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Influence of Daily Carbon Dioxide Exposure Duration and Root Environment on Soybean Response to Elevated Carbon Dioxide

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ABSTRACT

Little is known about effects of daily CO_2 enrichment duration and root environment on plant response to elevated CO_2. Two experiments were performed with Essex soybean (*Glycine max* L. Merr.) in open-top field chambers to address these questions. In one experiment, effects of 12 and 24 h d^{-1} exposures to double-ambient CO_2 were compared for plants grown in 14 L pots that were either insulated to moderate soil temperature or not insulated. Although never significant statistically, trends at some growth stages suggested that nighttime CO_2 enrichment contributed to growth and yield. Plants grew and yielded more in insulated than noninsulated pots, but there were no significant CO_2 enrichment × insulation interactions. In the second experiment, response to approximately 1.3, 1.6, and 1.9 times ambient CO_2 was compared for plants grown in the ground or 14 L pots. Enhancement of photosynthesis, growth, and yield by CO_2 enrichment was similar in pots and in the ground. Linear responses to different CO_2 concentrations were significant for all yield components in both root environments, whereas quadratic responses were significant for plants in pots but not for plants in the ground. Tests of proportionality of response for yield components showed no evidence of significant differences between plants in pots and in the ground except weight per 100 seeds. Seed yield enhancement at 1.9 times ambient CO_2 was 36% for plants in pots and 33% for plants in the ground. Overall, proportional response of soybean to CO_2 enrichment was relatively uniform in spite of large differences in baseline growth and yield.

NUMEROUS STUDIES have been performed since the 1970s to determine the impact of increased atmospheric CO_2 on agricultural and natural plant systems. Most have shown that CO_2 enrichment stimulates plant growth, in part through increased photosynthesis (see reviews by Bazzaz, 1990; Cure and Acock, 1986; Jarvis, 1989; Lawlor and Mitchell, 1991; Rogers et al., 1994; Strain and Thomas, 1992). The magnitude of response has often been highly variable however, even with the same species or cultivar. Causes for such variation may include differences in chamber environments, daily or seasonal CO_2 enrichment duration, or root environment.

Most studies have included CO_2 enrichment for 24 h d^{-1} to simulate ambient diurnal CO_2 conditions best. Some researchers (Li and Gupta, 1993; Mulchi et al., 1992; Sandhu et al., 1992) have not included CO_2 enrichment at night, perhaps because of the high cost of CO_2 coupled with the assumption that plant uptake of CO_2 is minimal at night. With open-air systems (Lewin et al., 1994; Prior and Rogers, 1995), CO_2 enrichment is not feasible when wind velocity is inadequate to disperse released CO_2 over plot areas. There are no reports of studies to show the effects of daily exposure duration on plant response to CO_2 enrichment.

The relevance of CO_2 enrichment studies on plants grown in pots is of major concern (Arp, 1991; Idso and Idso, 1994; Idso and Kimball, 1991; Jarvis, 1989; Lawlor and Mitchell, 1991; Strain and Thomas, 1992). A common contention is that limited root volume in small pots leads to reduced photosynthetic capacity due to feedback inhibition caused by imbalances in supply and demand of carbohydrates (Arp, 1991; Idso and Kimball, 1991; Thomas and Strain, 1991). Arp (1991) concluded that reduction in photosynthetic capacity is greater when an increased supply of carbohydrates is combined with small sink size (more feedback inhibition in small than in large pots) and suggested that feedback inhibition may not occur for plants in the ground. This contention was supported by Idso and Kimball (1991) who reported no decrease in CO_2-induced photosynthetic enhancement over 2 yr for sour oranges (*Citrus aurantium* L.) grown in the ground. However, an experiment by McConnaughay et al. (1993), showed that response to CO_2 was not necessarily decreased by small pots; growth and reproductive enhancement were greater in pots with high rather than low nutrient concentrations, regardless of total nutrient content or pot size. They concluded that pot size, shape, and nutrient status may lead to over- or underestimates of the CO_2 responses of plants grown in the field with standard agronomic practice. Also, Reekie and Bazzaz (1991) found no simple relationship between pot size and CO_2 response of four annual species. Hogan et al. (1991) suggested that, although studies with plants in large pots might better estimate innate capacity to respond to elevated CO_2, studies with plants in smaller pots might better estimate response under field conditions where biotic or abiotic factors may limit root growth. Sionit et al. (1984) reported that, although 'Bragg' soybean grown in large pots had lower overall rates of photosynthesis than plants grown in the ground, the proportional change

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Abbreviations: DAP, days after planting; NCER, net carbon exchange rate; g_s, stomatal conductance.

in photosynthesis due to CO_2 enrichment was greater for plants in pots than in the ground. There are no reports of studies to directly compare yield response of agronomic crops to CO_2 enrichment for plants grown in pots and in the ground.

Wheat straw is commonly used as mulch to decrease soil temperature fluctuation in experiments with pot-grown plants (Heagle et al., 1979a,b). We recently reported use of aluminized bubble wrap to insulate pots (Heagle et al., 1998). Effects of temperature fluctuation in pot root media on plant response to CO_2 enrichment has not been reported.

Our objectives were to: (i) determine the relative growth and yield response of soybean to 12 and 24 h d^{-1} CO_2 enrichment for plants grown in insulated or noninsulated pots and (ii) determine relative growth and yield response of soybean to CO_2 enrichment for plants grown in pots and in the ground.

MATERIALS AND METHODS

The experiments were performed with Essex (Smith and Camper, 1973) soybean during 1994 and 1995 at our field site 5 km south of Raleigh, NC. Plants were exposed to CO_2 at near-ambient O_3 concentrations in cylindrical, nonfiltered-air open-top chambers 3 m diam. × 2.4 m tall (Heagle et al., 1973). Dispensing and monitoring protocols have been described for CO_2 (Rogers et al., 1983) and for O_3 (Heagle et al., 1979c). Carbon dioxide was monitored at canopy height with infrared analyzers (LI 6252, LI-COR Inc., Lincoln, NE 68504), and O_3 was monitored at canopy height with UV analyzers (Model 49, Thermo Environmental Instruments, Inc., Franklin, MA 02038).

