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Yield vs. Quality trade-offs for wheat in response to carbon dioxide and ozone

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Abstract

Although it is established that there exist potential trade-offs between grain yield and grain quality in wheat exposed to elevated carbon dioxide (CO_2) and ozone (O_3), their underlying causes remain poorly explored. To investigate the processes affecting grain quality under altered CO_2 and O_3, we analysed 57 experiments with CO_2 or O_3 exposure in different exposure systems. The study covered 24 cultivars studied in 112 experimental treatments from 11 countries. A significant growth dilution effect on grain protein was found: a change in grain yield of 10% by O_3 was associated with a change in grain protein yield of 8.1% (R^2 = 0.96), whereas a change in yield effect of 10% by CO_2 was linked to a change in grain protein yield effect of 7.5% (R^2 = 0.74). Superimposed on this effect, elevated CO_2, but not O_3, had a significant negative effect on grain protein yield also in the absence of effects on grain yield, indicating that there exists a process by which CO_2 restricts grain protein accumulation, which is absent for O_3. Grain mass, another quality trait, was more strongly affected by O_3 than grain number, whereas the opposite was true for CO_2. Harvest index was strongly and negatively influenced by O_3, but was unaffected by CO_2. We conclude that yield vs. protein trade-offs for wheat in response to CO_2 and O_3 are constrained by close relationships between effects on grain biomass and less than proportional effects on grain protein. An important and novel finding was that elevated CO_2 has a direct negative effect on grain protein accumulation independent of the yield effect, supporting recent evidence of CO_2-induced impairment of nitrate uptake/assimilation. Finally, our results demonstrated that processes underlying responses of grain yield vs. quality trade-offs are very different in wheat exposed to elevated O_3 compared with elevated CO_2.

Keywords: carbon dioxide, grain mass, grain number, grain yield, growth dilution, harvest index, ozone, protein, starch, wheat

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Introduction

Global environmental change, and its effects on agriculture, has a multitude of aspects, which are closely intertwined. On a global scale, two of the most important changes in atmospheric composition are the rising concentrations of carbon dioxide (CO_2; IPCC, 2007) and tropospheric ozone (O_3; Dentener *et al*., 2006). Elevated CO_2 has the potential to stimulate yields in C_3 crops such as wheat, *Triticum aestivum* L. (Amthor, 2001; Tubiello & Ewert, 2002), one of the most important crops worldwide (Evans, 1993). On the other hand, moderately elevated O_3 can significantly reduce the yield of wheat and other crops (Feng *et al*., 2008; Fuhrer, 2009).

From a human nutrition perspective, not only the size of yield is of interest. Food security represents the availability, access and utilizations of food (FAO, 2001). The utilization aspect includes the appropriate nutritional content of the food. An important food security factor for wheat, potentially negatively affected by elevated CO_2, is the grain protein concentration (GPC). Crude protein (calculated from and directly proportional to the N concentration) is an important constituent of wheat grain, typically making up >10% of grain mass. Although reduced wheat yield by O_3 was associated with increased GPC (Fangmeier *et al*., 1999; Pleijel *et al*., 1999), CO_2 yield stimulation has been linked to reduced GPC (Cotrufo *et al*., 1998; Pleijel *et al*., 1999; Idso & Idso, 2001; Högy & Fangmeier, 2008; Taub *et al*., 2008).

Dilution of protein by, e.g. elevated CO_2 results if the increase in biomass accumulation is larger than the increase in N acquisition (Loladze, 2002). This effect is referred to as growth dilution and leads to reduced concentration of protein with increased yield (a common effect of elevated CO_2) and increased protein concentration with reduced yield (a common effect of elevated O_3). Failure of N acquisition to keep pace with growth enhancement can be caused by N becoming an increasingly scarce resource, by effects on the shoot system nitrate assimilation capacity or by the root system nutrient uptake capacity. It is important for the understanding of the processes behind effects on GPC if grain

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protein yield (GPY, the amount of grain protein accumulated per unit area), i.e. grain N acquisition, is changed by CO_2 and O_3. As a very large fraction of N in other parts of the plant is translocated to the grain of wheat during the maturation period (Simpson *et al.*, 1983), and nitrogen harvest index (the proportion of plant N in the grains at harvest) values are typically in the range of 80–90% (e.g. Barraclough *et al.*, 2010), it is reasonable to assume GPY as a proxy for N acquisition.

Mostly, meta-analyses of wheat response to CO_2 and/or O_3 have focussed on individual variables analysed one-by-one. In recent meta-analyses (Feng *et al.*, 2008; Högy & Fangmeier, 2008; Taub *et al.*, 2008), effects on GPC were tested independent of effects on grain yield (GY). Thus, functional relationships between effects of CO_2 and O_3 on plant growth (C acquisition) and grain protein accumulation (N acquisition) were not derived to elucidate the effects on GPC by these investigations. To understand the effects of environmental change on plants, investigation of the relationships between different response variables may provide further understanding of the processes involved. For example, by comparing the effect of, e.g. O_3 or CO_2 on GPY and GY, it can be directly inferred to what extent effects on GPC result entirely from growth dilution or also from other more direct effects on N acquisition, present also when GY was not affected, and growth dilution thus cannot explain the effect.

