Influence of Ozone Stress on Soybean Response to Carbon Dioxide Enrichment: I. Foliar Properties

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

ABSTRACT

Tropospheric O_3 can cause foliar injury, decreased growth, and decreased yield, whereas CO_2 enrichment generally causes opposite effects. Little is known about plant response to mixtures of O_3 and CO_2. Open-top field chambers were used to determine if foliar responses of soybean [*Glycine max* (L.) Merr.] to CO_2 enrichment are affected by O_3 stress and vice versa. Plants were grown in 14-L pots and exposed to four CO_2 and three O_3 concentrations in 12 combinations. The CO_2 treatments were ambient (366 μL L^{-1}) and three treatments with CO_2 added for 24 h d^{-1} at approximately 1.3, 1.6, and 2.0 times ambient. The O_3 treatments were charcoal-filtered air (CF), nonfiltered air (NF), and NF with O_3 added for 12 h d^{-1} (NF+), resulting in seasonal concentrations of approximately 20, 46, and 75 nL L^{-1}. Foliar effects of CO_2 enrichment were dependent on the amount of stress caused by O_3. In the CF treatment, plants were not stressed by O_3, and CO_2 enrichment caused chlorosis and decreased chlorophyll. In the NF and NF+ treatments, plants were stressed by O_3, and CO_2 enrichment suppressed chlorosis and increased chlorophyll. Ozone decreased specific leaf weight, increased foliar N and C, and decreased C/N ratios, whereas CO_2 caused opposite responses for these measures. Ozone increased foliar S and B but did not affect P or K concentrations. Conversely, CO_2 enrichment suppressed foliar S, B, P, and K concentrations. These interactions between O_3 and CO_2 emphasize a need to consider the amount of plant stress caused by O_3 in studies to measure effects of CO_2 enrichment.

Tropospheric O_3 concentrations have increased rapidly over the past 50 yr (Altshuller, 1987). Concentrations in many areas of the USA are now approximately twice as high as would exist without anthropogenic influence (Heck et al., 1994b). Ambient concentrations of O_3 in many areas can alter permeability of plant cell membranes, disrupt metabolism (Heath, 1988, 1996), decrease foliar chlorophyll and photosynthesis, change photosynthate allocation, and suppress growth and yield (Heagle, 1989; Miller, 1988). Dose-response models, coupled with economic analyses, indicate that O_3 effects on major agronomic crops cost the U.S. economy approximately $3 billion annually (Adams et al., 1987; Heck et al., 1984a,b; Lesser et al., 1990).

Tropospheric CO_2 concentrations have increased from an annual mean of 315 μL L^{-1} in 1958 to 350 μL L^{-1} in 1988, and further increases are expected (Allen, 1990). Plant responses to CO_2 enrichment are generally opposite responses to elevated O_3, and include increased photosynthesis and decreased stomatal conductance (Jones et al., 1984; Jones et al., 1985; Rogers et al., 1983a; Sionit et al., 1984), development of larger, thicker, and heavier leaves (Thomas and Harvey, 1983), increased branching, increased numbers of nodes (Allen et al., 1988; Rogers et al., 1984), changed root/shoot ratios (Idso et al., 1988), and increased growth and yield (Allen et al., 1988; Rogers et al., 1983a, 1984, 1986).

Carbon dioxide enrichment has been shown to increase or decrease foliar chlorophyll of various species whether chorophyll content is measured on a weight per weight (*w*/*w*) or weight per leaf area (*w*/*a*) basis. On a *w*/*w* basis, decreases were reported by Allen et al. (1988), Cave et al. (1980), Delucia et al. (1985), Heagle et al. (1993), and Rao et al. (1995), whereas increases were reported by Allen et al. (1988) and Vu et al. (1989). On a *w*/*a* basis, decreases were reported by Delucia et al. (1985), Houpis et al. (1988), Wulff and Strain (1981), and Wullschleger et al. (1992), whereas increases were reported by Chen and Sung (1990) and Pinter et al. (1994). Several studies showed no effects on a *w*/*a* basis (Cave et al., 1980; Havelka et al., 1984a,b; Reeves et al., 1994).

Decreased foliar chlorophyll accompanied by visible chlorosis is a common response of soybean to O_3 stress (Brennan et al., 1987; Miller et al., 1991; Reich et al., 1986). Effects of CO_2 enrichment on soybean chlorophyll have been variable, however. For the cultivar Bragg, the response (*w*/*w*) at 58 d after planting (DAP) was curvilinear, with highest chlorophyll content at intermediate CO_2 levels and less chlorophyll at 800 μL L^{-1} than at 330 μL L^{-1} (Allen et al., 1988). For Stonewall soybean, foliar chlorophyll (*w*/*a*) was not affected by 705 μL L^{-1} CO_2 as measured at the full bloom-early pod set stage (Reeves et al., 1994). Carbon dioxide enrichment caused foliar chlorosis of Bragg soybean, but chlorophyll per se was not measured (Rogers et al., 1986).

A recent review indicated that relative enhancing effects of CO_2 are greatest when resources limit growth, or when plants are grown in suboptimum environments, including those contaminated by air pollutants such as O_3 (Idso and Idso, 1994). An early report showed that CO_2 at 500 μL L^{-1} above ambient (approximately 850 μL L^{-1}) partially protected tobacco (*Nicotiana tabacum* L.), but not pinto bean (*Phaseolus vulgaris* L.), from foliar injury caused by exposure to O_3 (Heck and Dunning, 1967). Recent reports of experiments with concurrent exposures to CO_2 and O_3 showed that growth stimu-

A.S. Heagle, USDA-ARS, Air Quality - Plant Growth and Development Research Unit, 1509 Varsity Drive, Raleigh, NC 27606, and Dep. of Plant Pathology, North Carolina State Univ.; J.E. Miller, USDA-ARS, Air Quality - Plant Growth and Development Research Unit, 1509 Varsity Drive, Raleigh, NC 27606, and Dep. of Crop Science, North Carolina State Univ.; and F.L. Booker, Air Quality Research Unit, 1509 Varsity Drive, Raleigh, NC 27606, and 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 16 Dec. 1996. *Corresponding author (E-mail: asheagle@unity.ncsu.edu).

Published in Crop Sci. 38:113–121 (1998).

Abbreviations: DAP, days after planting; 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}; *w*/*a*, measured on a weight per area basis; *w*/*w*, measured on a weight per weight basis.

