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Effects of the root-knot nematode, *Meloidogyne incognita*, on the sensitivity of tomato to sulfur dioxide and ozone

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Abstract

Infection of plants with root-knot nematode leads to an increase in transpiration rate. We hypothesize that, in infected plants, the diffusive intake of gaseous pollutants would be greater and the interaction between the nematode and pollutant(s) would be governed by the degree of stomatal opening. Tomato plants infected with the root-knot nematode, *Meloidogyne incognita* were exposed to air containing 0, 50 or 100 ppb of SO_2 or O_3 for 5 h every third day on 27 occasions in 1988 and 1989. Plants exposed to the gases at 100 ppb had chlorotic and/or necrotic leaves, small shoots and roots, reduced leaf pigment levels and low yield, compared to untreated plants. Greater foliar injury developed on plants exposed to SO_2+O_3 mixture. Symptoms were even greater on nematode-infected exposed plants. *M. incognita* alone reduced tomato yield by 14.4% and induced a 3.6% increase in the width of stomatal pores and a 15.6% increase in the transpiration rate. A positive correlation was observed between stomatal pore width and rate of transpiration. Interaction between SO_2 and O_3 depended on the presence (significant) or absence (insignificant) of nematodes. Most effects of nematode infection and gas exposures (especially mixtures) were synergistic. Disease intensity (galls per root system) was increased, but nematode reproduction (egg masses per root system, eggs per egg mass) reduced on plants exposed to SO_2 and/or O_3. © 1997 Elsevier Science B.V.

Keywords: SO_2; O_3; Nematode; Interaction; Tomato; Stomata; Transpiration; Yield

1. Introduction

Air pollutants usually occur as mixtures (Reinert, 1984; Heagle et al., 1993; Khan and Khan, 1994c). The combustion sources that produce SO_2 also release other pollutants or their precursors. Internal combustion of petroleum emits, besides SO_2, oxides of nitrogen and nonmethane hydrocarbons which cause generation of tropospheric ozone (Ashmor and Bell, 1991). The multiple pollutants can have additive, synergistic or antagonistic effects (Khan and Khan, 1994b).

Sulfur dioxide and ozone frequently occur together and have been extensively studied for their phytotoxic effects on crop plants (Reinert, 1984; Heagle et al., 1993; Khan and Khan, 1993). Synergistic effects on yield from exposure to SO_2 and

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O_3 at low concentrations have been measured in spring rape (Adaros et al., 1991) and tomato (Khan and Khan, 1994b).

A few reports on interactions between air pollutants and plant parasitic nematodes are available but the interactive effects are variable (Weber et al., 1979; Shew et al., 1982; Bisessar and Palmer, 1984; Khan and Khan, 1993). Khan and Khan (1993) reported significantly greater galling and greater egg mass production by the root-knot nematode, *Meloidogyne incognita* race 1, on tomatoes exposed intermittently to 100 ppb SO_2. Nematode-infected plants developed greater SO_2-induced chlorosis and necrosis of leaves than non-infected plants exposed to 100 or 200 ppb SO_2. Tobacco plants exposed to 80 ppb ambient O_3 developed 20% more galling caused by *M. hapla* compared to the inoculated plants sprayed with an antioxidant, EDU (ethylenediurea) (*N*-[2-oxo-1-imidazolidinyl ethyl]-*N*-phenylurea) (Bisessar and Palmer, 1984). Ozone-injury on leaves also was greater on the nematode infected plants. However, for soybeans, intermittent exposures with O_3 and SO_2+O_3 mixtures inhibited development and reproduction of *Heterodera glycines* and *Paratrichodorus minor*, whereas *Belonolaimus longicaudatus* and *Aphelenchoides fragariae* remained unaffected (Weber et al., 1979). In another study (Shew et al., 1982), a mixture of 0.8 μl SO_2 l^{-1} and 0.2 $\mu l\,l^{-1}$ O_3 favoured the reproduction of *Pratylenchulus penetrans* on tomato. Nematode-infected plants developed more O_3 injury on foliage than uninfected plants.

Root-parasitic nematodes and gaseous air pollutants obviously occupy two different regions of the environment (soil and air). Hence, air pollutants are unlikely to affect nematodes directly. It is speculated, however, that after diffusion through stomata, the air pollutants may cause changes in the host plant that either facilitate or suppress the invasion and development of nematodes. Nematode infection may increase transpiration rates, leading to greater diffusion of air pollutants into leaves and consequently increased foliar injury. In the present study, the hypothesis is that, if a synergistic relationship develops between air pollutants and root infecting nematodes, stomata of the infected plants would open widely and transpire more water than noninfected plants. To test this hypothesis, the single and joint effects of SO_2, O_3 and the root-knot nematode, *Meloidogyne incognita* (Kofoid and White) Chitwood on tomato, *Lycopersicon esculentum* Mill. were investigated using intermittent exposures.

2. Materials and methods

2.1. Gas exposure system

The gas exposure system consisted of nine 90 cm × 90 cm × 120 cm chambers made of transparent fibreglass, with an exhaust duct (20 cm × 20 cm) at the top of each one and a vertical moveable door on the front. A blowing assembly was fitted at the bottom. These chambers have been described earlier (Khan and Khan, 1993). Sulfur dioxide was produced in a generator by the reaction of sodium sulfite (Na_2SO_3) and dilute sulfuric acid (Khan and Khan, 1993). Ozone was generated by ionizing oxygen in the presence of ultraviolet light (Khan and Khan, 1994b). The outlets (0.4 cm diameter) of the gas generators (Standard Appliances, Varanasi, India) were connected to the blower inlet of the chambers. The blower assembly, which was run at a constant speed, mixed the gas(es) with ambient air and dispensed them into the chamber. Desired concentrations of O_3 (50 and 100 ppb ± 10%) were obtained by calibrating the pumping rate of O_3 from the ionizing unit, which was controlled by an air flow meter fitted on the generator. Desired concentrations of SO_2 (50 and 100 ppb ± 10%) were obtained by preparing sodium sulfite solutions of different concentrations (Khan and Khan, 1993).

