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Todd, A. G.
Department of Horticultural Science University of Guelph

Ormrod, D. P.
Department of Horticultural Science University of Guelph

Hale, B. A.
Department of Horticultural Science University of Guelph

Goodyear, S. N.
Department of Horticultural Science University of Guelph

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TEMPERATURE EFFECTS ON TOMATO RESPONSE TO OZONE AT CONSTANT VAPOR PRESSURE DEFICIT

A. G. TODD, D. P. ORMROD, B. A. HALE and S. N. GOODYEAR

Department of Horticultural Science, University of Guelph
Guelph, Ontario N1G 2W1, Canada

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TODD A. G., ORMROD D. P., HALE B. A. and GOODYEAR S. N. *Temperature effects on tomato response to ozone at constant vapor pressure deficit.* BIOTRONICS **20**, 43–52, 1991. The increasing concentrations of "greenhouse" gases in the troposphere are of concern to environmentalists. One of these gases, ozone (O_3), is a serious agricultural phytotoxin. Increased temperatures resulting from elevated concentrations of the "greenhouse" gases could modify plant response to O_3. However, apparent temperature effects may be the result of vapor pressure deficit (*VPD*) differences. Young tomato plants (cv. New Yorker Special) were exposed to four maximum O_3 concentrations (0.04, 0.08, 0.12 and 0.16$\mu l\ l^{-1}$) delivered in an eight hour dynamic exposure profile at three temperatures (20, 25 and 30℃). Vapor pressure deficit effects were minimized by creating similar *VPD*'s at all temperatures. When this was done, modification of plant response to O_3 by temperature was found in only two of eleven variables examined, namely, stem fresh weight and specific leaf area. In these variables the plant response to O_3 at 25℃ was significantly greater than that at 20℃. In contrast, O_3 had significant effects regardless of temperature on all but one of the response variables. No temperature modification of response to O_3 was discovered in root fresh and dry weights, stem dry weight, leaf fresh and dry weights, leaf area, plant height, height : stem dry weight and root : shoot ratios. This suggests that in young tomato plants, over the range of temperatures studied, temperature has little effect on response to O_3. This reseach also demonstrated that future studies of the effect of temperature on plant response to air pollutants can and should be conducted with constant *VPD* regardless of temperature.

Key words : temperature ; ozone ; tomato ; vapor pressure deficit ; growth response

INTRODUCTION

Ozone (O_3) is a naturally occurring gas found in both the stratosphere and the troposphere (*16*). In the troposphere, anthropogenic sources of precursor compounds, including hydrocarbons and nitrogen oxides (NO_x), significantly increase the concentration of O_3 which may result in injury to plants. Recently, concern has developed over the increase in concentrations of the "greenhouse"

gases (carbon dioxide, methane, chlorofluorocarbons, nitrous oxide and O_3 in the troposphere. These gases may impede the reradiation of heat from the earth's surface, possibly increasing the temperature of the troposphere (*5*). It has been predicted that global temperatures could rise 1.5 to 4.5℃ in the 50 year period from 1986 to 2036 (*6, 15*) with much greater change in localized areas.

Several environmental factors, including temperature, have been investigated with respect to modifying plant response to O_3. Temperature has an influence on all physical and chemical aspects of physiological processes in plants including transpiration, nutrient uptake, photosynthesis and respiration. Experiments conducted to determine the effect of exposure temperature on O_3 response have found an inverse relationship between visual O_3 injury and temperature (*11*) with significantly more visual injury as temperature during exposure increases (*3, 4*) In all of these experiments percent relative humidity was used as the parameter to describe and standardize the water status of the air surrounding plants during exposure. This may not be the best parameter to use in studying the physiological response of plants to air pollutants since plants respond to the differential vapor pressure between saturated cell surfaces and the surrounding air. The vapor pressure deficit (*VPD*) describes the dryness of the air which determines evaporation rates and ultimately transpiration rates and stomatal opening. Percent relative humidity can be used as a measurement variable, but it must be recognized that with a constant *VPD* across temperatures, the percent relative humidities will differ (*18*). When previous experiments are examined with this in mind, it is apparent that temperature treatments were probably confounded with differential *VPD* levels so that, for the observed responses, temperature effects could not be separated from *VPD* effects.

