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Effects of the interaction between ozone and carbon dioxide on gas exchange, photosystem II and antioxidants in rice leaves

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Abstract

To understand the interactive effects of O_3 and CO_2 on rice leaves; gas exchange, chlorophyll (Chl) fluorescence, ascorbic acid and glutathione were examined under acute (5 h), combined exposures of O_3 (0, 0.1, or 0.3 cm^3 m^{-3}, expressed as O^0, $O^{0.1}$, or $O^{0.3}$, respectively), and CO_2 (400 or 800 cm^3 m^{-3}, expressed as C^{400} or C^{800}, respectively) in natural-light gas-exposure chambers. The net photosynthetic rate (P_N), maximum (F_v/F_m) and operating (F_q'/F_m') quantum efficiencies of photosystem II (PSII) in young (8^{th}) leaves decreased during O_3 exposure. However, these were ameliorated by C^{800} and fully recovered within 3 d in clean air ($O^0 + C^{400}$) except for the $O^{0.3} + C^{400}$ plants. The maximum PSII efficiency at 1,500 μmol m^{-2} s^{-1} PPFD (F_v'/F_m') for the $O^{0.3} + C^{400}$ plants decreased for all measurement times, likely because leaves with severely inhibited P_N also had a severely damaged PSII. The P_N of the flag (16^{th}) leaves at heading decreased under O_3 exposure, but the decline was smaller and the recovery was faster than that of the 8^{th} leaves. The F_q'/F_m' of the flag leaves in the $O^{0.3} + C^{400}$ and $O^{0.3} + C^{800}$ plants decreased just after gas exposure, but the F_v/F_m was not affected. These effects indicate that elevated CO_2 interactively ameliorated the inhibition of photosynthesis induced by O_3 exposure. However, changes in antioxidant levels did not explain the above interaction.

Additional key words: ascorbic acid; chlorophyll fluorescence; elevated CO_2; glutathione; net photosynthesis; *Oryza sativa*; ozone; quantum efficiency; respiration; stomatal conductance.

Introduction

Photochemical oxidants are generated as a result of complex photochemical reactions involving ultraviolet rays and the nitrogen oxides and hydrocarbons emitted from cars and factories during warm, sunny and windless weather. Up to 90% of photochemical oxidants are the secondary pollutant ozone (O_3) (Cabrera *et al*. 1988). The Japanese environmental quality standard for photochemical oxidants is set at < 0.06 cm^3 m^{-3}, and when it exceeds 0.12 and 0.24 cm^3 m^{-3}, local public bodies must, under the regulations, dispatch an oxidant warning and an alarm, respectively (Nakanishi *et al*. 2009). In the Kanto region of Japan, where rice is cultivated as a staple summer crop, 10–20 warnings are received every growing season, and hourly peak values are sometimes close to 0.2 cm^3 m^{-3} (Environ. Improve. Div. Tokyo Metro. Bureau Environ. 2005). Kobayashi (1999) estimated that O_3 decreased rice production by up to 10% in the Kanto region of Japan in 1981–1985. Thus, we need to understand the effects of acute (single or repeated) O_3 exposure on rice plant physiology, as a basis for reductions in dry-matter production and yield formation of this crop in the field where acute photochemical oxidants appear, along with chronic exposure.

Ozone is known to induce visible injury (Ishioh *et al*. 2005, Imai and Kobori 2008), destroy cellular ultrastructure (Toyama *et al*. 1989), inhibit net photosynthesis (P_N; Imai and Kobori 2008, Yamaguchi *et al*. 2008),

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Abbreviations: AA – ascorbic acid; C_i – intercellular CO_2 concentration; Chl – chlorophyll; DHA – dehydroascorbic acid; g_s – stomatal conductance; F' – steady fluorescence; F_0 – minimum flouorescence of dark-adapted state; F_0' – minimum fluorescence in the steady state; F_m – maximum fluorescence of dark-adapted state; F_m' – maximum fluorescence in the steady state; F_q' – difference between F_m' and F'; F_v – variable fluorescence; F_v' – variable fluorescence in the steady state; GSH – glutathione; GSSG – oxidized glutathione; P_N – net photosynthetic rate; PPFD – photosynthetic photon flux density; PSII – photosystem II; q_N – nonphotochemical quenching coefficient; q_P – photochemical quenching coefficient; R_D – respiration rate; RDS – redox state; ROS – reactive oxygen species.
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decrease the activity and content of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) (Ishioh and Imai 2005), and decrease the contents of Chl and carotenoids (Inada *et al*. 2008, Rai and Agrawal 2008) in leaves. It also changes photosynthate partitioning (Nouchi *et al*. 1995), and, ultimately, decreases the yield (Reid and Fiscus 2008, Yamaguchi *et al.* 2008, Rai *et al*. 2010) of rice. To maintain rice yield, we need to understand the role of O_3 in both carbon fixation and photochemical reactions. Fortunately, the use of Chl fluorescence measurements has become an established practice to diagnose changes in photosystem II (PSII) due to environmental stresses such as excessive light and water stress, and a number of studies on the effects of environmental stress on PSII have been conducted (Papageorgiou and Govindjee 2004). So far, however, there are few studies on the effect of O_3 on photochemical reactions in rice leaves, *i.e.*, PSII was adversely affected by chronic exposure (Rai and Agrawal 2008, Pang *et al*. 2009).

Concurrent with O_3 air pollution, the global atmospheric CO_2 concentration has increased from the pre-industrial value of about 280 $cm^3\ m^{-3}$ to 385.2 $cm^3\ m^{-3}$ in 2008, and this trend will continue as long as current trends in human activities persist (WMO WDCGG 2010). Because elevated CO_2 concentrations decrease stomatal conductance (g_s), they ameliorate O_3-induced injury by suppressing O_3 intake through the stomata (Booker and Fiscus 2005). Donnelly *et al*. (2000) showed that chronic O_3 (ambient plus 50 $cm^3\ m^{-3}$)-induced adverse effects on the photosynthetic activity and Chl content were ameliorated by elevated CO_2 (680 or 510 $cm^3\ m^{-3}$) in a spring wheat. By exposing rice leaves to combinations of O_3 and CO_2, Ishioh and Imai (2005) found that elevated CO_2 ameliorated the decline in P_N, Rubisco and Chl caused by O_3, and Imai and Kobori (2008) further examined the P_N, g_s, and P_N/C_i-curve and concluded that the amelioration of the O_3-induced decline in P_N was largely due to the decreased g_s under elevated CO_2. However, this was not the sole reason, as other components of photosynthetic process, such as the photosystem, were affected by O_3.

Therefore, to determine interactive effects between O_3 and CO_2 on key photosynthetic parameters, we examined the effects of O_3 and CO_2 on the P_N, g_s and PSII at the vegetative and reproductive stages of rice development. Furthermore, because there are reports for rice (Nouchi 1993) and *Arabidopsis thaliana* (Overmyer *et al*. 2000, Sasaki-Sekimoto *et al*. 2005) that antioxidants scavenge oxidative stressors, including O_3, we measured the contents of ascorbic acid and glutathione as major antioxidants in rice.

