Physiological Responses of One-year-old *Zelkova serrata* Makino Seedlings to Ozone in Open-top Chamber[1]

Hyun Seok Kim[2] and Kyung Joon Lee[2]

Open-top chamber 內에서 오존에 暴露시킨 1年生 느티나무(*Zelkova serrata* Makino) 苗木의 生理的 反應에 關한 硏究[1]

金炫錫[2] · 李景俊[2]

ABSTRACT

This study was conducted to evaluate resistance and physiological responses of *Zelkova serrata* Makino seedlings to ozone in open-top chamber.

One-year-old seedlings of *Zelkova serrata* were planted in pots in April and grown in greenhouse until August. The plants were transferred into two out-door open-top chambers with a dimension of 2.0 m in diameter and 2.0 m in height. First chamber served as a control and was supplied with ambient air. Ozone was added to the second chamber for 5 hours per day(10.00 AM-15.00 PM) for 23 consecutive days at 0.1 ppm. Each chamber housed 70 pots. Every two, three or five days after initiation of exposure, ten pots were randomly removed from the chamber and determined for the contents of chlorophyll a, b, total chlorophyll and β-carotene in the leaves. Photosynthesis and dark respiration were estimated by measuring CO_2 absorption in a gas exchange chamber and oxygen absorption by oxygen monitoring system, respectively. Superoxide dismutase(SOD) activity in the leaves was assayed by a xanthine oxidase method.

First visible injury of translucent(water-soaked looking) spots appeared on the leaves 14 days after the initial exposure, and ozone accelerated senescence of old leaves. Contents of chlorophyll a and b decreased by 17%, and 31%, respectively, in ozone treatment two days after exposure. The decrease in chlorophyll b was greater than that of chlorophyll a. Content of β-carotene in ozone treatment decreased by 25% two days after initiation of exposure, but the reduction was recovered with time. Photosynthesis decreased by 45%, and the respiration increased by 28% in the ozone treatment. SOD activity started to increase 4 days after beginning of exposure and increased by 285% 7 days after exposure, and decreased to the level below the control treatment with the advancement of the visible injury.

Key words: Zelkova serrata *Makino, ozone, open-top chamber, chlorophyll,* β-carotene, photosynthesis, *dark respiration, SOD(superoxide dismutase)*

要 約

이 연구는 open-top chamber내에서 오존 가스에 폭로시킨 느티나무(*Zelkova serrata* Makino)의 저

항성과 생리적 반응을 구명하기 위하여 수행되었다.

화분에 심어 4월에서 8월까지 온실에서 키운 1년생 느티나무 묘목을 야외에 설치한 두개의 open-top chamber(직경2m, 높이2m)에 70본씩 나누어 넣은 후, 한 chamber는 오존 가스 처리구로, 다른 chamber는 대조구로 실험에 이용하였다. 오존 가스 처리구에는 하루 5시간씩(10.00 AM-15.00 PM) 23일 간 0.1ppm 농도의 오존을 폭로하였으며, 대조구는 일반 대기에 노출시켰다. 오존 가스에 폭로 후 2일, 3일, 혹은 5일마다 각 chamber에서 10본씩의 묘목을 임의로 꺼내어 엽록소 a, b의 양, 총엽록소의 양, β-카로틴의 함량을 측정하였다. 광합성량은 가스교환 chamber 내에서 이산화탄소 흡수량으로 측정하였으며, 호흡량은 암흑에서 산소 흡수량으로 측정하였고, SOD(superoxide dismutase)는 xanthine oxidase방법으로 측정하였다.

오존 폭로 14일 후에 물에 젖은 듯한 반점의 가시적 피해가 나타나기 시작하였으며, 오존 가스는 성엽의 노화를 촉진시켰다. 오존에 폭로시킨 지 이틀 후에 엽록소 a, b의 양은 각각 17%, 31% 감소하였으며, 엽록소 b의 감소 속도가 엽록소 a의 감소 속도보다 빨랐다. β-카로틴은 오존에 폭로시킨 지 이틀 후에 25%가 감소하였으나, 시간이 지남에 따라 회복되는 것으로 나타났다. 광합성 능력은 오존 처리구에서 45% 감소하였고, 호흡량은 28% 증가하였다. SOD활성은 오존 폭로 4일 후부터 증가하여 7일 만에 대조구의 285%에 도달하였다가 이후 다시 감소하여 가시적 피해가 나타나는 시기부터 대조구보다 더 낮은 활성을 보였다.

