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Effects of elevated ozone on growth and yield of field-grown rice in Yangtze River Delta, China

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Abstract

With rapid industrialization and urbanization in the Yangtze Delta, China, the tropospheric ozone concentration has increased to levels that induce crop yield loss. Rice, a widely grown crop in China, was investigated in field-established, open-top chambers. Four treatments were used: charcoal-filtered air (CF), non-charcoal-filtered air (NF), and charcoal-filtered air with two levels of additional ozone (O_3-1 and O_3-2). The AOT40s (accumulated hourly mean ozone concentration above 40 ppbv) were 0, 0.91, 23.24, and 39.28 ppmv·h for treatment of CF, NF, O_3-1, and O_3-2, respectively. The rice height and biomass were reduced in the elevated ozone concentration. Less organic matter partitioning to roots under the elevated ozone significantly decreased rice root activity. The yield loss was 14.3% and 20.2% under O_3-1 and O_3-2 exposure, respectively. This was largely caused by a reduction in grain weight per panicle.

Key words: ozone; rice; growth; yield; Yangtze Delta

Introduction

Ozone (O_3) is a photochemical oxidant that occurs naturally in the earth's troposphere, and the background concentrations range from 0.025 to 0.05 ppmv (USEPA, 1996). In some sites, its concentration has dramatically increased over the last century and it has been suggested that the ground-level O_3 concentration is increasing at a rate of about 0.3% to 2% per year (Thompson *et al.*, 1992). Ozone in the lower atmosphere originates predominantly from oxidation and photolysis of nitrogen oxides emitted from vehicle exhaust fumes in urban areas (Welfare *et al.*, 1996). Thus, a high level of O_3, is usually accompanied by industrialization and urbanization, especially in mega cities of developing countries, where environmental standards have not been adequately established (Ariyaphanphitak *et al.*, 2005; David *et al.*, 1994). It has been reported that in some major cities of developing countries, such as, Pakistan (Ghauri *et al.*, 1991), Indonesia (Komaia and Ogawa, 1991; Pardede, 1991), and India (Pandey and Agrawal, 1992; Pandey *et al.*, 1992), the tropospheric O_3 concentrations have been elevated. Although the information on ground-level O_3 concentrations in China is limited, but the available data have suggested that O_3 concentrations can reach potentially damaging levels (Wang *et al.*, 2007b).

The Yangtze Delta in China is one of the world's continental scale Metro-Agro-Plexes (MAPs). As Chameides *et al*. (1994) has pointed out, its rapid growth has resulted in increased ground-level O_3, to pollution levels. Recent monitoring in the Yangtze Delta showed that 6.1%–10.4% of the hourly mean O_3 concentrations have exceeded 60 ppbv, and the peak O_3 concentration was as high as 196 ppbv (Zhou, 2004). Crop losses on account of O_3 have been estimated to be higher than 10% for wheat and more than 5% for rice (Feng *et al.*, 2003), and 20%–30% for wheat (Wang *et al.*, 2007a) based on dose-response relationships derived from experiments conducted in other regions.

O_3 is the primary atmospheric pollutant causing loss of crop yield (Maggs and Ashmore, 1998). O_3, alone or in combination with other pollutants, has accounted for approximately 90% of crop loss, induced by air pollution, in the U.S. (David *et al.*, 1994). Rice, as a major grain crop widely planted in East and Southeast Asia, has been investigated in terms of its response to O_3 pollution. Studies have shown that elevated O_3 decreases rice pigment content and photosynthetic rate, growth, biomass, and yield (Kobayashi *et al.*, 1995; Wahid *et al.*, 1995a, 1995b; Olszyk and Wise, 1997; Maggs and Ashmore, 1998) and O_3 stress induces *in vivo* oxidative injury and affects the activities of antioxidant enzymes (Jin *et al.*, 2000). Thai Jasmine rice cultivars exposed to elevated O_3 have suffered a significant reduction in filled seeds per panicle, root biomass and length, and grain yield (Ariyaphanphitak *et al.*, 2005). Kobayashi *et al.* (1995) has found a significant

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decrease in leaf blade area, leaf sheath and culm dry weight, and ratios of root:shoot in the cultivars Koshihikari and Nipponbare. However, little is known about the changes in rice yield components and root activity, when rice is exposed to elevated O_3 concentration.