Materials and methods

Plant materials and gas exposure: Japonica rice (*Oryza sativa* L. cv. Koshihikari) seeds were sown in plastic pots (diameter × height = 0.16 m × 0.19 m) containing 2.5 kg of dry soil and 12.5 g of chemical fertilizer (N, P_2O_5, K_2O = 8, 8, 8, %) and cultivated in a natural-light glasshouse with ventilation from early May to late August when the gas exposures began. Therefore, all plants received the same environmental conditions. Gas exposures were conducted at two growth stages with different set of plants. Just after the full expansion of the 7th leaf (Haun index = 7.0, Haun 1973) or the 16th leaf (flag leaf, Haun index = 16.0), the plants were transferred into 4 natural-light gas-exposure chambers (width × depth × height = 2 m × 2 m × 1.9 m: *S-2003A, Koito Industries*, Yokohama, Japan) and kept at 28/23°C (12-h day/12-h night), 60% RH and 400 $cm^3\ m^{-3}$ CO_2 ($O^0 + C^{400}$). Just after the full expansion of the 8th leaf or at heading, 5-h gas exposure treatments (8:00–13:00; local time) were applied under several combinations of O_3 and CO_2: $O^0 + C^{400}$ (control = clean air), $O^0 + C^{800}$, $O^{0.1}$ or $O^{0.3} + C^{400}$, and $O^{0.1}$ or $O^{0.3} + C^{800}$. O_3 was supplied by a high-voltage ozone generator using dry air (*MO-5A, Ozone System*, Tokyo, Japan), and CO_2 was supplied from cylinders containing liquid CO_2. These gases were injected into air that had been charcoal filtered. The concentrations of O_3 and CO_2 were measured and computer-controlled by an ultraviolet absorption-type O_3 analyzer (*EG-2001F, Ebara Jitsugyo*, Tokyo, Japan) and an infrared CO_2 analyzer (*ZRH, Fuji Electric Systems*, Tokyo, Japan), respectively. After the 5-h gas exposure, all the plants were kept in the same chamber for 3 d under 28/23°C 60% RH and 400 $cm^3\ m^{-3}$ CO_2 ($O^0 + C^{400}$).

Gas-exchange measurements: To compare the responses of leaves at different growth stages, *in situ* gas-exchange measurements were made on the 8th leaves (vegetative state of plant development) or the 16th leaves (heading) on the main stem. Measurements were made just before gas exposure (BE: 1–0 h before), during gas exposure (DE: 4–5 h from the start of gas exposure), just after gas exposure (AE-0: 0.1–1.1 h after) and 1 and 3 d after gas exposures (AE-1, AE-3). Two portable photosynthesis systems (*LI-6400XT, LI-COR*, Lincoln, NE, USA) were used, with 5 replicate plants in each chamber. Environmental conditions within the *LI-COR* cuvette during measurements were set at 28°C leaf temperature, 1.5 kPa VPD and 1,500 μmol $m^{-2}\ s^{-1}$ PPFD (mixed light from red and blue LEDs). Gas-exchange measurements of the 8th leaves also were conducted at 23°C leaf temperature and 1.5 kPa VPD in darkness (1–2 h after the beginning of dark period) to obtain respiration rate (R_D).

Chl fluorescence measurements were conducted immediately after the gas-exchange measurements using a fluorometer (*LI-6400-40, LI-COR*, Lincoln, NE, USA) attached to *LI-6400* system, with the same replicate plants

that were used for photosynthesis. Chl fluorescence measurements were made by applying 0.1, 7,000 and 1,500 μmol m^{-2} s^{-1} of measuring light, saturating pulse (flash) and actinic light, respectively. Before the fluorescence measurements, the leaves were kept in the dark for 10 min (to avoid the recovery from O_3 injury, Sonoike 2009) and then the minimum (F_0) and maximum (F_m) fluorescence were determined by irradiating the measuring light and saturating pulses. Thereafter, the steady fluorescence (F'), minimum fluorescence (F_0') and maximum fluorescence (F_m') in the steady state were determined under actinic light irradiation. The minimum fluorescence in the steady state (F_0') was determined during a brief interruption of actinic light irradiation in the presence of far-red light. The maximum quantum efficiency (F_v/F_m), operating efficiency (F_q'/F_m'), maximum efficiency at the given PPFD (F_v'/F_m') and the photochemical (q_p) and nonphotochemical (q_N) quenching coefficients of PSII were obtained using the following equations (Baker 2008, Sonoike 2009):

$$F_v/F_m = (F_m - F_0)/F_m$$
$$F_q'/F_m' = (F_m' - F')/F_m'$$
$$F_v'/F_m' = (F_m' - F_0')/F_m'$$
$$q_p = (F_m' - F')/(F_m' - F_0')$$
$$q_N = 1 - (F_m' - F_0')/(F_m - F_0)$$

Antioxidant measurements: Separate sets of plants were exposed to combined O_3 and CO_2 and used for measurement of antioxidant concentrations. The 7th and 8th leaves at vegetative stage or the 15th and 16th (flag) leaves at heading were collected 0, 1, and 3 d after gas exposures with 4 replicate plants in each chamber. Immediately after the measurements of fresh mass (FM), leaves were frozen in liquid N_2 and ground with a pestle and mortar by adding metaphosphoric acid to obtain leaf extracts. Because of the limited numbers of replicate plants, the amount of ascorbic acid (reduced form: AA; oxidized form: DHA) for the 7th- or 15th-leaf extract was determined by the hydrazine method (Roe *et al*. 1944) on a spectrophotometer (*Ubest-30, JASCO Co*., Tokyo, Japan), and the amount of glutathione (reduced form: GSH; oxidized form: GSSG) for the 8th- or 16th-leaf extract by the enzymatic recycling method (Mano *et al*. 2009) on a microplate reader (*MTP-450 (Lab), Corona Electric Co*., Ibaraki, Japan). The standard reagents used were L(+)-ascorbic acid (*Kanto Chemical Co., Inc*., Japan), glutathione (oxidized form, *Wako Pure Chemical Ind., Ltd*., Japan), and an enzyme kit consists of glutathione reductase, 5-5'-dithiobis(2-nitrobenzoic acid) and NADPH (*NWLSS™ Glutathione Assay, Northwest Life Science Specialities, LLC*, USA). The redox states (RDS) of ascorbic acid and glutathione were calculated as follows: AA/(AA + DHA) and GSH/(GSH + GSSG), respectively.

Statistical analysis: All data were subjected to a two-way analysis of variance (*ANOVA*) with a software package (*Excel Statistics 2004 for Windows, Social Survey Research Information Co*., Tokyo, Japan). Appropriate standard errors of the means (SE) were calculated for presentation with line diagram. The significance of the treatment effect was determined by *F*-test.

