Response of Soybeans to Chronic Doses of Ozone Applied as Constant or Proportional Additions to Ambient Air

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Cooperative investigations of the USDA-ARS and North Carolina State University. Journal Series Paper 9586 of the North Carolina Agricultural Research Service, Raleigh 27695-7601. This research was partly supported by an Interagency Agreement between the Environmental Protection Agency and the USDA, AD-12-F-1-490-2.

The use of trade names in this publication does not imply endorsement by the U.S. Department of Agriculture or the North Carolina Research Service of the products named, or criticism of similar ones not mentioned. Although this research was partly funded by the U.S. Environmental Protection Agency, it has not been subjected to Agency review; therefore, it does not necessarily reflect the views of the Agency and no official endorsement should be inferred.

Accepted for publication 29 July 1985.

ABSTRACT

Heagle, A. S., Lesser, V. M., Rawlings, J. O., Heck, W. W., and Philbeck, R. B. 1986. Response of soybeans to chronic doses of ozone applied as constant or proportional additions to ambient air. Phytopathology 76:51-56.

Field-grown soybeans (*Glycine max* (L.) Merrill 'Davis') were exposed to chronic doses of ozone (O_3) for 7 hr/day from shortly after emergence until maturity. The O_3 doses were applied by supplementing the O_3 present in unfiltered air in open-top field chambers. Ozone was added in constant or in variable amounts which were proportional to ambient O_3 concentrations. The two methods of addition gave similar seasonal mean O_3 concentrations but there were greater fluctuations and higher peak concentrations with the proportional method than with the constant-addition method. Regression of yield response on O_3 concentrations showed no significant differences between types of additions and was similar to that obtained for Davis soybean in 1981. Calculated yield reductions at the ambient O_3 concentration of 0.050 ppm (1 ppm v/v = 1 μl/L) by using the Weibull model for the two methods of O_3 addition combined was 10%, assuming 0.025 ppm as the natural O_3 concentration.

Additional key words: air pollution, yield effects.

One primary objective of the National Crop Loss Assessment Network (NCLAN) is to determine relationships between chronic doses (small concentrations for long periods) of ozone (O_3) and crop yield. The intent is to simulate seasonal ambient O_3 exposures likely to occur with different levels of air quality. The NCLAN field studies are performed in open-top chambers (4) with O_3 doses obtained by adding O_3 for 7 hr/day to the variable amounts of O_3 present in ambient air (5–8,11–14).

Adding constant incremental amounts of O_3 to ambient O_3 produces a series of O_3 concentration curves that follow the changes over time in ambient O_3 levels. The curves obtained for different treatments parallel each other at constant increments regardless of the ambient O_3 concentrations. Thus, on cloudy days when ambient O_3 concentrations are low, the added O_3 represents a greater proportion of the ambient O_3 than when the ambient O_3 concentration is high. Adding O_3 in different proportions to the ambient O_3 concentration would result in a series of O_3 concentration curves that become more divergent as the ambient O_3 level increases. Each incremental proportion retains a given proportion to the ambient O_3 concentration regardless of whether ambient O_3 is high or low. Proportional additions would result in a wider range of O_3 concentrations for a given treatment than would constant additions.

Concentration is more important than exposure duration in causing a plant response to short-term (several hours) exposures to O_3 (10,15) and sulfur dioxide (SO_2) (3). At equal doses (dose = concentration × exposure duration) variable concentrations of O_3 (17) or SO_2 (1,2,16) caused more plant response than did constant concentrations. However, there are no reports of similar experiments using seasonal exposures to low levels of O_3 in the field. This experiment was performed to determine whether O_3 dose-soybean yield relationships are affected by differences in O_3 concentration exposure dynamics which result from constant and proportional additions of O_3 in open-top field chambers.

