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Effects of elevated CO_2 on development and morphology of spring wheat grown in cooled and non-cooled open-top chambers

M. van Oijen[ABC], A.H.C.M. Schapendonk[B], M.J.H. Jansen[B], C.S. Pot[B], J. van Kleef[B] and J. Goudriaan[A]

[A]*Wageningen Agricultural University, Department of Theoretical Production Ecology, P.O. Box 430, 6700 AK Wageningen, The Netherlands.*
[B]*Research Institute for Agrobiology and Soil Fertility (AB-DLO), P.O. Box 14, 6700 AA Wageningen, The Netherlands.*
[C]*Author for correspondence; email: m.a.vanoijen@ab.dlo.nl*

Abstract. Facilities for studying effects of elevated CO_2 on crops affect the microclimate in the crop. Open-top chambers may increase temperature by 1–3°C compared to ambient conditions.

This paper describes a newly developed cooling system for open-top chambers. In 1995 and 1996, experiments were carried out to test the system and analyse the effects of temperature on crop phenological and morphological response to elevated CO_2. Spring wheat (*Triticum aestivum* L. cv. Minaret) was subjected to ambient and doubled CO_2 concentration in both cooled and non-cooled chambers.

The cooling system reduced temperature by 1.6–2.4°C, and this delayed maturity by 10 days. In contrast, elevated CO_2 did not affect phenological development. Elevated CO_2 reduced tiller density, green leaf number per tiller and specific leaf area, thereby reducing the capacity for light interception of the crop. Crop height growth before anthesis mainly responded to temperature, but after anthesis it was only affected by CO_2, indicating a shift from sink- to source-limited growth. For none of the parameters studied, a significant statistical interaction of CO_2 and temperature was found.

The cooling system proved effective. A temperature difference of about 2°C affected crop development and morphology more strongly than CO_2 doubling. However, the absence of CO_2-temperature interaction suggests that CO_2 effects may validly be investigated even without a cooling system.

Introduction

Cure (1985) showed in a literature review of eight papers, that grain yield of wheat (*Triticum aestivum* L.) may increase by 35 ± 14% due to CO_2 doubling. Seven of the papers report on data from growth chamber experiments. In a recently concluded international research programme, 'ESPACE-Wheat' (Hertstein *et al*. 1996), there was an attempt to assess the effects of CO_2 on spring wheat cv. Minaret in more field-like conditions, by using open-top chambers (OTCs). OTC experiments with at least two levels of CO_2 were carried out in eight European countries. Although OTCs are more field-like than growth chambers, the growing conditions in OTCs still deviate in many ways from ambient conditions (e.g. increased temperature, reduced light intensity, reduced relative air humidity, increased constancy of the windspeed pattern and increased spatial variability of rainfall; Heagle *et al*. 1988). It is generally assumed that these chamber effects do not modify the effects of elevated CO_2, but we found no support in the literature for this assumption.

The ongoing rise of the atmospheric concentrations of CO_2 and other greenhouse gases is expected to cause global warming. Interactive effects of CO_2 and temperature on crops thus are of interest, but until recently the interactions have received little study (Allen 1990). Also the fact that the instruments with which CO_2 effects are investigated, such as OTCs, tend to increase temperature above ambient levels, warrants closer investigation of the CO_2-temperature interaction. So far, in cereals, the interaction has only been studied in temperature gradient tunnels (e.g. Horie *et al*. 1995; Rawson 1995; Batts *et al*. 1996; Sanhewe *et al*. 1996) and growth chambers (e.g. Lawlor *et al*. 1993; McKee and Woodward 1994; Frank and Bauer 1996; Slafer and Rawson 1997). Both types of facilities deviate from field conditions in radiation pattern and fluctuations of humidity. Reports on the interaction between CO_2 and temperature in OTC-grown wheat are scarce, so Lawlor and Mitchell (1991) suggested that cooled OTCs should be used for this purpose. So far, however, no cooled OTCs have been used (Lawlor 1996). For this reason, we developed a cooling system for OTCs that can decrease OTC temperature to near-ambient levels. This paper describes the cooling system and two experiments in which it was used.

When OTCs are not cooled, the higher temperatures within may accelerate plant phenological development (Adaros *et al*. 1989). Anthesis of spring barley (*Hordeum vulgare* L.) was about 7–8 days earlier in OTCs than under ambient conditions (Buckenham *et al*. 1982), and maturity of spring wheat was reached 14 days earlier in OTCs than outside (Dijkstra *et al*. 1995). The morphology of the crop may also be affected by the use of OTCs: increased plant height is commonly observed (Heagle *et al*. 1988; Adaros *et al*. 1989) and for

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spring barley a 27% reduction in ear number at anthesis has been reported (Adaros *et al*. 1989). It is unclear whether crop development and morphology are also influenced by elevated CO_2. Rate of development towards flowering was positively correlated with CO_2 concentration in cowpea (*Vigna unguiculata* L.) but showed a negative correlation in sorghum (*Sorghum bicolor* L.) and soybean (*Glycine max* L.) (Ellis *et al*. 1995). The majority of reports on cereals show little effect of elevated CO_2 on development rate (Slafer and Rawson 1997). Few reports are available on the effects of elevated CO_2 on wheat morphology. Barnes *et al*. (1995) reported that CO_2 doubling increased tillering, but Batts *et al*. (1996) reported CO_2-induced extra tillering only in one of two experimental years. In view of the scarcity of reports and the possibility of interaction with effects caused by the use of OTCs, research on the interaction between temperature and CO_2 in OTCs would be useful.

This paper presents technical details of the newly developed cooling system for OTCs, and reports on results of the experiments with the facility, aimed at determining to what extent temperature dependent changes in development and morphology of spring wheat affect the response of the crop to elevated CO_2.

