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Impacts of O_3 and CO_2 Enrichment on Growth of Komatsuna (*Brassica campestris*) and Radish (*Raphanus sativus*)

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Summary

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In the present study, we investigated the effects of O_3 and CO_2 on the growth of crop plants komatsuna (*Brassica campestris*. cv. Rakuten) and radish (*Raphanus sativus*. cv. Akamaru), and to suggest prediction maps of growth-loss affected by O_3 under CO_2 enrichment in the Kanto district of Japan. Komatsuna and radish plants in controlled climate chambers were exposed to O_3 and CO_2 from two days after emergence for 30 days. The following reciprocal combinations were tested: four levels of O_3 concentrations at <5, 60, 90 or 120 nl l^{-1}, and two levels of CO_2 concentrations at 380 or 760 µl l^{-1}. In komatsuna, leaf area and the biomass of all tissue components were reduced by O_3, and elevated CO_2 significantly increased biomass. An interactive effect of O_3 and CO_2 on leaf area were observed, and the growth reduction rate as affected by O_3 under elevated CO_2 was lower than the rate under ambient-level CO_2 in a komatsuna. On the other hand, in radish, biomass of all tissue components were reduced by O_3, and CO_2 significantly increased biomass. An interactive effect of O_3 and CO_2 on leaf area was observed, however, the growth reduction rate as affected by O_3 under elevated CO_2 was similar to the rate under ambient-level CO_2. We conclude that the sensitivity of O_3 may change under an elevated CO_2 concentration in green vegetable such as komatsuna. It should be noted that the negative impact of O_3 on radish under CO_2 enrichment may become greater than in komatsuna. Our findings indicate that O_3 sensitivity would be different between the types of vegetables. Thus, The prediction maps of growth-loss as affected by O_3 under elevated CO_2 in the Kanto district of Japan were different between komatsuna and radish.

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Introduction

The increasing air pollutants, such as ozone (O_3) and atmospheric carbon dioxide (CO_2) are considered to be major environmental problems. Atmospheric CO_2 concentration is expected to be double in the 21st century (IPCC 2001). The concentrations of O_3 and CO_2 are predicted to rise about 0.5-2.0% per year (RUNECKLES & KRUPA 1994). In Japan, according to the ambient air quality data of Atmospheric Environmental Regional Observation System in National Institute for Environmental Studies, relatively high concentrations of O_3 have been detected, especially in urban areas such as the Kanto district, and its concentration has been rising. A high concentration of CO_2 has been demonstrated to have beneficial effects on plants, such as increased net photosynthetic rates which lead to an increase in plant growth (e.g. GRIME 1997). On the other hand, O_3 is recognized as a widespread phytotoxic air pollutant (CHAMEIDES & al. 1994), and many experimental studies have shown that O_3 causes reductions in photosynthetic activity and dry matter production of plants (e.g. FUHRER & al. 1997, SANDERMANN & al. 1997). KOBAYASHI 1988, 1999 have reported that ambient levels O_3 in urban areas of Japan may already be related to the reduction of growth and yield of some crops.

In Europe, critical levels of O_3 have been defined and exposure indices for O_3 have been constructed to define the relationship between O_3 exposure and plant responses and for use in modelling and mapping areas at risk across Europe by using the way of the accumulated exposure to O_3 above a threshold of 40 nl l^{-1} (AOT40) - crop response relationship. The concept of using the AOT to define O_3 exposure has been demonstrated to be appropriate for several types of vegetables (FUHRER & al. 1997). However, there have been few reports evaluating the influence of O_3 on Japanese crop cultivars using the concept of O_3 dose-response relationship (KOBAYASHI 1999).