In this study, with open-top chambers established in rice paddy, the authors have investigated the growth in different stages, the biomass of various plant parts, root activity, and yield component responses to elevated O_3 concentration, specifically focusing on the changes in yield components and root activity after elevated O_3 exposure.

1 Materials and methods

1.1 Experimental site

The experimental site is located at the Shuangqiao Farm (31°53′N, 121°18′E) in Jiaxing City, Zhejiang Province, about 100 km from Shanghai, China. This site is in the center of the Yangtze Delta which 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 warm wet summers. Mean temperature and annual precipitation are 15.5°C and 1199 mm, respectively. The prevailing cultivation rotation is wheat-rice or rape-rice.

1.2 Crop management

On 19 May 2006, rice (3694-Fan) seeds were sown in a seedling bed and then transplanted into experimental blocks on 20 June. These plots consisted of three rows, with four plots in each row. Between plots, the protective belts were approximately 3 m wide. Pure nitrogen (N) of 60 kg/hm^2, 60 kg/hm^2 of P_2O_5, and K_2O was applied before transplanting, and 69.3 kg/hm^2 of pure N was reapplied during the tillering and jointing stages. The crops were harvested on 25 October.

1.3 Ozone exposure

O_3 exposure began on 16 July when the rice was in the three-leaf stage and ended on 10 October when it was ripe. The fumigation lasted for 40 d except when it was raining. Twelve OTCs (open top chambers) (2.2 m in height and 2 m in diameter) consisted of an octagonal aluminum frame covered with transparent film. They had an improved, innovative, ozone distribution system. A rotatable transparent pipe with many small holes (diameter of 10 mm, at intervals of 10 cm) released either charcoal-filtered air (CF) or ozone above the crop canopy. Air was driven either by CF air or ozone plusing CF from a centrifugal blower. O_3 was generated from pure oxygen with the help of a high-voltage electric discharge (Yuyao Shenglete Company, Zhejiang, China). A series of solenoid valves, linked online with a programmable log controller (PLC K80S, LG, Korea), were used to control gas meters to provide oxygen, based on the established relationship between O_3 concentration within the OTC and the oxygen volume. O_3 concentrations within the OTCs were monitored by an O_3 analyzer (Model 9810B, Monitor Labs, Australia). O_3 concentration monitoring showed that O_3 concentrations were uniformly distributed, vertically and horizontally, within the OTC (Zheng *et al*., 2007). A data logger (21xL, Campbell Scientific Inc., UT, USA) stored O_3 concentration data and temperature within the OTCs, which was measured with a constantan-copper thermocouple (Type-T, Omega Engineering, CT, USA).

1.4 Experimental design

There were four treatments: charcoal-filtered air (CF), air without filters, that is non-charcoal-filtered air (NF), charcoal filtered air plus additional O_3 with diurnal variation (two levels, O_3-1 and O_3-2). In the O_3-1 treatment, the diurnal pattern of O_3 concentration was 50 ppbv from 9:00–10:00, 100 ppbv from 10:00–12:00, 150 ppbv from 12:00–14:00, 100 ppbv from 14:00–16:00, and 50 ppbv from 16:00–17:00. In the O_3-2 treatment, the diurnal pattern of O_3 concentration was 100 ppbv from 9:00–10:00, 150 ppbv from 10:00–12:00, 200 ppbv from 12:00–14:00, 150 ppbv from 14:00–16:00, and 100 ppbv from 16:00–17:00. Twelve chambers were used to yield three replicates for each treatment.

Critical levels for ozone were defined as a cumulative exposure over a threshold concentration for a given length of time (Ashmore and Wilson, 1994). Then this concept was adopted and the threshold concentration was set at 40 ppbv; the resulting index was termed the AOT40 (accumulated exposure over a threshold of 40 ppbv) (Fuhrer *et al.*, 1997). In this study, the AOT40 was 0, 0.91, 23.24, 39.28 ppmv·h for CF, NF, O_3-1, and O_3-2, respectively. Crop practices were adopted according to local agronomic customs, therefore, that water, fertilizer, pathogen, insect, and weed were not limiting factors.

