

Do open-top chambers overestimate the effects of rising CO₂ on plants? An analysis using spring wheat

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Abstract

The microclimate in facilities for studying effects of elevated CO₂ on crops differs from ambient conditions. Open-top chambers (OTCs) increase temperature by 1–3 °C. If temperature and CO₂ interact in their effect on crops, this would limit the value of OTC experiments. Furthermore, interaction of CO₂ and temperature deserves study because increases in atmospheric CO₂ concentration are expected to cause global warming.

This paper describes two experiments in which a recently developed cooling system for OTCs was used to analyse the effects of temperature on photosynthesis, growth and yield of spring wheat (*Triticum aestivum* L., cv. Minaret). Two levels of CO₂ were used (350 and 700 ppm), and two levels of temperature, with cooled OTCs being 1.6–2.4 °C colder than noncooled OTCs.

Photosynthetic rates were increased by elevated CO₂, but no effect of temperature was found. Cross-switching CO₂ concentrations as well as determination of A–C_i curves showed that plant photosynthetic capacity after anthesis acclimated to elevated CO₂. The acclimation may be related to the effects of CO₂ on tissue composition: elevated CO₂ decreased leaf nitrogen concentrations and increased sugar content. Calculations of the seasonal mean crop light-use efficiency (LUE) were consistent with the photosynthesis data in that CO₂ increased LUE by 20% on average whereas temperature had no effect. Both elevating CO₂ and cooling increased grain yield, by an average of 11% and 23%, respectively. CO₂ and temperature stimulated yield via different mechanisms: CO₂ increased photosynthetic rate, but decreased crop light interception capacity (LAI), whereas cooling increased grain yield by increasing LAI and extending the growing season with 10 days. The effects of CO₂ and temperature were not additive: the CO₂ effect was about doubled in the noncooled open-top chambers. In most cases, effects on yield were mediated through increased grain density rather than increased individual grain weights.

The higher growth response to elevated CO₂ in noncooled vs. cooled OTCs shows that a cooling system may remove a bias towards overestimating crop growth response to CO₂ in open-top chambers.

Keywords: CO₂, grain yield, open-top chambers, photosynthesis, spring wheat, temperature

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Introduction

Research on the effects of elevated CO₂ on plant growth is difficult to carry out under field-like conditions. Efficient fumigation requires shielding the plants from the environ-

ment by using open-top chambers (OTCs), greenhouses or growth chambers. In studies using free air CO₂ enrichment (FACE), conditions are less affected, but the wind pattern around the plants is still influenced which affects aerodynamic crop resistance and temperature difference between crop and atmosphere, and operation costs are higher due to extra gas usage (Kimball *et al.* 1997).

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Therefore, some modification of the plant growing environment is often accepted. OTCs have been used increasingly frequently, and presently constitute the major source of information on field crop responses to rising CO₂ (Heagle *et al.* 1988). When using OTCs it is generally assumed that, even if plants in OTCs grow differently than outside, their sensitivity to elevated CO₂ is not affected and can be reliably measured. However, no support for this assumption has been given.

Research on the effects of elevated CO₂ on wheat is of particular relevance as this crop is the most important food crop world-wide: at the present time, 16% of the world's arable land is sown to wheat, giving a yearly production of 544 million ton, or 21% of total food production from cereals, root crops and sugar crops (FAO 1997; data for 1995). In a review of growth chamber experiments, grain yield of wheat (*Triticum aestivum* L.) was shown to be increased by $35 \pm 14\%$ due to CO₂-doubling (Cure & Acock 1986). The major cause of the yield increase is stimulated photosynthesis. Analyses of short-term experiments in growth chambers show that leaf photosynthetic rate in wheat increases asymptotically with CO₂ concentration (Farquhar *et al.* 1980; Allen 1990). Model calculations predict an interaction between the CO₂ effect and temperature: CO₂ is expected to increase photosynthetic rate more strongly at high than at low temperatures (Farquhar *et al.* 1980; Long 1991). However, the calculations do not account for any plant adaptation to its environment during growth, and thus may only apply to the instantaneous effects of sudden changes in CO₂ and temperature. Moreover, as model parameterization was based on growth chamber experiments, the results may not be applicable to field crops.

Idso & Idso (1994), in a review of the literature between 1983 and 1992, reported a positive correlation between temperature and the response of plant growth rate to CO₂ doubling. Their review comprised 31 spp. in 42 studies, only one of which dealt with wheat. Averaging across species, the percentage increase due to CO₂ doubling increased with temperature at a rate of 3.6% per °C. Rawson (1995) found a value of 1.8% °C⁻¹ for the temperature dependency of CO₂ effects on wheat yield in his temperature gradient tunnel experiments. Wheeler *et al.* (1996) did not find a consistent interaction between CO₂ and temperature: the effect of elevated CO₂ on wheat biomass yield in gradient tunnels was increased by high temperature in one of two experimental years only. Interactive effects of CO₂ and temperature on wheat grain yield have not yet been studied in conditions of OTCs (Allen 1990; Lawlor 1996).

Temperature deserves attention in research on CO₂ effects because elevated CO₂ itself is expected to contribute to global warming, so the two factors may change simultaneously. Moreover, apart from the fact that facilit-

ies like OTCs typically have 1–3 °C higher than ambient temperatures (Heagle *et al.* 1988), temperature also shows climatic differences between locations that may affect crop responses. This is of special importance in multisite research programs where crop responses to elevated CO₂ are compared. The experiments reported here were part of such an international research programme, 'ESPACE-Wheat' (Hertstein *et al.* 1996), in which OTC experiments with at least two levels of CO₂ were carried out in eight European countries. In all experiments spring wheat cultivar Minaret was used. Several of the experiments included additional factors such as ozone, water availability, nitrogen availability or temperature.

