Comparative Responses of Container- versus Ground-Grown Soybean to Elevated Carbon Dioxide and Ozone

Fitzgerald L. Booker,* Joseph E. Miller, Edwin L. Fiscus, Walter A. Pursley, and Leonard A. Stefanski

ABSTRACT

In studies of CO_2–enrichment effects on plants, the applicability of results derived from experiments using container-grown plants for predictions of future crop performance in a CO_2–enriched atmosphere has been questioned. Concerns also have been expressed about plant growth studies with the air pollutant O_3 in pot-grown plants. Further, since elevated CO_2 and O_3 co-occur, studies are required with the combination of gases. In this 2-yr experiment, soybean [*Glycine max* (L.) Merr.] plants grown in large pots (15 and 21 L) and in the ground were exposed to mixtures of CO_2 and O_3 in open-top chambers. The CO_2 treatments were ambient and CO_2 enrichment of approximately 337 μmol mol^{-1} added 24 h d^{-1}. Ozone treatments were charcoal-filtered (CF) air (23 nmol mol^{-1}) and approximately 1.5 times ambient O_3 levels (71 nmol mol^{-1}) given 12 h d^{-1}. Relative effects of elevated CO_2 and O_3 on aboveground biomass and seed yield were quite similar for plants grown in pots compared with plants grown in the ground. Elevated CO_2 increased total seed mass and O_3 suppressed it to similar magnitudes in both rooting environments. Elevated CO_2 also reduced the toxic effects of O_3. Net photosynthesis (*A*) was similar while stomatal conductance (g_s) was higher in pot-grown compared with ground-grown plants, possibly due to better soil moisture status. The results indicated that planting density and rooting environment affected plant morphology, but relative responses of seed yield to elevated CO_2 and O_3 were not fundamentally different between soybean plants grown in large pots and in the ground in open-top chambers.

Most studies of elevated atmospheric CO_2 effects on agricultural and natural plant systems have shown that CO_2 enrichment stimulates plant growth (Ainsworth et al., 2002; Bazzaz, 1990; Cure and Acock, 1986; Drake et al., 1997; Jablonski et al., 2002; Rogers and Dahlman, 1993; Rogers et al., 1994). The degree of stimulation was often highly variable however, even with the same species or cultivar (Ainsworth et al., 2002; Fiscus et al., 2001; Kimball, 1983). In contrast, O_3 suppresses plant growth and, as with elevated CO_2, effects can vary among experiments (Heagle, 1989; Heck et al., 1983; Morgan et al., 2003). Other than differences among genotypes, causes for such variation in response may include differences in experimental protocols and plant growth environments.

In studies with CO_2 enrichment, the relevance of studies performed with container-grown plants to anticipated crop performance in a CO_2–enriched atmosphere has been questioned (Ainsworth et al., 2002; Idso and Idso, 1994; Jarvis, 1989; Lawlor and Mitchell, 1991). One concern has been that limited root volume in small pots might reduce photosynthetic capacity through carbohydrate source–sink imbalance (Arp, 1991; Thomas and Strain, 1991), and that feedback inhibition might occur to a lesser extent for plants in the ground. This possibility was supported by Idso (1999), who reported that enhanced standing biomass of several tree species grown in the ground was sustained for more than a decade by ambient plus 300 μmol CO_2 mol^{-1}, while results from container-grown tree species were highly variable. McConnaughay et al. (1993), however, showed that response to CO_2 was not always decreased by use of small pots. In their experiment, growth response to elevated CO_2 was greater in pots with high compared with low nutrient concentrations, regardless of total nutrient content or pot size. In another experiment with four annual species, Reekie and Bazzaz (1991) found that plant responses to CO_2 were not simply related to pot size. Only one study has compared plant growth and yield responses to CO_2 enrichment for plants grown in pots and plants grown in the ground (Heagle et al., 1999). In that study (Heagle et al., 1999), soybean was planted in 15-L pots and in the ground and treated with four concentrations of CO_2 in nonfiltered (NF) air in open-top field chambers. Even though the growth and final biomass of plants in the two rooting environments were somewhat different, relative growth and yield responses to elevated CO_2 were similar.

Concerns about the relevance of experiments to determine effects of O_3 with pot-grown plants also exist. Several published reports indicate little or no effect of rooting media volume on plant response to O_3, however (Heagle et al., 1979a, 1983, 1979c). Heagle et al. (1979a) found that four wheat (*Triticum aestivum* L.) cultivars had similar proportional suppression of seed yield by season-long exposure to O_3 when plants were grown in-ground or in 3.8-L pots. Also, the proportional injury and yield response of field corn (*Zea mays* L.) (Heagle et al., 1979c) and soybean (Heagle et al., 1983) to O_3 was similar with plants grown in 15-L pots or in the ground.

Because elevated CO_2 and O_3 co-occur in the troposphere, recent studies have been performed to determine effects of mixtures of these gases (reviewed in Morgan et al., 2003; Olszyk et al., 2000; Rudorff et al., 2000). Studies often showed that stimulation of growth and yield caused by CO_2 enrichment was greater when O_3 concentrations were also high (Booker et al., 2004; Fiscus et al., 1997, 2001; Heagle et al., 1998b, 2000; Mulchi et al., 1995). Field experiments with soybean

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Abbreviations: *A*, net photosynthesis; CF, charcoal-filtered; DAP, days after planting; g_s, stomatal conductance; NF, nonfiltered; PPFD, photosynthetic photon flux density.

grown in 15-L and 21-L pots revealed that twice-ambient CO_2 concentration protected plants from all O_3–induced stresses measured (Booker et al., 2004, 1997; Fiscus et al., 1997, 2002; Heagle et al., 1998a, 1998b; Miller et al., 1998; Reid and Fiscus, 1998). Research to determine whether plant culture conditions involved in container- vs. ground-grown plants affect the intensity of this elevated $CO_2 \times O_3$ interaction has not been reported for any plant species.

Our objective was to compare the effects of season-long exposure to elevated CO_2 and O_3, administered singly and in mixtures in open-top chambers, on gas exchange, aboveground growth, and yield of soybean grown in large pots and in the ground. The experiment was intended to ascertain whether results from previous elevated $CO_2 \times O_3$ experiments that used container-grown soybean plants (Booker et al., 2004, 1997; Fiscus et al., 1997; Heagle et al., 1998b; Miller et al., 1998; Reid and Fiscus, 1998) were representative of treatment responses of soybean plants grown in rows in the ground (Mulchi et al., 1995). To attain this objective, an experiment was conducted using open-top chambers during the 1999 field season to examine the effects of elevated CO_2 and O_3 on A, g_s, aboveground midseason growth, and yield of plants grown in 15-L pots and in the ground. In the 2000 field season, plants grown under similar conditions for other, related experiments provided yield data for plants grown in 21-L pots and in the ground. These latter data were included in this study to extend the database available for evaluating treatment effects.

MATERIALS AND METHODS

The experiments were performed with soybean cultivar Essex during 1999 and 2000 at a site 5 km south of Raleigh, NC. Seeds were treated with a commercial *Bradyrhizobium* preparation and planted on 26 May 1999 and 31 May 2000 in large, black plastic pots (15-L in 1999 and 21-L in 2000). The pots contained a 2:1:1 (v/v/v) mixture of sandy loam soil/sand/Metro Mix 200 (Scotts Sierra Horticultural Products Company, Marysville, OH)[1]. All pots were insulated with opaque, aluminized bubble-wrap (Reflectix, Incorporated, Markleville, IN) fit as a cylinder around the outside of each pot. Access to the soil by roots growing out of drainage holes in the pots was prevented by a sheet of black plastic covering the ground inside the open-top chambers containing potted plants. Pots were planted at four seeds per pot and were thinned to two plants per pot in mid-June and to one plant per pot in late June. In the 1999 experiment, there were 16 experimental pots per chamber, surrounded by 8 pots as borders. In the 2000 experiment, there were 13 experimental pots per chamber. After canopy closure, the potted plant densities were equivalent to 7.64 and 4.14 plants m^{-2} of ground area in the 1999 and 2000 experiments, respectively.

