Decrease in the photosynthetic rate of leaves of
Nex rotunda seedlings exposed to urban atmospheric condition

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Abstract: In order to quantify the sole effect of urban atmospheric condition on revegetation trees, we exposed potted *I. rotunda* seedlings in the central district of Fukuoka City, where air pollutant concentration was high and heat island effect was observed, and in a suburban nursery as control. The seedlings of both sites were subjected to identical sunlight and soil conditions. After 10-week exposure, we observed a significant reduction of the photosynthetic rate of leaves, but not of growth. The reduction of photosynthetic rate would be caused by highly concentrated air pollutant as well as heat island effect. The reduction was due to the reduced carboxylation efficiency and was moderated by the soil of higher nitrogen content.

Key words: air pollutant, heat island effect, *Ilex rotunda*, photosynthesis, exposure experiment

高木正博・玉泉幸一郎：都市内の大気環境への暴露によって低下したクロガネモチ苗木の光合成速度

摘要：都市環境が緑化樹に与える様々なストレス要因のうち、車が集中する都市部で必然的に悪化する大気環境（高い大気汚染物質濃度とヒートアイランド効果）の影響を知るために、福岡市の中心部とコントロールとして5 km 離れた郊外にクロガネモチの鉢苗を10 週間暴露した。暴露処理中は暴露個体とコントロールで同じ土壌水分条件と光環境を保った。暴露処理の後、単葉の光合成速度は暴露個体で有意に減少したが成長は差が認められなかった。この光合成速度の低下の原因として、高い濃度の汚染物質濃度の影響だけではなく高い気温と低い湿度といったヒートアイランド効果の影響も考えられた。光合成速度の減少は、気孔の阻害よりも二酸化炭素固定効率の低下によってもたらされていた。また土壌中窒素濃度が高いと光合成速度の低下は緩和された。

キーワード：大気汚染物質，ヒートアイランド効果，クロガネモチ，光合成，暴露実験

1. Introduction

Revegetation trees grown in urban areas suffer from many and concurrent environmental stresses, such as air pollutant, air of higher temperature and lower humidity, diminished sunlight, and poor nutrient and tread soil. The stresses were realized as decrease in growth and depression of physiological activities (Mansfield & Freer-Smith, 1981; Jim, 1992; Hodge & Boswell, 1993). The decrease in growth rate and chlorophyll content of revegetation trees in urban area compared to suburban was reported for *Ligustrum lucidum* (Cañas et al., 1997) and for *Delonix regia* and *Cassia fistula* (Pandey & Agrawal, 1994). The lowered photosynthetic rate of *Tilia euchlora* and *Elaeagnus angustifolia* in urban area was indicated by Gej & Gozlinski (1983).

Of the many stress factors affecting revegetation trees due to urbanization, atmospheric factors (i.e., air pollutants, higher temperature and lower humidity) are inherent in urbanization. Air pollutant concentration is necessarily higher in the districts with high vehicular traffic. Temperature and humidity of air are

affected by waste heat and heat radiated from concrete surfaces (heat island effect). In contrast, stresses caused by insufficient sunlight due to interception by surrounding buildings and poor soil condition could be improved by planning planting position or adequate arboricultural management. Therefore, it is important to separate and quantify the effect of atmospheric factors from that of the remediable factors, in order to investigate the cause of decrease in growth and depressed physiological activity of revegetation trees. This would be also useful in urban planning and selecting revegetation tree species.

The maximum photosynthetic rate of mature *Ilex rotunda* Thunb. in the central district of Fukuoka was lower than that of suburban *I. rotunda* (Takagi et al., 1996), although *I. rotunda* is known to be tolerant to air pollutants and poor sunlight condition. This depression is likely to be caused by the atmospheric stress factor, because the concentration of air pollutant in the district was extremely high (Statistical Bureau of the City of Fukuoka, 1995) and heat island effect was observed (Takagi et al., 1996). In this study, we grew potted *I. rotunda* seedlings in the central dis-

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tricts of Fukuoka and in a control suburban site under identical sunlight and soil conditions to investigate the sole effect of atmospheric stress factor, eliminating remediable stress factors. After a 10-week exposure, we compared the growth and photosynthesis capacity of the seedlings.