MATERIALS AND METHODS

Soybeans (*Glycine max* (L.) Merrill 'Davis') inoculated with a commercial preparation of *Rhizobium* were planted in a 0.4-ha field in rows spaced 1 m apart on 29 June 1982. Planting prior to this date was prevented by excess soil moisture. The soil was Appling sandy loam (clayey, kaolinitic, thermic, Typic hapludult) previously fertilized on 10 May 1982 according to soil-test recommendations. The herbicides alachlor [2-chloro-2′-6′-diethyl-*N*-(methoxymethyl)-acetanilide] at 5 L/ha and linuron [3-(3,4-dichlorophenyl)-1-methoxy-1-methylurea] at 1 kg/ha were applied at planting. Plants emerged on 3 July. Sixteen 3 × 3-m plots were chosen (eight for each of two blocks) based on uniformity of plant stand and soil appearance.

The plants in each of the two-row plots were thinned to one plant per 2.5 to 7.5 cm on 7 July to produce an average stand of 18 plants per meter of row. Soil tensiometers (Irrometer Company, Riverside, CA) were installed in each plot 5 cm from the west row at a depth of 30 cm. All plots were irrigated by using drip tubes when 50% of the plots showed tensiometer readings greater than 50% (−0.05 MPa). Between planting and crop maturity, 33.2 cm of rain fell and 19.4 cm (whole-plot equivalent) of irrigation water was applied. Insects and mites were controlled with one application of permethrin [(3-phenoxyphenyl) methyl (±) *cis*, *trans*-3-(2,2-dichloroethynyl)-2,2-dimethyl cyclopropanecarboxylate] and three applications of cyhexatin (tricyclohexyl hydroxystannane).

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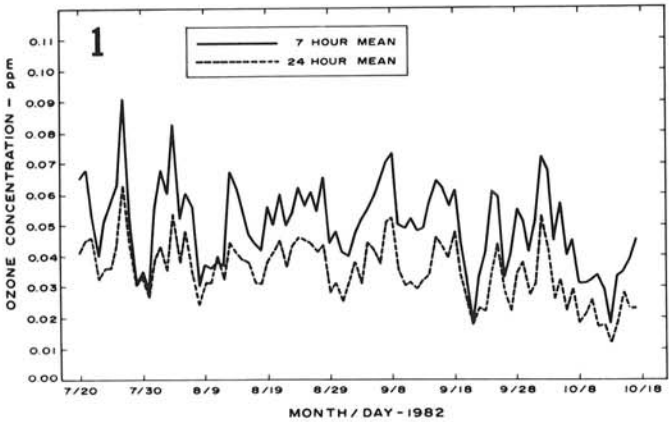