Effects of 12 vs. 24 h d^{-1} Carbon Dioxide Enrichment and Pot Insulation

Seeds were treated with a commercial Bradyrhizobium preparation and planted on 1 June 1994 in pots containing 14 L of a 2:1:1 mixture of sandy loam soil/sand/Metro Mix 220 (Scotts Sierra Horticultural Products Co., Marysville, OH 43041)[1]. The soil was obtained commercially and was probably Norfolk (a fine loamy, siliceous, thermic, pheric Typic Kandiuzult). Half of the plants were insulated with aluminized bubble wrap (ReflectixTM, Reflectix, Inc., Markleville, IN 40056) fit as a cylinder from ground level to the top of each pot. They were irrigated as required with drip tubes to prevent water stress and 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 acephate (Orthene 75 SP at 1.7 ml L^{-1} water), bifenthrin (Talstar 10 WP at 2.5 ml L^{-1} water), or avermectin (Avid, 0.15 EC at 0.03 ml L^{-1} water).

The design was three CO_2 treatments in each of two blocks, requiring six chambers. Two pot insulation treatments were the subplot. The CO_2 treatments were ambient (no CO_2 addition) and CO_2 enrichment of approximately 335 $\mu L\ L^{-1}$ ($\mu L\ L^{-1} = \mu mol\ mol^{-1}$) for 12 h d^{-1} (0600–1800 h EST) or 24 h d^{-1} (Table 1). Seedlings emerged on 5 June and were thinned to one per pot on 20 June. The CO_2 treatments began on 23 June and continued until 15 October, when plants in all treatments were at or past the R7 stage (Fehr and Caviness, 1977). The subplot treatment was insulated or noninsulated pots. Insulated and noninsulated pots were placed in a 2 × 2 Latin square in each of the four chamber quadrants. Two insulated and two noninsulated pots were also placed randomly in the southernmost row of each chamber. Root medium temperature was measured continuously on 57 d during the season. Thermocouples were placed at a depth of 10 cm in six pots per insulation treatment (two pots per insulation treatment in the northeast quadrant in one chamber for each CO_2 treatment). Because temperature was measured on 18 d during the first half of the season and 39 d during the last half, data from 11, 14, 18 July, 4, 11, 19, 26 August; and 2, 19, 16, 23, 30 September were used to estimate seasonal insulation effects on pot temperature. Temperature fluctuated more in noninsulated pots than in insulated pots. Mean (all dates combined) daily maximum and minimum temperatures in noninsulated pots were 30.0 and 20.4°C, respectively. Comparable values for insulated pots were 27.4 and 21.4°C, respectively. Mean (all dates combined) 24 h temperature was 24.9°C in noninsulated pots and 24.3°C in insulated pots.

Two plants per insulation treatment per plot were sampled for growth measures at 40, 57, and 89 d after planting (DAP). Plants were in the late vegetative stage at 40 DAP, between the R2 and R3 stages at 57 DAP, and at the mid-R6 stage at 89 DAP. The number, dry weight, and area of main stem and branch leaves, dry weight of main stem and branches, and dry weight of roots were measured at each harvest. At 89 DAP, number and dry weight of pods were also measured. The remaining four plants for each insulation treatment in the northern half of each plot were harvested on 14 November. Shoot weight (minus leaves and petioles), pod and seed number, and pod and seed weight were measured.

Analyses of variance were performed on the plot means for all variables for all harvest times. The main-plot factor was the CO_2-enrichment duration (0, 12, or 24 h) and the subplot factor was the insulation treatment. Contrasts were performed to determine if the magnitude of response to enrichment for 12 h d^{-1} differed from the magnitude of the response to enrichment for 24 h d^{-1}.

Effects of Root Environment–Pots vs. Ground

Essex seeds were planted in 14 L pots as described previously, and in the ground on 16 May 1995. All pots were insulated with Reflectix cylinders. The soil for plants in the ground was Appling (a clayey, kaolinitic, thermic, Typic Kanhapludult), fertilized the previous October to soil test recommendations.

The experimental design was three replicate blocks of four CO_2 concentrations for each root environment, requiring 12 nonfiltered-air chambers for plants in pots and 12 nonfiltered-air chambers for plants in the ground. The plot design for plants in the ground was two 3-m rows spaced 1 m apart. The plot design for plants in pots was 16 pots arranged in four rows of four pots each with plants in eight additional pots as borders.

Plants emerged on 20 May and were thinned on 30 May to one plant per pot and to approximately one plant per 5 cm of row. Plants in pots were irrigated and fertilized as described for the previous experiment. Plants in the ground were irrigated with a soaker hose installed parallel to each row at a distance of approximately 10 cm.

The CO_2 concentrations were ambient and approximately

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

Table 1. Monthly meteorological conditions, ambient ozone concentrations, and carbon dioxide concentrations during studies to determine effect of daily duration of carbon dioxide (CO_2) enrichment and root environment on soybean response to CO_2 enrichment at near-ambient ozone concentrations.

	1994—Daily enrichment duration, pot insulation experiment							1995—Pot-ground experiment						
	12–30 June	July	August	September	1–15 October	Season means		24–31 May	June	July	August	September	1–8 October	Season means
Mean max. temp. (°C)†	30	31	30	26	21	27		28	28	31	32	25	27	27
Mean min. temp. (°C)†	20	22	19	15	10	22		16	18	21	21	17	16	18
Mean % RH, 24 h‡	76	80	79	75	74	76		80	84	79	74	83	77	75
Mean total PAR, mol m^{-2} d^{-1}§	41	35	37	30	–	36		42	38	50	43	31	29	38
Rain, cm¶	8	16	13	7	13	57 (total)		3	26	5	9	8	12	63 (total)
Ozone conc., nL L^{-1}	57#	41	42	41	34	43		49	43	51	49	38	29	45
CO_2 added, µL L^{-1}							CO_2 added, µL L^{-1}							
0 h d^{-1}	360	368	356	388	387	370	0 h d^{-1}	365	367	364	361	369	382	366
12 h d^{-1} (1.9 × amb.)	663††	697	763	719	635	706	24 h d^{-1} (1.3 × amb.)	466	487	492	476	483	478	485
24 h d^{-1} (1.9 × amb.)	681	661	779	714	638	703	24 h d^{-1} (1.6 × amb.)	568	599	615	578	584	586	596
							24 h d^{-1} (1.9 × amb.)	659	725	740	680	688	677	708

† Temperatures for September 1995 measured 10 km north of field site.
‡ Relative humidity (RH) for 1995 measured 18 km west of field site.
§ PAR data collection for 1994 began on 20 June and ended on 12 September.
¶ Seasonal total irrigation for plants in pots was 251 and 302 L pot^{-1} in 1994 and 1995, respectively. Seasonal total irrigation for plants in the ground in 1995 was equivalent to approximately 25.4 cm of rain.
# Chamber ozone concentrations for 12 h d^{-1} (0800–2000 h EST). Ozone concentrations from 12 to 30 June were 46 nL L^{-1} in block 1 (which received charcoal filtered air from 10–16 June) and 65 nL L^{-1} in block 2 (which received nonfiltered air from 10–16 June)
†† Chamber CO_2 concentrations shown are 12 h d^{-1} (0800–2000 h EST). Night (2000–0800 h) CO_2 concentrations were 1.18 times daytime values for the 0 and 24 h d-addition treatments and 0.68 times the daytime values for the 12 h d^{-1} addition treatment. Carbon dioxide enrichment for 1994 in the 12 h d^{-1} treatment was for 24 h per day from 10 to 16 June.

