# Ozone and Sulfur Dioxide-Induced Changes in Soybean Growth

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Cooperative investigations of the U. S. Department of Agriculture, North Carolina State University, and the U. S. Environmental Protection Agency. Paper No. 5478 of the Journal Series of the North Carolina Agricultural Experiment Station, Raleigh.

The use of commercial names of equipment or projects in this publication does not imply endorsement by the USDA, USEPA, or the North Carolina Agricultural Research Service or criticism of similar ones not mentioned.

The authors extend their appreciation to J. O. Rawlings, Professor of Statistics for assistance in this study.

Accepted for publication 15 March 1980.

## ABSTRACT

REINERT, R. A. and D. E. WEBER. 1980. Ozone and sulfur dioxide-induced changes in soybean growth. Phytopathology 70:914-916.

The growth of soybean was inhibited by exposure to 490 $\mu g\ O_3/m^3$ (25 pphm) and 665 $\mu g\ SO_2/m^3$ (25 pphm), singly and in combination, when plants were exposed for 4 hr three times per week for 11 wk. The main effects of $O_3$ were a reduction of shoot, root, and plant dry weight measured at 5, 7, 9, and 11 wk. The main effects of $SO_2$ were a reduction of shoot dry weight at 7 wk and total plant growth at 11 wk. Sulfur dioxide contributed to the reduced growth in soybean in the absence of visible $SO_2$ injury. The effect of $SO_2$ and $O_3$ in combination on soybean growth was only additive. Treatments containing $O_3$ reduced the numbers and dry weight of root nodules of soybean, compared with treatments without $O_3$.

*Additional key words*: *Glycine max*, air pollution, pollutant interaction.

With increasing soybean (*Glycine max* [L.] Merr.) production there is need to better understand the factors affecting its growth and development. Air pollutants comprise one important group of factors that may affect soybean growth.

Recent tests showed that the foliage of many cultivars of soybean was sensitive to ozone ($O_3$) (6,11) and sulfur dioxide ($SO_2$)(6), but the amount of injury varied with cultivar. Growth of cultivars Dare and Hood was inhibited by $O_3$ and by an $O_3 + SO_2$ mixture, but the effects on growth were not correlated with the amount of visible injury to foliage (12). Ozone inhibited nodulation, (number and total weight) and synthesis of leghemoglobin in soybean cultivar Lee (10) as well as nodulation in cultivars Dare and Hood (7).

In field studies, yields (number and oven-dry weights of seeds) of Dare soybean exposed to 196 $\mu g\ O_3/m^3$ (10 pphm), or to a mixture of 196 $\mu g\ O_3/m^3$ and 262 $\mu g\ SO_2/m^3$(10 pphm) for 6 hr per day for 133 days were less than those of nonexposed controls. Yields of soybean were not different in plants exposed to 262 $\mu g\ SO_2/m^3$ or 98 $\mu g\ O_3/m^3$ from seeding to harvest (4). Davis (3) found a negative linear correlation between yields and foliar injury caused by experimental exposure of soybean cultivar Kino to $SO_2$ under field conditions.

The present investigations were designed to determine the cumulative impact of $O_3$ and $SO_2$, singly and in combination, on soybean dry weight changes through various stages of vegetative growth.

## MATERIALS AND METHODS

Four groups of 20 Dare soybean plants were grown from seed and transplanted singly to white silica sand in 13-cm-diameter pots (for the 5th-wk harvests) or 20-cm diameter pots (for the 7th-, 9th-, and 11th-wk harvests). Roots were inoculated with 200 mg of a commercial inoculum of *Rhizobium japonicum* Kirch as seedlings were transplanted. Plants were watered daily as needed. In addition each plant received approximately 100 ml of half-strength Hoagland's nutrient solution minus nitrogen twice each week. The soybeans were grown under 10-hr photoperiods of normal daylight, and flowering was inhibited by interrupting the night period with 3 hr of illumination from eight 150-W incandescent lamps. Greenhouse temperatures were 28 ± 7 C and relative humidity ranged 50–75%.

