Experimental Studies on the Effects of Ozone on Growth and Photosynthetic Activity of Japanese Forest Tree Species

Masahiro Yamaguchi*, Makoto Watanabe[1], Hideyuki Matsumura[2], Yoshihisa Kohno[2] and Takeshi Izuta[3]

Faculty of Agriculture, Tokyo University of Agriculture and Technology, Fuchu, Tokyo 183-8509, Japan
[1]Research Faculty of Agriculture, Hokkaido University, Sapporo, Hokkaido 060-8589, Japan
[2]Environmental Science Research Laboratory, Central Research Institute of Electric Power Industry, Abiko, Chiba 270-1194, Japan
[3]Institute of Agriculture, Tokyo University of Agriculture and Technology, Fuchu, Tokyo 183-8509, Japan

*Corresponding author. Tel: +81-42-367-5728, E-mail: masah_ya@cc.tuat.ac.jp

ABSTRACT

Ozone (O_3) is a main component of photochemical oxidants, and a phytotoxic anthropogenic air pollutant. In North America and Europe, the current concentration of O_3 has been shown to have significant adverse effects on vegetation. In this review, we summarize the experimental studies on the effects of O_3 on the growth and photosynthetic activity of Japanese forest tree species to understand the present knowledge and provide sound basis for future research toward the assessment of O_3 impacts on Japanese forest ecosystem. Since the 1990s, several Japanese researchers have conducted the experimental studies on the effects of ambient levels of O_3 on growth and physiological functions such as net photosynthesis of Japanese forest tree species. Although the sensitivity to O_3 of whole-plant growth is quite different among the species, it was suggested that the current ambient levels of O_3 in Japan are high enough to adversely affect growth and photosynthetic activity of Japanese forest tree species classified into high O_3 sensitivity group such as Japanese beech. The N load to soil has been shown to reduce the sensitivity to O_3 of Japanese larch and increase that of Japanese beech. To establish the critical level of O_3 for protecting Japanese forest tree species, therefore, it is necessary to take into account the N deposition from the atmosphere. There is little information on the combined effects of O_3 and other environmental factors such as elevated CO_2 and drought on growth and physiological functions of Japanese forest tree species. Therefore, it is necessary to promote the experimental study and accumulate the information on the combined effects of O_3 and any other abiotic environmental factors on Japanese forest tree species.

1. INTRODUCTION

Ozone (O_3) is a main component of photochemical oxidants and produced by photochemical reaction of volatile organic compounds (VOCs) and nitrogen oxide (NO_x) (ADORC, 2006; EPA, 2006a). Ozone adversely affects not only human health but also vegetation (ADORC, 2006; EPA, 2006a). The emissions of precursor for O_3 from Asian countries have rapidly increased since the 1970s and surpassed the emissions from North America and Europe in the mid-1990s (Ohara *et al*., 2007; Akimoto, 2003). This situation is expected to continue for at least next couple decades (Klimont *et al*., 2001). In the near future, therefore, the concentration of ground-level O_3 is expected to increase especially in Asian countries including Japan (Yamaji *et al*., 2008; Dentener *et al*., 2006; Derwent *et al*., 2002; Emberson *et al*., 2001).

Since the ambient levels of O_3 in the USA and Europe negatively affect growth and physiological functions such as photosynthesis of forest tree species, this gas is considered as one of the important factors relating to forest decline and tree dieback in the relevant regions (Bytnerowicz *et al*., 2004; Chappelka and Samuelson, 1998; Skärby *et al*., 1998; Sandermann *et al*., 1997). In Japan, relatively high concentrations of O_3 above $100 \, nL \, L^{-1}$ (ppb) have been frequently observed from spring to autumn in several mountainous areas (Kohno *et al*., 2007; Takeda and Aihara, 2007; Aihara *et al*., 2004; Maruta *et al*., 1999). Based on the results of the experimental studies and field surveys, it has been suggested that O_3 is an important environmental stress relating to the forest decline in Japan (Kume *et*

al., 2009; Suto *et al.*, 2008; Takeda and Aihara, 2007; Yamaguchi *et al.*, 2007b; Kohno *et al.*, 2005; Aihara *et al.*, 2004; Yonekura *et al.*, 2001a, b; Maruta *et al.*, 1999).

In Japan, pioneer studies on the effects of O_3 on woody plants were published in the 1970s. Nouchi *et al.* (1973a, b) and Matsushima *et al.* (1977) mainly focused on O_3-induced visible injury and changes in the ultrastructural characteristics of leaves of several tree species. Kuno (1980, 1979), Furukawa *et al.* (1983), Fujinuma *et al.* (1987) and Furukawa (1991) reported the effects of O_3 on growth or physiological functions such as net photosynthesis of street trees. Matsumoto *et al.* (1992) reported the effects of O_3 at remarkably high concentrations on needle gas exchange rates of Japanese cedar (*Cryptomeria japonica*). Since the 1990s, the experimental studies on the effects of ambient levels of O_3 on growth, phenological characteristics and physiological functions such as net photosynthesis of Japanese forest tree species have started. To provide sound basis for future research toward the assessment of O_3 impacts on Japanese forest ecosystems, in this review, we summarize the experimental studies hitherto reported on the effects of O_3 on growth and photosynthetic activity of Japanese forest tree species.

2. EFFECTS OF O_3 ON GROWTH OF JAPANESE FOREST TREE SPECIES

Table 1 indicates the summary of experimental studies on the effects of O_3 on Japanese forest tree species. Miwa *et al.* (1993) reported that the exposure of Japanese cedar seedlings to relatively high O_3 concentration (300 ppb) did not induce significant reduction in the whole-plant dry mass, but induced significant increase in the ratio of the above-ground dry mass to root dry mass (Top/Root ratio). Izuta *et al.* (1996) reported that the whole-plant dry mass and root dry mass of Japanese beech (*Fagus crenata*) seedlings were reduced by the exposure to ambient levels of O_3 (75 and 150 ppb). Yonekura *et al.* (2001a, b) also reported that the exposure to ambient level of O_3 (60 ppb) reduced dry masses of root, leaf and stem, the whole-plant dry mass and annual ring width of Japanese beech seedlings. Nakaji and Izuta (2001) and Nakaji *et al.* (2004) reported that dry masses of needles and fine roots and the whole-plant dry mass of Japanese red pine (*Pinus densiflora*) seedlings were reduced by the exposure to ambient level of O_3 (60 ppb). Aforementioned studies were conducted using the steady-state O_3 exposure system. Because there are seasonal and diurnal variations in tropospheric O_3 concentration (Khiem *et al.*, 2010; EPA, 2006a; Yamaji *et al.*, 2006), O_3 exposure

system with the variations in the atmospheric concentration of O_3 observed in the fields needs to be used for the evaluation of the realistic effects of O_3 on forest tree species. Matsumura *et al.* (1996) and Matsumura *et al.* (1998) conducted experimental studies on the effects of O_3 on the growth of several Japanese forest tree species using an O_3 exposure system with seasonal and diurnal variations in the atmospheric concentration of O_3. In the study of Matsumura *et al.* (1996), the seedlings of Japanese cedar, Japanese cypress (*Chamaecyparis obtusa*) and Japanese zelkova (*Zelkova serrata*) were exposed to O_3 at 0.4, 1.0, 2.0 and 3.0 times the ambient concentration (12-h (6:00-18:00) average concentration of O_3: 16, 39, 74 and 114 ppb, respectively). The whole-plant dry mass of Japanese zelkova exposed to 2.0 and 3.0 times the ambient concentration of O_3 and that of Japanese cedar exposed to 3.0 times the ambient concentration of O_3 were significantly lower than those exposed to 0.4 times the ambient concentration of O_3, while there was no significant effect of O_3 on the whole-plant dry mass of Japanese cypress. In the study of Matsumura *et al.* (1998), the seedlings of Japanese cedar, Nikko fir (*Abies homolepis*), Japanese white birch (*Betula platyphylla*) and Japanese zelkova were exposed to O_3 at 0.4, 1.0, 2.0 and 3.0 times the ambient concentration (12-h (6:00-18:00) average concentration of O_3: 18, 37, 67 and 98 ppb, respectively). The whole-plant dry mass of Japanese cedar, Japanese white birch and Japanese zelkova were decreased linearly with increasing the concentration of O_3, while that of Nikko fir was not. These results indicate that the sensitivity to O_3 of the whole-plant growth is quite different among the Japanese forest tree species.

