Effects of Daily Ozone Exposure Duration and Concentration Fluctuation on Yield of Tobacco

Allen S. Heagle, W. W. Heck, V. M. Lesser, and J. O. Rawlings

Plant pathologist, USDA-ARS, Department of Plant Pathology; plant physiologist, USDA-ARS, Department of Botany; statistician, Department of Statistics; and professor of statistics, Department of Statistics; North Carolina State University, Raleigh 27650.

Cooperative investigations of the USDA-ARS and the North Carolina State University. Journal Series Paper 10333 of the North Carolina Agricultural Research Service, Raleigh 27695-7601. Research partly supported by an Interagency Agreement between the Environmental Protection Agency and the USDA; Interagency agreement AD-12-F-1-490-2.

The use of trade names in this publication does not imply endorsement by the USDA or the North Carolina Agricultural Research Service of the products named, nor 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 and therefore does not necessarily reflect the views of the Agency and no official endorsement should be inferred.

We thank Robert Philbeck, Van Poole, and Fred Mowry for maintenance of dispensing and monitoring systems; Henry Marshall and William Collins for advice and assistance in tobacco culture; and Susan Spruill for computer programming.

Accepted for publication 3 December 1986.

ABSTRACT

Heagle, A. S., Heck, W. W., Lesser, V. M., and Rawlings, J. O. 1987. Effects of daily ozone exposure duration and concentration fluctuation on yield of tobacco. Phytopathology 77:856-862.

Flue-cured tobacco (*Nicotiana tabacum* 'McNair 944') was exposed to chronic doses of ozone (O_3) in open-top field chambers to determine the influence of frequency and magnitude of peak O_3 concentrations and daily exposure duration on tobacco yield response. The treatments were established by adding O_3 in amounts that were proportional to ambient O_3 concentrations or in constant amounts. The frequency of occurrence and level of peak O_3 concentrations were greater for each proportional-addition treatment than for the corresponding constant-addition treatments. However, the seasonal mean O_3 concentrations were nearly identical, and the yield response to O_3 was similar for both types of O_3 addition. Yield in plots receiving proportional addition of O_3 for 12 hr/day (1000 to 2200 hours EDT) was 10% less (three treatment levels combined) than in those receiving proportional addition for 7 hr/day (1000 to 1700 hours EDT). If other important crop species have a similar response to O_3 late in the afternoon, previous national crop loss estimates based on seasonal 7-hr/day O_3 exposures may be low.

Additional key words: air pollution, yield effects.

Knowledge of relationships between chronic doses of ozone (O_3) and crop yields is important for establishing air quality standards that are responsive to all segments of society. Ozone concentrations in ambient air fluctuate during a given day and from day to day, and the method of applying O_3 in studies to determine effects has long been a topic of interest. In many studies, the O_3 concentration is held constant during exposure, although in considering dose (concentration × exposure duration) for short-term exposures, the concentration may be more important than the exposure duration in causing plant response (8,10,16). For a given mean O_3 concentration, exposure regimes using variable O_3 levels caused greater effects on bean plants than those at constant O_3 levels (14). For a given mean O_3 concentration, chronic O_3 exposures with episodic diurnal concentration patterns that included some daily peaks at 0.18 ppm caused greater effects on growth of alfalfa than did those with a set diurnal concentration pattern with all daily peaks at about 0.12 ppm (11). These reports show the potential importance of peak O_3 concentrations, but little is known of their relative importance in field exposures that use ambient O_3 as a baseline dose and, therefore, contain numerous peak values.

A National Crop Loss Assessment Network (NCLAN) was developed to measure the effects of chronic doses of O_3 on yield of important crop species (9). Research at NCLAN field sites originally used constant additions of O_3 to nonfiltered air in open-top chambers (2) for 7 hr/day (1000 to 1700 hours EDT) (3,5–7,9,12,13). Because nonfiltered ambient air was used as the baseline for all O_3 additions, each seasonal O_3 regime was episodic, as determined by ambient O_3 concentrations. With constant O_3 additions, diurnal O_3 vs. time curves for each O_3 increment paralleled each other at constant increments regardless of the ambient O_3 concentrations (3,13). Ozone addition ended at 1700 hours because constant addition later in the day would result in high O_3 levels for some treatments that do not normally occur then.

As the NCLAN program developed, there was increased interest in developing a technique to dispense O_3 to open-top chambers in amounts proportional to ambient O_3 concentrations. This method was developed at Lawrence Livermore National Laboratories (Gail Bingham, Utah State University, *personal communication*). It uses an analog controller with adjustable offset and gain, an O_3 monitor, and an O_3 generator that can adjust the rate of O_3 production in response to electronic signals from the controller. In field tests with this method, each increment of O_3 addition resulted in a series of O_3 concentration curves that become more divergent as ambient O_3 increased (4). This method also resulted in a wider range of O_3 concentrations with higher peaks than resulted from constant O_3 addition (4). The O_3 dose-yield response relationship of soybeans was not changed significantly by the method of O_3 addition (constant vs. proportional-to-ambient) when the duration of O_3 addition was the same (7 hr). However, at higher O_3 levels there was some indication that the proportional O_3 additions caused a greater yield decrease than the constant additions (4). These results were based on one experiment, and similar tests for other crop species have not been performed. Thus, further work is needed to compare results obtained with constant and proportional O_3 addition. Because of improved dispensing technology, it is now feasible to dispense O_3 for longer daily periods. There are no reports of field studies to measure the effect of daily exposure duration on chronic O_3 dose-yield response relationships.

Our objectives were to compare the effects of seasonal 7-hr/day constant, seasonal 7-hr/day proportional, and seasonal 12-hr/day proportional O_3 additions on growth and yield of flue-cured tobacco (*Nicotiana tabacum* L.).

