Korean J. Ecol., 22(2): 79~83, 1999

Effects of Ozone on Crops and Protective Effects of Ethylenediurea as an Anti-Oxidant

Yun, Myoung-Hui and Woong Sang Lee

Department of Biological Science, Myongji University, Yongin, 449-728, Korea

ABSTRACT: Phytotoxic effects of ozone and ethylenediurea (EDU) on soybean (*Glycine max*) and spinach (*Spinacia oleracea*) were observed by using open-top field chamber system (OTC). Gas exchange rates (photosynthesis, stomatal conductance and transpiration rates) of soybean plants were decreased by 20% to 30% by ambient ozone and resulted in 30% reduction of seed yields. In OTC, ambient ozone and 0.12 μl/l O_3 decreased gas exchange rates of spinach by 25% to 40% and by 50%, respectively. The protective effect of EDU against ozone induced injury was obtained at 100 mg/l on soybean, and at 250 mg/l on spinach, respectively. The excessive application of EDU, however, inhibited photosynthesis, transpiration, and stomatal conductance without any specific visible damage.

Key Words: Ethylenediurea, Gas exchange rates, Ozone, Soybean, Spinach.

INTRODUCTION

Atmospheric gaseous pollutants such as nitrous oxide, ozone, and sulfur dioxide have been reported to act harmfully on plants. The concentration of air pollutants has been risen in the troposphere largely due to human activity including fossil fuel combustion by cars and land use conversion by deforestation (Krupa and Manning 1988).

Ozone is considered to have the greatest harmful effect on plant and increasing atmospheric ozone has become one of the major concerns worldwide (Lee 1988). Ozone, produced by photochemical reaction between nitrous oxide emitted by cars and ultraviolet radiation from the sun, has known to cause a great amount of crop yield loss (Heck *et al.* 1982) and forest decline (Koch *et al.* 1998). Atmospheric ozone can primarily cause visible injury including chlorosis, necrosis, and acceleration of aging. It has been reported to reduce the photosynthetic rate and to decrease growth rates which result in yield loss significantly even without visible symptoms (Heggestad 1988).

For past three decades, many investigators have evaluated various groups of chemical compounds to determine whether they would protect plants from ozone induced injury. Many studies have shown that ethylenediurea (EDU) has a great potential as a strong protectant against ozone injury (Reinhard and Manning 1992, 1993a, 1993b). EDU can be applied in several ways and various concentrations on different plant species to obtain the greatest effects because each plant species reacts in different ways to various treatments of EDU and ozone (Heagle 1989).

Although EDU has the great protective effects against ozone injuries, the mode of action is not completely understood (Carnahan *et al.* 1987). One of the reasons is that the mechanism of ozone injury on plants is not completely understood. In addition, EDU itself is known to be toxic when applied at a high concentration (Reinhard and Manning 1992, Heggestad 1988). Continued research to determine appropriate concentrations, times, and methods are needed not only to obtain optimal protection but also to provide fundamental data for understanding the mechanisms of effects of EDU and ozone.

The purpose of the present study was to evaluate the protective effect of EDU in soybean and spinach exposed to ambient ozone as well as increased ozone ozone levels using OTC.

MATERIALS AND METHODS

Glycine max and *Spinacia oleracea* were the model crops used in this study. The effects of ambient ozone and protective effect of EDU against ozone-induced injury were experimented at Yongin campus of Myongji Univesity from June to August in 1995 with soybean (*Glycine max*) which are sold commercially and eaten in Korea. Seeds were germinated in trays and transferred to open plots and open-top field chambers which are manufactured personally (Fig.1). To observe the effects of ambient ozone, one chamber was treated with activated-charcoal filtered air (CF) to eliminate ozone and the non-filtered chamber (NF) was treated with ambient air without filtering During the experiment, the average ozone concentration was 0.014 μl/l and the peak concentration was 0.07 μl/l in NF. Ozone concentration in CF was maintained less than 0.01 μl/l. Open-top field chambers (OTC) were operated for 2 months.