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

	1983						1994					
Variable†	June	July	Aug.	Sept.	Oct. 1–14	Season	June	July	Aug.	Sept.	Oct. 1–12	Season
Mean max. temp. (C)	32	34	32	30	23	31	30	31	30	26	21	27
Mean min. temp. (C)	19	23	21	18	9	20	20	22	19	15	10	22
Mean % RH (24 h)	71	71	73	74	76	72	76	80	79	75	74	76
Mean % RH (1000–1400 h EST)	56	59	57	58	60	58	67	68	66	62	64	64
Mean total PAR (mol m^{-2} d^{-1})†	47	45	42	36	31	42	41	35	37	30	–	36
Rain (cm)	1.2	7.5	7.2	10.1	2.0	28.0	8.2	15.7	12.8	6.6	13.4	56.7
Irrigation (L pot^{-1})	18	79	101	61	6	265	5	54	104	67	12	242
O_3 conc. 12-h means (0800–2000 EST) nl L^{-1}												
CF	30	21	18	14	14	20	30	22	17	18	18	20
NF	58	60	53	37	30	50	57	41	42	41	34	43
NF+	88	96	90	57	42	79	89	62	74	67	52	70
CO_2 conc. 12-h mean (0800–2000 EST)‡ μL L^{-1}												
ambient air	365	383	361	371	371	370	361	363	358	365	364	362
ambient air + 115 μL L^{-1}	474	515	477	475	459	482	464	474	496	485	456	480
ambient air + 230 μL L^{-1}	591	650	603	580	548	599	573	580	637	605	553	600
ambient air + 345 μL L^{-1}	697	789	721	686	636	713	673	694	771	716	651	716

† 1994 Photosynthetically active radiation (PAR) data collection began on 20 June and ended on 12 Sept. CF = charcoal filtered-air open-top chamber; NF = nonfiltered-air open-top chamber; NF+ = NF with ozone added for 12 h d^{-1} (0800–2000 h EST), RH = relative humidity. Ozone concentrations in ambient air were approximately 10% higher than in nonfiltered-air chambers. June O_3 concentrations for 10–30 June 1994. Mean O_3 concentrations for replicates of a given treatment were within 5 nL L^{-1} of the concentrations shown.

‡ 24-h mean CO_2 concentrations were approximately 9% higher than the 12-h concentrations shown. June CO_2 values for 9–30 June 1994. Mean CO_2 concentrations for replicate chambers of a given treatment were within 12 μL L^{-1} of the concentrations shown.

lation of radish (*Raphanus sativus* L.) (Barnes and Pfirrman, 1992), soybean (Mulchi et al., 1992), wheat (*Triticum aestivum* L.) (Mortensen, 1990; Rao et al., 1995), and tomato (*Lycopersicon esculentum* Miller) (Mortensen, 1992) caused by CO_2 at twice-ambient levels was greater for plants that were stressed by O_3 than for nonstressed plants. Carbon dioxide enrichment ameliorated O_3-induced foliar injury of white clover (*Trifolium repens* L.) after 4 wk of exposure, but the effect diminished after 8 wk of exposure (Heagle et al., 1993).

Only a few studies have been performed with multiple CO_2 and O_3 concentrations (Heagle et al., 1993; Mulchi et al., 1992); multiple concentrations of both gases are required to estimate interactive response curves. Temporal changes in visible foliar injury, foliar chlorophyll, and foliar nutrient content in response to elevated CO_2 + O_3 mixtures have not been reported. Our objective was to determine the degree of interaction between CO_2 and O_3 by measuring effects of chronic exposure on foliar response of soybean during vegetative and reproductive growth stages.

MATERIALS AND METHODS

The experiments were performed during 1993 and 1994 at our field site 5 km south of Raleigh, NC. Soybean seeds were treated with a commercial *Bradyrhizobium* preparation and planted in 30-cm-diam. by 29-cm-tall pots containing 14 L of a 2:1:1 mixture of sandy loam soil/sand/Metro Mix 220 (W.R. Grace Co. Cambridge, MA)[1] at pH 6.1. The soil was obtained commercially and was probably Appling (clayey, kaolinitic, thermic Typic Kanhapludult) topsoil. Plants were irrigated with drip tubes to prevent water stress and were fertilized at 14-d intervals with 1 L per pot of a water solution containing 2.5 g of soluble fertilizer (10:30:20, N:P:K). The initial fertilization also included 0.31 g L^{-1} of a micronutrient formulation (STEM, Peter's Fertilizer Products, W.R. Grace & Co., Fogelsville, PA). Insects and mites were controlled with up to six applications of acephate (*O*, *S*-dimethyl acetylphosphoramidothioate) (Orthene 75 SP at 1.7 mL L^{-1} water), bifenthrin {(2 methyl[1,1′-biphenyl]-3-yl) methyl 3-(2-chloro-3,3,3-trifluoro-1-propenyl)-2,2-dimethylcyclopropanecarboxylate} (Talstar 10 WP at 2.5 mL L^{-1} water), or avermectin (Avid, 0.15 EC at 0.03 mL L^{-1} water).

In both years, 24 open-top chambers (plots), 3-m diameter by 2.4-m tall, (Heagle et al., 1973) were used for two randomized replicates of 12 treatment combinations (four CO_2 treatments combined with three O3 treatments). Dispensing of CO_2 for 24 h d^{-1} and O_3 for 12 h d^{-1} (0800–2000 h EST) began within 3 d after plants emerged and continued through reproductive maturity. The CO_2 treatments were approximately 1.0, 1.3, 1.6, and 1.9 times ambient concentrations. The O_3 treatments were charcoal-filtered air (CF), nonfiltered air (NF), and NF with O_3 added in proportion to ambient O_3 (NF+). Ozone concentrations in the CF, NF, and NF+ treatments were approximately 0.4, 0.9, and 1.4 times ambient O_3. General dispensing and monitoring protocols have been described previously for O_3 (Heagle et al., 1979) and for CO_2 (Rogers et al., 1983b). Both gases were monitored 24 h d^{-1} at canopy height in the center of each chamber. In the present study, O_3 was monitored with UV analyzers (TECO Model 49), which were calibrated bi-weekly with a TECO Model 49 PS calibrator (Thermo Environmental Instruments, Inc., Franklin, MA). 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. Concentrations of CO_2 and O_3 for each treatment for each season are shown in Table 1. Reproductive stages at 5-d intervals for both seasons are shown in Table 2.

Sampling for estimates of foliar injury and measures of chlorophyll, carotenoids, and specific leaf weight were made

[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 2. Reproductive (R) stages of soybean cultivars at 5-d intervals in 1993 and 1994.†

Year	Planting date	Cultivar	Mean R stage DAP^{-1}											
			45	50	55	60	65	70	75	80	85	90	95	100
1993	24 May	Essex	–	1.0	2.0	2.0	2.6	3.7	4.7	5.0	5.5	5.9	6.0	6.0
1994	1 June	Essex	0.2	1.6	2.0	2.4	3.3	4.5	4.9	5.3	5.8	6.0	6.0	6.0
		Holladay	0.0	1.0	2.0	2.3	3.1	4.7	5.0	5.0	5.7	6.0	6.0	6.0
		NK 6955	0.0	0.6	1.8	2.0	2.4	3.2	4.3	5.0	5.0	5.4	5.6	5.9
			105	110	115	120	125	130	135	140	145	150	155	160
1993	24 May	Essex	6.0	6.0	6.0	6.0	6.3	6.8	7.0	7.0	7.0	–	–	–
1994	1 June	Essex	6.0	6.0	6.0	6.2	6.5	6.9	7.0	7.1	7.6	7.9	8.0	8.0
		Holladay	6.0	6.0	6.0	6.0	6.6	6.9	7.0	7.2	7.5	7.7	7.9	7.9
		NK	6.0	6.0	6.0	6.0	6.1	6.2	6.5	6.9	7.3	7.7	8.0	8.0

† All cultivars are maturity group V. Growth stages according to Fehr and Caviness (1977). R stage values interpolated from observations taken on 25 d in 1993 and 27 d in 1994. Values shown are combined means for plants in all treatments. Growth stage when exposures began at 7–9 DAP was between VE and VC for both years. Growth stages for Essex in 1993 at 15 and 44 DAP were V1 and V12, respectively.

between 0900 and 1200 h EST. Foliar injury was estimated visually as the percent chlorosis and necrosis in 5% increments (0–100%). Chlorophyll, carotenoid, and specific leaf weight measures were obtained from 1.5-cm-diam. leaf disks from individual main-stem trifoliolate leaves. Each leaf-disk sample was placed in a brown glass bottle containing 45 mL of 95% (*v*/*v*) ethanol. Samples were stored in the dark for 2 to 3 d and shaken daily. Absorbance was measured with a spectrophotometer at 470, 649, and 665 nm. Amounts of chlorophyll a, chlorophyll b, and total carotenoids were calculated as described previously (Lichtenthaler and Wellburn, 1983). Leaf disks were dried for 48 h at 60°C and weighed to obtain a measure of specific leaf weight (mg cm^{-2}).

