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Egyptian Plant Species as New Ozone Biomonitors

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ABSTRACT

The aim of this study was to test and select one or more highly sensitive, specific and environmentally successful Egyptian bioindicators for ozone (O_3). For that purpose more than thirty Egyptian species and cultivars were subjected between 1993 and 1995 to extensive screening studies under controlled environmental and pollutant exposure conditions to mimic the Egyptian environmental conditions and O_3 levels in urban and rural sites. Four plant species were found to be more sensitive to O_3 than the universally used O_3-bioindicator, tobacco Bel W3, under the Egyptian environmental conditions used. These plant species, Jew's mallow *(Corchorus olitorius* cv. local), white clover (*Trifolium repens* L. c.v. Masry), garden rocket (*Eruca sativa* c.v. local) and alfalfa (*Medicago sativa* L. c.v. local), ranked in order of decreasing sensitivity, exhibited typical O_3 injury symptoms faster and at lower O_3 concentrations than tobacco Bel W3.

Three parameters were tested in search of a reliable tool for the diagnosis and prediction of O_3 hidden damage prior to the appearance of visual foliar symptoms: pigments degradation, stomatal conductance (G_s) and net photosynthetic CO_2 assimilation (P_{net}). Pigment degradation was found to be unreliable in predicting species sensitivity to O_3. Evidence supporting stomatal conductance involvement in O_3 tolerance were found only in tolerant species. A good correlation was found between G_s,

restriction of O_3 and CO_2 influx into the mesophyll tissues, and P_{net}. Changes in P_{net} seemed to depend largely on fluctuations in G_s.

INTRODUCTION

Ozone (O_3) in the troposphere have substantial impacts on agriculture worldwide. It causes more plant damage than all the other air pollutants combined (Heagle, 1989 and Lefohn, 1991). Industrial activity in the twentieth century has lead to a rise in ozone level in urban regions and even in remote rural areas. Ozone is produced by photochemical reactions of the primary precursors such as hydrocarbons and nitrogen oxides (NO_x). Emissions of these precursors are increased by industrialization and the growing numbers of motor vehicles. Furthermore, O_3-producing photochemical reactions are favoured by high temperatures and high light intensities (Lefohn, 1991). In many developing countries like Egypt, urbanization and industrialization is rapidly growing. Urban population growth rates in Egypt reached 3.4% in 1990, also, the number of industrial plants and power stations has increased substantially over the last decade (1980-1990) (WHO/UNEP, 1992). Emissions of O_3 precursors resulting from cars and buses alone in Cairo have subsequently doubled over this decade (WHO/UNEP, 1992). Furthermore, the climate in Egypt is frequently suitable for the formation of high O_3 concentrations by photochemical reactions especially during the summer.

Relatively little is known about the ambient levels of O_3 in Egypt due to the high cost of using instrumental monitoring to assess O_3 levels in urban and rural areas. Few research groups have attempted to measure surface O_3 levels in Cairo. Nasralla and Shakour (1981), using the neutral KI method, reported an O_3 concentration of 100-200 ppb for most of the year, which increased during summer months (May-July) showing a peak hourly concentration of 500 ppb. In a latter report, they measured O_3 concentrations in a residential area of Cairo, using a UV photometer. They reported O_3 levels in the range of 55-70 ppb during the summer of 1989 (WHO/UNEP, 1992). The most recent publication was by Farag *et* *al.* (1993). They monitored O_3 in urban and rural sites around Cairo in 1991-1992 using UV photometers, and found mean O_3 concentrations of 100 ppb or more in rural and urban areas during the summer season.

Therefore, there is an increasing interest in developing techniques to monitor the levels of surface O_3 and to detect its effects on agriculture and urban ecosystems. A useful tool in such an effort would be a plant system that responds to ambient levels of O_3 with typical and characteristic symptoms (Weinstein *et al.*, 1990). Over the years, methods have been developed to use sensitive plant species as bioindicators for the detection, recognition and monitoring of airborne pollutants.

The use of plant biomonitors is considered an inexpensive, however, reliable supplement or substitute to very expensive monitoring systems employing sophisticated instruments (Manning & Feder, 1980). Perhaps the most important advantage of biomonitoring with plants over instrumental monitoring is the capacity of plants to absorb and integrate doses of pollutants over a wide range of environments. Thus, they are able to express the biological effect of a pollutant dose while integrating climatic, cultural and genetic factors into their response (Weinstein *et al.*, 1990).

The use of plant as bioindicators and biomonitors of ambient air quality has been thoroughly documented and reviewed in several publications (Manning & Feder, 1980; Steubing & Jager, 1982; Posthumus, 1982 and Toneijck & Posthumus, 1987). Some of the best defined examples of bioindicators plants for gaseous air pollutants include those used to detect hydrogen fluoride and ozone (Manning & Feder, 1980 and Heggastad, 1991). The most extensively used plant indicator for ozone has been the tobacco cultivar Bel-W3 (Heggestad, 1991), an extremely sensitive plant to O_3 which displays recognizable and typical injury symptoms almost everywhere it is grown. The tobacco indicator system was improved by comparing the response of Bel-W3 to the response of Bel-B, an O_3-tolerant cultivar. This tobacco system was successfully used in O_3 biomonitoring programs to detect the existence of phytotoxic level of tropospheric O_3 and to provide rough estimates of relative seasonal concentrations of O_3 in different cities in Europe, Canada and USA (Kromroy *et al.*, 1988; Tonneijck, 1989; Mignanego *et al.*, 1992; Runeckles & Bowen, 1993; Gimeno *et al.*, 1995 and Della Mea *et al.*, 1997). A wide variety of other plant species have been used as O_3-bioindicators. These plant species include white clover (Becker *et al.*, 1989 and Heagle *et al.*, 1995), radish (Kostka-Rick & Manning, 1993) beans (Guzy & Heath, 1993) watermelon (Gimeno *et al.*, 1993 and Ferandez-Bayon *et al.*, 1993) and cotton (Temple, 1990).

Plant response to air pollutants is dependent on a host of climatic, physiologic, edaphic and genetic factors which can alter the type and severity of foliar symptoms (Heck *et al.*, 1979). It is, therefore imperative that screening studies, designated to select suitable bioindicator plants, be conducted under the same environmental conditions in which they are going to be used as bioindicators. This would ensure that meaningful, reliable and repeatable dose-response relationships could be generated.

Air pollution monitoring in Egypt is extremely limited because of the problems associated with the high cost of instrumental monitoring and the unavailability of reliable biomonitors adapted to the Egyptian environment. The present study reports an attempt to develop Egyptian bioindicators for ozone and was designed to achieve the following objectives: 1) The screening of a large number of plant species and cultivars which are already suited and adapted to the Egyptian environmental conditions in order to select candidates for O_3 bioindication; 2) Testing of the selected plant material extensively under controlled environmental and pollutant exposure conditions in order to relate symptoms or changes in plant performance to particular O_3 doses; 3) testing plants at more than one stage of growth, bearing in mind the differential sensitivity to air pollution associated with plant age; and 4) testing the selected plant material in an attempt to find additional reliable diagnostic tools which can predict O_3 damage prior to the appearance of visual foliar injury such as pigment content, photosynthetic capacity and stomatal response.

MATERIALS AND METHODS

1. Plant Material

Sixteen Egyptian plant species and cultivars were selected from the major plant material currently cultivated in Egypt. These plant varieties were selected to include species reported in the literature to be O_3-sensitive and others which were listed as O_3-tolerant. A detailed listing of the plant species used with respect to common name, scientific name, cultivar(s) name(s), ozone sensitivity and literature references is presented in Table (1). The tobacco cultivars Bel W3 and Bel B which are the most widely used plant material in O_3 biomonitoring programs worldwide (Tonneijck, 1989; Mignanego *et al.*, 1992; Runeckles & Bowen 1993; and Gimeno *et al.*, 1995) were also included to test their relative sensitivity under the Egyptian environmental conditions used in this study, and to use them as reference to compare with the Egyptian species of unknown O_3-sensitivity. All the seeds of the Egyptian plant species were obtained from the Horticulture Research Station, Dokky, Egypt. Seeds were planted in 10-cm kord fiber pots containing Cornell mix, a peat/vermiculite mixture (1:2) supplied with Dolomatic Lime and Peters Uni-Mix Plus II to give a trace element range of 10-10-5. Plants were grown in a controlled-environment greenhouse under simulated Egyptian fall-winter, light, temperature and relative humidity conditions. The greenhouse was maintained at 24/18°C day/night temperatures, 55-65% relative humidity and a 14hr photoperiod.