Seeds also treated with a commercial *Bradyrhizobium* preparation were sown in the ground on 24 May 1999 and 31 May 2000. The soil for plants in the ground was a sandy loam (Appling, kaolinitic, thermic, Typic Hapludult). Ground-grown plants were planted in rows with 1-m spacing and with plant spacing of 5.5 cm (18 plants m^{-2}) and 7.7 cm (13 plants m^{-2}) in 1999 and 2000, respectively.

Ground plots were fertilized according to soil test recommendations with 132.4 kg K ha^{-1} on 18 May 1999 and on 17 May 2000. Pots were fertilized with an aqueous solution containing 2.5 g L^{-1} of soluble fertilizer (10–30–20, N–P–K) (Peters Professional, Scotts-Sierra Horticultural Products Company) six times during the season. The initial fertilization included micronutrients at 0.31 g L^{-1} (STEM, Scotts-Sierra Horticultural Products Company). Plants were irrigated as required to prevent visible signs of water stress. Pots were irrigated with drip tubes and plants in the ground were irrigated with a soaker hose installed parallel to each row at a distance of approximately 10 cm. Total irrigation throughout the 1999 experiment was 258 L pot^{-1} and 33 cm for plants in the ground; irrigation in the 2000 experiment was 419 L pot^{-1} and 5.3 cm for plants in the ground (see Table 1 for rainfall amounts). Plots were sprayed to control insects and spider mites on 2 Aug. 1999 and on 20 June, 28 June, 21 July, and 1 Sept. 2000 with bifenthrin [(2-methyl-1,1-biphenyl-3-y1)-

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

Table 1. Average monthly and seasonal meteorological conditions, and O_3 and CO_2 concentrations. Temperature and relative humidity (RH) are daytime averages [photosynthetic photon flux density (PPFD) > 50 μmol m^{-2} s^{-1}]. Chamber CO_2 and O_3 concentrations are 12 h d^{-1} (0800–2000 h) averages.

Parameter	Year	June	July	August	September	1–16 October	Season
Temperature, °C	1999	24	28	28	22	21	25
	2000	28	28	28	24	21	26
RH, %	1999	67	68	64	75	66	68
	2000	64	70	68	73	54	66
PPFD, mol m^{-2} d^{-1}	1999	37	42	40	29	25	35
	2000	43	38	40	29	32	36
Rain, cm†	1999	5	6	8	46	1	
	2000	17	9	16	23	0	
[O_3], nmol mol^{-1}							
Charcoal-filtered air	1999	24	29	26	19	15	24
1.5 × ambient O_3		70	77	91	66	59	75
Charcoal-filtered air	2000	29	24	20	14	20	22
1.5 × ambient O_3		66	72	65	62	68	67
[CO_2], μmol mol^{-1}							
Ambient	1999	380	366	370	372	386	373
Elevated		659	703	737	689	688	699
High		822	896	946	890	928	899
Ambient	2000	368	361	365	373	376	369
Elevated		700	754	730	714	686	717

† Seasonal total irrigation for plants in pots was 258 and 419 L pot^{-1} in 1999 and 2000, respectively. Seasonal total irrigation for plants in the ground was equivalent to approximately 33 and 5 cm of rain in 1999 and 2000, respectively.

methyl-3-(2-chloro-3,3,3-trifluoro-1-propenyl)-2,2-dimethylcyclopropanecarboxylate] (Whitmire Micro-Gen Research Laboratories, Incorporated, St. Louis, MO) at 2.6 mL L^{-1} water and abamectin (avermectin B_1) (Syngenta Crop Protection, Incorporated, Greensboro, NC) at 0.32 mL L^{-1} water.

Plants were exposed to mixtures of CO_2 and O_3 in cylindrical open-top chambers, 3 m diameter × 2.4 m tall. Gas dispensing and monitoring were conducted as described for CO_2 (Rogers et al., 1983) and O_3 (Heagle et al., 1979b). Carbon dioxide was monitored at canopy height with infrared analyzers (Model 6252, Li-Cor, Incorporated, Lincoln, NE), and O_3 was monitored at canopy height with UV analyzers (Model 49, Thermo Environmental Instruments, Incorporated, Franklin, MA). The CO_2 and O_3 analyzers were calibrated once every 2 wk.

The experimental design consisted of all combinations of two CO_2 treatments and two O_3 treatments. There were three replicate chambers for each rooting environment × CO_2 × O_3 combination in the 1999 experiment ($n = 24$). In the 2000 experiment, there were three replicate chambers for each in-ground × CO_2 × O_3 treatment combination but only two replicate chambers for each pot-grown × CO_2 × O_3 combination ($n = 20$). The CO_2 treatments were ambient (no CO_2 addition) and CO_2 enrichment of approximately 337 μmol mol^{-1} 24 h d^{-1} (Table 1). In the 1999 experiment, three additional chambers were included to test the effects of a higher CO_2 addition, approximately 530 μmol mol^{-1} added to ambient. Ozone treatments were CF air and NF air plus approximately 1.5 times ambient O_3 given 12 h d^{-1} (0800–2000 h) (Table 1). Both CO_2 and O_3 treatments were administered 7 d per week. The treatments began in mid-June and continued until mid-October, when plants in all treatments were at physiological maturity.

In the 1999 experiment, four pots per chamber and eight plants (four plants from each row) per chamber in the ground-grown plots were sampled for aboveground midseason biomass at 98 to 102 d after planting (DAP). The number and dry mass of leaves, stems, branches, and pods were measured. At 162 to 164 DAP in the 1999 experiment, the remaining 12 pots and two 80-cm row sections in each of two rows were harvested for yield measurements. At 146 to 149 DAP in the 2000 experiment, five pots and two 100-cm row sections in each of two rows were harvested for yield. At the two yield harvests, the number and dry mass of stems, branches, pods, and seeds were determined. Developmental stage was determined during reproductive development according to Fehr and Caviness (1977).

Net photosynthesis was measured at growth CO_2 and O_3 conditions on seven occasions during reproductive development with a portable photosynthesis system (Model 6200, Li-Cor, Incorporated) in the 1999 experiment. Measurements were made on the center leaflet of nonshaded main stem leaves at the second or third main stem node below the apex. Three plants were measured in each of two replicate chambers for each root environment × CO_2 × O_3 treatment combination. Measurements were made between 1000 and 1300 h when ambient photosynthetic photon flux density (PPFD) > 1000 μmol m^{-2} s^{-1}. Midday leaf conductance was measured on 22 occasions during reproductive development in the 1999 experiment on the abaxial and adaxial surfaces of upper canopy leaves with a steady state porometer (Model 1600M, Li-Cor, Incorporated) when weather conditions permitted (no precipitation after sundown on the previous day and PPFD > 800 μmol m^{-2} s^{-1}). Four plants were measured in each of two replicate chambers for each treatment combination. Leaf conductance measurements were corrected for the standard boundary layer conductance imposed by the instrument (2.7 mol m^{-2} s^{-1}, Li-Cor, Incorporated, 1600M Instruction Manual, Revision 6, 1989), and reported as g_s.