1.3, 1.6, and 1.9 times ambient CO_2 for 24 h d^{-1} (Table 1). Dispensing of CO_2 began on 24 May and ended on 9 October when all plants were at or beyond the R7 development stage.

Measurements of net carbon exchange rate (NCER) and stomatal conductance (g_s), were made at 72, 79, 92, 97, 106, and 111 DAP with a portable photosynthesis system (LI-6200, LI-COR Inc., Lincoln, NE 68504). Soybean reproductive stages were R2-4 on the first sampling date and had progressed to R6 for the last three sampling dates in both root environments. Measurements were made on the center leaflet of non-shaded main stem leaves at the second main stem node below the apex. Two to three plants in two replicate chambers for each CO_2 concentration were measured for each root condition except at 106 DAP, when only one replicate of pot-grown plants was sampled due to deteriorating light conditions. Measurements were made between 1000 and 1300 h EST, when ambient PAR exceeded 1000 µmol m^{-2} s^{-1}.

Four plants in pots from each chamber and four plants in 20 cm of each row at the south end of each chamber were sampled for growth measures when plants were in the R6 stage (118–120 DAP). Parameters measured were the same as those described for the 89 DAP harvest in the previous experiment. Because of intrinsic differences between pot and ground environments, it was not feasible to use the same sampling units in both environments. Eight plants in pots per plot and 16 to 19 (mean = 18) plants from each of four 90 cm sections of row per plot were harvested for yield measures on 17–18 October. Parameters measured were the same as described for the final harvest in the previous experiment.

Analyses of variance were performed on replicate (chamber) means for all harvests to test the root environment, CO_2, and CO_2 × root environment interaction effects. Regression analyses were performed separately for each root environment to estimate linear and quadratic responses to CO_2 concentration. All analyses were conducted with SAS software (SAS Institute, Cary, NC).

Analysis of variance for growth and yield measures showed that the pot and ground response functions were not equal. This was expected because sampling units differed (one plant per pot and 4–19 plants in the ground). There is no simple way to eliminate the sampling unit differences. However, if response to CO_2 is equal for pots and ground, then we would expect the two response functions to differ only by a constant of proportionality. This null hypothesis is equivalent to the hypothesis that the relative changes in response between levels of CO_2 at x and $x + \Delta$, are equal for both pots 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. In terms of the coefficients of quadratic models, the null hypothesis of proportionality is equivalent to proportionality between the sets of coefficients. That is, if $m_g(x) = g_0 + g_1x + g_2x^2$ and $m_p(x) = p_0 + p_1x + p_2x^2$, then the response functions differ by a constant of proportionality if, and only if, $p_0 = cg_0$, $p_1 = cg_1$ and $p_2 = cg_2$ for some constant c. This is a two-degree-of-freedom, nonlinear statistical hypothesis. An F-statistic for testing proportionality is obtained in the usual manner from the mean squared errors from the fit of full (no proportionality constraints) and reduced (proportionality constraints enforced) models (Bates and Watts, 1988). The method differs from the usual linear models test-statistic construction only in that the reduced model is nonlinear and is fit to the data using nonlinear least squares. Tests of proportionality were performed for all measured responses at the final harvest.

RESULTS

Environmental conditions during 1994 were relatively normal for our site, with no prolonged periods of rain or high temperatures (Table 1). Conversely, conditions in 1995 ranged from well-above-average rain during vegetative growth in June to unusually hot and dry during early and mid-reproductive stages in late July and August. Moderate moisture stress occurred during the af-

Table 2. Mean squares from analyses of variance of midseason measures of effects of carbon dioxide (CO_2) enrichment and root environment on growth and net carbon exchange rate of soybean in open-top field chambers.

Experiment	Days after planting	Source	df	Stem weight	Leaf wt.	Root wt.	Pod wt.	Leaf area	Specific leaf wt.
								thousands	
Diurnal enrichment duration and insulation	40	CO_2 (C)	2	0.26	3.29*	7.41	NA	105	0.957*
		Insulation (I)	1	1.18	2.91	5.3*	NA	599	0.758*
		Error a	2	0.24	0.09	0.54	NA	25	0.225
		C × I	2	2.14	9.88	2.69	NA	530	0.023
		Error b	3	0.97	2.87	0.39	NA	346	0.057
	57	CO_2 (C)	2	230	130	230	NA	4 191	0.275
		Insulation (I)	1	833	603*	834**	NA	57 151*	0.010
		Error a	2	9	10	7	NA	2 294	0.016
		C × I	2	29	16	29	NA	397	0.057
		Error b	3	62	40	5	NA	2 131	0.026
	89	CO_2 (C)	2	6 034	2 132*	529	244**	11 272	4.894*
		Insulation (I)	1	5 765*	1 228	871**	736	74 615*	1.213*
		Error a	2	818	75	92	0	3 562	0.112
		C × I	2	421	25	14	53	1 438	0.221
		Error b	3	409	149	3	437	4 089	0.052
Pot vs. ground (growth)	119	Root environ. (E)	1	1 242**	729**	NA	7 271**	35 046**	0.050
		CO_2 (C)	3	1 183**	149*	NA	1 974*	2 030	0.350*
		E × C	3	124	20	NA	139	1 146	0.100
		Error		–	–	–	–	–	–
				Net carbon exchange rate per days after planting					
				72 DAP	79 DAP	92 DAP	97 DAP	106 DAP	111 DAP
Pot vs. ground (NCER)	72–111	Root environ. (E)	1	23.6**	117.9**	3.6	55.7*	7.2	19.7
		CO_2 (C)	3	192.5**	178.0**	57.1*	46.7*	46.9*	44.9**
		E × C	3	7.7	2.6	16.6	21.2	4.8	23.9*
		Error†	8	2.1	5.8	7.3	7.9	5.4	5.3

*, ** Significant at 0.05 and 0.01 level of confidence, respectively.
† Error term degrees of freedom = 8 except at 79 and 92 DAP (df = 7) and at 106 DAP (df = 5) due to inadequate light intensity.

ternoon on several days in August 1995, in spite of frequent irrigation. Ambient O_3 concentrations were highest during June in 1994 and highest during July and August in 1995 (Table 1).

