Vegetation Injury from the Interaction of Nitrogen Dioxide and Sulfur Dioxide

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ABSTRACT

Six plant species were exposed for 4 hr to nitrogen dioxide and/or sulfur dioxide in greenhouse exposure chambers. Although concentrations of nitrogen dioxide below 200 pphm and concentrations of sulfur dioxide below 50 pphm caused no leaf injury, injury did develop when plants were exposed to mixtures of 5 to 25 pphm of each of the two gases. Leaf injury from either nitrogen dioxide or sulfur dioxide alone occurred as marginal and/or interveinal necrosis on each leaf surface (bifacial). Injury produced by a mixture of the two gases appeared as chlorotic and necrotic flecking on the upper surface of the interveinal areas of tomato, radish, oats, and tobacco. Reddish-brown lesions (stipple) developed on pinto bean and soybean leaves. Lower leaf-surface injury frequently occurred in the mixed-gas fumigations, with little or no upper surface injury. The concentrations of nitrogen dioxide plus sulfur dioxide which caused plant injury were similar to those found in urban areas, and may result in yield losses for plants grown under field conditions.
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Plants grown under field conditions are continuously exposed to an array of gaseous and particulate components in the atmosphere, many of which are phytotoxic. Most greenhouse and field studies have involved single gas exposures, but in order to recognize and understand better the effects of air pollutants on plants, it is necessary to study the response of plants to mixtures of pollutants.

Mixtures of sulfur dioxide and hydrogen fluoride or sulfur dioxide and hydrocarbons did not interact to injure gladioli (8). Thomas et al. (17) observed that plants grown in greenhouses equipped with water-spray scrubbers to remove sulfur dioxide developed more oxidant injury than comparable plants grown in greenhouses without the scrubbers. They suggested that this effect was due to an antagonism between the gases. The increased humidity in the greenhouse with the water scrubbers could have created an environment more favorable for the development of oxidant injury, and this may have accounted for their observations. Middleton et al. (12) found that a sulfur dioxide to ozone ratio of 6:1 produced on Pinto bean leaves symptoms associated with both gases, but when the ratio was decreased to 4:1, ozone appeared to interfere to some extent with the development of sulfur dioxide injury. Menser & Heggestad (11) found that either a 2-hr or 4-hr fumigation with 3 pphm ozone or 30 pphm sulfur dioxide did not injure tobacco plants, but when both gases were present, injury developed which was similar to that produced by ozone. They referred to this enhanced injury as synergistic action between the two gases. Enhanced injury to peanut (1) and white pine (4, 9) occurred when ozone and sulfur dioxide were present in an exposure mixture, each at concentrations of 10 pphm or less. In studies to determine the combinations of air pollutants that were producing undersurface injury to plants, Haagen-Smit et al. (6) exposed five plant species to a mixture of 40 pphm nitrogen dioxide, 10 pphm sulfur dioxide, and 400 pphm hydrocarbons in sunlight. Alfalfa was the only species injured, but the injury was not on the lower surface. When sulfur dioxide was removed from the reaction mixture, "typical smog injury" did develop.

The objectives of our study were (i) to determine if atmospheric concentrations of nitrogen dioxide and sulfur dioxide, which alone were not phytotoxic, would in combination injure plants, and if so, (ii) to describe the symptoms of the injury.

MATERIALS AND METHODS.– Two experiments were performed to study nitrogen dioxide-sulfur dioxide interactions. In the first experiment, tobacco (*Nicotiana tabacum* L. 'Bel W_3') was used. In the second, tobacco, bean (*Phaseolus vulgaris* L. 'Pinto'), tomato (*Lycopersicon esculentum* Mill. 'Roma VF'), radish (*Raphanus sativus* L. 'Cherry Belle'), oats (*Avena sativa* L. 'Clintland 64'), and soybean (*Glycine max* L. [Merr.] 'Hark') were used. All plants were grown in 10-cm diam pots containing a 1:1 mixture of peat-perlite, and watered daily with a half-strength nutrient solution modified to contain an iron chelate. Tobacco and tomato seeds were germinated in vermiculite and transplanted, and the remainder of the plants was seeded directly. Plants were grown in greenhouses supplied with charcoal-filtered air at about 29 ± 3 C day, 20 ± 1 C night temperatures, about 80% relative humidity, and with 12 hr at ca. 11,000 lux of supplemental lighting. Tobacco and tomato had from four to six leaves, and the other plants were 21 days old from seeding when they were exposed to the pollutants. Plants were exposed at the same time each day for 4 hr in greenhouse exposure chambers (7) at 29 ± 3 C, an average relative humidity of 60%, and a minimum light intensity of 11,000 lux.

