Elevated CO₂ Ameliorates O₃-Inhibition of Growth and Yield in Paddy Rice

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To elucidate the interaction between tropospheric O₃ and CO₂ in paddy rice, combined applications of O₃ (0–0.3 cm³ m⁻³) and CO₂ (380, 800 cm³ m⁻³) were performed at the vegetative stage or flowering to early maturing stage of rice plants using environmentally controlled chambers: O₃ decreases growth and yield, although CO₂ exhibits the opposite action. At the vegetative stage, elevated O₃ decreased the relative growth rate (RGR) and net assimilation rate (NAR) but elevated CO₂ ameliorated those effects. At the flowering to early ripening stage, elevated O₃ decreased dry matter production and yield with lowering of the percent maturity of grains, but the elevated CO₂ tended to offset these declines. The results indicate that elevated O₃ primarily inhibits its photosynthesis-related process of paddy rice. Elevated CO₂, which is predicted for the future, interactively ameliorates that inhibition.

Keywords : carbon dioxide, growth analysis, net photosynthesis, *Oryza sativa*, ozone, pollen fertility

INTRODUCTION

Photochemical oxidants appear on windless and sunny summer days during the rice growing season and decrease the yield of this staple food source by inhibiting net photosynthesis (P_N) and growth (Nouchi, 2001; Nakanishi et al., 2009). Rice crops growing around the fringe of urban areas, especially in the Kanto region of Japan, often temporarily receive elevated photochemical oxidants, sometimes close to 0.2 cm³ m⁻³ (Environ. Improve. Div., Tokyo Metro. Bureau Environ., 2005). Because O₃ constitutes about 90% of all photochemical oxidants, studies of O₃-induced injury of rice have been conducted, yielding substantial anatomical, physiological, and biochemical evidence (Nakamura et al., 1975; Jeong et al., 1980; Toyama et al., 1989; Nouchi, 1993). Nevertheless, because of the complex mechanisms and actions causing O₃-induced injury, no satisfactory implications or countermeasures exist for them (Nouchi, 2003).

On the other hand, the annual increment of atmospheric CO₂ concentration has been about 2 cm³ m⁻³ during the past decade. Its recent global-average value is approaching 387 cm³ m⁻³ (WMO WDCGG, 2011). Because CO₂ is a key substrate for photosynthesis, elevated CO₂ concentrations promote P_N. Subsequently, they increase the yield of many C₃-crop species including rice (Imai, 1995; Horie et al., 2000). Therefore, it is important to determine the manner in which such detrimental O₃ and beneficial CO₂ interact and thereby affect the production processes of rice because studies of the combined effects of O₃ and CO₂ on this staple food source are few (Ainworth, 2008).

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We surmised that the detrimental effects of O_3 would be ameliorated by elevated CO_2 because induced stomatal closure that occurs as a result of elevated CO_2 restricts the invasion of O_3 inside the leaf mesophyll (McKee et al., 1995; Jaspers et al., 2005) and increased intercellular CO_2 (C_i) promotes P_N (Imai and Kobori, 2008), provided that O_3 concentration is maintained within a range observed in the field. For this study, we exposed O_3 and CO_2 singly or in combination with rice crops during the vegetative stage or flowering to early maturation stage and observed their interactive effects on photosynthesis-related production processes. The stage of flowering to early maturation is important for rice yield determination because fertilization and rapid starch accumulation into grains occur (Murata and Matsushima, 1975; Hoshikawa, 1989). If O_3 attacks fertilization directly, then the decline of the set percentage of grains occurs more severely than that of either percent maturity or grain weight. The opposite is true if O_3 attacks indirectly through photosynthesis inhibition. Observations of yield and yield components enable us to evaluate the relative deterioration by O_3 and its amelioration by elevated CO_2 . To obtain detectable changes of growth and yield parameters, most gas exposure was applied for one or two weeks.

MATERIALS AND METHODS

Plant materials

A japonica paddy rice cultivar (*Oryza sativa* L. cv. Koshihikari) was used for these four experiments. Five germinated seeds were sown in each plastic Wagner-pot filled with soil and nutrients (Exps. 1, 2, and 4: 1/5000 a pots containing 2.5 kg of dry soil and 4 g of compound fertilizer [N, P_2O_5 , K_2O =15, 15, 15, %], Exp. 3: 1/2000 a pots containing 10 kg of dry soil and 8 g of the same compound fertilizer) and watered sufficiently under outdoor conditions. When plants had four fully expanded leaves (Haun index=4.0; Haun, 1973), they were thinned to one plant per pot and kept in submerged conditions (depth: 3 cm). At the vegetative growth (having 8 leaves on main culm: Exp. 1 and 2), flowering to early maturation (having 16 leaves on main culm: Exp. 3) or flowering (Exp. 4) stage, plants were subjected to gas-exposure treatments.