Analysis of variance was performed on the chamber means of all interim and final harvest variables for main effects and interactions of CO_2 and O_3 treatments using a completely randomized model (SAS Institute, 2001). Treatment effects and means for periodically measured plant response variables (reproductive stage, A, and g_s) were estimated using a repeated measures model in which chambers constituted the whole plots and sampling period was the repeated factor (SAS Proc Mixed) (Littell et al., 1996). The model included interactions between the whole plot factors and the effect of sampling period.

Previous analysis showed that the pot and ground response functions were not equal (Heagle et al., 1999), but this was expected because sampling units differed (one plant per pot compared with 14 to 17 plants sampled per meter of row in the ground). Thus, direct comparison of some response functions of pot-grown and ground-grown plants was not possible. However, if elevated CO_2, O_3, or their interaction had the same effect on pot- and ground-grown plants, then the two response functions would differ only by a constant. This null hypothesis is equivalent to the hypothesis that the relative changes in response between levels of CO_2 or O_3 at x and $x + \Delta$ are equal for both pot and ground data; that is,

$$[m_g(x + \Delta) - m_g(x)]/m_g(x) = [m_p(x + \Delta) - m_p(x)]/m_p(x)$$

where m_g and m_p denote the mean response functions for ground and pot data, respectively. This is a nonlinear statistical hypothesis. An F statistic for testing proportionality is obtained from the mean squared errors from the fit of full (no proportionality constraints) and reduced (proportionality constraints enforced) models (Bates and Watts, 1988). Tests of proportionality were performed on midseason biomass measurements obtained from the 1999 field season and on yield measurements from the 1999 and 2000 field seasons.

RESULTS

Environmental Conditions

The 1999 field season was generally hot and dry during June through August, but wet and cooler in September (Table 1). The 2000 field season was also hot, wetter during June and August, but drier in September 2000 than in 1999. Ozone concentrations were typical of the area, and the O_3 additions were 1.5 times (±1%) the average ambient concentration of 50 nmol mol^{-1}. Mean ambient CO_2 concentration during the experiment was 371 μmol mol^{-1}, and the elevated CO_2 treatment concentration averaged 708 μmol mol^{-1} (±0.5%) (Table 1). The additional high CO_2 treatment concentration averaged 899 μmol mol^{-1} (pot-grown plants only).

Growth

By 102 DAP in the 1999 experiment, aboveground vegetative and reproductive biomasses generally were enhanced by elevated CO_2 and suppressed by O_3 compared with controls (Table 2). Pot-grown plants were 15% shorter than plants grown in the ground, but had much greater aerial biomass, which was mainly due to greater production of branches and pods. Despite these differences, the proportional responses to O_3 and CO_2

Table 2. Growth and biomass at 98 to 102 d after planting of soybean exposed to mixtures of CO_2 and O_3 when grown in the ground and in 15-L pots in the 1999 experiment. Treatments were charcoal-filtered (CF) air-ambient CO_2 (CF-373), CF air-elevated CO_2 (CF-699), nonfiltered (NF) air plus O_3–ambient CO_2 (OZ-373), and NF air plus O_3–elevated CO_2 (OZ-699). Results from an additional CF air-high CO_2 treatment (CF-899) are also shown. Values are expressed on a per plant basis and are means ± SE of three replicate chambers for each treatment combination. Values in parentheses indicate percentage of CF-373 treatment for each rooting environment.

Rooting environment	Treatment	df	Height	Branch number	V stage	Pod number	Main leaves	Branch leaves	Main stem	Branch stem	Pods	Total above ground biomass
			cm				g					
Ground	CF-373		74 ± 2 (100%)	9 ± 1 (100%)	15 ± 1	138 ± 12 (100%)	12 ± 1 (100%)	8 ± 1 (100%)	15 ± 1 (100%)	12 ± 2 (100%)	31 ± 2 (100%)	78 ± 6 (100%)
	CF-699		77 ± 2 (104%)	11 ± 1 (122%)	15 ± 1	195 ± 12 (141%)	13 ± 1 (108%)	12 ± 1 (150%)	20 ± 1 (133%)	21 ± 2 (175%)	44 ± 2 (142%)	110 ± 6 (141%)
	OZ-373		67 ± 2 (90%)	8 ± 1 (89%)	14 ± 1	115 ± 12 (83%)	9 ± 1 (75%)	7 ± 1 (88%)	12 ± 1 (80%)	10 ± 2 (83%)	26 ± 2 (84%)	63 ± 6 (81%)
	OZ-699		75 ± 2 (101%)	11 ± 1 (122%)	15 ± 1	168 ± 12 (122%)	11 ± 1 (92%)	9 ± 1 (112%)	18 ± 1 (120%)	16 ± 2 (133%)	39 ± 2 (126%)	94 ± 6 (120%)
	source											
	CO_2	1	**	*	NS	**	*	*	***	***	***	***
	O_3	1	*	NS	NS	NS	**	NS	**	NS	*	*
	$CO_2 \times O_3$	1	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Pots	CF-373		61 ± 1 (100%)	18 ± 1 (100%)	16 ± 1	340 ± 15 (100%)	13 ± 1 (100%)	47 ± 2 (100%)	25 ± 1 (100%)	67 ± 4 (100%)	70 ± 4 (100%)	257 ± 8 (100%)
	CF-699		66 ± 1 (108%)	16 ± 1 (89%)	16 ± 1	385 ± 15 (113%)	17 ± 1 (131%)	51 ± 2 (108%)	31 ± 1 (124%)	81 ± 4 (121%)	90 ± 4 (128%)	285 ± 8 (111%)
	OZ-373		59 ± 1 (97%)	17 ± 1 (94%)	16 ± 1	315 ± 15 (93%)	11 ± 1 (85%)	35 ± 2 (75%)	21 ± 1 (84%)	56 ± 4 (84%)	64 ± 4 (91%)	189 ± 8 (74%)
	OZ-699		68 ± 1 (112%)	18 ± 1 (100%)	16 ± 1	411 ± 15 (121%)	14 ± 1 (108%)	55 ± 2 (117%)	33 ± 1 (132%)	95 ± 4 (142%)	96 ± 4 (137%)	293 ± 8 (114%)
	CF-899		66 ± 2 (108%)	17 ± 1 (94%)	16 ± 1	416 ± 5 (122%)	17 ± 1 (131%)	60 ± 2 (128%)	33 ± 2 (132%)	97 ± 4 (145%)	98 ± 2 (140%)	305 ± 7 (119%)
	source†											
	CO_2	1	***	NS	NS	**	**	***	***	***	***	***
	O_3	1	NS	NS	NS	NS	**	NS	NS	NS	NS	**
	$CO_2 \times O_3$	1	*	**	NS	NS	NS	**	**	*	NS	**
					Significance of test to reject proportionality of response for ground-grown and pot-grown plants, $P > F$‡							
			NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

* $P \leq 0.05$.
** $P \leq 0.01$.
*** $P \leq 0.001$.
NS, not significant at $P \leq 0.05$.
† Does not include CF-899 treatment.
‡ Test of the null hypothesis that the relative changes in response between treatments are equal in pots and ground data. NS implies that the response is proportional.

were remarkably similar, with some exceptions. Elevated CO_2 increased height, pod number, and all aboveground biomass variables in both ground- and pot-grown plants, while branch number was increased only in ground-grown plants. The O_3 treatment suppressed plant height and biomass of main stem leaves, stems, and pods of ground-grown plants, but only main stem leaf biomass was suppressed in pot-grown plants. Statistically significant $O_3 \times CO_2$ interactions were found only with pot-grown plants, in which case elevated CO_2 prevented the effects of O_3. In several cases, biomass was greater in the combined elevated CO_2 and O_3 treatments than in elevated CO_2 alone.