2. Sensitivity Screening Experiments:

Preliminary testing experiments were conducted in 1993 in the laboratory at the Faculty of Agriculture, Alexandria University, Egypt, in which more than 30 Egyptian species and cultivars were screened for O_3 sensitivity by exposing them to an acute ozone dose of 200-300 ppb for short durations (3-5 days) in continuously stirred tank reactors (CSTR's). From these experiments sixteen plant species were selected for further testing under controlled environmental conditions

Table (1) Selected plant species, their ozone sensitivity as reported in the literature and their respective Egyptian cultivars used in the present study.

Common name	Scientific name	O_3 sensitivity	References	Egyptian Cultivar
Common bean	*Phaseolus vulgaris* L.	Sensitive	US-EPA, 1976; Manning & Feder, 1980; Guzy & Heath, 1993	.Giza 3 Contender
Clover	*Trifolium alexandrinum* L.	Sensitive	Becker *et al.*, 1989 Heagle *et al.*, 1992	Masry
Alfalfa	*Medicago sativa* L.	Sensitive	Jacson & Hill,1970 US-EPA, 1976	Local var.
Spinach	*Spinacea oleracea* L.	Sensitive	US-EPA,1976; Posthumus,1982 Manning, 1993	Balady
Summer squash	*Cucurbita pepo*	Sensitive	Jacobson & Hill, 1970	Eskandarany
Tobacco	*Nicotiana tabacum* L.	Sensitive Tolerant	Manning & Feder, 1980 Heggestad, 1991	Bel-W3* Bel-B*
Lettuce	*Lactuca sativa* L.	Tolerant	US-EPA, 1976	(Romaine) Paris Island Cos (Stem) Balady
Cotton	*Gossypium barbadense* L.	Tolerant	US-EPA, 1976 Temple, 1990	Giza 70, Giza 12 Alexandria 4 , Hybrid
Garden rocket	*Eruca sativa*	Suspected sensitive	Personal observations	Local var.
Jew's mallow	*Corchorus olitorius*	Suspected sensitive	Personal observations	Local var.
Broad bean	*Vicia faba* L.	Suspected Tolerant	Personal observations	Romy
Okra	*Hibiscus esculentus*	Suspected Tolerant	Personal observations	Long Green

*Not Egyptian cultivars but internationally used for bioindication of O_3.

at Boyce Thompson Institute for Plant Research at Cornell University, USA, during the summers of 1994 and 1995.

The sensitivity of the different plants was tested at two or three growth stages: a) an early vegetative stage (18-20 days from seed); b) the active vegetative stage (32-34 days from seed) and c) the late vegetative stage (50-53 days from seed). Two different levels of O_3 (50 and 100 ppb) were chosen to mimic the ozone concentrations recently reported in rural and urban sites in Egypt by Farag *et al.* (1993). All ozone sensitivity experiments were repeated twice or three times with 6 individual plants as replicates for each treatment, in each of 2 horizontal flow fumigation chambers (n=60 for each plant variety).

3. Exposure to ozone

Plants were routinely removed from the greenhouse at the desired growth stage and placed in the fumigation chambers to stabilize 24 h before ozone exposure was to start. The horizontal-flow fumigation chambers were equipped with multi-vapour and high-pressure sodium lamps to provide a 14h photoperiod at a light intensity of 560-600 $\mu E\ m^{-2}s^{-1}$. Air temperature inside the fumigation chambers was 24°C day/18°C night and the relative humidity was maintained between 50-60%. Ozone was generated from pure oxygen in an electrical discharge generator (Griffin Technics Corp., USA) and added to the air entering the chamber continuously. One chamber was supplied with charcoal filtered air and was used as a control (cfa). Four chambers were used for O_3 exposure; two chambers were adjusted to each of the two O_3 concentrations 50 and 100 ppb and statistically considered as separate replicates. Air samples were drawn continuously throughout the fumigation period, by the help of solenoid valves, switching between the control and fumigation chambers every one minute. The levels of O_3 in the air samples drawn from all chambers was monitored using a UV-absorption ozone analyzer (Teco, Thermo Environmental Instruments Inc., USA) linked to a chart recorder. Plants were exposed to O_3 or cfa 6h per day for three or five consecutive days according to the experiment

4. Rating Leaf Injury:

Plant injury data was routinely collected and recorded every 24 h, at the beginning of the day before the start of fumigation (18 h after the end of the previous day's fumigation session). Recording of visible symptoms was cumulative and started on day 2 and ended on day 5. Injury was rated by visual estimation of the injured area on each leaf; and the percentage of leaf area injured according to a 0-100% scale, in 5% increments, was recorded. Dead or completely yellowed leaves were given a rating 100% injury. The rating system used to evaluate the extent of foliar damage caused by ozone is presented in Table (2).

Table (2): The rating system used for evaluating plant response to O_3 fumigation

Rating of injury	% injured leaf area	Injury index
None	0	0
Very slight	1-15	1
Slight	16-25	2
Moderate	26-50	3
Severe	51-75	4
Very severe	76-100	5

5 . Pigment Extraction and analysis:

The first fully expanded leaf was used for the quantitative analysis of pigment contents when plants were at the early vegetative stage (18 days from seed), and the second leaf was used when they were at the latter stages of growth (34 or 53 days from seed). Chlorophyll 'a' and 'b' and total carotenoids concentrations were measured in leaf extracts prepared with 96% (v/v) ethanol according to the extraction method of Knudson *et al.*, (1977). Absorbances of the chlorophyll extracts at 665, 649 and 470 were measured spectrophotometrically. The individual levels of chlorophyll 'a' (chl a), chlorophyll 'b' (chl b) and the total amount of carotenoids (car) were calculated from equations derived for the solvent ethanol by Lichtenthaler and Wellburn (1983). Samples for pigment extraction were collected 18 h after the end of the fumigation session on the first and fifth day of O_3 exposure

6. Leaf Gas Exchange and Stomatal Conductance

Measurements of net photosynthesis (P_{net}) and stomatal conductance (G_s) were made immediately post-fumigation on the first and third days of O_3 exposure. Nondestructive gas exchange measurements were taken on the first or the second fully expanded leaf when plants were 18 or 34 days old, respectively. Six plants per cultivar from each of the horizontal-flow chambers were used for gas exchange measurements. Measurements were made using a portable photosynthesis system (LI-6200, LI-COR Inc., Lincoln, NE, USA) equipped with differential infrared CO_2 analyzer (LI-6250) to monitor changes in CO_2 concentrations.

7. Statistical Analysis

Data were analysed statistically using ANOVA procedure of the "Statistica" software computer package. Three independent variables (plant age, ozone treatment, and day of sampling) were always tested against one dependent variable (e.g. Chlorophyll "a") at a time. L.S.D. values were calculated for significant differences.

RESULTS

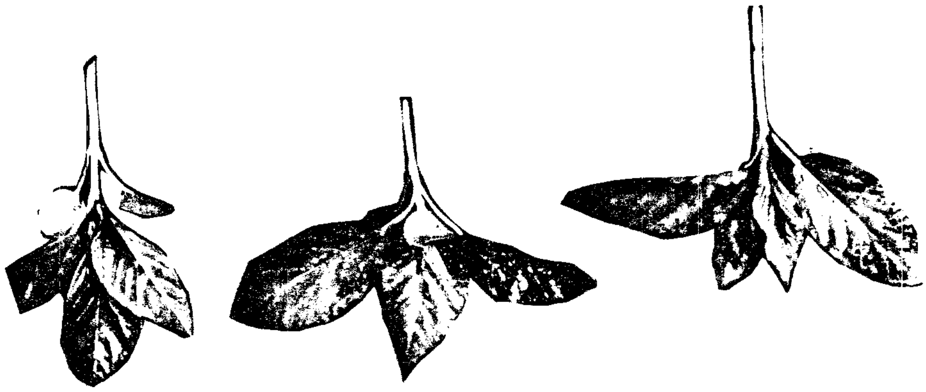
Visible Foliar Injury Symptoms:

Typical O_3-type foliar injury symptoms were observed on seven of the sixteen cultivars under study. Symptoms varied from interveinal chlorosis to small necrotic flecks or stipples which ranged in color, from yellow to tan or white to pigmented dark lesions, according to the plant species examined. Four Egyptian local cultivars were more sensitive to O_3 than the universally used O_3-bioindicator, tobacco cv. Bel W3. These cultivars were Jew's mallow (local var.), white clover (Masry), garden rocket (local var.) and alfalfa (local var.) (Table 3).

