Effects of elevated O₃ concentration on winter wheat and rice yields in the Yangtze River Delta, China

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ABSTRACT

The effects of a continuing rise of ambient ozone on crop yield will seriously threaten food security in China. In the Yangtze River Delta, a rapidly developing and seriously air polluted region in China, innovative open-top chambers have been established to fumigate winter wheat and rice in situ with elevated O₃. Five years of study have shown that the yields of wheat and rice decreased with increasing O₃ concentration. There were significant relationships between the relative yield and AOT40 (accumulated hourly O₃ concentration over 40 ppb) for both winter wheat and rice. Winter wheat was more sensitive to O₃ than rice. O₃-induced yield declines were attributed primarily to 1000-grain weight and harvest index for winter wheat, and attributed primarily to grain number per panicle and harvest index for rice. Control of ambient O₃ pollution and breeding of O₃ tolerant crops are urgent to guarantee food security in China.

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1. Introduction

Ozone (O₃) is a strong oxidative pollutant that is detrimental to crop yields, tree production and human health. Trends in tropospheric O₃ concentration have leveled off or decreased slightly in North America and Europe (IPCC, 2007), but the trends are increasing in east Asian countries, such as Japan (Naja and Akimoto, 2004), and China (Shao et al., 2006; Wang et al., 2007). Long-term monitoring has shown that annual mean ambient O₃ concentration increased by 0.23 ppb per year from 1995 to 2005 over Mt. Waliguan in Qinghai province, China (Deliger and Zhao, 2007). Results from 26 established global atmospheric chemistry-transport models driven by analyzed meteorological fields have shown that global annual mean surface O₃ concentrations will increase by 1.5 ppb (CLE scenario)–4.3 ppb (IPCC SRES A2 scenario) over the period 2000–2030 (Dentener et al., 2006). In China, rapid development of the economy and society and fast urbanization will cause ambient O₃ concentration to rise faster than in other countries due to increasing emissions of trace gases such as nitrogen dioxide and volatile organic compounds.

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Investigations into the effects of O₃ on crops have been carried out over four decades, especially in developed countries. For example, the US National Crop Loss Assessment Network (NCLAN) and European Open Top Chamber Programme (EOTCP) have shown significant yield losses of major crops in USA and Europe. Investigations of crop responses to elevated O₃ concentration in terms of phenology, physiology, production and yield have confirmed that elevated O₃ could accelerate crop senescence, decrease photosynthesis, reduce biomass and carbon allocation into roots, and cause yield loss (Morgan et al., 2003; Andersen, 2003; Fiscus et al., 2005; Ashmore, 2005; Feng et al., 2008; Feng and Kobayashi, 2009). Although there are differences in O₃ sensitivity among crops, there are a considerable number of crops sensitive to elevated O₃ concentrations and subject to yield loss. The effects of elevated O₃ concentrations on crop growth and yield are assessed generally by a cumulative exposure over a threshold concentration for a given length of time (Nussbaum et al., 1995; Fuhrer et al., 1997). The index AOT40 (accumulated exposure over a threshold ozone concentration of 40 ppb) (USEPA, 1996) was widely adopted in Europe to assess the effects of O₃. Mills et al. (2007) compiled AOT40–yield response functions for 19 crops in European regions from data available in 700 published papers and proceedings. Of these crops, wheat is an O₃-sensitive crop and rice is a moderately O₃-sensitive crop. By analyzing the

quantified relationship between crop yield and AOT40, this study can provide valuable data to optimize the coefficient of ozone dose–response in China and improve regional modeling studies that adopted the AOT40 index (e.g., Tian et al., 2011; Ren et al., 2007, 2011).

Wheat and rice are two globally important crops that are sensitive to O₃ stress (Mills et al., 2007). Rice is consumed by more than half of the world's current population (IRRI, 2002). Wheat is grown on about 200 million hectares in a wide range of environments, with an annual production of more than 619 million metric tons (FAO, 2007). Because they are widely planted, the responses of wheat and rice to elevated O₃ concentrations have been assessed in many countries (Pleijel et al., 1991; Kobayashi et al., 1995; Rai et al., 2007). Recently, the physiological, growth and yield responses of wheat and rice to elevated O₃ have been reviewed by Feng et al. (2008), Feng and Kobayashi (2009) and Ainsworth (2008), respectively. Ainsworth (2008) has reported that the mean rice yield would be decreased by 14% when exposed to 62 ppb O₃ (mean concentration) compared with charcoal-filtered air after meta-analyzing 18 studies. Feng et al. (2008) concluded that an elevated O₃ concentration would decrease wheat grain yield by 26% at a mean O₃ concentration of 77 ppb based on a database containing 53 peer-reviewed studies. Even with lower O₃ exposure (average 43 ppb, mean concentration), there was also a significant decrease in the grain yield of wheat (18%) (Feng and Kobayashi, 2009).