Early reproductive development in ground-grown plants was several days ahead of pot-grown plants, although by 69 DAP, plants in both rooting environments were at the R2 stage of development (Fig. 1, Table 3). Rooting environment effects were not statistically significant during the remainder of the experiment ($P >$ 0.05). Reproductive growth was accelerated by treatment with O_3 during stage R6 to R8 (Fig. 1). Elevated CO_2 partially suppressed this O_3 effect. The timing of late reproductive development and effect of O_3 were similar in the pot-grown and ground-grown plants.

Aboveground biomass partitioning was not extensively affected by elevated CO_2 or O_3 in either rooting environment (Table 4). However, with ground-grown plants, elevated CO_2 increased partitioning of biomass to branch stems compared with controls at the expense of main stem leaves. In pot-grown plants, O_3 increased partitioning to branch stem and pods.

Tests of proportionality supported the conclusion that plants grown in pots responded similarly to O_3 and CO_2 treatments compared with plants grown in the ground (i.e., the test to reject the null hypothesis of proportionality of response was not statistically significant for any plant growth variable) (Table 2).

Table 3. Probabilities of rooting environment, CO_2 and O_3 treatment effects on developmental stage (R-stage), net photosynthesis (*A*), and stomatal conductance (g_s) of plants grown in the ground or in 15-L pots between 52 and 147 d after planting (DAP) in the 1999 experiment.†

Effect	df	R-stage	df	*A*	df	g_s
Root environment	1	***	1	**	1	***
CO_2	1	NS‡	1	***	1	***
O_3	1	***	1	***	1	**
Root environment × CO_2	1	NS	1	**	1	**
Root environment × O_3	1	NS	1	NS	1	NS
$CO_2 \times O_3$	1	**	1	**	1	NS
Root environment × $CO_2 \times O_3$	1	NS	1	NS	1	NS
DAP	13	**	6	***	6	***
DAP × root environment	13	**	6	***	6	***
DAP × CO_2	13	NS	6	*	6	**
DAP × O_3	13	**	6	**	6	NS
DAP × $CO_2 \times O_3$	13	NS	6	NS	6	NS

* $P \leq 0.05$.
** $P \leq 0.01$.
*** $P \leq 0.001$.
† Does not include CF-899 treatment.
‡ NS, not significant at $P \leq 0.05$.

Harvest

The relative effects of elevated O_3 and CO_2 on yield (total seed mass) at final harvest were similar for plants grown in pots compared with plants grown in the ground in both years of the experiment (Tables 5 and 6; Fig. 2). Elevated CO_2 increased total seed mass while O_3 suppressed it compared with the control. Seed yield was increased 24% by elevated CO_2 in both pot-grown and ground-grown plants. Added O_3 lowered yield by 26% in 1999 and by 40% in 2000 for plants in both rooting environments, while yield increases of 15% occurred in the combined gas treatments. Increased yield with elevated CO_2 was primarily due to increased pod numbers in both pot- and ground-grown plants (Tables 5, 6). In elevated CO_2, mass per seed actually decreased slightly in ground-grown plants in both 1999 and 2000. Mass per seed was lower in 1999 but higher in 2000 in pot-grown plants treated with elevated CO_2. Elevated

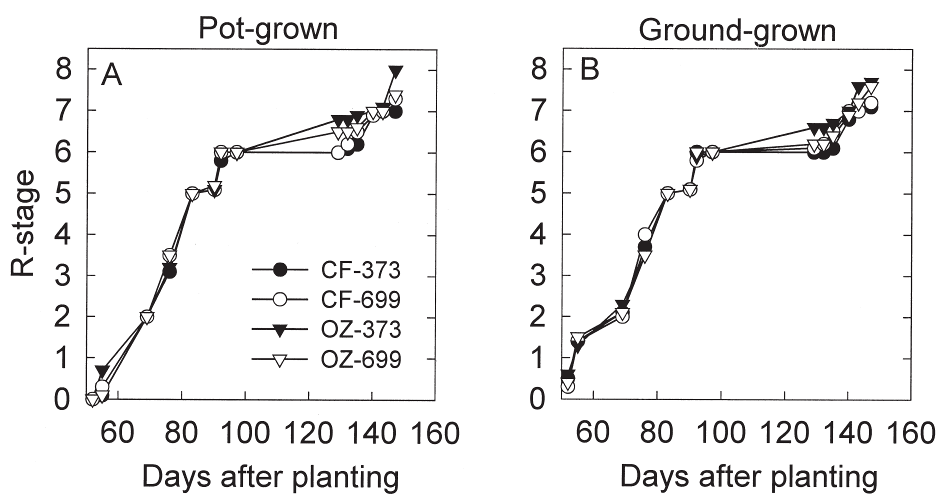

Fig. 1. Reproductive developmental stage (R-stage) for (A) pot-grown and (B) ground-grown Essex soybean treated with combinations of CO_2 and O_3 concentrations in the 1999 experiment. Values are means from three replicate chambers per treatment combination. Ozone treatments were charcoal-filtered (CF) air (24 nmol O_3 mol^{-1}) and nonfiltered (NF) air plus O_3 (75 nmol O_3 mol^{-1}). Carbon dioxide treatments were ambient (373 μmol CO_2 mol^{-1}) and elevated (699 μmol CO_2 mol^{-1}). Treatments were CF air-ambient CO_2 (CF-373), CF air-elevated CO_2 (CF-699), NF air plus O_3–ambient CO_2 (OZ-373), and NF air plus O_3–elevated CO_2 (OZ-699).

Table 4. Partitioning of biomass among organs (organ biomass/total aboveground biomass) of soybean at 98 to 102 d after planting as influenced by rooting environment, CO_2, and O_3 in the 1999 experiment. Treatments were charcoal-filtered (CF) air-ambient CO_2 (CF-373), CF air-elevated CO_2 (CF-699), nonfiltered (NF) air plus O_3–ambient CO_2 (OZ-373), and NF air plus O_3–elevated CO_2 (OZ-699). Results from an additional treatment with CF air-high CO_2 (CF-899) are also shown. Values are expressed on a per plant basis and are means ± SE of three replicate chambers for each treatment combination.

Rooting environment	Treatment	df	Main stem leaf	Branch leaf	Main stem	Branch stem	Pods
Ground	CF-373		0.15 ± 0.01	0.11 ± 0.01	0.20 ± 0.01	0.15 ± 0.01	0.40 ± 0.01
	CF-699		0.11 ± 0.01	0.11 ± 0.01	0.18 ± 0.01	0.19 ± 0.01	0.40 ± 0.01
	OZ-373		0.14 ± 0.01	0.10 ± 0.01	0.20 ± 0.01	0.15 ± 0.01	0.41 ± 0.01
	OZ-699		0.12 ± 0.01	0.10 ± 0.01	0.19 ± 0.01	0.18 ± 0.01	0.41 ± 0.01
	source						
	CO_2	1	**	NS	NS	*	NS
	O_3	1	NS	NS	NS	NS	NS
	$CO_2 \times O_3$	1	NS	NS	NS	NS	NS
Pots	CF-373		0.05 ± 0.01	0.18 ± 0.01	0.10 ± 0.01	0.26 ± 0.01	0.27 ± 0.02
	CF-699		0.06 ± 0.01	0.18 ± 0.01	0.11 ± 0.01	0.29 ± 0.01	0.32 ± 0.02
	OZ-373		0.05 ± 0.01	0.19 ± 0.01	0.11 ± 0.01	0.30 ± 0.01	0.34 ± 0.02
	OZ-699		0.05 ± 0.01	0.19 ± 0.01	0.11 ± 0.01	0.32 ± 0.01	0.33 ± 0.02
	CF-899		0.06 ± 0.01	0.20 ± 0.01	0.11 ± 0.01	0.32 ± 0.01	0.32 ± 0.01
	source†						
	CO_2	1	NS	NS	NS	NS	NS
	O_3	1	NS	NS	NS	*	*
	$CO_2 \times O_3$	1	*	NS	NS	NS	NS

* $P \leq 0.05$.
** $P \leq 0.01$.
NS, not significant at $P \leq 0.05$.
† Does not include CF-899 treatment.

