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Author(s)	Watanabe, Makoto; Hoshika, Yasutomo; Inada, Naoki et al.
Citation	Journal of agricultural meteorology, 71(3), 227-231 https://doi.org/10.2480/agrmet.D-14-00012
Issue Date	2015-09
Doc URL	https://hdl.handle.net/2115/60223
Type	journal article
File Information	71306(koike).pdf

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Full Paper

Journal of Agricultural Meteorology 71 (3): 227-231, 2015

Difference in photosynthetic responses to free air ozone fumigation between upper and lower canopy leaves of Japanese oak (*Quercus mongolica* var. *crispula*) saplings

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Abstract

To clarify the difference of ozone (O_3) sensitivity of photosynthesis in leaves located different canopy positions, we investigated photosynthetic responses of Japanese oak saplings to free air O_3 exposure, and compared the results with Siebold's beech saplings reported previously. The O_3 at 60 nmol mol^{-1} during daytime was fumigated for two growing seasons, from 6 August to 11 November 2011 and from 17 May to 10 November 2012. In June, August and October of 2012, we determined photosynthetic activity of upper and lower canopy leaves. The exposure to O_3 increased the ratio of intercellular CO_2 concentration to ambient CO_2 concentration in October, indicating stomatal sluggishness. We observed an O_3-induced, significant decrease in light-saturated net photosynthetic rate (A_{sat}) with no significant interaction between O_3 effect and canopy position for A_{sat}. Therefore, we conclude that the O_3 sensitivity of photosynthesis in upper and lower canopy leaves was similar in Japanese oak, while our previous study reported that the upper canopy leaves of Siebold's beech have a higher O_3 sensitivity than lower canopy leaves.

Key words: Free air ozone exposure, Japanese Oak, Light condition, Photosynthesis, Sensitivity to ozone.

1. Introduction

The increasing concentration of ozone (O_3) in the troposphere may lead to a high risk of decline in the productivity of vegetation (*e.g.* Matyssek and Sandermann, 2003; Sitch *et al.*, 2007; Koike *et al.*, 2013). The concentration of O_3 has been increasing since the Industrial Revolution (The Royal Society, 2008). A significant increase in O_3 concentrations in East Asia is predicted in the near future because of rapid increases in emissions of the main O_3 precursors, nitrogen oxides and volatile organic compounds (Ohara *et al.*, 2007; Yamaji *et al.*, 2008). It is therefore important to assess the risk of increasing O_3 concentrations in forest trees in East Asia (Kohno *et al.*, 2005; Watanabe *et al.*, 2010).

A free-air O_3 exposure experiment using 10-year-old saplings of Siebold's beech (*Fagus crenata*) and Japanese oak (*Quercus mongolica* var. *crispula*) has been conducted in Sapporo Experimental Forest in northern Japan since 2011 to facilitate better risk assessment of O_3 (Hoshika *et al.*, 2012a, b, 2013; Watanabe *et al.*, 2013, 2014). Japanese oak is a deciduous broad-leaved tree species, classified as a mid-successional species with long longevity of 200~300 years (Koike, 1988; Watanabe, 1994). Watanabe *et al.* (2013) reported an O_3-induced decline in the light-saturated net photosynthetic rate and an enhanced dark respiration rate in the sun leaves of Japanese oak, although the extent was small as compared to Siebold's beech.

When assessing the O_3 impact on carbon absorption capacity of whole tree or forest, it is important to understand how O_3 sensitivities differ between canopy positions. Light limitations within the canopy may alter the sensitivity of leaves to O_3 injury. Previous studies reported higher O_3 sensitivity under higher light condition in poplar (*Populus tristis* × *P. balsamifera*) and Siebold's beech, whereas the opposite trend has been found in sugar maple (*Acer saccharum*), and European beech (*Fagus sylvatica*) (Tjoelker *et al.*, 1993, 1995; Kitao *et al.*, 2009; Watanabe *et al.*, 2014). These reports indicate that the tendency of O_3 sensitivity between upper and lower canopy leaves is species specific. It is important to accumulate information on O_3 sensitivity with various light conditions among various tree species. A high photosynthetic tolerance to O_3 exposure in Japanese oak was evaluated only in upper canopy leaves with higher light condition (Watanabe *et al.* 2013). Therefore, it is necessary to evaluate O_3 sensitivity in shade leaves of Japanese oak in order to adequately understand the impact of O_3 on the carbon-absorption capacity in forests of this tree species.

