Ozone Stress, Carbon Dioxide Enrichment, and Nitrogen Fertility Interactions in Cotton

A. S. Heagle,* J. E. Miller, F. L. Booker, and W. A. Pursley

ABSTRACT

Ozone (O_3) in the troposphere can cause plant stress leading to foliar injury and suppressed growth and yield, whereas elevated CO_2 generally enhances growth and yield. Numerous studies have been performed to determine effects of O_3 and CO_2 separately, but relatively few have been performed to determine if O_3 can affect plant response to CO_2 or vice versa. Open-top field chambers were used to determine if such interactions occur for cotton (*Gossypium hirsutum* L.), which is relatively sensitive to O_3. Nitrogen nutrition is especially important in cotton production so N nutrition was included as an experimental factor. Plants were grown in 14-L pots at low, medium, and high soil N levels and exposed to three CO_2 and two or three O_3 treatments in all combinations during two seasons. The CO_2 treatments were ambient (370 $\mu L\ L^{-1}$) and two treatments with CO_2 added for 24 h d^{-1} at approximately 1.5 and 2.0 times ambient. In 1995, the O_3 treatments were charcoal filtered air (CF), and nonfiltered air (NF) with O_3 added for 12 h d^{-1} (NF+). In 1996, a NF treatment was also included to represent ambient O_3 conditions. The CF, NF, and NF+ treatments resulted in seasonal O_3 concentrations of approximately 23, 51, and 75 nL L^{-1}. Carbon dioxide enrichment generally stimulated growth and yield whereas O_3 exposure suppressed growth and yield. Stimulation induced by CO_2 increased as O_3 stress increased. For example, in 1995 at medium N, the percentage increase in yield caused by doubling CO_2 in CF air was 0%, but was 52% in NF+ air. Comparable values for 1996 were 23% in CF air and 140% in NF+ air. These interactions occurred for a range of soil N levels, and were probably caused by CO_2-induced prevention of O_3 stress. The results emphasize the need to consider $O_3 \times CO_2$ interactions to ensure correct interpretation of cause-effect relationships in CO_2 enrichment studies with crops that are sensitive to O_3.

Tropospheric O_3 concentrations in many areas of the world are approximately twice as high as preindustrial levels (Heck et al., 1984; U.S. EPA, 1996a). Atmospheric CO_2 concentrations are rising and are expected to double from current levels during the next century (Watson et al., 1990). Ozone is a strong oxidant that causes plant stress and lower yield (Heck et al., 1984), whereas CO_2 enrichment usually stimulates plant growth and yield (Allen, 1990; Cure and Aycock, 1986; Kimball et al., 1993). Most research to determine effects of O_3 and CO_2 on plants has been done without considering possible interactive effects of the two gases. Recent experiments with concurrent exposures to O_3 and CO_2 indicate that plant stress caused by O_3 is offset by CO_2 enrichment in several species (Barnes and Pfirrman, 1992; Heagle et al., 1993; Idso and Idso, 1994; Mortensen, 1990, 1992; Mulchi et al., 1992; Rao et al., 1995; Reinert and Ho, 1995; Reinert et al., 1997). A field study with soybean [*Glycine max* (L.) Merr.], using multiple concentrations of O_3 and CO_2, showed that stimulatory growth and yield responses of soybean to CO_2 enrichment were dependent on the amount of O_3 stress (Heagle et al., 1998a, 1998b; Miller et al., 1998). Because plant species and cultivars vary in response to elevated O_3 and CO_2, research to determine possible interactive effects of these gases is needed with additional species.

Cotton is sensitive to O_3 stress and CO_2 enrichment. Open-top field chamber experiments with cotton (Heagle et al., 1988; Temple et al., 1985; Heagle et al., 1986) indicate that cotton yield is decreased by approximately 12 to 21% by ambient concentrations of O_3 that occur in different areas of the USA (Heagle, 1989). Free air, and open-top chamber CO_2 enrichment studies in Arizona indicate that CO_2 at approximately 550 to 650 $\mu L\ L^{-1}$ increased cotton yield by approximately 40 to 60% compared with ambient CO_2 concentrations (Kimball et al., 1997; Kimball and Mauney, 1993; Mauney, et al., 1994; Pinter et al., 1996). Estimates of the negative effects of O_3 and positive effects of CO_2 singly on cotton yield using the model GOSSYM have been published (Reddy et al., 1989). A cotton growth simulation model ($COTCO_2$) has been developed with CO_2 concentration as a major influence on estimates of cotton growth and yield (Wall et al., 1994). Possible interactive effects of O_3 and CO_2 have never been reported for cotton. If such interactions occur for cotton, both models would benefit by adjustments to account for them.

Reports of effects of soil fertilizer rates on crop response to CO_2 enrichment have been mixed, depending on the crop and experiment. A summary of published reports indicated that levels of P and N that limited plant growth either increased, decreased, or had little effect on carbon exchange rate or dry weight response to CO_2 enrichment (Idso and Idso, 1994). Two studies involving effects of N nutrition on cotton response to CO_2 enrichment have been reported. In a nonreplicated greenhouse study, differences in soil N concentration did not affect the dry weight response of cotton exposed for 40 d to 640 $\mu L\ L^{-1}$ (Wong, 1979). In extensive field studies in Arizona, there were no significant interactions between soil N and CO_2 enrichment for cotton yield,

A.S. Heagle, USDA-ARS Air Quality - Plant Growth and Development Research Unit, 3908 Inwood Road, Raleigh, NC 27603 and Dep. of Plant Pathology, North Carolina State Univ; J.E. Miller and F.L. Booker, USDA-ARS Air Quality - Plant Growth and Development Research Unit, 3908 Inwood Road, Raleigh, NC 27603, and Dep. of Crop Science, North Carolina State Univ; W.A. Pursley, Dep. of Crop Science, North Carolina State Univ. Cooperative investigations of the USDA-ARS Air Quality Research Unit and the North Carolina State University. Funded in part by the North Carolina Agricultural Research Service. Received 8 June 1998. *Corresponding author (asheagle@unity.ncsu.edu).

Published in Crop Sci. 39:731–741 (1999).

Abbreviations: DAP, days after planting; NCER, net carbon exchange rate; g_s, stomatal conductance; C_i, internal CO_2 concentration; CF, open-top field chamber receiving charcoal filtered air; NF, open-top field chamber receiving nonfiltered air; NF+, open- top field chamber receiving nonfiltered air with O_3 added for 12 h d^{-1}.

although soil N and CO_2 enrichment both affected cotton yield (Kimball and Mauney, 1993).

Our objective was to determine if interactions between O_3 and CO_2 occur for cotton growth and yield, and if soil N levels affect such interactions. We examined the effects of season-long exposure to mixtures of O_3 and CO_2 on growth and yield of cotton grown at three N levels in open-top field chambers.

MATERIALS AND METHODS

General Procedures

The experiment was performed with cotton 'Deltapine 51' during 1995 and 1996 at our field site 5 km south of Raleigh, NC. Plants were exposed to mixtures of O_3 and CO_2 in open-top field chambers, 3-m diameter × 2.4 m tall (Heagle et al., 1973). Plants were grown in 15-L pots containing 14 L of a 2:1:1 mixture of sandy loam soil:sand:Metro-Mix 220 (Scotts Sierra Horticultural Products Co., Marysville, OH)[1] at pH 6.2. Pot temperature fluctuation was moderated with a sleeve (cylinder) which was composed of 0.6 cm-thick bubble wrap, coated on both sides with aluminum (ReflectixTM, Reflectix, Inc., Markleville, IN), fit tightly around each pot and secured with aluminum tape. Plants were irrigated with drip tubes at 4 L pot^{-1} as needed to prevent water stress. Irrigation frequency was less than once per week for seedlings and as often as once per day for large plants during hot sunny days. Total irrigation per pot for all treatment combinations was 194 L in 1995 and 143 L in 1996. One liter of a solution containing 0.8 g of acephate (*O*,*S*-dimethyl acetylphos-phoramidothioate) was applied as a soil drench to each pot 3 d after planting (DAP) both years to prevent thrips infestation. Metalaxyl [methyl-*N*-(2,6-dimethylphenyl)-*N*-(2-xylol)-DL-alaninate at 0.06 mL L^{-1} of water] and iprodione [3-(3,5-dichlorophenyl)-*N*-(1-methylethyl)2,4-dioxo-1-imidazoline-carboximide at 0.02 mL L^{-1} of water were applied both years as a soil drench to prevent seedling root disease. Acephate (at 1.7 mL L^{-1} water) or bifenthrin [(2-methyl-1,1-biphenyl-3-y1)-methyl-3-(2-chloro-3,3,3-trifluoro-1-propenyl)-2,2-dimethylcyclopropanecarboxylate at 2.6 mL L^{-1} water] were applied to foliage four or five times each season to prevent infestations of bollworms and other insects and mites. In 1995, an application of imidocloprid (1-[(6-chloro-3-pyridinyl)methyl]-*N*-nitro-2-imidazolidinimine at 1.8 mL L^{-1} water) on 27 July controlled aphids resistant to bifenthrin.

