Changes in Growth Rate and Nitrogen Content of Tomato Plants After Exposure to NO_2

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ABSTRACT

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Lycopersicon esculentum (tomato) plants were grown in sand culture under two different levels of nitrogen nutrition (28 mg/liter NO_3-N and 140 mg/liter NO_3-N) supplied in Hoagland's solution. One month after being transplanted, one group of plants was fumigated either with 0.47 mg/m^3 NO_2 for 80 hr or with 0.76 mg/m^3 for 164 hr and a second group received charcoal-filtered air. The stems and/or leaves were harvested immediately and/or 48 hr after fumigation. Tissue analyses (Kjeldhal method) indicated that total N content had increased following exposure to NO_2, particularly in the leaves of the 140 mg/liter NO_3-N treatment, as had fresh and dry weights. No NO_2 injury was apparent on any of the fumigated plants.

Additional key words: air pollution, nitrogen dioxide, *Lycopersicon esculentum*.

Nitrogen dioxide (NO_2) gas present in the ambient atmosphere originates from combustion processes in which heated nitrogen (N_2) and oxygen (O_2) combine to form nitric oxide (NO) which is readily oxidized to NO_2. Nitrogen dioxide is soluble in cold water and decomposes in hot water as per the following (4, 5):

$$2NO_2 + H_2O \rightarrow HNO_3 + HNO_2 \quad \text{eq. 1}$$

$$3HNO_2 \rightarrow HNO_3 + 2NO + H_2O \quad \text{eq. 2}$$

Atmospheric concentrations of NO_2, measured for approximately 10 yr in federal and state networks, show high concentrations in the Los Angeles area, but NO_2 concentrations in New Jersey (1) are reported to be below 0.19 mg/m^3 approximately 90% of the time. Although the higher concentrations of NO_2 exist only sporadically, levels presumably never reach a zero value.

Reports (3, 8, 15, 16, 17, 18) indicate that NO_2 is toxic or inhibitory to plants. Although Hill (7) has demonstrated that plants have the capacity to remove NO_2 from the atmosphere, the fate of NO_2 within the leaf has not been determined. If NO_2 enters plants and reacts according to equations 1 or 2, low atmospheric concentrations may not be detrimental to plant growth. The nitrogen may be absorbed and utilized for growth. If so, increased nitrogen content should be detected in plants exposed to NO_2.

The object of this investigation was to determine the effect of prolonged, low-level exposures to NO_2 on the growth of tomato (*Lycopersicon esculentum* L.) as indicated by increases in fresh and dry weights and to determine nitrogen content after exposure to NO_2.

MATERIALS AND METHODS

Cultural method.—Two-wk-old tomato seedlings (cultivar Rutgers) were transplanted in washed quartz sand in 9.5-liter plastic pots (two plants per pot). After growth for 1 wk in half-strength Hoagland's solution (6) containing 14 mg/liter nitrogen from $Ca(NO_3)_2$, the plants received full-strength Hoagland's solution containing 140 ml of 28 mg or 140 mg/liter NO_3-N every other day, and 140 ml of distilled water on alternate days, for 3 wk. At the end of this time the plants were exposed to NO_2.

Fumigation method.—Two 1.83 × 1.83 × 2.44-m glass chambers, located in the greenhouse, were equipped with a humidifier, air conditioner, and white fluorescent lamps to keep humidity, temperature, and light intensity relatively constant at 70%, 29 C, and 186 lux, respectively. A continuous air stream was introduced into the top of each chamber through a 10-cm diameter port equipped with a baffle to diffuse the air flow. Pumps along the base of the chamber removed air at the rate of one complete exchange every 45 sec. Before entrance into the chamber the air passed through a charcoal filter which absorbed SO_2, O_3, and most particulates. Nitrogen dioxide (purchased from Matheson Gas Products, East Rutherford, NJ 07073) in a pressurized cylinder containing 10% NO_2 and 90% $N_2$was introduced into the filtered air stream passing through the chamber. Gas flow from the cylinder was regulated with a Model 201 A rotameter (Matheson). Ports in the side of the chamber allowed for continuous sampling of the NO_2 concentrations with a Mast T. M. NO_2 meter calibrated

by the Saltzman chemical method (14). Eight to 14 uniform replicate plants of each nutritional treatment (four to seven pots) were placed in each of two chambers; one chamber served as a control into which only charcoal-filtered air was introduced, and the other received NO_2 which was introduced into the charcoal-filtered air stream.