Materials and methods

Experimental set-up

Two experiments were carried out, in 1995 and 1996, on light clay soil (pH 7.5, organic matter content 6%), in Wageningen, the Netherlands (51°58′ N, 5°40′ E). The experiments were done in a factorial set-up, with two levels of CO_2 and two levels of temperature, in three replicates, giving 12 OTCs in all, and a fifth treatment consisting of six ambient plots.

OTCs were equilateral hexagons of 1.95 m height, with 1.5 m between parallel sides, giving 1.95 m^2 soil area completely sown to spring wheat cv. Minaret. Chamber walls were made of 3 mm thick polycarbonate (LEXAN®), which is 88% transparent to photosynthetically active radiation, but absorbs UV-B. The OTCs were surrounded completely by 75 cm of additional wheat plants, of the same variety, to minimize border effects by irradiation from the sides. CO_2 was supplied via tubes on the soil surface and via an airbag at 1.3 m height. Air-exchange rate was approximately 3.6 times per minute. Additional tubes on the soil surface were used for irrigation whenever the topsoil dried out visibly. Fertilization was given slightly above common farming practice to prevent nutrient deficiency. Also, careful weed- and disease-control was maintained to ensure optimal crop growing conditions.

The plants were sown on 3 April 1995 and 21 March 1996. Sowing distance was 12.5 cm between rows and 3 cm (1995) or 2.5 cm (1996) within rows. Fifty percent emergence followed after 13 days in 1995 and after 20 days in 1996. Plant density, determined at anthesis, was lower in 1995 (203–218 plants m^{-2}) than in 1996 (220–251 plants m^{-2}).

Cooling system

The two levels of temperature were created by coupling the air-inlets of half of the OTCs to a water-cooled system. The cooling system consisted of one cooling tank with water pump, six radiators, and tubing for water circulation (Fig. 1). Water was cooled to about 7°C in the 4 m^3 cooling tank (type Alfa-Laval compact, designed for preserving milk). Cooling capacity of the tank was 11 kW at 32°C ambient temperature. The cooled water was pumped through isolated polyethylene tubes to the six radiators placed before the air-inlets of different OTCs. The water pump (type Iwaki MDH 32RV) had a capacity of 12 m water elevation at a throughput of 120 L min^{-1}. The radiators used (Opel-Kadett type A/C) were designed for cooling car engines. After passing through the radiators, water circulated back to the tank for re-cooling.

Climatic conditions

Ambient levels of incoming radiation were measured at 5-min intervals at 100 m from the OTC site. Extensive measurements of radiation patterns inside OTCs, at various positions and times, had shown an average reduction of light intensity in these OTCs of 25% compared to ambient measurements (Dijkstra *et al*. 1995). The average radiation level was lower in 1996 than in 1995 (Table 1).

Table 1. Climatic averages during the growing season (emergence to maturity)

[CO_2] of ambient CO_2-OTCs assumed equal to ambient plots

	Treatment	1995	1996
[CO_2] at 11 a.m. (μL^{-1})	Ambient CO_2/warm	366	379
	Ambient CO_2/cooled	366	379
	Elevated CO_2/warm	716	751
	Elevated CO_2/cooled	720	756
	Ambient plots	366	379
Temperature (°C)	Ambient CO_2/warm	18.0	16.8
	Ambient CO_2/cooled	16.4	15.8
	Elevated CO_2/warm	18.3	16.9
	Elevated CO_2/cooled	16.9	15.6
	Ambient plots	16.2	14.6
Radiation (MJ m^{-2} d^{-1})	Ambient CO_2/warm	13.9	12.5
	Ambient CO_2/cooled	14.0	12.2
	Elevated CO_2/warm	13.9	12.5
	Elevated CO_2/cooled	14.0	12.2
	Ambient plots	18.6	16.3
Vapour pressure at 9 a.m. (kPa)	Ambient plots	1.31	1.22

Temperature was measured at 5-min intervals in each of the OTCs and ambient plots, by thermocouples placed in the centre of the plots at 40 cm height under a small white sun cap. The measurements were assumed to characterize in-canopy temperature equally well across the different treatments. The assumption is likely correct when comparing different chamber treatments, which have the same air circulation pattern, but possible differences in canopy temperature gradients between chambers and ambient plots (Drake *et al*. 1989) were not assessed. Calibration of the thermocouples was checked weekly, and proved constant. The cooling system reduced the temperature in OTCs to levels slightly above those in ambient plots. 1996 was cooler than 1995 (Table 1).

For the elevated CO_2 treatment, pure CO_2 was added just before the air-inlet of the chambers, where it was thoroughly mixed with ambient air in the chamber ventilator. CO_2 concentrations were measured weekly, and needed adjustment only during the initial weeks of experimentation. In 1995, elevated CO_2 levels were 716 ± SEM 9.5 $\mu L\ L^{-1}$ in warm chambers, and 720 ± 6.7 $\mu L\ L^{-1}$ in cooled chambers (Table 1). In 1996, the elevated levels were 751 ± 6.7 $\mu L\ L^{-1}$ and 756 ± 9.7 $\mu L\ L^{-1}$ in warm and cooled chambers, respectively.