Results

Effects of O_3 and CO_2 on photosynthesis in the 8th leaves: The results for gas exchange and PSII are shown in Fig. 1. During gas exposure (DE), the P_N of the $O^{0.1} + C^{400}$, $O^{0.3} + C^{400}$ and $O^{0.3} + C^{800}$ plants decreased to 72, 45, and 91%, respectively, of the respective initial values. Just after gas exposure (AE-0), these further decreased to 49, 38, and 46%, respectively, of the initial values. However, in clean air ($O^0 + C^{400}$), the plants began to recover from O_3-induced decline, and 1 d after gas exposure (AE-1), the P_N values were 78, 60, and 83%, respectively, of the initial values. The P_N recovered further by 3 d after gas exposure (AE-3), when there was no significant difference between the $O^{0.1} + C^{400}$ and $O^0 + C^{400}$ plants. However, the inhibition of the P_N in the $O^{0.3} + C^{400}$ plants remained low from AE-1 to AE-3, and the P_N of the $O^{0.3} + C^{400}$ plants was 60% of the initial value at AE-3 (Fig. 1*A*). In the $O^0 + C^{800}$, $O^{0.1} + C^{400}$, $O^{0.1} + C^{800}$, $O^{0.3} + C^{400}$ and $O^{0.3} + C^{800}$ plants, the stomatal conductance (g_s) substantially decreased compared to that of the $O^0 + C^{400}$ plants. With the same O_3 concentration, the g_s of the C^{400} and C^{800} plants were similar at DE. At AE-0, the g_s stayed at the same level as those at DE, except that of the $O^{0.1} + C^{400}$ plants, which decreased further. The g_s recovered almost completely between AE-1 and AE-3, when there was no significant difference between any of the treatments, except the $O^{0.3} + C^{400}$ plants (Fig. 1*B*). As shown in Table 1, there were significant, direct effects of O_3 and CO_2 and also, interaction between these two factors on the P_N and g_s of the 8th leaves at DE. At AE-0, the effect of CO_2 was diminished because plants were kept in an air of C^{400}. However, a significant effect remained for CO_2 on the P_N and g_s remained at AE-1 and AE-3 because the leaves received $O^{0.1}$ treatment recovered well while those received $O^{0.3}$ treatment were severely damaged.

The photosystem II (PSII) measurements revealed responses similar to the P_N responses (Fig. 1*C–G*). The maximum quantum efficiency (F_v/F_m) of the $O^{0.1} + C^{400}$, $O^{0.3} + C^{400}$ and $O^{0.3} + C^{800}$ plants decreased to 92, 86, and 95%, respectively, of the respective initial values at DE. At AE-0, they decreased further to 91, 81, and 89%, respectively, of the initial values. In the $O^{0.1} + C^{400}$ and $O^{0.3} + C^{800}$ plants, the F_v/F_m recovered at AE-1, but that of the $O^{0.3} + C^{400}$ plants was 92 and 93% of the initial value on AE-1 and AE-3, respectively (Fig. 1*C*). The operating quantum efficiency (F_q'/F_m') decreased at DE and AE-0,

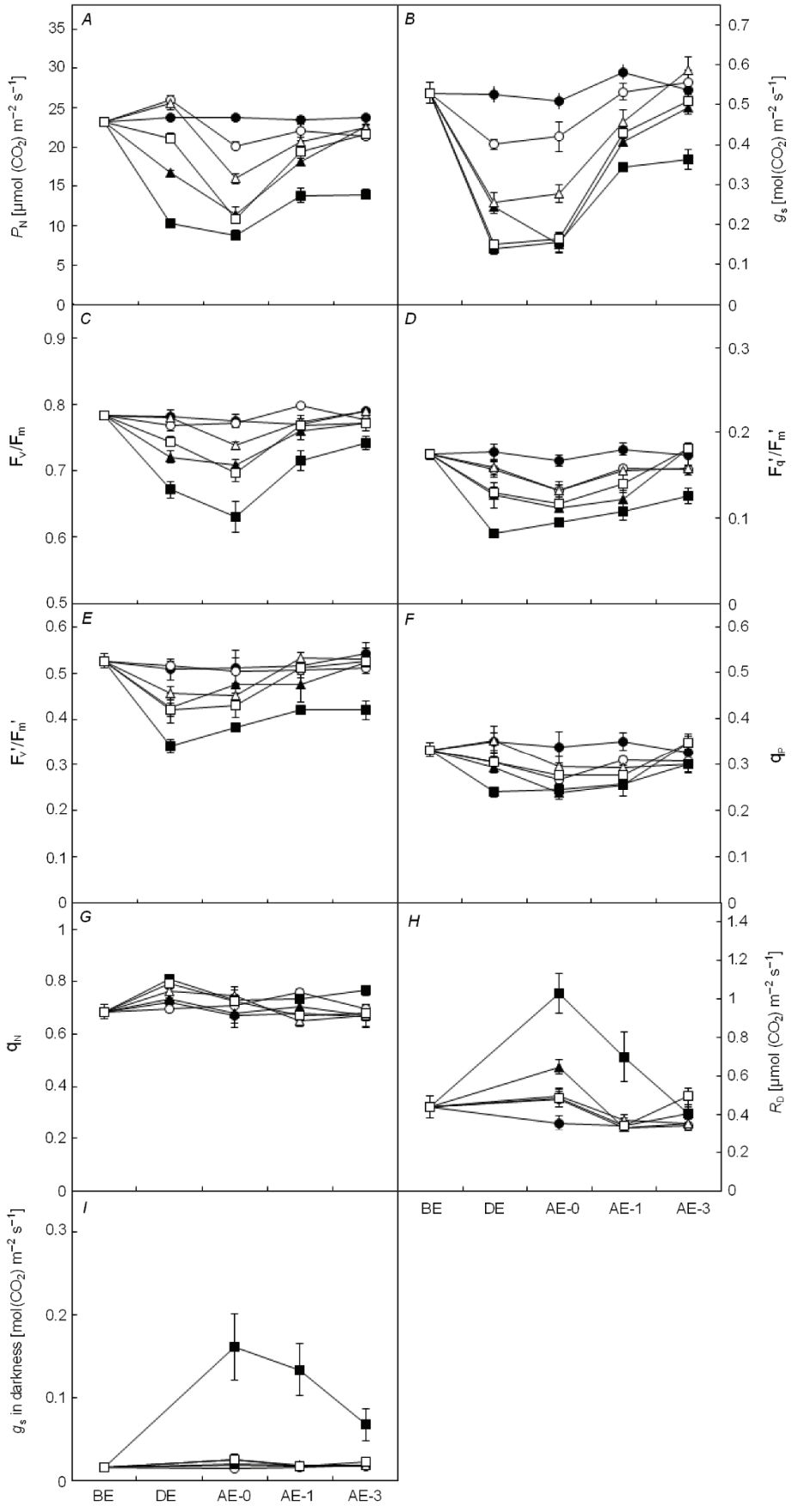

Fig. 1. Effects of O_3 and CO_2 on the net photosynthetic rate (P_N), stomatal conductance (g_s), and the maximum quantum efficiency (F_v/F_m), operating efficiency (F_q'/F_m'), maximum efficiency at 1,500 μmol m^{-2} s^{-1} PPFD (F_v'/F_m') , photochemical (q_p) and nonphotochemical (q_N) quenching coefficients of PSII, and the respiration rate (R_D) and g_s in darkness in the 8th leaves. *Vertical bars* indicate standard errors of the means (n = 5). BE, DE, AE-0, AE-1 and AE-3 indicate before, during, just after, and 1 d and 3 d after gas exposure, respectively. ●, $O^0 + C^{400}$; ○, $O^0 + C^{800}$; ▲, $O^{0.1} + C^{400}$; Δ, $O^{0.1} + C^{800}$; ■, $O^{0.3} + C^{400}$; □, $O^{0.3} + C^{800}$.

Table 1. Statistical analyses of the effects of O_3 and/or CO_2 on the gas exchange and chlorophyll fluorescence parameters of rice leaves shown in Fig. 1 and 2. DE, AE-0, AE-1 and AE-3 indicate during, just after, 1 d, and 3 d after the gas exposure, resp. *p<0.05, **p<0.01, ***p<0.001, n.s. – not significant by two-way *ANOVA*. F_q'/F_m' – operating quantum efficiency of PSII; F_v/F_m – maximum quantum efficiency of PSII; F_v'/F_m' – maximum efficiency at 1,500 μmol m^{-2} s^{-1} PPFD; g_s – stomatal conductance; P_N – net photosynthetic rate; q_N – nonphotochemical quenching coefficient; q_P – photochemical quenching coefficient; R_D – respiration rate.

