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Trends of Several Air Pollutants and the Effects of Ozone on the Plant Antioxidant System in *Platanus occidentalis* in Korea

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Abstract : This study investigated concentrations of the several air pollutants and compared antioxidative enzyme activities on *Platanus occidentalis* because this tree species is one of the widespread street trees in Korea. This species has been emerging the ambient air pollutants during its growing periods. The purpose of this study was to identify the relationship between air pollution on the tree species and antioxidant enzyme activities on the trees. O_3, NO_2, CO and SO_2 concentrations of several cities in Korea were compared for last decades. Among the air pollutants, O_3 and NO_2 concentrations in six big cities in Korea showed similar increasing trends during this period. In contrast, SO_2 and CO concentrations in the same cities dramatically decreased between 1994 and 2005. *Platanus occidentalis* trees were controlled to investigate, ascorbate peroxidase (APX) and glutathione reductase (GR) activity. Ozone exposure generally increased APX and GR activities of tree seedlings. It is a typical compensatory strategy of stressed trees.

Key words **:** *CO, compensation, concentration, NO_2, O_3, SO_2*

Introduction

Abiotic stress such as air pollution is among factors most limiting to plants productivity and survivorship (Baczek and Koscielniak, 2003; Meloni *et al.* 2003). Air pollution in Metro Seoul of Korea is becoming serious problem during last several decades. Especially, O_3 concentrations of cities are continue to rise as a direct consequence of anthropogenic reasons such as increasing number of automobiles. O_3 concentrations have risen by a further 2-3% by the end of the 2004. Many studies indicate that the decline in growth and photosynthesis observed in trees fumigated with ozone is often reduced when trees are raised at other air pollutants (Grantz *et al.* 2003, Lawson *et al.* 2002). However, little is investigated to the effects of air pollution on physiological and biochemical changes of street trees in Korea.

Among the air pollutants, ozone is one of the most widespread air pollutants in the world, particularly in industrialized areas and countries. This pollutant affects physiological processes directly or indirectly; directly, by affecting the major enzyme of photosynthesis, Rubisco; and indirectly, by affecting stomatal aperture (Fox and Mickler, 1996; Fredericksen *et al.*, 1996; Woo and Hinckley, 2005).

The number of ozone warnings in Korea has more than doubled to 126 in year 2005 due to dry and hot weather. The Ministry of Environment reported that ozone warnings have been issued 126 times nationwide in last year, compared with 48 in 2003 and 52 in 2000. Many researchers of nationwide reported that the frequent ozone warnings are attributed to hot and dry weather rather than worsened air pollution, adding that rain will cool down the temperature and help lower ozone concentration soon (Chung, 2005).

Platanus occidentalis is one of the most important tree species in world wide both as fast growing tree species in rural ecosystem, as well as being components of street trees in urban areas. *Platanus occidentalis* is commonly used in street trees in Korea due to well adapted behavior in harsh area. We surveyed the trends of several air pollutants in Seoul last decade and compared the antioxidant enzyme activities such APX and GR activities on *Platanus occidentalis*.

Materials and Methods

1. Concentration of air pollutants

O_3, CO, NO_2, and SO_2 concentrations of six metro cities in Korea were compared for last decades from 1992 to 2005. Ministry of Environment provided the air pollution data.

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2. Plants

Platanus occidentalis seedlings were planted into pots which contained peat, vermiculite and soil in a 1:1:1 ratio. Pots were watered daily and fertilizer was used twice per week. The temperature in the greenhouse was between 23 and 25°C, the relative humidity between 60 and 80% at seedling height. This study was conducted in the greenhouse at the University of Seoul.

3. Ozone exposure

Three replicates of seedlings were randomly assigned to each of ozone chambers and control. Ozone was generated by passing oxygen through a generator. Ozone concentrations were monitored with an ozone monitor at seedling height. Ozone concentrations varied from 150 ppb for 65 days.

4. APX and GR enzyme activity assay

Sample leaf pieces, 0.02 g (dry weight), were taken from a recently mature leaf of every individual in three sites. Each sample piece was immediately plunged into liquid nitrogen (< -80°C), and was ground in a mortar with a tissue homogenizer containing 30 mg insoluble PVPP and 2 ml CO_2-free extraction buffer to a fine powder. The extraction medium contained 100 nM BICINE (pH 8), 1 mM EDTA, 5 mM $MgCl_2$, 5 mM DTT, 0.02% BSA (w/v). The crude solution was transferred to a 1.5 ml micro-centrifuge tube, centrifuged for 30 seconds at 12,000×g (Model Marathon centrifuge 13 F/M; Fisher Scientific, 711 Forbes Ave., Pittsburgh PA), and supernatant retained on ice for the measurement of activity.