The plants were exposed to low NO_2 concentrations in two separate experiments. In the first experiment (Exp. I) NO_2 averaged 0.47 mg/m^3 for 80 hr, and in the second experiment (Exp. II) 0.75 mg/m^3 for 164 hr, under prevailing diurnal light conditions. For the second fumigation the chambers were reversed, so that the chamber which had served as the control in the first experiment became the NO_2 exposure chamber, and vice versa. The plants received nutrient solution minus a nitrogen source during exposure to NO_2 [Ca $(NO_3)_2$, the nitrogen source, was replaced by appropriate quantities of $CaSO_4$]. During fumigations, the relative humidity was kept at approximately 70 ± 5%, temperature at approximately 29 ± 2 C, and light at 186 lux.

Analytical method.—Tomato stems and/or leaves of eight fumigated and eight control plants from each NO_3-N treatment in Experiment I were harvested 48 hr after fumigation. In Experiment II, six fumigated and six controls from each NO_3-N treatment were harvested immediately after fumigation, and six fumigated and eight controls from each NO_3-N treatment were harvested 48 hr later. The samples were dried overnight in a 20 C forced-draft oven and dry weights recorded. The tissues were ground through a 0.50-mm (40-mesh) screen in a semi-micro Wiley Mill and 100 mg samples of tissues from each plant were analyzed for total nitrogen by the Pepkowitz and Shive modification of the micro-Kjeldhal method (13). Results are reported as mg N per 100 mg dry tissue. The data were analyzed for statistical significance by Student's *t*-test.

TABLE 1. Comparison of fresh and dry weights of leaves and stems of NO_2-fumigated and nonfumigated tomato plants from two NO_3-N treatments harvested 48 hr after exposure to 0.47 mg/m^3 NO_2 for 80 hr

Treatment with NO_3-N and NO_2 exposure	Plant weight[a] (g)			
	Fresh		Dry	
	Leaves	Stems	Leaves	Stems
NO_3-N, 28 mg/liter				
Control	1.33	0.87	0.18	0.06
NO_2-exposed	1.26	0.77	0.17	0.06
% of Control	95	89	94	100
NO_3-N, 140 mg/liter				
Control	3.70	3.00	0.46	0.19
NO_2-exposed	4.41	3.78	0.54	0.24
% of Control	119 y[b]	126	117 y	126

[a]Each value is the mean of eight replicate plants.
[b]Letter y denotes significant differences between NO_2-exposed and control (P = 0.01). No letter denotes no significant difference.

RESULTS

Effect of NO_3-N nutrition on the growth of tomato plants.—Tables 1 and 2 contain average fresh and dry weights of control and NO_2-exposed tomato plants in the two NO_3-nitrogen treatments. Plants that had received low nitrogen (28 mg/liter NO_3-N) were stunted, had purple leaf veins, and symptoms of nitrogen deficiency, especially in Experiment I. Plants that had received 140 mg/liter nitrogen were larger and the foliar parts were of a normal green color.

Effect of low NO_2 concentration on fresh and dry weight.—The fresh and dry weights of tomato leaves and stems from plants exposed to NO_2 were significantly greater than those of control plants grown without NO_2 exposure in the 140 mg/liter NO_3-N treatment ($P = 0.01$) (Table 1). Both fresh and dry weights of fumigated tissue were 13-26% greater than those of control values (Tables 1 and 2). However, there were no significant growth differences in the fresh or dry weights of NO_2 exposed or

TABLE 2. Comparison of fresh and dry weights of leaves and stems of NO_2-fumigated and nonfumigated tomato plants from two NO_3-N treatments harvested immediately or 48 hr after exposure to 0.75 mg/m^3 NO_2 for 164 hr

Treatment with NO_3-N and NO_2 exposure	Plant weight[a] (g)							
	Harvested following exposure to NO_2							
	Immediately				After 48-hr			
	Fresh		Dry		Fresh		Dry	
	Leaves	Stems	Leaves	Stems	Leaves	Stems	Leaves	Stems
NO_3-N, 28 mg/liter								
Control	0.79	0.57	0.13	0.05	1.08	0.86	0.17	0.07
NO_2-exposed	0.77	0.65	0.12	0.05	1.21	0.92	0.18	0.07
% of Control	98	114	92	100	112	107	106	100
NO_3-N, 140 mg/liter								
Control	4.67	3.96	0.64	0.31	5.61	5.16	0.77	0.45
NO_2-exposed	5.38	4.59	0.73	0.36	6.59	6.04	0.87	0.51
% of Control	115	116	114	116	117	117	113	113

[a]Each value is the mean of six-to-eight replicate plants. No letter denotes no significant difference between means.

