



## Increased [CO<sub>2</sub>] does not compensate for negative effects on yield caused by higher temperature and [O<sub>3</sub>] in *Brassica napus* L.

Georg Frenck<sup>a,b</sup>, Leon van der Linden<sup>a</sup>, Teis Nørgaard Mikkelsen<sup>a</sup>, Hans Brix<sup>b</sup>, Rikke Bagger Jørgensen<sup>a,\*</sup>

<sup>a</sup> Risø National Laboratory for Sustainable Energy, Biosystems Division, Technical University of Denmark, Frederiksborgvej 399, DK-4000 Roskilde, Denmark

<sup>b</sup> Department of Biological Sciences, Plant Biology, Aarhus University, Ole Worms Allé 1, DK-8000 Aarhus C, Denmark

### ARTICLE INFO

#### Article history:

Received 20 April 2010

Received in revised form 4 May 2011

Accepted 4 May 2011

#### Keywords:

Oilseed rape

*Brassica napus*

Crop yield

Carbon dioxide

Temperature

Ozone

### ABSTRACT

The projected changes of atmospheric composition and associated climatic parameters will challenge the agricultural production in ways, which existing crop populations have not previously experienced. Therefore, understanding the responsiveness to changes of multiple environmental parameters in existing genotypes is vital. In this study, the responses in yield and biomass production of four different cultivars of oilseed rape (*Brassica napus* L.) were tested under five different combinations of increased [CO<sub>2</sub>] (700 ppm), temperature (+5 °C) and [O<sub>3</sub>] (+40 ppb). Especially the multifactor treatments are relevant for predictions of the future production, as they mimic the multidimensional environmental changes that are expected within this century. All treatments were given the same amount of water, which mimicked future limited water availability e.g. in treatments with elevated temperature.

The biomass and yield parameters were found to be significantly cultivar dependent. However, in all cultivars elevated temperature caused a significant reduction in yield parameters, while biomass was not affected significantly. Elevated [CO<sub>2</sub>] increased the vegetative biomass significantly, but seed yield was only significantly enhanced in one of the four cultivars studied. Increased [O<sub>3</sub>] did not have significant effects on any of the cultivars. In general, the negative effects of a 5 °C temperature elevation on yield could not be compensated by elevated [CO<sub>2</sub>], when simultaneously applied in multifactor treatments. The evaluation of cultivar differences in productivity under elevated [CO<sub>2</sub>] in combination with increased temperatures and [O<sub>3</sub>] is necessary to derive a realistic prediction for the future food and biomass production and for the selection of cultivars providing an adaptation potential to environmental change. Our results suggest that future breeding of *B. napus* should be based on old cultivars, since more modern varieties seem to have lower potentials to respond to CO<sub>2</sub> and thus counteract the detrimental effects of yield reducing environmental factors such as temperature and O<sub>3</sub>.

© 2011 Elsevier B.V. All rights reserved.

### 1. Introduction

The concentration of carbon dioxide ([CO<sub>2</sub>]) in the world's atmosphere is steadily increasing. The projected levels of atmospheric [CO<sub>2</sub>] range from 500 to 1000 ppm (parts per million) by the end of the 21st century, depending on future emission rates of greenhouse gases (IPCC, 2007).

Since CO<sub>2</sub> and other greenhouse gases alter physical radiation properties and the energy balance of the atmosphere, they

influence the global temperature regime (McCarthy et al., 2001). Therefore, simultaneous to the increase in concentrations of greenhouse gases (e.g. CO<sub>2</sub>, CH<sub>4</sub>, N<sub>2</sub>O) global average surface temperature is projected to increase by as much as 1.8–4.0 °C within this century (IPCC, 2007).

In addition, a future increase in tropospheric ozone concentration [O<sub>3</sub>] is also most likely, since current anthropogenic emissions of precursors mediating ozone formation are predicted to increase (Fuhrer, 2009; Prather et al., 2001).

In the natural environment, none of these factors will change individually. Therefore, we need to understand how they act together and affect crop performance and productivity in an interactive way. The responses of plants to multiple interacting environmental change factors have been shown to produce changes in crop productivity, which were not always predictable from stud-

\* Corresponding author. Tel.: +45 2133 1109; fax: +45 4677 4160.

E-mail addresses: [gefr@risoe.dtu.dk](mailto:gefr@risoe.dtu.dk) (G. Frenck), [legl@risoe.dtu.dk](mailto:legl@risoe.dtu.dk) (L. van der Linden), [temi@risoe.dtu.dk](mailto:temi@risoe.dtu.dk) (T.N. Mikkelsen), [hans.brix@biology.au.dk](mailto:hans.brix@biology.au.dk) (H. Brix), [rijq@risoe.dtu.dk](mailto:rijq@risoe.dtu.dk) (R.B. Jørgensen).

**Table 1**  
Summary of experimental conditions. Target values (day/night values), achieved means and standard errors for the temperature, carbon dioxide and ozone regimes in the phytotron environment are shown for daily averages of all treatments. For carbon dioxide only daytime values are presented. Differences between the ambient and elevated regimes were highly significant for each factor ( $P < 0.001$ ).

	Temperature [ $^{\circ}$ C], day/night		Carbon dioxide [ppm]		Ozone [ppb]	
Target values:	19/12	24/17	385	700	20/20	60/20
Ctrl – control	•		•		•	
T – temperature		•	•		•	
C – CO <sub>2</sub>	•			•	•	
O – O <sub>3</sub>	•		•			•
CT – CO <sub>2</sub> + temperature		•		•	•	
CTO – CO <sub>2</sub> + temperature + O <sub>3</sub>		•		•		•
Statistics						
Mean	19.0/12.4	23.8/17.4	394.3	693.4	22.3/22.0	47.7/22.0
Standard error ( $\pm$ )	0.06/0.06	0.14/0.11	5.05	6.66	2.53/2.61	5.31/6.24

ies of single factor treatments (e.g. Luo et al., 2008; Qaderi et al., 2006). An increase in [CO<sub>2</sub>] for example, has the potential to offset reductions in photosynthesis due to drought and temperature stress (Long, 1991). Furthermore, the connection between the different processes involved in plant biomass production are not straightforward, since the complex networks of regulative interactions are linked to a multitude of limiting environmental conditions and biological parameters (Fuhrer, 2003; Körner, 2006). Hence, an increase of the photosynthetic rate, which often is the result of increased [CO<sub>2</sub>], cannot be directly linked to higher crop production and yield. Based on the results of experiments which aim to disentangle the combined effects of concurrent global change components, it is still difficult to draw generalized conclusions (Fuhrer, 2003).