Aforementioned experimental studies were conducted within one growing season. However, there are several reports concerning the carry-over effects of O_3 on perennial plants. For example, the exposure to O_3 during one growing season changes phenological characteristics such as delay in the timing of bud break, and reduces leaf number per bud and growth in the following growing season (Yonekura *et al.*, 2004; Oksanen and Saleem, 1999; Andersen *et al.*, 1997; Pearson and Mansfield, 1994). Therefore, multi-year experiments are crucial to assessing the degree of adverse effects of O_3 on the growth of forest trees (Ashmore, 1993). Matsumura (2001) conducted the multi-year experiments. Young trees of 14 species were exposed to charcoal-filtered air (CF) or non-filtered air (NF) for three growing seasons at two different sites in Kanto districts of Japan (Chiba Prefecture and Gunma Prefecture; 12-h (6:00-18:00) seasonal mean concentration of O_3 (from April to September) during the experimental period in CF treatments: 8 and 12 ppb,

Table 1. Summary of experimental studies on the effects of O$_3$ on Japanese forest tree species.

Reference	Facility	Location	Treatments	Duration	Species	Age	O$_3$ Effects (Combined effects)
Miwa et al. (1993)	OTC	Tokyo	CF, 100 ppb, 200 ppb, 300 ppb (4 h/day, 3 days/week) Combined with acid rain	12 weeks/year in each of 2 years	Japanese cedar (Cryptomeria japonica)	2 yr	No effect on whole-plant dry mass. Increased Top/Root ratio (Additive). Decreased Chl. (Counteractive).
Matsumura et al. (1996)	Environment-controlled greenhouse	Chiba	0.4, 1.0, 2.0, 3.0× ambient O$_3$ (12-h ave.: 16, 39, 74, 114 ppb)	24 weeks/year in each of 3 years	Japanese cedar	2 yr	Decreased whole-plant dry mass, RGR, NAR, A and R_d.
					Japanese cypress (Chamaecyparis obtusa)	2 yr	No effect.
					Japanese zelkova (Zelkova serrata)	1 yr	Decreased whole-plant dry mass, RGR, NAR, A and R_d. Increased Top/Root ratio.
Izuta et al. (1996)	Environment-controlled growth cabinet	Tokyo	CF, 75 ppb, 150 ppb (6 h/day, 3 days/week)	18 weeks	Japanese beech (Fagus crenata)	3 yr	Decreased root and whole-plant dry mass, RGR, NAR, A, CE and Chl.
Matsumura et al. (1998)	Environment-controlled greenhouse	Chiba	0.4, 1.0, 2.0, 3.0× ambient O$_3$ (12-h ave.: 18, 37, 67, 98 ppb) Combined with acid rain	20 weeks	Japanese cedar	1 yr	Decreased whole-plant dry mass, A and A_{max} (Additive). Increased Top/Root ratio (Synergetic).
					Nikko fir (Abies homolepis)	5 yr	No effect on whole-plant dry mass. Increased R_d (Additive). Increased Top/Root ratio (Synergetic).
					Japanese white birch (Betula platyphylla)	1 yr	Decreased whole-plant dry mass, A, A_{max} and CE (Additive). Increased Top/Root ratio (Synergetic).
					Japanese zelkova	1 yr	Decreased whole-plant dry mass, A, A_{max} and CE (Additive). Increased Top/Root ratio (Synergetic).
Nakaji and Izuta (2001)	Naturally-lit growth chamber	Tokyo	CF, 60 ppb (8 h/day) Combined with N load (0, 135, 405 kg N ha^{-1} year^{-1})	173 days	Japanese red pine (Pinus densiflora)	1 yr	Decreased needle, fine root and whole-plant dry mass (Additive). Decreased ratio of fine root dry mass to shoot dry mass (Additive). Decreased A, CE and Rubisco conc. (Additive). No effect on g_s.
Yonekura et al. (2001a)	Naturally-lit growth chamber	Tokyo	CF, 60 ppb (7 h/day) Combined with water stress	156 days	Japanese beech	4 yr	Decreased dry masses of bud, leaf, stem, root and whole-plant, A, A_{max}, CE and Rubisco conc. (Additive). No effects on QY and g_s.
Yonekura et al. (2001b)	Naturally-lit growth chamber	Tokyo	CF, 60 ppb (7 h/day) Combined with water stress	156 days	Japanese beech	3 yr	Decreased annual ring width, A, CE, A_{max}, F_v/F_m, ψ_{pre} in the leaves and starch grain size in chloroplasts (Additive). No effect on QY, g_s and R_d. Increased diameter of plasto-globuli in chloroplasts.
Matsumura (2001)	Tunnel-type OTC	Gumma and/or Chiba	CF, NF (12-h ave.: 12 and 37 ppb at Gumma) (12-h ave.: 8 and 26 ppb at Chiba) Combined with acid mist	2 or 3 growing seasons	Japanese red pine	1 or 2 yr	Decreased shoot, root and whole-plant dry mass (Additive).
					Japanese black pine (Pinus thunbergii)	2 yr	No effect on whole-plant dry mass.
					Japanese larch (Larix kaempferi)	2 yr	Decreased shoot, root and whole-plant dry mass (Additive).
					Norway spruce* (Picea abies)	6 yr	Decreased root dry mass (Additive). No effect on whole-plant dry mass.

Table 1. continued.

Reference	Facility	Location	Treatments	Duration	Species	Age	O_3 Effects (Combined effects)
					Japanese fir (*Abies firma*)	6 yr	Decreased root dry mass (Additive). No effect on whole-plant dry mass.
					Nikko fir	5 yr	Decreased root and whole-plant dry mass (Additive).
					Veitch's silver fir (*Abies veitchii*)	5 yr	Decreased shoot, root and whole-plant dry mass (Synergetic or Additive).
					Japanese cypress	2 yr	Increased shoot and whole-plant dry mass (Additive).
					Japanese cedar	1 or 2 yr	Decreased root and whole-plant dry mass (Additive).
					Populus maximowiczii	4 yr	Decreased root dry mass (Additive).
					Japanese white birch	1 yr	Decreased shoot, root and whole-plant dry mass (Additive).
					Quercus mongolica	4 yr	No effect on whole-plant dry mass.
					Japanese beech	1 yr	Decreased root and whole-plant dry mass (Synergetic or Additive).
					Japanese zelkova	1 yr	Decreased shoot, root and whole-plant dry mass (Additive).
Nakaji *et al.* (2004)	Naturally-lit growth chamber	Tokyo	CF, 60 ppb (7 h/day) Combined with N load (0, 90, 180 kg N ha⁻¹ year⁻¹)	2 growing seasons	Japanese red pine	1 yr	Decreased dry masses of stem and whole-plant, A and Rubisco conc. (Synergetic). No effect on activities of NR and NiR and conc. of inorganic-N, amino acid-N and protein-N.
Yonekura *et al.* (2004)	Naturally-lit growth chamber	Tokyo	CF, 60 ppb (7 h/day) Combined with water stress	1 year	Japanese beech (*Fagus crenata*)	3 yr	Acceleration of leaf abscision, delay in bud break and decreased leaf no. per bud (Additive).
Watanabe *et al.* (2005)	Naturally-lit growth chamber	Tokyo	CF, 60 ppb (7 h/day) Combined with water stress	2 growing seasons	Japanese beech	3 yr	Decreased A (Counteractive). Decreased conc. of Rubisco and Chl. (Additive).
Matsumura *et al.* (2005)	Square-greenhouse type OTC	Gunma	CF or 1.0, 1.5 × ambient O_3 (12-h ave.: 17, 43, 66 ppb) Combined with elevated CO_2 (12-h ave.: 377 and 544 ppm)	2 growing seasons	Japanese white birch (*Betula platyphylla*)	2 yr	Decreased whole-plant dry mass (Counteractive).
					Japanese mountain birch (*Betula ermanii*)	3 yr	No effect on whole-plant dry mass.
					Japanese beech	4 yr	Decreased shoot, root and whole-plant dry mass (Additive).
					Japanese red pine (*Pinus densiflora*)	2 yr	Decreased shoot and whole-plant dry mass (Additive).
					Japanese cedar (*Cryptomeria japonica*)	2 yr	No effect on whole-plant dry mass.
Watanabe *et al.* (2006)	Square-greenhouse type OTC	Gunma	CF or 1.0, 1.5, 2.0 × ambient O_3 (24-h ave.: 12, 43, 63, 84 ppb) Combined with N load (0, 20, 50 kg N ha⁻¹ year⁻¹)	2 growing seasons	Japanese red pine	2 yr	Decreased dry masses of needle, stem, root and whole-plant and A (Additive).
					Japanese larch (*Larix kaempferi*)	3 yr	Decreased dry masses of stem and whole-plant (Counteractive). Decreased dry masses of needle and root and A (Additive).

Table 1. continued.