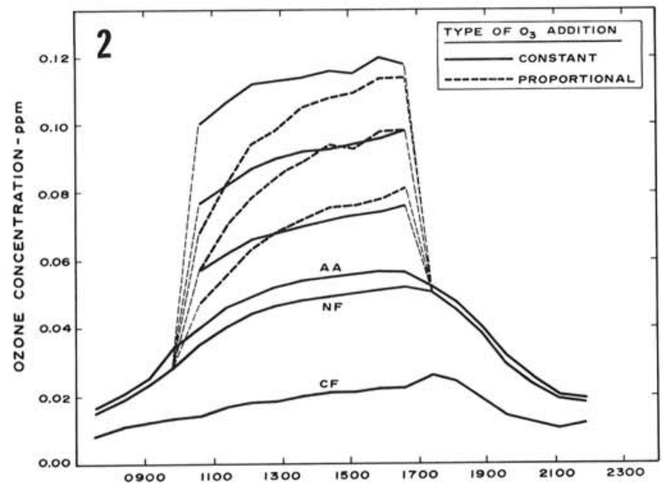

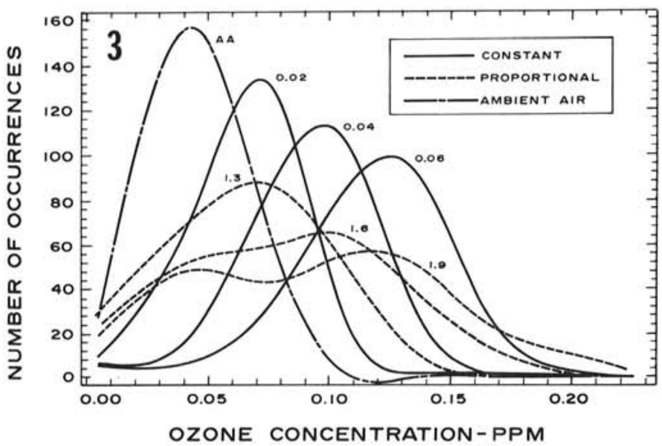

Figs. 1–3. Treatment patterns used in testing the responses of soybeans to constant or proportional additions of ozone (O_3) to ambient air. **1,** Daily 7-hr (1000 to 1700 hr EDT) and 24-hr mean O_3 concentrations (1 ppm v/v = 1 μl/L) in ambient air (AA) during the period of exposures (20 July–17 October). **2,** Seasonal (20 July–17 October 1982) diurnal fluctuation in concentration for the various O_3 treatments. CF = charcoal filtered-air chamber; NF = the common concentration for all nonfiltered-air chambers during the time of day when no O_3 was added; AA = ambient air concentration which was always slightly greater than the NF concentration; NF + 0.02, NF + 0.04, and NF + 0.06 ppm (1 ppm v/v = 1 μl/L) treatments are represented by the top three solid lines; NF × 1.3, NF × 1.6, and NF × 1.9 treatments are represented by the dashed lines. **3,** Frequency distributions for O_3 concentrations in AA or in open-top chambers resulting from constant O_3 additions of 0.02, 0.04, or 0.06 ppm (solid lines) or from O_3 additions which resulted in proportional-to-ambient (1.3, 1.6, or 1.9 times ambient) O_3 concentrations (dashed lines) (1 ppm v/v = 1 μl/L). An occurrence is defined as the last 1-min of a 3-min sample taken every 45 min during each daily 7-hr exposure period from 20 July to 17 October 1982. The mean frequency of three consecutive O_3 concentrations were plotted to give smooth transitions across concentrations.

Open-top chamber frames (4) were placed over 16 plots on 6 July, clear polyvinyl chloride-film panels were installed by 19 July, and chamber fans were run daily between 0600 and 2200 hours EDT from 19 July until the final harvest. The experimental design included eight chamber-O_3 treatments in each of two randomized blocks. Two of the treatments were charcoal-filtered air (CF) and nonfiltered air (NF) without added O_3. Three constant-addition treatments were established by adding 0.02, 0.04, or 0.06 ppm (1 ppm v/v = 1 μl/L) O_3 to ambient O_3 in NF chambers for 7 hr/day (1000–1700 hours EDT) by using the methods for dispensing and monitoring described previously (7). The three proportional-addition treatments were approximately 1.3, 1.6, or 1.9 times the amount of O_3 present in ambient air obtained by adding O_3 to NF chambers for 7 hr/day (1000–1700 hours EDT). For proportional additions, a Monitor Labs 8410 O_3 monitor, which continuously monitored ambient O_3, was used to regulate voltage output from an analog controller to a Griffin GTC-1A ozonizer. Relationships between ambient O_3 and ozonizer output as sequentially monitored in the chambers appeared to be linear.

Ozone exposures began on 20 July, when the first trifoliolate leaves were 75% expanded. Exposures continued for 7 hr each day until 17 October when plants in all treatments were physiologically mature. However, O_3 was not dispensed during periods of rainfall (a total of 24.5 exposure hours during the season) or when either of the O_3 dispensing systems malfunctioned (a total exposure of 43.3 hours during the season).

Two plots outside each chamber plot (companion plots) were used to measure yield as an indication of edaphic variability in the field for use in covariate analyses. Each companion plot was a 2-m row located 1.2 m east or 1.2 m west of the fan box of each chamber. All cultural practices for plants in the companion plots were the same as for those in the treatment plots.

Four 1-m sections of row (two in each of the two plot rows) were used for plant response measurements. Approximately 40 cm of row at both ends of each plot row served as border. Foliar injury was estimated as the percentage chlorosis and necrosis in 5% increments (0–100%) for individual trifoliolate leaves arising from the main stem of four randomly chosen plants (one for each 1 m of row per plot). Abscised leaves were rated as 100% injured. Estimates were made on 11 and 31 August and 23 September. On 11 August, injury was estimated for the eight oldest trifoliolates. On 31 August and 23 September, injury was estimated for the eight youngest leaves because of difficulty in locating and observing leaves at lower stem positions without disrupting plant canopies.