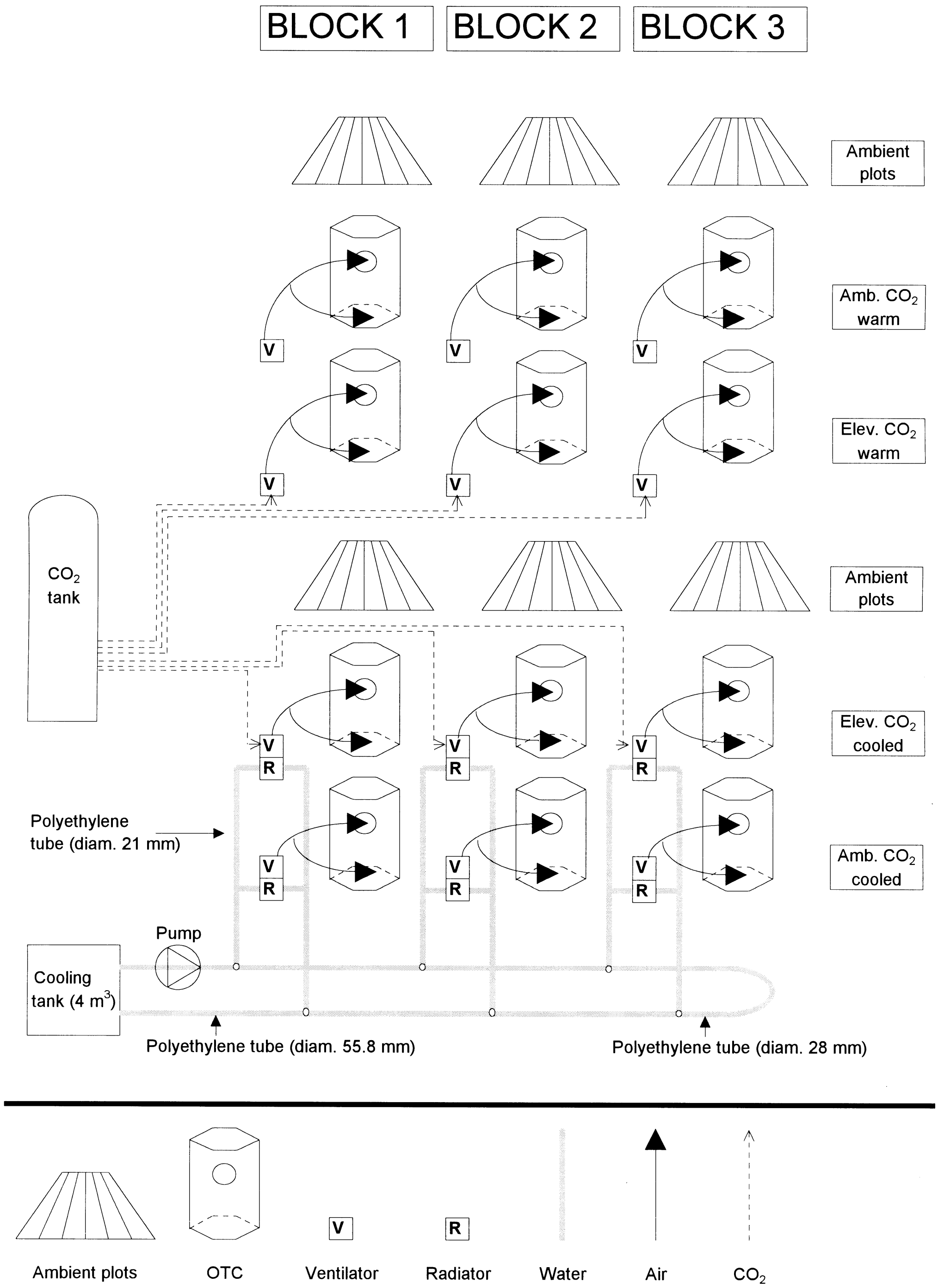

Fig. 1. Lay-out of the cooling system, with indication of the flows of cooling-water, air and CO_2. Flows of irrigation-water, to each plot, are left out. Note that, contrary to what is shown in the figure, the actual positions of OTCs and ambient plots were randomized within each block.

Ambient levels of atmospheric humidity were measured daily at 9 a.m. at 1800 m from the OTC site (Table 1). Measurements of air humidity inside chambers were carried out on 11 April 1995 (at an average ambient temperature during measurements of 17.3°C) and on 25 July 1995 (28.7°C). On these respective days vapour pressure was 1.15 ± SEM 0.006 kPa and 1.14 ± 0.008 kPa in warm chambers and 1.14 ± 0.008 kPa and 1.15 ± 0.007 kPa in cooled chambers. The differences were not statistically significant. Cooling did significantly increase relative humidity from 55 to 57% and from 27 to 29% on the two respective days, by decreasing saturating vapour pressure. Absence of cooling-induced condensation was verified by frequent checking of the radiators throughout both experiments; liquid water on the surface was never observed.

Plant measurements and statistical analyses

We assessed the extent to which temperature affected the developmental and morphological response of spring wheat to CO_2-doubling. Effects on development were assessed as changes in developmental stage (Tottman and Broad 1987), and changes in rate of chlorophyll loss during senescence. Effects on morphology were assessed as changes in number and height of tillers, number and specific area of green leaves, and percentage ground cover.

Every week, five plants were randomly chosen in each OTC and ambient plot, for non-destructive measurement of development stage and plant height. The developmental stage was recorded according to the decimal code of Tottman and Broad (1987).

Chlorophyll measurements were taken in 1996 only, from early June to crop maturity. Every week, in each plot, five top leaves were chosen randomly, and three chlorophyll measurements were taken per leaf using the Minolta SPAD-502 meter. The SPAD readings were converted to total chlorophyll contents by a calibration curve that related SPAD readings to spectrophotometrically measured contents of Chlorophyll *a* and *b* (Chl (mmol m^{-2}) = 0.0026 SPAD + 0.00024 $SPAD^2$; $r^2 = 0.99$).

At the end of anthesis (i.e. at decimal code of development DC69 in 1995 and DC70 in 1996), 0.5 m^2 was harvested from each plot, to determine morphological canopy characteristics: specific leaf area, number of green leaves per main stem and tiller density. End of anthesis, and the accompanying harvest, was at different dates depending on the growing conditions. In 1995, the harvests were 79, 87 and 86 days after planting for warm OTCs, cooled OTCs and ambient plots, respectively. In 1996, the respective harvests were 88, 95 and 96 days after planting.

The percentage of soil covered by green leaves was estimated visually at weekly intervals.

Statistical analysis was carried out separately for the 1995 and 1996 experiments. All measurements were analysed using analysis of variance (ANOVA) corresponding to the two-factorial randomized block designs used; the ambient plots were not included in the ANOVA's.