The main plot (chamber) treatments were mixtures of O_3 and CO_2 over a range of concentrations. Dispensing of CO_2 for 24 h d^{-1} and O_3 for 12 h d^{-1} (0800–2000 hours EST) for 7 d per week began less than 8 d after plants emerged and continued through maturity. General dispensing and monitoring protocols have been described previously for O_3 (Heagle et al., 1979) and for CO_2 (Rogers et al., 1983). Both gases were monitored 24 h d^{-1} at canopy height in the center of each chamber. Ozone was monitored with UV analyzers (TECO Model 49, Thermo Environmental Instruments, Inc., Franklin, MA) which were calibrated bi-weekly with a TECO Model 49 PS calibrator. Carbon dioxide was monitored with infrared analyzers (LI 6252, LI-COR Inc., Lincoln, NE), which were calibrated bi-weekly with pressurized tank CO_2 over the range of concentrations used in these experiments. Monthly and seasonal O_3 and CO_2 concentrations are described separately for each year in Table 1.

Three N concentrations (high, medium, and low) were obtained by incorporation of urea formaldehyde (38:0:0, N:P:K) at 2.04. 1.02, or 0.52 g L^{-1} of growth medium. Phosphorus (0:46:0, N:P:K) was incorporated at 1.02 g L^{-1} of growth medium, and micronutrients (Micromax, Scotts Sierra Horticultural Products Co.) were incorporated at a rate of 0.68 g L^{-1} of growth medium for all N levels. Potassium sulfate was supplied in six or seven (approximately bi-weekly) applications of a solution containing 1.4 g of K_2SO_4 L^{-1} at a rate of 1 L pot^{-1} for all N levels. Four pots for each N level were placed as a group in the northern and southern half of each chamber. We anticipated that different N rates would cause large differences in growth, and that large plants would shade smaller ones. To prevent confounding due to differential shading, the medium N level treatment group was always placed between the high or low N group, which were randomly assigned to the eastern or western position in the northern chamber half, and to the opposite sides in the southern chamber half.

Periodic nondestructive measures of growth and yield potential were made on one plant per N treatment in each chamber half on five dates each season, between 44 and 115 DAP. Height, number of nodes, and number of branches were recorded on all dates. The number of the first fruiting branch was recorded, and numbers of squares and bolls were counted at appropriate dates. At 49 DAP in 1995 and at 50 DAP in 1996, one plant for each N level was removed from each group of four plants to measure biomass dry weight (main stem, branches, main stem leaves, branch leaves, and roots), leaf areas (main stem and branch leaves separately), numbers (branches, main stem leaves, branch leaves, nodes, and squares), and height. At 45 and 78 DAP in 1995 and at 59 and 85 DAP in 1996, foliar injury was estimated visually as the percentage chlorosis and necrosis in 5% increments (0–100%) on main stem leaves of one plant per group of four plants.

Fluffy locks were harvested from the remaining six plants per N treatment from each chamber at 127, 136, 150, and 160 DAP in 1995 and at 120, 129, 143, and 150 DAP in 1996. Seedcotton (yield) was weighed for each harvest. It was bulked across harvests for each N treatment per chamber and ginned. Standard market quality analyses were performed for lint at the Louisiana State University Cotton Fiber Laboratory, Baton Rouge, LA. Seed quality was analyzed at the Hahn Laboratories Inc., Columbia, SC. Shoots (stems, branches, and empty locules) were harvested to obtain dry weights at 164 DAP in 1995 and at 151 DAP in 1996. For both years, analyses of variance were performed on chamber means (within whole plot, subplot, and sub-subplot treatments) with SAS software (SAS Institute, 1990). The whole-plot factor was the O_3 and CO_2 treatment combination, the sub plot factor was chamber position (north vs. south) and the sub-sub plot factor was N treatment.

1995

Seeds were planted on 23 May, and seedlings emerged on 28 May. Seedlings were thinned to two per pot at 13 DAP and to one per pot at 28 DAP. The whole plot design was all combinations of three CO_2 and two O_3 treatments. Carbon dioxide enrichment began on 2 June (10 DAP), and O_3 exposures began on 3 June. Dispensing of both gases continued until 4 October (134 DAP). The CO_2 treatments were ambient, and approximately 1.5 and 1.9 times ambient CO_2 concentrations. The O_3 treatments were charcoal-filtered air (CF = approximately 0.5 times ambient O_3) and nonfiltered air with O_3 added proportionally to the ambient O_3 concentration for 12 h d^{-1} (NF+ = approximately 1.6 times ambient O_3). The design required 16 chambers to provide three randomized replicates for all treatment combinations except for treatments

[1]The use of trade names in this publication does not imply endorsement by the North Carolina Agricultural Research Service or the USDA of the products named, nor criticism of similar ones not mentioned.

Table 1. Monthly meteorological conditions, O_3 concentrations, and CO_2 concentrations during studies to determine effects of O_3 and CO_2 interactions on cotton.

	1995						1996					
	June 2–30	July	August	Sept.	October 1–4	Season	May 22–31	June	July	August	Sept. 1–5	Season
Mean max. temp. (°C)†	26	33	30	26	26	28	26	29	29	27	25	27
Mean min. temp. (°C)†	18	22	21	17	16	19	17	20	21	19	19	19
Mean % RH (24 hr)‡	85	79	71	76	89	80	80	75	75	84	87	80
Mean solar radiation (MJ m^{-2} d^{-1})	14	23	20	14	12	18	21	24	20	17	10	20
Rain (cm)§	25	6	9	8	12	61	3	8	20	8	8	48
Ozone conc. (nL L^{-1})¶												
Ambient	43	50	51	39	28	45	49	57	52	46	27	51
CF	20	22	24	20	15	21	25	27	26	21	14	24
NF	–	–	–	–	–	–	47	56	53	47	27	51
NF+	69	84	76	59	40	71	81	82	79	76	46	78
Carbon dioxide conc. (μL L^{-1})#												
Ambient	366	362	362	372	383	369	381	370	368	375	381	372
A × 1.5	556	554	515	525	504	537	559	548	538	553	536	547
A × 1.9	723	730	668	690	631	700	729	723	714	736	711	724

† Temperatures for September 1995 measured 10 kilometers north of field site.
‡ Relative humidity (RH) for 1995 measured 18 kilometers west of field site.
§ Total drip irrigation per pot for all treatment combinations was 194 L in 1995 and 143 L in 1996. Rain for September 1996 does not include the amount deposited by Hurricane Fran on 6 September.
¶ Ambient is open-air, non-chamber concentration. Ozone concentrations are 12 h d^{-1} (0800–2000 hours EST) means. Values shown are within 8 nL L^{-1} of values for individual plots for a given ozone treatment.
# Carbon dioxide concentrations are 12 h d^{-1} (0800–2000 hours EST) means. Values shown are within 23 μL L^{-1} of values for individual plots at a given treatment level.

containing the 1.5 times ambient CO_2 concentration, which had two replicates.

1996

Seeds were planted on 13 May. Seedlings emerged on 19 May and were thinned to two per pot at 18 DAP and to one per pot at 28 DAP. The whole plot design was all combinations of three CO_2 treatments and three O_3 treatments. Carbon dioxide enrichment began on 21 May (8 DAP), O_3 exposures began on 22 May, and dispensing of both gases ended on 5 September (115 DAP). The CO_2 treatments were ambient and approximately 1.5 and 1.9 times ambient CO_2. The O_3 treatments were charcoal filtered air (CF), nonfiltered air (NF) and NF with O_3 added proportionally to the ambient O_3 concentration (NF+) for 12 h d^{-1} to obtain seasonal 12 h d^{-1} concentrations of approximately 0.5, 1.0, and 1.5 times ambient O_3, respectively. The design required 22 chambers to provide three randomized replicates for all treatment combinations except for treatments containing the mid-level O_3 and mid-level CO_2 treatments, which had two replicates.

In 1996, measurements of net carbon exchange rate (NCER), stomatal conductance (g_s), and transpiration were made at growth concentrations of O_3 and CO_2 for plants grown at the high N rate. Measures were made on 46, 49, 71, 74, and 78 DAP at midday when PAR exceeded 1000 μmol m^{-2} s^{-1} with a LI-6200 portable photosynthesis system (LI-COR). One leaf on each of two plants were sampled in each of two replicate plots for all combinations of CF and NF+ with the ambient and 1.9 times ambient CO_2 concentrations. Measures were made on leaves positioned one or two nodes below the uppermost fully expanded leaf on the main stem.

A hurricane (Fran) arrived during the evening of 5 September (115 DAP). Fran brought sustained wind velocity of over 60 mph (97 km h^{-1}) for approximately 6 h and 21 cm of rain. Electrical power was interrupted for 5 d. Many of the plastic chamber panels were separated partly from the chamber frames; a few were torn but chamber aluminum-channel frames were not damaged or moved. At 115 DAP, some fluffy locks were present in all chambers indicating that a high proportion of yield potential had been attained. Fortunately, plant stems were previously secured to a bamboo stake in each pot, and most were not severely damaged. However, plants considered to be damaged to the extent that yield would be compromised (shattered leaves, broken stems, detached bolls) were labeled. If any plant in a three-plant N group was considered damaged to that extent, the entire three-plant group was discarded. Chamber panels were replaced, and fans were restarted on 11 September and run until final harvest to maintain near-ambient meteorological conditions in the chambers. However, dispensing of O_3 and CO_2 was not resumed.

RESULTS

Weather conditions during the exposure periods for both years were within normal range for our location (Table 1). Solar radiation was much higher during the first month of growth in 1996 than in 1995. However, there were no seasonal differences in meteorological conditions or concentrations of O_3 or CO_2 considered large enough to cause major differences in response to O_3 or CO_2.

