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Protection of Agricultural Crops in Egypt Against Adverse Effects of Atmospheric Pollutants I. By Using of Ethylene Diurea

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Abstract: In the present study the damaging effects of ambient air on healthy of soybeans (*Glycine max* L. cv. Crawford) were assessed by comparison of three non-filtered air (NF) exposed plants in an open-top chambers (OTC's) and three Ambient Air (AA) plots exposed plants with three pure charcoal filtered air (CF) exposed plants in OTC's system. In parallels, another group of NF-OTC's, AA-plots and CF-OTC's were treated with EDU to reduce air pollution impacts on plant growth. This field experiment was conducted at rural site of Abo Kabeer, Sharkia (East of Egypt) for full-season exposures. Monthly concentrations of ambient O_3 have significant increase throughout the duration of soybean cycle till reaching 88 nL L^{-1} during August, while the concentrations of SO_2 and NO_2 are below toxicity levels. At the onset of the experiment, no significant difference between AA and NF air treatments was observed before and after of EDU applications. Also, no significant difference between CF/-EDU and CF/+EDU treatments for all soybean measurements. The total leaf area was decreased by 26 and 25% in case of NF/-EDU and AA/-EDU treatments, respectively. The 18% stimulation in Leaf Area Index (LAI) observed in the application of EDU under the AA plots treatments. The obtained results recorded an 8 and 6% more pods per plant in the NF/+EDU OTCs and AA/+EDU plots treatments, respectively. The EDU has improved nitrogen content in leaves by 26.1% in NF/+EDU and by 25.3% in the AA plots treatments. In general, mixed treatments from EDU soil drench and foliar spray proved all soybean growth and yield characters than its treatments singly. For an evaluation of these results, O_3 is only having adverse effects on agricultural crops in rural sites of Egypt. The absolute amounts of EDU protection against negative effects of O_3 could reach to 22.7% when using it as soil drench plus foliar spray.

Key words: Ozone, soybeans, EDU, growth, Egypt

INTRODUCTION

In many developing countries like Egypt, air pollution monitoring is extremely limited. Few research studies have been carried out at the National Research Center in Cairo on air pollution. Mainly measurements of gaseous pollutants are SO_2, NO_2 and photochemical oxidants. The annual mean guidelines for these pollutants were exceeded at urban sites in all cases (WHO/UNEP, 1992). Egypt has widespread conditions which are favorable for tropospheric O_3 formation. These conditions are due to large number of automobile, more burning of coal and straw and natural vegetation fires and the meteorological conditions associated with anticyclones, such as high solar radiation, high temperature and low wind speed (Nasrolla and Shakour, 1981; Madkour and Laurence, 2002).

Ozone (O_3) at the present levels may responsible for a significant detrimental effect on commercial yield and biological parameters in rural areas (Fuhrer, 2002; Crews, and Peoples, 2004; Piikki *et al*., 2004) Ambient O_3 levels in non-polluted areas are typically in the normal range of 20-40 nL L^{-1} (Sandermann, 1996.). Current levels are considered to be higher by a factor of 3-4 over 19th century concentrations (Sellden and Pleijel, 1995). Ozone levels were increased in Western Europe at high altitudes from around 1870 into the 1990s (Marenco *et al.*, 1994). In the United States, an O_3 monitoring station located at White-face Mountain in Adirondack State Park (New York) provides one of the longest series of O_3 measurements. Based on measurements from 1981 to 2002, it appears that O_3 levels in Egypt have been ranged between 55-90 ppb (WHO/UNEP, 1992; Ali *et al*., 2004).

There are number of chemical growth regulators and antioxidant/antiozonant is used to protect plants against O_3 damage (Kuehter and Flaglar, 1999; Ribas and Penuelas, 2000). Carnaham *et al.* (1978). reported that N-[2-(2-oxo-l-imidazolmidyl)ethyl]-N-phenylurea (EDU) is effective in protecting plants from O_3 injury when applied as soil drench or a foliar spray, moreover, O_3-susceptible plants were converted into highly tolerant ones. Recently, EDU application to bean plants grown at rural sites

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produced an increase of pods dry matter weight (Regnar-Joosten *et al.,* 1994). Moreover, O_3 injury and senescence can be retarded by retreating plants with EDU (Tonneijck and Van Dijk, 1997).

Legumes are recognized as being highly responsive and could be used as indicator plants to increasing concentrations of O_3 air pollution (Ali, 1993; Guidi *et al.*, 2000; Kanoun *et al.,* 2001; Madkour and Laurence, 2002). Legume plants grown under chronic O_3 conditions typically exhibit reduced rates of leaf photosynthesis, especially during their reproductive stages of growth (Krupa *et al.*, 1998). These results suggest that unchecked increases in tropospheric O_3 in the future will prevent O_3 sensitive crops, from maximizing their potential gains in productivity (Koch *et al.*, 2000; Weinstain *et al.*, 2001).

This experiment was conducted to examine: 1) the effects of increased atmospheric O_3, SO_2 and NO_2 on soybean plants; 2) the ability of the anti-oxidant (EDU) to protect plants from O_3, SO_2 and NO_2 injury.

MATERIALS AND METHODS

Experiment description: Field experiment was conducted during the mid of June, 2004 in rural area at a village close to Abo-Kabeer city, Sharkia province, Egypt. This site surrounded by a 2 meter high-screen from all sides to protect the plants in the AA-plots and in the OTC's against wind damage. Major air pollutants sources are absent in the vicinity of the site. Soil texture at the experimental location was loamy clay with pH 8.2 and was covered with anti-rooting plastic to suppress weed growth. Weeds were daily controlled by hand removal. Roots protruded from the plots, were also removed periodically.