During each exposure, a handy air sampler (Kimoto Electricals, Japan) was kept inside the chamber to measure gas concentrations. The absorbing media used in the sampler were sodium tetrachloromercurate and alkaline potassium iodine solutions for SO_2 and O_3, respectively. After sampling, the solutions were analysed colorimetrically (Anonymous, 1986). The air flow rate was measured at different points in the exhaust duct of the chamber using an electronic anemometer during exposures. Throughout the experiment, the air flow rate was maintained at 1.9 m s^{-1}. The air

(with or without gas) inside the chamber was replaced approximately seven times in 1 min. For the control or 0 ppb regime (without SO_2 and/or O_3), the same air flow rate ($1.9\,m\,s^{-1}$) was maintained. Ambient concentrations of SO_2 and O_3 were 8.3 ± 2.6 and 5.7 ± 3.1 ppb, respectively.

2.2. Treatments and plant culture

Two-week-old seedlings of tomato cv. Pusa Ruby were raised in sterilized soil from surface sterilized seeds and transplanted on 2nd October 1988 or 10th October 1989 into 180 15 cm diameter clay pots filled with 1.5 kg autoclaved soil (field soil and compost 3:1). One week after planting, 90 pots were inoculated with freshly hatched second-stage juveniles of *M. incognita* (2000 juveniles per pot). Plants were inoculated by pouring a suspension of nematodes (2000 juveniles plus water) into six small holes in the soil around the seedling. After inoculation, plants were exposed intermittently to three concentrations (0.0, 50 and 100 ppb) of SO_2 and O_3, singly and in all possible combinations for 5 h every third day over a period of two and a half (2.5) months (27 exposures per treatment). There were nine gas exposure treatments for each inoculated and each uninoculated regime (0.0+0.0, 50+0.0, 0.0+50, 100+0.0, 0.0+100, 50+50, 50+100, 100+50 and 100+100 ppb SO_2+O_3 respectively). There were 18 treatments in total, with 10 pots per treatment. All gas exposures were given for 5 h on the same day starting from 10.0 am to 3.0 pm. The pots of the control set (0.0+0.0) were also placed in an exposure chamber for 5 h with an air exchange rate of $1.9\,m\,s^{-1}$. This chamber was not connected to an O_3 or SO_2 generator, but received ambient air. To reduce chamber variation, treatments were rotated among the chambers. For example, if the 50+50 ppb SO_2+O_3 treatment was given in chamber 1, on the next occasion, chamber 2 was used for the same treatment. The control set was also rotated.

Plants were irrigated daily with tap water at 9:00 am. The pots were labelled according to treatment and replication, and placed in a greenhouse at a day/night temperature regime of 25/19°C with an 11 h photoperiod and a photon flux density of $450\,\mu mol\,m^{-2}\,s^{-1}$ supplied by a mixture of fluorescent tubes (40 W) and incandescent bulbs (200 W). The pots were arranged in rows (two rows per treatment) next to each other on two adjacent benches (18 rows per bench) in the greenhouse. Plants were watered daily at 9:00 am; average relative humidity was 65%. On days designated for gas exposure, pots were transferred to the exposure chambers at 10:00 am and returned to the greenhouse by 3:00 pm. Seventy-five days after the start of exposures, five plants of each treatment were harvested.

2.3. Symptoms, plant growth and yield

Plants were observed daily for the development of foliar injury and flower buds were counted. At harvest, the number of fruits per plant and the fruit weight per plant were determined. Roots were gently removed and washed. The shoots and roots of five plants per treatment were then dried at 60°C for 48 h and weighed. All the leaves of unharvested plants (five plants) were used to determine the percentage of foliar injury with a planimeter.

2.4. Foliar carotenoids and chlorophyll

Carotenoids and chlorophyll contents of leaves were determined following the procedures of Mackinney (1941) and MacLachlan and Zalik (1963). Fresh interveinal areas of the third leaflet of the third leaf of each branch (1 g from each replicate separately) were ground in 40 ml acetone (80%) with a mortar and pestle. The suspension was filtered through two Whatman filter papers (No. 1) in a Buchner funnel equipped with a suction pump. The filtrate was placed in a volumetric flask, and acetone was added to make 100 ml total volume. The filtrates were used to read percentage transmittance in a spectrophotometer at 480 and 510 nm for carotenoids (MacLachlan and Zalik, 1963) and 645 and 663 nm for chlorophyll (Mackinney, 1941).

2.5. Leaf stomata

Five middle-aged leaves were removed from each of the unharvested plants and immediately placed in FAA (formalin-acetic acid-alcohol). Leaf pieces 1 cm^2 in size from 10 leaves cut between the midrib

and the leaf margin halfway between the base and the apex were boiled in 40% HNO_3, to separate the epidermal peels. The peels were washed in water, stained with iron-alum and haematoxylin, dehydrated in an ethanol series, and mounted in canada balsam for microscopic examinations (Ghouse and Yunus, 1972). The stomata in each 1 cm^2 area of each leaf sample of each treatment were counted and the widths of stomatal pores were measured.

2.6. Nematode disease and reproduction

The intensity of root-knot disease and reproduction of *M. incognita* were determined by counting the galls and egg masses formed on the whole root system. To stain egg masses, roots were held in phloxine B solution (0.95 $g l^{-1}$ of tap water) for 20 min. Fecundity (number of eggs per egg mass) was determined by excising 20 egg masses carefully from each washed root system. The 20 egg masses of each group of five replicates (total of 100) of each treatment were blended in 1% NaOCl solution according to the procedure used by Khan and Khan (1994a) to estimate the number of eggs per egg mass. A sample from the egg suspension taken in a counting dish to count eggs was considered as one trial.

2.7. Transpiration rate

To measure the loss of water through foliage of tomato, the pots just after irrigating with water (50 ml per day for 25 days; 100 ml per day for next 25 days and 150 ml per day for the last 25 days) were weighed daily at 9:00 am. The pots were reweighed on the next day at the same time. A set of five pots similar in all respects but without plants were positioned side by side to determine the loss of water from the pot and soil surfaces. The loss of water through the foliage in 24 h was also expressed in terms of loss of water per gram of dry weight of the shoot.