12 vs. 24 h d^{-1} Carbon Dioxide Enrichment and Pot Insulation

Growth

Plant weights were generally higher at both CO_2 enrichment durations than with no enrichment, but the CO_2 effect was significant only for some response measures at some harvests (Table 2). Total plant weight response at each harvest (Fig. 1) reflects the growth response of individual plant parts. At 40 DAP, weight increases for plants exposed to enriched CO_2 were small, of similar magnitude for 12 and 24 h d^{-1} durations (insulation treatments combined), and significant only for leaf weight. At 57 DAP, a trend for CO_2-induced weight increase (Fig. 1) was not significant (Table 2). At 89 DAP, the leaf weight and pod weight response to CO_2 was significant (Table 2), and, although a clear trend for greater vegetative enhancement occurred for the 24 h compared to the 12 h duration (Table 2 and Fig. 1), the pod weight increase was 16% at both durations (Table 3). Contrasts between 12 and 24 h enrichment durations showed no significant differences for any response measure at any harvest.

Except at 40 DAP, plants generally grew faster in insulated than in noninsulated pots (Fig. 1) and the insulation effect was significant for several measures (Table 2). At 57 DAP, stem, leaf and root weights were 50, 47, and 39% greater, respectively in insulated pots than in non-insulated pots (CO_2 concentrations combined, data not shown). At 89 DAP, stem, leaf, root, and pod weights were 35, 26, 41, and 17% greater, respectively in insulated than in non-insulated pots (Table 3). However, there was never a significant CO_2 × insulation interaction (Table 2).

Yield

All yield components were increased by CO_2 enrichment (Tables 4 and 5) and the results were significant for pod weight, pod number, and stem weight. Plants in insulated pots yielded significantly more (44%) than plants in noninsulated pots (Tables 4 and 5). A trend for greater enhancement at 24 h than at 12 h for plants in insulated pots, but not in noninsulated pots, was not great enough to cause significant CO_2 × insulation interactions for any yield component. If values for insulated and noninsulated pots are combined, the degree of enhancement is similar for both enrichment durations. For example, seed weight increased by 17% for both enrichment durations (compared to ambient). Enhancement of stem weight was 61 and 64% for the 12 and 24 h enrichment durations, respectively, causing a 21 and 18% decline, respectively, in the harvest index (seed wt./stem wt.) (Table 5).

Root Environment–Pots vs. Ground

Net Carbon Exchange Rate and Stomatal Conductance

Carbon dioxide enrichment significantly increased NCER at all measurement dates, and the root environment effect was significant on three of the six dates (Table 2 and Fig. 2). At 72 DAP, NCER was greater

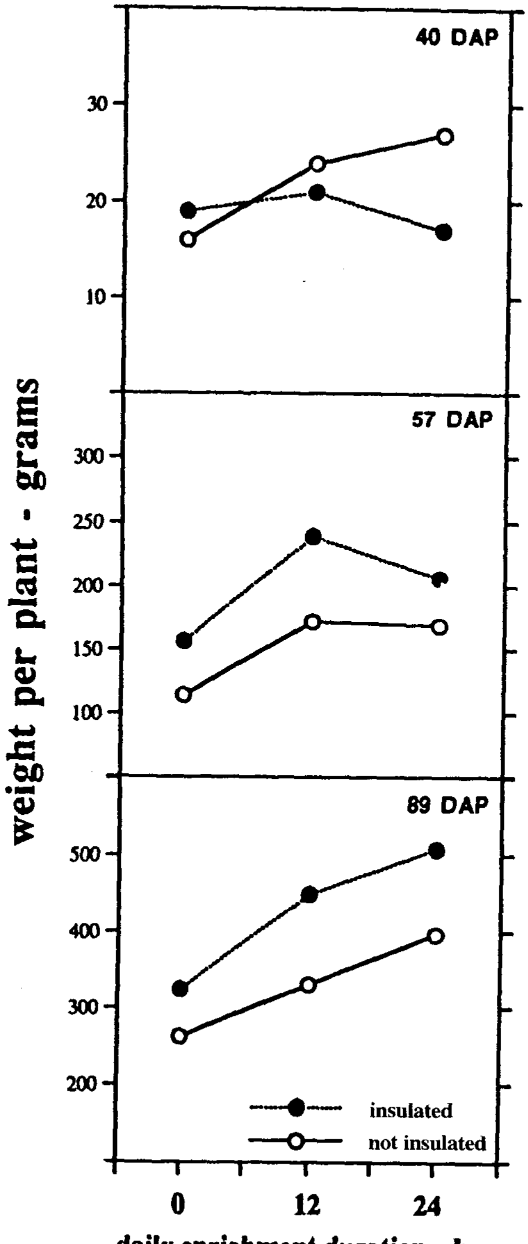

Fig. 1. Total plant dry weight at 40, 57, and 89 d after planting (DAP) of Essex soybean plants grown in insulated or noninsulated 14 L pots and exposed to double-ambient CO_2 for 0, 12, or 24 h d^{-1} in open-top field chambers.

overall for plants in the ground than for plants in pots. Conversely, NCER was higher for plants in pots at 79 and 97 DAP (Fig. 2). The root environment × CO_2 interaction was significant only at 111 DAP when CO_2 enrichment increased NCER for plants in pots but not for plants in the ground. Carbon dioxide enrichment generally decreased stomatal conductance (g_s) (Fig. 3) although the effect was significant statistically only at 106 and 111 DAP. Stomatal conductance was greater for plants in pots than in the ground at 79, 92, and 97 DAP but not at other measurement dates (Fig. 3). For all CO_2 concentrations and measurement dates combined, mean g_s was 2.3 cm s^{-1} for plants in pots and 1.8 cm s^{-1} for plants in the ground. The root environment effect on g_s was significant at 79, 92, and 97 DAP, but the root environment × CO_2 interaction was not significant at any measurement date.

Growth

At 119 DAP, CO_2 enrichment significantly increased weight of stems, leaves, pods, and specific leaf weight, but not leaf area (Tables 2 and 3). Total shoot weight (Fig. 4) indicates the response for individual plant parts. Partitioning of biomass was not affected by root environment. Biomass for main stem + branches, leaves, and pods (CO_2 concentrations combined) was 33, 17, and 50%, respectively, of total shoot weight. These values were almost identical (within 1%) in both root environments. Because plant responses were measured on different sized experimental units (one plant per pot vs. four plants per 20 cm of row), the statistically significant root environment effects (Table 2) were expected, but it was necessary to include root environment in the analysis of variance to obtain the correct error mean square for testing CO_2 and interaction effects. Although the percentage weight increases caused by CO_2 were greater for plants in pots than for plants in the ground (Table 3), the environment × CO_2 interaction was not significant for any growth measure (Table 2).