Leaf position	Time	Factor	P_N	g_s	F_v/F_m	F_q'/F_m'	F_v'/F_m'	q_p	q_N	R_D	g_s in darkness
8th	DE	O_3	***	***	***	***	***	*	*		
		CO_2	***	*	***	*	*	n.s.	n.s.		
		$O_3 \times CO_2$	***	**	***	*	n.s.	*	n.s.		
	AE-0	O_3	***	***	***	***	**	n.s.	n.s.	***	**
		CO_2	n.s.	n.s.	*	n.s.	n.s.	n.s.	n.s.	**	**
		$O_3 \times CO_2$	***	**	n.s.	**	n.s.	*	n.s.	***	**
	AE-1	O_3	***	***	**	***	n.s.	**	n.s.	*	**
		CO_2	**	n.s.	**	n.s.	*	n.s.	n.s.	n.s.	**
		$O_3 \times CO_2$	***	**	n.s.	**	n.s.	n.s.	*	*	**
	AE-3	O_3	***	**	**	n.s.	*	n.s.	n.s.	n.s.	*
		CO_2	***	**	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
		$O_3 \times CO_2$	***	n.s.	*	***	**	n.s.	n.s.	n.s.	*
16th	DE	O_3	***	***	n.s.	*	*	n.s.	**		
		CO_2	***	***	n.s.	*	**	*	*		
		$O_3 \times CO_2$	n.s.	n.s.	n.s.	n.s.	n.s.	**	n.s.		
	AE-0	O_3	***	***	n.s.	*	*	n.s.	n.s.		
		CO_2	***	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.		
		$O_3 \times CO_2$	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.		
	AE-1	O_3	***	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.		
		CO_2	***	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.		
		$O_3 \times CO_2$	**	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.		
	AE-3	O_3	*	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.		
		CO_2	n.s.	n.s.	*	n.s.	n.s.	n.s.	n.s.		
		$O_3 \times CO_2$	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.		

as did F_v/F_m. This damage was slightly reversed on AE-1, but the values for the $O^{0.1} + C^{400}$, $O^{0.3} + C^{400}$ and $O^{0.3} + C^{800}$ plants were still decreased to 70, 62, and 81%, of the respective initial values. On AE-3, the inhibition disappeared completely in the $O^{0.1} + C^{400}$ and $O^{0.3} + C^{800}$ plants, but the activity of the $O^{0.3} + C^{400}$ plants was still 28% lower than that of the control plants ($O^0 + C^{400}$) (Fig. 1*D*). The F_v'/F_m' of the $O^{0.1} + C^{400}$, $O^{0.3} + C^{400}$ and $O^{0.3} + C^{800}$ plants at DE decreased to 81, 65, and 80%, respectively, of the respective initial values at DE, but there was no significant difference between any of the treatments, except for the $O^{0.3} + C^{400}$ plants, from AE-0 to AE-3. On the other hand, the maximum quantum efficiency at 1,500 μmol m^{-2} s^{-1} (F_v'/F_m') of the $O^{0.3} + C^{400}$ plants was 73, 80 and 80% of the initial values at AE-0, AE-1 and AE-3, respectively (Fig. 1*E*). The photochemical quenching coefficient (q_p) decreased in the $O^{0.3} + C^{400}$ plants at DE and in the $O^{0.3} + C^{400}$ and $O^{0.1} + C^{400}$ plants at AE-0. The q_p of the $O^{0.1} + C^{400}$, $O^{0.1} + C^{800}$, $O^{0.3} + C^{400}$ and $O^{0.3} + C^{800}$ plants decreased to 77, 88, 77, and 83%, respectively, of the respective initial values at AE-1, but there was no significant difference between any of the treatments on AE-3 (Fig. 1*F*). The nonphotochemical quenching coefficient (q_N) of the $O^{0.3} + C^{400}$ plants was slightly high compared to that of the control at AE-3 (Fig. 1*G*). As a whole, the effects of O_3 and CO_2 on parameters of PSII were similar to the trends observed for P_N and g_s, but O_3 and CO_2 had less effect on q_p and q_N (Table 1).

The respiration rate (R_D) of the $O^{0.1} + C^{400}$ and $O^{0.3} + C^{400}$ plants at AE-0 increased to 147 and 234%, respectively, of the respective initial values (Fig. 1*H*). The g_s in darkness of the $O^{0.3} + C^{400}$ plants at AE-0 increased tenfold compared to that of the control