Ozone data were collected using a simple chemical method via the neutral buffered KI method (Saltzman and Gilbert, 1959). The SO_2 was monitored calorimetrically by using West and Gaeke method (Stern, 1977). The NO_2 was sampled and analyzed calorimetrically according to the modified Saltzman method (Jacobes, 1968). The monitoring station within a village at Abo-Kabeer city, Sharkia province, Egypt was placed over the roof of my house (one O_3 monitor and two bubblers for calorimetric determination of SO_2 and NO_2). The concentrations of O_3, SO_2 and NO_2 were recorded over the entire course of the experiment.

Plant cultivation and treatments: Using the design of Heagle *et al.* (1973), portable OTC's (2-m diameter x 2-m height) were constructed in split plot design to evaluate the effect of O_3, SO_2 and NO_2 on soybean plants. These OTC's are divided into four groups. One group are non-filtered chambers and non-treated with EDU (NF/-EDU) and another group of OTC's are also non-filtered and treated with EDU (NF /+EDU). The third group are carbon-filtered air (CF) chambers treated with EDU (CF/+EDU) and the last one are carbon-filtered air OTC's and non-treated with EDU (CF/-EDU). Ambient air plot (AA) was used to compare the responses of the chambers environment with and without treatment of EDU (AA/+EDU and AA/-EDU). Air filtration within the OTC's was carried out daily during the experiment using charcoal and was tested also daily to be effective around the ideal atmospheric conditions. Large numbers of soybean (*Glycine max* L. cv. Crawford) seeds were planted at June 16, 2005 in five lines per chamber or plots. Plots and OTC's were irrigated with tap water as required. After two weeks, growing plants were reduced to be fifty (10 for each line). A 200 mL of aqueous solution of EDU was applied four times (two weeks intervals) as a soil drench and/or foliar spray to the plants that grown in the chambers and to the plants that grown in the AA plot at July 11. Three cases of EDU treatments as following: the 1st one is 200 mL of EDU as a soil drench; the 2nd one is 200 mL of EDU as a foliar spray; the 3rd one is 100 mL of EDU as a soil drench + 100 mL of EDU as foliar spray. The EDU solution was prepared by suspending a 350 mg L^{-1} EDU in warm distilled water 24 h before application. The non-exposed plants to EDU (/-EDU) were received an equal volume of tap water. Maintenance and observations were carried out for all plots and OTC's on the same day each week.

Measurements of phenological events: Observations of soybean phenology were made for all plants at regular intervals from 1st week of seed germination to the last week of harvesting. The following time discriminated pathological events such as: dormant seed, seed break, shoot growth, flowers appear, pods appear and once pod fill and pod maturity (harvest). Mean values of all treatments and standard deviations were calculated.

Characteristics of green and dry harvest: A green (intermediate) harvest was performed after one week of each EDU exposure (July 18, August 07, August 27 and September 16). Five plants per EDU per treatment per chamber or plot were harvested and the leaves were divided into the following categories: healthy (green), senescent (yellowing) and injured (O_3-symptoms). At the same time, the biomass was determined by weighing the flesh of leaves, stems and roots of these 5-plants. Also, the individual leaf length was measured with a plastic ruler every 7 days during the growing season for all leaves per plant and using manual method individual leaf area could be calculated. Leaf Area Index (LAI) was then estimated from total leaf area per plant and number of plants per chamber per ground area in the OTC's. After each biomass, samples of these 5-plants were taken for analysis of total nitrogen content using the micro-Kjeldahl technique. A dry harvest (air dry) was carried out at the end of September when the pod was brown and contained loose seeds and then the dry biomass and pods characters (> 4 cm) were determined. Total lipids and total proteins of dry seeds were determined according to the extraction method of Bligh and Dyer (1959).

Data analysis: Statistical analyses were carried out using the SPSS BASE 10.0 (SPSS Inc., Chicago, IL) packages. One-way ANOVA was used to test for significant effects due to air quality and EDU treatment and their interaction, plant data for each harvest were subjected to normal procedures of analysis of variance. Results for the proportions of leaves that were healthy, senescent or injured were analysed with a generalized linear model for binomial distributions. Treatment means were compared pair-wise with a Student's t-test and differences were considered statistically significant when the $p<0.05$.

RESULTS

Pollutants concentrations: Over the entire course of the experiment, the charcoal filters typically prevent the chamber air pollutants concentrations to levels (16, 5 and 4 nL L^{-1} for O_3, SO_2, NO_2, respectively) close to normal ones that existing in the ambient non-polluted areas (Table 1). The resulting SO_2 and NO_2 concentrations in both AA-plot and NF- OTC's treatments were below threshold concentrations (18, 13 nL L^{-1} for SO_2, NO_2, respectively) which can't cause any phytotoxic effects on the investigated soybean plants (Table 1). The O_3 concentrations within the chambers and ambient air were higher than the threshold levels (the highest concentration in August), while phytotoxic effects occur due to their exposures (Table 1).