In the present study, we investigates differences in photosynthetic responses to O_3 between upper and lower canopy leaves of Japanese oak saplings and compares the results of Japanese oak responses with Siebold's beech grown at the same experimental site (Watanabe *et al.*, 2014).

2. Materials and methods

2.1 Study site and experimental design

The study site was Sapporo Experimental Forest of Hokkaido University, in northern Japan (43°04' N, 141°20' E, 15 m a.s.l.). Details of the study site and fumigation system have been described in Watanabe *et al.* (2013). The monthly average temperature and precipitation at the experimental site during the fumigation period in 2012 are shown in Figs. 1a and 1b. Average temperature and total amount of precipitation during the fumigation period were 17.3℃ and 727 mm, respectively. We prepared two plots, one for 'ambient' plot and another for elevated O_3. These two plots were about 20 m apart. Each plot was a rectangle of

Received; June 3, 2014.
Accepted; April 10, 2015.
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DOI: 10.2480/agrmet.D-14-00012

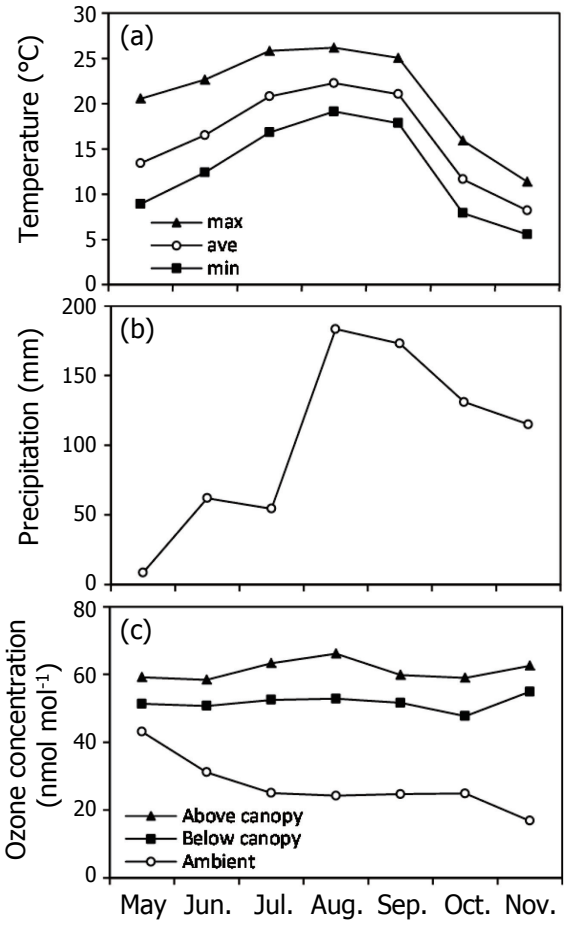


Fig. 1. Monthly temperature, precipitation and ozone concentration during daytime at the experimental site during the fumigation period in 2012. Ozone concentrations were monitored in above and below canopies of elevated ozone plot and above canopy of ambient plot. The ozone fumigation period in 2012 was from 17 May to 10 November.

sides 5.5 m × 7.2 m, and 5.5 m in height. The soil moisture in the root layer (20 cm depth) was measured by 10HS sensors equipped with an EM5b data logger (Decagon Devices, Pullman WA, USA) in each plot. The average soil moisture was 29% during the fumigation period in 2012. These values were close to the field capacity (32%), and there was no difference for the soil moisture between two plots. Ten seedlings of two-year-old Siebold's beech and Japanese oak were planted in May 2003 in each plot, which were grown under ambient conditions. We began the O_3 exposure experiment in 2011 when the trees were 10 years old. The mean tree height and stem diameter at breast height at the start of the O_3 fumigation were 3.3 ± 0.4 m and 26.7 ± 5.9 mm for Siebold's beech and 5.5 ± 0.7 m and 54.6 ± 13.9 mm for Japanese oak, respectively. The leaves were attached in a height range from 3 to 6 m. There was no significant difference between the plots in heights, stem diameters, or crown length.