Reference	Facility	Location	Treatments	Duration	Age	Species	O_3 Effects (Combined effects)
					2 yr	Japanese cedar	Decreased dry masses of needle, stem, root and whole-plant (Additive). No effect on A.
Yamaguchi *et al.* (2007a)	Square-greenhouse type OTC	Gunma	CF or 1.0, 1.5, 2.0 × ambient O_3 (24-h ave.: 13, 43, 64, 85 ppb) Combined with N load (0, 20, 50 kg N ha⁻¹ year⁻¹)	1 growing season	2 yr	Japanese beech	Decreased dry masses of stem, root and whole-plant (Additive). Decreased A, CE and conc. of Rubisco, TSP and nonpolar amino acid in leaves (Additive). Increased conc. of acidic amino acid in leaves (Additive).
Yamaguchi *et al.* (2007b)	Square-greenhouse type OTC	Gunma	CF or 1.0, 1.5, 2.0 × ambient O_3 (24-h ave.: 12, 43, 63, 84 ppb) Combined with N load (0, 20, 50 kg N ha⁻¹ year⁻¹)	2 growing seasons	2 yr	Japanese beech	Decreased dry masses of leaf, branch and whole-plant (Synergetic). Decreased dry masses of stem and root and R/S ratio (Additive). Decreased A and PNUE (Synergetic or Additive). Decreased activity or conc. of Rubisco (Synergetic). Increased g_s and N_{area} (Additive or Counteractive)
Watanabe *et al.* (2007)	Square-greenhouse type OTC	Gunma	CF or 1.0, 1.5, 2.0 × ambient O_3 (24-h ave.: 12, 43, 63, 84 ppb) Combined with N load (0, 20, 50 kg N ha⁻¹ year⁻¹)	2 growing seasons	2 yr	*Quercus serrata*	Decreased dry masses of leaf, stem, root and whole-plant, A and PNUE (Additive or Synergetic). Decreased E, WUE and conc. of Rubisco, TSP and Chl (Additive). Decreased Rubisco activity (Synergetic or Additive).
Watanabe *et al.* (2008)	Square-greenhouse type OTC	Gunma	CF or 1.0, 1.5, 2.0 × ambient O_3 (24-h ave.: 12, 43, 63, 84 ppb) Combined with N load (0, 20, 50 kg N ha⁻¹ year⁻¹)	2 growing seasons	2 yr	*Castanopsis sieboldii*	Decreased dry masses of leaf, stem and whole-plant (Additive). Increased 2nd-flush leaf dry mass (Synergetic). Decreased root dry mass and A (Synergetic or Additive). Increased no. of shed leaves and A (Additive).
Yamaguchi *et al.* (2010)	Square-greenhouse type OTC	Gunma	CF or 1.0, 1.5, 2.0 × ambient O_3 (24-h ave.: 12, 43, 63, 84 ppb) Combined with N load (0, 20, 50 kg N ha⁻¹ year⁻¹)	2 growing seasons	2 yr	Japanese beech	Decreased NR activity, TSP conc. and TSP/N ratio (Synergetic). Increased acidic amino acid conc. (Additive). No effects on inorganic-N conc., activities of NiR and GS and ratios of inorganic-N/N and amino acid-N/N.
Watanabe *et al.* (2010)	Environment-controlled growth cabinet	Tokyo	CF, 100 ppb (6 h/day, 3 days/week) Combined with elevated CO_2 (350 and 700 ppm)	18 weeks	2 yr	Japanese beech	Increased dry masses of 2nd-flush leaf, branch, coarse root and whole-plant and 2nd-flush leaf area (Synergetic). Increased fine root dry mass and LMR (Additive).

A: net photosynthetic rate; A_{max}: CO_2-saturated net photosynthetic rate; CE: carboxylation efficiency; CF: charcoal-filtered air treatment; Chl: chlorophyll concentration; E: transpiration rate; F_v/F_m: maximum quantum yield of photosystem II; GS: glutamine synthetase; g_s: stomatal diffusive conductance to H_2O; LMR: ratio of leaf mass to whole-plant dry mass; NAR: net assimilation rate; N_{area}: leaf N content per unit leaf area; NF: non-filtered air treatment; NiR: nitrite reductase; NR: nitrate reductase; OTC: open-top chamber; PNUE: photosynthetic nitrogen use efficiency; QY: quantum yield; R_d: dark respiration rate; RGR: relative growth rate; R/S ratio: ratio of root dry mass to shoot dry mass; Rubisco: ribulose 1,5-bisphosphate carboxylase/oxygenase; TSP: total soluble protein; WUE: water use efficiency; ψ_{pw}: leaf water potential at predawn. *: Norway spruce is not native to Japan.
Elevation, latitude and longitude of the locations: 60 m a.s.l., 35° 41'N and 139° 29'E for Tokyo, respectively; 25 m a.s.l., 35° 52'N and 140° 01'E for Chiba, respectively; 540 m a.s.l., 36° 28'N and 139° 11'E for Gunma, respectively.

Table 2. Classification of Japanese forest tree species into the O_3 sensitivity groups (after Kohno *et al.*, 2005, with permission).

O_3 sensitivity	Type		Species	Critical level (Daylight AOT40)
High	Broad-leaved	Deciduous	*Populus maximowiczii, Populus nigra*, Japanese beech, Japanese zelkova	8-15 ppm h
		Evergreen	*Castanopsis sieboldii*	
	Coniferous	Deciduous	Japanese larch	
		Evergreen	Japanese red pine	
Moderate	Broad-leaved	Deciduous	*Quercus serrata*, Japanese white birch	16-30 ppm h
		Evergreen	*Quercus myrsinaefolia, Cinnamomum camphora*	
	Coniferous	Evergreen	Nikko fir	
Low	Broad-leaved	Deciduous	*Quercus mongolica* var. *grosseserrata*	31 ppm h<
		Evergreen	*Lithocarpus edulis, Machilus thunbergii*	
	Coniferous	Evergreen	Japanese black pine, Japanese cedar Japanese cypress	

High O_3 sensitivity: The whole-plant dry mass increment was significantly reduced by the exposure to ambient level of O_3.
Moderate O_3 sensitivity: The whole-plant dry mass increment was significantly reduced by the exposure to 1.5 or 2.0 times ambient level of O_3.
Low O_3 sensitivity: The whole-plant dry mass increment was not significantly reduced by the exposure to 1.5 or 2.0 times ambient level of O_3.
Daylight AOT40: accumulated exposure over a threshold of 40 ppb O_3 during daylight hours from April to September (6 months).
Daylight hour: global radiation > 50 W m^{-2}.

respectively; those in NF treatments: 26 and 37 ppb, respectively). The ambient levels of O_3 reduced the whole-plant dry mass of Japanese red pine, Japanese larch (*Larix kaempferi*), Veitch's silver fir (*Abies veitchii*), Japanese white birch, Japanese beech and Japanese zelkova at the both sites. Kohno *et al.* (2005) summarized several results of experimental studies conducted for multiple growing seasons on the effects of O_3 on forest tree species (e.g. Matsumura, 2001; Matsumura and Kohno, 1999). The sensitivity of each tree species to O_3 was classified into 3 groups (high, moderate and low) based on the response of the whole-plant dry mass growth to O_3 (Table 2). For example, Japanese larch and Japanese beech have been classified into high O_3 sensitivity group; Japanese white birch and Nikko fir have been classified into moderate O_3 sensitivity group; Japanese cedar and Japanese cypress have been classified into low O_3 sensitivity group. Recently, Takeda and Aihara (2007) showed that O_3 negatively affects growth and photosynthetic parameters of Japanese beech grown under field condition at Tanzawa Mountains where the decline of Japanese beech forest has been reported. Kume *et al.* (2009) suggested the possibility that recent increase in the atmospheric concentration of O_3 is an important factor of Japanese beech decline at Mt. Tateyama based on the results of their field survey. These results and those obtained from the experimental studies clearly indicate that current ambient levels of O_3 in Japan are high enough to adversely affect growth of Japanese forest tree species especially in those classified into high O_3 sensitivity group such as Japanese beech.