After the third trifoliate come out, EDU was applied to the plants growing in open plots by spraying 500 ml of various concentrations of EDU (0, 100, 250, and 500 mg/l). EDU treatment was conducted once every two weeks and for a total of three times over a 2-months of the whole experiment period. After finishing the treatments, photosynthesis, stomatal conductance and transpiration were measured among treatments by using LI-6200 portable photosynthsis system (Li-Cor, Lincoln, NE, U.S.A.). Chlorophyll were extracted with dimethyl-formamide (Merk) and chlorophyll contents were determined by following Moran (1982). The dry weights were measured after oven-drying at 70℃ for 48 h.

Corresponding experiment was conducted with spinach which is known to be relatively tolerant to frost injury from October to December in 1995. Spinach seeds were geminated in 15 cm diameter pots in October 1. Plants were applied with ozone and EDU from November 15 for 15 days in OTC. Ozone concentrations in CF and NF were the same with soybean experiment, and ozone added chamber (OA) was maintained 0.12 μl/l ozone. EDU was applied twice to the plants in OA by spraying 500 ml of various concentrations of EDU (0, 100, 250, and 500 mg/l). In order to compare the membrane damage after chilling in plants predisposed to various levels of ozone and EDU, conductivity was measured after soaking 5 g of leaves in 40 ml of deionized water for 24 hrs in room temperature. Then the leaves were autoclaved in 121℃ for 15 min. and conductivity was measured again. The membrane damage rate was expressed by the percentage of the conductivity measured in room temperature compared with the autoclaved leaves (Jacobson *et al.* 1992).

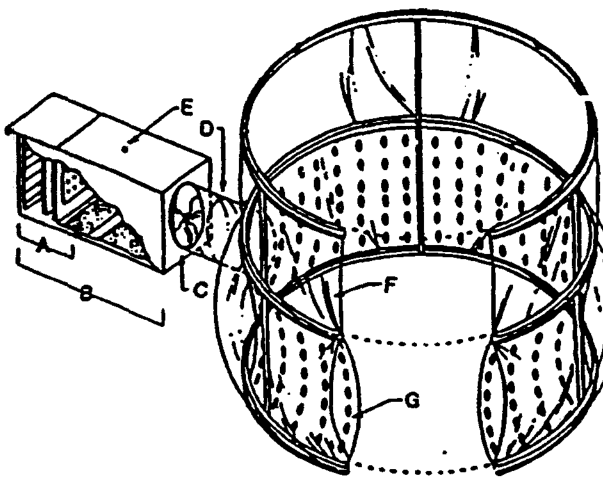

Fig. 1. Open-Top field chamber. (A) filters, (B) plenum, (C) blower, (D) air duct, (E) port of injection of ozone, (F) single-layer wall, (G) double-layer wall with perforations for air entry.

RESULTS

Net photosynthetic rate of soybean plants grown in NF was 5.49 μmol $m^{-2}s^{-1}$. This means approximately 50% decrease in photosynthesis by ambient ozone concentration compared with the photosynthetic rate of 10.37 μmol m^{-2} s^{-1} of plants grown in CF. Stomatal conductance of plants in NF was decreased by 30% compared with CF-plants. Total chlorophyll concentrations of soybean leaves was 30% less in NF-plants compared with CF-plants. While the plants in CF produced 74 pods, the plants in NF produced only 50 pods. The average dry weight showed the similar pattern as the number of soybean pods. The above results suggest that although there are no visible ozone-induced symptoms, the yield of soybean plants can be reduced by the ambient ozone (Table 1).