It has been suggested that growth stimulation by elevated CO_2 may counteract the negative effect of O_3 in a rather simplistic manner (e.g. Rudorff *et al.*, 1996). This may hold for effects on GPC, if they are mainly determined by whole-plant acquisition of resources (C, N) which may be affected in opposite directions by the two gases. However, if effects on GPC are controlled by effects on GY components [GM (grain mass), GN (grain number), HI (harvest index, the proportion of above-ground biomass in the grain at harvest)], or specific physiological processes influenced by one of the trace gases, but not the other, then the positive effect of elevated O_3 on GPC is not necessarily analogous with the negative effect on GPC by elevated CO_2. Although yield reduction by O_3 has been linked to a shorter duration of grain filling (Ewert & Pleijel, 1999; Gelang *et al.*, 2000), smaller GM (Piikki *et al.*, 2008) and lower HI (Pleijel *et al.*, 1995), GY stimulation by elevated CO_2 has been associated with an increase in GN rather than in GM (Jablonski *et al.*, 2002; Piikki *et al.*, 2008). As the response pattern in terms of yield components is different, it may be incorrect to assume that the effects of CO_2 and O_3 on GPC are simply opposing influences. Furthermore, there is evidence that elevated CO_2 inhibits nitrate assimilation in wheat (Bloom *et al.*, 2010), which may significantly reduce GPC in plants exposed to high concentrations of this gas. This effect is not directly related to the effect of CO_2 on yield and it is thus different from a pure growth dilution effect; it may be present in elevated CO_2 even in the absence of a growth stimulating effect. It is important to determine whether such yield-independent effect may explain part of the reduction in GPC typically observed in CO_2 experiments with wheat or not.

In this study, we make use of data from a large number of experimental studies presented in the scientific literature, from different countries and using different wheat cultivars and exposure systems, to investigate the effects of O_3 and CO_2 on the quantity (GY, GPY), composition (GPC, starch) and components (GN, GM, HI) of wheat GY and their mutual relationships. Our main objectives were to examine if effects of CO_2 and O_3 on GPC are constrained by (and thus predictable from) close relationships between primary effects on biomass accumulation and GY and secondary smaller effects on N acquisition, i.e. testing the growth dilution hypothesis, and if these relationships were similar in CO_2 and O_3 experiments. The following specific hypotheses were tested:

1 Growth dilution of grain protein by elevated CO_2 does not differ from the yield concentration effect on grain protein caused by elevated O_3.
2 In addition to growth dilution, there exists a negative effect of CO_2 on grain protein accumulation which is independent of its effect on yield.
3 Grain starch concentration is not significantly affected by O_3 or CO_2.
4 Effects of O_3 on GY are related primarily to effects on grain mass, whereas CO_2 primarily affects grain number.

Materials and methods

Database compilation

Results from 57 experiments, published in the peer review literature, were used. To be included, values of GPC and GY of an experiment had to derive from the same plants (location, harvest year, cultivar, treatment). GY had to be given as unit area yield (field experiments) or GY per pot (i.e. excluding GY per shoot data). The publications containing the data are given in Appendix S1. Table 1 presents the number of experiments and treatments included for different agronomic variables, for the whole data set and for CO_2 and O_3 separately. All experiments used for starch, GN, GM and HI were part of the population of experiments used for GPC and GY. Among the 112 exposure treatments (this number does not include the controls, see below), there were three with reduced CO_2, 60 with

Table 1 Number of experiments, treatments, countries and cultivars used for different agronomic variables

	Number of experiments	Number of treatments*	Number of countries	Number of cultivars†
Protein and Grain yield	**57** (*43*;20)	**112** (*63*;49)	**11** (*10*;6)	**24** (*17*;8)
Starch	**11** (*7*;7)	**28** (*13*;15)	**5** (*4*;3)	**6** (*5*;3)
Grain mass	**39** (*23*;20)	**88** (*41*;47)	**10** (*9*;6)	**16** (*12*;8)
Grain number	**38** (*22*;20)	**86** (*39*;47)	**10** (*9*;6)	**15** (*11*;8)
Harvest index	**32** (*18*;19)	**78** (*31*;47)	**8** (*6*;6)	**13** (*9*;7)
Total above-ground biomass				

Bold figures show the total number. Within brackets figures in italics show the number for CO_2 followed by the number for O_3. Note that six experiments included both CO_2 and O_3 treatments.

*The controls (nonfiltered air with approximately ambient O_3 and CO_2 concentrations) are not included in these figures.

†In one case the average of four cultivars.

elevated CO_2, 18 with reduced O_3 (filtered air was considered as reduced O_3) and 31 with elevated O_3.

Only pure CO_2 and O_3 treatments were included. In experiments with complete factorial designs including CO_2 and another variable (temperature, seven experiments; N, six experiments; water supply, one experiment), the elevated CO_2 treatments at a certain level of temperature, N or water were considered as separate data sets, using the corresponding nonelevated CO_2 treatment as the control. Data from treatments including both elevated CO_2 and O_3 were not used to avoid violation of the assumption of independence between replicates (the combination treatment would have been included in both the CO_2 and the O_3 data sets).

Both outdoor experiments and controlled environment experiments were included. Of the 57 experiments, 41 (21 CO_2; 15 O_3; 5 CO_2 and O_3) were open-top chamber (OTC) experiments, four free-air CO_2 enrichment (FACE) experiments (field-grown plants), four (only CO_2 experiments) were performed with self-ventilating chambers using field-grown plants and eight (only CO_2 experiments) were conducted with closed chamber systems using potted plants in all cases. Among the OTC experiments, 30 (10 CO_2; 15 O_3; 5 CO_2 and O_3) were made with field-grown plants and 11 (all CO_2) with pot-grown plants. For all chamber experiments, the nonfiltered air treatments with ambient [CO_2] and [O_3] were used as controls. For FACE experiments, ambient air plots without CO_2 fumigation were used as controls.