With regard to an expanding human population, maximizing agricultural productivity in changing environments becomes an important challenge. Due to the negligible geographical variability of [CO<sub>2</sub>], there is almost no natural adaptation to altered [CO<sub>2</sub>] for use in future crop breeding. To create a basis for future breeding programs it is therefore needed to analyse interactions between cultivar and environment and how this affects productivity and quality of crop plants under elevated [CO<sub>2</sub>] (Ainsworth et al., 2008a). The role of genetic diversity of crop plants in relation to responsiveness to global change components has been widely disregarded. The restricted, but growing, experimental evidence, though, reveals genotypic differences in production and quality responses of crop plants to altered [CO<sub>2</sub>] (Amthor, 1998; Shimono et al., 2009; Ziska et al., 2004) as also shown for ozone (Biswas et al., 2008) and temperature (Morrison and Stewart, 2002).

Here, four cultivars of oilseed rape (*Brassica napus* L.), differing in geographic origin and age, were exposed to different, including multifactorial combinations of elevated mean [CO<sub>2</sub>], [O<sub>3</sub>] and temperatures. Two ozone treatments – one applying ozone as single factor treatment and another with increased [CO<sub>2</sub>] and temperature in combination were also conducted.

## 2. Materials and methods

### 2.1. Experimental treatments

The responsiveness of four cultivars of oilseed rape (*B. napus* L.) to elevated concentrations of CO<sub>2</sub> (700 ppm), elevated daytime levels of O<sub>3</sub> (60 parts per billion, ppb) and elevated temperature (+5  $^{\circ}$ C above ambient) were studied in six chambers of the phytotron at the Technical University of Denmark. The treatment factors were imposed separately or in combination as indicated in Table 1. The six identical gastight chambers in the phytotron (width 6 m, depth 4 m, height 3 m) used in the study are electronically and physically separated with individual control of light, temperature, humidity, [CO<sub>2</sub>] and [O<sub>3</sub>]. The environmental conditions in the phytotron

as monitored during the study are summarized in Table 1. The control treatment (Ctrl) simulated presents Danish early summer daytime conditions. A representative O<sub>3</sub> background concentration of 20 ppb was provided day and night to all treatments. Elevated O<sub>3</sub> concentrations were applied as a moderate increase to 60 ppb during the day. Two fans mounted on each side of every chamber ensured air mixing. The relative humidity (RH) was kept at 55/70% (day/night) in all treatments. A 16/8 h day/night light regime was generated by 28 high pressure mercury and 14 halogen lamps (1000 W and 400 W each) per chamber as supplement to natural light entering through the transparent glass roof. The resulting net photosynthetic active radiation (PAR) averaged at approximately 520  $\mu$ mol photons m<sup>-2</sup> s<sup>-1</sup> at the canopy height (ca. 1 m). Sunrise and sunset were simulated within the first and last hour of day-light regime by a gradually changing light intensity.

### 2.2. Plant material and growth conditions

Certified seeds of four spring cultivars of *B. napus* were used. The cultivars differed in respect to their geographic origin and the year of release, indicating also a range of different genetic characteristics and backgrounds (Table 2). Plants were grown in 11 L pots each filled with 4 kg of a standard sphagnum substrate (Pindstrup Substrate No. 6, Pindstrup Mosebrug A/S, Denmark) supplemented with 10 g NPK fertilizer (21-3-10, Kemira Denmark A/S). The experimental population included 36 plants for each cultivar in every treatment and were established with a density of 64 individuals m<sup>-2</sup> (four plants per pot), which is in accordance with field density. The pots were placed on wheeled growing tables, separating the cultivars from each other on different tables. To avoid unilateral impacts and chamber specific biases, the tables holding the plants were rotated within the chambers on a weekly basis and among the chambers every second week. The plants and their corresponding treatment were moved to a new chamber, so that all six chambers were visited by a given treatment and its corresponding plant-badge at least one time during the growth period. All chambers were set to ambient conditions, and when this was attained, the tables from one chamber were moved into a new chamber and the corresponding treatment for that badge of plants was induced. After relocating plants between chambers, stabilization of treatments in the new chamber was accomplished within approximately 1 h. Also the relative position of a given populations within a chamber was changed every second week. For practical reasons, rotation between the chambers was discontinued after plants had reached full flowering, but within-chamber rotations were continued. Watering was carried out by a surface dripping system that delivered 4.4 L m<sup>-2</sup> day<sup>-1</sup>, independent of treatment, at the beginning of daytime regime. Any water exceeding field capacity during early developmental stages, when water uptake was still low, was allowed to drain from the soil. Water-

**Table 2**  
*B. napus* cultivars included in the experiment, their year of release, breeder organisation and country of origin.

Cultivar	Origin	Year of release	Breeder	Distribution area
Bolero	Germany	1997	Raps GbR	Southern and eastern Europe
Mary	Denmark	1978	DLF Trifolium A/S	Denmark
Mozart	Germany	1999	NPZ/Lembke	Baltic countries
Tanto	France	1990	INRA/Serasem	No information available

ing was reduced in a stepwise fashion, when the experimental populations had reached almost full maturation, as determined by leaf senescence (approximately the 3rd month after planting). To avoid pathogen infection (Long et al., 2006) all plants were treated with the fungicide CANTUS® (BASF AG, Germany) every second week during the first month after plants reached the two-leaf stage.

### 2.3. Harvest

The plants were kept in the growth chambers until pod and seed ripening was completed. At harvest, 10 plants per cultivar were selected at random and their number of pod, stem height and width were recorded, before the material was dried at 36°C in a forced ventilated oven (TU2, Heraeus, Germany). After drying, total seed yield and stem weight were determined. Seed yield per pod was derived from weighing the seeds of ten randomly chosen pods per plant (Numigral, Sinar Technologies, UK). Thousand Seed Weight (TSW) and seed number were subsequently calculated from these data. Harvest Index (HI) was calculated as the ratio of seed yield to total above ground dry matter.

### 2.4. Data treatment and statistical analysis

All statistical calculations were performed in R (version 2.11.1, R Development Core Team, 2010). Homoscedacity and normality were tested and improved by the following transformations: stem weight, log-transformed; seed number and total seed yield, square root-transformed; HI third power transformed. The responses were then analysed separately by fitting a linear mixed effects model with the following fixed terms able to be tested in the experimental design: Cultivar, TEMP, CO<sub>2</sub>, O<sub>3</sub>, TEMP:CO<sub>2</sub>, TEMP:Cultivar, CO<sub>2</sub>:Cultivar, O<sub>3</sub>:Cultivar, TEMP:CO<sub>2</sub>:Cultivar. Treatment was included as a grouping variable in order to nest error within the chamber and therefore treatment sharing plants to prevent elevation of Type I error by considering pseudo-replicates as true replicates. This statistical model was first fitted by the maximum likelihood method and backwards reduced using AIC comparison (function: stepAIC in MASS, Venables and Ripley, 'Modern Applied Statistics with S' (4th edition, 2002)). The reduced model was then refitted using restricted maximum likelihood and analysis of variance was performed. The model was then validated by inspection of residuals and normal quantile plots. To clarify the responses of the individual cultivars, the same procedure was performed on cultivar specific subsets of the data under exclusion of Cultivar and all its interactions in the fixed terms of the model.

