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Influence of ozone on the growth and yield of tomato (*Lycopersicon esculentum* Mill cv Rodeo)

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ABSTRACT

The influence of three ozone levels (control - 0 nl O_3 l^{-1}, 100 and 200 nl O_3 l^{-1}) were studied under controlled environmental conditions in sand culture and with five replicates. Elevated (100 and 200 nl O_3 l^{-1}) ozone levels produced well defined abnormal leaf characteristics such as wilting and drying, pink coloration, necrosis, pale green and chlorotic leaves. The leaf area and the dry matter production of plants were severely affected by both 100 and 200 nl O_3 l^{-1} levels. Ozone treatment also delayed phenological development of plants and the untreated plants (control) came into bearing 10 days earlier as compared with the 200 nl O_3 l^{-1} treated plants. Though the number of fruiting clusters were almost the same in all treatments, the untreated plants out yielded plants from the rest of the treatments in terms of total fruit number and fruit weight. Ozone treated plants also contained low levels of leaf chlorophyll indicating their rapid breakdown.

Key words: chlorophyll, dry matter, leaf abnormalities, ozone, phenology, tomato, yield

INTRODUCTION

Ozone (O_3) is a tri-atomic form of oxygen and is found largely in the stratosphere. In nature it is formed under the influence of ultra violet radiation of nitrogen oxides and carbon dioxide. These reactions take place in air and the end product is carried away by wind over long distances. Under wind still conditions high concentrations of O_3 can cause both physical and physiological damage to plants (Stadelman and Fuhrer 1986). It is a strong anti-oxidant with a standard redox potential of 2.08 volts and is presently considered as the most important phytotoxic "air polluter". Ozone is also the most reactive form of molecular oxygen and the fourth most powerful oxidizing agent known.

The harmful effects of O_3 were first observed by Richards *et al.* (1958) in the famous wine growing regions of California, where the damage were characterized by dark brown patches on the upper side of grape leaves (Vitis labruscae L.), and the symptom was described as "oxidant stipple". Maize, wheat, and sugar beets are some of the crops reported as O_3 sensitive species. Toxic effects on these plants were observed at O_3 concentrations as low as 40 parts per billion by volume (Fuhrer *et al.* 1989).

Ozone enters the plant through the stomata depending on diffusion gradients, and the flux depends on the concentration gradient between the leaf tissue and the leaf-air interface. The stomatal response of plants to increased O_3 levels varies mainly in two ways. In some plants the stomata close and prevent the diffusion of O_3 while others do not respond and the gas diffuses freely into leaf tissues (Butler and Tibbits 1976). The O_3 concentration in the inter-cellular spaces of leaves is almost zero, in plants grown in environments with elevated levels of O_3 (Laisk *et al.* 1989). This indicates that during its passage to inner tissues through the stomata or immediately after diffusion it is absorbed to the cell walls, derivatized or disintegrated. Because of its ionic resonance structure [$^{(-)}O\text{-}O^{(+)} = O$] its solubility in water is better than molecular oxygen. O_3 absorbed by cell wall, damages the cell wall integrity and is described as "ozonolysis" (Elstner 1984) and subsequently cause radical-induced lipid peroxidation (Halliwell and Gutteridge 1989; Orvar and Ellis 1997). The underlying physiological mechanism as to how O_3 affects the latter reaction is not clearly understood.

Organisms by nature are geared to protect the cell components from oxidative agents. The plant kingdom posses two interrelated mechanisms to