MATERIALS AND METHODS

This experiment was performed during 1983 in a 0.4-ha field of Appling sandy loam soil (clayey, kaolinitic, thermic, Typic hapludults) located 8 km south of Raleigh, NC. The field was treated on 3 May with isopropalin at 2.3 L/ha, diazinon at 4.7 L/ha, and metalaxyl at 3.1 L/ha.

Seedlings of flue-cured tobacco, *N. tabacum* cultivar McNair 944, were obtained from a commercial plant bed and were planted with a two-row planter in north-south rows spaced 105 cm on 18 May. Fertilizer (6-12-18, N-P-K) was applied in a band 10 cm from each row at planting according to soil test recommendations. On 19 and 20 May, McNair 944 seedlings selected for uniformity from the same commercial plant bed were planted by hand in 34 plots after removing seedlings planted on 18 May. Each plot contained two rows of five plants per row spaced 50 cm apart in the row. The 24 most uniform plots, each consisting of two rows with five plants each, were chosen to be included in the experiment on 23 May. Plant height and length and maximum width of all leaves for each of the 10 plants in the 24 plots were measured on 2 June for possible use in covariate analyses. Fertilizer (15-0-14, N-P-K) at a rate of 260 kg/ha was placed in a band 10 cm from each plot row on 9 June.

Soil tensiometers (Irrometer Company, Riverside, CA) were installed 10 cm from the west row, at depths of 20 cm in all plots and at 30 cm in half of the plots on 20 June. All plots were irrigated with approximately 2.5 cm of water (whole-plot basis) using drip tubes when tensiometer readings for more than half of the tensiometers at 20 cm were greater than 50% of scale (−0.05 MPa). Between planting and crop maturity, 18.7 cm of rain fell, and 26.9 cm of irrigation water was applied.

Insects were controlled with four applications of acephate and one application of carbofuran. Off-shoot-T (octanol and decanol) was applied to inhibit lateral shoot growth when terminal shoots were removed.

The experimental design was two replicates (randomized blocks) of 11 O_3 treatments in open-top chambers (2,6) and one ambient air (AA) treatment with no chamber. Two of the chamber O_3 treatments were charcoal-filtered air (CF) and nonfiltered air (NF) with no O_3 added. For three of the chamber treatments, an Orec ozonizer (Ozone Research and Development Corp., Phoenix, AZ) was used to add O_3 at constant amounts of approximately 0.02, 0.03, or 0.05 ppm, v/v (1 ppm, v/v = 1 μL/L) O_3 to the ambient O_3 in NF chambers for 7 hr/day (1000 to 1700 hours EDT) using methods for O_3 dispensing and monitoring described previously (6). Six chamber treatments were O_3 additions proportional to the amount of O_3 in ambient air. These treatments were established using a Monitor Lab O_3 monitor that continuously monitored ambient O_3. The voltage output from the O_3 monitor regulated voltage output from an analog controller that regulated the amount of O_3 produced by a Griffin GTC-1A ozonizer (4). The proportional O_3 treatments resulted in chamber O_3 concentrations of approximately 1.3, 1.5, and 1.8 times the amount of O_3 in ambient air. Each proportional O_3 treatment was applied for 7 hr/day (1000 to 1700 hours EDT) or for 12 hr/day (1000 to 2200 hours EDT) whenever the ambient O_3 concentration exceeded 0.03 ppm. We chose to add 5 hr of daily exposure in the afternoon because O_3 levels in ambient air are usually greater than 0.03 ppm from 1700 to 2200 hours EDT but not before 1000 hours EDT. Ozone was added from 2 June, when plants were about 20 cm tall with an average of five expanded leaves, and continued until 28 August.

Leaves considered harvestable (when partially yellow) were picked once each week starting on 27 June and continuing until 29 August. On 13 July, the terminal shoots of flowering plants were removed and these plants were treated the same day and 2 days later with Off-Shoot-T. This procedure was performed for the remainder of the plants when flowers began to open. Lateral shoots that developed were picked on 4 and 17 August. The remaining leaves, stems, and roots were harvested on 29 August. At each harvest, tissues from each plant were dried at 70 C for at least 2 days and weighed. For this study, marketable leaves were defined as yellowed leaves with stem insertion greater than 15 cm above the ground.

Statistical analyses. An analysis of variance (ANOVA) was conducted for marketable leaf weight (yield) in all plots to determine the effect of block, treatment, plant position (row and position within row), and position × treatment interaction. This analysis was performed using all 10 plants per plot and using six plants (three center plants per row) per plot. The effect of the initial measures of plant height and leaf length × width (leaf area) on marketable weight was determined in the ANOVA. Each covariate individually increased the precision of the analysis as measured by the reduction in experimental error, but there was no advantage in using both. Therefore, the effectiveness of pretreatment leaf area as a covariate was assessed in fitting dose-response models.

Polynomial dose-response models and a nonlinear model based on the Weibull probability distribution of sensitivities (15) were used to characterize the effect of O_3 dispensing method on yield response to O_3 in all open-top-chamber plots. Lack-of-fit tests were done for the polynomial and Weibull models, and only models that adequately described the dose-response relationships were used in further analyses. Comparison of the residual sum of squares from the models were made in all tests to determine the homogeneity of the response over dispensing methods. Homogeneity of the O_3 dose-response models was determined for the three methods of dispensing using both 7-hr and 12-hr seasonal mean O_3 concentrations as the independent variable. This was accomplished by developing both full and reduced dose-response models for both the polynomial and Weibull. The full model had a common response between the O_3 concentrations in the CF and NF treatments but allowed a divergence in response for each of the three 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 three dispensing methods above the NF treatment. Pairwise tests of the homogeneity of 7-hr proportional vs. 7-hr-constant models, 7-hr constant vs. 12-hr-proportional models, and 7-hr proportional vs. 12-hr-proportional models were also made.

