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Effect of Ozone Stress on Different Growth Stages of Potato (*Solanum tuberosum*)

By

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With 2 Figures

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Summary

MINA U., KUMAR P. & VARSHNEY C. K. 2010. Effect of ozone stress on different growth stages of potato (*Solanum tuberosum*). – Phyton (Horn, Austria) 49 (2): 253–266, with 2 figures.

In the present study responses of different growth stages of potato to ozone exposure were investigated. Potato plants of vegetative (30 day old plants (DOPs)), reproductive (45 DOPs) and post reproductive (60 DOPs) growth stage were exposed to 75ppb and 150ppb ozone concentration for 12 days, 2 hr per day in Controlled Environment Dynamic Chambers. Ozone exposure induced appearance of leaf injury symptoms as well as reduced growth and yield of potato plants of all the three growth stages. Between plants of three growth stages ozone injury on leaves (from 75 ppb and 150 ppb ozone exposer) was maximum in 45 DOPs, followed by 60 DOPs and 30 DOPs. Among plants of all the three growth stages maximum reduction in growth and yield parameters was observed in plants exposed at reproductive (45 DOPs) growth stage. Reduction in growth parameters

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i.e., shoot length, total leaves, total leaflets and shoot biomass of exposed plants over unexposed plants of different growth stages were statistically non significant. Reduction in average number of tubers/plant 45 DOPs exposed to 75 ppb and 150 ppb ozone were reduced 54% and 69% respectively as compared to unexposed plants. Fresh weight of tubers/plant in 45 DOPs exposed to 75 ppb and 150 ppb was reduced by 48% and 68% respectively as compared to unexposed plants.

Zusammenfassung

MINA U., KUMAR P. & VARSHNEY C. K. 2010. Effect of ozone stress on different growth stages of potato (*Solanum tuberosum*). [Auswirkungen von Ozonstress auf verschiedene Wachstumsphasen der Kartoffel (*Solanum tuberosum*)]. – Phyton (Horn, Austria) 49 (2): 253–266, mit 2 Abbildungen.

In dieser Arbeit wurden die Reaktionen von *Solanum tuberosum* verschiedener Entwicklungsstadien auf Ozonexposition untersucht. Kartoffelpflanzen der vegetativen (30 Tage alte Pflanzen = TAP), reproduktiven (45 TAP) und post-reproduktiven (60 TAP) Phase wurden in Umwelt-Simulationskammern Ozongehalten von 75 ppb und 150 ppb täglich zwei Stunden lang für 12 Tage ausgesetzt. Ozon bewirkte Blattschäden, verminderten Wachstum und verminderten Ertrag bei allen Entwicklungsstadien. 45 TAP zeigten die intensivsten Schäden an den Blättern (sowohl bei 75, als auch bei 150 ppb Ozon-Exposition), gefolgt von den 60 TAP und 30 TAP. Die größte Verminderung des Wachstums und auch des Ertrages wurde bei Pflanzen der reproduktiven Phase (45 TAP) gefunden. Die Abnahme der Wachstumsparameter (Sprosslänge, Blattanzahl, Spross- und Biomasse) war bei exponierten und nicht exponierten Pflanzen aller Wachstumsphasen statistisch nicht signifikant. Die Verminderung der durchschnittlichen Knollenanzahl pro Pflanze 45 TAP, ausgesetzt 75 ppb und 150 ppb Ozon ist um 54% beziehungsweise 69% geringer als bei nicht exponierten Pflanzen. Das Frischgewicht der Knollen pro Pflanze 45 TAP (75 ppb und 150 ppb) ist gegenüber nicht exponierten Pflanzen um 48% und um 68% reduziert.