Ground area-based GY was reported for 31 experiments. For each of these experiments, a linear regression between GPC and GY was calculated. Using the regression of each experiment, the average reduction in GPC for an increase in GY of 1 ton ha^{-1} was calculated.

Concepts for comparing effects and evaluating growth dilution

To facilitate comparison between experiments and treatments, relative scales for the experimental effects on yield (grain, total above-ground), yield components (GN, GM, HI) and yields of protein and starch were used. The relative effect of a certain treatment on a certain variable in an experiment can be expressed as (Pleijel & Danielsson, 2009):

$$\frac{A_{i,j,k}}{A_{ref,j,k}} = 1 + \alpha_{ij,k}, \tag{1}$$

where $A_{i,j,k}$ is the value of variable j of treatment i in experiment k, $A_{ref,j,k}$ is the value of variable j of the control in experiment k, and $\alpha_{i,j,k}$ represents the relative effect on the variable j of treatment i in relation to the control of experiment k. Controls were only used to calculate experimental effects: by definition α is zero for all variables in the control. To investigate growth dilution effects, one should start by comparing the accumulation of grain N (indicated by GPY) with the accumulation of biomass (indicated by GY). If data are plotted with the relative effect on GPY on the y-axis and relative effect on GY on the x-axis, and the result is a linear regression with a slope significantly smaller than unity, this can be interpreted as a significant growth dilution effect. If a direct negative effect on nitrogen uptake, not related to the effect on GY, exists in addition to the growth dilution effect, there will be a significant intercept in a plot between the relative effects on GPY and GY. The magnitude of effects on other variables (GM, GN, HI) can also be compared using the relative scale defined by Eqn (1).

If the relative effect on GPY is α_{gpy}, and the relative effect on GY is α_{gy}, the relative effect on GPC α_{gpc} is:

$$\alpha_{gpc} = \frac{1 + \alpha_{gpy}}{1 + \alpha_{gy}} - 1. \tag{2}$$

Assuming a linear relationship between α_{gpy} and α_{gy}, the relationship between α_{gpc} and α_{gy} will be nonlinear according to Eqn (2). This is exemplified by the broken lines in Fig. 1. The straight blue line in Fig. 1 represents the case that GPY is influenced by a constant factor between 0 and 1 (0.7 in the example) in relation to GY, leading to growth dilution of grain protein when GY is stimulated and a higher concentration of grain protein when GY is reduced. The corresponding relationship between GPC and GY (based on Eqn 2) is given by the broken blue line. The solid yellow line adds to the relationship shown by the solid blue line in a specific effect that reduces grain protein accumulation at any GY by a constant fraction (0.2 in the example), for example a specific physiological effect on grain N acquisition by CO_2 or O_3.The broken yellow line shows the relationship between GPC and GY that

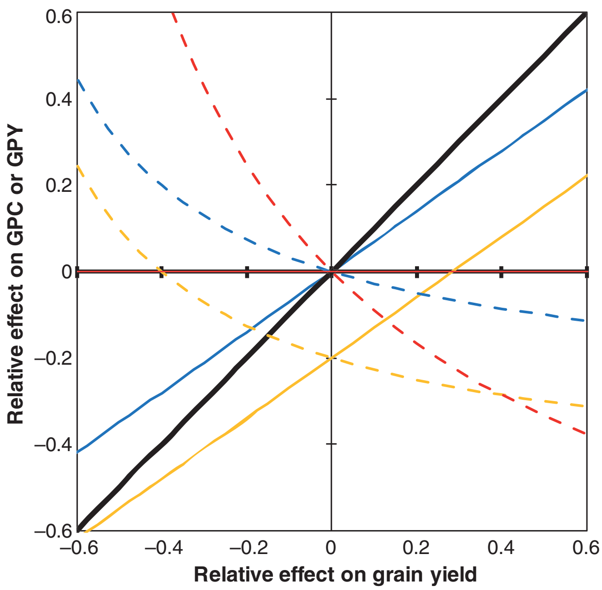

Fig. 1 Three possible cases for the relationships between grain protein yield (GPY) or grain protein concentration (GPC) and grain yield (GY) under the influence of environmental factors such as O_3 and CO_2. The straight blue line represents the case that the accumulation of GPY is influenced by a factor (between 0 and 1) in relation to the accumulation of GY, leading to reduced GPC when GY is stimulated, and enhanced GPC when GY is reduced. The corresponding relationship between GPC and GY (based on Eqn 2) is given by the broken blue line. The solid yellow line adds to the relationship shown by the solid blue line a specific effect that reduces N accumulation at any GY by a constant fraction, e.g. a specific physiological effect on N uptake by elevated CO_2 or O_3.The broken yellow line shows the relationship between GPC and GY that follows. The third case, given by the solid red line following the *x*-axis, represents the case that GPY is constant and is not influenced by any effects on GY. The corresponding relationship of GPC with GY is shown by the broken red line.

follows. The third case, defined by the solid red line following the *x*-axis, represents the case that GPY is not influenced by effects on GY. This is the assumption that Loladze (2002) used to show potential effects of growth dilution of elevated CO_2 on human nutrition. The corresponding relationship of GPC with GY is shown by the broken red line. It can be noted that the solid blue line represents a relationship where grain N acquisition increased, but to a lesser extent than biomass accumulation, whereas the solid red line represents an extreme case where a stimulation of GY depends only on increased nitrogen use efficiency.