Jew's mallow the most sensitive species showed foliar injury symptoms after only one fumigation session (6 hr) at the lowest O_3 level (50ppb). Jew's mallow was most sensitive to O_3 at the early vegetative stage (18 days) than at the active vegetative stage (32 days). Typical O_3 symptoms on Jew's mallow are exhibited in Plate (1) and consisted of small, brown to black pigmented stipples on the upper leaf surface. The stipples increased and coalesced into larger lesions in the following days after repeated exposure. Stipples were more abundant on the tip and margins of the leaves. At the 50 ppb O_3 exposure level, the symptoms were sometimes different. The upper surface of the leaf acquired a clearly defined bumpy texture caused by the swelling of groups of cells. Later, these cells died and turned into dark lesions.

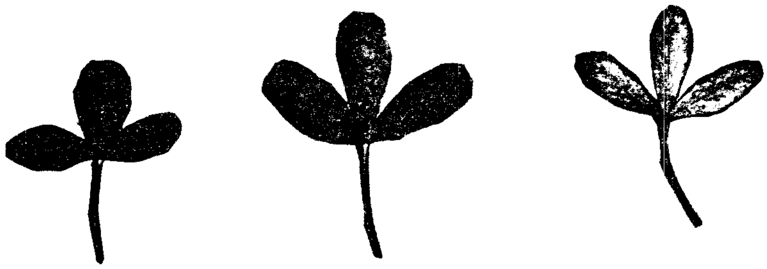
White clover was highly sensitive to O_3 at the early vegetative stage (18 days). Symptoms on clover leaves were typical ozone flecking after one fumigation session (6 hr) at the two O_3 levels used (50 and 100 ppb). It consisted of small white or tan necrotic areas on the upper surface due to the death of palisade cells. The necrotic lesions spread and merged quickly, turning into bifacial intreveinal bleaching and necrosis (Plate 2).

Garden rocket ranged third in O_3-sensitivity because it developed symptoms only after two or three fumigation sessions. However, at that time the symptoms were acute (injury index 3-5) and were observed on leaves fumigated at the lower (50 ppb) and higher (100 ppb) O_3 concentrations. Injured leaves showed reddish-purple pigmentation on the upper leaf surface. The pigmented chlorosis was interveinal and it



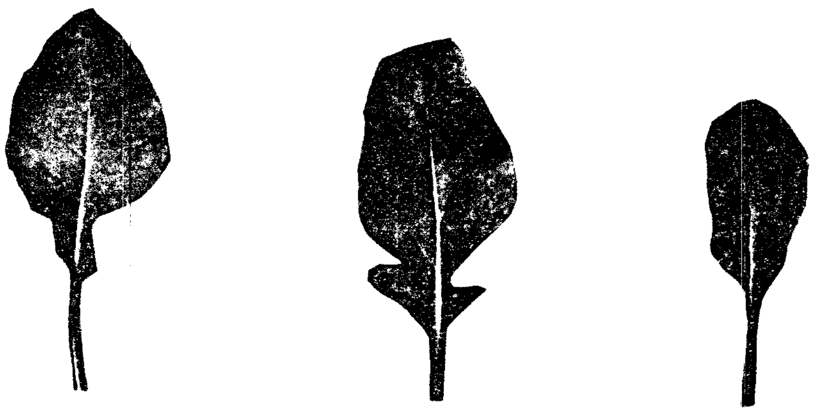
Control **50 pbb** **100 ppb**

Plate 1: O_3-induced injury symptoms on Jew's mallow (Local)



Control **50 pbb** **100 ppb**

Plate 2: O_3-induced injury symptoms on white clover (Masry)



Control **50 pbb** **100 ppb**

Plate 3: O_3-induced injury symptoms on garden rocket (Local)

Control **50 pbb** **100 ppb**

Plate 4: O_3-induced injury symptoms on alfalfa (local)

spread to give the leaf a purplish mottled appearance intercepted with green veins (Plate 3).

The local alfalfa cultivar was also found to be sensitive to O_3 at both levels studied. The symptoms of O_3 injury were observed after two fumigation sessions, and consisted of the commonly encountered upper-surface light yellow-green chlorosis and necrosis which tended to concentrate between the larger veins. At the low O_3 level (50 ppb), the small lesions were usually bounded by the smallest veins resulting in small angularly shaped yellow-green areas surrounded by large areas of green tissues. At the higher O_3 level (100 ppb), injury was more acute and leaves developed large, yellow-green chlorotic areas with many irregular islands of normal green tissues scattered through them (Plate 4).

Tobacco Bel W3 ranged fifth in sensitivity to O_3 when tested under the Egyptian environmental conditions adopted in this study. Foliar injury symptoms developed on the oldest leaves of 50-53 days old plants, after two fumigation sessions at the 100ppb level, and after three O_3 exposure episodes at the 50 ppb level. Typical "weather fleck" symptoms were observed and recorded (Plate 5). The symptoms were characterized by numerous small lesions on the upper leaf surface of the fully expanded leaves. The individual flecks consisted of small lesions of dead tissue caused by the death of group of palisade cells. The lesions were white-tan and had a rounded or angular shape.

The two cultivars of lettuce used in this study belonged to different lettuce groups; Paris Island Cos belongs to the Romaine lettuce group, and Balady belongs to the Stem lettuce group. Those two cultivars were found to be less sensitive to O_3 than the previously discussed species and were ranged "intermediate". Paris Island Cos was found to be more sensitive to O_3 than Balady at the active vegetative stage (32 days). However, Balady was more sensitive at the late vegetative stage (50-53 days). Foliar injury consisted of large bifacial light green to yellow chlorotic areas at the tip and margins of older leaves. Injury spread towards the middle of the leaf with repeated fumigation, and small dark brown necrotic lesions occurred bifacially all over the chlorotic areas (Plates 6 & 7).

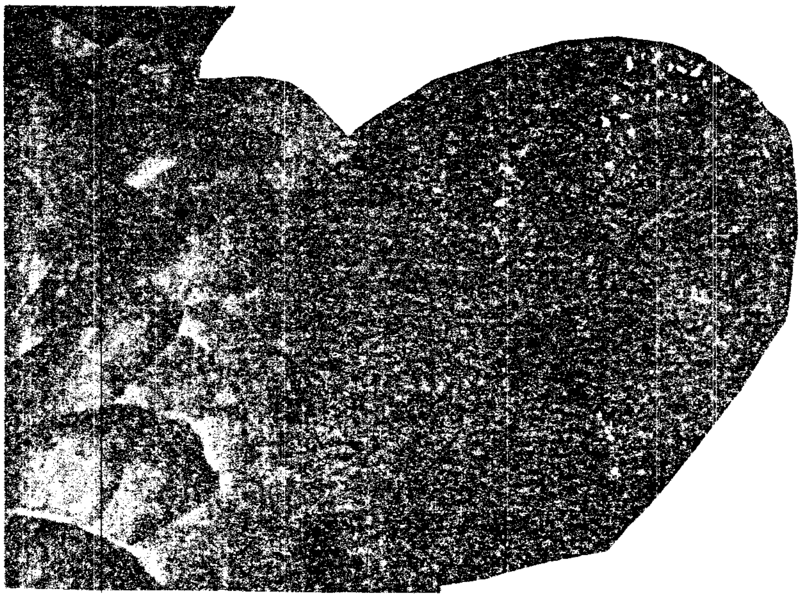


Plate 5: O_3-induced injury symptoms on tobacco (Bel W3)

Control **50 pbb** **100 ppb**

Plate 6: O_3-induced injury symptoms on Romaine lettuce (Paris Island Cos)

Control **50 pbb** **100 ppb**
Plate 7: O_3-induced injury symptoms on Stem lettuce (Balady)

Sensitivity screening:

From the data presented in Table (3) it was concluded that the four species, Jew's mallow (local), white clover (Masry), garden rocket (local) and alfalfa (local) were the most O_3 sensitive species tested. These species used at the early vegetative stage (18 days from seed) were more sensitive to O_3 fumigation at concentrations as low as 50 ppb than the known O_3-bioindicator tobacco cv. Bel W3 grown under Egyptian environmental conditions. These four cultivars were selected and designated as candidates for ozone bioindication in Egypt.