Carbon Exchange Rate and Stomatal Conductance

At ambient CO_2, net carbon exchange rates (NCER) and stomatal conductance (g_s) were lower in the NF+ treatment than in the CF treatment at all measurement dates. Conversely, at twice ambient CO_2, NCER and g_s were virtually identical at both O_3 levels. This $O_3 \times CO_2$ interaction for NCER and g_s occurred at all measurement times and is typified by the responses at 49 and 71 DAP (Fig. 1).

Foliar Injury

Ozone caused foliar injury (chlorosis, bronzing, and reddening) as estimated at both dates in both seasons (Tables 2 and 3, Fig. 2), and elevated CO_2 caused significant reddening for all but the second estimate in 1995. Older leaves were injured more by O_3 than younger leaves, whereas CO_2-induced reddening was more severe on younger leaves. Foliar symptoms caused by both

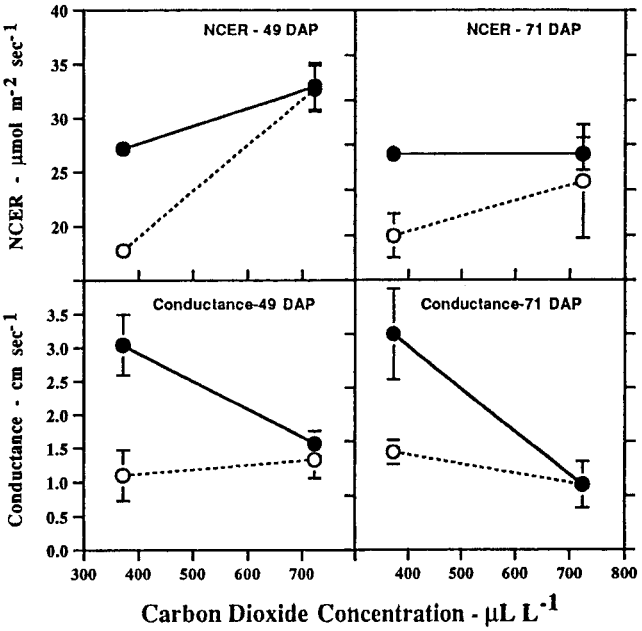

Fig. 1. Midday net carbon exchange rate (NCER) and stomatal conductance (g_s) of cotton leaves from plants at the high N level on 1 June (49 DAP and 23 July (71 DAP) in 1996. All measures were made at treatment (growth) concentrations of O_3 and CO_2. Each value is the mean of eight readings (two readings on one leaf on two plants in two plots). Bars show standard errors. Standard errors for NCER less than 1.0 are hidden by plot symbols—solid circle = charcoal filtered air (CF); open circle = nonfiltered air with ozone added (NF+).

gases increased as the season progressed in both years. Increased chlorosis due to N deficiency in the low N treatment was probably the cause for significant N effects on injury (Tables 2 and 3).

Carbon dioxide enrichment suppressed O_3 injury, and the $O_3 \times CO_2$ interaction was always significant (Tables 2 and 3, Fig. 2). For example, at ambient CO_2, mean foliar injury in the NF+ treatment was typically 2 to 3 times greater than in the CF treatment, but at twice ambient CO_2, foliar injury in all O_3 treatments was almost the same (Tables 2 and 3, Fig. 2).

Midseason Growth

Plants exposed to NF or NF+ air were generally smaller than plants exposed to CF air, but the O_3 effect was significant only for certain measures. In 1995, height, main stem leaf area, and shoot (stem, branches, leaves, flowers) weight were significantly smaller in NF+ air than in CF air (Table 2). In 1996, the O_3 effect was significant only for root weight and number of leaves (Table 3).

Carbon dioxide enrichment generally stimulated growth, and the CO_2 effect was significant for shoot and root weight in both years and for plant height and leaf area in 1996 but not in 1995 (Tables 2 and 3). Although there was a general trend for CO_2-induced suppression of O_3 effects, the $O_3 \times CO_2$ interaction was significant only for number of bolls at 101 DAP in 1996 (Tables 2 and 3).

Table 2. Foliar injury and growth responses of cotton grown at three N levels to mixture of O_3 and CO_2 in 1995.

O_3 treatment	O_3 conc.	CO_2 conc.	N trt‡	Foliar injury† 45 DAP	Foliar injury† 78 DAP	Height §	Main stem leaf area§	Branch leaf area§	Shoot wt§	Root wt§	Nodes	Branches	Leaves	Squares	Bolls 72 DAP	Bolls 115 DAP
	nL L^{-1}	µL L^{-1}		%		cm	cm^2		g		Number§					
CF	21	369	Low	9	32	59	1490	568	25.0	8.2	11.8	9.7	32	4.7	2.3	7.3
			Medium	8	32	74	1825	1626	41.6	7.5	12.8	14.0	48	13.0	4.5	14.3
			High	8	23	73	2244	2451	52.1	8.8	13.3	15.5	59	16.7	7.8	18.8
		537	Low	8	53	58	1283	729	29.5	7.6	11.5	9.8	33	5.0	3.0	7.8
			Medium	9	48	74	2062	1952	58.3	9.7	12.5	11.5	47	7.8	4.3	13.5
			High	8	46	73	2620	2764	68.9	10.2	13.5	16.3	60	11.0	9.0	19.0
		700	Low	9	57	60	1259	648	32.6	9.2	11.7	10.5	33	5.0	3.0	9.0
			Medium	10	58	75	1931	1865	61.6	9.9	12.8	13.0	52	10.2	3.5	14.5
			High	7	50	75	2200	2549	65.7	8.9	13.0	15.3	59	14.5	9.3	34.3
NF+	71	369	Low	39	81	60	1181	680	19.3	5.0	12.0	10.2	29	7.2	3.0	5.3
			Medium	36	83	67	1612	1428	31.2	5.3	13.2	12.5	43	11.5	7.5	11.0
			High	31	78	67	1888	1954	35.6	5.4	12.8	15.3	52	13.3	8.5	21.5
		537	Low	24	62	60	1256	823	28.1	7.2	11.8	12.0	34	4.8	2.8	6.5
			Medium	16	65	71	1754	1927	48.5	10.1	13.3	13.3	52	8.5	6.0	13.8
			High	13	54	74	2037	2709	55.7	8.8	12.5	15.8	64	15.8	11.3	20.0
		700	Low	16	60	58	1281	729	30.3	8.5	12.0	11.7	36	4.5	3.5	9.5
			Medium	10	56	71	1898	1952	58.4	11.1	13.0	13.0	52	10.2	5.3	13.8
			High	9	51	71	1961	2297	54.5	7.5	13.2	14.2	57	12.0	8.5	16.8
	Significance levels from analyses of variance; * and ** significant at $P \leq 0.05$ and 0.01, respectively.															
	Source	df														
	O_3	1		**	**	*	*	ns	**	ns	ns	ns	ns	ns	ns	ns
	CO_2	2		**	ns	ns	ns	ns	**	*	ns	ns	*	ns	ns	ns
	$O_3 \times CO_2$	2		**	**	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
	N	2		**	ns	**	**	**	**	ns	**	**	**	**	**	**
	$O_3 \times N$	2		**	ns	*	*	ns	*	ns	ns	ns	ns	ns	ns	ns
	$CO_2 \times N$	4		ns	ns	ns	ns	ns	*	ns	ns	ns	ns	ns	ns	ns
	$O_3 \times CO_2 \times N$	4		ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

† Injury is defined as % visible chlorosis, necrosis and reddening. Values are the mean per leaf for mainstem leaves at nodes 3 to 9 for the 45 DAP estimate and nodes 3 to 13 for the 78 DAP estimate.

‡ Low, medium, and high N were obtained by incorporating urea formaldehyde (38:0:0, N:P:K) at a rate of 0.52, 1.02, and 2.04 g L^{-1} respectively, of growth medium.

§ Except for foliar injury and number of bolls, all response measures were taken at 49 DAP. Shoot wt. = weight of stem, branches, leaves and flowers. Each value for growth and boll measures is the mean per plant of six plants (2 plants in 3 replicate chambers) except for mid-level carbon dioxide treatments, for which each value is the mean of four plants (2 plants in 2 replicate chambers).

Nitrogen fertilization stimulated growth, number of squares, and number of bolls in both years (Tables 2 and 3). The N effect was significant for all growth measures, number of squares, and number of bolls in both years except for root weight in 1995 (Tables 2 and 3).

The CO_2 effect on growth responses was similar at all N levels with a few exceptions. For example, in 1995 the only significant $CO_2 \times N$ interaction was for shoot weight; the weight response to CO_2 was greater for plants grown at medium N than at high or low N (Table 2). In 1996, the only $CO_2 \times N$ interactions were for numbers of leaves, squares, and bolls for which the CO_2 effect was generally greater with increased N (Table 3). None of the variables showed significant $O_3 \times CO_2 \times N$ interactions in either year.

Data are not shown for the nondestructive measures at five dates between 44 and 115 DAP each season, because the responses are generally typified by growth responses shown for the destructive harvest at 49 or 50 DAP (Tables 2 and 3). Nitrogen fertilization significantly ($P \leq 0.05$) increased plant height, numbers of nodes, numbers of branches, and decreased the position of the first flowering node at each nondestructive measurement date in both seasons. Elevated CO_2 significantly increased plant height on all five dates in both years. Although CO_2 enrichment did not affect numbers of nodes or branches in 1995, it significantly increased number of nodes at 73 DAP in 1996 and number of branches at 58 and 73 DAP in 1996. Ozone significantly increased the number of nodes at 91 and 115 DAP in 1995 and at 73 DAP in 1996, but did not significantly affect branch numbers in either year.

