

Effects of Nitric Oxide, Nitrogen Dioxide and Nitric Oxide-Nitrogen Dioxide Pretreatments on the Sensitivity of Tomato cv. Rutgers to Ozone

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Introduction

Plant research with gaseous mixtures has revealed pollutant-plant interactions which profoundly effect plant response. These pollutant-plant interactions can be characterized as being synergistic (response significantly greater than additive effects of plants exposed to single pollutants), additive, or antagonistic (response less than additive effect of plants exposed to single pollutants).

Studies of these interactions are of considerable importance since plants growing in the ambient environment (unlike those of most laboratory experiments) usually are exposed to more than one pollutant simultaneously or sequentially over a period of 24 hours. Laboratory research has indeed confirmed that simultaneous exposures to gaseous mixtures such as O_3 and SO_2 can significantly effect plant response. (2, 4, 9).

Most studies of interaction effects have focused on simultaneous exposures to gaseous mixtures. In many ambient atmospheric situations, however, concentrations of specific pollutants show fluctuations which are periodic, reaching maximum levels at predictable hours of the day. In urban areas which have significant auto emissions, peak ozone levels occur at midday. This ozone peak is preceded by peaks in nitrogen dioxide (circa 10:00 am) and nitric oxide (circa 8:00 am). Therefore, under ambient conditions, plants may not only be exposed to more than one pollutant simultaneously, but are also exposed to some pollutants sequentially.

This study was designed to determine the response of test plants to sequential exposures to nitric oxide, nitrogen dioxide and ozone at several exposure levels and sequences. Specific emphasis was placed on the determination of the effects of pre-exposure to nitric oxide, nitrogen dioxide and nitric oxide following by nitrogen dioxide on symptom development in plants subsequently exposed to phytotoxic levels of ozone.

Materials and Methods

Tomato seedlings, *Lycopersicon esculentum* cv. Rutgers were grown in 10 cm diameter plastic pots containing a 1:1:1 mixture of peat, perlite and loam soil supplemented with a 12:12:12 water soluble fertilizer. These seedlings were grown in an environmental chamber with a 27/17°C day/night temperature regime and a 14-hour photoperiod. Relative humidity was uncontrolled ranging from 40-60% during the light period and 60-80% during the dark period. Cool white fluorescent and incandescent lamps provided a light intensity of 8.93×10^4 ergs $cm^{-2} sec^{-1}$ at the plant surface. Plants were refertilized with a 12:12:12 slow release granular fertilizer mix (Osmocote) 2 weeks after transplanting. Plants grown to the second nine-leaflet state were utilized for all exposures.

Exposures were conducted in two 10 cubic foot volume plexiglass exposure chambers. Air flow through the chambers was approximately 15 cubic feet per

minute. During exposures, exposure chamber temperature and relative humidity were maintained at $27 \pm 2^\circ\text{C}$ and $70 \pm 2\%$, respectively. Light intensity of $10.7 \times 10^{-4} \text{ ergs}^{-2} \text{ sec}^{-1}$ was provided by cool white fluorescent and incandescent lamps above the exposure chambers. Ozone was produced for all exposures by an Alron high voltage ozone generator. Ozone concentrations were monitored during exposure by a Mast oxidant meter calibrated with the Potassium Iodide-Boric Acid Method (3). Prepared nitric oxide (0.1%)-nitrogen and nitrogen dioxide (0.2%)-nitrogen gas mixtures were used for nitric oxide and nitrogen dioxide exposures, respectively. The desired exposure concentrations were achieved by further diluting these gas mixtures with room air which was drawn into the exposure chambers. Nitrogen dioxide concentrations were monitored by drawing a 30 minute six liter air sample through a bubbler containing absorbing reagent. The sample was analyzed by using the Sodium Arsenite Method (1). Nitric oxide concentrations were also monitored by the same method. For nitric oxide determinations it was necessary to convert the nitric oxide into nitrogen dioxide by drawing the air sample through a U-tube containing a chromium trioxide medium.

As a part of the experimental design all plants used in this study were subjected to a phytotoxic exposure of 0.20 ppm/3 hours ozone. Half of these plants were, in addition, pretreated with non-phytotoxic exposures of nitric oxide and nitrogen dioxide at several exposure levels and sequences. These included (1) 1 ppm NO/3 hrs. (2) 0.35 ppm NO/3 hrs. (3) 1 ppm NO₂/3hrs. (4) 0.35 ppm NO₂/3 hrs and (5) 0.35 ppm NO/2 hrs. followed by 0.35 ppm NO₂/2 hrs. Plants which were exposed to ozone but were not pretreated with nitric oxide or nitrogen dioxide served as controls. In preliminary exposures to the above levels of nitric oxide and nitrogen dioxide these gases were observed not to be phytotoxic, that is, no visible symptoms were produced from such exposures.

Because of limited chamber space available, only four tomato plants were exposed per chamber for a given exposure. One chamber was used for sequential exposures to nitric oxide, nitrogen dioxide and ozone; plants in the second chamber were only exposed to ozone. To provide sufficient numbers of plants for statistical evaluation, each of the five experiments was conducted three times and the data pooled. In each case sequential exposures were paired with exposures to ozone alone.