2. Materials and methods

2.1 Plant material

I. rotunda is a board-leaved evergreen tree, the major urban revegetation tree species in Fukuoka and was chosen as the city tree. Two-year-old commercially available seedlings of *I. rotunda* were planted in 3.3 L volume ceramic pots containing a 7:3 mixture of granite clay and porous gravel (Kanumatsuti). All seedlings were grown outdoors in the nursery of the Kyushu University for three months from mid April 1997. The nursery was in a suburban areas of the city, 1 km from heavy traffic streets and thus free from car exhaust. Watering was carried out as necessary. Granulated fertilizer (JA, Kumiai urea containing IB Kasei No. S1), which is effective over a few months, was supplied to half of the seedlings at planting at 2 g nitrogen per pot. The remaining seedlings were not supplied with the additional fertilizer. On 13 July, after measuring stem size and photosynthetic rates, the seedlings of both fertilizing groups were divided into exposure and control treatment respectively, so that the average photosynthesis rate for both treatment was almost equal. The half of the seedlings subjected to the exposure treatment were transferred to the central districts of the city described below. The remaining seedlings were allowed to continue growing in the nursery as control. Four sets of the seedlings were thus obtained, consisting of five seedlings each.

2.2 Study site and exposure treatment

The transferred seedlings were exposed to urban atmospheric conditions for 10 weeks at a median strip in the central district of Fukuoka, 5 km south from the nursery. Fukuoka (33°35'N, 130°24'E) is the central commercial city in western Japan and has a population of 1.2 million. The street runs north to south, is 45 m in width with six lanes, and is situated between buildings of 30 to 40 m in height. Traffic flow over a 12 h daytime period on the street was 30,000 cars. The pots were placed in an open space on the southern edge of the median strip. At this median strip, the duration of direct sunlight irradiating the seedlings was restricted by buildings on the east and west. In order to obtain irradiation time equal to that in the central district, we hung shading sheets on the west and east sides of the seedlings in the nursery.

During exposure, water of the same quality and quantity was given to the exposed and the control seedlings every day except on rainy days. Concentrations of NO₂ and SO₂ were measured since these gases

were directly exhausted from cars. Mean NO₂ and SO₂ concentrations for 24 h were measured using a passive NO₂ and SO₂ sampler (Green blue, Handy SONOX) three times at weekly intervals. Temperature and humidity of the air were recorded using a temperature and humidity probe and recorded at 10-min intervals (Sato Keiryoki, SKL-2000TH) during the exposure. The diurnal course of PAR for both the exposed and the control seedlings were measured using the PAR sensors (Koito Industries, IKS-25) on 20 August. These sensors and samplers were set just above the seedlings. On 23 September, seedlings were transferred to the laboratory of the Kyushu University for growth and photosynthesis measurements and chemical analysis.

2.3 Measurement of seedling properties

The photosynthesis rate (P) of leaves of the seedlings was measured before and after the exposure using a portable photosynthesis and transpiration measurement system (ADC, LCA-3). Before the exposure, P for three leaves per seedling was measured in the nursery under full sunlight at ambient CO₂ concentrations. Following the exposure, the response of P to varying CO₂ concentration under light saturated condition were measured with a single leaf attached to the seedling. Three leaves per seedling, three seedlings per treatment for a total of nine leaves per treatment were measured. The CO₂ concentration supplied to the leaves was controlled by a mass flow valve that mixed pure CO₂ gas gasified from liquid CO₂ and CO₂-free air passed through soda lime. Leaf temperature was measured with a thermistor in a chamber and maintained at 25±0.5°C by a peltier element optionally attached under the chamber. Light was provided by a metal halide lamp (OSRAM, HQI-TS 70W/NDL) and set to 1,200 μmol m⁻² s⁻¹ in PAR. Humidity was set to 1.3±0.1 kPa in VPD by turning a valve controlling the humidity of inlet gas to a leaf chamber of the LCA-3.

From the response curve, P and stomatal conductance at 350 μmol mol⁻¹ CO₂ concentration were used for analysis and called P_{350} and g_{s350} , respectively. Intercellular CO₂ concentration was calculated after von Caemmerer & Farquhar (1981). The slope of the initial linear portion of the response curve of P to intercellular CO₂ concentration was defined as carboxylation efficiency (K_c) (Farquhar & Sharkey, 1982). The P was measured under dark and 350 μmol mol⁻¹ CO₂ concentrations and called dark respiration rate (R_d). Regulation capacity of stomata (q_g) was estimated as the ratio of g_{s350} to minimal stomatal conductance during dark respiration (Kammerbauer et al., 1987). The effect of the exposure treatment on growth of all seedlings was indicated as the ratio of the increment of the size of stem during the exposure to that of the beginning of the exposure. The stem size (D₀²H) was evaluated by the product of the height and squared diameter at soil

surface. The total nitrogen of the soil in all pots was analyzed after the photosynthesis measurement with 1 g dried soil using a CN coder (Yanako, MT-500). The soils of the each treatments were sampled from the all pots and pooled and mixed for the analysis respectively. Non parametric two-way ANOVA was used to compare each parameter between the exposed and the control seedlings and between the fertilized and the unfertilized seedlings.

