# Postprint: Response of Stomatal Characteristics in Six Landscaping Tree Species to Ozone Dose

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## Abstract

An open-top chamber (OTC) system was employed to investigate the effects of five ozone (O3) concentrations on stomatal density, aperture, and size in six urban greening tree species, namely poplar genotypes '546' (Populus deltoides cv. '55/56' × P.deltoides cv. 'Imperial' ) and '107' (P.euramericana cv. '74/76' ), Fraxinus chinensis, Platanus orientalis, Robinia pseudoacacia, and Sophora japonica. The results demonstrated that stomatal density, aperture, and size all decreased significantly with increasing O3 concentrations, and significant differences existed among tree species for all stomatal characteristic indices. Significant interactive effects were detected among O3 treatment, tree species, and sampling period, as well as between O3 treatment and tree species, and between sampling period and tree species. All stomatal indices of the six tree species exhibited a significant linear negative correlation with O3 dose (AOT40, the accumulated value of hourly O3 concentrations exceeding 40 nmol/mol) ($P < 0.05$), decreasing significantly with increasing O3 concentrations. This study provides theoretical evidence for in-depth research on the adaptation of leaf tissue structure and function in urban plants to ground-level O3 pollution against the backdrop of environmental change.

## Full Text

### Stomatal Characteristics and Ozone Dose-Response Relationships for Six Greening Tree Species

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**Abstract**

Open-top chambers were used to investigate the stomatal characteristics (i.e., stomatal density, aperture, and size) of six greening tree species in response to different ozone exposure treatments over one growing season. The selected species, representing a wide range of woody species used for urban greening in Beijing, included *Populus deltoides* × *P. euramericana* '55/56' , *P. deltoides* '74/76', *P. euramericana* '107', *Fraxinus chinensis* 'Imperial', *Platanus orientalis*, *Robinia pseudoacacia*, and *Sophora japonica*. One-year-old seedlings showed significant variation in stomatal characteristics among species. Generally, stomatal density, aperture, and size all decreased significantly with increasing ozone concentrations. There were significant differences in stomatal characteristics among the six tree species, and significant interaction effects among tree species, ozone treatments, and sampling times. All six species showed a significant negative linear correlation ($P < 0.05$) between stomatal parameters and the ozone dose index AOT40 (accumulated hourly mean O concentration over a threshold of 40 nmol/mol during daylight hours). Among the linear relationships between stomatal parameters and AOT40, poplar showed the maximum slope, and the order of O sensitivity was: *Populus* > *Platanus orientalis* > *Fraxinus chinensis* > *Robinia pseudoacacia* > *Sophora japonica*. Our results provide new evidence for research on the effects of ground-level O pollution on leaf tissue structure and functions of greening plants under environmental change.

**Keywords:** ozone; dose; stomatal characteristics; woody species; linear relationship

## 1. Introduction

Ground-level ozone (O ) is considered one of the most phytotoxic air pollutants due to its significant damaging effects on plants. Ozone concentration is rising at a rate of approximately 0.5%–2% per year over the mid-latitudes of the northern hemisphere due to rapid industrialization and urbanization during the last three decades. High levels of O occur during summertime in most parts of China, and are expected to increase further as NO precursors continue rising. According to monitoring data from the Ministry of Environmental Protection, hourly average summer concentrations in Beijing reach 60 nmol/mol, with peaks up to 300 nmol/mol—far exceeding the critical load for sensitive tree species.

Urban trees play an irreplaceable role in maintaining urban ecological balance and mitigating environmental problems. However, elevated O concentrations cause a series of damage symptoms in trees, including visible foliar injury, reduced photosynthetic rates, sluggish or malfunctioning stomatal responses, increased water consumption, altered biomass allocation patterns, and decreased resistance to pests and environmental stresses, thereby reducing the potential of urban trees to improve ecological conditions.