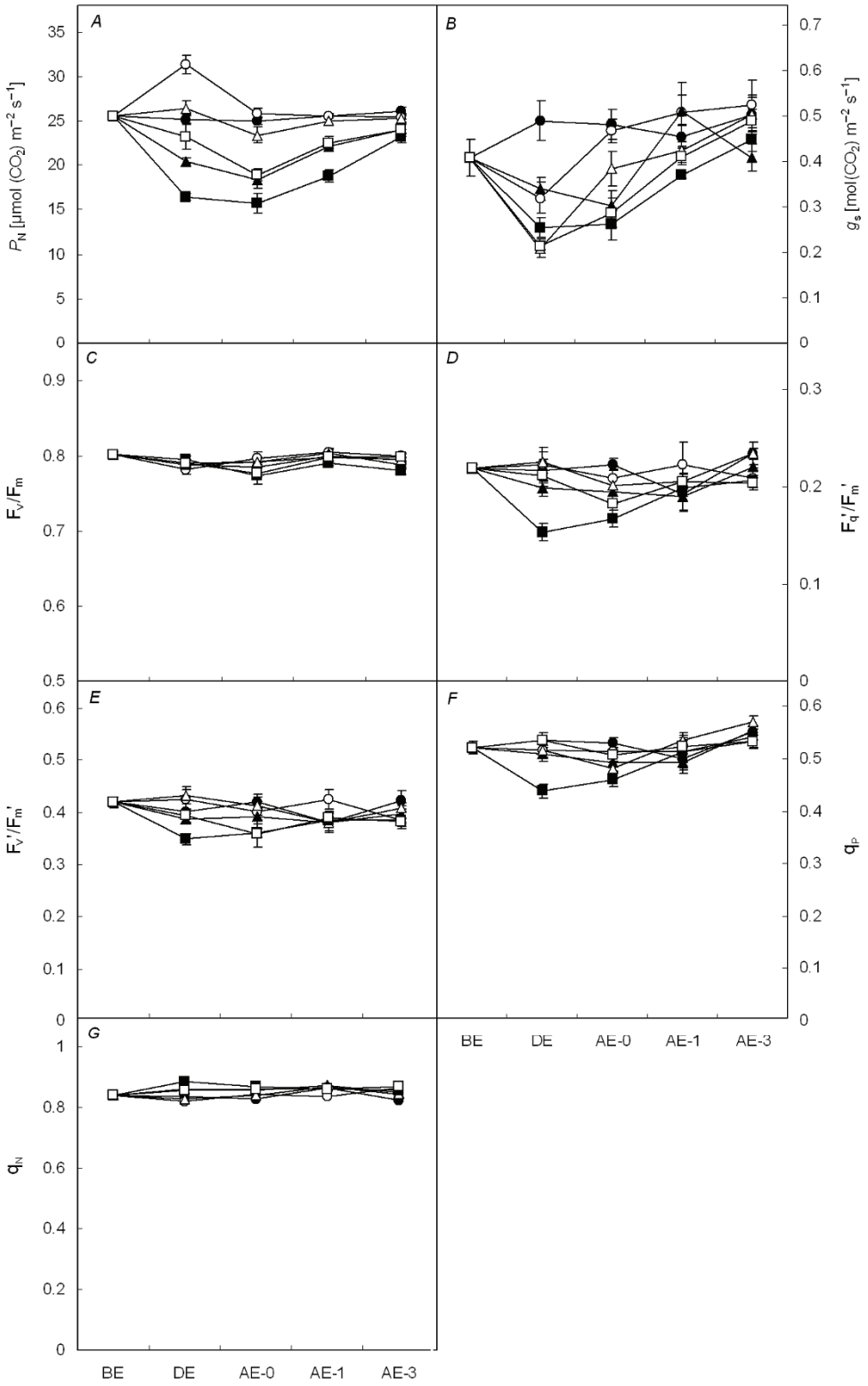

Fig. 2. Effects of O_3 and CO_2 on the net photosynthetic rate (P_N), stomatal conductance (g_s), and maximum quantum efficiency (F_v/F_m), operating efficiency (F_q'/F_m'), maximum efficiency at 1,500 μmol m^{-2} s^{-1} PPFD (F_v'/F_m'), and photochemical (q_p) and nonphotochemical (q_N) quenching coefficients of PSII in the flag leaves. V*ertical bars* indicate standard errors of the means (n = 5). BE, DE, AE-0, AE-1 and AE-3 indicate before, during, just after, and 1 d and 3 d after gas exposure, respectively. ●, $O^0 + C^{400}$; ○, $O^0 + C^{800}$; ▲, $O^{0.1} + C^{400}$; Δ, $O^{0.1} + C^{800}$; ■, $O^{0.3} + C^{400}$; □, $O^{0.3} + C^{800}$.

($O^0 + C^{400}$), and this tendency was maintained until AE-3 (Fig. 1*I*). Statistical analyses indicated that the effects of O_3 and CO_2 on R_D and g_s in darkness at AE-0 and AE-1 were similar to those of P_N and g_s in light but the R_D recovered almost completely at AE-3 (Table 1).

Effects of O_3 and CO_2 on photosynthesis in the 16th (flag) leaves: As shown in Fig. 2, the P_N of the $O^{0.1} + C^{400}$, $O^{0.3} + C^{400}$ and $O^{0.3} + C^{800}$ plants decreased at DE and AE-0, and recovered at AE-1 and AE-3. However, the P_N of the $O^{0.3} + C^{400}$ plants at AE-3 decreased to only 91% of the initial value (Fig. 2*A*). The g_s of all treatments decreased at DE, but this decrease was completely reversed at AE-1 (Fig. 2*B*). The F_v/F_m did not differ significantly among treatments at any measurement time (Fig. 2*C*). The F_q'/F_m' of the $O^{0.3} + C^{400}$ and $O^{0.3} + C^{800}$ plants decreased at AE-0, but there was no significant difference between the other treatments (Fig. 2*D*). The F_v'/F_m' of the $O^{0.3} + C^{400}$ plants decreased to 86% of the initial value, and this occurred in the $O^{0.3} + C^{800}$ plants at AE-0 as well. However, there was no significant difference between any of the plots from AE-1 to AE-3 (Fig. 2*E*). The q_p in the $O^{0.3} + C^{400}$ plants decreased to 84 and 88% of the initial value at DE and AE-0, respectively. However, no significant difference was observed between the control and the $O^{0.3} + C^{400}$ plants at AE-1 (Fig. 2*F*). Table 1 showed that the 16th leaves had similar trend with the 8th leaves in the P_N but the g_s was less affected and the PSII was almost unaffected by O_3 and CO_2 as seen in Fig. 2.

Effects of O_3 and CO_2 on the antioxidants: Just before the gas exposures, the total ascorbic acid (Total), reduced ascorbic acid (AA) and dehydroascorbic acid (DHA) contents [mmol kg^{-1}(FM)] and the redox state (RDS) of ascorbic acid (RDS = AA/Total) in the 7th leaves were 5.73, 2.90, 2.83 and 0.50, respectively, and those in the 15th leaves were 8.46, 0.78, 7.68, and 0.10, respectively. Table 2 shows that the total ascorbic acid content in the 7th leaves was not affected at AE-0, but the contents of the $O^{0.1} + C^{400}$, $O^{0.3} + C^{400}$, and $O^{0.3} + C^{800}$ plants decreased significantly at AE-1 compared to those before gas exposures (BE). The RDS of ascorbic acid in the $O^{0.1} + C^{400}$, $O^{0.3} + C^{400}$, and $O^{0.3} + C^{800}$ plants at AE-1 decreased to 55, 51, and 55%, respectively, of the BE. The $O^{0.1} + C^{400}$ and $O^{0.3} + C^{800}$ plants recovered from this decrease by AE-3, but the $O^{0.3} + C^{400}$ plants did not. The total ascorbic acid content in the 15th leaves was not affected by gas exposures. The RDS of ascorbic acid in the $O^{0.3} + C^{400}$ plants decreased at AE-1, but recovered at AE-3. There was no interactive effect between O_3 and CO_2 in both 7th and 15th leaves for any parameter, except RDS of the 7th leaves at AE-3.

The total glutathione (Total), reduced glutathione (GSH) and oxidized glutathione (GSSG) contents [μmol kg^{-1}(FM)] and the RDS of glutathione (RDS = GSH/Total) in the 8th leaves just before the gas exposures were 66.0, 48.5, 17.5, and 0.72, respectively, and those in the 16th (flag) leaves were 139.1, 117.3, 21.8, and 0.84, respectively. Table 3 shows that the total glutathione content in the 8th leaves in the $O^{0.3} + C^{400}$ at AE-3 increased to 165% of BE. The decrease in RDS and concomitant increase in GSSG occurred with O_3 exposures in the $O^{0.1} + C^{400}$ and $O^{0.3} + C^{400}$ plants at AE-0 and AE-1. However, these were recovered by AE-3. The total glutathione content in the 16th leaves of the $O^{0.3} + C^{400}$ plants at AE-1 increased to 144% of the BE, and that of the $O^{0.3} + C^{800}$ plants at AE-3 was also higher than the BE. As seen in ascorbic acid, there was no interactive effect between O_3 and CO_2 in both 8th and 16th leaves, except total glutathione and GSH of the 16th leaves at AE-1 (Table 3).