Results

Effectiveness of the cooling system

In both years, throughout the season, daily average temperature in non-cooled OTCs was well above that in ambient plots (Fig. 2). On average, it was 2.8°C higher than in the ambient plots. In contrast, temperature in cooled OTCs closely followed the time course of temperature in ambient plots, but cooled chambers were still slightly warmer than ambient plots (0.4°C in 1995 and 1.2°C in 1996).

The effectiveness of the cooling system increased with ambient plot temperature due to increased temperature difference between cooling water and atmosphere (Fig. 3*A*). Therefore, in a typical diurnal course (Fig. 3*B*), temperature in cooled OTCs was continuously below that of warm OTCs, but it always remained higher than in ambient plots except for the middle part of the photoperiod.

Phenology

Crop development was not affected by CO_2, but it was delayed by cooling (Table 2). In cooled OTCs, the time course of development, quantified by the decimal code, closely followed crop development in ambient plots (Fig. 4). In both experimental years, maturity (decimal code DC90) was reached about 10 days later in cooled OTCs and ambient plots than in warm OTCs (Fig. 4).

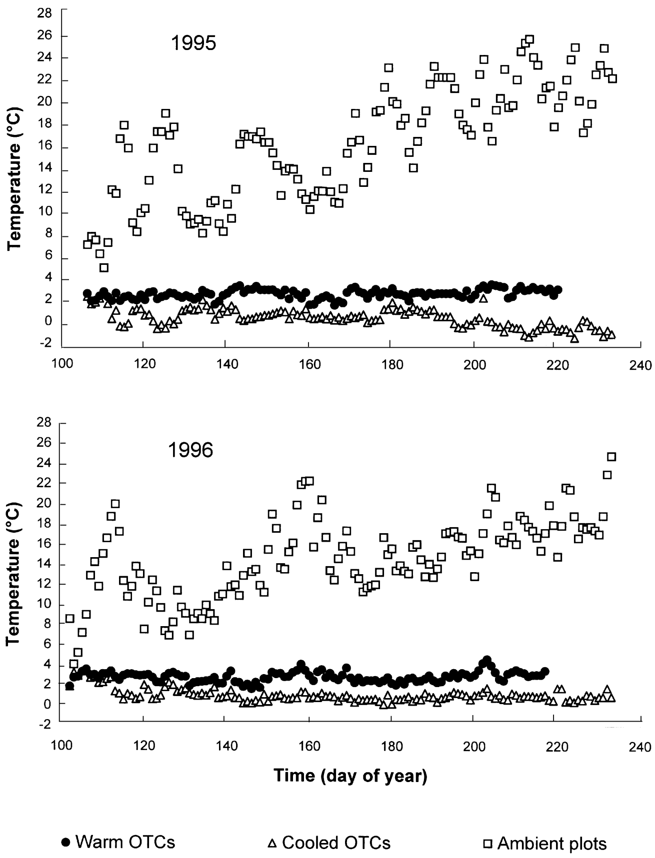

Fig. 2. Seasonal courses of daily average temperature for ambient plots (*n*=6), and of temperature offset (i.e. difference with ambient plots) for warm OTCs (*n*=6) and cooled OTCs (*n*=5).

Table 2. Decimal code of crop development (Tottman and Broad 1987)

Treatment	1995 June 12	1995 July 14	1996 June 12	1996 July 9
Ambient CO_2/warm	58	82	65	79
Ambient CO_2/cooled	43	77	59	76
Elevated CO_2/warm	57	82	67	80
Elevated CO_2/cooled	41	79	59	75
Ambient plots	46	78	57	73

Leaf chlorophyll content

Upper leaves from warm OTCs had similar chlorophyll contents as leaves from cooled OTCs, but leaf senescence (i.e. loss of chlorophyll) started about a week earlier (Fig. 5). Leaves from ambient plots stayed green about 2 days longer. Elevated CO_2 reduced chlorophyll content by about 5% throughout the vegetative period, but did not affect the onset of the rapid decline due to senescence (Fig. 5).