Introduction

Tropospheric ozone (O_3) is considered to be the most important phytotoxic air pollutant in many parts of Europe, Asia, North and Central America and the Far East (ASHMORE & BELL 1991, EMBERSON & al. 2001, KRUPA & al. 2000). Its concentrations in the troposphere of the countries of northern hemisphere have increased by 1–2% per year (CHAMEIDES & al. 1994). Nearly one-quarter of Earth's surface is currently at risk from tropospheric ozone in excess of 60ppb during mid-summer, with even greater concentrations occurring locally (FOWLER & al. 1999). Ozone is a phytotoxic air pollutant with potential to affect potato tuber yield (CRAIGON & al. 2002, VANDERMEIREN & al. 2005) and to cause early haulm senescence in potato crops (VANDERMEIREN & al. 2005). Most of the negative impacts of ozone on potato have been reported from United States and European nations. Studies on phytotoxic effect of ozone on potato crop from Asian re-

gion are almost lacking. Though elevated tropospheric ozone levels significantly affecting the growth and yield of crops in Baghdad (KANBOUR & al. 1987), Pakistan (WAHID & al. 2001), China (ZHENG & al. 1998) and India (VARSHNEY & ROUT 2003) of Asian continent as well. In India potato is grown in almost all the states, nearly 80% of the crop is grown in Indo Gangetic Plains comprising Punjab, Haryana, Uttar Pradesh, Bihar and West Bengal. Potato contributes about 1.23% to the total production from agriculture and allied activities in India (SHARMA 2002).

It has been also reported that the phytotoxicity of ozone varies greatly between species and cultivars (BLACK & al. 2000) as well as with the exposed growth stage of the plant (MEYER & al. 1997, GELANG & al. 2001). The magnitude of ozone effects on growth and yield of any crop related directly to the timing of exposure. In wheat, ozone sensitivity is higher between flowering and seed maturity than before anthesis (MEYER & al. 1997, GELANG & al. 2001). Similar growth stages dependent effects on yield and growth were also shown in bean (VANDERMEIREN & al. 1995), strawberry (DROGOUDI & ASHMORE 2002), tomato and alfalfa (YOUNGLOVE & al. 1994). However, studies with respect to response of different growth stages of potato to ozone exposure are also lacking. The objective of the present study was to investigate the response of different growth stages of Indian variety of potato and, effect of ozone exposure on growth, yield and carbon allocation of potato crop plants of different growth stage. This will help in identification of sensitive growth stage of potato as well as give us an idea of approximate losses in crop yield if exposed to ozone stress at detrimental growth stage.

Material and Methods

Experimental Site

Experiments in controlled environment dynamic chambers with pot grown potato plants were performed at the ecological garden of School of Environmental Sciences, Jawaharlal Nehru University. The experimental site is in the university campus surrounded with dense vegetation areas without major local air pollution sources.

Plant Material and Growth Conditions

Disease free- certified seed tubers (*Solanum tuberosum* var. Kufri Jyoti) were obtained from National seed corporation, IARI. Seed tubers were sown in earthen pots (size 23cm × 23cm, containing uniform mixture of garden soil and vermiform compost in the ratio of 3:1). The plants were watered as per requirements to maintain proper soil moisture.

Controlled Environment Dynamic Chambers and Ozone Exposure

Controlled environment dynamic chambers used were of 1m high, 1m long and 1m broad dimensions. Inside each chamber a small fan was attached for proper circulation of air. Ozone was generated from oxygen by electric discharge in ozone

generator (BARC Model). For monitoring ozone concentration of chambers, gas samples from the exit port of the chamber were analysed with the help of Continuous Ozone Monitor Model-ML9810B (Monitor Labs, USA). Ozone in ambient air was monitored by KIMOTO handy sampler (model HS-7) according to BYERS & SALTZMAN 1959 method.

Exposure Schedule

Potato plants were exposed at 30, 45 and 60 days old stage, representing vegetative stage, reproductive stage and post reproductive stage respectively. Potato plants at each growth stage were exposed to 12 fumigation cycles of 75ppb and 150ppb O_3 concentration for 12 days for 2h /day in controlled environment dynamic chambers.

