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The Growth and Development of *Vigna radiata* in Filtered and Unfiltered Open Top Chambers

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ABSTRACT

In this study, mungbean [*Vigna radiata* (L.) Wilczek] was exposed to charcoal filtered air (FA), unfiltered air (UFA) and ambient air (AA) treatments in open-top chambers (OTCs). The vegetative growth and development assessed periodically at three different growth stages spending a period of three months. The growth and development of bean showed distinct different responses to O_3 and NO_2 depending upon treatments (FA, UFA & AA). Early growth suppression and slow increments at reproductive stage were clearly evident in plants under ambient air conditions. An extended vegetative growth phase in FA treatment lead to delay in onset of appeared earlier in AA and UFA treatments by a difference of 7 and 5 days, respectively as compared to pollution free treatment (FA).

Key Words: *Vigna radiata*; Air pollutants; Open top chambers; Canopy development; Reproductive development

INTRODUCTION

With the continuous growth in population, urbanization, industrialization and transportation, the number and amount of chemicals that damage environment and other toxic substances entering the biosphere have increased steadily both in the developed and the developing countries particularly during the last two decades (Wahid, 2003). In research on the effects of air pollution on plants, it is important to investigate plant responses at pollutant concentrations, which are representative of ambient conditions. Ambient air pollution has been shown to reduce the growth and economic yield of a wide range of major agricultural crops in North America and Europe (Ashmore *et al*., 2004). Such effects are currently attributed largely to the secondary photo-oxidant ozone (O_3), which is widespread in many rural areas. The formation of Ozone is influenced by major emissions of its precursors, nitrogen oxides (NO) and hydrocarbons, of which the motor vehicle exhaust and home furnaces are the most important source (Chhatwal *et al*., 1989). However in some areas nitrogen dioxide (NO_2), sulphur dioxide (SO_2) and other air pollutants are also important in terms of crop yield (Meyer *et al*., 2000; Wahid *et al*., 2001; Calatayud *et al*., 2004; Ashmore, 2005).

A recent report on air pollution in twenty major cities, more commonly from developing countries, highlighted indications of rapidly deteriorating air quality, although in many time reliable monitoring data are limited (WHO/UNEP, 1992). The consequent air pollution problems are serious, due to the absence of the kind of controls imposed on emissions in the developed world. However, it is more difficult to investigate the long-term effects of ambient pollutant concentrations on crops growing in the field. Various experimental systems have been developed in an attempt to resolve this problem. For example, open-air exposure systems have been described, which remain in place throughout the growing season (Greenwood *et al.*, 1982; Mcleod *et al.*, 1985). Such systems enable the effects of pollutant concentrations above ambient to be investigated, but are unsuitable for sub-ambient to be investigations. Under conditions of low wind speed, air exclusion system has successfully been used (Olszyk *et al*., 1986). The open top chambers (OTCs) used in this study were based on the Bell and Ashmore design (1986), which has been used extensively in air-filtration studies at a number of locations in Southeast England over the last sixteen years. These chambers are particularly suitable for use in the developing world like Pakistan.

This study aimed to assess the effects of ambient air pollutant on the growth and development of bean (*Vigna radiata* L. Wilczek) at a location on the outskirts of Lahore, Botanical Garden, University of the Punjab, Lahore, Pakistan.

MATERIALS AND METHODS

The open top chambers used in this study were constructed at Botanical Garden, University of the Punjab, Quaid-e-Azam Campus, Lahore, Pakistan according to the design of Bell and Ashmore (1986), which has been wildly used in air filtration studies in England and many other European countries, may also suitable for developing countries like Pakistan. The open top chambers were 1.5 m in diameter and height, consisting of an aluminum framework with transparent polyethylene walls, with air supplied at three changes per minute via vertical manifolds. Four chambers were ventilated with ambient unfiltered air (UFA), while the other four were ventilated with air that had been passed through an activated charcoal filter (FA). Both chambers were equipped with prefilters to remove dust. Four open-plots (AA) without any chamber were also used to grow plants in ambient conditions. Small doorways in each chamber for watering and growth assessment purposes were present. The fans and filters were sheltered in order to minimize rise in temperature of the air entering the chambers. Microclimatic measurements i.e., light intensity, air temperature and relative humidity were carried out inside and outside the chambers with the help of portable light and temperature humidity probe at 0800, 1200 and 1600 h daily in the center of open-top chamber and in ambient field plot at crop height. Air pollutants were measured in charcoal filtered air chambers and unfiltered air chambers. Ozone concentrations were determined at crop height using neutral buffered potassium iodide method (Saltzman & Gilbert, 1959) on every alternate day (three times in a week) between 1000-1600 h. NO_2 concentrations were determined by using diffusion tubes method (Atkins *et al.*, 1978) on weekly basis.