Gas exposure

Four natural-light, environment-controlled chambers (width $\times$ depth $\times$ height=2 m $\times$ 2 m $\times$ 1.9 m; S-2003A; Koito Industries Ltd., Yokohama, Japan) were used for gas exposure. Ozone was supplied by a high-voltage O_3 generator using dry air (MO-5A; Ozone System, Tokyo, Japan) and CO_2 was supplied from liquid CO_2 cylinders. These gases were injected into air that had been filtered through activated charcoal layers. The O_3 and CO_2 concentrations were measured and were computer-controlled using an ultraviolet absorption-type O_3 analyzer (EG-2001F; Ebara Jitsugyo Co., Ltd., Tokyo, Japan) and an infrared CO_2 analyzer (ZRC-1DF51; Fuji Electric Systems, Co., Ltd., Tokyo, Japan), respectively. The actual concentration of O_3 in the control treatment (O^0) was lower than $0.003 \text{ cm}^3 \text{ m}^{-3}$. Day (12 h)/night (12 h) temperatures were maintained at 28/23°C; the relative humidity was 60%.

The carbon dioxide concentration was maintained during all day at 380 (400) or $800 \text{ cm}^3 \text{ m}^{-3}$ throughout the treatment period; O_3 was exposed during the 12-h-daytime period (6:30–18:30 in local time) in Exps. 1–3, and during 4-h-daytime (8:30–12:30) in Exp. 4. In Exp. 1, plants of the vegetative stage were exposed to 0, 0.05, 0.10, and $0.20 \text{ cm}^3 \text{ m}^{-3}$ O_3 (expressed respectively as O^0 , $O^{0.05}$, $O^{0.1}$, and $O^{0.2}$) under $380 \text{ cm}^3 \text{ m}^{-3}$ CO_2 (C^{380}) for 1 week and afterward, kept in clean, ambient air ($O^0 + C^{380}$) for the subsequent 1 week in each chamber. In Exp. 2, plants at the vegetative stage were exposed to combinations of O_3 (O^0 or $O^{0.1}$) with ambient or elevated CO_2 (380 or $800 \text{ cm}^3 \text{ m}^{-3}$; C^{380} or C^{800}) for 1 week and afterward, kept in a clean, ambient air for the subsequent 1 week in each chamber. In Exp. 3, plants at the flowering to early maturation stage were exposed to combinations of O_3 (O^0 or $O^{0.1}$) and CO_2 (C^{380} or C^{800}) for (1) the whole 2 weeks, (2) the first 1 week period, or (3) the second 1 week period. Subsequently, they were kept under outdoor

conditions until maturity. In Exp. 4, plants at the flowering stage were exposed to combinations of O₃ (O⁰, O^{0.1} or O^{0.3}) and CO₂ (C⁴⁰⁰ or C⁸⁰⁰).

Sampling and plant growth analysis

Ten plants in Exp. 1 and 12 plants in Exp. 2, respectively, were harvested at Day 0 (start of gas exposure), Day 7 (termination of gas exposure), and Day 14 (1 week after the termination of gas exposure). At harvest, leaves were detached and their area was measured using an automatic area meter (AAM-8; Hayashi Denko Co., Ltd., Tokyo, Japan). Thereafter, the whole plant-body was oven-dried at 85°C for 48 h and weighed after cooling to room temperature in desiccators. Using data for the dry weight and leaf area, the relative growth rate (RGR), net assimilation rate (NAR), and leaf area ratio (LAR) were calculated as follows.

$$\begin{aligned} \text{RGR} &= (\ln W_2 - \ln W_1) / (t_2 - t_1) \\ \text{NAR} &= \{(W_2 - W_1)(\ln L_2 - \ln L_1)\} / \{(t_2 - t_1)(L_2 - L_1)\} \\ \text{LAR} &= \{(L_2 - L_1)(\ln W_2 - \ln W_1)\} / \{(\ln L_2 - \ln L_1)(W_2 - W_1)\} \end{aligned}$$

In those equations, W , t and L respectively denote the whole-plant dry weight, time of sampling, and leaf area.