After exposure, plants were returned to the environmental chamber. Symptom development was evaluated 96 hours after exposure. Total symptom severity was determined by the % leaf area exhibiting flecking, mottling and bifacial necrosis. In addition, symptom severity based on bifacial necrosis was also determined as % of the leaf area injured. The data were statistically evaluated by employing Student's t-test. An alpha level of .05 was accepted as significant.

Results

The effects of nitric oxide, nitrogen dioxide, and nitric oxide followed by nitrogen dioxide pretreatments on symptom development in tomato plants which were subsequently exposed to a phytotoxic ozone dose are summarized in Table 1. Plants pre-exposed to nitric oxide, 0.35 ppm/3 hrs. (experiment 2) and nitrogen dioxide, 1.00 ppm/3 hrs. (experiment 3) were observed to show a significant antagonistic effect on symptom development in plants which were later exposed to phytotoxic ozone levels. On the other hand, no significant differences were observed in experiments 1 (1.00 ppm NO/3 hrs.), 4 (0.35 ppm NO₂/3hrs.) and 5 (0.35 ppm NO/2 hrs. followed by 0.35 ppm NO₂/2 hrs.). The apparent antagonistic effects as indicated in

TABLE I. *Effect of nitric oxide, nitrogen dioxide, and nitric oxide - nitrogen dioxide pretreatments on ozone-induced foliar injury on tomato. N = 12*

Experiment	Pollutant concentration (ppm) and exposure duration						% leaf area injured
	NO	hrs.	NO ₂	hrs.	O ₃	hrs.	
1	1.00	3	—	—	0.20	3	15.4
	—	—	—	—	0.20	3	24.0
2**	0.35	3	—	—	0.20	3	27.9
	—	—	—	—	0.20	3	37.7
3*	—	—	1.00	3	0.20	3	25.7
	—	—	—	—	0.20	3	45.0
4	—	—	0.35	3	0.20	3	16.5
	—	—	—	—	0.20	3	17.9
5	0.35	2	0.35	2	0.20	3	10.9
	—	—	—	—	0.20	3	18.0

the means of experiments 1 and 5 were not statistically significant at the 0.05 alpha level.

Results presented in Table I are based on total leaf area injured which included symptoms of flecking, mottling, and bifacial necrosis. When the effect on the development of bifacial necrosis, the severest form of injury, was considered results similar to those reported in Table I were obtained.

Discussion

Results of experiments 2(0.35 ppm NO/3 hrs.) and 3 (1.00 ppm NO₂/3 hrs.) indicate that pretreatment with gases such as nitric oxide and nitrogen dioxide which themselves do not induce visible injury, can antagonize the phytotoxic effects of ozone in sequential exposures. These results however, were not supported by the results of experiments 1 (1.00 ppm NO/3 hrs.), 4 (0.35 ppm NO₂/3 hrs.) and 5 (0.35 ppm NO/2 hrs followed by 0.35 ppm NO₂/2 hrs.). The results of experiment one are particularly anomalous, as this exposure did not result in a statistically significant antagonistic response whereas exposure to a smaller dose, 0.35 ppm NO/3 hrs. did. On the other hand, the higher dose of NO₂, experiment 3 resulted in an antagonistic response and the smaller dose did not. Based on dose-response considerations the results of experiments 3 and 4 could be expected but those of 1 and 2 are anomalous.

What is the basis of the anomaly observed in NO pretreatments? It is probable that this is due at least in part to the large variability in data collected. The large data variability in these experiments may have been due to the limitations placed on the study by exposure chamber capacity. To provide sufficient numbers for statistical evaluation 3 exposures were conducted for each experiment. These exposures were conducted on different days. The variation in symptom response from day to day as evidenced in control plants was considerable despite attempts to minimize this day to day variation by controlling pre-exposure environmental growth and exposure conditions.

Antagonistic effects of pollutant gases have been previously reported by Heagle and Johnston (2). In studies with soybean Heagle and Johnston observed that simultaneous exposure to mixtures of SO₂ and O₃ could result in an an-

tagonistic effect when injury from either gas singly was severe. They suggested that the O_3 in the exposure mixtures of $SO_2 + O_3$ could at times protect soybeans from SO_2 . The observation by Heagle and Johnston that antagonism was observed only when plant injury was severe was evaluated in this study by assessing leaf area which exhibited the severest symptoms, bifacial necrosis. Similar results were obtained when symptom development was evaluated on % leaf area injured by bifacial necrosis and total % leaf area injured. Apparently then antagonism in the study reported here was not related to symptom severity.

Unlike the studies of Heagle and Johnston which utilized simultaneous exposures to phytotoxic gases, this study utilized sequential exposures to non-phytotoxic levels of NO and NO_2 followed by an exposure to phytotoxic levels of ozone. In experiments 2 and 3 such exposures apparently produced an antagonistic effect on ozone-induced symptom developed. What is the nature of this antagonism? Antagonistic mechanisms could include physiological changes in NO and NO_2 exposed plants such as partial stomatal closure and/or biochemical changes which protect plants from ozone. No experimental evidence on antagonistic mechanisms is, however, available.

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