Global Change Biology

Global Change Biology (2011) 17, 2697–2706, doi: 10.1111/j.1365-2486.2011.02400.x

Effects of elevated ozone concentration on yield of four Chinese cultivars of winter wheat under fully open-air field conditions

XINKAI ZHU[1]*, ZHAOZHONG FENG†[1], TAOFANG SUN*, XIAOCHENG LIU*, HAOYE TANG†‡, JIANGUO ZHU‡, WENSHAN GUO* and KAZUHIKO KOBAYASHI†

**Jiangsu Provincial Key Lab of Crop Genetics and Physiology/Wheat Research Institute, Yangzhou University, Yangzhou 225009, China, †Graduate School of Agricultural and Life Sciences, The University of Tokyo, 1-1-1 Yayoi, Bunkyo, Tokyo 113-8657, Japan, ‡State Key Laboratory of Soil and Sustainable Agriculture, Institute of Soil Sciences, Chinese Academy of Sciences, Nanjing 210008, China*

Abstract

Four modern cultivars of winter wheat (*Triticum aestivum* L.) were grown under elevated ozone concentration (E-O_3) in fully open-air field conditions in China for three consecutive growth seasons from 2007 to 2009. Results indicated that a mean 25% enhancement above the ambient ozone concentration (A-O_3, 45.7 p.p.b.) significantly reduced the grain yield by 20% with significant variation in the range from 10% to 35% among the combinations of cultivar and season. The varietal difference in the yield response to E-O_3 became nonsignificant when the ANOVA was done by omitting one cultivar which showed unstable response to E-O_3 among the seasons. The reduction of individual grain mass accounted mostly for the yield loss by E-O_3, and showed significant difference between the cultivars. The response of relative yield to E-O_3 was not significantly different from those reported in China, Europe and India on the basis of experiments in open-top chambers. Our results thus confirmed the rising threat of surface O_3 on wheat production worldwide in the near future. Various countermeasures are urgently needed against the crop losses due to O_3 such as mitigation of the increase in surface O_3 with stricter pollution control, and enhancement of the wheat tolerance against O_3 by breeding and management.

Keywords: China, cultivar, grain mass, ozone, winter wheat, yield

Received 24 October 2010 and accepted 12 December 2010

Introduction

Tropospheric ozone (O_3) is currently considered as the most phytotoxic air pollutant at regional to hemispheric scales due to the significant damages to agricultural crops, forest trees and natural vegetation (Fuhrer & Booker, 2003; Ashmore, 2005; Feng *et al.*, 2008; Fuhrer, 2008; Booker *et al.*, 2009; Feng & Kobayashi, 2009). The global relative yield loss induced by current ambient [O_3] was estimated to be in the range between 7% and 12% for wheat (Van Dingenen *et al.*, 2009). Economic losses in four major crops (wheat, soybean, rice and maize) were estimated to range from 14 to 26 billion US dollars on the basis of world market prices for the year 2000, and about 40% of this damage was occurring in China and India (Van Dingenen *et al.*, 2009). In the absence of appropriate emission controls over the O_3 precursors, O_3 concentrations are projected to increase significantly by the middle of this century (The Royal Society, 2008), suggesting that significant yield loss and cost to the world agriculture will grow in the coming a few decades.

Wheat (*Triticum aestivum* L) is the second largest food crop with an annual production of more than 650 million metric tons and harvested area of over 200 million hectares worldwide (FAO, 2008). Nearly two-thirds of the world population depends on this crop for their primary diet. Global wheat production must continue to increase by 2% annually until 2020 to meet the increasing demands driven by growing human population and prosperity (Singh *et al.*, 2007). However, wheat is among the O_3-sensitive crops (Mills *et al.*, 2007; Feng & Kobayashi, 2009), and many studies in the past have shown that elevated [O_3] negatively affects the growth and yield of the crop in interaction with other environmental and genetic factors (e.g. Heagle *et al.*, 1997; Pleijel *et al.*, 2006; Sarkar & Agrawal, 2010; Feng *et al.*, 2011).

A meta-analysis of 53 published studies investigating O_3 effects on wheat between 1980 and 2007 indicated that elevated [O_3] (72 p.p.b. on average) have decreased grain yield by 29% and above ground biomass by 18%

[1]Contributed equally to this work.

Correspondence: Wenshan Guo, e-mail: guows@yzu.edu.cn; Kazuhiko Kobayashi, fax 81 3 5841 5186, e-mail: aclasman@mail.ecc.u-tokyo.ac.jp

in wheat when compared with plants grown in charcoal-filtered air (13 p.p.b. [O_3] on average) (Feng *et al.*, 2008). Another meta-analysis reviewing the effects of current ambient [O_3] on yield and yield component of wheat revealed that about 42 p.p.b. [O_3] has significantly reduced grain yield by 12%, grain mass by 6.2%, and grain number per ear by 5.9%, respectively, as compared with those in base [O_3] including charcoal-filtered and nonfiltered [O_3] of 14 p.p.b. on average (Feng & Kobayashi, 2009). However, these results are based on experiments in greenhouses and chambers, which have been reported to overestimate (Nussbaum & Fuhrer, 2000) or underestimate (Piikki *et al.*, 2008) the impacts of elevated [O_3] on plants.

In recent years, Free Air Concentration Enrichment (FACE) with O_3 has been applied in soybean (Morgan *et al.*, 2006; Betzelberger *et al.*, 2010) and rice (Pang *et al.*, 2009; Shi *et al.*, 2009). When the seasonal mean 8h-[O_3] was increased from 42.1 to 71.9 p.p.b., soybean yields were decreased by 17%, but the variation in the yield response ranged from 8 to 37% among the cultivars and years (Betzelberger *et al.*, 2010). It has been suggested that soybean yields are negatively affected by elevated [O_3] to a greater extent in FACE than the prediction based on chamber results (Morgan *et al.*, 2006). To date, however, no observations have been made on yield loss in wheat exposed to elevated [O_3] under fully open-air conditions.

In the present study, four winter wheat cultivars were grown under elevated [O_3] with a FACE system for three consecutive growing seasons from 2007 to 2009. Our aims in this paper are (1) to determine whether there was a difference among four cultivars in the yield response to O_3, (2) to identify the yield component(s) that are responsible for the yield loss, and (3) to compare the yield response to elevated [O_3] with those reported in chambers elsewhere (Feng *et al.*, 2003; Mills *et al.*, 2007; Sarkar & Agrawal, 2010). The findings of the study are also discussed with respect to their implications for wheat production in China and the world.