3. Results

3.1 Exposure condition

Daily mean concentrations of NO_2 and SO_2 were higher in the urban district than in the control nursery. The average NO_2 for three measurements was $120(\pm 17 \text{ SE}) \text{ nmol mol}^{-1}$ in the urban district and $30(\pm 2 \text{ SE}) \text{ nmol mol}^{-1}$ in the nursery. The concentrations of SO_2 was $41(\pm 4 \text{ SE}) \text{ nmol mol}^{-1}$ in the urban district and $4(\pm 1 \text{ SE}) \text{ nmol mol}^{-1}$ in the nursery. The heat island effect was observed in the urban district. The average, minimum and maximum of air temperature during the exposure was higher in the urban district than in the control nursery (Table 1). The average, minimum and maximum of air humidity during the treatment was lower in the urban district than the control nursery. In the urban district, the sunlight irradiated seedlings was for only five hours due to the interception by the surrounding buildings. An identical diurnal course of PAR was obtained at the nursery by hanging shading sheets beside the seedlings (Fig. 1).

Table 1 Average, minimum and maximum of air temperature ($^{\circ}\text{C}$) and air humidity (%) in urban district of Fukuoka City and in suburban control district during study period

表-1 試験地と対照区とした郊外の暴露期間中の気温 ($^{\circ}\text{C}$) と湿度 (%) の最小値, 最大値および平均値

	Air temperature		Air humidity	
	Urban	Control	Urban	Control
Average	29.9	27.4	62.5	75.6
Minimum	23.4	19.1	29.6	40.1
Maximum	41.4	37.0	95.6	99.9

Table 2 Probability of 2×2 factorial analysis for gas exchange parameters and stem size increment of *Ilex rotunda* seedlings after 10-week exposure to urban atmospheric condition and suburban control condition

表-2 都市大気環境と対照区の郊外に 10 週間暴露したクロガネモチ鉢苗のガス交換特性と成長の暴露処理と施肥処理による二元分散分析

	Exposure	Fertilize	Interaction
Photosynthetic rate (P_{350})	$p < 0.01$	n.s.	n.s.
Calboxylation efficiency (K_c)	$p < 0.001$	$p < 0.01$	n.s.
Dark respiration rate (R_d)	$p < 0.05$	$p < 0.01$	$p < 0.01$
Stomatal conductance (g_{s350})	n.s.	n.s.	$p < 0.01$
Stomata regulation capacity (q_g)	$p < 0.05$	n.s.	n.s.
Stem size increment (ΔD_0^{2H})	n.s.	$p < 0.001$	n.s.

n.s., not significant.

Fig. 1 Diurnal courses of PAR in urban district of Fukuoka City (thin line) and in suburban control site (bold line) on August 27, 1997. The latter was modified by hanging shading sheets beside the seedlings to obtain sunlight conditions identical to those of the urban seedlings.

図-1 1997年8月27日における福岡市中心部(細線)と対照区とした郊外(太線)のPARの日変化
対照区では中心部と同じ日変化を得るために鉢苗の両側に寒冷紗をぶら下げた。

3.2 Seedling characteristic

The average and standard errors of seedling characteristics are shown in Fig. 2. The results of non parametric two-way ANOVA of the seedling characteristics are shown in Table 2. The relative size increment of the stem (ΔD_0^{2H}) did not differ significantly between the exposed and the control seedlings. In contrast, P_{350} and K_c decreased significantly for both fertilized and unfertilized groups due to the exposure to the urban atmosphere. P_{350} decreased by 30% for the unfertilized group and by 20% for the fertilized group. The decrease in K_c was by 27% for both groups. Nitrogen rich soil moderated the decrease in P_{350} . R_d differed significantly with exposure and fertilizing treatment, but the interaction was also significant. The effects of the exposure and fertilizing treatment on g_{s350} were insignificant and interactive. The g_{s350} of the exposed seedlings

Fig. 2 Photosynthetic rate (P_{350}), carboxylation efficiency (K_c), dark respiration rate (R_d), stomatal conductance (g_{s350}), stomata regulation capacity (q_g) and stem size increment (ΔD_0^2H) of fertilized and unfertilized *Ilex rotunda* seedlings of the urban district (exposed) and of suburban control site (control) after 10-week exposure. Bars indicate standard errors.

図-2 10週間の暴露期間終了後の暴露個体と対照個体の光合成速度 (P_{350}), カルボキシル化効率 (K_c), 暗呼吸速度 (R_d), 気孔コンダクタンス (g_{s350}), 気孔開閉能力 (q_g) および幹成長速度 (ΔD_0^2H)
縦棒は標準誤差を示す。

was higher in the fertilized groups and lower the in unfertilized groups. The q_g of both the fertilized and unfertilized groups decreased significantly due to exposure.