RESULTS

Ozone concentrations. Ambient O_3 concentrations varied daily depending on regional and local weather patterns. Daily 7-hr (1000 to 1700 hours EDT) mean O_3 concentrations exceeded 0.08 ppm on 21 of the 82 days from 2 June to 22 August (Fig. 1). Seasonal (2

June to 28 August) 7-hr and 12-hr/day mean O_3 concentrations in ambient air and for the various chamber treatments are shown in Table 1. The mean seasonal 7-hr/day O_3 concentrations for the ambient air, nonfiltered air, and charcoal-filtered air treatments were 0.068, 0.057, and 0.029 ppm, respectively. For the constant 7-hr O_3 additions, the seasonal values show mean additions to ambient O_3 of 0.014, 0.029, and 0.047 ppm. The proportional treatments from 1000 to 1700 hours averaged 1.3, 1.5, and 1.8 times the AA concentration for both the 7-hr and 12-hr additions (Table 1). The 12-hr/day seasonal means show that proportional treatments of 1.3, 1.5, and 1.8 times the ambient O_3 concentrations also occurred for the 12-hr additions (Table 1). The 7-hr and 12-hr proportional treatments resulted in slightly higher seasonal 7-hr mean O_3 values at each O_3 increment than did the constant 7-hr treatments (Table 1).

Seasonal mean diurnal curves for O_3 concentrations (Fig. 2) show that 0.03 ppm was exceeded in AA from approximately 0920 to 2235 hours EDT. These curves also show that for each level of 7-hr constant O_3 addition (three uppermost solid lines), a relatively constant deviation from the AA O_3 concentration occurred. Conversely, for each proportional addition treatment, the relative deviation from the AA O_3 concentration changed as the AA O_3 concentration changed. With increasing AA O_3 concentration (from 1000 to 1300 hours), the curves for the proportional treatments (Fig. 2; dashed lines) show an increasing divergence from the AA curve and from each other, whereas the reverse was true when AA O_3 concentrations were decreasing (from 1700 to 2200 hours). Frequencies of occurrence for different O_3 concentrations for AA and for each level of O_3 addition for each

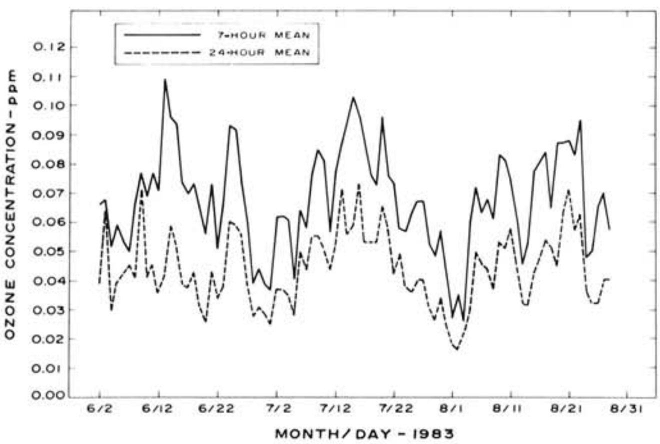

Fig. 1. Daily 7-hr (1000 to 1700 hours EDT) and 24-hr mean O_3 concentrations (1 ppm, v/v = 1 μL/L) in ambient air during the period of exposures (2 June to 22 August).

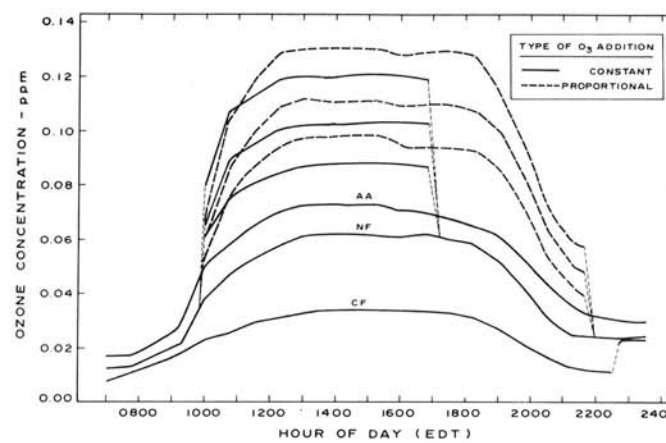

Fig. 2. Seasonal (2 June to 22 August) mean diurnal fluctuation in O_3 concentration for the various O_3 treatments. CF = Charcoal-filtered-air chamber; NF = the common concentration for all nonfiltered-air chambers during the time of day when O_3 was not added; AA = ambient air concentrations. NF + 0.02, NF + 0.03, and NF + 0.05 ppm treatments are represented by the top three solid lines: The 12-hr proportional (NF × 1.3, NF × 1.5, and NF × 1.8) treatments are represented by the dashed lines. The 7-hr proportional treatment concentrations (not shown) followed the same course as the 12-hr proportional treatments between 1000 and 1700 hours EDT.

