Growth of Radish and Marigold Following Repeated Exposure to Nitrogen Dioxide, Sulfur Dioxide, and Ozone

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ABSTRACT

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Radish and marigold plants were exposed to 0.3 ppm of nitrogen dioxide (NO_2), sulfur dioxide (SO_2), and/or ozone (O_3) nine times during a 3-wk period. No interactions among NO_2, SO_2, and O_3 were detected in measurement of radish foliage and root dry weight. Treatments containing O_3 reduced radish foliage and root (hypocotyl) dry weight 356 and 531 mg/plant, respectively. Interactions among NO_2, SO_2, and O_3 occurred in shoots and roots of marigold. SO_2 alone reduced marigold shoot and root dry weight, but this effect was reversed in the presence of O_3. The suppressive effect of SO_2 on root weight was also reversed by NO_2. Treatments containing SO_2 reduced dry flower weight 0.17 g/plant, but effects of the pollutant interactions observed in shoots and roots were not present.

Additional key words: air pollutants, *Rhaphanus sativus, Tagetes patula*

Chronic stress to crop plants may occur in the environment from phytotoxic air pollutants at low concentrations over long periods of time. However, repeated exposures at higher, peak concentrations may also occur over short periods of time in both urban and occasionally rural areas near stationary sources of pollutants. These repeated exposures at peak concentrations may be continuous for several days or be intermittent.

Experimental exposures of two-pollutant mixtures involving chronic and repeated exposures have been reviewed (5). Sulfur dioxide (SO_2) from combustible fuel sources and ozone (O_3) produced photochemically are the two pollutants most frequently found in mixture in ambient atmospheres. Because nitrogen dioxide (NO_2) is also produced from combustible fuels, it is a third pollutant to consider in pollutant mixture studies.

Assessment of interaction between SO_2 and O_3 has appeared in several publications. Tingey et al (7) found that the effects of SO_2 and O_3 on radish root weight were less than additive after chronic exposure. Chronic exposure of tobacco and alfalfa (8) to SO_2 and O_3 resulted in weight changes equal to the additive effect of the single pollutants (tobacco) and less than the additive effects of the single pollutants (alfalfa). Single acute exposures of radish to SO_2 and O_3 resulted in changes in root (hypocotyl) weight that were not different from the individual pollutant effects (8).

More recently, the effects of NO_2, SO_2, and O_3 were studied using radish. Reinert and Gray (4) found that the three pollutants were independent of each other in producing smaller radish roots (hypocotyl) and foliage after a single exposure at 0.2 and 0.4 ppm of each pollutant. Further tests (Sanders and Reinert, *unpublished data*) involving only three repeated exposures of radish at different ages to 0.3 ppm of each pollutant, singly and in mixture, showed a greater than additive reduction in root weight from SO_2 and O_3, averaged over the presence and absence of NO_2.

Because plants respond differently among species to mixtures of pollutants in both single and repeated exposure experiments, we designed a study involving nine repeated exposures over a 3-wk period using radish and marigold as test plants. The objectives of the study were to determine whether NO_2, SO_2, and O_3 effects were dependent or independent of each other in terms of dry weight changes in each species and to determine whether the two species differed in their response to mixtures of the three pollutants after numerous exposures.

MATERIALS AND METHODS

Plants were grown in a commercial preparation of peat, perlite, and vermiculite; steam-pasteurized soil; and sand (4:2:1). Five to 10 radish (*Raphanus sativus* L. 'Cherry Belle') seeds were seeded directly into 24 plastic pots measuring 10 cm in diameter. Radish seedlings were thinned to one plant per pot before pollutant fumigation. Marigold (*Tagetes patula* L. 'King Tut') seeds were planted in vermiculite. Individual seedlings were transplanted to 24 plastic pots measuring 10 cm in diameter 7 days after seeding. Plants were grown in a greenhouse supplied with charcoal-filtered air. Environmental conditions and cultural practices were similar to those previously described (4).