1993 Experiment

Essex (Smith and Camper, 1973) seeds were planted in each of 30 pots per chamber on 24 May. Seedlings emerged on 29 May and were thinned to one per pot on 8 June. Wheat straw was used as a mulch around and between pots to moderate temperatures. Twelve plants in the southern half of each chamber were used for periodic response measures. Seven plants in the northern half were used for final yield measures (Heagle et al., 1998). The remaining 11 plants served as borders. Carbon dioxide enrichment began on 31 May and O_3 exposures began on 1 June. Dispensing of both gases continued until 14 October.

Estimates of foliar injury and sampling for chlorophyll, carotenoids, and specific leaf weight were made on main-stem leaves at five 2-d periods (one replicate per day). Sampling periods and leaf positions (above the primary node) sampled were as follows: 43 to 44 DAP, leaves 2, 4, 6; 57 to 58 DAP, leaves 5, 7, 9, 11, 13; 71 to 72 and 92 to 93 DAP, leaves 7, 9, 11, 13, 15; 113 to 114 DAP, leaves 9, 11, 13, 15. Each sample for each leaf position and plot consisted of six leaf disks (one for each of three leaflets per trifoliolate on two plants). Sampling to measure foliar starch content was done at 87 DAP; eight 2.2-cm-diam. disks were taken from main-stem leaves at position 13 on each of three plants per plot between 1500 and 1600 h EST. Samples were freeze-dried and analyzed to determine starch content by the UV method (Boehringer Mannheim Corp, Indianapolis, IN, 46250).

Foliar nutrients were quantified for all main-stem leaves from two plants per plot at the last four sampling periods; leaves were oven-dried to constant weight and composited to produce one sample per plot.

1994 Experiment

Essex, Holladay (Burton et al., 1996), and Northrup King 6955 (NK 6955) seeds were planted on 1 June in four pots per cultivar per chamber. The cultivars were arranged in four rows of three pots each per chamber with the cultivar positions randomized within each row in each chamber. Twelve pots of Essex served as border plants. Pot temperature was moderated with a sleeve (cylinder) composed of 0.6-cm-thick bubble wrap, coated on both sides with aluminum (Reflectix, Reflectix, Inc., Markleville, IN), tightly fit around each pot and secured with aluminum tape. Plants emerged on 7 June and were thinned to one per pot on 20 June. Carbon dioxide enrichment began on 9 June, and O_3 exposures began on 10 June. Dispensing of CO_2 and O_3 continued until 12 October.

Estimates of foliar injury and sampling for chlorophyll content and specific leaf weight were made at 62 to 63 DAP (leaf positions 9 and 11) and 91 DAP (leaf positions 10 and 12) for one plant per cultivar per replicate chamber. Samples for chlorophyll and specific leaf weight measures consisted of three disks per leaf.

STATISTICS

Analyses of variance were performed on replicate means for foliar injury, total chlorophyll and starch (*w*/*a*), chlorophyll a/b ratio, leaf–disk weight, and foliar C and nutrients (*w*/*w*). Each harvest for the 1993 season was analyzed separately with a factorial model with O_3 and CO_2 as main effects. To address the relationship between O_3, CO_2, and harvest date, sub-plot analysis was used for each season. For 1993, O_3 and CO_2 were the whole-plot factors and the harvest date was the sub-plot factor. For 1994, O_3 and CO_2 were the whole-plot factors, cultivar was first sub-plot, and harvest date was the sub-sub-plot factor. All analyses were conducted with SAS software (SAS Institute, 1990).

RESULTS

In 1993, the seasonal mean maximum temperature was 4°C higher and relative humdity was lower than in 1994. Solar irradiation and seasonal mean O_3 concentrations were higher in 1993 than in 1994, mostly because of differences during July and August (Table 1). Carbon dioxide concentrations were similar both years (Table 1).

Foliar Injury, Chlorophyll, Starch, and Specific Leaf Weight

Increasing O_3 concentrations increased foliar injury, decreased chlorophyll and starch content (*w*/*a*), in-

Table 3. Mean squares and significance levels from analyses of variance for foliar injury, total chlorophyll, chlorophyll a/b ratio, leaf-disk weight, C, C/N ratio, and nutrient composition of Essex soybean exposed to mixtures of O_3 and in 1993.

Source	df†	Foliar injury	Chlorophyll	Chlorophyll a/b ratio	Leaf-disk wt	df‡	C	N	C/N	P	K
		%	μg cm^{-2}		mg cm^{-2}		g kg^{-1}			g kg^{-1}	
O_3	2	8 597**	414**	0.82**	2 510**	2	145**	35**	5.8	0.00	1.03
CO_2	3	506**	25**	0.25*	3 184**	3	236**	202**	28.9**	1.45**	39.42**
$O_3 \times CO_2$	6	470**	28**	0.24*	67	6	9	21*	4.7*	0.05	2.14*
error a	11	46	4	0.06	98	11	27	69	1.6	0.11	2.06
Harvest (H)	4	4 512**	433**	2.34**	7 056**	3	1 349**	23 634**	286.5**	10.34**	293.1**
$O_3 \times H$	8	110**	8	0.09	376**	6	12	157	3.5	0.46**	4.85**
$CO_2 \times H$	12	24	3	0.08	96	9	21	119	2.2	0.15	2.11*
$O_3 \times CO_2 \times H$	24	58**	4	0.20	82	18	18	67	1.9	0.14	1.22

	df††	S	Ca	Mg	Mn	Cu	Zn	Fe	B
		g kg^{-1}			mg kg^{-1}				
O_3	2	0.35**	75**	0.4	549**	0.08	74	1 568**	40**
CO_2	3	0.98**	1	0.2	225	0.88**	48	820	45**
$O_3 \times CO_2$	6	0.03	9	0.7*	80	0.18	85	215	14
error a	11	0.01	4	0.2	91	0.04	97	346	6
Harvest (H)	3	3.35**	1 187**	4.2**	1 180**	17.86**	159	9 497**	345**
$O_3 \times H$	6	0.03	10**	0.5	116	0.13	49	199	15*
$CO_2 \times H$	9	0.04	70**	0.4	137	0.10	82	308	6
$O_3 \times CO_2 \times H$	18	0.02	4	0.3	129	0.14**	85	238	5

† Degrees of freedom for injury, total chlorophyll, chlorophyll a/b ratio, and leaf weight.
‡ Degrees of freedom for C, C/N ratio and foliar nutrients.
*,** Significant at the 0.05 and 0.01 level, respectively.

creased the chlorophyll a/b ratio, and suppressed specific leaf weight (Tables 3–6). These main effects of O_3 decreased as the CO_2 level increased, however. Conversely, the overall effects of CO_2 were suppressed foliar injury, increased chlorophyll, decreased chlorophyll a/b ratio, and increased specific leaf weight. These main effects on injury, chlorophyll, and the chlorophyll a/b ratio were evident only in the NF and NF+ treatments, whereas main effects on specific leaf weight and starch occurred at all O_3 levels. Changes in chlorophyll a/b ratios were always caused by changes in chlorophyll b. Effects of each gas individually were cumulative, causing significant harvest date effects and significant harvest × O_3 and harvest × CO_2 interactions (Tables 3, 5). Carotenoid concentrations (data not shown) showed the same trends as total chlorophyll responses and will not be discussed further.