느티나무 묘목은 오존에 의하여 비교적 민감하게 피해를 받으며, SOD 활성은 오존 폭로에 대하여 초기 방어기작으로 나타나는 생리적 지표라고 결론 짓는다.

INTRODUCTION

Ozone(O_3) is one of the most important photo-chemical phytotoxicants that affect plant growth. Increased ambient level of O_3 results from human activities. Automobile exhaust is a primary indirect source. In large cities, internal combustion engines exhaust tons of waste hydrocarbons and nitrogen oxides into the air. The mixture of these gases is converted into ozone by high energy of ultraviolet light during the day time (Leighton, 1961).

Ozone is a reactive oxidant and destroys various cellular components. Many studies have reported the decrease in chlorophyll content in ozone-exposed plants(Knudson *et al.*, 1977; Fernandez-Bayon *et al.*, 1993). Sakaki *et al.*, (1983) fumigated spinach plants(*Spinacia olercea* L. cv. New Asia) with ozone in light and observed destruction of chlorophylls and carotenoids in the leaves. Chlorophyll a and carotenoids in leaves started to break down 6-8 hours after the commencement of 0.5 ppm ozone fumigation.

Reduced photosynthesis as a result of exposure to ozone has been reported (MacDowall, 1965; Reich, 1983; Fernandez-Bayon *et al.*, 1993; Sheng

et al., 1993). Amundson *et al.*(1986) reported a linear relationship between levels of O_3 and a reduction of net photosynthetic assimilation of CO_2. Ozone reduced the light-saturated rate of CO_2 uptake, and it was suggested that the apparent carboxylation inefficiency appeared to be the initial cause of decline in photosynthesis (Farage *et al.*, 1991).

Hoigne and Bader(1975) reported that ozone degraded into superoxide, hydrogen peroxide, and hydroxyl radicals. And ozone produced singlet oxygen from reactions with biological molecules(Kanofsky and Sima, 1991). The oxygen-detoxifying enzymes have been suggested as an improtant factor of defense mechanism. Superoxide dismutase(SOD) was induced in red spruce, loblolly pine and Scots pine(Tandy *et al.*, 1989). Many others reported the similar results(Lee and Benett, 1982; Decleire *et al.*, 1984). But, spinach leaves exposed to ozone exhibited decreased SOD and catalase activities, though ascorbate peroxidase activity increased (Sakaki *et al.*, 1983; Tanaka *et al.*, 1988)

The objective of this study was to understand the physiological responses of *Zelkova serrata* trees to ozone in open-top chamber. Particularly, critical duration of ozone exposure to induce

visible injury was determined. In addition, superoxide dismutase was quantified to understand mechanism involved in resistance of trees to ozone exposure.

MATERIALS AND METHODS

Plant Materials and Ozone Treatments

On April 25ʼ 1994, one-year-old seedlings of *Zelkova serrata* Makino were planted in pots(2l in capacity) with a mixture of peatmoss, vermiculite, compost and sand in the ratio of 1 : 1 : 1 : 1 (v/v). All plants were well watered through out the experiment and equally fertilized at the beginning of the experiment. During May through August, all plants were kept in ambient air in partially shaded greenhouse.

On August 21, 1994, the plants were transferred into fumigation chambers. The two open-top chambers covered with translucent polyethylene film had a dimension of 2.0 m in diameter and 2.0 m in height and were set in open field. One chamber served as a control and was supplied with ambient air. Ozone concentration in the ambient air fluctuated from less than 0.01ppm in the morning to 0.01-0.02ppm in the afternoon. Ozone was generated by an ozone generator(R-CAN Distribution Inc. Canada, 0.5 g O_3 /hr in capacity) and added to the second chamber for 5 hours per day(10.00 AM-15.00 PM) to keep the O_3 concentration at 0.1 ppm at canopy level. The air in the chamber was continuously mixed by a blowing fan. The fumigation lasted for 23 days. The top of the chambers was covered with a screen which blocked 60% of natural daylight. Each chamber housed 70 pots. Ten pots were randomly removed from the chamber every two or three days and were measured for the followings.

