International Journal of Botany

ISSN: 1811-9700

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International Journal of Botany 4 (1): 117-122, 2008
ISSN 1811-9700

Effect of Air Pollution and Ethylene Diurea on Broad Bean Plants Grown at Two Localities in KSA

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Abstract: The primary objectives of this investigation were to examine the interactive effects of air quality treatments and ethylene-diurea (EDU) on physiological characteristics of broad beans (*Vicia faba* L. cv. Lara) during its whole growth. Ethylene-diurea (EDU) was used as a factor to evaluate O_3 pollution impact on plant growth. Leaf samples were collected from upper canopy positions six times (pre-EDU addition, week after four EDU's addition, at the time of harvesting). Maximal differences in leaf carbohydrate and pigments were observed in response to EDU applications. Significant reduction were noted for air quality treatments regarding carbohydrate and pigment fractions. Minimal differences were found for first EDU application while maximal ones were recorded at 200 mg L^{-1} of treatments. The EDU treatments stimulated carbohydrate and pigment contents at the upper canopy position with higher levels. The stimulation in leaf carbohydrates by the EDU treatment, compared to the non-treated EDU, provides a rational explanation for the counteracting effects of EDU against moderate exposures to O_3.

Key words: Leaf biochemical contents, O_3 air pollution, EDU additions, broad bean

INTRODUCTION

Atmospheric ozone (O_3) is part and parcel of global climate change. Although ozone at the ground level is a greenhouse gas, it plays a minor role in regulating our air temperature, contributing only about 7% to the total warming effect (Krupa, 1997; NARSTO, 2000; Krupa *et al*., 2001). Numerous investigators have shown that chronic, whole growth season or whole life cycle exposures to O_3 can result in losses of marketable yield in crops and reductions in growth and productivity of species (USEPA, 1996; Hassan *et al*., 1999; McGrath, 2000; Kanoun *et al*., 2001; Ali *et al*., 2002). Finlayson-Pitts and Pitts (1999) and Ali (2003) proved that ambient concentrations of O_3 are only present at levels, which have been reported to have significant detrimental negative effects on commercial yield and biological parameters of great importance at rural sites.

Legumes especially *Vicia faba* L. cv. Lara 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 (Mersie *et al*., 1994; 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; Weinstein *et al*., 2001).

There are number of chemical growth regulators and antioxidant/antiozonant are used to protect plants against O_3 damage (Gatta *et al*., 1997; Kuehler 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 caused an increase in the dry matter weight of pods (Pitcher *et al*., 1992; Kostka-Rick and Manning, 1993; Joosten *et al*., 1994). Similar results were reported by Hassan *et al.* (1995) and Ali (2003). They found that broad bean, radish and turnip plants treated with EDU had higher growth and yield than untreated ones. Moreover, O_3 injury and senescence can be retarded by retreating plants with EDU (Whitaker *et al*., 1990; Tonneijack and Van Dijk, 1997).

Experiment was conducted to examine the use of the anti-ozonant ethylene diurea (EDU) be able to induce ozone O_3 tolerance in broad bean plants. In parallel, the objectives of the current investigation were to gain additional information on changes in leaf pigment and carbohydrate contents within the broad beans canopy during all growth stages which also corresponds to the periods when grain yield reductions were found to correlate with chronic O_3 exposures.

MATERIALS AND METHODS

Experimental materials: At the beginning of December (2/12/2005), forty healthy of equal size broad bean seeds were planted in three pots (1 m diameter) at growth chamber of Botany Department, College of Sciences, King Saud University and at King Fahad Street. The plants were grown in rows 0.6 m apart and spaced 0.1 m apart within the rows. There were eight rows of plants per pot. Parallel, applications of EDU solution (250 mg L^{-1}) in pots soil were carried out at two-week intervals in three pots for these two localities starting after thirty days from seed germination.

Foliage leaf samples were collected from both localities within the canopy before EDU application. After these sample collections, EDU drench in soil will start. The leaf samples were collected from all treatments a week after each EDU applications. One more leaf samples collection was done at the time of harvesting. A sample consisted of terminal leaflets randomly selected from five plants per treatments.

Ozone, nitrogen dioxide and sulfur dioxide concentrations in the study localities were measured during vegetative growth periods (December, June, February, March) of broad bean plants multigases meter (Greywolf, Sweden).

Physiological characteristics: Three grams of oven dry plant powder of each studied plants was mixed with 5 mL of 2% phenol solution and 10 mL of 30% trichloracetic acid (TCA). The mixture was shaken and kept overnight in a refrigerator, then filtered. The filtrate containing soluble sugars (monosaccharides and disaccharides was made up to a known volume with distilled water. The residue containing insoluble sugars (polysaccharide) was collected and dried down at 80°C till constant weight was obtained. Direct reducing value (reducing sugars) was estimated according to the method of Nelson (1944) as modified by Naguib (1964). Total reducing value (disaccharides) and polysaccharides were estimated according to the method of Naguib (1964). Estimation of total carbohydrates was determined according to the method adopted by AOAC (1960).