Plants in the four 1-m rows of the treatment plots were harvested on 8 November. Pods were removed by hand and the number of filled pods per plant were counted. Pods for each 1-m row sample were combined and weighed before threshing. Seed weight per 1 m of row and weight of one 100-seed sample (8.1% moisture content) from each 1 m of row were measured. Plants in the companion plots were harvested on 22 November. They were threshed as one sample per plot and seeds were weighed.

Statistical analyses. The nature of variation within the field was determined with yield data from the companion plots. Estimates of yield for each treatment plot which would have occurred without O_3 treatment were predicted by the average of the two companion plot rows per treatment plot and by calculating fitted response surface values. The fitted values were obtained by regressing the companion plot yield on the field position coordinates of the companion plots.

Analyses of variance of the foliar injury and yield data from the treatment plots were performed. In these analyses, the effect of blocks, O_3 treatments, 1-m row positions within treatment plots (position), and position × treatment interaction were determined. The arc sine transformation was used on injury proportions to stabilize variances.

Polynomial dose-response models and a nonlinear model based on the Weibull probability distribution of sensitivities (18) were used to characterize the yield response to O_3 and the effect of constant or proportional O_3 addition for the chamber treatments. The Weibull model was used because it is flexible enough to accommodate the range of responses likely to occur from chronic exposure to O_3. Also, if responses of cultivars or species are similar, it can easily be used to consolidate response curves from different experiments into a single model (8,18).

If the method of dispensing affected the dose-response curves, the effect would occur above the NF level for treatments with O_3 added. Thus, two types of models (reduced and full) were obtained for both the polynomial and Weibull models. The "full" models had a common response between the O_3 concentrations in the CF and NF treatments but allowed a divergence in response for the two dispensing methods above the NF treatment. The "reduced" models also had a common response between O_3 concentrations in the CF and NF treatments but did not allow for a divergent response for the two dispensing methods above the NF treatment. The comparison of the "reduced" and "full" models provided the test of "no difference" for the two dispensing methods.

RESULTS

Ozone concentrations. Daily fluctuations in 7- and 24-hr mean O_3 concentrations in ambient air (AA) are shown in Fig. 1. The ambient O_3 levels for 1982 were slightly lower than for previous years (5,6), but the same trend toward decreased levels as the season progressed was apparent. The mean seasonal 7 hr/day O_3 concentrations for the AA, NF, and CF treatments were 0.050, 0.044, and 0.019 ppm, respectively (Table 1). The difference between the NF and AA concentrations was caused by degradation of O_3 in the NF chamber air-handling system. For the constant-addition treatments, the seasonal 7 hr/day O_3 doses for the NF + 0.02, NF + 0.04, and NF + 0.06 treatments were 0.066, 0.086, and 0.109 ppm, respectively (Table 1). For the proportional additions at 1.3, 1.6, and 1.9 times ambient, the seasonal 7-hr means were 0.065, 0.081, and 0.092 ppm, respectively. These values were within 6% of our target values. That constant-additions and proportional-to-ambient additions were achieved is shown by the seasonal mean diurnal fluctuations for AA and the various chamber treatments in Fig. 2. During the 7-hr exposure period, curves for the constant-addition treatments (Fig. 2; three uppermost solid lines) closely paralleled the AA concentration curve. However, the curves for the proportional-addition treatments (Fig. 2; dashed lines) show an increasing divergence from the AA curve and from each other as the O_3 concentration in AA increased. For each level of proportional addition, the highest and second highest 7 or 1 hr/day

TABLE 1. Mean concentrations of O_3 measured during studies to determine the response of soybeans to constant or proportional additions of O_3 to ambient-air O_3 concentrations