Twenty plants (five for each treatment) from each group were exposed to 490 $\mu g\ O_3/m^3$ (25 pphm) and 655 $\mu g\ SO_2/m^3$ (25 pphm), singly and combination, or to charcoal-filtered air for 4-hr periods on 3 alternate days per week in exposure chambers adapted for greenhouse use (5). Plants remained in the chambers under continuous circulation of charcoal-filtered air between exposure periods. Temperature and humidity in the chambers were similar to greenhouse conditions. Ozone concentrations were monitored by Mast $O_3$ analyzers which were calibrated to 1% buffered KI. Sulfur dioxide was measured by a Davis $SO_2$ analyzer. Because $SO_2$ interferes with the measurement of $O_3$, a chromium trioxide scrubber (8) was used to remove $SO_2$ from the $O_3$ sample probe of the chamber receiving both $O_3$ and $SO_2$.

Twenty soybean plants, five from each pollutant treatment, were harvested at 5, 7, 9, and 11 wk after seedlings were transplanted. The experiment was replicated twice. Following each harvest the amount and type of visible injury was evaluated. Dry weights of roots and shoots, plant height, and numbers and dry weights of nodules were determined. Data were analyzed at each harvest by analysis of variance, and treatment sum of squares was partitioned to test for $O_3$ and $SO_2$ main effects and the $SO_2 \times O_3$ interaction effect (2).

## RESULTS

The foliar injury symptoms due to the $O_3$ and $SO_2 + O_3$ treatments were similar to those previously reported (4,12). The amount of injury on soybean leaves was generally 5% greater in the $SO_2 + O_3$ treatment than the $O_3$ treatment after 7 wk. The average $O_3$-injury per leaf ranged from approximately 65–75% of the leaf surface injured from the 5th to the 11th wk. Soybean plants exposed to $SO_2$ alone did not develop visible injury following 11 wk of growth.

The growth of soybean (expressed as shoot, root, and plant dry weight) was evaluated four times from age 5–11 wk. Mean weights at each harvest for each growth variable are given in Table 1. The values for the main and interaction effects are also given and are expressed in grams dry weight. As seen in Table 1, the main effect of $O_3$ is the average difference (g) of the means for the $O_3$ treatments at

0031-949X/80/09091403/$03.00/0

the low ([$O_3$] − [Control]) and high ([$SO_2 + O_3$] − [$SO_2$]) levels of $SO_2$ (2). A similar relationship holds for determining the main effect of $SO_2$. The two differences averaged to give the main effect are defined as the simple effects. The interaction effect is one-half the difference (g) of the simple effects of $O_3$, 1/2 ([{$O_3$+$SO_2$} − {$SO_2$}] − [{$O_3$} − {Control}]), or $SO_2$ and measures the failure of the effect of one pollutant to be consistent at different levels of the second pollutant (2). If this interaction effect and the two main effects all have the same sign (Table 1), then the pollutants are interacting synergistically. However, if the interaction effect has a sign opposite that of the main effects the pollutants are interacting antagonistically.

Shoot, root, and plant dry weight was significantly less after 5 wk in those treatments containing $O_3$ contrasted with those treatments that did not contain $O_3$, as shown by a negative weight difference (Table 1). The inhibition of shoot growth doubled at each harvest through 11 wk (−0.35, −1.42, −3.48 and −6.23 g), and the inhibition of root growth nearly doubled through 9 wk. The main effect of $SO_2$ on shoot growth also doubled through the 9th wk; the effect was significant at 7 wk ($P$ = 0.01) and 11 wk ($P$ = .10). Sulfur dioxide began to significantly inhibit root growth at 9 wk ($P$ = .10) and was inhibitory ($P$ = .10) to shoot, and total plant growth after the fourth harvest at 11 wk.

There were no significant interaction effects of the combined pollutants ($SO_2 \times O_3$) after any of the four harvests, but the interaction effects appeared slightly antagonistic in early stages of growth and slightly synergistic in later stages of growth.

The effects of the two pollutants singly, and in combination on nodulation are given in Table 2. The main effect of $O_3$ was significant ($P$ = 0.05) after 9 wk of growth. Prior to that stage of soybean development there were more than three times as many nodules on control plants than on $O_3$-treated plants, but the nodule numbers were highly variable. Nodule weights were less variable and the main effect of $O_3$ was significant at each harvest (Table 2). There were no main effects of $SO_2$ on nodule development and no significant interacting effects of the combined pollutants on either nodule number or nodule weight.