3. EFFECTS OF O_3 ON THE PHOTOSYNTHETIC ACTIVITY AND OTHER RELATED FUNCTIONS OF JAPANESE FOREST TREE SPECIES

Izuta *et al.* (1996) and Matsumura *et al.* (1996) reported that relative growth rates (RGRs) of Japanese beech, Japanese cedar and Japanese zelkova were reduced by the exposure to O_3. In their studies, the O_3-induced reductions in net assimilation rate (NAR) and net photosynthetic rate of the leaves or needles were reported. These results indicate that the O_3-induced reduction in the growth was mainly due to that in the net photosynthetic rate of the leaves or needles. It was also reported that the exposure to O_3 reduced the net photosynthetic rate of the leaves or needles of Japanese white birch, Japanese red pine, Japanese larch, *Q. serrata* and *C. sieboldii* (Watanabe *et al.*, 2008, 2007, 2006; Nakaji *et al.*, 2004; Nakaji and Izuta, 2001; Matsumura *et al.*, 1998). When net photosynthetic rate was reduced by the exposure to O_3, simultaneous reductions in the carboxylation efficiency (CE), CO_2-saturated net photosynthetic rate (A_{max}) and/or maximum quantum yield of photosystem (PS) II (F_v/F_m), and increase in the stomatal diffusive conductance to water vapor (g_s) in the leaves or needles of Japanese forest tree species were also observed (Yamaguchi *et al.*, 2007a; Watanabe *et al.*, 2005; Nakaji and Izuta,

2001; Yonekura *et al.*, 2001a, b; Matsumura *et al.*, 1998, 1996; Izuta *et al.*, 1996). It was documented that the exposure to O$_3$ reduced the concentration and activity of ribulose 1,5-bisphosphate carboxylase/oxygenase (Rubisco) and chlorophyll concentration in the leaves or needles of Japanese forest tree species (Watanabe *et al.*, 2007, 2005; Yamaguchi *et al.*, 2007a, b; Nakaji *et al.*, 2004; Nakaji and Izuta, 2001; Yonekura *et al.*, 2001a; Izuta *et al.*, 1996; Miwa *et al.*, 1993). Yonekura *et al.* (2001b) reported that the O$_3$-induced reduction in net photosynthetic rate was firstly due to the reduction in the quantity and/or activity of Rubisco in the leaves of Japanese beech. Therefore, there is a possibility that the exposure to O$_3$ firstly reduces the capacity of carbon fixation in the chloroplasts resulting in the reduction in net photosynthetic rate of the leaves or needles of Japanese forest tree species.

Proteins such as Rubisco in the leaves or needles represent the predominant N fraction (Feller, 2004; Spreitzer and Salvucci, 2002). Ozone exposure has been shown to reduce the concentration of total soluble protein (TSP) in the leaves of *Q. serrata* and Japanese beech (Watanabe *et al.*, 2007; Yamaguchi *et al.*, 2007a, b). Watanabe *et al.* (2007) and Yamaguchi *et al.* (2007b) reported that the exposure to O$_3$ reduced photosynthetic nitrogen use efficiency (*PNUE*) in the leaves of *Q. serrata* and Japanese beech. In the case of Japanese beech, the exposure to O$_3$ did not significantly affect N concentration in the leaves, suggesting that O$_3$ induces alterations in foliar N metabolism and also a reduction in the availability of N for photosynthesis in the leaves (Yamaguchi *et al.*, 2007b). There is limited information on the effects of O$_3$ on N metabolism in the leaves or needles of Japanese forest tree species (Yamaguchi *et al.*, 2010, 2007a; Nakaji *et al.*, 2004). Nakaji *et al.* (2004) reported that the exposure to O$_3$ did not significantly affect the activities of nitrate reductase (NR) and nitrite reductase (NiR) and concentrations of inorganic N compounds (NO$_3^-$, NO$_2^-$ and NH$_4^+$) and free amino acid in the needles of Japanese red pine. On the other hand, Yamaguchi *et al.* (2010, 2007a) reported the O$_3$-induced inhibition of resorption of N from the leaves in autumn, reductions in the NR activity and the ratio of TSP concentration to N concentration and increase in the concentration of acidic amino acid in the leaves of Japanese beech. At the present time, it is unclear how O$_3$ affects N metabolism in the leaves of Japanese forest tree species. To clarify the mechanisms underlying the detrimental effects of O$_3$ on Japanese forest tree species, therefore, further research concerning the effects of O$_3$ on physiological functions such as foliar N metabolism is required.

4. COMBINED EFFECTS OF O$_3$ AND OTHER ABIOTIC ENVIRONMENTAL FACTORS ON JAPANESE FOREST TREE SPECIES

Izuta (2002, 1998) and Izuta *et al.* (2001) reviewed experimental studies on the combined effects of O$_3$ and simulated acid rain on Japanese forest tree species. Recently, it was pointed out that the interactive effects of O$_3$, N deposition, elevated carbon dioxide (CO$_2$) and climate change such as drought stress must be key issues to predict forest future in the changing environment (Paoletti *et al.*, 2010). In this section, we focused on the combined effects of O$_3$ and N load to soil, elevated CO$_2$ or drought on growth, photosynthetic activity and other related functions of Japanese forest tree species.

4.1 Nitrogen Load to Soil

Atmospheric deposition of N to terrestrial ecosystems has been increasing with elevated anthropogenic emissions of N since the industrial revolution (Richter *et al.*, 2005; Galloway *et al.*, 2004, 2003; IPCC, 2001). Because N is a limiting nutrient for plant growth in terrestrial ecosystems (Vitousek and Howarth, 1991), an increase in N input to forest ecosystems generally stimulates tree growth. However, many researchers suggested that excessive deposition of N such as nitrate and ammonium from the atmosphere to forest ecosystems might induce soil acidification, modify tree nutrient status and increase the sensitivity of trees to other environmental stresses such as gaseous air pollutants (Aber *et al.*, 1989; Schulze, 1989; Nihlgård, 1985).

Based on the monitoring data and estimations of O$_3$ concentration and atmospheric N deposition in East Asia (Network Center for EANET, 2011; Yamaji *et al.*, 2006; Kohno *et al.*, 2005), there is the possibility that forest tree species are adversely affected not only by O$_3$, but also by excessive N deposition in East Asian countries including Japan. In the experimental studies of Watanabe *et al.* (2008, 2007, 2006) and Yamaguchi *et al.* (2010, 2007b), seedlings of *Q. serrata*, Japanese beech, *C. sieboldii*, Japanese red pine, Japanese larch and Japanese cedar were grown in potted soil supplied with N as NH$_4$NO$_3$ solution at 0, 20 and 50 kg ha^{-1} year^{-1} and simultaneously exposed to charcoal-filtered air or O$_3$ at 1.0, 1.5 and 2.0 times the ambient concentration for two growing seasons (24-h seasonal mean concentration of O$_3$ (from April to September) during the experimental period: 12, 43, 63 and 84 ppb, respectively). Watanabe *et al.* (2008, 2007, 2006) reported the additive effects of O$_3$ and N load on growth of the seedlings of *Q. serrata*, *C. sieboldii*,

Japanese red pine and Japanese cedar. On the other hand, significant interactive effects of O_3 and N load on growth were detected in Japanese larch and Japanese beech (Yamaguchi *et al.*, 2007b; Watanabe *et al.*, 2006). The relationships between relative whole-plant dry mass increment of Japanese larch or Japanese beech and daylight AOT40 of O_3 (accumulated exposure over a threshold of 40 ppb during daylight hours (global radiation > 50 W m^{-2}), see Fig. 1) were shown
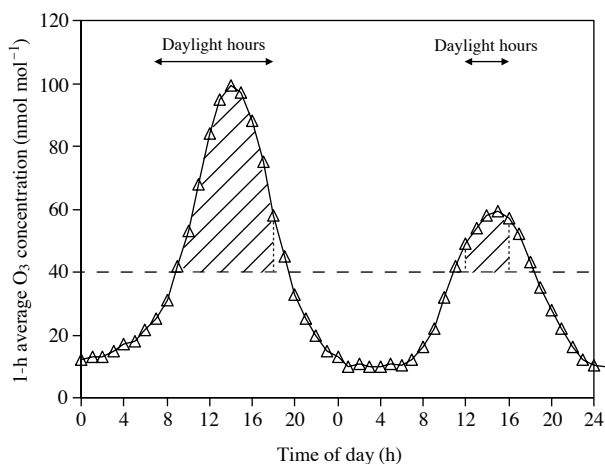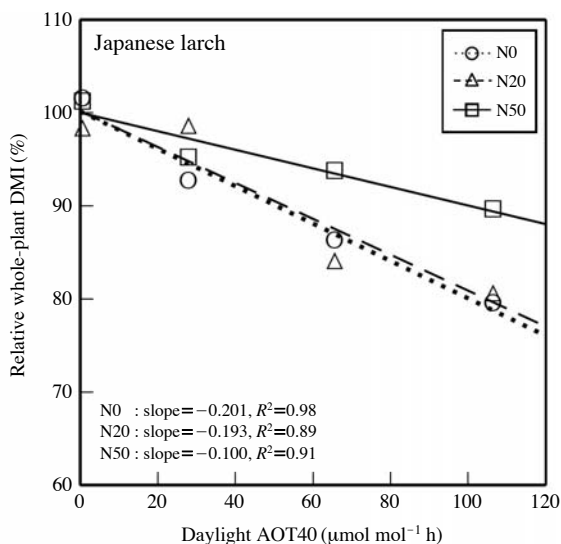
in Fig. 2. Daylight AOT40 is the sum of the difference between the hourly mean O_3 concentration and 40 ppb for all daylight hours (shaded area in Fig. 1) within a specified time period (from April to September in this case). The calculation of the relationships was based on the method of Karlsson *et al.* (2004). The coefficient of determination (R^2) obtained from linear regression analysis and the slope of regression line in each N treatment are indicated in Fig. 2. The absolute value of the slope of regression line indicates the sensitivity to O_3 of whole-plant dry mass growth of the seedlings. While the N load to soil reduced the sensitivity to O_3 of whole-plant dry mass growth of Japanese larch (Watanabe *et al.*, 2006), it increased that of Japanese beech (Yamaguchi *et al.*, 2007b). These results indicate that the combined effect of O_3 and N load on growth is quite different among the Japanese forest tree species.