Yield and Final Shoot Weight

Plants exposed to elevated O_3 were generally smaller with fewer bolls, lower seed-cotton weight (yield), and lower final shoot weight (weight of stem, branches, and empty locules) than plants in CF air for both years. The O_3 effect was significant for yield in both years and for boll number in 1995 (Table 4). Conversely, plants exposed to elevated CO_2 generally had more bolls and weighed more than plants at ambient CO_2. The CO_2 effect was significant for final shoot weight in 1995 and for boll numbers, yield, and shoot weight in 1996. As occurred for some midseason measures, the apparent

Table 3. Foliar injury and growth responses of cotton grown at three N levels to mixtures of O_3 and CO_2 in 1996.

O_3 treatment	O_3 conc.	CO_2 conc.	N trt‡	Foliar injury† 59 DAP	Foliar injury† 85 DAP	Height §	Main stem leaf area§	Branch leaf area§	Shoot wt§	Root wt§	Nodes	Branches	Leaves	Squares	Bolls 73 DAP	Bolls 101 DAP
	nL L^{-1}	µL L^{-1}		%		cm	cm^2		g		number§					
CF	24	372	Low	24	49	53	1487	1247	34.6	6.2	12.2	13.0	38	21.0	8.8	8.8
			Medium	23	45	60	1759	1799	43.8	6.1	13.5	13.8	48	25.7	14.0	12.5
			High	19	44	62	2018	2646	52.7	6.1	14.0	14.5	59	30.2	14.0	17.8
		547	Low	38	53	55	1438	1412	51.3	7.7	12.3	12.5	42	18.0	7.3	8.5
			Medium	24	52	66	2049	2457	74.9	8.1	12.3	11.8	52	23.8	12.0	14.8
			High	26	54	72	2158	3053	80.0	9.5	13.8	15.0	66	37.5	19.5	24.5
		724	Low	35	58	62	1869	2077	66.4	8.2	12.3	13.3	45	19.8	9.5	9.8
			Medium	33	56	72	2159	2834	83.5	8.9	13.0	14.0	58	27.3	15.0	17.8
			High	30	54	70	2375	3779	93.3	9.5	13.5	15.2	75	35.8	20.0	21.0
NF	51	372	Low	47	70	53	1543	1439	34.0	5.8	13.3	14.3	43	17.3	11.0	8.3
			Medium	41	67	66	1904	2281	48.6	6.5	13.3	14.5	56	30.3	10.5	12.0
			High	35	70	65	1971	2551	51.1	7.0	13.8	15.0	61	32.5	17.8	20.3
		547	Low	44	63	59	1580	1517	48.2	7.8	12.3	13.5	44	17.5	7.8	9.0
			Medium	27	52	71	2019	2789	69.4	8.2	13.5	14.3	61	28.5	18.5	16.5
			High	24	62	67	2270	3411	78.3	9.3	14.3	15.5	74	37.5	18.5	22.0
		724	Low	41	60	63	1695	1909	64.5	8.9	12.3	12.5	45	18.3	9.0	9.5
			Medium	26	61	74	1999	2671	81.1	9.0	12.8	13.3	57	27.5	15.3	17.3
			High	30	55	75	2338	3779	98.4	11.3	14.0	15.5	77	45.5	22.8	24.5
NF+	78	372	Low	73	92	56	1324	1370	27.7	4.5	12.0	12.7	38	18.8	11.5	6.0
			Medium	78	94	65	1546	2331	42.0	4.6	12.8	13.8	54	31.7	11.0	9.8
			High	79	96	62	1850	2883	46.5	5.5	13.5	13.3	61	38.8	16.8	13.0
		547	Low	48	81	60	2006	2542	64.8	8.2	12.8	14.3	58	27.0	8.5	10.3
			Medium	36	78	67	2156	2397	58.9	6.9	14.8	13.8	57	29.8	14.5	14.8
			High	33	65	72	2262	3138	66.6	7.2	12.8	13.8	67	34.3	21.5	26.8
		724	Low	38	71	63	1861	2073	62.6	7.9	12.3	12.2	51	22.8	9.3	11.0
			Medium	27	70	76	2398	3425	86.3	9.0	13.7	13.8	67	35.3	13.3	14.8
			High	27	71	74	2560	4198	99.1	10.3	13.7	13.2	83	45.2	19.8	24.0
	Significance levels from analyses of variance; * and ** significant at $P \leq 0.05$ and 0.01, respectively.															
	Source	df														
	O_3	2		**	**	ns	ns	ns	ns	*	ns	ns	*	ns	ns	ns
	CO_2	2		**	**	**	**	**	**	**	ns	ns	ns	ns	ns	**
	$O_3 \times CO_2$	4		**	**	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	*
	N	2		**	*	**	**	**	**	**	**	**	**	**	**	**
	$O_3 \times N$	4		*	ns	ns	ns	ns	ns	ns	*	ns	ns	ns	ns	ns
	$CO_2 \times N$	4		**	ns	ns	ns	ns	ns	ns	ns	ns	*	*	**	**
	$O_3 \times CO_2 \times N$	8		ns	**	ns	ns	ns	ns	ns	*	ns	ns	ns	ns	ns

† Injury is defined as % visible chlorosis, necrosis and reddening per leaf for mainstem leaves at nodes 3 to 9 for the 59 DAP estimate and nodes 3 to 13 for the 85 DAP estimate.

‡ Low, medium, and high N were obtained by incorporating urea formaldehyde (38:0:0, N:P:K) at a rate of 0.52, 1.02, and 2.04 g L^{-1} respectively, of growth medium.

§ Except for foliar injury and number of bolls, all response measures were taken at 50 DAP. Shoot wt. = weight of stem, branches, leaves and flowers. Each value for growth and boll measures is the mean per plant of six plants (2 plants in 3 replicate chambers) except for mid-level O_3 and CO_2 treatments, for which each value is the mean of four plants (2 plants in 2 replicate chambers).

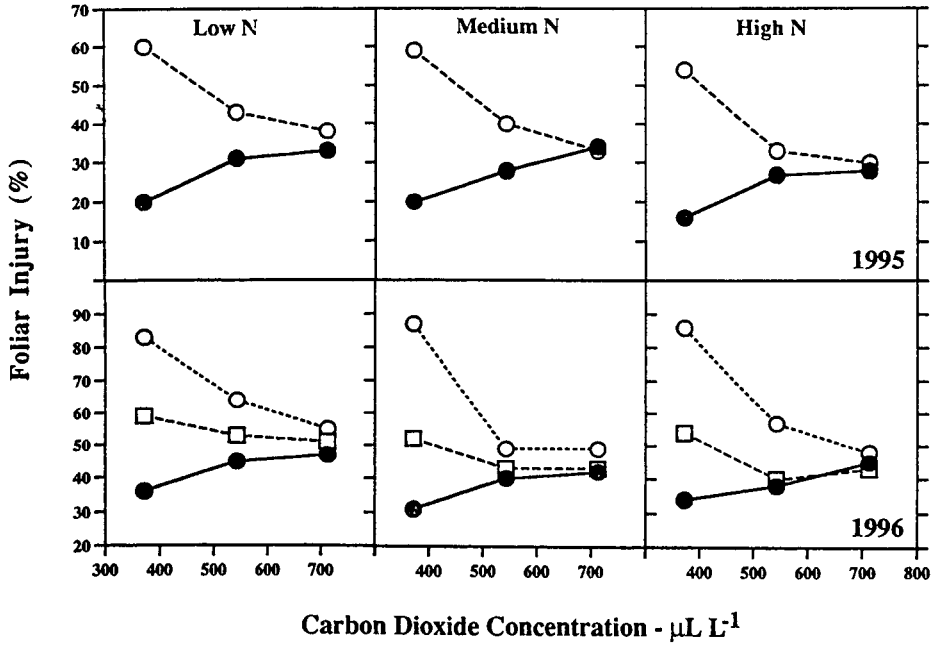

Fig. 2. Mean percentage foliar injury (chlorosis, reddening, and necrosis) per leaf on cotton plants grown at three N levels and exposed to mixtures of O_3 and CO_2 in 1995 (top row) and 1996 (bottom row). Each point is the mean for two injury estimate dates using data from Tables 2 and 3. Solid circle = charcoal filtered-air chamber (CF); open square = nonfiltered-air chamber (NF); open circle = NF with O_3 added for 12 h d^{-1} (NF+). Ozone and CO_2 concentrations for each treatment are shown in Table 1.

stimulatory effect of elevated CO_2 was much greater on plants stressed by O_3 than for plants grown in CF air (Table 4). The $O_3 \times CO_2$ interaction was significant for yield in both years, for final shoot weight in 1995, and for number of bolls in 1996. For example, in 1995 for the medium N treatment, the percentage increase in yield caused by doubling CO_2 in CF air was 0%, but was 52% in the NF+ treatment (Table 4, Fig. 3). Comparable values for 1996 were 23% in CF air and 140% in the NF+ treatment.