The 24 plants of each species were randomly assigned to eight pollutant treatments (charcoal-filtered air for the control, NO_2, SO_2, O_3, $NO_2 + SO_2$, $SO_2 + O_3$, $NO_2 + O_3$, and $NO_2 + SO_2 + O_3$) and exposed nine times to 0.3 ppm of each pollutant for 3 hr each time. Radish plants were 5 days old at the first exposure and 23 days old at the ninth exposure. Marigold plants were exposed 6 hr at each exposure to 0.3 ppm of each pollutant and were 14 days old at the first exposure and 32 days old at the ninth exposure. The experiment using both species was repeated a second time.

The air pollutants were dispensed into continuously stirred tank reactor exposure chambers (2) located in the greenhouse.

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Table 1. Mean square values from the analysis of variance of radish and marigold response variables[a]

		Radish			Marigold, dry weight		
		Dry weight[b]		Fresh weight			
Source	d.f.	Foliage	Root	Root	Shoot	Root	Flower
Replication	1	0.132	1.193	72.307	1.565*	0.688**	0.011
Air pollutants	7	2.525**	5.295**	233.135*	0.845	0.561	0.113
NO_2	1	0.426	0.028	10.865	0.072	0.140	0.234
SO_2	1	0.294	1.658	79.787	0.006	0.004	0.384*
$NO_2 \times SO_2$	1	0.200	0.055	2.522	0.284	0.981**	0.000
O_3	1	15.244**	33.380**	1,459.753**	0.022	0.040	0.008
$NO_2 \times O_3$	1	0.349	0.269	23.898	0.799	0.006	0.020
$SO_2 \times O_3$	1	0.016	0.861	44.878	3.869**	2.640**	0.089
$NO_2 \times SO_2 \times O_3$	1	1.145	0.367	0.238	0.861	0.118	0.056
Error	7	0.343	0.550	35.118	0.686	0.204	0.085
Residual	32	0.287	0.238	7.182	0.181	0.080	0.028
Total	47						

[a] The experimental design for each species consisted of two replications, eight pollutant treatments, and a three-plant experimental unit for a total of 48 plants. Levels of significance: $P = 0.05$ (*) and $P = 0.01$ (**).
[b] Mean square values and error mean square for radish foliage and root (hypocotyl) dry weight have been multiplied by 10^1.

O_3 was generated with a Welsbach generator by silent electrical discharge in dry oxygen. SO_2 and NO_2 were dispensed from separate tanks that contained 1% of each gas in dry N_2. O_3 and NO_2 were monitored with chemiluminescence monitors and SO_2 was monitored with a flame photometric analyzer. (Pollutants were monitored continuously on a time-shared basis.)

The percentage of visible injury (chlorosis and necrosis) of the total plant leaf surface for each species was estimated at 5% increments (0–100% scale) 2 days after the final exposure. Radish and marigold plants were harvested 30 and 58 days, respectively, from seeding and were oven-dried at 70 C for 72 hr, after which dry weights of shoots, roots, and flowers (marigold) were recorded. Radish root (hypocotyl) fresh weight was recorded before drying. Data from radish and marigold were evaluated separately using analysis of variance. The experiment consisted of two replications, eight pollutant treatments, and three plants per experimental unit for a total of 48 plants of each species. A 2 × 2 × 2 factorial arrangement of the eight pollutant treatments was used to determine interaction among the three pollutants. The overall treatment sum of squares was partitioned into an orthogonal set of contrasts representing the main and interaction sums of squares, and differences were determined by an *F*-test (1,3).