Nakaji and Izuta (2001), Nakaji *et al.* (2004) and Watanabe *et al.* (2006) reported that the N load to soil did not change the degree of O_3-induced reduction in net photosynthetic rate in the needles of Japanese red pine and Japanese larch. In contrast, the degrees of O_3-induced reduction in net photosynthetic rate of *Q. serrata*, Japanese beech and *C. sieboldi* became high with increasing the amount of N load to soil (Watanabe *et al.*, 2008, 2006; Yamaguchi *et al.*, 2007b). To clarify the mechanisms underlying the combined effects of O_3 and N load on net photosynthesis of Japanese forest tree species, Yamaguchi *et al.* (2010, 2007b) investigated the effects of O_3 and N load on the concentration
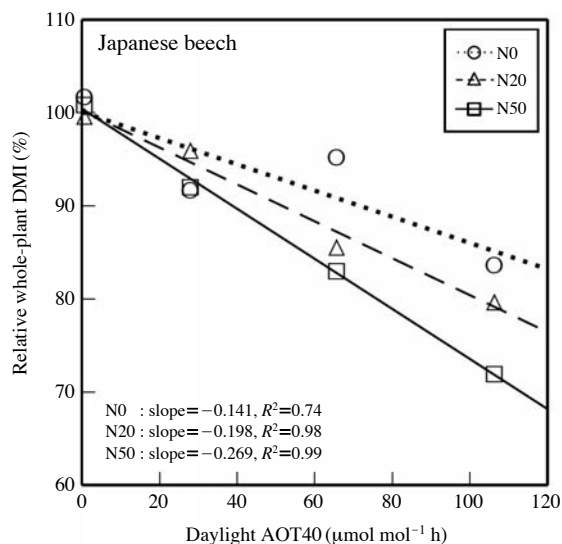

Fig. 1. Conceptual diagram for the calculation of daylight AOT40 of O_3 (accumulated exposure over a threshold of 40 ppb, nmol mol^{-1} h or µmol mol^{-1} h). Shaded area contributes to daylight AOT40. Daylight hour: global radiation > 50 W m^{-2}.

Fig. 2. The relationships between relative whole-plant dry mass increment (DMI) of Japanese larch and Japanese beech seedlings per one growing season and daylight AOT40 of O_3. The seedlings were grown in the soil supplied with N as NH_4NO_3 solution at 0 (N0), 20 (N20) or 50 kg ha^{-1} year^{-1} (N50) and simultaneously exposed to charcoal-filtered air or O_3 at 1.0, 1.5 and 2.0 times ambient concentration. Data source: Watanabe *et al.* (2006) and Yamaguchi *et al.* (2007a, b).
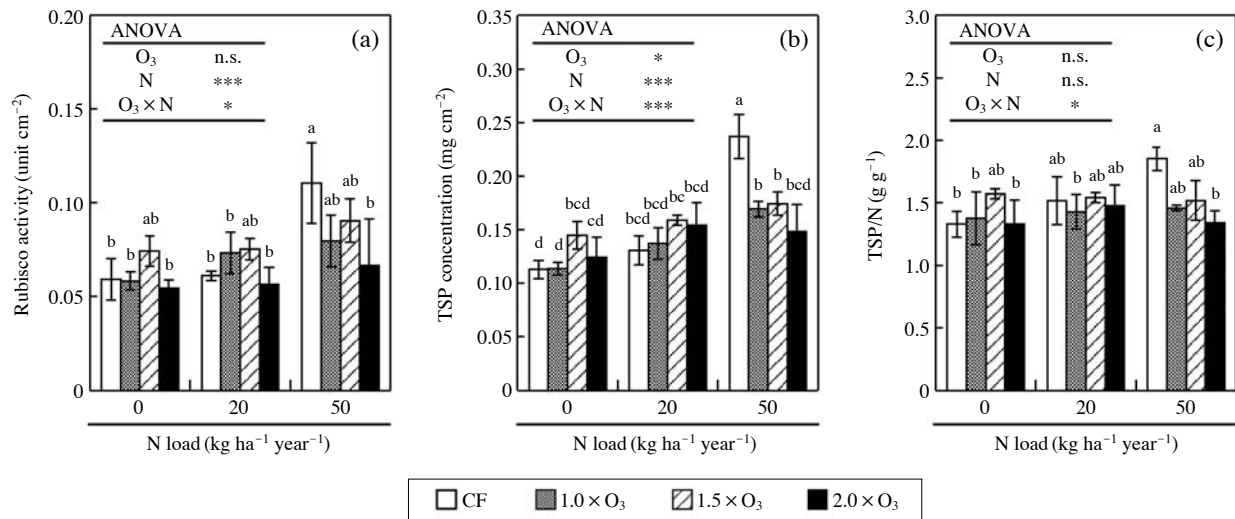
Fig. 3. Effects of O_3 and N load on activity of ribulose 1,5-bisphosphate carboxylase/oxygenase (Rubisco), concentration of total soluble protein (TSP) and ratio of TSP to leaf N content per unit leaf area (TSP/N) in the leaves of Japanese beech. The standard deviation is given by vertical bar. Two-way ANOVA: *$p < 0.05$, ***$p < 0.001$, n.s.=not significant. Different letters above the bar indicate significant difference among the 12 treatments (Tukey's HSD test, $p < 0.05$). Data source: Yamaguchi *et al.* (2010, 2007b).

and activity of Rubisco, enzyme activity of N metabolism and concentrations of amino acid and soluble protein in the leaves of Japanese beech seedlings. In their studies, the seedlings of Japanese beech were grown in the soil supplied with N as NH_4NO_3 solution at 0, 20 or 50 kg ha^{-1} year^{-1} and simultaneously exposed to charcoal-filtered air (CF) or O_3 at 1.0, 1.5 and 2.0 times ambient concentration. The exposure to O_3 significantly reduced the concentration and activity of Rubisco in the leaves of the seedlings grown in relatively high N load treatment, but not in relatively low N load treatment (Fig. 3a). This result indicates that the interactive effect of O_3 and N load on net photosynthetic rate is mainly attributed to the difference in the degrees of O_3-induced reduction in the amount of Rubisco among the N treatments (Yamaguchi *et al.*, 2007b). Furthermore, the exposure to O_3 reduced the concentration of TSP and the ratio of TSP concentration to leaf N concentration in relatively high N load treatment, but not in relatively low N load treatment (Fig. 3b and c). Therefore, Yamaguchi *et al.* (2010) concluded that the exposure to O_3 reduced the allocation of N to soluble protein in the leaves of Japanese beech seedlings grown under relatively high N load, but did not in the leaves of the seedlings grown under a relatively low N load.

4. 2 Elevated CO_2 and Drought

Elevated CO_2 and drought are well known to affect the sensitivity of forest tree species to O_3 (e.g. EPA,

2006b). Unfortunately, there is little information on the combined effects of O_3 and elevated CO_2 or soil water stress on Japanese forest tree species (Watanabe *et al.*, 2010, 2005; Matsumura *et al.*, 2005; Yonekura *et al.*, 2001a, b).

Matsumura *et al.* (2005) reported that the effect of elevated CO_2 on O_3-induced reduction in growth was counteractive in Japanese white birch seedlings, while not in the Japanese mountain birch (*Betula ermanii*), Japanese beech, Japanese red pine and Japanese cedar seedlings. This result indicates that the combined effect of O_3 and elevated CO_2 is different among Japanese forest tree species. On the other hand, Watanabe *et al.* (2010) reported that the simultaneous exposure to O_3 and elevated CO_2 induced marked growth stimulation of Japanese beech seedlings as compared with those exposed to elevated CO_2. Yonekura *et al.* (2001a, b) reported the additive effects of O_3 and soil water stress on the growth of Japanese beech seedlings. On the other hand, Watanabe *et al.* (2005) reported that chronic soil water stress counteracted the negative effects of O_3 on net photosynthesis of the leaves of Japanese beech seedlings. Combined effects of O_3 and other environmental factors such as elevated CO_2 and drought on growth and physiological functions of Japanese forest tree species are still poorly understood. Therefore, it is necessary to promote the experimental study and accumulate the information on the combined effects of O_3 and any other abiotic environmental factors on growth, physiological functions and nutrient

status of Japanese forest tree species.