Materials and methods

Experiment site

The experiment was conducted in Xiaoji town, Jiangdu county, Jiangsu province, China (119°42′E, 32°35′N). This site has been in continuous cultivation for more than 1000 years with rice-wheat or rice-rapeseed rotations. The soil is Shajiang Aquic Cambosols with a sandy-loamy texture. Relevant soil properties were described by Shi *et al.* (2009): sand (1–0.05 mm) 9.2%, silt (0.05–0.001 mm) 65.7%, clay (<0.001 mm) 25.1%, bulk density 1.2 g cm^{-3}, soil organic C (SOC) 1.5%, total N 1.59 g kg^{-1}, total P 1.23 g kg^{-1}, available P 10.4 mg kg^{-1}, and pH 6.8. The site, at 5 m above the sea level in elevation, sits in the subtropical marine climatic zone with mean annual precipitation of 1100–1200 mm, mean annual temperature of 16 °C, total annual sunshine hours of >2000 h, and a frost-free period of >230 days. During the growth from 1st March (around turning green stage) to final harvest, mean daily maximum and minimum temperature were 20.9° and 10.2 °C in 2007, 20.6° and 10.1 °C in 2008, and 20.2° and 9.5 °C in 2009, respectively (Fig. 1a, b, c). Mean daily maximum photosynthetic photon flux density (PPFD) was 1266 μmol m^{-2} s^{-1} in 2007, 1376 μmol m^{-2} s^{-1} in 2008 and 1343 μmol m^{-2} s^{-1} in 2009 (Fig. 1d, e, f). Accumulated precipitation was 194, 171 and 136 mm in 2007, 2008 and 2009, respectively, for a 3-month period from March to May (Fig. 1g, h, i).

Ozone fumigation

Three 240 m^2 plots were subjected to elevated [O_3] (hereinafter, E-O_3) and three equal size plots were maintained at ambient [O_3] (hereinafter called A-O_3). The target [O_3] for the E-O_3 plots was 50% higher than the ambient [O_3]. Any one of the E-O_3 plots was separated from the other plots by at least 70 m to avoid cross-contamination. The experimental design was based on completely randomized plots allocated to either A-O_3 or E-O_3, and split into subplots of wheat cultivars. In the E-O_3 plots, crops were grown within 14 m in diameter octagons with a perimeter of eight 6 m ABS pipes. A mixed gas consisting of about 5% O_3 and 95% O_2 was produced by an O_3 generator (KCF-BT0.2, Jiangsu Koner Ozone Co. Ltd, Yangzhou, China). Using a mass flow controller, the O_3/O_2 mixture was released in a stream of compressed air into the plots through the ABS pipes positioned at about 50 cm above the canopy height. The [O_3] at the center of each plot was measured every 20 s by an O_3 analyzer (model 49C, Thermo Environmental Instruments, Franklin, MA, USA). On the basis of the wind direction and wind speed, O_3 was released achieving the elevation of [O_3] within ± 15% of the set point for 90% of the time, and within ± 20% of the set point for 95% of the time. Ozone fumigation ran from 09:00 hours to sunset when ambient [O_3] was higher than 20 p.p.b. When leaves were wet from fog, dew or rain, the O_3 release was halted as has been done with soybean (Morgan *et al.*, 2006) and aspen (Karnosky *et al.*, 2007). When ambient [O_3] was higher than 170 p.p.b., the target [O_3] was fixed at 250 p.p.b. to avoid extraordinarily high [O_3]. Such situations were very rare, however, the 10-min mean [O_3] in E-O_3 plots had a seasonal maximum of 176 p.p.b. and a 99.5 percentile of 142 p.p.b. averaged across the three replicates throughout the 3 years' fumigation period. In the ambient plots, plants were grown under ambient [O_3] without perimeter pipes. The duration of ozone fumigation was different from year to year. For the three growth seasons, O_3 fumigation was conducted for periods from 14 April to 22 May in 2007, from 5 March to 26 May in 2008, and from 1 March to 24 May in 2009.

Plant material

We used four modern cultivars: Yannong 19 (strong-gluten wheat, hereafter called Y19), Yangmai 16 (medium-gluten wheat, hereafter called Y16), Yangmai 15 (weak-gluten wheat, hereafter called Y15) and Yangfumai 2 (weak-gluten wheat, hereafter called Y2) in three consecutive growing seasons: 2006–2007, 2007–2008 and 2008–2009. Standard cultivation

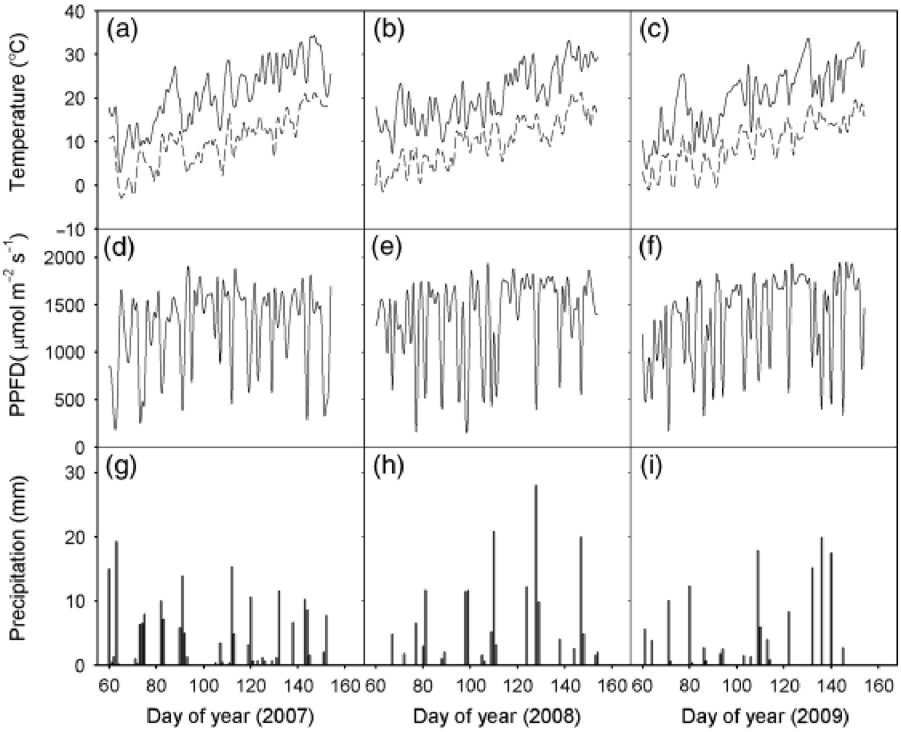

Fig. 1 Meteorological data collected at the experiment site during the 2007, 2008 and 2009 growth seasons. Maximum (black line) and minimum (grey line) daily temperature in 2007 (a), 2008 (b) and 2009 (c) seasons, daily maximum photosynthetic photon flux density (PFPD) in 2007 (d), 2008 (e) and 2009 (f) seasons, and daily precipitation in 2007 (g), 2008 (h) and 2009 (i) seasons.

practices common to the region were followed in all experimental plots. Seeds of the four cultivars were sown in subplots (11 m^2 each), which were distributed randomly in each plot of A-O_3 and E-O_3. For the 2006–2007 growing season, the subplots were distributed randomly in the ring, but the position was the same across all the rings (A-O_3 and E-O_3). To better estimate the possible effects of position within the rings, the subplots were distributed randomly within and across the rings in 2007–2008 and 2008–2009 growing seasons. E-O_3 had no effect on the flowering date for all varieties investigated. However, the maturity date was 1–3 days in O_3-treated plants earlier than ambient ones for three growth seasons excluding Y19 at 2007 with 6 days earlier. Relative to other three varieties, Y19 showed 5 days later at flowering date, but only 1/2 days later at maturity date. For Y2, Y15 and Y16, they showed similar phenology at either A-O_3 or E-O_3.