CO_2 increased stem mass in both pot- and ground-grown plants by about 40% overall, which was greater than the effects on seed biomass and seed:stem mass ratios. The effect of elevated O_3 on seed biomass was due to reductions in pod numbers and mass per seed in both rooting environments in 1999 and 2000 (Tables 5 and 6). Overall, seeds per pod were not strongly affected by O_3. Statistically significant $O_3 \times CO_2$ interactions were found mostly with pot-grown plants in 1999, but they occurred in both pot-grown and ground-grown plants in 2000. In each instance, elevated CO_2 partially prevented the injurious effects of O_3.

Again, tests of proportionality supported the conclusion that plants grown in pots responded similarly to O_3 and CO_2 treatments compared with plants grown in the ground (Tables 5, 6). Seed yield on an areal basis also indicated that the pattern of responses to the treatment gas combinations was similar between plants grown in large pots and in the ground (Table 7). Most of the rooting environment × gas treatment interactions were not statistically significant. The rooting environment × CO_2 interaction in the 1999 experiment was statistically significant because the yield increase in plants treated with elevated CO_2 was 3% higher in pot-grown compared with ground-grown plants.

The possibility that CO_2 concentrations higher than those typically used in field experiments would lead to even greater increases in growth was tested with CF air-treated plants grown in pots. Treatment with 899 μmol CO_2 mol^{-1} did not promote additional growth or yield beyond that caused by treatment with 699 μmol CO_2 mol^{-1}. In fact, a general suppression of growth occurred (Table 5).

Photosynthesis and Stomatal Conductance

The A and g_s of upper canopy leaves were measured periodically during reproductive growth in the 1999 experiment (Table 3; Fig. 3 and 4). On average, A was 7% higher in pot-grown plants compared with ground-grown plants. The highest A values attained during the measurement period were of similar magnitude for plants in the two rooting environments, although seasonal patterns were slightly different; i.e., peak A in ambient CO_2–treated plants was reached later in the growing season in ground-grown compared with pot-grown plants. In the CF-699 treatment, average A for all measurement dates combined was not different in pot-grown compared with ground-grown plants (30.3 ± 0.4 and 29.8 ± 0.9 μmol m^{-2} s^{-1}, respectively), although on a relative basis, A in the CF-699 treatment was higher in ground-grown plants (Table 3; Fig. 3). Ozone generally suppressed A of plants grown in both rooting environments, although the O_3 effect did not occur until 93 DAP in the ground-grown plants (Table 3; Fig. 3). Net photosynthesis in the OZ-699 treatment was similar to A in the CF-699 treatment in both rooting environments (Table 3; Fig. 3).

Stomatal conductance was 33% higher in potted plants compared with ground-grown plants (Table 3; Fig. 4). Elevated CO_2 suppressed g_s compared with plants grown at ambient CO_2 in both rooting environments, although the effect was more pronounced in pot-grown plants. Added O_3 lowered average g_s for pot-grown plants by 18%, whereas g_s in ground-grown plants was about equal in the CF and O_3 treatments in ambient CO_2 (Fig. 4). Stomatal conductance was quite low in plants grown in the CF-899 treatment compared with the other CO_2–added treatments, even though A was similar among all elevated CO_2 treatments.

DISCUSSION

It has been suggested that A, and thus plant growth response to elevated CO_2, would be limited in pot-grown

Table 5. Final yield at 162 to 164 d after planting for soybean exposed to mixtures of CO_2 and O_3 when grown in the ground and in 15-L pots in the 1999 experiment. Treatments were charcoal-filtered (CF) air-ambient CO_2 (CF-373), CF air-elevated CO_2 (CF-699), nonfiltered (NF) air plus O_3–ambient CO_2 (OZ-373), and NF air plus O_3–elevated CO_2 (OZ-699). Results from an additional treatment with CF air-high CO_2 (CF-899) are also shown. Values for ground-grown plants are based on an 80-cm row section while values for pot-grown plants are expressed on a per plant basis. Values are means ± SE of three replicate chambers for each treatment combination. Values in parentheses indicate percentage of CF-373 treatment for each rooting environment.

Rooting environment	Treatment	Pod number	Pod mass	Total seed mass	Mass 100 seed^{-1}	Seed number	Seed number/pod number	Seed mass/pod mass	Total stem mass	Seed mass/stem mass
			g	g	g				g	
Ground	CF-373	1570 ± 48 (100%)	741 ± 29 (100%)	548 ± 20 (100%)	17 ± 1	3271 ± 105 (100%)	2.08 ± 0.01	0.74 ± 0.01	177 ± 9 (100%)	3.1 ± 0.1
	CF-699	2146 ± 48 (137%)	930 ± 29 (126%)	678 ± 20 (124%)	16 ± 1	4338 ± 105 (133%)	2.02 ± 0.01	0.73 ± 0.01	247 ± 9 (140%)	2.7 ± 0.1
	OZ-373	1429 ± 48 (91%)	570 ± 29 (77%)	417 ± 20 (76%)	14 ± 1	2898 ± 105 (89%)	2.03 ± 0.01	0.73 ± 0.01	126 ± 9 (71%)	3.3 ± 0.1
	OZ-699	2139 ± 48 (136%)	876 ± 29 (118%)	629 ± 20 (115%)	15 ± 1	4230 ± 105 (129%)	1.99 ± 0.01	0.72 ± 0.01	230 ± 9 (130%)	2.7 ± 0.1
	source									
	CO_2	***	***	***	NS‡	***	**	***	***	***
	O_3	NS	**	**	***	*	*	*	**	NS
	$CO_2 \times O_3$	NS	NS	NS	*	NS	NS	NS	NS	NS
Pots	CF-373	339 ± 7 (100%)	174 ± 3 (100%)	125 ± 2 (100%)	18 ± 1	713 ± 19 (100%)	2.10 ± 0.06	0.72 ± 0.01	41 ± 1 (100%)	3.1 ± 0.1
	CF-699	420 ± 7 (124%)	221 ± 3 (127%)	155 ± 2 (124%)	18 ± 1	892 ± 19 (125%)	2.13 ± 0.06	0.70 ± 0.01	59 ± 1 (144%)	2.6 ± 0.1
	OZ-373	298 ± 7 (88%)	130 ± 3 (75%)	91 ± 2 (73%)	14 ± 1	629 ± 19 (88%)	2.12 ± 0.06	0.70 ± 0.01	30 ± 1 (73%)	3.1 ± 0.1
	OZ-699	407 ± 7 (120%)	205 ± 3 (118%)	144 ± 2 (115%)	16 ± 1	882 ± 19 (124%)	2.17 ± 0.06	0.71 ± 0.01	52 ± 1 (127%)	2.8 ± 0.1
	CF-899	411 ± 10 (121%)	217 ± 8 (125%)	150 ± 6 (120%)	18 ± 1	850 ± 33 (119%)	2.07 ± 0.02	0.69 ± 0.01	59 ± 2 (144%)	2.5 ± 0.2
	source†									
	CO_2	***	***	***	**	***	NS	*	***	***
	O_3	**	***	***	***	*	NS	**	***	NS
	$CO_2 \times O_3$	NS	**	***	**	NS	NS	**	NS	NS
			Significance of test to reject proportionality of response for ground-grown and pot-grown plants, $P > F$‡							
		NS	NS	NS	*	NS	*	NS	NS	NS

* $P \leq 0.05$.
** $P \leq 0.01$.
*** $P \leq 0.001$.
NS, not significant at $P \leq 0.05$.
† Does not include CF-899 treatment.
‡ Test of the null hypothesis that the relative changes in response between treatments are equal in pots and ground data. NS implies that the response is proportional.