Measurement of Chlorophyll a and b in Leaves

Half gram of fresh leaves were excised from both control plants and ozone-treated plants, and homogenized in 80% acetone. And the acetone extracts were stored in a dark refrigerator. After 48 hours of storage, they were filtered and the filtrates were determined for absorption at 663 and 645 nm for chlorophyll a and b, respectively, according to the methods of Mackinney(1941).

Measurement of β -Carotene in Leaves

Concentration of β -carotene was determined according to the methods of AOAC(1970). One gram of leaves was ground with 40 ml acetone, 60 ml hexane and 0.1g magnesium carbonate for 5 minutes. After filtration, the flask containing residue was rinsed by 25 ml acetone for 2 times and by 25 ml hexane. And 100 ml of distilled water was poured to wash out acetone from the extract. After separating the aqueous layer, the supernatant was poured to the 100 ml flask with 9ml acetone and filled to 100 ml with hexane. The content of β -carotene was determined for the absorption at 436nm.

Estimation of Dark Respiration of the Leaves

Dark respiration was measured by the amount of oxygen absorbed from unit leaf area(cm^2) per hour under controlled environment. Ten leaf discs 6 mm in diameter were punched from excised leaves. The discs were dipped immediately into distilled water and kept in the dark in a chamber at 25℃. Four milliliters of 50mM potassium-phosphate buffer solution(pH 7.2) containing 0.5 mM $MgCl_2$ and 0.1mM $CaSO_4$, and 2ml of 0.625M $NaHCO_3$ were used as a reaction solution. Dark respiration per unit leaf surface area per hour (mole O_2 hr^{-1} cm^{-2}) was measured by Oxygen Monitoring System(YSI 5300).

Measurement of Photosynthetic Rate

The photosynthetic rate was determined by the amount of carbon dioxide absorption using Koito Photosynthesis analyzer system, KMC-1500. The whole plant was inserted into a cuvette, stabilized for 30 minutes, and changes in CO_2 concentration in the cuvette was monitored at constant temperature of 25℃, 30l/min of air flow rate, and at light intensity of about 30,000 lux.

Preparation of SOD Extracts and Enzyme Assays

Leaves were prepared by homogenization in

grinding medium(0.1mM Na₂-EDTA, 50mM potassium phosphate buffer, pH 7.8) and followed by centrifugation at 20,000g for 15min to remove particulate matter(Beauchamp and Fridovich, 1971).

Superoxide dismutase(SOD) was assayed by the NBT(nitro blue tetrazolium)-xanthine oxidase method of Beauchamp and Fridovich(1971). The reaction mixture was composed of 40μ M NBT, 32μ M xanthine, and 2ml leaf extract. The reaction was initiated by addition of 18g xanthine oxidase. The initial rate of the reaction was determined as an increase of absorbance at 530 nm for 120 sec.

Quantification of Proteins

Proteins were determined using the bicinchoninic acid protein assay(Smith *et al.*, 1985). One gram sodium bicinchoninate (BCA), 2g sodium carbonate, 0.16g sodium tartrate, 0.4g NaOH and 0.95g NaHCO₃ were brought to 100 ml with water. And 0.4g CuSO₄5H₂O was dissolved in 10ml water. Two solutions were mixed at 100:2 ratio to make SWR(Standard Working Reagent), and 1 ml SWR and $20-\mu\ell$ sample were mixed and incubated for 30 min at 60℃. After cooling, the absorbance was read at 562nm. The protein content was used to express SOD activity in the leaves.

RESULTS AND DISCUSSION

During the experimental period of five months, starting from May until September, background ozone concentration in the air in Suwon ranged from minimum of 0.01ppm to maximum 0.02ppm. Therefore, charcoal filter was not used in this experiment due to low background ozone.

