# Response of Chronically Ozonated Soybean Plants to an Acute Ozone Exposure

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## ABSTRACT

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The effects of chronic ozone ($O_3$) exposures on the sensitivity of soybean to a subsequent acute exposure to $O_3$ were measured. Plants were exposed to low concentrations ($\leq$0.10 ppm $O_3$) or to charcoal-filtered air (0.0 ppm $O_3$) for 6 hr/day on 5, 10, or 15 consecutive days (chronic dose). One day after the last chronic exposure, plants were exposed for 3 hr to 0.20 ppm of $O_3$ (acute dose) or to filtered air. Plants exposed to chronic doses of $O_3$ below the visible injury threshold ($\leq$0.06 ppm) were more sensitive to the acute $O_3$ dose than were plants exposed to filtered air. Plants exposed to chronic $O_3$ doses above the visible injury threshold ($\geq$0.06 ppm) were less susceptible to the acute $O_3$ dose than were plants exposed to filtered air. The predisposition toward greater sensitivity to the acute $O_3$ dose tended to be cumulative over time and was partially reversed by treatment with filtered air prior to the acute exposure.

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

Most areas of the eastern United States experience oxidant air pollution, of which ozone ($O_3$) is the principal phytotoxic component. Plants grown in the East are commonly exposed during the daylight hours to $O_3$ concentrations that range from 0.05 to 0.10 ppm (1 ppm = 1,960 $\mu g/m^3$). Regional meteorological conditions occasionally result in $O_3$ concentrations in the range of 0.10 to 0.20 ppm in agricultural areas (3).

Research workers attempting to define acceptable limits for $O_3$ in agricultural and urban areas have performed dose-response studies with many plant species. Most studies have utilized only acute exposures (short-term exposures at relatively high concentrations); a few have utilized chronic exposures (long-term exposures at relatively low concentrations). Few studies have considered the effects of both chronic and acute exposures, even though acute oxidant pollution episodes are often interspersed with the lower oxidant levels. Most dose-response studies begin with plant culture in greenhouses equipped with activated-charcoal air-filtration systems that usually reduce $O_3$ concentrations to $<$0.02 ppm. Results presented in several reports suggest that small differences in $O_3$ concentration during plant growth can greatly affect plant sensitivity to acute $O_3$ exposure (4,7–9). Ozone in the range of 0.02–0.10 ppm partially protected plants from injury due to subsequent acute $O_3$ exposures in some cases (7–9), and increased plant injury in others (4,7–9).

More information on the effects of chronic levels of $O_3$ on subsequent sensitivity of plants to acute $O_3$ levels is needed to better predict the effects of fluctuating ambient $O_3$ levels and to better define agriculturally acceptable levels of oxidant air pollution.

Our objective was to determine the effects of chronic levels of $O_3$ during plant growth on the sensitivity of soybeans to a subsequent acute $O_3$ exposure.

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## MATERIALS AND METHODS

Seeds of soybeans, *Glycine max* (L.) Merr. 'Dare,' were treated with inoculant of *Rhizobium japonicum* and planted in a mixture of Pro-Mix BX:sand:soil (2:2:1, v/v) in 15-cm-diameter plastic pots. Pro-Mix BX (Premier Brands, Inc., New York, NY 10017) is a mixture of peat and perlite with added nutrients. Plants were grown in a greenhouse with charcoal-filtered air and wet-pad cooling. Ozone concentrations in the greenhouse were less than 0.01 ppm (1,960 $\mu g/m^3$) during plant growth, except for an occasional peak of 0.02 ppm during the summer. Seedlings were thinned from four to one plant per pot 7 days after planting and fertilized at 5-day intervals with 150 ml of a solution containing 15 g of soluble fertilizer (6-25-13, N-P-K with micronutrients) (VPHF, Miller Chemical and Fertilizer Corp., Hanover, PA 17331) per 3.8 L of water starting 10 days after planting.