The two lettuce cultivars were ranged "intermediate" in terms of ozone tolerance. Their O_3-sensitivity was comparable to the tobacco cv. Bel W3.

The Egyptian common bean cultivars Giza 3 and Contender, were originally selected for this study because of the sensitivity of bean cultivars to O_3 (USEPA, 1976), and their widespread use as O_3-

bioindicators (Manning & Feder, 1980). Both these cultivars were found to be tolerant to ozone at the two growth stages tested. The Egyptian cultivars of two other species which were reported to be sensitive to ozone exposure, summer squash (Jacobson & Hill, 1970) and spinach (Posthumus, 1982) did not develop any visible foliar symptoms in response to five days of O_3 fumigation (6 h/day) at 50 or 100 ppb O_3.

The four cotton cultivars (Giza 12, Giza 70, Alexandria 4 and Hybrid), the broad bean cultivar (Romy) and the okra cultivar (Long Green) which were selected as O_3-tolerant bioindicators were found to be also tolerant under the environmental conditions used in this study. No symptoms were detected on any of those cultivars at the two growth stages studied after 5 days of exposure (6 h/day) to O_3 at levels common in urban (100 ppb) and rural (50 ppb) areas in Egypt (Farag *et al.*, 1993).

Leaf Pigment content:

The effect of ozone fumigation for 5 days on the level of chlorophyll 'a' are presented in Table (4). The sensitive plant species showed varying degrees of chlorophyll 'a' loss. The magnitude of this loss did not correlate with their sensitivity order, which was arranged on the basis of the extent of foliar injury. However, the extent of chlorophyll 'a' destruction was higher in sensitive and intermediate species than in tolerant ones. Greater chlorophyll degradation was recorded on sensitive plants which were exposed to O_3 at the active vegetative stage (32 days from seed) than on similar plants fumigated at the early vegetative stage (18 days from seed). Chlorophyll loss was equally recorded on most of the tolerant species under study especially those exposed to O_3 at an older age (32 days). Although the bean cultivar "Contender" and the cotton cultivars "Giza 12 and "Giza 70" showed an increase in the level of chlorophyll 'a' when exposed to O_3 at the age of 18 days, they responded diffrerently when older plants (32 days) were fumigated.

Plant species responded differently to the increase in O_3 concentration. Sensitive species with the exception of Jew's mallow, the most sensitive species, showed an increased loss in chlorophyll 'a' in response to the higher O_3 level (100 ppb). On the other hand, the tolerant

Table (3): Progressive appearance of visible foliar injury on O_3-sensitive species in response to O_3 fumigation with 2 levels (50 and 100 ppb) for five days (6h/day) at different plant ages.

Plant Species & cultivar	Plant age (days from seed)	% leaves injured						% injured leaf area						Injury index[@]					
		O_3 (50 ppb)			O_3 (100 ppb)			O_3 (50 ppb)			O_3 (100 ppb)			O_3 (50 ppb)			O_3 (100 ppb)		
		d 2	d 3	d 5*	d 2	d 3	d 5	d 2	d 3	d 5	d 2	d 3	d 5	d2	d3	d5	d2	d3	d5
Jew's mallow (local)	18	30.76	34.11	38.66	72.22	77.30	83.00	10.0	22.00	25.00	40.00	65.00	75.00	1	2	2	3	4	5
	32	11.10	11.10	16.65	22.22	27.75	38.33	4.5	12.5	14.5	18.5	22.00	35.00	1	1	1	2	2	3
Clover (Masry)	18	18.66	27.50	45.83	53.33	56.66	60.00	5.00	28.00	40.00	20.00	65.00	78.50	1	3	3	2	4	5
	32	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	0	0	0	0	0	0
Garden rocket (local)	18	NS	45.00	61.26	NS	46.66	55.71	NS	40.00	50.00	NS	70.00	90.00	0	3	4	0	4	5
	32	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	0	0	0	0	0	0
Alfalfa (local)	18	NS	37.75	50.00	NS	46.66	60.00	NS	10.00	22.50	NS	31.66	39.16	0	1	2	0	3	3
	32	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	0	0	0	0	0	0
Tobacco (Bel W3)	32	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	0	0	0	0	0	0
	53	NS	NS	14.28	NS	43.33	60.00	NS	NS	7.50	NS	21.66	48.33	0	0	1	0	2	3
Romanie lettuce (PIC)	18	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	0	0	0	0	0	0
	32	9.16	13.33	18.33	23.72	30.34	33.11	10.83	11.66	13.50	15.00	31.25	40.00	1	1	1	2	3	3
Stem lettuce (Balady)	18	NS	NS	20.00	NS	26.11	26.11	NS	NS	6.66	NS	8.33	18.33	0	0	1	0	1	2
	32	NS	NS	NS	10.55	21.11	31.66	NS	NS	NS	5.83	26.00	33.00	0	0	0	1	3	3

[@] Injury index (see Table 1). NS= no symptoms.

*Observations were taken before the start of the fumigation period on the second, third and fifth day (18 hrs from the end of the previous fumigation period).

Species are sorted in order of descending sensitivity.

Results are averaged from 3 different experiments with 6 plant replicates for each individual treatment

Table (4): Effect of ozone fumigation on chlorophyll 'a' content of fully expanded leaves of different plant species differing in ozone sensitivity.

Plant Species & Cultivar	Ozone Level (ppb)	Chlorophyll a (µg/mg dry weight)#			
		18 days@		32 days@	
		d 1	d 5	D 1	d 5
Jew's mallow (Local)	cfa	7.80a	6.81ab	23.24a	19.74b
	50	7.65a	6.32b	21.65a	17.06c
	100	7.58a	6.16b	21.45a	17.32c
Clover (Masry)	cfa	13.06a	9.32b	7.65a	5.11b
	50	11.76a	7.56c	4.35b	1.96c
	100	10.71b	5.50d	4.84b	1.18c
Garden rocket (Local)	cfa	8.30a	5.02b	4.74a	3.91a
	50	7.11a	3.44c	4.78a	1.84b
	100	7.88a	3.42c	5.03a	1.27b
Alfalfa (Local)	cfa	8.87a	6.96b	4.07a	4.36a
	50	7.28ab	3.58c	2.73a	2.34bc
	100	9.09a	5.97b	2.58a	1.29c
Tobacco* (Bel W3)	cfa	6.19a	2.78b	9.70a	7.91ab
	50	2.13b	1.26b	7.22b	4.72c
	100	4.06a	1.38b	6.32b	3.42c
Romanie lettuce (Paris Island Cos)	cfa	12.48a	8.65b	7.66a	6.98a
	50	11.57a	7.11b	4.54b	3.94b
	100	11.74a	6.30c	7.11a	3.12b
Stem lettuce (Balady)	cfa	11.56a	11.05a	8.44a	6.88b
	50	10.57a	7.40b	5.58bc	3.75d
	100	10.35a	9.85a	6.48b	5.25c
Common bean (Giza 3)	cfa	8.20a	4.66bc	3.88a	3.71a
	50	4.59c	2.97d	2.29b	2.20b
	100	5.37bc	2.90d	2.86ab	2.21b
Common bean (Contender)	cfa	5.27a	3.57b	21.08a	21.75a
	50	4.86a	3.64b	19.42b	18.56b
	100	5.57a	3.61b	22.28a	14.14c
Broad bean (Romy)	cfa	14.82a	8.89c	9.72a	8.64a
	50	13.28a	7.96c	5.74b	2.35c
	100	12.02b	7.21c	4.74bc	3.25bc
Cotton(Giza 12)	cfa	27.23a	15.70b	4.69a	4.35a
	50	28.02a	15.87b	3.55a	2.71a
	100	27.69a	17.41b	3.09a	3.14a
Cotton (Giza 70)	cfa	17.24a	13.32b	3.04b	2.97b
	50	18.08a	13.89b	5.60b	3.28b
	100	18.73a	14.47b	3.51b	2.44b

Results are averaged from two different experiments with 6 plants replicates for each treatment.

@ Samples were collected 18 hr after the end of the fumigation period on the first and fifth day.