To model crop yield response to environmental change, and to facilitate breeding crop cultivars for high yields, the yield components are of interest. Available investigations have shown that elevated O₃ concentrations decreased the mass of individual grains and the grain number of rice by 5% and 20%, respectively (Ainsworth, 2008), and individual grain weight and grain number per ear of wheat by 18% and 11%, respectively (Feng et al., 2008).

As the most populous country in the world, China's grain supply is critical to global food security. If crop yields are threatened by elevated O₃ concentrations, there would be a negative impact on future grain production both in China and worldwide. Available ambient O₃ monitoring data have implied that the O₃ concentrations were sufficiently high to reduce rice and wheat yield significantly in some developed regions of China (Wang et al., 2007), such as the Yangtze River Delta (Zhou, 2004; Wang et al., 2005) and the Pearl River Delta (Shao et al., 2006). Crop losses due to O₃ concentrations in China have been estimated to be as much as 23% (Chameides et al., 1999; Aunan et al., 2000; Wang and Mauzerall, 2004; Wang et al., 2005). However, these estimates are usually questionable because the dose–response relationships used in those estimations were derived from experiments conducted in USA and Europe where crop cultivars, soil and climate environments, and management practices are different from those in China.

Recently, a few studies have been carried out to investigate the crop responses to elevated O₃ concentrations in China. Feng et al. (2003) studied the effects of elevated O₃ concentration on pot-planted wheat and rice in North China. Shi et al. (2009) and Zhu et al. (2011) investigated the effects of O₃ on rice and winter wheat yields, respectively, for four cultivars under open-air field conditions. However, it is not clear whether the dose–response relationships based on single season investigations or only two treatments are reliable predictors of long-term crop losses under elevated O₃ concentrations. In this study, by analyzing the experimental data for 5 years, we assessed wheat and rice losses under elevated O₃ concentrations and their relationship to changes in yield components. This is the first multiple-year study to establish dose–response relationships for wheat and rice under field conditions in order to assess and predict crop loss in China more reliably.

2. Material and methods

2.1. Site description

The experimental site is located at the Shuangqiao Farm (31°53'N, 121°18'E) in Jiaxing City, Zhejiang Province, about 100 km southwest of Shanghai, China's largest city. This site is in the center of the Yangtze Delta and is one of the most important crop production areas in China. It is influenced by the Asian monsoon climate system, with cold dry winters and hot wet summers. Mean temperature and annual precipitation are 15.5 °C and 1200 mm, respectively. The soil developed from peatland 30–40 years ago is silt clay. Generally, rice and winter wheat were grown in fields in rotation, rice being grown from July to November, and wheat from November to May of the following year.

2.2. Open top chamber

The octagonal open-top chamber (OTC, 2.2 m in height and 2 m in diameter) had aluminum alloy frames covered with polyethylene plastic film, with an improved innovative O₃ distribution system (Zheng et al., 2007). The air or charcoal-filtered air was delivered by a centrifugal blower into a vertical plastic duct (diameter of 11 cm), where O₃ was added, then into a horizontally rotatable transparent pipe (diameter of 5 cm) above the crop canopy and released from many small holes (diameter of 10 mm at an interval of 10 cm) situated facing 45° downward on the pipe. The rotatable pipe was connected to the duct by a custom-designed shaft, propelled by the reaction force from released air and raised with crop growth. O₃ was supplied from an O₃ generator from pure oxygen by the high-voltage electric discharge method (Yuyao Shenglete Company, Zhejiang, China). The target O₃ concentration was obtained by a mass flow controller (custom-made by Shengye Company, Beijing, China) regulating oxygen entering the O₃ generator based on the established relationship between O₃ concentration within the OTC and oxygen volume. A series of solenoid valves, linked online with a programmable Log Controller (PLC K80S, LG, Korea) was employed to switch gas entering the O₃ analyzer (Model 9810B, Monitor Labs, Australia) from each OTC sequentially. The O₃ concentration within each chamber was monitored sequentially for 5 min. A data logger (CR1000, Campbell Scientific Inc., UT, USA) was connected to store O₃ concentration data and to monitor temperature within the OTCs using constantan-copper thermocouples. We checked the O₃ concentration data recorded by the data logger every day and regulated the oxygen volume entering the O₃ generator if the target O₃ concentrations within the OTC were not achieved. Monitoring has shown that O₃ concentrations were evenly distributed vertically and horizontally within the OTC. The difference in mean temperature between the inside and outside of the OTC was less than 1.87 °C (Zheng et al., 2007). Measurements with a Li-190 quantum sensor (Li-Cor Environmental, Lincoln, Nebraska, USA) showed that photosynthetic active radiation was reduced by less than 15% within the OTC compared with the outside.

