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Photosynthetic Rates and Antioxidant Enzyme Activity of *Platanus occidentalis* Growing under Two Levels of Air Pollution along the Streets of Seoul

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We investigated the net photosynthetic rates and antioxidative enzyme activity in *Platanus occidentalis* trees growing on two separate streets in Seoul, and representing different degrees of air pollution. In general, concentrations of SO_2, NO_2, and PM10 decreased from May to September. The photosynthetic rate was reduced significantly on the street with higher levels of pollution. Moreover, activities of two antioxidative enzymes, ascorbate peroxidase and glutathione reductase, were greater in May along the more polluted street. These data suggest that *P. occidentalis* growing in highly polluted environments may increase their antioxidant enzyme activity to compensate for and to minimize the damage from this stress.

Keywords: antioxidative enzymes, stress compensation

Stress due to environmental pollutants can cause major evolutionary changes in plant species (Kangasjarvi et al., 1994; Elkarmi and Eideh, 2006), affecting the selection process and occasionally causing the loss of sensitive genotypes, while enhancing the survival of resistant genotypes. In urban environments, trees play an important role in improving air quality by taking up gases and particles (Beckett et al., 2000). These street trees intercept a greater percentage of aerosols than do the shorter vegetation, resulting in a higher deposition rate of gaseous pollutants and particulates. However, their uptake rates depend upon many factors, e.g., leaf-surface conditions, depth of the boundary layer, and stomatal openness.

Abiotic stresses such as air pollution can limit plant productivity and survival (Meloni et al., 2003; Vollenweider et al., 2003; Perrings et al., 2005). Over the past few decades, this pollution has become a serious problem in Seoul, Korea. In particular, O_3 concentrations continue to rise as a direct consequence of human activity. Emissions from automobiles have not been successfully restricted and, by the end of 2004, ambient SO_2 and O_3 concentrations had risen by 4 to 5%. The Ministry of Environment, Korea, has reported that 126 ozone warnings were issued nationwide during 2005, compared with only 48 in 2003 and 52 in 2000. These more-frequent ozone warnings may possibly be attributed to hot and dry weather, rather than increased air pollution, such that in years with higher rainfall, the environment would experience a cooling effect and lower density of ozone (Chung, 2005).

Ozone exposure has been correlated with decreased plant growth and photosynthesis, and these processes are reduced further by additional air pollutants (Lawson et al., 2002; Grantz et al., 2003; Vollenweider and Günthardt-Goerg, 2005). In response to abiotic stress, the mitochondria and chloroplasts increase production of reactive oxygen species (ROS), including superoxide, H_2O_2, and hydroxyl radicals (Lascano et al., 2001; Fornazier et al., 2002; Meloni et al., 2003). To limit the damage caused by these ROS, trees activate complex defense systems that incorporate low-molecular-mass antioxidants as well as antioxidative enzymes such as ascorbate peroxidase (APX, EC 1.11.1.1), superoxide dismutase (SOD, EC 1.15.1.1), and glutathione reductase (GR, EC 1.6.4.2). APX, considered the most important peroxidase for H_2O_2 detoxification, is distributed throughout the cell. It catalyzes the reduction of H_2O_2 to H_2O, using ascorbate as an electron donor in the first step of the ascorbate-glutathione cycle (Wohlgemuth et al., 2002; Parida et al., 2004).

The physiological and biochemical effects of air pollution on street trees in Seoul remain to be investigated. *Platanus occidentalis* is a fast-growing species that can survive in harsh environments. It is planted along Korean streets and is also a component of rural ecosystems. The objective of this study was to determine the effects of ambient air pollution on the antioxidant systems of *P. occidentalis*. We surveyed this species at two sites in Seoul that represent different degrees of air pollution, and compared their rates of net photosynthesis as well as APX and GR activities.