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protect themselves from toxic oxygen species. They are either (1) enzymatic processes e.g. super oxide dismutases (Salin 1988) or (2) antioxidants (ethylene-di-urea, -tocopherol and ascorbic acid) which inactivate these toxic oxygen species (Scarpa et al. 1985; Tappel 1968; Varshney and Rout 1998). O_3 induces histo-pathological damages in cell walls (Pell and Weissberger 1976) mainly through increasing the permeability of cell walls. These changes increases the passive entrance of both inorganic and organic solutes through the wall to the inner compartments (Heath 1988). Such changes also affect the trans-membrane transport systems (Castillo and Heath 1990). Loss in cell membrane qualities increases the loss of osmotically active substances and affects the cell wall potential (Heath 1988). With the loss of turgor in stomatal cells, the stomata are closed and as a consequence, the assimilate efficiency of plants is reduced (Hsiao and Lauchli 1986). Ozone also inhibits net photosynthetic rate (Matyssek *et al.*1991); carbohydrate and carbon metabolism (Robinson and Rowland 1996); changes the chlorophyll structure (Toyama *et al.* 1984), the disintegration of the chlorophyll (Ormrod et al. 1990) and increases the dark respiration (Wallin *et al.* 1990). The phytotoxic effects of O_3 on plants includes abnormal dark green leaves (Mudd and Freeman 1977), chlorosis, premature necrosis (Domergue and Louget 1980) and leaf fall (Keller 1986).

There is a dearth of knowledge on the harmful-effects of this vital substance on plant growth and development. Therefore, the present work was planned to study the effects of O_3 growth, phenology, yield parameters and chlorophyll content of tomato plants under controlled environmental conditions.

MATERIALS AND METHODS

Experimental design

The study was designed as a pot experiment using a complete randomized block design with three O_3 levels (0 nl O_3 l^{-1} - control, and treating plants with 100 nl O_3 l^{-1} and 200 nl O_3 l^{-1}), and each level replicated five times making a total of 15 pots.

Plant material and growth conditions

Week old tomato (Lycopersicon esculentum Mill. cv. Rodeo) seedlings were transplanted in plastic containers (2 plants pot^{-1}) containing sand. The containers were filled with a total of 10 kg of sand of three particle sizes. The lower 6-cm was filled with particles of 6-7 mm, the middle 12-cm 0.5-0.75 mm particles and the top 6-cm with particle sizes of 1.5-2.2 mm. Before planting, the sand was washed thoroughly to leach any detrimental substances, which might be inhibitory to normal plant growth. The transplanted seedlings were kept under glass house conditions for 4 days so as to relieve from the shocks following transplanting and the development of new roots. At the beginning of the experiment the pots were arranged on trolleys and taken to a controlled growth chamber (Type Uvikon), pre-programmed for long day conditions (16-hr light and 8-hr darkness). The temperature was maintained at 28°C during the light period and 24°C during the dark period. The light intensity of the chamber was about 700 mol m^{-2} s^{-1}. The relative humidity was kept at 80%. The plants were supplied with a Hoagland's nutrient solution, and the leachate was collected from below in plastic containers fixed to the bottom of each pot. The leachate was recycled every day. When all of the solution was used the plants were given tap water. In order to measure the soil moisture tension of the medium, a tensiometer was inserted into each pot, in the fine sand layer as close to the plant as possible. The plants were given nutrient solution or water when the moisture tension reached 40 kPa. Care was taken that all plants received one liter of the nutrient solution weekly.

Ozone treatment

The plants were exposed to O_3 inside an airtight Plexiglas cabin of 150 x 100 x 180 cm (L x B x H), stationed inside a separate environmental chamber with the same conditions as indicated above. An oxygen cylinder was first fixed to the oxygen inlet port of an ozone generator (Fischer model 500). Oxygen entering the generator moves along an electro-chemical path and is converted to O_3. Ozone thus produced was then passed through active carbon and finally into the cabin with pressurized normal air (24 m^3 $hour^{-1}$). Several test runs were done to ensure that the instruments were operating satisfactorily before the commencement of the experiment. Air samples were also taken from the cabin at random intervals at plant height and analyzed using an O_3 analyzer (Monitor Labs 8810). The deviations from the expected O_3 concentration were always less than 5%.

The plants were treated with O_3 two weeks after transplanting, over a period of 8 hours. Thereafter, the plants were transferred from the plexiglass cabin to the growth chamber.