The objective of the present study was to examine, in light of predicted greenhouse gas effects in increasing environmental temperatures, the question of whether there is a demonstrable effect of temperature on O_3 response when differential *VPD's* among temperatures are minimized during O_3 exposure.

MATERIALS AND METHODS

Plant culture

Tomato plants (*Lycopersicon esculentum* Mill cv. New Yorker) were grown from seed in 10 cm diameter plastic pots containing a 1 : 1 : 1 mixture of sphagnum peat moss : vermiculite : perlite (Pro-mix BX). Five seeds were sown per pot, the soil thoroughly wetted, and the pots placed in a germination cabinet at a constant temperature of 27℃ until seedling emergence. When the seedlings emerged the pots were placed in Conviron Model EY15 growth chambers with the following environmental conditions: day/night temperatures, 25/20 ± 2℃; relative humidity, 70 ± 5%; photosynthetically active radiation (*PAR*), 325 ± 10 μmole $m^{-2}s^{-1}$ at the top of the canopy as monitored by a quantum meter (LI-COR model LI-185); and a 16 h photoperiod from 0600 to 2200 h. Irradiance input wattage was 81% from cool-white fluorescent tubes and 19% from incandescent

lamps. Plants were irrigated as required with half strength Hoagland's complete nutrient solution (*13*). Five day after emergence seedlings were thinned to one per pot. The plants were moved daily within and among four growth chambers during the pre- and post-exposure periods according to a pre-determined schedule each day to minimize within and among chamber effects.

Experimental design

This experiment was designed to test the effect of exposure temperature, when the *VPD* of the air is standardized, on the growth of young tomato plants exposed to four peak O_3 concentrations. The configuration of the continuously stirred tank reactor (CSTR) chambers used in this experiment required that a split-plot design be used. There were four CSTR's in one temperature controlled room which meant that only one temperature treatment could be studied at any one time for each group of four O_3 treatments. Exposure temperature treatments were the whole-plot factor with O_3 concentration as the split-plot factor with four tomato plants as sub-samples in each sub-plot. There were three temperatures (20, 25 and 30°C) and four peak concentrations of O_3 (0.04, 0.08, 0.12 and 0.16 $\mu l\ l^{-1}$) delivered in a dynamic concentration pattern. Each replicate consisted of three randomly assigned temperature blocks separated at random in time. The experiment was conducted three times over several months to provide three independent replicates.

Exposure procedure

Approximately 16 h prior to exposure 20 plants were removed from growth chamber and 16 of these were selected on the basis of uniformity. Nondestructive covariate measuremeants were taken to reduce plant to plant variation in the statistical analysis of treatment effects (*20*) and four plants were randomly assigned to each CSTR. The covariates measured were height, plastochron index, and planar leaf area. Height was measured from the bud axil of the lowest cotyledon to the bud axil of the largest leaf smaller than 20 mm, measured from the axil to the tip of the terminal leaflet. Plastochron index was calculated by the method of Erickson and Michelini (*7*). Planar leaf area was determined by placing a clear plastic sheet with a one cm grid on it directly above the plant and counting the number of grid intersections that overlapped leaf tissue.

Plants were placed in the CSTR's at approximately 2200 h for acclimation before the start of exposure at 1100 h the following day. Environmental conditions were similar to those in the growth cabinets except that irradiance input wattage in the CSTR's was from 50% high pressure sodium lamps and 50% metal halide lamps rather than from fluorescent and incandescent lamps. Pots were placed in large dishes on the morning of the exposure and irrigated to excess to ensure that plants had enough water to supply all transpiration requirements throughout the O_3 exposure.

Ozone was generated by a high voltage corona discharge generator and analyzed by a Dasibi Model 1003 AH monitor. The O_3 concentration in each of

the CSTR's was monitored and controlled by a shared-time computer program (*10*).