In a corresponding open-plots experiment with soybean plants, the average photosynthetic rate of the control plants was 11.88 μmol m^{-2} s^{-1}. The photosynthetic rates increased in plants treated with 100 mg/l EDU up to 14.5 μmol m^{-2} s^{-1}, on the other hand, these rates were reduced in plants treated with either 250 mg/l or 500 mg/l EDU by 10.30 μmol m^{-2} s^{-1} and 9.95 μ mol m^{-2} s^{-1}, respectively. However, the transpiration rate increased not only in the plants treated with 100 mg/l EDU by up to 36%, but also in the plants treated with 250 mg/l EDU by up to 20%. Only the plants treated with 500 mg/l EDU showed a reduction of transpiration by 26%. Very similar to transpiration, stomatal conductance increased in the plants treated either 100 mg/l or 250 mg/l EDU by up to 30% to 50%, but it was reduced in the plants treated with 500 mg/l EDU. While the plants treated with 100 mg/l EDU showed 20% higher chlorophyll concentrations (4.53 mg/g) compared with control plants, how- ever, the plants treated with either 250 mg/l, or 500 mg/l EDU decreased by 10% to 20% (Table 2). Taken together all these data, it shows that the most protective effects can be obtained in soybean plants when 100 mg/l EDU solution is applied under the present ambient ozone concentration.

In spinach, leaves exposed to 0.12 μl/l ozone showed necrotic lesions. Corresponding to this visible symptom, photosynthetic rate reduced to 5.19 μ mol m^{-2} s^{-1}, which represented 50% reduction of initial photosynthetic rate of 10.83 μmol m^{-2} s^{-1} after 10 days. This photosynthetic rate maintained until the termination of experiment. On the other

Table 1. Changes in net photosynthesis (Pn), stomatal conductance (Cs), chlorophyll content (Chl), number of pods, and soybean dry weight (Dwt) after two month-exposure of soybean plants to activated-charcoal filtered air (CF) or non-filtered air (NF)

	Pn (μ mol $m^{-2}s^{-1}$)	Cs (cm/s)	Chl (mg/g)	Pods (number)	Dwt (g)
CF	10.37**	1.87*	4.7*	74*	28.2*
NF	5.49	1.34	3.2	50	17.5

*, ** represent the statistical difference at 0.05 and 0.01 probability levels, respectively.

Table 2. Effects of EDU on net photosynthesis (Pn), transpiration (Tr), stomatal conductance (Cs), and chlorophyll content (Chl) in soybean plants exposed to ambient ozone for two months in open plots. Values are means and standard errors of 3 samples

EDU	Pn (μmol $m^{-2}s^{-1}$)	Tr ($mgH_2O\ m^{-2}s^{-1}$)	Cs (cm/s)	Chl (mg/g)
0	11.88±0.553	0.97±0.066	0.88±0.115	3.63±0.20
100	14.50±0.679	1.32±0.049	1.34±0.069	4.53±0.26
250	10.30±0.550	1.16±0.038	1.17±0.122	3.19±0.12
500	9.95±1.240	0.72±0.137	0.78±0.199	2.99±0.43

hand, the average photosynthetic rate of the spinach grown in NF maintained the initial rate for 10 days, however, it decreased by 40% of the initial rate at the end of this experimentation. However, visible injury was not observed in NF-plants. The average photosynthetic rate of the plants in CF maintained the initial rate for 10 days and increased slightly up to 11.52 μ mol $m^{-2}s^{-1}$, but it was not statistically significant (Fig. 2). Transpiration rates showed the similar trend with photosynthetic rates. Transpiration rate reduced 25% of initial rate in NF-plants and 50% in OA-plants, however, transpiration did not changed in CF-plants (Fig. 3).

The best protective effect against 0.12 μl/l ozone was observed in spinach plants treated with both 250 mg/l EDU twice and 500 mg/l EDU once in photosynthetic rate and transpiration. EDU treated plants showed 20-50% increase in photosynthesis and transpiration compared with control plants (Table 3).

Chlorophyll contents and biomass decreased by 20 to 40% in only spinach plants grown in OA. Relative conductivities of the NF-plants and OA-plants were about 60% higher than CF-plants, which means that ozone caused the membrane damage during summer and reduced the cold tolerance (Table 4).