Plant Analysis

Throughout the course of experiment plants were assessed for their growth and development. Non-destructive growth measurements included shoot length, total number of leaves and visual estimates of ozone damage were monitored regularly. The latter was assessed as the total number of leaves exhibiting ozone induced speckling > 10% of their total leaf area. Destructive harvest of unexposed and exposed plants was carried out twice for each treatment. First harvesting (harvest I) immediately following 12 days of ozone exposure of each growth stage in which half of unexposed and exposed plants were harvested and, final harvest (harvest II) at maturity of remaining half of unexposed and exposed plants of each growth stage. At harvest I unexposed and exposed plants of each growth stage were assessed for changes in morphological and biochemical parameters includes – shoot length, total number of leaves/leaflets, injured leaves/leaflets, shoot biomass, root biomass, total chlorophyll content, carotenoids content and membrane permeability. At harvest II unexposed and exposed plants of each growth stage were assessed for changes in growth and yield parameters with respect to each crop. Growth parameters were aboveground and belowground biomass. Yield parameters of potato assessed were: tubers/plant and fresh weight of tuber /plant.

Photosynthetic Pigments

Total chlorophyll and carotenoids content were estimated. 0.5 gm of fresh leaves were homogenised in 20 ml of 80% acetone (acetone: water v/v) in a pre-chilled mortar and pestle. The homogenate filtered through two layers of cheesecloth. The filtrate centrifuged at 3000g for 15 minutes in Janetzki refrigerated centrifuge (model K-24) at 24°C. The supernatant was decanted and the volume made up to 25 ml with 80% acetone. Precautions were taken to shield the chlorophyll extract from bright light. The optical density of the chlorophyll extract measured at 480, 645, 663 and 510 nm wavelength using Jasco spectrophotometer (model-7800UV/Vis). Total chlorophyll and carotenoid were determined by using the formulae described by MACLACHLAN & ZALIK 1963.

Membrane Permeability

At harvest I membrane permeability of unexposed and exposed potato plants leaves were measured by observing ion leakage in leaf discs via conductivity meter.

For ion leakage ten leaf discs cut to a size of 1cm in diameter were placed in a vial containing 25ml ultra pure water. Conductivity was measured after 2h and then vials were stored at –50°C overnight. Next day, after thawing vials were shaken for 2h and the conductivity was measured again. Measurements on frozen and thawed represent the conductivity of the total ion content in the tissue. Membrane permeability is expressed as a total ion leakage in terms of micro mho/ cm^2 (LANCE & TING 1973).

Experimental Design and Statistical Analysis

A factorial design representing three different growth stages of the potato crops and two ozone (O_3) concentrations (75 ppb and 150 ppb) in controlled environment dynamic chambers along with unexposed plants, randomized in three blocks was used. The seven treatments comprise unexposed plants, 1^{st} growth stage: 75 ppb O_3, 1^{st} growth stage: 150 ppb O_3, 2^{nd} growth stage: 75 ppb O_3, 2^{nd} growth stage: 150 ppb O_3, 3^{rd} growth stage: 75 ppb O_3 and 3^{rd} growth stage: 150 ppb O_3. For each treatment and in each chamber eight pots were kept. Pots with plants were arranged on bench in three randomized complete blocks. Within each block, growth stage represents the main plot and ozone concentrations (ambient; ambient + 75 ppb; ambient + 150 ppb) represent the subplot.

All untransformed data from both harvests were subjected to univariate ANOVA.

Results

Climatic Condition and Ozone Concentration

Generally the conditions were warm and sunny during the experiment period (October 2002-January 2003). During the experimental period surface ozone in ambient environment of Delhi on many occasions exceeded 40 ppb levels (prescribed threshold for crops) (Fig. 1). In February 2003, the average hourly concentration in ambient environment of experimental site (ecology garden of School of Environmental Sciences, Jawaharlal Nehru University) was 36.7 ppb. Usually at daytime ozone concentrations were typically high as compared to night time at experimental site. The daily maximum varied 27–33°C and the daily minimum temperature range is 9–20°C. Sky was clear on most of the days of experiment. The mean temperature during the experimental period was 20°C.