5. CRITICAL LEVEL OF O_3 FOR PROTECTING JAPANESE FOREST TREE SPECIES

The ambient levels of O_3 in Japan have been shown to adversely affect growth and photosynthetic activity of Japanese forest tree species especially in those classified into high O_3 sensitivity group as mentioned above. In Europe, the concept of critical level has been developed to prevent long-term injury and damage of air pollutants to the receptors such as plants (Mills *et al.*, 2010). The critical levels for vegetation are defined as the concentration, cumulative exposure or cumulative stomatal flux of atmospheric pollutants above which direct adverse effects on sensitive vegetation may occur according to present knowledge (Mills *et al.*, 2010). At the present time, to define the concentration-based critical levels for O_3, AOT40 has been adopted for use within the United Nations Economic Commission for Europe (UNECE) Convention of Long-Range Trans-boundary Air Pollution (CLRTAP) and the European Union (Mills *et al.*, 2010; Ashmore *et al.*, 2004). As a result of much efforts directing to establishing the critical level of O_3, critical level for forest trees has been defined as 5 μmol mol^{-1} h (ppm h) of daylight AOT40 accumulated over a six-month growing season (from April to September) associated with a 5% growth reduction per one growing season for sensitive deciduous tree species native to Europe such as European beech (*Fagus sylvatica*) and European birch (*Betula pendula*) (Mills *et al.*, 2010; Karlsson *et al.*, 2004). Because the vegetation and climatic condition in Japan is quite different from that in Europe, critical level of O_3 for forest tree species in Europe is not directly applicable to that in Japan (Kohno *et al.*, 2005). Kohno *et al.* (2005) proposed that provisional critical level of O_3 for Japanese forest tree species classified into the high O_3 sensitivity group such as Japanese larch and Japanese beech is 8-15 ppm h of daylight AOT40 accumulated over one growing season (from April to September) associated with a 10% reduction in the increment of the whole-plant dry mass per one growing season (Table 2). However, N deposition from the atmosphere should be taken into account to evaluate the critical level of O_3 for protecting Japanese forest tree species, because the sensitivities of Japanese larch and Japanese beech to O_3 are influenced by the amount of N load to soil (Yamaguchi *et al.*, 2007b; Watanabe *et al.*, 2006). Furthermore, as indicated by Matsumura *et al.* (2005) and Watanabe *et al.* (2010), it is necessary to take into account the en-

vironmental factors such as atmospheric CO_2 concentration to evaluate the critical level of O_3. To establish the critical level of O_3 for protecting Japanese forest tree species, therefore, further research concerning the combined effects of O_3 and other abiotic environmental factors on the growth of Japanese forest tree species is required.

Ozone enters the leaf through the stomata and then injures cellular components such as plasma membrane (Nouchi, 2002). Since the real impacts of O_3 mainly depend on the amount of O_3 reaching the sites of damage within the leaf, cumulative flux or uptake of O_3 through the stomata and associated response functions are suitable for mapping and quantifying impacts of O_3 at the local and regional scale (Mills *et al.*, 2010). Therefore, atmospheric concentration-based critical level of O_3 expressed as AOT40 can be used only for estimating the risk of damage. The approach based on the O_3 flux into leaves or needles requires the development of mathematical models to estimate stomatal O_3 uptake primarily from the knowledge of stomatal responses to environmental factors (e.g. Emberson *et al.*, 2000a, b). At the present time, however, there is limited information on stomatal flux of O_3 into the leaves or needles of Japanese forest tree species (Hoshika *et al.*, 2009). Therefore, it is necessary to promote the research toward the modeling of stomatal flux of O_3 for the final purpose of mapping and quantifying the impacts of O_3 on Japanese forest tree species.

6. CONCLUSION AND PERSPECTIVES

Based on the results obtained from the experimental studies, the current levels of O_3 in Japan are high enough to adversely affect growth of Japanese forest tree species with relatively high O_3 sensitivity such as Japanese beech. To protect Japanese forest, therefore, we need to establish the critical level of O_3, primarily using AOT40 index, for Japanese forest tree species with consideration of other abiotic environmental factors affecting the sensitivity to O_3 such as N deposition from the atmosphere. In addition to the estimating the risk of damage of O_3 using AOT40 index, it is necessary to quantify the impacts of O_3 on Japanese forest tree species. For this purpose, it is necessary to promote the research toward the modeling of stomatal flux of O_3 into the leaves or needles of Japanese forest tree species. Furthermore, Kohno *et al.* (2005) pointed out whether results obtained from experimental studies on the effects of O_3 on the growth of Japanese forest tree species using the seedlings could be applicable to the evaluation of O_3-induced adverse effects on the growth of mature trees grown under natural conditions

or not. To understand and evaluate the actual impacts of O₃ on the growth and physiological functions of Japanese forest tree species grown in the field, therefore, further research is required for the scaling effects of O₃ from seedlings to mature forest trees.

ACKNOWLEDGEMENTS

REFERENCES

Aber, J.D., Nadelhoffer, K.J., Steudler, P., Melillo, J.M. (1989) Nitrogen saturation in Northern forest ecosystems. Bioscience 39, 378-386.

ADORC (Acid Deposition and Oxidant Research Center) (2006) Tropospheric ozone: A growing threat. ADORC (Acid Deposition and Oxidant Research Center), Niigata, Japan, pp. 1-16.

Aihara, K., Aso, T., Takeda, M., Koshiji, T. (2004) Actual condition of forest decline and approach (II) The phenomena of forest decline at Tanzawa Mountain in Kanagawa prefecture. Journal of Japan Society for Atmospheric Environment 39, A29-A39. (in Japanese)

Akimoto, H. (2003) Global air quality and pollution. Science 302, 1716-1719.

Andersen, C.P., Wilson, R., Plocher, M., Hogsett, W.E. (1997) Carry-over effects of ozone on root growth and carbohydrate concentrations of ponderosa pine seedlings. Tree Physiology 17, 805-811.

Ashmore, M. (1993) Critical levels for ozone. In Critical levels of air pollutants for Europe (Ashmore, M. and Wilson, R.B. Eds), Egham, U. K, pp. 20-47.

Ashmore, M., Emberson, L., Karlsson, P.E., Pleijel, H. (2004) New directions: A new generation of ozone critical levels for the protection of vegetation in Europe. Atmospheric Environment 38, 2213-2214.

Bytnerowicz, A., Godzik, B., Grodzinska, K., Fraczek, W., Musselman, R., Manning, W., Badea, O., Popescu, F., Fleischer, P. (2004) Ambient ozone in forests of the Central and Eastern European Mountains. Environmental Pollution 130, 5-16.

Chappelka, A.H., Samuelson, L.J. (1998) Ambient ozone effects on forest trees of the Eastern United States: A review. New Phytologist 139, 91-108.

Dentener, F., Stevenson, D., Ellingsen, K., van Noije, T., Schultz, M., Amann, M., Atherton, C., Bell, N., Bergmann, D., Bey, I., Bouwman, L., Butler, T., Cofala, J., Collins, B., Drevet, J., Doherty, R., Eickhout, B., Eskes, H., Fiore, A., Gauss, M., Hauglustaine, D., Horowitz, L., Isaksen, I.S.A., Josse, B., Lawrence, M., Krol, M.,

Lamarque, J.F., Montanaro, V., Müller, J.F., Peuch, V.H., Pitari, G., Pyle, J., Rast, S., Rodriguez, J., Sanderson, M., Savage, N.H., Shindell, D., Strahan, S., Szopa, S., Sudo, K., van Dingenen, R., Wild, O., Zeng, G. (2006) The global atmospheric environment for the next generation. Environmental Science and Technology 40, 3586-3594.

Derwent, R., Collins, W., Johnson, C., Stevenson, D. (2002) Global ozone concentrations and regional air quality. Environmental Science and Technology 36, 379-382.

Emberson, L.D., Wieser, G., Ashmore, M.R. (2000a) Modelling of stomatal conductance and ozone flux of Norway spruce: comparison with field data. Environmental Pollution 109, 393-402.

Emberson, L.D., Ashmore, M.R., Cambridge, H.M., Simpson, D., Tuovinen, J.-P. (2000b) Modelling stomatal ozone flux across Europe. Environmental Pollution 109, 403-413.

Emberson, L.D., Ashmore, M.R., Murray, F., Kuylenstierna, J.C.I., Percy, K.E., Izuta, T., Zheng, Y., Shimizu, H., Sheu, B.H., Liu, C.P., Agrawal, M., Wahid, A., Abdel-Latif, N.M., van Tienhoven, A.M., de Bauer, L.I., Domingos, M. (2001) Impacts of air pollutants on vegetation in developing countries. Water, Air, & Soil Pollution 130, 107-118.