The clay pots of 36 cm diameter were used in this study. The sieve garden soil and sand were mixed in the ratio of 3:1 before filling in the pots. After preparation of pots, they were arranged in rows and were labeled for different treatments. Certified seeds of (*Vigna radiata* L.) (varieties NM-92 & NM-51) were obtained from Mutation Breeding Division, NIAB, Faisalabad. Healthy surface sterilized seeds of (*Vigna radiata* L.) were selected and soaked in sterilized distilled water for 12 h to initiate better germination and for early seedlings development. After complete germination, number of seedlings per pot was reduced to four carefully by thinning to ensure that these plants were of equal size and vigor. Then all the pots were shifted in open-top chambers and in ambient field plots for their respective air treatments.

The data for growth and development recorded at different growth stages and analyzed by analysis of variance (ANOVA). Duncan's New Multiple Range test at 5% level was used to test the significance of treatment mean (Steel & Torrie, 1960).

RESULTS

Microclimate and pollutant concentrations. Microclimatic conditions within open-top chambers (OTCs) were significantly different from those prevailing in the ambient environment (Table I). The average ambient air temperature during the season was 1.11^0C higher inside the OTCs, while relative humidity value was 1.19% higher inside the chambers as compared to the outside atmosphere. On the contrary light level was reduced by 1.35% inside the OTCs slight increase in temperature inside the chambers was possibly due to the heating effect of air blowers and may not be related to low light level inside the chambers.

The level of pollutant gases (NO_2 & O_3) during the whole growing season remained much higher in unfiltered air as compared to the Charcoal filtered air (Table II). Average NO_2 concentration during the season was 26.91 nL L^{-1} in UFA, while 11.73 nL L^{-1} in FA. The seasonal mean ozone (O_3) concentration was 61.60 nL L^{-1} in UFA and 8.30 nL L^{-1} in FA open-top chambers.

Vegetative development. The plants in the OTCs grew taller than those in the ambient air treatments. Data recorded clearly show that shoot growth was significantly enhanced in pollution free environment (FA) right from the seedlings phase of growth and this initial advantage over ambient (AA) and unfiltered (UFA) treatments was maintained throughout the growth period. Early growth depressions and slower further increments were clearly evident in plants under ambient air conditions. Chamber grown *Vigna radiata* plants had higher fresh weight of vegetative parts than comparable plants grown in UFA and AA sub-plots were observed from the early growth phases. Increase in dry weight yield of shoot in AA in the intermediate growth weeks apparently was due to early onset of reproduction phase in this treatment as by the final harvest stage their dry shoot weights reduced by 37.06% as compared to FA treatment. The top growth differences in AA and UFA were non-significant ($P > 0.05$).

There were no significant differences in the number of leaves and branches, which formed on UFA and AA, treated plants. However, individual leaves on charcoal filtered chamber grown plants progressively earlier than comparable leaves on AA plants. The average number of branches was also high in FA as compared to UFA and AA plants (Table III). Certain other aspects of vegetative development were also altered when *Vigna radiata* were grown inside OTCs. For example; FA plants developed larger leaf area and dark green colour as compared to UFA and AA plants. The leaves of chamber-grown plants were thinner than those of plant grown in AA sub-plots. The effect of various air pollutants on plants showing chlorosis or leaves abscission flecks on upper surface of leave (due to O_3) and leave bleaching (due to NO_2) were much more seen in AA plotted plants (data not shown).

Reproductive development. The reproductive phase of development commenced earlier in AA plants (40 d.a.e.) than chamber grown both treatments (43 d.a.e. in UFA & 49 d.a.e. in FA plants). An extended vegetative growth phase in FA treatment lead to delay in onset of appeared earlier in AA and UFA treatments, respectively. During initial phase (flowering), plants growing in FA developed more flowers than those growing in UFA and AA. However, no significant differences between UFA and AA plants have been seen (Fig. 1). The distribution of flowers and pods on the plant was monitored throughout reproductive development. Generally, a higher number of pods formed on FA compared to UFA and AA plants (Table IV).