Effects of EDU on phenological events: At the onset of the experiment, all seeds broke after 3-5 days and vegetative growth after 16-20 days after sowing seeds in soil (Table 2). Little significant differences were recorded before flower appears. On the other hand, air pollutants had significant adverse effect when flowers appear in comparing to CF/-EDU and CF/+EDU in soil drench and/or foliar spray. The EDU started to improve the soybean functions when pods appear, while more reductions were recorded in AA/-EDU and NF/-EDU treatments in case of all EDU treatments but better especially in case of mixed EDU treatments. Similar data were observed during pods fill. The air pollutants

Table 1: Mean concentrations of O_3, SO_2 and NO_2 (nL L^{-1}) over the entire course of soybean plants life cycle grown in open top chambers (OTC's) carbon filtered air (CF), ambient air plots (AA) and OTC's non-filtered air (NF) in the East of Egypt

Treatments	CF/ -EDU	CF/ +EDU	NF/ -EDU	NF/ +EDU	AA/ -EDU	AA/ +EDU
O_3 concentration						
May	16^{a}	16^{a}	81^{b}	81^{b}	81^{b}	81^{b}
June	16^{a}	16^{a}	85^{c}	85^{c}	85^{c}	85^{c}
July	16^{a}	16^{a}	86^{c}	86^{c}	85^{c}	85^{c}
August	16^{a}	16^{a}	88^{cd}	88^{cd}	88^{cd}	88^{cd}
September	16^{a}	16^{a}	87^{cd}	87^{cd}	87^{cd}	87^{cd}
October	16^{a}	16^{a}	86^{c}	86^{c}	86^{c}	86^{c}
SO_2 concentration						
May	5^{a}	5^{a}	13^{b}	13^{b}	12^{b}	12^{b}
June	5^{a}	5^{a}	15^{c}	15^{c}	14^{c}	14^{c}
July	5^{a}	5^{a}	16^{c}	16^{c}	14^{c}	14^{c}
August	5^{a}	5^{a}	18^{cd}	18^{cd}	16^{cd}	16^{cd}
September	5^{a}	5^{a}	17^{cd}	17^{cd}	16^{cd}	16^{cd}
October	5^{a}	5^{a}	16^{c}	16^{c}	14^{c}	14^{c}
NO_2 concentration						
May	4^{a}	4^{a}	10^{b}	10^{b}	9^{b}	9^{b}
June	4^{a}	4^{a}	10^{b}	10^{b}	10^{b}	10^{b}
July	4^{a}	4^{a}	11^{b}	11^{b}	11^{b}	11^{b}
August	4^{a}	4^{a}	13^{c}	13^{c}	13^{c}	13^{c}
September	4^{a}	4^{a}	13^{c}	13^{c}	13^{c}	13^{c}
October	4^{a}	4^{a}	12^{bc}	12^{bc}	12^{bc}	12^{bc}

CF = Carbon filtered Air, NF = Non-filtered air, AA = Ambient Air, +EDU = treated with ethylene-diurea, -EDU = Non-treated with ethylene-diurea. Values in rows or columns followed by the same letters are not significantly different

Table 2: The EDU effects on mean phenological events (days) of soybean plants growing in OTCs under effects of air pollutants

Treatments	CF/ -EDU	CF/ +EDU	NF/ -EDU	NF/ +EDU	AA/ -EDU	AA/ +EDU
EDU as soil drench						
Seed dormancy (days)	3^{a}	3^{a}	3^{a}	3^{a}	3^{a}	3^{a}
Seed break (days)	5^{a}	5^{a}	6^{a}	6^{a}	6^{a}	6^{a}
Shoot growth (days)	20^{a}	20^{a}	16^{b}	16^{b}	17^{b}	17^{b}
Flower appear (days)	45^{a}	45^{a}	30^{b}	35^{c}	30^{b}	35^{c}
Pod appear (days)	66^{a}	66^{a}	40^{b}	52^{c}	40^{b}	52^{c}
Pod fill (days)	78^{a}	78^{a}	60^{b}	75^{c}	60^{b}	75^{c}
Harvest time (days)	102^{a}	102^{a}	78^{b}	95^{c}	78^{b}	95^{c}
EDU as foliar spray						
Seed dormancy (days)	3^{a}	3^{a}	3^{a}	3^{a}	3^{a}	3^{a}
Seed break (days)	5^{a}	5^{a}	6^{a}	6^{a}	6^{a}	6^{a}
Shoot growth (days)	20^{a}	20^{a}	16^{b}	16^{b}	17^{b}	16^{b}
Flower appear (days)	45^{a}	45^{a}	30^{b}	37^{c}	30^{b}	37^{c}
Pod appear (days)	66^{a}	66^{a}	40^{b}	55^{c}	40^{b}	55^{c}
Pod fill (days)	78^{a}	78^{a}	60^{b}	78^{c}	60^{b}	78^{c}
Harvest time (days)	102^{a}	102^{a}	78^{b}	96^{c}	78^{b}	96^{c}
EDU as soil drench + foliar spray						
Seed dormancy (days)	3^{a}	3^{a}	3^{a}	3^{a}	3^{a}	3^{a}
Seed break (days)	5^{a}	5^{a}	6^{a}	6^{a}	6^{a}	6^{a}
Shoot growth (days)	20^{a}	20^{a}	16^{b}	16^{b}	17^{b}	16^{b}
Flower appear (days)	45^{a}	45^{a}	30^{b}	40^{c}	30^{b}	40^{c}
Pod appear (days)	66^{a}	66^{a}	40^{b}	62^{c}	40^{b}	62^{c}
Pod fill (days)	78^{a}	78^{a}	60^{b}	77^{c}	60^{b}	77^{c}
Harvest time (days)	102^{a}	102^{a}	78^{b}	99^{c}	78^{b}	99^{c}