TABLE 1. Concentrations of O_3 measured during exposures of tobacco to constant or proportional additions of O_3 to ambient O_3

Type of O_3 addition	7-hr/day values (ppm)[a]			1-hr/day values (ppm)[a]			12 hr/day[b]
	Seasonal means[c]	Highest[d]	Second highest[d]	Seasonal means[c]	Highest[d]	Second highest[d]	Seasonal means[c]
None							
AA[e]	0.068	0.109	0.103	0.078	0.131	0.122	0.063
CF	0.029	0.051	0.048	0.037	0.064	0.063	0.028
NF	0.057	0.093	0.087	0.067	0.120	0.114	0.052
Constant 7-hr							
NF + 0.02	0.082	0.122	0.118	0.094	0.152	0.139	0.067
NF + 0.03	0.097	0.139	0.139	0.111	0.171	0.152	0.076
NF + 0.05	0.115	0.159	0.154	0.129	0.183	0.177	0.086
Proportional 7-hr							
NF × 1.3	0.087	0.146	0.135	0.104	0.176	0.162	0.070
NF × 1.5	0.103	0.177	0.166	0.123	0.207	0.194	0.080
NF × 1.8	0.123	0.196	0.195	0.147	0.227	0.223	0.091
Proportional 12-hr							
NF × 1.3	0.089	0.162	0.141	0.106	0.184	0.168	0.082
NF × 1.5	0.102	0.178	0.167	0.123	0.203	0.192	0.095
NF × 1.8	0.121	0.209	0.199	0.146	0.227	0.221	0.112

[a] For the daily period from 1000 to 1700 hours EDT. 1 ppm, v/v = 1 μL/L.
[b] For the daily period from 1000 to 2200 hours EDT.
[c] Values for the period from 2 June to 22 August 1983. Each value is the mean from two replicate plots.
[d] Highest and second highest peak values are defined as the highest and second highest mean, respectively, of two consecutive recorded concentrations from 2 June to 22 August. Concentrations were recorded each 45 min using time-shared sequential monitoring with 3-min samples for each of 15 locations. This definition precluded occurrence of both peaks on 1 day.
[e] AA = Ambient air, CF = charcoal-filtered air, and NF = nonfiltered air.

type of O_3 regime are shown in Figure 3. With proportional additions (Fig. 3B and C) the range of O_3 concentrations was greater than that for the constant additions (Fig. 3A). Concentration frequencies for the 7- and 12-hr proportional additions were nearly identical for the period from 1000 to 1700 hours (Fig. 4A). When 12 hr (1000 to 2200 hours) of data were included in calculating concentration frequencies (Fig. 4B), the frequency of low concentrations was greater than that for high concentrations for both 7-hr addition regimes because data were included for 5 hr when no O_3 was added. Similar relationships to

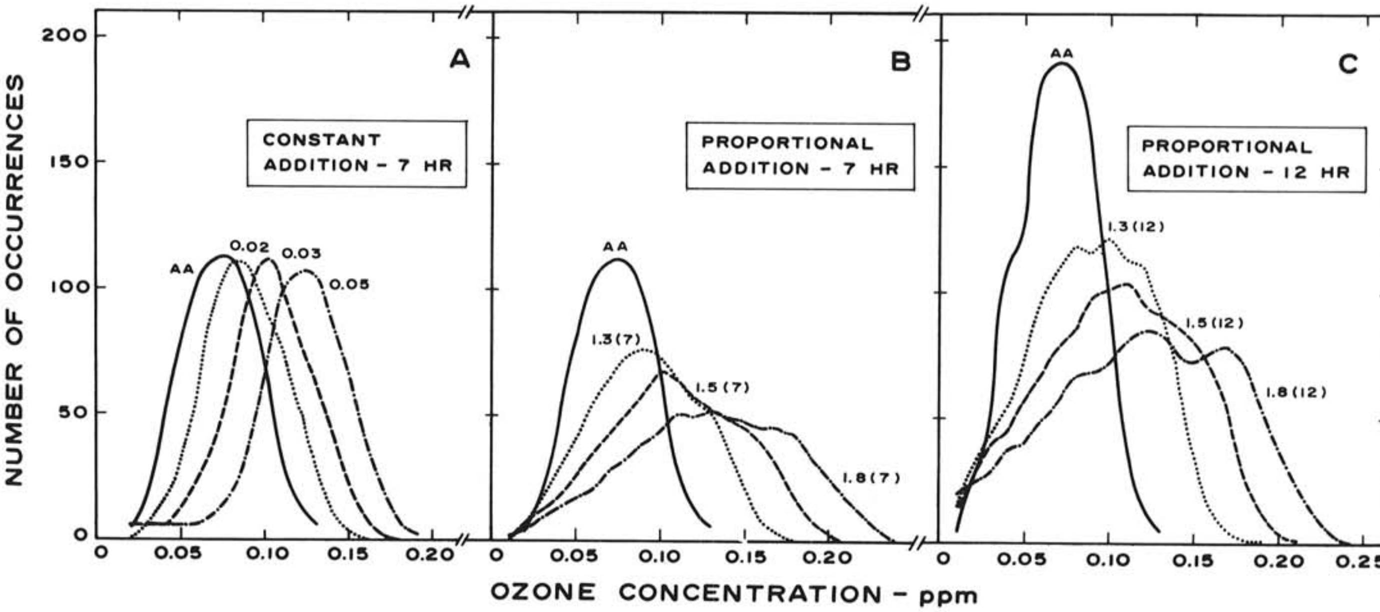

Fig. 3. Seasonal frequency of occurrence for O_3 concentrations in ambient air (AA) and in open-top chambers; **A,** With constant O_3 addition for 7 hr/day showing data from 1000 to 1700 hours EDT; **B,** With proportional O_3 addition for 7 hr/day using data from 1000 to 1700 hours; and **C,** With proportional O_3 addition for 12 hr/day using data from 1000 to 2200 hours EDT. An occurrence is defined as the concentration during the last minute of a 3-min sample taken every 45 min during each daily exposure period from 2 June to 22 August 1983. The mean frequency of three contiguous O_3 concentrations (in 0.01 ppm intervals) was plotted to give smooth transitions across concentrations.