MATERIALS AND METHODS

Study Sites

A pair of streets were selected from 2 of the 25 districts of Seoul. These less polluted (Yangje street; 37° 28'N, 127° 03'E) and more polluted (Jongno street; 37° 34'N, 126° 59'E) sampling sites are both 120 m above sea level, although the former is surrounded by higher land. The trees along these streets were planted 20 years ago. We sampled *P. occidentalis* trees (5 from each street) of similar size in order to minimize any physiological variations, and

Table 1. Height and DBH of *P. occidentalis* sampled from Yangje and Jongno streets in Seoul, Korea. Asterisks indicate mean and standard deviation from 5 samples.

Location	Age (years)	Height (m)	Diameter at breast height (cm)
Yangje	20	16.3 ± 2.2*	22.03 ± 1.5*
Jongno	20	13.6 ± 1.8*	24.41 ± 2.6*

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determined their average height and diameter at breast height (DBH; Table 1).

Air Pollution Data

Monthly air pollution data were collected from government meteorological stations for the study sites to compare the concentrations of particulate matter ≤ 10 microns in size (PM10), as well as ambient levels of NO_2 and SO_2. All data represented values monitored for Year 2005.

Photosynthetic Rate

Branches were gathered from the lower crowns, and their positions were marked with ribbons to ensure similar locations for all branches. Sampling was carried out in the early mornings of 23 to 25 May, 22 to 25 July, and 20 to 22 September 2005. The branches were brought to the laboratory, re-cut under water, and provided with a water supply. After this initial preparation, the leaves were acclimated for 2 h at 15°C under a photon flux density of 200 μmol m^{-2} s^{-1} and a relative humidity of >60%. The net photosynthetic rate (P_N) was measured with a portable, open-circuit infrared gas analyzer (Li-6400; LI-COR, USA) connected to a cuvette. The gas exchange system was calibrated the day before measurements were taken, using a known CO_2 concentration. Photosynthetic rates for each tree were measured three times during the study period. Artificial illumination was supplied by a red-blue LED light source attached to the sensor head. Irradiance-response curves were determined at 20°C and 360 ± 10 μmol mol^{-1} CO_2. Leaves were acclimated for 3 min prior to their measurement, at the following photon flux densities: 0, 30, 50, 80, 100, 500, 800, 1000, 1500, and 2000 μmol m^{-2} s^{-1}. The data were subjected to regression analysis of photosynthetic rate against light intensity.

Antioxidative Enzyme Activity

Pieces of recently matured leaves (0.02 g dry weight) were taken from each tree and immediately plunged into liquid nitrogen, then ground to a fine powder using a tissue homogenizer that contained 30 mg insoluble PVPP and 2 cm^3 CO_2-free extraction buffer [100 nM BICINE (pH 8), 1 mM EDTA, 5 mM $MgCl_2$, 5 mM DTT, and 0.02 % (w/v) BSA]. The homogenate was transferred to a 1.5-mL microfuge tube and centrifuged for 30 s at 12,000g (Model Marathon centrifuge 13 F/M; Fisher Scientific, USA), and the supernatant was retained on ice. The APX activity assay was performed as described by Nakano and Asada (1981). Briefly, the 1.5-mL reaction, which contained 50 mM phosphate buffer (pH 6.0), 0.1 μM EDTA, 0.5 mM ascorbate, 1.0 mM H_2O_2, and 0.05 mL of the supernatant, was initiated by the addition of H_2O_2, and oxidation of the ascorbate was measured by absorbance at 290 nm for 1 min. Enzyme activity was quantified using the molar extraction of ascorbate (2.8 mM cm^{-1}), and the results were expressed in units per mg protein (Neto et al., 2006). GR activity at 25°C was determined as described previously, by measuring the rate of NADPH oxidation as the decrease in absorbance at 340 nm (Parida et al., 2004). Briefly, the 1-mL reaction, which contained 100 mM Tris-HCl (pH 7.8), 21 mM EDTA, 0.005 mM NADPH, 0.5 mM oxidized glutathione (GSSG), and the supernatant, was initiated by the addition of NADPH.