Discussion

Consistent with previous observations in rice leaves (Imai and Kobori 2008), P_N was inhibited by $O^{0.1}$ and $O^{0.3}$ but ameliorated by C^{800} in the 8th leaves (Fig. 1*A*), and one of the causal factors was stomatal closure due to elevated CO_2, which limited the O_3 intake (McKee *et al*. 1997, Mullholand *et al*. 1997, Booker and Fiscus 2005). At the same O_3 concentration, g_s was similar irrespective of CO_2 concentration. However, at the same CO_2 concentration, g_s was lower at higher O_3 concentration (Fig. 1*B*). Therefore, the limitation of O_3 uptake through the stomata cannot fully explain the O_3-induced decrease in P_N and its amelioration by elevated CO_2, as shown by the larger decline of P_N under $O^{0.3}$ than under $O^{0.1}$ at the same C_i (Imai and Kobori 2008). Mesophyll dysfunction, including the inactivation of Rubisco (Ishioh and Imai 2005) and/or the photosystem (Rai and Agrawal 2008), could be other reasons. The O_3-induced decrease in P_N started to attenuate at AE-1, and almost disappeared at AE-3, except in the $O^{0.3} + C^{400}$ plants (Fig. 1*A*). A similar situation was found in the case of g_s, and, therefore, we assumed that one reason why P_N did not recover for a long time after gas exposure, as in the $O^{0.3} + C^{400}$ plants, was stomatal dysfunction (Fig. 1*B*), in which O_3 adversely affects the osmotic adjustment of the guard cells toward the inhibition of the K^+ channel (Torsethaugen *et al*. 1999). In accordance with the observation by Imai and Kobori (2008), the abnormally high g_s in the $O^{0.3} + C^{400}$ plants in darkness (Fig. 1*H*), which was equivalent to that under illumination (Fig. 1*B*) supports the above-mentioned assumption.

Though the effects of O_3 on parameters of PSII were not as large compared to those of gas exchange, the F_q'/F_m' in the 8th leaves of the $O^{0.1} + C^{400}$ and $O^{0.3} + C^{800}$ plants decreased from DE to AE-1, but that of the $O^{0.3} + C^{400}$ plants decreased until AE-3 (Fig. 1*D*). Similarly, the F_v'/F_m' in the $O^{0.1} + C^{400}$ and $O^{0.3} + C^{800}$ plants decreased

Table 2. Effects of O_3 and CO_2 on the ascorbic acid content [mmol kg^{-1}(FM)] and its redox state (RDS) in the 7th and 15th leaves, $n = 4$. *p<0.05, **p<0.01, ***p<0.001, n.s. – not significant, by two-way *ANOVA*. AA – ascorbic acid; DHA – dehydroascorbic acid; Total = AA + DHA.

Leaf position	O_3	CO_2	Just after gas exposure (AE-0)				1 d after gas exposure (AE-1)				3 d after gas exposure (AE-3)			
	[cm^3 m^{-3}]		Total	AA	DHA	RDS	Total	AA	DHA	RDS	Total	AA	DHA	RDS
7th	O^0	C^{400}	5.20	2.86	2.34	0.57	6.69	3.36	3.33	0.50	5.58	3.13	2.44	0.56
		C^{800}	6.71	3.60	3.11	0.54	6.48	3.22	3.25	0.49	7.26	4.06	3.20	0.56
	$O^{0.1}$	C^{400}	5.28	2.96	2.32	0.60	4.08	1.17	2.91	0.28	2.60	1.45	1.14	0.56
		C^{800}	5.86	3.14	2.72	0.54	5.69	2.67	3.02	0.50	5.54	2.77	2.77	0.50
	$O^{0.3}$	C^{400}	4.25	2.26	1.98	0.56	2.98	0.75	2.09	0.26	2.81	0.73	2.08	0.25
		C^{800}	6.03	3.26	2.77	0.58	2.87	0.90	1.97	0.28	2.72	1.22	1.50	0.44
15th	O^0	C^{400}	8.40	0.79	7.61	0.11	7.23	0.95	6.27	0.13	5.67	0.74	4.93	0.12
		C^{800}	7.21	0.80	6.41	0.11	6.64	0.90	5.74	0.13	5.80	0.50	5.30	0.11
	$O^{0.1}$	C^{400}	7.72	0.69	7.03	0.09	6.68	0.40	6.28	0.06	6.49	0.72	5.77	0.11
		C^{800}	7.19	0.86	7.04	0.11	6.50	0.52	5.98	0.10	6.47	0.81	5.66	0.13
	$O^{0.3}$	C^{400}	6.40	0.72	5.69	0.13	8.70	0.43	8.27	0.05	5.09	1.39	3.71	0.27
		C^{800}	6.86	0.68	6.17	0.10	5.96	0.57	5.40	0.09	6.30	0.58	5.72	0.11
ANOVA		Factor												
	7th	O_3	n.s.	n.s.	n.s.	n.s.	***	***	*	*	**	***	n.s.	***
		CO_2	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	*	*	n.s.	n.s.
		$O_3 \times CO_2$	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	**
	15th	O_3	n.s.	n.s.	n.s.	n.s.	n.s.	*	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
		CO_2	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
		$O_3 \times CO_2$	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

Table 3. Effects of O_3 and CO_2 on the glutathione content [μmol kg^{-1}(FM)] and its redox state (RDS) in the 8^{th} and flag (16^{th}) leaves, n = 4. *p<0.05, **p<0.01, ***p<0.001, n.s. – not significant, by two-way *ANOVA*. GSH – glutathione; GSSG – oxidized glutathione; Total = GSH + GSSG.

Leaf position	O_3	CO_2	Just after gas exposure (AE-0)				1 d after gas exposure (AE-1)				3 d after gas exposure (AE-3)			
	$cm^3\ m^{-3}$]		Total	GSH	GSSG	RDS	Total	GSH	GSSG	RDS	Total	GSH	GSSG	RDS
8^{th}	O^0	C^{400}	69.4	51.7	17.7	0.75	65.6	49.8	15.7	0.71	58.7	39.7	19.0	0.62
		C^{800}	65.4	46.3	19.2	0.71	68.4	48.7	19.6	0.67	67.3	40.6	26.7	0.60
	$O^{0.1}$	C^{400}	68.2	29.1	39.1	0.39	62.9	28.1	34.8	0.40	66.4	40.9	25.5	0.56
		C^{800}	54.2	27.4	26.8	0.48	59.1	36.8	22.3	0.61	66.5	46.3	20.2	0.68
	$O^{0.3}$	C^{400}	71.6	30.8	40.8	0.40	57.6	24.6	33.0	0.42	109.1	78.2	31.0	0.73
		C^{800}	69.8	31.2	38.6	0.45	63.9	34.6	29.3	0.50	62.4	43.0	19.4	0.60
16^{th}	O^0	C^{400}	137.4	116.3	21.1	0.85	133.3	114.2	19.1	0.86	136.2	112.1	24.1	0.82
		C^{800}	135.2	115.2	20.0	0.85	134.0	113.5	20.5	0.84	134.3	113.6	20.7	0.84
	$O^{0.1}$	C^{400}	128.8	96.1	32.7	0.75	124.9	91.4	33.5	0.73	155.3	128.4	26.8	0.82
		C^{800}	132.6	115.8	16.8	0.87	147.2	124.4	22.8	0.83	139.0	113.4	25.5	0.82
	$O^{0.3}$	C^{400}	133.4	92.1	41.3	0.68	199.9	152.6	47.3	0.76	189.6	149.2	40.4	0.78
		C^{800}	138.7	100.2	38.4	0.71	140.9	93.3	47.7	0.66	175.8	131.0	44.8	0.75
ANOVA		Factor												
	8^{th}	O_3	n.s.	n.s.	**	**	n.s.	n.s.	*	n.s.	n.s.	n.s.	n.s.	n.s.
		CO_2	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
		$O_3 \times CO_2$	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	16^{th}	O_3	n.s.	n.s.	**	**	*	n.s.	**	*	***	*	**	n.s.
		CO_2	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
		$O_3 \times CO_2$	n.s.	n.s.	n.s.	n.s.	*	*	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

