A comparison of physiological responses among four cultivars of soybean (*Glycine max* (L.) Merr.) to single and combined exposure of ozone and peroxides

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Abstract

Crop response to future air pollution stress is important information for crop production in the future. Ozone (O_3) and peroxides are recognized as significantly damaging air pollutants to plants. Therefore, the present research aimed to investigate the single and combined effects of O_3 and peroxides on leaf injury and physiological responses, and to compare the soybean cultivar sensitivities under exposure to single O_3 and combined O_3 and peroxides. Two Japanese soybean cultivars, Tachinagaha (TC), and Chamame (CM) and two Thai soybeans, A75 and Sorjor 5 (SJ5) were selected as plant materials. Four treatment plots were set up, including a control plot (C plot: free O_3 and peroxides), O_3 50 ppbv (O plot), O_3 50 ppbv and peroxides 2–3 ppbv (OP1 plot) and O_3 50 ppbv and peroxides 4–5 ppbv (OP2 plot). We found that combined O_3 and several ppbv peroxides (OP2 plot) caused more severe damage than the OP1 plot and single O_3 (O plot) to leaf injury, chlorophyll content and photosynthetic rate, and reduced total dry weight and pod dry weight. In combined O_3 and peroxides exposure, SJ5 showed the most sensitive cultivar in leaf injury, photosynthetic rate, biomass and pod dry weight, while CM showed less sensitivity for photosynthetic rate and pod dry weight.

Key words: Ozone, Peroxides, Photosynthetic rate, Soybean, Visible leaf injury.

1. Introduction

Ozone (O_3) is a secondary pollutant that has been reaching high concentrations in the Earth's troposphere (Voulgarakis *et al.*, 2011). It has been recognized as one of the most damaging air pollutants (Tompson, 1992) in recent years. In the last century, the concentrations of O_3 increased up to 80 ppbv (Wang *et al.*, 2006) and have been increasing in many places in the world (Voulgarakis *et al.*, 2011). Since O_3 formation in the air is accelerated in higher air temperature conditions, it tends to reach a level that causes numerous

decreases in growth and yield under global warming conditions. Ozone is highly reactive, then it binds to plasma membranes and alters metabolism. As a result, chloroplast thylakoid membranes are injured, ribulose-1,5-bisphosphate carboxylase/ oxygenase (Rubisco) is degraded and stomatal aperture is poorly regulated (Fiscus *et al.*, 2005; Lu *et al.*, 2009). Long-term exposure to ozone can lead to the reduction of growth and yield. Crop losses by O_3 damage in 2000 have been estimated as \$1.8 to \$3.9 billion in the United States and \$3.0 to \$5.5 billion in China (Dingenen *et al.*, 2009). However, Takami *et al.* (2003) reported that severe forest decline was seen in the Oku-Nikko Mountains in Japan where O_3 concentration was not as high. They also reported that peroxides concentration in the area was high. Then the severe plant damage in

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these areas cannot be fully explained by the effects of O_3 alone.

Peroxides always coexist with O_3 in the air by the reaction of O_3 and volatile organic compounds (VOC). Atmospheric peroxides generally comprise hydrogen peroxide (H_2O_2) and organic peroxides such as methyl hydroperoxide (MHP) and hydroxymethyl hydroperoxide (HMHP) (Hatakeyama *et al.*, 1993; Takami *et al.*, 2003). In fact, combined O_3 and peroxides are much more harmful to some vegetables and other plants than single O_3 (Chen *et al.*, 2005; Chen *et al.*, 2010). Recent investigations showed that peroxides concentrations have been increasing (Chen *et al.*, 2008; Chutteang *et al.*, 2012). Terry *et al.* (1995) suggested that peroxides can damage DNA, lipids, proteins, and plasma membrane that may impact productivity by a reduced photosynthetic rate, inhibited translocation and declined biomass.