APX activity was assayed according to Nakano and Asada (1981). The reaction mixture (1.5 ml) contained 50 mM phosphate buffer (pH 6.0), 0.1 M EDTA, 0.5 mM ascorbate, 1.0 mM H_2O_2 and 50 μL enzyme extract. The reaction was started by the addition of H_2O_2 and ascorbate oxidation measured at 290 nm for 1 min. Enzyme activity was quantified using molar extinction for acorbate (2.8 mM^{-1} cm^{-1}) and the results expressed in mol H_2O_2 μmin^{-1} g^{-1} DM, taking into consideration that 2 mol ascorbate is required for reduction of 1 mol H_2O_2 (Neto *et al.* 2005).

Glutathione reductase activity was determined at 25°C by measuring the rate of NADPH oxidation as the decrease in absorbance at 340 nm. The reaction mixture (1 ml) consisted of 100 mM Tris-HCl (pH 7.8), 21 mM EDTA, 0.005 mM NADPH, 0.5 mM oxidized glutathione (GSSG) and the enzyme. NADPH was added to start the reaction (Parida *et al.* 2004).

Results and Discussion

1. Concentration of air pollutants

Ozone and NO_2 concentration in six big cities in Korea showed similar increasing trends (Figure 1 and 2). Especially, ozone is one of the increasing air pollutants in the big cities these days. Ozone concentration is strongly related to high temperature and light intensity in the city especially ultra violet (UV). High temperature and UV stimulated the ambient ozone concentration. Due to a dramatic increase in Korea's temperature during summer over the last several decades from global warming, O_3 concentrations have increased and created serious environmental and plant growth problems. Fig-

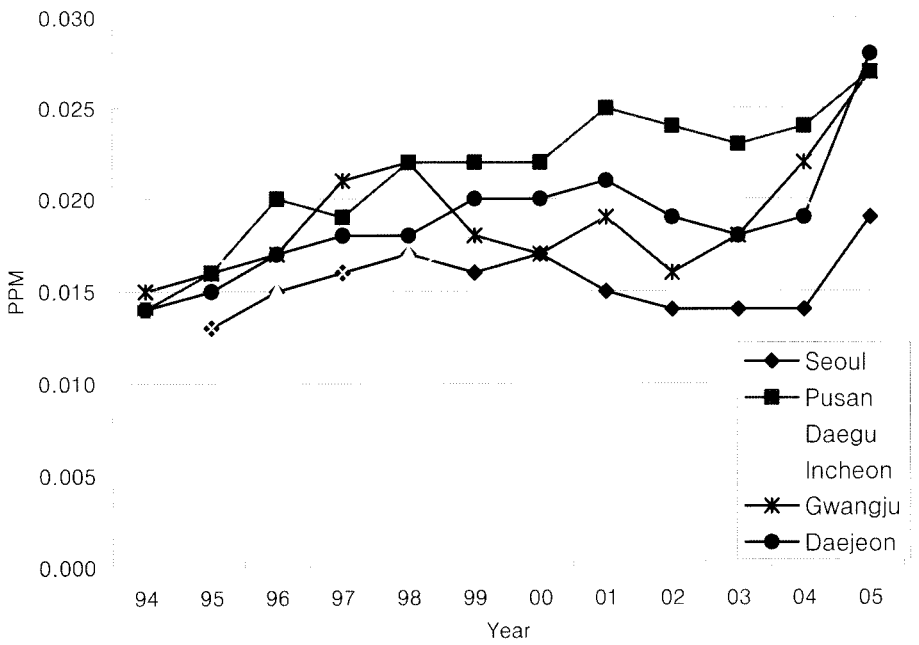

Figure 1. Changes of the O_3 concentration in six metro cities in Korea.

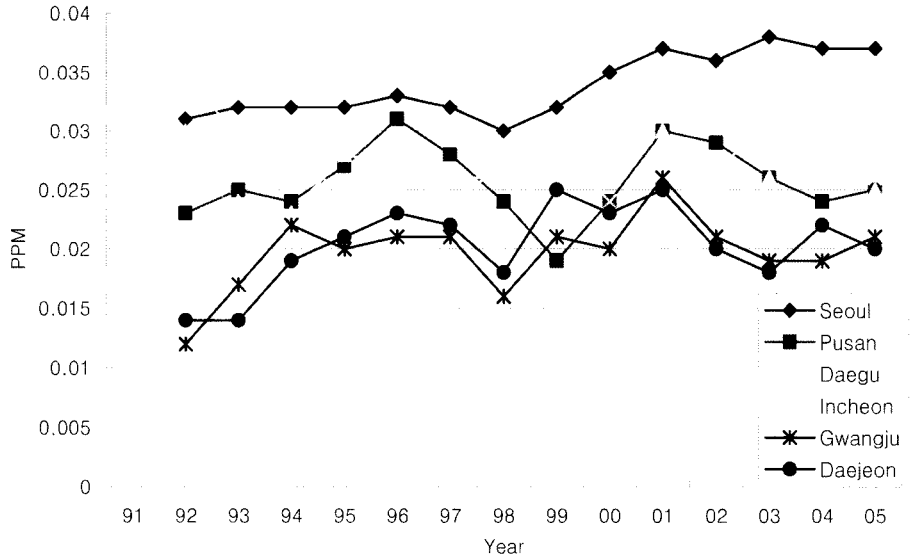

Figure 2. Changes of the NO_2 concentration in six metro cities in Korea.