2.3. Ozone treatments

There were 12 OTCs established in the field with four treatments of O₃ and three replicates randomly assigned to each treatment. All chambers were separated by a guard block (2 m × 3 m). Charcoal filtered air (CF) and non-filtered air (NF) were the control treatments. Two levels of elevated O₃ treatments were applied by adding O₃ into charcoal filtered air as the O₃-1 and O₃-2 treatments. The target O₃ concentrations were 70–100 ppb for the O₃-1 treatment and 150–200 ppb for the O₃-2 treatment, varying with year and crop (Table 1). Two modes of fumigation were applied: constant O₃ exposure and diurnal variation of O₃ exposure. In the former mode, constant O₃ concentrations were applied daily for 8 h (09:00–17:00) for the O₃-1 and O₃-2 treatments. In the latter mode, ambient diurnal variations in O₃ concentrations were simulated in the growing seasons for rice in 2006 and for winter wheat in 2006 and 2007. In the O₃-1 treatments: 50 ppb O₃ for 09:00–10:00, 100 ppb O₃ for 10:00–12:00, 150 ppb O₃ for 12:00–14:00, 100 ppb O₃ for 14:00–16:00, and 50 ppb O₃ for 16:00–17:00; in the O₃-2 treatments: 100 ppb O₃

Table 1
The crop management and O₃ exposure of winter wheat.

		2004	2006	2007	2008
Cultivar		Yangmai185	Jia002	Jia002	Jia002
Crop management	Sowing	Oct. 15	Nov. 7	Nov. 15	Nov. 18
	Harvesting	May 23	May 16	May 28	May 31
Exposure	Mode	Constant	Diurnal variation	Diurnal variation	Constant
	Beginning	Mar. 15	Mar. 13	Mar. 26	Mar. 20
Target Concentrations (ppb)	End	May 18	Apr. 28	May 19	May 13
	O ₃ -1	100	100	75	100
	O ₃ -2	200	150	150	150
	treatment				

Table 2The crop management and O₃ exposure of rice.

		2004	2005	2006	2007	2008
Cultivars		Jiahua 2	Jiahua 2	Fan 3694	Fan 3694	Fan 3694
Crop management	Sowing	May 15	May 27	May 15	May 25	May 26
	Transplanting	Jul. 2	Jul. 1	Jun. 20	Jun. 29	Jul. 4
	Harvest	Oct.28	Oct. 25	Oct. 25	Nov. 2	Nov. 12
Exposure	Mode	Constant	Constant	Diurnal variation	Constant	Constant
	Beginning	Jul. 21	Jul. 25	Jul. 17	Jul. 29	Jul. 25
	End	Oct. 10	Oct. 14	Oct. 10	Oct. 11	Oct. 19
Target Concentration (ppb)	O ₃ -1 treatment	100	75	100	100	100
	O ₃ -2 treatment	200	150	150	150	150

for 09:00–10:00, 150 ppb O₃ for 10:00–12:00, 200 ppb O₃ for 12:00–14:00, 150 ppb O₃ for 14:00–16:00, and 100 ppb O₃ for 16:00–17:00. Fumigations were performed every day except when it was raining. The timings of exposure are shown in Tables 1 and 2.

2.4. Crop management

Winter wheat (*Triticum aestivum* L.) and rice (*Oryza sativa* L.) seeds were provided by Jiaxing Academy of Agriculture using locally adapted cultivars (Tables 1 and 2). The cultivars of wheat and rice were changed in 2006 because local cultivars were also changed. All crop management practices, such as sowing, transplanting (rice), harvesting, and fertilizing, were adopted according to local agronomic customs so that water, fertilizer, pathogens, insects and weeds were not limiting factors. Compound fertilizer (N/P₂O₅/K₂O = 15:15:15) was applied at a rate of 78 g/m² for each OTC before transplanting (rice) or sowing (winter wheat), and urea (N content, 46.5%) was applied at a rate of 21 g/m² for each OTC during the jointing stage and the booting stage (rice) or tillering stage (winter wheat).

2.5. Yield

Before harvesting each crop, 15 plants were sampled from each OTC for yield determination. The yield components (floret, panicle and grain for wheat, and panicle, first and second rachis, and grain for rice) were separated for counting or weighing. Yield loss due to elevated O₃ concentrations for each year was calculated as the relative yield, which is the percentage of yield for each treatment compared with the yield under CF treatment.