The O_3 concentration in Asia ranges from 5 to 80 ppbv (Chen *et al.*, 2008; Kang, 2002). The concentrations of gas-phase H_2O_2 are found in ranges of 0.02 to 6 ppbv in Japan. (Sakugawa *et al.*, 1990; Takami *et al.*, 2003; Chutteang *et al.*, 2012). The peroxide concentrations were in the order of several ppbv in Nagano Prefecture, Japan (Chen *et al.*, 2008). Chen *et al.* (2010) reported that exposure to O_3 of 50 ppbv in combination with peroxides of 2-3 ppbv caused an extremely severe decrease in the leaf photosynthetic rate and much higher foliar injury of radish than that of 100 ppbv O_3 alone.

Soybean (*Glycine max* (L.) Merr) is an important crop species that is sensitive to O_3 (Morgan *et al.*, 2003). Heagle *et al.* (1998) reported that a moderately high level of O_3 (60-100 ppbv) in the mid-Atlantic states of the USA may cause as much as a 40% loss of soybean yield. However, the sensitivity of plants to O_3 and air pollutants varies among genotypes (Akhtar *et al.*, 2010; Betzelberger *et al.*, 2010). Genetic variation in O_3 sensitivity was commonly observed in several plant species, for example snap bean (Reitnart and Eason, 2000), tomato (Temple, 1990), potato (Heagle *et al.*, 2003), and soybean (Burkey and Carter, 2009). However, there is no information about cultivar sensitivity of soybeans under combined exposure of O_3 and peroxides. Moreover the physiological responses to combined O_3 and peroxides are necessary to investigate future crop productivity assessment since atmospheric peroxides concentration will increase in the

future. Hence the objectives of the present research are to investigate the single and combined effects of O_3 and peroxides on leaf injury and physiological responses, and to compare the soybean cultivar sensitivities.

2. Materials and Methods

2.1 Plant materials

Two Japanese soybeans, Tachinagaha (TC) and Chamame (CM), and two Thai soybean cultivars, A75 and Sorjor 5 (SJ5) were used. The experiments were conducted two times from December 2010 to March 2011 and December 2011 to March 2012. In these two experiments, methods and procedures were the same. Soybean seeds were seeded in plastic pots of 0.8-L on 16 December 2010 and 22 December 2011 filled with Cumulic Andosols (Melanudand) soil. Fertilizer (N-P-K=14-14-14) was applied at 5 g per pot before seeding, and pest control was also applied. The plants were grown under charcoal-filtered air in a glass house for 20 days before the exposure experiments, and the exposure was begun at the third trifoliate leaf stage. Ten plants in ten pots were randomly selected for each treatment. The potted plants were watered twice a day before and after exposure every day.

2.2 Exposure treatments

Four outdoor growth chambers (Koito Ltd., Yokohama, Japan), in which the air temperature (day: 06.00 to 18.00 25°C; night: 18.01 to 05.59 18°C) and the relative humidity (70%) were controlled, were used. The air in the chambers was homogenized by fans. The four experimental plots were the control plot that is O_3 and peroxides free (C plot), 50 ppbv O_3 plot (O plot), 50 ppbv and 2-3 ppbv peroxides (+ α -pinene) plot (OP1 plot), and 50 ppbv O_3 and 4-5 ppbv peroxides (+ α -pinene) plot (OP2 plot). The C plot was controlled with charcoal-filtered air. Ozone was generated from air by a silent electrical discharge O_3 generator (MO-5A, Nihon Ozone Ltd., Kagoshima, Japan). The oxides of nitrogen were completely removed by water filter. The concentration of O_3 was regulated by an automatic controlling system (HMC-2000/S; Koito Ltd., Yokohama, Japan) and monitored every 90 seconds. This measurement was performed automatically and continuously. Peroxides were produced by a similar formation in the atmosphere, namely by the reaction of O_3 with vaporized α -pinene in the chamber (Chen *et al.*, 2005, 2010). The gaseous peroxides in

each chamber were sampled with a Pyrex glass mist chamber (Cofer *et al.*, 1985) three times a day in the morning, early afternoon, and late afternoon. The concentration of peroxides was measured by a portable RQflex 10 reflectometer (MERCK KGaA, Darmstadt, Germany). Details of the above procedures are explained in Chen *et al.* (2005, 2010) and Chutteang *et al.* (2012). The exposure was conducted from 9:00 to 16:00 every day.