Ozone Injury

75 ppb and 150 ppb ozone exposure caused visible injury in exposed plants of all the three growth stages in the form of reddish brown lesions on the upper surface of leaflets which later turns into necrotic lesions (Fig. 2a, b). Initially, the medium and lower canopy of plant showed the most injury, although at the end it is appeared on most of the canopy. These lesions increase in size as the fumigation progresses. Folding of leaflets margins was also observed (Fig. 2c). The extent of injury was variable among growth stages. In 30 DOPs due to 75 ppb and 150 ppb ozone exposure leaflet injury were 33% and 49% respectively. In 45 DOPs it was

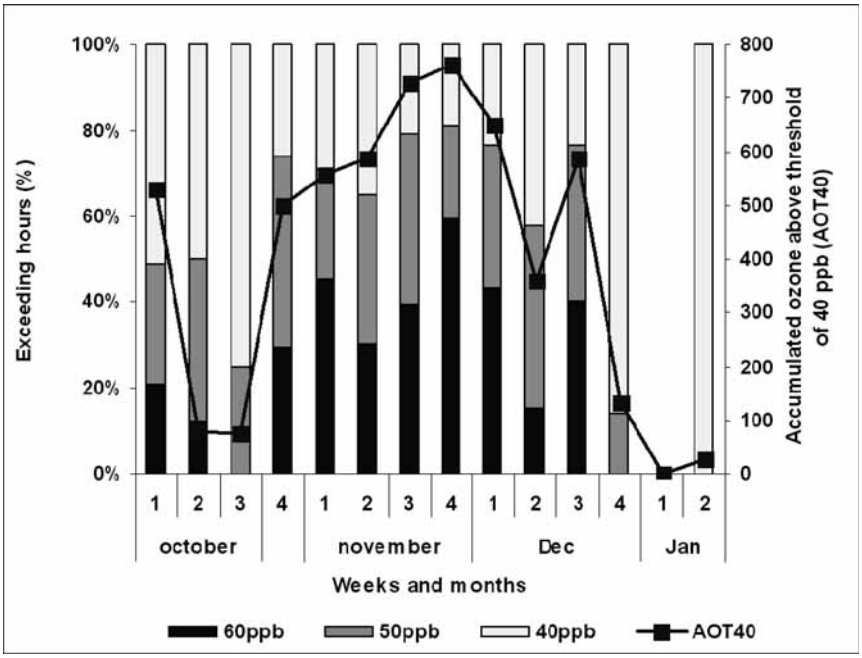


Fig. 1. Ozone in Delhi above plants threshold levels (Oct 2002–Jan 2003).

82% and 66% due to 75 ppb and 150 ppb ozone exposure respectively. In 60 DOPs it was 42% and 56% due to 75 ppb and 150 ppb ozone exposure respectively (Table 1).

Shoot Length

At harvest I in 30 DOPs of potato exposed to 75 ppb and 150 ppb ozone, shoot length was reduced by 27% and 30% respectively as compared to unexposed plants. Shoot length of 75 ppb and 150 ppb exposed 45 DOPs of potato were reduced by 38% and 52% respectively as compared to unexposed plants. Reductions in shoot length of unexposed and exposed 60 DOPs of potato due to 75 ppb and 150 ppb ozone were 8% and 14% respectively (Table 1).

Total Number of Leaves and Leaflets

At harvest 1, total number of leaves in 75 ppb and 150 ppb exposed 30 DOPs were reduced by 43% and 52% respectively as compared to unexposed plants. In exposed 45 DOPs, 75 ppb and 150 ppb ozone exposure reduced total number of leaves by 19% and 43% respectively as compared to unexposed plants. Total number of leaves in 75 ppb and 150 ppb exposed 60 DOPs were 22% and 27% less respectively compared to un-

Table 1. Growth response of three growth stages of potato to 12 days ozone exposure.