The photosynthetic pigments (chlorophyll a, chlorophyll b and carotenoids) were determined six times seasonally, according to methods described by Metzner *et al.* (1965).

Statistical analyses: Leaf carbohydrate, total N, photosynthetic pigments and total lipids results were analyzed using a randomized complete block design having two replicates, two moisture and five air quality treatments. All statistical analyses were performed using the SPSS BASE 11.0 (SPSS Inc., Chicago, IL) packages. Means were separated using LSD at $p<0.05$ unless otherwise specified in the tables.

RESULTS AND DISCUSSION

The seasonal average over plant growing months equaled 86, 33 and 38 nl L^{-1}, for studied gases respectively which was higher in KFS than values of KSU. Several prolonged periods of cloudy weather particularly during first and the second weeks of June with very low (42, 15 and 12 nl L^{-1}) for KFS build up in the atmosphere. The ambient O_3 levels were increased gradually over study years. The KSU lowered the ambient O_3 to be ranged between 11 to 16 nl L^{-1} (Table 1).

Soluble, insoluble and total leaf carbohydrate results for broad beans exposed to increased atmospheric O_3 in combination with NO_2 and SO_2 conditions during its whole growth are shown in Table 2. Carbohydrate results combined over treatments for the KSU and KFS were totally different. Before EDU levels application in the soil during the growing season of broad bean, the increased atmospheric O_3 significantly impacted on gradual reduction of leaf carbohydrate contents. Plants grown under restricted moisture conditions typically exhibited lower leaf carbohydrate levels than for well-watered conditions.

In all cases of EDU treatments, the soluble, insoluble and total leaf carbohydrate contents were impacted by the EDU treatments imposed throughout the growth of the plants (Table 2). There were trends significantly of higher improvement in total carbohydrate contents during flowering (3rd addition of EDU) and early pod fill (4th addition of EDU) and lower improvement in contents during 1st addition of EDU and late pod fill (before harvesting) compared to CF controls. Likewise, supporting trends for lower insoluble and total carbohydrate levels in response to EDU + O_3 treatment were found during flowering, early pod fill and late pod fill under non-irrigated conditions.

Table 1: Variations in O_3, NO_2 and SO_2 at two localities in KSA

Localities	O_3	NO_2	SO_2
KSU			
December	16	8	9
June	11	9	9
February	22	12	11
March	36	17	16
KFS			
December	45	18	12
June	42	15	12
February	78	25	28
March	86	33	38

KSU = King Saud University, KFS = King Fahad Street

Table 2: The effect of air quality treatments and EDU on carbohydrate fractions (mg g^{-1}) of broad bean leaves at two localities in KSA

	KSU-30	KSU-45	KSU-60	KSU-75	KSU-90	KSU-110	KFS-30	KFS-45	KFS-60	KFS-75	KFS-90	KFS-110	LSD
Treatments	days												$p<0.05$
O_3 effect only													
Monosaccharides	9.6	9.0	8.8	8.5	8.2	8.0	9.9	9.2	9.0	8.9	8.5	8.2	2.3
Disaccharides	8.6	8.1	8.0	8.0	8.0	8.0	8.9	8.6	8.2	8.0	8.0	8.0	4.2
Polysaccharides	10.5	10.0	9.8	9.5	9.4	9.1	11.0	11.0	11.0	10.1	10.1	10.0	2.3
Total	28.7	27.1	26.6	26.0	25.6	25.1	29.8	28.8	28.2	27.0	26.6	26.2	4.3
O_3 × EDU effect													
Monosaccharides	9.6	10.2	10.8	11.2	11.2	10.6	9.9	10.0	10.6	10.8	11.2	9.9	3.0
Disaccharides	8.6	9.4	9.7	10.1	10.5	10.6	8.9	9.1	9.6	9.9	10.5	9.9	4.6
Polysaccharides	10.5	11.5	11.7	13.7	13.9	12.5	11.0	11.9	11.8	12.4	13.4	12.5	5.3
Total	28.7	31.1	32.2	34.0	34.6	33.7	29.8	31.0	32.0	34.1	35.1	32.3	6.7

KSU = King Saud University, KFS = King Fahad Street, LSD = Least Significant Difference, EDU = Ethylene diurea

Table 3: The effect of air quality treatments and EDU on pigment fractions (μg g^{-1}) of broad bean leaves at two localities in KSA