Yield

Pod and seed weight, pod and seed number, and stem weight increased to the same extent with increased CO_2 in both root environments (Table 5). The statistically significant root environment effect was caused by measuring yield on different units. Carbon dioxide enrichment significantly increased all yield components except 100-seed weight which increased with CO_2 enrichment only for plants in pots (Table 5). The ANOVA indicated a significant environment × CO_2 interaction for all measures, perhaps because of the large root environment effect that was an artifact of the experimental design. Root environment is confounded with the difference in sampling units for the two environments and this complicates the analysis of these data. The key issue is whether the response to CO_2 is proportionally equal in the two environments. This was addressed directly by testing proportionality of response curves (linear or quadratic) for plants in the two root environments (Table 4). For all yield components except 100-seed weight and harvest index, regression analyses showed significant linear responses to CO_2 enrichment in both root environments. Quadratic responses were significant for plants in pots but not for plants in the ground (Table 4). The significant quadratic component for plants in pots was due to decreased response at 708 μL L^{-1} compared to response at 596 μL L^{-1} (Table 5). Plot means for seed weight at incremental CO_2 concentrations (Fig. 5) reflect response trends for all yield components except 100-seed weight. Seed yield enhancement at double-ambient CO_2 (708 vs. 366 μL L^{-1y}) was 36% for plants in pots and 33% for plants in the ground (Table 5 and Fig. 5). Pod and seed number enhancement at double ambient CO_2 was less for plants in pots than for plants in the ground (Table 5).

The *F*-statistics for the tests of proportionality for

Table 3. Effect of diurnal duration of carbon dioxide (CO_2) enrichment and root environment on growth response of Essex soybean to CO_2 enrichment at near-ambient ozone concentrations in open-top field chambers.

Experiment	Root environment	Days after planting	Daily hours of CO_2 addition	12 h d^{-1} CO_2 conc.	Main stem and branch weight	Leaf wt.	Root wt.	Pod wt.	Leaf area	Specific leaf wt.
				μL L^{-1}	g				cm^2	mg cm^{-2}
Enrichment duration and installation	Pots† (not insulated)	89	0	370	96 (21)	57 (10)	31 (0.2)	78 (11)	12 221 (1846)	4.60 (0.16)
			12	706	126 (32)	77 (20)	42 (9.0)	86 (19)	11 355 (3080)	6.92 (0.30)
			24	703	154 (31)	100 (6)	51 (0.8)	93 (13)	14 747 (1859)	6.83 (0.54)
	Pots† (insulated)		0	370	117 (17)	72 (7)	45 (1.7)	91 (10)	16 056 (619)	4.45 (0.30)
			12	706	178 (6)	102 (6)	59 (7.9)	110 (14)	17 583 (1434)	5.83 (0.15)
			24	703	213 (45)	121 (12)	71 (1.5)	103 (17)	19 647 (1978)	6.16 (0.03)
Pots vs. ground	Pots‡ (insulated)	119	0	366	68 (5)	40 (2)	23 (0.1)	110 (5)	10 964 (295)	3.68 (0.08)
			24	485	90 (3)	46 (1)	29 (2.2)	129 (1)	11 379 (239)	4.04 (0.05)
			24	596	99 (4)	50 (1)	30 (0.8)	142 (6)	11 839 (154)	4.20 (0.05)
			24	708	107 (3)	53 (1)	31 (0.2)	157 (5)	11 656 (105)	4.55 (0.13)
	Ground§		0	366	102 (10)	56 (4)	NA	158 (13)	13 735 (1456)	4.07 (0.20)
			24	485	102 (11)	52 (7)	NA	152 (19)	12 732 (1434)	4.08 (0.12)
			24	596	119 (9)	61 (3)	NA	177 (10)	14 013 (242)	4.37 (0.19)
			24	708	130 (5)	64 (4)	NA	192 (18)	15 077 (1528)	4.31 (0.17)

† Each value is the mean per plant (with SE in parentheses) of four plants (two in each of two replicate plots).
‡ Each value is the mean per plant (with SE) of 12 plants (four in each of three replicate plots).
§ Each value is the mean per four plants (with SE) of 12 plants (four plants in 20 cm of row from each of three replicate plots).

various responses are reported in Table 4, in the row labeled "test of proportionality." Tests of proportionality indicated no significant response difference between plants in pots or in the ground for any yield component except for 100-seed weight (Table 4). Weight of 100 seeds increased with increased CO_2 for plants in pots but showed an opposite trend for plants in the ground. Thus, with the exception of 100-seed weight, there is no statistically significant evidence to reject the hypothesis of proportionally equal response to CO_2 in the two environments. As with any hypothesis test, however, failure to reject the null hypothesis should be interpreted in light of the possibility that the data may not be sufficient (i.e., lack power) to detect departures from the null hypothesis.

DISCUSSION

Although most research indicates that CO_2 enrichment significantly increases crop yields, a wide range in responses has been reported. For example, for soybean in different experiments, double ambient CO_2 was estimated to cause yield responses ranging from a 134% increase to a 7% decrease (Rogers et al., 1986). Much of this variation may have been caused by differences in soil moisture stress; the apparent response to CO_2

Table 4. Summary statistics for effects of diurnal duration of carbon dioxide (CO_2) enrichment and root environment on yield response of soybean to CO_2 enrichment at near-ambient ozone concentration in open-top field chambers.

