



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

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ABSTRACT

Ozone (O₃) is a main component of photochemical oxidants, and a phytotoxic anthropogenic air pollutant. In North America and Europe, the current concentration of O₃ has been shown to have significant adverse effects on vegetation. In this review, we summarize the experimental studies on the effects of O₃ 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 O₃ impacts on Japanese forest ecosystem. Since the 1990s, several Japanese researchers have conducted the experimental studies on the effects of ambient levels of O₃ on growth and physiological functions such as net photosynthesis of Japanese forest tree species. Although the sensitivity to O₃ of whole-plant growth is quite different among the species, it was suggested that the current ambient levels of O₃ in Japan are high enough to adversely affect growth and photosynthetic activity of Japanese forest tree species classified into high O₃ sensitivity group such as Japanese beech. The N load to soil has been shown to reduce the sensitivity to O₃ of Japanese larch and increase that of Japanese beech. To establish the critical level of O₃ 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 O₃ and other environmental factors such as elevated CO₂ 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 O₃ and any other abiotic environmental factors on Japanese forest tree species.

Key words: Ozone, Japanese forest tree species, Growth, Photosynthetic activity, Critical level

1. INTRODUCTION

Ozone (O₃) 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 O₃ 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 O₃ 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 O₃ 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 O₃ 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 O₃ 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 O₃ on woody plants were published in the 1970s. Nouchi *et al.* (1973a, b) and Matsushima *et al.* (1977) mainly focused on O₃-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 O₃ on growth or physiological functions such as net photosynthesis of street trees. Matsumoto *et al.* (1992) reported the effects of O₃ 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 O₃ 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 O₃ impacts on Japanese forest ecosystems, in this review, we summarize the experimental studies hitherto reported on the effects of O₃ on growth and photosynthetic activity of Japanese forest tree species.

2. EFFECTS OF O₃ ON GROWTH OF JAPANESE FOREST TREE SPECIES

Table 1 indicates the summary of experimental studies on the effects of O₃ on Japanese forest tree species. Miwa *et al.* (1993) reported that the exposure of Japanese cedar seedlings to relatively high O₃ 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 O₃ (75 and 150 ppb). Yonekura *et al.* (2001a, b) also reported that the exposure to ambient level of O₃ (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 O₃ (60 ppb). Aforementioned studies were conducted using the steady-state O₃ exposure system. Because there are seasonal and diurnal variations in tropospheric O₃ concentration (Khiem *et al.*, 2010; EPA, 2006a; Yamaji *et al.*, 2006), O₃ exposure

system with the variations in the atmospheric concentration of O₃ observed in the fields needs to be used for the evaluation of the realistic effects of O₃ on forest tree species. Matsumura *et al.* (1996) and Matsumura *et al.* (1998) conducted experimental studies on the effects of O₃ on the growth of several Japanese forest tree species using an O₃ exposure system with seasonal and diurnal variations in the atmospheric concentration of O₃. 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 O₃ at 0.4, 1.0, 2.0 and 3.0 times the ambient concentration (12-h (6:00-18:00) average concentration of O₃: 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 O₃ and that of Japanese cedar exposed to 3.0 times the ambient concentration of O₃ were significantly lower than those exposed to 0.4 times the ambient concentration of O₃, while there was no significant effect of O₃ 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 O₃ at 0.4, 1.0, 2.0 and 3.0 times the ambient concentration (12-h (6:00-18:00) average concentration of O₃: 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 O₃, while that of Nikko fir was not. These results indicate that the sensitivity to O₃ 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 O₃ on perennial plants. For example, the exposure to O₃ 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 O₃ 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 O₃ (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 O₃ on Japanese forest tree species.