2.3 Evaluation of visible leaf injury, SPAD index and chlorophyll content

Visible foliar injury was evaluated on first trifoliate leaves every two to three days after exposure. Three lateral leaves at the same position for each plant from four plants per plot were evaluated. Visible injury was expressed by its intensity of 0, 1, 2, 3 and 4, which represent no injury, chlorosis < 50% of individual leaf area, chlorosis > 50% of individual leaf area, necrosis < 50% of individual leaf area and necrosis > 50% of individual leaf area, respectively. The SPAD index was measured by a chlorophyll meter (SPAD502, Minolta Camera Co., Ltd., Japan) every 2-3 days. Chlorophyll content was evaluated by the equation from the correlation of the SPAD index and total chlorophyll content for each cultivar. Total chlorophyll contents were measured from leaf disc extract (Moran, 1982). The equation of TC cultivar is $y = 0.0107x - 0.07$ ($n = 30$), CM cultivar is $y = 0.0142x - 0.08$ ($n = 25$), SJ5 cultivar is $y = 0.0095x - 0.05$ ($n = 27$), and A75 cultivar is $y = 0.0513x - 0.10$ ($n = 25$).

2.4 Measurements of leaf gas exchange

Leaf gas exchange was measured on the first trifoliate leaf by a photosynthesis measurement system (LI6400-40 by LI-COR, Nebraska, U.S.A.) at 10 (1st measurement), 20 (2nd measurement), and 30 (3rd measurement) days after exposure in the second experiment from four plants in each plot and cultivar. The leaf chamber environment conditions were set to optimal conditions for stomatal opening and light saturation condition that are under PAR 1500 $\mu\text{mol PPFD m}^{-2} \text{ s}^{-1}$, CO_2 400 $\mu\text{molCO}_2 \text{ mol}^{-1}$, temperature 25°C, and RH 65-70%. The light-saturated photosynthetic rate (A), transpiration rate (E), stomatal conductance (gs), and intercellular CO_2 concentration (Ci) were evaluated. The 1st and 3rd measurements were performed under sunny conditions, but the 2nd measurement was under cloudy conditions, so in the present research the results of the 1st and 3rd measurements

were used in the case of correlations between A and gs and Ci.

2.5 Measurement of biomass

The biomasses of root, leaf, stem, and pod were measured on Day 50 (CM) and on Day 55 (A75, TC and SJ5) when the growth stage was R6 and the pod contained green seed in the pod cavities (Fer and Caviness, 1977). Four plants in each plot and cultivars were randomly selected. We could not continue the experiment further because some plants started to die due to severe damage. Each organ was weighed after oven drying at 80°C for 3 days.

2.6 Data analysis

The present research experiments were conducted in two years, and these data sets were combined and averaged since no significant difference was observed. Thus all results shown in the present article were based on the two-year data sets except leaf gas exchange. Statistical data analysis was performed with R 2.13.1 Software. Analysis of variance (ANOVA) was used to test the effects of experimental plots and cultivars. Tukey's HSD test was performed to identify significant differences in a cultivar. Sample numbers were eight plants per plot per cultivar.

3. Results

3.1 Visible foliar injury

In the O plot, the silver stipples were found in the early stage after fumigation and they turned to brown spot, and necrosis gradually appeared. However, brown spot and necrosis appeared much earlier in the OP1 and OP2 plots than the O plot. Some typical symptoms of visible foliar injury in combined O_3 and peroxides (OP2) are shown in Fig. 1. The intensity of visible foliar injury of four soybean cultivars showed a similar pattern in which the OP2 plot caused more severe damage than those of the OP1 plot and O plot (Fig. 2). SJ5 was more sensitive than CM, A75 and TC in the OP1 and OP2 plots. In the O plot, A75 cultivar was less sensitive than TC, SJ5 and CM (Fig. 3).

3.2 Chlorophyll content

The total chlorophyll content of the OP1 and OP2 plots showed a different response to that of the O plot. And the O plot showed two step responses to the O_3 dose while the OP1 and OP2 plots showed a linear response to peroxide dose as shown in Fig. 4. At first, the total chlorophyll content of the O plot was gradually decreased and then rapidly decreased. The total

Fig. 1. A typical symptom of visible leaf injury under combined O_3 and peroxides after 30 days of exposure in OP2 plot.