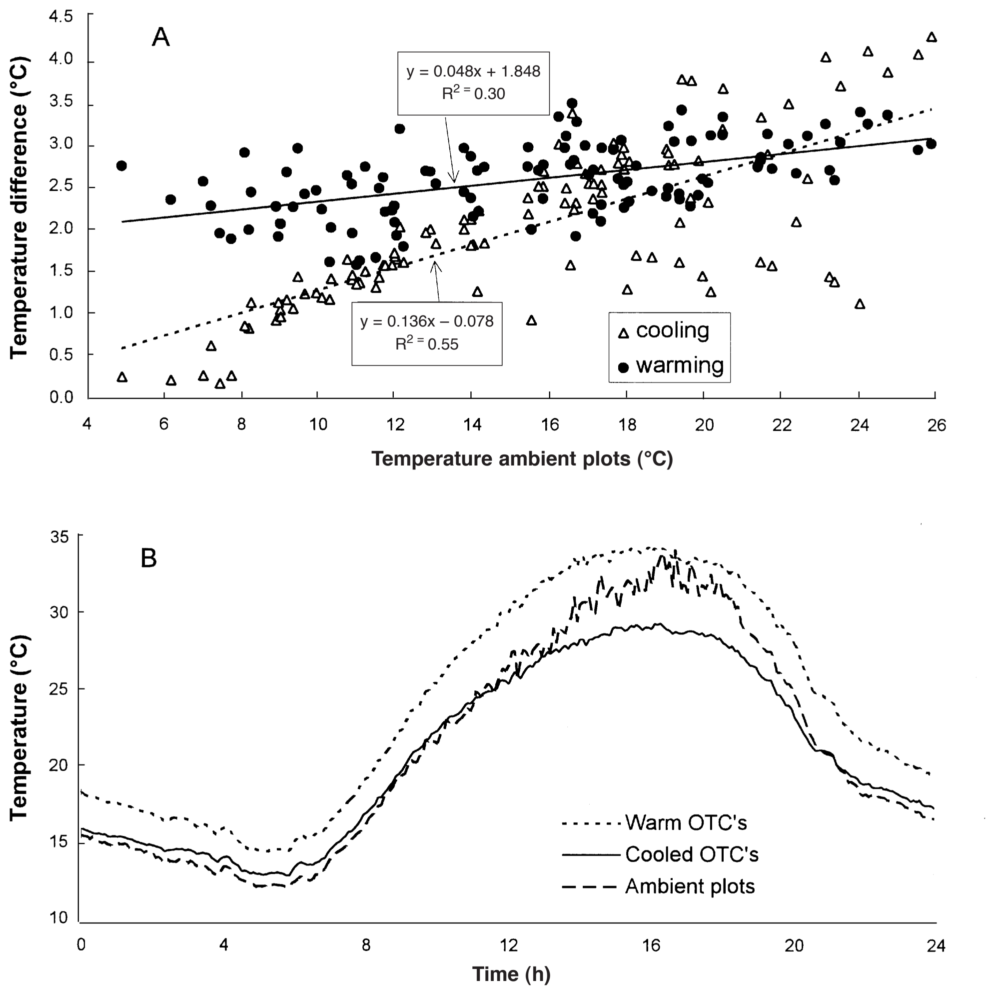

Fig. 3. Effectivness of the cooling system. *A*. All daily values for 1995 of the temperature-difference between warm OTCs and ambient plots (= 'warming') and between warm OTCs and cooled OTCs (= 'cooling') vs temperature of ambient plots; *B*. Typical example of a diurnal curve of temperature for ambient plots, warm OTCs and cooled OTCs (5 August 1995).

Ground cover

In both years, percentage ground cover was highest in the ambient plots (Fig. 6). The difference between ambient plots and OTCs, which already appeared during the first two months after planting, was especially pronounced in 1995 (Fig. 6). Elevated CO_2 had a small negative effect on ground cover (Fig. 6). Cooling, on the other hand, increased the percentage of ground cover relative to warm OTCs (Fig. 6). Cooling not only increased the maximum level of ground cover but also its duration, but levels were still lower than in ambient plots. These observations on ground cover were consistent with those on developmental stage (Fig. 4) and chlorophyll content (Fig. 5) in that cooling delayed crop maturity by about 10 days, whereas CO_2 had little effect.

Leaf measurements

There was no consistent effect of temperature on the number of green leaves on main stems at anthesis: cooling caused a decrease in 1995, but an increase in 1996 (Table 3). Elevated CO_2 consistently decreased the number of green leaves per main stem by 4 to 9%. In both years, plants from ambient plots had the lowest number of green leaves per main stem.

Specific leaf area (SLA) was increased by cooling (2 to 6%) and decreased by elevated CO_2 (2 to 5%), but the effects were not statistically significant (Table 3).

Tiller measurements

In both years, tiller density in OTCs was lower than in ambient plots (Table 3). However, cooling had a positive