In this study we report on the experiments in Wageningen, The Netherlands, which were the only ones where the interaction between CO₂ and temperature was studied. The experiments were intended to clarify whether OTCs are appropriate instruments to assess CO₂ effects, in spite of chamber-induced warming, and thus to assess whether differences between sites in observed response of wheat photosynthesis, growth and yield to elevated CO₂ were influenced by differences in temperature. Temperature was manipulated by means of a recently developed cooling system for OTCs (Van Oijen *et al.* 1998). Both cooled and noncooled OTCs were used to create two temperature levels, representative of ambient conditions and normal OTCs, respectively.

Materials and methods

Experimental set-up

Two experiments were carried out on a light clay soil, near Wageningen, The Netherlands, in 1995 and 1996. The experiments were done in a factorial set-up, with two levels of CO₂ and two levels of temperature, in three replicates, giving 12 OTCs in all. Additionally there were six ambient plots.

OTCs were hexagonal, with 1.5 m between parallel sides, giving 1.95 m² soil area completely sown to spring wheat cv. Minaret. Chamber walls were made of 3 mm polycarbonate (LEXAN®), which is 88% transparent to photosynthetically active radiation, but absorbs UV-b. Additional wheat plants were sown outside the OTCs to prevent irradiation from the sides.

The two levels of temperature were created by coupling the air inlets of half of the OTCs to a water-cooled system developed at AB-DLO (Van Oijen *et al.* 1998). The system consisted of a 4-m³ cooling tank (type Alfa-Laval compact), in which water was cooled down to 7 °C, tubing for water circulation, and six radiators (Opel-Kadett type A/C). The cooling system decreased temperature effectively (see 'Climatic conditions' below) and measurements before and during the growing season showed

Table 1 Details of the experiments

	Treatment	1995	1996
Sowing date	all	April 3	March 21
Emergence (50%)	OTCs	April 16	April 10
	ambient plots	April 16	April 11
Anthesis harvest (0.5 m ²)	warm OTCs	June 21 (DC69) *	June 17 (DC70)
	cooled OTCs	June 29 (DC69)	June 24 (DC70)
	ambient plots	June 28 (DC69)	June 25 (DC70)
Maturity harvest (0.8 m ²)	warm OTCs	August 8 (DC92)	August 5 (DC92)
	cooled OTCs	August 22 (DC92)	August 19 (DC92)
	ambient plots	August 21 (DC92)	August 20 (DC92)
Plant density (# m ⁻²)	all	203–218	220–251

*DC = decimal code of wheat development (Tottman & Broad 1987)

that cooling did not affect absolute air humidity, so drying of incoming air by condensation was prevented. Note, however, that relative humidity did increase, but solely as a result of the lowered temperature.

CO₂ was supplied via tubes on the soil surface and via an airbag at 1.3 m height. Additional tubes on the soil surface were used for irrigation whenever the topsoil dried out visibly. In both years, fertilizer was given on the basis of preplanting soil samples to 0.3 m deep, in three split applications at levels slightly above common farming practice to prevent any nutrient deficiency. In 1995, total applied amounts of N, P₂O₅ and K₂O were 21.1, 9.3 and 16.7 g m⁻², respectively, and in 1996 the corresponding figures were 15.3, 9.6 and 3.6 g m⁻². Also, manual weeding and up to monthly fungicide application were carried out to ensure optimal crop growing conditions. Dates of sowing were 3 April 1995 and 21 March 1996. Emergence followed after 13 days in 1995 and after 20 days in 1996 (Table 1).

Further details of OTCs and cooling system are given elsewhere (Van Oijen *et al.* 1998).

Climatic conditions

1995 had a higher average radiation level than 1996: 18.6 MJ global radiation m⁻² d⁻¹ vs. 16.3 MJ m⁻² d⁻¹ in ambient plots. Values in OTCs were about 25% lower.

1995 was warmer than 1996: 16.2 °C compared to 14.6 °C in ambient plots. In both years, the temperature in noncooled OTCs was on average 2.8 °C higher than in the ambient plots. The cooling system effectively reduced the temperature in the OTCs, but cooled chambers were still slightly warmer than ambient plots (0.4 °C in 1995 and 1.2 °C in 1996). In 1995, one of the six cooled chambers was on average 1.6 °C warmer than the ambient plots. This chamber thus was intermediate in temperature between noncooled and cooled treatments, and plant growth and yield was intermediate as well.

Average ambient CO₂-levels were 365–380 ppm in both

years, and elevated CO₂-levels were 716–720 ppm in 1995 and 751–756 ppm in 1996.

Measurements and statistical analyses

On 6 and 20 July 1995, photosynthetic rates of flag leaves were measured *in situ* on plants inside OTCs and ambient plots, by means of a portable leaf chamber analyser (LCA; Analytical Development Co. (ADC) (UK). An incandescent lamp, cooled by a fan, was used to ensure equal saturating light levels for all measurements, of 1800 μmol m⁻² s⁻¹ photosynthetically active photon flux density. Readings were taken after leaf chamber and lamp had been in place for at least one minute. On 6 July plants were only measured at the CO₂ concentration at which they were growing. On 20 July this was repeated, but measurements after cross-switching the two CO₂ concentrations were added.

In 1996, photosynthetic rates were measured over a longer period (June and first half of July). For these measurements whole plants were carefully taken from the field and brought to a laboratory. Photosynthetic rates and stomatal conductance of upper leaves were measured at various CO₂ concentrations, to determine A–C_i curves. Per leaf curve about one hour enclosure in the measuring cuvette was required. During the measurements, temperature was 19.6 ± SD 0.5 °C and humidity was 9.6 ± SD 1.2 mbar.