The target O_3 concentration above the canopy for O_3 fumigation was 60 nmol mol^{-1} during daylight hours. The O_3 fumigation lasted 98 days from 6 August to 11 November 2011 and 178 days from 17 May to 10 November 2012. The O_3 concentration above the canopy was monitored continually in two minutes interval by an O_3 monitor (Mod. 202, 2B Technologies, Boulder CO, USA). Additionally, the O_3 concentration below the canopy was monitored using the same type of O_3 monitor during the second growing season. Figure 1c shows the average monthly daytime O_3 concentration above and below the canopy at an elevated O_3 plot and above the canopy of the ambient plot during the fumigation period in 2012. The mean ± standard deviations of one-hour average O_3 concentration during daylight hours in the O_3 fumigated plot during 2012 was 61.5 ± 13.0 nmol mol^{-1} (above canopy) and 51.2 ± 12.2 nmol mol^{-1} (below canopy); that in the ambient plot was 27.5 ± 11.6 nmol mol^{-1}. Ambient O_3 concentration above 60 nmol mol^{-1} was observed for 5 days (16 hours total), while the ambient O_3 concentration did not exceed 70 nmol mol^{-1} during the fumigation period.

2.2 Measurement of leaf gas exchange rate

The gas exchange rates of the first flush leaf were measured during 10–16 June, 26–31 August, and 9–16 October 2012 using an open gas exchange system (LI-6400, Li-Cor Inc., Lincoln, NE, USA). Five saplings were selected randomly in each plot (*i.e.* ambient and elevated O_3). We measured the gas exchange rate for two first flush leaves from each tree selected: one leaf was from the upper canopy (5~6 m) and the other was from the lower canopy (3~4m). The relative light intensity in the lower canopy was 10~15% as compared to that of the above canopy. We determined net photosynthetic rate (A_{sat}), stomatal conductance to water vapor (G_s), and the ratio of intercellular CO_2 concentration to ambient CO_2 concentration (C_i/C_a) under saturating photosynthetic photon flux (PPF) at 1500 μmol m^{-2} s^{-1}. The CO_2 concentration in the chamber, leaf temperature, and leaf-to-air vapor pressure deficit (VPD) during measurement were 380 μmol mol^{-1}, 25.0 ± 0.5℃ and 1.5 ± 0.2 kPa, respectively. In August and October, we also analyzed the light response of the net photosynthetic rate after the aforementioned measurement. We determined the net photosynthetic rate at each step of PPF (*i.e.* at 100, 75, 50 and 0 μmol m^{-2} s^{-1}). Then, we calculated dark respiration rate (R) and apparent quantum yield (φ, Lambers *et al.* 2008) as a slope of regression line between PPF and the net photosynthetic rate. The gas exchange rates were measured between 0800 and 1500 hours. In the preliminary examination, we observed little difference between morning and afternoon measurements in the same leaf. After the gas exchange rate had been measured, we collected leaf samples in order to determine the leaf mass per area (LMA). These leaf samples were dried in an oven for 5 days at 70℃ and then weighed. The LMA was calculated as leaf dry mass divided by leaf area.

2.3 Statistical analysis

Statistical analyses were run using R software, version 2.15.0 (R Development Core Team, 2012). The effects of O_3, canopy position, and measurement time (June, August, and October) on each parameter were tested by repeated measure analysis of variance (repeated measure ANOVA).

3. Results and discussion

Figure 2 shows LMA of upper and lower canopy leaves of Japanese oak saplings in June, August, and October 2012. The LMA in upper canopy leaves was 96.6% higher than that in lower

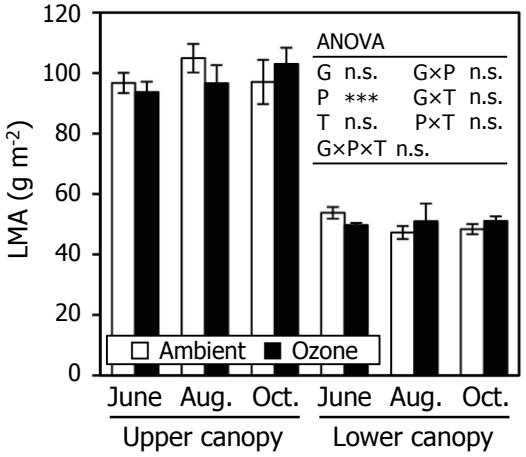


Fig. 2. Leaf mass per area (LMA) in upper and lower canopy leaves of Japanese oak sapling grown under ambient and elevated concentrations of ozone. The error bar shows SE (n = 5). Repeated Measures ANOVA: G gas treatment; P difference of canopy position; T time course, *** $P < 0.001$; n.s. not significant.

canopy leaves as an average across the O_3 treatments and measurement periods. There was no significant effect of O_3 or measurement time, or interactions between gas treatment, canopy position, and measurement time for the LMA. LMA is a representative parameter for estimating acclimation of leaf to given light condition (*e.g.* Kitaoka *et al.*, 2009; Kitao *et al.*, 2009; Watanabe *et al.*, 2014). The clear difference of LMA between upper and lower canopies supports the different light conditions between upper and lower canopies.