Linear regressions were statistically tested for homogeneity of *Y*-intercepts and slopes between different groups (CO_2 vs. O_3 experiments or contrasting experiments with field-grown and potted plants) using ANCOVA in SAS software version 9.3.1 (SAS Institute, Cary, NC, USA). A significant difference in *Y*-intercept can be readily interpreted only if the slope is not significantly different between groups. Furthermore, the possible deviation of the regressions from a 1 : 1 relationship was tested according to Underwood (1997). Effects were considered statistically significant at $P < 0.05$.

Results

GPY and GPC in relation to GY

For both gases, the slopes of the linear relationships between the effect on GPY and the effect on GY deviated significantly and negatively from unity (Table 2), thus showing significant growth dilution of grain protein (Fig. 2). Slopes were closer to unity than to zero, indicating that the magnitude of effects of CO_2 and O_3 on GY was strongly controlled by N acquisition. The growth dilution effect for grain protein was somewhat stronger for CO_2 (slope: 0.75) than for O_3 (slope: 0.81), but the difference was not statistically significant (Table 2). The intercepts of CO_2 (-0.072 ± 0.038; intercept $\pm$ 95% confidence interval; $P < 0.001$) and O_3 (close to zero, nonsignificant) relationships were significantly different ($P < 0.001$). Thus, the relationship for O_3 exemplifies the solid blue line of Fig. 1, whereas the CO_2 relationship mimics the solid yellow line in Fig. 1. FACE experiments (not shown specifically in the figures) did not differ from chamber experiments, having an average increase in GPY of 8% for an average GY stimulation of 12%.

A number of interaction experiments were used in the analysis, where the different levels of the cofactor was considered as an independent dataset in the present analysis. Data points belonging to these experiments were marked with different colours in Fig. 2. Statistical tests showed that no significant interactions existed between CO_2 on the one hand, and N supply ($P = 0.798$) or temperature ($P = 0.096$), on the other.

The response pattern was more consistent for O_3 than for CO_2 as shown by the higher R^2 value for O_3 (0.96) than for CO_2 (0.74; Fig. 2). This could not be attributed to the fact that the CO_2 experiments were more diverse in terms of exposure systems, as the R^2 values remained essentially the same when only including OTC experiments with field-grown plants (0.96 for O_3 and 0.72 for CO_2).

The lines in Fig. 3 follow Eqn (2) using the regressions exhibited in Fig. 2 and essentially mimic the broken blue (O_3) and yellow (CO_2) lines of Fig. 1. Thus, O_3 and CO_2 affected the N : biomass relationship of wheat grain differently. In addition to the growth dilution effect common to O_3 and CO_2, there was a negative effect on GPC for CO_2, but not O_3, which was independent of the effect on GY. The inclusion of filtered air

Table 2 Results of analysis of covariance (ancova) of different *Y* variables as a function of treatment groups (CO_2 vs. O_3 or potted vs. field-grown plants) and *X* variables (covariates)

Variables compared (*Y* vs. *X*)	Groups compared	*P*-values: Covariate	Group (intercepts)	Covariate × Group (slopes)	Significance for deviation from the 1 : 1 relationship	Corresponding figure
Protein yield vs. grain yield	CO_2 vs. O_3	**<0.001**	**<0.001**	0.410	O_3: <**0.001** CO_2: <**0.001**	2
Protein yield vs. grain yield	CO_2 potted vs. CO_2 soil-grown	**<0.001**	0.312	0.122	Pots: <**0.05** Soil: <**0.001**	4
Starch yield vs. grain yield	CO_2 vs. O_3	**<0.001**	0.298	0.526	O_3: ns CO_2: ns	5
Grain mass vs. grain yield	CO_2 vs. O_3	**<0.001**	0.884	**<0.001**	O_3: <**0.001** CO_2: <**0.001**	6
Grain number vs. grain yield	CO_2 vs. O_3	**<0.001**	0.954	**<0.001**	O_3: <**0.001** CO_2: ns	7
Harvest index vs. total above-ground biomass	CO_2 vs. O_3	**<0.001**	0.813	**<0.001**	O_3: <**0.001** CO_2: <**0.001**	8

Main effects of Covariate or Treatments can be readily interpreted only if the Slope term (Covariate × Group interaction) was not statistically significant. Relationships were also tested for deviation from the 1 : 1 relationship according to Underwood (1997). *P*-values ≤ 0.05 are indicated by bold font.