Table 2. Mean dry weight and significant factorial effects of radish and marigold plant parts after exposure to various pollutants[a]

	Radish			Marigold		
	Dry weight (mg)		Fresh weight (g)	Dry weight (g)		
Treatment	Foliage	Root	Root	Shoot	Root	Flower
Control	594	746	15.13	3.35	1.88	0.70
NO_2	569	607	13.09	3.11	1.63	0.81
SO_2	394	467	10.94	2.34	1.05	0.37
$NO_2 + SO_2$	646	482	8.26	2.94	1.56	0.61
O_3	182	28	0.58	2.73	1.39	0.56
$NO_2 + O_3$	245	94	1.64	2.51	1.29	0.73
$SO_2 + O_3$	201	29	0.53	3.39	1.69	0.54
$NO_2 + SO_2 + O_3$	150	27	0.39	2.94	1.97	0.56
Main and interaction effects[b]						
NO_2	60	−15	−0.95	−0.08	0.11	0.14
SO_2	−50	−118	−2.58	−0.02	0.02	−0.18*
$NO_2 \times SO_2$	41	22	−0.46	0.15	0.29**	0.00
O_3	−356**	−531**	−11.07**	−0.04	0.06	−0.03
$NO_2 \times O_3$	−54	47	1.41	−0.26	−0.02	−0.04
$SO_2 \times O_3$	12	85	1.93	0.57**	0.47**	0.09
$NO_2 \times SO_2 \times O_3$	−98	−55	−0.14	−0.27	−0.10	−0.07

[a] Each weight value is a mean of six plants: two replications and a three-plant experimental unit. Both radish and marigold were exposed nine times to 0.3 ppm each of nitrogen dioxide (NO_2), sulfur dioxide (SO_2), and ozone (O_3), alone and in mixture.
[b] The linear additive model used to evaluate these effects assumes that fixed treatment effects sum to zero. The main and interaction effects represent the deviation in the plant's response from zero on a per plant basis. The following examples (N = NO_2, S = SO_2, O = O_3, CON = control) show how the main and interaction effects are determined using the eight pollutant treatment means:
Main effect of O_3 = 1/4[(NSO) − (NS) + (SO) − (S) + (NO) − (N) + (O) − (CON)];
Interaction of $SO_2 \times O_3$ = 1/4[(NSO) − (NS) + (N) − (NO) + (SO) − (S) + (CON) − (O)];
Interaction of $NO_2 \times SO_2 \times O_3$ = 1/4[(NSO) − (NS) + (N) − (NO) + (S) − (SO) + (O) − (CON)].
Levels of significance: $P = 0.05$ (*) and $P = 0.01$ (**).

RESULTS

Radish. O_3 symptoms ranged from upper-surface necrotic flecks to large, bifacial necrotic areas. SO_2 symptoms appeared as small necrotic lesions. Pollutant combinations containing O_3 resulted in O_3-type symptoms. Visible injury from the $NO_2 + O_3$, $SO_2 + O_3$, and $NO_2 + SO_2 + O_3$ treatments appeared to be less than additive as compared with the individual pollutants. The $NO_2 + SO_2$ treatment induced greater than additive injury.

The analysis of variance indicated that there was a significant O_3 effect on dry weight of foliage and fresh and dry weight of roots (hypocotyls) that was consistent regardless of the presence and absence of NO_2 or SO_2 (Table 1). For example, radish root dry weight was reduced an average of 531 mg in plants exposed to pollutant treatments containing O_3 as compared with plants exposed to pollutant treatments without O_3 (Table 2). Ozone treatments also significantly reduced the dry weight of foliage. No main effect of NO_2 or SO_2 occurred, nor were there any significant interactions among the three pollutants in terms of foliage and root dry weight.

Marigold. No visible injury resulted from the NO_2 treatment. Small, upper-surface necrotic lesions on fully expanded leaves characterized O_3 injury. Treatments containing O_3 induced visible injury similar to that from O_3 alone. Bleached, bifacial lesions occurring along the leaf margins were characteristic of SO_2 injury. Visible injury from the NO_2 + SO_2, SO_2 + O_3, and NO_2 + SO_2 + O_3 treatments was less than additive as compared with the individual pollutants.

Injury from the $NO_2 + O_3$ treatment was slightly greater than additive.