Seeds were hand-sown with a basic seeding density of 2.25 million ha^{-1} and a row space of 25 cm on 5 November 2006, 15 November 2007 and 19 November 2008. Inorganic fertilizers were applied with the same amount and at the same developmental stages among three growth seasons. Nitrogen was applied as urea ($N = 46\%$) and diammonium phosphate at a total rate of 210 kg N ha^{-1}, which was split into basal application at planting (60% of the total N), side-dressings at early tillering (only urea, 10% of the total N) and elongation (30%) stages. P and K were applied as diammonium phosphate and potassium chloride, respectively, at a rate of 90 kg P_2O_5 ha^{-1} and 90 kg K_2O ha^{-1} which were split-applied with 60% at planting and 40% at elongation stage, respectively.

Harvesting

At maturity, grain yield was determined for all plants from a 2 m^2 patch in the middle of each subplot (excluding plants in the borders). Grain yield components, i.e. the number of ears per square meter (average over 2 m^2), the number of grains per ear (averaged for 50 ears), and 1000 grain mass (three replicates) in each subplot were also determined at crop maturity.

Calculation of O_3 exposure dose

AOT40 (accumulated daytime [O_3] above threshold of 40 p.p.b.) is usually calculated for 90 days centering on the anthesis date (Pleijel *et al*., 2007), but all the wheat varieties used in this study reached maturity around 30 days after anthesis. We therefore decided to calculate the AOT40 for 75 days from 44 days before to 30 days after anthesis date. When no O_3 release was performed, ambient [O_3] was used to calculate AOT40 in E-O_3 plots. In 2007, data of ambient [O_3] were missing for the period before O_3 fumigation, and, hence, averages of the ambient [O_3] in 2008 and 2009 seasons were used in the daily AOT40 calculation for that period of 2007.

Statistical analysis of the crop performance under elevated [O_3]

The observations on crop yield and yield components were averaged, when needed, for each subplot and subjected to ANOVA with a mixed linear model to detect the effects of the O_3 treatment (ambient vs. elevated O_3 contrast), cultivar, season, and subplot location within the rings, as well as their interactions. In the model, the ring was assigned to random effect, and the other variables to fixed effects. Tukey–Kramer HSD test was used to compare yield and yield components between O_3 levels, seasons and cultivars. A difference between the means was considered significant when $P \leq 0.05$. The statistical analysis was done by using JMP software (SAS Institute, Cary, NC, USA).

Comparison of the yield response with other studies

For the comparison of the yield sensitivity to E-O_3 with the other studies (Feng *et al*., 2003; Mills *et al*., 2007; Sarkar & Agrawal, 2010), yield loss per unit increase of AOT40 was estimated with the same mixed linear model as above but having AOT40 among independent variables in place of the A-O_3 vs. E-O_3 contrast. A 95% confidence interval (CI) was constructed for the relative yield sensitivity to O_3. For the comparison with our results, we digitized Fig. 2 of Mills *et al*., (2007) and Fig. 7 of Sarkar & Agrawal (2010) by using GRAFULA software (Wesik SoftHaus, St. Petersburg, Russia), and fit to the respective dataset a linear model:

$$RY = 1s\,\mathrm{AOT40},$$

where *RY* is relative yield, and s is the relative yield sensitivity to AOT40. For Sarkar & Agrawal (2010), the two varieties were pooled since no significant effects of variety or variety × AOT40 interaction were detected. A 95% CI was constructed for the relative yield sensitivity in the respective studies (Mills *et al*., 2007; Sarkar & Agrawal, 2010), and compared with the interval estimate in this study. The model fitting was done by using JMP software (SAS Institute, USA). The comparison was also done with the relative yield sensitivity to AOT40 for Chinese cultivars in Feng *et al*. (2003), who reported only the point-estimate of the sensitivity s.

Results

O_3 exposure

Table 1 shows the O_3 doses across the 75 days from 44 days before to 30 days after anthesis date for cultivar Y16. Other cultivars had different anthesis date, and, hence, slightly different O_3 doses. As mentioned before, we estimated [O_3] data (the mean 7-h [O_3] (M7) and AOT40) before fumigation in 2007, so other variables in 2007 as shown in Table 1 were not compared with other two growth seasons. Because the O_3 fumigation was discontinued under certain conditions as mentioned before, the effective increase in M7 was 23%, 25% and 28% in 2007, 2008 and 2009, respectively. The AOT40 in E-O_3 was increased significantly above that in A-O_3 by 89.2%, 97.3% and 122% for 2007, 2008, and 2009, respectively (Table 1). The daily [O_3] (M7) across three growth seasons showed much higher at reproductive stage of winter wheat than vegetative stages, especially in May (Fig. 2). The mean daily maximum ozone concentration in E-O_3 was 29.8% and 33.3% higher than that in A-O_3 in 2008 and 2009, respectively (Table 1).

Grain yield

Since the subplot location had no significant effects nor significant interactions with any of the factors considered, it was omitted from the subsequent analyses. E-O_3 significantly reduced the grain yield from 0.78 to 0.62 kg m^{-2} (20.2%) when averaged across four cultivars and three growing seasons (Table 2, Fig. 3). Yields were significantly higher in 2008 than those in 2007 which were significantly higher than those in 2009 ($P < 0.05$). There was significant difference in grain yield among the cultivars with the highest being Y15 and the lowest being Y19 ($P < 0.05$) on average across the three seasons, but the varietal difference in yield varied by season (Table 2, Fig. 3). The yield response to O_3 showed no significant interaction with cultivar ($P = 0.112$, Table 2), but the O_3 × cultivar × season interaction was highly significant ($P = 0.007$, Table 2). A detailed analysis of

Table 1 Mean O_3 concentration and AOT40 (accumulated daytime [O_3] above a threshold of 40 ppb) from 44 days before to 30 days after anthesis date for variety Y16 in each treatment (A-O_3: ambient [O_3] treatment, and E-O_3: elevated [O_3] treatment) for three growth seasons