Carbon dioxide enrichment suppressed effects of O_3 at all harvests each year (Tables 4, 6), and $O_3 \times CO_2$ interactions for injury and chlorophyll were significant for both years (Tables 3, 5). Mean effects on Essex leaves for the five harvests combined in 1993, and for Essex, Holladay, and NK-6955 for the two harvests combined in 1994, show the overall nature of the $O_3 \times CO_2$ interactions in each season (Fig. 1). For example, CO_2 enrichment suppressed foliar injury and increased chlorophyll content in the NF and NF+ treatments, but increased injury and decreased chlorophyll in the CF treatment (Fig. 1). The $O_3 \times CO_2$ interaction for the chlorophyll a/b ratio (Table 3) occurred because the

Table 4. Foliar injury, total chlorophyll, chlorophyll a/b ratio, and leaf-disk weight at 43, 57, 71, 92, and 113 d after planting for Essex soybean exposed to mixtures of O_3 and CO_2.

O_3 conc.†	CO_2 conc.‡	Foliar injury - % per leaf§					Total chlorophyll - μg cm^{-2}¶					Chlorophyll a/b ratio					Leaf-disk weight - mg cm^{-2}¶					Starch- μg cm^{-1}¶
		43	57	71	92	113	43	57	71	92	113	43	57	71	92	113	43	57	71	92	113	57
nL L^{-1}	μL L^{-1}																					
20	370	9	4	6	16	27	37	44	52	51	38	2.61	2.58	3.12	2.46	2.83	2.91	2.30	3.08	3.77	3.41	1.12
	482	16	9	2	15	24	34	49	54	49	31	2.91	2.45	2.79	2.41	2.86	2.80	2.66	4.03	4.56	4.11	1.55
	599	9	7	11	29	34	35	48	47	43		2.66	2.49	2.96	2.47	3.12	3.41	2.76	3.81	4.91	4.38	1.79
	713	14	13	14	24	44	38	42	44	40	25	2.68	2.55	2.97	2.49	2.89	3.58	2.73	3.80	5.00	4.16	1.83
50	370	17	23	25	45	65	34	39	39	40	18	2.55	2.56	2.92	2.53	3.52	2.46	2.49	2.80	3.22	3.05	0.45
	482	12	20	9	22	54	36	42	47	46	25	2.58	2.51	2.91	2.54	2.96	2.81	2.37	3.17	3.74	3.23	0.89
	599	18	13	14	22	55	32	44	48	44	18	3.09	2.50	3.18	2.43	3.17	2.97	2.69	3.39	4.02	3.60	1.21
	713	22	15	18	32	47	37	45	43	39	21	2.42	2.46	3.02	2.69	3.09	3.10	2.80	3.66	3.87	4.10	1.95
79	370	56	43	47	67	86	19	32	28	23	9	4.48	2.67	3.53	2.65	3.31	2.58	2.49	2.80	3.12	3.00	0.21
	482	36	32	40	45	66	29	37	35	36	18	2.52	2.59	3.46	2.57	3.44	3.02	2.78	2.86	3.58	3.55	0.40
	599	35	35	29	38	60	30	35	37	33	19	2.74	2.50	2.94	2.56	3.14	3.09	2.60	3.38	3.79	3.81	0.79
	713	34	28	24	41	67	31	38	38	35	15	2.64	2.59	3.07	2.56	3.53	3.39	2.78	3.68	3.66	3.77	1.46

† Seasonal 12 h d^{-1} mean concentrations of 20, 50, and 79 nL L^{-1} were measured in the CF, NF, and NF+ treatments, respectively.
‡ Seasonal 12 h d^{-1} mean concentrations in chambers receiving ambient air (370 μL L^{-1}) and ambient air with CO_2 added for 24 h d^{-1}.
§ Each foliar injury value is the mean of 12 to 22 mainstem trifoliolate leaves (3 to 5 leaves, 2 plants, 2 replicates) for samples taken at successive days after planting (DAP) as follows: 43–44 DAP, leaves 2, 4, 6; 57–58 DAP, leaves 5, 7, 9, 11, 13; 71–72 DAP and 92–93 DAP, leaves 7, 9, 11, 13, 15; 113–114 DAP leaves 9, 11, 13, and 15.
¶ Each chlorophyll and leaf-disk weight value is the mean of 36 to 60 1.5 cm-diam. leaf disks (3 disks from each of the 12 to 20 leaves as described for foliar injury). Each starch value is the mean of 48 2.2 cm-diam. disks (8 disks, 3 plants, 2 replicates).

Table 5. Mean squares and significance levels from analyses of variance for foliar injury, total chlorophyll, and leaf-disk weight of Essex, Holladay, and Northrup King 6955 soybean exposed to mixtures of O_3 and CO_2 in 1994.

Source	df	Foliar injury %	Chlorophyll μg cm^{-2}	Leaf-disk wt. mg cm^{-2}
O_3	2	6 030**	2 900**	5.1**
CO_2	3	374	283*	4.4**
$O_3 \times CO_2$	6	1 424**	251*	0.8
Error a	12	152	74	0.4
Cultivar (V)	2	215	435**	11.8**
$O_3 \times V$	4	23	192**	0.3
$CO_2 \times V$	6	125	32	0.3
$O_3 \times CO_2 \times V$	12	109	24	0.6
Error b	24	95	27	0.4
Harvest (H)	1	62 708**	1 468**	25.8**
$O_3 \times H$	2	1 013**	723**	1.8**
$CO_2 \times H$	3	189	66	0.9*
$V \times H$	2	88	66	1.1*
$O_3 \times CO_2 \times H$	6	435*	149**	0.3
$O_3 \times V \times H$	4	30	53	0.2
$C \times V \times H$	6	84	18	0.2
$O_3 \times C \times V \times H$	12	62	19	0.2

*,** Significant at the 0.05 and 0.01 level, respectively.

CO_2-induced decrease was consistent only at 79 μL L^{-1} O_3 and the O_3-induced increase was consistent only at ambient CO_2 (Table 4). Carbon dioxide enrichment increased specific leaf weight uniformly at all O_3 levels in 1993 (Table 3, Fig. 1).