Nitrogen fertilization increased boll numbers, yield and final shoot weight, and the N effect was significant for all measures for both years (Table 4). In 1995, the high N rate caused rank vegetative growth at all CO_2 concentrations, and boll numbers and yield decreased with CO_2 enrichment at high N, but not at other N levels (Table 4, Fig. 3), which explains the $CO_2 \times N$ interaction for boll numbers and yield (Table 4). In 1995, the proportional increase of final shoot weight because of N fertilization was greater than the proportional increase in yield as reflected by the decreased harvest index. The significant $CO_2 \times N$ and $O_3 \times CO_2 \times N$ interactions in 1995 may have occurred because final shoot weight response to CO_2 was greater at the high N than at low N in the CF treatment but not in the NF+ treatment. In 1996, rank growth did not occur at high N and, although the $CO_2 \times N$ interaction was significant for all measures (Table 4), the cause for the interaction depended on the response measure. For example, the effect of CO_2 generally increased as N level increased for number of bolls and yield (Table 4, Fig. 3). For final shoot weight, the effect of N on the CO_2 response was more dependent on the O_3 and CO_2 combination (Table 4).

Significant $O_3 \times N$ interactions occurred for several measures in both years (Table 4) but the interactions were inconsistent and their biological relevance is unclear. For example, N caused a greater increase in shoot weight in the NF+ than in the CF treatment in 1995, but the reverse was true in 1996 (Table 5). In 1996, the $O_3 \times N$ interaction for boll numbers and yield was due to a curvilinear response to N; N caused more stimulation in the NF treatment than in the CF or NF+ treatments (Table 4).

Quality of Lint and Seed

For both years, O_3 decreased micronaire and yellowness, whereas CO_2 caused the opposite effects (Tables 5 and 6). Each gas offset the effects of the other causing the significant $O_3 \times CO_2$ interactions for micronaire and yellowness in both years (Tables 5 and 6). A trend for O_3-induced increase in brightness, and for CO_2-induced decrease in brightness occurred both years. These effects were significant for O_3 in 1996 and for CO_2 and the $O_3 \times CO_2$ interaction both years. An O_3-induced decrease in elongation occurred in 1995 (Table 5) but not in 1996 (Table 6). Ozone significantly increased fiber length (HVI upper half mean) in 1995 with a similar, but nonsignificant, trend in 1996. Carbon dioxide enrichment significantly decreased fiber length in both years. Although the CO_2 effect on fiber length was greater at NF+ than at CF in both years, the $O_3 \times CO_2$ interaction was significant only in 1995. None of the effects of N on lint quality were consistent over years (Tables 5 and 6).

Seed quantity was adequate for only one replicate sample for quality analyses. Therefore, no analysis of

Table 4. Yield components and final shoot weight for cotton grown at three N levels and exposed to mixtures of O_3 and CO_2 in 1995 and 1996.

				1995†					1996†				
Ozone treatment	Ozone conc.‡	Carbon dioxide conc.‡	N trt‡	Number of bolls	Seed-cotton weight (yield)	Shoot weight	Harvest index§		Number of bolls	Seed-cotton weight (yield)	Shoot weight	Harvest index§
	nL L^{-1}	μL L^{-1}			g					g		
CF	23	371	Low	25	91	124	0.73		26	121	135	0.89
			Medium	43	159	248	0.64	¶	40	179	193	0.93
			High	68	274	396	0.69	¶	56	275	227	1.21
		542	Low	25	93	132	0.70	¶	25	120	142	0.85
			Medium	51	163	279	0.58	¶	46	202	230	0.88
			High	69	224	448	0.50		71	312	308	1.01
		712	Low	27	93	138	0.67		32	135	158	0.85
			Medium	48	158	265	0.60		54	221	253	0.87
			High	66	229	456	0.50	¶	72	307	312	0.99
NF	51	371	Low	–	–	–	–		24	105	100	1.04
			Medium	–	–	–	–		40	161	159	1.02
			High	–	–	–	–		56	218	200	1.09
		542	Low	–	–	–	–		24	105	150	0.70
			Medium	–	–	–	–		52	213	233	0.92
			High	–	–	–	–		70	300	285	1.05
		712	Low	–	–	–	–	#	24	117	185	0.63
			Medium	–	–	–	–	#	60	236	277	0.85
			high	–	–	–	–	¶	79	326	342	0.95
NF+	75	371	Low	15	51	76	0.67		18	64	82	0.78
			Medium	33	94	148	0.64		25	91	124	0.73
			High	64	215	287	0.75		41	140	178	0.79
		542	Low	22	82	120	0.68		30	121	136	0.89
			Medium	48	157	238	0.66		44	170	189	0.90
			High	57	211	511	0.41		73	286	278	1.03
		712	Low	29	96	143	0.67	¶	32	141	184	0.77
			Medium	45	143	273	0.52	¶	54	219	245	0.89
			High	55	177	525	0.34		72	288	306	0.94
	Significance levels from analyses of variance; * and ** significant at $P \leq 0.05$ and 0.01, respectively.											
	Source	df-1995						df-1996				
	O_3	1		**	**	ns	ns	2	ns	**	ns	*
	CO_2	2		ns	ns	**	**	2	**	**	**	ns
	$O_3 \times CO_2$	2		ns	*	**	ns	4	*	**	ns	**
	N	2		**	**	**	**	2	**	**	**	**
	$O_3 \times N$	2		ns	ns	*	ns	4	**	**	*	**
	$CO_2 \times N$	4		**	**	**	**	4	**	**	**	*
	$O_3 \times CO_2 \times N$	4		ns	ns	*	ns	8	ns	ns	ns	**

† Each value is the mean three-plant total of six 3-plant samples (one sample, 2 chamber locations, 3 chambers) except for mid-level CO_2 and mid-level O_3 treatments for which each value is the mean of four 3-plant samples (1 sample, 2 chamber locations, 2 chambers) except for deletions as indicated for 1996; seed cotton is the sum of lint and seed weight. Shoot weight is the sum of stem, branches, and empty locules weight.

‡ Ozone and CO_2 concentrations are two-year means; for yearly O_3 and CO_2 concentrations see Table 1. Low, medium, and high N were obtained by incorporating urea formaldehyde (38:0:0, N:P:K) at a rate of 0.52, 1.02, and 2.04 g L^{-1} respectively, of growth medium.

§ Harvest index is defined as the ratio of seed-cotton weight to shoot weight.

¶ Hurricane Fran caused deletion of one 3-plant sample.

Hurricane Fran caused deletion of two 3-plant samples.

variance test was possible for seed quality factors. Seed quality responses were consistent over years and with other response measures, however. For both years, trends were for O_3-induced decreases in percentage oil, quality index, and grade and for increases in ammonia and percentage fatty acids, whereas CO_2 tended to cause the opposite effects (Tables 5 and 6). Moreover, CO_2 enrichment appeared to prevent the effects of O_3. For example, in 1995 at ambient CO_2 for the N treatments combined, percentage oil was 20.3% in the CF treatment and 16.8 % in the NF+ treatment. At twice ambient CO_2, the comparable values were 20.4 and 20.2%.

DISCUSSION

Suppression of cotton growth and yield by O_3 was prevented, or partially prevented, by CO_2 enrichment. One consequence of this interaction is that apparent CO_2-induced enhancement was often greater for plants stressed by O_3 than for plants in CF air. These results, and previous reports showing the high sensitivity of cotton to O_3 (Heagle et al., 1986; Heagle et al., 1988; Temple et al., 1985), may help explain the large CO_2-induced yield increases previously reported for cotton. In field studies, CO_2 enrichment at approximately 550 to 650 μL L^{-1} increased yield of cotton grown in ambient O_3 by approximately 40 to 60% (Kimball et al., 1997; Kimball and Mauney, 1993; Mauney et al., 1994; Pinter et al., 1996). In the present studies at Raleigh, NC, twice-ambient CO_2 induced yield increases averaging 40% for cotton grown at near ambient O_3 concentrations in NF chambers, whereas the comparable increase for plants grown at low O_3 in CF chambers was only 15%. Seasonal O_3 concentrations in many cotton production areas are similar to seasonal O_3 concentrations near Raleigh, NC (U.S. EPA, 1996b). Therefore, it is apparent that the

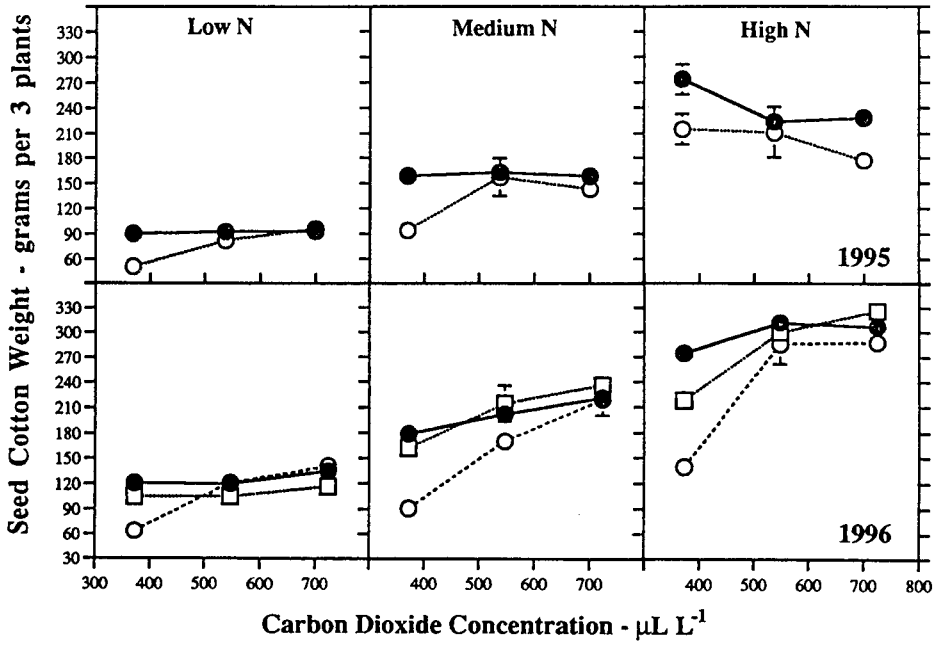

Fig. 3. Seed-cotton weight per three plants of cotton grown at three N levels and exposed to mixtures of O_3 and CO_2 in 1995 (top row) and 1996 (bottom row). Values from Table 4. Bars show standard errors. Standard errors less than 20 g are hidden by plot symbols—solid circle = charcoal filtered-air chamber (CF); open square = nonfiltered-air chamber (NF); open circle = NF with O_3 added for 12 h d^{-1} (NF+).

degree of O_3 stress must be considered when interpreting results of CO_2 enrichment studies with cotton.

