Injury and Yield Responses of Soybean to Chronic Doses of Ozone and Sulfur Dioxide in the Field

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ABSTRACT

Beginning 14 days after emergence, 'Dare' soybean plants were covered by chambers and exposed for 6 h per day to ozone (O_3), sulfur dioxide (SO_2) and a mixture of these gases. The chamber treatments were carbon filtered air (CF), 5 pphm O_3 (low O_3), 10 pphm O_3 (high O_3), 10 pphm SO_2 (SO_2), and 10 pphm O_3 + 10 pphm SO_2 (mix). Plants were also grown outside in ambient air (AA). Injury, growth, and yield of plants were evaluated 43, 92, and 133 days after exposures began. Sulfur dioxide alone or in the mix did not significantly affect these responses. Low O_3 caused injury and defoliation but did not significantly reduce growth or yield. High O_3 and the mix caused injury and defoliation and reduced growth and yield. Injury was usually somewhat greater, and yield somewhat less, in the mix than in the high O_3, but these differences were not statistically significant. The results show that soybean can sustain some ozone injury without loss of yield. The results suggest that, unless acute episodes occur which cause extensive foliar injury, soybean yield will not be reduced in areas with seasonal daily 6-h averages of less than 5 pphm O_3 or 10 pphm SO_2.

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Ozone (O_3) and sulfur dioxide (SO_2) are two of the most important plant pathogenic air pollutants. Phytotoxic effects have been reviewed for O_3 (4, 9, 13) and for SO_2 (1, 2, 4). Most reports concern foliar injury produced by individual pollutants administered in greenhouse exposures. A few reports discuss pollution effects on plant growth and yield. Ambient photochemical oxidants (primarily O_3) reduced the growth or yield of citrus (16), grapes (15), and sweet corn (3). Early reports suggest that SO_2 can reduce growth and yield of crops only when leaves are injured. For example, Hill and Thomas (10) showed a linear relationship between foliar injury induced by SO_2 and yield of alfalfa.

Plants in polluted air are often exposed to mixtures of O_3 and SO_2. Mixtures of O_3 and SO_2 produced greater than additive foliar injury on tobacco (11, 18), white pine (6), alfalfa, and radish (18) but not on several other herbaceous species (18).

Ozone and SO_2 can also interact to affect plant growth and yield. In a greenhouse a mixture of O_3 and SO_2 (5 pphm each) (at 25 C and 760 mg Hg, 1 pphm $O_3 = 19.6\ \mu g\ O_3/m^3$ and 1 pphm $SO_2 = 26.2\ \mu g\ SO_2/m^3$) soybean growth decreased, whereas similar exposure to the same concns of those gases individually caused no decrease in growth (19). Growth of radish (17) and tobacco (12) in greenhouses was also decreased by a mixture of O_3 and SO_2, but the effects were not greater than the additive effects of the single gases.

There are no reports of field research on the effects of regulated doses of O_3 and SO_2 in mixtures on plant injury, growth, and yield. This paper deals with soybeans grown in field chambers exposed to O_3 and SO_2, alone and in a mixture, in doses that occur and may be exceeded in the air surrounding some urban and selected rural areas.

MATERIALS AND METHODS.— Seeds of soybean, *Glycine max* 'Dare' were sown in rows (120 cm apart) on 11 May 1971 in sandy clay loam soil near Raleigh, North Carolina. Soil moisture was maintained close to field capacity during the growing cycle and weeds were controlled by hand cultivation. Insects were controlled with two spray applications of carbaryl (1-napthyl methylcarbamate) on 2 June and 16 June. Mites were controlled with one application of dicofal [1, 1-*bis* (*p*-chlorophenyl)-2, 2, 2-trichloroethanol] on 14 July. Twelve days after emergence, twenty-four plots of two 3-m rows each, were selected on the basis of uniform plant appearance. The plants were thinned to about one plant per 5 cm.