Nitrogen dioxide and sulfur dioxide were diluted in nitrogen in high pressure tanks and metered into the exposure chambers. In the first tobacco experiment, nitrogen dioxide was measured with a Mast ozone meter (Mast Development Company, 2212 East 12th Street, Davenport, Iowa), calibrated for nitrogen dioxide, and equipped with a sulfur dioxide scrubber (14); in the second experiment, an Atlas (Atlas Electric Devices Company, 4114 North Ravenswood Avenue, Chicago, Ill.) colorimetric nitrogen dioxide analyzer was used. Both instruments were standardized by a manual nitrogen dioxide method (19). In both experiments, sulfur dioxide was measured with a Davis (Davis Instruments, 47 Halleck Street, Newark, N. J.) conductimetric sulfur dioxide analyzer standardized to the West-Gaeke method (19).

The six cultivars were represented in each chamber during an exposure, and three pollutant treatments were used each day. In the initial exposures, single gases were used to establish the injury thresholds of the most sensitive species, and to observe the injury symptoms. After establishing these thresholds, plants were exposed to mixtures (5 to 50 pphm) of nitrogen dioxide and sulfur dioxide (1 pphm NO_2 = 18.8 $\mu g/m^3$ and 1 pphm SO_2 = 26.2 $\mu g/m^3$, both at 25 C and 760 mm Hg). These concentrations when the gases were tested singly were below the acute injury thresholds of the cultivars.

Leaf injury was assessed 2 days after exposure as the percentage area of each leaf showing chlorosis and/or necrosis. The upper and lower surfaces were rated separately. Injury on each leaf was visually estimated in 5% increments (0-100% scale), and an injury index was computed on the basis of the average injury to the three most severely injured leaves/plant, except for pinto bean on which only the two primary leaves were observed. The injury index included a three-leaf average (two leaves for Pinto bean) even if any or all leaves showed no injury. The number of plants used in computing a mean treatment injury is shown in the appropriate tables.

RESULTS.—Injury to the broad-leaf plants (Fig. 1-A, B) exposed to either nitrogen dioxide or sulfur dioxide at concentrations equal to or exceeding 200 pphm nitrogen dioxide or 50 pphm sulfur dioxide for 4 hr appeared as a bifacial, marginal, and/or interveinal necrosis. Oats developed tipburn and necrotic streaking of the leaf blade. Frequently, when soybeans were exposed to sulfur dioxide concentrations greater than 50 pphm, bifacial necrotic spots and profuse undersurface red pigmentation developed in the primary leaves and were often found in the leaflets of the trifoliate leaf. Injury symptoms observed from the single gas exposures were usually similar to those previously described (2, 3, 16).

Foliar injury developed on tobacco (experiment 1) when the concentrations of nitrogen dioxide and sulfur dioxide were each equal to or exceeded 10 pphm for the 4-hr exposure period (Table 1). The mixture of gases caused foliar injury at significantly lower concentrations than the single gases alone. Injury appeared as a diffuse, white, upper-surface fleck, which intensified as the concentration was increased to 25 pphm of nitrogen dioxide or 50 pphm sulfur dioxide.

TABLE 1. Injury to tobacco exposed to mixtures of nitrogen dioxide and sulfur dioxide[a]

NO_2[b] pphm	SO_2,[b] pphm	Leaf Injury %[c]	No. observations
5	5	0	8
5	25	0	4
10	10	26	16
20	10	60	4
25	5	0	4
25	25	68	4
25	50	100	4

[a] Threshold injury concentration for nitrogen dioxide and sulfur dioxide in 4-hr exposures were 200 pphm and 50 pphm, respectively.