The analysis of variance of marigold weight variables did not indicate any significant difference among air pollutant treatments before partitioning (Table 1). The partitioning of the treatments revealed that pollutant effects could be described on the basis of interactions. The reductions in shoot and root weight caused by SO_2 alone (30 and 44%, respectively) and by O_3 alone (19 and 26%, respectively) were significantly reversed when SO_2 and O_3 were both present, regardless of whether NO_2 was present or absent. The weight loss in roots caused by SO_2 was also reversed by the presence of NO_2 regardless of the presence or absence of O_3. Thus, there was a significant antagonistic effect of one pollutant on the effect of another; the weight loss caused by SO_2 depended on the presence of NO_2 or O_3. These interaction effects did not occur on flower weight. Treatments containing SO_2 significantly reduced flower weight 0.18 g/plant (Table 2).

DISCUSSION

Designing experiments in the greenhouse to simulate doses from chronic exposure is difficult because of the infinite number of choices available involving pollutant concentration, duration, and frequency of exposure. We have attempted in this study to stress radish and marigold with repeated exposures (three times per week for three weeks) to NO_2, SO_2, and O_3 at concentrations slightly above ambient to identify different responses of species to pollutant mixtures. We found that the two species were different in their responses and that the pollutants did not interact to produce weight changes in radish but did interact to produce weight changes in marigold.

Several recent studies have reported the effects of NO_2, SO_2, and O_3 alone and in mixture, using radish as a test plant (4,6). Each study was run under slightly different conditions: single exposure at different pollutant concentrations and exposure duration (4); three exposures for 1 wk at three ages of plants (Sanders and Reinert, *unpublished data*); and single exposures of different ratios of three concentrations of three pollutants in mixture (6). The first study (4) demonstrated the independent effects of exposure duration (3 and 6 hr) and equal concentrations (0.2 and 0.4 ppm) of NO_2, SO_2, and O_3 alone and in mixture on radish foliage and root (hypocotyl) weight. In the second study (Sanders and Reinert, *unpublished data*), three repeated exposures of radishes at different ages to SO_2 and O_3 resulted in a greater than additive reduction in radish root (hypocotyl) weight. In the present study, the effects of the three pollutants on weight changes were again independent. In the third study (6), where all three pollutants were present in mixtures at different concentration ratios ranging from 0.1–0.4 ppm, the influence on radish root (hypocotyl) weight of one pollutant was dependent on the concentration of the other two pollutants. The nature of the interaction was antagonistic (less than additive) when the concentration of any one of the three pollutants was increased (6). In the case of radishes, the dependence or independence of the three pollutants in causing weight changes depends on several factors: pollutant concentration, plant age, the ratio and concentration of one pollutant to another, and single vs. repeated exposures.

King Tut marigold demonstrated a different concept of pollutant interaction. Sanders and Reinert (*unpublished data*) found less than additive $SO_2 \times O_3$ interaction on root weight and less than additive $NO_2 \times SO_2$ interaction on root weight. These effects occurred when marigold plants of different ages were exposed three times. In the present study, effects of SO_2 on root weight were strongly dependent on the presence of both NO_2 and O_3, and the effects of SO_2 on shoot weight were dependent on the presence of O_3 after nine exposures.

Care must be taken in interpreting results and making generalizations from different experimental designs. Radish and marigold responded differently to repeated exposure to each of the three pollutants alone and in mixture. Biomass reductions in radish were only produced by treatments containing O_3 and were not significantly influenced by NO_2 and SO_2. With marigold, the effects of one pollutant were dependent on the presence of a second pollutant. Interaction of the pollutants were numerous, and weight changes of marigold plant parts were dependent on these interactions. King Tut was less sensitive to NO_2 and O_3 than to SO_2. Thus, it may be extremely difficult to estimate yield loss and economic impact of air pollutant mixtures on numerous plant species. Finally, the potential increased use of fossil fuels, producing greater amounts of NO_2 and SO_2, requires that a greater data base be developed to determine how NO_2 and SO_2 modify ambient oxidant effects on crop plants grown under both greenhouse and field conditions.

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