Plants were exposed to $O_3$ in nine cylindrical chambers, 1.22 m tall by 1.07 m in diameter, covered with clear Teflon film (5). Ozone was produced by electrostatic discharge in dry oxygen. Concentrations in the chambers were sequentially monitored with a chemiluminescence $O_3$ monitor (Model 8410A, Monitor Labs Inc., San Diego, CA 92121) calibrated according to the 1% KI method (2). Supplemental lighting (8.7 klx) was supplied during exposures by 400-W multivapor lamps (General Electric Co., 159 Madison Ave., New York, NY 10016). Plants were randomly placed within chambers and different chambers were used for each $O_3$ treatment on successive exposure days.

Three days after the acute exposure, foliar injury (chlorosis, stippling, and necrosis) was estimated for each unifoliolate and trifoliolate leaf (5% increments, 0–100%). Seven days after the acute exposure, the leaf area (LI-COR Portable Area Meter, Lincoln, NE 68504) and dry weight (70 C, 72 hr) of shoots (stems and leaves) and roots were measured.

The data were analyzed by analyses of variance. Where warranted by significant $F$ tests ($P \leq 0.05$), least significant difference (LSD) tests were used to identify significant differences among the means.

**Pretreatment with different ozone concentrations.** Plants were exposed to 0.00 (controls), 0.03, 0.06, or 0.09 ppm of $O_3$ for 6 hr per day (1000–1600 hours EST) on 10 consecutive days starting 14 days after planting. During the acute exposure treatment, which occurred on the 24th day after planting, plants were exposed to 0.00 (controls) or 0.20 ppm of $O_3$ for 3 hr (1100–1400 hours EST). There were five plants for each treatment. This experiment was performed during November and was repeated in January. Temperature and relative humidity during growth and exposures ranged from 20 to 22 C and 15 to 60%, respectively. At 14 and 24 days after planting, the first trifoliolate leaves were estimated to be 50 and 100% expanded, respectively.

An experiment was performed in July that was identical to those performed in November and January, except that $O_3$ concentrations were 0.00 (controls), 0.02, 0.04, 0.06, 0.08, and 0.10 ppm for the chronic pretreatment exposures with six plants per treatment. Temperature and relative humidity ranged from 21 to 37 C and 30 to 90%, respectively. Also, plants grew much more rapidly in July than in November and January. At 14 and 24 days after planting, the first trifoliolate leaves were estimated to be 75 and 100% expanded, respectively.

**Pretreatment at different intervals.** Plants were exposed to 0.00 (controls) or 0.04 ppm of $O_3$ for 6 hr per day (0900–1500 hours EST) for 5, 10, or 15 days. The 5-day exposures were on days 14–18, 19–23, and 24–28 after planting (Table 2). The 10-day exposures were on days 14–23, 19–28, or 14–18, plus 24–28 after planting (Table 2). The 15-day exposure was on days 14–28 after planting (Table 2). The acute exposure treatment was on day 29 after planting; plants from each pretreatment group were exposed for 3 hr (1100–1400 hours EST) to 0.00 (controls) or 0.20 ppm of $O_3$. There were four plants in each chronic and acute treatment combination. This experiment was performed in February and was repeated in April. Greenhouse and exposure chamber temperatures and relative humidity ranged from 21 to 28 C and 30 to 80%, respectively. The first, second, and third trifoliolate leaves were estimated to be 50, 10, and 0% expanded, respectively, at 14 days after planting, and all were completely expanded at 29 days after planting.

## RESULTS

**Pretreatment with different ozone concentrations.** The leaf injury, leaf area, and dry weight response patterns were similar each time this experiment was repeated. Therefore, we will show only data from the July test (Table 1).

Foliar injury symptoms from acute and chronic exposures to $O_3$ resembled those previously described (10). Unifoliolate and first trifoliolate leaves were injured more severely than were younger leaves. For chronic exposures, the threshold concentration of $O_3$ for visible foliar symptoms was between 0.03 and 0.06 ppm (Table 1).

The amount of foliar injury caused by the acute exposure at 0.20 ppm $O_3$ was dependent on the $O_3$ concentrations used in the chronic exposures. When visible injury from chronic exposure either was not apparent or only slight (0–1%), injury caused by the combined chronic and acute exposures was usually greater than additive (Table 1). When injury from chronic exposure was moderate to severe (7–53%), the injury caused by the combined chronic and acute exposures was usually less than additive.