In Exp. 3, six plants were harvested at maturity (45 days after flowering) and sorted into ear (panicle), leaf blade, leaf sheath + stem, root, and dead leaf fractions. Then they were oven-dried at 85°C for 48 h and weighed after cooling to room temperature in desiccators. Regarding the main-culm ear, the rough rice grains were selected in the NaCl solution (s. g. = 1.06), and fully fertile grains were obtained. Infertile grains were separated into sterile (without ovary development) and imperfect (partial ovary development) fractions by fingering. Thereafter, they were oven dried at 85°C for 48 h and weighed.

Pollen germination at flowering

In Exp. 4, healthy flowers opened within 5 min in the control treatment (O⁰ + C⁴⁰⁰), which were chosen randomly from 10 plants, were sampled and the pollen grains were pulled down immediately onto agar medium (1% agar with 12% sucrose and 100 cm³ m⁻³ boric acid) in Petri dishes and exposed to combinations of O₃ (O⁰, O^{0.1} or O^{0.3}) and CO₂ (C⁴⁰⁰ or C⁸⁰⁰) for 20 min. Their germination was assessed using an optical microscope.

RESULTS

Effect of O₃ exposure on the growth at vegetative stage (Exp. 1)

Figure 1 presents effects of O₃ concentrations (0, 0.10, 0.15, and 0.20 cm³ m⁻³) on RGR, NAR, and LAR at the vegetative stage of rice plants. During 1 week of O₃ exposure, RGR was suppressed with increasing O₃ concentration; NAR showed a similar change with RGR, but LAR was opposite. Successive growth for 1 week in clean air (O⁰ + C³⁸⁰) after O₃ exposure showed that RGR and NAR tended to recover, although LAR did not follow.

Effect of O₃ and CO₂ exposures on the growth at vegetative stage (Exp. 2)

The effects of O₃ and CO₂ on RGR, NAR, and LAR are presented in Fig. 2. During gas exposure, RGR was affected strongly through the effects on NAR. The effect on RGR was the greatest in the O⁰ + C⁸⁰⁰ treatment according to the promotion of NAR, which implied the promotion of P_N. In contrast, RGR of the O^{0.1} + C³⁸⁰ treatment was the smallest because of the inhibition of NAR. However, RGR of the O^{0.1} + C⁸⁰⁰ treatment was similar to that of the control treatment (O⁰ + C³⁸⁰), indicating that the inhibitory effect of O₃ was ameliorated by the promotion of the NAR under elevated CO₂. The LAR was little affected by either O₃ or CO₂. The RGR of the O⁰ + C³⁸⁰ treatment recovered from O₃-inhibition during the growth in clean air (O⁰ + C³⁸⁰) because of the recovery of NAR, but that of the O⁰ + C⁸⁰⁰ treatment declined substantially because of the decline of NAR.

Fig. 1 Growth analysis of rice plants during and after O₃ exposure, each for 1 week (Exp. 1). ■: Plants grown under O₃ exposure for 1 week. □: Plants grown under clean air (O⁰ + C³⁸⁰) for 1 week successively after O₃ exposure.

Fig. 2 Growth analysis of rice plants during and after the O₃ and CO₂ exposure, each for 1 week (Exp. 2). ■: Plants grown under the combination of O₃ and CO₂ for 1 week. □: Plants grown under clean air (O⁰ + C³⁸⁰) for 1 week successively after gas exposure. O⁰, O^{0.1}: 0 and 0.1 cm³ m⁻³ O₃. C³⁸⁰, C⁸⁰⁰: 380 and 800 cm³ m⁻³ CO₂.

Effects of O₃ and CO₂ exposures during the flowering to early maturation stage on the dry-matter production, yield, and yield components (Exp. 3)

Effects of 1 or 2 weeks of gas exposure on the dry-matter production, yield and yield components are presented in Table 1. By 2 weeks of successive O₃ exposure under ambient CO₂ (O^{0.1} + C³⁸⁰), the total plant weight decreased 16.3% when compared to that of the control treatment (O⁰ + C³⁸⁰). However, under elevated CO₂ (O^{0.1} + C⁸⁰⁰), it was not decreased. In the O⁰ + C⁸⁰⁰ treatment, it tended to increase (6.7%). Responses of the ear and leaf-sheath + culm weights resembled those of the total plant weight. The root dry weight and the harvest index (ear weight/total plant weight) were fundamentally unaffected by O₃ exposure. Examination of the yield components for the main culm revealed that successive 2-week exposure of O₃ (O^{0.1}) suppressed the percentage of setting and maturity of rice grains (6.2 and 16.1%, respectively), but elevated CO₂ (C⁸⁰⁰) substantially ameliorated suppression by O₃. Throughout the exposure treatments, the percent set was higher than the percent maturity, and O₃ inhibition was greater in the latter, especially for 2-week successive exposure of O₃.