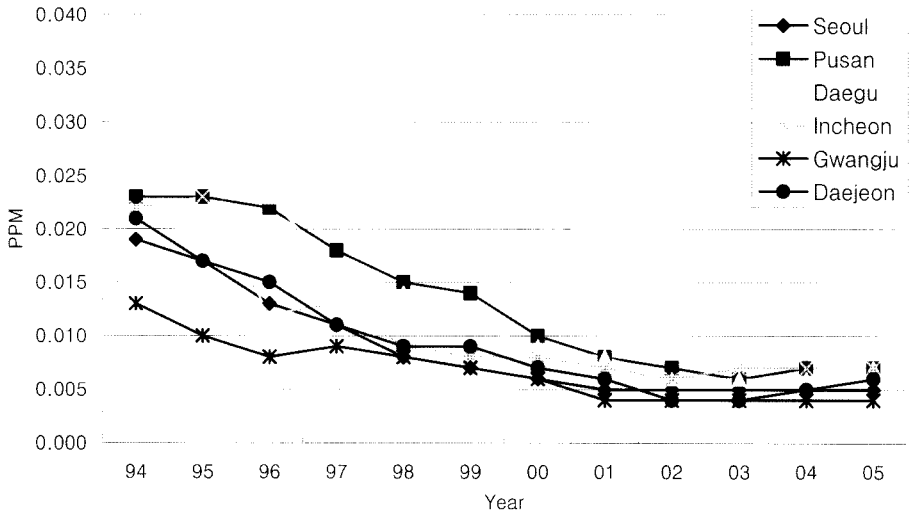

Figure 3. Changes of the SO_2 concentration in six metro cities in Korea.

ure 1 shows that O_3 concentration in Korea generally increased between 1994 and 2005. Among the cities, O_3 concentration of Pusan showed higher values than those of other cities. In terms of NO_2, the values of Seoul were highest in six cities (Figure 2).

Figure 3 illustrates the SO_2 concentration trend in Korea during last decade. Over the past several decades, the Korean government has actively involved movements to reduce sulphur dioxide emissions from automobiles and industrial sources. Government data show that SO_2 concentration in Korea dramatically decreased between 1994 and 2005. Since active SO_2 controls of the government including local community were implemented in every year, SO_2 emissions have declined each year (Figure 3). In addition, CO concentration in six big cities in Korea showed similar decreasing trends with SO_2 (Figure 4).

SO_2 concentration in Seoul showed greatest decreasing trends during 14 years (Figure 3). Incheon city showed largest CO reduction during this period. The trends of air pollutants in Korea are similar to advanced-country model. Generally, primary air pollutants such as SO_2 and CO were very high in developing country because these air pollutants have directly been produced from automobiles, industrial facilities and power plants.

2. APX and GR enzyme activities

Response of enzyme activities to ozone was obvious between ozone treated and control in this study (Figure 5 and 6). APX and GR activities of ozone treated seedlings had significant increase as a result of ozone exposure. Leaf APX and GR activities were higher in both ozone treated seedlings than those in control. In addition, *Platanus occidentalis* seedlings did not show early visible damage on the leaf during ozone exposure (Woo *et al.*, 2004b).