Date	G	pH	TA	$[CO_2]$	$[O_2]$	HAT	NH_4-N	PO_4-P	PAR
	$\mu S.cm^{-1}$		$meq.l^{-1}$	mM	$mg.l^{-1}$		$\mu g.l^{-1}$		%
U. stygia									
25 MAY	158 ± 7	6.26	0.85	1.08 ± 0.09	7.5 ± 0.5	9.2	0.0	12.2	--
	135-175	*6.18-6.40*		*0.82-1.33*	*6.0-8.6*				
2 JUN	192 ± 4	6.28	0.94	1.00 ± 0.10	6.0 ± 0.5	14.1	--	--	--
	177-197	*6.22-6.43*		*0.75-1.22*	*4.4-7.0*				
8 JUN	160 ± 7	6.12	0.82	1.08 ± 0.12	--	13.1	0.0	21.1	40.1 ± 4.5
	133-170	*6.16-6.41*		*0.77-1.38*					*30.3-56.7*
U. intermedia									
25 MAY	113 ± 2	6.12	0.62	1.18 ± 0.09	4.6 ± 0.4	29.4	9.3	18.3	--
	106-118	*6.03-6.23*		*0.91-1.44*	*3.5-6.1*				
2 JUN	184 ± 3	6.12	1.04	1.75 ± 0.10	1.3 ± 0.1	44.6	--	--	--
	174-192	*6.06-6.19*		*1.52-2.03*	*1.1-1.6*				
8 JUN	139 ± 5	6.03	0.81	1.74 ± 0.14	--	37.6	0.0	46.8	27.9 ± 2.1
	127-155	*5.98-6.17*		*1.33-2.03*					*21.6-33.8*

exposed plants (Table 1). Numbers of leaflet were also less in exposed potato plants as compared to unexposed plants at all growth stages. Reduction in number of leaflets in exposed potato plants was in the range of 12-47% as compared to respective unexposed plants (Table 1).

Photosynthetic Pigments

Reduction in total chlorophyll of 30, 45 and 60 DOPs exposed to 75 ppb ozone was 28%, 31% and 41% respectively, and due to 150 ppb ozone exposure it was 48%, 49% and 59% respectively as compared to unexposed plants. Carotenoid content of 30, 45 and 60 DOPs exposed to 75 ppb were reduced by 39%, 24% and 27% respectively, and due to 150 ppb ozone exposure the reduction were 44%, 29% and 55% respectively as compared to their respective unexposed plants (Table 3).

Membrane Permeability

At harvest I the membrane permeability of 30, 45 and 60 DOPs exposed to 75 ppb was 40%, 45% and 50% more respectively, and due to 150 ppb ozone it was 80%, 55% and 56% more respectively as compared to unexposed plants (Table 3).

Biomass

At harvest I reductions in shoot biomass of 30, 45 and 60 DOPs exposed to 75 ppb ozone were 33%, 14% and 17% respectively, and due to

Table 2. Growth and yield of exposed and unexposed potato plants of three growth stages at maturity.

Parameter	Growth stage (Day old plants)	Treatment		
		Unexposed	75 ppb	150 ppb
Tubers/plant	30	20.5 ± 3.7	20.3 ± 2.3	14.3 ± 2.5
	45	26.0 ± 1.8	12.0 ± 3.9	8.0 ± 1.3
	60	28.2 ± 2.5	17.3 ± 1.4	13.5 ± 1.1
Tuber fresh wt. /plant (g)	30	1463 ± 239	1105 ± 68	98.0 ± 97
	45	1668 ± 167	864 ± 329	527 ± 144
	60	1984 ± 22	1396 ± 337	1249 ± 102
Shoot biomass (g)	30	25.4 ± 5	20.8 ± 1.6	14.5 ± 2.4
	45	23.8 ± 7.6	11.3 ± 3.5	7.2 ± 1.6
	60	19.9 ± 1.9	17.3 ± 4.4	12.6 ± 2.1
Belowground biomass (g)	30	349 ± 63	247 ± 46	197 ± 20
	45	439 ± 63	225 ± 72	133 ± 65
	60	510 ± 83	326 ± 98	190 ± 75

150 ppb ozone were 50%, 25% and 26% respectively as compared to unexposed plants. Below ground biomass of 30, 45 and 60 DOPs exposed to 75 ppb ozone was reduced by 60%, 50% and 46% respectively, and due to 150 ppb ozone it was 62%, 68% and 68 % respectively as compared to unexposed plants (Table 1).

