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Effect of Pollution and Ethylene-diurea on bean plants grown in KSA

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Abstract

The primary objectives of this investigation were to examine the interactive effects of three air quality treatments, ethylene-diurea (EDU) and two irrigation conditions on physiological characteristics of kidney beans (Phaseolus vulgaris L.) during its whole growth. These plants were grown in 15-open top chambers (OTC's). Ethylene-diurea (EDU) was used as a factor to evaluate O_3 pollution impact on plant growth. The air quality treatments consisted of charcoal filtered (CF) air, nonfiltered (NF) air and ambient air (AA) were irrigated and non- irrigated. 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, N contents, pigments and total lipids were observed in response to moisture conditions in presence and absence of EDU applications. Significant reduction were noted for air quality treatments regarding carbohydrate and pigment fractions but not for all cases of leaf N and lipid contents under O_3 effects only. Minimal differences were found for first EDU application while maximal ones were recorded of studied treatment at 200 mg l^{-1}. The EDU treatments stimulated carbohydrate and pigment contents at the upper canopy position with higher levels for both NF and AA compared to untreated conditions. The NF and AA treatments caused lower total carbohydrate and pigment contents in the canopy position before harvesting of EDU applications. The stimulation in leaf carbohydrates by the EDU treatment, compared to the non-treated EDU of AA and NF treatments, provides a rational explanation for the counteracting effects of EDU against moderate exposures to O_3 regarding grain yields in C_3 plants.

Keywords: Leaf contents, Moisture relations, EDU additions, pollution, Kidney bean.

1. INTRODUCTION

Atmospheric pollution is part and parcel of global climate change. Although certain gases 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 Phaseolus vulgaris are recognized as being highly responsive and could be used as indicator plants to increasing concentrations of 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; Regner-Joosten, et al., 1994). Similar results were reported by Hassan et al. (1995) and Ali (2003). They found that kidney 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 kidney bean plants. In parallel, the objectives of the current investigation were to gain additional information on changes in leaf N, total lipid, pigment and carbohydrate contents within the kidney 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.

2 MATERIALS AND METHODS

2.1 Experimental Materials

Kidney beans (*Phaseolus vulgaris* L. Giza 3) were grown in a continuous rotation in 2-m diameter open top chambers (OTC's) and 2-m height (Heagle et al., 1973). The field site was located at a rural site at Almikhwah, Al- Baha, KSA, on loamy clay soil. The plant samples growing in a split-plot arrangement in twelve-OTC's which constituted two complete replicates of three air quality treatments (CF = charcoal filtered air; NF = non-filtered; AA = ambient air) and two soil moisture regimes (well-irrigated vs. non-irrigated). Six of the OTC's were equipped as non-irrigated treatments. The OTC's equipped as 3-CF (well-irrigated), 6-NF-NEDU (well-irrigated vs. non-irrigated) and 6-NF-EDU (well-irrigated vs. non-irrigated).

At the beginning of March, forty healthy of equal size kidney bean seeds were planted in each OTCs, and similarly sown outdoors under a transparent plastic roof as ambient air treatment (AA). 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 chamber. Each chamber was equipped with an overhead sprinkler irrigation unit. One application of EDU as a soil drensh were carried out at two-week intervals in 100, 200 mg l-1 solutions, respectively starting after thirty days from seed germination.

Foliage leaf samples were collected from one location within the canopy started on April before foliar spray EDU application to plants. After these sample collections, EDU treatments 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 concentrations in study site were measured during vegetative growth periods (March, April, May, June) of kidney bean plants over its life cycle using AEROQUAL series-S200 Monitor version 4 with removable multi-sensors heads (Air Monitors Limited, UK).

2.2 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 80oC 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).

Total N contents in the oven dry leaf sample were analyzed before EDU applications, after four EDU applications, and before harvesting using the micro-Kjeldahl technique.

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

The total lipids were extracted six times seasonally, according to the method of Bligh and Dyer (1959).