CF = Carbon filtered Air, NF = Non-filtered air, AA = Ambient Air, +EDU = treated with ethylene-diurea, -EDU = Non-treated with ethylene-diurea. Values in rows followed by the same letters are not significantly different

were observed during pods fill. The air pollutants

Table 3: The effects of EDU exposures (after 1st application) on green harvest characteristics of soybean (*Glycine max* L. cv. Crawford) grown under air pollution treatments at Abo Kabeer, Sharkia province, Egypt

Treatments	CF/-EDU	CF/+EDU	NF/-EDU	NF/+EDU	AA/-EDU	AA/+EDU
EDU as soil drench						
Healthy leaves number	33.8^{a}	33.8^{a}	21.0^{b}	25.0^{c}	21.0^{b}	25.0^{c}
Injured leaves number	1.1^{a}	1.1^{a}	6.3^{b}	4.9^{b}	6.3^{b}	4.9^{b}
Senescent leaves number	1.8^{a}	1.8^{a}	9.0^{b}	6.4^{c}	9.0^{b}	6.4^{c}
Green leaves biomass (g)	1.61^{a}	1.61^{a}	1.12^{b}	1.53^{a}	1.12^{b}	1.53^{a}
Green stems biomass (g)	4.71^{a}	4.71^{a}	3.66^{b}	4.12^{a}	3.66^{b}	4.12^{a}
Green roots biomass (g)	0.65^{a}	0.65^{a}	0.49^{b}	0.60^{a}	0.49^{b}	0.60^{a}
Total green biomass (g)	6.97^{a}	6.97^{a}	5.27^{b}	6.25^{a}	5.27^{b}	6.25^{a}
Leaf area (cm^2)	4.71^{a}	4.71^{a}	3.65^{b}	3.96^{b}	3.65^{b}	3.96^{b}
Leaf area index (LAI)	1.18^{a}	1.18^{a}	0.99^{b}	1.10^{a}	0.99^{b}	1.10^{a}
Total nitrogen content of leaves (%)	0.88^{a}	0.88^{a}	0.66^{b}	0.73^{a}	0.66^{b}	0.73^{a}
Total nitrogen content of stems (%)	0.96^{a}	0.96^{a}	0.78^{b}	0.82^{a}	0.78^{b}	0.82^{a}
Total nitrogen content of roots (%)	0.71^{a}	0.71^{a}	0.53^{b}	0.61^{a}	0.53^{b}	0.61^{a}
Total nitrogen content of plant (%)	2.55^{a}	2.55^{a}	1.97^{b}	2.16^{a}	1.97^{b}	2.16^{a}
EDU as foliar spray						
Healthy leaves number	33.8^{a}	33.8^{a}	21.0^{b}	26.6^{c}	21.0^{b}	26.6^{c}
Injured leaves number	1.1^{a}	1.1^{a}	6.3^{b}	4.3^{b}	6.3^{b}	4.3^{b}
Senescent leaves number	1.8^{a}	1.8^{a}	9.0^{b}	5.4^{c}	9.0^{b}	5.4^{c}
Green leaves biomass (g)	1.61^{a}	1.61^{a}	1.12^{b}	1.65^{a}	1.12^{b}	1.65^{a}
Green stems biomass (g)	4.71^{a}	4.71^{a}	3.66^{b}	4.22^{a}	3.66^{b}	4.22^{a}
Green roots biomass (g)	0.65^{a}	0.65^{a}	0.49^{b}	0.68^{a}	0.49^{b}	0.68^{a}
Total green biomass (g)	6.97^{a}	6.97^{a}	5.27^{b}	6.55^{a}	5.27^{b}	6.55^{a}
Leaf area (cm^2)	4.71^{a}	4.71^{a}	3.65^{b}	4.11^{b}	3.65^{b}	4.11^{b}
Leaf area index (LAI)	1.18^{a}	1.18^{a}	0.99^{b}	1.10^{a}	0.99^{b}	1.10^{a}
Total nitrogen content of leaves (%)	0.88^{a}	0.88^{a}	0.66^{b}	0.78^{a}	0.66^{b}	0.78^{a}
Total nitrogen content of stems (%)	0.96^{a}	0.96^{a}	0.78^{b}	0.88^{a}	0.78^{b}	0.88^{a}
Total nitrogen content of roots (%)	0.71^{a}	0.71^{a}	0.53^{b}	0.68^{a}	0.53^{b}	0.68^{a}
Total nitrogen content of plant (%)	2.55^{a}	2.55^{a}	1.97^{b}	2.34^{a}	1.97^{b}	2.34^{a}
EDU as soil drench + foliar spray						
Healthy leaves number	33.8^{a}	33.8^{a}	19.0^{b}	28.7^{c}	19.0^{b}	28.7^{c}
Injured leaves number	1.1^{a}	1.1^{a}	8.1^{b}	3.0^{a}	8.1^{b}	3.0^{a}
Senescent leaves number	1.8^{a}	1.8^{a}	9.2^{b}	4.6^{c}	9.2^{b}	4.6^{c}
Green leaves biomass (g)	1.61^{a}	1.61^{a}	1.02^{b}	1.33^{c}	1.02^{b}	1.33^{c}
Green stems biomass (g)	4.71^{a}	4.71^{a}	3.16^{b}	4.12^{a}	3.16^{b}	4.12^{a}
Green roots biomass (g)	0.65^{a}	0.65^{a}	0.43^{b}	0.60^{a}	0.43^{b}	0.60^{a}
Total green biomass (g)	6.97^{a}	6.97^{a}	4.61^{b}	6.05^{a}	4.61^{b}	6.05^{a}
Leaf area (cm^2)	4.71^{a}	4.71^{a}	3.19^{b}	4.16^{a}	3.19^{b}	4.16^{a}
Leaf area index (LAI)	1.18^{a}	1.18^{a}	0.89^{b}	1.08^{a}	0.89^{b}	1.08^{a}
Total nitrogen content of leaves (%)	0.88^{a}	0.88^{a}	0.61^{b}	0.81^{a}	0.61^{b}	0.81^{a}
Total nitrogen content of stems (%)	0.96^{a}	0.96^{a}	0.71^{b}	0.89^{a}	0.71^{b}	0.89^{a}
Total nitrogen content of roots (%)	0.71^{a}	0.71^{a}	0.50^{b}	0.61^{c}	0.50^{b}	0.61^{c}
Total nitrogen content of plant (%)	2.55^{a}	2.55^{a}	1.82^{b}	2.31^{a}	1.82^{b}	2.31^{a}

