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Stomata Density and Size of *Acer palmatum* to the Elevated Ozone

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Abstract : *Acer palmatum* was selected and its responses to elevated ozone were subsequently measured during growing periods. Ozone concentration of this study was compared to the calculated AOT40 value. *A. palmatum* had significantly many but small stomata size to the ozone stress. The length of stomata of *A. palmatum* was reduced from 5.6 to 5.0 μm to the ozone exposure. However, the number of stomata (density) was increased from 102 to 131 in the 500×500 μm leaf area.

Key words **:** Acer palmatum, *air pollution, ozone, AOT40, stomata density, stomata length*

Introduction

In urban environments, trees play an important role in the improvement of air quality, through uptake of gases and particles. In particular, street trees intercept a greater percentage of aerosols than shorter vegetation, resulting in a higher deposition rate of gaseous pollutants and particulates. However, uptake rates depend upon many factors such as leaf surface condition, boundary layer and stomatal openness.

Generally, the responses of facing a stressful environment are determined by investigating various parameters. First, visible foliar injuries provide clues to recognize the effects of ozone on many plant species. One of the main responses is foliar stipple, which is characterized by small, discolored patches of cells on leaf adaxial surfaces and is caused by the loss of chlorophyll (Tonneijck and van Dijk, 2002; Neufeld *et al.*, 2006). Furthermore, in the responses such as leaf ontogeny, leaf area or leaf final area under environmental stress can vary according to species-specific characteristics (Tichà, 1985; Gnthardt-Goerg *et al.*, 1993). In addition, environmental conditions can contribute to the final leaf size as well as leaf differentiation such as tissue structure and stomatal density, which can affect later control of leaf gas-exchange (Hinckley and Braatne, 1994; Frey *et al.*, 1996).

This study was performed to establish basic information on the *Acer palmatum* responding to elevated ozone. The main study purposes are 1) to determine the relevance of ozone exposure protocol in this study in comparison with to AOT 40, and 2) to observe the leaf stomata density and size to the elevated ozone.

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Material and Method

1. Plants and ozone exposure protocol

Two-year-old *Acer palmatum* seedlings were selected for ozone fumigation in a natural environmental chamber (25±1.0°C/day, 22±1.0°C/night, 65±5% RH). Ozone exposure followed the protocol presented in Figure 1: its concentration was gradually increased from 50 to 110 nmol mol^{-1}. All plants underwent ozone treatment for 40 days (June 6 to July 15), preceded by two-month accliminationre acclimated in the greenhouse for two months before the ozone fumigation. During ozone fumigation, the position of each plant was changed everyday in order to avoid measurement errors from their space.

The effect of exerted by ozone on plants is decided by a complicated correlation of various factors such as species, growing stages and vegetation during ozone treatment (Cho, 2007). AOT40 has been used as an index of ozone effect on forests and crops in Europe and it is the calculated value of the accumulating ozone concentration over 40 ppb during day hours, as shown in the following formula:

$$AOT40 = \sum_{j=i}^{j=i+90} \sum_{k \in day} (c_{i,k} - 40)$$

where Ci,k indicates the ozone concentration for j days and k hours and "k∈day" means the sum of day hours. It has been shown that crops are negatively affected by ozone when AOT 40 is over 3,000 nmol mol^{-1}-hr (Cho, 2007; Fuhrer *et al.*, 1997). In addition, UN/ECE (2001)

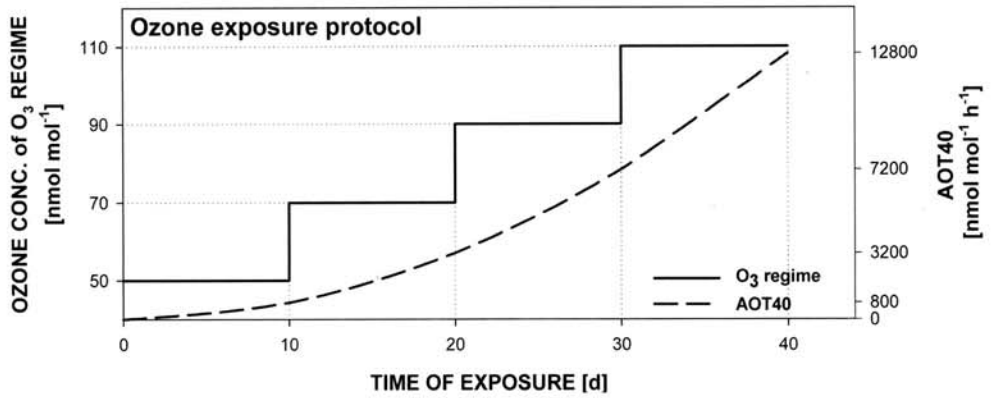

Figure 1. Ozone exposure protocol. The numbers on the X-axis represent the date of each research. The numbers on the left and right sides of the Y-axis represent the ozone concentration and AOT40 calculated at every 10 d, respectively.

has reported that AOT40 level over 10,000 nmol mol^{-1}-hr of AOT 40 had a harmful influence on forest. In applying the concept of AOT 40 to this study, we summed up the ozone concentration over the 8 hours of (the hours of ozone treatment) during the study and assumed the ozone concentration was below 40 nmol mol^{-1} following the greenhouse accilimation period before the study commenced. We compared each point of ozone concentration according to the ozone protocol to the value calculated by AOT 40 (Figure 1).