	7 hr/day values (ppm)[a]			1 hr/day peak values (ppm)[a]		
Treatment	Seasonal[b]	Highest[c]	Second highest[c]	Seasonal[b]	Highest[c]	Second highest[c]
No addition						
AA[d]	0.050	0.091	0.083	0.062	0.102	0.099
CF	0.019	0.035	0.033	0.028	0.045	0.042
NF	0.044	0.081	0.069	0.056	0.098	0.089
Constant addition						
NF + 0.02	0.066	0.104	0.098	0.080	0.120	0.110
NF + 0.04	0.086	0.124	0.123	0.106	0.141	0.140
NF + 0.06	0.109	0.168	0.147	0.134	0.192	0.176
Proportional addition						
NF × 1.3	0.065	0.119	0.113	0.088	0.139	0.136
NF × 1.6	0.081	0.154	0.143	0.111	0.193	0.169
NF × 1.9	0.092	0.200	0.164	0.126	0.220	0.192

[a] For the daily period from 1000 to 1700 hours EDT. 1 ppm (v/v) = 1 μl/L.
[b] Values for the period 20 July to 17 October 1982. Each value is the mean from two replicate plots.
[c] The highest and second highest peak values are defined as the highest and second highest mean, respectively, of two consecutive recorded concentrations from 20 July to 17 October. Concentrations were recorded for 3-min samples taken at 45-min intervals by using time-shared sequential monitoring for each of 15 locations. This definition precluded occurrence of both peaks on a single day.
[d] AA = ambient air, CF = charcoal-filtered air, and NF = nonfiltered air.

TABLE 2. Foliar injury and yield of cultivar Davis soybeans exposed to chronic doses of O_3 added in constant or proportional amounts to O_3 in ambient air[a]

Type of O_3 addition	Seasonal 7 hr/day mean O_3 conc. (ppm)	Foliar injury (%)[b]			Filled pods per meter of row (no.)	Weight per meter of row (g)		100-seed weight (g)
		11 Aug	31 Aug	12 Sept		Filled pods	Seeds	
None								
CF[c]	0.019	29	0	6	1,279	622	468	16.9
NF	0.044	30	1	12	1,374	637	478	16.2
Constant								
NF + 0.02	0.066	41	9	22	1,153	475	354	14.6
NF + 0.04	0.086	48	9	25	1,138	435	319	13.4
NF + 0.06	0.109	58	22	50	915	323	240	13.2
Proportional								
NF × 1.3	0.065	40	6	19	1,288	537	399	14.3
NF × 1.6	0.081	44	12	30	1,096	434	324	14.0
NF × 1.9	0.092	48	21	46	934	347	259	13.5

[a] Each value is the mean of eight samples (four 1-m-of-row samples in each of two chambers).
[b] Each value is the mean of 32 trifoliolate leaves (eight leaves on four plants). Mean injury was estimated for the oldest eight leaves per plant on 11 August and for the youngest eight leaves per plant on 31 August and 12 September. An arcsin transformation (radians) was done on the percent foliar injury. The regression equations of percent injury on O_3 dose for the three consecutive dates were: $Y = 100[\sin(0.468 + 3.38x)]^2$, $y = 100[\sin(4.27x)]^2$, $y = 100[\sin(7.19x)]^2$, respectively. The standard error for the intercept in the first equation was 0.033; the standard errors of the three slope coefficients were 0.44, 0.17, and 0.28, respectively.
[c] CF = charcoal-filtered air and NF = nonfiltered air.

TABLE 3. Equations for the regression of seed yield of cultivar Davis soybeans on chronic doses of O_3 added in constant or proportional amounts to the O_3 in ambient air

Polynomial models[a]	
Constant addition[b]	$y = 549 - 2{,}723\,x$ (16) (226)
Proportional addition	$y = 426 + 3{,}637\,x - 59{,}814\,x^2$ (28) (1,174) (10,482)
Ignoring method of addition (reduced model)	$y = 357 + 9{,}514\,x - 200{,}060\,x^2 + 945{,}529\,x^3$ (64) (3,987) (68,869) (352,771)
Full (segmented) polynomial models[c]	$y = 469 - 44\,x$ (for $x \leq 0.044$ ppm) (33) (891)
	$y = 469 - 3{,}630\,(x - 0.044)$ (for $x > 0.044$ and constant addition) (33) (362)
	$y = 469 - 4{,}112\,(x - 0.044)$ (for $x > 0.044$ and proportional addition) (33) (467)
Weibull models[d]	
Constant addition	$y = 490 \exp[-(x/0.126)^{2.17}]$ (20, 0.007, 0.44)
Proportional addition	$y = 479 \exp[-(x/0.103)^{3.89}]$ (12, 0.003, 0.67)
Ignoring method of addition	$y = 492 \exp[-(x/0.121)^{2.26}]$ (21, 0.006, 0.43)