The trends shown in Fig. 1 occurred in both years for all but the first harvest dates (43 and 62 DAP, respectively) when the CO_2-amelioration of O_3 injury was not yet apparent in the NF treatment (Tables 4 and 6). This might have caused the $O_3 \times CO_2 \times$ harvest date interaction for injury in 1993 (Table 3), and a similar interaction for injury and chlorophyll in 1994 (Table 5). Significant $O_3 \times CO_2 \times$ harvest date interactions for injury and chlorophyll were caused partly by differences in the proportional response rather than by changes in trends over time. Ozone caused less of a decrease in chlorophyll content of NK 6955 than of Essex or Holladay (Table 6) which probably accounts for the significant cultivar $\times$ O_3 interaction (Table 5).

Foliar Elements

Increasing concentrations of O_3 and CO_2 affected C concentration and often affected nutrient concentrations (Table 3). Similar trends occurred at all harvest dates except for a few instances, which will be discussed. Because $O_3 \times$ harvest and $CO_2 \times$ harvest interactions were rare, means for the four harvest dates combined

Table 6. Foliar injury, chlorophyll, and specific leaf weight at 62 and 91 days after planting (DAP) of Essex, Holladay, and Northrup King (NK) 6955 soybean exposed to mixtures of O_3 and CO_2 in 1994.

O_3 conc. 12 h d^{-1} mean nL L^{-1}	CO_2 conc. 12 h d^{-1} mean μL L^{-1}	Cultivar	Foliar injury†		Total chlorophyll‡		Specific leaf wt.‡	
			62 DAP	91 DAP	62 DAP	91 DAP	62 DAP	91 DAP
			% per leaf		μg cm^{-2}		mg cm^{-2}	
18	362	Essex	0	19	51	64	3.20	4.06
	480		0	30	58	55	3.48	4.01
	600		6	41	59	56	3.33	3.87
	716		8	49	59	42	3.98	4.15
42	362		1	53	47	34	2.32	2.41
	480		0	40	55	54	2.48	3.40
	600		5	28	52	52	2.84	4.44
	716		0	46	52	51	2.50	3.68
69	362		26	83	38	20	2.85	2.64
	480		15	73	45	25	2.98	2.93
	600		5	44	48	31	2.49	3.30
	716		8	70	52	36	3.24	3.35
18	362	Holladay	0	26	47	52	2.70	3.87
	480		6	36	44	56	3.02	4.39
	600		1	54	54	50	3.20	4.48
	716		0	48	52	40	3.25	4.67
42	362		13	63	43	37	2.61	3.16
	480		4	36	44	50	3.47	4.81
	600		0	41	49	46	3.11	4.15
	716		4	51	48	41	3.09	3.78
69	362		30	80	33	13	2.95	2.55
	480		20	74	38	17	3.08	3.49
	600		13	61	47	26	3.07	3.92
	716		4	58	46	30	3.14	3.68
18	362	NK-6955	0	8	43	51	2.97	4.58
	480		1	16	48	47	3.32	5.33
	600		1	46	45	45	3.40	5.10
	716		11	70	45	29	5.47	6.23
42	362		1	55	47	45	3.34	3.78
	480		0	18	48	50	3.63	4.81
	600		4	43	47	46	3.78	5.43
	716		4	35	46	45	3.45	5.10
69	362		28	85	34	19	3.08	3.16
	480		13	70	45	36	3.70	5.62
	600		8	50	43	40	3.68	4.53
	716		8	51	43	37	3.55	3.68

† Each injury value is the mean of four trifoliolate leaves (2 leaves, 2 replicates).
‡ Each chlorophyll and specific leaf weight value is the mean of 12 1-cm-diam. disks (3 disks, 2 leaves, 2 replicates).

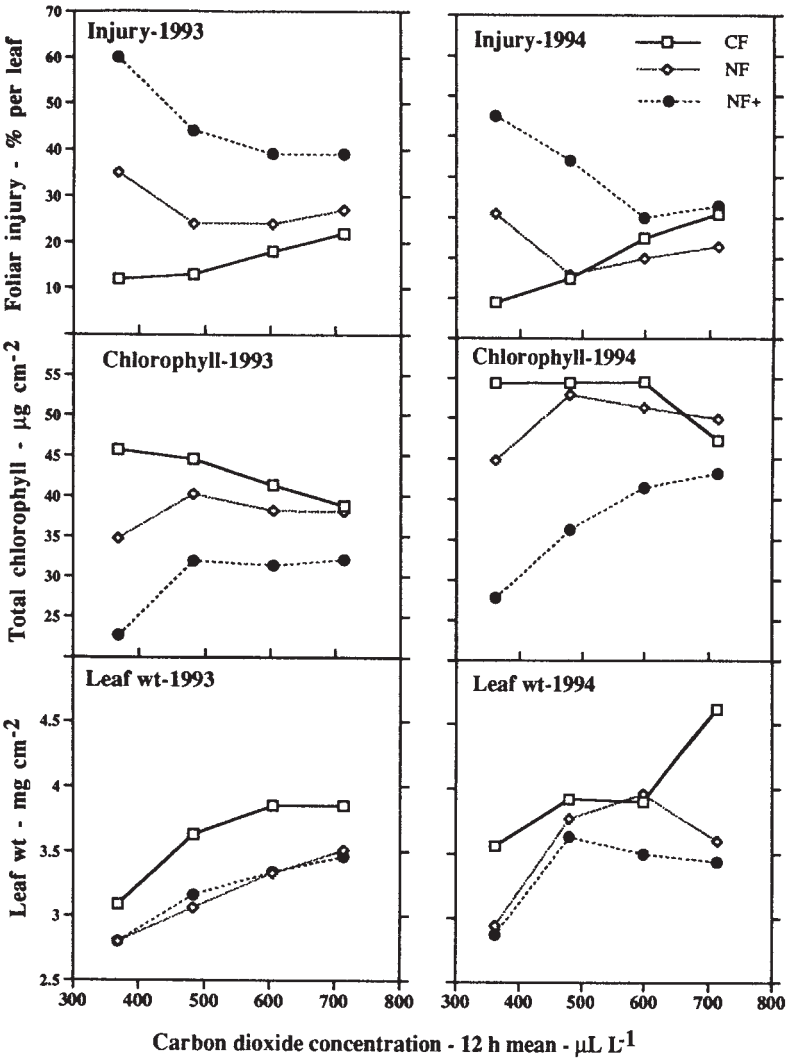

Fig. 1. Foliar injury, total foliar chlorophyll content, and specific leaf weight of Essex soybean exposed to mixtures of CO_2 and O_3 in 1993 and 1994. CF = charcoal filtered air, NF = nonfiltered air, NF+ = NF with O_3 added for 12 h d^{-1}. Seasonal mean O_3 concentrations for each treatment for each year are shown in Table 1. Values shown are combined means for all harvests using data in Table 4 for 1993 and data in Table 6 for 1994.

are shown for selected elements in Fig. 2 and 3. At all harvests, exposure to increasing concentrations of O_3 generally increased foliar N and C, whereas CO_2 enrichment decreased N and C (Table 3, Fig. 2). Because the proportional changes in N were greater than the proportional changes in C, the net effect was for CO_2 enrichment to increase significantly the C/N ratio, while O_3 tended to decrease it ($P \leq 0.059$) (Fig. 2).