Determination of chlorophyll

Leaf samples for chlorophyll determination were taken at 48 and 72 hours after treatment. The three most mature leaves were selected and sampled using a cork borer leaf discs (50 mm^2) were collected and their fresh weight was determined. The chlorophyll extraction was done as described by Moran and Porath (1980) where the leaf discs were immersed in 10 ml of N,N - Dimethyl formamide. The extraction took place in darkness at room temperature over a period of 24 hours. The extinction of the samples were determined with a spectrophotometer (Uvikon 819, Kontron Instruments), using wavelengths of 647 and 664.5 nm (maximum absorption of chlorophyll b and a respectively). The concentration of chlorophyll a and b were calculated as described by Inskeep and Bloom (1985) using the following extinction coefficients.

$C_{chl\,a} = 12.70\,E_{664.5} - 2.79\,E_{647}$; $C_{chl\,b} = -4.62\,E_{664.5} + 20.70\,E_{647}$ and

$C_{chl\,a+b} = 8.08\,E_{664.5} + 17.91\,E_{647}$

Growth and dry weight measurements

All plants were harvested on the same day. Plant height was recorded just before the harvests. The individual plant parts were separated. Leaf area of healthy green leaves was measured using a Licor Model 3100 area meter. The dry weights of individual plant parts (including fruits) were determined after drying to a constant weight at a temperature of 105°C in an oven.

Statistical analysis

Statistical analysis was done by SAS (Statistical Analysis System, SAS Institute, Carey, North Carolina, USA).

RESULTS AND DISCUSSION

Twelve hours after the treatments were imposed a very slight degree of wilting was observed as a result of O_3 treatment. The wilted appearance of mature leaves disappeared in 24 hours. Forty-eight hours thereafter both the young and under-developed leaves of plants treated with O_3 turned chlorotic. Some of the young, fully expanded leaves turned pink. It is thought that, the appearance of pink colour in leaves treated with O_3, is a symptom leading to premature senescence, and is mainly due to increased levels of anthocyanin production (Pauls and Thompson 1980; Toyama *et al.* 1985; Matyssek et al. 1991). The chlorotic appearance was most likely due to the breakdown or disintegration of leaf pigments above all the chlorophylls. Tenga and Ormrod (1990) also showed the diminished greenness of tomato leaves exposed to ozone as observed in the present study. However, the post exposure recovery of greeness as observed by Tenga and Ormrod (1990) was not established in the present study and the chlorotic nature of leaves remained, although, leaves appearing about 4 weeks after the treatments were imposed appeared normal and healthy.

Three to four days after treatment, some of the young fully expanded leaves developed numerous brown necrotic spots. Schraudner *et al.* (1998) in their experiments conducted with an ozone sensitive tobacco cultivar Bel W3 showed that, a 5h exposure of plants to 150 nl O_3 l^{-1} caused localized necrotic lesions. The leaf damages observed in tobacco cultivar Bel W3 were due to ozone derived reactive oxygen intermediates, mainly H_2O_2 and, to a lesser extent due to superoxide anion radicals.

In contrast, leaves close to the base of the treated plants appeared evenly necrotic and were shed within 2-3 days. Work conducted by Greitner and Winner (1988) with soybeans and Hasler *et al.* (1990) with different grape varieties also concluded that the susceptibility of older leaves, especially those close to senescence are more prone to ozone than mature functional leaves. The immature unopened leaves at the apical meristem wilted and dried off only at the highest O_3 level of 200 nl O_3 l^{-1}. Most of the latter abnormal leaves were shed within a week after treatment. As compared with the control plants, plants exposed to both ozone levels, produced smaller, pale green leaves and they were often shriveled. The above symptoms suggest that at a younger stage, the leaves are more susceptible to O_3 than mature functional leaves of tomato plants. The leaf abnormalities observed in plants exposed to O_3 were more at the higher ozone level of 200 nl O_3 l^{-1}.

Elevated O_3 concentrations reduced the leaf area by 18% and 27% for 100 and 200 nl O_3 l^{-1} levels respectively, as compared with the unexposed plants (Table 1). This indicates that O_3 caused leaf damage

Table 1. The effect of three O_3 levels on leaf area and total dry weight of tomato plants at the termination of the study (mean ± standard error).