Reference	Facility	Location	Treatments	Duration	Species	Age	O ₃ Effects (Combined effects)
Miwa <i>et al.</i> (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 (<i>Cryptomeria japonica</i>)	2 yr	No effect on whole-plant dry mass. Increased Top/Root ratio (Additive). Decreased Chl. (Counteractive).
Matsumura <i>et al.</i> (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 (<i>Chamaecyparis obtusa</i>)	2 yr	No effect.
					Japanese zelkova (<i>Zelkova serrata</i>)	1 yr	Decreased whole-plant dry mass, RGR, NAR, A and R _d . Increased Top/Root ratio.
Izuta <i>et al.</i> (1996)	Environment-controlled growth cabinet	Tokyo	CF, 75 ppb, 150 ppb (6 h/day, 3 days/week)	18 weeks	Japanese beech (<i>Fagus crenata</i>)	3 yr	Decreased root and whole-plant dry mass, RGR, NAR, A, CE and Chl.
Matsumura <i>et al.</i> (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 (<i>Abies homolepis</i>)	5 yr	No effect on whole-plant dry mass. Increased R _d (Additive). Increased Top/Root ratio (Synergetic).
					Japanese white birch (<i>Betula platyphylla</i>)	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 (<i>Pinus densiflora</i>)	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 <i>et al.</i> (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 <i>et al.</i> (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 plastoglobuli in chloroplasts.
Matsumura (2001)	Tunnel-type OTC	Gunma and/or Chiba	CF, NF (12-h ave.: 12 and 37 ppb at Gunma) (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 (<i>Pinus thunbergii</i>)	2 yr	No effect on whole-plant dry mass.
					Japanese larch (<i>Larix kaempferi</i>)	2 yr	Decreased shoot, root and whole-plant dry mass (Additive).
					Norway spruce* (<i>Picea abies</i>)	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 (<i>Abies firma</i>)	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 (<i>Abies veitchii</i>)	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).
					<i>Populus maximowiczii</i>	4 yr	Decreased root dry mass (Additive).
					Japanese white birch	1 yr	Decreased shoot, root and whole-plant dry mass (Additive).
					<i>Quercus mongolica</i>	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 <i>et al.</i> (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 <i>et al.</i> (2004)	Naturally-lit growth chamber	Tokyo	CF, 60 ppb (7 h/day) Combined with water stress	1 year	Japanese beech (<i>Fagus crenata</i>)	3 yr	Acceleration of leaf abscission, delay in bud break and decreased leaf no. per bud (Additive).
Watanabe <i>et al.</i> (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 <i>et al.</i> (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 (<i>Betula platyphylla</i>)	2 yr	Decreased whole-plant dry mass (Counteractive).
					Japanese mountain birch (<i>Betula ermanii</i>)	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 (<i>Pinus densiflora</i>)	2 yr	Decreased shoot and whole-plant dry mass (Additive).
					Japanese cedar (<i>Cryptomeria japonica</i>)	2 yr	No effect on whole-plant dry mass.
Watanabe <i>et al.</i> (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 (<i>Larix kaempferi</i>)	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	Species	Age	O ₃ Effects (Combined effects)
					Japanese cedar	2 yr	Decreased dry masses of needle, stem, root and whole-plant (Additive). No effect on A.
Yamaguchi <i>et al.</i> (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	Japanese beech	2 yr	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 <i>et al.</i> (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	Japanese beech	2 yr	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 <i>et al.</i> (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	<i>Quercus serrata</i>	2 yr	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 <i>et al.</i> (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	<i>Castanopsis sieboldii</i>	2 yr	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 <i>et al.</i> (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	Japanese beech	2 yr	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 <i>et al.</i> (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	Japanese beech	2 yr	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}: CO₂-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 H₂O; 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_{pred}: 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 O₃ sensitivity groups (after Kohno *et al.*, 2005, with permission).

O ₃ sensitivity	Type		Species	Critical level (Daylight AOT40)
High	Broad-leaved	Deciduous	<i>Populus maximowiczii</i> , <i>Populus nigra</i> , Japanese beech, Japanese zelkova	8-15 ppm h
		Evergreen	<i>Castanopsis sieboldii</i>	
	Coniferous	Deciduous	Japanese larch	
		Evergreen	Japanese red pine	
Moderate	Broad-leaved	Deciduous	<i>Quercus serrata</i> , Japanese white birch	16-30 ppm h
		Evergreen	<i>Quercus myrsinaefolia</i> , <i>Cinnamomum camphora</i>	
	Coniferous	Evergreen	Nikko fir	
Low	Broad-leaved	Deciduous	<i>Quercus mongolica</i> var. <i>grosseserrata</i>	31 ppm h <
		Evergreen	<i>Lithocarpus edulis</i> , <i>Machilus thunbergii</i>	
	Coniferous	Evergreen	Japanese black pine, Japanese cedar	
			Japanese cypress	

High O₃ sensitivity: The whole-plant dry mass increment was significantly reduced by the exposure to ambient level of O₃.