	O_3 concentration (ppb)									
	7 h means (M7)*		12 h means (M12)†		24 h means (M24)		Mean 1 h peak (M1-p)		AOT40 (ppm h)	
	A-O_3	E-O_3	A-O_3	E-O_3	A-O_3	E-O_3	A-O_3	E-O_3	A-O_3	E-O_3
2007	46.4‡	56.9‡	45.8§	61.3§	29.8§	37.1§	67.6§	94.3§	7.8‡	14.7‡
2008	46.0	57.6	41.5	50.3	27.6	30.9	60.5	78.5	8.3	16.4
2009	44.6	57.3	38.8	48.6	25.6	30.3	56.6	75.5	6.8	15.1

*7 h: 0900–1600 h.
†12 h: 0700–1900 h.
‡For calculation of M7 and AOT40 in 2007, [O_3] record was missing for the period before O_3 fumigation, hence the averages of the ambient [O_3] in 2008 and 2009 seasons was used for that period.
§M12, M24 and M1-p in 2007 were calculated only for the period after the commencement of O_3 fumigation until harvest.

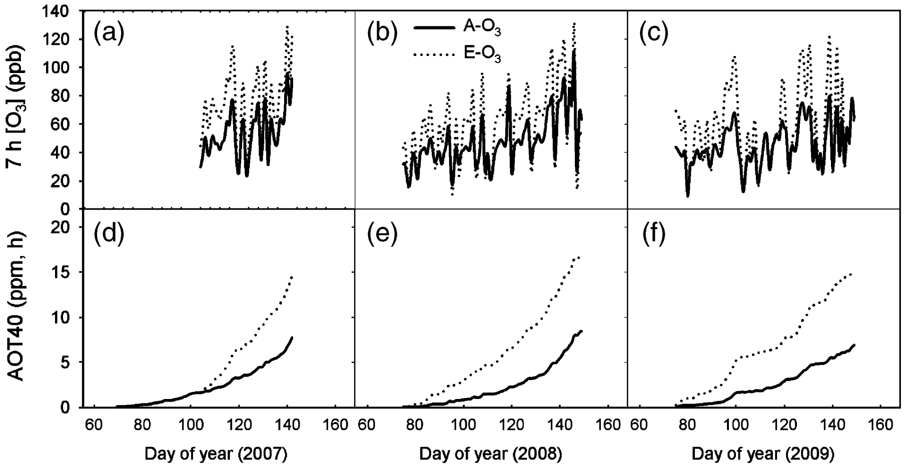

Fig. 2 Seven-hour daily mean [O_3] (M7) for ambient [O_3] (A-O_3) and elevated [O_3] (E-O_3) in 2007 (a), 2008 (b) and 2009 (c) seasons, and AOT40 (accumulated daytime [O_3] above a threshold of 40 p.p.b.) in 2007 (d), 2008 (e) and 2009 (f) seasons.

Table 2 Analysis of variance for grain yield and its components in response to elevated [O_3]

Effects	F/*P**	Grain yield	Grain mass	Number of grains per ear	Number of ears per area	Number of grains per area
O_3	F	79.64	51.04	10.30	3.36	0.01
	P	0.0009	0.0020	0.0326	0.1409	0.9309
Cultivar (C)	F	52.96	29.24	77.24	43.59	3.20
	P	<0.0001	<0.0001	<0.0001	<0.0001	0.0323
$O_3 \times C$	F	2.11	6.26	3.45	5.51	3.77
	P	0.1123	0.0012	0.0244	0.0027	0.0172
Season (S)	F	151.13	3.32	58.44	147.19	144.62
	P	<0.0001	0.0453	<0.0001	<0.0001	<0.0001
$O_3 \times S$	F	11.34	0.63	1.75	0.83	1.56
	P	0.0001	0.5392	0.1860	0.4413	0.2215
$C \times S$	F	6.59	9.72	16.41	10.54	5.88
	P	<0.0001	<0.0001	<0.0001	<0.0001	0.0001
$O_3 \times C \times S$	F	3.44	0.47	4.34	0.76	2.82
	P	0.0072	0.8268	0.0016	0.6058	0.0207

*F is the F-value, and *P* is the *P*-value for each effect in ANOVA.

the results showed that this interaction was due to the unstable yield response to E-O_3 in Y19, in which E-O_3 reduced the yield by 18.7%, 34.7% and 10.1% in 2007, 2008 and 2009, respectively. When this cultivar is omitted, the ANOVA showed no $O_3 \times$ cultivar $\times$ season interaction at all ($P = 0.859$), while the $O_3 \times$ cultivar ($P = 0.109$) or $O_3 \times$ season ($P = 0.220$) interaction remained nonsignificant.

Yield components

E-O_3 significantly reduced individual grain mass by 19.2% and grain number per ear by 3.52%, whereas its effect on ear number per m^2 was nonsignificant ($P = 0.141$, Table 2) when averaged across the four cultivars and three growth seasons (Table 2, Figs. 4, 5 and 6). When the latter two yield components are combined into total grain number per m^2, no effect of O_3 was observed at all ($P = 0.931$, Table 2). Thus the grain mass reduction was the dominant response to E-O_3 among the yield components. The effects of E-O_3 did not vary by season in any of the yield components (Table 2), whereas all the yield components showed significantly different response to E-O_3 between cultivars (Table 2). On average across the three seasons, the decrease in individual grain mass due to E-O_3 was significantly larger in Y2 and Y19 (22–25%) than in Y16 and Y15 (14–16%); the contrast between the O_3-induced grain mass reduction in the former two cultivars and that in the latter two cultivars was highly significant ($P = 0.0002$).

A significant decrease in grain number per ear was found in Y2 (6.86%), whereas no effect was found in Y16 (0.32%). The significant interaction: $O_3 \times$ cultivar $\times$ season