from DE to AE-0, but that of the $O^{0.3} + C^{400}$ plants decreased until AE-3 (Fig. 1*E*). On the other hand, the q_p of the $O^{0.1} + C^{400}$ and $O^{0.3} + C^{400}$ plants decreased compared to that of the control ($O^0 + C^{400}$) at AE-0, and the q_p of the $O^{0.1} + C^{400}$, $O^{0.3} + C^{400}$ and $O^{0.3} + C^{800}$ plants decreased at AE-1 (Fig. 1*F*). Therefore, the initial factor of the decrease in F_q'/F_m' in the $O^{0.1} + C^{400}$ and $O^{0.3} + C^{800}$ plants at DE and AE-0 was ascribed mainly to the F_v'/F_m', and the second factor continued to AE-1 was the q_p: O_3 first disrupted the PSII, and then adversely affected the downstream of plastoquinone A (Q_A) in photosynthetic electron transport. The decrease in F_v'/F_m' from DE to AE-3 by the $O^{0.3} + C^{400}$ plants indicated severe disruption of PSII by O_3. The P_N of the $O^{0.3} + C^{400}$ plants did not recover for a long time due to the damage sustained by PSII. Consequently, as the PSII is one of the most vulnerable parts to O_3 exposure, the recovery of PSII from the O_3-induced damage also indicates the recovery in other parameters of photosynthesis.

In the 16th leaves, P_N decreased as a result of O_3 exposure, and the reduction due to O_3 was ameliorated by C^{800} (Fig. 2*A*), as also observed in the 8th leaves (Fig. 1*A*). However, the damage sustained by the 16th leaves was less, and they recovered faster than the 8th leaves: the decreased P_N in the $O^{0.3} + C^{400}$ plants recovered almost completely at AE-3. Also, the disruption of the PSII in the $O^{0.3} + C^{400}$ plants was less and the 16th leaves (Fig. 2*C–F*) recovered faster than in the 8th leaves (Fig. 1*C–F*). Since the F_q'/F_m' in the $O^{0.3} + C^{800}$ plants decreased at AE-0 and that of the $O^{0.3} + C^{400}$ plants decreased at DE, the decrease in the former treatment was ascribed to the decrease in F_v'/F_m', and the decrease in the latter treatment to decreases in both the $F_v'/F_m'$and the q_p. Only the PSII was suppressed in the $O^{0.3} + C^{800}$ plants, whereas both the PSII and the downstream reactions of Q_A were suppressed in the $O^{0.3} + C^{400}$ plants. However, as the F_v/F_m values were not significantly different between the control and $O^{0.3}$ plants, the damage of the PSII in the 16th leaves was recovered faster than that in the 8th leaves. Also, the q_N of the $O^{0.3}$ plants increased more than in the control at AE-3 both in the 8th and 16th leaves, indicating the treatments with more damage and late recovery consumed an excessive energy. This manifested as heat dissipation derived from the decreased energy consumption for the carbon fixation reaction.

Rao *et al*. (1995) reported that in wheat the activation of the ascorbate-glutathione cycle is induced by O_3 exposure, based on increases in DHA and GSSG. However, McKee *et al*. (1997) found no increase in ascorbic acid or glutathione content caused by elevated CO_2 during fumigation of wheat with air containing moderately elevated O_3. In our study, the RDS of ascorbic acid and glutathione decreased as a result of O_3 exposure where photosynthesis was severely impeded, but these changes occurred at different times, because the recovery of AA was slower than that of GSH (Tables 2, 3). We considered that, (*1*) ascorbic acid and glutathione might detoxify the ROS in different way(s) besides the ascorbate-glutathione cycle, and/or (*2*) changes in RDS did not seem to harmonize irrespective of active ascorbate-glutathione cycle operation, since each one molecule of DHA and GSH react but their amounts are very different in the rice leaves. Indeed, the amount of ascorbic acid in rice leaves was far greater than that of glutathione (Inada *et a*l. 2008). Furthermore, the elevated CO_2 did not compensate for the decline of these antioxidant levels (Table 2, 3). Therefore, changes in antioxidant levels in our experiment did not explain the amelioration of photosynthesis by elevated CO_2 to O_3 exposure.

In the rice plant, Ishioh *et al*. (2005) observed that at the reproductive stage, the detrimental effects of O_3-fumigation on the P_N and dry mass were less than those at the vegetative stage. In the current experiment, the responses of the P_N in the 8th and the 16th (flag) leaves to O_3 and CO_2 were substantially different (Fig. 1, Table 1), probably due to differences in leaf thickness and inclination angle. The leaves become thicker with increasing height of the leaf position (Hoshikawa 1989), *i.e*. the 16th leaves are thicker than the 8th ones. Because the concentration of antioxidants per FM is higher in the 16th leaves (Tables 2, 3), it is easy to anticipate that the inhibition of the P_N and related processes in those leaves is less than in the 8th leaves due to higher contents of antioxidants per unit leaf area in the 16th leaves. On the other hand, the inclination angle of rice leaves becomes steeper at later growth stages (Ito *et al*. 1973). Because the inclination angle of the 16th leaves is steeper than that of the 8th leaves, the amount of incident light on the 16th leaves is smaller than on the 8th ones. Consequently, the probability of photoinhibition in the older (16th) leaves would be smaller than in the younger (8th) leaves, as reported by Heath (1994) in *Phaseolus vulgaris*. In wheat, Mulholland *et al*. (1997) observed that the O_3-induced inhibition of the P_N was less pronounced in the 8th (flag) leaves than in the 5th to 7th leaves, and they ascribed this to the greater content of active Rubisco and the absence of shade-acclimation of the flag leaves.

In summary, the photosynthesis mechanism and related processes in rice plants growing near urban areas occasionally sustain damage due to acute exposure to photochemical oxidants (especially O_3), leading to a decrease in the potential yield. However, since O_3-induced injury is ameliorated by elevated CO_2, the injury may be reduced in the future provided that the background O_3 level does not rise significantly. Therefore, it is important to ascertain whether or not such responses actually occur during the lifecycle of the rice plants with elevated CO_2 (Olszyk and Wise 1997), and to analyze the plant hormones which mediate oxidative signal transduction (Morita *et al*. 2002, Baier *et al*. 2005).

References

Baier, M., Kandlbinder, A., Golldack, D., Dietz, K.–J.: Oxidative stress and ozone: perception, signaling and response. – Plant Cell Environ. **28**: 1012-1020, 2005.

Baker, N.R.: Chlorophyll fluorescence : A probe of photosynthesis *in vivo*. – Annu. Rev. Plant Biol. **59**: 89-113, 2008.

Booker, F.L., Fiscus, E.L.: The role of ozone flux and antioxidants in the suppression of ozone injury by elevated CO_2 in soybean. – J. Exp. Bot. **56**: 2139-2151, 2005.

Cabrera, H., Dawson, S.V., Stromberg, C.: A California air standard to protect vegetation from ozone. – Environ. Pollut. **53**: 397-408, 1988.

Donnelly, A., Jones, M.B., Burke, J.I., Schnieders, B.: Elevated CO_2 provides protection from O_3 induced photosynthetic damage and chlorophyll loss in flag leaves of spring wheat (*Triticum aestivum* L., cv. 'Minaret'). – Agric. Ecosyst. Environ. **80**: 159-168, 2000.

Environ. Improve. Div., Tokyo Metropolitan Government Bureau Environ.: [Review report on the photochemical oxidant measures.] – J. Jap. Soc. Atmos. Environ. **40**: A65-A77, 2005. [In Jap.]