Table 6. Final yield at 146 to 149 d after planting for soybean exposed to mixtures of CO_2 and O_3 when grown in the ground and in 21-L pots in the 2000 experiment. Treatments were charcoal-filtered (CF) air-ambient CO_2 (CF-369), CF air-elevated CO_2 (CF-717), nonfiltered (NF) air plus O_3–ambient CO_2 (OZ-369), and NF air plus O_3–elevated CO_2 (OZ-717). Values for ground-grown plants are based on an 100-cm row section while values for pot-grown plants are expressed on a per plant basis. Values are means ± SE of three replicate chambers for each in-ground treatment combination, and two replicate chambers for each pot-grown treatment combination. Values in parentheses indicate percentage of CF-369 treatment for each rooting environment.

Rooting environment	Treatment	Pod number	Pod mass	Total seed mass	Mass 100 seed^{-1}	Seed number	Seed number/pod number	Seed mass/pod mass	Total stem mass	Seed mass/stem mass
			g						g	
Ground	CF-369	1973 ± 119 (100%)	868 ± 51 (100%)	621 ± 36 (100%)	16 ± 1	3878 ± 205 (100%)	1.97 ± 0.04	0.72 ± 0.01	285 ± 12 (100%)	2.2 ± 0.1
	CF-717	2353 ± 119 (119%)	1042 ± 51 (120%)	756 ± 36 (122%)	15 ± 1	4878 ± 205 (126%)	2.07 ± 0.04	0.73 ± 0.01	375 ± 12 (132%)	2.0 ± 0.1
	OZ-369	1457 ± 119 (74%)	528 ± 51 (61%)	379 ± 36 (61%)	12 ± 1	3041 ± 205 (78%)	2.11 ± 0.04	0.72 ± 0.01	146 ± 12 (51%)	2.6 ± 0.1
	OZ-717	2258 ± 119 (114%)	942 ± 51 (108%)	676 ± 36 (109%)	15 ± 1	4587 ± 205 (118%)	2.04 ± 0.04	0.72 ± 0.01	326 ± 12 (114%)	2.1 ± 0.1
	source									
	CO_2	***	***	***	*	***	NS	NS	***	**
	O_3	*	**	**	***	*	NS	NS	***	*
	$CO_2 \times O_3$	NS	*	*	***	NS	NS	NS	**	NS
Pots	CF-369	412 ± 8 (100%)	198 ± 12 (100%)	139 ± 11 (100%)	17 ± 1	815 ± 18 (100%)	1.98 ± 0.02	0.70 ± 0.01	49 ± 5 (100%)	2.9 ± 0.2
	CF-717	448 ± 8 (109%)	247 ± 12 (125%)	175 ± 11 (126%)	19 ± 1	927 ± 18 (114%)	2.08 ± 0.02	0.70 ± 0.01	77 ± 5 (157%)	2.3 ± 0.2
	OZ-369	310 ± 8 (75%)	123 ± 12 (62%)	82 ± 11 (59%)	14 ± 1	604 ± 18 (74%)	1.96 ± 0.02	0.66 ± 0.01	28 ± 5 (57%)	2.9 ± 0.2
	OZ-717	480 ± 8 (116%)	238 ± 12 (120%)	164 ± 11 (118%)	18 ± 1	918 ± 18 (113%)	1.92 ± 0.02	0.69 ± 0.01	71 ± 5 (145%)	2.3 ± 0.2
	source									
	CO_2	***	**	**	*	***	NS	NS	**	*
	O_3	**	*	*	NS	**	*	NS	*	NS
	$CO_2 \times O_3$	***	*	NS	NS	**	NS	NS	NS	NS
			Significance of test to reject proportionality of response for ground-grown and pot-grown plants, $P > F$†							
		NS	NS	NS	NS	NS	NS	NS	NS	NS

* $P \leq 0.05$.
** $P \leq 0.01$.
*** $P \leq 0.001$.
NS, not significant at $P \leq 0.05$.
† Test of the null hypothesis that the relative changes in response between treatments are equal in pots and ground data. NS implies that the response is proportional.

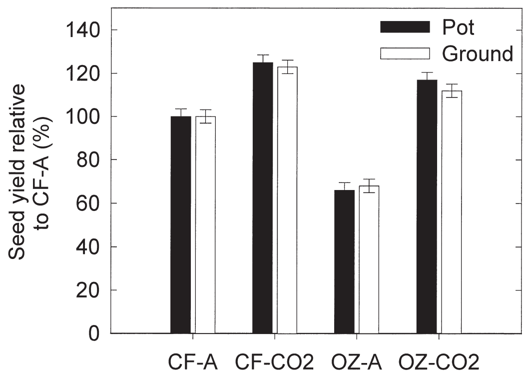

Fig. 2. Relative seed yields combined for both years of the experiment (1999 and 2000) for Essex soybean grown in large pots or in the ground. Plants were treated with combinations of CO_2 and O_3 concentrations throughout the two growing seasons. Ozone treatments were charcoal-filtered (CF) air (22 to 24 nmol O_3 mol^{-1}) and nonfiltered (NF) air plus O_3 (67 to 75 nmol O_3 mol^{-1}). Carbon dioxide treatments were ambient (369 to 373 μmol CO_2 mol^{-1}) and elevated (699 to 717 μmol CO_2 mol^{-1}). Treatments were CF air-ambient CO_2 (CF-A), CF air-elevated CO_2 (CF-CO2), NF air plus O_3–ambient CO_2 (OZ-A), and NF air plus O_3–elevated CO_2 (OZ-CO2). Relative treatment effects on seed yield for pot-grown and ground-grown plants are shown as a percentage of their respective control treatment (CF-A). Values are means ± SE.

plants by photosynthetic feedback due to inadequate sink size (Arp, 1991; Thomas and Strain, 1991). McConnaughay et al. (1996) pointed out, however, that many studies on sink size confound the effects of limited nutrient supply and limited root volume. Factors that principally control sink activity—soil moisture, mineral nutrition, and developmental stage—might be more important influences on plant growth than the rooting environments considered here. In analyzing elevated CO_2 studies on trees for effects of pot size on gas-exchange, Curtis (1996) found that pot size was often confounded with both the duration of CO_2 exposure period and treatment facility used. However, in long-term (>50 d) studies conducted in open-top chambers, the effect of elevated CO_2 on *A* was greater in plants grown in 10- to 25-L pots compared with plants grown in-ground, whereas rooting environment had no effect on the decrease in g_s induced by elevated CO_2 in unstressed plants (Curtis, 1996). Concern about container- versus ground-grown plants might be better directed toward evaluation of water supply, nutrient availability, root biomass/root volume relationships, planting density, PPFD, temperature, and exposure system used. As demonstrated in our study, when culture conditions for plant growth were optimized as much as possible for an experiment conducted in open-top field chambers, yield responses to the various treatments were similar between pot- and ground-grown soybean (Fig. 2). Values of *A* in the elevated CO_2 treatments were close in pot-grown and ground-grown plants (Fig. 3), suggesting that photosynthetic gas exchange responses to elevated CO_2 were similar in plants in the two rooting environments. However, the higher g_s in potted plants compared with ground-grown plants may reflect a more favorable water status in pot-grown plants. The higher g_s might also have been a factor involved in the suppression of *A* by O_3 in the pot-grown plants that occurred earlier in the growing season than in ground-grown plants. The higher g_s would increase O_3 uptake by the leaves with greater subsequent injury (Fiscus et al., 1997; McKee et al., 1997).