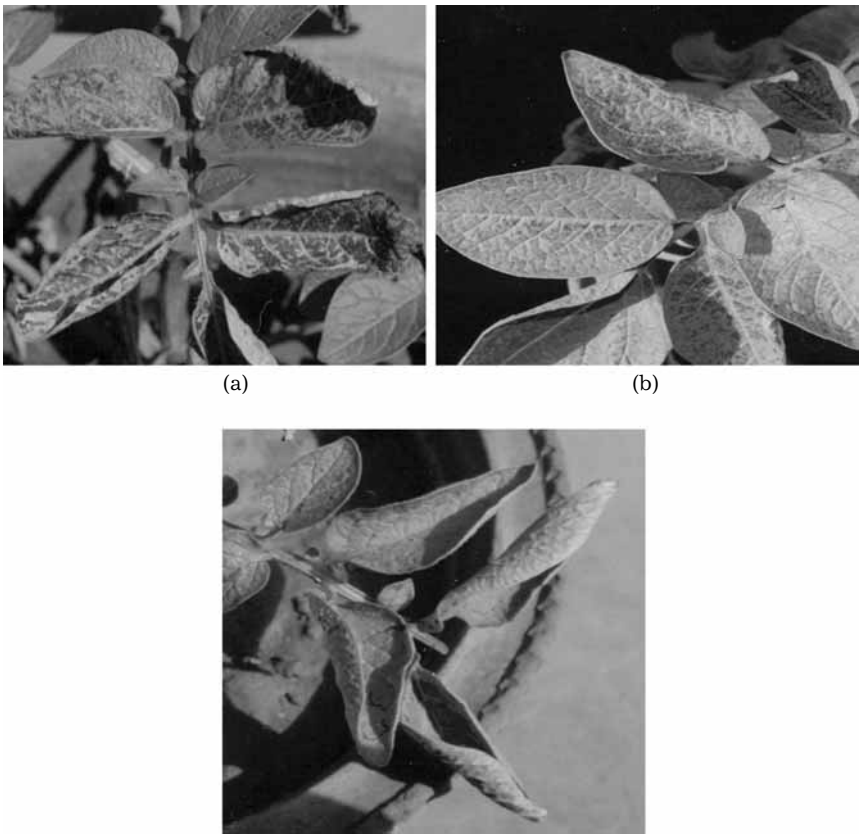
At harvest II reductions in shoot biomass of 30, 45 and 60 DOPs exposed to 75 ppb ozone were 18%, 53% and 13% respectively, and due to 150 ppb ozone were 42%, 69% and 38% respectively as compared to their unexposed plants (Table 2). Below ground biomass of 30, 45 and 60 DOPs exposed to 75 ppb ozone was reduced by 29%, 48% and 36% respectively, and due to 150 ppb ozone it was 44%, 69% and 36 % respectively as compared to unexposed plants.

Yield

Average number of tubers/plant in 30, 45 and 60 DOPs exposed to 75 ppb ozone were reduced by 0.1%, 54% and 39% respectively as compared to unexposed plants. In 150 ppb ozone exposed 30, 45 and 60 DOPs reductions in average number of tuber/plant were 30%, 69% and 52% respectively, as compared to unexposed plants. Fresh weight of tubers/plant in 30, 45 and 60 DOPs exposed to 75 ppb was reduced by 24%, 48% and 30% respectively as compared to unexposed plants, and due to 150 ppb it was reduced by 33%, 68% and 37% respectively as compared to unexposed plants (Table 2).

Discussion

In India potato crop is grown during the season of spring and autumn coinciding with high ozone levels in the ambient air (Mittal & al. 2007).



(a) (b)

(c)

Fig. 2. a + b: Ozone injury symptoms on potato leaves. c: Leaflets curling in potato due to ozone stress.

Ozone injury symptoms on potato crop in India were first time reported by BAMBAWALA 1986. Thereafter different studies reported ozone phytotoxicity on some of the Indian crops such as cereals (wheat and rice) pulses and horticultural crops (AGARWAL & RAO 1983, AGARWAL & al. 1987, KHAN & KHAN 1994, VARSHNEY & ROUT 1998, EMBERSON & al. 2001, ROUT 2003) but no attempt has been made to quantify impact of ozone exposure on potato crop. In the present study growth and yield performance of the potato plants exposed to two different levels of ozone at different growth stages were assessed. Results of the study showed that the growth performance of unexposed plants was better as compared to exposed plants at different growth stage.