Experiment	Source	DF	Pod wt. g	Seed wt. g	Pod no.	Seed no.	100-seed wt. g	Stem wt. g	Harvest index-seed wt./stem wt.
			Mean squares from analysis of variance*,**						
			thousands	thousands	thousands	thousands		thousands	
Diurnal enrichment duration and pot insulation	CO_2	2	3.57*	0.95	4.28*	6.7	2.86	1.3*	0.942
	Insulation	1	8.39*	4.83*	40.11*	123.9*	0.01	1.6*	0.197
	Error a	2	0.46	0.21	0.47	4.5	0.08	0.07	0.124
	CO_2 × insulation	2	0.35	0.45	0.25	6.5	0.30	0.03	0.099
	Error b	3	0.38	0.37	0.66	3.6	0.91	0.02	0.123
			Regression analysis estimates (standard errors)†						
Pot vs. ground	Ground								
	Intercept		230 (240.1)	167 (184.7)	722 (690.7)	1120 (1099.0)	17.8 (5.82)	39 (128.6)	2.45 (0.87)
	Linear		1.5 (0.9)**	1.2 (0.7)**	2.0 (2.7)**	5.3 (4.3)**	0.002 (0.02)	0.7 (0.5)**	−0.001 (0.003)
	Quadratic (10^5)		−70 (80)	−60 (70)	−26 (249)	−100 (400)	−0.6 (2)	−30 (50)	0.05 (0.03)
	Pots								
	Intercept		−153 (64.2)	−120 (48.2)	−128 (127.8)	−350 (263.7)	10.2 (3.53)	−56 (16.6)	3.00 (0.67)
	Linear		1.1 (0.2)**	0.9 (0.2)**	1.7 (0.5)**	3.8 (1.02)**	0.02 (0.03)**	0.4 (0.06)**	−0.002 (0.003)*
	Quadratic (10^5)		−90 (20)**	−70 (20)**	−132 (46)*	−300 (90)**	−2 (1)	−30 (6)**	0.1 (0.2)
			Test of proportionality (*F* statistic/*P* value *F*) based on 2, 18 DF						
			0.65/0.536	0.66/0.523	0.42/0.665	1.19/0.326	6.53/0.007**	0.24/0.791	0.31/0.736

*, ** Significant at the 0.05 and 0.01 level, respectively.
† Although quadratic models were never a better fit than linear models for plants in the ground, quadratic models are presented for both media to allow comparison. Quadratic estimates and standard error values have been multiplied by 100 000.

Table 5. Effect of diurnal duration of carbon dioxide (CO_2) enrichment and root environment on yield response of Essex soybean to CO_2 enrichment in open-top field chambers at near-ambient ozone concentrations.

Experiment	Root medium	Daily h of CO_2 addition	12 h d^{-1} CO_2 conc.	Pod wt.	Pod no.	Seed wt.	Seed no.	Seed 100-wt.	Stem wt.	Seed wt./stem wt.
			µL L^{-1}	g		g		g	g	
Diurnal enrichment duration-insulation	Pots† (not insulated)	0	362	195 (8)	367 (21)	142 (6)	778 (11)	18.2 (0.6)	40 (2)	3.52 (0.04)
		12	706	248 (11)	420 (26)	173 (6)	856 (25)	20.2 (0.2)	65 (6)	2.66 (0.11)
		24	703	232 (0)	401 (9)	153 (5)	790 (19)	19.4 (1.1)	70 (8)	2.16 (0.27)
	Pots† (insulated)	0	362	239 (1)	466 (12)	174 (2)	948 (24)	18.3 (0.3)	59 (1)	2.95 (0.01)
		12	706	274 (3)	523 (4)	183 (3)	947 (26)	19.2 (0.2)	101 (3)	1.81 (0.48)
		24	703	307 (29)	532 (12)	217 (26)	1071 (103)	19.6 (0.06)	92 (1)	2.32 (0.21)
Pots vs. ground	Pots‡ (insulated)	24	366	143 (4)	303 (11)	105 (4)	623 (25)	16.9 (0.2)	44 (0.3)	2.40 (0.07)
		24	485	182 (6)	365 (17)	133 (4)	754 (32)	17.7 (0.2)	59 (0.6)	2.27 (0.07)
		24	596	210 (9)	394 (15)	151 (7)	809 (28)	18.7 (0.3)	68 (2.6)	2.25 (0.07)
		24	708	201 (2)	386 (4)	143 (1)	774 (18)	18.5 (0.5)	67 (1.9)	2.14 (0.05)
	Ground§	24	366	699 (24)	1399 (45)	514 (19)	2889 (67)	17.8 (0.7)	241 (14.9)	2.13 (0.06)
		24	485	786 (34)	1642 (37)	578 (28)	3371 (61)	17.2 (0.6)	270 (4.2)	2.14 (0.10)
		24	596	900 (7)	1774 (84)	656 (3)	3839 (134)	17.2 (0.6)	331 (7.4)	1.98 (0.05)
		24	708	942 (15)	1994 (88)	685 (11)	4228 (147)	16.3 (0.4)	341 (13.5)	2.02 (0.10)

† Each value (SE in parentheses) is the mean per plant of eight plants (four in each of two plots). Pots were insulated with a cylinder of aluminized bubble wrap (Reflectix) or were not insulated.
‡ Each value (SE in parentheses) is the mean of 24 plants (eight plants in each of three replicate plots).
§ Each value (with SE in parentheses) is the mean of 12 90-cm sections of row (four 90-cm sections in each of three replicate plots).

enrichment increases when CO_2 enrichment decreases water stress (Rogers et al., 1986). We recently reported a similar phenomenon with soybean stressed by O_3; growth and yield response to CO_2 enrichment increased as O_3 stress increased (Heagle et al., 1998; Miller et al., 1998). The leading hypothesis to explain this interaction