Measurements on five dates from 46 to 78 DAP showed suppression of NCER by O_3 (NF+ treatment) in plants exposed to ambient CO_2, but not in plants exposed to elevated CO_2 (49 and 71 DAP shown in Fig. 1). Elevated CO_2 has also been shown to prevent O_3 suppression of NCER in soybean (Reid and Fiscus, 1998). In CF air, elevated CO_2 caused small increases in NCER at 46 and 49 DAP, but this did not occur at 71, 74, or 78 DAP. Similar results were found with soybean as plants aged (Miller, 1998, personal communica-

Table 5. Lint and seed quality for cotton grown at three N levels and exposed to mixtures of O_3 and CO_2 in 1995.

				Lint									Seeds†				
								3.2-mm gauge		Color							
Ozone treatment	Ozone conc.	Carbon dioxide conc.	N treatment‡	Lint in seed-cotton†	Micronaire	HVI Upper half	Uniformity index	Strength	Elongation	Brightness (Rb-%)	Yellowness (+b)	Final grade	% Ammonia	% Oil	% Free fatty acids in oil	Quantity index	Grade
	nL L^{-1}	μL L^{-1}		%													
CF	21	369	Low	42	5.12	1.11	84.6	25.9	10.6	77.4	8.0	31.0	2.62	20.3	0.4	102	102
			Medium	43	4.69	1.11	84.8	28.5	10.9	75.1	8.5	34.3	2.66	20.2	0.3	102	102
			High	42	4.89	1.12	85.2	30.8	10.1	74.2	8.5	34.3	3.00	20.4	0.8	105	105
		537	Low	42	5.07	1.10	85.4	29.3	10.1	75.6	8.6	31.0	2.40	20.9	0.3	103	103
			Medium	43	4.90	1.10	85.7	29.1	10.3	74.4	8.3	36.0	2.54	20.6	0.5	103	103
			High	42	4.67	1.13	85.3	30.5	10.1	72.7	8.2	41.0	3.02	19.8	0.5	102	103
		700	Low	42	5.00	1.10	84.1	27.5	10.3	76.9	8.4	31.0	2.37	20.4	0.4	101	101
			Medium	43	4.92	1.09	84.2	27.4	10.6	76.4	8.3	31.0	2.52	20.9	0.3	104	104
			High	42	4.89	1.09	83.8	30.5	10.1	72.8	8.6	41.0	2.97	19.9	0.5	102	103
NF+	71	369	Low	39	3.97	1.15	83.2	29.0	9.2	79.4	7.1	31.0	3.94	17.3	0.7	98	98
			Medium	39	3.56	1.16	84.0	31.7	9.1	78.5	6.9	34.3	3.93	16.2	0.9	93	94
			High	39	3.73	1.13	84.2	30.7	9.6	75.3	7.7	37.7	3.93	17.0	1.0	97	97
		537	Low	41	4.68	1.11	85.3	30.0	9.7	78.4	8.0	31.0	3.05	19.7	0.3	102	102
			Medium	41	4.82	1.09	85.4	28.7	9.7	76.3	8.0	36.0	2.83	20.8	0.3	105	105
			High	41	4.94	1.11	85.6	30.8	8.9	70.5	8.7	41.5	3.16	19.9	0.5	104	104
		700	Low	42	4.87	1.10	83.7	27.7	9.8	77.4	7.9	31.0	2.80	20.1	0.3	102	102
			Medium	42	4.96	1.09	84.7	29.4	9.5	74.9	8.3	37.7	2.72	20.7	0.4	104	104
			High	42	4.91	1.11	84.6	28.6	9.7	71.6	8.4	41.0	3.11	19.8	0.5	103	103
	Significance levels from analyses of variance; * and ** significant at $P \leq 0.05$ and 0.01, respectively.†																
	Source	df															
	O_3	1			**	*	ns	ns	**	ns	**	ns					
	CO_2	2			**	**	**	ns	ns	**	**	ns					
	$O_3 \times CO_2$	2			**	**	ns	ns	*	*	**	ns					
	N	2			ns	ns	ns	**	ns	**	**	**					
	$O_3 \times N$	2			ns	ns	ns	ns	ns	**	*	ns					
	$CO_2 \times N$	4			ns	ns	ns	ns	ns	ns	ns	ns					
	$O_3 \times CO_2 \times N$	4			ns	ns	ns	ns	ns	*	ns	ns					

† Seed quantity was adequate for only one bulked sample per treatment combination for quality analyses, and percentage lint was calculated for bulked samples. Therefore, no analysis of variance test was performed for seed quality factors or percentage lint.

‡ Low, medium, and high N were obtained by incorporating urea formaldehyde (38:0:0, N:P:K) at a rate of 0.52, 1.02, and 2.04 g L^{-1} respectively, of growth medium.

tion), and this may be the result of acclimation of NCER to elevated CO_2 (Drake et al., 1997). In the present study, the apparent stimulation of NCER by CO_2 enrichment was consistently greater for plants stressed by O_3 than for plants exposed to CF air. In actuality, much of the increase in NCER with CO_2 enrichment is due to elevated CO_2 preventing O_3 suppression of NCER. This pattern of $CO_2 \times O_3$ interaction is similar to that found for growth and yield in the present study with cotton and with yield of other species such as soybean (Heagle et al., 1998a, 1998b). Both elevated CO_2 and elevated O_3 (NF+ treatment) suppressed stomatal conductance (g_s). Elevated CO_2 suppresses g_s by increasing internal CO_2 concentrations (C_i) (Mott, 1988). Ozone indirectly suppresses g_s by suppressing NCER, which presumably leads to higher C_i (Fiscus et al., 1997). These results are consistent with the hypothesis that CO_2 enrichment decreases O_3 effects on NCER by partially closing stomates, thereby decreasing O_3 flux into the leaves. Other mechanisms for CO_2 amelioration of O_3 stress may be involved, however. For example, CO_2 enrichment may cause biochemical changes that increase O_3 detoxification or enhance repair of O_3 injury (Allen, 1990). Further work is needed to determine mechanisms of CO_2 amelioration of O_3 stress.

The wide range of soil N levels in the present experiment affected response to CO_2 enrichment under some O_3 and CO_2 combinations but not others. In 1995, the $CO_2 \times N$ interaction for yield was caused mainly by rank growth in the high N treatment where CO_2 enrichment decreased yield. At low and medium N in 1995, the response to CO_2 enrichment was similar, and agrees with results from free-air carbon dioxide enrichment studies where yield enhancement caused by CO_2 enrichment for cotton grown at limiting N levels was not significantly different from that for cotton grown at adequate N levels (Kimball and Mauney, 1993). Rank growth did not occur at high N in 1996 and, although significant $CO_2 \times N$ interactions occurred, the cause depended on the response measure and the O_3 and CO_2 combination. For example, boll number and yield response to CO_2 generally increased as N increased, but this was not true for shoot weight. Moreover, these trends for N effects on boll number and yield response to CO_2 were not as evident in the NF+ treatment as in the CF and NF treatment. Nevertheless, at medium and high N levels in 1996, yield response to CO_2 was usually similar. Overall, our results suggest that the N effect on cotton response to CO_2 enrichment will be small over the range of soil N likely to occur in cotton production. Neverthe-

Table 6. Lint and seed quality for cotton grown at three N levels and exposed to mixtures of O_3 and CO_2 in 1996.