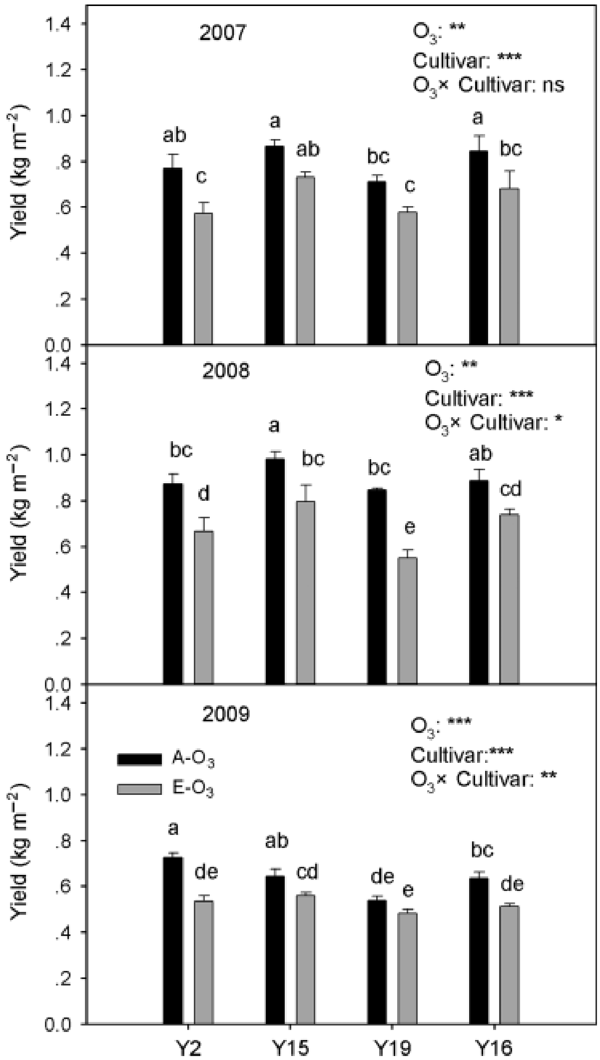

Fig. 3 Yield of winter wheat under ambient [O_3] (A-O_3) and elevated [O_3] (E-O_3) (mean ± SD, $n = 3$). Within each year, bars without a same letter are significantly different from each other at $P < 0.05$. For ANOVA results, *,**,***Significant difference at $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively. ns, not significant.

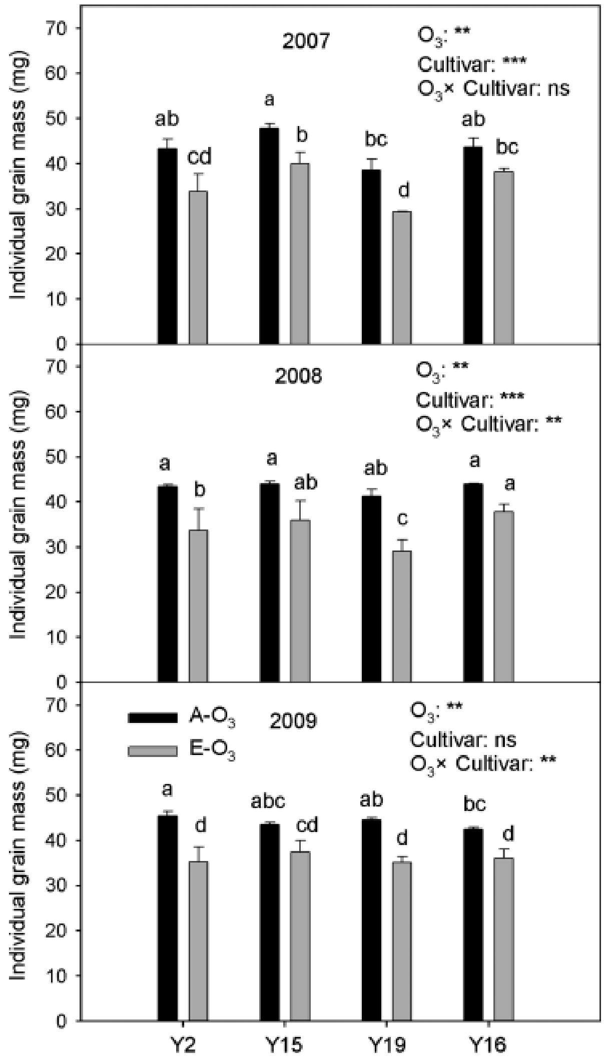

Fig. 4 Effects of ozone (ambient [O_3] treatment (A-O_3) and elevated O_3 treatment (E-O_3)) on individual grain mass of winter wheat (mean ± SD, $n = 3$). See Fig. 3 for explanation of the symbols.

(Table 2), disappeared when Y19 was omitted from the analysis, while the other results were quite similar except the only weak significance ($P = 0.085$) for the effect of E-O_3 on grain number per ear. Thus the responses of the yield components to E-O_3 were stable among three seasons in all cultivars but Y19.

Comparison of yield response to elevated [O_3] with other studies

As noted above, the cultivar Y19 exhibited large year-to-year variation in the response of grain yield and yield components to O_3, and, hence, was omitted from the analysis below. The ANOVA showed that only the season × cultivar interaction is significant with all other interactions being non-significant. The yield sensitivity to AOT40 was therefore estimated with the model based on AOT40, cultivar, season, and season × cultivar interaction (Table 3). Across the three cultivars (Y15, Y16 and Y2), grain yield changed by −0.0197 (kg m^{-2} ppm h^{-1}) to a unit increase of AOT40. Considering the estimation error (SE = 0.00279, kg m^{-2} ppm h^{-1}), the 95% CI is constructed for the yield sensitivity as [−0.027, −0.012] (kg m^{-2} ppm h^{-1}), which is divided by the intercept: 0.961 (kg m^{-2}) (Table 3) to yield the 95% CI of the *relative* yield sensitivity: [−0.028, −0.013] (ppm h^{-1}).

The yield loss sensitivity: −0.013 (ppm h^{-1}) for a Chinese winter wheat cultivar planted in pots and exposed to elevated [O_3] in open-top chambers (Feng

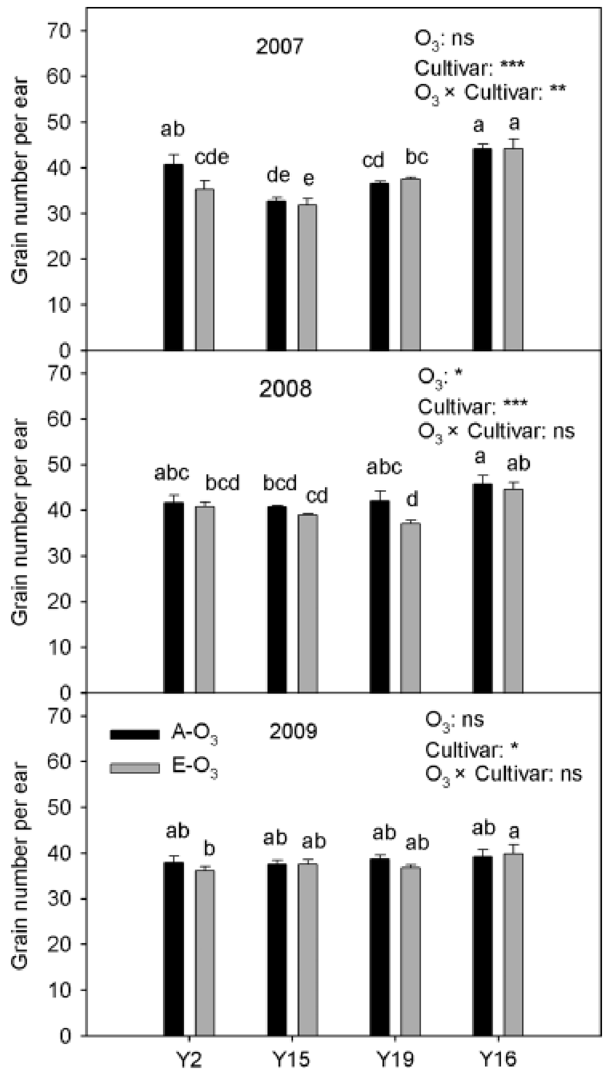

Fig. 5 Effects of ozone (ambient [O_3] treatment (A-O_3) and elevated O_3 treatment (E-O_3)) on grain number per ear of winter wheat (mean $\pm$ SD, $n = 3$). See Fig. 3 for explanation of the symbols.