Temperature and O_3 treatments were initiated at 1100 h and lasted until 1900 h. A dynamic exposure profile with a peak concentration at 1500 h was used to mimic ambient ozone profiles typical of an agricultural area in Southern Ontario (*9*). The concentrations of O_3 used encompassed an average background concentration, the maximum acceptable concentration [averaged over a 1 h period as defined by the Canadian National Ambient Air Quality Objectives (*8*)] and the United States standard for oxidant concentration in the atmosphere (*17*). Vapor pressure deficit levels during each exposure were monitored every half hour and manually adjusted as necessary. The *VPD* used in this experiment was determined by the range of *VPD* that is optimal for plant growth and the driest air that could normally be expected in the exposure chambers without dehumidification. The optimal *VPD* range for plant growth is 0.5-1.0 kPa (*14*) and the highest *VPD* that could be reliably obtained in the CSTR's was approximately 0.8 kPa. A steam injection system similar to that used by Heck *et al.*, (*12*) was used for the experiment. The temperatures and *VPD*'s achieved over the course of the experiment are listed in Table 1. The half hour monitoring and adjustment schedule used in this experiment differed from the Heck *et al.* (*12*) exposure procedure for which the humidity was set once at the beginning of the experiment.

Table 1. Dry-bulb temperatures and vapor pressure deficits achieved.

Temperature treatment	Dry-bulb temperature(℃) Mean[z]	Standard deviation
20	19.7	±0.48
25	25.1	±0.40
30	30.2	±0.46
	Vapor pressure deficit (kPa)	
20	0.76	±0.07
25	0.82	±0.06
30	0.86	±0.13

[z] Each mean is derived from 48 observations.

Harvest procedure

All plants were harvested 36 h after exposure. Although this period was arbitrarily chosen, results from similar experiments have shown that this is sufficient time for expression of growth reduction due to treatment effects (*9*). Plant height, leaf, stem and root fresh weights and leaf area were recorded. Leaf area was determined by a LI-COR model LI-3100 area meter. Leaves, stems and roots were then oven dried to constant weight at 70 ± 5℃ for 48 h and the dry weights recorded.

Data analysis

The first step in the analysis was to test that the date satisfied the assumptions underlying statistical examination (*21*). All date were found to satisfy these assumptions. The date were then analyzed by regression combined with analysis of covariance using the General Linear Models procedure of SAS (*21*). The full multiple regression model for the beginning of the analysis included terms for variation due to covariates, replication, temperature, O_3, O_3 × temperature, $(O_3)^2$ and $(O_3)^2$ × temperature. To accommodate the split-plot design, the error term for the whole-plot (replicate × temperature) was also included in the model. The analysis of the full model was inspected to discover which of the three covariates should be retained. A covariate was kept at $P \leq 0.20$ because even at this probability level it reduces the error sums of squares enough to provide a better estimation of treatment effects than if it were removed from the model. The covariates retained were planar leaf area for root fresh weight, root dry weight, leaf fresh weight, leaf dry weight, leaf area and height per unit of stem dry weight, and initial height, for harvest height, stem fresh weight and stem dry weight. There were no covariates significant at $P \leq 0.20$ for the derived variables root: shoot ratio and specific leaf area. The plastochron index was not useful as a covariate.

After removal of nonsignificant covariates, the full model split-plot analysis was rerun. This model then was reduced by removing nonsignificant terms ($P \leq 0.05$ for linear and quadratic effects and $P \leq 0.15$ for interactions) one at a time. A reduced model was retained if a lack of fit test indicated that it was not significantly different, at $P \leq 0.10$, from the full model. Pairwise comparisons were made between treatment combinations that comprised model interaction terms to determine which, if any, were significant at $P \leq 0.05$. If there were none, the interaction term was removed from the model and the new reduced model tested for lack of fit. The root fresh weight, stem fresh weight, and specific leaf area models were initially found to contain O_3 × temperature interactions at $P \leq 0.15$. After further analysis this interaction term was removed from the root fresh weight model. When the model reduction was complete, a common intercept for each temperature was determined by summation of average replicate, replicate × temperature effects for each temperature and the covariate mean multiplied by its parameter estimate as determined by SAS using the solutions option of the General Linear Models procedure (*21*). In the absence of an O_3 × temperature interaction, and if the intercepts were not significantly different ($P \leq 0.05$), the estimated effects of temperature were averaged to create a common intercept.