Visible Injuries

On September 4, 14 days after initiation of ozone exposure, translucent(water-soaked looking) spots in ozone treated leaves were observed, which could be caused by the leaking of water from the cells into the intercellular spaces. These were the first visible injury. With time the water-soaked spots changed into the dark brown

spots, and the size of spots increased with time. Older leaves in the lower part of crown turned yellow and showed symptom of early senescence.

Zelkova serrata seedlings may be classified as a sensitive woody plant based on the first visible injury observed after 14 days of ozone exposure at 0.1ppm. Resistant species such as Acer rubrum may not respond to ozone up to 0.3ppm(Lee and Hinckley, 1995).

Leaf Chlorophyll Contents

Fig. 1 shows the contents of chlorophylls a, b and total chlorophyll. Exposure to O₃ for 23 days significantly (P<0.05) reduced leaf concentrations of chlorophyll a, b and total chlorophyll in comparison to leaves maintained in ambient air.

Chlorophyll a content was reduced right after ozone exposure and maintained the lower level through the experimental period. Knudson *et al.* (1977) reported that maximum reduction occurred 4 days after fumigation, and the reduction of chlorophyll was not recovered.

There was a tendency for greater reduction in chlorophyll b than a. It was a similar result to Knudson *et al.*(1977) and Fernandez-Bayon *et al.*(1993). It is assumed that chlorophyll b with aldehyde group would be oxidized more easily than chlorophyll a with methane group.

Greenstock and Miller(1975) reported that trion and L-ascorbate, which were the scavengers of O_2^-, effectively protected pigments from the destruction. Ouannes and Wilson(1968) presented
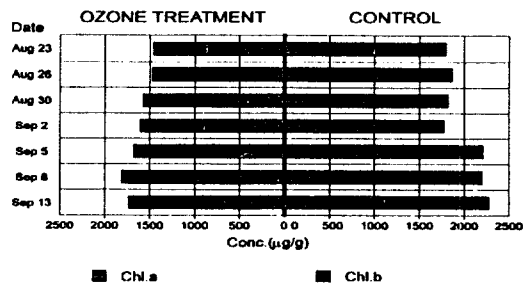

Fig. 1. Concentration of chlorophylls in the leaves of 1-yr.-old *Zelkova serrata* seedlings exposed to 0.1 ppm ozone in open-top chamber. Ozone exposure was initiated on Aug. 22.
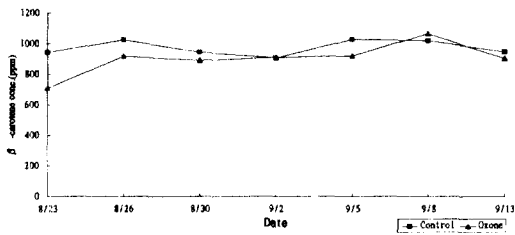
Fig. 2. Concentration of β-carotene in the leaves of 1-yr.-old *Zelkova serrata* seedlings exposed to 0.1 ppm ozone in open-top chamber. Ozone exposure was initiated on Aug. 22.

Fig. 3. Dark respiration of leaf discs of *Zelkova serrata* exposed to 0.1 ppm ozone in open-top chamber. Ozone exposure was initiated on Aug. 22.

Fig. 4. Photosynthesis of 1-yr.-old *Zelkova serrata* seedlings exposed to 0.1 ppm ozone in open-top chamber. Ozone exposure was initiated on Aug. 22.

that DABCO, a scavenger of $^1O_2$, had essentially no effect on the destruction of pigments, and D_2O, which lengthened the lifetime of $^1O_2$(Merkel *et al.*, 1972), had no effect. Benzoate and formate, the OH scavengers(Harbour and Bolton, 1978), had no effect on the destruction of chlorophyll a and carotenoids. These results suggested that O_2^- played an important role in the destruction of these pigments(Sakaki *et al.*, 1983).

β-Carotene Content

Fig. 2 shows the content of β-carotene during the period of ozone exposure. The β-carotene content in ozone treatment was lowest right after ozone exposure, but recovered to normal level at the later stage. This result was different from that of Sakaki *et al.*(1983) who reported that the total carotenoid contents of spinach in ozone treatment was stayed in lower level throughout the experiment. Special difference might have been involved in the difference in the response to ozone.