In both years there was a harvest of 0.5 m² per plot at the end of flowering (decimal code of development DC69 or DC70) and a final harvest of 0.8 m² per plot after ripening (DC92) (Table 1). Leaves, stems and ears were separated, counted, dried for 24 h at 70 °C followed by 3 h at 105 °C, then weighed and analysed for their content of total nitrogen, nitrate, sugars and total carbon. The anthesis harvest included measurement of specific leaf area to calculate LAI. The final harvest included a count of number of grains per ear.

All data were statistically analysed by two-factorial

analysis of variance, corresponding to the two-factorial randomized block designs used; ambient plots were left out of the analysis. The single chamber at intermediate temperature in 1995 was also left out. The two experiments (1995 and 1996) were analysed separately.

Estimation of cumulative light interception and crop light-use efficiency

Cumulative interception by the canopy of photosynthetically active radiation (PARCUM, MJ m⁻²) was estimated as the integral over time of the daily product of incoming PAR and fractional light interception. Fractional light interception was assumed to be equal to fractional ground cover by green foliage, which itself was visually estimated at weekly intervals from two different observation angles per plot (Van Oijen *et al.* 1998), and linearly interpolated to yield daily values. Crop light-use efficiency (LUE, g MJ⁻¹) was calculated as the ratio of accumulated above-ground biomass at maturity (g m⁻²) and PARCUM.

Results

Photosynthesis

On 6 July 1995 (day of the year 187) photosynthesis measurements were taken only at the [CO₂] the plants had received during growth. Plants grown and measured at elevated CO₂ showed an increase of light-saturated photosynthetic rate of about 30% (Fig. 1a). In contrast, temperature had no effect. On 20 July, only leaves from cooled OTCs and ambient plots could be measured, because leaf senescence had already progressed too far in the 'warm' OTCs. At this date, each plant was measured both at 350 and at 700 ppm CO₂. The measurements again showed a stimulus of about 30% by elevated CO₂, when the plants were measured at the [CO₂] they experienced during growth. In contrast, a sudden switch from the pretreatment [CO₂] of 350 ppm to 700 ppm had a much bigger effect than the 30% increase observed at continuously elevated CO₂. When leaves grown at 350 ppm were subjected to 700 ppm, photosynthetic rates increased by about 70% (Fig. 1b). Vice versa, a switch from 700 to 350 ppm caused a decrease of 50% (Fig. 1b). This indicates that photosynthetic capacity of plants grown at elevated CO₂ had acclimated to elevated CO₂.

In 1996, A-C_i curves were followed in time. An overall gradual decrease of the curves was observed from the earliest measurements at days 154–155 onwards, but there were clear differences between the treatments (Fig. 2). Effects of both CO₂ and temperature on A-C_i curves were small until days 178–179, when

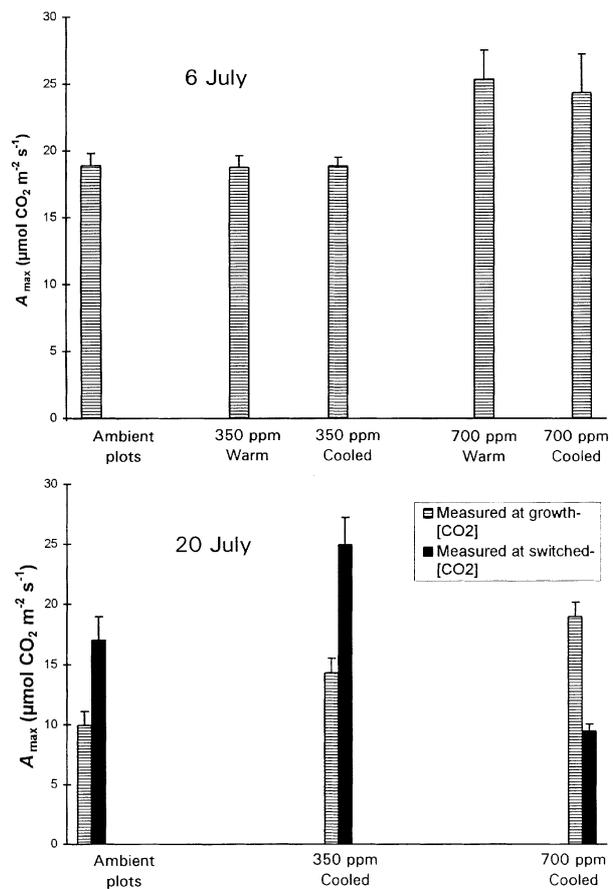


Fig. 1 Net photosynthetic rates at light saturation (A_{max}) of flag leaves in 1995. Error bars indicate standard errors of the means. (a) measurements of 6 July, using the CO₂ concentrations at which the plants were grown; (b) measurements of 20 July, using both the CO₂ concentrations at which the plants were grown and a temporarily switched CO₂ concentration (350 → 700 ppm or vice versa).

leaves from warm OTCs showed signs of decreasing photosynthetic capacity (Fig. 2). Effects of elevated CO₂ only became clear during the grain-filling period, from day 189–190 onwards, when decreased photosynthetic capacity in plants from OTCs at elevated CO₂ was observed (Fig. 2).

Growth and yield

Cooling delayed the phenological development of the plants, leading to later anthesis (Table 1) and to about 10 days later end-of-grain-filling in both years (Fig. 3). Elevating CO₂ had no significant effect on development.