[b] 1 pphm NO_2 = 18.8 $\mu g/m^3$ and 1 pphm SO_2 = 26.2 $\mu g/m^3$, both at 25 C and 760 mm Hg.

[c] Average percentage leaf injury to the three most severely injured leaves/plant.

The plant species exposed to the gas mixtures in experiment 2 developed leaf injury when exposed to all combinations of concentrations equaling or exceeding 10 pphm of each gas for 4 hr, with the exception of tomato and oats (Table 2). All species except tomato developed a trace of injury at concentrations of 5 pphm of each gas. From 0 to 16% injury was observed in each plant species when one of the toxicants was held at 5 pphm and the other toxicant was changed from 5 to 25 pphm. Maximum foliar injury developed on all species at either 10:10 or 15:10 pphm of nitrogen dioxide and sulfur dioxide, respectively. As the concentrations of both gases were increased, foliar injury was reduced. This reduction in injury did not appear to be correlated with environmental factors. Soybean appeared to be the species most sensitive to the gas mixtures; tomato, the most tolerant.

Leaf symptoms associated with the gas mixtures were catagorized into upper and lower surface injury. The upper surface injury appeared as small discrete interveinal necrotic flecks and/or chlorotic spots on the leaves of most species (Fig. 1-C, D). Pinto bean and soybean often accumulated a dark, reddish-brown pigment in the cells on the upper leaf surface (stipple). The upper leaf surface injury in most species was similar to ozone injury. In tobacco, ozone fleck or lesions appeared slightly larger than the upper surface flecking from the mixed gas exposures. This differentiation is difficult, however, without plants exposed to ozone only.

Injury due to the pollutant mixtures also developed on the lower leaf surface of radish, soybean, Pinto bean, and tobacco independent of the upper surface injury. Radish developed the most