The trends for leaf area followed those for foliar injury. When the chronic treatment slightly (0–2%) decreased the rate of leaf expansion (growth), the decreased growth attributable to the acute treatment tended to be relatively large (Table 1). When the chronic treatment caused a significantly decreased leaf growth (23–31%), the reverse was true (Table 1).

For shoot dry weight, there were no cases of significantly greater than additive effects of the combined treatments (Table 1). However, the contribution of the acute treatment to the total decrease in the growth rate became less as the effect of the chronic treatments increased (Table 1). For example, the chronic treatment at 0.02 or 0.04 ppm singly caused less than 10% weight loss and the mean contribution to weight loss of the acute treatment for the combined exposures was 17%. The chronic pretreatment at 0.08 or 0.10 ppm caused 19 or 27% loss, respectively, and the mean contribution of the acute treatment was only 3% (Table 1).

**Pretreatment at different intervals.** The effect of chronic exposure to $O_3$ on injury from the acute $O_3$ treatment (0.20 ppm) was not statistically significant for individual leaves. However, statistical analysis for total injury per plant (the sum of the injury to individual leaves per plant) showed that the chronic effect and chronic × acute interaction were statistically significant, $P = 0.05$. Plants pretreated at 0.04 ppm of $O_3$ were usually injured more by the acute treatment than were those pretreated in filtered air (Table 2). The results indicate that the effects of pretreatment at 0.04 ppm of $O_3$ on sensitivity to the acute exposure were cumulative and, in some cases, partially reversible. Injury from exposure to 0.20 ppm of $O_3$ was usually greater on plants exposed to 0.04 ppm of $O_3$ for 10 or 15 days than on plants exposed for 5 days (Table 2). Injury was usually greater on plants exposed to 0.04 ppm of $O_3$ on the 5 days immediately prior to the acute exposure than for plants allowed to recover in filtered air prior to the acute exposure.

## DISCUSSION

Changes in chronic $O_3$ concentration as small as 0.02 ppm during plant growth may cause relatively large changes in sensitivity to subsequent $O_3$ exposures. In the present study, the sensitivity of soybeans to an acute $O_3$ exposure was increased by chronic

TABLE 1. Injury and growth responses of soybean to chronic pretreatment and acute exposures to ozone[a]

| Ozone pretreatment[a] (ppm) | Injury per treatment (%) | | | | | | Leaf area ($cm^2$) per treatment | | | Dry weight (g) per treatment | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Unifoliolate leaves | | | First trifoliolate leaves | | | | | | | | |
| | Chronic pretreatment[b] | Chronic plus acute (0.20 ppm $O_3$)[c] | Difference from additive[d] | Chronic[b] | Chronic and acute[c] | Difference from additive[d] | Chronic[b] | Chronic and acute[c] | Reduction (%) due to acute exposure[e] | Chronic[b] | Chronic and acute[c] | Reduction (%) due to acute exposure[e] |
| 0.00 | 0 | 34 | 0 | 0 | 40 | 0 | 877 | 762 | 13 | 4.49 | 3.63 | 19 |
| 0.02 | 0 | 46 | +12 | 0 | 59 | +19 | 869 | 695 | 20 | 4.34 | 3.50 | 19 |
| 0.04 | 1 | 54 | +19 | 1 | 38 | − 3 | 860 | 703 | 18 | 4.28 | 3.62 | 15 |
| 0.06 | 15 | 50 | + 1 | 33 | 40 | −33 | 763 | 686 | 10 | 3.62 | 3.22 | 11 |
| 0.08 | 34 | 43 | −25 | 46 | 53 | −33 | 678 | 673 | 1 | 3.19 | 3.02 | 5 |
| 0.10 | 53 | 62 | −25 | 53 | 59 | −34 | 607 | 620 | −2 | 2.82 | 2.79 | 1 |
| FLSD ($P = 0.05$) | 16.1 | | | 10.6 | | | 59 | | | 0.27 | | |

[a] Chronic exposures were 6 hr daily on day 14 through 23 after planting. The acute treatment was for 3 hr at 0.20 ppm of $O_3$ on day 24 after planting.
[b] Mean effect caused by chronic pretreatment exposures only.
[c] Mean effect caused by 0.20 ppm of $O_3$ for 3 hr on day 24 after planting plus the effect of chronic exposures.
[d] Additive injury is defined as the amount of injury caused by chronic pretreatment alone (no acute exposure) plus injury caused by the acute exposure alone (no chronic exposure).
[e] Percent reduction = [1 − (effect after chronic plus acute exposure ÷ effect after chronic exposure only)] × 100.