RESULTS

On both streets, concentrations of the three main pollutants (SO_2, NO_2, and PM10) decreased between May and

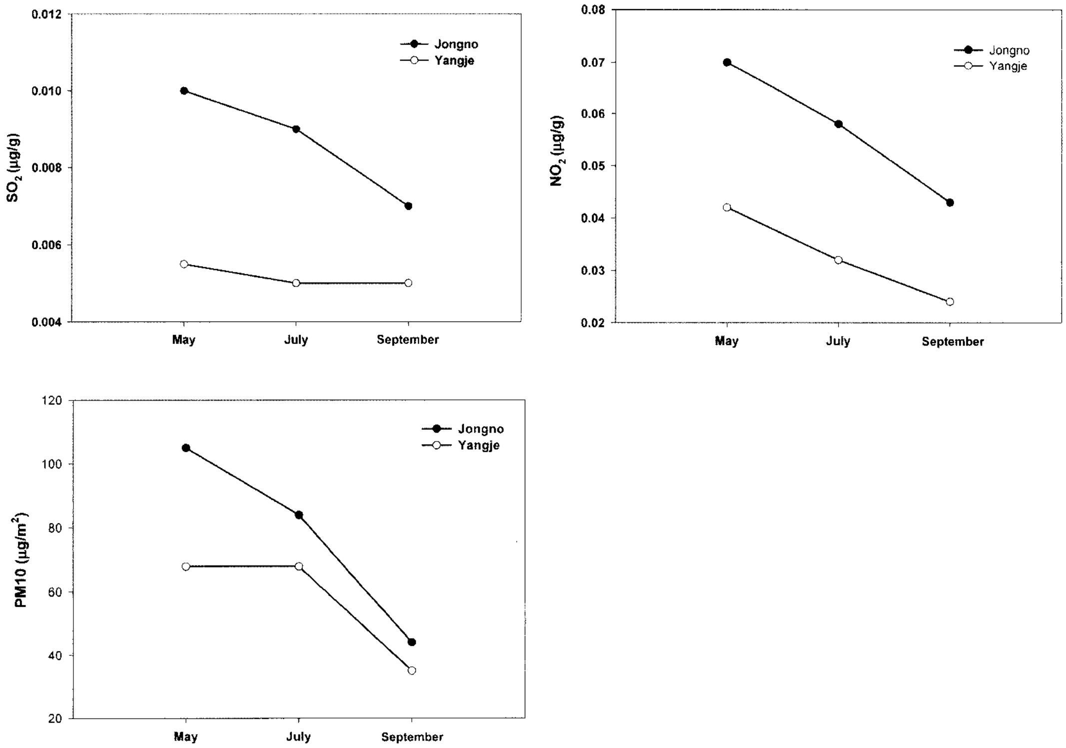

Figure 1. Changes in concentrations of SO_2, NO_2, and PM10 during growing season (recorded in May, July, and September) for Jongno (●) and Yangje (○) streets in Seoul.