2.6. Statistical analysis

An analysis of variance (ANOVA) was used to test for mean differences between treatments ($N = 3$ chambers). The Fishers Protected LSD test was used for post-hoc examination of treatment differences. Statistical analysis was performed with the

statistical package SPSS (SPSS Inc., Chicago, IL, USA). The relationships among relative yield, yield component and AOT40 value were analyzed for each crop over 4 or 5 growing seasons using Excel 2003.

3. Results

3.1. Winter wheat

Elevated O₃ significantly reduced winter wheat yield (Table 3). Yields decreased by 8.5–58% and 40–73% compared with CF control for O₃-1 and O₃-2 treatments, respectively, with the annual variations in O₃ concentrations and AOT40. Nevertheless, significant exponential and linear regressions were fitted between relative yield and AOT40 for 4 years (Fig. 1A) for all data (Equation (1)) and for all data except the highest AOT40 in the first year (Equation (2)), respectively.

$$RY = 100 \exp(-0.0255 \text{ AOT40}) \quad (R^2 = 0.846) \quad (1)$$

$$RY = 100 - 2.2795 \text{ AOT40} \quad (R^2 = 0.859) \quad (2)$$

The wheat yield components, such as number of florets, number of grains per panicle, 1000-grain weight and harvest index were also impacted by elevated O₃ (Table 3). Except for a lack of statistical analysis in 2004 due to handling of the harvested plants from chambers under the same treatment together, the number of grains

Table 3The yield components of winter wheat after exposure to different O₃ treatments.

Years	Treatments	AOT40 (ppm h)	No. of florets per panicle	No. of grains per panicle	1000-grain weight (g)	Harvest index (%)	Yield per plant (g)
2004	CF	0	15.6	25.81	45.75	50.43	1.29
	NF	3.82	15.67	27.82	46.5	55.19	1.41
	O ₃ -1	22.61	14.13	23.37	27.18	29.28	0.54
	O ₃ -2	61.91	13.21	19.65	12.56	31.16	0.35
2006 ($N = 3$)	CF	0	17.93 ± 0.74	46.90 ± 3.93a	37.43 ± 1.37a	47.01 ± 0.53a	1.52 ± 0.07a
	NF	2.5	17.71 ± 0.95	46.24 ± 4.25a	32.01 ± 0.78ab	47.07 ± 2.21a	1.35 ± 0.17ab
	O ₃ -1	14.27	16.87 ± 0.81	33.50 ± 3.29b	29.71 ± 1.20b	43.61 ± 3.42ab	1.10 ± 0.15b
	O ₃ -2	24.22	16.71 ± 0.34	24.13 ± 3.75c	24.05 ± 0.75c	35.58 ± 3.20b	0.91 ± 0.05b
ANOVA	<i>F</i>		2.063	5.47	18.256	2.875	3.286
	<i>P-value</i>		0.184	0.024	0.001	0.103	0.079
2007 ($N = 3$)	CF	0	15.45 ± 1.26a	39.41 ± 4.03a	43.62 ± 1.90a	58.72 ± 5.13a	1.65 ± 0.37a
	NF	0.21	15.85 ± 1.30a	40.58 ± 7.50a	42.16 ± 0.73ab	60.33 ± 2.67a	1.61 ± 0.40a
	O ₃ -1	1.58	14.95 ± 0.81ab	36.59 ± 4.03b	41.74 ± 0.49b	56.41 ± 5.69a	1.51 ± 0.33a
	O ₃ -2	9.17	14.08 ± 1.13b	32.39 ± 5.22b	25.63 ± 1.03c	43.35 ± 7.96b	0.99 ± 0.26b
ANOVA	<i>F</i>		5.311	3.743	138.506	33.188	13.542
	<i>P-value</i>		0.003	0.011	0	0	0
2008 ($N = 3$)	CF	0.1	16.37 ± 0.73a	36.55 ± 1.33a	43.05 ± 2.98a	50.45 ± 2.49a	1.57 ± 0.11a
	NF	0.15	16.65 ± 0.16a	35.50 ± 1.59a	44.69 ± 1.67a	48.85 ± 1.17a	1.55 ± 0.04a
	O ₃ -1	15.32	13.87 ± 1.14b	27.49 ± 4.55b	31.25 ± 2.74b	39.12 ± 5.53b	0.86 ± 0.15b
	O ₃ -2	27.67	13.73 ± 0.31b	21.44 ± 2.21c	27.25 ± 2.34b	25.30 ± 4.25c	0.58 ± 0.02c
ANOVA	<i>F</i>		15.233	20.402	36.265	28.492	84.129
	<i>P-value</i>		0.001	<0.001	<0.001	<0.001	<0.001

Note: Data are “means ± standard errors”; and values with different letters in the same column for each year designate significant difference among the O₃ treatments ($P < 0.05$).