CF = Carbon filtered Air, NF = Non-filtered air, AA = Ambient Air, +EDU = treated with ethylene-diurea, -EDU = Non- treated with ethylene-diurea. Values in rows followed by the same letter(s) are not significantly different.

accelerated the harvest time of soybean plants grown under the treatments of AA/-EDU and NF/-EDU, while the four applications from EDU would appear to protect the plants from rapidity of senescence in all cases of EDU applications (Table 2).

Effects of EDU on green harvest characteristics: The 1st EDU application was stimulated the healthy leaves of soybean plants in both NF and AA treatments by 12% and 12.6%, respectively (Table 3), while it stimulated by 15.1 and 15%, respectively after 4th EDU application, as compared with corresponding NF/-EDU treatments (Table 4). The fast growing in the number of injured and senescent leaves under pollution conditions during the life cycle of soybean. Significant increases in total plant biomass under AA/+EDU and NF/+EDU in comparing with AA/-EDU and NF/+EDU after 1st and 4th EDU treatments in case of all EDU treatments but better especially in case of mixed EDU treatments (Table 3 and 4). Only significant effect of the both treatments was noticed on the biomass of individual plant organs in September 21 (Table 4). Total green biomass in July 21 increased by 11.8% in NF/+EDU and by 12% AA/+EDU as compared to –EDU treatments (Table 4). The best protection of EDU to soybean were found in total biomass measured in September 21 where increased by 13% in NF/+EDU (Table 4). The highly damage rate due to pollutants stress reached to 66% in September 21 of both AA/–EDU and NF/–EDU treatments. The leaf area in July 21 was decreased by 12% in case of NF/-EDU and

Table 4: The effects of EDU exposures (after 4th application) on green harvest characteristics of soybean (*Glycine max* L. cv. Crawford) grown under air pollution treatments at Abo Kabeer, Sharkia province, Egypt