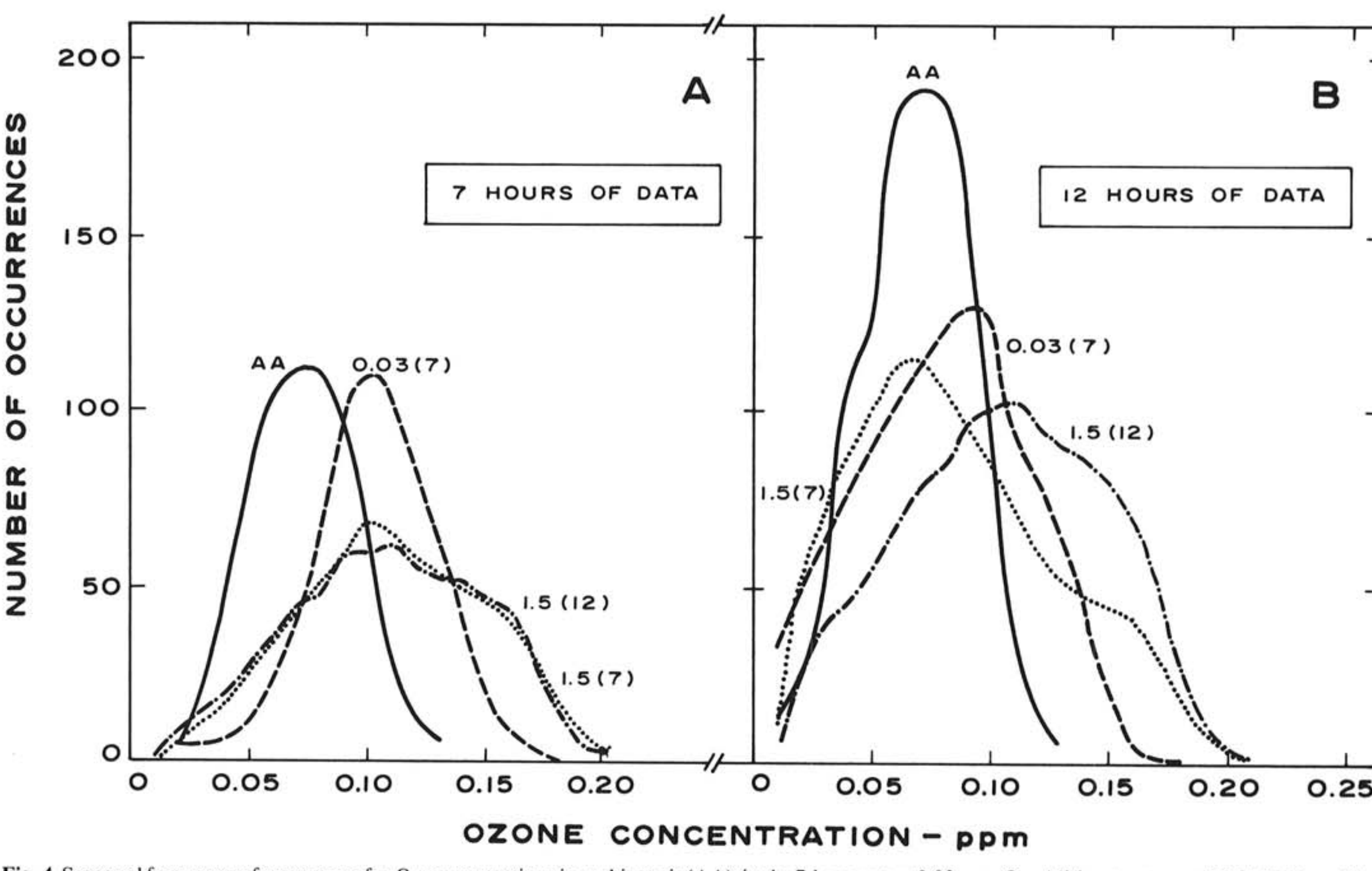

Fig. 4. Seasonal frequency of occurrence for O_3 concentrations in ambient air (AA), in the 7-hr constant 0.03 ppm O_3 addition treatment (NF + 0.03), and in 7- and 12-hr proportional (NF × 1.5) addition treatments (1.5–7 and 1.5–12, respectively); **A,** Frequencies for 7-hr daily (1000 to 1700 hours EDT); **B,** Frequencies for 12-hr daily (1000 to 2200 hours EDT).

ambient air O_3 concentrations occurred for low and high constant and proportional additions (data not shown).

Growth and yield. For the treatments with O_3 added, more leaf tissue was chlorotic and thus harvested during the first six harvests than for the treatments with no O_3 added. After harvest six, accumulative marketable leaf weight (yield) in the low O_3 treatments surpassed that in the high O_3 treatments (Fig. 5). Relationships for accumulative yields between the 12-hr proportional additions and the CF, NF, and AA treatments are shown in Figure 5 and are similar to those obtained with other types of O_3 addition (data not shown). The early leaf senescence induced by O_3 probably resulted in the trends shown in Table 2 for suppressed height and weight with increasing O_3. The percentage decreases for most weight parameters at each O_3 increment for 7-hr constant additions were within 2% of those for 7-hr proportional additions (Table 2).

The relationships between the various weight measures were fairly constant across O_3 treatments. For example, the ratio of total leaf weight to marketable leaf weight ranged from 1.10 in the NF treatment to 1.27 in the NF 12 × 1.8 treatment (Table 2). For this reason, we limited statistical analyses to effects of O_3 on marketable leaf weight. The ANOVA indicated that O_3 treatment, plant position, and plant position × treatment interaction had significant effects on yield when either six or 10 plants per plot were used in the analysis. The ANOVA results were similar when six or 10 plants were used, although the analysis with 10 plants resulted in a 25% improvement in the estimate of pure error (residual mean square error) over that obtained using six plants per plot. Thus, all 10 plants were used to obtain the plot means that were used in regression analyses.