2.8. Statistical analysis

Means of observations for each treatment were calculated separately for both years. Since all ten pots were exposed to the same treatment each year, the experiment was replicated over time. The mean measure of each regime for each year was used as one replicate, hence, there were two replicates for each treatment. A three factor analysis of variance (nematode $\times$ SO_2 $\times$ O_3) was conducted using orthogonal polynomials to determine whether or not a significant regression relationship exists (Montgomery, 1984). Sulphur dioxide and ozone were further partitioned into linear and quadratic components of regression and significance was tested at three probability levels, namely $P=0.05$, 0.01 and 0.001. To ascertain the nature of interaction, figures are drawn on means of the two replicates for SO_2 and O_3 within each level of nematode treatment, i.e. inoculated and uninoculated.

3. Results

3.1. Foliar injury

Sulphur dioxide at 100 ppb caused a mild leaf chlorosis, leaves later turned brown. At 50 ppb, no foliar injury could be attributed to SO_2. Ozone, however, at both the concentrations (50 and 100 ppb) caused small necrotic lesions in the intercostal areas of leaves. Analysis of variance indicated that linear and quadratic components for main effects of SO_2 and O_3 were significant at $P=0.01$ (Table 1). Plants exposed to both SO_2 and O_3 exhibited greater foliar injury than was expected from the individual effects (Figure 1). There were 34.7, 21.7 and 11.9% increases in the injury on the plants exposed to SO_2+O_3 mixtures at 50+50, 50+100 and 100+50 ppb, respectively, compared to the sum of individual effects. At 100 ppb of each gas in the mixture, the plants, however, sustained 11.2% less injury for $SO_2 \times O_3$ interactions. Foliar injury was greatest in treatments receiving nematode inoculation as well as gas exposure (Figure 1), but there was no difference in the time of appearance of foliar injury in inoculated versus uninoculated plants. All gas treatments inoculated with the root-knot nematode had greater chlorosis and necrosis than the sum of injuries measured separately for the gases. However, this effect was not statistically significant, either linearly or quadratically at $P=0.05$ (Table 1).

Table 1
Mean of squares for single and joint effects of SO_2 and O_3 and root-knot nematode on foliar injury and dry matter production

		Foliar injury		Shoot dry weight		Root dry weight	
Source of variation	DF	MS	F-value	MS	F-value	MS	F-value
Nematode (NEM)	1	17.60	13.64***	7.65	588.46***	0.60	115.38**
SO_2	2						
SO_2 linear (SL)	1	63.9	49.53***	1.93	148.46**	0.26	50.00***
SO_2 quadratic (SQ)	1	41.2	31.93***	0.06	4.61*	0.008	1.54
O_3	2						
O_3 linear (OL)	1	118.0	91.47***	2.47	190.0***	0.35	67.31***
O_3 quadratic (OQ)	1	97.5	75.58***	0.23	17.69***	0.0025	0.48
$SO_2 \times O_3$	4						
SL × OL	1	88.8	68.83***	0.006	0.46	0.0006	0.11
SL × OQ	1	3.05	2.36	0.005	0.38	0.00003	0.0058
SQ × OL	1	2.8	2.17	0.001	0.077	0.00013	0.025
SQ × OQ	1	2.3	1.78	0.001	0.077	0.00003	0.0058
Nematode × SO_2	2						
Nem × SL	1	0.3	0.23	0.03	2.31	0.0067	1.29
Nem × SQ	1	0.01	0.0007	0.002	0.15	0.0003	0.058
Nematode × O_3	2						
Nem × OL	1	0.0004	0.0003	0.09	6.92*	0.002	0.38
Nem × OQ	1	0.0013	0.001	0.003	0.23	0.001	0.19
Nematode × $SO_2 \times O_3$	4						
Nem × SL × OL	1	2.03	1.57	0.106	7.92*	0.0006	0.11
Nem × SL × OQ	1	0.035	0.027	0.075	5.77*	0.00003	0.0058
Nem × SQ × OL	1	0.088	0.068	0.092	7.08*	0.00013	0.025
Nem × SQ × OQ	1	0.075	0.058	0.037	2.85	0.00003	0.0058
Error	18	1.29	—	0.013	—	0.0052	—

Values marked with asterisks are significant at $P=0.05$ (*), 0.01 (**) or 0.001 (***), otherwise not significant at $P=0.05$.

3.2. Dry matter production and yield

Sulfur dioxide or ozone at 100 ppb or root-knot nematode reduced the dry weights of shoots and roots (Figs. 1 and 2). Percent reduction was greater with the nematode than the gases. The main effects of these three factors were statistically significant at $P=0.001$. However, for root dry weight, only linear effects were significant (Table 1). The gas mixtures at the tested concentrations caused greater suppressions in dry weights but their interactive effects were not significant. Nematode infection of gas-exposed plants resulted in a greater reduction in shoot dry weights than the sum of reductions caused by the gases and nematode separately (Figs. 1 and 2). This effect was significant ($P=0.05$) for nematode times O_3 linear and nematode times SO_2 times O_3, but not significant ($P=0.05$) for the nematode times SO_2 quadratic times O_3 quadratic (Table 1).

Flower production in relation to gas exposure or nematode inoculation was uninfluenced, but fruit-set was considerably affected, especially at 100 ppb concentration. Main effects of the nematode and SO_2/O_3 were linearly significant (Table 2). Adverse effects on fruit-set were exacerbated in the presence of nematodes and the SO_2-O_3 mixture, being greatest (15.6%) at 100 + 100 ppb. ANOVA, however, did not reveal a significant interaction (Table 2).