After EDU treatment twice, chlorophyll contents, biomass, and relative conductivity showed the best protection in plants treated with 250 mg/l EDU (Table 5).

Table 3. Effects of EDU on net photosynthesis (Pn) and transpiration rates (Tr) in spinach plants exposed to 0.12 μl/l ozone. Values are means and standard errors of 3 samples

EDU (mg/l)	Pn (μ mol $m^{-2}s^{-1}$)		Tr ($mgH_2O\ m^{-2}s^{-1}$)	
	1X[a]	2X[b]	1X[a]	2X[b]
0	5.19±0.195	5.29±0.355	0.72±0.101	0.67±0.088
100	5.15±0.263	5.99±0.913	0.60±0.058	0.68±0.073
250	5.87±0.549	6.46±0.173	0.80±0.058	1.03±0.145
500	6.79±0.131	4.35±0.259	0.90±0.058	0.67±0.033

[a] represent EDU treatment once
[b] represent EDU treatment twice

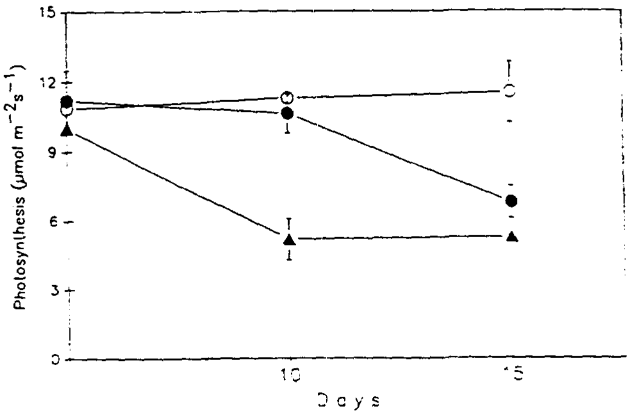

Fig. 2. Changes in photosythesis of spinach during 15 days of exposure to activated-charcoal filtered chamber (-○-), non-filtered chamber (-●-), and ozone-added chamber (-▲-). Bars represent standard error (n=3).

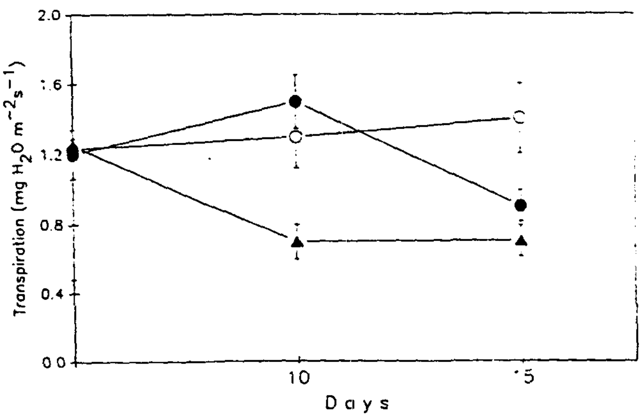

Fig. 3. Changes in transpiration of spinach during 15 days of exposure to activated-charcoal filtered chamber (-○-), non-filtered chamber (-●-), and ozone-added chamber (-▲-). Bars represent standard error (n=3).

DISCUSSION

Soybean plants that were grown in nonfiltered chamber showed the reductions in photosynthesis, transpiration, stomatal conductance, chlorophyll

Table 4. Changes in chlorophyll content (Chl.), biomass, and relative conductivity of spinach exposed to activated-charcoal filtered air (CF), non-filtered air (NF), and ozone-added air (OA) for 15 days. Values are means and standard errors of 3 samples

Treatment	Chl. (mg/g)	Biomass (g)		Relative conductivity (%)
		Stem	Root	
CF	3.22±0.228	1.27±0.137	0.19±0.019	1.20±0.182
NF	3.67±0.199	1.33±0.234	0.17±0.012	1.87±0.082
OA	2.67±0.182	0.77±0.027	0.13±0.010	1.96±0.087