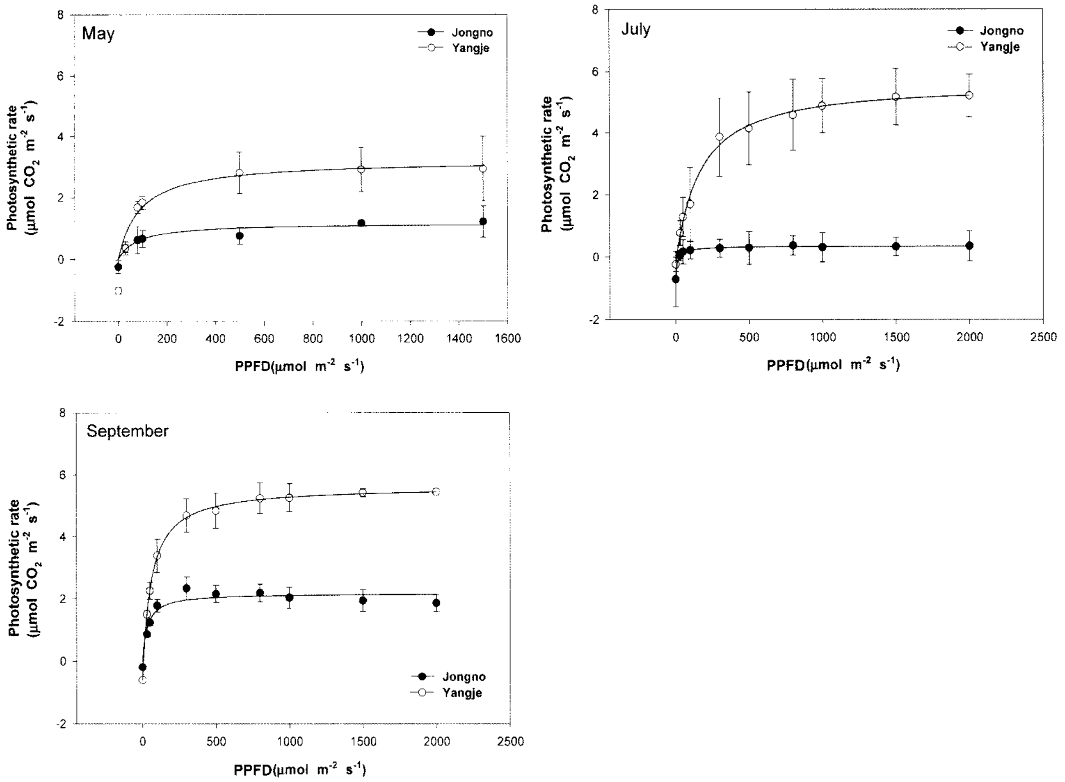

Figure 2. Irradiance-response curves for net photosynthesis rate (P_N) in *P. occidentalis* grown along Jongno (●) and Yangje (○) streets in Seoul. Bars indicate standard deviation. PPFD, Photosynthetic Photon Flux Density.

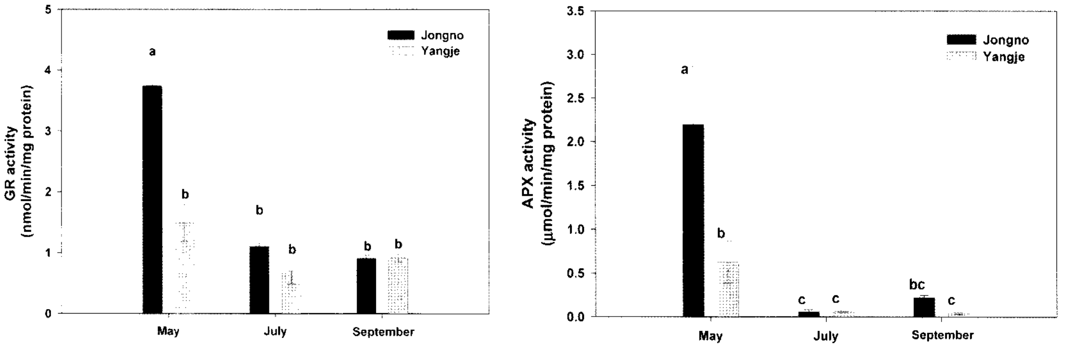

Figure 3. Comparison of GR and APX activities in leaves of *P. occidentalis* grown along Jongno and Yangje streets in Seoul. Bars indicate standard deviation. Enzyme activity is expressed as units per mg of protein. Values followed by different letters indicate means are significant at 5% level.

September (Fig. 1). The net photosynthetic rate was significantly correlated with those air conditions. P_N values for all samples obtained from the Yangje site were higher than those from Jongno (Fig. 2). In July and September, *P. occidentalis* trees from Yangje street (less polluted) had higher P_N values than in May (6 and 3 μmol CO_2 m^{-2} s^{-1}, respectively). The regression line for photosynthetic rate against light intensity was significantly lower from Jongno street (more polluted), and the depression of the irradiance-response curve was most severe in July. Thus, the rates of carbon assimilation were reduced from those observed from the less polluted site during the same period. These data suggest that, in urban areas, air pollution may be the major factor affecting the photosynthetic rate of *P. occidentalis*.

