Effects of Sulfur Dioxide and Nitrogen Dioxide on Vegetative Growth of Soybeans

C. I. Klarer, R. A. Reinert, and J. S. Huang

Graduate assistant; plant pathologist and professor, Agricultural Research Service, U.S. Department of Agriculture; and plant pathologist and associate professor, respectively, Department of Plant Pathology, North Carolina State University, Raleigh 27650.

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ABSTRACT

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Greenhouse grown soybeans, *Glycine max* 'Bragg,' inoculated with *Rhizobium japonicum* were exposed to sulfur dioxide (SO_2) (0.0, 0.2, 0.3 ppm) and nitrogen dioxide (NO_2) (0.0, 0.1, 0.2 ppm) singly and in combination. Three-hour exposures were initiated at 3 wk postemergence and continued every other day. Plants were harvested after 15 exposures. Number and fresh weight of nodules and fresh and dry weight of stems, leaves, and roots were recorded. A red pigmentation and stippling developed on leaves after the initial exposure to 0.3 ppm SO_2 and all $SO_2 + NO_2$ mixtures. A chlorotic mottle developed in plants after several exposures to $SO_2 + NO_2$ mixtures. The interaction of SO_2 and NO_2 caused a significant weight reduction in all plant parts measured except the stems. Linear reductions in weight were related to pollutant-dose. Variables measured generally showed linear reductions in weight due to the interaction of the pollutants.

Additional key words: air pollutants, dose response, pollutant mixtures.

Sulfur dioxide (SO_2) and nitrogen dioxide (NO_2) are major gaseous pollutants and may occur together in the atmosphere. Effects of the single pollutants on vegetation are fairly well documented (2,12). Recent studies, however, suggest that the two gases do not act independently when present together, but frequently interact to influence plant response. Evidence of this interaction includes increased visible injury (13) and decreased plant growth (1,3,4,8).

Soybean, *Glycine max* (L.) Merr., has been shown to be sensitive to SO_2 and NO_2 mixtures. Tingey et al (13) exposed several plant species to SO_2 and NO_2 (0.05–0.25 ppm) mixtures for 4 hr. Visible injury developed on the majority of plant species exposed to these mixtures even though these concentrations were below the visible injury thresholds for the individual gases. The visible injury was more similar to ozone (O_3) injury than to injury from SO_2 or NO_2 alone.

Soybeans were exposed to SO_2 and NO_2, singly and in combination, for several weeks, in two separate field studies (1,8). In one study (8) a zonal air pollution system was used in which SO_2 concentrations ranged from 0.14 to 0.42 ppm and NO_2 concentrations ranged from 0.12 to 0.37 ppm. Ambient O_3, however, was present and may have influenced the plant's response to SO_2 and NO_2. In the other study (1) open-top chambers were used to expose plants to 0.0, 0.1, or 0.3 ppm SO_2 with or without the addition of 0.1 ppm NO_2. In both studies, the plants exposed to combinations of SO_2 and NO_2 showed greater yield reductions than those exposed to the single pollutants.

None of these previous studies were designed to explain the dose-response relationship of SO_2 and NO_2 mixtures. Since the concentrations and proportions of SO_2 and NO_2 are constantly changing in the atmosphere, the knowledge of the effects of a range of concentrations of SO_2 and NO_2 is important.

The objective of the research reported here was to describe the response of vegetative soybean growth to a factorial series of SO_2 and NO_2 concentrations in the absence of O_3.

MATERIALS AND METHODS

Bragg soybean seeds, inoculated with commercial *Rhizobium japonicum* (Nitragin Co., Milwaukee, WI), were planted in 38-cm-diameter pots (five seeds per pot) containing a mixture of steam-pasteurized soil and sand (1:1, v/v) amended with 3.53 kg of dolomitic lime per 0.76 m^3 of mix. Plants were grown at 24–30 C and a 12-hr photoperiod in a greenhouse equipped with charcoal filters with supplemental lighting of 15 Klux provided by 1,000-W multivapor lamps. Seedlings were thinned to two plants per pot 7 days after planting and then to one plant per pot 14 days after planting. Beginning at 2 wk after planting, plants were fertilized twice a week with 100 ml of Hoagland's solution minus nitrogen (6).

