

Carbon Dioxide Enhancement Effects in Container- versus Ground-Grown Soybean at Equal Planting Densities

Edwin L. Fiscus,* Fitzgerald L. Booker, Jean-Jacques B. Dubois, Thomas W. Rufty, Joseph W. Burton, and Walter A. Pursley

ABSTRACT

Prior work showed that CO₂ enhancement ratios (ER) were similar for plants grown in open-top chambers (OTCs) whether grown in the ground or in insulated containers aboveground. Per plant comparisons were suspect since the ground-grown plants were cultivated in rows at normal densities making it difficult to separate the effects of plant competition from the variables of interest. Soybean [*Glycine max* (L.) Merr. cv. Essex] was grown in the ground and in aboveground containers in OTCs in ambient and elevated CO₂ at equal planting densities. The hypothesis was that at equal densities, container- and ground-grown plants would exhibit both equivalent ERs and equivalent per plant yields. Although the only differences in net photosynthetic rate (A_n ; $\mu\text{mol m}^{-2} \text{s}^{-1}$) and conductance to water vapor (g_s ; $\text{mol m}^{-2} \text{s}^{-1}$) were due to CO₂ and container- and ground-grown plants had similar ERs (mean = 20%), per plant yields were still less in the container-grown plants at both levels of CO₂ (mean = -17%). Reproductive measures, except mass per seed, as well as total stem biomass were significantly reduced in the containers. High CO₂ increased seed oil concentration and the level of fatty acid saturation. The only observed environmental difference was higher daytime root zone temperatures in containers (2–6°C). The robust ERs suggest that neither above- nor below-ground resource limitations was the cause of the yield discrepancies.

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Abbreviations: A_n , net photosynthetic rate ($\mu\text{mol m}^{-2} \text{s}^{-1}$); CF, charcoal filtered; DAP, days after planting; DTR, diurnal temperature range; ER, CO₂ enhancement ratio [mass at elevated CO₂ (700 $\mu\text{mol CO}_2 \text{ mol}^{-1} \text{ air}$)/mass at ambient CO₂ (370 $\mu\text{mol CO}_2 \text{ mol}^{-1} \text{ air}$)]; g_s , stomatal conductance to water vapor ($\text{mol m}^{-2} \text{s}^{-1}$); OTC, open-top chamber; PAR, photosynthetically active radiation; R, reproductive; T_{max} , maximum daily temperature; T_{min} , minimum daily temperature, V, vegetative.

THE RELEVANCE OF using container-grown plants to assess the potential plant growth, yield, and physiological potential of future environments has been questioned (Ainsworth et al., 2002; Idso and Idso, 1994; Jarvis, 1989; Lawlor and Mitchell, 1991). The possibility that use of container-grown plants may confound the issue of predicting future productivity is anything but clear (Jarvis, 1989; Lawlor and Mitchell 1991; Reekie and Bazzaz 1991; Thomas and Strain 1991; McConnaughay et al., 1993; Idso and Idso, 1994; Heagle et al., 1999; Ainsworth et al., 2002; Booker et al., 2005). It has been suggested that the restrictions imposed on root growth by the volume of the containers may inhibit net photosynthetic rate (A_n ; $\mu\text{mol m}^{-2} \text{s}^{-1}$) and limit potential

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productivity increases (Arp and Drake, 1991; Thomas and Strain, 1991). However, McConnaughay et al. (1993) showed that the CO₂ enhancement ratio (ER) was not related specifically to container size but more to the nutrient concentrations, as opposed to the total quantity, available in those containers. Reekie and Bazzaz (1991) also found that ER was not simply related to container size. In addition, in two previous studies on soybean of the effects of elevated CO₂ on container- vs. ground-grown plants it was demonstrated that the ER for seed yield was the same regardless of cultural method (Heagle et al., 1999; Booker et al., 2005). Direct comparisons on a per plant basis were difficult to perform because ground-grown plants were cultured in rows at standard planting densities while the density of the container-grown plants was limited by the container diameter. Thus competition for aerial and soil resources that might limit the growth, productivity, and ER of individual ground-grown plants was different for container-grown plants. Also, plants in both rooting environments were irrigated, but container-grown plants were regularly treated with soluble fertilizer to minimize shortages of mobile nutrients. Soil temperatures and gradients likely differed between the two rooting environments as well but were not monitored.

The major objective in this study was to determine whether the yield, yield components, and ER in container- and ground-grown plants differed when grown at the same density. The specific hypothesis tested in this experiment was that container- and ground-grown plants cultured at equal planting densities will not only exhibit similar ERs for yield but also will exhibit similar yields on a per plant basis.

MATERIALS AND METHODS

Experiments were conducted at the Air Quality Education Unit field site 5 km south of Raleigh, NC (35° 43' N, 78° 40' W). Elevation was 107 m above sea level and the soil consisted of about 30 cm of Norfolk sandy loam (fine-loamy, kaolinitic, thermic Typic Kandiudult) overlying an Appling sandy loam (fine, kaolinitic, thermic Typic Kanhapludult).