The total nitrogen concentration of the pooled soil sample of the fertilized group was higher than that of the unfertilized group after exposure. The nitrogen concentrations for the fertilized groups were 0.34 g kg^{-1} and 0.32 g kg^{-1} ; concentrations for the unfertilized groups were 0.21 g kg^{-1} and 0.20 g kg^{-1} , for the exposed and the control seedlings, respectively. These concentrations were lower than that of planting pits of *I. rotunda* in Fukuoka. The range of the concentration of 13 locations throughout Fukuoka was from 0.1 to 2.0 g kg^{-1} (Takagi and Gyokusen, unpublished data).

4. Discussion

Exposure to urban air condition for 10 weeks reduced the photosynthetic rate of *I. rotunda* seedlings but did not affect the growth. Depressed photosynthetic capacity of mature trees in urban areas was observed for *I. rotunda* (Takagi et al., 1996) and for *Tilia euchlora* and *Elaeagnus angustifolia* (Gej & Gozliniski, 1983). However, these studies could not determine whether the cause of depression was inherent factors in urbanization (atmospheric conditions) or remediable factors by arboricultural management (sunlight and soil condition). In this study, the effects of the remediable factors on the exposed seedlings were eliminated, and the P_{350} of the exposed seedlings

was reduced. Therefore, we could indicate that urban atmospheric conditions alone depressed the photosynthetic capacity of *I. rotunda*.

One reason that the seedling growth was not significantly affected by the exposure but the photosynthetic capacity was reduced might be the short duration of the exposure. Although the P_{350} decreased by 30% for the unfertilized seedlings and by 20% for the fertilized seedlings, the ΔD_0^2H were not significantly different between the exposure seedlings and control. Decreased carbon assimilation, however, should cause decreased mass increment. Therefore, this undisturbed growth irrespective of carbon gain may be the evidence of tolerance to the stresses of *I. rotunda*.

Both the higher concentration of air pollutant and the heat island effect would be the cause of the decrease in P_{350} . Kammerbauer et al. (1987) concluded that the 30% decrease in photosynthetic rate of *Picea abies* exposed at a highway border for 10 weeks was due to reduced carboxylation efficiency. Whitlow et al. (1992) observed water deficit of leaves of *Tilia cordata* and *Fraxinus pennsylvanica* brought about by the higher air temperature and lower humidity of the urban atmosphere in New York, USA. Water stress reduces carboxylation efficiency (Lambers et al., 1998). However, dry condition can protect plant from air pollution. Lower relative humidity closed stomata thus less ozone was able to enter leaves (Treshow & Anderson, 1989). Further research will be needed to understand the mechanism of impaired photosynthesis

by urban atmosphere to revegetation trees.

For the most part of the reduction in P_{350} by the exposure to the urban atmosphere was due to the depressed K_c . However, the degree of reduction in P_{350} differed between the fertilizing treatments; 30% decrease for the unfertilized group and 20% decrease for the fertilized group, although the degree of reduction in K_c did not differ between the fertilizing treatments; 27% decrease for both groups. Thus the difference of P_{350} between the fertilizing treatments would be due to the difference of stomatal conductance. For the fertilized seedlings, the decreased photosynthetic capacity due to the reduced carboxylation efficiency was moderated by the improved stomatal opening. In contrast, for the unfertilized seedlings, that was accelerated by the reduced stomatal opening. The reason for the differing stomatal behavior with fertilizing treatments was unclear. However, significantly reduced q_g due to the exposure indicated that the urban atmospheric condition was injurious to stomata. Wolfenden & Mansfield (1991) indicated that guard cells of stomata could be disrupted by SO_2 causing stomata to remain open. Hirano et al. (1991) showed that dust that fell into stomata reduced stomatal conductance and prevented stomata from closing.

We did not measure ozone concentration in this study, because we thought that its concentration did not differ between the urban and the control site as distinctly as compared with that of NO_2 and SO_2 since it is a secondary generated air pollutant. The photochemical oxidant concentration monitored by the City office indicated that differences of its concentration throughout the city were parallel to those of NO_2 and SO_2 (Statistical Bureau of the City of Fukuoka, 1998). Ozone is known to depress physiological activities of plants. Therefore, ozone might be one of the culprits causing the reduced photosynthetic capacity of the exposed seedlings.

Not only the P_{350} but also *in situ* photosynthetic rate of the urban exposed seedlings would be lower than those in the nursery. *In situ* photosynthetic rate is affected by environmental factors. Among these factors, CO_2 concentration, air temperature and humidity might not be the same in the urban district and the control nursery. However, Takagi et al. (1998) found that, in spite of having a higher CO_2 concentration, the urban district caused reduced *in situ* photosynthetic rates in mature planted *I. rotunda* compared to the control nursery site due to lower air humidity.

Two suggestions concerning selection of revegetation tree species and their arboricultural management could be derived from this study. First, tolerance to heat island effect as well as air pollution is important as selection criteria of revegetation species. Second,

nutritious soil can moderate depression of photosynthetic capacity due to air pollutant.

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