated atmospheric CO<sub>2</sub> (Chaudhuri *et al.* 1990b). A similar discrepancy, but in the opposite direction, was observed in response to O<sub>3</sub> exposure under ambient CO<sub>2</sub> conditions (oz), in which seasonal total intercepted radiation and  $\epsilon_s$  were reduced by 17 and 16%, respectively, whereas biomass and grain yield at maturity were decreased by 35 and 30%. This decrease in radiation interception may well have originated from the observed acceleration of leaf senescence, but this effect is too small to explain the much larger reduction in grain yield; similar discrepancies have been reported for *Glycine max* L. (Unsworth *et al.* 1984). The possibility that O<sub>3</sub> may exert additional directly detrimental effects on sensitive reproductive stages or processes, thereby affecting grain development and yield, is considered further below.

Elevated atmospheric CO<sub>2</sub> concentrations provided partial protection against O<sub>3</sub>-induced injury at the individual leaf level, since foliar damage was significantly reduced relative to plants exposed under ambient CO<sub>2</sub> (Figs 2a,b; 3a–b). A similar protective influence has been observed in previous work, in which CO<sub>2</sub>-induced reductions in stomatal conductance were postulated to reduce the effective O<sub>3</sub> dose, thereby limiting damage to the photosynthetic apparatus (McKee *et al.* 1995). The promotion of antioxidant production would provide an alternative protection mechanism (Rao *et al.* 1995), particularly in older leaves in view of previous reports that the antioxidant repair capacity may be light-dependent, and hence influenced by the radiation environment experienced by individual leaves (Foyer 1993). The reductions in  $\epsilon_s$  in the oz550 and oz680 treatments of 0.3 and 6% relative to the corresponding ambient O<sub>3</sub> c550 and c680 CO<sub>2</sub> treatments were small compared to the substantial yield losses induced by O<sub>3</sub> (cf. oz680 vs. c680; Table 3), these yield losses were again too large to be explained



by the reductions in accumulated seasonal radiation interception resulting from O<sub>3</sub> damage. The more limited foliar injury observed under elevated CO<sub>2</sub> suggests that the O<sub>3</sub>-induced yield losses would have been substantially smaller than those obtained, particularly in the oz680 treatment, if these were mediated solely by effects on vegetative organs and assimilate production. These observations therefore suggest that O<sub>3</sub> may affect specific processes *directly* in addition to the *indirect* effects mediated through injury to the vegetative component. Such effects might act at the level of pollen viability, fertilization, embryo development and abortion or abscission of reproductive structures. Indeed, previous studies of oilseed rape (*Brassica napus* L.; Bosac *et al.* 1993, 1994) and the related species Wisconsin Fast Plant (*Brassica campestris* L.; Stewart *et al.* 1996) have demonstrated that single or repeated exposures to 100 nmol mol<sup>-1</sup> O<sub>3</sub> may induce a range of direct effects including reduced pollen germination and tube growth, abscission of flowers or pods, abortion of seeds within pods and decreases in seed numbers and size. Thus flowering may represent a stage of wheat development during which elevated CO<sub>2</sub> cannot provide any ameliorative benefit by reducing O<sub>3</sub> fluxes to the site of action; indeed short-term exposures of wheat to O<sub>3</sub> around anthesis have been shown to decrease yields significantly (Mulchi *et al.* 1986). Some residual benefit of elevated CO<sub>2</sub>, perhaps resulting from increased assimilate availability, was nevertheless observed since yields in oz550 and oz680 were higher than those in the chAA control plants (Table 3; Fig. 5).

## Conclusions

Minaret exhibited a linear increase in biomass and yield in response to season-long elevation of atmospheric CO<sub>2</sub> under ambient O<sub>3</sub> conditions, but the response was apparently nonlinear in the presence of elevated O<sub>3</sub>. Plants grown under elevated O<sub>3</sub> and ambient CO<sub>2</sub> were shorter than control plants, and exhibited accelerated senescence of all leaves, including the flag. Although elevated CO<sub>2</sub> provided some protective benefit against O<sub>3</sub> exposure at the individual leaf and canopy levels, substantial reductions in final yields were nevertheless obtained. These yield losses were too large to be attributable to O<sub>3</sub>-induced impairment of canopy development and radiation interception, suggesting that alterations in assimilate production and/or partitioning and possible deleterious effects on reproductive processes such as pollen viability, fertilization and early seed development may have an important role in determining the severity of O<sub>3</sub>-induced yield losses.

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