Estimation of yield components such as number of pod per plant, number of seed per pod, seed yield per plant and 100 seeds weight were significantly high in FA plants than UFA and AA plants (Table IV). Similarly, size of pod

Table I. Mean monthly microclimatic variation inside and outside the Open-top Chambers

Month	Air temperature (°C)			Relative humidity (%)			Light intensity (Lux x 1000)		
	Inside	**Outside**	**Difference**	**Inside**	**Outside**	**Difference**	**Inside**	**Outside**	**Difference**
March	24.13	23.00	+1.12	48.16	46.87	+1.29	31.54	32.36	-1.32
April	30.50	29.39	+1.11	38.49	37.26	+1.23	39.46	40.77	-1.31
May	37.38	36.26	+1.12	32.62	31.42	+1.19	41.46	42.75	-1.29
June	40.04	38.95	+1.09	21.06	20.01	+1.05	43.11	44.39	-1.28

Table II. Monthly mean ambient air pollutant concentrations (nL L^{-1}) in the Open- top Chambers (OTCs)

Pollutants	OTCs	Monthly mean				Seasonal mean
		March	**April**	**May**	**June**	
	FA	8.12	8.12	8.22	8.66	8.30
O_3	UFA	52.26	62.30	64.17	64.22	61.60
	AA	52.29	62.33	64.20	64.24	
	FA	12.24	12.91	11.56	10.21	11.73
NO_2	UFA	29.40	28.98	26.20	23.07	26.91
	AA	29.43	28.99	26.22	23.09	

OTCs: Open top chambers FA: Filtered Air UFA: Unfiltered Air AA: Ambient Air

Table II. Vegetative growth responses under various air treatments

Genotype	Air treatment	Canopy height (cm)			Shoot dry weight (g)			Number per plant								
								Green leaf			Abscised leaf			Branches		
		8W	**10W**	**13W**	**8W**	**10W**	**13W**	**8W**	**10W**	**13W**	**8W**	**10W**	**13W**	**8W**	**10W**	**13W**
	FA	11.8a	14.6c	19.1a	0.76a	1.09c	2.56a	17a	23a	14a	1b	1c	2c	8a	10a	10a
NM-92	UFA	10.0b	15.0b	16.5b	0.29b	1.15b	1.93b	11b	15b	10b	1b	2b	3b	5b	7b	7b
	AA	9.9b	15.5a	16.2b	0.27b	1.28a	1.89c	10b	14b	8c	2a	3a	4a	5b	6c	6c
	LSD	0.23	0.01	0.39	0.12	0.04	0.03	1.61	1.73	1.41	0.01	0.02	0.11	0.59	0.81	0.81
	FA	11.2a	14.1b	18.4a	0.65a	0.99c	2.09a	16a	21a	13a	1b	1b	2c	7a	9a	9a
NM-51	UFA	9.7b	14.8a	15.8b	0.49b	1.06b	1.81b	10b	14b	8b	1b	2a	3b	5b	6b	6b
	AA	9.5b	15.0a	15.7b	0.47b	1.11a	1.78c	9b	13b	7c	2a	2a	4a	4c	6b	6c
	LSD	0.37	0.24	0.27	0.06	0.03	0.01	1.09	1.27	0.81	0.01	0.03	0.11	0.04	0.01	0.01

Treatment means followed by different letters in the same column are significantly different from one another according to Duncan's multiple range test at P=0.05. LSD: Least significant differences

Table IV. Changes in some yield characteristics of *Vigna radiata* exposed to different air treatments at maturity

Genotype	Air treatment	Pod plant^{-1}	Pod size plant^{-1} (cm)	No. of seed plant^{-1}	Seed yield plant^{-1} (mg)	100 seed wt. (mg)
	FA	19a	6.4a	8.90a	16892a	11175a
NM-92	UFA	14b	4.8b	6.69b	8835b	9998b
	AA	13b	4.5b	6.33b	7875c	9785c
	FA	17a	5.8a	7.94a	12765a	10545a
NM-51	UFA	12b	4.6b	5.66b	6135b	9102b
	AA	11b	4.3b	5.30b	5389c	8854c

Treatment means followed by different letters in the same column are significantly different from one another according to Duncan's multiple range test at P=0.05

formed on each plant was significantly smaller in UFA and AA plants. However, seed ripening occurred about a week later in filtered air (FA) treatment.

DISCUSSION

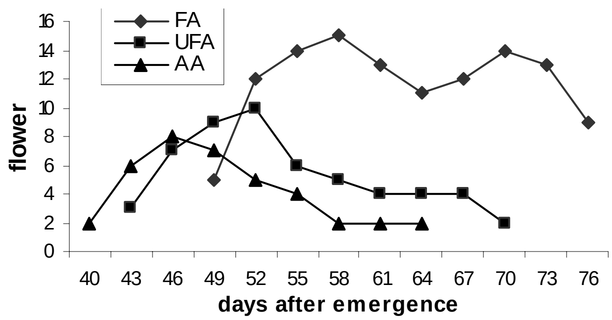
The quantification of the effect of the chambers on *Vigna radiata* growth and development formed an integral part of the air pollution experiments at Lahore, Pakistan. Parameters such as temperature, light intensity and relative humidity were routinely monitored in ambient air, unfiltered and filtered air chambers (OTCs). The reported differences in these microclimatic conditions will be discussed in relation to the observed changes in the growth and development of *Vigna radiata*.