Soybean [*Glycine max* (L.) Merr. cv. Essex] was grown in charcoal-filtered (CF) open-top field chambers (OTCs). Seeds were treated with *Bradyrhizobium* (Rhizo-Flo, Becker Underwood Inc., Ames, IA) and planted on 1 June 2004. Four seeds were planted at a depth of about 2 cm in all treatments. All treatments were thinned to two plants per location on 17 June and to one per location on 29 June. The result for both ground- and container-grown plants was a 4 by 4 plant grid with plants on 41-cm centers giving a final density of nine plants m⁻². Plants in all treatments were drip irrigated, with one emitter per plant, as required to prevent visible signs of water stress.

Plants were grown in 21-L black plastic containers 29 cm high with 35 cm and 29.5 cm top and bottom diameters, respectively. Containers were filled with a 2:1:1 mixture of sandy loam soil/sand/Metro Mix 200 (Scotts Sierra Horticultural Products Company, Marysville, OH) limed to raise the

pH to 6.2. Each container was insulated by wrapping it with aluminized bubble wrap (Reflectex Inc., Markleville, IN). The containers were placed on black plastic sheeting to prevent any roots growing out of the drainage holes from penetrating the soil. Plants were fertilized with 1 L of a solution containing 2.5 g L⁻¹ soluble fertilizer (10–30–20) (Peters Professional, Scotts Sierra Horticultural Products Company) seven times during the season. The initial fertilization included soluble trace elements mix micronutrients at a rate of 0.31 g L⁻¹.

Ground-grown plants were arranged in the same pattern and planting density as the container-grown plants. The field plot was fertilized according to soil test recommendations at a rate of 134 kg K ha⁻¹ on 25 March. Plants received 50 cm of supplemental irrigation throughout the season.

Both container-grown and ground-grown plants were sprayed on 11 June with Talstar One (bifenthrin; (2-methyl-1,1-biphenyl-3-yl)-methyl 3-(2-chloro-3,3,3-trifluoro-1-propenyl)-2,2-dimethylcyclopropanecarboxylate; 7.9%) at a rate of 5.2 mL L⁻¹ to control army worms (*Spodoptera* spp.); 25 June with Orthene 75S (Acephate; O,S-dimethylacetylphosphoramidothioate) at a rate of 4 mL L⁻¹ to control thrips (*Thrips* spp. and *Frankliniella* spp.); 6 July with Ridomil Gold EC (Mefenoxam; (R)-2-[(2,6-dimethylphenyl)-methoxyacetyl-amino]-propionic acid methyl ester; 47.6%) at a rate of 8.5 mL L⁻¹ in a soil drench of 1 L plant⁻¹ to control *Phytophthora*; 27 August with Talstar One at a rate of 2.6 mL L⁻¹ to control army worms and grasshoppers (*Melanoplus* spp.); and 24 September with Provado 1.6 (Imidacloprid; 1-[(6-chloro-3-pyridinyl) methyl]-N-nitro-2-imadazolidinimine) at a rate of 1.8 mL L⁻¹ to control aphids (*Aphis* spp. and *Myzus persicae*).

Both container-grown and ground-grown plants were exposed to elevated CO₂ in cylindrical OTCs 3 m in diameter and 2.4 m tall (Heagle et al., 1979). Carbon dioxide was dispensed from 8 June through 18 October when the plants in all treatments reached physiological maturity. Gas dispensing and monitoring were conducted as described by Rogers et al. (1983). Chamber CO₂ and O₃ concentrations were continuously sampled at canopy height and measured with infrared gas analyzers (model 6252, Li-Cor, Inc., Lincoln, NE) and UV analyzers (model 49, Thermo Environmental Instruments, Inc., Franklin, MA) that were calibrated every 2 wk. All chambers received charcoal-filtered (CF) air and CO₂ was added as necessary. Ambient CO₂ and O₃ were also monitored with the same system. The matrix of treatments consisted of: ambient CO₂ with container-grown plants (CF370C); ambient CO₂ with ground-grown plants (CF370G); elevated CO₂ with container-grown plants (CF700C); and elevated CO₂ with ground-grown plants (CF700G).

In three of the treatments (CF370G, CF370C, and CF700C), copper-constantan thermocouples were embedded in the rooting medium within 2 cm of the seedling stem and at a depth of about 20 cm in both the container- and ground-grown plants. Soil temperatures were monitored continuously and means stored at 5 min intervals. Data files were subsequently scanned for maximum (T_{max}), minimum (T_{min}), and mean daily soil temperatures. Ambient air temperature, photosynthetically active radiation (PAR), and water vapor pressure were also monitored at a central location on the site.

Net photosynthetic rate (A_n) and stomatal conductance to water vapor (g) were measured at growth CO₂ during 8 d from

21 July to 22 September, 50 to 113 d after planting (DAP), with an infrared gas analyzer system (model 6200, Li-Cor, Inc., Lincoln, NE).

Vegetative (V) and reproductive (R) growth stages (Fehr and Caviness, 1977) were recorded. The V stage observations were made every 3 to 4 d beginning 14 June and continuing until 2 August when plants were no longer adding main stem nodes. The R stage observations began on 16 July and were made daily during flowering. Observations were then made weekly from pod formation through maturity (23 July–16 November). All plants were harvested on 19 November by cutting at the soil line. Pod number, pod weight, total seed weight, hull weight, mean seed weight, and stem and branch weight were then determined. Seed number was calculated as well as seed/pod ratio and harvest index.