TABLE 3. Comparison of total nitrogen content of leaves of NO_2-fumigated and nonfumigated tomato plants from two NO_3-N treatments harvested immediately and/or 48 hr after NO_2 exposures of 0.47 mg/m^3 for 80 hr or 0.75 mg/m^3 for 164 hr

Treatment with NO_3-N and NO_2 exposure	Nitrogen content[a] (mg/100 mg dry wt.)		
	0.47 mg NO_2/m^3/80 hr	0.75 mg NO_2/m^3/164 hr	
	48-hr harvest	Immed. harvest	48-hr harvest
NO_3-N, 28 mg/liter			
Control	1.76	1.39	1.15
NO_2-exposed	1.78	1.84	1.53
% of Control	101	132 y[b]	133
NO_3-N, 140 mg/liter			
Control	2.51	2.05	1.93
NO_2-exposed	2.67	2.56	2.28
% of Control	106 y	125 y	118 y

[a]Each value is the mean of six-to-eight replicate plants.

[b]Letter y denotes significant differences between NO_2-exposed and controls ($P = 0.01$). No letter denotes no significant difference between the means.

nonexposed plants grown in the 28 mg/liter NO_3-N solution.

Effect of low NO_2 concentration on total nitrogen content.—Table 3 indicates that there was a highly significant ($P = 0.01$) increase in nitrogen content of the NO_2-fumigated leaf tissues from plants that received the 140 mg/liter NO_3-N treatment in both experiments. A significant increase ($P = 0.01$) also was observed in Experiment II in N content in the leaves of plants grown in the 28 mg/liter NO_3-N solutions (Table 3).

DISCUSSION

Previous exposures of *Nicotiana glutinosa* to high concentrations of NO_2 (1.9 to 2.9 mg/m^3 for 3 hr) caused injury on leaves of plants grown at an optimum NO_3-N level (140 mg/liter) in the nutrient solution, but not on the foliage of plants grown at very low NO_3-N levels (14 mg/liter) (J. Troiano and I. A. Leone, *unpublished*). Decreased response to gases other than NO_2 in nitrogen-deficient plants previously has been observed by Leone and Brennan (10, 11, 12) who also reported that plants grown at an optimal level of N-nutrition were injured more than plants grown with higher levels of N after exposure to O_3 or SO_2.

In the present experiment, low NO_2 exposures of 0.47 mg/m^3 for 80 hr or 0.76 mg/m^3 for 164 hr apparently caused no foliage injury in tomato plants grown in solutions with eigher low (28 mg/liter) or optimal (140 mg/liter) levels of NO_3-N nutrition.

Spierings (15) and Taylor and Eaton (17) reported growth decreases in tomato seedlings exposed to levels of NO_2 similar to those used in Experiment I (0.47 mg/m^3 NO_2) wherein plants in the 140 mg/liter NO_3-N solution showed greater growth after exposure to NO_2. These investigators used 10-day-old tomato seedlings, potted in soil. In our experiments, however, the tomatoes were grown in sand culture, received nutrient solution regularly, and were fumigated after 1 mo of growth. Thus, age of plants, type of culture, time of year, and nutrition may alter the effects of exposure to NO_2 on growth.

Although other investigators have reported increases of total N in plants exposed to NO_2 (15, 18) this phenomenon was not related to increased growth rate. In our study there was increased fresh and dry weight (significant at $P = 0.01$ in Experiment I) as well as increased N content as a result of exposure to NO_2. Conceivably, if N content is increased in a plant by exposure to NO_2 and the N is used for biochemical synthesis, growth should be increased. Evidence of increased synthesis recently has been indicated by reports of increases in ribulose 1, 5-diphosphate carboxylase activity and chlorophyll content in 14-day-old pea seedlings 6 days after exposure to 1.9 mg/m^3 NO_2 (9).

We can offer the following three hypotheses as possible explanations of how N from NO_2 fumigation might become available for plant utilization: (i) the increased N accumulation in leaves could have resulted from stimulated uptake of extraneous NO_3-N through the roots. Even though the cultures were flushed before fumigation with N-free solutions, and received N-free solutions during fumigation, residual NO_3 in the roots or on sand particles could have supplied a source of N. Another source of N for root absorption could have been by dissolution and oxidation of the NO_2 gas within the sand culture medium. In a review, Bohn (2) indicated that soil can absorb NO_2 which subsequently can be converted biologically to the nitrate ion. (ii) exposure to NO_2 might have increased the rate of translocation of N compounds from roots to above-ground parts. (iii) the increased N content after exposure to NO_2 could have resulted from direct absorption by leaves and its utilization in metabolism either before or after conversion to another molecular form.

Utilization of NO_2 in plant metabolism provides a sink for this pollutant and warrants further investigation by the use of isotopically labeled NO_2. However, there are other reasons for further inquiry into the metabolic fate of NO_2 in plants. Effects on peas, pinto beans, oats, radish, soybean, tobacco, and tomato by low concentrations of two or more gas mixtures, none of which causes apparent damage per se (synergism), have been reported to result from concentrations of NO_2 and SO_2 commonly

occurring in the ambient air (9, 19). The role and fate of the individual pollutants should first be evaluated before data on synergistic effects can be interpreted.

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