## DISCUSSION

The inhibitory main effects of $SO_2$ and $O_3$ on soybean growth were continuous throughout the 11 wk of growth. The inhibitory effects of $SO_2$ on shoot and plant growth occurred without appearance of macroscopic injury to foliage. Ozone has been shown to limit the early growth of soybean (12) and to cause lower seed yield in treated soybean than in untreated control plants (4). The effects of $SO_2$ on soybean growth and yield also were studied. However, in these studies (4,12) treatment contrasts to determine the main effects of the two pollutants were not made. If this had been done, perhaps the $SO_2$ impact could have been better characterized.

The lower dry weight of shoots in plants that received treatments containing $O_3$ can be accounted for partly by the amount of foliar injury, but the growth of roots was inhibited almost as much as

TABLE 1. Mean vegetative growth of soybean exposed to $O_3$ and $SO_2$, singly and in combination after four harvest periods and estimates of the main and interaction effects of $SO_2$ and $O_3$[a]

| Variable | Treatments | Harvest (wk) 5 | 7 | 9 | 11 |
|---|---|---|---|---|---|
| | | Means[b] | | | |
| Shoot dry wt. (g) | Control | .99 | 2.63 | 7.21 | 13.14 |
| | $SO_2$ | .84 | 2.11 | 6.61 | 11.17 |
| | $O_3$ | .64 | 1.04 | 4.22 | 6.88 |
| | $SO_2 + O_3$ | .49 | .86 | 2.65 | 4.96 |
| | | Main and interaction effects[c] | | | |
| | $SO_2$ | − .148 | − .354** | −1.083 | −1.946w |
| | $O_3$ | − .348*[d] | −1.418** | −3.477** | −6.231** |
| | $SO_2 \times O_3$ | + .002 | + .090w | − .479 | + .027 |
| | | Means | | | |
| Root dry wt. (g) | Control | .55 | .89 | 1.85 | 3.02 |
| | $SO_2$ | .47 | .73 | 1.47 | 2.24 |
| | $O_3$ | .22 | .34 | .90 | 1.82 |
| | $SO_2 + O_3$ | .19 | .23 | .44 | .92 |
| | | Main and interaction effects[c] | | | |
| | $SO_2$ | − .062 | − .136 | − .420w | − .836w |
| | $O_3$ | − .303** | − .523** | − .985* | −1.263* |
| | $SO_2 \times O_3$ | + .024 | + .023 | − .021 | − .064 |
| | | Means | | | |
| Plant dry wt. (g) | Control | 1.54 | 3.52 | 9.06 | 16.16 |
| | $SO_2$ | 1.30 | 2.84 | 8.08 | 13.41 |
| | $O_3$ | .86 | 1.38 | 5.12 | 8.70 |
| | $SO_2 + O_3$ | .68 | 1.09 | 3.10 | 5.88 |
| | | Main and interaction effects[c] | | | |
| | $SO_2$ | −.211* | − .481 | −1.503 | − 2.783w |
| | $O_3$ | − .651** | −1.943** | −4.462** | −7.494** |
| | $SO_2 \times O_3$ | + .027 | + .192 | − .052 | − .037 |

[a]Concentrations were 25 pphm of each gas singly or in combination. Plants were exposed 4 hr, three times per week for the duration of time through each harvest.

[b]Each mean represents the average of 10 plants from two replicated experiments.

[c]The linear additive model to evaluate these effects assumes that fixed treatments sum to zero. The main and interaction effect differences represent the grams of weight change per plant from zero.

[d]Asterisks and w represent levels of significance as follows: $P$=0.10 (w), $P$ = 0.05 (*), and $P$ = 0.01 (**).

TABLE 2. Mean number and weight of nodules and plant height of soybean exposed to $O_3$ and $SO_2$ singly and in combination after four harvest periods and estimates of the main and interaction effects of $SO_2$ and $O_3$[a]