The above-ground biomass yield was increased by cooling in both years at both harvests, at both levels of CO₂ (Table 2). The increases were statistically significant except for the intermediate harvest in 1996. Biomass was also increased by elevated CO₂, but

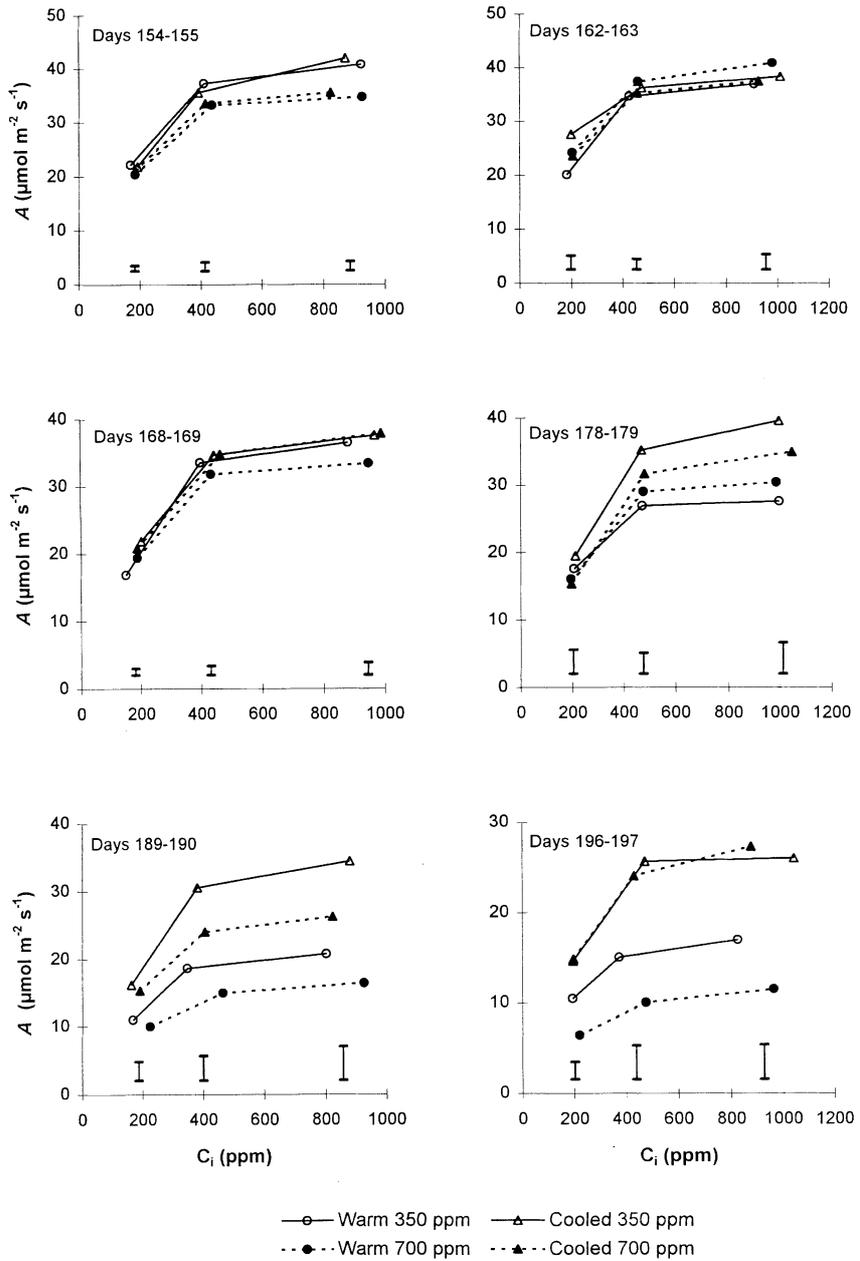


Fig. 2 Weekly measurements of photosynthetic rates vs. internal CO₂ concentration of upper leaves between day of the year 154–155 and 196–197 in 1996. Per treatment, three leaves were measured at ambient CO₂ concentrations of 355 ± 3.8 (SD), 702 ± 5.2 and 1301 ± 8.9 ppm. For each measurement period, and for each of the three ambient CO₂ concentrations, analyses of variance were carried out to determine the standard errors of difference (d.f. = 6) shown under the curves.

less strongly than by cooling, and only statistically significantly in 1995. The harvest index was not affected by treatments in 1995, but it was decreased by elevated CO₂ in 1996, almost nullifying any effect of CO₂ on grain yield.

Dry grain yield (g m⁻²) is the product of individual grain weight (g grain⁻¹) and grain density (grains m⁻²). Grain density can be analysed further as the product of ear density (ears m⁻²) and the number of grains per ear. All these yield components were determined at the final harvest (Table 2). In both years, grain yield was increased by cooling, but more strongly so at ambient CO₂ (1995: 30% 1996: 29%) than at elevated

CO₂ (21%; 10%) (Table 2). This suggests a CO₂–temperature interaction which, however, was not statistically significant ($P = 0.19$). The yield increases were caused to greater extent by increased grain density than by increased grain weight, except for the elevated CO₂ treatment in 1996, where only grain weight was increased by cooling. Effects on grain density were most pronounced, and statistically significant, in 1995. Cooling increased grain density in 1995 mainly by increasing numbers of grains per ear, and in 1996 mainly through higher ear density.