## 3. Results

The general pattern of response to elevated temperatures for all cultivars was a reduction in the total number of seeds and the mass of seeds per pod (Fig. 1a and b), which gave a significant reduction in seed yield per plant (Table 3, Fig. 1c). However, when the trends were viewed cultivar-wise, a decreased seed yield associated with reductions in total seed number and seed mass per pod was only significant for the cultivars 'Bolero' and 'Mary' (Table 4). For the cultivar 'Mozart' the reductions in seed yield per plant were not

significant in environments with higher temperatures, even though it also exhibited the significant reductions in the total number of seeds and the seed mass per pod under such conditions. Yield in 'Tanto' did not respond to any of the treatments applied (Table 4).

Stem biomass was not significantly affected by higher growing temperatures in any of the cultivars tested here, although the temperature response of stem weight was found to exhibit a significant cultivar-dependency (Table 3, Fig. 1e). The significant reductions in total seed yield under elevated temperatures resulted in a significantly reduced HI (Tables 3 and 4, Fig. 1f).

Generally elevated [CO<sub>2</sub>] increased the total number of seeds and seed yield (Table 3, Fig. 1a and d). However, when the data were analysed cultivar-wise, the increase in yield was only significant in 'Mary' (Table 4).

As to the vegetative biomass under elevated [CO<sub>2</sub>] significant increases of stem weights were found (Table 3). However, when analysed cultivar-wise only 'Bolero' presented significant higher stem biomass (Table 4).

Ozone was found to have no effects on yield or stem weight in any of the cultivars. 'Tanto', however, exhibited a significant reduction of HI in treatments with elevated [O<sub>3</sub>] (Table 4), a response which significantly separated this cultivar from the remaining ones.

The four different cultivars tested here exhibited a strong variability in seed yield independent from the treatment applied (Table 3, Fig. 1d). The yields achieved per plant in the control treatment had a range from 12.8 g in 'Bolero' to 6.1 g in 'Tanto'. This treatment-independent variation in yield was associated with significant differences in seed number per plant and seed mass per pod, while TSW did not significantly differ between the cultivars (Fig. 1c). However, no differential responses of seed yield to the applied treatments were revealed for the different *B. napus* accessions investigated here.

Also for stem biomass no differences were found for the four cultivars. Therefore the cultivar specific pattern of HI apparently only was affected by the cultivar-dependent determination of seed yield.

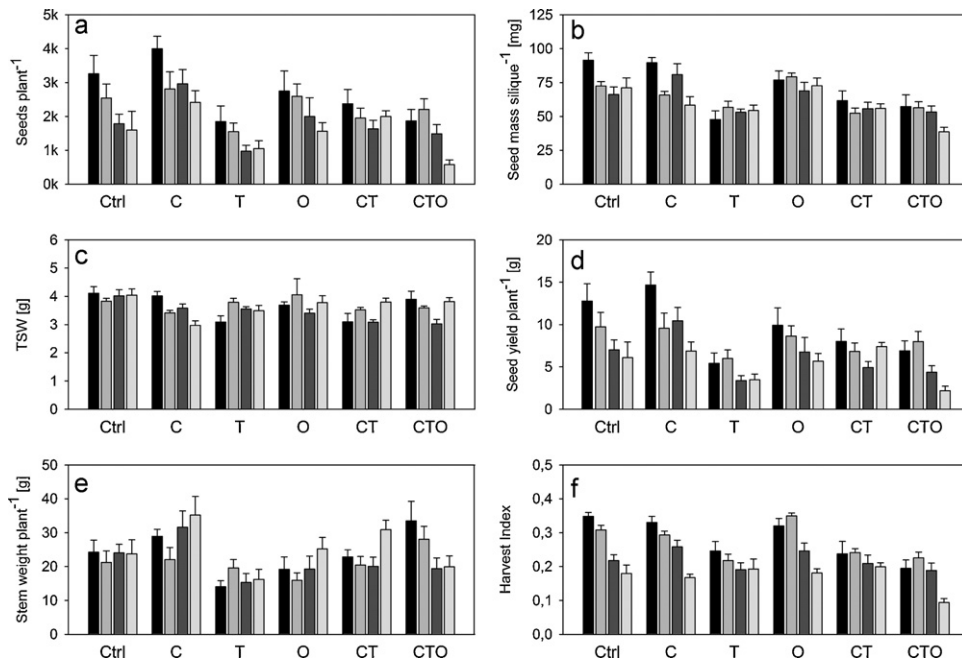
The temperature caused decline in yield dominated its CO<sub>2</sub> driven stimulation: The average yield tended to be reduced in both treatments simultaneously applying elevated [CO<sub>2</sub>] and higher temperatures (CT, CTO, Fig. 2a). The additional application of ozone generally led to further reduced yields (CTO, Fig. 2a). For stem biomass, the positive effects of elevated [CO<sub>2</sub>] and the negative effects of increased temperature tended to equalize, leading to a similar stem biomass production as under control conditions, when applied together (Fig. 2b).

Table 5 summarizes mean values of yield and biomass parameters.

## 4. Discussion

### 4.1. Temperature effects

Generative parameters were decreased under elevated temperature conditions in all *B. napus* cultivars tested, and this also resulted in a decreased seed yield of 38–58%. At supra-optimal temperatures biomass accumulation in plants typically declines as a consequence of (a) reduced rates of photosynthesis (Jones, 1992;



**Fig. 1.** Mean values  $\pm$  standard error of measured yield and biomass parameters in four different *B. napus* cultivars (■, Bolero; ▨, Mozart; ■, Mary; □, Tanto) grown under two temperature regimes (19/12 and 24/17 °C), two CO<sub>2</sub> levels (385 and 700 ppm) and two ozone regimes (20/20 and 20/60 ppm) in a phytotron environment, (number of plants = 10). Treatment abbreviations according to Table 1; TSW – Thousand Seed Weight, HI – Harvest Index.

**Table 3**

Analysis of variance (ANOVA, *F*-values) results for effects of temperature (TEMP), carbon dioxide (CO<sub>2</sub>) and ozone (O<sub>3</sub>) and their interactive effects on yield and biomass parameters on the four *B. napus* cultivars grown under two temperature (19/12 °C and 24/17 °C), two CO<sub>2</sub> (385 and 700 ppm) and two ozone regimes (20/20 and 20/60 ppm) in a phytotron environment, (*n* = 10). Only effects represented in the lowest AIC model are shown. Significance indices: *P* < 0.1; \**P* < 0.05; \*\**P* < 0.01; \*\*\**P* < 0.001; TSW – Thousand Seed Weight, HI – Harvest Index.