RESULTS AND DISCUSSION

Equations describing the growth response of tomato in this experiment (Table 2) indicate that there were few temperature effects on growth response to O_3 in an environment in which vapor pressure deficit was standardized. There was no effect of temperatire on tomato root fresh weight response to O_3. There

Table 2. Final (reduced model) equations describing growth response of tomato to O_3 ($\mu l\ l^{-1}$) and temperature (℃).

Variable	Temperature	O_3 Response Equation	Significance[y]
Root fresh weight (g plant^{-1})	n.s.	$1.40+1.68\ (O_3)$	0.005
Root dry weight (g plant^{-1})	n.s.	$0.088-0.10\ (O_3)$	0.003
Stem fresh weight (g plant^{-1})	20	$2.6-0.08\ (O_3)$ a[z]	n.s.
	25	$2.8-2.66\ (O_3)$ b	0.003
	30	$2.8-2.04\ (O_3)$ ab	0.015
Stem dry weight (g plant^{-1})	n.s.	$0.128-0.07\ (O_3)$	0.014
Plant height(mm)	n.s.	$69+127\ (O_3)-580\ (O_3)^2$	0.005
Height/stem dry weight (mm g^{-1})	n.s.	$576+517\ (O_3)$	0.0001
Leaf fresh weight (g plant^{-1})	20	$8.0+11.9\ (O_3)-116\ (O_3)^2$a	0.039
	25	$8.6+11.9\ (O_3)-116\ (O_3)^2$b	0.039
	30	$8.7+11.9\ (O_3)-116\ (O_3)^2$b	0.039
Leaf dry weight (g plant^{-1})	n.s.	$0.80-0.79\ (O_3)$	0.0001
Leaf area (cm^2 plant^{-1})	n.s.	$314-340\ (O_3)$	0.0001
Specific leaf area (cm^2 g^{-1})	20	$392+61\ (O_3)$b	n.s.
	25	$402-151\ (O_3)$a	0.025
	30	$399-98\ (O_3)$ab	n.s.
Root:shoot ratio(g g^{-1})	n.s.	n.s.	

[y] Indicates significance of the linear or quadratic coefficient; n.s. is non-significant, $P=0.05$.

[z] Equations within each response variable followed by the same letter are not significantly different according to a t-test at $P=0.05$.

was a significant O_3 effect but it was similar at all temperatures. The root dry weight response pattern to O_3 and temperature was similar to that of root fresh weight. There was an effect of temperature on the response of stem fresh weight to O_3, indicated by the significant difference in the slope of the line describing response to O_3 at 20℃ relative to the response at 25℃ (Fig. 1). The slope of the line describing growth response to O_3 was much greater at 25℃ than at 20℃ and significantly different from zero, while the slope of the line describing stem fresh weight response to O_3 at 20℃ was not significantly different from zero. The slopes of the lines at 25 and 30℃ were similar numerically but only the slope describing response to O_3 at 25℃ was significantly different from that at 20℃. There was no effect of temperature on response to O_3 of stem dry weight. Stem dry weight was significantly reduced as O_3 concentration increased.

