Responses of radish (*Raphanus sativus* L.) plants at different growth phases to ozone exposure

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Received 29 October 2008; received in revised form 29 April 2009; accepted 12 June 2009.

Abstract

Three growth phases of radish (*Raphanus sativus* var. 'Pusa chetki') namely, early vegetative phase (EVP, 20 days); late vegetative phase (LVP, 35 days); and reproductive phase (RP, 50 days) were exposed to two ozone concentrations (75 and 150 ppb) in closed top dynamic chambers for 12 days. Ozone treated plants exhibited visible injuries (white spots mainly on the upper surface of leaves). Significant (p<0.0) reductions in shoot length (4 to 35 %), shoot biomass (4 to 62 %), total number of flowers (10 to 44 %), and hypocotyl biomass (40 to 80 %) were also observed. Ozone exposure was more detrimental during EVP compared to LVP and RP. Results of this study also showed that 150 ppb of ozone exerted more harmful effects at all growth phases than 75 ppb.

Keywords: Ground level ozone, Ozone injury symptoms, Membrane permeability

Introduction

Ground level ozone (O_3) is regarded as one of the most important phytotoxic pollutants (Ashmore and Bell, 1991). Its concentration in the atmosphere is rising at an annual rate of 0.5 % (Hertstein et al., 1995). High ambient O_3 levels adversely affect growth and yield of many crops (Varshney and Rout, 1998; Zhang et al., 1998). The magnitudes of such effects are dependent on the sensitivity of the crops to O_3, severity, frequency, duration of O_3 exposure (Zhang et al., 1998), and the stage of plant growth (Gelang et al., 2001). Although previous studies have shown that O_3 seriously affects the growth, physiological processes, biomass, and yield of radish plants (Pleijel et al., 1999), experimental studies on the effects of O_3 stress on different growth phases are lacking. A study was, therefore, undertaken to investigate the effects of O_3 exposure on different growth phases of radish and to assess the magnitude of phytotoxicity of different ozone concentrations.

Materials and Methods

The experiment was conducted at the Ecological Garden of Jawaharlal Nehru University, New Delhi, India (76°50' to 77°23' E and 28°12' to 28°53'N) from September 2002 to January 2003. The ambient temperature during the experimental period ranged from 5 to 34.3°C (mean = 22°C). Ambient O_3 was monitored from September 2002 to January 2003 between 9:00 to 16:00 h using a Continuous Ozone Monitor (Model ML-9810b, Monitor labs, USA). Detection limit and precision of the continuous monitor was 1 ppb and 2 ppb respectively. Rainfall, irradiance, and wind speed during the experimental period varied from 0 to 1.3 $mm{\cdot}day^{-1}$, 8781.2 to 20124.7 $kJ{\cdot}m^{-2}$, and 0.5 to 1.1 $m{\cdot}s^{-1}$ respectively. The accumulated dose over a threshold of 40 ppb (AOT40) was calculated for the total crop growth period as the sum of the differences between hourly ozone concentration (in ppb) and the threshold of 40 ppb for each daylight (solar radiation greater than 50 Wm^{-2}) hour

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in which ozone concentration exceeded 40 ppb.

Radish (*Raphanus sativus* var. 'Pusa chetki') plants were raised from seeds (procured from National Seed Corporation, New Delhi) in earthen pots (size 23 × 23 cm, containing a uniform mixture of garden soil and vermiform compost in 1:1 ratio). Four radish seeds were sown in each pot and were thinned down to two plants per pot, 10 days after germination. The plants were watered twice a week throughout the experimental period (25 September 2002 to 31 January '03). No fertilizer or pesticide was applied.