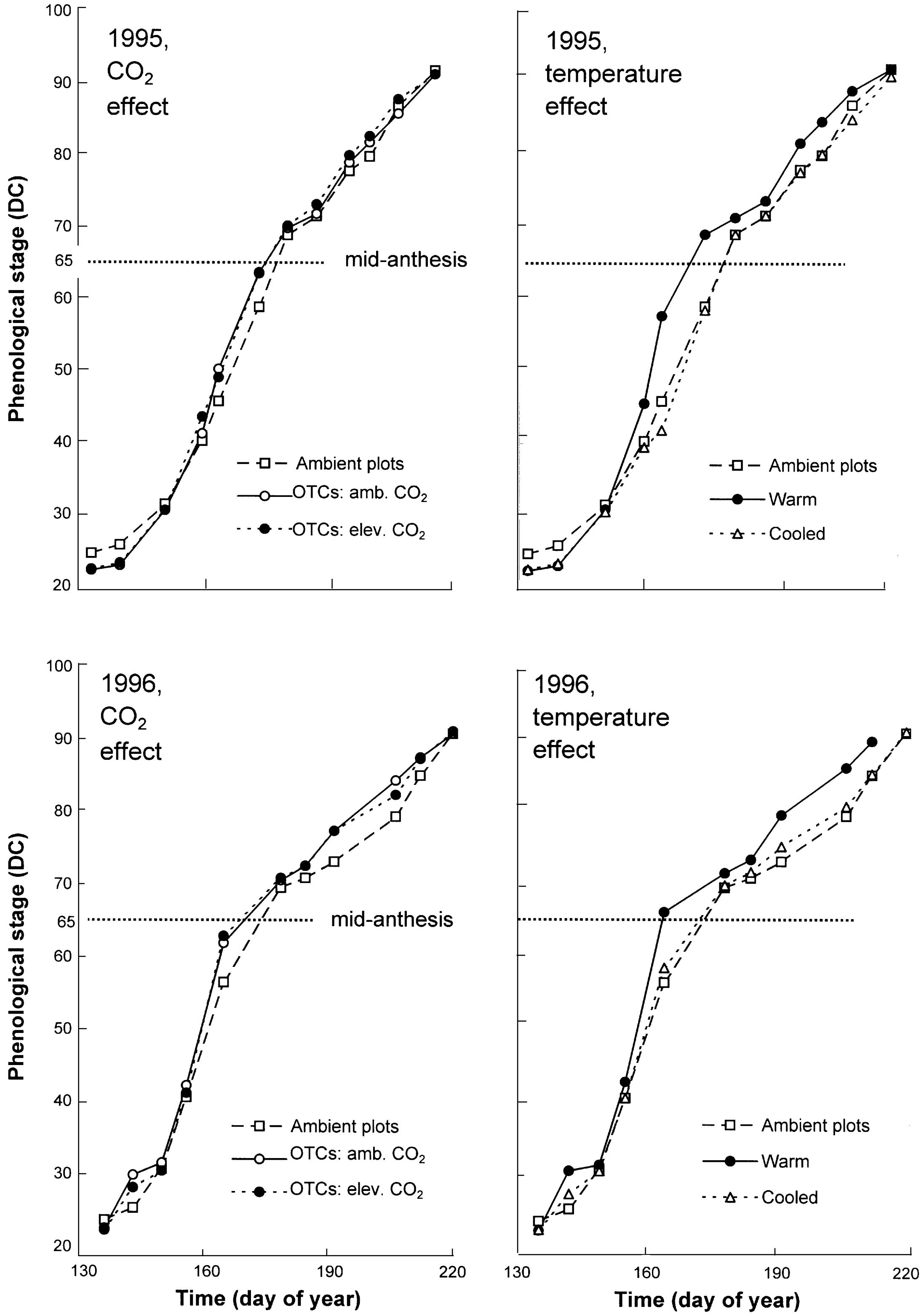

Fig. 4. Phenological development in 1995 and 1996, quantified as the increase in decimal code of development (DC; Tottman and Broad 1987) vs time. The lines connecting points in the graph are added for clarity; they are not intended to suggest that the developmental scale is continuous.

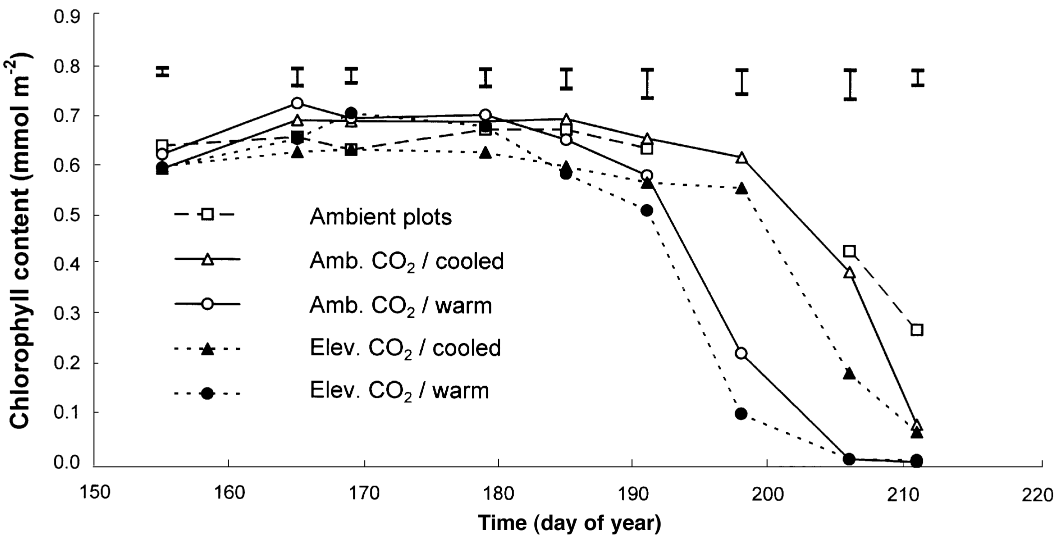

Fig. 5. Chlorophyll content of upper leaves vs time in 1996. Data were taken by means of SPAD-measurement. Vertical bars above data indicate standard errors of difference for the four OTC treatments (*df* = 6).

Table 3. Canopy characteristics at the end of anthesis

OTC treatments with ambient CO_2 are labelled '350', those with elevated CO_2 '700'. The rightmost three columns show results from analyses of variance for the four OTC-treatments only (i.e. ambient plots left out). Statistical significance of CO_2, temperature- and interaction effects is indicated as ** ($P < 0.01$), * ($P < 0.05$) or n.s. (not significant)

Year	Variable	Treatment					Statistical effects		
		350/ warm	350/ cooled	700/ warm	700/ cooled	amb plots	CO_2	temp.	CO_2× temp.
1995	Tiller density (# plant^{-1})	2.31	2.87	2.27	2.73	3.16	n.s.	**	n.s.
	Tiller density (# m^{-2})	432	606	495	570	666	n.s.	**	*
	Green leaves (# per main stem)	4.73	4.07	4.4	3.87	3.77	*	**	n.s.
	SLA (cm^2 g^{-1} d.m.)	204	208	197	203	202	n.s.	n.s.	n.s.
1996	Tiller density (# plant^{-1})	2.55	3.04	2.35	2.70	2.91	n.s.	*	n.s.
	Tiller density (# m^{-2})	620	665	587	632	728	n.s.	n.s.	n.s.
	Green leaves (# per main stem)	3.26	3.55	3.12	3.24	3.07	**	**	**
	SLA (cm^2 g^{-1} d.m.)	248	254	235	250	240	n.s.	n.s.	n.s.

effect, and mitigated the differences between OTCs and ambient plots. CO_2 had little effect on tiller density.

In both years, plants in cooled OTCs initially were shorter than plants in warm OTCs, but after day 180 this was reversed (Fig. 7). This was caused by a late period of higher growth rates in cooled OTCs, which after day 160 still elongated by an additional 20–25 cm, whereas in warm OTCs this was only 10–15 cm more. In 1995, all plants in chambers elongated less than plants of ambient plots, but in 1996 only chambered plants at ambient CO_2 remained smaller than ambient plot plants. The effect of CO_2 was less pronounced than the effect of temperature. Only at the end of the growing season were plants grown in elevated CO_2 taller than plants grown in ambient CO_2 for both temperature levels (Fig. 7).