Komatsuna (Japanese mustered spinach; *Brassica campestris*) and radish (*Raphanus sativus*) are extensively grown in the Kanto district of Japan. These vegetables are mature quite rapidly, in about one month, enabling the cultivation several times in one year. Thus, it is important to evaluate the influence of O_3 on these vegetables. Furthermore, these two types of vegetable are different, i.e. komatsuna is green vegetable and radish is root vegetable. Thus, the response of growth under elevated O_3 and CO_2 would be different between komatsuna and radish.

The objective of this study was to investigate the impacts of O_3 and that of CO_2 on the growth of two types of vegetables, green vegetable (komatsuna) and a root vegetable (radish), and to relate their responses to the O_3 dose concept. Especially, we evaluated whether the AOT40 - crop growth response relationship would change under CO_2 enrichment. In addition, we constructed prediction maps of growth-loss as affected by O_3 under ambient level or elevated CO_2 in the Kanto district of Japan.

Material and Methods

Komatsuna (*Brassica campestris*. cv. Rakuten) and radish (*Raphanus sativus*. cv. Akamaru) plants were exposed to four different concentrations of O_3 and two levels of CO_2 concentrations in controlled climate chambers. Seeds in each vegetables were germinated in water for 2 days and then sown in 1.4-L plastic pots (11 cm in depth and 15 cm in diameter) filled with black soil. They were then grown in controlled climate chambers (KG-50HLAW-S, Koito Co. Japan) for 30 days. During the growth period, the air temperature in the chambers was maintained at 25.0 ± 1.0°C and 18.0 ± 1.0°C in the daytime from 0700 to 1700 h and from 1900 to 0500 h at night, respectively. The temperature was gradually increased from 18.0 to 25.0°C between 0500 and 0700 h and gradually decreased from 25.0 to 18.0°C between 1700 and 1900 h. Relative air humidity in the chambers was maintained at 65 ± 5%.Plants were subjected to one of eight pollutant regimes: two CO_2 treatments in combination with four O_3 treatments. Plants were exposed to two levels of CO_2 concentrations, i.e., 380 μl l^{-1} (ambient CO_2 level) and 760 μl l^{-1} (twice-ambient CO_2 level) during a 24–hour period. Pure CO_2 was used, and its concentration was monitored continuously at 6-minute intervals with a CO_2 analyzer (WMA-3, PP systems, USA). The four levels of O_3 described above were charcoal-filtered air (<5 nl l^{-1} O_3; CF), 60 nl l^{-1} O_3, 90 nl l^{-1} O_3 and 120 nl l^{-1} O_3 between 1000 and 1500 h, and the levels of O_3 was gradually increased from 0 to target O_3 concentrations, such as 60, 90 or 120 nl l^{-1}, between 0830 and 1000 h, and gradually decreased from target O_3 concentrations to 0 nl l^{-1} between 1500 and 1630 h. The concentration of O_3 at the plant canopy height in each treatment was continuously monitored at 6-minute intervals with a UV absorption O_3 analyzer (Model 1100, Dylec Inc., Japan). The O_3 was generated from ambient air with an electrical discharge O_3 generator (MO-5A, Nippon Ozone Co., Japan). During the 30-days experimental period, the accumulated exposure to O_3 over a threshold of 40 nl l^{-1} (AOT40) in the treatments of CF, 60, 90 and 120 nl l^{-1} were 0, 3.24, 8.30 and 14.88 μl l^{-1} h, respectively. Thirty days after planting, all plants were harvested to determine leaf area, leaf number and dry mass of top (leaf and stem) and root.