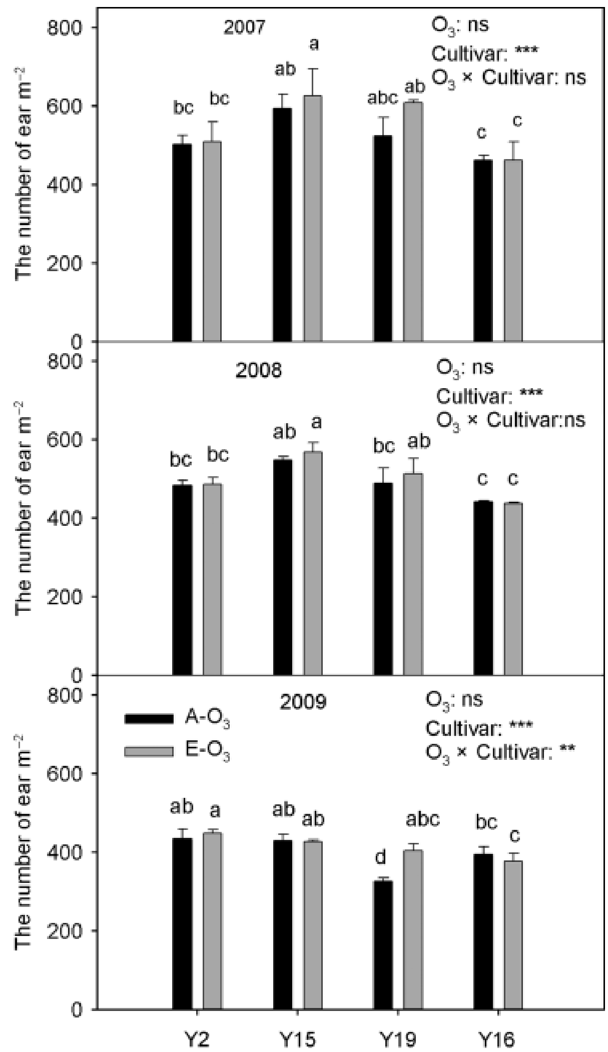

Fig. 6 Effects of ozone (ambient [O_3] treatment (A-O_3) and elevated O_3 treatment (E-O_3)) on ear number per unit area of winter wheat (mean $\pm$ SD, $n = 3$). See Fig. 3 for explanation of the symbols.

et al., 2003) is right at the upper boundary of our 95% CI. Mills *et al*. (2007) have calculated the relative yield sensitivity to AOT40 as -0.0161 (ppm h^{-1}), which is within the 95% CI in this study. Our estimate based on the digitized data yielded a 95% CI for the yield sensitivity as [-0.018, -0.015] (ppm h^{-1}), which is contained within that of this study. Sarkar & Agrawal (2010) have given a higher sensitivity of -0.03 (ppm h^{-1}) than those in our study and Mills *et al*. (2007), while the 95% CI of the sensitivity: [-0.033, 10.024] (ppm h^{-1}), overlaps with that of this study.

Discussion

In this study with FACE-Ozone, the mean 25% enhancement of [O_3] from A-O_3 (45.7 p.p.b.) to E-O_3 (57.3 p.p.b.) reduced wheat yield by about 20%, whereas a meta-analysis of non-FACE results indicated the wheat yield loss from 15 to 27% (95% CI) in elevated [O_3] (65 p.p.b.) relative to the base [O_3] (<26 p.p.b.) (Feng & Kobayashi, 2009). The FACE-Ozone results thus show apparently higher sensitivity in wheat yield to elevated [O_3] than the non-FACE results do. However, the meta-analysis gave a smaller yield loss estimate than that from dose-response function, possibly because the former included observations in greenhouses and controlled growth chambers as well as those in open-top chambers whereas the latter was derived from only open-top chamber experiments (Feng & Kobayashi, 2009). It has been found that the effects of elevated [O_3] on wheat growth tend to be less in indoor growth chambers than in open-top chambers (Feng *et al*., 2008).

Table 3 Estimated parameters of the linear model for the yield response to O_3.

Model: Yield = intercept + s AOT40 + cultivar effect + season effect + cultivar × season interaction

	Intercept ($kg\,m^{-2}$)	S ($kg\,m^{-2}\,ppm\,h^{-1}$)	Cultivar	Season	Cultivar × season
Estimate (SE)	0.961 (0.034)	−0.0197 (0.0028)	–	–	–
DF	4.24	4.27	40.13	42.48	39.98
P-value	< 0.0001	0.0017	< 0.001	< 0.001	0.0003

The quantitative analysis indeed showed no significant difference between the yield sensitivity to AOT40 in this field study and that in each of the chamber studies (Feng *et al*., 2003; Mills *et al*., 2007; Sarkar & Agrawal, 2010). It is noteworthy that the yield sensitivity is significantly different among the three chamber studies tested here beyond the 95% CIs. Our field study has thus supported the results of the chamber experiment, but the 95% CI with two-fold difference in the relative yield sensitivity has not allowed us to detect the difference between the open-top chamber and field studies. A continuation of the FACE-Ozone experiment with more than one level of E-O_3 should facilitate narrowing of the CI, which would enable a more precise testing of the open-top chamber results against the field observations.

