Plant Physiol. (1966) 41, 132-135

Suppression of Plant Growth by Nitrogen Dioxide[1]

O. C. Taylor and F. M. Eaton
Air Pollution Research Center and Department of Soils and Plant Nutrition, University of California, Riverside, California

Received May 19, 1965.

Summary. *Nicotiana glutinosa* and pinto bean seedlings (*Phaseolus vulgaris*) were exposed for short periods (3 days or less) to high concentrations of NO_2 (4.11–20.53 mg/m^3 to compare the resulting leaf lesions with ozone damage produced at concentrations of 0.43 to 0.86 mg/m^3. Although the same physiological age leaf tissue was damaged by both toxicants, damage caused by NO_2 was unlike that caused by ozone.

Pinto bean (*Phaseolus vulgaris*) and Pearson improved tomato (*Lycopersicon esculentum*) seedlings were continuously exposed for 10 to 22 days, to low concentrations of NO_2 (less than 1.03 mg/m^3). These exposures caused significant growth suppression, increase in green color (total chlorophyll content), and distortion of leaves.

Nitrogen oxides are emitted to the atmosphere in large quantity from the many combustion sources in our mechanized society. The estimated daily emission for Los Angeles in 1963 was 660 tons of which approximately 60 % was from vehicular sources (1). Maximum measured concentrations exceeded 3.0 ppm (6.16 mg/m^3) at times and reached a peak of 3.93 ppm (8.07 mg/m^3) NO_x, but generally concentrations were well below 0.5 ppm (1.027 mg/m^3) with the highest levels occurring during the night (2). The involvement of NO_2 in photochemical reactions in the atmosphere was responsible for reduction in concentration during daylight hours. Because of this reaction Thomas (9) stated that it was unlikely that atmospheric concentrations of nitrogen oxides will ever accumulate to a level that will cause significant plant damage.

Benedict and Breen (4) found that the most sensitive weeds treated in their experiments required 20 ppm (41.06 mg/m^3) of NO_2 to cause damage. According to Middleton et al. (7) pinto bean plants were much more sensitive to NO_2 and required exposure to only 3.0 ppm (6.15 mg/m^3) for 4 to 8 hours to be visibly damaged. Low concentrations of NO_2, approximately equivalent to concentrations in the Los Angeles atmosphere, applied continuously for successive days, caused marked chlorosis of basal leaves of *Nicotiana glutinosa* (5). This chlorotic symptom on the lower more mature leaves may or may not have been accompanied by accelerated leaf abscission.

The studies reported here were attempts to determine the effect of continuously applied low nonlesion inducing concentrations of NO_2 on plant growth, chlorophyll content, and the cation-anion relationship within plants.

[1] This work was supported in part by grant number AP-270 from the Division of Air Pollution, Bureau of State Services, Public Health Service.

Materials and Methods

Pearson improved tomato (*Lycopersicon esculentum*) and pinto beans (*Phaseolus vulgaris*) seedlings for the experiments were grown in a uniform soil mix of equal parts peat and loamy top soil. Tomato seeds were germinated in vermiculite, and single plants were transplanted to 1 liter plastic pots containing 600 g of the soil mix and fumigations were started when the third and fourth true leaves appeared. Five pinto bean seeds were planted in each pot and subsequently thinned to a single healthy seedling. Fumigations were started 6 days after the bean seeds were planted, when the 2 primary bean leaves had a total area of about 25 cm^2.

A dual fumigation chamber was located in a larger glass enclosed chamber equipped with refrigerated air conditioning to facilitate temperature control. The experiments were run in natural sunlight with partial shade. The plant exposure chambers were approximately 0.3 m^3 in volume and large enough to accomodate 20 replications per treatment. A common air supply was first filtered through activated charcoal to remove oxidant air pollutants and the air stream was divided equally between the treatment and control sections of the chamber. NO_2 was continuously added and thoroughly mixed with the air stream entering the treatment chamber. NO_2 was diluted and pressurized with prepurified nitrogen gas in a stainless steel tank to facilitate the introduction of small amounts of NO_2 necessary to maintain the desired NO_2 concentration in the exposure chamber. Concentrations of NO_2 were continuously monitored with a recording analyzer using Saltzman solution (8). The chambers were designed so that the treatment and control plants could be interchanged and plants rerandomized every second or third day to minimize the effect of position on growth response.