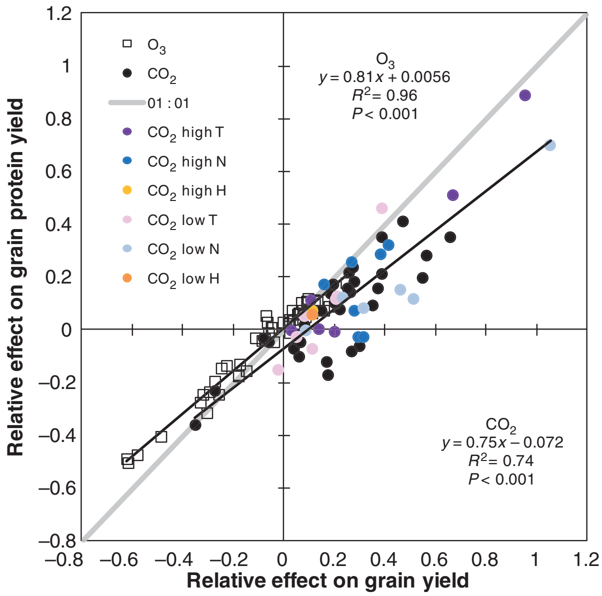

Fig. 2 The relationship between the relative effect on grain protein yield and the relative effect on grain yield for wheat exposed to altered [CO_2] or [O_3]. Coloured points indicate interaction experiments between CO_2 and temperature (blue), nitrogen (green) and water (orange) with the lighter hue of each colour representing the lower level of the interaction variable.

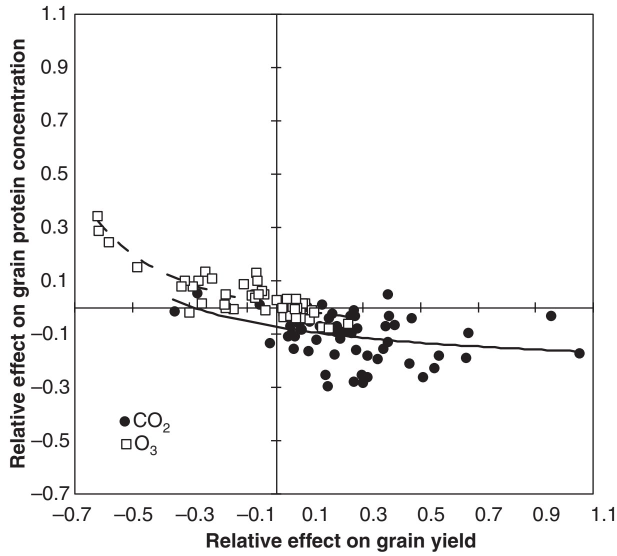

Fig. 3 Effect on relative grain protein concentration in relation to the relative effect on grain yield by O_3 and CO_2.The broken (O_3) and solid (CO_2) lines were derived from Eqn (2) using the slopes and intercepts of the linear regressions in Fig. 2. They are analogous to the blue and yellow broken lines in Fig. 1.

treatments that mostly caused increased yield, and a few experiments with reduced CO_2 concentration that caused reduced yield, resulted in a relatively large overlap of relative effects on GY by CO_2 and O_3. Consideration of this range of overlapping data in Fig. 3 strengthens the conclusion that the effect by CO_2 on GPC is shifted downwards compared with effects of O_3.

For the 31 experiments where area-based yields and protein concentrations of field-grown crops were reported, an average crude protein concentration reduction of 1.2 percent-units GPC for an increase in GY of 1 ton ha^{-1} was obtained, similar to the 1.12% found by Bogard *et al.* (2010) in an experiment studying a range of wheat varieties grown in different environments in France.

In Fig. 4, the relationships between effects on GPY and effects on GY in CO_2 experiments are shown

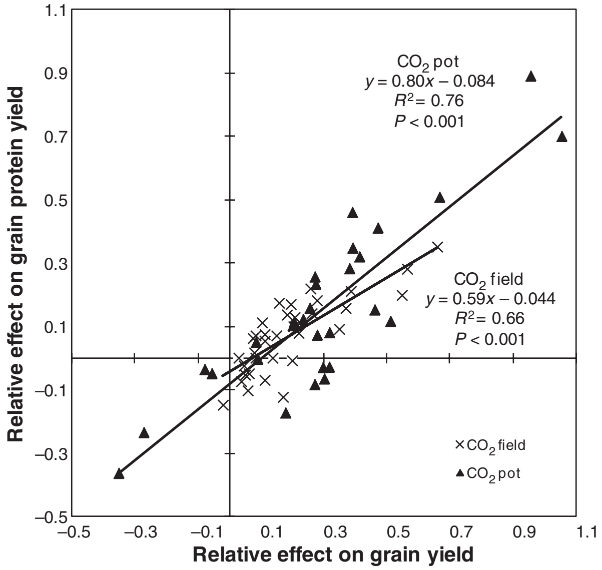

Fig. 4 Relationships between the relative effect on grain protein yield and the relative effect on grain yield for wheat exposed to altered $[CO_2]$ in experiments using field-grown plants and experiments using pot-grown plants.

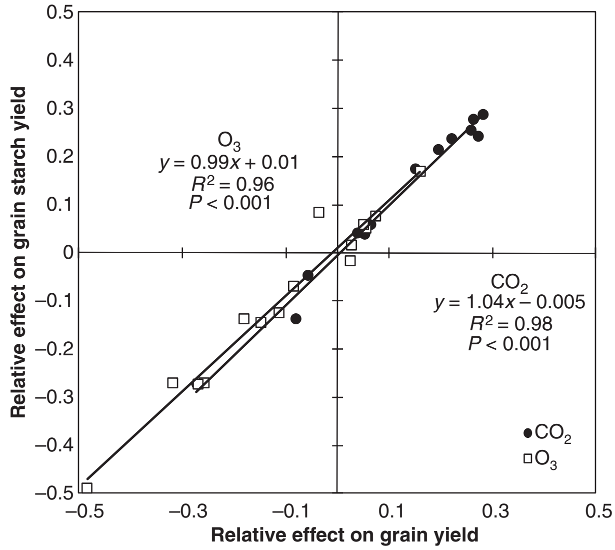

Fig. 5 Relationships between the relative effect on grain starch yield and the relative effect on grain yield for wheat exposed to altered $[CO_2]$ or $[O_3]$.

separately for field-grown and pot-grown plants. Although the slope was larger for potted plants, the difference between the two relationships was not statistically significant (Table 2) and the two data populations overlap to a large extent. Nor was the type of exposure system used (FACE, OTC, glasshouses, self-ventilating chambers, air tunnels, growth chambers) a significant source of variation in relationships between GPY and GY (P = 0.183; data not shown).