For evaluating the prediction maps of growth-loss affected by O_3 during summer in the Kanto district of Japan based on the method of O_3 dose (AOT40) - growth response relationship. the data of O_3 concentration in June, July and August from 1990 to 2000 in the Kanto district (Tokyo Metropolitan, Saitama Prefecture, Kanagawa Pref., Chiba Pref., Gunma Pref., Tochigi Pref. and Ibaraki Pref.) of Japan were collected from the 'Hourly Ambient Air Quality Data File' of the Atmospheric Environmental Regional Observation System at the National Institute for Environmental Studies. Data of photochemical oxidant (O_x) concentration was considered as data of O_3 concentration because its monitoring system recorded only O_x concentration, but most O_x is O_3. The number of monitoring stations of O_x concentration in Tokyo, Saitama, Kanagawa, Chiba, Gunma, Tochigi and Ibaraki were 43, 59, 40, 67, 17, 20 and 31, respectively. At each monitoring station, average AOT40 was calculated by using the data of hourly O_3 concentrations for 9 hours (from 0800 to 1700 hours local time) during June, July and August between 1990 and 2000. Furthermore, at each monitoring point, AOT40 for which the average hourly O_3 concentration increases 30 nl l^{-1} was estimated. Because the O_3 concentration rose about 0.29 nl l^{-1} per year between 1980 and 2000 in the Kanto district, 30 nl l^{-1} O_3 is equivalent to the total increase for about 100 years. ArcGIS™ 8 (Arc View 8 and Spatial Analyst, ESRI Japan., Japan) was used for analysis and mapping of these data. Tension-type of spline methods for estimation and interpolation of the values of O_3 concentration among monitoring points were used.

The statistical analyses were performed with the SPSS statistical package (SPSS 10.0.5J, SPSS Japan Inc., Japan). Means were calculated from the three replicates, each replicate consisting of six seedlings. The growth relative to growth at CF treatment was tested for the best liner fit against the O_3 dose (AOT40).

Results and Discussion

The O_3 caused significant reductions in leaf area and biomass of all tissue components of komatsuna and radish (Table 1). Many studies have shown that O_3 causes reductions of leaf area and dry matter production of plants because of the reduction in photosynthetic activity, such as net photosynthetic rate (FUHRER & al. 1997). However, a significant reduction of leaf number, and a significant increase of S/R ratio were revealed only in komatsuna plant. The O_3 has previously been shown to decrease carbohydrate distribution from leaves to roots (MCCOOL & MENGE 1983). Thus, the S/R ratio in komatsuna was increased by exposure to O_3. A tendency for S/R ratio of radish plant under ambient-level CO_2 to increase due to O_3 was also observed.

Table. 1. Vegetative responses of komatsuna and radish to mixtures of O_3 and CO_2. Shoot dry mass (DM) and S/R ratio shows the dry mass of leaf and stem, and the ratio of shoot DM to root DM, respectively. Root DM in radish is included turnip and root. ANOVA: * $p<0.05$, ** $p<0.05$, *** $p<0.05$.

Species	Treatment		Measurments					
	CO_2 (µl l^{-1})	O_3 (nl l^{-1})	Leaf No.	Leaf Area (cm^2)	Shoot DM (g)	Root DM (g)	Total DM (g)	S/R ratio (g g^{-1})
Komatsuna	380	<5	7.2	139.8	0.76	0.31	1.07	2.42
		60	7.1	107.7	0.63	0.26	0.89	2.46
		90	6.0	94.1	0.53	0.20	0.73	2.59
		120	6.1	71.3	0.39	0.15	0.53	2.57
	760	<5	7.2	177.4	1.19	0.46	1.65	2.56
		60	7.4	170.9	1.10	0.42	1.53	2.60
		90	6.9	160.6	0.98	0.38	1.37	2.60
		120	6.6	122.7	0.85	0.31	1.15	2.77
	ANOVA test							
	O_3		*	**	**	**	*	*
	CO_2		ns	***	***	***	***	ns
	$O_3 \times CO_2$		ns	*	ns	ns	ns	ns
Radish	380	<5	6.3	90.2	0.31	0.63	0.95	0.49
		60	6.3	66.7	0.28	0.59	0.87	0.48
		90	6.3	61.9	0.24	0.39	0.63	0.60
		120	6.1	59.1	0.19	0.36	0.55	0.54
	760	<5	6.5	115.6	0.44	1.45	1.89	0.30
		60	6.4	97.7	0.36	1.34	1.70	0.27
		90	6.5	93.1	0.29	1.15	1.44	0.25
		120	6.4	87.7	0.25	0.88	1.14	0.28
	ANOVA test							
	O_3		ns	**	***	***	*	ns
	CO_2		ns	***	***	***	***	**
	$O_3 \times CO_2$		ns	**	ns	ns	ns	ns