*Tobacco cultivars were exposed to ozone and sampled 32 and 53 days from seed.

& Means followed by different letters are significantly different at the 5% level within the same plant species and the same age.

species lost more chlorophyll 'a' due to 5 days of O_3 fumigation at the rate of 50 ppb than at the rate of 100 ppb. The cotton cv. "Giza 70" and the bean cv. "Contender" were the only exceptions.

Statistical analysis of the data presented in Table (4) showed a significant effect (1% level) of O_3 level, plant age as well as sampling date on the level of chlorophyll 'a' both in the sensitive and in the tolerant plant species studied. The interactions between each two individual variables and all three variables combined were also significant at the 5% level in all but few cases.

Chlorophyll 'b' content was affected by O_3 fumigation in a trend similar to that followed by chlorophyll 'a' (Table 5). Plant species which were ranged sensitive, lost more chlorophyll 'b' than tolerant species. The extent of chlorophyll 'b' degradation was higher at the active vegetative stage (32 days) than at the early vegetative stage (18 days) in both the sensitive and the tolerant species. Higher O_3 levels (100 ppb) caused an increase in chlorophyll 'b' loss mainly in the sensitive species with the exception of Jew's mallow. On the contrary, tolerant species lost more chlorophyll 'b' in response to the lower O_3 concentrations (50 ppb) except for the bean cv. "Contender" and the cotton cv. "Giza 70".

The effects of O_3 level, plant age, and days of sampling on chlorophyll 'b' content were all statistically significant at the 1% level both in the sensitive and in the tolerant species. The tolerant tobacco cv. Bel B showed no significant effects with all the three variables tested. The interaction between O_3 level and plant age was also significant at the 5% or 1% level in most of the species under study. Total carotenoids levels are reported in Table (6). The data presented show that in sensitive and intermediate species, greater decrease in total carotenoids was observed when plants were fumigated at the age of 32 days from seed than at 18 days. Some tolerant species followed the same trend e.g. bean cv. "Contender" and "Giza 3" and the broad bean cv. "Romy". The degree of carotenoid loss did not always reflect the level of sensitivity to O_3. On the other hand, different O_3 levels had different effects on the carotenoid levels. The low O_3 exposure concentration (50 ppb) caused higher carotenoids loss from leaves of sensitive, intermediate and tolerant

Table (5): Effect of ozone fumigation on chlorophyll 'b' content of fully expanded leaves of different plant species differing in ozone sensitivity.

Plant species &Cultivar	Ozone Level (ppb)	Chlorophyll b (μg/mg dry weight)#			
		18 days@		32 days	
		d 1	d 5	d 1	d 5
Jew's mallow (Local)	cfa	2.35a	2.07a	15.94a	10.62b
	50	2.29a	2.02a	10.98b	7.32c
	100	2.31a	1.89a	12.48b	9.32d
Clover (Masry)	cfa	4.90a	2.94b	2.37a	1.56a
	50	4.16ab	2.21b	1.50a	0.83b
	100	3.84ab	1.74b	1.60a	0.50b
Garden rocket (Local)	cfa	4.47a	1.61c	1.61a	1.38a
	50	2.81b	1.26c	1.61a	0.71b
	100	3.03b	1.24c	1.64a	0.59b
Alfalfa (Local)	cfa	3.51a	2.44b	1.37a	1.49a
	50	2.72a	1.26c	0.90a	0.92a
	100	3.45a	2.09b	0.86a	0.58b
Tobacco* (Bel W3)	cfa	2.77a	1.13b	2.97a	2.59a
	50	0.92bc	0.53c	2.15a	1.49ab
	100	1.88b	0.72c	1.84ab	1.26b
Romaine lettuce (Paris Isl. Cos)	cfa	4.11a	3.87a	2.26a	2.18a
	50	3.86a	2.53b	1.29ab	1.49ab
	100	3.83a	2.15b	1.91a	1.06b
Stem lettuce (Balady)	cfa	4.62a	3.55ab	4.46a	2.32b
	50	4.00a	2.48b	2.56b	1.16c
	100	3.96a	2.30b	2.79b	1.65bc
Common bean(Giza 3)	cfa	4.09a	4.25a	1.45a	1.69a
	50	3.64b	1.36c	0.83b	0.84b
	100	3.25b	1.57c	1.08b	0.87b
Common bean (Contender)	cfa	4.29a	2.33b	8.58a	9.09a
	50	4.96a	3.33ab	9.93a	6.65b
	100	3.92a	2.03b	7.83b	4.07c
Broad bean (Romy)	cfa	5.83a	3.73b	4.14a	3.76a
	50	5.22a	3.34b	2.39b	1.36b
	100	5.33a	3.41b	1.99b	1.83b
Cotton (Giza 12)	cfa	16.17b	9.23d	1.51a	1.39a
	50	23.75a	13.57c	1.16a	0.86a
	100	24.75a	14.14c	0.98a	1.04a
Cotton (Giza 70)	cfa	10.44c	8.07d	0.92a	0.94a
	50	14.99b	11.58b	1.69a	0.97a
	100	19.53a	13.09b	1.11a	0.71a

\# Results are averaged from two different experiments with 6 plants replicates for each treatment.

@ Samples were collected 18 hr after the end of the fumigation period on the first and fifth day.

*Tobacco cultivars were exposed to ozone and sampled 32 and 53 days from seed

& Means followed by different letters are significantly different at the 5 % level within the same plant species and the same age.

Table (6) Effect of ozone fumigation on total carotenoids content of fully expanded leaves of different plant species differing in ozone sensitivity.

Plant Species & Cultivar	Ozone Level (ppb)	Total Carotenoids (µg/mg dry weight)#			
		18 days@		32 days@	
		d 1	d 5	d 1	d 5
Jew's mallow (Local)	cfa	1.97a	1.86a	2. 02a	1.34b
	50	1.74a	1.85a	1.86a	1.24b
	100	1.95a	1.55a	1.87a	1.25b
Clover (Masry)	Cfa	1.67a	1.77a	1.00a	1.05a
	50	1.57a	1.82a	1.05a	0.41b
	100	1.02b	1.28ab	0.81ab	0.46b
Garden rocket (Local)	cfa	2.06a	1.70b	1.34a	0.95ab
	50	1.62b	0.93c	1.15a	0.66b
	100	1.64b	0.97c	1.12a	0.79b
Alfalfa (Local)	cfa	1.78a	1.44a	0.99a	0.70a
	50	0.68b	0.73b	0.72a	0.55b
	100	0.44b	0.72b	0.68a	0.39b
Tobacco (Bel W3)*	cfa	2.04a	0.99bc	1.45a	1.57a
	50	0.94bc	0.55c	0.53b	0.87b
	100	1.44b	0.76c	0.90b	0.66b
Romanie lettuce (Paris Isl. Cos)	cfa	1.49b	2.31a	1.85a	1.46a
	50	0.56c	1.88ab	1.15b	0.42c
	100	0.41c	1.86ab	1.87a	0.76bc
Stem lettuce (Balady)	cfa	2.76a	1.43bc	2.57a	1.24b
	50	2.00a	0.93c	1.14b	0.74c
	100	2.21ab	1.08c	1.08b	0.88bc
Common bean (Giza 3)	cfa	2.20a	1.58b	1.16a	1.21a
	50	1.68b	0.96c	0.69b	0.69b
	100	1.84b	1.02c	0.89b	0.69b
Common bean (Contender)	cfa	1.89a	1.00c	1.32a	0.93b
	50	1.72a	1.46b	1.20a	1.02b
	100	1.76a	1.26bc	1.24a	0.88b
Broad bean (Romy)	cfa	1.78a	2.20a	2.70a	1.63b
	50	1.92a	1.89a	1.31b	0.44c
	100	2.19a	2.01a	1.17b	0.76c
Cotton (Giza 12)	cfa	4.23a	2.42b	1.30a	1.15a
	50	1.77bc	1.01c	1.06a	0.90a
	100	1.62c	0.93c	0.93a	0.95a
Cotton (Giza 70)	cfa	4.27a	3.30b	0.96a	0.98a
	50	2.73b	2.11c	1.39a	0.96a
	100	1.17d	0.90d	1.02a	0.81a

Results are averaged from two different experiments with 6 plants replicates for each treatment.

@ Samples were collected 18 hr after the end of the fumigation period on the first and fifth day.