Beginning at 3 wk postemergence, plants were exposed to three concentrations of SO_2 (0.0, 0.2, and 0.3 ppm) and three concentrations of NO_2 (0.0, 0.1, and 0.2 ppm) singly and in all possible combinations for 3 hr every other day, alternating mornings and afternoons. Exposures were made under greenhouse conditions using continuously stirred-tank-reactor exposure chambers (5). Sulfur dioxide was dispensed from a tank containing 1% SO_2 in nitrogen (N_2) and was monitored by a flame photometric SO_2 analyzer (Meloy Labs, Springfield, VA). Nitrogen dioxide was dispensed from a tank containing 1% NO_2 in N_2 and monitored by a chemiluminescence nitrogen oxides analyzer (Monitor Labs, Inc., San Diego, CA). Each gas analyzer was calibrated with a portable model 8500 calibrator (Monitor Labs). Both gases were monitored in each charcoal-filtered air exposure chamber, whether or not they were part of the treatment.

Plants were harvested after 7 wk and 15 exposures. Roots were washed and nodules were removed, counted, and weighed. Leaves,

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stems, and roots were oven dried at 58 C for 48 hr and then weighed.

The treatment design was a 3 × 3 factorial with a total of nine treatments and an experimental unit of six plants per treatment. Two replications of the experiment were conducted 2 wk apart from mid-April until mid-June. Main and interaction effects of SO_2 and NO_2 were tested for significance using analysis of variance. Treatment sums of squares were partitioned into linear and curvilinear effects and tested for significance at $P = 0.01$ and 0.05.

RESULTS

A chlorotic mottle of the interveinal tissues began developing on the lower leaves of plants exposed to the $SO_2 + NO_2$ combination treatments after four exposures. As the exposures continued, the mottle increased in severity and developed on younger foliage. After 15 exposures, interveinal tissues of the older leaves (leaves 1–3) were yellow, while the interveinal tissues of the younger leaves (leaves 5–7) were mottled a faint, yellow-green. No differences were seen in the severity of the mottle among the four $SO_2 + NO_2$ mixture treatments. This mottle was not evident on plants exposed to SO_2 or NO_2 alone.

A red pigmentation and stippling developed on the underside of the lower leaves (leaves 1–3) in the 0.3 ppm SO_2 treatment and in all four $SO_2 + NO_2$ treatments. This stippling was present on some plants after the first exposure to SO_2 alone. As the exposures continued, the stippling did not develop on any new foliage. However, in the $SO_2 + NO_2$ treatments the stippling increased in intensity and also developed on the upper surfaces of the lower leaves.

Pollutants affected plant dry weight, nodule number, and nodule fresh weight (Table 1). SO_2 had a significant effect on all variables measured, whereas NO_2 only had a significant effect on root dry weight and nodule number and fresh weight. The interaction of SO_2 and NO_2 in mixture was significant in all plant variables measured except stem weight. When sums of squares were partitioned into linear and curvilinear components, only linear components were significant, with the exception of the SO_2 linear × NO_2 curvilinear interaction which was significant for nodule number. No significant effect of the pollutants on average nodule size (nodule weight divided by nodule number) was observed. Results for fresh weights of root, leaf, and stem were consistent with the dry weight results.

TABLE 1. Analysis of variance mean square values for linear and curvilinear effects of exposure to SO_2 and NO_2 on soybean leaf, stem, and root dry weight and on number and fresh weight of nodules[a]

Factor	df	Leaf wt (g)	Stem wt (g)	Root wt (g)	Nodule wt (g)	Number
Rep	1	11.38	2.15	0.25	1.01	259,014
SL[b]	1	29.93*	3.62*	20.63**	5.46**	160,540**
SC	1	1.90	0.08	1.29	0.21	773
NL	1	13.46	0.41	5.30**	4.73**	172,872**
NC	1	0.56	0.09	0.56	0.34	2,454
SL × NL	1	20.62*	0.53	3.17**	3.60*	3,279
SL × NC	1	0.93	0.09	1.07	0.05	36,305*
SC × NL	1	4.83	0.45	0.16	0.04	1
SC × NC	1	1.46	0.01	0.12	0.12	892
Error	8	3.04	0.27	0.25	0.42	5,262
Residual	90	1.11	0.11	0.24	0.33	10,119

[a] Beginning at 3 wk postemergence, soybeans were exposed to SO_2 (0.0, 0.2, 0.3 ppm) and/or NO_2 (0.0, 0.1, 0.2 ppm) for 3 hr every other day for a total of 15 exposures.

[b] Symbols represent the following: S = SO_2, N = NO_2, L = linear, and C = curvilinear. Levels of significance: $P = 0.05$ (*) and $P = 0.01$ (**).