Grain yield was also increased by elevated CO₂. The effect was stronger in warm OTCs (1995: 20%; 1996:

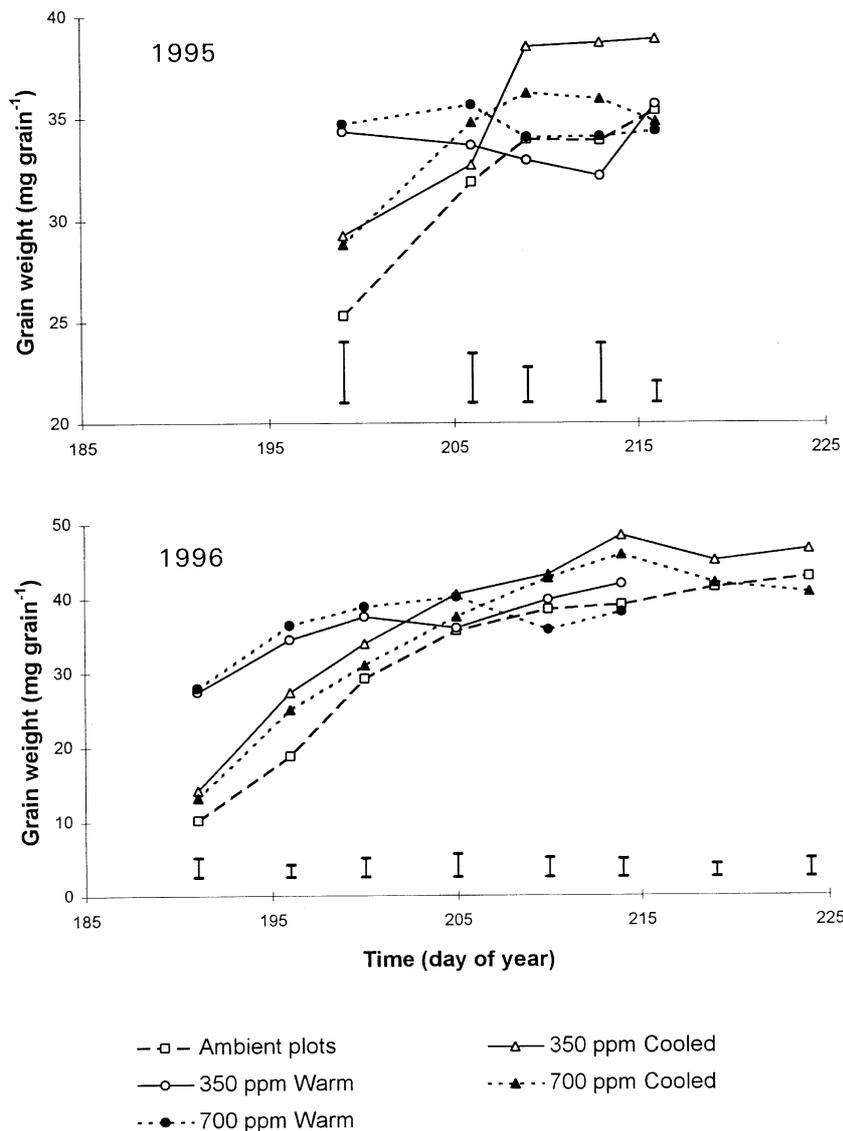


Fig. 3 Grain growth curves. Vertical bars below data indicate standard errors of difference for the four OTC treatments (d.f. = 6, except last two measurement dates of 1996: d.f. = 2).

14%) than in cooled OTCs (11%; - 2%) (Table 2). CO₂ had little effect on individual grain weight but grain density was increased, except in the cooled OTCs in 1996. In the warm OTCs, the effect of CO₂ on grain density was split equally between increased grain number per ear and increased ear density. In cooled OTCs, however, elevated CO₂ especially increased the number of grains per ear.

Grain yield was higher in ambient plots than in any of the OTC treatments, except for the cooled OTCs in 1996, which reached similar yield levels (Table 2). High yields in ambient plots were fully explained by high grain densities, which in turn were caused by high ear densities. Grain yields in ambient plots were not higher than in cooled OTCs in 1996 because of lower individual grain weights.

Nutrient relations

Elevated CO₂ decreased levels of nitrogen and nitrate and increased sugar levels (Table 3). However, elevating CO₂ did not increase carbon content of grains and stems, which generally was about 45% irrespective of organ type, treatment and year. Cooling had similar effects as elevating CO₂ in that nitrogen concentrations were reduced.

Light-use efficiency and light interception

Elevated CO₂ reduced cumulative light interception (PARCUM) in cooled OTCs by 10% in 1995 and by 5% in 1996, but only by 4% and 2%, respectively, in warm OTCs (Table 4). These observed reductions of PARCUM were small when compared to the opposite

Table 2 Data from destructive harvests. The statistical effects describe results from ANOVA's for the four OTC-treatments only (i.e. ambient plots left out). For 1995, One chamber with deviant temperature was left out of the analysis (see Materials and Methods). Statistical significance of CO₂-, temperature- and interaction- effects is indicated as ** ($P < 0.01$), * ($P < 0.05$) or NS (not significant)