Increasing O_3 increased foliar concentrations of S and B but did not affect P or K concentrations. Conversely, CO_2 enrichment decreased P, K, S, (Table 3, Fig. 3) and B (Table 3). The CO_2-induced decrease of foliar P diminished as the season progressed, causing the significant harvest date $\times$ CO_2 effect (Table 3). A tendency for O_3 to decrease foliar K was greater at Harvests 1 and 2 than at Harvests 3 and 4, and caused the significant $O_3 \times$ harvest date interaction for K (Table 3). Increasing O_3 concentrations increased foliar Ca, Mn, and Fe. At 20, 50, and 79 nL L^{-1} O_3 (for the CO_2 treatments combined), Ca content was 21.5, 23.5, and 24.5 g kg^{-1}, respectively, Mn content was 66, 72, and 74 mg kg^{-1}, respectively, and Fe content was 96, 103, and 111 mg kg^{-1}, respectively. Carbon dioxide enrichment did not affect Ca, Mn, and Fe (data not shown). Ozone-induced decrease of foliar P and B diminished as the season progressed causing a significant $O_3 \times$ harvest date interaction for each element (Table 3).

Significant $O_3 \times CO_2$ interactions occurred for foliar concentrations of N, K, Mg, and the C/N ratio (Table 3). Nitrogen concentrations were routinely lower in leaves exposed to 20 nL L^{-1} O_3 than at higher O_3 levels for all but the ambient CO_2 treatment (Fig. 2). Also, except for the N response at ambient CO_2, the difference in foliar N across the O_3 treatments diminished as CO_2 concentrations increased (Fig. 2). The $O_3 \times CO_2$ interaction for K may have resulted because CO_2 enrichment caused an almost linear suppression of foliar K at 50 and 79 nL L^{-1} O_3 but not at 20 nL L^{-1}, where the CO_2 effect was almost constant at all enrichment levels (Fig. 3). Foliar Mg content was highly irregular with no appar-

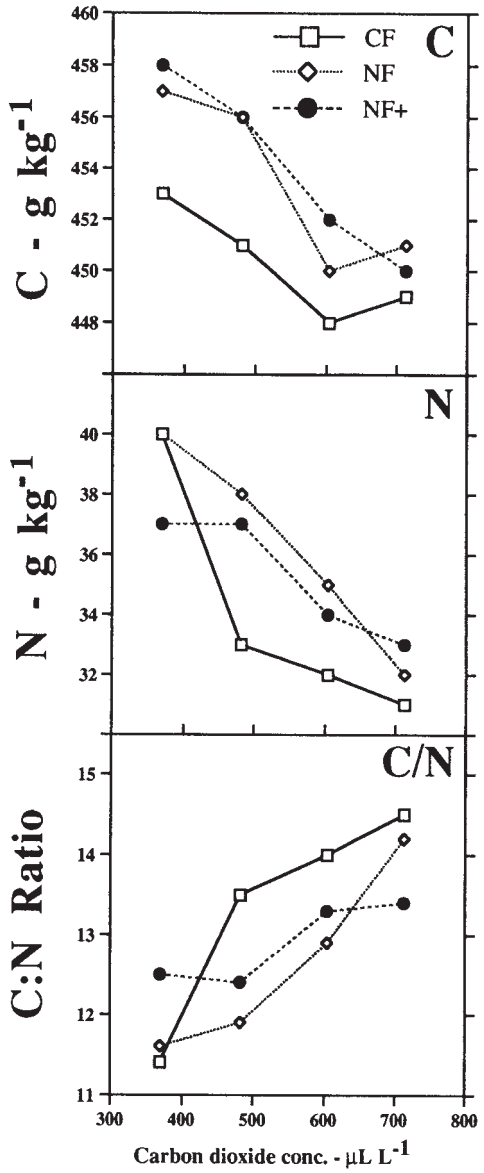

Fig. 2 Foliar C and N concentrations and C:N ratios for all mainstem trifoliolate leaves of Essex soybean exposed to mixtures of CO_2 and O_3 in 1993. Each value is the mean of eight composite samples (2 samples collected at 57, 71, 92, and 113 days after planting).

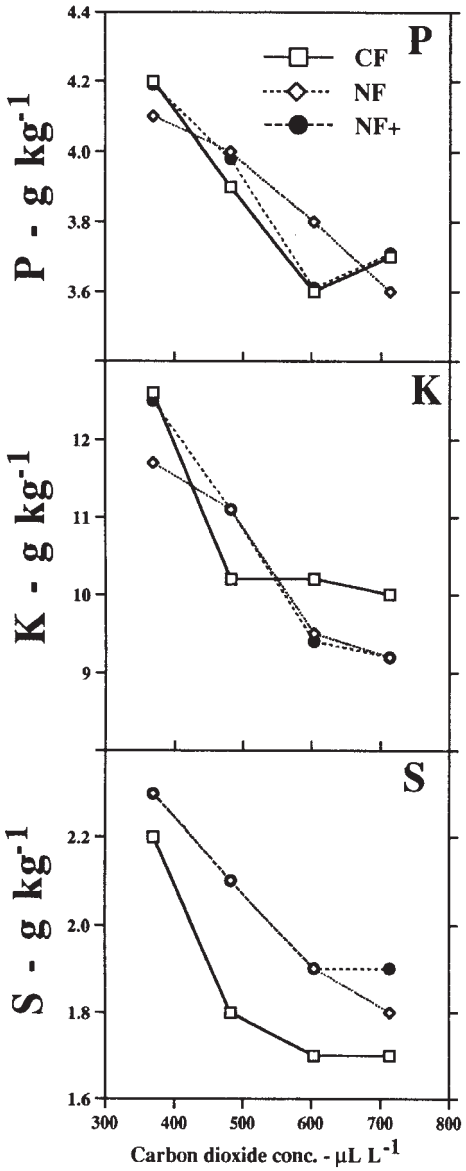

Fig. 3. Foliar P, K and S concentrations for all mainstem trifoliolate leaves of Essex soybean exposed to mixtures of CO_2 and O_3 in 1993. Each value is the mean of eight composite samples (2 samples collected at 57, 71, 92, and 113 days after planting).

ent relationship with O_3 or CO_2 concentrations. Thus, we were unable to assign a probable reason for the significant $CO_2 \times O_3$ interaction for Mg. The $CO_2 \times O_3$ interaction for the C/N ratio (Table 3) occurred because the O_3-induced suppression occurred at all CO_2 levels except ambient (Fig. 2).

DISCUSSION

These results may help explain variable chlorophyll responses to CO_2 enrichment reported previously. Most research showing decreased chlorophyll was done in controlled environments (Cave et al., 1980; Delucia et al., 1985; Wulff and Strain, 1981), whereas work showing neutral effects or increased chlorophyll was done in the field [e.g., Alabama (Reeves et al., 1994), Arizona (Pinter et al., 1994), Delaware (Havelka et al., 1984a,b), Florida (Vu et al., 1989), and Taiwan (Chen and Sung, 1990)]. Differential responses from these studies may have been caused by differences in rooting volume, light intensity, or the amount of stress caused by O_3. Each of these factors were probably greater for field studies than for controlled-environment studies. Arp (1991) presented correlative evidence for a hypothesis that CO_2 enrichment generally decreases foliar chlorophyll content when rooting volume is limiting to growth. However, because our results show variable chlorophyll responses to CO_2 enrichment with the same rooting volume and light intensity, chlorophyll increases reported for field studies may have been caused by CO_2-induced amelioration of O_3 effects rather than by CO_2 enrichment per se.