Mean dry weights of the individual nine treatments (Table 2) indicated that NO_2, in the absence of SO_2, generally caused a slight increase in dry weight, while SO_2, in the absence of NO_2, generally caused a slight decrease compared to the charcoal-filtered-air control. The presence of both gases caused a greater decrease in weight than either SO_2 or NO_2 alone, indicating that the effect of both gases was dependent on the presence of the other gas.

Sulfur dioxide and NO_2 did not have similar effects on leaf and stem weight. Sulfur dioxide alone caused a reduction in stem weight, but had little effect on leaf weight. The interaction of SO_2 and NO_2 was significant only in reducing leaf weight; however, a similar trend was present for stem weight.

DISCUSSION

The red pigmentation and stippling observed on the lower leaves was similar to the injury reported by Tingey et al (13). The yellowing and mottling seen in the older foliage of plants after several exposures to gas mixtures corresponded to the increased senescence observed on soybeans exposed at the pod-filling stage and in the presence of ambient O_3 (1). In our study, plants were exposed prior to flowering in the absence of ambient O_3. These different studies suggest that soybeans are sensitive to SO_2 and NO_2 mixtures over various developmental stages, although the influence of ambient O_3 on plant response needs to be defined.

Exposure to SO_2 and NO_2 suppressed soybean root growth more than the growth of stems or leaves. A similar response had been found with roots of radish plants exposed to SO_2, NO_2, and O_3 (10). Reduction in translocation of photosynthate by SO_2 (9) and inhibition of photosynthesis by SO_2 and NO_2 (7) have been reported. Decreased synthesis of photosynthates and decreased allocation to root tissue may explain the greater impact on roots than on foliage when plants were exposed to SO_2 and NO_2.

Numbers and fresh weight of nodules were reduced by the pollutant mixtures. The reductions in nodule weight and nodule number may have been influenced by the reduction in root weight. Ratios of root weight to nodule number or nodule weight were analyzed and were significantly different. This indicated that changes in root weight and nodule number and weight were not consistent over all treatments. Reduction in nodule weight and number may have influenced nitrogen fixation and had an impact on growth, since *R. japonicum* provided the major source of nitrogen to the plants. No visible symptoms of nitrogen deficiency were observed.

TABLE 2. Effects of SO_2 and NO_2 on soybeans. Treatment means for soybean dry weights and for nodule fresh weight and number[a]

Variable	Std. error	NO_2 (ppm)	O_2 (ppm) 0.0	0.2	0.3
Leaf wt (g)	0.50	0.0	8.85	9.05	9.01
		0.1	9.25	8.25	7.65
		0.2	9.65	7.18	7.45
Stem wt (g)	0.15	0.0	3.19	3.18	2.98
		0.1	3.20	2.87	2.78
		0.2	3.35	2.76	2.79
Root wt (g)	0.14	0.0	3.34	3.02	2.99
		0.1	3.54	2.32	2.22
		0.2	3.41	2.24	2.08
Nodule wt (g)	0.18	0.0	5.44	5.55	5.45
		0.1	5.40	5.16	4.74
		0.2	5.55	4.84	4.51
Nodule number	20.9	0.0	767	707	692
		0.1	740	648	593
		0.2	651	612	608

[a] Beginning at 3 wk postemergence, soybeans were exposed to SO_2 and/or NO_2 for 3 hr every other day for a total of 15 exposures.

Data from previous studies of the effects of SO_2 and NO_2 on plants have not provided a clear analysis of the dose-response relationships, either because only two levels of NO_2 were used (1), or a zonal air pollution system was used for dispensing the pollutants (8) which does provide a system for filtering ambient O_3 and excluding O_3 from the SO_2 and NO_2 effects. By using three different concentrations of both SO_2 and NO_2 under controlled conditions, we were able to describe a dose-response relationship of SO_2 and NO_2 on soybean vegetative growth. In all cases, with one exception, the variables measured showed a linear response to all the pollutants. The only other report (11) describing the dose-response relationship of SO_2 and NO_2 included O_3 in the treatments. In both studies, low concentrations of the pollutants were used in studying the dose-response relationships. At higher concentrations, SO_2 and NO_2 may have a greater independent impact, and the synergistic interaction of the two gases and their linear effect may not hold true. More studies are needed to describe the dose-response surface of SO_2 and NO_2 over a wider range of concentrations and plant species, genotypes and growth stages.

Results of this study emphasize the importance of studying the effects of combinations of SO_2 and NO_2, rather than just the single pollutant effects. It shows that the effect of each pollutant is dependent on the presence of the other pollutant. These results also emphasize the importance of NO_2 emissions, for which no secondary standards exist, since in the presence of SO_2, NO_2 contributed to a significant reduction in soybean biomass.

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