Fig. 2. O_3 dose responses of visible leaf injury in four soybean cultivars
The vertical bars of symbols are standard deviations.

chlorophyll content of the OP1 and OP2 plots rapidly decreased from just after exposure (Fig. 4). In the case of the O plot, CM showed the most sensitivity, while A75 and SJ5 seem to be less sensitive. In the OP1 and OP2 plots, SJ5 and CM showed high sensitivity, while TC showed less sensitivity (Fig. 4). The order of cultivar sensitivity in the total chlorophyll content in the O, OP1 and OP2 plots was similar to those of visible leaf injury.

3.3 Leaf gas exchange

Figure 5 shows a decrease in the net photosynthetic rate. The OP2 plot showed a much more highly reduced net photosynthetic rate than the OP1 and O plots at 10 and 20 days after exposure. SJ5 was the most sensitive in the O, OP1 and OP2 plots except on Day 30. TC showed less sensitivity in the O plot, but CM showed less sensitivity in both the OP1 and OP2 plots (Fig. 5).

Fig. 3. Comparison of visible leaf injury sensitivity
The vertical bars of symbols are standard deviations.

Fig. 4. Comparison of decrease in total chlorophyll content (Chl).

Fig. 5. Percentage of net photosynthetic rate (A) to C plot of four soybean cultivars
The data were measured only in the second year experiment.

Correlations between A and g_s and C_i are shown in Fig. 6. The A versus g_s relationship in the C plot is shown by the dotted line (—) circle in Fig. 6a and its position in the figure did not change as shown in Fig. 6c. This means that the A versus g_s relationship in the C plot was almost the same for 20 days from 10 days and 30 days after exposure. Whereas in the OP1 and OP2 plots, shown respectively by a two-dot chain line (---) circle and solid line (—) circle, both A and g_s values of all cultivars decreased during 20 days. This means that A values of all cultivars decreased followed by the decrease in g_s when the exposure continued for more 20 days. In this case the decreases in A and g_s values in the OP2 plot were more than the OP1 plot.

As for the position of the A versus g_s relationship in the O plot shown by the dashed line (---) circle, A and g_s values of some cultivars changed greatly during 20 days, and then the A versus g_s relationship became a positive correlation at 30 days after exposure as shown in Fig. 6c.

In the case of the C plot, the A versus C_i relationship did not change during 20 days as shown in Fig. 6b and

Fig. 6d. Whereas in the OP1 and OP2 plots, A of all cultivars decreased but C_i increased for 20 days. Especially this tendency of the OP2 plot was much greater than the OP1 plot. In the O plot such tendency was much smaller than the OP1 plot (See Fig. 6b and Fig. 6d). As a whole, in the O plot, A was reduced by g_s , but not by C_i . In the OP plots, A was reduced by both low g_s and high C_i .

We also found that the sensitive cultivar, SJ5, showed the highest g_s value in the C, O and OP1 plots at 10 days after exposure and became lowest at 30 days after exposure. Whereas tolerant cultivars, TC and CM, g_s showed lower values in each plot except the OP2 plot at 10 days after exposure as shown in Fig. 6a. As for A versus C_i , SJ5 showed the highest C_i in each C, O and OP1 plot groups at 10 days after exposure (see Fig. 6b). The present research results showed that the sensitive cultivar (SJ5) highly reacted with single O_3 and combined O_3 and peroxides much more than tolerant cultivars (TC and CM).

3.4 Biomass

The biomasses of four soybean cultivars after 50

Fig. 6. Correlations between net photosynthetic rate (A) and stomatal conductance (gs) (Fig. 6a and Fig. 6c) and CO₂ concentration in intercellular space (Ci) (Fig. 6b and Fig. 6d) of four soybean cultivars C and S show CM and SJ5 cultivars, respectively. The data were measured only in the second year experiment.