O_3 level (nl O_3 l^{-1})	Leaf area (cm^2 $plant^{-1}$)	Plant dry weight(g)
Control	605.23^a ± 9.50	245.36^a ± 10.12
100	501.89^b ±12.22	205.01^b ± 11.20
200	445.76^c ±13.65	180.14^c ± 6.58

Values followed by the same letter within a column are not significant at P = 0.05, as determined by the Duncan's multiple range test.

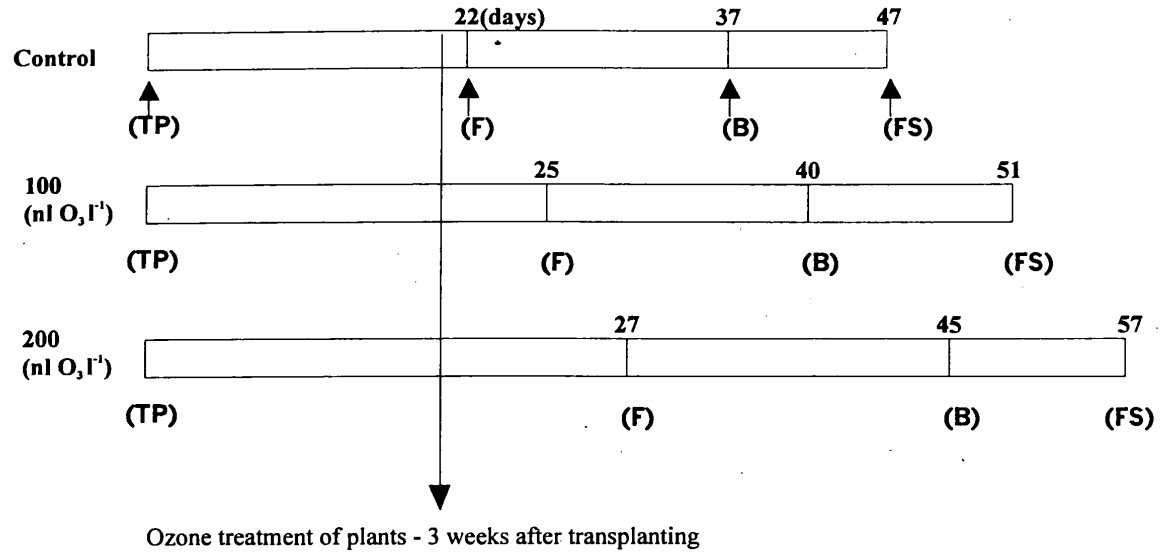

Fig. 1. The phenology of tomato plants as affected by three ozone levels. (TP- transplanting; F- flowering; B- blooming; FS- fruitset

as shown already through leaf fall and necrosis. The reduction in leaf area in O_3 treated plants would have been also due to reduced rate of leaf production and appearance. Work done in this vital area is obscure. The plant dry weight too showed a similar trend as the leaf area (Table 1). Since leaves play a major role in carbon assimilation, disturbances to leaf growth and their function have a direct effect on photosynthesis. This is reflected mainly by the dry matter production of the plant. Though the effects of elevated levels of O_3 on the rate of photosynthesis was not determined in this study, experiments conducted both under controlled environment chambers and under field conditions have proved the negative effects of O_3 on photosynthesis (Wallin *et al.*1990; Aben *et al.* 1990; Hill and Littlefield 1969).

During the reproductive period, a high number of flower abscission prior to blooming was observed in plants exposed to O_3 and the reduced number of fruits e.g. plants treated with 200 nl $O_3 l^{-1}$ had reduced fruit yield by 50% compared to control.

The time taken from transplanting to observation of flower buds, flower bud stage and blooming, as well as between blooming and fruit set are given in Fig. 1.