Sulfur dioxide or O_3 at 100 ppb or root-knot nematode decreased the yield (weight of fruits per plant) by about 13–14% (Fig. 2), being linearly significant at $P=0.001$ (Table 3). Interaction of SO_2 and O_3 was not significant at $P=0.05$, although, a greater yield decline was recorded with such treatments (Fig. 2). Nematode infection, however, exacerbated the injurious effects of the gases singly or in mixture, leading to a significantly greater decrease in yield. Nematode-infected plants

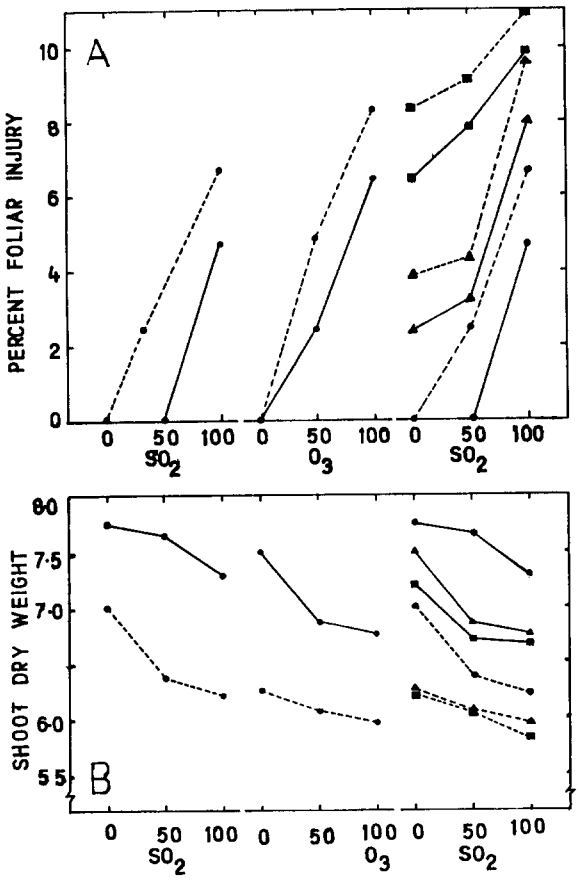

Fig. 1. (A) Floiar injury caused by SO_2 and/or O_3 to tomato plants inoculated with the root-knot nematode, *Meloidogyne incognita* or uninoculated. (B) Effects of SO_2 and/or O_3 on shoot dry weight of tomato plants inoculated with the root-knot nematode, *Meloidogyne incognita*, or uninoculated. (----) With nematode; (——) without nematode; • 0 ppb O_3; ▲ 50 ppb O_3; ■ 100 ppb O_3.

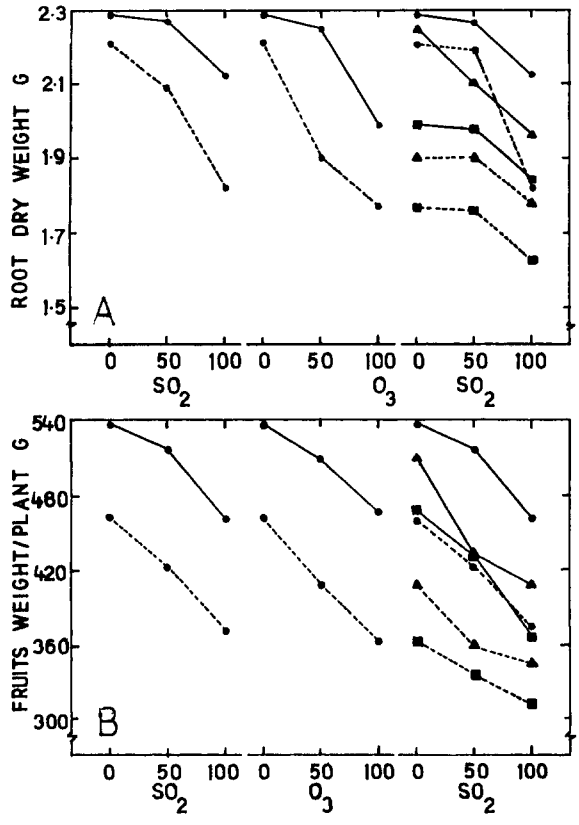

Fig. 2. Effects of SO_2 and/or O_3 on root dry weight (A) and weight of fruits/plants (B) of tomato plants inoculated with the root-knot nematode, *Meloidogyne incognita*, or uninoculated. (----) With nematode; (——) without nematode; • 0 ppb O_3; ▲ 50 ppb O_3; ■ 100 ppb O_3.

exposed to 50+50 ppb mixture exhibited an 9.4% additional decline in the yield, compared to the sum of individual effects of the nematode and gases. For this interaction, only linear effects of nematode times SO_2/O_3 and nematode times SO_2 linear times O_3 linear were significant at $P=0.05$ (Table 3). Mean fruit weight of tomato was also adversely affected, and main effects of nematode and O_3 linear were significant at $P=0.001$ and of SO_2 linear at $P=0.05$ (Table 3). Joint treatments of the gases plus nematodes were not significant except for nematode times SO_2 linear times O_3 linear $P=0.05$). There was a 4–6.7% additional decrease in the mean fruit weight of the plants received SO_2, O_3 and nematode, being maximum with 50+50 ppb mixture (Figure 3).

3.3. Foliar pigments

Infection of plants with *M. incognita* caused a significant ($P=0.05$) negative effect on the carotenoid content of leaves, whereas neither gas produced such effects (Table 4). Gas mixtures, however, caused a considerable decrease (5.2–9.8%) in the leaf carotenoids, being greater at 100+100 ppb, compared to the control (Fig. 3). Treatments including the nematode and gases either singly or mixed caused a greater decrease in the carotenoids than expected from the individual

Table 2
Mean of squares for single and joint effects of SO_2, O_3 and root-knot nematode on flower production and fruit-setting

		Flowers/plant		Fruits/plant	
Source of variation	DF	MS	F-value	MS	F-value
Nematode (NEM)	1	0.11	0.06	9.0	5.36*
SO_2	2				
SO_2 linear (SL)	1	4.17	2.27	12.0	7.14*
SO_2 quadratic (SQ)	1	1.39	0.75	0.68	0.40
O_3	2				
O_3 linear (OL)	1	6.0	3.26	6.0	4.57*
O_3 quadratic (OQ)	1	1.39	0.75	0.08	0.05
$SO_2 \times O_3$	4				
SL × OL	1	0.063	0.03	0.022	0.01
SL × OQ	1	0.021	0.01	0.021	0.01
SQ × OL	1	0.021	0.01	0.021	0.01
SQ × OQ	1	0.007	0.003	0.009	0.005
Nematode × SO_2	2				
Nem × SL	1	0.042	0.02	0.42	0.25
Nem × SQ	1	0.014	0.007	0.021	0.01
Nematode × O_3	2				
Nem × OL	1	0.056	0.04	0.17	0.10
Nem × OQ	1	0.042	0.02	0.021	0.01
Nematode × $SO_2 \times O_3$	4				
Nem × SL × OL	1	0.063	0.03	0.062	0.03
Nem × SL × OQ	1	0.021	0.01	0.021	0.01
Nem × SQ × OL	1	0.021	0.01	0.021	0.01
Nem × SQ × OQ	1	0.007	0.004	0.007	0.004
Error	18	1.84	—	1.68	—

Values marked with asterisks are significant at $P=0.05$ (*); otherwise not significant at $P=0.05$.

effects, but ANOVA did not reveal a significant interaction (Table 4).