Effect of O₃ and CO₂ on the pollen germination at flowering (Exp. 4)

As Table 2 shows, the percentage pollen germination decreased concomitantly with increasing O₃ levels under ambient CO₂ (C⁴⁰⁰). However, under elevated CO₂ (C⁸⁰⁰), the pollen germination was not inhibited by elevated O₃ (O^{0.1}, O^{0.3}). These results indicate clearly that elevated CO₂ protected O₃ inhibition for pollen germination.

DISCUSSION

Figure 1 shows that the RGR of rice plants was progressively inhibited by elevated O₃

Table 1 Effects of O₃ and CO₂ exposure during flowering to early-maturing stages of rice on the dry weight, yield and yield components at maturity (Exp. 3).^z

Treatment	Duration of exposure	Dry weight/plant (g)																							
		Total	Ear	Leaf-blade	Leaf-sheath + culm	Root	Dead leaf	Harvest index	Single-ear weight (g) ^y	Grain numbers ^y	1000-grain weight (g) ^y	Percent set ^y	Percent maturity ^y												
O ⁰ +C ³⁸⁰	Whole 2 weeks	93.42	a ^x	40.03	a	8.57	a	30.39	a	5.53	a	8.90	a	0.43	a	2.84	a	163.3	a	22.34	a	89.7	bc	79.8	a
O ^{0.1} +C ³⁸⁰		78.22	b	33.16	b	8.32	a	22.09	b	5.53	a	9.12	a	0.42	a	2.43	c	156.7	ab	22.25	a	83.5	c	63.7	b
O ^{0.1} +C ⁶⁰⁰		93.81	a	39.90	ab	8.53	a	29.74	a	6.01	a	9.63	a	0.43	a	2.50	bc	149.2	b	20.98	b	94.1	ab	78.6	ab
O ⁰ +C ⁶⁰⁰		99.70	a	44.45	a	7.65	a	31.67	a	6.23	a	9.70	a	0.45	a	2.82	ab	150.5	ab	22.40	a	97.2	a	91.7	a
O ⁰ +C ³⁸⁰	First 1 week	90.86	a	38.39	ab	9.20	a	30.23	a	5.30	a	7.74	a	0.42	a	2.81	a	151.2	a	22.55	a	95.8	a	89.9	a
O ^{0.1} +C ³⁸⁰		82.47	b	34.92	b	8.59	a	26.20	b	5.29	a	7.47	a	0.42	a	2.75	a	152.0	a	22.15	ab	94.7	a	90.1	a
O ^{0.1} +C ⁶⁰⁰		93.69	a	39.86	ab	8.75	a	30.48	a	5.69	a	8.91	a	0.43	a	2.84	a	155.2	a	22.62	a	95.1	a	88.0	a
O ⁰ +C ⁶⁰⁰		96.68	a	41.62	a	8.52	a	31.46	a	5.98	a	9.10	a	0.43	a	2.70	a	148.5	a	21.61	b	96.1	a	93.7	a
O ⁰ +C ³⁸⁰	Second 1 week	94.24	a	39.59	a	8.89	ab	30.23	a	5.98	a	9.55	ab	0.42	a	2.72	a	151.2	a	21.82	a	96.0	a	84.5	a
O ^{0.1} +C ³⁸⁰		86.08	b	36.36	a	7.96	b	25.56	b	5.82	a	10.38	a	0.42	a	2.62	a	150.7	a	21.79	a	96.8	a	72.2	b
O ^{0.1} +C ⁶⁰⁰		91.94	ab	38.98	a	9.39	a	30.71	a	5.48	a	7.38	b	0.42	a	2.78	a	157.8	a	21.72	a	95.6	a	83.3	a
O ⁰ +C ⁶⁰⁰		93.49	a	39.11	a	8.66	ab	32.03	a	5.97	a	7.72	b	0.42	a	2.75	a	149.8	a	22.08	a	93.4	a	87.8	a

^z Before and after the gas exposures, plants were kept under ambient conditions.^y Values for the main culm.^x In each column of each exposure duration, the means followed by the same letter are not significantly different at the 0.05 probability level.

Table 2 Effects of O₃ and CO₂ exposure on the percentage pollen germination of rice *in vitro* (Exp. 4).