Table 1. Changes of stomata density according to unit area (500×500 μm) and length in species under ozone stress. The numbers in parentheses are standard deviation (SD). Data was analyzed using One-way ANOVA between control and treatment at the significance level of 0.05. Different letters indicate different means at 5% significance level.

Species	Treatments	Number of stomata (500×500 μm)	Length of stomata (μm)
A. palmatum	Control	102.3 (2.63)b	5.6 (1.21)a
	Ozone treatment	131.0 (11.40)a	5.0 (1.89)b

2. Statistical analysis

Statistical analysis of the effect of ozone on the stomata density and size were examined using One and Two-way ANOVA. The significant difference between treatment and control was determined with Duncan's multiple range test (DMRT) was performed. Differences were considered significant at $p<0.05$.

Results and Discussion

The leaf surfaces were observed with SEM to investigate the changes in the stomata features and shape, and the leaf abaxial surfaces under the elevated ozone (Figure 2). Stomata density and length were measured to investigate the changes after ozone exposure. The stomata density of *A. palmatum* was increased under the ozone treatment (Table 1). The length of *A. palmatum* was statistically decreased under the elevated ozone (Table 1).

One of the main roles of stomata is allowing the passage of CO_2, which is the essential material for carbon assimilation. The quality of a plant's physiological activity depends on the plant's ability to control its stomata. In other words, it is possible to estimate a plant's vitality by measuring stomata characteristics. Narrower aperture through closed stomata is linked to higher stomata resistance and lower stomatal conductance.

In this study, *A. palmatum* had significantly many but

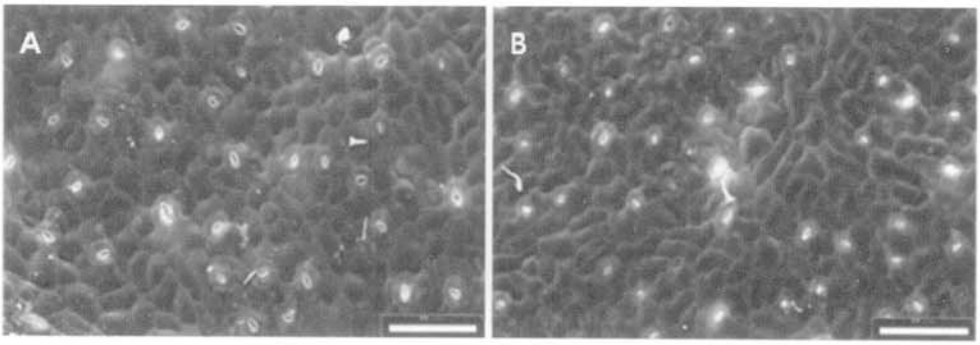

Figure 2. Stomatal shapes on leaf abaxial surfaces of *A. palmatum*. The left (A) and right columns (B) represent control under ambient air and treatment under elevated ozone, respectively (Bar-20 μm).

small stomata size to the ozone stress (Table 1 and Figure 2). Another study also reported the wide variations in the stomata size and frequency among species and genotypes. *Populus maximowiczii* × *P. nigra*, *Acer negundo*, and *Acer saccharium* had many small stomata. *Populus deltoids*, *Populus nigra*, and *Ginkgo biloba* had few but large stomata (Kozlowski and Parllardy, 1997).

Furthermore, Stomata changes will be a good indicator for the environmental changes because plants generally close their stomata under air pollution. Excess levels of ozone inside leaves have been reported to change stomatal conductance, as controlled by the density, size and aperture of stomata (Samuelson, 2001; Elagöz *et al.*, 2006). In this study, *A. palmatum* showed decreased stomata size. However, the increased stomata density was found on the *A. palmatum*. A higher stomatal density is related to greater sensitivity to ozone and thicker palisade mesophyll tissues have been found in sensitive individuals than in insensitive ones (Evans *et al.*, 1996).

Plants exhibit not only common changes such as stomata closure and decreased photosynthesis but also species-specific characteristics based on genetic and ontogenetic features such as various changes on the leaf abaxial surfaces and stomata density responding to elevated ozone. Based on these results, we plan to investigate the antioxidant mechanism of each plant in order to more clearly elucidate the physiology. In addition, these accumulated and integrated results from each plant will be helpful to make an index for measuring a plant's responses to elevated ozone (Tausz *et al.*, 2007). To provide further proof of the relationship between this ozone protocol and AOT40, more research on a greater variety of species using the AOT40 index is required. We will investigate several protocols with varying concentration and duration in comparison with AOT40 to confirm the correlation between visible injuries and AOT40 values, and the absence of any overlapped effect, by examining a range of ozone concentrations and exposure durations.

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