Temperature did not affect plant height response to O_3. There was a significant quadratic effect of O_3 on plant height which was similar at all temperatures (Table 2, Fig. 2). The response equation contained a positive linear coefficient and a negative quadratic coefficient. This indicates that at low concentrations of O_3 there was an increase in plant height with increasing O_3 and at higher concentrations a decrease. The turning point from increase in plant height to decrease appears to be at a peak O_3 value of about 0.12 $\mu l\ l^{-1}$. There was no effect of temperature on the response to O_3 of the height per unit stem dry weight. There was, however, a significant positive effect of O_3, suggesting

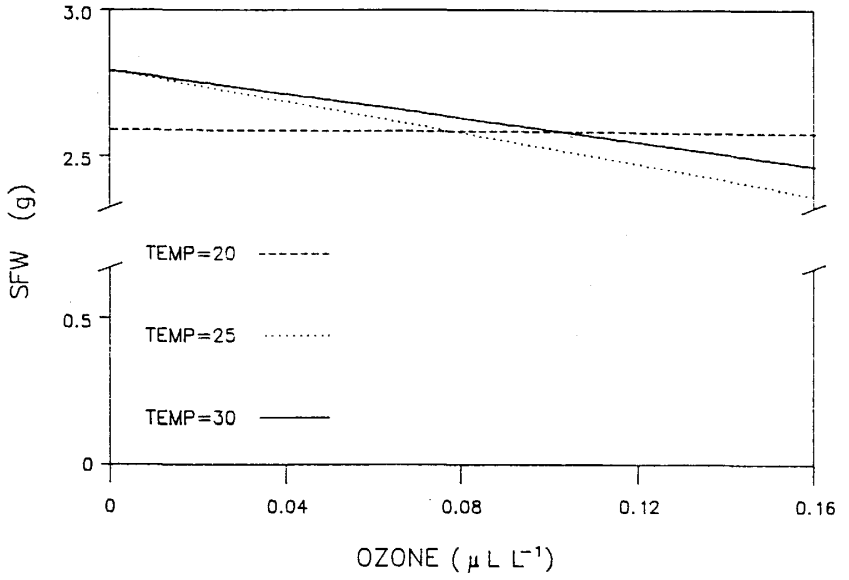

Fig. 1. Stem fresh weight (*SFW*) response to O_3 at 20, 25 and 30°C.

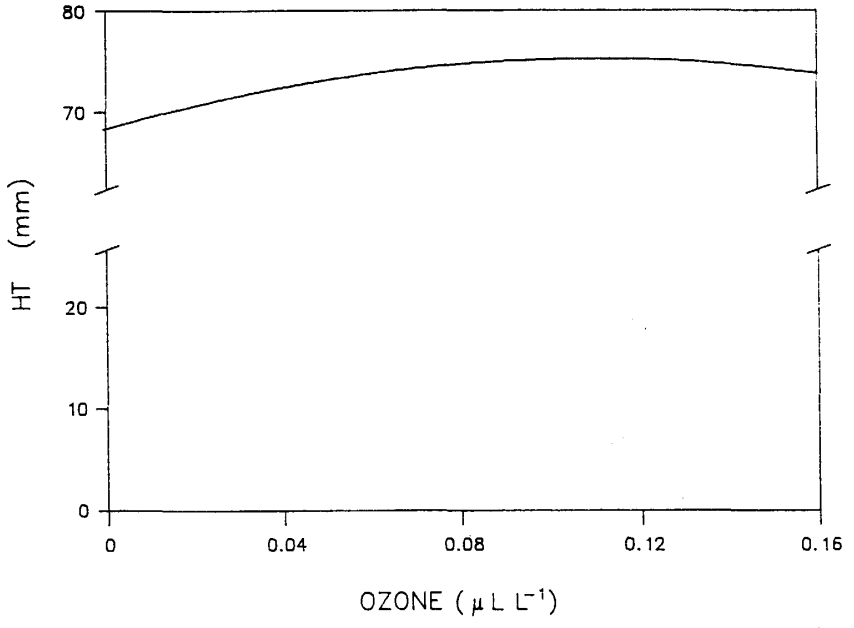

Fig. 2. Plant height (*HT*) response to O_3.

that O_3-treated plants are more spindly. Taken with the stimulation of height previously reported (*19*) this provides further evidence that low concentrations of O_3 stimulate cell elongation in the stem tissue of some species, producing an apparent stimulation of elongation growth. There are other reports indicating a growth stimulation in response to low O_3 concentrations (*1*).