1.5 Biomass and yield measurement

Height and biomass were measured on 5 August, 10 September, and 8 October. At the final harvest on 25 October, eight plants were collected randomly from each chamber. Rice panicles and leaves were separated from the shoot, and the dry weight of every part was determined by oven-drying at 70°C to constant weight. The number of panicles per plant, length of panicles, and the number of grains per panicle, primary and secondary rachis, was measured at harvest.

1.6 Root activity

Root activity was measured using the TTC (triphenyl tetrazolium chloride) method (Chen, 2003) and expressed as the deoxidization ability (mg/(g·h)). Dehydrogenase was expressed as the deoxidized TTC quantity, which was an index of root activity. In brief, 0.5 g of root sample was immerged in 10 ml mixed solution of equal quantities (0.4%) of TTC and phosphate buffer, and kept in the dark for 1–3 h at 37°C. Then 2 ml of 1 mol/L H_2SO_4 was added to stop the reaction. The immerged roots were ground and transferred into a tube with ethyl acetate cleaning solution to a total volume of 10 ml. The solution absorbance was read as 485 nm, with a spectrophotometer.

1.7 Statistical analysis

Treatment means were statistically compared using the statistical package SPSS (SPSS Inc., Chicago, IL, USA). One-way ANOVA was used to determine statistically significant differences in relative dry weight of various plant parts and yield components among treatments. Two-factor analysis was used to assess the O_3 effect on biomass following the exposure period. Path coefficient analysis measured the direct influence of one variable on another and also separated the correlation coefficient into components of direct and indirect effects (Rodríguez *et al*., 2001). A detailed path coefficient analysis could help relate he contributor of various factors to yield loss.

2 Results

2.1 Growth and biomass

The impact of elevated O_3 concentration on rice height increased with the duration of fumigation (Fig.1a). A significant reduction in rice height appeared on the first sampling date, less than 20 d after initiation of exposure, in the higher O_3 concentration (O_3-2). No significant reduction was observed under the lower O_3 treatment (O_3-1), even at the final harvest.

Relative to CF exposure, total rice biomass was reduced by 20.1%–27.7% and 8%–24% under O_3-1 and O_3-2 exposures, respectively (Fig.1b). There were significant differences between O_3-2 and CF or NF treatments.

Elevated O_3 altered biomass partitioning. The measurements before 8 October showed no significant differences in biomass partitioning to roots and panicles among the treatments, although there was a trend toward decreasing percentage of root and panicle biomass with higher O_3 concentration (Table 1). At the final harvest, the biomass partitioning to root and panicle significantly decreased under higher O_3 concentration. The percentage of leaf biomass increased significantly from 10 September (Table 1).

Two-way ANOVA showed that both time and O_3 concentration affected the total and component biomass of rice (Table 2). However, the interactive effects of time and O_3 concentration were only significant for panicle biomass.

Higher O_3 concentration (O_3-2) decreased root dry weight by 24.7%–42.8%, and shoot dry weight by 18.9%–26.8% in this study, indicating that roots were affected

Table 1 Relative dry weight of various plant parts to whole plants exposed to different ozone fumigations (%)

	5 Aug.	10 Sep.	8 Oct.	25 Oct.
Root relative dry weight				
CF	21.1±2.1 a	14.6±0.7 ab	18.1±3.6 a	11.1±0.9 a
NF	20.1 ±0.6 a	15.6±0.4 a	17.6±3.0 a	10.2±0.3 ab
O_3-1	23.0±1.6 a	14.8±0.9 a	17.5±3.9 a	8.9±0.6 b
O_3-2	19.5±2.1 a	12.4±1 b	14.5±0.8 a	9.6±0.7 ab
Stem relative dry weight				
CF	47.91.7 a	52.9±0.9 a	29.6±1.5 a	38.7±0.9 a
NF	48.30.7 a	50.6±0.5 ab	29.7±3.5 a	35.1±0.2 a
O_3-1	44.51.3 a	49.9±1.5 b	28.4±2.8 a	36.6±1.5 a
O_3-2	45.71.5 a	49.7±0.6 b	32.7±3.6 a	39.4±2.9 a
Foliage relative dry weight				
CF	31.1±0.6 a	23.2±0.8 a	14.1±1.9 a	13.0±0.9 a
NF	31.5±0.4 a	23.6±0.5 a	13.9±1.3 a	13.1±0.4 a
O_3-1	32.5±1.0 a	27.9±1.4 b	16.7±1.6 ab	15.4±0.7 b
O_3-2	29.0±5.8 a	30.6±0.9 b	20.8±2.6 b	17.4±0.9 b
Panicle relative dry weight				
CF	0	9.3±0.1 a	31.9±2.6 a	37.2±2.0 a
NF	0	10.3±0.4 a	31.6±4.1 a	41.7±0.6 a
O_3-1	0	7.3±0.7 b	29.4±3.3 a	39.1±2.0 ab
O_3-2	0	7.3±0.3 b	24.9±2.2 a	33.6±3.1 b