Respiration and Photosynthesis

Fig. 3 shows the amount of oxygen absorption of leaf discs in the dark. The dark respiration of plants in ozone treatment was not much different from control plants at the beginning of ozone exposure, but showed difference 10 days after exposure. The ozone-treated leaves showed 28% higher respiration than untreated leaves. This is similar to the result of Reich(1983). Effects of O_3 exposure on dark respiration declined with increasing leaf age(Reich, 1983).
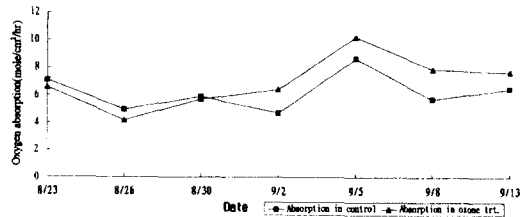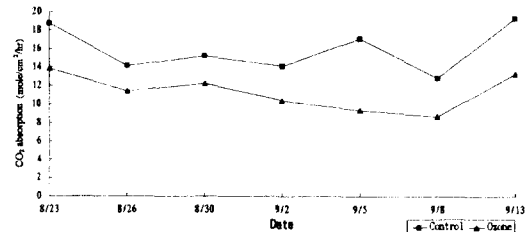
Fig. 4 shows the amount of carbon dioxide fixation measured by open cuvette method. The carbon dioxide fixation was reduced by ozone treatment starting one day after ozone exposure. And the reduction reached maximum 14 days after ozone exposure, and was approximately 40% at the later stage of the ozone exposure.

Farage *et al.*(1991) reported that decrease in the efficiency of carboxylation appeared to be the initial cause of decline in photosynthesis *in vivo* following acute O_3 fumigation. This meant a decrease in the activity and quantity of ribulose-1,5-bisphosphate carboxylase/oxygenase(Rubisco). This was similar to those of others(Pell and Pearson, 1983; Landry and Pell, 1993), and ozone reduced photosynthesis of older leaves greater than that of younger leaves (Reich, 1983).

Changes in photosynthetic rate can indirectly influence stomatal conductance, by altering the intercellular CO_2 concentration, and thereby affect the flux of O_3 to the leaf interior(Sheng *et al.*, 1993). The attainment of equilibrium rate for net photosynthesis has been observed during exposure to moderate or low concentration of O_3

(Taylor *et al.*, 1982; Yang *et al.*, 1983) in a variety of plants. This equilibrium condition implies a balance between pollutant flux and the cells' capacity for detoxification and repair of metabolic or cytological lesions(Sheng *et al.*, 1993).

Superoxide Dismutase Activity

Fig. 5 shows the activity of superoxide dismutase(SOD). SOD level remained same one day after ozone exposure. However, activity of SOD started to increase 4 days after ozone exposure and reached the peak 8 days after the initiation of exposure. On August 30, the activity of SOD in ozone treatment was approximately three times larger than that in control. After the peak, SOD in ozone treatment rapidly decreased to the level below the control treatment with the advancement of visible injury.

Superoxide dismutase, which dismutates O_2^- to H_2O_2 and O_2, has been implicated in O_3 tolerance because increased activity of this enzyme was observed in plants treated with the O_3 protectant, ethylenediurea(Lee and Bennett, 1982). Declier *et al.*(1984) and Tandy *et al.*(1989) also supported that. But, McKersie *et al.*(1982), and Matters and Scandalios(1987) reported conflicting results with the hypothesis that SOD was directly involved in O_3 tolerance. Sakaki *et al.*(1983) reported that ozone reduced SOD activity. But present experiment proved that SOD activity increased at first and then decreased. It implies
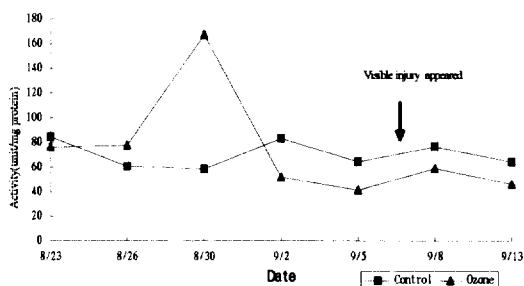

Fig. 5. Activity of SOD(superoxide dismutase) during the 23 days of exposure to 0.1 ppm ozone in open-top chamber. Ozone exposure was initiated on Aug 22. Data with one($P<0.1$), or two($P<0.05$) asterisks indicate statistically significant difference between the two treatments on the same date.

that at first stage of ozone exposure SOD activity increased as a resistance mechanism to ozone, but further ozone exposure possibly destroyed this protection mechanism.