Stomata are crucial regulatory organs for gas exchange processes such as photosynthesis and transpiration. Their characteristics reflect plant responses and adaptations to environmental changes and stress. As a stress factor, O enters plants primarily through stomata, making stomatal features a key determinant of O uptake and subsequent damage. While several studies have reported effects of O on stomatal characteristics of urban tree species, results have been inconsistent—some found O significantly reduced stomatal aperture but not density, while others reported increased stomatal density. These discrepancies likely arise from differences in experimental duration, O concentrations, and leaf developmental stages.

Establishing dose-response relationships based on different O concentration gradients is an effective method for quantitatively evaluating O damage. Given that tree growth is slow and visible injury symptoms take years to manifest, using physiological indicators—particularly sensitive ones like stomatal characteristics—to develop dose-response relationships is a practical approach. This study selected six common urban tree species in northern China to investigate their stomatal responses to different O concentrations, aiming to provide a scientific basis for screening pollution-resistant species and protecting urban trees from O damage.

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## 2. Materials and Methods

**2.1 Study Site and Experimental Materials** The experiment was conducted in Tangjiabao Village, Yanqing District, Beijing (40°45 N, 115°97 E), located northeast of downtown Beijing. The area has a continental monsoon climate in the transition zone between temperate and mid-temperate, semi-arid and semi-humid regions, with cold winters and cool summers. The mean annual temperature is 10.2°C, mean annual precipitation is 567 mm, and annual sunshine hours average 2800 h.

Six urban tree species were selected: three poplar genotypes (*Populus deltoides* × *P. euramericana* '55/56' , *P. deltoides* '74/76' , and *P. euramericana* '107' ), *Fraxinus chinensis* 'Imperial' , *Platanus orientalis*, *Robinia pseudoacacia*, and *Sophora japonica*. In May 2016, uniform one-year-old seedlings were transplanted into 20-cm diameter pots filled with a 1:1 mixture of local soil and peat moss. Seedlings were allowed to acclimate for two weeks before ozone treatments began.

**2.2 Experimental Design** Seedlings of similar height and basal diameter were selected and placed in open-top chambers (OTCs) to acclimate to chamber conditions. The OTCs were regular octagonal columns (3.0 m height). Four ozone treatments were established: CF (charcoal-filtered ambient air, [O ] < 40 nmol/mol), NF (non-filtered ambient air), NF20 (NF + 20 nmol/mol O ), NF40 (NF + 40 nmol/mol O ), and NF60 (NF + 60 nmol/mol O ). Each treatment had three replicates.

Ozone concentrations were monitored using a Model 49i ozone analyzer (Thermo Scientific, Franklin, MA, USA). Fumigation ran daily from 08:00 to 18:00 (10 h) on non-rainy days. Temperature and humidity inside and outside chambers were monitored using a CR1000 data logger (Campbell Scientific, North Logan, Utah, USA). During the fumigation period, internal temperatures averaged 1.7°C higher and humidity 4.5% lower than external conditions.

**2.3 Sampling and Measurements** Leaf samples were collected at two time points: early August and early September. For each species, three seedlings per treatment were sampled. Three to five mature, healthy leaves from the middle-upper canopy were collected between 9:00–11:00. The abaxial surface near the midrib was cleaned, and clear nail polish was applied. After drying, the film was peeled off with tweezers, mounted on slides, and observed under a microscope.

Stomatal density was determined by counting stomata in microscope images using DinoCapture 2.0 software. Stomatal length (parallel to the stomatal pore) and width (perpendicular to the pore) were measured using a micrometer. Stomatal aperture was calculated as: aperture = × (length/2) × (width/2). For each image, 10–15 clear stomata were measured and averaged.

**2.4 Data Analysis** Data were analyzed using SPSS 18.0 software. Three-way ANOVA was performed to examine main effects and interactions of O treatment, sampling time, and tree species on stomatal parameters. Tukey' s Honestly Significant Difference (HSD) test was used for pairwise comparisons. Linear relationships between stomatal characteristics and AOT40 (accumulated hourly O concentration >40 nmol/mol) were analyzed using General Linear Model and Spearman' s correlation. Significance was set at $P < 0.05$. Data are presented as mean ± SD.