The values are means ± SE (n = 3). Results followed by different letters are statistically significant at the 0.05 level. CF, NF are the same as in Fig.1.

Table 2 Two factor analysis of O_3 and time for biomass

F value	Root	Stem	Leaf	Panicle	Total
Time	13.2***	30.4***	17.3***	179.4***	46.6***
O_3	3.2*	6.6***	0.258649	9.6***	5.3**
Time and O_3	0.602	0.720	1.264	2.5*	0.949

*, **, *** Values are significant at 0.05, 0.01, and 0.001 level of probability, respectively.

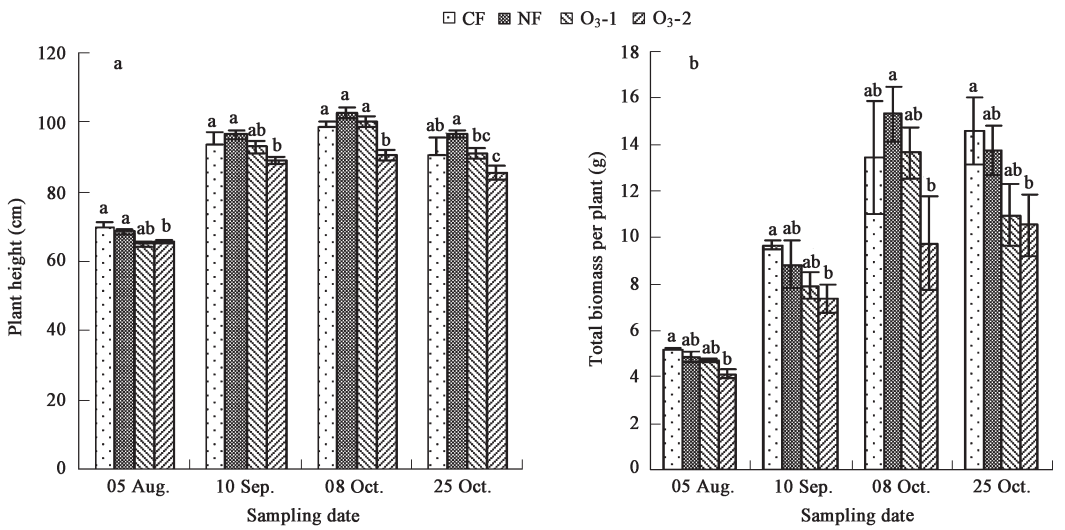

Fig. 1 Effects of elevated O_3 on plant height (a) and dry weight of whole plants (b) exposed to different ozone concentrations at different sampling times. Values are means ± SE (n = 24). Different letters show significance at the 0.05 level for each sampling time. CF: charcoal-filtered air; NF: non-charcoal-filtered air.