After the final harvest, percentage seed oil, protein, and fatty acid composition were determined. Oil concentration of seeds was measured by a pulsed proton NMR using a Maran pulsed NMR (Resonance Instruments, Witney, Oxfordshire, UK) by the Field Induction Decay-Spin Echo procedure of Rubel (1994). Oil concentration and moisture concentration were measured and oil (% dry weight) was determined by correcting for moisture concentration.

Protein concentration of the soybean meal was determined by the Dumas combustion method (Jung et al., 2003) that consists of converting all of the N in a sample to nitrogen oxides through combustion at 800 to 1000°C, then reducing the oxides to N₂ gas which is measured by a thermal conductivity detector. Samples were oven dried overnight at 80°C. Then, 0.2-g samples were prepared in tin foil packets for combustion analysis in a LECO FP-425 Nitrogen Determinator (LECO Corp., St Joseph, MI). Protein (%) was calculated from N values using: Protein (%) = 6.25 N (%).

Soybean meal samples (1 g) were extracted for 12 h in 3 mL of solvent (chloroform: hexane: methanol, 8:5:2 v/v/v) in stoppered glass test tubes. Fatty acid methyl esters of the lipid extracts were prepared by transesterification using sodium methoxide. The samples were analyzed by gas chromatography using an HP 6890 GC (Agilent Technologies, Inc., Wilmington, DE) equipped with a DB-23 (30 m by 0.53 mm) column (Agilent Technologies, Inc., Wilmington, DE). Operating conditions were 1 µL injection volume, a 20:1 split ratio, and He carrier gas flow of 0.1 cm³ s⁻¹. Temperatures were 250, 200, and 275°C for the injector, oven and flame ionization detector, respectively. Chromatograms were analyzed using HP ChemStation software. Calibrations of fatty acids were developed using authentic fatty acid methyl esters (AOCS RM-6, Sigma-Aldrich, St Louis, MO).

The experimental design was a randomized complete block with three replications and one block per replication with the OTC as the experimental unit. Measurements for plants from the same OTC on a single date were treated as subsamples and averaged. The OTC was the repeated unit (subject) when measurements were repeated over time (T_{\min} , T_{\max} , A_n , and g_s). Treatment factors comprised CO₂ and rooting environment, each at two levels (370 or 700 µmol CO₂ mol⁻¹ air, and ground [G] or container [C], respectively), in a factorial arrangement. Owing to instrument limitations the rooting environment temperature data consisted of only three combinations of the

two factors, CO₂ (370 and 700 µmol CO₂ mol⁻¹ air) and rooting environment (C or G) in the following three combinations: CF370G, CF370C, CF700C.

Analysis of variance for all biomass and harvest data was conducted using the GLM procedure of the SAS System for Windows, version 9.1.3. (SAS Institute, 2004). Root zone temperature, conductance, photosynthesis, V stage, and R stage data were analyzed using repeated measures methods implemented in the procedure MIXED of the SAS System (SAS Institute, 2004; Littell et al., 2000, 2006). Root zone temperature measurements were spaced evenly in time, and the optimal variance-covariance structure for them was found to be heterogeneous, a first order autoregressive structure, using both Akaike's and Schwartz's information criteria. Repeated observations were spaced unevenly for all the other repeated variables, and a spatial power covariance structure was used, as generalization of the first order autoregressive that allows for the uneven spacing (Littell et al., 2006). All repeated measures analyses were conducted first using discrete time models, where no trend over time was taken into account (i.e., with time as a classification variable), then with continuous time models, where polynomial effects of gradually higher order were considered for trends over time.

RESULTS

The seasonal 12 h mean O₃ concentration was 40 nmol mol⁻¹ in the atmosphere surrounding the OTCs and 18 nmol mol⁻¹ in the OTCs (Table 1). The seasonal 12 h mean CO₂ concentration was 379 µmol CO₂ mol⁻¹ air in the ambient CO₂ chambers and 707 µmol CO₂ mol⁻¹ air in the elevated CO₂ chambers. Mean temperatures and vapor pressures were slightly higher in July than in June and declined through the rest of the season. Mean daily PAR was similar in June and July followed by a steady decline through the rest of the season.

Only time had a significant effect on V stage when DAP were considered discrete, and first and second order polynomial effects of DAP were the only significant effects when DAP was taken as continuous. Analysis of variance was also performed on data for DAP 78 alone, and no effect of planting method on final V stage was found. Of course, the final R stages for all treatments are the same, but there was no detectable difference among treatments in the time required to reach that stage.

Photosynthetic rates and conductance values clearly segregated according to CO₂ (Fig. 1). Data were unevenly spaced, and a spatial covariance structure was used. In addition, because substantial heterogeneity existed in the variance structure of the four treatment cells, a separate variance-covariance matrix was fitted for each. Under the discrete time model significant effects were indicated for CO₂, DAP, and the interaction between them. There was also a mild rooting environment by DAP interaction for A_n ($p = 0.0103$) and g_s ($p = 0.0106$) (Table 2) owing to detectable differences in photosynthesis and conductance between the two rooting environments on some individual

Table 1. Mean \pm SE of monthly and seasonal meteorological conditions and CO₂ and O₃ concentrations. Temperature, photosynthetically active radiation (PAR), and water vapor pressures are daytime means for the environment outside the open top chambers (OTCs). Data for any 5-min period in which the average PAR >50 $\mu\text{mol m}^{-2} \text{s}^{-1}$ was included in the daytime means and the cumulative daily PAR. CO₂ and O₃ concentrations are 12 h d⁻¹ (0800–2000 h) averages inside the OTCs.