	KSU-30	KSU-45	KSU-60	KSU-75	KSU-90	KSU-110	KFS-30	KFS-45	KFS-60	KFS-75	KFS-90	KFS-110	LSD
Treatments	days												$p<0.05$
O_3 effect only													
Chlorophyll a	1.13	1.13	1.11	1.11	1.10	1.08	1.16	1.16	1.13	1.10	1.10	1.05	0.23
Chlorophyll b	1.02	1.02	1.00	1.01	1.10	1.10	1.13	1.13	1.11	1.10	1.10	1.10	0.22
Carotenoids	0.95	0.95	0.95	0.88	0.98	0.90	1.02	1.02	1.01	0.96	0.96	0.92	0.10
Total	3.10	3.10	3.06	3.00	3.18	3.08	3.31	3.31	3.52	3.16	3.16	3.07	0.11
O_3 × EDU effect													
Chlorophyll a	1.13	1.25	1.31	1.32	1.39	1.39	1.16	1.31	1.36	1.36	1.30	1.21	0.02
Chlorophyll b	1.02	1.14	1.14	1.20	1.22	1.22	1.13	1.30	1.18	1.21	1.20	1.20	0.06
Carotenoids	0.95	1.00	1.00	1.00	1.02	1.02	1.02	1.02	1.00	1.00	1.00	1.00	0.11
Total	3.10	3.39	3.45	3.52	3.63	3.63	3.31	3.63	3.54	3.57	3.50	3.41	0.27

KSU = King Saud University, KFS = King Fahad Street, LSD = Least Significant Difference, EDU = Ethylene Diurea

Consistently higher chlorophyll a, chlorophyll b and total pigment contents were found at KFS in response to the EDU + O_3 treatments (Table 3), especially during pre-flowering and early podfill (1st, 2nd, 3rd of EDU addition). The increases were concomitant to the stimulation in photosynthetic activities throughout the canopy caused by the higher EDU concentrations drench in soil for both localities. Also, results show decreases in pigment contents in the upper canopy position in response to the non-EDU treatment, compared to the EDU, during late vegetative growth. The EDU treatment counteracted the negative effects of O_3 on leaf pigments in the upper canopy leaves during flowering and early pod fill; however, as noted above, the levels for the EDU treatment were typically below those for the abnormal treatments. The plants in the EDU treatment at The delay in maturation noted for the KFS or KSU treatments under both EDU treatments (Table 3).

There were progressively lower carbohydrate levels with depth into the canopy and age of plant which was due to the combination of lower photosynthesis rates in response to lower light levels and to aging of lower canopy leaves. Exposure to chronic O_3 levels induced faster leaf aging, resulting in premature senescence. Pell and Pearson (1983) reported reduced ribulose 1,5-biphosphate carboxylase (rubisco) contents in O_3 stressed leaves as they aged. There were patterns for gradual higher carbohydrate levels for AA treatments under EDU effects, which likely related to stimulation in photosynthesis due to increased sink capacity and demand associated with podfill. However, during late pod fill (Table 2), carbohydrate levels returned to values slightly typical of those observed during late vegetative growth and flowering of KSU (Table 2, 3), which relate to the combination of decreased sink demand as pods neared maturity and increased leafage for the determinate plants being examined. Chernikova *et al.* (1998) modeled the impact of air quality and moisture regime on gaseous flux characteristics for leaves in these studies and reported significantly lower photosynthesis rates in both cultivars caused by reduced stomatal conductance under the restricted moisture regime compared to well-watered plants.

The lower carbohydrate levels were a result of reductions in photosynthesis rates caused by chronic O_3 exposures, the utilization of photosynthate in repair processes for cellular components damaged by the toxic products of O_3 and enhanced aging of leaves due to O_3 exposures (Chernikova, 1997; Leblanc, 1998). Chronic exposure to O_3 affects photosynthesis processes in the following ways: (1) damage to cellular proteins in membranes which cause leakage of ions and fluids that result in reduced stomatal conductance, (2) damage to enzymes including rubisco and (3) reductions in chlorophyll contents and leaf area expansion during canopy development, thereby reducing the canopy photosynthetic capacity (Chernikova, 1997; Pell and Pearson, 1983; Mulchi *et al.*, 1992).