Four replications of six treatments were randomized over the twenty-four plots. Thirteen days after emergence, plants in five treatments were enclosed in field exposure chambers covered with clear Teflon film. The incoming air in all chambers was filtered continuously through activated charcoal. Enclosed plants were exposed to: i) carbon-filtered air (CF), ii) 5 pphm O_3 (low O_3), iii) 10 pphm O_3 (high O_3), iv) 10 pphm SO_2 (SO_2), or v) 10 pphm O_3 + 10 pphm SO_2 (mix). The gases were added for 6 h/day (0800 to 1400 hr EST) from 14 days after emergence until maturity. Plants in the sixth treatment were not enclosed (AA).

Exposure chamber design.— Previously described chambers 2.4 × 2.4 m square by 2.1 m high were used in two replicates (8). Cylindrical chambers 3 m in diam, and 2.4 m high were used for the other two replicates (7). The cylindrical chambers (Fig. 1) were modified from those previously described (7) by using panels covered with clear 5.08×10^{-2} mm (2-mil)-thick Teflon film (Fig. 1-I, J, H), adding an exhaust duct (Fig. 1-G) and adding an exhaust fan and an impregnated activated charcoal exhaust filter to remove O_3 and SO_2 (Fig. 1-K). The square chambers were modified from those previously described (8) by adding a similar exhaust system (Fig. 1-G, K).

The O_3-generating system, described previously (8) and the SO_2-dispensing system were identical in both types of chambers. The SO_2-dispensing system was located in the filter box (Fig. 1-C) (details not shown) and consisted of a tank of liquid SO_2, a pressure regulator 7.03×10^{-4} kg-force/mm² (1 PSIG), a toggle valve, a solenoid valve, and a micro-metering valve (CV=0.0008) which regulated the amount of SO_2 entering the chamber. The solenoid valve was used with a 24-h timer to control the exposure period automatically. The toggle valve allowed the SO_2 to be turned on or off without changing the metering valve setting.

The O_3 concns in the chambers were monitored continuously with twelve Mast oxidant sensors (Mast Development Company, Davenport, Iowa) located in an air-conditioned building. A single constant source of O_3 produced by ultraviolet light was used to calibrate the sensors. The concn of O_3 at this source was determined (±1.5 pphm) periodically and this value was corrected to a 1% neutral KI standard (20). Each Mast sensor was equipped with a variable resistor, which allowed direct recorder readout of O_3 concn at the calibration source for 18 hr a day and