[a] y = yield (g) of seeds per meter of row; $x = O_3$ dose characterized as the seasonal 7 hr/day mean concentration (ppm). Standard errors for each parameter estimate are shown in parentheses.

[b] The quadratic equation (with standard errors in parentheses) for constant additions was $y = 500\,(31) - 694\,x\,(1{,}063) - 15{,}901\,x^2\,(8{,}145)$.

[c] The segmented polynomial model provides for a linear response ($b_1 = -43.8\,x$) to O_3 levels ≤ 0.044 ppm and a different linear response above 0.044 ppm for constant addition, $b_c = -3{,}650$, and for proportional addition, $b_p = -4{,}112$, and $x = O_3$ concentration.

[d] For the Weibull model ($y = \alpha \exp[-x/\sigma]^c$); y = estimated yield of seeds (g) per meter of row; $x = O_3$ dose as seasonal 7 hr/day mean concentration (ppm); α = maximum seed yield at 0 ppm O_3; $\sigma = O_3$ concentration at which α is reduced by 63%; and c is a dimensionless shape parameter. Standard errors in parentheses are for α, σ, and c, respectively.

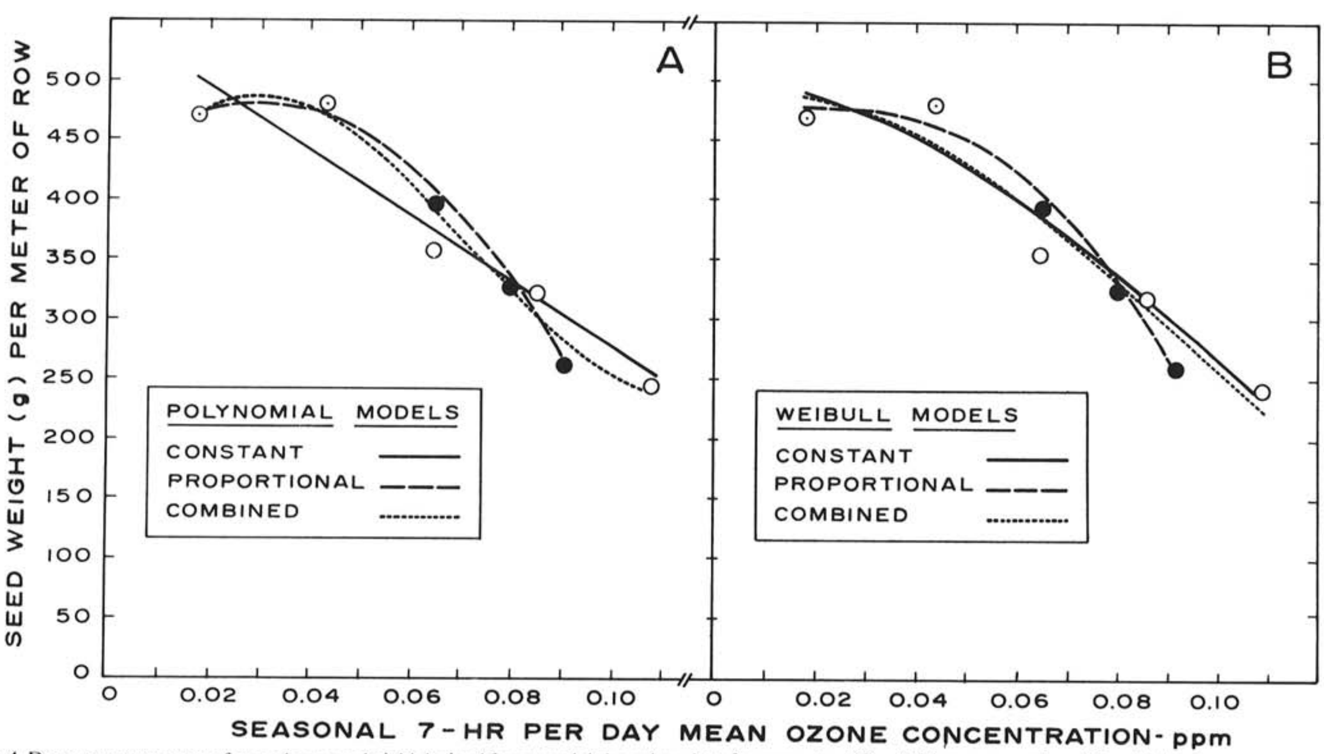

Fig. 4. Dose-response curves for soybean seed yield derived from models based on data from constant O_3 addition, proportional O_3 additions, or constant and proportional O_3 additions combined: **A,** polynomial models; **B,** Weibull models. Model formulas are shown in Table 3. ⊙ = Measured yields for charcoal-filtered and nonfiltered air treatments; o = measured yields for constant-addition treatments; • = measured yields for proportional-addition treatments. (1 ppm v/v = 1 μl/L).

O_3 concentrations were higher than for the comparable constant-addition treatments (Table 1). As expected, the frequency of occurrence of relatively high O_3 concentrations was greater in the proportional than in the constant-addition treatments for each level of O_3 addition (Fig. 3). The O_3 concentration in the NF × 1.9 treatment was above 0.175 ppm 7% of the time during the 7-hr exposure periods but for the NF + 0.06 treatment, this was true only about 3% of the time. The constant-addition treatments had the effect of clustering the frequency of occurrence of incremental O_3 concentrations over a narrow range of concentrations while the reverse was true for the proportional additions (Fig. 3). The greater the constant addition, the higher the concentration for which the cluster occurred, although the clustering was greater for the lowest constant addition.