One of our aims in this study was to test if there is a varietal difference in wheat yield response to elevated [O_3]. Although no significant difference was observed between cultivars in the yield response to E-O_3, varietal difference was evident in the response of individual grain mass, whose decline was mostly responsible for the yield loss. Numerous studies have shown that O_3 reduces yield of wheat by decreasing ear numbers, grain numbers per ear, and individual grain mass (see review by Black *et al*., 2000). Our result is consistent with the result of a meta-analysis based on chamber experiments in that reduced individual grain mass is the major cause of the yield loss to O_3 (Feng *et al*., 2008; Feng & Kobayashi, 2009). Individual grain mass is under genetic control, and is affected by occurrence of stresses only during the grain filling stage. In the same FACE-Ozone experiment as this study, Feng *et al*. (2011) have observed varietal differences in the response of photosynthetic capacity in flag leaves to elevated [O_3]. E-O_3 significantly reduced chlorophyll contents and photosynthetic rate in flag leaves and accelerated premature senescence of the leaves during grain filling stage. These changes occurred earlier in cultivar Y2 than Y16 (Feng *et al*., 2011). The declining photosynthate supply during the grain filling stage should have resulted in reduced grain mass more in Y2 than Y16. It was indeed observed that E-O_3 reduced the grain filling rate, but had no effects on the duration of grain filling (X. K. Zhu, unpublished data). Feng *et al*. (2010) have suggested the contribution of apoplastic ascorbate to the varietal difference in the flag leaf responses to E-O_3, which has been reported to protect the leaf tissue in many plants against the oxidative damages from ozone (e.g. Conklin & Barth, 2004).

The varietal difference in wheat responses to O_3 from leaf tissue to the yield component indicates the possibility of breeding ozone-tolerant cultivars. The varietal difference in wheat responses to [O_3] may also account at least partly for the difference in the relative yield response between the experiments with different varieties in different locations, e.g. a Chinese cultivar in Feng *et al*. (2003), European and American cultivars in Mills *et al*. (2007), and Indian cultivars in Sarkar & Agrawal (2010). These three experiments had yield sensitivities to [O_3] distinctly different from each other. Field-based experiments require the use of cultivar(s) adapted to local climate, and, hence, a comparison of results among the experiments inherently includes the effects of the genetic variability.

As noted above, the impact of elevated [O_3] on grain filling appears to be critical to the yield loss, which is consistent with the findings of Pleijel *et al*. (1998). They exposed field-grown spring wheat to the same O_3 dose (2500 p.p.b. h above 40 p.p.b.) before and after the onset of anthesis, and found that O_3 exposure after anthesis reduced the grain yield more than that before it (Pleijel *et al*., 1998). The yield loss sensitivity could therefore vary by the seasonal pattern of [O_3]. For example, an experiment, in either open-top chambers or FACE, would result in a lower yield loss sensitivity to [O_3], if the seasonal trend in [O_3] is less pronounced than that in this study, which had higher ambient [O_3] during grain filling than the earlier periods (Fig. 2).

In this study, when the cultivar Y19 is omitted, there was no significant difference between the seasons in the grain yield response to E-O_3, despite the much shorter period of exposure to E-O_3 in 2007 than in 2008 and 2009. This could be explained by the increasing trend in ambient [O_3] across the growing season (Fig. 2). Ambient [O_3] was low earlier in the season, and, hence, the delay of the O_3 exposure in 2007 has not resulted in much smaller O_3 elevation over the ambient [O_3] than the other seasons (Table 1).

Another determinant of the yield response to [O_3] is local climate. It has been reported that the yield losses are better described with stomatal flux-based index of O_3 than the concentration-based index: AOT40, in wheat and potato (Pleijel *et al*., 2007). This means that the yield sensitivity to surface [O_3] is subjected to the influence of climatic variables, particularly air and soil moisture that alter leaf stomatal conductance.

Because of the possible involvements of genetic variability, seasonal [O_3] patterns, and local climate in the yield sensitivity to [O_3], a reliable estimation of the crop loss due to elevated [O_3] at a large spatial scale would require field-based experiments conducted at multiple locations to cover the region of concerns. The multisite experiments have indeed been done in the USA (Heck *et al*., 1984) and Europe (Pleijel *et al*., 2007), whereas none has ever existed in Asia.

This study has supported, within the estimation error, the wheat yield sensitivity to elevated [O_3] observed in open-top chamber experiments, which constituted a basis of the projections of crop losses for the future (Wang & Mauzerall, 2004; Van Dingenen *et al*., 2009). Considering the projections of large crop losses to elevated [O_3], and the regional as well as global importance of wheat crop, it is surprising that we do not have even a plan for the networked experiments across Asia.

Whereas AOT40 is usually calculated for 90 days centering on the anthesis date (Pleijel *et al*., 2007), the wheat varieties used in this study reached maturity 30 days after anthesis date. Because accumulating AOT40 beyond the 30 days made no sense, we calculated it across 75 days from 44 days before through to 30 days after the anthesis date, or from 16 March to 29 May for Y16 and Y2 in 2008 and 2009 seasons with some differences for other cultivars and seasons. We could have begun the integration of [O_3] above 40 p.p.b. by 15 days earlier, and, thereby, extended the period of integration to 90 days. This should have, however, made little difference in the seasonal AOT40, since ambient [O_3] was very low before mid-March. The fixed time window of 90 days has indeed been altered to a shorter time period under warmer climate in Europe (Pleijel *et al*., 2007).

It is noteworthy that the subplot location within the main plots had no interaction with O_3 in wheat yield in this study. Within a FACE ring, there is inherently a gradient of [O_3], which is higher near the windward side of the O_3-emitting tubes, and the season-long mean would exhibit a bowl-shaped distribution of the concentration as reported for FACE-CO_2 (Okada *et al*., 2001). The [O_3] gradient may not have been large enough to affect the yield response to E-O_3 significantly, or such variation was averaged out across the subplots when the plant samples were harvested.

Conclusions

Our results thus confirmed the rising threat of surface [O_3] on wheat production in the near future. A more precise prediction would require continuation of the existing experiment with FACE-Ozone, and setting up of experiments with a similar design in other locations where wheat is a critical component for food security. Although the varietal difference was non-significant in the yield response to elevated [O_3], the decline of individual grain mass, which was most responsible for the yield loss, exhibited clear difference between the cultivars. This result indicates a possibility of breeding for higher tolerance in wheat to elevated [O_3]. The physiological and biochemical traits associated with the varietal difference (Feng *et al*., 2010, 2011) could serve as useful indicators of inherited O_3 tolerance for the breeding efforts. More importantly than the adaptive efforts, however, controls of the release of ozone precursors must be urgently imposed or strengthened to curb the rapid increase in surface [O_3] in this region and thereby reduce the risks to crop production, natural vegetation and human health.

Acknowledgements

This study was supported by the Global Environment Research Fund (C-062) of the Ministry of Environment, Japan, Postdoctoral Fellowship (P09120) and the Grant-in-aid for Scientific Research program (Scientific Research 21248030) both of the Japan Society for the Promotion of Science. It was also supported by the International S & T Cooperation Program of China (2009DFA31110), the Knowledge Innovation Program of Chinese Academy of Sciences (KZCX2-EW-414) and the Instrument Developing Project of the Chinese Academy of Sciences (YZ0603). We are grateful to Professor Liu G. for his technical support in the free-air ozone release system.