2.3 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 10.0 (SPSS Inc., Chicago, IL) packages. Means were separated using LSD at P< 0.05 unless otherwise specified in the tables.

3. RESULTS AND DISCUSSION

Monthly means of only study period for annual O_3 concentrations for both ambient and chamber air quality regimes are contained in Table 1. The average over plant growing months (March, April, May, June) O_3 concentration for ambient conditions equaled 86 nl l-1 which was somewhat higher than values determined to non-filtered air in chamber equaled 83 nl l-1 at the rural study site. Several prolonged periods of cloudy weather particularly during first and the second weeks of March with very low (<60 nl l-1) build up in the atmosphere likely caused lower than normal results (included in calculations but not appear in Table 1). The ambient O_3 levels were increased gradually over study period, while non-filtered O_3 levels were stable. The charcoal filters lowered the ambient O_3 inside the chambers to be ranged between 14 to 16 nl l-1 of O_3 levels.

Table 1: Variations in O_3 concentrations (ppb) during study period at a rural site in KSA.

O_3 Concentrations/months	Ambient air	Non-filtered air	Carbon filtered air
March	56	47	17
April	59	48	18
May	60	49	18
June	67	58	17

Soluble, insoluble and total leaf carbohydrate results for kidney beans exposed to increased atmospheric O_3 in combination with two irrigation conditions during its whole growth are summarized in Tables 2A,2B. Carbohydrate results combined over treatments for the AA and NF were slightly similar. Before foliar spray EDU application during the growing season of kidney 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 foliar spray EDU treatments, the soluble, insoluble and total leaf carbohydrate contents were impacted by the EDU treatments imposed throughout the growth of the plants (Tables 2A,2B). There were trends significantly of higher improvement in total carbohydrate contents during flowering and early podfill and lower improvement in contents during 1st addition of EDU and late podfill (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 podfill and late podfill under non-irrigated conditions.

Consistently higher chlorophyll a, chlorophyll b and total pigment contents were found at irrigated and non-irrigated soil in response to the EDU + O_3 treatments (Tables 3A,3B), especially during pre-flowering flowering and early podfill. The increases were concomitant to the stimulation in photosynthetic activities throughout the canopy caused by the higher EDU concentrations drench in soil for non-filtered chambers. Also, results show decreases in pigment contents in the upper canopy position in response to the NF + O_3 treatment, compared to the CF control, 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 podfill; 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 NF matured similarly to those in the CF treatments with plants in the AA treatments remaining green for over one week longer than the CF controls in the NF and two weeks longer than the CF controls in the AA treatments. The delay in maturation noted for the NF or AA + O_3 treatments under both moisture treatments (Tables 3A,3B).

Table 2a: The effect of three air quality treatments and two irrigation conditions on carbohydrate fractions (mg/g) of kidney bean leaves in presence and absence of EDU at a rural site in KSA.

Treatments	CF	NF-30d	NF-45d	NF-60d	NF-75d	NF-90d	NF-110d
O_3 effect only							
Irrigated soil							
Monosaccharides	13.5	9.6	9.0	8.8	8.5	8.2	8.0
Disacchacrides	13.0	8.6	8.1	8.0	8.0	8.0	8.0
Polysaccharides	18.5	10.5	10.0	9.8	9.5	9.4	9.1
Total	45.0	28.7	27.1	26.6	26.0	25.6	25.1
Nonirrigated soil							
Monosaccharides	13.2	8.9	8.1	8.0	8.0	8.0	8.0
Disacchacrides	11.3	8.0	8.0	8.0	7.8	7.2	7.1
Polysaccharides	14.2	9.9	9.2	9.0	9.0	9.0	9.0
Total	38.7	26.8	25.3	25.0	24.8	24.2	24.1
O_3 x EDU effect							
Irrigated soil							
Monosaccharides	13.5	9.6	10.2	10.8	11.2	11.2	10.6
Disacchacrides	13.0	8.6	9.4	9.7	10.1	10.5	10.6
Polysaccharides	18.5	10.5	11.5	11.7	13.7	13.9	12.5
Total	45.0	28.7	31.1	32.2	34.0	34.6	33.7
Nonirrigated soil							
Monosaccharides	13.2	8.9	9.2	9.9	9.7	9.6	8.9
Disacchacrides	11.3	8.0	9.2	9.5	9.7	9.6	9.0
Polysaccharides	14.2	9.9	9.9	10.2	11.4	11.5	10.9
Total	38.7	26.8	28.3	29.6	30.8	30.7	28.8
LSD $P<0.05$	2.3	3.0	4.6	3.3	3.5	6.7	5.3