EPA (Environmental Protection Agency) (2006a) Air quality criteria for ozone and related photochemical oxidants Vol. I, U.S. Environmental Protection Agency, Research Triangle Park, NC, USA, pp. E-1-34.

EPA (Environmental Protection Agency) (2006b) Air quality criteria for ozone and related photochemical oxidants Vol. III, U.S. Environmental Protection Agency, Research Triangle Park, NC, USA, pp. AX9-87-155.

Feller, U. (2004) Proteolysis. In Plant Cell Death Processes (Noodén, L.D. Eds.), Elsevier Science, California, USA, pp. 107-124.

Fujinuma, Y., Furukawa, A., Totsuka, T., Tazaki, T. (1987) Uptake of O₃ by various street trees. Environment Control in Biology 25, 31-39.

Furukawa, A., Katase, M., Ushijima, T., Totsuka, T. (1983) Inhibition of photosynthesis of poplar species by ozone. Journal of the Japanese Forestry Society 65, 321-326.

Furukawa, A. (1991) Inhibition of photosynthesis of *Populus euramericana* and *Helianthus annuus* by SO₂, NO₂ and O₃. Ecological Research 6, 79-86.

Galloway, J.N., Aber, J.D., Erisman, J.W., Seitzinger, S.P., Howarth, R.W., Cowling, E.B., Cosby, B.N. (2003) The nitrogen cascade. Bioscience 53, 341-356.

Galloway, J.N., Dentener, F.J., Capone, D.G., Boyer, E.W., Howarth, R.W., Seitzinger, S.P., Asner, G.P., Cleveland, C.C., Green, P.A., Holland, E.A., Karl, D.M., Michaels, A.F., Porter, J.H., Townsend, A.R., Vörösmarty, C.J. (2004) Nitrogen cycles: past, present, and future. Biogeochemistry 70, 153-226.

Hoshika, Y., Hajima, T., Shimizu, Y., Takigawa, M., Omasa, K. (2009) Effect of growing season on ozone

stomatal flux for deciduous forests in East Asia. Eco-Engineering 21, 3-8.

IPCC (Intergovernmental Panel on Climate Change) (2001) Climate change 2001: the scientific basis, University Press, Cambridge, pp. 196.

Izuta, T. (1998) Ecophysiological responses of Japanese forest tree species to ozone, simulated acid rain and soil acidification. Journal of Plant Research 111, 471-480.

Izuta, T. (2002) Studies on the effects of ozone and acid deposition on Japanese crop plants and forest tree species. Journal of Japan Society for Atmospheric Environment 37, 81-95. (in Japanese with English summary)

Izuta, T., Umemoto, M., Horie, K., Aoki, M., Totsuka, T. (1996) Effects of ambient levels of ozone on growth, gas exchange rates and chlorophyll contents of *Fagus crenata* seedlings. Journal of Japan Society for Atmospheric Environment 31, 95-105.

Izuta, T., Matsumura, H., Kohno, Y., Shimizu H. (2001) Experimental studies on the effects of acid deposition on forest tree species. Journal of Japan Society for Atmospheric Environment 36, 137-155. (in Japanese with English summary)

Karlsson, P.E., Uddling, J., Braun, S., Broadmeadow, M., Elvira, S., Gimeno, B.S., Thiec, D.L., Oksanen, E., Vandermeiren, K., Wilkinson, M., Emberson, L. (2004) New critical levels for ozone effects on young trees based on AOT40 and simulated cumulative leaf uptake of ozone. Atmospheric Environment 38, 2283-2294.

Khiem, M., Ooka, R., Hayami, H., Yoshikado, H., Huang, H., Kawamoto Y. (2010) Process analysis of ozone formation under different weather conditions over the Kanto region of Japan using the MM5/CMAQ modelling system. Atmospheric Environment 44, 4463-4473.

Klimont, Z., Cofala, J., Schöpp, W., Amann, M., Streets, D.G., Ichikawa, Y., Fujita S. (2001) Projections of SO_2, NO_x, NH_3 and VOC emissions in East Asia up to 2030. Water, Air, & Soil Pollution 130, 193-198.

Kohno, Y., Matsumura, H., Ishii, T., Izuta T. (2005) Establishing critical levels of air pollutants for protecting East Asian vegetation-A challenge. In Plant responses to air pollution and global change (Omasa, K., Nouchi, I. and De Kok, L.J. Eds), Springer-Verlag, Tokyo, pp. 243-250.

Kohno, Y., Suto, H., Ishii, T., Aihara, K., Uchiyama Y. (2007) Concentration and AOT40 of ozone in Tanzawa Mountains and its potential effect on Japanse beech forests. In Results of the Scientific Research on the Tanzawa Mountains (The Research Group of the Tanzawa Mountains Eds), Hiraoka Environmental Science Laboratory, Sagamihara, Kanagawa, Japan, pp. 383-395. (in Japanese with English summary)

Kume, A., Numata, S., Watanabe, K., Honoki, H., Nakajima, H., Ishida M. (2009) Influence of air pollution on the mountain forests along the Tateyama-Kurobe Alpine route. Ecological Research 24, 821-830.

Kuno, H. (1979) Effects of photochemical oxidant on the growth of poplar cuttings I. Journal of Japan Society for Air Pollution 14, 265-274. (in Japanese with English summary)

Kuno, H. (1980) Effects of photochemical oxidant on the growth of poplar cuttings II. Journal of Japan Society for Air Pollution 15, 155-162. (in Japanese with English summary)

Maruta, E., Shima, K., Horie, K., Aoki, M., Dokiya, Y., Izuta, T., Totsuka, T., Yokoi, Y., Sakata, T. (1999) Forest decline of *Fagus crenata* at Mt. Hinokiboramaru (Tannzawa, Kanagawa Prefecture, Japan) and acid deposition. Environmental Sciences 12, 241-250. (in Japanese)

Matsumoto, Y., Maruyama, Y., Morikawa, Y., Inoue, T. (1992) Some negative results of simulative acid mist and ozone treatments to *Cryptomeria japonica* D. Don seedlings in explanation of mature *C. japonica* decline in the Kanto plains in Japan. Japanese Journal for Forest Environment 34, 85-97. (in Japanese with English summary)

Matsumura, H. (2001) Impacts of ambient ozone and/or acid mist on the growth of 14 tree species: an open-top chamber study conducted in Japan. Water, Air, & Soil Pollution 130, 959-964.

Matsumura, H., Aoki, H., Kohno, Y., Izuta, T., Totsuka T. (1996) Effects of ozone on dry weight growth and gas exchange rate of Japanese cedar, Japanese cypress and Japanese zelkova seedlings. Journal of Japan Society for Atmospheric Environment 31, 247-261. (in Japanese with English summary)

Matsumura, H., Kobayashi, T., Kohno, Y. (1998) Effects of ozone and/or simulated acid rain on dry weight growth and gas exchange rates of Japanese cedar, Nikko fir, Japanese white birch and Japanese zelkova seedlings. Journal of Japan Society for Atmospheric Environment 33, 16-35. (in Japanese with English summary)

Matsumura, H., Kohno, Y. (1999) Impact of O_3 and/or SO_2 on the growth of young trees of 17 tree species: an open-top chamber study conducted in Japan. In Critical Levels for Ozone-Level II, Environmental Documentation No. 115 (Fuhrer, J. and Achermann, B. Eds), Swiss Agency for Environment, Forest and Landscape (SAFEL), Bern, Switzerland, pp. 187-192.

Matsumura, H., Mikami, C., Sakai, Y., Murayama, K., Izuta, T., Yonekura, T., Miwa, M., Kohno, Y. (2005) Impacts of elevated O_3 and/or CO_2 on growth of *Betula platyphylla*, *Betula ermanii*, *Fagus crenata*, *Pinus densiflora* and *Cryptomeria japonica* seedlings. Journal of Agricultural Meteorology 60, 1121-1124.

Matsushima, J., Kawai, T., Oodaira, T., Sawada, T., Nouchi, I. (1977) Comparisons of fine structures of zelkova leaves with no visual injury fumigated with ozone, nitrogen dioxide, sulfur dioxide and ethylene. Journal of Japan Society for Air Pollution 11, 360-369. (in Japanese with English summary)

Mills, G., Pleijel, H., Büker, P., Braun, S., Emberson, L., Harmens, H., Hayes, F., Simpson, D., Grünhage, L., Karlsson, P.-E., Danielsson, H., Bermejo, V., Fernandez, I.G. (2010) Mapping critical levels for vegetation-Revision undertaken in summer, 2010, to include

new flux-based critical levels and response functions for ozone. In Mapping Manual 2004 (Spranger, T. Ed), UNECE Convention on Long-range Transboundary Air Pollution, pp. III-4-57.