C plot : TC (■) CM (◆) A75 (▲) SJ5 (●)
O plot : TC (■) CM (◆) A75 (▲) SJ5 (●)
OP1 plot : TC (□) CM (◇) A75 (△) SJ5 (○)
OP2 plot : TC (■) CM (◆) A75 (▲) SJ5 (●)

days (CM cultivar) and 55 days (TC, A75 and SJ5 cultivars) exposure are shown in Table 1. In TC cultivar, the total dry weights of the C and O plots were not significantly different, and the OP1 plot was not clearly different from the O plot, whereas a 44% decrease in total dry weight and a 91% decrease in pod dry weight than the C plot were found in the OP2 plot. Moreover root dry weight significantly reduced in the OP2 plot. The results of CM cultivar showed that the dry weight of all organs was significantly different among the four plots. The total dry weight was not different between the C and O plots, while both the OP1 and OP2 plots caused a 29% and 55% reduction in the total dry weight than the C plot. Both the OP1

and OP2 plots caused a 47% and 49% reduction in the total dry weight of A75 and 45% and 59% in that of SJ5, respectively. As for the total dry weight in the O plot, TC showed the most tolerant cultivar and the other three cultivars showed no significant difference. In the OP1 and OP2 plots, TC was less sensitive while SJ5 was the most sensitive cultivar. Considering pod dry weight, we found that SJ5 was the most decreasing cultivar in O (30%), OP1 (61%), and OP2 (96%), while CM was the least decreasing cultivar in O (14%), OP1 (45%) and OP2 (52%). This may imply that SJ5 was the most sensitive cultivar on total dry weight and pod dry weight, while CM was the less sensitive cultivar on pod dry weight (Table 1).

Table 1. Effect of O₃ and peroxides on dry weight of four soybean cultivars (Mean of eight replicates $\pm$ SD.)

Cultivars Plots		Dry weight (g plant ⁻¹)						
		Root	Petiole	Stem	Leaf	Flower	Pod	Total
TC	C	1.88±0.59 ^a	0.34±0.19	1.15±0.42	1.48±0.78	0.12±0.14	2.80±0.81 ^a	7.77±1.29 ^a
	O	1.81±0.67 ^a	0.31±0.15	1.02±0.42	1.41±0.67	0.10±0.14	2.35±0.95 ^{ab}	6.99±1.17 ^{ab}
	OP1	1.51±0.27 ^{ab}	0.29±0.06	1.09±0.32	1.14±0.27	0.17±0.13	1.45±0.85 ^b	5.65±1.65 ^{bc}
	OP2	1.11±0.40 ^b	0.29±0.09	1.04±0.16	1.49±0.29	0.16±0.06	0.25±0.17 ^c	4.33±0.80 ^c
ANOVA		**	ns	ns	ns	ns	**	**
CM	C	1.18±0.19 ^{ab}	0.35±0.17 ^a	1.01±0.22 ^a	1.31±0.67 ^a	–	3.73±0.66 ^a	7.58±1.82 ^a
	O	1.24±0.20 ^a	0.23±0.05 ^{ab}	0.90±0.13 ^a	0.87±0.27 ^{ab}	–	3.22±0.24 ^a	6.45±0.49 ^{ab}
	OP1	0.91±0.18 ^b	0.29±0.08 ^a	0.86±0.17 ^a	1.25±0.17 ^a	–	2.07±0.62 ^b	5.37±1.18 ^b
	OP2	0.58±0.16 ^c	0.12±0.04 ^b	0.47±0.10 ^b	0.46±0.20 ^c	–	1.79±0.44 ^b	3.42±0.86 ^c
ANOVA		**	**	**	**		**	**
A 75	C	1.57±0.16 ^a	0.34±0.17 ^a	1.19±0.47 ^a	1.78±0.91 ^a	–	4.27±0.39 ^a	9.15±1.95 ^a
	O	1.34±0.21 ^a	0.30±0.12 ^{ab}	1.13±0.19 ^a	1.45±0.60 ^{ab}	–	3.49±0.55 ^a	7.70±0.73 ^a
	OP1	0.74±0.24 ^b	0.20±0.06 ^b	0.72±0.19 ^b	0.86±0.33 ^b	–	2.26±0.47 ^b	4.78±1.06 ^b
	OP2	0.81±0.14 ^b	0.25±0.07 ^{ab}	0.90±0.23 ^{ab}	1.13±0.36 ^{ab}	–	1.55±0.48 ^b	4.63±0.59 ^b
ANOVA		**	*	*	**		**	**
SJ 5	C	1.59±0.61 ^a	0.50±0.35	1.12±0.52 ^a	1.71±0.88	0.00±0.01	3.01±0.12 ^a	7.92±1.97 ^a
	O	1.42±0.38 ^a	0.44±0.25	1.10±0.46 ^{ab}	1.64±0.78	0.01±0.02	2.11±0.90 ^b	6.72±1.11 ^a
	OP1	0.87±0.19 ^b	0.34±0.15	0.77±0.18 ^b	1.21±0.37	0.01±0.03	1.17±0.35 ^c	4.37±1.11 ^b
	OP2	0.68±0.22 ^b	0.34±0.09	0.79±0.15 ^{ab}	1.27±0.22	0.02±0.03	0.11±0.06 ^d	3.25±0.57 ^b
ANOVA		**	ns	**	ns	ns	**	**