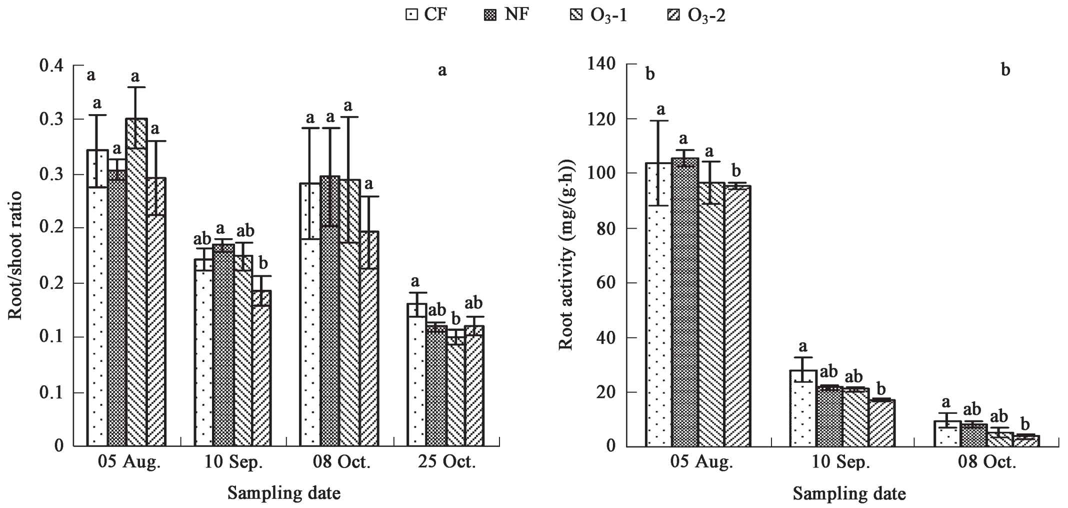

Fig. 2 Effects of elevated O_3 on root: shoot ratio (a) and root activity (b) at different sampling times exposed to ozone. Values are means ± SE (n = 3). Treatments are described in Fig.1. Different letters indicate significant differences at the 0.05 level for each time point.

more seriously than shoots after exposure to elevated O_3. This could also be seen from the decreasing trends in the ratio of root to shoot, with increasing O_3 concentration (Fig.2a). Besides reduction in root biomass, root activity was significantly reduced by higher O_3 concentration (O_3-2) as compared to CF and NF treatments (Fig.2b).

2.2 Yield

As compared to the CF treatment, yield per square meter was decreased by 14.3% and 20.2%, respectively. Higher O_3 concentration (O_3-2) significantly decreased all rice characteristics listed in Table 3 except the number of panicles per plant. Compared to CF, in O_3-2, the primary and secondary rachis of panicles were decreased by 8.5% and 27.2%, respectively; grain number per panicle and full filled seeds by 19.5% and 4.3%; harvest index, 1000-grain wt, and grain wt per panicle by 11.9%, 7%, and 23%, respectively.

In this study, primary rachis, filled seed percentage, harvest index and 1000-grain weight did not contribute significantly to rice yield loss at elevated O_3 concentration. Secondary rachis showed the largest positive direct effect on grain yield per plant (p = 1.1323), and the number of panicles per plant on grain yield per panicle (p = 0.6527). The direct effect of number of grains per panicle was negative (p = –0.6786), but the indirect effect through secondary rachis on grain yield per panicle was positive (p = 1.1096) which was even larger than the direct effect (Table 4). This result showed that decrease in secondary rachis was the main factor leading to yield loss because of O_3 exposure. The number of panicles per plant and the number of grains per panicle also contributed to yield loss with O_3 exposure.

Table 4 Path coefficient analysis, direct effects (underlined) and indirect effects, for the characteristics of grain yield per plant

	Number of grains per panicle	Number of panicles per plant	Secondary rachis
Number of grains per panicle	<u>–0.6786</u>	0.4877	1.1096
Number of panicles per plant	–0.5069	<u>0.6527</u>	0.7439
Secondary rachis	–0.6650	0.4288	<u>1.1323</u>

3 Discussion

Exposure to elevated O_3 concentration for 40 d caused considerable suppression of rice growth and yield, which was consistent with the reports in other countries, for example, Thailand (Ariyaphanphitak *et al*., 2005), Japan (Nouchi *et al*., 1991; 1995), Pakistan (Wahid *et al*., 1995a; Maggs and Ashmore, 1998), and USA (Kats *et al*., 1985). The loss of rice yield in this study was lower than the result of Feng's study in the same area (Feng *et al*., 2003), which was perhaps caused by different rice varieties and O_3 fumigation. In this study, rice stem biomass under O_3-2 exposure was decreased by 26.9%, whereas, the height

Table 3 Yield responses of rice exposed to CF, NF, and O_3 in open top chambers for 8 h/d for 40 d in 2006