*Tobacco cultivars were exposed to ozone and sampled 32 and 53 days from

& Means followed by different letters are significantly different at the 5 % level within the same plant species and the same age.

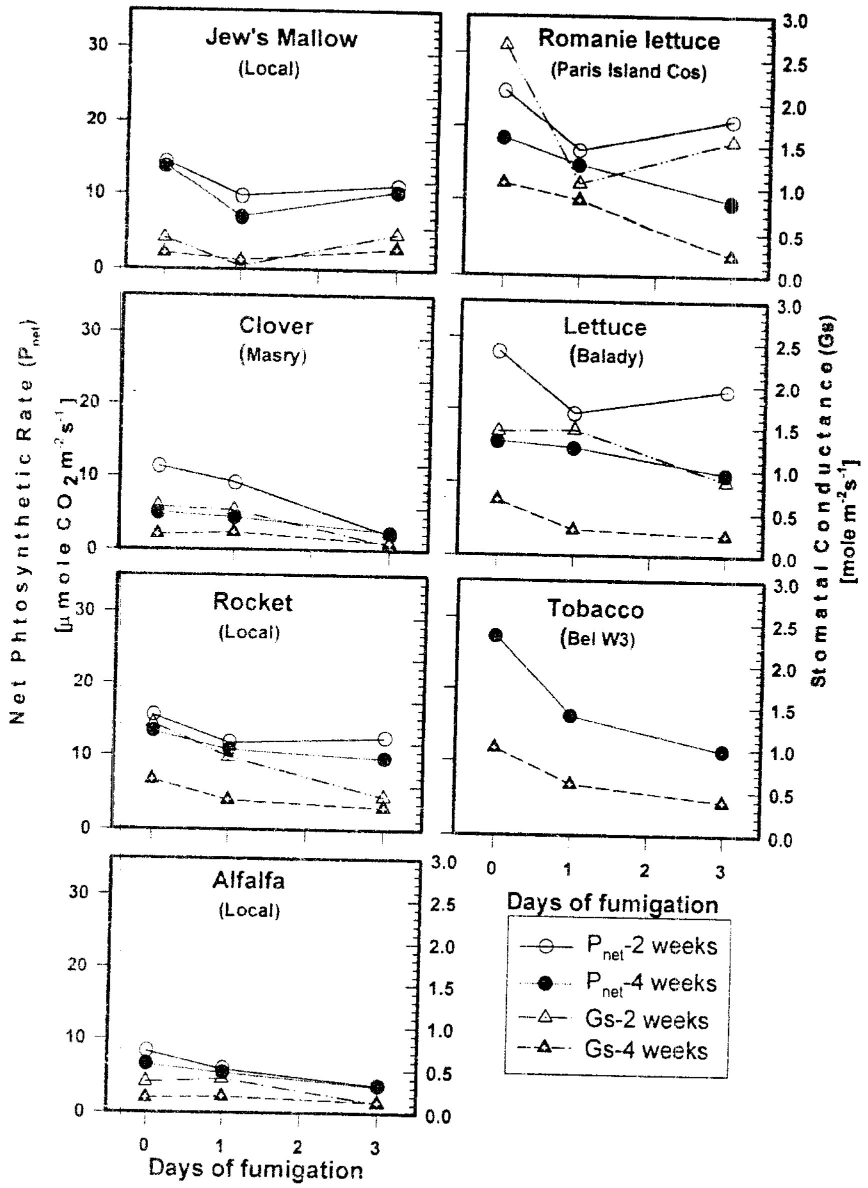


Figure (1). Time course changes in the net photosynthetic rate and stomatal conductance upon exposure of O_3-sensitive species to ozone. [Plants were exposed to 100 ppb O_3 for three consecutive days (6 hr per day). Photosynthesis and gas exchange measurements were taken before ozone exposure began and during last hour of the fumigation session on the first and third days of exposure].

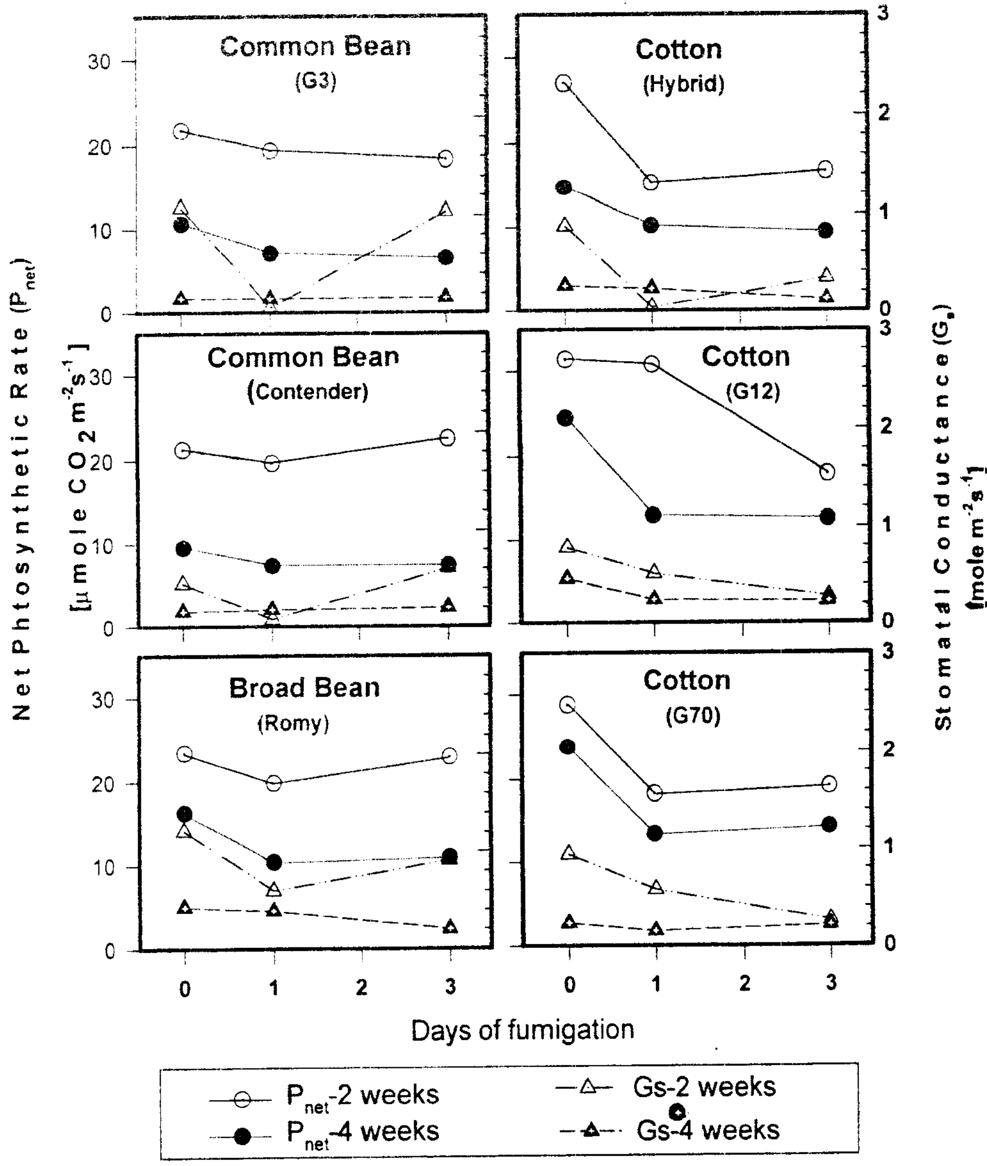


Figure (2): Time course changes in the net photosynthetic rate and stomatal conductance upon exposure of O_3-tolerant species to ozone. [Plants were exposed to 100 ppb O_3 for three consecutive days (6 hr per day). Photosynthesis and gas exchange measurements were taken before ozone exposure began and during last hour of the fumigation session on the first and third days of exposure].

species than the high O_3 concentrations (100 ppb). Alfalfa and tobacco cv. Bel W3 were the only sensitive species to differ from that trend. All the three variables (O_3 level, plant age and sampling day) when tested individually had significant effects on the carotenoids concentration at the 1% level. Few of the interactions between the individual variables had significant effects.