For O_3 exposure, three closed top transparent fiber glass chambers (1 × 1 × 1m) were placed in a wire netted cage. Each chamber had an inlet at the base connected to an O_3 generator (BARC Model, Mumbai) and an outlet at the top of the chamber connected to air suction pump to serve as an exit port. A small electric fan of 25 x 22 cm was fixed inside the chamber to ensure uniform mixing of air. Three growth phases of radish plants namely, early vegetative phase (EVP; 20 day old plants), late vegetative phase (LVP; 35 day old plants), and reproductive phase (RP; 50 day old plants) were exposed to two O_3 concentrations (75 and 150 ppb) for 2 h per day for 12 days inside the chambers from 12:00 to 14:00 h. O_3 concentrations were standardized by regulating flow rate of the generator. The control plants were also kept inside the chamber for 2 h and treated with ambient air using a vacuum pump. O_3 injury was estimated as the total number of leaves exhibiting visual injury symptoms over > 10 % of their total leaf area. For each treatment, destructive sampling was done after 12 days of O_3 exposure and after seed formation (135 days after sowing; harvests I and II respectively). Membrane permeability (expressed as electrolyte leakage) was also recorded in exposed and control plants by the method of Evans and Ting (1973).

Pots with radish plants were arranged on the bench in three randomized complete blocks. Within each block, the growth phases represented the main plots and O_3 concentrations the subplots. Each growth phase x O_3 concentration treatment combination had ten pots (3 x 3 x 10 = 90 pots). All data for the both the harvests were subjected to univariate ANOVA test using SPSS software version 14.0.

Results and Discussion

Ambient ozone concentrations

Ambient O_3 concentrations of Delhi were high during October and November and low during December and January (Fig. 1). O_3 levels exceeded 40 ppb threshold (AOT40) from September to February, except January. Furthermore, in the month of November, O_3 level crossed 60 ppb levels. The high concentrations of O_3 during September to November could be attributed to high light intensity and long sunshine hours, when temperatures were above 16°C. Low concentrations were during December and January (winter), attributed to the low light intensity, low temperature, and frequent episodes of fog formation. Previous studies from Delhi

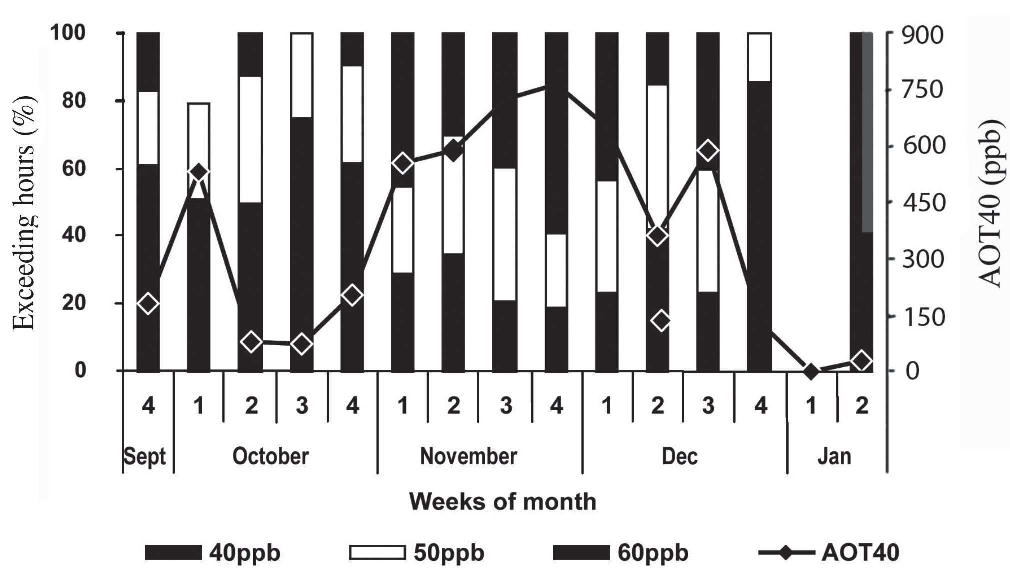

Figure 1. Ambient levels of ozone in Delhi during the experimental period (Sept. 2002 to Jan. 2003) exceeding AOT (accumulated over threshold) values.

have also reported similar monthly variations (Mittal et al., 2007).