The position effect was caused by a linear trend toward larger plants in the north than in the south part of the plots. We suspect that chamber effects on light were responsible for these differences because previous work has shown a mean decrease in photosynthetically active radiation of about 10% at the north positions and about 15% at the south positions (6). With or without

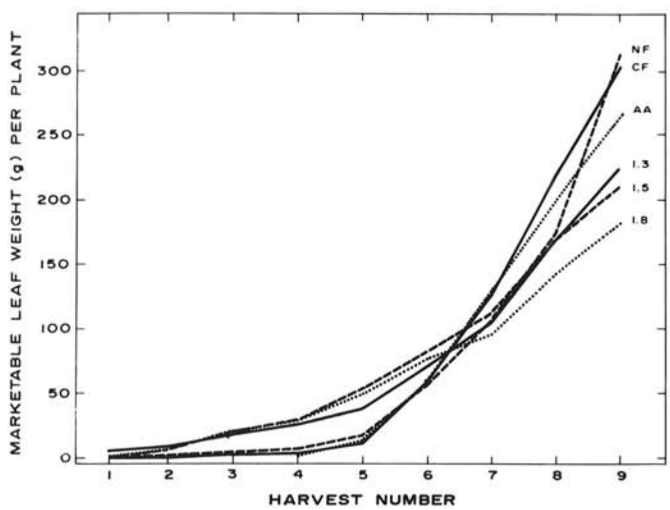

Fig. 5. Accumulative dry weight of marketable tobacco leaves per plant for nine weekly harvests for plants in ambient air (AA) and in open-top chambers receiving charcoal-filtered air (CF), nonfiltered air (NF), and 12-hr/day proportional O_3 additions resulting in seasonal 12-hr/day mean O_3 concentrations at 1.3, 1.5, and 1.8 times greater than AA concentrations.

TABLE 3. Regression equations for marketable leaf weight of tobacco using seasonal 7-hr/day or seasonal 12-hr/day mean O_3 concentrations as the independent variable for all O_3 treatments

	Covariate used[a]	Covariate not used
Equations derived by using 7-hr means[b]		
Polynomial	$y = 363 - 1{,}373x$ (10) (101)	$y = 364 - 1{,}373x$ (11) (116)
Weibull	$y = 322 \exp[-(x/0.165)^{2.12}]$ (13) (0.010) (0.42)	$y = 326 \exp[-(x/0.168)^{1.97}]$ (16) (0.015) (0.46)
Equations derived by using 12-hr means[b]		
Polynomial	$y = 152 + 9{,}851x - 177{,}435x^2 + 825{,}209x^3$ (52) (2,663) (4,041) (187,770)	$y = 366 - 1{,}681x$ (11) (143)
Weibull	$y = 335 \exp[-(x/0.144)^{1.71}]$ (18) (0.008) (0.35)	$y = 338 \exp[-(x/0.145)^{1.66}]$ (22) (0.009) (0.40)

[a] Covariate was the mean leaf length × width per plant (cm) measured before dispensing began. The models account for the covariate adjusted to the mean length × width measure.

[b] Regressions calculated using 7-hr (1000 to 1700 hr EDT) or 12-hr (1000 to 2200 hr EDT) seasonal mean O_3 concentrations. y = Estimated marketable leaf weight (dry weight–grams per plant); x = O_3 concentration (ppm). Standard errors for parameter estimates are given in parentheses. For the Weibull model ($y = \alpha \exp[-x/\sigma]c$); y = estimated marketable leaf weight (dry weight–grams per plant); x = O_3 concentration (ppm); α = maximum marketable leaf weight at 0 ppm O_3; σ = 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.

TABLE 2. Growth and yield response of tobacco to chronic doses of O_3 added in constant or proportional amounts to ambient O_3[a]

Type of O_3 addition	Plant height (cm)	Plant weights (g/plant) Total	Root	Stalk	Leaf weight Total	Leaf weight Marketable
None						
AA[b]	147	550	88	148	314	267
CF	151	595	95	157	343	304
NF	155	609	97	165	347	316
Constant						
NF + 0.02	144	504	75	137	292	255
NF + 0.03	143	494	78	134	281	238
NF + 0.05	138	432	63	121	248	201
Proportional 7-hr						
NF7 × 1.3	147	515	80	145	290	250
NF7 × 1.5	142	492	78	137	276	232
NF7 × 1.8	141	447	66	129	253	207
Proportional 12-hr						
NF12 × 1.3	143	453	68	124	262	224
NF12 × 1.5	141	462	71	130	262	213
NF12 × 1.8	135	397	54	112	232	183

[a] Each value is the mean per plant of 20 plants (five plants in two rows of two plots).

[b] AA = Ambient air, CF = charcoal-filtered air, and NF = nonfiltered air.

the use of pretreatment leaf area as a covariate, no single directional trend in the chambers (east-west or north-south) could account for the position × treatment interaction.

Yield in the 12-hr proportional treatments was less at each level of addition than for either type of 7-hr addition (Table 2). However, tests of the homogeneity of dose-response models comparing all three methods of O_3 addition and comparing pairwise methods of O_3 addition indicated that the response curves were not significantly different. This was true when either the 7-hr or 12-hr/day seasonal mean O_3 concentration was used as the independent variable. Therefore, data for all three O_3 addition methods were combined in the dose-response models shown in Table 3 and Figure 6. The use of the initial leaf length × width covariate improved the estimates of standard error but did not noticeably change the model parameters (Table 3). Tests for lack of fit when the covariate was omitted from this model indicated that a linear model was adequate when either the 7-hr or 12-hr mean was used in the polynomial model.