There were distinct differences in morphology of the pot-grown and ground-grown plants. Although pot-grown plants were slightly shorter than ground-grown plants, they had a nearly identical number of main stem nodes. Branch counts and total biomass were much greater on pot-grown plants. At about 100 DAP (early R6), the pot-grown plants had more than twice the biomass of ground-grown plants, although planting density of pot-grown plants was less than half that of ground-grown plants (Table 2). Calculations of biomass partitioned to leaves, stems, and pods illustrated that about twice as much biomass was partitioned to branches in pot-grown vs. ground-grown plants, with lesser amounts in main stems and main stem leaves. At final harvest (R8), however, the seed-to-stem ratio was similar for plants grown in the two rooting environments in the 1999 experiment, while ratios were more variable in the 2000 experiment (Tables 5, 6).

Table 7. Effect of rooting environment on yield responses to elevated CO_2 and O_3 on an areal basis. Values are means ± SE. Treatments were charcoal-filtered (CF) air-ambient CO_2 (CF-A), CF air-elevated CO_2 (CF-CO_2), nonfiltered (NF) air plus O_3–ambient CO_2 (OZ-A), and NF air plus O_3–elevated CO_2 (OZ-CO_2).

	Rooting environment	Treatment	Seed yield
			g m^{-2}
	1999		
	ground	CF-A	685 ± 22
		CF-CO_2	847 ± 22
		OZ-A	521 ± 22
		OZ-CO_2	786 ± 22
	pot	CF-A	958 ± 22
		CF-CO_2	1186 ± 22
		OZ-A	695 ± 22
		OZ-CO_2	1103 ± 22
Source			
Rooting environment			***
CO_2			***
O_3			***
$CO_2 \times O_3$			***
Rooting environment × CO_2			**
Rooting environment × O_3			NS
Rooting environment × $CO_2 \times O_3$			NS
	2000		
	ground	CF-A	621 ± 36
		CF-CO_2	756 ± 36
		OZ-A	379 ± 36
		OZ-CO_2	676 ± 36
	pot	CF-A	574 ± 44
		CF-CO_2	723 ± 44
		OZ-A	338 ± 44
		OZ-CO_2	679 ± 44
Source			
Rooting environment			NS
CO_2			***
O_3			***
$CO_2 \times O_3$			**
Rooting environment × CO_2			NS
Rooting environment × O_3			NS
Rooting environment × $CO_2 \times O_3$			NS

* $P \leq 0.05$.
** $P \leq 0.01$.
*** $P \leq 0.001$.
NS, not significant at $P \leq 0.05$.

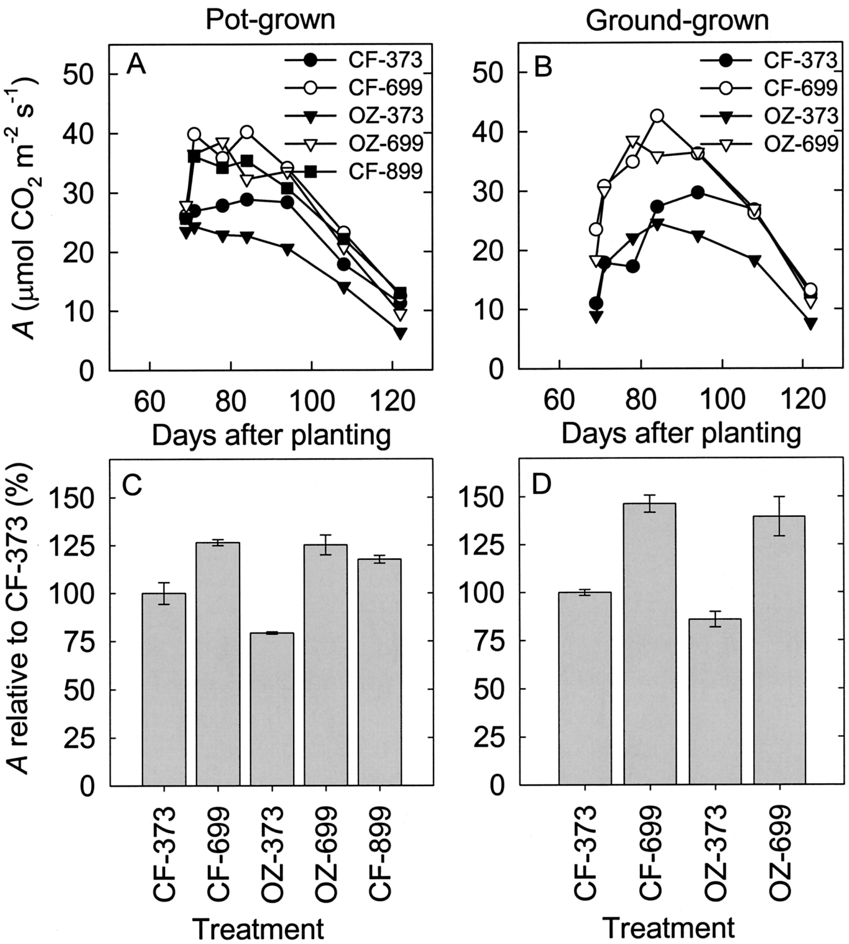

Fig. 3. Net photosynthesis (*A*) of upper-canopy leaves of pot-grown (A) and ground-grown plants (B) exposed to combinations of CO_2 and O_3 concentrations throughout the growing season in the 1999 experiment. Relative treatment effects on average *A* are shown as percentage of control (CF-373) in pot-grown (C) and ground-grown plants (D). Ozone treatments were charcoal-filtered (CF) air (24 nmol O_3 mol^{-1}) and nonfiltered (NF) air plus O_3 (75 nmol O_3 mol^{-1}). Carbon dioxide treatments were ambient (373 μmol CO_2 mol^{-1}), elevated (699 μmol CO_2 mol^{-1}), and high (899 μmol CO_2 mol^{-1}, pot-grown plants only). Treatments were CF air-ambient CO_2 (CF-373), CF air-elevated CO_2 (CF-699), CF air-high CO_2 (CF-899), NF air plus O_3–ambient CO_2 (OZ-373), and NF air plus O_3–elevated CO_2 (OZ-699). Values are means from three replicate chambers per treatment combination.