Table 5. Changes in chlorophyll content (Chl.), biomass, and relative conductivity of spinach treated with 0.12 μl/l ozone and EDU. Values are means and standard errors of 3 samples

EDU (mg/l)	Chl. (mg/g fwt.)	Biomass (g)		Relative conductivity (%)
		Leaf	Root	
0	2.67±0.182	0.77±0.027	0.13±0.010	1.96±0.087
100	2.34±0.037	0.67±0.064	0.11±0.012	1.77±0.260
250	3.36±0.261	0.82±0.076	0.16±0.008	1.51±0.081
500	2.13±0.170	0.36±0.035	0.05±0.012	1.81±0.098

contents, and biomass compared to the plants that were grown in activated-charcoal filtered chamber. Nevertheless, there were no visible ozone-induced symptoms such as chlorotic or necrotic lesion. Effects of ambient ozone were observed in winter wheat (*Triticum aestivum*) by Kohut *et al.* (1987) and in Williams soybean by Heggestad *et al.* (1986). They reported that ambient ozone induced 33% yield loss in winter wheat (*Triticum aestivum*) and 10% reduction in Williams soybeans.

In this study, growth of plants treated with non-filtered air decreased compared to plants grown in open plots (data not shown). It appears that even though open-top field chambers designed to provide the similar environment to natural one, there could be some inhibiting effects by chambers (Heagle *et al.* 1983). We suggest that the advanced comparative experiments are required to measure the chamber effects more precisely.

Fiscus *et al.* (1995) compared the growth of crops among open-top field chambers. Chronic ozone treatments caused continuous decreasing in symplastic volume, specific leaf mass, and tissue elasticity. Moreover, even though midday turgor of leaf increased by 32%, and stomatal competency increased as well, leaf conductances decreased without changes in xylem pressure potential. Gantz and Zeiger (1987) also reported that photosynthetic rate was reduced remarkably in mesophyll cells isolated from soybean leaves after being exposed to ozone in dark conditions. In addition, Makay *et al.* (1987) exposed sevenday-old wheat to 0.5 μl/l of ozone for 6 hours to observe the typical ozone induced visible symptoms such as chlorosis and necrotic lesion in leaves. They reported a decrease in phospholipid, a major component of cell membranes, however, fatty acid content increased. Sheng *et al.* (1993) exposed three cultivars of soybeans, 'Dare', 'Essex.' and 'Williams 82' to 0.2 μl/l of ozone for 4 hrs. They reported distinct reductions in gas exchange rates such as in net photosynthesis by 30% and in stomatal conductance by 70%.

Protective effects of EDU in soybeans were observed in the plants repeatedly treated with 100 mg/l solution. On the other hand, inhibition of growth was observed in the plants treated with either 250 mg/l or 500 mg/l. These phytotoxic effects of EDU itself occurring when applied excessively were also reported by Reinhard and Manning (1992). After applying 150 mg/l of EDU to radish, they observed leaf margin necrosis and spoonful up-rolling symptoms in EDU treated leaves specifically. Heggestad (1988) reported ear weight of cotton treated with EDU was reduced by 10% in charcoal filtered chamber and by 19% in non-filtered chamber.

In spinach, known as one of chilling stress tolerant crops, the protective effects of EDU were observed with a slightly different pattern. The most distinct protective effect was obtained in plants either treated with 500 mg/l EDU once or 250 mg/l EDU twice. However, the application of 500 mg/l EDU twice caused inhibition of growth by excessive EDU. Nevertheless, there was no visible symptoms induced by EDU. On the other hand, treatment of 100 mg/l EDU did not show any effects on growth of spinach plants.

In conclusion, this study showed that ambient and 0.12 μl/l ozone reduced gas exchange rates, chlorophyll contents, and biomass in soybean and spinach. However, EDU application can recover the reduction of the above parameters. This study suggests that application of EDU can be used to protect crop plants against various ozone damages, however, the careful studies are required to determine the optimum EDU concentration for the best protection from the ozone damages.