Net Photosynthesis and Stomatal Conductance:

The rate of CO_2 assimilation (P_{net}) and stomatal conductance (G_s) were followed in all species under study during a three day fumigation period at the rate of 100 ppb O_3, 6 h per day. The results are presented in Figures (1 & 2). Ozone fumigation caused a significant ($P<0.01$) and gradual reduction in the rate of CO_2 assimilation in the five O_3-sensitive species, Jew's mallow, clover, rocket, alfalfa and tobacco Bel W3, which ranged in magnitude from 20 to 80% decrease. This was accompanied by a parallel decrease in stomatal conductance (30-85%) which indicated significant ($P<0.01$) closure of stomata in response to O_3 fumigation in those species.

There was no appreciable difference in the response of plants treated at the early vegetative stage (18 days) and plants treated at the active vegetative stage (32 days) with respect to the decrease in P_{net} or G_s. Older plants had initially lower P_{net} rates and stomatal openings which decreased in response to O_3 treatment with a range comparable to younger plants. The two intermediate lettuce cultivars showed a similar range of parallel decline (significant at $P<0.01$) in both P_{net} and G_s only with plants which received O_3 at the age of 32 days, when both cultivars were more susceptible to ozone treatment. Sharper reductions in P_{net} (47%) and G_s (77%) were observed with Paris Island Cos which was reported earlier to be more sensitive to O_3 than Balady (28% and 63%, respectively) at this stage of growth. When lettuce plants were fumigated at the early vegetative stage a different trend of response was observed. A marked decrease in net photosynthesis (32 and 31%) was the result of the first O_3 exposure episode, but when exposure to O_3 was repeated for two more days, plants seemed to recover and the rate of P_{net} increased (loss 16% and 19%) but did not reach the initial values. Stomatal conductance closely paralleled the P_{net} decrease and increase only in "Paris Island Cos" but not in "Balady".

The three bean cultivars, which belonged to two different species of beans, were found to be tolerant to O_3 and showed similar responses during the three days of fumigation. Younger plants (18 days) were more resistant to decrease in photosynthetic CO_2 assimilation than older plants (32 days). P_{net} was depressed by approximately 11%, 8% and 15% in "Giza 3", "Contender" and "Romy", respectively, after the first day of O_3 exposure in 18 days plants. Plants recovered afterwards to levels very close or even greater than the initial P_{net} values. The same trend was found with G_s which clearly points to a close correlation between the decrease in stomatal opening, the reduction in CO_2 flux and the decline in net photosynthesis rates. However, older plants showed more P_{net} decrease when fumigated with O_3 than younger plants. Reduction in P_{net} was significant ($P<0.01$) and ranged from 22% for "Contender" to 39% for "Giza 3" and 33% for "Romy".

The depression in P_{net} was not accompanied with a matching decline in stomatal conductance in those cultivars. Only in the broad bean cultivar "Romy" a decrease in G_s (53%) was observed at the end of three days fumigation. On the contrary, both the common bean cultivars showed an increase in G_s i.e. an opening in stomates in response to O_3.

Cotton cultivars showed significant ($P<0.01$) and gradual reductions in both P_{net} and Gs values during the three days of O_3 exposure. No difference in the magnitude of response to O_3 was observed between plants of the two age groups tested. The decrease in CO_2 assimilation capacity seemed to be due to an increase in stomatal resistance (decrease in G_s) and a consequent restriction of CO_2 influx into the leaf.

DISCUSSION

The present study represent the first attempt at screening and testing Egyptian plant species in order to select candidates to be used as bioindicators for O_3 pollution in Egypt. Four candidates were found to exhibit hypersensitive reactions faster and at lower O_3 concentrations than the universally used O_3-bioindicator tobacco Bel-W3 under Egyptian environmental conditions. These four species, ranked in decreasing order of sensitivity, are: Jew's mallow (local), white clover (Masry), garden rocket (local) and alfalfa (local).

The four selected species exhibited clear-cut and repeatable injury symptoms when exposed to O_3 concentrations similar to those found in urban and rural areas in Egypt. Therefore, they obey the first criteria of a good indicator plant according to Manning and Feder (1980). They can thus, act as chemical sensors which detect the presence of ozone in the air. Posthumus (1982), suggested five guidelines for the selection of an indicator species all of which are met by those candidates selected during the study: (1) the species should be sensitive to the pollutant at a level below the sensitivity of vegetation of economic importance, (2) the specific pollutant induced markings should be characteristic and easily observed; (3) the species should grow from a terminal shoot; (4) the species should be widely spread (grow in a host of environments); and (5) the species should be present throughout the growing season.

The fact that these species are sensitive to O_3 at an early stage of growth (18 day from seed) is considered an added benefit since they are easily grown from seed and easily replaced by new plants due to the short time required to grow them to their sensitive age. In addition, they do not have any special cultural requirements that would complicate their fast propagation.

Therefore, we recommend those four species for further field testing to ascertain their worth under natural conditions in order for them to be designated as O_3 bioindicators in regional or national air biomonitoring programs in Egypt.

In an attempt to find reliable tools for diagnosis which can predict O_3 damage prior to the appearance of visual foliar injury, pigments degradation was followed through five days of fumigation with O_3 at the rate of 50 and 100 ppb (6 h per day). Ozone treatment induced premature senescence manifested as significant decrease in chlorophylls 'a' and 'b' and carotenoids which did not correlate very well with the appearance of visible symptoms. Jew's mallow, the most O_3-sensitive species, showed signs of necrosis and/or chlorosis shortly after only one O_3 exposure episode (100 ppb for 6 h) and exhibited the most extensive leaf injury symptoms after two fumigation sessions (75%). However, this same species showed mild pigments loss (16% only) when compared to less visibly damaged species like white clover, alfalfa or tobacco Bel W3. Furthermore, the loss of chlorophylls 'a' and 'b' did not reflect the extent of symptoms severity. On the contrary, in sensitive species, older plants (32 days) which exhibited no or few symptoms showed more chlorophylls 'a' and 'b' degradation than younger plants (18 days) exhibiting severe symptoms. This discrepancy could be explained if we trace the early events leading to lesion formation. It is an acceptable fact that O_3, once in the leaf interior, would rapidly react with components of the cell wall and apoplastic fluid and form oxygen free radicals such as the superoxide anions and H_2O_2 and others (Heath, 1994). O_3-derived oxyradicals may be scavenged by low molecular weight antioxidants in the apoplast, such as ascorbic acid and polyamines (Langbartels, *et al.*, 1991). Concentrations of activated oxygen species that exceed the antioxidative capacity of the apoplast may attack plasma membranes and cause peroxidation reactions which trigger the signal chain of events leading to wound reactions such as stress ethylene formation and lesion development. The loss of the plasma membrane semipermeability as a result of lipid peroxidation can occur remarkably fast at high O_3 doses or very low free radicals scavenging ability. In this case, rapid membrane injury may lead to such rapid protoplast collapse that unregulated proteolysis, due to tonoplast rupture, could lead to rapid unregulated cell death (Pell *et al.*, 1997). It is our belief that the discrepancy between the severity of foliar injury and degradation of chlorophylls 'a' and 'b' in sensitive plants could be ascribed to very weak antioxidative systems the capacity of which is exceeded by the amount of free radicals generated by O_3 uptake. It follows that the attack on plasmalemma from active oxygen radicals is more pronounced and cell damage spreads faster in the form of necrosis rather than chlorosis. On the other hand, with smaller levels of free radicals (due to less acute O_3 doses or to a more active antioxidant system) necrotic responses are often absent. Instead foliage exhibits signs of accelerated senescence in the form of pigment loss. In these cases, cell death is minimal due to the ability of the plasma membrane to sustain the stress caused by lower concentration of active oxygen species (Pell *et al.*, 1997).

The above discussion may also explain our observation that the lower O_3 level (50 ppb) caused more chlorophylls 'a' and 'b' and carotenoids degradation than (100 ppb), especially in tolerant plant species which did not exhibit foliar symptoms in response to O_3 exposure. This could also be ascribed to effective antioxidant systems in these species, which enable the scavenging of free oxyradicals in the apoplast, thus decreasing their concentration to a level which does not destroy the plasmalemma. A low level of oxyradicals, whether due to the exposure to chronic levels of O_3 or to efficient antioxidation activity in the plant, are more likely to diffuse within the cell reaching the thylakoid membranes and attacking them. Peroxidation of the thylakoids results in the loosening of those membranes and the gradual loss of pigment molecules.