A_{sat}, G_s, φ and R in upper canopy leaves were significantly higher than those in lower canopy leaves, while there was no significant effect of canopy position for C_i / C_a (Table 1 and Fig.3). It is a general trend that photosynthetic capacity in upper canopy leaves is higher than that in lower canopy leaves for efficient carbon gain (*e.g.* Lambers et al. 2008; Kitao *et al.* 2009; Watanabe *et al.* 2014). There were significant interactions between canopy position and time course for A_{sat} and G_s. Decreases of A_{sat} and G_s with time course in lower canopy leaves were rather earlier than those in upper canopy leaves.

We found significant interaction between gas treatment and time course for C_i / C_a. Although there was no difference of C_i / C_a between gas treatments in June and August, the C_i / C_a under elevated O_3 condition in October was higher than that under ambient condition. Hoshika *et al.* (2013) also reported elevated O_3-induced higher C_i / C_a in late growing season in Siebold's beech sapling at the same experimental site with the present study, indicating O_3-induced acceleration of the loss of stomatal control with leaf age, called the 'stomatal sluggishness' (Paoletti and Grulke, 2010; Hoshika *et al.*, 2012a).

There was no significant interaction between gas treatment and canopy position for all parameters (Table 1 and Figs. 2 and 3), indicating no difference in O_3 sensitivity between canopy positions for photosynthetic traits of Japanese oak. In Siebold's beech, on the other hand, upper canopy leaves with high LMA showed a clear decrease in A_{sat} and an increase in R under elevated O_3 while there was no effect of O_3 on A_{sat} and R in the lower canopy leaves (Watanabe *et al.*, 2014). In both species, there was no significant effect of O_3 on φ.

A comparison of O_3 sensitivity between tree species based on photosynthesis of only upper canopy leaves may lead to error because variability of O_3 sensitivity within a canopy is species

Table 1. Net photosynthetic rate (A_{sat}), stomatal conductance (G_s) and ratio of intercellular CO_2 concentration to ambient CO_2 concentration (C_i / C_a) under photosynthetic photon flux at 1500 μmol m^{-2} s^{-1} in upper and lower canopy leaves of Japanese oak sapling grown under ambient and elevated concentrations of ozone.

		A_{sat} (μmol m^{-2} s^{-1})		G_s (mol m^{-2} s^{-1})		C_i / C_a	
Position	Time	Ambient	Ozone	Ambient	Ozone	Ambient	Ozone
Upper	June	15.3 (1.0)	16.0 (0.7)	0.25 (0.03)	0.23 (0.01)	0.69 (0.02)	0.65 (0.02)
	August	15.8 (0.6)	14.0 (1.4)	0.34 (0.01)	0.27 (0.01)	0.72 (0.01)	0.71 (0.02)
	October	9.7 (0.5)	7.5 (0.9)	0.20 (0.02)	0.23 (0.02)	0.70 (0.01)	0.79 (0.03)
Lower	June	10.3 (0.6)	8.7 (0.9)	0.16 (0.01)	0.14 (0.01)	0.68 (0.02)	0.68 (0.02)
	August	6.9 (0.7)	5.8 (0.9)	0.16 (0.02)	0.14 (0.02)	0.75 (0.02)	0.76 (0.04)
	October	5.0 (0.5)	3.4 (0.7)	0.09 (0.01)	0.09 (0.01)	0.73 (0.03)	0.77 (0.05)
Repeated Measure ANOVA							
Gas		*		n.s.		n.s.	
Position		***		***		n.s.	
Time		***		***		***	
Gas × Position		n.s.		n.s.		n.s.	
Gas × Time		n.s.		n.s.		**	
Position × Time		**		*		n.s.	
Gas × Position × Time		n.s.		n.s.		n.s.	

Standard error is shown in parentheses (n = 5).
Repeated Measures ANOVA: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; n.s. not significant.