ACKNOWLEDGEMENTS

This paper was supported by Korea Research Foundation in 1994 (Grant No. 04-D-0124)

LITERATURE CITED

Carnahan, J.E., E.L. Jenner and E.K. Wat. 1987. Prevention of ozone injury to plants by a protectant chemical. Phytopathology 68: 1225-1229.

Fiscus, E.L, F.S. Booker, J.E. Miller and C.D. Reid. 1995. Response of soybean leaf water relations to tropospheric ozone. Can. J. Bot. 73: 517-626.

Gantz, D.A. and E. Zeiger. 1987. Stomatal response to light and leaf-air water vapor pressure difference show similar kinetics in sugarcane and soybean. Plant Physiology 81: 865-868.

Heagle, A.S., M.B. Letchworth and C.A. Mitchell. 1983. Injury and yield response of peanuts to chronic doses of ozone in open-top field chambers. Phytopathology 73: 551-555.

Heagle, A.S. 1989. Ozone and crop yield. Annu. Rev. Phytopathology 27: 397-423.

Heck, W.W., O.C. Tayor, R. Adams, G. Bingham, H. Miller, E. Perston and L. Weinstein. 1982. Assesment of crop loss from ozone. JAPCA 32:353-361.

Heggestad, H.E. 1988. Reduction in soybean seed yields by ozone air pollution. J. Air Pollut. Control Assoc. 38: 1010- 1041

Heggestad, H.E., J.H. Bennet, E.H. Lee and L.W. Douglass. 1986. Effects of increasing doses of sulfur dioxide and ambient ozone on tomato: Plant growth, leaf injury, elemental composition, fruit yields, and quality. Phytopathology 76: 1338-1344.

Jacobson, J.S., L.I. Heller, S.J. L'Hirondelle and J.P. Lassoie. 1992. Phenology and cold tolerance of *Picea rubens* Sarg. seedlings exposed to sulfuric and nitric acid mist. Scan. J. For. Res. 7: 331-344.

Koch, J.R., A.J. Scherzer, S.M. Eshita and K.R. Davis. 1998. Ozone sensitivity in hybrid poplar is correlated with a lack of defense-gene activation. Plant Physiology 118: 1243-1252.

Kohut, R.J., R.G. Amundson, J.A. Laurence, L. Colavito, P. Leuken and P. King. 1987. Effects of ozone and sulfur dioxide on yield of winter wheat. Phytopathology 77: 71-74.

Krupa, S.V. and W.J. Manning. 1988. Atmospheric ozone: formation and effects on vegetation. Environ. Pollut. 50: 101-137.

Lee, W.S. 1988. Growth response and drought susceptibility of forest trees exposed to simulated acidic rain and ozone. Ph. D. Dissertation. VPI. and SU, Blacksburg, VA, USA. 192.

Mackay, C.E., L. Senaratna, B.D. McKersie and R.A. Fletcher. 1987. Ozone induced injury to cellular membranes in *Triticum aestivum* and protection by triazole S-3307. Plant Cell Physiology 27: 1271-1278.

Moran, R. 1982. Formulate for determination of chlorophyllous pigments extracted with N,N-Dimethylformamide. Plant Physiology 69: 1379-1381.

Reinhard, K.R. and W.J. Manning. 1992. Effects and interaction of ozone and the anti-ozonant EDU at different stages of radish (*Raphanus sativus*) development. J. Experimental Botany 43: 1621-1631

Reinhard, K.R. and W.J. Manning. 1993a. Dose-response studies with ethylenediurea (EDU) and radish. Environ. Pollut. 79: 249-260.

Reinhard, K.R. and W.J. Manning. 1993b. Dynamics of biomass partitioning in field grown radish varieties, treated with ethylenediurea. Environ. Pollut. 80: 133-145.

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 fumigations. Environ. Pollut. 80: 45-52.

(*Received December 8, 1998*)