The general patterns in leaf carbohydrates in the plants during podfill in response to air quality treatments typically paralleled those for leaf photosynthesis and grain yields (Mulchi *et al*., 1992). The stimulation of leaf carbohydrate and pigment contents by the EDU treatment, compared to the KSU and KFS + non-EDU treatments, provide a rational explanation for the counteracting effects commonly reported for grain yields in C_3 plants in response to elevated atmospheric O_3in combination with moderate exposures to EDU levels. The number of pods (Ali, 2003) and sink capacity (Leblanc, 1998) established during pod set are likely closely linked to photosynthate levels in the plants. Likewise, seeds per plant and seed wt. 100^{-1}, the primary components of grain yield per plant, all parallel the leaf carbohydrate results for the air quality treatments (Mulchi *et al*., 1992; Leblanc, 1998). However, considering that the carbohydrate levels in the EDU treatment were consistently lower than were found in the CF treatment, these results confirm suggestions that exposure to chronic high O_3 (i.e., 80±5 nl O_3 L^{-1}), even in the presence of levels of EDU, will likely limit C_3 plants from attaining their maximum potential benefits regarding yields (Mulchi *et al.*, 1992, 1995; Rudorff *et al*., 1996). As a consequence, efforts to limit or reduce atmospheric O_3 concentrations as EDU levels rise in the future should be maintained and strengthened, especially in developing countries, in order to promote high levels of productivity in C_3 crops to feed an expanding world population.

Leaves under low O_3 concentrations (KSU) likely exhibit higher levels of transpiration initially due to higher stomatal conductance which results in faster water loss from the soil than plants grown under elevated EDU and/or O_3 concentrations. As moisture stress increases, stomatal conductance decreases which reduces the uptake of CO_2 by leaves resulting in lower carbohydrate levels. Additional research is needed regarding the interactive effects of gaseous exposures on water relations in plants, especially under restricted moisture conditions (Chernikova *et al*., 1998; Leblanc, 1998).

Factors which influence canopy photosynthesis, such as light levels, chlorophyll contents, atmospheric CO_2 concentrations, moisture relations, leafage and sink demand for photsynthate are all directly or indirectly affected by the air quality treatments and were addressed in the carbohydrate section. Several additional comments appear in order concerning plant N levels. First, factors such as elevated atmospheric CO_2 and adequate soil moisture in soils promote photosynthesis rates, leaf area expansion and general biomass accumulation in plants. Exposure to elevated O_3 and/or restricted soil moisture levels limits such processes in plants (Krupa *et al*., 2001). Processes which promote canopy expansion and biomass accumulation likewise enhance the N storage capacity and vice versa. Furthermore, those factors which promote above ground biomass development in plants such as soybeans typically affect the size and number of nodules or the capacity of plants to fix N_2. Therefore, when examining the influence of air quality treatments on leaf carbohydrate and N concentrations, the effects on total leaf and nodule biomass must be considered because they serve to buffer changes in leaf carbohydrate and N contents.

Second, factors which have positive influences on the total photosynthetic capacity not only influence leaf carbohydrate contents but total pre-pod N storage capacity and N_2 fixation capacity via their affects on nodule development. Plants capable of maintaining high rates of photosynthate supply to developing pods and nodules are more capable of meeting the N demands by developing pods than plants being subjected to stressful conditions. Plants subjected to stress induced by chronic exposures to elevated O_3, or less than adequate soil moisture levels, exhibit smaller canopy development, which limits both photosynthetic capacity and activities. Such reductions in photosynthesis reduce both carbohydrate contents and N reserves in leaves and N_2 fixation capacity of the plants as evidenced by smaller size and number of nodules (Pausch *et al*., 1996; Mulchi *et al*., 1992). Additional research is needed in the present studies concerning the impact of air quality treatments on nodule biomass and specific nodule activities throughout the vegetative and reproductive growth of the plants.

The biochemical mechanism by which EDU protects plants against O_3 is hard to identify (Lee *et al*., 1997; Brunschon-Harti *et al*., 1995; Eckardet and Pell, 1996). There are many mechanisms have been suggested but all are contradictory (Stevens *et al*., 1988; Whitaker *et al*., 1990; Miller *et al*., 1994). Higher activities of certain scavenger enzymes along with several antioxidants could be the agents that protect plants against O_3 (Bowler *et al*., 1992; Larson, 1995; Wellburn and Wellburn, 1997). Bennett *et al.* (1984) reported that catalase 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 (Bowler *et al*., 1992; Lee *et al*., 1997). EDU prevent the loss of glutathione reductase in ozonated leaves and retained its concentration as high as control plants.

Regarding glutathione, EDU counteract the inhibitory effect of O_3 and maintained the ratio of reduced glutathione. This is in agreement with the results of Lee *et al.* (1997), who stated that EDU-treated bean tissues (EDU+O_3) maintained high levels of total glutathione and had high reduced/oxidized glutathione than ozonated leaves. Therefore, it is expected that reduced/oxidized glutathione to be high in EDU-treated leaves after fumigation with O_3, especially glutathione reductase activity of EDU-treated leaves was high under O_3 stress. The lower ratio of reduced/oxidized glutathione in ozonated leaves was associated with the decline in reduced glutathione content. So it is clear that EDU can maintain glutathione and superoxide dismutase under O_3 stress, or may be even synthesize more molecules (Lee *et al.*, 1997; Tonneijack and Van Dijk, 1997).

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