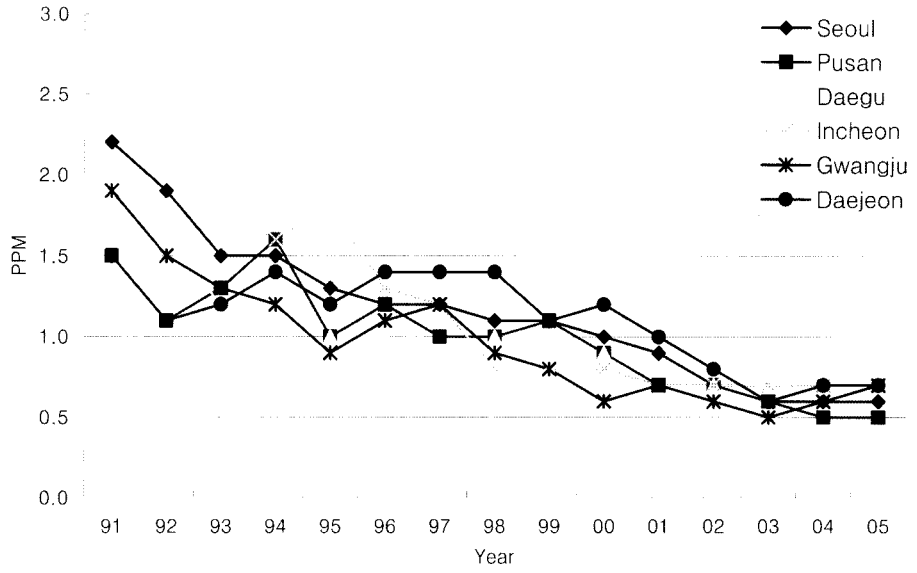

Figure 4. Changes of the CO concentration in six metro cities in Korea.

An increase of the antioxidative enzyme activities under air pollution stresses could also be indicator for a build-up of a protective mechanism to reduce oxidative damage triggered by stress experienced by trees in the air polluted streets (D'Haese *et al.*, 2005; Wohlgemuth *et al.*, 2002). In this study, increased APX and GR activities of *Platanus occidentalis* on ozone treatment suggested that some environmental stresses may spread to streets trees in cities.

Plants frequently make physiological adjustments to an environmental stress. Reducing root-to-shoot ratio and accelerated rates of leaf maturation are examples of these adjustments. Many changes in a plant physiology and growth, such as those caused by air pollution, are the results of biological compensatory responses to an environmental stress (Woo and Hinckley, 2005; Winner, 1994; Mooney *et al*, 1988). The main strategy of compensation for stress in plants is to minimize damage from stress. Therefore, air pollution can reduce biomass and photosynthesis but trees may increase their antioxidative enzyme activity for different leaves in order to maintain detoxification of ROS (Conklin and Barth, 2004; Karnosky *et al.*, 2005).

Resistance to air pollution is strongly associated with antioxidative enzyme activities (Parida *et al.* 2004). In tolerant trees, APX and GR activities was found to be higher, enabling trees to protect themselves against the oxidative stress. In this study, APX and GR activities significantly increased in *Platanus occidentalis*. This higher APX and GR activities suggested the typical compensatory stategy (Neto *et al.* 2005). *Platanus occidentalis* in ozone treatment should need to be maintained relatively a high APX and GR activities under air pollution stress. This response may be an example of a strategy to adjust or compensate for air pollution stress (Hurst *et al.*, 2004).

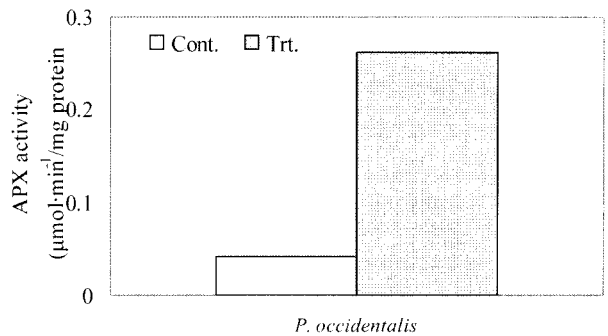

Figure 5. Effects of O_3 on activities of ascorbate peroxidase (APX) in *Platanus occidentalis*. Cont. and Trt. mean control and O_3 treatment, respectively (Woo *et al.*, 2004a).

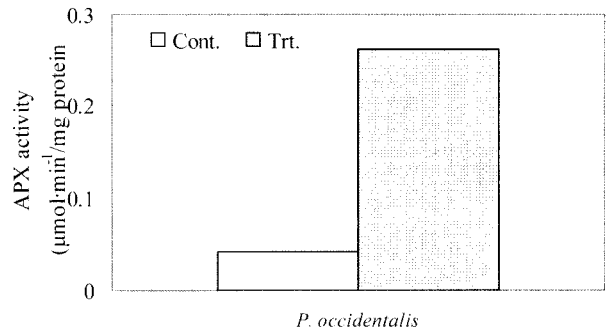

Figure 6. Effects of O_3 on activities of glutathione reductase (GR) in *Platanus occidentalis*. Cont. and Trt. mean control and O_3 treatment, respectively (Woo *et al.*, 2004b).

Conclusion

The purpose of this study was to identify the relation-

ship between air pollution and antioxidant enzyme activities on the tree species. Ozone and NO_2 concentrations in six big cities in Korea showed similar increasing trends during last decade. In contrast, SO_2 and CO concentrations in the same cities dramatically decreased between 1994 and 2005. *Platanus occidentalis* trees were controlled to investigate, ascorbate peroxidase (APX) and glutathione reductase (GR) activity. Ozone exposure generally increased APX and GR activities of tree seedlings. It is a typical compensatory strategy of stressed trees.

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