Parameter	June	July	August	September	October	Season
Temperature (°C)	24.8 \pm 0.41	25.9 \pm 0.21	23.8 \pm 0.25	21.9 \pm 0.38	16.7 \pm 0.31	22.6 \pm 0.31
Cumulative daily PAR (mol m ⁻² d ⁻¹)	46.5 \pm 3.20	46.7 \pm 3.00	38.7 \pm 3.80	29.1 \pm 4.20	22.7 \pm 3.50	36.7 \pm 0.74
Vapor pressure (kPa)	2.47 \pm 0.17	2.60 \pm 0.10	2.44 \pm 0.12	2.21 \pm 0.14	1.63 \pm 0.11	2.27 \pm 0.13
Supplemental irrigation						
Ground plants (cm) [†]	1.12	25.98	12.02	4.35	6.40	49.8
Containers (L container ⁻¹)	4	81	104	97	42	328 [‡]
Mean CO ₂ ($\mu\text{mol CO}_2 \text{ mol}^{-1}$ air)						
Ambient	375 \pm 0.8	370 \pm 0.7	372 \pm 0.8	377 \pm 0.9	389 \pm 0.3	375.6 \pm 0.6
Elevated	723 \pm 6.8	705 \pm 3.7	711 \pm 5.9	698 \pm 6.2	700 \pm 4.0	707.2 \pm 2.3
Mean O ₃ (nmol mol ⁻¹)	21.1 \pm 0.6	22.9 \pm 1.0	16.2 \pm 0.7	15.3 \pm 0.5	15.4 \pm 0.5	18.4 \pm 0.6

[†]Rainfall equivalent. In addition to the irrigations there was a total of 68.7 cm of precipitation during the experiment.

[‡]The daily mean (2.16 L d⁻¹) is similar to the seasonal means of measured daily water use (1.74 L d⁻¹) of container soybean in previous experiments (Booker et al., 2004).

days. This effect was small and inconsistent from day to day and consequently was not detected when continuous effects of time were taken into consideration. When continuous polynomial trends of time were included, no evidence of effects beyond a second degree polynomial trend of time was found, and interaction of CO₂ with time was only detectable for the linear trend. Hence, data indicated separate linear trends over time for 370 and 700 $\mu\text{mol CO}_2 \text{ mol}^{-1}$ air, and separate y-intercepts, but a common quadratic coefficient (Table 2).

There were significant effects of both CO₂ and rooting environment for all the harvest variables except for the biomass per 100 seeds (Table 3). However there were no CO₂ by rooting environment interaction effects for any of the variables. Seed yield per plant was significantly increased by 18 and 22% by elevated CO₂ in container- and ground-grown plants, respectively. However the yield was significantly decreased in container plants compared to ground plants at both CO₂ levels (average 16.6%). Since the mass per seed was unchanged and the number of seeds per pod were within 2% of each other in all the treatments, the decrease in seed number in container plants may be attributed almost entirely to a reduced number of pods per plant. Stem biomass at harvest also showed substantial increases in response to elevated CO₂ with 42 and 41% increases in ground- and container-grown plants, respectively, which was approximately double the increase in seed yield. As with seed yield the stem biomass was significantly reduced in the container-grown plants by an average 19% over both CO₂ levels.

Since no temperature data were available for ground-grown plants at 700 $\mu\text{mol CO}_2 \text{ mol}^{-1}$ air, the effects of varying CO₂ on rooting environment T_{min} (Fig. 2B, Table 4) were analyzed for containers only. There was no detectable difference in root zone T_{min} between the two levels of CO₂. When testing for effects of rooting envi-

ronment on T_{min}, CF370C and CF700C were aggregated, but whether they were, or whether differences between ground and containers were tested only at CF370, there was no detectable effect of rooting environment on T_{min}.