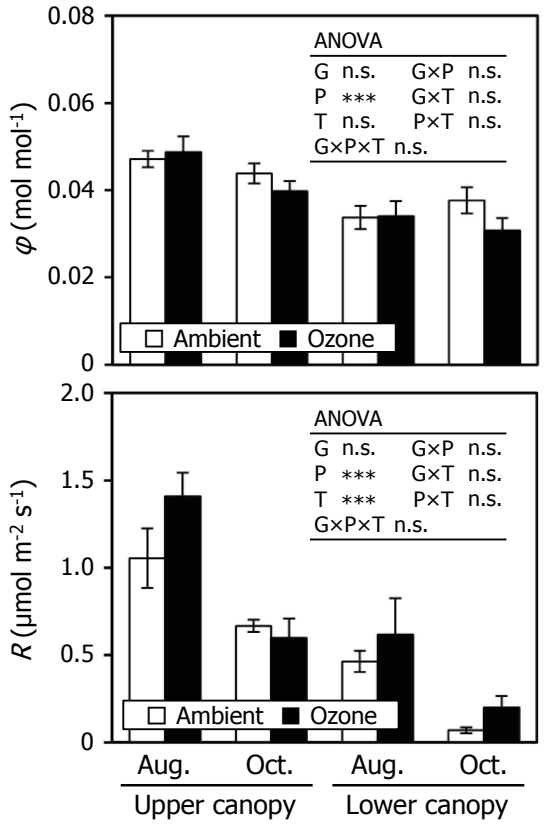


Fig. 3. Apparent quantum yield (φ) and dark respiration rate (R) in upper and lower canopy leaves of Japanese oak sapling grown under ambient and elevated concentrations of ozone. The error bar shows SE (n = 5). Repeated Measures ANOVA: G gas treatment; P difference of canopy position; T time course, *** $P < 0.001$; n.s. not significant.

specific. Actually, when O_3 sensitivity is compared between the Japanese oak and Siebold's beech based on the photosynthesis of only upper canopy leaves, Siebold's beech is considered to be a sensitive tree species. However, focusing on only lower canopy leaves gives the opposite result. Evaluating O_3 sensitivity of biomass productivity requires an integrated approach.

Stomatal O_3 uptake is one of the most important factors in evaluating the effects of O_3 on leaf photosynthesis. Patterson *et al.* (2000) demonstrated lower stomatal O_3 uptake in leaves of understory trees (2~9 m in height) than that in overstory trees (12~23 m in height) for black cherry, red maple and northern red oak trees in Great Smoky Mountains National Park, USA. In the present study, G_s under saturated light condition was lower in the lower canopy leaves as compared to leaves in upper canopy (Table 1). Additionally, lower canopy leaves were usually shaded by upper canopy leaves. Therefore, G_s, and thereby stomatal O_3 uptake, in lower canopy leaves would be lower than those in upper canopy leaves. This indicates an adverse effect per unit O_3 uptake on photosynthesis might be higher in the lower canopy leaves.

We did not monitor O_3 concentration in the lower canopy at ambient plot. In general, O_3 concentration in the lower canopy is lower than that above canopy (*e.g.* Patterson *et al.* 2000; Erzyzanowski, 2004). However, the concentration of ambient O_3 in the study site was low and therefore we consider the effect of ambient O_3 was not significant in both upper and lower canopy leaves.

There were major variations in the environmental conditions among the previous studies that evaluated O_3 sensitivity (Tjoelker *et al.*, 1993, 1995; Kitao *et al.*, 2009; Watanabe *et al.* 2014); therefore, uncertainties concerning the comparison of the specificity of each tree species were raised. On the other hand, the results of the Japanese oak in the present study and those of Siebold's beech in Watanabe *et al.* (2014) were obtained from the same experimental site in the same growing season. Additionally, relative light intensities in lower canopy were similar in the two species, around 10~15%. Therefore, our results strongly confirm the species specificity in the difference of O_3 sensitivity between upper and lower canopy leaves even in the same environmental condition. As a next step, the clarification of mechanisms of the difference between species is needed.

Acknowledgments

This study was supported by the Environment Research and Technology Development Fund (B-1105) of the Ministry of the Environment, Japan (grant to T. Koike), and a Grant–in–Aid from the Japan Society for the Promotion of Science through its Type B program (to T. Koike, 23380078 and 26292075), Young Scientists B (to M. Watanabe, 24710027 and 15K16136, and to Y. Hoshika, 24780239) and Young Scientists for research abroad (to Y. Hoshika). We appriciate for relevant suggestions and comments from the editor and anonymous two reviewers, which helped us to improve the manuscript.

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