CF = Carbon filtered Air, NF = Non-filtered air, AA = Ambient Air, LSD = Least significant difference, EDU = Ethylene diurea, d=day.

The impact of restricted moisture treatments in combination with air quality treatments typically show non-significant difference of N levels in leaves with those of well-watered conditions (Tables 4A,4B). However, the impact of restricted moisture treatments does not appear to be uniform across the four EDU treatments. There were progressively lower leaf N concentrations with increased of O_3 concentrations. The highest value for NF + EDU + O_3 occurring during early podfill being 48.2 mg l^{-1} and the smallest within-rest of treatments. There were no significant differences between O_3 singly and in combination with EDU concerning foliar N levels. There were significantly higher in foliar N mean values combined over moisture and air quality treatments for the EDU plots compared to plants grown under non-EDU conditions, especially starting at 100 mg l^{-1} and still with no change.

Table 2b: The effect of three air quality treatments and two irrigation conditions on carbohydrate fractions (mg/g) of kidney bean leaves in presence and absence of EDU at a rural site in KSA.

Treatments	CF	AA-30d	AA-45d	AA-60d	AA-75d	AA-90d	AA 110d
O_3 effect only							
Irrigated soil							
Monosaccharides	13.5	9.9	9.2	9.0	8.9	8.5	8.2
Disacchacrides	13.0	8.9	8.6	8.2	8.0	8.0	8.0
Polysaccharides	18.5	11.0	11.0	11.0	10.1	10.1	10.0
Total	45.0	29.8	28.8	28.2	27.0	26.6	26.2
Nonirrigated soil							
Monosaccharides	13.2	8.9	8.6	8.2	8.0	8.0	8.0
Disacchacrides	11.3	8.2	8.2	8.0	8.8	8.2	8.1
Polysaccharides	14.2	10.5	10.1	10.0	9.9	9.5	9.2
Total	38.7	30.5	26.9	26.2	26.7	25.7	25.3
O_3 x EDU effect							
Irrigated soil							
Monosaccharides	13.5	9.9	10.0	10.6	10.8	11.2	9.9
Disacchacrides	13.0	8.9	9.1	9.6	9.9	10.5	9.9
Polysaccharides	18.5	11.0	11.9	11.8	12.4	13.4	12.5
Total	45.0	29.8	31.0	32.0	34.1	35.1	32.3
Nonirrigated soil							
Monosaccharides	13.2	8.9	8.9	9.8	9.9	9.5	8.9
Disacchacrides	11.3	8.2	9.7	9.9	10.1	9.2	8.9
Polysaccharides	14.2	10.5	10.9	11.2	11.2	11.2	10.8
Total	38.7	27.8	29.5	30.9	31.2	29.9	28.6
LSD $P<0.05$	3.2	3.0	4.3	3.2	4.6	4.3	3.5

CF = Carbon filtered Air, NF = Non-filtered air, AA = Ambient Air, LSD = Least significant difference, EDU = Ethylene diurea, d=day.