The tomato and bean plants were cut off at the cotyledonary node when harvested. Chlorophyll was determined by the method described by Arnon (3) using a wavelength of 652 mμ.

Results

In preliminary experiments exposure of *Nicotiana glutinosa* to 4.72 mg/m³ NO_2 for 8 hours and 40 minutes caused leaf damage. The collapsed and subsequently dead bleached tissue was most prominent at the apex and along the margin of the leaves (fig 1) and most severe on older leaves near the base of the plants. Shorter exposure (5 hrs) to 4.93 mg/m³ NO_2 did not cause visible damage on the *N. glutinosa* plants. Similarly plants of Maryland variety, Catterton, and 2 strains of cigar wrapper tobacco, Bel-W-3 (ozone sensitive) and Bel-B (ozone resistant), were not visibly damaged by exposure to 4.93 mg/m³ NO_2 for 4 days. Nine-day-old pinto bean seedlings did not develop visible symptoms until they were exposed to 20.53 mg/m³ NO_2 for 4 hours.

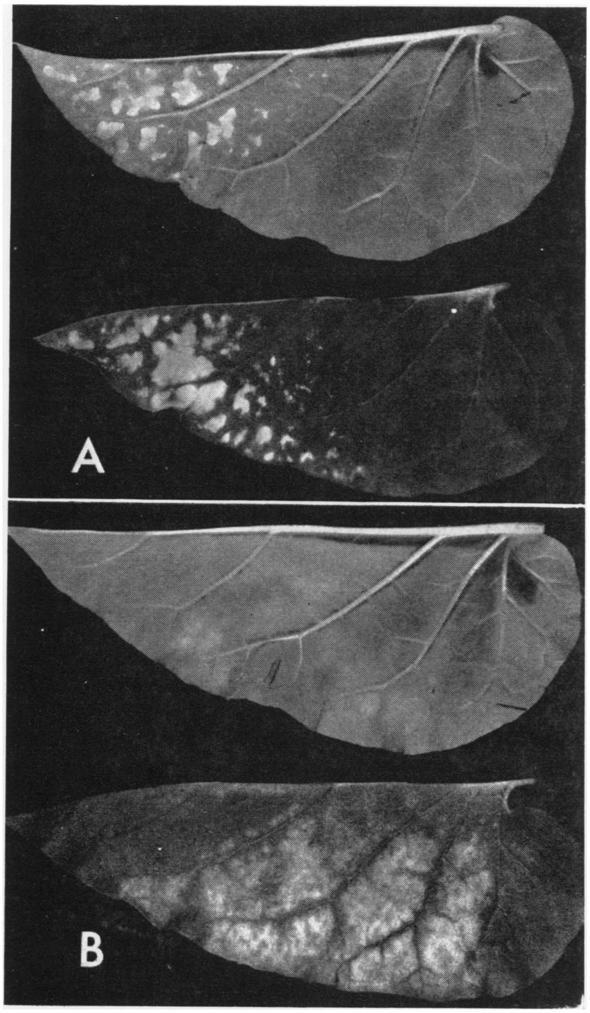

FIG 1. *Nicotiana glutinosa* leaves. A, Necrotic lesions caused by 4.72 mg/m³ NO_2. B, Bleaching of leaves caused by 0.6 mg/m³ ozone.

In the 3 experiments with pinto bean plants an attempt was made to maintain NO_2 concentrations between 0.61 mg/m³ and 0.82 mg/m³, but during the night the concentrations often increased to approximately 1.03 mg/m³ with no appreciable change in flow of air or NO_2. Apparently the rate of NO_2 absorption by the plants declined significantly at night. Continuous exposure of bean plants to the low concentrations of NO_2 caused a gradual but decided change in plant appearance without causing visible necrosis. The most pronounced change was the downward cuping and darker green leaf color of treated plants (fig 2). Overall suppression of growth was evident after 1 week to 10 days (table I). The effect of NO_2 on color of the plants was substantiated by the significant increase in total chlorophyll content based on both fresh and dry leaf weights (table I).