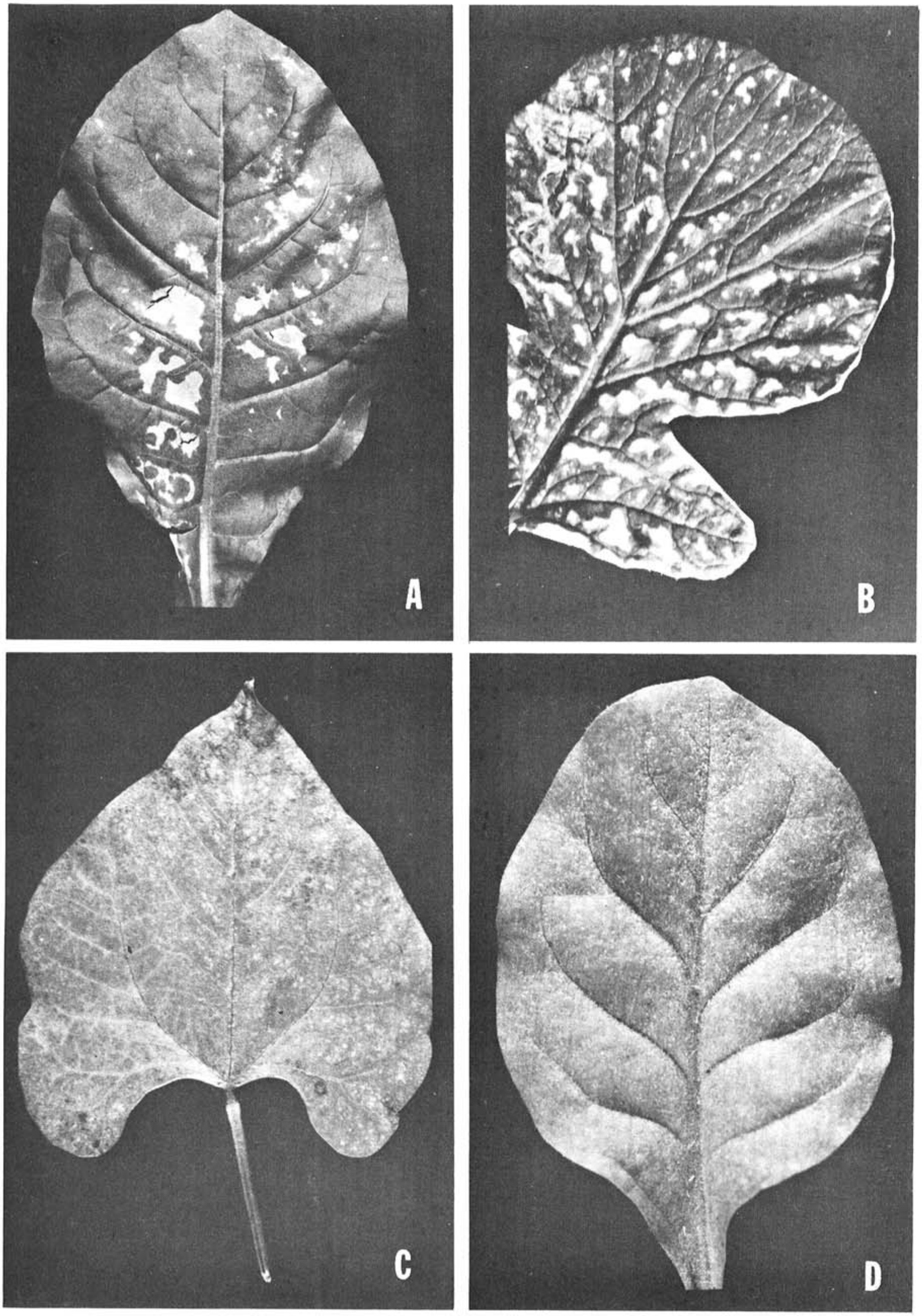
A
B
C
D

TABLE 2. Injury to the upper leaf surface of plants exposed to mixtures of nitrogen dioxide and sulfur dioxide[a]

		Leaf injury[b] (%)						
NO_2, pphm	SO_2, pphm	Pinto bean	Oats	Radish	Soybean	Tobacco	Tomato	No. Observations
5	5	2	1	1	2	1	0	7
5	10	0	0	0	0	0	0	4
5	20	1	0	0	6	2	0	3
5	25	1	3	0	7	1	1	4
10	5	0	0	1	1	9	0	3
10	10	11	27	27	35	11	1	3
15	10	24	12	24	20	18	17	4
15	25	4	0	4	1	6	0	3
20	20	16	10	6	9	4	0	2
25	5	0	0	13	2	16	0	2

[a] Threshold injury concentrations for nitrogen dioxide and sulfur dioxide in 4-hr exposures were 200 and 50 pphm, respectively.

[b] Average percentage leaf injury to the three most severely injured leaves/plant except for bean, which was based on injury to the two primary leaves.

undersurface injury, and often displayed more injury on the lower than on the upper leaf surface (Table 3). A reddish-brown pigment accumulated in the lesions on the lower leaf surface of Pinto bean and soybean, whereas in radish and tobacco, the lower surface injury appeared as a silvering of the interveinal area.

DISCUSSION.—Pigmented lesions, upper-surface bleaching, bifacial necrosis, chlorosis, and chlorotic stippling or mottling are symptoms associated with ozone and the oxidant complex. Ozone-sulfur dioxide mixtures are also known to cause flecking and necrotic bleaching in tobacco (11). All of these symptoms were observed, to some degree, in the plant species exposed to mixtures of nitrogen dioxide and sulfur dioxide.

Some injury from the nitrogen dioxide-sulfur dioxide exposures also resembled certain types of injury reported for sulfur dioxide. Barrett & Benedict (2) reported silvering on the lower surface of alfalfa and cotton after chronic exposures to sulfur dioxide or to injury threshold sulfur dioxide concentrations when the humidity was high. We found a similar type of undersurface silvering in radish and tobacco leaves. An intensive red pigmentation and undersurface stippling was observed in Pinto bean and soybean following exposures to sulfur dioxide above 50 pphm, and to the mixtures of sulfur dioxide and nitrogen dioxide.