	TEMP	CO <sub>2</sub>	O <sub>3</sub>	TEMP × CO <sub>2</sub>	Cultivar	TEMP × Cultivar	CO <sub>2</sub> × Cultivar	O <sub>3</sub> × Cultivar	TEMP × CO <sub>2</sub> × Cultivar
Seeds plant <sup>-1</sup>	<b>32.57*</b>	<b>14.63.</b>	3.65	–	<b>10.52***</b>	–	–	<b>2.27.</b>	–
Seed mass pod <sup>-1</sup>	<b>89.23**</b>	0.01	–	–	<b>5.18**</b>	2.1	<b>4.22**</b>	–	–
TSW	3.51	1.72	–	1.69	1.75	<b>3.18*</b>	<b>2.67*</b>	–	<b>3.39*</b>
Seed yield plant <sup>-1</sup>	<b>38.65*</b>	<b>10.37.</b>	3.85	–	<b>11.8***</b>	–	–	–	–
Stem weight plant <sup>-1</sup>	2.25	<b>26.94*</b>	–	–	1.17	<b>2.46.</b>	–	–	–
HI	<b>27.82*</b>	–	0.2	–	<b>60.04***</b>	<b>19.52***</b>	–	<b>6.04***</b>	–

Significant values are given in bold.

Stone, 2001; Barnabás et al., 2008), (b) reduced light interception due to shortened life cycle caused by faster development (Stone, 2001) and (c) elevated rates of respiration (Atkin and Tjoelker, 2003). Furthermore, supra-optimal temperature conditions can

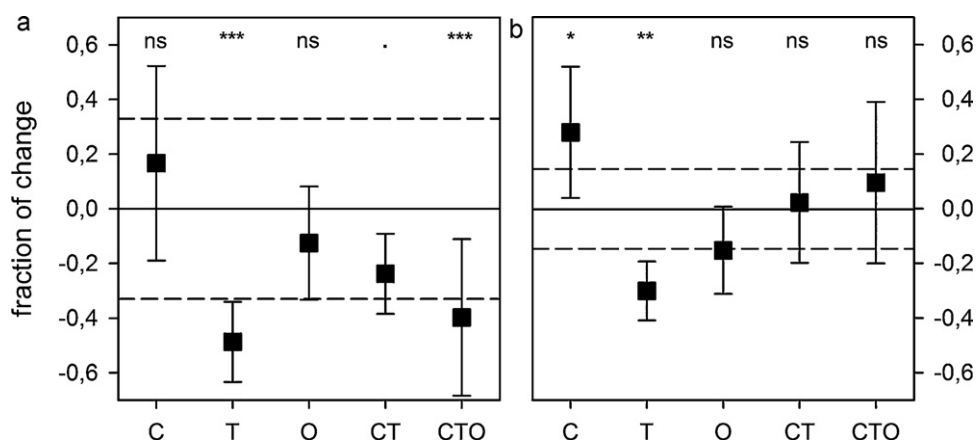
lead to fewer and/or malformed and/or smaller organs in plants in consequence of reduced productivity and impaired developments (Stone, 2001; Barnabás et al., 2008). Biomass parameters were less responsive to the treatments than yield, and in agreement with that

**Table 4**

Analysis of variance (ANOVA, *F*-values) results for effects of carbon dioxide (CO<sub>2</sub>), temperature (TEMP) and ozone (O<sub>3</sub>) and their interactive effects ("") on yield and biomass parameters on four different *B. napus* cultivars grown under two temperature (19/12 °C and 17/24 °C), two CO<sub>2</sub> (385 and 700 ppm) and two ozone regimes (20/20 and 20/60 ppm) in a phytotron environment (*n* = 10). Significance indices: *P* < 0.1; \**P* < 0.05; \*\**P* < 0.01; \*\*\**P* < 0.001. Only effects represented in the lowest AIC model are shown. TSW – Thousand Seed Weight; HI – Harvest Index.

	TEMP	CO <sub>2</sub>	O <sub>3</sub>	TEMP:CO <sub>2</sub>	TEMP	CO <sub>2</sub>	O <sub>3</sub>	TEMP:CO <sub>2</sub>
<i>B. napus</i> cv. 'Bolero'					<i>B. napus</i> cv. 'Mary'			
Seeds plant <sup>-1</sup>	<b>10.65.</b>	2.83	1.97	–	<b>9.93.</b>	<b>8.71.</b>	–	–
Seed mass pod <sup>-1</sup>	<b>31.42*</b>	2.26	–	–	<b>16.07*</b>	2.43	–	–
TSW	3.7	–	–	–	7.77	3.42	4	–
Seed yield plant <sup>-1</sup>	<b>16.21*</b>	3.67	–	–	<b>16.81*</b>	<b>6.11.</b>	–	–
Stem weight plant <sup>-1</sup>	0.06	<b>11.41*</b>	–	–	5.03	5.14	–	–
HI	<b>43.78**</b>	–	4.3	–	<b>6.22.</b>	–	–	–
<i>B. napus</i> cv. 'Mozart'					<i>B. napus</i> cv. 'Tanto'			
Seeds plant <sup>-1</sup>	<b>5.81.</b>	–	–	–	1.37	0.8	1.55	–
Seed mass pod <sup>-1</sup>	<b>34.89*</b>	4.03	3.69	–	<b>8.35.</b>	2.57	–	–
TSW	–	3.66	–	–	0.55	3.83	–	<b>15.78.</b>
Seed yield plant <sup>-1</sup>	4.4	–	–	–	–	–	–	–
Stem weight plant <sup>-1</sup>	–	3.11	–	–	1.55	4.18	–	–
HI	<b>18.5.</b>	0.8	–	2.08	–	<b>5.82.</b>	<b>7.08.</b>	–

Significant values are given in bold.



**Fig. 2.** Relative response of (a) average seed yield and (b) stem biomass responses  $\pm 95\%$  confidence interval for four different *B. napus* cultivars grown under two temperature regimes (19/12 and 24/17 °C), two CO<sub>2</sub> levels (385 and 700 ppm) and two ozone regimes (20/20 and 20/60 ppm) in a phytotron environment ( $n = 10$ ) in relation to control conditions (solid horizontal line)  $\pm 95\%$  confidence interval (broken horizontal line). Significances given for the difference of plants sharing the corresponding treatment and plants in the control treatment and plants sharing the corresponding treatment: ns – not significant;  $P < 0.1$ ; \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ . Treatment abbreviations according to Table 1.

**Table 5**

Mean values and standard deviations (SD) of measured yield and biomass parameters in four different *B. napus* cultivars grown under two temperature regimes (19/12 and 24/17 °C), two CO<sub>2</sub> levels (385 and 700 ppm) and two ozone regimes (20/20 and 20/60 ppm) in a phytotron environment ( $n = 10$ ). C – elevated [CO<sub>2</sub>], T – elevated temperature, O, elevated [O<sub>3</sub>], Ctrl – control treatment; TSW – Thousand Seed Weight, HI – Harvest Index.