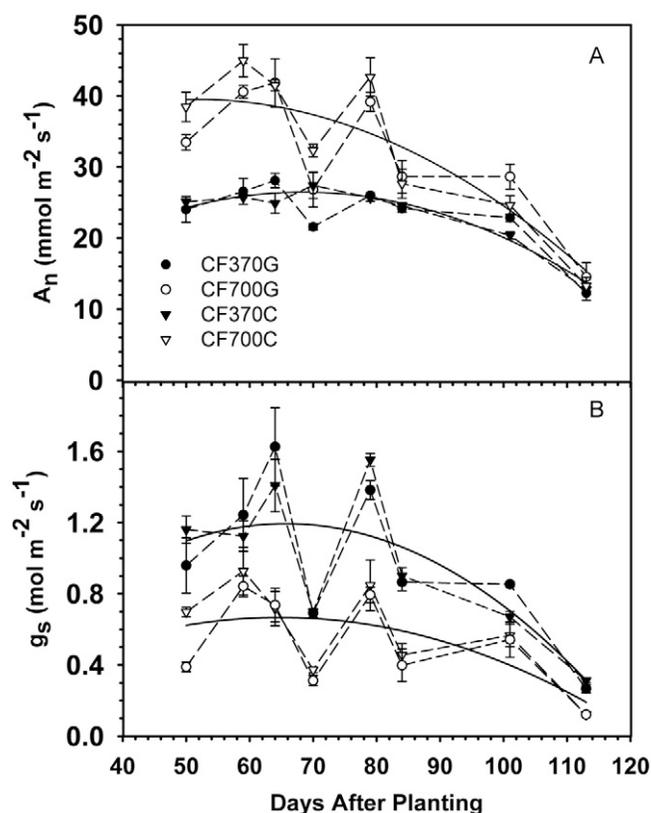


Figure 1. (A) Net photosynthetic rates and (B) stomatal conductance values obtained during the reproductive period for both container- and ground-grown soybean in open-top chambers. Bars are \pm SE, and the smooth lines are the quadratic fit of the data with the coefficients given in Table 2. Treatments were all charcoal-filtered air (CF) with plants grown in the ground (G) or in 21 L containers (C) at current ambient (370 $\mu\text{mol CO}_2 \text{ mol}^{-1}$ air) or elevated (700 $\mu\text{mol CO}_2 \text{ mol}^{-1}$ air) CO₂ concentrations.

Table 2. Tests of effects for the full model, discrete time, and tests for polynomial trends of time on photosynthesis and stomatal conductance with regression coefficients for the appropriate quadratic fits.

	Photosynthesis		Conductance	
Discrete time, full model				
Effect	DF	Pr > F	DF	Pr > F
CO ₂	1	†	1	†
Rooting environment	1	NS [‡]	1	NS
CO ₂ by rooting environment	1	NS	1	NS
DAP	7	†	7	†
CO ₂ by DAP	7	†	7	***
Rooting environment by DAP	7	**	7	*
CO ₂ by rooting environment by DAP	7	NS	7	NS
Continuous time, reduced model				
Effect	DF	Pr > F	DF	Pr > F
CO ₂	1	†	1	†
DAP	1	†	1	***
DAP by CO ₂	1	†	1	**
DAP by DAP	1	†	1	†
Intercept				
CF370	-3.5738		0.0651	
CF700	22.4418		-0.8173	
Linear coefficients				
CF370	0.875		0.03717	
CF700	0.6618		0.04302	
Common quadratic coefficient	-0.00641		-0.00031	

P* ≤ 0.05.*P* ≤ 0.01.****P* ≤ 0.001.†*P* ≤ 0.0001.‡NS, not significant at *P* ≤ 0.05.

Analysis of the effects of elevated CO₂ on rooting environment T_{max} and the diurnal temperature range (DTR) (Fig. 2A, 2C, Table 4) were similar to each other in all respects. However there was a significant effect of rooting environment on T_{max} and DTR, with *p* < 0.001 whether both levels of CO₂ were aggregated for data from containers or whether the effect of rooting environment was tested for CO₂ = 370 μmol CO₂ mol⁻¹ air only. There also was a significant interaction of rooting environment and time (*p* < 0.0001) but, although there were significant decreasing linear trends over the period, there was no interaction between the linear trends for the two rooting environments. The slopes of the cooling from August to October were not significantly different for the two rooting environments.

Analysis of variance on the bean protein and oil data (Table 5) revealed significant 3 to 4% increases in seed oil concentration as a result of the elevated CO₂ treatment in both rooting environments and no significant direct effect of rooting environment nor any interaction between the two. The oil production (mass) per plant increased in ground- and container-grown plants in response to elevated CO₂ by 27 and 14%, respectively. When grown

in containers oil production decreased compared to ground-grown plants by 25 and 33% when grown in 370 and 700 μmol CO₂ mol⁻¹ air, respectively. Other than the 3 to 4% increase in percentage oil in the seeds most of the increased oil production per plant was due to increased seed production. There was only a weak rooting environment effect on the protein concentrations showing a slightly elevated value (1.2%) in the container grown plants most of which appeared to occur at elevated CO₂. Results for the fatty acid concentration were mixed with all but 18:2 showing significant effects of elevated CO₂. The effects were also mixed for 16:0 with a decline in both ground and containers and a barely significant effect of rooting environment, with containers somewhat lower. Fatty acid 18:0 increased in both ground and containers with a highly significant increase in containers at both levels of CO₂. Fatty acid 18:1 declined in ground-grown but increased in container-grown plants and 18:3 decreased in both ground- and container-grown plants. Only 18:1 and 18:2 failed to show any significant direct effect due to rooting environment while 18:1, 18:2, and 18:3 all showed strong CO₂ by rooting environment interactions. Fatty acid 18:3 also showed strong direct CO₂ and rooting environment effects with the CO₂ causing small decreases in 18:3 in both ground- and container-grown plants. The shifts in seed oil composition noted here, particularly the 4.2% decline in 18:3 fatty acids coupled with a 3.5% increase in 18:1, reduced the overall degree of unsaturation in the elevated CO₂ plants by about 9.5%.