Year	Variable	Treatment					Statistical effects			
		350/ warm	350/ cooled	700/ warm	700/ cooled	amb. plots	CO ₂	temp.	CO ₂ temp.	
1995	<i>Intermediate harvest at DC69</i>									
	Above-ground d.m. (g m ⁻²)	472	767	626	827	1080	*	**	NS	
	Ear d.m. (no grains) (g m ⁻²)	89	159	120	161	180	NS	**	NS	
	Stem d.m. (g m ⁻²)	267	423	368	485	604	**	**	NS	
	Leaf d.m. (g m ⁻²)	95	131	106	121	204	NS	*	NS	
	LAI (m ² m ⁻²)	1.96	2.71	2.10	2.46	4.11	NS	**	NS	
	Senesced d.m. (g m ⁻²)	21	54	32	60	92	NS	**	NS	
	<i>Final harvest at DC92</i>									
	Above-ground d.m. (g m ⁻²)	985	1271	1174	1413	1666	*	**	NS	
	Harvest index (g g ⁻¹)	0.49	0.49	0.49	0.49	0.45	NS	NS	NS	
	Grain d.m. (g m ⁻²)	479	624	575	694	758	*	**	NS	
	Grain weight (mg grain ⁻¹)	30.0	32.8	32.8	32.7	32.0	*	*	*	
	Grain density (# m ⁻²)	16000	19000	17500	21200	23700	*	**	NS	
	Grain density (# ear ⁻¹)	34.3	37.4	36.0	41.0	37.3	*	**	NS	
	Ear d.m. (no grains) (g m ⁻²)	131	151	142	174	190	*	**	NS	
	Ear density (# m ⁻²)	467	508	487	517	636	NS	NS	NS	
	Stem d.m. (g m ⁻²)	276	362	346	418	528	*	**	NS	
	Senesced d.m. (g m ⁻²)	98	134	112	128	191	NS	*	NS	
	1996	<i>Intermediate harvest at DC70</i>								
		Above-ground d.m. (g m ⁻²)	951	1016	1028	1065	1165	NS	NS	NS
Ear d.m. (no grains) (g m ⁻²)		246	229	260	227	225	NS	NS	NS	
Stem d.m. (g m ⁻²)		491	552	565	609	640	*	NS	NS	
Leaf d.m. (g m ⁻²)		156	175	139	151	193	NS	NS	NS	
LAI (m ² m ⁻²)		3.86	4.45	3.25	3.80	4.64	NS	NS	NS	
Senesced d.m. (g m ⁻²)		59	60	63	77	107	NS	NS	NS	
<i>Final harvest at DC92</i>										
Above-ground d.m. (g m ⁻²)		1272	1660	1553	1710	1671	NS	*	NS	
Harvest index (g g ⁻¹)		0.57	0.56	0.53	0.53	0.55	**	NS	NS	
Grain d.m. (g m ⁻²)		721	927	822	908	912	NS	*	NS	
Grain weight (mg grain ⁻¹)		38.5	41.6	36.0	40.1	36.3	*	**	NS	
Grain density (# m ⁻²)		18700	22300	22900	22600	25200	NS	NS	NS	
Grain density (# ear ⁻¹)		43.4	41.3	48.3	42.0	38.7	**	**	*	
Ear d.m. (no grains) (g m ⁻²)		126	168	161	182	181	*	**	NS	
Ear density (# m ⁻²)		432	542	475	537	650	NS	*	NS	
Stem d.m. (g m ⁻²)		302	413	396	471	435	*	**	NS	
Senesced d.m. (g m ⁻²)		122	153	175	149	144	NS	NS	NS	

effects of CO₂ on LUE, and not statistically significant. Cooling, on the other hand, significantly increased PARCUM by 19–28% at both CO₂ levels in both years.

LUE responded differently in that cooling had no effect, whereas elevating CO₂ increased LUE by 8–24% (Table 4).

Discussion

Effects of elevated CO₂ and temperature on photosynthesis and LUE

Elevated CO₂ stimulates photosynthesis more at high than at low temperature (Farquhar *et al.* 1980; Long 1991).

The main physiological reason for this is the fact that elevated CO₂ decreases the rate of photorespiration, which is relatively high at high temperature. Our data afford only a limited test of this interaction between temperature and CO₂, as only the photosynthesis measurements of 1995 were carried out on-site at the different temperature and CO₂ levels at which the plants were growing. These measurements showed no effects of temperature at all, irrespective of CO₂ level (Fig. 1a). However, the two-degree temperature difference between cooled and warm OTCs may have been too small to reveal any such effect. Delgado *et al.* (1994) used a slightly

Table 3 Nutrient concentrations at intermediate and final harvest. Statistical significance is indicated as in Table 1. One chamber was excluded from the 1995-data because of deviant temperature (see Table 1 and text). All concentrations are expressed on the basis of dry matter

Year	Variable	Treatment					Statistical effects			
		350/ warm	350/ cooled	700/ warm	700/ cooled	amb. plots	CO ₂	temp.	CO ₂ temp.	
1995	<i>Intermediate harvest at DC69</i>									
	Leaf N (g kg ⁻¹)	39.0	39.1	34.4	33.1	37.6	**	NS	NS	
	Leaf nitrate (g kg ⁻¹)	0.5	0.5	0.4	0.1	1.5	NS	NS	NS	
	Leaf sugars (g kg ⁻¹)	64.3	60.9	77.4	81.6	53.1	NS	NS	NS	
	Stem N (g kg ⁻¹)	14.8	11.3	13.1	9.6	12.2	**	**	NS	
	Stem nitrate (g kg ⁻¹)	1.5	1.0	1.0	0.3	1.7	*	*	NS	
	Stem sugars (g kg ⁻¹)	78.4	91.5	114.0	146.7	104.1	**	*	NS	
	<i>Final harvest at DC92</i>									
	Grain N (g kg ⁻¹)	21.3	18.8	17.6	15.7	21.9	**	*	NS	
	Grain sugars (g kg ⁻¹)	32.9	38.9	34.5	40.0	34.5	NS	**	NS	
	Grain C (g kg ⁻¹)	449.7	446.7	445.3	446.0	448.0	NS	NS	NS	
	Stem N (g kg ⁻¹)	2.1	1.6	1.8	1.6	2.8	*	**	*	
	Stem sugars (g kg ⁻¹)	10.1	9.0	14.8	11.0	8.7	NS	NS	NS	
	Stem C (g kg ⁻¹)	446.3	448.0	449.3	443.7	443.3	NS	NS	NS	
	1996	<i>Intermediate harvest at DC70</i>								
Leaf N (g kg ⁻¹)		42.0	39.5	33.1	33.5	41.4	**	NS	NS	
Leaf nitrate (g kg ⁻¹)		1.5	1.1	0.2	0.6	1.5	*	NS	NS	
Leaf sugars (g kg ⁻¹)		59.7	49.5	91.1	61.6	44.2	**	**	*	
Stem N (g kg ⁻¹)		11.2	10.1	7.6	8.0	11.2	**	NS	NS	
Stem nitrate (g kg ⁻¹)		2.0	1.5	0.5	0.7	2.0	*	NS	NS	
Stem sugars (g kg ⁻¹)		151.0	158.3	179.0	185.7	156.3	NS	NS	NS	
<i>Final harvest at DC92</i>										
Grain N (g kg ⁻¹)		20.6	20.4	18.1	17.7	20.9	*	NS	NS	
Grain sugars (g kg ⁻¹)		32.2	35.9	32.6	37.4	38.2	NS	*	NS	
Grain C (g kg ⁻¹)		456.7	457.0	455.3	456.0	458.6	*	NS	NS	
Stem N (g kg ⁻¹)		2.8	2.6	2.2	2.6	4.6	NS	NS	NS	
Stem sugars (g kg ⁻¹)		11.2	11.1	9.7	11.3	7.4	NS	NS	NS	
Stem C (g kg ⁻¹)		466.0	461.7	465.0	459.7	463.2	NS	*	NS	