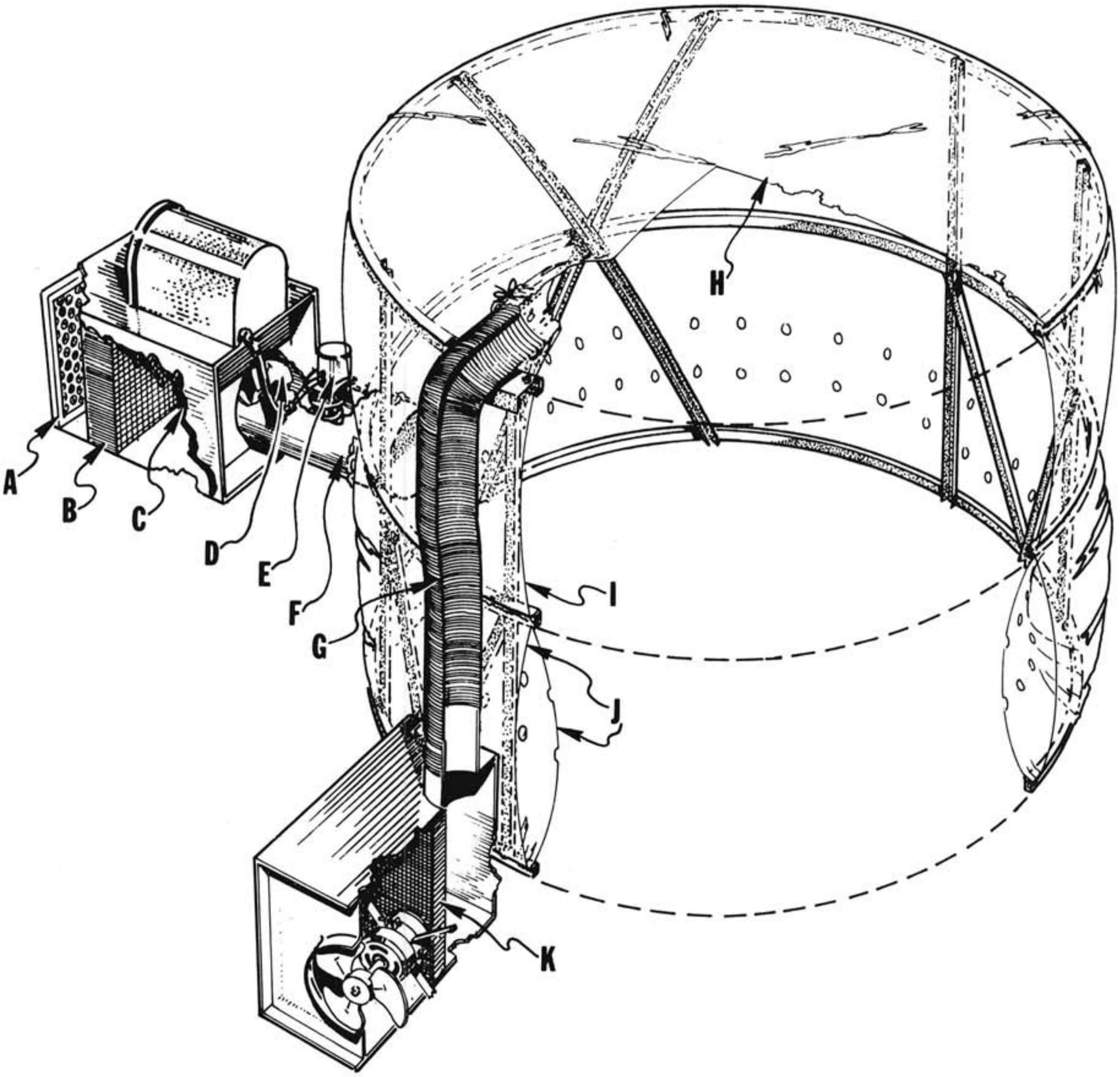

Fig. 1. Cylindrical field exposure chamber. (A) particulate filter; (B) activated charcoal filter; (C) location of SO_2 dispensing system (details not shown); (D) axial fan; (E) ultraviolet lamp; (F) galvanized steel duct; (G) exhaust duct; (H) clear Teflon top panel; (I) clear Teflon side panel; (J) double-layered clear Teflon duct panel: (K) activated charcoal-impregnated exhaust filter.

in the chambers for 6 hr a day during exposures. With this arrangement we were able to use twelve Mast sensors to determine a single O_3 concn at the calibration source and to identify and replace malfunctioning sensors before each daily exposure. Chromium trioxide scrubbers (14) were used to remove SO_2 when O_3 was monitored in the mix chambers. Sulfur dioxide concns were measured continuously (±2 pphm) with flame photometric SO_2 analyzers (Melpar LL-1100-IBM) located in an air-conditioned building.

Shaded iron-constantan thermocouples continuously monitored chamber and ambient temp. No differences in temp occurred between the two types of chambers. The temp differential between chamber air and ambient air on hot (>30 C) cloudless days reached a maximum of about 5 C for several hr, but was typically 1.5 to 3 C. No temp differentials were found at night. Chamber and ambient relative humidity (RH) were measured continuously at plant height with three wide-range humidity transducers (Hygrodynamics-15-702). No differences in RH were found between the two types of chambers. The RH was about 2-3% higher in the chambers than in ambient air when the ambient wind velocity exceeded about 4.47 m/s (10 mph). When wind velocity was less than that, RH in the chambers was the same as in ambient air.

Plant injury, growth, and yield.— Plants were evaluated 43, 92, and 133 days after exposures began (harvests one, two and three, respectively). Plants were in full bloom at 43 days, most pods were filled at 92 days and most plants were mature at 133 days. In harvests one and two, five randomly selected plants per plot were cut at ground level. In harvest three, 25 plants per plot were cut (five samples, consisting of five randomly selected plants each).

In harvests one and two, the percentage defoliation and the percentage necrosis and chlorosis of the upper surface of attached trifoliate leaves arising from the main stem were estimated in increments of 5% (0-100%). Abscised leaves were rated 100% injured. We did not attempt separate estimates of necrosis, chlorosis, and defoliation caused by the pollutants from those caused by normal senescence. In harvest one, the plant fresh wt and the number of nodes with opened flowers/plant were determined. In harvests two and three, plant fresh wt, the number of pods, and the fresh weight of pods/plant were determined.