				Lint									Seeds†				
								3.2-mm gauge		Color							
Ozone treatment	Ozone conc.	Carbon dioxide conc.	N treatment‡	Lint in seedcotton†	Micronaire	HVI Upper half	Uniformity index	Strength	Elongation	Brightness (Rb-%)	Yellowness (+b)	Final grade	% Ammonia	% Oil	% Free fatty acids in oil	Quantity index	Grade
	nL L^{-1}	µL L^{-1}		%													
CF	24	372	Low	47	5.00	1.12	83.2	25.3	9.8	78.4	8.6	24.3	2.42	20.8	0.8	103	103
			Medium	47	5.00	1.10	83.1	26.6	9.4	79.1	8.6	24.3	2.51	21.5	0.3	106	106
			High	47	4.93	1.12	83.0	26.1	9.5	79.7	8.7	21.0	2.64	21.8	0.3	108	108
		547	Low	47	5.20	1.10	83.0	26.4	9.5	80.2	8.6	21.0	2.33	21.4	0.3	105	105
			Medium	46	4.85	1.12	84.7	25.2	10.1	79.6	8.5	21.0	2.25	21.4	0.4	104	104
			High	49	4.75	1.09	82.1	25.9	10.1	77.4	8.6	31.0	2.42	21.9	0.3	107	107
		724	Low	49	4.93	1.09	82.7	25.5	9.8	78.9	8.6	21.0	2.23	21.2	0.3	103	103
			Medium	46	4.77	1.07	82.1	26.5	10.0	78.2	8.7	21.0	2.23	21.6	0.3	105	105
			High	46	4.80	1.07	82.7	27.4	9.8	78.4	8.9	21.0	2.47	21.5	0.3	106	106
NF	51	372	Low	46	4.65	1.15	82.9	27.5	9.5	81.7	7.9	21.0	2.84	20.6	0.3	104	105
			Medium	45	4.35	1.10	81.1	27.1	9.7	81.6	8.0	21.0	2.91	20.1	0.3	103	103
			High	45	3.70	1.15	82.6	29.2	9.5	82.5	8.0	16.0	3.25	18.0	0.4	97	97
		547	Low	46	4.95	1.10	81.8	26.2	9.4	79.3	8.3	21.0	2.44	21.3	0.5	105	105
			Medium	46	4.90	1.08	81.5	24.0	9.4	78.7	8.7	21.0	2.38	21.2	0.3	104	104
			High	46	4.80	1.10	82.1	26.2	9.8	79.4	8.4	21.0	2.59	21.5	0.3	107	107
		724	Low	46	5.10	1.11	82.9	26.3	9.4	79.8	8.8	21.0	2.23	20.7	0.3	101	101
			Medium	46	4.90	1.08	82.9	26.6	9.6	78.5	8.9	21.0	2.26	21.5	0.3	105	105
			High	46	4.80	1.06	81.9	27.4	10.1	78.7	8.5	26.0	2.49	21.1	0.3	104	105
NF+	78	372	Low	46	3.10	1.18	82.9	30.7	9.6	81.7	7.9	21.0	3.69	15.1	0.5	88	88
			Medium	44	3.10	1.16	81.4	29.8	9.3	81.2	8.1	16.0	3.85	14.8	1.1	87	88
			High	45	2.75	1.15	80.2	29.3	9.5	81.0	8.3	21.0	3.99	13.7	1.0	84	84
		547	Low	46	4.40	1.12	82.6	26.7	9.8	80.5	8.3	21.0	2.67	19.8	0.4	100	100
			Medium	45	4.20	1.11	82.0	28.4	9.6	80.3	8.1	21.0	2.73	19.6	0.3	100	100
			High	45	3.75	1.13	82.3	29.9	9.7	80.4	8.3	21.0	2.95	17.7	0.5	94	94
		724	Low	46	4.70	1.10	83.1	26.1	9.7	79.8	8.6	21.0	2.42	10.6	0.3	102	102
			Medium	45	4.75	1.07	81.9	26.1	9.5	79.7	8.5	21.0	2.46	21.4	0.3	105	106
			High	46	4.25	1.09	82.2	28.8	9.6	79.3	8.6	21.0	2.61	20.4	0.3	102	103
		Significance levels from analyses of variance; * and ** significant at $P \leq 0.05$ and 0.01, respectively; #, significant at $P \leq 0.06$.†															
		Source	df														
		O_3	2		**	ns	ns	**	ns	**	**	ns					
		CO_2	2		**	**	ns	ns	ns	**	**	ns					
		$O_3 \times CO_2$	4		**	ns	ns	ns	ns	**	#	ns					
		N	2		**	ns	ns	ns	ns	ns	ns	ns					
		$O_3 \times N$	4		ns	ns	ns	ns	ns	ns	ns	ns					
		$CO_2 \times N$	4		ns	ns	ns	ns	ns	ns	ns	ns					
		$O_3 \times CO_2 \times N$	8		ns	ns	ns	ns	ns	ns	ns	ns					

† Seed quantity was adequate for only one bulked sample per treatment combination for quality analyses, and percentage lint was calculated for bulked samples. Therefore, no analysis of variance test was performed for seed quality factors or percentage lint.

‡ Low, medium, and high N were obtained by incorporating urea formaldehyde (38:0:0, N:P:K) at a rate of 0.52, 1.02, and 2.04 g L^{-1} respectively, of growth medium.

less, the interactions between soil N, O_3 stress, and CO_2 enrichment are complex and require more study.

Evidently, O_3-induced increase in fiber length (HVI upper half mean) and brightness (Rd) improved fiber quality but this was offset by O_3-induced decreases in micronaire and uniformity. This may explain why overall fiber grade was not affected by O_3 either year. Carbon dioxide enrichment did cause a decrease in fiber grade in 1995, possibly because of decreased fiber length.

The $O_3 \times CO_2$ interactions reported in the present study are similar to those reported for soybean (Heagle et al., 1998a, 1998b; Miller et al., 1998, Mulchi et al., 1992). Moreover, they occurred for a range of N fertilizer rates and for two seasons, showing they can occur over a wide range of growth conditions. These $O_3 \times CO_2$ interactions occurred at concentrations of O_3 that exist in many areas of the world showing the potential for misinterpreting the cause for plant response to CO_2 enrichment when the O_3 stress level is not known. Estimates of food and fiber production in the CO_2-enriched world of the future depend partly on accurate input to model algorithms considering CO_2 effects on plant yield. A better understanding of $O_3 \times CO_2$ interactions is needed to improve estimates provided by such models.

ACKNOWLEDGMENTS

We thank Stephanie Horton, Mary-Catherine Smith, Brenda Cleveland, Jeff Jenco, Lloyd Jones, Adrian Moon, and Heather Pratt for technical assistance, Robert Philbeck for construction and maintenance of dispensing and monitoring systems, Fred Mowry for data acquisition software, Len Stefanski and Erin Blankenship for statistical advice and analyses, and Barbara Shew and Steve Shafer for manuscript review.

REFERENCES

Allen, L.H. 1990. Plant responses to rising carbon dioxide and potential interactions with air pollutants. J. Environ. Qual. 19:15–34.

Barnes, J.D., and T. Pfirrmann. 1992. The influence of CO_2 and O_3, singly and in combination, on gas exchange, growth and nutrient status of radish (*Raphanus sativus* L.). New Phytol. 121:403–412.

Cure, J.D., and B. Aycock. 1986. Crop responses to carbon dioxide doubling: a literature survey. Agric. Forest Meteorol. 38:127–145.

Deusen, H., and C. Faerber. 1995. A cotton valuation model: a proposal to determine the utility value of cotton fibres. p. 431–433. *In* D.A. Richter and J. Armour (ed.) Proc. 1995 Beltwide Cotton Conferences, San Antonio, TX. 4–7 Jan. 1995. National Cotton Council, Memphis, TN.

Drake, B.G., M.A. Gonzales-Meler, and S.P. Long. 1997. More efficient plants: A consequence of rising atmospheric CO_2? Annu. Rev. Plant Physiol. Plant Mol. Biol. 44:609–639.

Fiscus, E.L., C.D. Reid, J.E. Miller, and A.S. Heagle. 1997. Elevated CO2 reduces O3 flux and O3-induced yield losses in soybeans: Possible implications for elevated CO2 studies. J. Exp. Bot. 48: 307–313.

Heagle, A.S. 1989. Ozone and crop yield. Annu. Rev. Phytopathology 27:397–423.

Heagle, A.S., D.E. Body, and W.W. Heck. 1973. An open-top field chamber to assess the impact of air pollution on plants. J. Environ. Qual. 2:365–368.

Heagle, A.S., W.W. Heck, V.M. Lesser, J.O. Rawlings, and F.L. Mowry. 1986. Injury and yield response of cotton to chronic doses of ozone and sulfur dioxide. J. Environ. Qual. 15:375–382.

Heagle, A.S., J.E. Miller, and F.L. Booker. 1998a. Influence of ozone stress on soybean response to carbon dioxide enrichment. I. Foliar properties. Crop Sci. 38:113–121.

Heagle, A.S., J.E. Miller, W.W. Heck, and R.P. Patterson. 1988. Injury and yield response of cotton to chronic doses of ozone and soil moisture deficit. J. Environ. Qual. 17:627–635.

Heagle, A.S., J.E. Miller, W.A. Pursley, and F.L. Booker. 1998b. Influence of ozone stress on soybean response to carbon dioxide enrichment: III. Yield and seed quality. Crop Sci. 38:128–134.

Heagle, A.S., J.E. Miller, D.E. Sherrill, and J.O. Rawlings. 1993. Effects of ozone and carbon dioxide mixtures on two clones of white clover. New Phytol. 123:751–762.

Heagle, A.S., R.B. Philbeck, H.H. Rogers, and M.B. Letchworth. 1979. Dispensing and monitoring O_3 in open-top field chambers for plant effects studies. Phytopathology 69:15–20.

Heck, W.W., W.W. Cure, J.O. Rawlings, L.J. Zaragoza, A.S. Heagle, H.E. Heggestad, R.J. Kohut, L.W. Kress, and P.J. Temple. 1984. Assessing impacts of ozone on agricultural crops: I. Overview. J. Air Pollution Control Assoc. 34:729–735.