Fig. 1. The exponential (for all data) and linear regressions (for all apart from the highest AOT40 data in first year) of relative crop yield to CF control and AOT40 (accumulated daily mean O_3 concentration above 40 ppb during fumigation): (A) winter wheat, (B) rice.

per panicle, 1000-grain weight and harvest index significantly decreased with increasing O_3 exposure (Table 3). Significantly, linear relationships across cultivars and years between relative yield and relative yield components to CF treatment showed that 1000-grain weight ($P < 0.01$) and harvest index ($P < 0.01$) were the primary factors impacting yield loss under elevated O_3 (Fig. 2).

3.2. Rice

Rice yields were reduced significantly under elevated O_3 across cultivars, years and exposure protocols (Table 4). The mean yields decreased by 10–34% and 16–43% compared with CF control for O_3 -1 and O_3 -2 treatments, respectively, with the annual variations in O_3 concentrations and AOT40. Significant exponential and linear regressions were fitted between relative yield to CF treatment and AOT40 for 5-year periods for all data (Equation (3)) and for all data

except the highest AOT40 in the first year (Equation (4)) (Fig. 1B), respectively.

$$RY = 100 \exp(-0.0092 AOT40) \quad (R^2 = 0.756) \quad (3)$$

$$RY = 100 - 0.9489 AOT40 \quad (R^2 = 0.828) \quad (4)$$

The rice yield components were reduced under elevated O_3 concentrations (in O_3 -1 and O_3 -2 treatments), which varied with O_3 exposure and seasons (Table 4). The O_3 effects were significant for harvest index in 5 years, 1000-grain weight and number of second rachis in 4 years, and number of first rachis and number of grain per panicle in 3 years. For 5-year periods across cultivars and exposure protocols, the significant linear relationships between relative yield and relative yield components to CF treatment showed that the

Fig. 2. The linear relationships between relative yield and relative yield components to CF control for winter wheat in 4 year O_3 experiments: (A) 1000-grain weight, (B) Number of grains per panicle, (C) Number of florets, and (D) Harvest index.

Table 4
The yield components of rice after exposure to different O₃ treatments.