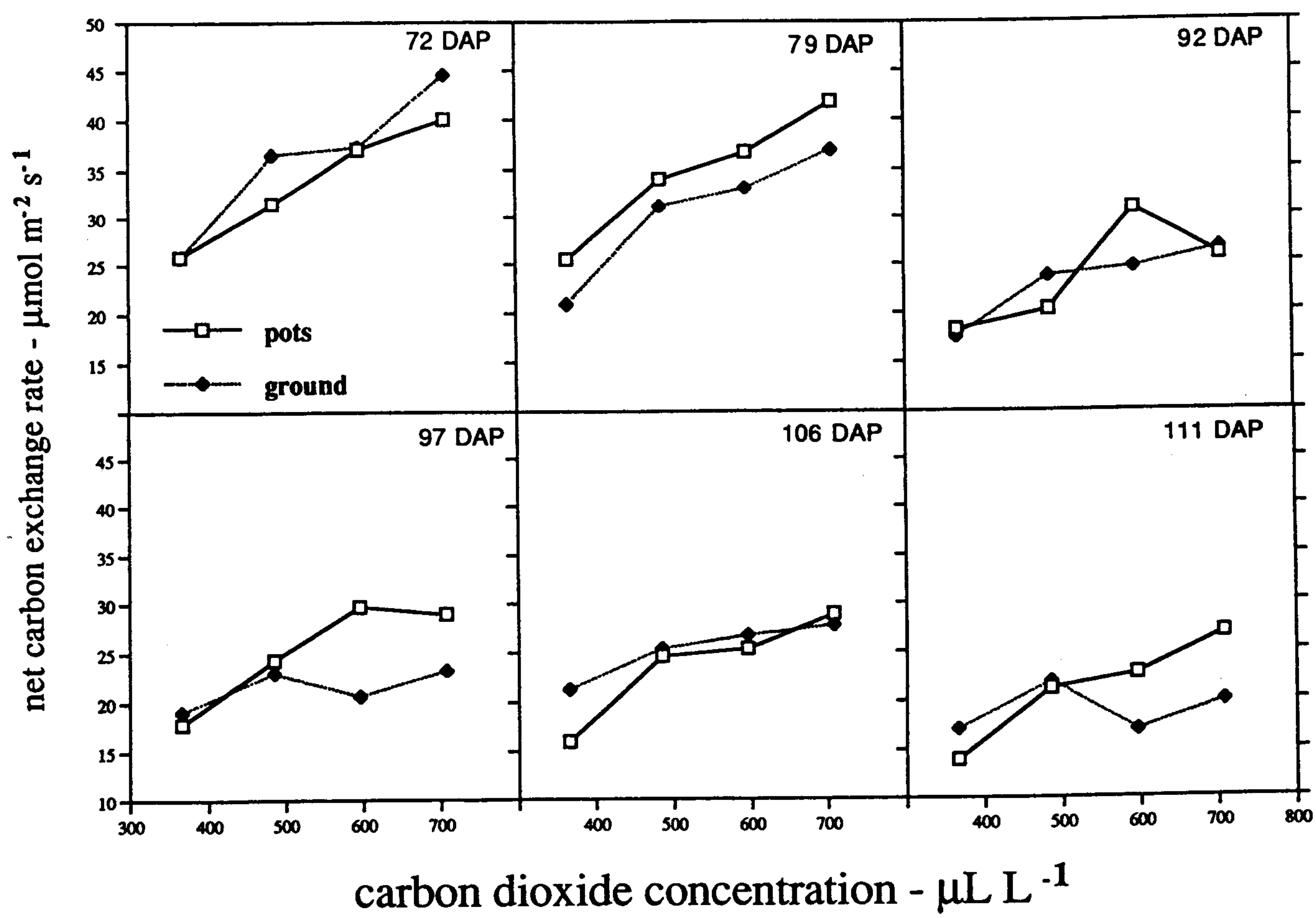

Fig. 2. Net carbon exchange rates at 72, 79, 92, 97, 106, and 111 d after planting (DAP) for Essex soybean grown in pots or in the ground and exposed to different concentrations of CO_2 for 24 h d^{-1} in open-top field chambers.

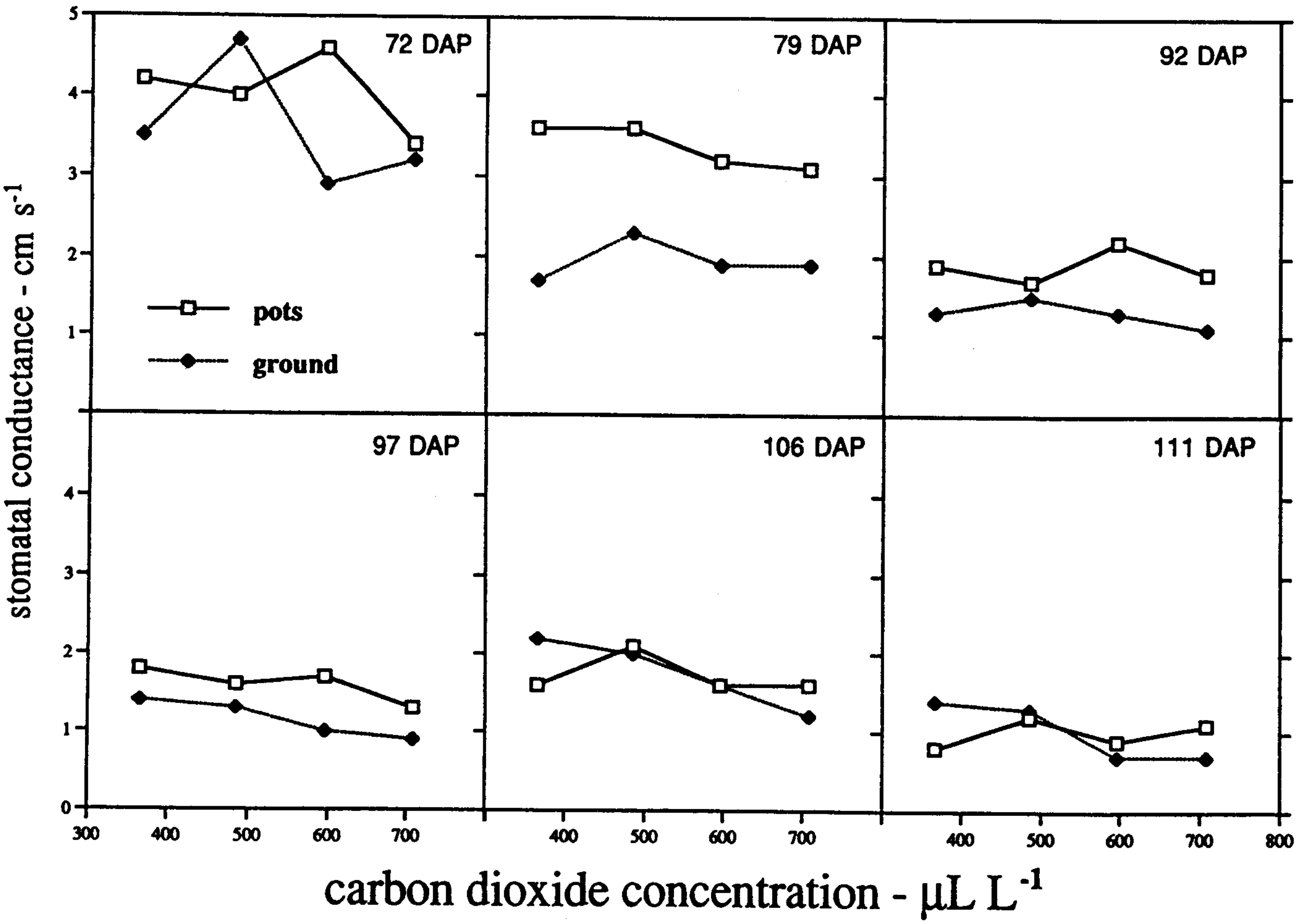

Fig. 3. Stomatal conductance at 72, 79, 92, 97, 106, and 111 d after planting (DAP) for Essex soybean grown in pots or in the ground and exposed to different concentrations of CO_2 for 24 h d^{-1} in open-top field chambers.

is that both stresses are ameliorated, at least partly, because CO_2 enrichment decreases stomatal conductance, which decreases transpiration and O_3 uptake (Fiscus et al., 1997).