Response	Treatment	Ctrl		C		T		O		CT		CTO	
		Cultivar	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean
Pods plant <sup>-1</sup>	Bolero	148	±21	183	±17	108	±20	126	±24	130	±21	123	±19
	Mozart	138	±21	149	±24	114	±15	19	±24	137	±17	163	±20
	Mary	108	±16	142	±16	70	±11	107	±29	92	±11	92	±13
	Tanto	96	±27	170	±23	73	±14	101	±15	143	±9	56	±10
Seeds plant <sup>-1</sup> (×1000)	Bolero	3.3	±0.5	4.0	±0.4	1.9	±0.5	2.8	±0.6	2.4	±0.4	1.9	±0.3
	Mozart	2.5	±0.4	2.8	±0.5	1.6	±0.3	2.6	±0.4	2.0	±0.3	2.2	±0.3
	Mary	1.8	±0.3	3.0	±0.4	1.0	±0.2	2.0	±0.6	1.6	±0.3	1.5	±0.3
	Tanto	1.6	±0.6	2.4	±0.4	1.1	±0.2	1.6	±0.3	2.0	±0.2	0.6	±0.1
Seeds pod <sup>-1</sup>	Bolero	22.2	±1.1	21.8	±1.0	15.3	±1.9	20.6	±1.8	18.1	±1.1	14.3	±1.1
	Mozart	18.5	±0.8	18.9	±0.7	13.7	±1.0	19.8	±0.5	14.3	±0.9	14.7	±1.1
	Mary	16.7	±1.5	21.7	±1.8	14.3	±0.8	19.1	±1.4	17.7	±1.4	16.4	±1.4
	Tanto	16.3	±1.4	16.5	±1.0	14.9	±1.2	17.0	±0.6	14.4	±0.9	9.7	±0.9
Seed mass pod <sup>-1</sup> (10 <sup>-2</sup> ) [g]	Bolero	9.2	±0.6	9	±0.4	4.8	±0.6	7.7	±0.7	6.2	±0.7	5.7	±0.9
	Mozart	7.2	±0.3	6.6	±0.3	5.7	±0.4	7.9	±0.3	5.2	±0.4	5.6	±0.4
	Mary	6.6	±0.6	8.1	±0.8	5.3	±0.2	6.9	±0.6	5.6	±0.5	5.3	±0.4
	Tanto	7.1	±0.7	5.8	±0.6	5.4	±0.4	7.3	±0.6	5.6	±0.3	3.9	±0.3
TSW [g]	Bolero	4.1	±0.2	4.0	±0.2	3.1	±0.2	3.7	±0.1	3.1	±0.3	4.0	±0.3
	Mozart	3.8	±0.1	3.4	±0.1	3.8	±0.1	4.1	±0.6	3.5	±0.1	3.6	±0.1
	Mary	4.0	±0.2	3.6	±0.2	3.6	±0.1	3.4	±0.1	3.1	±0.1	3.0	±0.2
	Tanto	4.0	±0.2	3.0	±0.2	3.5	±0.2	3.8	±0.2	3.8	±0.1	3.8	±0.1
Seed yield plant <sup>-1</sup> [g]	Bolero	12.8	±2.0	14.7	±1.5	5.4	±1.2	9.9	±2.1	8.0	±1.5	6.9	±1.2
	Mozart	9.7	±1.7	9.6	±1.8	6.0	±1.0	8.6	±1.2	6.8	±1.0	8.0	±1.2
	Mary	7.0	±1.2	10.4	±1.6	3.4	±0.6	6.8	±1.7	4.9	±0.7	4.4	±0.8
	Tanto	6.1	±1.9	6.9	±1.1	3.5	±0.7	5.7	±0.9	7.4	±0.5	2.2	±0.5
Stem height [cm]	Bolero	145	±3.4	178	±2.2	127	±5.4	150	±5.6	156	±5.4	198	±11.4
	Mozart	130	±4.2	147	±5.2	149	±1.9	147	±5.3	156	±5.2	168	±8.3
	Mary	165	±3.3	198	±9.0	142	±7.3	163	±4.9	177	±11.3	174	±9.9
	Tanto	157	±6.7	187	±7.9	150	±7.8	163	±5.7	185	±5.5	188	±5.8
Stem width [cm]	Bolero	1.3	±0.1	1.3	±0.1	1.1	±0.0	1.0	±0.1	1.4	±0.1	1.4	±0.1
	Mozart	1.3	±0.1	1.3	±0.1	1.2	±0.1	1.3	±0.1	1.2	±0.1	1.3	±0.1
	Mary	1.1	±0.1	1.4	±0.1	1.1	±0.1	1.1	±0.1	1.2	±0.1	1.2	±0.1
	Tanto	1.1	±0.1	1.2	±0.1	1.0	±0.1	1.1	±0.0	1.3	±0.0	1.0	±0.1
Stem weight plant <sup>-1</sup> [g]	Bolero	23.5	±3.6	28.2	±2.1	13.6	±1.8	18.5	±3.7	22.2	±2.1	32.8	±5.8
	Mozart	20.5	±3.4	21.5	±3.6	19.0	±2.5	15.3	±2.2	19.9	±2.5	27.5	±3.8
	Mary	23.0	±2.6	30.7	±4.8	14.5	±2.6	18.4	±3.8	19.4	±2.7	18.7	±3.1
	Tanto	22.9	±4.2	34.6	±5.4	15.7	±2.9	24.4	±3.4	30.3	±2.8	19.4	±3.2
HI [%]	Bolero	35	±1	33	±2	25	±3	32	±2	24	±4	19	±3
	Mozart	31	±1	29	±1	22	±2	35	±1	24	±1	23	±2
	Mary	22	±2	26	±2	19	±2	25	±2	21	±3	19	±2
	Tanto	18	±2	17	±1	19	±3	18	±1	20	±1	9	±1

Batts et al. (1997) reported that the adverse effects of temperature on crop productivity appear to be more severe for yield than for total biomass. In crops, the efforts of breeding generally focus on improvements of yield rather than tolerance of plants to suboptimal conditions (Barnabás et al., 2008). Therefore the prevailing pattern of carbon allocation in crops favours harvestable biomass fractions, usually the seeds, which in turn are therefore more susceptible to reductions, when conditions become suboptimal.

The high responsiveness of yield parameters to increased temperature may also be well explained by the multitude of developmental processes, which are negatively affected under warmer conditions. In cereals Barnabás et al. (2008) described that increased temperatures affected generative processes negatively, e.g. flower initiation, embryo sac and pollen formation, flowering, fertilisation, seed setting and seed and grain filling were affected. In the present study we also found generative traits negatively affected. During the last phase in the reproductive cycle, the seed filling, the final seed weight is determined by rate and duration of photoassimilate production, mobilisation and translocation to the seeds ultimately determining total yield (Barnabás et al., 2008). As higher temperature accelerates plant development, the seed-filling period shortens. However, the acceleration of the rate of seed filling may counterbalance the shortened seed-filling period to retain similar seed yield (Dupont and Altenbach, 2003), however, this trend was not observed in our study with a +5 °C increase in temperature.

As an expression of that yield was more affected by elevated temperature than biomass was, HI was significantly affected by temperature in all cultivars except 'Tanto'.