Years	Treatments	AOT40 (ppm h)	No. of grain per panicle	No. of first rachis per panicle	No. of second rachis per panicle	1000-grain weight (g)	Harvest index (%)	Yield per plant (g)
2004 (N = 3)	CF	0	107.90 ± 8.12a	11.50 ± 3.44a	20.70 ± 3.65a	27.70 ± 1.01a	53.48 ± 2.04a	2.72 ± 0.21a
	NF	0.91	102.60 ± 8.41a	10.00 ± 0.82a	17.60 ± 4.59a	27.12 ± 0.83a	54.13 ± 1.34a	2.78 ± 0.20a
	O ₃ -1	29.472	85.80 ± 9.33b	9.10 ± 0.95b	11.40 ± 3.19b	25.74 ± 1.09b	50.52 ± 2.11a	2.21 ± 0.22b
	O ₃ -2	82.603	55.70 ± 6.49c	8.10 ± 0.74c	7.70 ± 2.85c	24.39 ± 0.62c	45.98 ± 2.01b	1.62 ± 0.13c
	ANOVA	F	41.355	5.572	25.678	1.782	2.545	5.195
	P-value		0.000	0.003	0.000	0.228	0.129	0.028
2005 (N = 3)	CF	0	153.00 ± 7.39	12.63 ± 0.41	25.13 ± 1.77a	23.14 ± 0.26	52.66 ± 2.00a	2.92 ± 0.14a
	NF	0	145.40 ± 3.35	12.00 ± 0.53	23.17 ± 0.70ab	22.71 ± 0.05	52.04 ± 1.00ab	2.76 ± 0.13ab
	O ₃ -1	4.3	139.10 ± 6.83	12.06 ± 0.11	22.46 ± 1.10ab	22.83 ± 0.46	51.19 ± 0.01ab	2.62 ± 0.04ab
	O ₃ -2	6.93	132.50 ± 7.83	11.70 ± 0.36	20.63 ± 1.26b	22.54 ± 0.25	48.99 ± 1.01b	2.44 ± 0.10b
	ANOVA	F	1.817	1.082	2.151	0.745	2.351	3.479
	P-value		0.222	0.410	0.172	0.555	0.148	0.070
2006 (N = 3)	CF	0	146.80 ± 7.56ab	14.33 ± 0.48a	22.88 ± 1.96ab	25.61 ± 0.27a	42.49 ± 0.02ab	5.37 ± 0.60a
	NF	3.14	155.20 ± 6.78a	14.79 ± 0.26a	25.33 ± 1.52a	24.87 ± 0.46ab	46.35 ± 0.01a	5.63 ± 0.44a
	O ₃ -1	23.24	133.50 ± 5.96bc	14.04 ± 0.34ab	19.67 ± 1.61bc	25.03 ± 0.33a	42.38 ± 0.02ab	4.14 ± 0.41ab
	O ₃ -2	39.28	118.20 ± 5.67c	13.12 ± 0.70b	16.65 ± 1.39c	23.81 ± 0.28b	37.08 ± 0.04b	3.52 ± 0.59b
	ANOVA	F	6.128	4.619	7.067	4.745	2.996	2.394
	P-value		0.004	0.037	0.012	0.035	0.095	0.044
2007 (N = 3)	CF	0.4	106.20 ± 15.30	11.44 ± 0.42	15.14 ± 2.03	24.61 ± 1.80a	45.58 ± 1.63a	2.41 ± 0.23a
	NF	0.5	112.90 ± 11.00	11.65 ± 0.45	16.35 ± 2.67	23.81 ± 0.94a	47.06 ± 2.03a	2.63 ± 0.30a
	O ₃ -1	13.83	103.40 ± 8.87	10.69 ± 0.53	14.86 ± 2.04	17.91 ± 2.92b	41.42 ± 1.78b	1.86 ± 0.22ab
	O ₃ -2	32.08	99.70 ± 15.00	10.85 ± 0.80	13.75 ± 3.37	15.06 ± 1.08b	39.49 ± 1.75b	1.49 ± 0.28b
	ANOVA	F	0.418	1.945	0.717	18.623	12.683	4.396
	P-value		0.745	0.201	0.569	0.001	0.002	0.042
2008 (N = 3)	CF	0.01	137.13 ± 14.66a	13.40 ± 0.91a	21.86 ± 2.21a	23.43 ± 0.73a	47.34 ± 2.21a	3.17 ± 0.43a
	NF	0.27	133.07 ± 3.21a	13.36 ± 0.18a	21.01 ± 0.43a	23.77 ± 0.48a	45.13 ± 2.69a	3.04 ± 0.27a
	O ₃ -1	22.77	106.79 ± 11.40a	12.56 ± 0.77ab	15.63 ± 2.52b	20.53 ± 0.14b	40.90 ± 0.75b	2.08 ± 0.33b
	O ₃ -2	52.84	104.11 ± 8.89b	12.01 ± 0.69b	14.14 ± 2.70b	17.35 ± 1.36c	40.65 ± 1.16b	1.80 ± 0.04b
	ANOVA	F	8.211	2.823	9.492	41.017	9.213	14.981
	P-value		0.008	0.007	0.005	0.000	0.006	0.001

Note: Data are “means ± standard errors”; and values with different letters in the same column for each year designate significant difference among the O₃ treatments ($P < 0.05$).

number of grain per panicle ($P < 0.01$) and harvest index ($P < 0.01$) are the primary factors impacting yield loss under elevated O₃ concentrations (Fig. 3). Other components such as number of first rachis ($P < 0.05$), number of second rachis ($P < 0.05$) and 1000-grain weight ($P < 0.05$) were also affected by O₃, possibly influencing yield loss.

4. Discussion

4.1. Yield losses of rice and wheat due to elevated O₃ concentration

The yield loss for winter wheat was higher than for rice under both O₃-1 and O₃-2 treatments (Tables 3 and 4). Mills et al. (2007) had classified wheat as a sensitive crop and rice as a moderately sensitive crop by general linear model analysis between yield loss and AOT40. Meta-analyses have shown that wheat yield loss was 26% at mean 7-h or 8-h O₃ concentrations of 72 ppb with a range of 30–200 ppb across all of the reported studies on effects of chronic O₃ treatment (Feng et al., 2008) and rice yield loss was 14% at a mean O₃ concentration of 62 ppb when compared with rice grown in charcoal filtered air (Ainsworth, 2008).