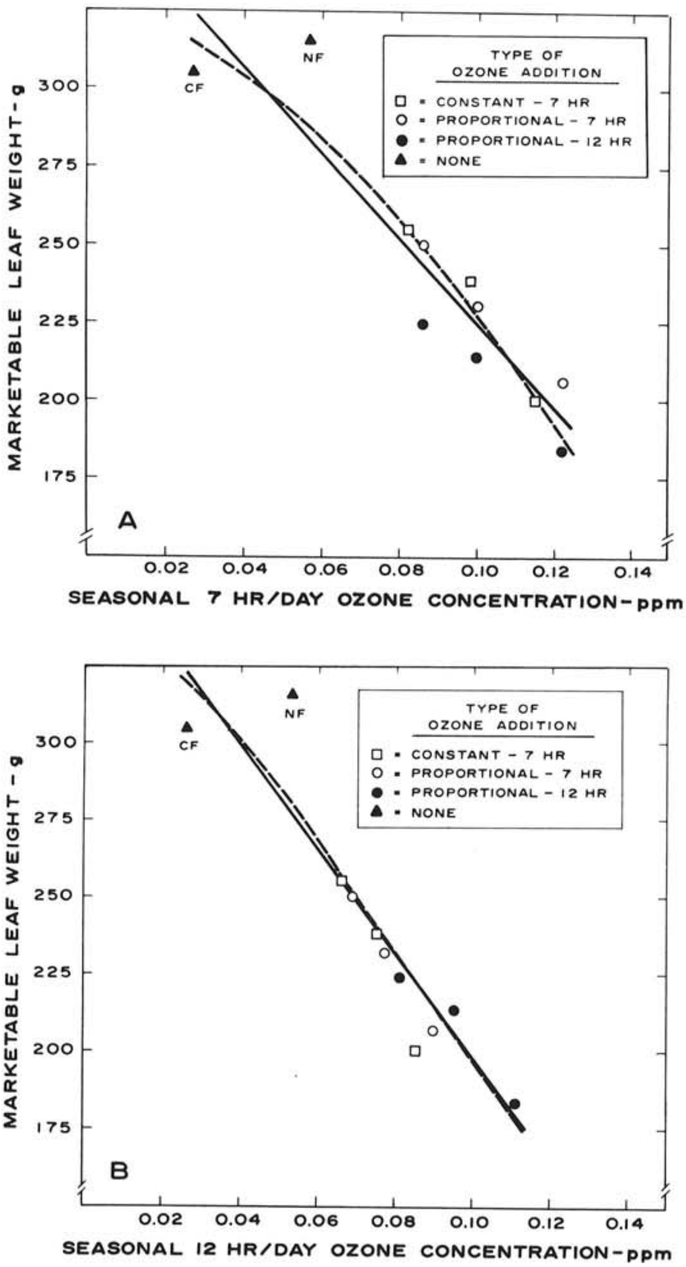

Fig. 6. Dose-response curves for dry weight of marketable tobacco leaves per plant using combined data for all types of O_3 additions with a polynomial (——) or Weibull (-----) model. Model formulas are shown in Table 3 (covariate used). ▲ Measured yield for CF and NF treatments; □ measured yield for constant addition treatments; o measured yield for proportional 7-hr addition treatments; and • measured yield for proportional 12-hr addition treatments; **A,** Using seasonal 7-hr/day mean O_3 concentrations (Table 1) as the independent variable; **B,** Using seasonal 12-hr/day mean O_3 concentrations (Table 1) as the independent variable.

DISCUSSION

National assessments of crop loss due to ambient levels of O_3 (7,9) were made from dose-response relationships obtained from 1980 to 1983 at five regional locations where protocols included additions of O_3 for 7 hr/day. The 12-hr proportional method of O_3 addition was tested because it represents a significant improvement in protocol to determine the response of plants to O_3. This method was not available in previous studies that used constant O_3 additions for 7 hr/day during the daily period (1000 to 1700 hours EDT) when O_3 levels in ambient air are usually highest (Fig. 2) and when plants are usually most sensitive. The results reported here comparing effects of 7- and 12-hr exposures were a major reason that NCLAN adopted 12-hr/day proportional additions starting in 1984. Other than for this cultivar of tobacco, there are no data to compare the effects of daily 7-hr and 12-hr O_3 exposures on crop yield. Further work is needed to determine if similar responses occur with other major crop species. If this is the case, some adjustment to previous national estimates of crop loss would be in order.

A major objective of this study was to determine whether differences in the frequency and magnitude of peak O_3 concentrations are a factor in O_3 dose-yield response relationships. We tested this premise by comparing results in the 7-hr constant and 7-hr proportional O_3 additions. For each of three addition levels, the seasonal 7-hr/day mean O_3 concentrations were similar for both types of addition (Table 1). However, the proportional additions resulted in greater daily 7-hr-peak and 1-hr-peak concentrations (Table 1, Fig. 3) than did the constant additions. The O_3 dose-response relationships for tobacco were not significantly changed by differences in the frequencies, magnitudes, and duration of peak O_3 concentrations in the ranges used in this study. These results are similar to those reported for soybean (4). These ranges probably span those likely to occur in ambient air in most parts of the world. Because differences in peak O_3 concentrations did not significantly affect the yield response of soybean or tobacco, it is our opinion that a seasonal mean O_3 concentration is a better exposure statistic to use as the independent variable in regression analyses of O_3 dose-yield response studies than an hourly peak concentration. There are two recent reports using NCLAN data that support the use of seasonal means (1,7). Whereas our combined analyses slightly favor the 7-hr mean, the seasonal 12-hr mean O_3 statistic appeared to bring the observed yield values more in concordance with the regression line (Fig. 6). This suggests a need to determine whether this is true for NCLAN data on other crops.