Our measurements of foliar starch at 87 DAP are

consistent with the hypothesis that CO_2-induced decreases in foliar chlorophyll are caused by starch accumulation that disrupts chloroplasts (Wulff and Strain, 1981; Cave et al., 1980; Delucia et al., 1985), although other hypotheses have been suggested. A general reduction of the overall photosynthetic apparatus in response to CO_2 enrichment may have caused decreased chlorophyll in ponderosa pine (*Pinus ponderosa* Douglas ex P. Lawson & Lawson) (Houpis et al., 1988). Tripp et al., (1991) showed that CO_2-induced chlorophyll decrease in tomato leaves can occur without increased starch content. A CO_2-induced limitation of transpiration might cause decreased N uptake (Vessey et al., 1990; Conroy and Hocking, 1993), which could contribute to chlorophyll loss.

Carbon dioxide enrichment ameliorated all measured stress responses to O_3. Mechanisms may be related to decreased stomatal conductance, which would decrease O_3 entering leaves; stomatal conductance was decreased significantly by CO_2 enrichment throughout the season (Fiscus et al., 1997). Another possible mechanism may be related to increased CO_2 assimilation (net photosynthesis), which resulted in more substrate and energy to prevent or repair injury (Allen, 1990; Kramer, 1991; Rao et al., 1995) . Carbon dioxide enrichment increased net CO_2 exchange rates of plants exposed to elevated O_3 throughout the 1993 season (Miller et al., 1998).

Carbon dioxide enrichment suppressed foliar concentrations of N, P, K, S, Cu, and B, but did not significantly affect concentrations of Zn, Ca, Mg, Mn, or Fe. These effects were similar to those reported previously for white clover (Heagle et al., 1993). Prior and Rogers (1995) reported a similar P response in soybean. It is not clear why our results differ substantially from those reported for Stonewall soybean, wherein N was the only element significantly affected by CO_2 enrichment (Reeves et al., 1994). Changes in foliar nutrients did not appear to adversely affect soybean growth and yield in the present study (Miller et al., 1998; Heagle et al., 1998a,b). Nevertheless, implications of nutrient responses presented here for plant growth and yield under present and future CO_2 and O_3 scenarios remain largely unknown. More research is needed on foliar responses of plants to $CO_2 \times O_3$ mixtures at a range of soil nutrient levels.

Our results clearly show that responses to CO_2 enrichment are dependent on the amount of O_3 present, and that the response to O_3 is dependent on the concentration of CO_2. Until recently, however, most research on effects of CO_2 enrichment has been done without considering stress caused by O_3. Interactions between CO_2 and O_3, similar to those reported here, probably have occurred in previous research. Tropospheric O_3 concentrations vary widely at different locations throughout the world, causing different amounts of plant stress. Research done where O_3 stress is great could show greater effects of CO_2 enrichment (by amelioration of O_3 stress) than work done where O_3 stress is absent. Although tropospheric O_3 has not been measured in most previous studies, estimates of past and present amounts of O_3 stress can be developed for many plant species grown in most areas of the USA and for much of the rest of the world. This knowledge, with further quantification of interactive effects of O_3 and CO_2, may serve to adjust previous reports of effects of CO_2 enrichment.

ACKNOWLEDGMENTS

We thank Brenda Cleveland, Jeff Jenco, Stephanie Horton, Colleen Hudak, and Mary-Catherine Smith for technical assistance; Robert Philbeck for construction and maintenance of dispensing and monitoring systems; Fred Mowry for data acquisition software; Len Stefanski and Kurex Sidik for statistical advice and analyses; and Barbara Shew and Steve Shafer for manuscript review.

REFERENCES

Adams, R.M., J.D. Glyer, and B.A. McCarl, 1987. The NCLAN economic assessment: approach, findings and implications. *In* W.W. Heck, O.C. Taylor, and D.T. Tingey (ed.) Assessment of crop loss from air pollutants. Proc. Int. Conf. Raleigh, NC. 25–29 Oct. 1987. Elsevier, London.

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

Allen., L.H., Jr., J.C.V. Vu, R.R. Valle, K.J. Boote, and P. Jones. 1988. Nonstructural carbohydrates and nitrogen of soybean grown under carbon dioxide enrichment. Crop Sci. 28:84–94.

Altshuller, A.P. 1987. Atmospheric chemistry and long-range transport. *In* W.W. Heck, O.C. Taylor, and D.T. Tingey (ed.) Assessment of crop loss from air pollutants. Proc. Int. Conf. Raleigh, NC. 25–29 Oct. 1987. Elsevier, London.

Arp, W.J. 1991. Effects of source-sink relations on photosynthetic acclimation to elevated CO_2. Plant Cell Environ. 14:869–875.

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.

Brennan, E., I. Leone, B. Greenhalgh, and G. Smith. 1987. Chlorophyll content of soybean foliage in relation to seed yield and ambient ozone pollution. J. Air Pollut. Control Assoc. 37:1429–1433.

Burton, J.W., T.E. Carter, and E.B. Huie. 1996. Registration of 'Holladay' soybean. Crop Sci. 36:467.

Cave, G., L.C. Tolley, and B.R. Strain. 1980. Effect of carbon dioxide enrichment on chlorophyll content, starch content and starch grain structure in *Trifolium subterraneum* leaves. Physiol. Plant. 51: 171–174.

Chen, J.J., and J.M. Sung. 1990. Gas exchange rate and yield responses of virginia-type peanut to carbon dioxide enrichment. Crop Sci. 30:1085–1089.

Conroy, J., and P. Hocking, 1993. Nitrogen nutrition of C_3 plants at elevated atmospheric CO_2 concentrations. Physiol. Plant. 89: 570–576.

Delucia, E.H., T.W. Sasek, and B.R. Strain. 1985. Photosynthetic inhibition after long-term exposure to elevated levels of atmospheric carbon dioxide. Photosynth. Res. 7:175–184.

Fehr, W.R., and C.E. Caviness. 1977. Stages of soybean development. Iowa State Univ. Agric. Home Econ. Exp. Stn. Spec. Rep. 80.

Fiscus, E.L., C.D. Reid, J.E. Miller, and A.S. Heagle. 1997. Elevated CO_2 reduces O_3 flux and O_3-induced yield losses in soybeans: Possible implications for elevated CO_2 studies. J. Exp. Bot. 48:307–313.

Havelka, U.D., R.C. Ackerson, M.G. Boyle, and V.A. Wittenbach. 1984a. CO_2-enrichment effects on soybean physiology. I. Effects of long-term CO_2 exposure. Crop Sci. 24:1146–1150.

Havelka, U.D., V.A. Wittenbach, and M.G. Boyle. 1984b. CO_2-enrichment effects on wheat yield and physiology. Crop Sci. 24: 1163–1168.