| Variable | Treatments | Harvest (wk) 5 | 7 | 9 | 11 |
|---|---|---|---|---|---|
| | | Means[b] | | | |
| Number of nodules | Control | 152 | 118 | 179 | 170 |
| | $SO_2$ | 113 | 114 | 159 | 179 |
| | $O_3$ | 52 | 37 | 92 | 92 |
| | $SO_2 + O_3$ | 51 | 31 | 82 | 91 |
| | | Main and interaction effects[c] | | | |
| | $SO_2$ | − 19.6 | − 5.4 | − 15.3 | + 4.6 |
| | $O_3$ | − 81.1 | − 81.8w | − 81.9*[d] | − 83.9* |
| | $SO_2 \times O_3$ | + 18.9 | − .8 | + 4.8 | − 4.9 |
| | | Means | | | |
| Nodule weight (mg) | Control | 91 | 218 | 441 | 721 |
| | $SO_2$ | 68 | 195 | 450 | 703 |
| | $O_3$ | 31 | 61 | 272 | 477 |
| | $SO_2 + O_3$ | 28 | 57 | 200 | 427 |
| | | Main and interaction effects[c] | | | |
| | $SO_2$ | − 13.2 | − 13.4 | − 31.3 | − 33.4 |
| | $O_3$ | − 50.0** | −147.4** | −208.7* | −259.7w |
| | $SO_2 \times O_3$ | + 9.2 | + 9.2 | − 40.4 | − 15.8 |
| | | Means | | | |
| Plant height (cm) | Control | 23.9 | 59.2 | 93.9 | 138.4 |
| | $SO_2$ | 18.0 | 40.0 | 66.8 | 89.0 |
| | $O_3$ | 29.1 | 47.8 | 83.8 | 111.1 |
| | $SO_2 + O_3$ | 21.8 | 37.0 | 56.3 | 81.2 |
| | | Main and interaction effects[c] | | | |
| | $SO_2$ | − 6.58* | −14.96* | −27.28w | −78.67** |
| | $O_3$ | + 4.53 | − 7.24 | −10.30 | −17.50* |
| | $SO_2 \times O_3$ | − .76 | + 4.22 | − .25 | − 9.78 |

[a]Concentrations were 25 pphm of each gas singly or in combination. Plants were exposed 4 hr, three times per week for the duration of time through each harvest.

[b]Each mean represents the average of 10 plants from two replicated experiments.

[c]The linear additive model to evaluate these effects assumes that fixed treatments sum to zero. The main and interaction effect differences represent the grams of weight change per plant from zero.

[d]Asterisks and w represent levels of significance as follows: $P$= 0.10 (w), $P$= 0.05 (*), and $P$ = 0.01 (**).

shoot growth. It is unlikely that $O_3$ penetrated the soil, since $O_3$ breaks down as it passes through columns of sand, peat, and gravel mixes to depths of 2 to 4 cm (1). Thus, there is little chance for $O_3$ to injure roots directly. Tingey et al (12) suggested that inhibition of root growth by $O_3$ probably results from suppression of the translocation of photosynthate to roots as discussed by Wardlaw (13). The root systems reduced by lack of photosynthates would be less efficient in the absorption and translocation of nutrients.

There were no significant interaction effects of $SO_2 \times O_3$ after each harvest. The interaction of the combined pollutants can only be described as additive, since the total change in growth from the two pollutants combined did not differ significantly from the sum of the single effects. However, during the early growth of soybean the interaction effect was a positive difference indicating antagonism or a sign change opposite that of the negative main effects of $SO_2$ or $O_3$. As the soybean matured the interaction effect was a negative difference indicating synergism or a sign change similar to main effects of $SO_2$ or $O_3$. The interpretation of the combined pollutant effects in our study differs from the results of other research. Tingey et al (12) stated that "the growth reductions (in soybean) resulting from $SO_2 + O_3$ treatment were greater than the additive reductions of the single gases" (5 pphm $O_3$ and 5 pphm $SO_2$). Heagle et al (4) concluded that, "the mix of 10 pphm $O_3$ and 10 pphm $SO_2$ caused more plant damage than the additive effects of each gas separately at 10 pphm." However, these conclusions were not based on a statistical test for interaction effects. Perhaps these conclusions would have been different if the treatment sum of squares were partitioned to test for main and interaction effects.

The inhibition on nodulation of soybean by $O_3$ in this study confirms previous reports (7,10). Reduction of nodulation by $O_3$ may be caused indirectly by increased presence of inhibitors or by reduced supply of growth substances to the root (9).

The results of this study provided information on rates of soybean growth under stress to $O_3$ and $SO_2$, singly and in combination. The changes in biomass observed through several harvests demonstrate the cumulative impact of these pollutants on soybean development. It showed that a chronic dose of 25 pphm $O_3$ was sufficient to reduce soybean growth and nodulation throughout the development. It shows that a chronic dose of 25 pphm $O_3$ is sufficient to reduce soybean growth and nodulation throughout the development of the plant, and that $SO_2$ at 25 pphm produced a smaller effect that was increasingly significant ($P = 0.10$) as the soybean plant matured.

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