Grain starch in relation to GY

For both CO_2 and O_3, very strong linear relationships with slopes close to unity, not significantly different from a hypothetical 1 : 1 relationship, were obtained between the effects on starch yield and GY (Fig. 5). Intercepts were not statistically significant and the effect of CO_2 did not differ from that of O_3 (Table 2). Thus, the effect on starch yield was in direct proportion to the effect on GY and there was no indication of the dilution effects observed being specifically associated with starch. In the case of starch accumulation, the effects of O_3 and CO_2 were similar, as opposed to the other responses covered by this study.

GM and GN in relation to GY

For O_3, there was a strongly significant association between effects on GM and GY (Fig. 6). The corresponding relationship for CO_2 was considerably

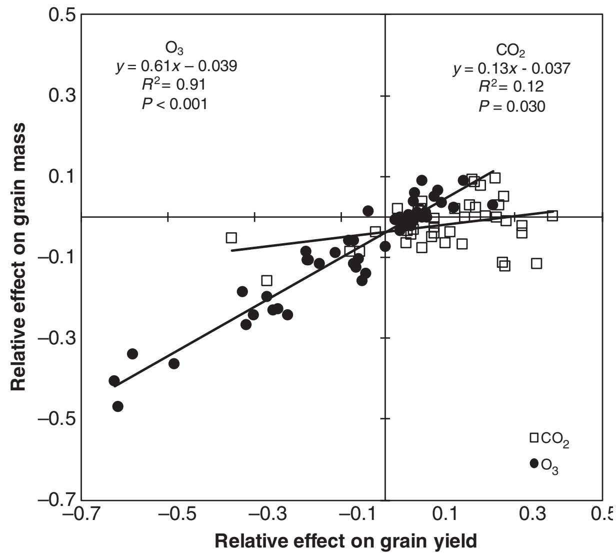

Fig. 6 Relationships between the relative effect on grain mass and the relative effect on grain yield for wheat exposed to altered $[CO_2]$ or $[O_3]$.

weaker (Fig. 6), although statistically significant (Table 2). For the effect of GN in relation to GY (Fig. 7), the effect of CO_2 was much stronger than for O_3, although there was a significant relationship also for O_3. The effect of CO_2 was strongly and significantly different from that of O_3 for both GM and GN (Table 2). All relationships except the GN-to-GY relationship for CO_2 represented significant deviations from a hypothetical 1 : 1 relationship (Table 2).

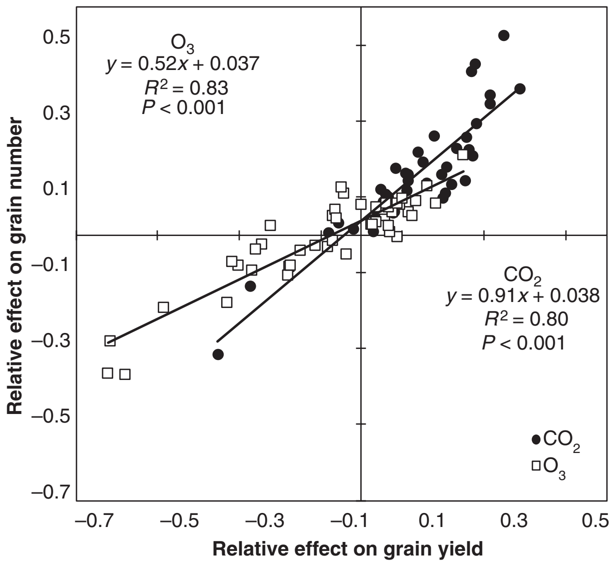

Fig. 7 Relationships between the relative effect on grain number and the relative effect on grain yield for wheat exposed to altered [CO_2] or [O_3].

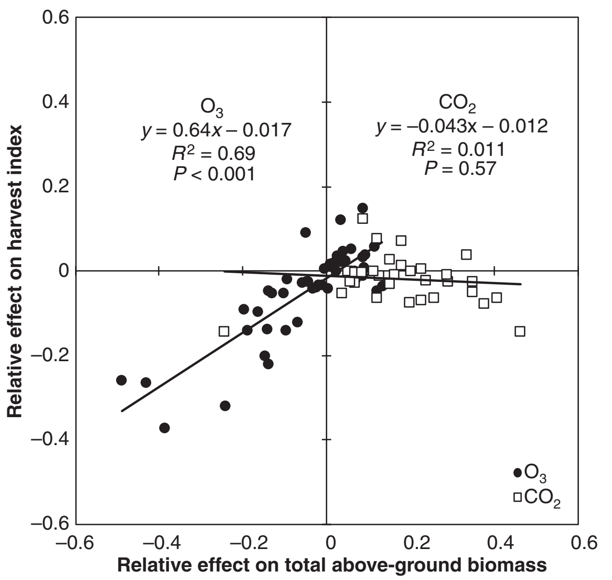

Fig. 8 Relationship between the relative effect on harvest index and the relative effect on total above-ground biomass for wheat exposed to altered [CO_2] or [O_3].