Moderate O₃ sensitivity: The whole-plant dry mass increment was significantly reduced by the exposure to 1.5 or 2.0 times ambient level of O₃.

Low O₃ sensitivity: The whole-plant dry mass increment was not significantly reduced by the exposure to 1.5 or 2.0 times ambient level of O₃.

Daylight AOT40: accumulated exposure over a threshold of 40 ppb O₃ 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 O₃ 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 O₃ on forest tree species (e.g. Matsumura, 2001; Matsumura and Kohno, 1999). The sensitivity of each tree species to O₃ was classified into 3 groups (high, moderate and low) based on the response of the whole-plant dry mass growth to O₃ (Table 2). For example, Japanese larch and Japanese beech have been classified into high O₃ sensitivity group; Japanese white birch and Nikko fir have been classified into moderate O₃ sensitivity group; Japanese cedar and Japanese cypress have been classified into low O₃ sensitivity group. Recently, Takeda and Aihara (2007) showed that O₃ 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 O₃ 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 O₃ in Japan are high enough to adversely affect growth of Japanese forest tree species especially in those classified into

high O₃ sensitivity group such as Japanese beech.

3. EFFECTS OF O₃ 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 O₃. In their studies, the O₃-induced reductions in net assimilation rate (NAR) and net photosynthetic rate of the leaves or needles were reported. These results indicate that the O₃-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 O₃ 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 O₃, simultaneous reductions in the carboxylation efficiency (CE), CO₂-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 O₃ 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 O₃-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 O₃ 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 O₃ reduced photosynthetic nitrogen use efficiency (PNUE) in the leaves of *Q. serrata* and Japanese beech. In the case of Japanese beech, the exposure to O₃ did not significantly affect N concentration in the leaves, suggesting that O₃ 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 O₃ 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 O₃ did not significantly affect the activities of nitrate reductase (NR) and nitrite reductase (NiR) and concentrations of inorganic N compounds (NO₃⁻, NO₂⁻ and NH₄⁺) and free amino acid in the needles of Japanese red pine. On the other hand, Yamaguchi *et al.* (2010, 2007a) reported the O₃-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 O₃ affects N metabolism in the leaves of Japanese forest tree species. To clarify the mechanisms underlying the detrimental effects of O₃ on Japanese forest tree species, therefore, further research concerning the effects of O₃ on physiological functions such as foliar N metabolism is required.

4. COMBINED EFFECTS OF O₃ 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 O₃ and simulated acid rain on Japanese forest tree species. Recently, it was pointed out that the interactive effects of O₃, N deposition, elevated carbon dioxide (CO₂) 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 O₃ and N load to soil, elevated CO₂ 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 O₃ 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 O₃, 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 NH₄NO₃ solution at 0, 20 and 50 kg ha⁻¹ year⁻¹ and simultaneously exposed to charcoal-filtered air or O₃ at 1.0, 1.5 and 2.0 times the ambient concentration for two growing seasons (24-h seasonal mean concentration of O₃ (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 O₃ 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 $> 50 \text{ W m}^{-2}$), see Fig. 1) were shown

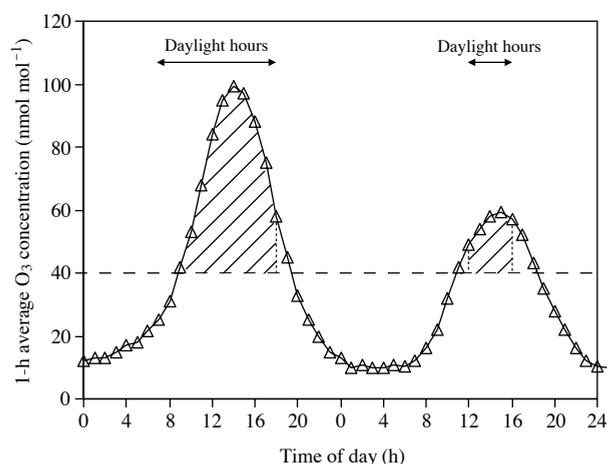
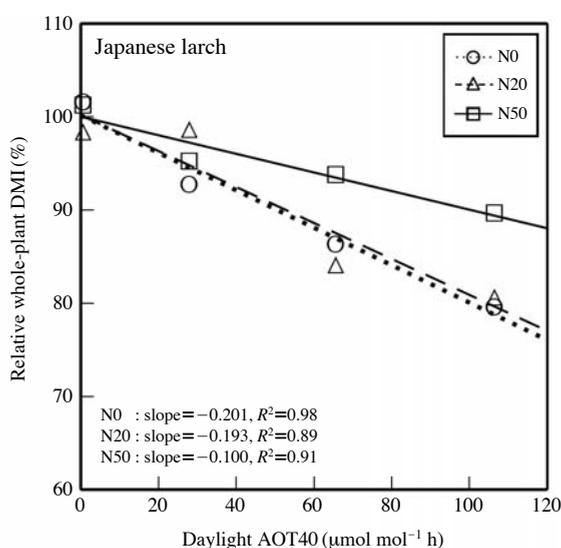