Many open-top chambers studies at concentrations, which were elevated above ambient air, have shown significant effects of pollutants on both Physiological development and yield (Mulchi *et al*., 1988; Miller *et al*., 1988). In present study, significant pollutant effects were detected at high concentrations. e.g., seasonal average of O_3 conc: 8.30 nL L^{-1} for FA and 61.60 nL L^{-1} for UFA, while NO_2 11.73 nL L^{-1}, 26.91 nL L^{-1} for FA and UFA chambers, respectively.

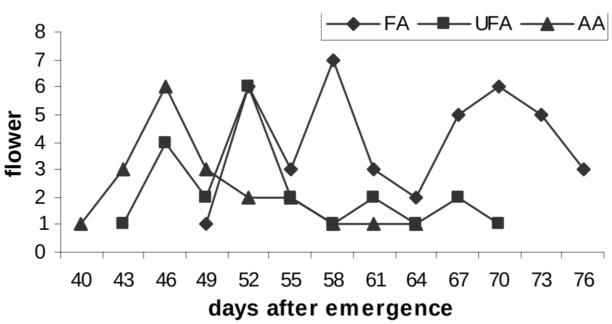
The intermittent different harvests indicated that ambient level of air pollutants influenced growth and development during the period of rapid shoot elongation and leaf emergence. For example; between 28 and 42 d.a.e., the net assimilation rate was 15% lower for UFA plants than for FA plants, indicating a change in the efficiency of usage of

Fig. 1. Effect of ambient air pollution on flowers with the passage of time in filtered air chambers (FA), unfiltered air chambers (UAF) and Ambient air pilot (AA)

(a) Flower/plant



(b) Abscised flower/plant



photosynthate in these plants. The relative growth rates of branches leaves were also lower for UFA than for FA plants during this period (Table III). Other studies have also reported effects of pollutants on these parameters (Miller, 1988).

The increased leaf size of FA plants was confirmed by incident radiation intercepted by the crop. Plants canopy grow higher in FA than plants growing in UFA. Similar increases in canopy for *Glycine max* growing in FA resulted from increased vegetative growth, increased growth efficiency and delay senescence (Unsworth *et al*., 1984). Although it was not possible to calculate the efficiency of conversion of intercepted microclimate and pollutant (O_3 & NO_2) into dry matter, it is likely that ambient levels of air pollution altered these parameters.

Use of charcoal filters increased size of the leaves associated with reproductive structures in *Vigna radiata* (Ahmed, 1995). This most probably resulted in an increase in assimilate translocation to developing more flowers and pods on FA plants compared to those on UFA plants. It is possible that a reduction in assimilate supply in UFA plants was sufficient to cause a reduction in either the initiation or subsequent development of flowers in this treatment. There was, however no evidence of a delay in the onset of flowering in UFA plants as has been described in other studies, where plants were exposed to low concentration of pollutants (Whitmore & Mansfield, 1983; Ashmore *et al*., 1988). The difference in flower number between the two treatments was carried through to early pod development. Thus, at this stage, the canopy of FA plants was able to supply sufficient assimilate to maintain more pods than of UFA plants. This probably reflected the location of the additional pods on the FA plants, since pods development on the upper racemes of *Vigna radiata* are least likely to survive up to maturity due to an inability to complete effectively for assimilates (Peat, 1983).

Many studies have indicated that concentration of air pollutants cause changes in the partitioning of dry matter within plants (Cooley & Manning, 1987; Mooney & Winner, 1988). When the shoots were separated into the leaves, stems and reproductive structures, it was found that changing the pollutant regime and had no significant effect on the allocation of dry matter between these organs. There were nevertheless indirect evidence of a shift in the distribution of the dry matter within both the reproductive structures and the leaves of the plants. For example, the upper leaves of FA plants were larger than those of UFA plants and during pod development there were more pods on the upper racemes of FA plants. Thus it is possible that air pollutants caused a change in the efficiency of mobilization of reserves in these organs.

The physiological basis of the differences in the growth of *Vigna radiata*, were observed during the season requires further study. The evidence suggests that the high concentration of air pollutants may have altered the photosynthetic efficiency of the canopy during the period of rapid shoot elongation and leaf expansion (growth phases). A reduction in efficiency would have subsequently reduced photo-assimilates available for pod retention and swelling and may explain the lower pod numbers on UFA plants.

Results from the current study confirm the view of Heagle *et al.* (1988) that there is need to perform experiments specifically designed to determine whether the chamber itself influences the crop response to pollutants. This would necessitate detailed investigations of the effect of small changes in microclimate parameters (e.g., 1.11^{o}C increase in temperature, 1.19% high relative humidity in OTCs) on pollutant uptake and would facilitate the development of a mathematical model, which could be used in the extrapolation of results from OTC experiments to the open-field environment.

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