The effects of air quality treatments were non-significant for means combined over air quality and moisture regimes (Table 5A,5B); however, there were shifts in relative lipids levels within the plants among air quality treatments from late vegetative growth to late podfill (100 mg l^{-1} – pre-harvesting). For example, the highest foliar lipids levels during flowering were found in the NF + O_3 + EDU treatments under irrigated conditions; whereas, this treatment exhibited the lowest lipids contents in AA + O_3 + EDU during early podfill with these trends being most consistent in the non-watered plots. The O_3 pollution exhibited significant lower in lipid contents in NF or AA + O_3 treated plants under both moisure regimes while non-significant response to EDU treatments for the two irrigation conditions in compared to those ones before EDU treatments. Foliar contents for the two air quality treatments (NF, AA) without EDU application in soil were similar except for soluble and total carbohydrates during the last sample date which related to differential maturity. Also, typically higher responses of leaf contents were observed in the high moisture treatments compared to restricted moisture conditions. With respect to air quality treatments under EDU effects, compared to non-EDU treatments, the NF and AA treatments generally increased carbohydrates and pigments with the largest increase occurring in the 200 mg l^{-1} of EDU. Significantly lower total carbohydrate, pigment, N and lipid contents were found in leaves in the NF and AA treatment of non-EDU during all recorded measurements.

Table 3a: The effect of three air quality treatments and two irrigation conditions on pigment fractions (µg/g) of kidney bean leaves in presence and absence of EDU at a rural site in KSA.

TREATMENTS	CF	NF-30D	NF-45D	NF-60D	NF-75D	NF-90D	NF 110D
O_3 EFFECT ONLY							
IRRIGATED SOIL							
CHLOROPHYLL A	1.51	1.13	1.13	1.11	1.11	1.10	1.08
CHLOROPHYLL B	1.23	1.02	1.02	1.00	1.01	1.10	1.10
CAROTENOIDS	1.01	0.95	0.95	0.95	0.88	0.98	0.90
TOTAL	3.75	3.10	3.10	3.06	3.00	3.18	3.08
NONIRRIGATED SOIL							
CHLOROPHYLL A	1.32	1.12	1.12	1.12	1.12	1.12	1.05
CHLOROPHYLL B	1.12	1.05	1.05	1.05	1.01	1.01	1.01
CAROTENOIDS	1.01	0.88	0.88	0.88	0.88	0.88	0.88
TOTAL	3.45	3.05	3.05	3.05	3.01	3.01	2.94
O_3 X EDU EFFECT							
IRRIGATED SOIL							
CHLOROPHYLL A	1.51	1.13	1.25	1.31	1.32	1.39	1.39
CHLOROPHYLL B	1.23	1.02	1.14	1.14	1.20	1.22	1.22
CAROTENOIDS	1.01	0.95	1.00	1.00	1.00	1.02	1.02
TOTAL	3.75	3.10	3.39	3.45	3.52	3.63	3.63
NONIRRIGATED SOIL							
CHLOROPHYLL A	1.32	1.12	1.22	1.22	1.25	1.25	1.29
CHLOROPHYLL B	1.12	1.05	1.21	1.21	1.25	1.25	1.25
CAROTENOIDS	1.01	0.88	1.01	1.01	1.01	1.01	1.01
TOTAL	3.45	3.05	3.44	3.44	3.51	3.51	3.55
LSD P<0.05	0.23	0.02	0.11	0.03	0.15	0.27	0.22

CF = Carbon filtered Air, NF = Non-filtered air, AA = Ambient Air, LSD = Least significant difference, EDU = Ethylene diurea, d=day.

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-biophosphate 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 podfill (Table 1), carbohydrate levels returned to values slightly typical of those observed during late vegetative growth and flowering of CF control (Tables 1,2,3,4), 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 and reported significantly lower photosynthesis rates in both cultivars caused by reduced stomatal conductance under the restricted moisture regime compared to well-watered plants.

Table 3b: The effect of three air quality treatments and two irrigation conditions on pigment fractions (µg/g) of kidney bean leaves in presence and absence of EDU at a rural site in KSA.