Treatments	CF/-EDU	CF/+EDU	NF/-EDU	NF/+EDU	AA/-EDU	AA/+EDU
EDU as soil drench						
Healthy leaves number	33.8^{a}	33.8^{a}	19.0^{b}	27.3^{c}	19.0^{b}	27.3^{c}
Injured leaves number	1.1^{a}	1.1^{a}	8.0^{b}	2.2^{c}	8.0^{b}	2.2^{c}
Senescent leaves number	1.8^{a}	1.8^{a}	9.3^{b}	6.8^{c}	9.3^{b}	6.8^{c}
Green leaves biomass (g)	1.61^{a}	1.61^{a}	1.12^{b}	1.56^{a}	1.12^{b}	1.56^{a}
Green stems biomass (g)	4.71^{a}	4.71^{a}	3.66^{b}	4.09^{a}	3.66^{b}	4.09^{a}
Green roots biomass (g)	0.65^{a}	0.65^{a}	0.49^{b}	0.60^{a}	0.49^{b}	0.60^{a}
Total green biomass (g)	6.97^{a}	6.97^{a}	5.27^{b}	6.25^{a}	5.27^{b}	6.25^{a}
Leaf area (cm^2)	4.71^{a}	4.71^{a}	3.65^{b}	4.16^{a}	3.65^{b}	4.16^{a}
Leaf area index (LAI)	1.18^{a}	1.18^{a}	0.99^{b}	1.12^{a}	0.99^{b}	1.12^{a}
Total nitrogen content of leaves (%)	0.88^{a}	0.88^{a}	0.66^{b}	0.78^{a}	0.66^{b}	0.78^{a}
Total nitrogen content of stems (%)	0.96^{a}	0.96^{a}	0.78^{b}	0.86^{a}	0.78^{b}	0.86^{a}
Total nitrogen content of roots (%)	0.71^{a}	0.71^{a}	0.53^{b}	0.65^{a}	0.53^{b}	0.65^{a}
Total nitrogen content of plant (%)	2.55^{a}	2.55^{a}	1.97^{b}	2.29^{a}	1.97^{b}	2.29^{a}
EDU as foliar spray						
Healthy leaves number	33.8^{a}	33.8^{a}	19.3^{b}	27.7^{c}	19.3^{b}	27.7^{c}
Injured leaves number	1.1^{a}	1.1^{a}	8.0^{b}	2.5^{c}	8.0^{b}	2.5^{c}
Senescent leaves number	1.8^{a}	1.8^{a}	9.3^{b}	6.1^{c}	9.3^{b}	6.1^{c}
Green leaves biomass (g)	1.61^{a}	1.61^{a}	1.12^{b}	1.56^{a}	1.12^{b}	1.56^{a}
Green stems biomass (g)	4.71^{a}	4.71^{a}	3.66^{b}	4.09^{a}	3.66^{b}	4.09^{a}
Green roots biomass (g)	0.65^{a}	0.65^{a}	0.49^{a}	0.60^{a}	0.49^{a}	0.60^{a}
Total green biomass (g)	6.97^{a}	6.97^{a}	5.27^{b}	6.25^{a}	5.27^{b}	6.25^{a}
Leaf area (cm^2)	4.71^{a}	4.71^{a}	3.65^{a}	4.16^{c}	3.65^{a}	4.16^{c}
Leaf area index (LAI)	1.18^{a}	1.18^{a}	0.99^{b}	1.12^{a}	0.99^{b}	1.12^{a}
Total nitrogen content of leaves (%)	0.88^{a}	0.88^{a}	0.66^{b}	0.85^{a}	0.66^{b}	0.85^{a}
Total nitrogen content of stems (%)	0.96^{a}	0.96^{a}	0.78^{b}	0.78^{a}	0.78^{b}	0.78^{a}
Total nitrogen content of roots (%)	0.71^{a}	0.71^{a}	0.53^{b}	0.66^{a}	0.53^{b}	0.66^{a}
Total nitrogen content of plant (%)	2.55^{a}	2.55^{a}	1.97^{b}	2.29^{a}	1.97^{b}	2.29^{a}
EDU as soil drench + foliar spray						
Healthy leaves number	33.8^{a}	33.8^{a}	19.3^{b}	31.1^{c}	19.3^{b}	31.1^{c}
Injured leaves number	1.1^{a}	1.1^{a}	8.0^{b}	1.1^{a}	8.0^{b}	1.1^{a}
Senescent leaves number	1.8^{a}	1.8^{a}	9.3^{b}	4.1^{c}	9.3^{b}	4.1^{c}
Green leaves biomass (g)	1.61^{a}	1.61^{a}	1.12^{b}	1.56^{a}	1.12^{b}	1.56^{a}
Green stems biomass (g)	4.71^{a}	4.71^{a}	3.66^{b}	4.42^{a}	3.66^{b}	4.42^{a}
Green roots biomass (g)	0.65^{a}	0.65^{a}	0.49^{b}	0.60^{a}	0.49^{b}	0.60^{a}
Total green biomass (g)	6.97^{a}	6.97^{a}	5.27^{b}	6.58^{a}	5.27^{b}	6.58^{a}
Leaf area (cm^2)	4.71^{a}	4.71^{a}	3.65^{a}	4.45^{c}	3.65^{a}	4.45^{c}
Leaf area index (LAI)	1.18^{a}	1.18^{a}	0.99^{b}	1.13^{a}	0.99^{b}	1.13^{a}
Total nitrogen content of leaves (%)	0.88^{a}	0.88^{a}	0.66^{b}	0.86^{a}	0.66^{b}	0.86^{a}
Total nitrogen content of stems (%)	0.96^{a}	0.96^{a}	0.78^{b}	0.87^{a}	0.78^{b}	0.87^{a}
Total nitrogen content of roots (%)	0.71^{a}	0.71^{a}	0.53^{b}	0.66^{a}	0.53^{b}	0.66^{a}
Total nitrogen content of plant (%)	2.55^{a}	2.55^{a}	1.97^{b}	2.29^{a}	1.97^{b}	2.29^{a}

CF = Carbon filtered Air, NF = Non-filtered air, AA = Ambient Air, +EDU = treated with ethylene-diurea, -EDU = Non- treated with ethylene-diurea. Values in rows followed by the same letter(s) are not significantly different.

by 11.8% in case of AA/-EDU treatments, respectively in comparing to CF treatments. Significant effects of the EDU largely on individual leaf area in September 21 being 13%, while LAI was 12% in the OTCs treated with NF or AA treatments. The total nitrogen for all plant organs was higher in soybean plants grown under NF/+EDU or AA/+EDU than those grown under ambient conditions. On the other hand, the highly decreases in total N were recorded in soybean stems (by 81%) of AA and NF treatments. Despite the consistent and significant increases for above results after the 4th EDU application in soil supporting soybean, the best improvement was recorded after 1st EDU drench of NF open top chambers being 11% in case of all EDU treatments but better especially in case of mixed EDU treatments (Table 4).

Effects of EDU on dry harvest characteristics: The mean effects of EDU exposures on dry harvest characteristics of soybean plants under air pollution treatments (Table 5). No significant difference either between CF/+EDU and CF/-EDU or NF/-EDU and AA/-EDU, or NF/+EDU and AA/+EDU treatments for all dry harvest data. Significant and dramatic effect of the EDU treatment was observed on the pods produced subjected to AA or NF air conditions. The EDU couldn't record a protection for organs and total dry biomass, seed oils and pod length of soybeans. The best results for EDU effects for soybean yield, seed weight, seed proteins and pods per plants in case of all EDU treatments but better especially in case of mixed EDU treatments. A 88.6% pollutants damage in pods number per plant were found in the NF-OTCs or

Table 5: The effects of EDU exposures on dry harvest (in September 29) characteristics of soybean (*Glycine max* L. cv. Crawford) grown under air pollution treatments at Abo Kabeer, Sharkia province, Egypt