greater temperature range (4 °C), but also found no significant interaction between CO₂ and temperature effects on leaf photosynthesis in winter wheat. In our experiments, the only noticeable effect of high temperature on photosynthesis was the earlier onset of postanthesis decline in photosynthetic capacity, as observed in the 1996 A–C_i measurements. This presumably was caused by accelerated development rather than a direct temperature effect on photosynthesis.

The results for photosynthesis are consistent with the absence of temperature effects on LUE (Table 4), which can be viewed as a measure of whole-season average canopy photosynthetic capacity. LUE was calculated on the basis of above-ground biomass only, but it is a good measure for total production capacity because root biomass, left in the soil after the final harvest, only constituted about 4% of total biomass (35–50 g m⁻² for all treatments; Arp *et al.* 1997). Even when correcting this for root loss (typically about one-half of wheat root

growth has already decayed by the end of the growing season; Swinnen 1994), LUE is still dominated by above-ground growth.

Elevating CO₂ increased photosynthetic rates and LUE, but the increase was constrained in both years by postanthesis reduction of photosynthetic capacity (Figs 1b, 2). The reduction may have been caused by downregulated Rubisco activity, as Sicher & Bunce (1997) found a close correlation between gradually diminishing CO₂ effects on photosynthesis and decreasing Rubisco activity in flag leaves of winter wheat. Downregulation of Rubisco is also consistent with the 12–21% reduced leaf nitrogen concentrations in the elevated CO₂ treatments at the anthesis harvest (Table 3) since Rubisco levels in wheat flag leaves are approximately proportional to leaf nitrogen content (Evans 1983). Rubisco constituted 64–70% of soluble protein in wheat, grown at various levels of CO₂, temperature and nitrogen supply (Delgado *et al.* 1994). In our experiments, the decrease in leaf nitrogen levels

Table 4 Cumulative intercepted photosynthetically active radiation (PARCUM, MJ m⁻²) and crop light-use efficiency (LUE, g biomass MJ⁻¹ intercepted PAR)

Year	Variable	Treatment					Statistical effects		
		350/ warm	350/ cooled	700/ warm	700/ cooled	amb. plots	CO ₂	temp.	CO ₂ temp.
1995	PARCUM (MJ m ⁻²)	298	381	287	341	624	NS	**	NS
	LUE (g MJ ⁻¹)	3.31	3.34	4.09	4.14	2.67	**	NS	NS
1996	PARCUM (MJ m ⁻²)	377	464	370	442	638	NS	**	NS
	LUE (g MJ ⁻¹)	3.38	3.58	4.19	3.86	2.62	*	NS	NS

was not the result of dilution in the canopy, as leaf biomass was generally decreased rather than increased by elevated CO₂ (Table 2). The mechanism underlying photosynthetic downregulation at elevated CO₂ is not well understood. Elevated sugar levels, as also observed in our experiments (Table 3) may trigger the effect (Bowes *et al.* 1996). However, a direct negative feedback of increased sugar levels on photosynthetic rates, without affecting Rubisco, cannot be excluded either (Neales & Incoll 1968).

Measurements of photosynthesis in ambient plots were only carried out in 1995, and showed equal or decreased photosynthetic rates at light saturation compared to cooled OTCs at 350 ppm CO₂ (Fig. 1). This was consistent with the low values of LUE for ambient plots, observed in both years (Table 4), but it was surprising in view of the normal to high leaf nitrogen concentrations, which suggested no lowering of photosynthetic capacity. Study of chamber effects on within-tissue nitrogen allocation may be needed for clarification.

Effects of elevated CO₂ and temperature on grain yield

Two-degree cooling increased grain yield by 10–30%. As described above, cooling did not effectuate this increase via a stimulation of photosynthesis. Instead, cooling increased LAI (Table 2) and lengthened the growing season (Table 1). Both effects strongly enhanced cumulative light interception while light-use efficiency was unchanged (Table 4).

In most cases, grain yield was also increased by CO₂-doubling, but never by more than 20%, and in the cooled OTCs in 1996 even a reduction of 2% was observed. Contrary to cooling, CO₂-doubling did not cause any yield increase via an effect on LAI (in fact, LAI was generally decreased by elevated CO₂), or on season length (there was no effect of CO₂ on the rate of phenological development), but only by stimulating photosynthesis. Consequently, elevated CO₂ increased productivity despite a decrease in light interception, through an increase in light-use efficiency.