Evidence is increasing to show that plant response to CO_2 enrichment is not necessarily affected by baseline plant growth rates. For example, growth and yield of plants grown in open-top field chambers is often different than that of plants grown outside. This was true for cotton and winter wheat, but proportional response to CO_2 enrichment was similar for plants in both environments (Kimball et al., 1995). The present experiments add to this evidence. Although baseline growth and yield were significantly affected by various root environments, the proportional yield response to CO_2 enrichment was similar under all conditions. The present experiment also showed that seasonal effects on baseline yield did not greatly affect proportional response; for soybean grown in insulated pots at ambient CO_2, seed yield was 66% greater in 1994 than in 1995, presumably because of prolonged hot and dry conditions in July and August of 1995. In spite of this large seasonal difference in baseline yield, seed yield enhancement at double ambient CO_2 was 25% in 1994 and 36% in 1995. Differences in baseline growth and yield do not appear to have

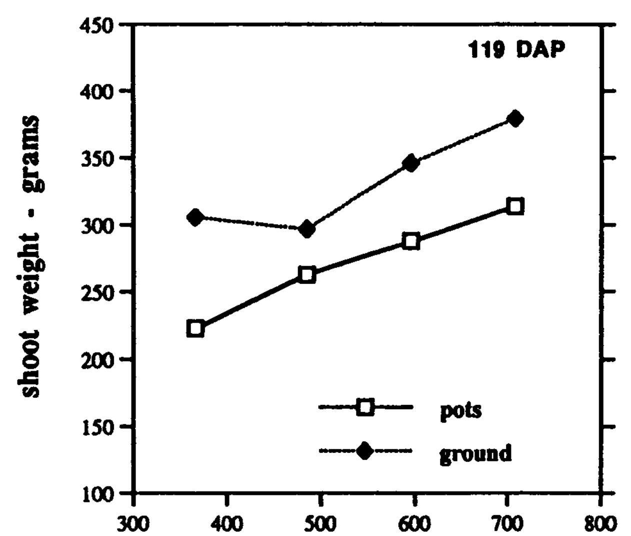

Fig. 4. Shoot dry weight of Essex soybean at 119 DAP grown in pots or in the ground and exposed to different concentrations of CO_2 for 24 h d^{-1} in open-top field chambers.

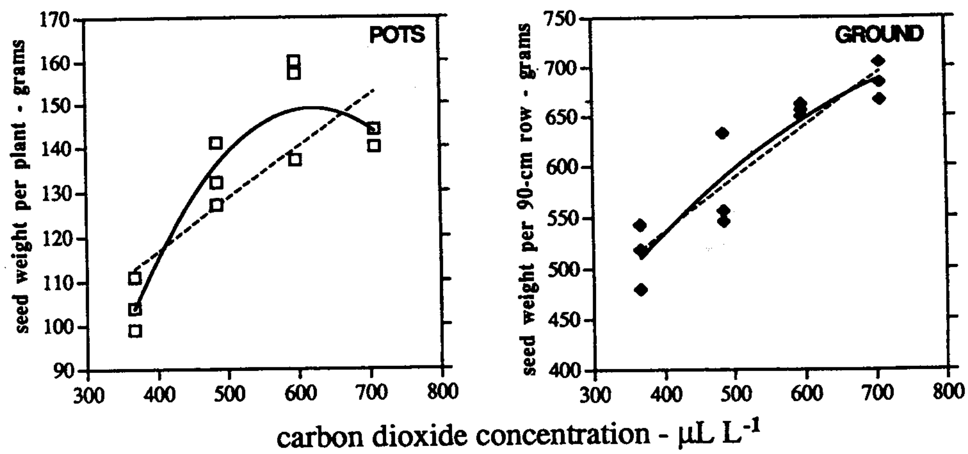

Fig. 5. Plot means for seed yield response to CO_2 enrichment for Essex soybean grown in pots or in the ground and exposed to different concentrations of CO_2 in open-top field chambers. Seed yield for plants in pots at 703 µL L^{-1} CO_2 was 144 g in two plots. Quadratic response curves are indicated by solid lines, linear response curves are indicated by dashed lines. Coefficients for the quadratic response curves are given in Table 4. Equations for the linear response curves do not appear in Table 4 and are: for plants in pots; 70 + 0.117x; and for plants in the ground; 328 + 0.520x. The ratio of the highest to the lowest value on the Y axis is the same (1.9) for both root environments.

major effects on plant response to O_3 either. Soybean, field corn, and winter wheat response to elevated O_3 was similar for plants grown in pots or in the ground (Heagle et al., 1983, 1979a,b).

Because so many experiments to measure plant response to CO_2 enrichment are performed with plants in pots, we sought to compare response for soybean grown in large pots and in the ground. Per plant yield for plants in pots was greater than for plants in the ground, presumably because of less plant-to-plant competition for nutrients, moisture, space, and light in pots than in the ground. With so many edaphic and meteorological variables involved, it was impossible, and never our intention, to pinpoint specific factors that might affect growth per se or response to CO_2. Rather, we were only interested in whether proportional responses differed in the two rooting conditions. Results from the present experiment and previous experiments with O_3, strongly suggest that experiments with large pots provide relevant results in experiments to determine effects of air quality on plants. The use of pots allows precise control of root media, which is required for experiments involving differences in nutrition or other edaphic factors, and which is very important for factorial experiments requiring 12 to 16 plots (chambers) for a single replication.

Cost of CO_2 is a major reason for the high overall costs of CO_2 enrichment experiments. This may be why some researchers have provided CO_2 enrichment only during daylight hours. Enrichment at night in open-air systems is often not feasible when wind is not adequate to disperse dispensed CO_2. Although statistical contrasts between responses in the 12 and 24 h enrichment durations never showed significant differences, the experimental design may have been inadequate to detect them. At 89 DAP there was a clear trend for greater growth enhancement at 24 h than at 12 h. The same was true for seed yield in insulated pots, but not for yield in noninsulated pots. Therefore, these results should be considered only as a first effort to address the question. Debate will continue on the suitability of various experimental protocols to characterize plant response to atmospheric gases. The wide range in soybean response to CO_2 enrichment under experimental conditions indicates that a wide range in response will also occur under field conditions, emphasizing the need to identify reasons for such variation.

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