More effects were detected on chlorophyll than on carotenoids (Fig. 4). The main effects of SO_2, O_3 (linear) or nematode were significant at $P=0.001$ (Table 4). The quadratic component for the O_3 effect was also significant ($P=0.01$). Mixtures at the tested concentrations caused a considerably greater decrease in leaf chlorophyll content. This interaction of SO_2 and O_3 was, however, significant only for linear components at $P=0.05$ (Table 4). Nematode infection exacerbated the injurious effect of the gases singly or mixed, leading to a greater decrease in chlorophyll than predicted from their individual effects (Fig. 4). The interaction was significant for nematode times SO_2 linear ($P=0.001$), nematode times O_3 linear and nematode times SO_2 linear times O_3 linear ($P=0.01$) (Table 4).

3.4. Leaf stomata and transpiration

Root-knot nematode, SO_2 or O_3 at 100 ppb, acting alone caused a decrease in the number of stomata per cm^2 of lower leaf surface, which was linearly significant for SO_2 ($P=0.05$) and O_3 ($P=0.01$) (Table 5). These treatments, however, induced an increase in the width of stomatal pores (Fig. 4), which was significant (linear) at $P=0.001$ (Table 5). In the joint treatments of nematodes and gases whether singly or mixed, the decrease in stomatal count and increase in pore width were greater, but no combination was statistically significant at $P=0.05$. Relatively greater effects were detected on pore width than on stomata number. Sulfur dioxide or O_3 at 50 ppb or their mixture (50+50 ppb) promoted stomatal opening (width) of nematode-inoculated plants by 9.5, 11.1 and

Table 3
Mean of squares for single and joint effects of SO_2, O_3 and root-knot nematode on yield of tomato

Source of variation	DF	Fruit weight/plant		Mean fruit weight	
		MS	F-value	MS	F-value
Nematode (NEM)	1	64009.0	70.0***	127.0	48.85***
SO_2	2				
SO_2 linear (SL)	1	37763.0	41.29***	19.0	7.31*
SO_2 quadratic (SQ)	1	3555.0	3.89	3.08	1.18
O_3	2				
O_3 linear (OL)	1	27611.0	30.19***	58.0	22.31***
O_3 quadratic (OQ)	1	807.0	0.88	7.03	2.70
$SO_2 \times O_3$	4				
SL × OL	1	2678.0	2.92	1.8	0.69
SL × OQ	1	165.0	0.18	0.014	0.005
SQ × OL	1	109.4	0.12	0.05	0.019
SQ × OQ	1	72.5	0.08	0.03	0.01
Nematode × SO_2	2				
Nem × SL	1	4194.0	4.59*	6.45	2.48
Nem × SQ	1	958.7	1.05	0.30	0.11
Nematode × O_3	2				
Nem × OL	1	4490.7	4.91*	10.06	3.87
Nem × OQ	1	512.9	0.56	0.08	0.03
Nematode × $SO_2 \times O_3$	4				
Nem × SL × OL	1	7446.0	8.14*	14.62	5.62*
Nem × SL × OQ	1	2551.2	2.79	0.30	0.11
Nem × SQ × OL	1	1390.2	1.52	0.19	0.07
Nem × SQ × OQ	1	24.1	0.03	0.19	0.07
Error	18	914.6	—	2.60	—

Values marked with asterisks are significant at $P=0.05$ (*), 0.01 (**) or 0.001 (***); otherwise not significant at $P=0.05$.

15.0%, respectively. The greatest increases, i.e. 13.7, 15.2 and 17.5%, respectively, were recorded at 100 ppb of SO_2 and O_3 singly or mixed.

Transpiration rates (water loss through foliage, ml $24\,h^{-1}$) were higher in plants exposed to the gases and/or inoculated with the nematode (Figure 5), but this effect was significant only for main effects of nematodes ($P=0.01$) and O_3 linear ($P=0.05$) (Table 6). Water loss was much greater, when it was expressed as ml g^{-1} dry shoot (Figure 5). Sulfur dioxide and ozone at 50 and 100 ppb caused an increase of 3.8 and 10.7%, and 6.2 and 12.4%, respectively. Nematodes also accelerated (15.6%) water loss, compared to the control. The main effects of the gases (linear) and nematodes were statistically significant at $P=0.001$ (Table 6). Water loss was greatest in joint treatments of gases and nematodes. Significant interactions were recorded for nematode times gas linear ($P=0.05$), nematode times SO_2 linear times O_3 ($P=0.01$), nematode times SO_2 linear times O_3 quadratic and nematode times SO_2 quadratic times O_3 linear ($P=0.05$) (Table 6).