Treatment	Germination (%)
O ⁰ + C ⁴⁰⁰	44.4 ± 3.6a ^z
O ⁰ + C ⁸⁰⁰	42.4 ± 1.0a
O ^{0.1} + C ⁴⁰⁰	37.2 ± 1.8bc
O ^{0.1} + C ⁸⁰⁰	44.1 ± 3.0a
O ^{0.3} + C ⁴⁰⁰	36.3 ± 5.0c
O ^{0.3} + C ⁸⁰⁰	40.7 ± 4.8ab

^z In a column, the means followed by the same letter are not significantly different at the 0.05 probability level.

concentration and that this resulted from the decline of NAR (a rough estimate of photosynthesis). When plants were transferred into clean air (O⁰ + C³⁸⁰), both RGR and NAR changed toward recovery, although LAR did not. These results imply that acute exposure of O₃ has detrimental effects on rice plants through photosynthesis-related processes (Nouchi, 2001; Ishioh and Imai, 2005).

Under combined gas exposure of O₃ and CO₂, the detrimental effects of O₃ on RGR and NAR were ameliorated by elevated CO₂ (O^{0.1} + C⁸⁰⁰; Fig. 2). This effect was manifested through the promotion of P_N and decreased stomatal conductance by elevated CO₂ (Imai and Kobori, 2008; Kobayakawa and Imai, 2011): The former was ascribed to increased intercellular CO₂ concentration (C_i), which maintained the Rubisco activity (Ishioh and Imai, 2005) and the latter to limit the invasion of O₃ into leaf mesophyll (McKee et al., 1995; Jaspers et al., 2005). However, when plants were kept under ambient CO₂ level (O⁰ + C³⁸⁰) after the CO₂-enrichment (O⁰ + C⁸⁰⁰; Fig. 2), both RGR and NAR declined substantially. This aftereffect resulted from the phenomenon known as *photosynthetic acclimation* induced by elevated CO₂ (Imai and Murata, 1978).

Through exposure of elevated O₃ during flowering to early maturation, rice plants suffered damage not only to dry-matter production, but also to the yield and yield components, especially when the exposures were prolonged (Table 1). The decline of percentage maturity by prolonged O₃ exposure was greater than that of the percent set, indicating that the fertilization was performed under partial suppression but that the grain growth (including single-ear weight) was greatly suppressed because of the decreased photoassimilate supply attributable to the elevated O₃. The fact that the effect of O₃ on total plant weight was greater than that of the percent maturity (Table 1) supports our inference that the inhibitory effect of O₃ on photosynthesis-related processes (measured as NAR) is greater than that of fertilization. Furthermore, we had a new recognition that the period of flowering to the early maturation stage of paddy rice was crucial in determining the rice yield (Murata and Matsushima, 1975; Hoshikawa, 1989) because only 2 weeks' exposure of 0.1 cm³ m⁻³ O₃ decreased the ear weight, which was equivalent to the grain yield, as high as 17.2% (Table 1).

Table 2 shows that the pollen germination, which decreased with elevated O₃ concentration, is almost entirely offset under elevated CO₂ (C⁸⁰⁰). Therefore, we infer a protective effect of elevated CO₂ on fertilization processes, although we did not examine the pollen-tube growth and later processes. In tobacco plants, a 5.5 h exposure of 0.1 cm³ m⁻³ O₃ engendered a 40–50% reduction of pollen germination and a 50% reduction of pollen-tube growth (Feder, 1968). The implications of the protective effect of elevated CO₂ against O₃ on pollen germination remain unknown.

Substantial concentration-dependent visible injury of leaves caused by O₃ exposure has been reported at the vegetative stage of rice (Imai and Kobori, 2008). In contrast, only slight and non-visible injury of flag-leaves and ears (panicles), respectively, by O₃ exposure were observed at the flowering to early maturation stage in the current experiment (data not shown). These were ascribed to thicker mesophyll (more antioxidants) and higher erect-angle (less light interception) of the flag-leaves (Kobayakawa and Imai, 2010) and sporadic arrangement of stomata on the hull

(Hoshikawa, 1989). However, irrespective of visible symptoms, rice production was affected by the interaction between O₃ and CO₂. Previous reports related to the dry matter production and yield in other field crops such as wheat (Rudorff et al., 1996), soybean (Booker et al., 2005), and peanut (Burkey et al., 2007) support our present results, although such interaction has not been observed in potatoes (Lawson et al., 2001).

In conclusion, under the environmental conditions that can be observed in the field, the detrimental effect of O₃ would be greater for photosynthesis-related processes than during fertilization. Although short-period O₃ exposure (1–2 weeks) injures the production processes of rice plants, elevated CO₂ ameliorates them if it occurs concomitantly. Therefore, under future higher CO₂ circumstances, detrimental effects of O₃ would be curtailed unless it is increased quickly.

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