Idso, K.E., and S.B. Idso. 1994. Plant responses to atmospheric CO_2 enrichment in the face of environmental constraints: a review of the past 10 years research. Agric. Forest Meteorol. 69:153–203.

Kimball, B.A., and J.R. Mauney. 1993. Response of cotton to varying CO_2, irrigation, and nitrogen: Yield and growth. Agron. J. 85: 706–712.

Kimball, B.A., J.R. Mauney, F.S. Nakayama, and S.B. Idso. 1993. Effects of increasing atmospheric CO_2 on vegetation. Vegetatio. 104/105:65–75.

Kimball, B.A., P.J. Pinter, Jr., G.W. Wall, R.L. Garcia, R.L. LaMorte, P. Jak, K.F.A. Frumau, and H.F. Vugts. 1997. Comparisons of responses of vegetation to elevated CO_2 in free-air and open-top chamber facilities. p. 113–130. *In* L. Allen, Jr. and M.B. Kirkam (ed.) Advances in carbon dioxide effects research. ASA Spec. Pub. no. 61. ASA, CSSA, SSSA, Madison, WI.

Mauney, J.R., B.A. Kimball, P.J. Pinter, Jr., R.L. Lamorte, K.F. Lewin, J. Nagy, and G.R. Hendrey. 1994. Growth and yield of cotton in response to a free-air carbon dioxide enrichment (FACE) environment. Agric. Forest Meteorol. 70:49–67.

Miller, J.E., A.S. Heagle, and W.A. Pursley. 1998. Influence of ozone stress on soybean response to carbon dioxide enrichment: II. Biomass and development. Crop Sci. 38:122–128.

Mortensen, L.M. 1990. Effects of ozone on growth of *Triticum aestivum* L. at different light, air humidity and CO2 levels. Norwegian J. of Agric. Sci. 4:343–348.

Mortensen, L.M. 1992. Effects of ozone concentration on growth of tomato at various light, air humidity and carbon dioxide levels. Scientia Horticulturae. 49:17–24.

Mott, K.A. 1988. Do stomata respond to CO_2 concentrations other than intercellular? Plant Physiol. 86:200–203.

Mulchi, C.L., L. Slaughter, M. Saleem, E.H. Lee, R. Pausch, and R. Rowland. 1992. Growth and physiological characteristics of soybean in open-top chambers in response to ozone and increased atmospheric CO2. Agric., Ecosystems Env. 38:107–118.

Pinter, P.J., B.A. Kimball, R.L. Garcia, G.W. Wall, D.J. Hunsaker, and R.L. LaMorte. 1996. Free-air CO_2 enrichment: Responses of cotton and wheat crops. p. 215–249. *In* G.W. Koch and H.A. Mooney (ed.) Carbon dioxide and terrestrial ecosystems. Academic Press, San Diego.

Rao, M.V., B.A. Hale, and D.P. Ormrod. 1995. Amelioration of ozone-induced oxidative damage in wheat plants grown under high carbon dioxide. Plant Physiol. 109:421–432.

Reddy, V.R., D.N. Baker, and J.M. McKinion. 1989. Analysis of effects of atmospheric carbon dioxide and ozone on cotton yield trends. J. Environ. Qual. 18:427–432.

Reid, C.D., and E.L. Fiscus. 1998. Effects of elevated (CO_2) and/or ozone on limitations to CO_2 assimilation in soybean (Glycine max). J. Exp. Bot. 49:885–895.

Reinert, R.A., and M.C. Ho. 1995. Vegetative growth of soybean as affected by elevated carbon dioxide and ozone. Environ. Pollut. 89:89–96.

Reinert, R.A., G. Palmer, and J. Barton. 1997. Growth and fruiting of tomato as influenced by elevated carbon dioxide and ozone. New Phytol. 137:411–420.

Rogers, H.H., W.W. Heck, and A.S. Heagle. 1983. A field technique for the study of plant responses to elevated carbon dioxide concentrations. J. Air Pollution Control Assoc. 33:42–44.

SAS Institute. 1990. SAS/STAT user's guide: Statistics, Version 6, 4th Edition. SAS Institute Inc. Cary, NC.

Temple, P.J., O.C. Taylor, and L.F. Benoit. 1985. Cotton yield responses to ozone as mediated by soil moisture and evapotranspiration. J. Environ. Qual. 14:55–60.

U.S. Environmental Protection Agency. 1996a. Air Quality Criteria for Ozone and Related Photochemical Oxidants. EPA/600-90/004bF, National Center for Environmental Assessment, Office of Research and Development, Research Triangle Park, NC.

U.S. Environmental Protection Agency. 1996b. Review of National Ambient Air Quality Standards for Ozone: Assessment of Scientific and Technical Information. EPA-452/R-96-007,OAQPS Staff Paper, Office of Air Quality Planning and Standards, Research Triangle Park, NC.

Wall, G.W., J.S. Amthor, and B.A. Kimball. 1994. $COTCO_2$: A cotton growth simulation model for global change. Agric. Forest Meteorol. 70:289–342.

Watson, R.T., H. Rodhe, H. Oeschegerm, and U. Siegenthaler. 1990. Greenhouse gases and aerosols. p. 1–40. *In* T.J. Houghton, G.J. Jenkins, J.J. Ephraums (ed.) Climate change. The IP:CC scientific assessment. Cambridge University Press, Cambridge, MA.

Wong, S.C. 1979. Elevated atmospheric partial pressure of CO_2 and plant growth. I. Interactions of nitrogen nutrition and photosynthetic capacity in C_3 and C_4 plants. Oecologia (Berl.) 44:68–74.

TURFGRASS SCIENCE

Golf Ball Deceleration Measuring System to Evaluate Surface Uniformity on Golf Course Greens

A. M. Rist, M. F. Kocher, W. W. Stroup, R. E. Gaussoin,* R. D. Grisso, and R. C. Shearman

ABSTRACT

Surface uniformity is an important component of golf course putting greens. Presently, there is no quantitative method to measure the surface uniformity of a putting green. The objective of this research was to develop a quantitative method to measure the uniformity of the surface of a golf course green. A Stimpmeter was attached to the end of a plastic tunnel. Photoelectric switches spaced at uniform intervals were activated by the ball as it rolled through the tunnel. As each switch was activated, a single-board computer recorded the time data. The time and distance data were used to determine average acceleration at five locations along the length of the ball roll. An analysis procedure, using data collected from various mowing treatments, was developed to determine if surface uniformity differences were detected. The method developed effectively identified uniformity differences among the mowing treatments.

Surface uniformity is an important characteristic of a golf green. The Stimpmeter, introduced by the United States Golf Association (USGA), is the current method used to measure uniformity (Radko et al., 1981; Thomas, 1983; Oatis, 1990). Instructions for correct usage have been outlined by the USGA (1977) and Beard (1982). Typical ball roll distances are between 180 and 370 cm. Greens are said to be uniform if ball roll distances from green to green on a single course are within 15 cm (Thomas, 1983). This uniformity refers to green to green uniformity and not within green uniformity. The Stimpmeter provides one measurement that averages turf characteristics affecting ball roll distance along the entire length of the ball roll. Several measurements of the speed of a golf ball are needed as the ball crosses the putting surface in order to obtain an indication of the uniformity of the surface within a single ball roll. From physics, we know that a uniform surface will cause an object to decelerate at a uniform rate. A uniform putting surface then should cause the ball to decelerate at a uniform rate.

The Stimpmeter releases a golf ball with an initial velocity of $\approx$1.9 m s^{-1}, and a ball roll of 180 cm lasts $\approx$2 s, while a ball roll of 300 cm lasts $\approx$3.3 s. In searching for equipment to make several time, distance, or speed measurements along a ball roll, the equipment will need to handle low speeds, and have a rapid response time.

Radar Doppler velocimetry is a method commonly used to measure the speed of a baseball pitch ($\approx$40 m s^{-1}). A radar beam reflected off a baseball moving toward the radar beam source has a different frequency than the original signal. The frequency difference depends on the speed of the baseball. Therefore, the speed of the baseball can be determined by measuring the frequency change of the reflected radar signal. Similar radar units have also been used to measure speeds of vehicles in traffic. Richardson et al. (1984) determined that dual-beam radar systems used for measuring agricultural tractor speeds have errors on the order of 3%. Sokol (1984) indicated a radar unit developed for measuring agricultural tractor speeds has errors no greater than 3% in the 0.11 to 0.89 m s^{-1} range, and no greater than 2% in the 0.89 to 19.7 m s^{-1} range; however, the radar unit described used data integration times in the range of 0.5 to 1 s, so it could not be relied on to make several measurements during a ball roll from a Stimpmeter.

A.M. Rist, R.E. Gaussoin, and R.C. Shearman, Horticulture Dep., Univ. of Nebraska-Lincoln, Lincoln, NE 68583-0724; M.F. Kocher and R.D. Grisso, Biological Systems Engineering Dep., Univ. of Nebraska-Lincoln, Lincoln, NE 68583-0726; W.W. Stroup, Biometry Dep., University of Nebraska-Lincoln, Lincoln, NE 68583-0712. Contribution no. 11940 of the Nebraska Agric. Research Div., Inst. of Agric. and Natural Resources, Univ. of Nebraska-Lincoln. Received 28 May 1998. *Corresponding author (rgaussoin1@unl.edu).

Published in Crop Sci. 39:741–745 (1999).