DISCUSSION

Overall, these experiments confirm in most respects the previous work showing equal ERs for plants grown in the ground and in large insulated containers aboveground (Heagle et al., 1999; Booker et al., 2005). However, using equal planting densities for both container and ground plants allows more direct comparisons on a per plant basis. When plants were grown at equal densities the seed yield per plant was significantly less when grown in containers as opposed to the ground with pod number, pod biomass, and seed number 12 to 15% lower. In the container plants elevated CO₂ served only to bring the yields up to the same level as the ambient CO₂ plants grown in the ground even though the relative ERs were similar. This latter observation supports the notion that ERs seem to be relatively indifferent to baseline growth and yield (Heagle et al., 1999). Possible exceptions to this may occur when the baseline is suppressed by unrecognized co-occurring stresses (i.e., high atmospheric O₃ concentrations or water deficits) the effects of which are subject to amelioration by elevated CO₂ (Fiscus et al., 1997, 2002; Kimball et al., 2002). In such cases the ER would be proportional

Table 3. Yield of Essex soybean grown in ground or in 21-L containers at the same planting density (nine plants m⁻²) and exposed to ambient or elevated CO₂ concentrations. Treatments were (i) charcoal-filtered (CF) air-ambient CO₂ (CF370); (ii) CF air plus 330 μmol CO₂ mol⁻¹ air (CF700). Values are per plant means ± SE of three replicate chambers for each treatment combination. Analysis of variance was performed on actual data except for stem biomass and seed/pod ratio, in which analysis was performed on log transformed data. Harvest index is defined as the ratio of seed biomass to shoot biomass. The CO₂ enhancement ratio is the parameter at 700 μmol CO₂ mol⁻¹ air divided by the parameter at 370 μmol CO₂ mol⁻¹ air. The seed:pod ratio is the seed biomass divided by the pod biomass.

Treatment	Pod number	Pod biomass	Seed number	Seed per pod	Seed biomass	100 seed biomass	Stem biomass	Seed/pod ratio	Harvest index
		g				g		w/w	
CF370G	402 ± 23	226 ± 14	848 ± 49	2.11 ± 0.016	172 ± 11	20.22 ± 0.33	59.11 ± 4.26	0.767 ± 0.009	0.607 ± 0.003
CF700G	498 ± 29	278 ± 16	1048 ± 53	2.10 ± 0.016	210 ± 12	19.77 ± 0.49	83.94 ± 8.31	0.753 ± 0.003	0.580 ± 0.006
CF370C	354 ± 5	197 ± 1	735 ± 12	2.07 ± 0.006	146 ± 1	19.84 ± 0.31	48.18 ± 0.47	0.737 ± 0.003	0.593 ± 0.003
CF700C	402 ± 9	238 ± 5	830 ± 22	2.07 ± 0.012	172 ± 4	20.71 ± 0.30	67.73 ± 2.32	0.720 ± 0.006	0.563 ± 0.003
Source									
CO ₂	**	**	**	NS [†]	**	NS	**	**	***
Rooting environment	**	**	***	*	**	NS	*	‡	*
CO ₂ by rooting environment	NS	NS	NS	NS	NS	NS	NS	NS	NS
CO ₂ enhancement ratio									
Ground	1.24	1.23	1.24	1.00	1.22	0.98	1.42	0.98	0.96
Container	1.14	1.21	1.13	1.00	1.18	1.04	1.41	0.98	0.95

* $P \leq 0.05$.

** $P \leq 0.01$.

*** $P \leq 0.001$.

[†]NS, not significant at $P \leq 0.05$.

[‡] $P \leq 0.0001$.

to the degree of yield suppression by the co-occurring stress. Also, the fact that rooting environment had direct effects on the stem biomass and all of the harvest parameters except for individual seed weight (Table 3) suggests a significant physiological effect of the elevated root zone temperature, restricted rooting volume, or both on individual plant seed yields. Direct temperature effects were also noted in previous comparisons of soybean (Heagle et al., 1999) where plants cultured in insulated pots grew faster and yielded 44% more seed mass than those grown in non-insulated pots.

There was no difference in T_{\min} between treatments. Since T_{\min} usually occurred at night it seems reasonable to suppose that the ground and container root zone temperatures converged as temperatures equilibrated in the absence of additional solar radiative heating. The seasonal mean root zone DTR in container-grown plants, however, was about 1.9°C higher than in ground-grown plants (Fig. 1) while the actual values ranged between about 2 and 6°C driving T_{\max} to around 26 to 29°C. During the daylight hours, when T_{\max} always occurred, one might expect higher T_{\max} in the containers both because they were more exposed to solar radiation and because they had a smaller mass to buffer the temperature changes. In addition, the use of a soil/sand/Metro-mix combination in the containers in the current study may have resulted in differences in the heat and/or water-holding capacity between the ground and the container mixture. Though we have no direct information about what processes might be influenced by the elevated T_{\max} , in these experi-

ments there are several possibilities. Past experiments using hydroponic culture have indicated that the optimal root temperature for soybean growth is about 24°C, and both root and shoot growth were negatively impacted as root temperature increased beyond the optimum (Rufty et al., 1981; Wright et al., 1999) as they may have here. Those experiments examined growth responses during the vegetative phase and shoot growth inhibitions of 10 to 17% were found, about the same order as those observed here with reproductive components. Given the mean difference in T_{\max} between container and ground plants of 1.9°C (Table 4) we can calculate that some enzymatic or membrane limited process (possibly catabolic) could be increased over that temperature range by 14 to 30%, depending on the specific Q_{10} for that process or system. Perhaps increased respiratory losses in the roots could account for the differences. It has also been demonstrated that root growth at higher rooting environment temperatures results in increased fatty acid saturation in new roots that can, in turn, result in decreased root conductance (Markhart et al., 1980). Thus, water flow to the shoot might be reduced causing a marginal reduction in stomatal conductance which may in turn raise leaf (and pod) temperatures via a decline in the evaporative cooling of transpiration.