On the contrary, significant increase of leaf area and biomass of komatsuna and radish due to CO_2 exposure (Table 1) is attributable to an increase in photosynthetic rate (McKEE & al. 1995, KIM & al. 2003). The influence of elevated CO_2 on total biomass was positive by 54% in komatsuna and 98% in radish. In radish, a reduction of S/R ratio was observed, and as a result, the turnip-root (storage root) was larger than other organs under elevated CO_2 concentration, and the positive effect of CO_2 on total biomass was larger than other crops (KIMBALL 1983). This would be due to the development of sink capacity of the turnip-root in radish, and large storage root would not show down-regulation of photosynthesis under elevated CO_2 (USUDA & SHIMOGAWARA 1998).

An interactive effect of O_3 and CO_2 on leaf area in both plants were observed (Table 1). Recent studies have shown that the combined effects of O_3 and CO_2 are not simply additive, and elevated CO_2 has been shown to protect against ozone-induced reduction in growth and physiology, but the effects these two gases together on plant growth and physiology are often contradictory (REINERT & HO 1995, McKEE & al. 1997, REID & al. 1998). In the present study, the effect of O_3 growth under elevated CO_2 was different between komatsuna and radish (Fig. 1). In radish, the growth reduction rate as affected by O_3 under elevated CO_2 was similar to the rate under ambient-level CO_2. On the other hand, the growth reduction rate affected by O_3 under elevated CO_2 was lower than the rate under ambient-level CO_2 in komatsuna. These results suggest that in a root vegetable like radish,

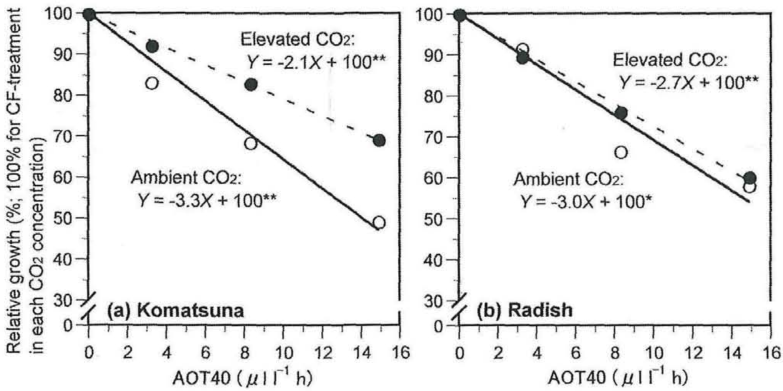

Fig. 1. Dose-response relationship between AOT40 and relative growth of (a) komatsuna and (b) radish on a relative basis to the growth for CF-treatment (<5 nl l^{-1} O_3) in each CO_2 concentration. The data of total biomass originated from Table 1. The AOT40 in the treatments of CF, 60, 90 and 120 nl l^{-1} O_3 were 0, 3.24, 8.30 and 14.88 μl l^{-1} h, respectively. Open circle: ambient CO_2 (380 μl l^{-1}), Closed circle: elevated CO_2 (760 μl l^{-1}).

the turnip-root biomass accounts for the large total biomass and that increase of growth would be large under elevated CO_2 because of strong carbon sink persists. However, the negative effect of O_3 on root growth would also be large. Thus, the response of growth to O_3 exposure may not change under CO_2 enrichment. On the contrary, in a green vegetable like komatsuna, in which leaf biomass accounts for large total biomass, elevated CO_2 would protect against the ozone-induced reduction in growth.