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## 3. Results

**3.1 Effects of O on Stomatal Density** Three-way ANOVA showed that O concentration, tree species, and their interaction significantly affected stomatal density ($P < 0.0001$), while sampling time alone did not ($P = 0.1498$). The interaction between sampling time and species was marginally significant ($P = 0.0660$). All six species showed significant linear decreases in stomatal density with increasing AOT40 at both sampling times ($P < 0.05$). The declining slope was steeper in September than in August for all species.

At NF60 (AOT40: 29.9-40.7 mol/mol), stomatal density decreased by 26-55% compared to CF. *Platanus orientalis* and *Sophora japonica* showed significant differences between sampling times, with densities decreasing by 14-35% from August to September.

**3.2 Effects of O on Stomatal Aperture** O concentration, sampling time, and species all significantly affected stomatal aperture ($P < 0.0001$), with significant interactions between O and species ($P < 0.0001$) and sampling time and species ($P < 0.0001$). All species showed significant linear decreases in stomatal aperture with increasing AOT40 at both times ($P < 0.05$). The declining slope was steeper in September than August.

Sampling time significantly affected poplar and ash ($P < 0.05$) but not other species. At NF (AOT40: 8.3-11.4 mol/mol), stomatal aperture of *Robinia pseudoacacia* and *Sophora japonica* was already significantly reduced.

**3.3 Effects of O on Stomatal Size** O concentration, sampling time, and their two-way interactions significantly affected stomatal size ($P < 0.01$). All species showed significant linear decreases in stomatal size with increasing AOT40 ($P < 0.05$), except for the two poplar genotypes in August. The declining slope was steeper in September than August for all species.

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## 4. Discussion

Stomatal attributes (number, size, and function) are highly sensitive to environmental changes. Ozone effects on plants are cumulative, and damage to stomatal properties intensifies over time. This study found that with increasing O concentration and fumigation duration, stomatal density, aperture, and size of all six species decreased significantly, indicating cumulative O effects. These changes likely reflect a trade-off where plants allocate more resources to maintenance survival rather than growth under stress.

Previous studies have reported inconsistent effects of O on stomatal density. Some found no significant effect, while others reported increased density. These discrepancies may relate to differences in fumigation duration, concentration, and leaf developmental stage. Stomatal formation occurs early in leaf development, so O concentration changes have greater impacts on young leaves. While adult leaf stomatal number is genetically controlled, our results show that even in mature leaves, high O can reduce stomatal density, possibly by damaging stomatal apparatus cells and epidermal cell development.

Stomatal aperture determines O entry into plants. Our finding that O reduced both aperture and size aligns with most previous research. However, some studies report stomatal sluggishness or failure to close under O stress. This variation reflects species-specific O sensitivity. More sensitive species may experience collapsed detoxification systems and impaired guard cell function at the same O concentration, preventing stomatal closure and increasing damage.

Significant interactive effects existed between O treatment and species, and between sampling time and species, for all stomatal attributes. This arises from inherent interspecific differences in stomatal properties, which also change with phenology. Species O sensitivity is genotype-specific, with more sensitive species showing earlier physiological responses. Our sensitivity ranking (*Populus* > *Platanus* > *Fraxinus* > *Robinia* > *Sophora*) aligns with previous studies.

While this study analyzed linear relationships between stomatal characteristics and AOT40, future research should quantify other physiological indicators (photosynthesis, water relations, antioxidant systems) to better understand O impacts. Plants can both regulate stomatal opening short-term and control stomatal development long-term to adapt to environmental changes. Long-term experiments are needed to understand cumulative O effects and plant adaptation across different developmental stages.

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## 5. Conclusion

Stomatal characteristics of the six urban tree species were highly sensitive to elevated O concentrations. Increasing O significantly reduced stomatal density, aperture, and size, with significant interactions among O treatment, species, and sampling time. The species-specific responses likely reflect inherent differences in stomatal properties and developmental stages between peak and late growth periods. This study provides theoretical evidence for understanding how urban plant leaf structure and function adapt to ground-level O pollution under environmental change.

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*Note: Figure translations are in progress. See original paper for figures.*