Fig. 1. Conceptual diagram for the calculation of daylight AOT40 of O_3 (accumulated exposure over a threshold of 40 ppb, $\text{nmol mol}^{-1} \text{ h}$ or $\mu\text{mol mol}^{-1} \text{ h}$). Shaded area contributes to daylight AOT40. Daylight hour: global radiation $> 50 \text{ W m}^{-2}$.



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

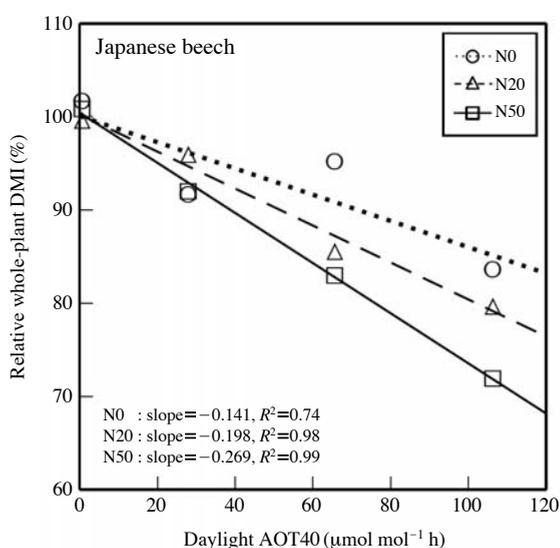


Fig. 2. The relationships between relative whole-plant dry mass increment (DMI) of Japanese larch and Japanese beech seedlings per one growing season and daylight AOT40 of O_3 . The seedlings were grown in the soil supplied with N as NH_4NO_3 solution at 0 (N0), 20 (N20) or 50 $\text{kg ha}^{-1} \text{ year}^{-1}$ (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).