Sheng *et al.*(1993) suggested that SOD activity was not a limiting factor in O_3 detoxification, and that ozone might be involved in the production of superoxide as a secondary reaction product, after initial interference with normal electron flow systems in organelles. But biochemical tolerance might be more related to maintaining an adequate supply of primary electron acceptors than to the removal of oxyradicals.

It may be concluded that one-year-old *Zelkova serrata* Makino seedlings are susceptible to ozone, based on the observation that they showed first visible injury 14 days after initiation of ozone exposure at 0.1ppm. Ozone reduced photosynthesis and increase respiration in the leaves. The SOD activity increased during the first 8 days after exposure and decreased to the level below the control treatment. Therefore, SOD may be considered as a physiological indicator for early defense against ozone.

REFERENCES

1. Amundson, R.G., R.M. Raba, A.W. Schoettle, and P.B. Reich. 1986. Response of soybean to low concentrations of ozone: II. Effects on growth, biomass allocation, and flowering. J. Environ. Qual. 15: 161-167.
2. AOAC. 1970. Official Methods of Analysis of the Association of Official Analytical Chemists. W. Horwitz, ed. pp.1015. AOAC, Washington D.C.
3. Beauchamp, C. and I. Fridovich. 1971. Superoxide dismutase: Improved assays and an assay applicable to acrylamide gels. Analytical Biochemistry 44:276-287.
4. Chevrier, N., F. Sarhan and Y.S. Chung. 1988. Oxidative damages and repair in Euglena gracilis exposed to ozone: I. SH group and lipids. Plant Cell Physiol. 29(2):321-327.
5. Decleire, M., W DeCat, L. deTemmermen and H. Baeten. 1984. Changes of peroxi-

dase catalase and superoxide dismutase activities in ozone-fumigated spinach leaves. J. Plant Physiol. 116:147-152.

6. Farage, P.K., S.P. Long, E.G. Lechner and N.R. Baker. 1991. The sequence of change within the photosynthetic apparatus of wheat following short-term exposure to ozone. Plant Physiol. 95:529-535.

7. Fernandez-Bayon, J.M., J.D. Barnes, J.H. Ollerenshaw, and A.W. Davison. 1993. Physiological effects of ozone on cultivars of watermelon(*Citrullus lanatus*) and muskmelon (*Cucumis melo*) widely grown in Spain. Environ. Pollut. 81:199-206.

8. Greenstock, C.L. and R.W. Miller. 1975. The oxidation of tiron by superoxide anion: Kinetics of the reaction in aqueous solution and in chloroplast. Biochem. Biophys. Acta 396:11-16.

9. Harbour, J.R. and J.R. Bolton. 1978. The involvement of the hydroxyl radical in the destructive photooxidation of chlorophylls *in vivo* and *in vitro*. Photochem. Photobiol. 28:231-234.

10. Heath. R.L. 1980. Initial events in injury to plants by air pollutants. Annu. Rev. Plant. Physiol. 31:395-431.

11. Heytler, P.G. 1969. Polarographic measurement of respiration and photosynthesis. Fed. Proc. 28:533.

12. Hoigne, J. and H. Bader. 1975. Ozonation of water: role of hydroxyl radicals as oxidizing intermediates. Science 190:782-784.

13. Kanofsky, J.P. and P. Sima. 1991. Singlet oxygen production from the reactions of ozone with biological molecules. J. Biol. Chem. 266:9039-9042.

14. Knudson, L.L., T.W. Tibbitts, and G.E. Edwards. 1977. Measurement of ozone injury by determination of leaf chlorophyll concentration. Plant Physiol. 60:606-608.

15. Landry, L.G. and E.J. Pell. 1993. Modification of rubisco and altered proteolytic activity in O_3-stressed hybrid poplar(*Populus maximowizii* x *trichocarpa*). Plant Physiol. 101:1355-1362.