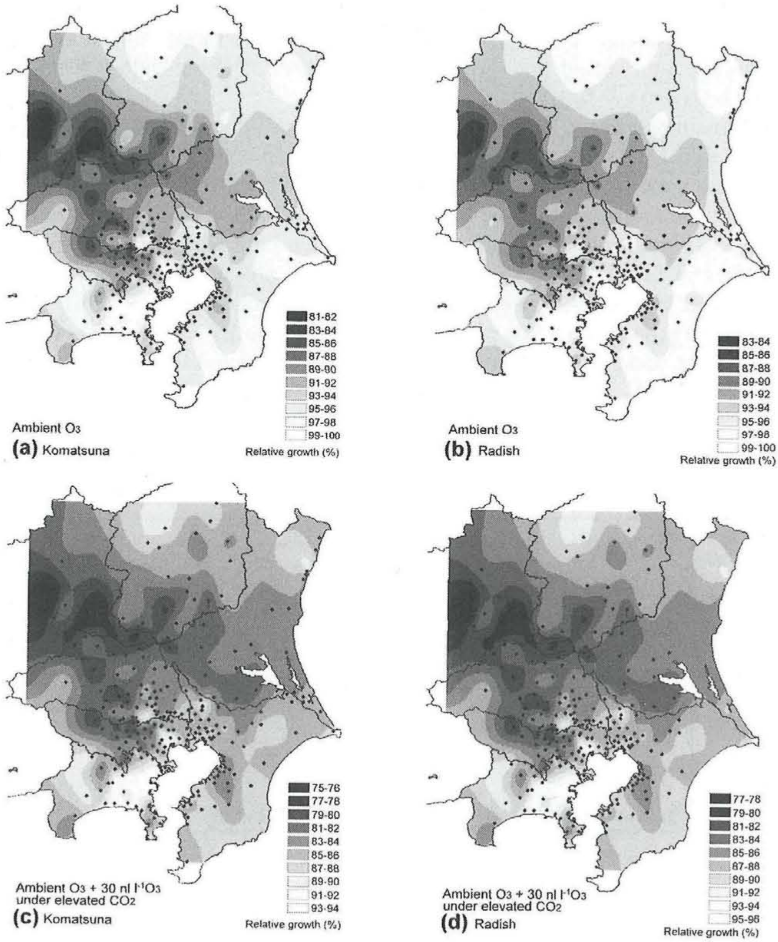

Fig. 2. Prediction map of growth-loss in komatsuna and radish in Kanto district of Japan by using the way of AOT40 - growth response relationship (Fig.1). (a) Ambient O_3 level (Average 30-days-AOT40 in June, July and August during 1990 to 2000) on growth of komatsuna. (b) Ambient O_3 level on growth of radish. (c) Elevated O_3 (30-days-AOT40 computed the prediction of increasing of 30 nl l^{-1} O_3) and CO_2 (elevated CO_2 (760 µl l^{-1} CO_2)) on growth of komatsuna. (d) Elevated O_3 and CO_2 on growth of radish.

Prediction maps of growth-loss as affected by O_3 in the Kanto district of Japan in summer based on the results of Fig. 1 are shown in Fig. 2. The growth of komatsuna and radish was reduced by about 5-20% by ambient-level O_3 (Fig. 2-a, b). In the area of Tokyo, Saitama and Gunma, a relatively higher reduction rate of biomass was observed. On the other hand, according to the prediction of growth-loss under elevated (twice-ambient) CO_2 and an increase in 30 $\mu l\ l^{-1} O_3$, the reduction rate of biomass would be higher almost everywhere in the Kanto area, and its would be about 5% higher compared with ambient-level of O_3. We constructed prediction maps with a simple method in this study, but improvement of the method of analysis and other environmental factors, such as temperature, are required to raise the accuracy of prediction.

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