References

Ashmore MR (2005) Assessing the future global impacts of ozone on vegetation. *Plant, Cell and Environment*, **28**, 949–964.

Betzelberger AM, Gillespie KM, Mcgrath JM, Koester RP, Nelson RL, Ainsworth EA (2010) Effects of chronic elevated ozone concentration on antioxidant capacity, photosynthesis and seed yield of 10 soybean cultivars. *Plant, Cell and Environment*, **33**, 1569–1581.

Black VJ, Black CR, Roberts JA, Stewart CA (2000) Impact of ozone on the reproductive development of plants. *New Phytologist*, **147**, 421–447.

Booker F, Muntifering R, McGrath M *et al*. (2009) The ozone component of global change: potential effects on agricultural and horticultural plant yield, product quality and interactions with invasive species. *Journal of Integrative Plant Biology*, **51**, 337–351.

Conklin PL, Barth C (2004) Ascorbic acid, a familiar small molecule intertwined in the response of plants to ozone, pathogens, and the onset of senescence. *Plant, Cell and Environment*, **27**, 959–970.

FAO (2008) Statistical database. Available at: http://faostat.fao.org (accessed 3 July 2008).

Feng Z, Jin M, Zhang F, Huang Y (2003) Effects of ground-level ozone (O_3) pollution on the yields of rice and winter wheat in the Yangtze River Delta. *Journal of Environmental Sciences*, **15**, 360–362.

Feng Z, Kobayashi K (2009) Assessing the impacts of current and future concentrations of surface ozone on crop yield with meta-analysis. *Atmospheric Environment*, **43**, 1510–1519.

Feng Z, Kobayashi K, Ainsworth EA (2008) Impact of elevated ozone concentration on growth, physiology and yield of wheat (*Triticum aestivum* L): a meta-analysis. *Global Change Biology*, **14**, 2696–2708.

Feng Z, Pang J, Kobayashi K, Zhu J, Ort DR (2011) Differential responses in two varieties of winter wheat to elevated ozone concentration under fully open-air field conditions. *Global Change Biology*, **17**, 580–591.

Feng Z, Pang J, Nouchi I, Kobayashi K, Yamakawa T, Zhu J (2010) Apoplastic ascorbate contributes to the differential ozone sensitivity in two varieties of winter wheat under fully open-air field conditions. *Environmental Pollution*, **158**, 3539–3545.

Fuhrer J (2008) Ozone risk for crops and pastures in present and future climates. *Naturwissenschaften*, **96**, 173–194.

Fuhrer J, Booker F (2003) Ecological issues related to ozone: agricultural issues. *Environment International*, **29**, 141–154.

Heagle AS, Miller JE, Pursley WA (2000) Growth and yield responses of winter wheat to mixtures of ozone and carbon dioxide. *Crop Science*, **40**, 1656–1664.

Heck WW, Cure WW, Rawlings JO *et al*. (1984) Assessing impacts of ozone on agricultural crops: II. Crop yield functions and alternative exposure statistics. *Journal of Air Pollution Control Association*, **34**, 810–817.

Karnosky DF, Werner H, Holopainen T *et al*. (2007) Free-air exposure systems to scale up ozone research to mature trees. *Plant Biology*, **9**, 181–190.

Mills G, Buse A, Gimeno B, Bermejo V, Holland M, Emberson L, Pleijel H (2007) A synthesis of AOT40-based response functions and critical levels of ozone for agricultural and horticultural crops. *Atmospheric Environment*, **41**, 2630–2643.

Morgan PB, Mies TA, Bollero GA, Nelson RL, Long SP (2006) Season-long elevation of ozone concentration to projected 2050 levels under fully open-air conditions substantially decreases the growth and production of soybean. *New Phytologist*, **170**, 333–343.

Nussbaum S, Fuhrer J (2000) Difference in ozone uptake in grassland species between open-top chambers and ambient air. *Environmental Pollution*, **109**, 463–471.

Okada M, Lieffering M, Nakamura H, Yoshimoto M, Kim HY, Kobayashi K (2001) Free-air CO_2 enrichment (FACE) using pure CO_2 injection: system description. *New Phytologist*, **150**, 251–260.

Pang J, Kobayashi K, Zhu J (2009) Yield and photosynthetic characteristics of flag leaves in Chinese rice (*Oryza sativa* L) varieties subjected to free-air release of ozone. *Agriculture Ecosystems and Environment*, **132**, 203–211.

Piikki K, De Temmerman L, Högy P, Pleijel H (2008) The open-top chamber impact on vapour pressure deficit and its consequences for stomatal ozone uptake. *Atmospheric Environment*, **42**, 6513–6522.

Pleijel H, Danielsson H, Emberson L, Ashmore MR, Mills G (2007) Ozone risk assessment for agricultural crops in Europe: further development of stomatal flux and flux-response relationships for European wheat and potato. *Atmospheric Environment*, **41**, 3022–3040.

Pleijel H, Danielsson H, Gelang J, Sild E, Sellden G (1998) Growth stage dependence of the grain yield response to ozone in spring wheat (*Triticum aestivum* L). *Agriculture, Ecosystems and Environment*, **70**, 61–68.

Pleijel H, Eriksen AB, Danielsson H, Bondesson N, Sellden G (2006) Differential ozone sensitivity in an old and a modern Swedish wheat cultivar—grain yield and quality, leaf chlorophyll and stomatal conductance. *Environmental and Experimental Botany*, **56**, 63–71.

Sarkar A, Agrawal SB (2010) Elevated ozone and two modern wheat cultivars: an assessment of dose dependent sensitivity with respect to growth, reproductive and yield parameters. *Environmental and Experimental Botany*, **69**, 328–337.

Shi G, Yang L, Wang Y *et al*. (2009) Impact of elevated ozone concentration on yield of four Chinese rice cultivars under fully open-air field conditions. *Agriculture, Ecosystems and Environment*, **131**, 178–184.

Singh RP, Huerta-Espino J, Sharma R, Joshi AK, Trethowan R (2007) High yielding spring bread wheat germplasm for global irrigated and rainfed production systems. *Euphytica*, **157**, 351–363.

The Royal Society (2008) *Ground-level ozone in the 21st century: future trends, impacts and policy implications*. Science Policy Report 15/08. The Royal Society, London

Van Dingenen R, Dentener FJ, Raes F, Krol MC, Emberson L, Cofala J (2009) The global impact of ozone on agricultural crop yields under current and future air quality legislation. *Atmospheric Environment*, **43**, 604–618.

Wang X, Mauzerall DL (2004) Characterizing distributions of surface ozone and its impact on grain production in China, Japan and South Korea: 1990 and 2020. *Atmospheric Environment*, **38**, 4383–4402.