Immediately after harvest three, pods were removed from the plants. Pods were counted, weighed, placed in paper bags, and air-dried for 14 days at about 25 C and 40% RH. After drying, the seeds were removed from the pods

TABLE 1. Response of soybean plants to 43, 92, or 133 daily 6-h exposures to O_3 and SO_2 alone or in a mixture [v]

Plant response	Number of daily 6-h exposures	Effect per treatment [w]					
		Ambient air (AA)	Charcoal-filtered air (CF)	Sulfur dioxide 10pphm (SO_2) [x]	Ozone 5 pphm (low O_3) [x]	Ozone 10 pphm (high O_3)	Ozone + sulfur dioxide - 10 pphm each (mix)
Defoliation/plant (%) [y]	43	31 b c	16 d	21 c d	35 a b	46 a	46 a
	92	53 b c	40 d	45 c d	49 c d	60 a b	67 a
Mean injury/leaf (%) [z]	43	39 b c	31 c	36 b c	44 b	69 a	72 a
	92	57 c	50 c	57 c	69 b	87 a	96 a
Plant fresh wt (gm)	43	216 a	208 a	215 a	157 a	73 b	99 a b
	92	472 a	439 a	481 a	352 a b	214 b	215 b
	133	206 a	199 a	218 a	156 a b	70 b c	55 c
Flowering nodes/plant	43	26 b c d	34 a b	39 a	30 a b d	19 d	24 c d
Pods/plant	92	260 a	179 a b c	208 a b	169 b c d	130 c d	107 d
	133	227 a	186 a	203 a	210 a	120 b	101 b
Pod fresh wt/plant (g)	92	192 a	165 a	171 a	144 a b	96 b c	83 c
	133	112 a	96 a	99 a	92 a	46 b	36 b
Seeds/plant	133	445 a	375 a	382 a	390 a	214 b	178 b
Seed harvest wt/plant (g)	133	73 a	60 a	60 a	58 a	27 b	22 b
Seed oven-dry wt/plant (g)	133	67 a	57 a	57 a	55 a	26 b	21 b

[v] Plants in all but the AA and CF treatments were exposed for 6-h/day beginning 14 days after emergence. Plants exposed to ambient air (AA) were not covered by chambers; those in the (CF) treatment were exposed continuously to carbon-filtered air.

[w] Each value is the mean of five plants in each of four replicates in 43- and 92-day exposures and of five samples of five plants in each of four replicates in 133-day exposures. Means in the same line followed by the same letter are not significantly different ($P = 0.05$) according to Tukey's test.

[x] 1 pphm $SO_2 = 26.2\ \mu g\ SO_2/m^3$ of air at 760 mmHg and 25 C. 1 pphm $O_3 = 19.6\ \mu g\ O_3/m^3$ of air at 760 mm Hg and 25 C.

[y] Percentage of trifoliate leaves arising from the main stem that were abscised. (Includes abscission due to normal senescence).

[z] Mean percentage chlorosis or necrosis on trifoliate leaves arising from the main stem. (Includes chlorosis and necrosis from normal senescence). Abscised trifoliates received an injury rating of 100%.

and the number and weight of seeds/plant determined. Seeds were then dried for 2 days at 70 C to determine their oven-dry weight.

Analyses of variance were performed on the data and significant treatment mean differences were determined using Tukey's method (5).

RESULTS.—*Pollutant concentrations.*— The mean O_3 concns in the four replicates of the 5 pphm (low O_3) treatment ranged from 4.6 to 5.2 pphm and in the 10 pphm (high O_3) treatment from 8.9 to 10.3 pphm. The treatment means were 5.0 and 9.5 pphm, respectively. The mean O_3 concn in the replicates of the 10 pphm O_3+10 pphm SO_2 (mix) treatment ranged from 9.2 to 10.5 pphm with a treatment mean of 9.5 pphm. The mean SO_2 concn in the replicates of the 10 pphm SO_2 (SO_2) treatment ranged from 9.4 to 10.0 pphm and in the mix treatment from 9.3 to 10.1 pphm. The treatment means were 9.7 and 9.8, respectively.