Although the climate, cultivars and exposure periods varied each year, the combined winter wheat dataset could be represented by a single regression equation of relative yield plotted against AOT40 (Fig. 1A). The same was true for the rice yield data (Fig. 1B). This may be because the soil conditions, water and nutrient supply were sufficient for winter wheat and rice growing in our study under all conditions. Although crop cultivars were switched in 2006 due to local practice, the two cultivars for wheat and rice were bred from the same strains and may have the same degree of sensitivity to O₃. In this study, we have developed an O₃ exposure system that simulates the diurnal variation of ambient O₃, and it was used in 2006 for wheat and rice and 2007 for wheat. Unfortunately, the

system was not stable and was difficult to maintain. So the constant O₃ exposures were resumed in later experiments. It is difficult to assess the differences in the response of wheat and rice to AOT40 between the constant and diurnal pattern of O₃ exposures because of yearly variations in exposure duration and dose in this study. Emberson et al. (2009) have reported that there were no obvious differences between the sensitivities of Asian winter and spring wheat varieties planted in different countries. Analysis of variance and generalized linear modeling (GLIM) showed that there were no statistically significant differences between the slope of the regressions for the data from the USA and EU for each of the crops studied (Mills et al., 2007). When compared with the slope of Mills' functions, our results showed that wheat and rice were more sensitive to O₃ in the Yangtze Delta of China when AOT40 was larger than 1.5 ppm h for wheat and 10.7 ppm h for rice, respectively (Fig. 4). The greater sensitivity of wheat and rice to O₃ in the Yangtze Delta where wheat and rice grow without water and nutrient stresses than that predicted from North American dose–response relationships has been reported by Emberson et al. (2009). Compared with our study, the wheat and rice used in North China by Feng et al. (2003) were less sensitive to O₃ (Fig. 4). In addition to growing method (pot vs. field) and climate (semi-humid vs. humid), the difference in germplasm resource may be a factor in explaining the differences in O₃ sensitivities of cultivars from the Yangtze Delta versus North China. The contribution of genetic variation will require further investigation.

4.2. Responses of yield components to elevated O₃ concentrations

Crop yield is affected by a series of components depending on crop architecture and inflorescence structure. For winter wheat, the number of florets and number of grains per plant were significantly reduced by elevated O₃ concentrations (Table 3). Compared with

Fig. 3. The linear relationships between rice relative yield and relative yield components to CF control for rice in 5 year O_3 experiments. (A) 1000-grain weight, (B) Number of secondary rachis, (C) Number of first rachis, (D) Number of grains per panicle, and (E) Harvest index.

the CF control, the number of florets and number of grains per panicle were reduced for all 4 years, by 8.5% and 17.5% for O_3 -1 treatment, and by 11.8% and 32.9% for O_3 -2 treatment, respectively. In the review by Feng and Kobayashi (2009), the number of florets was reduced by 6% at a mean O_3 concentration of 65 ppb. For rice, the numbers of grains, first rachis and second rachis per panicle were reduced for all 5 years, by 12.7%, 8.0%, and 20.0% for O_3 -1 treatment, and by 22.3%, 12.2%, and 30.5% for O_3 -2 treatment, respectively (Table 4). In the review by Ainsworth (2008), for rice, the number of grains was reduced by 20% at a mean O_3

concentration of 62 ppb. Shi et al. (2009) reported that the number of florets per panicle was significantly reduced, but individual grain mass was only slightly reduced for sensitive rice cultivars under elevated O_3 exposure in fully open-air field conditions.

Grain weight per plant is one of the yield components most critical to crop yield. The elevated O_3 concentration could significantly reduce grain weight because of decreased photosynthesis and increased oxidative stress. This study showed that the 1000-grain weights decreased by 18.6% and 37.2% for winter wheat (Table 3), and by 10.1% and 17.3% for rice (Table 4), in O_3 -1 and O_3 -2

Fig. 4. Comparison of exposure–response relationships among this study, Feng et al. (2003) and Mills et al. (2007) for (A) winter wheat, and (B) rice.

treatments, respectively. In recent reviews, grain weights losses of 18% for wheat (Feng and Kobayashi, 2009) and 5% for rice (Ainsworth, 2008) were reported.

Many yield components were reduced along with total yield under elevated O₃ conditions (Tables 3 and 4). Based on the relationships between relative yield and relative component value (Figs. 2 and 3), the results indicated that, for wheat, 1000-grain weight and harvest index were more sensitive to O₃ concentration than other components, and, for rice, the number of grains per panicle and harvest index were also seriously impacted by elevated O₃ concentrations.

4.3. Implications for estimating O₃-induced crop loss

The 5-year studies strongly support the hypothesis that winter wheat and rice yields are decreasing with increasing O₃ concentrations in China. The AOT40-based exposure–response equations derived from these experiments could be used to estimate current and future O₃-induced grain losses confidentially. Higher yield losses relative to AOT40 were found in our study compared with those in the study by Feng et al. (2003) and in the synthesis by Mills et al. (2007). This indicates that yield loss due to increased O₃ concentration might be higher than previous estimates.

The exposure–response equation, as an essential tool for estimating crop loss due to increasing O₃ concentration, has generally been obtained from open-top chamber (OTC) experiments because OTCs can be built economically, operated easily, and have stable O₃ concentrations. Nevertheless, there are some deficiencies when estimating crop loss based on OTC-derived exposure–response functions.