Miwa, M., Izuta, T., Totsuka, T. (1993) Effects of simulated acid rain and/or ozone on the growth of Japanese cedar seedlings. Journal of Japan Society for Air Pollution 28, 279-287. (in Japanese with English summary)

Nakaji, T., Izuta T. (2001) Effects of ozone and/or excess soil nitrogen on growth, needle gas exchange rates and Rubisco contents of *Pinus densiflora* seedlings. Water, Air, & Soil Pollution 130, 971-976.

Nakaji, T., Kobayashi, T., Kuroha, M., Omori, K., Matsumoto, Y., Yonekura, T., Watanabe, K., Utriainen, J., Izuta, T. (2004) Growth and nitrogen availability of red pine seedlings under high nitrogen load and elevated ozone. Water, Air, & Soil Pollution: Focus 4, 277-287.

Network Center for EANET (2011) Acid Deposition Monitoring Network in East Asia (EANET) Data report 2009, Network Center for EANET, Niigata, Japan, pp. 15-206.

Nihlgård, B. (1985) The ammonium hypothesis-an additional explanation to the forest dieback in Europe. AMBIO 14, 2-8.

Nouchi, I. (2002) Responses of whole plants to air pollutants. In Air pollution and plant biotechnology-Prospects for phytomonitoring and phytoremediation (Omasa, K., Saji, H., Youssefian, S. and Kondo, N. Eds), Springer-Verlag Tokyo, Tokyo, Japan, pp. 3-39.

Nouchi, I., Odaira, T. (1973a) Influence of ozone on plant pigments. Journal of Japan Society for Air Pollution 8, 120-125. (in Japanese with English summary)

Nouchi, I., Odaira, T., Sawada, T., Oguchi, K., Komeji, T. (1973b) Plant ozone injury symptoms. Journal of Japan Society for Air Pollution 8, 113-119. (in Japanese with English summary)

Ohara, T., Akimoto, H., Kurokawa, J., Horii, N., Yamaji, K., Yan, X., Hayasaka, T. (2007) An Asian emission inventory of anthropogenic emission sources for the period 1980-2020. Atmospheric Chemistry and Physics Discussions 7, 6843-6902.

Oksanen, E., Saleem, A. (1999) Ozone exposure results in various carry-over effects and prolonged reduction in biomass in birch (*Betula pendula* Roth). Plant, Cell and Environment 22, 1401-1411.

Paoletti, E., Schaub, M., Matyssek, R., Wieser, G., Augustaitis, A., Bastrup-Birk, A.M., Bytnerowicz, A., Günthardt-Goerg, M.S., Müller-Starck, G., Serengil, Y. (2010) Advances of air pollution science: From forest decline to multiple-stress effects on forest ecosystem services. Environmental Pollution 158, 1986-1989.

Pearson, M., Mansfield, T.A. (1994) Effects of exposure to ozone and water stress on the following season's growth of beech (*Fagus sylvatica* L.). New Phytologist 126, 511-515.

Richter, A., Burrows, J.P., Nüβ, H., Granier, C., Niemeier, U. (2005) Increase in tropospheric nitrogen dioxide over China observed from space. Nature 437, 129-132.

Sandermann, H., Wellburn, A.R., Heath, R.L. (1997) Forest decline and Ozone: Synopsis. In Forest decline and Ozone (Sandermann, H., Wellburn, A.R. and Heath, R.L. Eds), Springer-Verlag Berlin Heidelberg, Germany, pp. 369-377.

Schulze, E.-D. (1989) Air pollution and forest decline in a spruce (*Picea abies*) forest. Science 244, 776-783.

Skärby, L., Ro-Poulsen, H., Wellburn, F.A.M., Sheppard, L.J. (1998) Impacts of ozone on forests: a European perspective. New Phytologist 139, 109-122.

Spreitzer, R.J., Salvucci, M.E. (2002) Rubisco: Structure, regulatory interactions, and possibilities for a better enzyme. Annual Review of Plant Biology 53: 449-475.

Suto, H., Hattori, Y., Tanaka, N., Kohno, Y. (2008) Effects of strong wind and ozone on localized tree decline in the Tanzawa Mountains of Japan. Asian Journal of Atmospheric Environment 2, 81-89.

Takeda, M., Aihara, K. (2007) Effects of ambient ozone concentrations on beech (*Fagus crenata*) seedlings in the Tanzawa Mountains, Kanagawa Prefecture, Japan. Journal of Japan Society for Atmospheric Environment 42, 107-117. (in Japanese with English summary)

Vitousek, P.M., Howarth, R.W. (1991) Nitrogen limitation on land and in the sea: How can it occur? Biogeochemistry 13, 87-115.

Watanabe, M., Yonekura, T., Honda, Y., Yoshidome, M., Nakaji, T., Izuta, T. (2005) Effects of ozone and soil water stress, singly and in combination, on leaf antioxidative systems of *Fagus crenata* seedlings. Journal of Agricultural Meteorology 60, 1105-1108.

Watanabe, M., Yamaguchi, M., Iwasaki, M., Matsuo, N., Naba, J., Tabe, C., Matsumura, H., Kohno, Y., Izuta, T. (2006) Effects of ozone and/or nitrogen load on the growth of *Larix kaempferi*, *Pinus densiflora* and *Cryptomeria japonica* seedlings. Journal of Japan Society for Atmospheric Environment 41, 320-334.

Watanabe, M., Yamaguchi, M., Tabe, C., Iwasaki, M., Yamashita, R., Funada, R., Fukami, M., Matsumura, H., Kohno, Y., Izuta, T. (2007) Influences of nitrogen load on the growth and photosynthetic responses of *Quercus serrata* seedlings to O$_3$. Trees 21, 421-432.

Watanabe, M., Yamaguchi, M., Matsumura, H., Kohno, Y., Izuta, T. (2008) Effects of ozone on the growth and photosynthesis of *Castanopsis sieboldii* seedlings grown under different nitrogen loads. Journal of Agricultural Meteorology 64, 143-155.

Watanabe, M., Umemoto-Yamaguchi, M., Koike, T., Izuta, T. (2010) Growth and photosynthetic response of *Fagus crenata* seedlings to ozone and/or elevated carbon dioxide. Landscape and Ecological Engineering 6, 181-190.

Yamaguchi, M., Watanabe, M., Matsuo, N., Naba, J., Funada, R., Fukami, M., Matsumura, H., Kohno, Y., Izuta, T. (2007a) Effects of nitrogen supply on the sensitivity to O$_3$ of growth and photosynthesis of Japanese beech (*Fagus crenata*) seedlings. Water, Air, & Soil Pollution: Focus 7, 131-136.

Yamaguchi, M., Watanabe, M., Iwasaki, M., Tabe, C., Matsumura, H., Kohno, Y., Izuta, T. (2007b) Growth

and photosynthetic responses of *Fagus crenata* seedlings to O_3 under different nitrogen loads. Trees 21, 707-718.

Yamaguchi, M., Watanabe, M., Matsumura, H., Kohno, Y., Izuta, T. (2010) Effects of ozone on nitrogen metabolism in the leaves of *Fagus crenata* seedlings under different soil nitrogen loads. Trees 24, 175-184.

Yamaji, K., Ohara, T., Uno, I., Tanimoto, H., Kurokawa, J., Akimoto, H. (2006) Analysis of the seasonal variation of ozone in the boundary layer in East Asia using the Community Multi-scale Air Quality model: What controls surface ozone levels over Japan? Atmospheric Environment 40, 1856-1868.

Yamaji, K., Ohara, T., Uno, I., Kurokawa, J., Pochanart, P., Akimoto, H. (2008) Future prediction of surface ozone over east Asia using Models-3 Community Multiscale Air Quality Modeling System and Regional Emission Inventory in Asia. Journal of Geophysical Research 113, D08306.

Yonekura, T., Dokiya, Y., Fukami, M., Izuta, T. (2001a) Effects of ozone and/or soil water stress on growth and photosynthesis of *Fagus crenata* seedlings. Water, Air, & Soil Pollution 130, 965-970.

Yonekura, T., Honda, Y., Oksanen, E., Yoshidome, M., Watanabe, M., Funada, R., Koike, T., Izuta, T. (2001b) The influences of ozone and soil water stress, singly and in combination, on leaf gas exchange rates, leaf ultrastructural characteristics and annual ring width of *Fagus crenata* seedlings. Journal of Japan Society for Atmospheric Environment 36, 333-351.

Yonekura, T., Yoshidome, M., Watanabe, M., Honda, Y., Ogiwara, I., Izuta T. (2004) Carry-over effects of ozone and water stress on leaf phenological characteristics and bud frost hardiness of *Fagus crenata* seedlings. Trees 18, 581-588.