At both sites, the APX and GR activities generally decreased over time (Fig. 3), with the highest activities for both being recorded in May. Furthermore, these activities were significantly higher at the Jongno site during May and September (APX), and May (GR), than at the Yangje site.

DISCUSSION

A correlation between decreased photosynthesis and chronic air pollution has been observed previously (Samuelson and Kelly, 2000; Karnosky et al., 2005). Accordingly, the P_N values for *P. occidentalis* from the more polluted site (Jongno street) were significantly lower than those from the Yangje site (Fig. 2). Street trees experience seasonal changes in pollution stress; at Jongno, the highest level was recorded in May. Thus, relative to the less polluted site, a greatly reduced P_N was observed over time for Jongno. Although P_N values at the Yangje site remained high throughout the study period, the lowest irradiance-response curve was found in May (Fig. 2), corresponding to a higher level of some pollutants during that month. These data suggest that a greater concentration of air pollutants may be linked to the decrease in photosynthesis.

The mechanism by which air-pollution stress inhibits net photosynthesis remains unclear, but some of its features may include stomatal closure, less CO_2 available to the leaves, and inhibited carbon fixation (Robinson et al., 1998). However, others have reported that the decline in biomass measured under ozone stress is independent of stomatal conductance, and that the reduction in photosynthesis is determined by other physiological characteristics, such as altered Rubisco activity and dark respiration (Koch et al., 1998, 2000; Samuelson and Kelly, 2000; Gravano et al., 2003; Hurst et al., 2004). Nonetheless, photosynthetic rate remains a reliable indicator of plant functioning in response to pollutant stress, and a high P_N is one of the main characteristics used when selecting air pollution-resistant species for placement in urban areas (Reich et al., 1983, 1984). Likewise, we considered P_N to be the most important variable evaluated in this study.

Potential strategies used by plants to compensate for the high levels of environmental stress from air pollution include the following: reduction in root:shoot ratios; accelerated rates of leaf maturation; and, most importantly, mechanisms to minimize damage (Mooney et al., 1988; Winner, 1994; Bielenberg et al., 2002; Karnosky et al., 2005). High-level exposure to pollutants causes chloroplasts to function at a very high level of excitation energy, which increases the generation of ROS and induces oxidative stress (Meloni et al., 2003). Such elevated ROS production is correlated with a rise in the activities of antioxidative enzymes such as APX and GR (Lascano et al., 2001; Parida et al., 2004), which then enables the trees to reduce the oxidative damage triggered by those ROS (Wohlgemuth et al., 2002; D'Haese et al., 2005). Thus, increased antioxidative enzyme activity in the leaves is strongly associated with their resistance to air pollution (Parida et al., 2004). Here, the highest level of pollutants recorded during the study was measured in May from Jongno samples. Correspondingly, during that month, APX and GR activities were significantly higher in the leaves of *P. occidentalis* along that more polluted street (Fig. 1, 3). The difference in values for antioxidative enzyme activity between the two sites declined and became less significant during later months, concurrent with pollution levels that were lessening and becoming increasingly similar between sites. Enhanced APX and GR activities are typical responses to air-pollution stress (Neto et al., 2006). Thus, the markedly higher amounts of these enzymes measured from the leaves of *P. occidentalis* from Jongno street in May corresponded to greater pollution, suggesting a plant response to the environmental stress. Most plant leaves are young and emerging at the beginning of the active growing season (Karnosky et al., 2005), making them better able to cope with abiotic stresses such as air pollution.

In summary, we compared photosynthesis rates and the activities of APX and GR between populations of *P. occidentalis* of similar size and age that were situated on more polluted and relatively less polluted streets in Seoul. In general, P_N declined and antioxidant activities rose with increasing air pollution. The more polluted site (Jongno) had the highest level of air contaminants, the lowest P_N values, and generally greater APX and GR activities. In addition, the high enzyme activities were recorded at the more polluted location during the most polluted month (May), suggesting that air pollution affects the growth of *P. occidentalis*, but also that those plants make physiological adjustments to compensate for that environmental stress.

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