The primary errors come from altered growing conditions within the OTC relative to ambient conditions. The greenhouse effects of OTC have been criticized for many years. The OTC systems we used in this study could distribute O₃ evenly and stably, and exchange air between the inside and outside two to three times per minute (Zheng et al., 2007), so the environmental differences between the OTC and actual growing condition have been partially mitigated. The air temperature inside the OTC was 2 °C higher than outside when ambient temperature was above 30 °C in summer (Zheng et al., 2007). The light inside the OTC was also reduced by about 10% and air humidity was altered sometimes. Because our results were expressed as the relative yield and their component values under O₃ treatment compared with those under CF treatment using the same-designed OTCs, the OTC effects were avoided to some degree. O₃-FACE (Ozone-free air control enrichment) experiments have been carried out in USA, Europe and China (Shi et al., 2009), but unfortunately, it is difficult to derive dose–response functions from these data because only one treatment and one control are deployed in most FACE experiments.

In the first year experiment, the target O₃ concentration was set as high as 200 ppb above the ambient O₃ concentration. Excluding the first year's results, significant linear relationships were obtained between wheat and rice yields and AOT40, and would be better than the logarithmic relationships derived from all ranges of O₃ exposure. The exposure–response functions based on a large span of O₃ concentrations probably introduced errors in estimation of crop loss.

Because of rainy days, unstable ambient O₃ concentrations and the high activity of O₃ molecules, O₃ concentrations within the OTCs were achieved with small bias to the set concentrations. From Table 5, it is seen that the mean O₃ concentration under CF treatment ranged from 7.0 ppb to 20.3 ppb after ambient air had been charcoal-filtered, less than that under NF treatment (in the range 17.4 ppb–27.7 ppb). As compared with the target concentrations in Tables 1 and 2, mean O₃ concentrations achieved in O₃-1 and O₃-2 treatments (Table 5) are less than the target concentrations. Because of frequent rainy days, the mean O₃ concentrations

Table 5

Mean (±SD) O₃ concentration (ppb) within the OTCs during the O₃ fumigation in 2007 and 2008.

Year	Crop	CF	NF	O ₃ -1	O ₃ -2
2007	Wheat	11.6 ± 5.1	27.7 ± 6.4	51.5 ± 3.5	98.4 ± 3.5
2008	Wheat	20.3 ± 9.5	27.7 ± 2.8	96.5 ± 4.1	142.7 ± 8.3
2007	Rice	19.7 ± 4.6	22.6 ± 1.3	69.6 ± 4.9	118.6 ± 5.3
2008	Rice	7.0 ± 3.7	17.4 ± 4.5	82.2 ± 4.3	138.3 ± 6.6

achieved were as much as 36% less than the target concentration in 2007. So, exposure–response functions based on accumulated O₃ concentrations (e.g., AOT40) would be more meaningful.

Our experiments were conducted without stresses such as nutrient or water deficiencies, diseases and pests, etc. It is not clear what the changes in O₃-induced crop yield loss under natural stresses would be. So studies of the effects of O₃ on crops in combination with multiple climatic, soil and water factors are necessary in the future.

5. Conclusions

Yields of winter wheat and rice, the most important grain crops in China and the world, are being threatened by rising ambient O₃ concentrations caused by rapid economic development and urbanization. Our experiments over 5 years have confirmed that winter wheat and rice were sensitive to elevated O₃ levels in China. The AOT40-based dose–response relationships showed that wheat and rice yield losses in our study were more serious than in previous reports both domestically and from abroad, which means that there would be more crop yield loss under O₃ stress in China and O₃ pollution would cause more damage to Chinese food security than previously assumed.

Our study also confirmed that winter wheat was more sensitive to O₃ than rice. In addition, the response of yield components to O₃ concentration would change with crop type. Grain mass is the most O₃-sensitive component for winter wheat but grain number is the most sensitive component for rice. This would make more sense for crop selection, cultural practices and breeding to reduce O₃-caused crop loss.

Because of the wide variability in crops, cultural practices and climate overall of China, it is not sufficient to estimate national O₃ effects on crop yield based on the experimental data obtained from one or two sites. In our experimental site, water and fertilizer were optimal for crop growth, and winter wheat and rice were likely more sensitive to O₃. In order to scale up the experimental results to the national scale, the response of winter wheat and rice to O₃ under stresses such as poor nutrient availability, drought, or temperature stress should be investigated. In addition, other crops such as maize, soybean, etc. should be studied to evaluate the effects of O₃ on the overall Chinese cropping system.

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