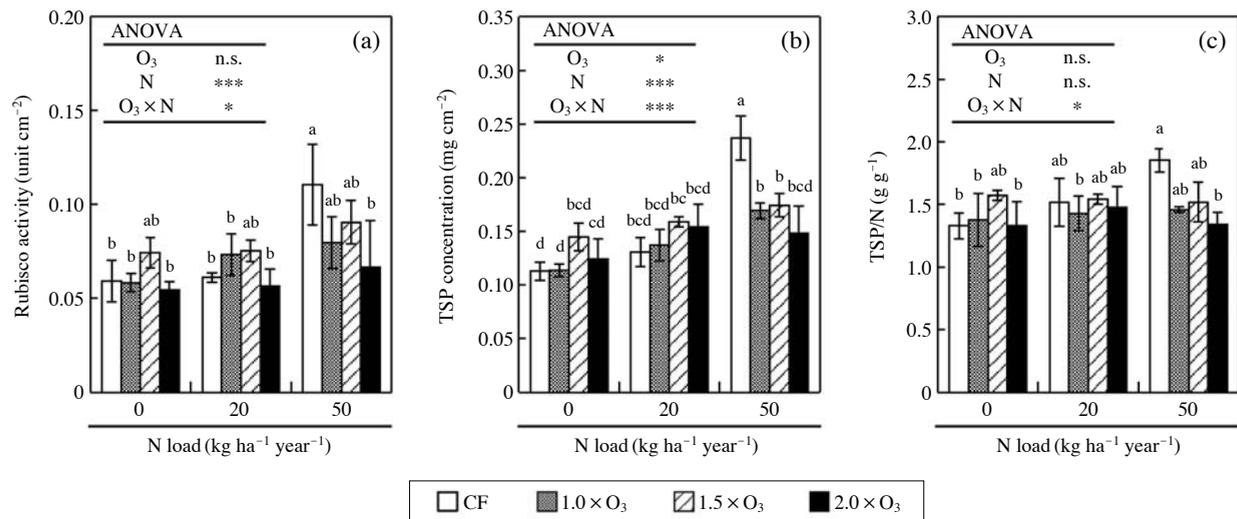


Fig. 3. Effects of O₃ and N load on activity of ribulose 1,5-bisphosphate carboxylase/oxygenase (Rubisco), concentration of total soluble protein (TSP) and ratio of TSP to leaf N content per unit leaf area (TSP/N) in the leaves of Japanese beech. The standard deviation is given by vertical bar. Two-way 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 NH₄NO₃ solution at 0, 20 or 50 kg ha⁻¹ year⁻¹ and simultaneously exposed to charcoal-filtered air (CF) or O₃ at 1.0, 1.5 and 2.0 times ambient concentration. The exposure to O₃ 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 O₃ and N load on net photosynthetic rate is mainly attributed to the difference in the degrees of O₃-induced reduction in the amount of Rubisco among the N treatments (Yamaguchi *et al.*, 2007b). Furthermore, the exposure to O₃ 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 O₃ 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 CO₂ and Drought

Elevated CO₂ and drought are well known to affect the sensitivity of forest tree species to O₃ (e.g. EPA,

2006b). Unfortunately, there is little information on the combined effects of O₃ and elevated CO₂ 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 CO₂ on O₃-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 O₃ and elevated CO₂ is different among Japanese forest tree species. On the other hand, Watanabe *et al.* (2010) reported that the simultaneous exposure to O₃ and elevated CO₂ induced marked growth stimulation of Japanese beech seedlings as compared with those exposed to elevated CO₂. Yonekura *et al.* (2001a, b) reported the additive effects of O₃ 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 O₃ on net photosynthesis of the leaves of Japanese beech seedlings. Combined effects of O₃ and other environmental factors such as elevated CO₂ 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 O₃ and any other abiotic environmental factors on growth, physiological functions and nutrient

status of Japanese forest tree species.

5. CRITICAL LEVEL OF O₃ FOR PROTECTING JAPANESE FOREST TREE SPECIES

The ambient levels of O₃ in Japan have been shown to adversely affect growth and photosynthetic activity of Japanese forest tree species especially in those classified into high O₃ 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 O₃, 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 O₃, 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 O₃ 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 O₃ for Japanese forest tree species classified into the high O₃ 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 O₃ for protecting Japanese forest tree species, because the sensitivities of Japanese larch and Japanese beech to O₃ 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 CO₂ concentration to evaluate the critical level of O₃. To establish the critical level of O₃ for protecting Japanese forest tree species, therefore, further research concerning the combined effects of O₃ 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 O₃ mainly depend on the amount of O₃ reaching the sites of damage within the leaf, cumulative flux or uptake of O₃ through the stomata and associated response functions are suitable for mapping and quantifying impacts of O₃ at the local and regional scale (Mills *et al.*, 2010). Therefore, atmospheric concentration-based critical level of O₃ expressed as AOT40 can be used only for estimating the risk of damage. The approach based on the O₃ flux into leaves or needles requires the development of mathematical models to estimate stomatal O₃ 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 O₃ 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 O₃ for the final purpose of mapping and quantifying the impacts of O₃ on Japanese forest tree species.

6. CONCLUSION AND PERSPECTIVES

Based on the results obtained from the experimental studies, the current levels of O₃ in Japan are high enough to adversely affect growth of Japanese forest tree species with relatively high O₃ sensitivity such as Japanese beech. To protect Japanese forest, therefore, we need to establish the critical level of O₃, primarily using AOT40 index, for Japanese forest tree species with consideration of other abiotic environmental factors affecting the sensitivity to O₃ such as N deposition from the atmosphere. In addition to the estimating the risk of damage of O₃ using AOT40 index, it is necessary to quantify the impacts of O₃ on Japanese forest tree species. For this purpose, it is necessary to promote the research toward the modeling of stomatal flux of O₃ 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 O₃ on the growth of Japanese forest tree species using the seedlings could be applicable to the evaluation of O₃-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.

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