#### 4.2. CO<sub>2</sub> effects

In all cultivars of *B. napus* an increased stem height was found, when grown at elevated [CO<sub>2</sub>], but only in 'Bolero' the biomass was significantly increased. The direct initial stimulating effect of increased levels of [CO<sub>2</sub>] on carbon fixation and biomass production is well described and documented (Ainsworth and Long, 2005). Even through, limited sink capacity acclimates photosynthetic capacity (Körner, 2006), plant productivity is usually higher under elevated [CO<sub>2</sub>] than under ambient conditions during longer periods of exposure. Franzaring et al. (2008) also reported increases in oil seed rape stem and shoot dry weight under increased [CO<sub>2</sub>] in the cultivar 'Campino' as revealed by a free air carbon dioxide enrichment (FACE) study. Mean vegetative biomass responded positively in several winter cultivars of oilseed seed rape providing the same trend as the present experiment (Johannessen et al., 2002).

Only in the cultivar 'Mary' was the yield significantly affected under elevated CO<sub>2</sub> in our study. In *B. napus* cv. 'Campino' neither a change in number nor dry weight of reproductive organs was found for mature plants under increased [CO<sub>2</sub>] (Franzaring et al., 2008). The authors explain these findings by an apparent temporal discontinuity of the induced advantage of relative CO<sub>2</sub> throughout the development. Reekie et al. (1998) stated that any CO<sub>2</sub> advantage on growth in *B. napus* is offset through time and development, due to absence of carbon storage organs and thus reduced sink strength. Crop development under increased concentrations of CO<sub>2</sub> was reported to be accelerated in several studies (see Franzaring et al., 2008), implying that the gain of additionally provided carbon is reduced by a shortened period for biomass accumulation. This pattern of faster development might be derived from higher canopy temperatures in crop plant stands as a result of decreased stomatal conductance and, in turn, depressed transpirational cooling of the canopy at high [CO<sub>2</sub>] (Führer, 2003). Therefore, the initial stimulation of photosynthesis cannot directly be extrapolated to higher crop yields under conditions of increased [CO<sub>2</sub>] (Körner, 2006). In contrast, Qaderi and Reid (2005) found significant enhancements (ca. +50%) of seed yield in *B. napus* cv. '46A65' in a chamber study

where they increased [CO<sub>2</sub>] from 360 ppm to 740 ppm. Correspondingly, 'Mary' increased both total seed number and seed yield under increased [CO<sub>2</sub>]. It has been shown earlier, that older crop cultivars, such as 'Mary' have a higher potential for exploitation of elevated [CO<sub>2</sub>] (Ziska et al., 2004) indicating that recent breeding efforts so far have not been able to promote [CO<sub>2</sub>] responsiveness in new crop cultivars (Amthor, 1998), and instead have reduced their CO<sub>2</sub> response potential (Ainsworth et al., 2008a).

#### 4.3. Ozone effects

In the present study none of the *B. napus* cultivars were affected significantly by elevated [O<sub>3</sub>], however, this might be explained by the moderate increase in ozone applied, which is within the range experienced in natural environments. Deleterious effects of O<sub>3</sub> on generative processes have been reviewed by Black et al. (2000); e.g. reductions in the numbers of pods and seeds were reported under high [O<sub>3</sub>]. The present study of oilseed rape cultivars indicate that they hold sufficient capacity for detoxification of O<sub>3</sub> and associated reactive oxygen species (ROS). Ollerenshaw et al. (1999) also found a significant depression of growth rate in only one cultivar of oilseed rape out of four tested, supporting the idea of varietal differences in the response to O<sub>3</sub> in *B. napus*. Genotype specific responses to ozonation are well described for a number of crop and wild species (Fiscus et al., 2005) including the close relative cabbage (*Brassica oleracea* L., Calatayud et al., 2002).

#### 4.4. Interaction between environmental parameters

Only a small number of studies investigated the interactive effects between multiple drivers of environmental change on crop yield, even though the projected environmental changes predict simultaneous increase in greenhouse gas concentrations, temperature and changed patterns of precipitation (Houghton et al., 2001). Qaderi et al. (2006) focused on the implications of elevated [CO<sub>2</sub>], temperature and drought on oilseed rape physiology. To the authors' knowledge, there has been no study so far incorporating the combined effects of elevated [CO<sub>2</sub>], [O<sub>3</sub>] and temperatures on crop yield, though, ozone is emerging as one of the possible stressors limiting potential yield gain of elevated [CO<sub>2</sub>] in climate change scenarios (e.g. Ainsworth et al., 2008b; Challinor et al., 2009; Dermody et al., 2008; Fiscus et al., 2005). Since both gases independently may influence physiological processes of plants, the interplay of those factors and temperature was investigated in their ultimate consequences, and that is yield production. It was shown here, that under the combined effects of elevated [CO<sub>2</sub>] and temperature their individual effects become equalized during the process of stem biomass and yield accumulation. Generally, elevated [CO<sub>2</sub>] could not ameliorate the adverse effects of higher temperatures on seed yield to such extents, that yield was maintained at levels achieved under control conditions. The interactions of increased temperatures and elevated [CO<sub>2</sub>] on photosynthetic biochemistry (e.g. Long, 1991) were also shown in one cultivar of oilseed rape, where adverse temperature effects on physiological responses were ameliorated by elevated [CO<sub>2</sub>] (Qaderi et al., 2006). Due to the relatively smaller number of developmental processes involved, this compensation is logically stronger for vegetative biomass than for yield. Since temperature not only affects plant productivity at the processes level of carbon fixation, but also has an impact on developmental processes, plant yield is affected by temperature to a degree that the positive effects of CO<sub>2</sub> cannot compensate for. When [CO<sub>2</sub>] and temperatures were elevated concurrently in rice (664 ppm CO<sub>2</sub>, +8 °C increase; Ziska et al., 1996), it resulted in the negation of yield enhancements induced by elevated [CO<sub>2</sub>] alone. Yield was even reduced below control quantities in the majority of experiments in wheat, when both environmental

factors were elevated (reviewed by Amthor, 2001). It was shown here that temperature reduced total seed number in all cultivars except 'Tanto', and thereby possibly reduced exploitation of seeds as carbon storage 'sinks' under simultaneously elevated  $[\text{CO}_2]$ . Thus seed yield was reduced.

The effect of adding  $[\text{O}_3]$  to combined conditions of elevated  $[\text{CO}_2]$  and temperature further decreased the yield.

In the future periodic drought stress could be a factor of vital importance for plant production. In the present experiment, however, we only indirectly manipulated the level of drought stress by administering the same amount of water to all treatments. The amount of water was set as appropriate for an optimal development under ambient conditions, but e.g. in the treatments with elevated temperature adult plants likely experienced moderate drought stress. In future multifactor experiments in RERAF more controlled drought stress treatments and measurements will be applied, but this was not possible here due to resource limitations.