Treatments	CF	AA-30d	AA-45d	AA-60d	AA-75d	AA-90d	AA 110d
O_3 effect only							
Irrigated soil							
Chlorophyll a	1.51	1.16	1.16	1.13	1.10	1.10	1.05
Chlorophyll b	1.23	1.13	1.13	1.11	1.10	1.10	1.10
Carotenoids	1.01	1.02	1.02	1.01	0.96	0.96	0.92
Total	3.75	3.31	3.31	3.52	3.16	3.16	3.07
Nonirrigated soil							
Chlorophyll a	1.32	1.13	1.13	1.13	1.10	1.10	1.10
Chlorophyll b	1.12	1.12	1.12	1.12	1.10	1.10	1.10
Carotenoids	1.01	0.96	0.96	0.96	0.95	0.95	0.90
Total	3.45	3.21	3.21	3.21	3.15	3.15	3.10
O_3 x EDU effect							
Irrigated soil							
Chlorophyll a	1.51	1.16	1.31	1.36	1.36	1.30	1.21
Chlorophyll b	1.23	1.13	1.30	1.18	1.21	1.20	1.20
Carotenoids	1.01	1.02	1.02	1.00	1.00	1.00	1.00
Total	3.75	3.31	3.63	3.54	3.57	3.50	3.41
Nonirrigated soil							
Chlorophyll a	1.32	1.13	1.28	1.28	1.26	1.27	1.29
Chlorophyll b	1.12	1.12	1.25	1.25	1.25	1.26	1.22
Carotenoids	1.01	0.96	1.00	1.00	1.01	1.01	1.00
Total	3.45	3.21	3.53	3.53	3.52	3.54	3.51
LSD P<0.05	0.23	0.22	0.22	0.15	0.21	0.12	0.18

CF = Carbon filtered Air, NF = Non-filtered air, AA = Ambient Air, LSD = Least significant difference, EDU = Ethylene diurea, d=day.

Table 4a : The effect of three air quality treatments and two irrigation conditions on total nitrogen (mg/g) contents of kidney bean leaves in the presence and absence of EDU at a rural site in KSA.

Treatments	CF	NF-30d	NF-45d	NF-60d	NF-75d	NF-90d	NF 110d
O_3 effect only							
Irrigated soil	50.2	46.4	46.0	45.8	45.1	45.0	45.0
Nonirrigated soil	48.5	45.3	45.3	45.0	45.1	45.0	45.0
O_3 x EDU effect							
Irrigated soil	50.2	46.4	47.7	47.9	48.2	48.5	47.0
Nonirrigated soil	48.5	45.3	45.9	46.2	46.4	46.3	46.3
LSD P<0.05	2.2	2.1	2.1	2.1	2.0	2.2	2.1

CF = Carbon filtered Air, NF = Non-filtered air, AA = Ambient Air, LSD = Least significant difference, EDU = Ethylene diurea, d=day.

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; Heath, 1988). 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; 3) reductions in chlorphyll contents and leaf area expansion during canopy development, thereby reducing the canopy photosynthetic capacity (Chemikova, 1997; Pell and Pearson, 1983; Mulchi et al., 1992).

Table 4b: The effect of three air quality treatments & two irrigation conditions on total nitrogen (mg/g) contents of kidney bean leaves in presence and absence of EDU at a rural site in KSA.

TREATMENTS	CF	AA-30D	AA-45D	AA-60D	AA-75D	AA-90D	AA 110D
O_3 EFFECT ONLY							
IRRIGATED SOIL	50.2	47.3	47.0	46.8	46.2	46.0	45.5
NONIRRIGATED SOIL	48.5	45.6	45.0	45.0	45.1	45.0	45.0
O_3 X EDU EFFECT							
IRRIGATED SOIL	50.2	47.3	48.5	48.9	48.9	48.2	47.1
NONIRRIGATED SOIL	48.5	45.6	45.9	46.2	46.3	46.1	45.9
LSD P<0.05	4.2	4.2	2.3	2.3	2.2	2.3	2.3

CF = Carbon filtered Air, NF = Non-filtered air, AA = Ambient Air, LSD = Least significant difference, EDU = Ethylene diurea, d=day.

Table 5a: The effect of three air quality treatments and two irrigation conditions on total lipid (%) contents of kidney bean leaves in presence and absence of EDU at a rural site in KSA.