The similarities between the injury from nitrogen dioxide-sulfur dioxide mixtures and ozone injury may explain why the occurrence of fleck-type injury and other associated symptoms of ozone injury in tobacco, for example, are not always correlated with high photochemical oxidant or ozone concentrations in the field. Similarly, some of the injury responses

TABLE 3. Injury to radish: a comparison of upper and lower leaf surface injury induced by nitrogen dioxide-sulfur dioxide

NO_2, pphm	SO_2, pphm	Leaf injury[a] (%) Upper surface	Lower surface
10	5	1	17
10	10	27	11
20	20	6	16
25	5	13	36

[a] Average percentage injury of the three most severely injured leaves/plant.

from nitrogen dioxide-sulfur dioxide mixtures were suggestive of sulfur dioxide exposures. These similarities make field diagnosis of injury due to specific toxicants very difficult.

For several years, nitrogen dioxide and sulfur dioxide have been measured on a continuous basis in several large cities (5, 20). The values (Table 4) indicate the percentage of time the average hourly concentrations exceeded the indicated concentrations. Additional measurements, reported from suburban Cincinnati, showed that the daily 2-hr average maximum concentrations of nitrogen dioxide ranged from 5 to 45 pphm, whereas, for the same sampling period, daily 2-hr average maximum sulfur dioxide concentrations varied from 5 to 15 pphm (13). These reported concentrations of nitrogen dioxide and sulfur dioxide indicate a widespread occurrence of possible phytotoxic combinations of these gases. The interaction of nitrogen dioxide and sulfur dioxide, therefore, could cause foliar injury in

Fig. 1. A) Bel W_3 tobacco exposed to 150 pphm SO_2 for 4 hr. Leaf has bifacial interveinal necrosis. **B)** Cherry Belle radish exposed to 700 pphm NO_2 for 4 hr. Leaf has interveinal and marginal bifacial necrosis. **C)** Pinto bean exposed to 10 pphm NO_2 and 10 pphm SO_2 for 4 hr. Both fleck and stipple injury symptoms can be seen on same leaf. **D)** Bel W_3 tobacco exposed to 10 pphm NO_2 and 5 pphm SO_2 for 4 hr. Necrotic lesions can be seen along veins and in interveinal area near the leaf tip.

TABLE 4. Atmospheric concentrations of nitrogen dioxide and sulfur dioxide in selected metropolitan areas exceeded 1, 10, and 30% of the time

	Concentrations (pphm) which exceed a given percentage of times[a]					
	Nitrogen dioxide 1962-68 (5)			Sulfur dioxide 1962-67 (20)		
City	1%	10%	30%	1%	10%	30%
Chicago	12	7	5	65	33	16
Cincinnati	9	5	4	17	7	3
Denver	10	6	4	6	3	2
Los Angeles	24	11	6	8	4	2
Philadelphia	11	7	4	45	21	9
St. Louis	8	5	3	25	11	5
San Francisco	18	8	6	7	3	1
Washington, D.C.	10	6	4	21	10	6

[a] Average hourly concentrations.

many areas of the United States. Many combustion sources emit sufficient quantities of both gases to cause ambient concentrations equal to those used in these studies. This means that plant injury from exposure to a mixture of these gases could result, even though the gases from a pollution source considered independently may not appear to be harmful.

Since significant amounts of plant injury were associated with the nitrogen dioxide-sulfur dioxide gas mixtures reported in this paper, it seems probable that significant adverse effects on plant growth and yield should also be observed. Sulfur dioxide from smelting processes has been shown to reduce growth of certain tree species (10). Taylor & Eaton (15) showed that continued fumigation with nitrogen dioxide reduces growth of tomato. Recently, Tingey et al. (18) showed that growth and yield reductions in radish exposed to mixtures of ozone and sulfur dioxide were at most additive, and in several cases significantly less than additive, when compared with the effects of the single pollutant (18). Long-term studies on growth and yield of plants exposed to mixtures of nitrogen dioxide and sulfur dioxide are needed to develop a complete understanding of the effects of air pollutants and pollutant mixtures on growth and yield of high-quality food and fiber crops.

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