Pearson improved tomato seedlings responded to low concentrations of NO_2 in much the same way as the bean seedlings (table II). Except for the dry weight of plant material in 1 experiment, the suppression of growth was significant or highly significant in all experiments. Characteristically the NO_2 treated tomato plants were much darker green than control plants and the leaflets had a strong tendency to curve downward (fig 2). Total nitrogen content of the tomato plants was not affected by the NO_2 treatments and the trend indicated a slight decrease in nitrate nitrogen (table III). The apparent reduction in nitrates was greater when frozen tissue was analyzed and compared to oven dried tissue, suggesting that 65° temperature of the drying oven may have changed the form of nitrogen in the tissue. There

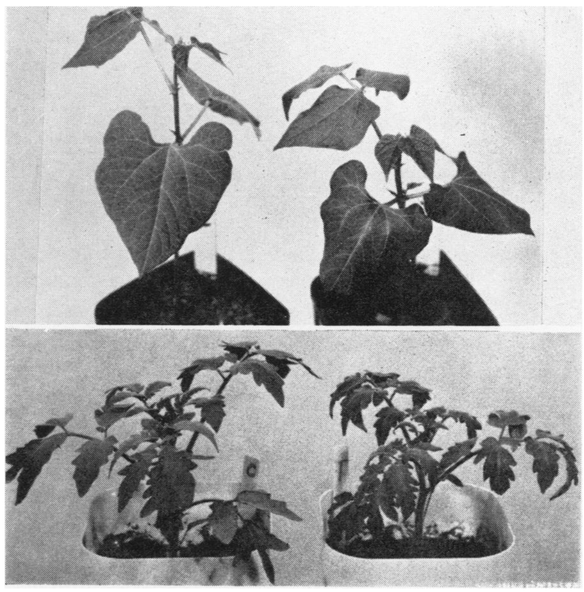

FIG. 2. Distortion of foliage, suppressed growth and intensified green color of tomato and pinto bean seedlings exposed for 19 days to NO_2. Left, Control plants grown in activated charcoal filtered air. Right, Plants grown in atmosphere containing 0.62 mg NO_2 per m³.

Table I. *Effect of NO_2 Exposure on Growth Rate of Pinto Bean Seedlings and Chlorophyll Content of Primary Leaves*

Statistical analysis by t test. N = 20.

Days exposed	Conc NO_2 mg/m[3]	Fr wt (g)		Dry wt (g)		Chlorophyll content (mg/g) Fr wt		Chlorophyll content (mg/g) Dry wt	
		NO_2	Check	NO_2	Check	NO_2	Check	NO_2	Check
10	0.62	1.41	1.94*	0.110	0.141*	2.26	1.84*	25.4	22.9*
19	0.62	3.85	4.53*	0.382	0.423*	2.25	1.90*	24.95	24.58 N.S.
11	0.62	1.76	2.06*	0.195	0.208 N.S.	3.01	2.40*	27.12	23.52*

N.S. Signifies no significant difference.
* Significant difference at 1 % level.

Table II. *Effect of NO_2 Exposure on Growth of Pearson Improved Tomato Plants Expressed as Fresh Weight, Dry Weight and Area of Fifth True Leaf*

Statistical analysis by t test. N = 20.

Days exposed	Conc NO_2 mg/m[3]	Fr wt (g)		Dry wt (g)		5th Leaf area (dm[2])	
		NO_2	Check	NO_2	Check	NO_2	Check
10	0.31–0.43	14.3	18.0*	0.10	0.15*	...	...
14	0.53–1.17	2.3	2.8**	0.17	0.18 N.S.	...	...
19	0.82–1.03	8.7	10.6*	0.66	1.04**	...	...
22	0.27–0.53	6.22	7.94**	0.47	0.59**	0.50	0.62**

N.S. Signifies no significant difference.
* Significant difference at 5 % level.
** Significant difference at 1 % level.