Leaf fresh weight showed no significant effect of temperature on the

response to O_3. The equations describing leaf frash weight response to O_3 contained linear and quadratic coefficients (Table 2). The coefficients were a positive linear and a negative quadratic, indicating that at low concentrations of O_3 (≤ 0.07 μl l^{-1}) there was a small increase in leaf fresh weight with increasing O_3 and at higher concentrations a decrease in leaf fresh weight. There was a significant effect of temperature on leaf fresh weight. Plants exposed to O_3 at 20℃ had significantly less leaf fresh weight than those exposed at 25 and 30℃. No effect of temperature on the response to O_3 was found in tomato leaf dry weight. There was a significant response to O_3 alone that was similar at all temperatures. There was no temperature effect on the response of leaf area to O_3. This lack of an effect of temperature alone differed from the response of leaf fresh weight. There was a significant effect of O_3 on leaf area which was the same at all temperatures.

The response of specific leaf area to O_3 was significantly affected by temperature. At 25℃ specific leaf area was significantly reduced by increasing O_3 concentration. The leaves were thicker or more dense and weighed more per unit area than leaves at 20℃.

The results of this experiment indicate there was little effect of exposure temperature on tomato growth response to O_3 across the temperature range of 20–30℃ when *VPD* was held essentially constant. The tomato growth response variable stem fresh weight and the derived variable specific leaf area were the only ones, out of the 11 analyzed, for which a temperature $\times$ O_3 interaction was found. This appears to contradict the finding of Heck *et al.* (*11*) who found that visual injury in pinto bean and tobacco decreased as exposure temperature increased. There are important differences between that experiment and this one. One difference is that in such earlier experiments visual injury was used as an indicator of potential growth reduction. Visual injury probably works well at higher concentrations of O_3, such as were used in those experiments, but may not be very useful for detecting differences in plant response to O_3 at the much lower concentrations typical of ambient conditions and more frequently used in current research.

Another very important difference between past experiments with temperature and O_3 is that in the present experiment the *VPD* was held essentially constant across temperatures. Previous research either did not consider this important environmental parameter or did not control it. Dunning *et al.* (*4*) reported that visual injury increased with temperature, depending on dose and species. There was no information reported on the air moisture content. In the work of Heck *et al.* (*11*) humidity during exposure was not reported. In a similar experiment, where an inverse relationship between temperature and visual injury in tobacco was also noted (*2*), relative humidity was held constant at 85% across exposure temperatures ranging from 10 to 32.3℃. When the *VPD* for each temperature is calculated from this information there was a large spread between the *VPD* at 10℃ versus 32℃. Dunning and Heck (*3*) also reported a significant increase in foliar injury on pinto bean as temperature increased from 21 to 32℃ with a constant relative humidity across

temperature. These reports suggest the possibility of a *VPD* effect along with, or perhaps mistaken for, a temperature effect.

Reference to reports of experiments that have examined the effect of humidity on plant response to O_3 provides information on a probable reason for the temperature effects reported by Heck *et al.* (*11*) and Cantwell (*2*). Injury in pinto bean and tobacco has been shown to increase significantly as %RH was increased (*3*). There were four humidity levels used in that experiment; 45, 60, 75 and 90%. The exposure temperature was 27℃ which allows the *VPD*'s to be calculated for each %RH as 1.96, 1.43, 1.07 and 0.36 kPa respectively. Therefore, as *VPD* decreased (increasing % RH) plant injury increased. When taken together, the varying *VPD*'s in previous experiments and the modification of plant response to O_3 by humidity, the possibility of a *VPD* modification of plant response to ozone, with or without a temperature modification of response, becomes more obvious in the re-analysis of the past attempts to study possible temperature $\times$ O_3 interactions.

The results from this study, in the context of earlier reports, suggest that *VPD* probably plays an important role in determining sensitivity to O_3 with temperature playing a less important role. To avoid confounding temperature and *VPD* in temperature effects studies in the future, such studies should treat *VPD* as another environmental variable that is controlled across temperatures.

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