In both years, CO₂-doubling increased grain yield more in the warm treatment (14%, 20%) than in the cold treatment (–2%, 11%). These results are consistent with literature reviews, which show that crop yield increase due to elevated CO₂ tends to increase with temperature (e.g. Idso & Idso 1994). There are two probable causes for the higher response to CO₂ in warm OTCs compared to cooled OTCs. First, warming may increase the sensitivity to CO₂ of photosynthesis, as explained above (Farquhar *et al.* 1980), although our study did not provide supporting evidence for this effect. Second, warming leads to crops with low LAI (Table 2), in which any stimulation of leaf growth will lead to significantly increased light interception and thus to extra growth enhancement. Also, the majority of leaves in small canopies tend to be light-saturated, which enhances their responsiveness to CO₂. In our experiments, elevated CO₂ reduced rather than increased LAI and cumulative light interception, but the reduction was smallest in the low-LAI warm OTCs. The lower yield response to elevated CO₂ in cooled OTCs was not related to impaired sink formation, as all yield components including grain density (Table 2) and tiller density (Van Oijen *et al.* 1998) were increased by cooling.

We conclude that the use of OTCs in CO₂ experiments will lead to overestimation of CO₂ response relative to ambient conditions outside OTCs, because chamber warming will decrease LAI and, possibly, increase leaf photosynthetic sensitivity to elevating CO₂. In our experiments, average yield response to CO₂-doubling was twice as high in normal OTCs as in cooled OTCs.

The two years did not give identical results. Effects of cooling, effects of CO₂, and yield differences between ambient plots and OTCs, were all smaller in 1996 than in 1995. A greater number of measurements showed statistically significant effects of CO₂ and temperature in 1995 than in 1996, especially at the anthesis harvest. The smaller effect of cooling in 1996 is partly explained by smaller temperature differences between cooled and warm OTCs. The other differences in effects between years, however, may have been due to the higher ambient

temperatures in 1995. This may directly have increased the CO₂ effect on photosynthesis, as explained above, but may also have aggravated the negative effects on yield of OTC-induced warming, with consequently greater differences between OTCs and ambient plots and greater response to cooling. This view is supported by the overall higher yield levels in 1996, indicating that temperatures were closer to optimal in that year. This was also evident from the observation that individual grain weights as well as the number of grains per ear were on average 20% and 15% higher, respectively, in 1996 than in 1995 (Table 2). We conclude that overestimation in OTCs of CO₂ effects on yield may be especially large at sites where the investigated crop species already in ambient plots experiences a temperature that is above optimum.

When we combine the information presented in Tables 2 and 3, we find that elevated CO₂ led to 21–33 g m⁻² extra sugars at anthesis. After anthesis vegetative growth ceases, so these sugars will become available for grain growth. However, elevated reserve levels did not constitute the major source of increased grain growth at elevated CO₂. Grain growth was stimulated by elevated CO₂ by a margin more than twice the size of the increase in sugars (except in the cooled treatment in 1996). In spite of the observed downregulation of photosynthesis, the major cause of increased grain yield in our experiments thus was increased photosynthetic rate during grain-filling, rather than increased pre-anthesis build-up of reserves.

Nutrient relations

Elevating CO₂ decreased nitrogen content and increased sugar levels in leaves, stems and grains. This is consistent with many earlier reports (see Drake *et al.* 1997). The decrease in stem N concentration may have been the result of dilution by the extra dry matter at elevated CO₂: at anthesis, average stem dry mass per unit ground area, across both temperatures and years, was 433 g m⁻² at 350 ppm CO₂, and 507 g m⁻² at 700 ppm (Table 2). However, the plants probably downregulated nitrogen uptake as well: average total nitrogen amount in the foliage (stems + leaves) at anthesis was decreased from 10.5 to 9.0 g N m⁻² when CO₂ was doubled (data derived from combining Tables 2 and 3). Although the downregulation of uptake may be related to the photosynthetic acclimation and the resulting low yield response to elevated CO₂ observed in our experiments, it may also be beneficial to the plants in postponing exhaustion of soil nutrients in a CO₂-enriched environment. On the other hand, decreased grain N concentrations may be unwanted because of reduced grain quality for baking and other purposes.

The increase in sugar levels at elevated CO₂ was likely

the result of both direct stimulation of carbohydrate production in photosynthesis, and the indirect effect of decreased nitrogen concentrations leading to lowered maintenance respiration rates.

Cooling had similar effects as elevated CO₂ on stems and grains, i.e. decreased N-contents and increased sugar levels, but in this case only reduced maintenance respiration is a likely explanation. However, cooling did not increase sugar levels in leaves. Possibly this was related to increased sugar use for foliage growth which was stimulated in cooled OTCs, but detailed carbon balance studies are required to clarify this.

Concluding remarks

1 Cooling removed part of the differences between chamber-grown plants and plants from ambient plots. Because chamber warming is a common phenomenon in all OTC types (Heagle *et al.* 1988), a cooling system may be a useful attribute in any OTC experiment. In our experiments, however, most measurements still showed some difference between ambient plots and OTCs (Table 2). This was also the case for the cooled OTCs at 350 ppm CO₂, which were expected to resemble the field situation best. Chamber effects thus are not fully accounted for by the temperature increase that had been corrected by the cooling system. Other chamber effects than temperature elevation, such as light reduction and suppression of UV-b, may have to be corrected as well.

2 The higher response to elevated CO₂ in noncooled vs. cooled chambers suggests that temperature differences between sites in different climatic regions may equally cause differences in CO₂ effects.

3 Photosynthetic acclimation to elevated CO₂ did not become pronounced until well after anthesis. This contrasts with observations that elevated CO₂ already at anthesis had noticeably reduced tiller density, number of green leaves per tiller, and specific leaf area (Van Oijen *et al.* 1998). We conclude that in spring wheat morphological adaptations at the canopy level to elevated CO₂ may precede physiological adaptation at the leaf level. Together the adaptations explain why elevated CO₂ only marginally increased grain yield.

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