#### 4.5. Responsiveness of cultivars and implications for crop breeding

Many of the measured yield and production parameters, exhibited a significant cultivar specific response pattern. Mean seed yield differed between the cultivars as much as 3.6:1 (Fig. 1d, CTO). Under ambient conditions and when  $[\text{CO}_2]$  was applied as single factor 'Bolero' produced the highest yield, but in other treatments 'Bolero' was inferior. In parallel with this Morrison and Stewart (2002) reported different heat stress responses for reproductive growth parameters in different *Brassica* sp. cultivars.

It will become a significant challenge to maintain and improve the food supply of the growing world population in simultaneously changing environments on limited land resources and in completion with bioenergy production (Ainsworth et al., 2008a; Heaton et al., 2008). The potentially stressful environmental conditions that threaten crop productivity stimulate breeding efforts towards cultivars able to sustain high productivity in suboptimal settings. The positive effects of rising  $[\text{CO}_2]$  on crop yield offer a unique new opportunity for increasing agricultural productivity and lowering the risks of yield losses. One of the main foci for breeding programs intending to provide highly productive germplasm, will be found in the maintenance of 'sink' capacity allowing maximal exploitation of elevated  $[\text{CO}_2]$  under co-occurring stressful environmental conditions (e.g. substantially increased temperature), which might impair developmental processes. Describing the cultivar variation of crop cultivars in their  $\text{CO}_2$  exploitation potential and its interactions with other factors of global change can help to reduce uncertainties of yield security in future decades.

Further, future research will need to explain differences in  $[\text{CO}_2]$  exploitation potential of crops related to the age of the cultivars, since past breeding efforts did not realize the selection of more  $\text{CO}_2$ -responsive cultivars and apparently even decreased yield potentials at elevated  $[\text{CO}_2]$  in modern cultivars (Ainsworth et al., 2008a; Amthor, 1998). There are, in accordance with the present study, a multitude of evidence that older cultivars have a higher potential for yield improvements at high  $[\text{CO}_2]$  than modern ones (Johannessen et al., 2002; Manderscheid and Weigel, 1997; Ziska et al., 2004). Future research should therefore focus on which properties and characteristics of crop populations were lost during recent breeding and led to the lack of  $\text{CO}_2$ -responsiveness of modern cultivars.

## 5. Conclusions

We found that the negative effects of a 5 °C temperature elevation on yield could not be compensated by elevated  $[\text{CO}_2]$ ,

when applied together in multifactor treatments mimicking future growth scenarios. The yield, which was cultivar dependent, tended to decrease further when  $[\text{O}_3]$  was elevated in addition to  $[\text{CO}_2]$  and temperature. Our results also suggest that future breeding of *B. napus* should be based on old cultivars, since modern varieties seem to have lower potentials to respond to  $\text{CO}_2$  and thus counteract the detrimental effects of yield reducing environmental factors such as temperature and  $\text{O}_3$ . As material for future breeding, it should be prioritized to screen a large array of cultivars to determine cultivar specific effects to the coming climate changes. Experiments should be multifactorial and include not only abiotic, but also biotic stressors like fungal diseases. In addition to plant productivity also plant quality should be measured.

## Acknowledgments

We thank Bente A. Andersen, Lis Brandt and Poul T. Sørensen (Risø-DTU) for practical assistance on cultivation and harvest procedures. Technical support regarding the growth facility was kindly provided by Esben Højrup and Allan A. Murphy (Risø-DTU). Georg Frenck was partly supported by ISOBIS (International School of Biodiversity Science) throughout the period of this work, as well as the NordForsk Network, 'Sustainable primary production in a changing climate', also contributed to the project. The  $\text{CO}_2$  used in this study was generously donated by Air Liquide Danmark A/S.

## References

- Ainsworth, E.A., Beier, C., Calfapietra, C., Ceulemans, R., Durand-Tardif, M., Farquhar, G.D., Godbold, D.L., Hendrey, G.R., Hickler, T., Kaduk, J., Karnosky, D.F., Kimball, B.A., Körner, C., Koornneef, M., Lafarge, T., Leakey, A.D.B., Lewin, K.F., Long, S.P., Manderscheid, R., Mcneil, D.L., Mies, T.A., Miglietta, F., Morgan, J.A., Nagy, J., Norby, R.J., Norton, R.M., Percy, K.E., Rogers, A., Soussana, J.-F., Stitt, M., Weigel, H.-J., White, J.W., 2008a. Next generation of elevated  $[\text{CO}_2]$  experiments with crops: a critical investment for feeding the future world. *Plant, Cell & Environment* 31, 1317–1324.
- Ainsworth, E.A., Long, S.P., 2005. What have we learned from 15 years of free-air  $\text{CO}_2$  enrichment (FACE)? A meta-analytic review of the responses of photosynthesis, canopy properties and plant production to rising  $\text{CO}_2$ . *New Phytologist* 165, 351–372.
- Ainsworth, E.A., Rogers, A., Leakey, A.D.B., 2008b. Targets for crop biotechnology in a future high- $\text{CO}_2$  and high- $\text{O}_3$  world. *Plant Physiology* 147, 13–19.
- Amthor, J.S., 1998. Perspective on the relative insignificance of increasing atmospheric  $\text{CO}_2$  concentration to crop yield. *Field Crops Research* 58, 109–127.
- Amthor, J.S., 2001. Effects of atmospheric  $\text{CO}_2$  concentration on wheat yield: review of results from experiments using various approaches to control  $\text{CO}_2$  concentration. *Field Crops Research* 73, 1–34.
- Atkin, O.K., Tjoelker, M.G., 2003. Thermal acclimation and the dynamic response of plant respiration to temperature. *Trends in Plant Science* 8, 343–351.
- Barnabás, B., Jäger, K., Fehér, A., 2008. The effect of drought and heat stress on reproductive processes in cereals. *Plant, Cell & Environment* 31, 11–38.
- Batts, G.R., Morison, J.I.L., Ellis, R.H., Hadley, P., Wheeler, T.R., 1997. Effects of  $\text{CO}_2$  and temperature on growth and yield of crops of winter wheat over four seasons. *European Journal of Agronomy* 7, 43–52.
- Biswas, D.K., Xu, H., Li, Y.G., Sun, J.Z., Wang, X.Z., Han, X.G., Jiang, G.M., 2008. Genotypic differences in leaf biochemical, physiological and growth responses to ozone in 20 winter wheat cultivars released over the past 60 years. *Global Change Biology* 14, 46–59.
- Black, V.J., Black, C.R., Roberts, J.A., Stewart, C.A., 2000. Tansley review no. 115. Impact of ozone on the reproductive development of plants. *New Phytologist* 147, 421–447.
- Calatayud, A., Alvarado, J.W., Barreno, E.v.a., 2002. Differences in ozone sensitivity in three varieties of cabbage (*Brassica oleracea* L.) in the rural Mediterranean area. *Journal of Plant Physiology* 159, 863–868.
- Challinor, A.J., Ewert, F., Arnold, S., Simelton, E., Fraser, E., 2009. Crops and climate change: progress, trends, and challenges in simulating impacts and informing adaptation. *Journal of Experimental Botany* 60, 2775–2789.
- Dermody, O., Long, S.P., McConaughay, K., DeLucia, E.H., 2008. How do elevated  $\text{CO}_2$  and  $\text{O}_3$  affect the interception and utilization of radiation by a soybean canopy? *Global Change Biology* 14, 556–564.
- Dupont, F.M., Altenbach, S.B., 2003. Molecular and biochemical impacts of environmental factors on wheat grain development and protein synthesis. *Journal of Cereal Science* 38, 133–146.
- Fiscus, E.L., Booker, F.L., Burkey, K.O., 2005. Crop responses to ozone: uptake, modes of action, carbon assimilation and partitioning. *Plant, Cell & Environment* 28, 997–1011.