3.5. Root-knot disease and reproduction of the nematode

Root-knot nematode, *M. incognita* caused severe galling on tomato roots (Fig. 6). Gas exposures enhanced the severity of gall development, leading to an increase of 4.7 and 16.4% in the number of galls per root system, which was linearly significant for SO_2 ($P=0.05$) and O_3 ($P=0.01$) (Table 7). Nematode reproduction of nematodes was,

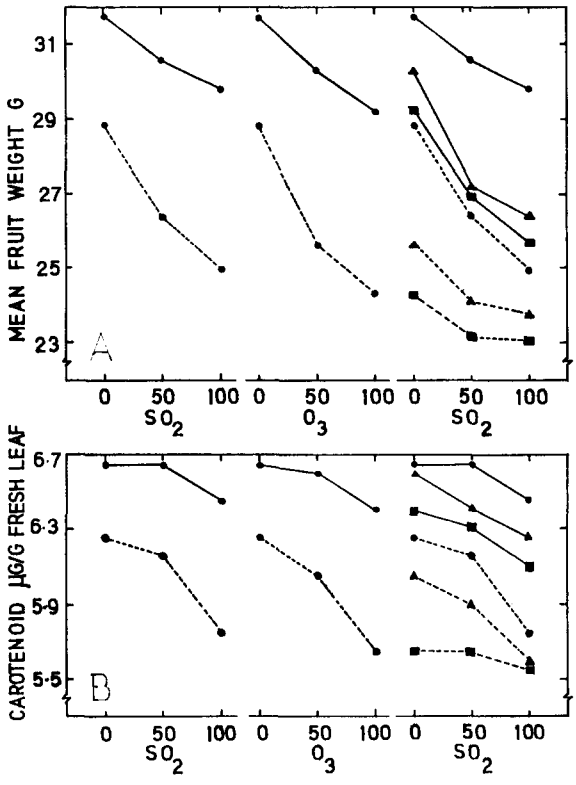

Fig. 3. Effects of SO_2 and/or O_3 on mean fruit weight (A) and leaf carotenoid (B) of tomato plants inoculated with the root-knot nematode, *Meloidogyne incognita*, or uninoculated. (----) With nematode; (——) without nematode; • 0 ppb O_3; ▲ 50 ppb O_3; ■ 100 ppb O_3.

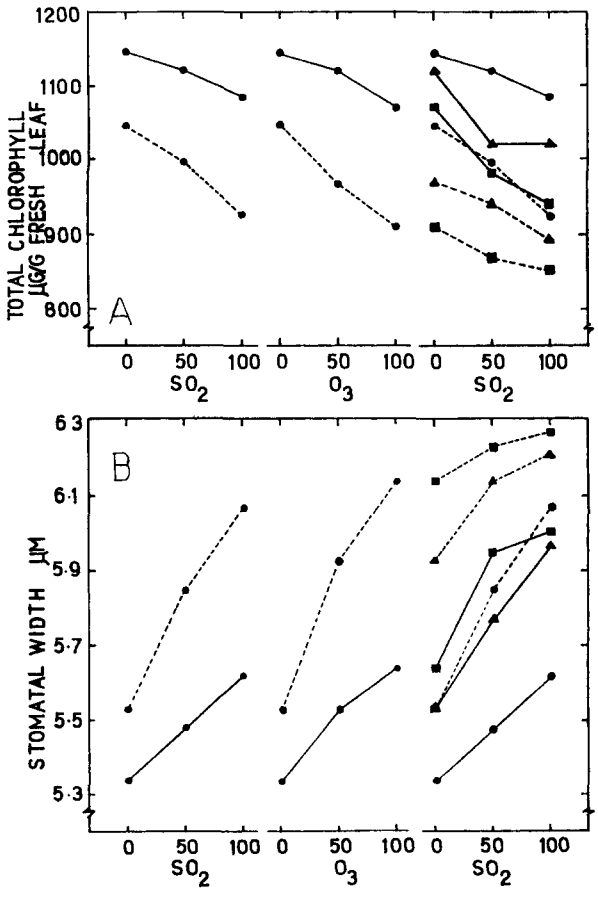

Fig. 4. Effects of SO_2 and/or O_3 on leaf chlorophyll (A) and pore width of stomata (B) of tomato plants inoculated with the root-knot nematode, *Meloidogyne incognita*, or uninoculated. (----) With nematode; (——) without nematode; • 0 ppb O_3; ▲ 50 ppb O_3; ■ 100 ppb O_3.

however, suppressed by exposures to gases (Figure 6). Linear components of the main effects of the gases were statistically significant ($P=0.001$) for number of egg masses per root system and eggs per egg mass (fecundity) (Table 7). Interaction for SO_2 linear times O_3 linear was detected for both variables at $P=0.05$ (Table 7). Mixtures of SO_2 and O_3 at 50+100, 100+50 and 100+100 ppb decreased egg mass production by 21, 22.3 and 40.2%, respectively. Corresponding values for fecundity were 18.6, 23.1 and 31.1%.

4. Discussion

Sulfur dioxide and ozone, like other air pollutants, diffuse inside the leaf through open stomata. The most obvious phytotoxic effects of air pollutants are leaf chlorosis and necrotic foliar lesions (Olszyk and Tibbitts, 1981; Pratt et al., 1983). Synergistic interactive effects of SO_2 and O_3 on foliar injury and leaf chlorophyll, observed in the present study agree with the results of others (Menser and Heggestad, 1966; Pratt et al., 1983; Khan and Khan, 1994b). Pratt et al. (1983) recorded greater reduction in soybean leaf pigment owing to exposure of SO_2 (200 and 400 ppb)+O_3 (8O and 100 ppb), compared to the sum of their individual effects. Synergism between SO_2 and O_3 in causing visible injury has been observed on crop plants like tobacco (Menser and Heggestad, 1966), tomato (Khan and Khan, 1994b), etc. Phytotoxic

Table 4
Mean of squares for single and joint effects of SO_2, O_3 and root-knot nematode on carotenoid and total chlorophyll of tomato leaves

Source of variance	DF	Carotenoids		Total chlorophyll	
		MS	F-value	MS	F-value
Nematode (NEM)	1	0.29	5.82*	62948.0	103.30***
SO_2	2				
SO_2 linear (SL)	1	0.16	3.16	89323.0	146.62***
SO_2 quadratic (SQ)	1	0.07	1.40	1013.0	1.66
O_3	2				
O_3 linear (OL)	1	0.14	2.80	81084.0	133.10***
O_3 quadratic (OQ)	1	0.0006	0.01	6012.0	9.87**
$SO_2 \times O_3$	4				
SL × OL	1	0.01	0.20	3423.7	5.62*
SL × OQ	1	0.21	0.42	850.0	1.39
SQ × OL	1	0.21	0.42	827.0	1.36
SQ × OQ	1	0.007	0.14	567.0	0.93
Nematode × SO_2	2				
Nem × SL	1	0.152	3.04	29686.0	49.0***
Nem × SQ	1	0.0017	0.034	1931.1	3.17
Nematode × O_3	2				
Nem × OL	1	0.110	2.2	6865.68	11.27**
Nem × OQ	1	0.00014	0.0028	217.0	0.36
Nematode × $SO_2 \times O_3$	4				
Nem × SL × OL	1	0.159	3.18	5293.0	8.69**
Nem × SL × OQ	1	0.045	0.91	1248.9	2.05
Nem × SQ × OL	1	0.0033	0.06	682.3	1.12
Nem × SQ × OQ	1	0.0033	0.06	219.3	0.36
Error	18	0.05	—	609.2	—