Foliar injury. The only statistically significant effect on injury was the O_3 treatment. The range in the amount of injury (chlorosis and necrosis) of the lowest eight leaves on 11 August was small compared to that estimated for the upper eight leaves on 12 September (Table 2). Injury increased with O_3 concentration and with duration of exposure, and it more than doubled from 31 August to 12 September. Regression equations relating O_3 concentrations to transformed foliar injury values over all chamber O_3 treatments combined are shown in a footnote of Table 2.

Yield. There were significant O_3-treatment and position effects within chambers. However, there were no significant treatment × position interactions and subsequent analysis of yield data used the mean over the four positions for each treatment plot. Analyses of the plot means were used to assess the importance of the covariate yield data obtained from the companion plots. The covariate obtained from the fitted companion plot response surface was marginally effective in reducing the experimental error. The averaged companion plot covariate was not effective. Neither covariate proved to be effective in reducing the standard errors of the fitted dose-response models and consequently covariate data were not used in the final analyses.

The position effect was caused by larger plants with greater yields (approximately 15%) in the north half than in the south half of the chambers. The number and weight of filled pods, weight of seeds per 1 m of row (seed yield), and weight of 100 seeds (seed size) declined with increasing O_3 concentrations above 0.044 ppm (Table 2). Seed weight was from 73 to 75% of pod weight regardless of the O_3 treatment. For most treatments, the effects of O_3 on seed yield were related to fewer filled pods (and therefore fewer seeds) and smaller seeds. For example, in the NF + 0.02 treatment, the number of filled pods was 10% less, seed size was 14% less, and seed yield was 24% less than for the CF treatment. This relationship of percentage seed yield loss equalling the percentage loss of filled pods plus the percentage loss in weight per 100 seeds also held true for the other O_3 addition treatments. For the low and moderate O_3 levels (NF + 0.02, NF + 0.04, NF × 1.3, and NF × 1.6), the effects on seed size were somewhat greater than the effects on number of filled pods. However, the reverse was true for the NF + 0.06 and NF × 1.9 treatments.