Another factor possibly contributing to lower soybean reproductive efficiency in containers may have been associated with decreased rooting volume. Several studies have shown that container size can influence plant growth (Richards and Rowe, 1977; Carmi and Heuer, 1981; Sionit et al., 1984; NeSmith and Duval, 1998; Kharkina

Table 4. Mean \pm SE root zone temperatures for Essex soybean grown in ground (G) or in 21-L containers (C) at the same planting density (nine plants m^{-2}) and exposed to ambient or elevated CO_2 concentrations. T_{min} is the daily temperature minimum, T_{max} the daily temperature maximum, and DTR the diurnal temperature range. Treatments were: (a) charcoal-filtered (CF) air-ambient CO_2 (CF370); (b) CF air plus $330 \mu mol CO_2 mol^{-1}$ air (CF700).

Treatment	T_{min}	T_{max}	DTR
	°C		
CF370G	22.81 \pm 1.68	24.49 \pm 0.10	1.68 \pm 0.04
CF700G	–	–	–
CF370C	22.81 \pm 3.59	26.41 \pm 0.13	3.59 \pm 0.08
CF700C	22.85 \pm 3.35	26.20 \pm 0.12	3.35 \pm 0.06
Source			
Rooting environment	NS†	‡	‡
Date	‡	‡	‡
Rooting environment by date	‡	‡	‡

†NS, not significant at $P \leq 0.05$.

‡ $P \leq 0.0001$.

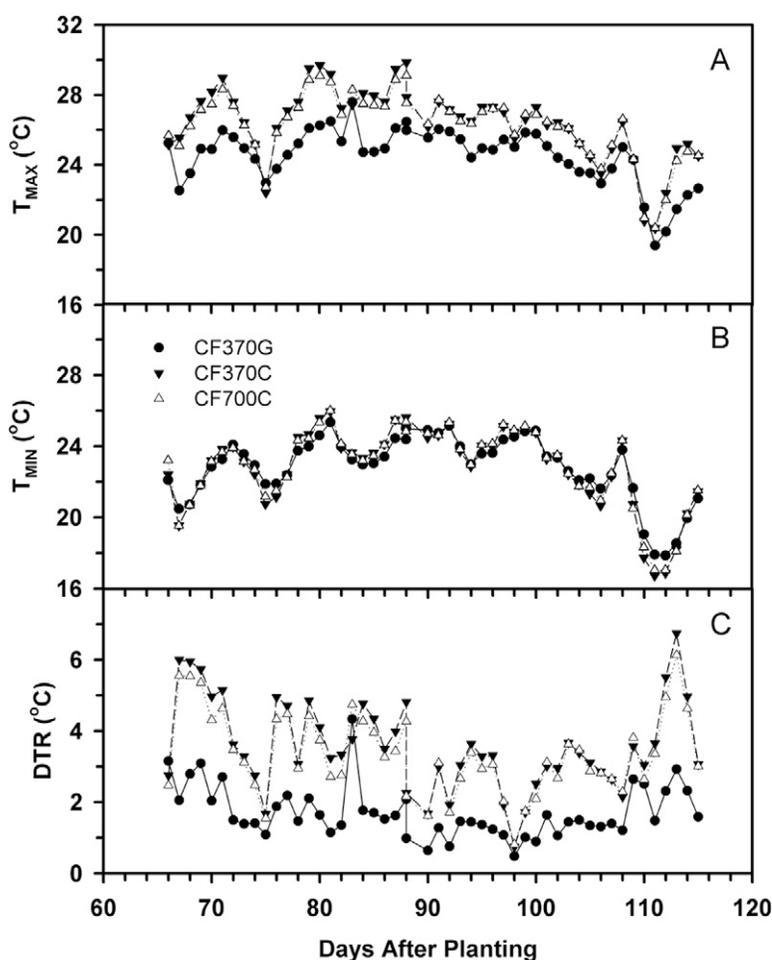


Figure 2. (A) Diurnal root zone maximum, (B) minimum, and (C) diurnal temperature range (DTR). Treatments were all charcoal-filtered air (CF) with plants grown in the ground (G) or in 21-L containers (C) at current ambient ($370 \mu mol CO_2 mol^{-1}$ air) or elevated ($700 \mu mol CO_2 mol^{-1}$ air) CO_2 concentrations. All temperatures were measured at a depth of about 20 cm.

et al., 1999). The root restriction evidently is not directly related to water or nutrient stress, in general, but might be due to decreased cytokinin production in root tissues and reduced cytokinin availability in the shoot (Carmi and Heuer, 1981; Krizek et al., 1985; Peterson et al., 1991; McConnaughay et al., 1993; Bar-Tal et al., 1995). While we cannot know the exact cause for reduced growth in container plants in our own study, care was taken to ensure that abundant water and nutrients always were available to the plants, so water and nutrient stresses were unlikely and indeed not indicated by the g_s and A_n data (Fig. 1) nor by the ER. Finally, it could be argued that growth and yield were not limited by above- or below-ground or container resources since plants in both rooting environments exhibited robust ERs. Clearly much more detailed observations are needed to clarify this question.