Furthermore, oxygen free radicals are implicated in inducing the production of compounds such as ethylene which act as signals or elicitors driving the nucleus towards accelerated cell senescence manifested as pigment loss, reduction in photosynthetic CO_2 assimilation and decrease in protein and RNA levels (Pell *et al.*, 1997). Effects at the nucleus site also include the induction of de novo synthesis of enzymes of the cell defense mechanism such as superoxide dismutase and glutathione reductase (Madkour, 1998).

The net photosynthetic CO_2 assimilation was another parameter studied in the course of this study. Plant leaves posses other mechanisms to protect against ozone-induced foliar damage besides their antioxidative system. Ozone enters the leaf through the open stomata, thus a major control point is via stomotal closure or decrease in stomotal conductance. The reduction in CO_2 assimilation (P_{net}) in all the sensitive species studied was accompanied by closely related levels of stomatal closure (G_S). Therefore making it safe to conclude that their sensitivity to ozone was not caused by lack of stomatal response. The closure of stomates as a defense mechanism is aimed at controlling O_3 diffusion into the stomatal chamber in order to decrease O_3 concentrations within the leaf mesophyll. Nevertheless, it is apparent from the results of P_{net} and G_S that the restriction in stomatal conductance (G_S) and the subsequent decrease in CO_2 flux into the leaf was the main contributing factor to the reduction in P_{net} observed in those species. The sensitivity of these species is possibly caused by the absence or the ineffectiveness of an antioxidative defense mechanism. These results were in agreement with those reported on bean species (Guzy & Heath, 1993) and on spinach (Robinson & Rowland, 1996).

Intermediate and tolerant species exhibited lower reductions in P_{net} than those recorded with sensitive species. The changes in photoassimilation were always paralleled by similar changes in stomatal conductance (G_S). This lead us to conclude that stomatal closure plays an important role in conferring resistance in those plant species by as a stress avoidance mechanism (Lefohn, 1991). Nevertheless, the protective effect of the antioxidative system on plasma and other intracellular membranes could not be discounted (Heath, 1994). The involvement of the enzymes superoxide dismutase and glutathione reductase in the defense mechanism against ozone was investigated by the first author and reported elsewhere (Madkour, 1998).

Stomatal closure in response to O_3 was ascribed to a stimulation of the signal phyto- hormones ethylene (C_2H_4) and abscissic acid (ABA) both of which are known to drive leaf early senescence and were suggested to be involved in stomatal closure (not proven for C_2H_4) (Heath, 1994 and Lefohn, 1991). Also the action of stomatal opening and closure depends upon the interactive process of the relative water potential difference between the guard and subsidiary cells. Any loss of permeability induced by the effect of O_3 on the membranes in these cells would alter the ionic balance and cause either closure or opening of stomates (Heath, 1994).

The present study obtained data supporting the selection of the Egyptian cultivars of Jew's mallow (local), white clover (Masry), garden rocket (local), and alfalfa (local) as bioindicator candidates for O_3 in Egypt. These species are recommended for further field testing before their subsequent use in biomonitoring networks. It was concluded that although pigments degradation was not a good parameter for prediction of symptoms severity or species sensitivity (since O_3 induced pigment loss was observed in both sensitive and tolerant species alike), It could be used as a way to predict the extent of foliar early senescence before the appearance of any visual symptoms. This study found evidence supporting stomatal involvement in ozone tolerance in resistant species only and a good correlation was found between stomatal conductance, gas influx into the mesophyll and the rate of photosynthetic CO_2 assimilation.

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الملخص العربى

استخدام أنواع نباتية مصرية لأول مرة كنباتات دالة على وجود ملوث الهواء أوزون

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تعتبر النباتات أكثر الكائنات الحية حساسية كمستقبلات لملوثات الهواء . و تستجيب النباتات للتلوث بظهور أعراض مرضية محددة جدا و مميزة لكل ملوث و هذه الصفة يمكن استغلالها بأن تختار نباتات ذات حساسية واضحة لملوث معين كنباتات دالة على هذا الملوث. أى كوسيلة للاستدلال على وجود هذا الملوث فى الهواء و للتعرف على درجة التلوث و كذلك كوسيلة لمراقبة ظهور الملوث فى منطقة معينة.

و إذا علمنا أن أجهزة مراقبة تلوث الهواء الاوتوماتيكية أجهزة غاية فــى الحساسية و تتعرض للأعطال بسهولة شديدة تحت ظروف الدراسة الحقلية ممــا يستوجب معه أن تجرى لها صيانة على فترات متقاربة، ذلــك بالإضافــة إلــى ارتفاع أسعارها ، فإنه يتضح لنا ما لهذه النباتات من أهمية كأدوات بديلة لمراقبـة تلوث الجو حيث

تتميز بانخفاض السعر و بأنها يسهل توافرها و إعادة زراعتها كلما احتاج الأمــر على مدار العام نظرا لاعتدال الظروف المناخية فى مصر.

هدف هذه الدراسة هو انتخاب وتوفير عدد من الأنــواع أو الأصنــاف النباتية المصرية الصالحة للزراعة تحت الظروف البيئية المصرية لاســتخدامها كنباتات دالة على التلوث بالأوزون فى برامج مراقبة تلــوث الــهواء بواســطة دارسى و مراقبى تلوث الهواء فى مصر . و لتحقيق هذا الهدف فإنه تم اختبــار أكثر من ثلاثين نوعا و صنفا نباتيا مصريا خلال الفــترة مــن ١٩٩٣ و حتــى ١٩٩٥بهدف اختيار انسبها كنباتات دالة على التلوث بالأوزون. أخضعت هــذه النباتات لاختبارات معملية مكثفة تم خلالها التحكم فى كل من الظروف الطبيعيــة (حرارة و رطوبة و طول الفترة الضوئيــة) بحيــث تمــاثل الظــروف البيئيــة المصرية و فى كمية و جرعة التعريض للأوزون بحيث تماثل التركيزات التــى تسود فى أجواء المدن والريف المصرى. اثبت البحث أن أربعة أجنــاس نباتيــة مصرية تتمتع بحساسية للأوزون تفوق حساسية الدخان صنف Bel W3 المســتخدم عالميا كدالة للأوزون فى برامج مراقبة تلوث الهواء. هذه الأجناس مرتبة ترتيبـا تنازليا من حيث شدة حساسيتها للأوزون هى: الملوخية- البرســيم المصــرى- الجرجير-البرسيم الحجازى. كانت استجابة هذه النباتــات و ظــهور أعــراض التسمم بالأوزون عليها أسرع و نتجت من تركيزات أقل انخفاضا من تلك التــى تسبب نفس الاستجابة فى نبات الدخان. يوصى البحث باعتبار هــذه الأجنــاس النباتية الأربعة نباتات دالة للأوزون فى برامج مراقبة تلوث الهواء فــى مصــر على أن يسبق ذلك تجربتها حقليا للتأكد من استجابتها تحت الظروف الطبيعية.

فى محاولة للتوصل إلى اختبار أو أكثر يمكنها التنبــؤ بتــأثيرات الأوزون قبــل ظهور الأعراض درست المؤشرات التالية: تحلل و تكسير الصبغــات النباتيــة، اتساع فتحة الثغر، و كفاءة التمثيل الحيوى لثانى أكسيد الكربون. انتهى البحــث إلى أن اختبار تحلل الصبغات النباتية لا يصلح كمؤشر علــى مــدى حساســية النباتات للأوزون و لكنه يعتبر مؤشر جيد للتنبؤ ببداية دخول النبات فــى حالــة

الشيخوخة المبكرة خاصة تلك التى لا تكون مصحوبة بظهور أعراض مرضية و فى حالة النباتات المقاومة للأوزون. أثبتت التجارب أن اتساع فتحة الثغر يمكن اعتباره من الأسباب المشجعة لصفة المقاومة للأوزون فقط فى حالة النباتات المقاومة. كما ثبت بالتجربة أن هناك ارتباط وثيق بين اتساع فتحة الثغر و تقييد حركة الغازات مثل الأوزون و ثانى أكسيد الكربون إلى النسيج الوسطى فى الورقة و كفاءة التمثيل الحيوى لثانى أكسيد الكربون. فقد وجد أن التغيرات الملاحظة فى كفاءة التمثيل الضوئى تعتمد اعتمادا واضحا على التغير فى مدى اتساع الثغر.