Values marked with asterisks are significant at $P=0.05$ (*), 0.01 (**) or 0.001 (***); otherwise not significant at $P=0.05$.

effect of both the gases in causing foliar injuries was further enhanced on nematode infected plants.

Gaseous air pollutants can injure the guard cells of stomata and render them permanently open (Black and Unsworth, 1980; Bennett et al., 1992; Khan and Khan, 1994b). Owing to the wider opening of pores, tomato plants transpired more water. The nematode infection also induced a wider opening of stomata and increased transpiration rates. A stimulatory effect on transpiration caused by root-knot nematode infection has been reported for tobacco and soybean (Odihirin, 1971; Mjuge and Estey, 1978). However, a decrease in stomatal count caused by the gases or nematodes was so marginal that it could not influence transpiration rates.

The nematode infection alone caused an increase of 3.6 and 15.6% in pore width of stomata and water loss ml g^{-1} dry shoot, respectively. Wider stomatal pores and higher transpiration rates caused by nematode must have accelerated the uptake of the SO_2/O_3, as there is a direct correlation between gas diffusion and transpiration rate (Black and Unsworth, 1980). Probably for this reason, even 50 ppb of either gas caused the measurable visible injury and yield reductions in infected plants. Another factor which appeared to be responsible for the synergistic effects of gas-nematode treatments is the higher gall density on exposed plants. This suggested that the gases favoured the penetration and development of the nematode juveniles. The effect may have resulted from unknown translocatable change(s) induced by the gases. However, both parameters of nematode reproduction (egg mass production and fecundity) were low in the exposed plants, especially in the treat-

Table 5
Mean of squares for single and joint effects of SO_2, O_3 and root-knot nematode on number and width of stomata

Source of variation	DF	Stomatal count		Stomatal width	
		MS	F-value	MS	F-value
Nematode (NEM)	1	2833611	1.26	1.04	61.18***
SO_2	2				
SO_2 linear (SL)	1	11063626	4.92*	0.69	40.59***
SO_2 quadratic (SQ)	1	2123517	0.94	0.02	1.18
O_3	2				
O_3 linear (OL)	1	21206400	9.42**	0.92	54.12***
O_3 quadratic (OQ)	1	2572668	1.14	0.05	2.94
$SO_2 \times O_3$	4				
SL × OL	1	21243	0.009	0.025	1.47
SL × OQ	1	9718	0.004	0.001	0.06
SQ × OL	1	13974	0.006	0.003	0.18
SQ × OQ	1	377	0.0002	0.001	0.06
Nematode × SO_2	2				
Nem × SL	1	60100	0.026	0.003	0.18
Nem × SQ	1	1995	0.0009	0.001	0.06
Nematode × O_3	2				
Nem × OL	1	26400	0.011	0.001	0.06
Nem × OQ	1	56336	0.025	0.001	0.06
Nematode × $SO_2 \times O_3$	4				
Nem × SL × OL	1	332640	0.15	0.059	3.48
Nem × SL × OQ	1	710290	0.32	0.010	0.59
Nem × SQ × OL	1	211603	0.09	0.007	0.41
Nem × SQ × OQ	1	10421	0.005	0.002	0.12
Error	18	2250391	—	0.017	—

Values marked with asterisks are significant at $P=0.05$ (*), 0.01 (**) or 0.001 (***); otherwise not significant at $P=0.05$.

ments where growth reductions were much higher. This indicates that at the egg laying stage, nematode females could not obtain sufficient nutrients owing to poor health of the host plant or owing to low allocation of photosynthates to roots (Tingey et al., 1973).

This investigation reveals statistically significant linear effects of SO_2, O_3 and nematodes on the tested variables. Stomatal opening and transpiration rate governed the response of tomato plants to these pollutants. A slight increase in pore width accompanied an increase in the transpiration rate. Nematode infection apparently accelerated transpiration rates and increased the diffusive intake of gaseous pollutants, which determined the interaction between the gases. For this reason, effects of SO_2 and O_3 were significant (synergistic/antagonistic) on inoculated plants, whereas the effects were insignificant (additive) on uninoculated plants. As a result of this, SO_2+O_3 at a concentration as low as 50 ppb caused visible injury and 9.4% greater yield reduction (total 32.6%) in infected plants. In most urban areas, SO_2 and/O_3 concentrations generally occur at or around 50 ppb. If fields exposed to such levels are infested with root-knot nematodes, the agricultural productivity is likely to be reduced further owing to synergistic effects.

Acknowledgements

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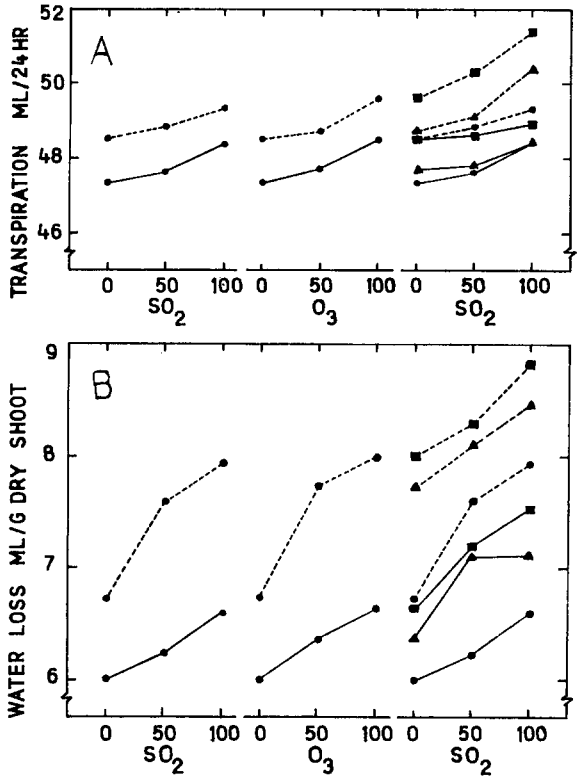

Fig. 5. Effects of SO_2 and/or O_3 on transpiration rates (loss of water through foliage) ml $24\,h^{-1}$ (A) and ml g^{-1} dry leaf (B) of tomato plants inoculated with the root-knot nematode, *Meloidogyne incognita*, or uninoculated. (----) With nematode; (——) without nematode; • 0 ppb O_3; ▲ 50 ppb O_3; ■ 100 ppb O_3.