Treatments	CF/-EDU	CF/+EDU	NF/-EDU	NF/+EDU	AA/-EDU	AA/+EDU
EDU as soil drench						
Dry leaves biomass (g)	1.00^{a}	1.00^{a}	0.88^{b}	0.92^{b}	0.88^{b}	0.92^{b}
Dry stems biomass (g)	2.99^{a}	2.99^{a}	2.24^{b}	2.44^{b}	2.24^{b}	2.44^{b}
Dry roots biomass (g)	0.45^{a}	0.45^{a}	0.33^{b}	0.36^{b}	0.33^{b}	0.36^{b}
Total dry biomass (g)	4.44^{a}	4.44^{a}	3.45^{b}	3.72^{b}	3.45^{b}	3.72^{b}
Grain yield (g m^{-2})	222.5^{a}	222.5^{a}	180.1^{b}	212.0^{c}	180.1^{b}	212.0^{c}
Seed weight (g 100^{-1})	18.5^{a}	18.5^{a}	13.0^{b}	15.6^{c}	13.0^{b}	15.6^{c}
Seed protein (%)	44.2^{a}	44.2^{a}	40.1^{b}	43.5^{a}	40.1^{b}	43.5^{a}
Seed oil (%)	21.9^{a}	21.9^{a}	19.1^{b}	19.9^{b}	19.1^{b}	19.9^{b}
Pod length (cm)	12.5^{a}	12.5^{a}	11.2^{a}	11.6^{a}	11.2^{a}	11.6^{a}
Pods per plant	62.2^{a}	62.2^{a}	55.4^{b}	60.2^{c}	55.4^{b}	60.2^{c}
EDU as foliar spray						
Dry leaves biomass (g)	1.00^{a}	1.00^{a}	0.88^{b}	0.94^{b}	0.88^{b}	0.94^{b}
Dry stems biomass (g)	2.99^{a}	2.99^{a}	2.24^{b}	2.42^{b}	2.24^{b}	2.42^{b}
Dry roots biomass (g)	0.45^{a}	0.45^{a}	0.33^{b}	0.36^{b}	0.33^{b}	0.36^{b}
Total dry biomass (g)	4.44^{a}	4.44^{a}	3.45^{b}	3.72^{b}	3.45^{b}	3.72^{b}
Grain yield (g m^{-2})	222.5^{a}	222.5^{a}	180.1^{b}	215.0^{c}	180.1^{b}	215.0^{c}
Seed weight (g 100^{-1})	18.5^{a}	18.5^{a}	13.0^{b}	16.3^{c}	13.0^{b}	16.3^{c}
Seed protein (%)	44.2^{a}	44.2^{a}	40.1^{b}	43.8^{a}	40.1^{b}	43.8^{a}
Seed oil (%)	21.9^{a}	21.9^{a}	19.1^{b}	20.3^{b}	19.1^{b}	20.3^{b}
Pod length (cm)	12.5^{a}	12.5^{a}	11.2^{a}	11.9^{a}	11.2^{a}	11.9^{a}
Pods per plant	62.2^{a}	62.2^{a}	55.4^{b}	60.6^{c}	55.4^{b}	60.6^{c}
EDU as soil drench + foliar spray						
Dry leaves biomass (g)	1.00^{a}	1.00^{a}	0.88^{b}	0.99^{a}	0.88^{b}	0.99^{a}
Dry stems biomass (g)	2.99^{a}	2.99^{a}	2.24^{b}	2.83^{a}	2.24^{b}	2.83^{a}
Dry roots biomass (g)	0.45^{a}	0.45^{a}	0.33^{b}	0.41^{a}	0.33^{b}	0.41^{a}
Total dry biomass (g)	4.44^{a}	4.44^{a}	3.45^{b}	4.22^{a}	3.45^{b}	4.22^{a}
Grain yield (g m^{-2})	222.5^{a}	222.5^{a}	180.1^{b}	218.9^{a}	180.1^{b}	218.9^{a}
Seed weight (g 100^{-1})	18.5^{a}	18.5^{a}	13.0^{b}	17.7^{a}	13.0^{b}	17.7^{a}
Seed protein (%)	44.2^{a}	44.2^{a}	40.1^{b}	43.9^{a}	40.1^{b}	43.9^{a}
Seed oil (%)	21.9^{a}	21.9^{a}	19.1^{b}	21.0^{a}	19.1^{b}	21.0^{a}
Pod length (cm)	12.5^{a}	12.5^{a}	11.2^{a}	12.0^{a}	11.2^{a}	12.0^{a}
Pods per plant	62.2^{a}	62.2^{a}	55.4^{b}	61.6^{a}	55.4^{b}	61.6^{a}

CF = Carbon filtered Air, NF = Non-filtered air, AA = Ambient Air, +EDU = treated with ethylene-diurea, -EDU = Non- treated with ethylene-diurea. Values in rows followed by the same letter(s) are not significantly different

AA-plots untreated with EDU and 11% more pods number per plant in the NF-OTCs or AA-plots treated with EDU. In parallel, seed weight was 12% more in plants grown under the NF/+EDU treatment and 12.2% on plants grown under the AA/+EDU treatment. Big large variations in the yields and seed proteins among plants in the presence and absence of EDU were observed. The best protection better especially in case of mixed EDU treatments. Generally from all data, the EDU could protect soybean plants from O_3 damage not exceeded than 22.7% (Table 5).

DISCUSSION

The information on the effect of air pollution on plants in the Mediterranean area is extremely scant and fragmentary (Fumagalli *et al.*, 2003). The threat from photochemical pollutants to crops in Egypt has not been investigated, although some studies have been conducted in other Mediterranean countries such as Italy (Schenone and Lorenzin, 1992), Greece (Barnes *et al.*, 1990; Velissariou *et al.*, 1992a, b; Saitanis *et al.*, 2001, 2003) and Spain (Martin *et al.*, 1991; Reinert *et al.*, 1992; Fernandez-Bayon *et al.*, 1993).