Mean yield measured in the NF treatment was higher than that in the CF treatment (Table 2), although replicate yield values for the two treatments overlapped. Yield in the two replicates of the CF treatment was 297 and 311 g per plant, whereas that in the NF treatment was 305 and 327 g. The differences were probably caused by edaphic factors rather than by any effect of O_3. All regression models (Table 3) predicted yield that was higher than observed for the CF treatment and lower than observed for the NF treatment (Fig. 6). At higher O_3 levels, the model predictions were close to observed values. Predictions for percentage yield loss (compared with CF) based on the models showed standard errors of less than 5%. However, predicted losses were greater than losses calculated from observed values because the predicted CF values were higher than observed CF values.

In these types of studies, crops were supplied with adequate soil moisture throughout the season. Soil moisture deficit often causes decreased stomatal conductance, decreased uptake of O_3, and, as a result, decreased effects caused by a given level of ambient O_3. Soil moisture deficit generally occurs at some time during each season in North Carolina, so the losses shown here may be higher than would usually occur in nonirrigated tobacco.

We recognize the difficulty in making inferences from one year of field research. Yearly variation in climate always affects health and yield of crop plants. However, our objectives were to characterize a relative yield response to different O_3 regimes. Thus, the actual mean yield was not critical. The concern is with possible interactions between the environment and O_3 response that we cannot rule out in this study. There is evidence that the effects of seasonal climate on relative plant response to seasonal O_3 exposure may not be large. Results with soybeans using different cultivars, at different sites, and in different years show a fairly uniform relative yield response to given O_3 doses (3,4,7). Thus, while we would expect some variance in the relative yield response of tobacco to O_3 over years, we expect it would be minor, especially because our protocols include controlled levels of soil moisture. Research should be performed to determine whether the results reported here with tobacco are similar to those for important food and fiber crops.

LITERATURE CITED

1. Cure, W. W., Sanders, J. S., and Heagle, A. S. 1986. Crop yield response predicted with different characterizations of the same ozone treatments. J. Environ. Qual. 15:251-254.
2. Heagle, A. S., Body, D. E., and Heck, W. W. 1973. An open-top field chamber to assess the impact of air pollution on plants. J. Environ. Qual. 2:365-368.
3. Heagle, A. S., Heck, W. W., Rawlings, J. O., and Philbeck, R. B. 1983. Effects of chronic doses of ozone and sulfur dioxide on injury and yield of soybeans in open-top field chambers. Crop Sci. 23:1184-1191.
4. Heagle, A. S., Lesser, V. M., Rawlings, J. O., Heck, W. W., and Philbeck, R. B. 1987. Response of soybeans to chronic doses of ozone applied as constant or proportional additions to ambient air. Phytopathology 77:51-56.
5. Heagle, A. S., Letchworth, M. B., and Mitchell, C. 1983. Injury and yield responses of peanuts to chronic doses of ozone in open-top chambers. Phytopathology 73:551-555.
6. Heagle, A. S., Philbeck, R. B., Rogers, H. H., and Letchworth, M. B. 1979. Dispensing and monitoring ozone in open-top field chambers for plant-effects studies. Phytopathology 69:15-20.
7. Heck, W. W., Cure, W. W., Rawlings, J. O., Zaragosa, L. J., Heagle, A. S., Heggestad, H. E., Kohut, R. J., Kress, L. W., and Temple, P. J. 1984. Assessing impacts of ozone on agricultural crops: II. Crop yield functions and alternative exposure statistics. J. Air Pollut. Control Assoc. 34:810-817.
8. Heck, W. W., Dunning, J. A., and Hindawi, I. J. 1966. Ozone: Nonlinear relationship of dose and injury on plants. Science 151:577-578.
9. Heck, W. W., Taylor, O. C., Adams, R. M., Bingham, G., Miller, J. E., Preston, E. M., and Weinstein, L. H. 1982. Assessment of crop loss from ozone. J. Air Pollut. Control Assoc. 32:353-361.
10. Heck, W. W., and Tingey, D. T. 1971. Ozone time-concentration model to predict acute foliar injury. Pages 249-255 in: Proceedings of the Second International Clean Air Congress. H. M. Englund and W. T. Beery, eds. Academic Press, New York.
11. Hogsett, W. E., Tingey, D. T., and Holmann, S. R. 1985. A programmable exposure control system for determination of the effects of pollutant exposure regimes on plant growth. Atmos. Environ. 19:1-11.
12. Kohut, R. J., and Laurence, J. A. 1983. Yield response of red kidney bean *Phaseolus vulgaris* to incremental ozone concentrations in the field. Environ. Pollut. Ser. A 32:233-240.
13. Kress, L. W., and Miller, J. E. 1983. Impact of ozone on soybean yield. J. Environ. Qual. 12:276-281.
14. Musselman, R. C., Oshima, R. J., and Gallavan, R. E. 1983. Significance of pollutant concentration distribution in the response of 'Red Kidney' beans to ozone. J. Am. Soc. Hort. Sci. 108:347-351.
15. Rawlings, J. O., and Cure, W. W. 1984. The Weibull function as a dose-response model for air pollution effects on crop yields. Crop Sci. 25:807-814.
16. Reinert, R. A., and Nelson, P. V. 1979. Sensitivity and growth of twelve elatior begonia cultivars to ozone. HortScience 14:747-749.