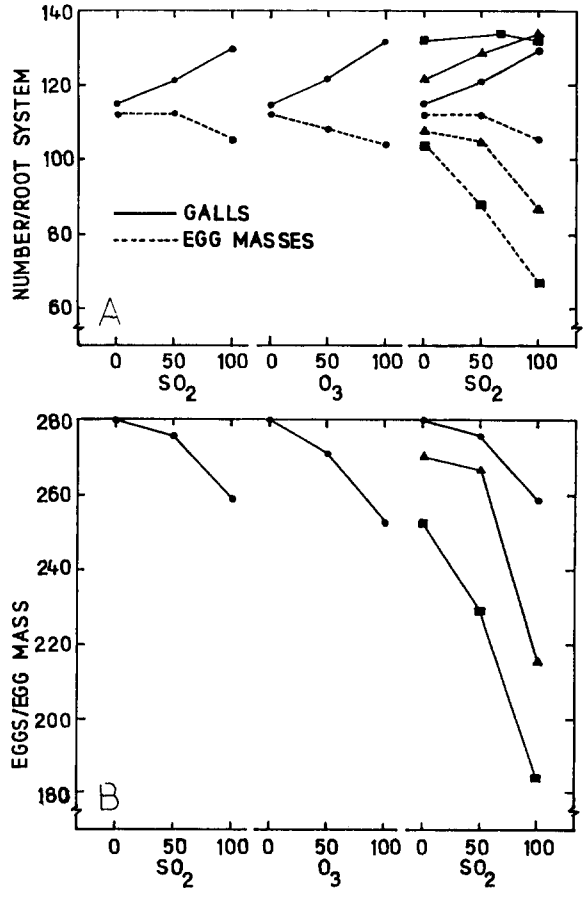

Fig. 6. Effects and SO_2 and/or SO_2 on the gall formation and egg mass production (A) and fecundity (number of eggs per egg mass) (B) of root-knot nematode, *Meloidogyne incognita*, on tomato roots. • 0 ppb O_3; ▲ 50 ppb O_3; ■ 100 ppb O_3.

Table 6
Mean of squares for single and joint effects of SO_2, O_3 and root-knot nematode on transpiration and water loss per gram of dry shoot

Source of variation	DF	Transpiration (ml per day)		Water loss per gram of dry shoot	
		MS	F-value	MS	F-value
Nematode (NEM)	1	20.1	11.04**	14.1	113.70***
SO_2	2				
SO_2 linear (SL)	1	6.6	3.63	3.74	30.16***
SO_2 quadratic (SQ)	1	0.68	0.37	0.017	0.14
O_3	2				
O_3 linear (OL)	1	9.2	5.05*	4.69	37.82***
O_3 quadratic (OQ)	1	0.33	0.18	0.18	1.45
$SO_2 \times O_3$	4				
SL × OL	1	0.27	0.39	0.0096	0.078
SL × OQ	1	0.24	0.13	0.00023	0.0018
SQ × OL	1	0.61	0.33	0.017	0.137
SQ × OQ	1	0.12	0.07	0.008	0.064
Nematode × SO_2	2				
Nem × SL	1	0.96	0.53	0.558	4.50*
Nem × SQ	1	0.57	0.31	0.0045	0.036
Nematode × O_3	2				
Nem × OL	1	0.96	0.53	0.636	5.13*
Nem × OQ	1	0.005	0.003	0.0005	0.004
Nematode × SO_2 × O_3	4				
Nem × SL × OL	1	0.60	0.33	1.271	10.25**
Nem × SL × OQ	1	0.010		0.766	6.18*
Nem × SQ × OL	1	0.17	0.005	0.813	6.56*
Nem × SQ × OQ	1	0.017	0.009	0.04	0.32
Error	18	1.82	—	0.124	—

Values marked with asterisks are significant at $P=0.05$ (*), 0.01 (**) or 0.001 (***); otherwise not significant at $P=0.05$.

Table 7
Mean of squares for single and joint effects of SO_2 and O_3 on number of galls and egg masses per root system and eggs per egg mass of root-knot nematode, *Meloidogyne incognita*

Source of variation	DF	Galls		Egg masses		Eggs/egg mass	
		MS	F-value	MS	F-value	MS	F-value
SO_2	2						
SO_2 linear (SL)	1	234.0	7.96*	1452.0	30.0***	7057.0	52.47***
SO_2 quadratic (SQ)	1	1.36	0.05	93.4	1.93	667.0	4.96
O_3	2						
O_3 linear (OL)	1	341.0	11.60**	1610.0	33.26***	7551.0	56.14***
O_3 quadratic (OQ)	1	2.8	0.09	17.4	0.36	78.0	0.58
$SO_2 \times O_3$	4						
SL × OL	1	105.0	3.57	465.0	9.61*	1081.0	8.04*
SL × OQ	1	12.0	0.41	16.8	0.35	203.0	1.51
SQ × OL	1	34.0	1.16	0.38	0.008	63.4	0.47
SQ × OQ	1	0.34	0.11	1.04	0.02	15.0	0.11
Error	9	29.4	—	48.4	—	134.5	—

Values marked with asterisk are significant at $P=0.05$ (*), 0.01 (**) or 0.001 (***); otherwise not significant at $P=0.05$.

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