Discussion

Effects of CO_2 and temperature on crop development

We found no evidence of accelerated development or senescence due to elevated CO_2. Neither the progressive increase in decimal code of development, nor the post-anthesis gradual decrease in chlorophyll content and ground cover, were affected by CO_2. This is consistent with the common observation that phenological development of wheat is strongly dependent on temperature, but independent of atmospheric concentration of CO_2 (Batts *et al.* 1996). However, some reports on CO_2-induced acceleration of wheat development exist: Marc and Gifford (1984) found anthesis to be 3 days earlier at doubled CO_2, and Sicher and Bunce (1997) concluded from reduced post-anthesis

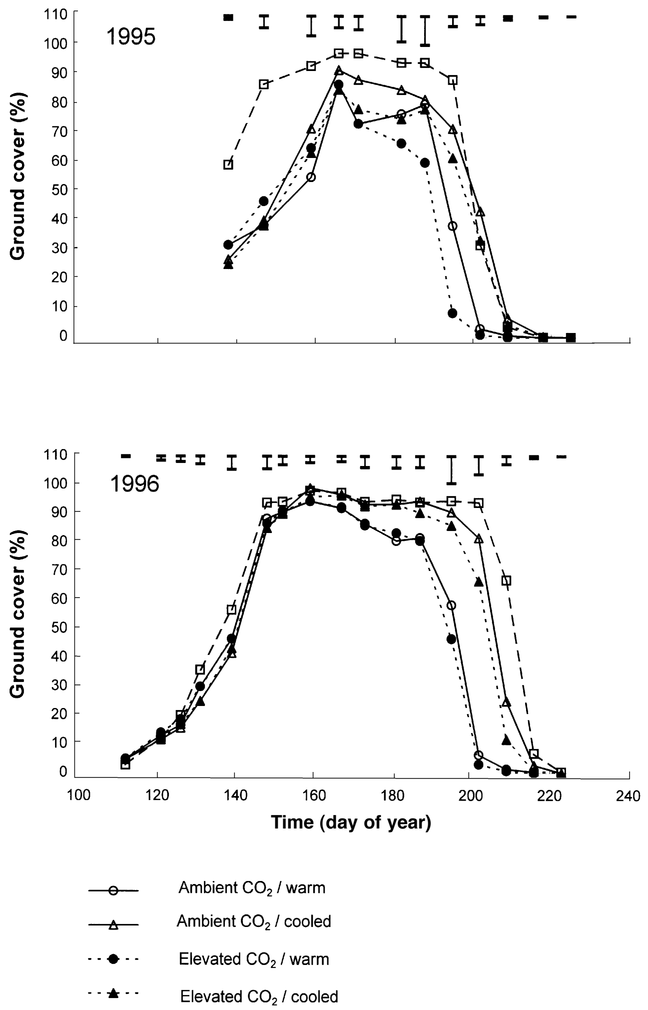

Fig. 6. Seasonal courses of ground cover by green foliage in 1995 and 1996. Vertical bars above data indicate standard errors of difference for the four OTC treatments (*df* = 6).

chlorophyll contents in flag leaves of OTC-grown winter wheat that senescence was accelerated at doubled CO_2. The data of Sicher and Bunce (1997) may, however, be interpreted differently. The chlorophyll content was equally reduced at their first measurement date, two months before maturity, as near maturity itself (always about 20% reduction, which exceeds the reduction seen in our experiments). The change in chlorophyll content may, thus, be the result of a stable level of down-regulation rather than earlier senescence. Our results agree with the findings of Slafer and Rawson (1997), who concluded that CO_2 does not influence development in wheat to a degree relevant to agronomy.

In contrast to CO_2, temperature strongly affected development rate. Compared to ambient plots, OTCs without cooling caused accelerated senescence and earlier maturity. Cooling largely, but not completely, prevented the acceleration of development which is consistent with the fact that the average temperature in cooled OTCs was slightly above that of ambient plots. In wheat, the rate of phenological development is known to be linearly related to daily average temperature irrespective of the thermal amplitude (Wardlaw *et al.* 1980; Slafer and Rawson 1995).

Effects of CO_2 and temperature on canopy morphology

Elevated CO_2 did affect the morphology of the wheat crop. In both years, and at both temperature levels, elevated CO_2 slightly reduced tiller density per plant, contrary to findings by earlier researchers (e.g. Sionit *et al.* 1981). In fact, the general pattern of crop response to elevated CO_2 was a reduced investment in production and maintenance of foliage: besides tiller density, number of green leaves per main stem and SLA were all slightly reduced. These changes in morphological parameters may be considered as acclimation at the canopy level to the improved conditions for crop photosynthesis. It would thus constitute an additional acclimation mechanism besides the more commonly reported down-regulation of leaf photosynthesis (e.g. Allen 1990; McKee and Woodward 1994; Sicher and Bunce 1997). The canopy acclimation occurred at both temperature levels, and is therefore probably not an artefact due to chamber warming. The reduced investment in foliage at elevated CO_2 caused a lowering of the percentage ground cover, especially during the second half of the growing season.

The relatively high temperature in non-cooled OTCs affected crop morphology similarly as elevated CO_2 in that tillering, SLA and ground cover were all reduced. Temperature differed from CO_2 in its effect on the number of green leaves per stem. Warm OTCs were the treatments with highest leaf number in 1995 but with the lowest in 1996. The inconsistency may be the result of the fact that measurement of green leaf number is very sensitive to the developmental stage at which the data are taken. Although measurements of leaf number were carried out at the same developmental stage, across all treatments, rather than on specific calendar dates, any error of a few days may have affected the number of green leaves still present. Note that this complication did not occur for the two CO_2 treatment levels, which were measured at the same day because their development was equally fast. The relationship we found between tillering and temperature is consistent with earlier literature. Fischer (1979) found that wheat tillering is strongly determined by the environmental conditions during a thermal time period of 350–400°Cd preceding anthesis. At high temperatures the duration of this sensitive period, in days, is shortened, and plants will tiller less abundantly (Fischer 1979). In the cooled OTCs, tillering and ground cover approached the levels observed in ambient plots.