TABLE 2. The response of soybean plants to 0.20 ppm of ozone following 15 days of interrrupted or continuous exposure to 0.04 ppm of ozone

| Timing of 6-hr exposures to 0.04 (+) or 0.00 (−) ppm of ozone[a] | | | Percentage foliar injury[b] | | |
|---|---|---|---|---|---|
| Days 14–18 | Days 19–23 | Days 24–28 | Chronic[c] effect | Chronic plus acute effect | Differences from additive[d] |
| − | − | − | 0 | 37 | 0 |
| + | − | − | 1 | 39 | + 1 |
| − | + | − | 0 | 48 | +11 |
| − | − | + | 0 | 47 | +10 |
| + | + | − | 1 | 43 | + 5 |
| + | − | + | 0 | 53 | +16 |
| − | + | + | 0 | 50 | +13 |
| + | + | + | 1 | 53 | +15 |
| FLSD | | | | 7.6 | |

[a] Plants were exposed to 0.00 (−) or 0.04 (+) ppm of $O_3$ for 6 hr (chronic exposures) on the days indicated. On the 29th day, plants were exposed to 0.00 or 0.20 ppm of $O_3$ for 3 hr (acute).

[b] Percentage injury is the mean of injury per leaf on the three oldest trifoliolate leaves per plant.

[c] The chronic effect is defined as percentage injury from exposure to 0.00 or 0.04 ppm of $O_3$ prior to the acute exposure.

[d] The additive effect is defined as the chronic effect plus the acute effect (37%), which was the mean percentage injury from the acute exposure (plants not pretreated at 0.04 ppm of $O_3$).

exposure to $O_3$ doses that caused little or no visible injury, and was decreased by chronic exposure to $O_3$ doses that caused moderate to severe injury (>5%). Previous studies (4,7) have shown a similar pattern of response for tobacco (*Nicotiana tabacum* L.). The acute response patterns observed after chronic pretreatment at different time intervals suggest that the increase in sensitivity is cumulative over time and is partially alleviated by interrupting the $O_3$ exposure for a period of time with exposure to filtered air. Runeckles and Rosen (9) noted similar trends in their experiments with beans (*Phaseolus vulgaris* L. 'Pure Gold Wax').

The changes in sensitivity may involve alterations in stomatal behavior (6,9). Exposure that caused little or no visible injury may have caused the stomates to close more slowly in response to a subsequent high $O_3$ concentration. Exposure to $O_3$ doses that caused visible injury may have caused partial stomatal closure (6) resulting in less $O_3$ entering the leaf during a subsequent exposure. Runeckles and Rosen (9) demonstrated a partial stomatal closure response pattern with beans, with no visible injury caused by chronic exposures. Other processes that could influence the observed sensitivity changes include alterations of cellular membranes, the slow buildup of toxic substances, or the depletion or increase of substances that impart resistance. The visible injury threshold, the growth reduction threshold, and the abrupt transition from increased to decreased sensitivity being at approximately the same chronic dose suggest that the mechanisms for these responses may be closely related.

The differences in plant sensitivity to $O_3$ that depend on the previous history of exposure may have important implications for the analysis of experiments in air pollution effects research. The sensitivity of plants grown in greenhouses or growth chambers supplied with $O_3$-free air may be quite different from that of plants grown in the field. Research workers should be aware that filtered-air control treatments may not be realistic in relation to field conditions and that results of dose-response experiments with plants grown in charcoal-filtered air prior to exposure may be misleading (1). Monitoring and reporting of background levels of air pollutants in plant growth facilities used for air pollution research should be a standard procedure.

The changes in plant sensitivity to $O_3$ discussed in this report were caused by $O_3$ concentrations that commonly occur in the ambient air of the eastern United States (<0.10 ppm). More detailed information on the effects of ambient levels of $O_3$ and other pollutants, singly and in combination, on injury, growth, and yield is necessary to assess the relevance of present air quality standards and current research efforts.

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