During 12 day exposure, exposed plants of all the three growth stages exhibited visible ozone injury symptoms. The symptoms observed on po-

Table 3. Photosynthetic pigments and membrane permeability of potato plants of three growth stages after 12 days ozone exposure.

Parameter	Growth stage (Day old plants)	Treatment		
		Unexposed	75 ppb	150 ppb
Total chlorophyll (mg/g fresh leaf)	30	3.6 ± 1.2	2.6 ± 1.1	1.9 ± 0.9
	45	3.4 ± 1.2	2.0 ± 0.8	1.4 ± 0.5
	60	3.5 ± 1.1	2.4 ± 0.7	1.8 ± 1.0
Carotenoids (mg/g fresh leaf)	30	1.8 ± 1.1	1.3 ± 0.5	1.2 ± 0.6
	45	1.7 ± 0.9	1.0 ± 0.3	0.7 ± 0.1
	60	1.1± 0.5	0.8 ± 0.01	0.6 ± 0.03
Membrane permeability ($\mu mho/cm^2$)	30	0.05 ± 0.01	0.07 ± 0.01	0.09 ± 0.02
	45	0.31 ± 0.19	0.45 ± 0.27	0.48 ± 0.1
	60	0.19 ± 0.2	0.40 ± 0.1	0.46 ± 0.1

tato leaves were similar to those described in other controlled experiments with different potato cultivars exposed to ozone; cv Bintje (DeTemmerman & al. 2002); cv Superior (Heagle & al. 2003); cv Desiree and cv Nicola (Sanz & al. 2001). Onset of ozone injury symptoms was early in plants exposed to 150ppb ozone concentration. Between plants of three growth stages ozone injury (from 75 ppb and 150 ppb ozone) was maximum in leaves of 45 DOPs, followed by 60 DOPs leaves and 30 DOPs leaves. Calvo & al. 2009 also reported that younger potato plants took longer for visible symptoms to develop than for the oldest ones. This supports the hypothesis that growth stage of plant is playing important role in determining the onset of visible injury symptoms. Leaf injury in exposed plants of different growth stages was statistically significant ($P \leq 0.01$).

Appearance of ozone injury symptoms and senescence of potato leaves after exposure reduced green area available for photosynthesis. The reduction in photosynthetic pigments i.e., total chlorophyll and carotenoids in exposed growth stages of potato plants were 28–59%, and 23–55% respectively (Table 2). The decline in pigments content during ozone exposure in present study were similar to potato cv. Hela in which 6 week exposure of 65 ppb for 24 h/day reduced chlorophyll content by 25% (Kollner & Krause 2000). A reduced photosynthetic leaf area and decline in photosynthetic pigments content may induce steady decline in plant growth and vitality, by affecting exposed plants energy requirements for growth. Alteration in permeability of leaf cell membrane of exposed plant was observed as compared to unexposed plants. Membrane permeability in 30, 45 and 60 DOPs exposed to 75 ppb was 40%, 45% and 50% more respectively, whereas due to 150 ppb ozone it was 80%, 55% and 56% more respectively as compared to unexposed plants. Increase in permeability of exposed potato plants of three growth stages suggests that ozone has a direct effect on membrane function by altering permeability characteristics. Soldatini & al. 1998 also reported that ozone act as a powerful oxidant

modifying the structure and function of biological membranes, leading to membrane leakage.

Harvest I observations shows reduction in growth performance of 75 ppb and 150 ppb ozone exposed plants of different growth stages. Reductions in growth parameters were- shoot length (8–52%); total leaves (18–52%); aboveground biomass (13–50%), and belowground biomass (46–68%). Immediately following the 12 days ozone exposure reductions found to be more in belowground biomass as compared to aboveground biomass of plants. Reduction in growth parameters i.e., shoot length, total leaves, total leaflets and shoot biomass of exposed plants over unexposed plants of different growth stages were non significant.