There was a small (3–4%) but highly significant increase in the seed oil concentration due to elevated CO_2 with no price exacted from the protein concentration (Table 5). When the increase in seed oil concentration is multiplied by the ER for seed yield, the mass of oil produced per plant rises 27 and 14% in ground- and container-grown plants, respectively. However, as planting density is increased to more normal values we would expect individual plant production to decline exponentially because of competition for limited resources and for overall crop productivity to reach levels consistent with the law of constant yield in agricultural systems (Barnes, 1977). The previous study by Booker et al. (2005) which showed an average in-ground yield ER of 23% on a land area basis suggests that the oil production increase would be comparable even if there were not also an increase in seed oil concentration. Indeed without considering the increase in seed production under elevated CO_2 , the 3 to 4% increase in oil per seed would amount to a very substantial increase in oil production on a regional, national, or international scale. According to the National Agricultural Statistical Service (2006) about 84.1 Tg of soybean were produced in the United States during 2005 suggesting that an increase of 3.5% of seed oil concentration could result in an additional 2.9 Tg of seed oil in a future climate with CO_2 concentrations well above current ambient levels. In addition, considering commonly observed ERs, that number could rise to more than 20 Tg.

CONCLUSIONS

Cultural methods had little detectable effect on photosynthesis, stomatal conductance, or ER when plants were grown at equal densities. Container plants had higher soil T_{max} and subsequently larger DTRs that were correlated with decreased yields in both ambient and elevated CO_2 . If the only experimental result desired is to know the relative ER for a

Table 5. Percentage (w/w) seed oil, protein, and fatty acid composition of Essex soybean grown in ground (G) or in 21-L containers (C) at the same planting density (nine plants m⁻²) and exposed to ambient or elevated CO₂ concentrations. Treatments were: (a) charcoal-filtered (CF) air-ambient CO₂ (CF370); (b) CF air plus 330 μmol CO₂ mol⁻¹ air (CF700). Values are per plant means ± SE of three replicate chambers for each treatment combination. Oil mass was calculated as total seed weight per plant × % Oil.

Treatment	Oil	Protein	Oil mass	16:0	18:0	18:1	18:2	18:3
	— % dw —		g plant ⁻¹	— % oil —				
CF370G	20.04 ± 0.06	39.5 ± 0.16	41.58 ± 3.29	11.2 ± 0.04	3.2 ± 0.01	18.9 ± 0.08	57.9 ± 0.05	8.8 ± 0.04
CF700G	20.60 ± 0.05	39.1 ± 0.18	52.85 ± 4.66	11.0 ± 0.03	3.2 ± 0.02	18.6 ± 0.09	58.4 ± 0.06	8.8 ± 0.04
CF370C	19.98 ± 0.06	39.6 ± 0.15	31.24 ± 0.92	11.1 ± 0.04	3.3 ± 0.02	18.1 ± 0.17	58.5 ± 0.12	8.9 ± 0.08
CF700C	20.69 ± 0.09	39.8 ± 0.21	35.58 ± 0.92	11.0 ± 0.03	3.4 ± 0.01	19.6 ± 0.21	57.8 ± 0.16	8.3 ± 0.06
Source								
CO ₂	†	NS†	**	†	†	†	NS	†
Rooting environment	NS	*	†	*	†	NS	NS	***
CO ₂ by rooting environment	NS	NS	NS	NS	NS	†	†	†
Contrasts								
CF370G vs. CF700G	†	NS	**	†	**	NS	***	NS
CF370C vs. CF700C	†	NS	NS	***	**	†	†	†
CF370G vs. CF370C	NS	NS	*	*	†	†	†	NS
CF700G vs. CF700C	NS	*	†	NS	†	†	†	†

**P* ≤ 0.05.

***P* ≤ 0.01.

****P* ≤ 0.001.

†*P* ≤ 0.0001.

†NS, not significant at *P* ≤ 0.05.

particular crop, soybean at least, then it would seem that either rooting environment or nearly any planting density would serve. However, understanding the absolute differences resulting from the cultural methods reported here and elsewhere could significantly advance our grasp of the overall processes involved with plant responses to elevated CO₂.

Elevated CO₂ increased the oil mass per plant by 27% due to two factors: a large yield ER and an additional increase in oil concentration per seed. Whether or not the increase per seed under elevated CO₂ will persist at higher planting densities without input of additional resources is unknown. However, since increased seed production per unit land area with elevated CO₂ is commonly reported, it is likely that increased oil production per unit land area will occur in future environments. Finally, some of the temperature related questions raised here might be addressed more effectively in future if the container temperature range could be constrained to equal that of the ground.

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