HI in relation to total above-ground biomass

The O_3 relationship between the effects on HI and total above-ground biomass was strongly significant, whereas there was no effect of CO_2 (Fig. 8). This difference, which was statistically significant (Table 2), indicates that the yield stimulation by CO_2 affects reproductive and vegetative parts of the above-ground biomass approximately equally, whereas O_3 strongly and negatively influences the proportion of above-ground biomass converted to grains. Both relationships had slopes significantly different from unity (Table 2).

Discussion

This study showed that over a broad range of experimental conditions and wheat cultivars, effects on GPY changed at a slower rate than the corresponding changes in GY under altered CO_2 or O_3, as shown by the slopes of the regressions in Fig. 2 being significantly smaller than unity. Thus, there was as significant growth dilution of grain protein by both trace gases. The slopes of the relationships between effects on GPY and GY were similar and not significantly different for CO_2 and O_3, essentially supporting our first hypothesis: growth dilution of grain protein by elevated CO_2 does not differ from the yield concentration effect on grain protein caused by elevated O_3. This dilution effect was not a specific treatment effect on starch accumulation, as treatment effects on starch accumulation were directly proportional to effects on GY accumulation for both CO_2 and O_3 treatments (Fig. 5), supporting our third hypothesis: grain starch concentration is not significantly affected by O_3 or CO_2.

Another important and novel finding was that the intercepts of the relationships between GPY and GY were significantly different for CO_2 and O_3, with elevated CO_2 treatment, unlike O_3, exhibiting significant reductions in GPY and GPC in the absence of effects on GY. This was consistent with our second hypothesis: in addition to growth dilution, there exists a negative effect of CO_2 on grain protein accumulation that is independent on the effect on yield. Elevated CO_2 may reduce plant N acquisition through various mechanisms (Taub & Wang, 2008; and references therein; Cramer *et al.*, 2008; Bloom *et al.*, 2010). A plausible explanation of the negative effects on GPY and GPC under CO_2 exposure, also when effects on GY were small or zero, is the adverse effect of elevated CO_2 on nitrate assimilation in wheat observed by Bloom *et al.* (2010). At CO_2-induced GY stimulation above 10%, however, plants in elevated CO_2 were able to take up more N and had higher GPY than plants growing under ambient CO_2, possibly as a results of larger transfer of C below ground (to roots, decomposers and mycorrhiza) causing larger nutrient uptake capacity under elevated CO_2. Positive effects of CO_2 on N acquisition have also been observed in trees (Finizi *et al.*, 2007). Our results thus do not support the admittedly simplified assumption by Loladze (2002) that the accumulation of grain components like protein is constant under GY variation caused by CO_2.

The response pattern of yield components was very different between CO_2 and O_3. GY stimulation by CO_2 was primarily associated with increased GN, whereas effects of O_3 on GY were more strongly associated with variation in GM compared with GN. These results were in line with earlier observations (e.g. Jablonski *et al.*, 2002; Piikki *et al.*, 2008) and our fourth hypothesis: effects of O_3 on GY are related primarily to effects on grain mass, whereas CO_2 primarily affects grain number. The contrasting responses to O_3 and CO_2 are likely to be explained by different processes being influenced. Elevated O_3 is known to negatively affect the duration of the leaves (Grandjean & Fuhrer, 1989) and consequently the duration of grain filling (Gelang *et al.*, 2000) through premature senescence, resulting in smaller grains. Elevated CO_2 can, on the other hand, by stimulating photosynthesis, promote the development of a larger number of seeds (Jablonski *et al.*, 2002). GM is considered an important quality trait in wheat, which is obviously more strongly affected by O_3 than by CO_2.

Also, the pronounced effect on HI by O_3 can be explained by premature senescence (Pleijel *et al.*, 1995): the above-ground biomass established before development of O_3 damage supports grain filling during a shorter period. The absence of CO_2 effects on HI suggests that CO_2 stimulates the production of nonreproductive and reproductive parts of the above-ground biomass to the same extent.

High GM could have a negative effect on GPC, by primarily enhancing protein-poor endosperm (Jenner *et al.*, 1991). GM was only weakly influenced by elevated CO_2 (Fig. 6) and effects on GM can therefore not explain effects by CO_2 on GPY and GPC to any large extent. Elevated O_3 affected both GPY and GM in similar ways, and a link can therefore not be excluded.

A reduction in HI can lead to a shift in distribution of N in the above-ground biomass from grain to straw/leaves. CO_2 affected GPY, but not HI. HI was thus not important for CO_2 effects on GPY. The O_3 effect on HI could, however, explain negative effects on GPY under elevated O_3, although it seems more likely that lower GPY was simply a consequence of reduced N uptake capacity of smaller plants. To summarize, there were substantial differences between the effects of CO_2 and O_3 on GM, GN and HI, but the slopes of the regressions for CO_2 and O_3 in Fig. 2 were similar and not significantly different. Thus, the differential effects on yield components by CO_2 and O_3 do not seem to have a strong effect on growth dilution of grain protein.