Haun, J.R.: Visual quantification of wheat development. – Agron. J. **65**: 116-119, 1973.

Heath, R.L.: Possible mechanisms for the inhibition of photosynthesis by ozone. – Photosynth. Res. **39**: 439-451, 1994.

Hoshikawa: The Growing Rice Plant. –Nobunkyo, Tokyo 1989. [In Jap.]

Imai, K., Kobori, K.: Effects of the interaction between ozone and carbon dioxide on gas exchange, ascorbic acid content, and visible leaf symptoms in rice leaves. – Photosynthetica **46**: 387-394, 2008.

Inada, H., Yamaguchi, M., Satoh, R., Hoshino, D., Nagasawa, A., Negishi, Y., Nouchi, I., Kobayashi, K., Izuta, T.: Effects of ozone on photosynthetic components and radical scavenging system in leaves of rice (*Oryza sativa* L.). – J. Agric. Meteorol. **64**: 243-255, 2008.

Ishioh, T., Imai, K.: [Effects of atmospheric ozone and carbon dioxide concentrations on gas exchanges, contents of Rubisco and chlorophyll in leaves of lowland rice.] – Proc. Kanto Branch,Crop Sci. Soc. Jap. **20**: 54-55, 2005. [In Jap.]

Ishioh, T., Kobori, K., Imai, K.: The detrimental effect of tropospheric O_3 on lowland rice is meliorated by elevated CO_2. – In: Toriyama, K., Heong, K. L., Hardy, B.,(ed.): Rice is Life: Scientific Perspectives for the 21st Century. Pp 557-560. IRRI, Los Baños 2005.

Ito, A., Udagawa, T., Uchijima, Z.: [Phytometrical studies of crop canopies. II. Canopy structure of rice crops in relation to varieties and growing stage.] – Jap. J. Crop. Sci. **42**: 334-342, 1973. [In Jap.]

Kobayashi, K.: [Assessing the impacts of tropospheric ozone on agricultural production.] – J. Jap. Soc. Atmos. Environ. **34**: 162-175, 1999. [In Jap.]

Mano, J., Khorobrykh, S., Amako, K.: [Reactive oxygen species and antioxidants associated with photosynthesis.] – Low Temp. Sci. **67**: 179-195, 2009. [In Jap.]

McKee, I.F., Eiblmeier, M., Polle, A.: Enhanced ozone-tolerance in wheat grown at an elevated CO_2 concentration: ozone exclusion and detoxification. – New Phytol. **137**: 275-284, 1997.

Morita, S., Tanaka, K.: Detoxification of active oxygen species and tolerance in plants exposed to air pollutants and CO_2. – In: Omasa, K., Saji, H., Youssefian, S., Kondo, N. (ed.): Air Pollution and Plant Biotechnology. Prospects for Phytomonitoring and Phytoremediation. Pp. 253-267. Springer-Verlag, Tokyo 2002.

Mulholland, B.J., Craigon, J., Black, C.R., Colls, J.J., Atherton, J., Landon, G.: Impact of elevated atmospheric CO_2 and O_3 on gas exchange and chlorophyll content in spring wheat (*Triticum aestivum* L). – J. Exp. Bot. **48**: 1853-1863, 1997.

Nakanishi, J., Shinozaki, H., Inoue, K.: [Ozone – Photochemical Oxidant.] – Maruzen Co., Tokyo 2009. [In Jap.]

Nouchi, I.: Changes in antioxidant levels and activities of related enzymes in rice leaves exposed to ozone. – Soil. Sci. Plant Nutr. **39**: 309-320, 1993.

Nouchi, I., Ito, O., Harazono, Y., Kouchi, H.: Acceleration of ^{13}C-labelled photosynthate partitioning from leaves to panicles in rice plants exposed to chronic ozone at the reproductive stage. – Environ. Pollut. **88**: 253-260, 1995.

Olszyk, D.M., Wise, C.: Interactive effects of elevated CO_2 and O_3 on rice and *flacca* tomato. – Agr. Ecosyst. Environ. **66**: 1-10, 1997.

Overmyer, K., Tuominen, H., Kettunen, R., Betz, C., Langebartels, C., Sandermann, H., Jr, Kangasjärvi, J.: Ozone-sensitive Arabidopsis *rcd1* mutant reveals opposite roles for ethylene and jasmonate signaling pathways in regulating superoxide-dependent cell death. – Plant Cell **12**: 1849-1862, 2000.

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

Papageorgiou, G.C., Govindjee. (ed.): Chlorophyll *a* Fluorescence. A Signature of Photosynthesis. Advances in Photosynthesis and Respiration. Vol. 19. – Springer, Dordrecht 2004.

Rai, R., Agrawal, M.: Evaluation of physiological and biochemical responses of two rice (*Oryza sativa* L.) cultivars to ambient air pollution using open top chambers at a rural site in India. – Sci. Total Environ. **407**: 679-691, 2008.

Rai, R., Agrawal, M., Agrawal, S.B.: Threat to food security under current levels of ground level ozone: A case study for Indian cultivars of rice. – Atmos. Environ. **44**: 4272-4282, 2010.

Rao, M.V., Hale, B.A., Ormrod, D.P.: Amelioration of ozone-induced oxidative damage in wheat plants grown under high carbon dioxide (Role of antioxidant enzymes). – Plant Physiol. 109: 421-432, 1995.

Reid, C.D., Fiscus, E.L.: Ozone and density affect the response of biomass and seed yield to elevated CO_2 in rice. – Global Change Biol. **14**: 60-76, 2008.

Roe, J.H., Oesterling, M.J.: The determination of dehydro-ascorbic acid and ascorbic acid in plant tissues by the 2,4–dinitrophenylhydrazine method. – J. Biol. Chem. **152**: 511-517, 1944.

Sasaki-Sekimoto, Y., Taki, N., Obayashi, T., Aono, M., Matsumoto, F., Sakurai, N., Suzuki, H., Hirai, M. Y., Noji, M., Saito, K., Masuda, T., Takamiya, K.-I., Shibata, D., Ohta, H.: Coordinated activation of metabolic pathways for antioxidants and defence compounds by jasmonates and their roles in stress tolerance in Arabidopsis. – Plant J. **44**: 653-668, 2005.

Sonoike, K.: [Basics of the measurements of photosynthesis by pulse amplitude modulation.] – Low Temp. Sci. **67**: 507-524,

2009. [In Jap.]
Torsethaugen, G., Pell, E.J., Assman, S.M.: Ozone inhibits guard cell K^+ channels implicated in stomatal opening. – Proc. nat. Acad. Sci. USA **96**: 13577-13582, 1999.
Toyama, S., Yoshida, M., Niki, T., Ohashi, T., Koyama, I.: Studies on ultrastructure and function of photosynthetic apparatus in rice cells. IV. Effects of low dose and intermittent fumigation of ozone on the ultrastructure of chloroplasts in rice leaf cells. – Jap. J. Crop Sci. **58**: 664-672, 1989.
WMO WDCGG: Data Summary No. 34. Pp. 1-13. JMA (Japan), Tokyo 2010.
Yamaguchi, M., Inada, H., Satoh, R., Hoshino, D., Nagasawa, A., Negishi, Y., Sasaki, H., Nouchi, I., Kobayashi, K., Izuta, T.: Effects of ozone on the growth, yield and leaf gas exchange rates of two Japanese cultivars of rice (*Oryza sativa* L.) – J. Agr. Meteorol. **64**: 131-141, 2008.