Heagle, A.S. 1989. Ozone and crop yield. Ann. Rev. Phytopathol. 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., J.E. Miller, W.A. Pursley, and F.L. Booker. 1998. Influ-

ence of ozone stress on soybean to 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. Phytopathol. 69:15–20.
Heath, R.L. 1988. Biochemical mechanisms of pollutant stress. *In* W.W. Heck, O.C. Taylor, and D.T. Tingey (ed.) Assessment of crop loss from air pollutants. Proc. Int. Conf. Raleigh, NC. 25–29 Oct. 1987. Elsevier, London.
Heath, R.L. 1996. Lipid metabolism and oxidant air pollutants. *In* M. Yunis and M. Iqbal (ed.) Plant response to air pollution. John Wiley & Sons, Chichester, England.
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. 1984a. Assessing impacts of ozone on agricultural crops: I. Overview. J. Air Pollut. Control Assoc. 34:729–735.
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. 1984b. Assessing impacts of ozone on agricultural crops: II. Crop yield functions and alternate exposure statistics. J. Air Pollut. Control Assoc. 34:810–817.
Heck, W.W., and J.A. Dunning. 1967. The effects of ozone on tobacco and pinto bean as conditioned by several ecological factors. J. Air Pollut. Control Assoc. 17:112–114.
Houpis, J.L., K.A. Surano, S. Cowles, and J.H. Shinn. 1988. Chlorophyll and carotenoid concentrations in two varieties of *Pinus ponderosa* seedlings subjected to long-term elevated carbon dioxide. Tree Physiol. 4:187–198.
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. For. Meteorol. 69:153–203.
Idso, S.B., B.A. Kimball, and J.R. Mauney. 1988. Effects of atmospheric CO_2 enrichment on root:shoot ratios of carrot, radish, cotton and soybean. Agric. Ecosyst. Environ. 21:293–299.
Jones, P., L.H. Allen, Jr., J.W. Jones, K.J. Boote, and W.J. Campbell. 1984. Soybean canopy growth, photosynthesis, and transpiration responses to whole-season carbon dioxide enrichment. Agron. J. 76:633–637.
Jones, P., J.W. Jones, and L.H. Allen, Jr. 1985. Carbon dioxide effects on photosynthesis and transpiration during vegetative growth in soybeans. Proc. Soil Crop Sci. Soc. Fla. 44:129–134.
Kramer, G.F., E.H. Lee, and R.A. Rowland. 1991. Effect of elevated CO_2 concentration on the polyamine levels of field-grown soybean at three O_3 regimes. Env. Pollut. 73:137–152.
Lesser, V.M., J.O Rawlings, S.E. Spruill, and M.C. Summerville. 1990. Effects of ozone on agricultural crops: Statistical methodologies and estimated dose-response relationships. Crop Sci. 30:148–155.
Lichtenthaler, H.K., and A.R. Wellburn. 1983. Determinations of total carotenoids and chlorophylls a and b of leaf extracts in different solvents. Biochem. Soc. Trans. 11:591–592.
Miller, J.E. 1988. Effects on photosynthesis, carbon allocation, and plant growth. *In* W.W. Heck, O.C. Taylor, and D.T. Tingey (ed.) Assessment of crop loss from air pollutants. Proc. Int. Conf. Raleigh, NC. 25–29 Oct. 1987. Elsevier, London.
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.
Miller, J.E., W.A. Pursley, S.F. Vozzo, and A.S. Heagle. 1991. Response of net carbon exchange rate of soybean to ozone at different stages of growth and its relation to yield. J. Environ. Qual. 20: 571–575.
Mortensen, L.M. 1990. Effects of ozone on growth of *Triticum aestivum* L. at different light, air humidity and CO_2 levels. Norwegian J. 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. Sci. Hortic. 49:17–24.
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 CO_2. Agric. Ecosyst. Environ. 38:107–118.
Pinter, P.J., Jr., S.B. Idso, D.L. Hendrix, R.R Rokey, R.S. Rauschkolb, J.R. Mauney, B.A. Kimball, G.R. Hendrey, K.F. Lewin, and J. Nagey. 1994. Effect of free-air CO_2 enrichment on the chlorophyll content of cotton leaves. Agric. For. Meteorol. 70:163–169.
Prior, S.A., and H.H. Rogers. 1995. Soybean growth response to water supply and atmospheric carbon dioxide enrichment. J. Plant Nutr. 18:617–636.
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. 109:21–432.
Reeves, D.W., H.H. Rogers, S.A. Prior, C.W. Wood, and G.B. Runion. 1994. Elevated atmospheric carbon dioxide effects on sorghum and soybean nutrient status. J. Plant Nutr. 17:1935–1954.
Reich, P.B., A.W. Schoettle, R.M. Raba, and R.G. Amundson. 1986. Response of soybean to low concentrations of ozone: I. Reductions in leaf and whole plant net photosynthesis and leaf chlorophyll content. J. Environ. Qual. 15:31–36.
Rogers, H.H., G.E. Bingham, J.D. Cure, J.M. Smith, and K.A. Surano. 1983a. Responses of selected plant species to elevated carbon dioxide in the field. J. Environ. Qual. 12:569–574.
Rogers, H.H., J.D. Cure, and J.M. Smith. 1986. Soybean growth and yield response to elevated carbon dioxide. Agric. Ecosyst. Environ. 16:113–128.
Rogers, H.H., J.D. Cure, J.R. Thomas, and J.M. Smith. 1984. Influence of elevated CO_2 on growth of soybean plants. Crop Sci. 24:361–366.
Rogers, H.H., W.W. Heck, and A.S. Heagle. 1983b. A field technique for the study of plant responses to elevated carbon dioxide concentrations. J. Air Pollut. Control Assoc. 33:42–44.
SAS Institute, 1990. SAS user's guide: Statistics. 4th ed. SAS Inst., Cary, NC.
Sionit, N., H.H. Rogers, G.E. Bingham, and B.R. Strain. 1984. Photosynthesis and stomatal conductance with CO_2-enrichment of container- and field-grown soybeans. Agron. J. 76:447–451.
Smith, T.J., and H.M. Camper. 1973. Registration of Essex soybean. Crop Sci. 13:495.
Thomas, J.F., and C.N. Harvey. 1983. Leaf anatomy of four species grown under continuous CO_2 enrichment. Bot. Gaz. 144:303–309.
Tripp, K.E., M.M. Peet, D.H. Willits, and D.M. Pharr. 1991. CO_2-enhanced foliar deformation of tomato: Relationship to foliar starch concentration. J. Am. Soc. Hortic. Sci. 116:876–880.
Vessey, J.K., L.T. Henry, and C.D. Raper, Jr. 1990. Nitrogen nutrition and temporal effects of enhanced carbon dioxide on soybean growth. Crop Sci. 30:287–294.
Vu, J.C.V., L.H. Allen, Jr., and G. Bowes. 1989. Leaf ultrastructure, carbohydrates and protein of soybeans grown under ambient CO_2 enrichment. Environ. Exp. Bot. 29:141–147.
Wulff, R.D., and B.R. Strain. 1981. Effects of CO_2 enrichment on growth and photosynthesis in *Desmodium paniculatum*. Can. J. Bot. 60:1084–1091.
Wullschleger, S.D., R.J. Norby, and D.L. Hendrix. 1992. Carbon exchange rates, chlorophyll content, and carbohydrate status of two forest tree species exposed to carbon dioxide enrichment. Tree Physiol. 10:21–31.