TREATMENTS	CF	NF-30D	NF-45D	NF-60D	NF-75D	NF-90D	NF 110D
O_3 EFFECT ONLY							
IRRIGATED SOIL	22.3	20.4	20.2	20.0	19.6	19.4	19.5
NONIRRIGATED SOIL	22.1	20.1	20.1	19.9	19.6	19.1	19.5
O_3 X EDU EFFECT							
IRRIGATED SOIL	22.3	20.4	21.4	21.9	21.1	21.4	19.6
NONIRRIGATED SOIL	22.1	20.1	21.1	21.8	21.0	21.1	19.4
LSD P<0.05	1.5	1.5	1.3	2.2	1.3	1.3	1.3

CF = Carbon filtered Air, NF = Non-filtered air, AA = Ambient Air, LSD = Least significant difference, EDU = Ethylene diurea, d=day.

Table 5b: The effect of three air quality treatments and two irrigation conditions on total lipid (%) contents of kidney bean leaves in presence and absence of EDU at a rural site in KSA.

TREATMENTS	CF	AA-30D	AA-45D	AA-60D	AA-75D	AA-90D	AA 110D
O_3 EFFECT ONLY							
IRRIGATED SOIL	22.3	20.2	20.0	20.2	19.9	19.2	19.1
NONIRRIGATED SOIL	22.1	20.0	20.0	20.2	19.5	19.0	19.4
O_3 X EDU EFFECT							
IRRIGATED SOIL	22.3	20.2	21.2	22.0	21.5	21.3	19.4
NONIRRIGATED SOIL	22.1	20.0	21.1	22.1	21.0	21.2	19.2
LSD P<0.05	1.3	1.3	1.5	1.5	1.6	1.5	1.1

CF = Carbon filtered Air, NF = Non-filtered air, AA = Ambient Air, LSD = Least significant difference, EDU = Ethylene diurea, d=day.

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 CF and NF + non-EDU or AA + 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_3 in 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 raise 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 very low O_3 concentrations (CF) 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).

In order to gain a better understanding of the factors which produce shifts in leaf biochemical contents during podfill, it is beneficial to review some of the processes known to be involved concerning N and carbohydrate storage and utilization in soybean. As reviewed by Shibes et al. (1987), Harper (1994) and Layzell and Maloney (1994), about a third of the daily photosynthate products are consumed in respiratory processes with the remaining two-thirds partitioned into processes involving biomass accumulation including root and nodule development. Several factors influence the rates of N_2 fixation in soybean nodules: 1) size and number of effective nodules; 2) strain ofRhizobium bacteria and bacteroid volume present; 3) nodule leghemoglobin, an O_3 -binding protein, contents; 4) fertilizer NO_3^{-1} concentration; 5) phloem sap supply; and 6) external O_3 concentrations. The product of N_2 fixation (NH4-1) in nodules is transferred to the ureides allantoin and allanoic acid which are transported via the xylem to other parts of the plant to meet the demand for N in the form of amino acids for protein synthesis. Prior to flowering, the N is also stored in paraveinal mesophyll cells as glycoproteins and other leaf proteins with about half of the soluble proteins as rubisco. Prior to flowering and throughout pod development, an active supply of photosynthate into pholem sap to roots and nodules is necessary to meet the high energy demand for N_2 fixation.

Factors which influence canopy photsynthesis, 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 & 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 (Blower et al., 1992; Larson, 1995; Wellburn & 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 (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 to oxidized one high as control plants (CF). 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 & Van Dijk, 1997).

4. Conclusion

The EDU treatment counteracted the negative effects of O_3 on leaf characters in the upper canopy leaves during flowering and early podfill; however, as noted above, the EDU treatment were typically below those for the abnormal treatments. The plants in the EDU treatment at NF matured similarly to those in the CF treatments with plants in the AA treatments remaining green for over one week longer than the CF controls in the NF and two weeks longer than the CF controls in the AA treatments. The delay in maturation noted for the NF or AA + O_3 treatments under both moisture treatments

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