Predictions for the current century suggest that CO_2 concentrations will continue to rise. In addition, it is highly likely that O_3 concentrations will increase, but in a pattern much more geographically variable than for CO_2. There are only a few studies available for assessing the interactive influences of CO_2 and O_3 on GY and quality. In the ESPACE-Wheat research programme, interactive effects between CO_2 and O_3 on GY and above-ground biomass (Bender *et al.*, 1999) were investigated in experiments from different European countries. Statistically significant interactions between the two gases were found in two of 13 experiments for GY and in no experiment for aboveground biomass. This indicates that interaction between CO_2 and O_3 on yield dilution of protein is not very important in wheat. However, few of these experiments reported effects on GPC. Pleijel *et al.* (2000) did not observe any interactive effects on GPC in an experiment with two levels of CO_2 and three levels of O_3. Fangmeier *et al.* (1997), on the other hand, reported a significant $CO_2 \times O_3$ interaction on wheat GPC and GPY ($P = 0.013$), but only at the higher of two N application levels.

From the results of the present study, it seems valid to assume that for O_3, the observed effect on GPC is essentially a growth dilution effect. Thus, the effect of O_3 on GPC depends on the magnitude of the effect on GY. The negative effect of elevated CO_2 on GPC is caused by growth dilution in combination with a direct, yield-independent, effect on N acquisition. Effects of atmospheric change on wheat GY, GPY and GPC will be the net result of the effects of CO_2 (increased GY, decreased GPC) and O_3 (decreased GY and GPY, increased GPC), where the latter will vary much more geographically than the former. Areas such as China and India (Van Dingenen *et al.*, 2009), may experience large and increasing yield losses due to O_3 over the next few decades, probably accompanied by reduced GPY, but increased GPC. In areas that will experience small changes in O_3, such as less densely populated areas with climates not promoting O_3 formation strongly, CO_2 effects may dominate over O_3 effects, resulting in increased GY and reduced GPC. The effect on GPY will depend on the magnitude of CO_2-induced GY stimulation. Where yield stimulation by CO_2 is large and the O_3 effect is small, GPY will most likely increase and GPC decrease, whereas both GPY and GPC may be negatively affected in areas where GY stimulation by elevated CO_2 is small. Thus, some combinations of CO_2 and O_3 could significantly aggravate global problems of human nutrition and food security. The likely adverse effect of CO_2 on nitrate assimilation is especially serious, as it will always tend to reduce GPY and GPC and it is beyond doubt that CO_2 concentrations will continue to rise for at least several decades from now.

It is important that modelling of future global change effects on crop production, including CO_2 and O_3 impacts, takes into account the dynamics of the effects demonstrated by this study. As few $CO_2 \times O_3$ interaction experiments have considered crop quality, there is a need for further experimental investigation into the significance of possible interaction effects between the two gases, which both have the potential to strongly influence food security over large parts of the world over the coming century.

Kibite & Evans (1984) concluded that environmental factors are of key importance for GPC in wheat and that genetic factors have a limited potential to influence GPC. Thus, the possibility to enhance GPC through plant breeding may be limited, although not completely absent (e.g. Bnejdi & El Gazzah, 2010; Bogard *et al*., 2010). The conclusion of Kibite & Evans (1984) is generally consistent with the pattern obtained in the present study, where close relationships between effects on GPY and GY, especially for O_3, were found over a wide range of genetic variation represented by a large number of cultivars.

Our study demonstrates that effects of O_3 and CO_2 were different: CO_2 affected GPC more strongly in relation the effect on GY as there was a negative effect on GPY and GPC superimposed on the growth dilution effect. The large variation in the response of GPY vs. GY to CO_2 may represent genetic variation, which could be explored in breeding programmes. The results of our study thus call for further investigation of the extent to which plant breeding can achieve not only a substantial decoupling of GY and GPC but also of stimulating grain N acquisition under rising CO_2, thus counteracting the effect represented by the negative intercept in Figs 2 and 3. As these negative intercepts may be caused by CO_2 impairing nitrate assimilation (Bloom *et al*., 2010), the influence of the choice of fertilizer (oxidized or reduced N) on the CO_2 effect on plant N acquisition should be investigated. The variation in CO_2 effect on GPY and GPC in our study could partly depend on the use of different types of N fertilizers. This is, however, not possible to assess based on the information reported in most of the studies. If plant breeding or choice of fertilizer cannot counteract the negative effects by CO_2 on plant N acquisition, potential future crop yield stimulation by elevated CO_2 comes at the expense of reduced quality (GPC) and thus reduced food security.

This study highlights the need for always relating effects on GPC to the corresponding effects on GY and GPY in any analysis of the effects of environmental or genetic factors on yield vs. quality trade-offs in crops.

Conclusions

The most important conclusions from this study can be summarized as follows.

There was a significant growth dilution effect on grain protein both under CO_2 exposure and O_3 exposure. Unlike O_3, elevated CO_2 caused an additional, yield-independent, negative effect on GPY and GPC. Effects on starch yield varied in direct proportion to effects on GY, indicating that growth dilution of grain protein is not an effect of altered starch accumulation. Grain mass was more strongly affected by O_3 than grain number, whereas the opposite was true for CO_2. CO_2 and O_3 affected the yield components of wheat in different ways, which shows that yield stimulation by CO_2 cannot simply be viewed as the opposite of the yield reduction by O_3. This study has strong implications for future food security as atmospheric change may lead to negative effects on GY and quality that should be considered in breeding programs, N fertilizer research and modelling of the effects of future atmospheric change on crops.

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