Table III. *Effect of NO_2 Exposure for 19 days on Cation, Nitrate and Total Nitrogen Content of Pearson Improved Tomato Seedlings*

	Ca	Mg	K	Na	Total Cation	NO_3–Nitrogen Oven Dried	NO_3–Nitrogen Quick Frozen	Total nitrogen Oven Dried
	Meq/g of plant tissue							
Control	105	57	127	11	300	37.7	36	380
Treated	96	51	123	11	281	35.9	23	388

was also a trend toward a reduction in Mg, Ca and K content of tissue exposed to NO_2.

Discussion

Acute symptoms on bean, tomato and tobacco seedlings, induced by exposure to high concentrations ($\geqslant$ 4.93 mg/m[3]) of NO_2, resemble necrotic lesions caused by SO_2 or by excessive concentrations of ozone.

The increase in total chlorophyll content, resulting in a deep green coloration of foliage of bean and tomato seedlings fumigated with NO_2 stands in contrast with the findings of Bush et al. (5) after fumigating plants with engine exhausts. Their description of chronic symptoms of *N. glutinosa* resembled in many ways the symptoms obtained by exposure to very low concentrations of ozone. The chlorotic symptoms might be regarded as reasonable because of the oxidizing potential of NO_2, but there was no evidence of such a reaction in our experiments with bean and tomato plants. The effectiveness of NO_2 in oxidizing buffered potassium iodide solution is approximately 10 % that of ozone (6). This same oxidizing relationship was evident in the production of visible necrotic lesions which were produced by 0.54 to 0.64 mg/m[3] ozone. About 10 times as much NO_2 (5.13–7.18 mg/m[3]) was needed to cause damage comparable to ozone.

The symptoms of damage described by Bush (5) and the acute symptoms observed in these investigations suggest that NO_2 damage is caused by the oxidizing properties of the toxicant. By contrast, the increase in chlorophyll per unit weight and the characteristic leaf curvature caused by NO_2 exposure in these investigations have no resemblance to previously described symptoms of oxidant, NO_2 or ozone damage. There was a suggestion of inverse relation between the increased chlorophyll and reduction in plant weight and leaf size: i.e. chlorophyll per leaf was not changed.

Literature Cited

1. Anon. 1964. NO_x from autos varies from community to community. The Clean Air Quarterly

(Publ. by State of Calif. Dept. of Public Health, Bureau of Air Sanitation). 8 (3): 4–5.

2. ANON. 1962. Oxides of nitrogen assume new importance in air pollution. The Clear Air Quarterly (Publ. by State of Calif. Dept. of Public Health, Bureau of Air Sanitation). 6 (1): 1–4.

3. ARNON, D. I. 1948. Copper enzyme in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. Plant Physiol. 24 (1): 1–15.

4. BENEDICT, H. M. AND W. H. BREEN. 1955. Use of weeds as a means of evaluating vegetation damage caused by air pollution. Proc. Natl. Air. Pollution Symp., 3rd Symp., Passadena, Calif.

5. BUSH, A. F., R. A. GLATER, J. DYER, AND G. RICHARDS. 1962. The effects of engine exhaust on the atmosphere when automobiles are equipped with afterburners. Dept. Eng. Report No. 62-63. Univ. of Calif., L. A. p 1–35.

6. LODGE, J. P. AND C. XINTERAS. 1963. Methods of analysis of polluted atmospheres in the U. S. Intern. J. Air Water Pollution 7: 79–90.

7. MIDDLETON, J. T., E. F. DARLEY, AND R. F. BREWER. 1958. Damage to vegetation from polluted atmospheres. J. Air Pollution Control Assoc. 8: 9–15.

8. SALTZMAN, B. E. 1954. Colorimetric microdetermination of nitrogen dioxide in the atmosphere. Anal. Chem. 26: 1949–55.

9. THOMAS, M. D. 1961. Effects of air pollution on plants. World Health Organization. Monogr. Ser. No. 46: 233–78.