Symptoms of ozone injury in radish

Visible O_3 injury symptoms initially appeared in the form of blisters mainly on the upper surfaces of the older leaves, which subsequently turned into white spots. At 75 ppb O_3 exposure 19, 23, and 13% lesions were noted on 20, 35, and 50 days old plants respectively. At 150 ppb, however, 20, 35, and 50 days-old radish plants developed 42, 36, and 7% lesions (Table 1). This is consistent with the trends reported by Kostka-Rick and Manning (1993). The lower percentage of injury (7%) in the 50 day old plants exposed to 150 ppb was mainly on account of senescence and abscission of leaves as well as availability of less number of leaves for manifestation of injury symptoms.

Growth of plants

O_3 treated plants exhibited significant reductions in shoot length and total number of leaves as compared to control plants during different growth phases (Table 1). As expected, higher dose (150 ppb) had a more pronounced negative effect on the growth parameters at all phases, compared to 75 ppb exposure. Highest reduction in shoot length (15 to 20%) at harvest I was observed for the 20 day old plants (EVP). However, at harvest II, EVP exhibited a relatively lower magnitude of reduction (4 to 13%). Growth phases x O_3 doses interaction was significant for shoot length at harvest II, but not earlier (Table 2). The total numbers of flowers and pods were also significantly lower in the O_3 treated plants compared to control plants at harvest II (Table 1). Twenty day old plants had lower magnitude of reductions in number of flowers (10 to 11%) as opposed to 50 day plants (25 to 44%; Table 1). Pod setting also was highest in EVP treatment followed by LVP and RP.

In general, EVP of radish was more sensitive to O_3 stress, compared to LVP and RP. For e.g, 50 to 62% reductions in shoot and 60 to 80% hypocotyl biomass were noted during EVP (harvest I). However, at harvest II, only 4 to 13% reduction in shoot and 40 to 46% reduction hypocotyl biomass were noted for the EVP (Table 1). Data in Table 1 also indicate that O_3 treated plants exhibited higher reduction in shoot biomass later in the life cycle. Although there may be some recovery in the growth of aboveground parts during all three growth phases, the negative effects on hypocotyl biomass persisted till seed set. This observation supports the hypothesis that O_3 exposure induces alterations in carbon allocation among shoot and hypocotyl at all growth phases. Probably more carbon is diverted for recovery of aboveground organs at the cost of hypocotyl.

Although greatest reductions in shoot and hypocotyl biomass were observed during EVP following O_3 treatments initially, they exhibited greater recovery at harvest II. Implicit in this is that O_3 exposure during LVP and RP may cause more yield losses compared to EVP, implying lower chances of recovery. Previous studies on beans and winter wheat corroborate this (Younglove et al. 1994). The significant reductions in shoot length, shoot biomass, total number of flowers, leaves, and pods observed in O_3 treated plants, persisted even after a gap of 70 days, implying a 'carryover phenomenon', whereby O_3 exposure influences growth responses during a subsequent period. Similar results were observed in sensitive and resistant white clover cultivars too (Fumagalli et al., 2003).

Membrane permeability

O_3 treated plants exhibited consistently high membrane permeability (18 to 50 %) compared to control plants (Table 1). O_3 being a powerful oxidant, may alter the structure and function of biological membranes, which explains the increased membrane leakage (Plazek et al., 2000). Changes in membrane permeability of exposed radish plants provide further support to the hypothesis that O_3 not only induces developmental acclimatization (biomass allocation) but also physiological acclimatization.

Results of this study show that O_3 exposure exerts detrimental effects on different growth phases of radish.

Table 1. Selected morphological characteristics of radish plants exposed to two levels of ozone during three growth phases of radish for 12 days.