- Franzaring, J., Högy, P., Fangmeier, A., 2008. Effects of free-air CO<sub>2</sub> enrichment on the growth of summer oilseed rape (*Brassica napus* cv. Campino). *Agriculture, Ecosystems & Environment* 128, 127–134.
- Fuhrer, J., 2003. Agroecosystem responses to combinations of elevated CO<sub>2</sub>, ozone, and global climate change. *Agriculture, Ecosystems & Environment* 97, 1–20.
- Fuhrer, J., 2009. Ozone risk for crops and pastures in present and future climates. *Naturwissenschaften* 96, 173–194.
- Heaton, E.A., Flavell, R.B., Mascia, P.N., Thomas, S.R., Dohleman, F.G., Long, S.P., 2008. Herbaceous energy crop development: recent progress and future prospects. *Current Opinion in Biotechnology* 19, 202–209.
- Houghton, J.T., Ding, Y., Griggs, D.J., Noguer, M., van der Linden, P.J., Dai, X., Maskell, K., Johnson, C.A., 2001. IPCC Report on Climate Change 2001: The Scientific Basis. Cambridge University Press, New York.
- IPCC, 2007. In: Solomon, S., Qin, D., Manning, M., Marquis, M., Averyt, K., Tignor, M.M.B., Miller, H.L., Chen, Z. (Eds.), *Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Third Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge University Press, Cambridge, UK.
- Johannessen, M.M., Mikkelsen, T.N., Jørgensen, R.B., 2002. CO<sub>2</sub> exploitation and genetic diversity in winter varieties of oilseed rape (*Brassica napus*); varieties of tomorrow. *Euphytica* 128, 75–86.
- Jones, H.G., 1992. *Plants and Microclimate: A Quantitative Approach to Environmental Plant Physiology*. Cambridge University Press, Cambridge, UK.
- Körner, C., 2006. Plant CO<sub>2</sub> responses: an issue of definition, time and resource supply. *New Phytologist* 172, 393–411.
- Long, S.P., 1991. Modification of the response of photosynthetic productivity to rising temperature by atmospheric CO<sub>2</sub> concentrations – has its importance been underestimated. *Plant, Cell & Environment* 14, 729–739.
- Long, S.P., Ainsworth, E.A., Leakey, A.D.B., Nosberger, J., Ort, D.R., 2006. Food for thought: lower-than-expected crop yield stimulation with rising CO<sub>2</sub> concentrations. *Science* 312, 1918–1921.
- Luo, Y., Gerten, D., Maire, G.L., Parton, W.J., Weng, E., Zhou, X., Keough, C., Beier, C., Ciais, P., Cramer, W., Dukes, J.S., Emmett, B., Hanson, P.J., Knapp, A., Linder, S., Nepstad, D., Rustad, L., 2008. Modeled interactive effects of precipitation, temperature, and [CO<sub>2</sub>] on ecosystem carbon and water dynamics in different climatic zones. *Global Change Biology* 14, 1986–1999.
- Manderscheid, R., Weigel, H.J., 1997. Photosynthetic and growth responses of old and modern spring wheat cultivars to atmospheric CO<sub>2</sub> enrichment. *Agriculture, Ecosystems & Environment* 64, 65–73.
- McCarthy, J.J., Canziani, O.F., Leary, N.A., Dokken, D.J., White, K.S., 2001. *IPCC Report on Climate Change 2001: Impacts, Adaptation and Vulnerability*. Cambridge University Press, New York.
- Morrison, M.J., Stewart, D.W., 2002. Heat stress during flowering in summer brassica. *Crop Science* 42, 797–803.
- Ollerenshaw, J.H., Lyons, T., Barnes, J.D., 1999. Impacts of ozone on the growth and yield of field-grown winter oilseed rape. *Environmental Pollution* 104, 53–59.
- Prather, M., Ehhalt, D., Dentener, F., Derwent, R., Dlugokencky, E., Holland, E., Isaksen, I., Katima, J., Kirchoff, V., Matsun, P., Midgley, P., Wang, M., 2001. Atmospheric chemistry and greenhouse gases. In: Houghton, J.T., Ding, Y., Griggs, D.J., Noguer, M., van der Linden, P.J., Dai, X., Maskell, K., Johnson, C.A. (Eds.), *Climate Change 2001: The Scientific Basis. Contribution of Working Group I to the Third Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge University Press, Cambridge, UK, pp. 241–280.
- Qaderi, M.M., Kurepin, L.V., Reid, D.M., 2006. Growth and physiological responses of canola (*Brassica napus*) to three components of global climate change: temperature, carbon dioxide and drought. *Physiologia Plantarum* 128, 710–721.
- Qaderi, M.M., Reid, D.M., 2005. Growth and physiological responses of canola (*Brassica napus*) to UV-B and CO<sub>2</sub> under controlled environment conditions. *Physiologia Plantarum* 125, 247–259.
- R Development Core Team, 2010. *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0. URL: <http://www.R-project.org>.
- Reekie, E.G., MacDougall, C., Wong, I., Hickleton, P.R., 1998. Effect of sink size on growth response to elevated atmospheric CO<sub>2</sub> within the genus *Brassica*. *Canadian Journal of Botany* 76, 829–835.
- Shimono, H., Okada, M., Yamakawa, Y., Nakamura, H., Kobayashi, K., Hasegawa, T., 2009. Genotypic variation in rice yield enhancement by elevated CO<sub>2</sub> relates to growth before heading, and not to maturity group. *Journal of Experimental Botany* 60, 523–532.
- Stone, P., 2001. The effects of heat stress on cereal yield and quality. In: A.S. B. (Ed.), *Crop Responses and Adaptations to Temperature Stress*. Food Products Press, Binghamton, NY, USA, pp. 243–291.
- Ziska, L.H., Manalo, P.A., Ordonez, R.A., 1996. Intraspecific variation in the response of rice (*Oryza sativa* L.) to increased CO<sub>2</sub> and temperature: growth and yield response of 17 cultivars. *Journal of Experimental Botany* 47, 1353–1359.
- Ziska, L.H., Morris, C.F., Goins, E.W., 2004. Quantitative and qualitative evaluation of selected wheat varieties released since 1903 to increasing atmospheric carbon dioxide: can yield sensitivity to carbon dioxide be a factor in wheat performance? *Global Change Biology* 10, 1810–1819.