It is well known that air pollution can cause significant damage to agricultural crops, but still there is a lack of interest and awareness regarding O_3 air pollution problems in Egypt. Monthly concentrations of ambient O_3 treated soybean plants for full season in rural site were recorded significant increase in O_3 concentrations during hot months (high levels in August), while other gases (SO_2, NO_2) below toxic levels. Sulphur dioxide and nitrogen oxides do not seem to be relevant since the measured concentrations were low. These results of both gases agree with WHO/UNEP (1992) and Hassan (1999) studies. Then, the resulting O_3 levels were sufficiently high to cause substantial injury to the bean leaves. These O_3-induced effects are similar to that obtained by Tonneijck and Van Dijk (1997).

Ozone, like other gaseous air pollutants, enters the leaves of plants primarily through the stomata (Bynterowicz *et al.*, 2003). The O_3 gas can affect cuticular lipids by transforming the lipid from a crystalline array of fine tubules to one that takes on the appearance of a melted amorphous structure (Danielsson *et al*., 2003). These changes have been observed to the stomatal opening and as a result of CO_2 uptake and subsequent photosynthesis and growth could be reduced. Thus, whether based on genetics, stage of development, or environmental conditions, the rate ability to produce cuticular lipids could impact the efficiency of the cuticular layer relative to excluding O_3 air pollutants (Bynterowicz *et al*., 2003).

Effects of EDU, ozone and their interaction on growth and yield should be considered in evaluation of the differential responses of EDU-treated and non-EDU-treated bean plants. Increases responses of both caused by EDU were only observed at the dry harvest, not at the green harvest when the pods were ready for market. EDU applications as soil drench plus foliar spray enhanced average pod yield by up to 30 % after 11-12 weeks of exposure, independent of the nature of pollutants (Kostka-Rick and Manning, 1993). EDU is only protects, or partially protects, bean against adverse affects of ozone but that it might also affects on its physiology and growth (Miller *et al*., 1994). However, measurements of ambient ozone revealed that plants experienced most ozone between the start of the experiment and the first harvest. EDU delayed the process of senescence in the bean plants regardless of whether this senescence was caused by ozone or by natural ageing, while non-significant increase of EDU on CF/+EDU in comparing to CF/-EDU treatments. There is evidence that low levels of EDU may have some phytotoxic effects on plants in absence of O_3 (Heagle, 1989), while EDU at high concentrations without any effects on growth or yield (Chanway and Runckles, 1984). On the other hand, EDU has improved the damaged growth or yield in presence of high O_3 (Table, 3-5). These confirms that EDU as an antioxidant has senescence-delaying capacities (Bennett *et al*., 1984). Similar yield increases resulting from protection against ozone by EDU have been reported for navy beans grown in Ontario (Toivonen *et al*., 1982) and for snap beans in Italy (Schenone *et al*., 1995). The reduced yield in non-EDU-treated plants compared with that in treated plants in these studies was observed in the presence of ozone-induced foliar injury and has been attributed to ambient ozone. Variability in environmental conditions, harvest time, bean cultivar, soil nature and EDU doses might be difficult to compare my results with respect to yield effects to those of other studies. The biomass allocation is changed after exposure to O_3 at the expense of reproductive organs or the roots, in plants. This, therefore, generally includes plants where seeds or subterranean hypocotyls are the economic yield (Hassan *et al*., 1995). There is good evidence about major effects of O_3 on the yield of crops in the Egypt (Nasralla and Shakour, 1981; WHO/UNEP, 1992; Ali *et al*., 2002). The changes in dry/fresh matter and biomass production in response to O_3 may have important implication under Egyptian field conditions, where O_3-induced diversion of resources to the shoot at the expense of the root could increase the sensitivity of plants to exacerbate nutritional disorders.

Injury results from ozone exposure and the protective effect of EDU have a good relation might be due to the environmental conditions. These factors such as temperature, relative humidity and the presence of other air pollutants also might influence plant responses to ozone (Pasqualini *et al*., 2001) and possibly, to EDU. Environmental conditions might influence the effect of EDU in protecting plants against injury. Thus, Regner-Joosten *et al.* (1994) showed that the amount of EDU absorbed by the roots of bean plants and the concentration of this chemical in the plants were proportional to the rate of transpiration.

The biochemical mechanism by which EDU protects plants against O_3 is hard to identify (Lee *et al*., 1997). There are many mechanisms have been suggested but all are contradictory (Miller *et al*., 1994). Higher activities of certain scavenger enzymes along with several antioxidants could be the agents that protect plants against O_3 (Wellburn and Wellburn, 1997). Bennett *et al.* (1984) reported that catalse and peroxidases can act to regulate injurious oxyradical and peroxyl concentrations in cells to determine equilibrium rates. Superoxide dismutase extracted from EDU-treated and EDU-untreated controls had the same activity as that extracted from EDU-treated plants after fumigation with O_3 and this further the earlier suggestion of Bennett *et al.* (1984) that EDU protection is a biochemical rather than biophysical. Superoxide dismutase may be present as a copper-zinc or a manganese-containing enzyme located in the chloroplast of green leaves and thus could be easily washed off from thylakoids (Lee *et al*., 1997). EDU prevent the loss of glutathione reductase in ozonated leaves and retained its concentration as high as control plants.

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