Controlled plants showed early flowering and fruit set as compared with plants exposed to O_3 (Fig. 1). This suggests that plants exposed to ozone shows delayed phenological development. The significance of this observation is that harvesting of O_3 treated tomato plants is possible at a later date.

The effect of treating plants with O_3 on selected yield parameters of tomatoes is shown in Table 2. Treating plants with O_3 did not effect the number of fruit clusters per plant, however, it reduced both the number of fruits as well as the fresh weight of fruits per plant. This could be due to several reasons. Exposure of plants to O_3 could effect the number of flowers per plant in two ways. Firstly, it is not known whether treating plants with O_3 reduces the number of flower primordia during the pre-flowering stage. This aspect needs further study. Secondly, O_3 treatment promoted pre-mature senescence of flowers. However, fruits harvested from the O_3 treated plants appeared normal and were same as those of the control. The reduced fruit weight in the treated plants is attributed to the disturbances caused

Table 2. The effect of three O_3 levels on selected yield parameters of tomato plants (mean ± standard error).

O_3 level (nl $O_3 l^{-1}$)	No. of fruiting clusters plant^{-1}	No. of fruits plant^{-1}	Total fresh weight of fruits plant^{-1}, g.
Control	5.2^a ± 0.20	24.8^a ± 0.37	1784.0^a ± 12.54
100	4.9^a ± 0.20	17.2^b ± 0.58	1633.8^b ± 9.48
200	5.0^a ± 0.24	12.8^c ± 0.37	1504.0^c ± 13.01
CV(%)	4.62	9.56	1.81

Values followed by the same letter within a column are not significant at P = 0.05, as determined by the Duncan s multiple range test.

Table 3. The effect of three ozone levels on the chlorophyll content, g m^{-2} of tomato plants (mean ± standard error).

O_3 level (nl $O_3 l^{-1}$)	Chlorophyll a	Chlorophyll b	Chlorophyll a:b
Control	0.492^a ± 0.012	0.240^a ± 0.014	2.07^a ± 0.094
100	0.325^b ± 0.006	0.170^b ± 0.004	1.91^a ± 0.038
200	0.215^c ± 0.010	0.130^c ± 0.010	1.64^b ± 0.082

Values followed by the same letter within a column are not significant at P=0.05,as determined by the Duncan's multiple range test.

during the vegetative growth stage especially to leaves and thereby the photosynthetic capacity of plants as a whole as has been already shown due to reduced leaf area and functional capacity of leaves. Leaf necrosis, chlorosis and eventually leaf shed would have played a major role in this respect.

The leaves of plants treated with O_3 contained reduced amounts of both chlorophyll (chl) a and b (Table 3). The decrease in chl a content was more than chl b. This reduction in chlorophyll content of plants, in response to O_3, is a common feature and is mostly due to the powerful oxidizing ability of ozone (Grandjean and Fuhrer 1989; Havranek *et al.* 1990; Reich *et al.* 1986, Tenga and Ormrod 1990). The latter workers are in the opinion that ozone could presumably stop new synthesis of chlorophyll in young leaves. This was established even in the present study, where new leaves appearing in the treated plants was pale looking as compared with the controlled plants. In contrast to work done with soybean (Brennan *et al.* 1987) and red spruce (Alscher *et al.* 1989) has shown that the chlorophyll content of ozone treated plants was same as the control. Studies conducted by Eamus *et al.* (1990) with Norwegian spruce showed an increase in chlorophyll content of O_3 treated plants. However the reasons, if any, for elevated chlorophyll levels in O_3 treated plants in the latter study is not clear.

CONCLUSIONS

On the basis of the data obtained in this study it is evident that vegetative characters, phenological development and tomato yield parameters are affected by ozone.

An ambient increase in ozone level in the plant's environment should have a significant bearing on the activity of some essential enzymes and the metabolism of some important organic substances. They might in fact act as "scavengers" and could mitigate the effects of ozone.

Further, it is of utmost importance to focus scientific studies in search of simple and inexpensive ozone mitigating strategies, which could counteract the ill effects of ozone.

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