16. Lee, E.H. and J.H. Bennett. 1982. Super-

oxide dismutase. A possible protective enzyme against ozone injury in snap beans(*Phaseolus vulgaris* L.). Plant Physiol. 69: 1444-1449.

17. Lee, K.J. and T.M. Hinckley. 1995. Physiological responses of poplars and red maple to ozone under different potassium and water regimes. Manuscript in preparation.

18. Leighton, P.A. 1961. Photochemistry of Air Pollution. Academic Press, New York. 300pp.

19. MacDowall, F.D.H. 1965. Stages of ozone damage to respiration of tobacco leaves. Can. J. Bot. 43:419-427.

20. Mackinney, G. 1941. Absorption of light by chlorophyll solutions. J. Biol. Chem. 140: 315-322.

21. Matters, G.L. and J.G. Scandalios. 1987. Synthesis of isozymes of superoxide dismutase in maize leaves in response to O_3, SO_2 and elevated O_2. J. Expt. Bot. 38:842-852.

22. Mckersie, B.D., W.D. Beversdorf, and P. Hucl. 1982. The relationship between ozone insensitivity, lipid-double antioxidants and dismutases in Phaseolus vulgaris. Can. J. Bot. 60:2686-2691.

23. Merkel, P.B., R. Nilsson, and D.R. Kearns. 1972. Deuterium effects on singlet oxygen lifetimes in solutions: A new test of singlet oxygen reactions. J. Am. Chem. Soc. 94:1030-1031.

24. Ouannes, C and T. Wilson. 1968. Quenching of singlet oxygen by tertiary aliphatic amines: Effect of DABCO. F. Am. Chem. Soc. 90:6527-6528.

25. Pell, E.J. and N.S. Pearson. 1983. Ozone-induced reduction in quantity of ribulose-1,5-bisphosphate carboxylase in alfalfa foliage. Plant Physiol. 51:378-381.

26. Reich, P.B. 1983. Effects of low concentrations of O_3 on net photosynthesis, dark respiration, and chlorophyll contents in aging hybrid poplar leaves. Plant Physiol. 73:291.

27. Rich, S. 1964. Ozone damage to plants. Annu. Rev. Phytopathol. 2:253-266.

28. Sakaki, Takeshi, Noriaki Kondo, and Kiyoshi Sugahara. 1983. Breakdown of photosynthetic

pigments and lipids in spinach leaves with ozone fumigation: Role of active oxygens. Physiol. Plant. 59:28-34.

29. Sheng, W.S., B.I. Chevone, and J.L. Hess. 1993. Photosynthetic inhibition and superoxide dismutase activity in soybean cultivars exposed to short-term ozone fumigation. Environ. Pollut. 80: 45-52.

30. Smith, P.K., R.I. Krohn, G.T. Hermanson, A.K. Malia, F.H. Gartner, M.D. Provenzano, E.K. Fujimoto, N.M. Goeke, B.J. Olson, and D.C. Klenk. 1985. Measurement of protein using bicinchoninic acid. Anal. Biochem. 150:76-85.

31. Tanaka, K., H. Saji, and N. Kondo. 1988. Immunological properties of spinach glutathione reductase and inductive biosynthesis of the enzyme with ozone. Plant Cell Physiol.

29:637-642.

32. Tandy, N.E., R. T. Di Giulio and C.J. Richardson. 1989. Assay and electrophoresis of superoxide dismutase from red spruce (*Picea rubens* Sarg.), loblolly pine(*Pinus taeda* L.), and Scots pine(*Pinus sylverstris* L.). A method for biomonitoring. Plant Physiol. 90:742-748.

33. Taylor, G.E., D.T. Tingey, and H.C. Ratsch. 1982. Ozone flux in *Glycine max* (L) Merr: sites of regulation and relationship to leaf injury. Oecologia 53:179-186.

34. Yang, Y.S., J.M. Skelly, B.I. Chevone, and J.B. Birch. 1983. Effects of short-term ozone exposure on net photosynthesis, dark respiration and transpiration of three eastern white pine clones. Environ. Int. 9:265-269.