Treatment	CF	NF	O_3-1	O_3-2
Number of primary rachis	14.33±0.48 a	14.79±0.26 a	14.04±0.34 ab	13.12±0.7 b
Number of secondary rachis	22.88±1.96 ab	25.33±1.52 a	19.67±1.61 bc	16.65±1.39 c
Number of panicles per plant	2.08±0.18 a	2.04±0.14 a	1.83±0.15 a	1.75±0.24 a
Number of grains per panicle	146.8±7.56 ab	155.2±6.78 a	133.5±5.96 bc	118.2±5.67 c
1000-grain wt (g)	25.61±0.27 a	24.87±0.46 ab	25.03±0.33 a	23.81±0.28 b
Harvest index	0.41±0.02 ab	0.46±0.005 a	0.42±0.02 ab	0.36±0.04 b
Filled seed (%)	92±0.70 ab	94±0.70 a	89±1.90 ab	88±3.0 b
Grain wt per panicle (g)	2.6±0.33 a	2.8±0.03 a	2.2±0.23 ab	2.0±0.42 b

The values are means ± SE (n = 24). Results followed by different letters are statistically significant at the 0.05 level.

Table 5 Effects of ozone on yield components in different studies

Country	Characteristics						Reference
	Number of grains per panicle	Number of fully-filled seeds	Number of panicles per plant	Harvest index	1000-grain wt (g)	Grain wt per panicle (g)	
Thailand	–	**	–	–	**	–	Ariyaphanphitak *et al.*, 2005
Pakistan	**	**	**	**	**	**	Wahid *et al.*, 1995a
Pakistan	ns	ns	ns	ns	ns	**	Maggs and Ashmore, 1998
Japan	**	–	*increased	ns	**	–	Kobayashi *et al.*, 1995
China	**	**	ns	**	**	**	This study

–: not measured component; ns: not significant; *, ** significant at 0.01 and 0.05 level, respectively.

was decreased by 8.3% relative to CF treatment. Higher reduction in biomass than height is not in agreement with the previous work, demonstrating that the effects of O_3 on rice shoot length were more serious than those on shoot biomass (Ariyaphanphitak *et al*., 2005).

Biomass partitioning to leaves was always higher in the elevated O_3 treatment than in the CF treatment, especially at the high ozone level (O_3-2). This increment was attributed to greater reduction in stem, root, and panicle biomass, caused by O_3 exposure (Table 1). This result was in good agreement with the previous reports by Ariyaphanphitak (2005). The decreasing biomass partitioning to roots resulted in a decline in root/shoot ratio as reviewed by Cooley and Manning (1987) and Nouchi *et al*. (1991). Elevated O_3 decreased root growth through alteration of photosynthetic partitioning, and the inhibition of photosynthetic translocation altered root physiological functions such as respiration and absorbing water and nutrients (Hofstra *et al*., 1981; Ito *et al*., 1985; Nouchi *et al*., 1991). Reduction in root activity reflected a decline in root function after elevated O_3 exposure. A similar result was reported by Nouchi *et al*. (1991), who found that O_3 exposure at 100 ppbv tended to inhibit NH_4^+-N uptake.

Different studies have reported that O_3 has significant effects on yield components, such as, the number of fully-filled seeds, 1000-grain weight, and grain weight per panicle (Table 5). However it is difficult to tell which parameter is the most sensitive to O_3, as varieties, O_3 fumigation concentrations, and cultivation practices differ among studies. Yield components examined in this study have all been correlated to grain yield per panicle except for the harvest index and 1000-grain weight. Path analysis shows that secondary rachis have the largest direct effect on grain yield per panicle when rice is exposed to O_3. The number of grains per panicle and the number of panicles per plant also have a significant direct effect on grain yield per panicle, but the indirect effects through secondary rachis are greater than the direct effects. It can be concluded that a decrease in the number of secondary rachis is the most important characteristic causing yield loss, when rice is exposed to O_3.

4 Conclusions

This study has conducted the response of the growth and yield loss of rice to elevated O_3 concentration. The plant height and total biomass of rice were reduced in elevated O_3 concentration. Elevated O_3 also altered biomass partitioning, and less organic matter partitioning to roots significantly decreased root activity, which would influence the water and nutrient supplies of crop production. The yield components were significantly reduced by O_3, including the number of primary and secondary rachis, the number of grains per panicle, 1000-grain weight and so on. The yield of rice was decreased under both O_3 exposures, which was largely caused by a reduction in grain weight per panicle.

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