Despite the fact that plants in the two rooting environments in both years of the experiment differed morphologically, their responses to elevated CO_2 and O_3 were remarkably similar. The hypothesis of proportionality of response was found for all reported variables, with the exception of mass per seed and seeds per pod in the 1999 experiment. Total seed biomass is the most important commercial variable for soybean, and the similarity in response for this variable in pot-grown and ground-grown plants was striking (Fig. 2). It was not surprising that seed yield expressed on an areal basis (Table 7) differed between rooting environments given that culture conditions such as planting density, growth media composition, fertilization, and irrigation methods were different for pot-grown compared with ground-grown plants. Nevertheless, the relative effects of elevated CO_2 and O_3 were not fundamentally different between plants grown in the two rooting environments. This indicates that elevated CO_2 and O_3 experiments that use container-grown soybean plants can be representative of treatment responses of ground-grown plants.

In our study, the elevated CO_2 increase in yield (24%) was within the overall relative responses found by two meta-analyses of soybean (Ainsworth et al., 2002; Jablonski et al., 2002). Unlike previous studies that reported high variability in yield responses to elevated CO_2 (Ainsworth et al., 2002; Fiscus et al., 2001; Kimball, 1983), our results indicated consistent effects of elevated CO_2 and O_3 on plant growth and yield in pot-grown and ground-grown plants.

The suppressive effects of O_3 on *A*, biomass, and yield for plants grown in pots and in the ground were typical of those reported in previous chronic O_3 studies (Heagle, 1989; Heck et al., 1983; Morgan et al., 2003). Average g_s was lower in pot-grown plants treated with O_3 compared with the control but not in ground-grown plants. These responses were likely related to later development of inhibitory effects of O_3 on *A* in ground-grown plants compared with pot-grown plants (Fig. 3). Lower *A* often leads to lower g_s primarily through feedback effects of intercellular CO_2 concentrations (Fiscus et al., 1997; Long and Naidu, 2002; Reich et al., 1985).

Elevated CO_2 lessened or prevented the toxic effects of O_3, as is often found with soybean (Booker et al.,

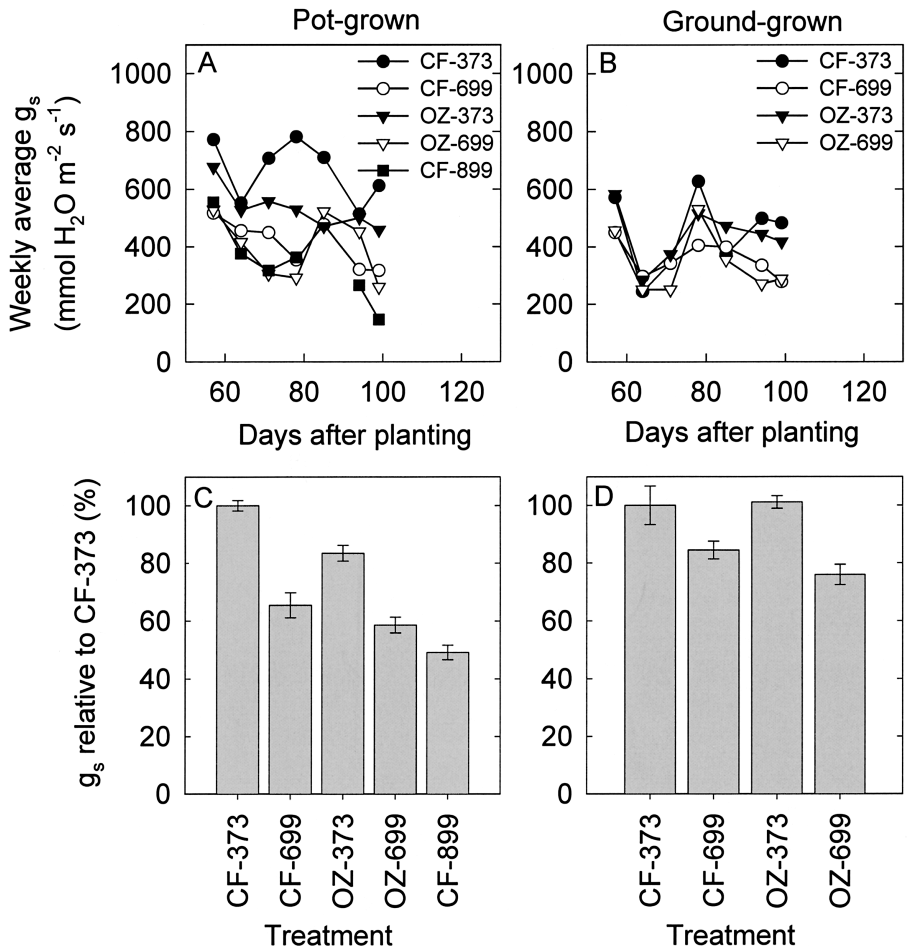

Fig. 4. Weekly average stomatal conductance (g_s) of leaves of pot-grown (A) and ground-grown plants (B) exposed to combinations of CO_2 and O_3 concentrations throughout the growing season in the 1999 experiment. Relative treatment effects on average g_s are shown as percentage of control (CF-373) in pot-grown (C) and ground-grown plants (D). Ozone treatments were charcoal-filtered (CF) air (24 nmol O_3 mol^{-1}) and nonfiltered (NF) air plus O_3 (75 nmol O_3 mol^{-1}). Carbon dioxide treatments were ambient (373 μmol CO_2 mol^{-1}), elevated (699 μmol CO_2 mol^{-1}), and high (899 μmol CO_2 mol^{-1}, pot-grown plants only). Treatments were CF air-ambient CO_2 (CF-373), CF air-elevated CO_2 (CF-699), CF air-high CO_2 (CF-899), NF air plus O_3–ambient CO_2 (OZ-373), and NF air plus O_3–elevated CO_2 (OZ-699). Weekly average g_s values are shown at the midweek days after planting (Wednesday). Values are means from three replicate chambers per treatment combination.

2004, 1997; Fiscus et al., 1997, 2001, 2002; Heagle et al., 1998b; Miller et al., 1998; Morgan et al., 2003; Mulchi et al., 1995; Reid and Fiscus, 1998) and other crops (Cardoso-Vilhena et al., 2004; Fiscus et al., 2002; Olszyk et al., 2000; Rudorff et al., 2000). The protective effect of elevated CO_2 against O_3 injury occurred in both pot-grown and ground-grown plants to similar extents presumably due in large part to lower O_3 uptake. The decrease in g_s with elevated CO_2, which occurred in both pot-grown and ground-grown plants, would decrease O_3 uptake and subsequent injury (Allen, 1990; Cardoso-Vilhena et al., 2004; Fiscus et al., 1997, 2001; McKee et al., 1997; Morgan et al., 2003). Increased availability of carbon skeletons with elevated CO_2 also might enhance defense and repair mechanisms that contribute to the protective effect (Allen, 1990).

The $CO_2 \times O_3$ interaction suggests the possibility that the stimulation of growth and yield in some elevated CO_2 studies might be due in part to suppression of ambient O_3 injury. Clearly, future studies of elevated CO_2 effects on plants should consider the potential interaction with ambient O_3. This study demonstrated that experiments done to investigate these interactions using plants grown in large pots yielded results that were not fundamentally different from those done with plants grown in the ground. Furthermore, results from studies comparing responses of plants treated in open-top chambers with those treated in free-air CO_2 enrichment (FACE) systems found that relative responses to elevated CO_2 were similar for the two methodologies (Kimball et al., 2002, 1997), thus extending the applicability of the results reported herein. Additional pot-ground studies are required to sort out effects of rooting volume, physical presence of a container, soil medium, temperature and moisture, nutrient availability, and planting density on plant responses to elevated CO_2 and O_3 to determine the factors that most critically influence plant growth and yield in these experiments.

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