CM was harvested on 50 days after exposure, while other cultivars were harvested on 55 days after exposure. Two-way ANOVA: **P<0.01, * P<0.05, NS=not significant. Values with different letters are significantly different at P<0.05.

4. Discussion

4.1 Visible foliar injury

Several scientists found that soybean had genetic variation in O₃ responses (Foy *et al.*, 1995; Robinson and Britz, 2000; Morgan *et al.*, 2003). Burkey and Carter (2009) reported that ancestral lines of soybean in the USA and Canada showed wide ranges of visible leaf injury to high O₃. The symptom of visible leaf injury in the O, OP1 and OP2 plots was similar to that in Burkey and Carter (2009). The intensity of visible leaf injury showed severe damage in combined O₃ and peroxides more than single O₃. Similar results were found by Chen *et al.* (2010), Chutteang *et al.* (2011) and Na-ngern *et al.* (2011). Moreover the sensitivity of visible leaf injury was different among four soybean cultivars and the orders of cultivar sensitivity were different between single O₃ and combined O₃ and peroxides. The cultivar sensitivity was identified as three groups consisting of tolerance, moderated tolerance,

and sensitivity in both single O₃ and combined O₃ and peroxides. This implies that some injury mechanisms may be different between single O₃ and combined O₃ and peroxides.

4.2 Chlorophyll content

Chen *et al.* (2010) reported that the total chlorophyll content of Japanese radish was reduced by the fumigation of combined 50 ppbv O₃ and sub-ppbv peroxides and by a high level of O₃ concentration. Similar results were found in the present research that a much more severe reduction was observed in the OP1 and OP2 plots than the O plot (Fig. 4). This implies that low O₃ concentrations cause less reduction in chlorophyll content but sub-ppbv peroxides concurrent with 50 ppbv O₃ caused severe damage in photosynthetic pigment. Similar results were found by Inada *et al.* (2008) that a low O₃ condition did not reduce chlorophyll content in two Japanese rice cultivars.

4.3 Leaf gas exchange

As for soybean, Robinson and Britz (2000) revealed

that short term exposure to a moderate level of ozone did not significantly affect CO₂ assimilation and stomatal conductance per unit leaf area. Fiscus *et al.* (2005) reported that reduction in CO₂ fixation under long-term O₃ exposure is mainly restricted by Rubisco activity. The present result showed a similar result in TC whose cultivar was less sensitive. The net photosynthetic rate slightly decreased at 10 days after single O₃ exposure and highly reduced at 30 days after exposure. On the other hand, the relative net photosynthetic rate to C plot of sensitive cultivar (SJ5) was about 40% at 10 days after exposure by single O₃. The OP2 plot showed an extremely severe decrease in the net photosynthetic rate. A similar result was found in Japanese radish by Chen *et al.* (2010). However, we cannot explain the reason why a more severe reduction of the photosynthetic rate occurred in OP plots than the O plot. Reid *et al.* (1998) reported that the chronic O₃ condition of 80 ppbv O₃ affected carbon fixation by decreasing the activity and content of Rubisco in soybean. This implies that 50 ppbv O₃ may not cause a severe decrease in carbon fixation, but combined peroxides and O₃ are very harmful and cause the significant reduction of carbon fixation.