Defoliation and leaf injury.— Injury was first observed in both the high O_3 and mix about one wk after exposures began. The symptoms resembled classical O_3 injury; white to tan flecking on the upper leaf surface and general chlorosis of older leaves. The injury increased with continuing exposures, resulting in extensive leaf necrosis, and abscission (Table 1). About one-and-a-half to two times as much leaf injury was present in the high O_3 and mix treatments than in the CF and SO_2 treatments; CF- and SO_2-treated plants appeared normal throughout the experiment (Table 1).

Symptoms of O_3 injury were first observed in the low O_3 about 2 wk after exposures began. Continuing exposures gradually caused premature chlorosis, senescence, and abscission of older leaves. Plants in low O_3 were injured less than those in high O_3 and mix; low O_3-treated plants resembled those in AA which were injured by ambient O_3. The values for injury and defoliation in the low O_3 and AA were usually intermediate between those in the CF and SO_2 and those in the high O_3 and mix (Table 1).

Sulfur dioxide, either alone or in the mix, did not significantly affect injury or defoliation. No symptoms of SO_2 injury were observed.

Plant fresh weight and flowering.— Plants were stunted and development of axillary trifoliates was greatly decreased in the high O_3 and mix treatments. They weighed less than half as much and had about half as many flowering nodes as plants in the CF and SO_2 (Table 1).

Plants in the low O_3 weighed about three fourths as much as those in the AA, CF and SO_2 but the decrease was not significant.

Sulfur dioxide alone or in the mix did not significantly affect plant growth or flowering (Table 1).

Pod number and fresh weight.— Plants in the mix had fewer and lighter pods (pod yield) than those in the high O_3 but the decreases were not significant (Table 1). Pod yield was less in the high O_3 than in the low O_3 but the decreases were significant only in the final harvest (Table 1). Pod yield in the low O_3 was usually similar to that in the AA, CF and SO_2.

Seed number and weight.— Plants in the mix had fewer and lighter seeds (seed yield) than those in the high O_3 but the decreases were not significant (Table 1). Seed yield in the high O_3 and mix was significantly less than in any other treatment.

In the most practical measure of soybean yield (seed weight/plant after harvest), there was more than a twofold decrease in the high O_3 and mix when compared to any of the other treatments (Table 1). Seed yield in the SO_2 and CF was similar. Seed yield in the AA was greater than in the CF, SO_2 or low O_3 but the differences were not significant.

DISCUSSION.— The threshold concentration of O_3 for significant inhibition of growth and yield of Dare soybeans in this study and in the greenhouse (19) was between 5 and 10 pphm. The threshold would likely be higher however, with a more resistant soybean variety, higher if the soil moisture content were lower and lower, if the exposure time were longer than 6 h.

In our exposures 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 but the differences were not statistically significant. It is possible that significant synergistic effects are more likely when one or both gases are present at concns near the injury threshold for each. For example, a mixture of 5 pphm each of O_3 and SO_2 caused more than additive decreases in the growth of Dare and Hood soybeans in the greenhouse (19). It is not known whether such a mixture would cause similar effects under field conditions.

The lowest concentration of ozone (5 pphm) and resultant dose (concn × time) in our experimental exposures can be exceeded in the air surrounding many urban areas. For example, near Raleigh, North Carolina, a relatively nonpolluted area of the Southeast, the mean daily O_3 concns for 6 h (0800-1400 hr EST) for July and August in 1970, 1971 and 1972 were 6.3, 4.4, and 6.7 pphm. Comparative SO_2 concns were less than 1.0 pphm.

More studies are needed to determine the effects of individual pollutants and of various ratios of different pollutants in mixtures under field conditions. Knowledge gained from studies of this kind, coupled with accurate monitoring of important pollutant concns in major agricultural areas will give us a better determination of the extent of pollution damage to plants. Such knowledge will show whether further research is needed to minimize pollution damage to plants. It will also enable us to define intelligent air pollution control standards that balance the costs of control with the direct and indirect costs of pollution.

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