Parameter	Growth phases	O_3 concentrations (ppb)		
		Control	75	150
12 days after O_3 exposure				
Shoot length (cm)	EVP	23.5±1.2	19.8±0.8	18.7±0.4
	LVP	28.2±1.4	25.0±1.7	24.2±1.6
	RP	32.6±3.3	29.8±0.8	28.9±1.7
Total number of leaves	EVP	9.6±0.5	8.4±0.2	8±0.3
	LVP	8.8±2.7	8.6±0.8	8.8±3.7
	RP	14±0.6	11.5±4.3	12.8±1.6
Injured Leaves	EVP	NA	1.6±0.5	3.4±0.3
	LVP	NA	2.0±0.8	3.2±0.5
	RP	NA	1.6±0.4	1.0±0.1
Shoot biomass (g)	EVP	0.8±0.07	0.4± 0.05	0.3±0.05
	LVP	2.1±0.3	1.2±0.5	0.7±0.02
	RP	4.2 ±1.3	2.6±0.2	2.4±0.03
Hypocotyl biomass (g)	EVP	0.5 ±0.05	0.2±0.03	0.1±0.02
	LVP	1 ±0.01	0.6±0.11	0.4±0.01
	RP	1.5±0.1	0.9±0.06	0.5±0.03
After seed formation (135 days after sowing)				
Shoot length (cm)	EVP	41.2 ±2.3	34.1±1.5	34±1.7
	LVP	46 ±2.8	31 ±1.9	30±0.8
	RP	40 ±1.5	31.6±1.3	35±1.4
Total number of leaves	EVP	21.6 ±2.5	16±0.7	19±0.8
	LVP	22.1 ±1	15±0.1	14.3±4.3
	RP	24 ±0.9	14±0.4	15±0.3
Shoot biomass (g)	EVP	19.3 ±1	18.5±1.2	16.8±1.7
	LVP	18.0 ± 0.9	14.7±2.1	12.7±0.4
	RP	18.2 ± 1.9	12.5±1.8	11.7±1.2
Hypocotyl biomass (g)	EVP	12.1 ± 0.2	7.2±0.3	6.5±0.4
	LVP	1 ±0.01	0.6±0.11	0.4±0.01
	RP	1.5±0.1	0.9±0.06	0.5±0.03
Total numbers of flowers	EVP	456±30.5	403±2.5	514±29.4
	LVP	186.5±19.1	124.5±9.7	140±12.3
	RP	382.5±21.8	166±7.5	189.3±12.4
Total numbers of pods	EVP	254±20.1	213±11.5	313±2.9
	LVP	138.5±94.0	82±9.5	93.4±12.2
	RP	200.5±22.3	134.5±10.5	167.5±14.2
Membrane permeability	EVP	0.40±0.03	0.47±0.02	0.50±0.09
	LVP	0.34±0.1	0.44±0.1	0.55±0.16
	RP	0.36±0.05	0.45±0.04	0.50±0.2

NA: not available. Means are followed by standard errors. EVP (early vegetative phase), LVP (late vegetative phase), and RP (reproductive phase) represent 20, 35, and 50 days old plants.

Table 2. F-ratio and significance level of ANOVA test for different parameters of radish.

Parameters	Growth stages	Treatments	Growth stages x treatments
Harvest I (12 days after O_3 exposure)			
Shoot length	94.2***	19.1***	0.132NS
Total leaves	116.9***	30.6***	2.1NS
Injured leaves	4.1**	3.3**	29.8***
Shoot biomass	155.9***	4.8**	39.3***
Hypocotyl biomass	172.7***	56.5***	5.2**
Total chlorophyll	64.7***	36.5***	5.7**
Carotenoids	408.0***	100.1***	7.7***
Harvest II (After seed formation, i.e., 135 days after sowing)			
Shoot length	21.5***	71.9***	16.4***
Total leaves	15.4***	24.4***	0.5NS
Shoot biomass	1.4NS	200.5***	0.8NS
Hypocotyl biomass	10.7***	688.2***	7.1***
Total flowers	14.8***	90.9***	8.5***
Total pods	5.0**	17.6***	1.5NS

Level of significance: ** = $p<0.01$; *** = $p< 0.001$; NS = not significant.

Early growth phases are particularly sensitive to O_3 exposure compared to the late phases. Nevertheless, better recovery from O_3 stress was observed at the early growth phases compared to late phases, implying that characterization of ontogenic shifts in O_3 sensitivity may help in the development of mitigation strategies. This study has also shown that 150 ppb O_3 is more detrimental to radish plants than 75 ppb O_3.

Acknowledgements

The fellowship provided by the University Grant Commission (UGC), New Delhi to Usha Mina during the period of research work is gratefully acknowledged.

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