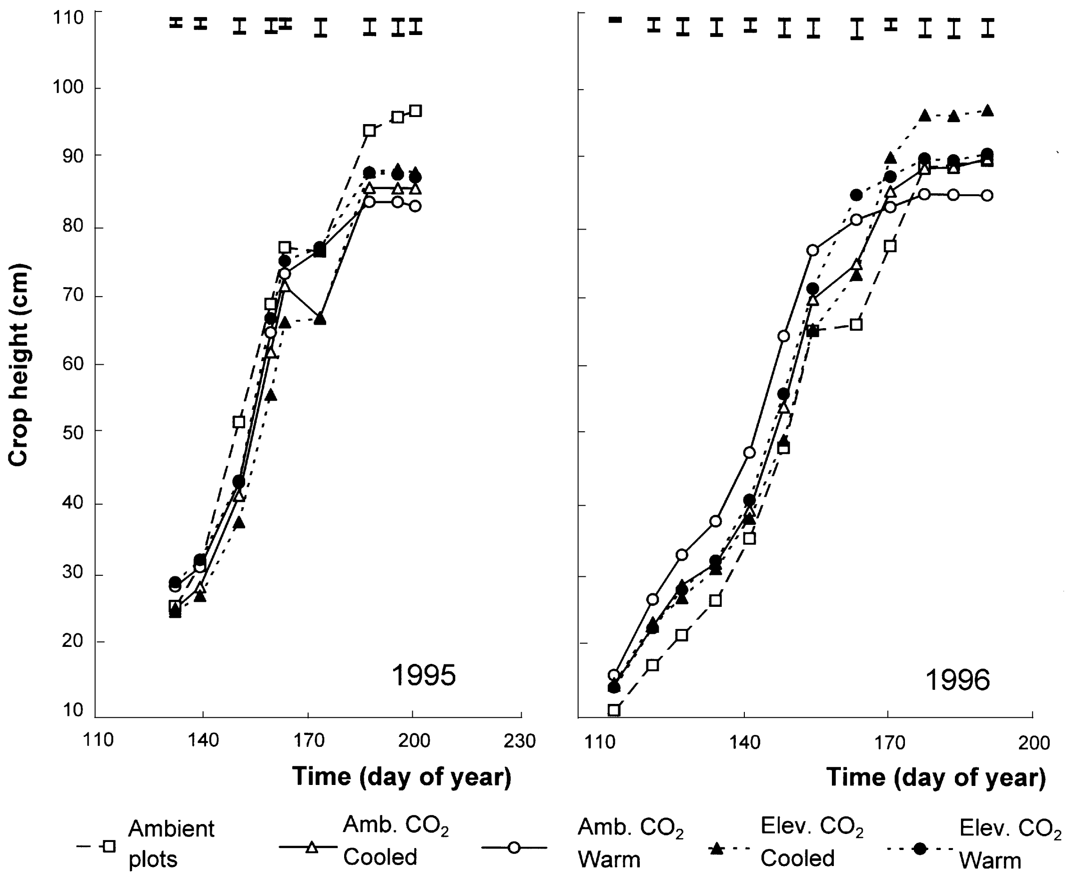

Fig. 7. Plant height growth in 1995 and 1996. Vertical bars above data indicate standard errors of difference for the four OTC treatments (*df* = 6).

With respect to plant height, cooling did not consistently diminish the differences between chamber-grown plants and plants from ambient plots. In 1995, crops of ambient plots were higher than the OTC crops, whereas the opposite occurred in 1996. The cause for the difference between the years is not clear, but possibly the low radiation levels in 1996, together with the slightly higher plant density, caused some extra height growth due to etiolation in the OTCs where light intensity was about 25% lower than in the ambient plots. Crop height growth before anthesis was mainly stimulated by high temperature, whereas after anthesis elevated CO_2 stimulated height growth. This may reflect a shift from sink-limited to source-limited foliage growth, as commonly observed in wheat (Goudriaan and Van Laar 1994).

Cooling as a means to minimize chamber effects

The cooling system maintained the average temperature in OTCs at near-ambient levels. Diurnal courses of temperature, however, were not equal in cooled OTCs and ambient plots. Cooling was more effective in reducing daily maximum temperature than minimum temperature, so daily thermal amplitudes were smaller in cooled OTCs than in ambient plots. However, the use of a cooling system with OTCs did reduce most of the differences between plants grown in OTCs and plants of ambient plots, both regarding development rate and regarding tillering and other morphological aspects of the canopy. Cooling thereby restored ground cover to near-ambient levels.

Note that the average temperatures during the growing seasons in the different treatments, as reported in Table 1, underestimate the extent of cooling. Due to the realised temperature differences, the seasons differ in length. In cooled OTCs maturity was delayed, so the growing seasons progressed further into the warm summer days of August. Therefore, the average growing season temperatures only differ 1.0–1.6°C between treatments, whereas the average cooling capacity on days that all treatments were still present was 1.6–2.4°C.

At both temperature levels, CO_2 effects on development were absent and CO_2 effects on morphology pointed to a similar level of canopy acclimation. We conclude that cooling, in spite of its effects on crop development and morphology, did not significantly alter crop responses to elevated CO_2.

Concluding remarks

(1) Even the small degree of warming in non-cooled OTCs (about 2°C) affected crop morphology and development more strongly than CO_2 doubling. Cooling effectively minimized the differences between chamber-grown and field-grown plants.

(2) Temperature did not interact with CO_2 on morphological and developmental processes. Thus OTC experiments, aimed at elucidating crop responses to elevated CO_2, are likely to be valid in this respect and not biased because of chamber effects.

(3) The present study does not exclude the possibility that chamber effects predispose the plants to a different photosynthetic and growth physiological response to elevated CO_2. A study of interactive effects of CO_2 and temperature on photosynthesis and yield of spring wheat will be presented elsewhere (van Oijen *et al.* 1998).

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