The full (segmented) polynomial and full Weibull models provided for an effect of O_3 between the CF (0.019 ppm) and NF (0.044 ppm) treatments which was not significantly different from zero, and separate regressions for each type of addition above NF. The responses beyond 0.044 ppm for both types of addition was linear for the polynomial model (Table 3). Tests of homogeneity of the response between the two O_3 addition methods, which allowed for either a linear, quadratic, cubic, or Weibull response, showed no significant differences between the methods of O_3 addition. However, a linear polynomial model provided the best fit to data from the constant-addition treatment, a quadratic polynomial model provided the best fit for the proportional treatments, and a cubic polynomial model provided the best fit ignoring the method of addition (Table 3, Fig. 4). For comparison, the quadratic polynomial for the constant-addition treatments is shown in a footnote of Table 3.

Tests for lack-of-fit for the Weibull and polynomial models (ignoring the method of O_3 addition) presented in Table 3 indicated that both were adequate. The tests indicated that the four-parameter polynomial model provided a better fit to the data than the three-parameter Weibull model. However, additional yield loss would be expected beyond the highest dose of the observed curve which the Weibull model, but not the polynomial model, suggests. Both models estimated similar yield losses (considering 0.025 ppm as the control) for all O_3 levels tested. For example, for mean concentrations of 0.06, 0.08, and 0.10 ppm, the polynomial model estimated yield losses of 15, 34, and 48%, while the Weibull model estimated losses of 16, 31, and 46%.

DISCUSSION

Loss of soybean yield probably resulted from an accumulation of small daily effects of O_3 over the growing season. It is unlikely that a single daily dose would decrease yield without previous and subsequent doses. The slope of the dose-response curve for the proportional treatments was greater than that for the constant treatments (Fig. 4). However, the differences in peak concentrations (Table 1) and concentration distribution frequencies (Fig. 3) for the two methods of O_3 addition were not enough to significantly change the dose-response relationships (Table 3). An extension of the dose range on either side of those compared here might result in significantly different relationships. One of our purposes was to compare dose-response relationships obtained from NCLAN studies using 7 hr/day constant O_3 additions with those developed from proportional O_3 additions. The protocol of constant 7 hr/day O_3 addition was adopted by NCLAN before the technology of proportional O_3 addition was developed. Probably, it would be better to perform O_3 dose-yield response studies using proportional O_3 additions whenever O_3 concentrations in AA are greater than those considered to be background. If ambient O_3 levels were high enough that proportional additions occasionally caused moderate or severe foliar injury, a single exposure could affect yield. In this case, the type of O_3 addition might affect dose-response relationships.

Despite the late planting and shortened growth and exposure period in 1982, results were similar to those obtained in 1981. In 1981, the measured seed yield of cultivar Davis soybeans at seasonal mean 7 hr/day O_3 concentrations of 0.069 and 0.086 ppm over 111 days of exposure was 23 and 34% less, respectively, than yield in the CF treatment (5). In 1982, the measured seed yield at 0.066 and 0.086 ppm O_3 over 90 days of exposure was 24 and 32% less, respectively, than yield in the CF treatment (Table 2). With the seasonal 7 hr/day mean O_3 concentration (dose) as the independent variable in regression analyses, seasonal dose and yield response relationships were similar for both years.

The similarities in results for 1981 and 1982 indicate that for chronic exposures to ambient levels of O_3, the seasonal mean O_3 concentration is a better way to characterize the exposure level (dose) than is the use of ppm-hours (hours of exposure × concentration in ppm). With ppm-hours (dose) as the independent variable, the doses at any given O_3 concentration would be 23% higher in 1981 than in 1982 and the dose-response relationships for 1981 would be different from those in 1982. For both years, exposures began within 17 days of soybean emergence and ended at physiological maturity. A decrease in the proportion of the period from emergence to maturity for which plants are exposed would probably cause a decrease in the magnitude of the effects but this premise has not been tested.

Results of this and previous research at Raleigh, NC, and elsewhere in the United States indicate that O_3 at ambient concentrations causes soybean yield loss. A combination Weibull model which used nine data sets predicted a soybean yield loss of 12% at a seasonal 7 hr/day mean O_3 concentration of 0.05 ppm (9).

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