Harvest II shows reduction in growth and yield parameters of 75 ppb and 150 ppb ozone exposed plants of three growth stages were- aboveground biomass 13–70%, belowground biomass 29–70% and tuber yield 25–68% (Table 2). Reductions in yield parameters (tubers/plant, tuber biomass and belowground biomass) of exposed plants of three-growth stage are statistically significant ($P \leq 0.001$) (Table 4). During post exposure period, belowground biomass in exposed potato plants has shown recovery, whereas aboveground biomass has not. This indicates apparent strategic adaptation in exposed potato plants i.e., most of the photosynthate appears to be diverted for belowground stem growth (tubers) not aboveground shoot. In present study reductions in number of tubers and tuber yield of plants of different growth stages as compared to unexposed plants were similar to KOLLNER & KRAUSE 2000 but in contrast of HARE & MOORE 1988 and CLARKE & al. 1990 observations. KOLLNER & KRAUSE 2000 observed reduction in tuber yields of Hela cv. of potato up to 25% from ozone exposure of 65 ppb for 6 weeks. Wherease HARE & MOORE 1988 and CLARKE & al. 1990 reported that ozone affects on potato yield were not observed until 75% foliage damage.

In terms of growth stages, the growth of 75 ppb and 150 ppb exposed 30 DOPs of potato (represents vegetative stage) was relatively better as compared to 45 (reproductive stage/tuber initiation stage) and 60 DOPs (post reproductive stage/ tuber bulking stage). Between 45 DOPs and 60 DOPs, growth and yield of exposed 60 DOPs were comparatively better. 45 DOPs of potato were in tuber initiation stage happens to be a critical stage, any type of stress at this stage will adversely affect crop yield. Leaflets injury and reduction in tuber yield in exposed plants of 45 day old stage clearly indicates sensitiveness of tuber initiation stage to ozone stress. Injured leaflets reduce the rate of photosynthesis, and reduced availability of photosynthate at tuber initiation stage will decrease number of tubers/plant and fresh wt. of tubers/plant. It has been also reported that ozone exposure in potato generally induces premature leaf senescence, so reducing green leaf area available for assimilate production and hence

decreases the number of tuber and tuber yield (FINNAN & al. 2002). In 60 DOPs tuber filling occurs rapidly and thus exhibited less impact on number of tubers/plant and total fresh wt. of tubers/plant. High demand of carbon for high yield may be affected by exposure to ozone stress at different growth stages.

Table 4. F-ratio and significance level of ANOVA test for potato.

Parameter	Growth stage	O_3	Growth stage x O_3
Harvest I			
Shoot length	13.2***	9.4***	1.8 ns
Total leaves	21.9***	9.2***	0.8 ns
Total leaflets	28.7***	8.3**	1.0 ns
Injured leaves	10.8***	32.1***	6.1***
Tubers/plant	23.5***	2.1***	5.6***
Tubers fresh wt. /plant	15.1***	6.4**	14.9*
Tubers biomass	13.1***	6.1**	6.3***
Aboveground biomass	11.4***	5.6**	0.1ns
Belowground biomass	14.1***	6.3**	14.6***
Total chlorophyll	8.7***	20.3***	0.12ns
Carotenoids	18.6***	18.9***	0.8 ns
Membrane permeability	13.4***	6.8***	5.1**
Harvest II			
Tubers/plant	5.2*	46.9***	5.6**
Tuber fresh wt./plant	20.9***	48.7***	2.8*
Aboveground biomass	7.1**	25.1***	2.1ns
Belowground biomass	5.3**	44.8***	2.1*

Level of significance: * = P<0.05; ** = P<0.01; *** = P< 0.001; ns = non significant

Results of the experiment are of practical consideration in view of global importance of potato crop. Potato is the fourth most important food crop on a global scale after wheat, rice and maize as well as for food security of world. Significant reduction in potato yield after ozone exposure at different growth stages in present study indicates that sensitivity and carryover effect of ozone stress in different growth stages of potato varies, but crop yield is highly sensitive to ozone stress. In future yield losses in sensitive potato crop cultivars grown in different agro climatic regions of India may observed on account of elevated surface ozone levels in atmosphere.

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