We found that the sensitive cultivar had a larger *g_s* value than that of the tolerant cultivar in each experimental plot. But these tendencies were not shown at 30 days after exposure as shown in Fig. 6a and Fig. 6c. This may be because the leaves were becoming senescent at 30 days after exposure. Flux of O₃ and other gases into the leaf is controlled by stomatal conductance (Fiscus *et al.*, 1997). As for the results mentioned above, O₃ and peroxides have a higher possibility of entering into the leaf in sensitive plants than tolerant cultivar and cause more severe damage. Fiscus *et al.* (2005) suggested that tolerant plants will close the stomata to protect against the absorption of O₃ while sensitive plants cannot regulate the stomata. Our results support the suggestion of Fiscus *et al.* (2005).

The present research results also suggest as shown in Fig. 6 that the net photosynthetic rate of soybean leaf under 50 ppbv O₃ alone was reduced by the reduction of *g_s*, but those of combined 50 ppbv O₃ and peroxides reduced by both *g_s* and *C_i* that relates to carbon fixation.

4.4 Biomass

O₃ exposure results in decreased photosynthetic carbon assimilation (Morgan *et al.*, 2003;

Betzelberger *et al.*, 2010). The present research results showed that total dry weight was not significantly decreased under 50 ppbv O₃, which is an average O₃ concentration in Japan. Obviously, the combined O₃ and high peroxides condition caused decreases in the total dry weight of more than 40% and pod dry weight of more than 50% in four soybean cultivars. A similar research result was reported by Chen *et al.* (2010) that the combined effect of O₃ and 2-3 ppbv peroxides reduced the biomass of Japanese radish by about 30%. Generally, biomass is primarily regulated by source and sinks strength. Then high reduction in biomass would be caused by more severe damage to visible leaf injury and a higher decrease in the photosynthetic rate in combined O₃ and peroxides than single O₃. Peroxides and O₃ can oxidize some protein and enzyme involved in photosynthesis (Chen *et al.*, 2005; Lu *et al.*, 2009). Then the structure and function of the photosynthetic organ were destroyed after the exposure of the soybean plant to combined O₃ and peroxides. The chlorophyll content was also degraded and the stomatal aperture could not be regulated. These caused a significant decline in the photosynthetic rate and synthesis of dry matter, resulting in a considerable decrease in biomass. TC cultivar showed less reduction in the total dry weight but a high decrease in pod dry weight while CM which showed more reduction in the total dry weight than that of TC but could maintain pod productivity (Table 1). Similar results were found by Betzelberger *et al.* (2010) who suggested that there were various mechanisms of tolerance to high O₃ concentration within soybean germplasm. For future high O₃ and peroxides condition, SJ5 may not be a suitable cultivar since reduction in crop yield is high, but CM may have less damage to production and would be a suitable cultivar. Our results showed that CM could maintain biomass and pod yield, although it induced more severe damage on leaf injury and a greater decrease in the total chlorophyll content.

In terms of dry matter distribution, TC, A75 and SJ5 showed that dry matter of pod was decreased, while the fraction of dry matter of leaf, petiole and stem dry mass was increased in OP plots. Marcelis (1996) demonstrated that the dry matter partitioning among the sinks of a plant is primarily regulated by the sink organs and depends on plant genetics and environment. Sensitive cultivars could not produce pods and seeds, but could produce leaf and stem biomass.

5. Conclusion

Our results showed that single O₃ caused leaf injury, chlorophyll degradation, low stomatal conductance, and low photosynthetic rate, but single O₃ did not cause more total biomass reduction than the C plot. Several ppbv peroxides coexisting with 50 ppb O₃ induced a significant increase in leaf injury, and also a significant reduction in net photosynthetic rates and biomass than single 50 ppbv O₃. It was found that SJ5 is the most sensitive cultivar while CM was the least sensitive in both single O₃ and combined O₃ and peroxides from the perspective of pod yield. It was also found that the sensitive cultivar showed higher g_s and higher C_i than tolerant cultivars in each experimental plot on A versus g_s and A versus C_i relationships after 10 days of exposure.

In conclusion, the true injury and stress on physiological function in the field cannot be evaluated if peroxides are ignored, and the information about the cultivar sensitivity should be studied in more detail for future conditions of increasing peroxides and O₃ concentrations under global warming.

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