Combined Effects of Elevated Atmospheric Carbon Dioxide and Ozone on Soybean Whole-Plant Water Use

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ABSTRACT / Increasing concentrations of atmospheric trace gases such CO_2 and O_3 might affect agricultural crop productivity in as yet unforeseen ways. A critical issue is how changes in these trace gas concentrations will affect agricultural hydrologic cycles. In order to address part of this question, a study was undertaken to test the effects of elevated atmospheric CO_2 and O_3 on whole-plant water loss. In a 2-year experiment, pot-grown soybean [*Glycine max* (L.) Merr., Essex] was exposed from emergence to maturity to reciprocal treatments of elevated CO_2 and O_3 in open-top field chambers. Plants were treated with charcoal-filtered air (CF) (18 nmol O_3 mol^{-1}) or CF air plus O_3 (72 nmol mol^{-1}) in combination with ambient (369 μmol mol^{-1}) or elevated CO_2 (718 μmol mol^{-1}). Whole-plant water loss was monitored by mea-

suring mass changes of potted plants placed on electronic balances in each chamber. Plants on the balances were harvested weekly to determine leaf area, and were replaced with another plant previously exposed to similar treatment conditions. Seasonal average daily whole-plant water loss was lower by 17% and 28% in plants treated with elevated CO_2 and O_3, respectively, compared with the control. Water loss from plants treated with both elevated CO_2 and O_3 was 22% lower than the control. Elevated CO_2 treatments suppressed water loss per unit leaf area by 28%, whereas water loss per unit leaf area was not significantly affected by the O_3–ambient CO_2 treatment. Water use efficiency for seed production was increased 45% in the elevated CO_2 treatments, but was not significantly affected by the O_3 treatments. These results suggest that whole-plant water use was lowered by elevated CO_2 because of decreased stomatal conductance despite 9% more leaf area. Decreased whole-plant water loss and seed production in the O_3-ambient CO_2 treatment was caused by suppressed growth and 28% less leaf area. Overall, elevated CO_2 counteracted the effects of O_3 on water use, growth, and yield. These results indicate that elevated CO_2 should help to buffer soybean crops against O_3 injury and water stress.

With increasing atmospheric concentrations of trace gases such as CO_2 and O_3 (Houghton and others 1996), a critical issue is how these changes will affect agricultural hydrologic cycles. An important question that arises is how whole-plant water loss will be affected by increasing CO_2 and O_3 levels. Results from a variety of controlled environment, greenhouse, and field experiments as well as crop model calculations indicate that the answer is not clear (Kimball and others 1993, Boote and others 1997, Polley 2002).

Elevated CO_2 typically lowers leaf conductance and transpiration of crop plants but increases leaf temperature, biomass, and leaf area (Kimball and others 1993,

Ainsworth and others 2002). Thus, the reduction in plant water loss caused by lower transpiration can be offset by increased leaf areas per unit ground area and higher leaf temperatures, which increase the leaf-to-air-vapor pressure gradient (Kimball and others 1993, Polley 2002). As a result, seasonal water use per unit of land area may be minimally affected by increasing concentrations of CO_2 (Kimball and others 1993, Boote and others 1997, Polley 2002). In contrast, production water use efficiency (WUE, the economic yield per unit water transpired) usually is improved by CO_2-enrichment (Polley 2002).

Ozone tends to suppress leaf conductance, biomass, and leaf area of crop plants (Miller 1988, Unsworth and Hogsett 1996, Olszyk and others 2000, Rudorff and others 2000). However, effects on conductance can be subtle, inconsistent, and subject to O_3-induced ontogenetic changes (Tingey and others 1994, Fiscus and others 1997). The resulting effect on seasonal whole-plant water use is unclear. Open-top field chamber

studies with alfalfa (*Medicago sativa* L.), cotton (*Gossypium hirsutum* L.), and bush bean (*Phaseolus vulgaris* L.) indicated that cumulative evapotranspiration was not significantly affected by O_3 (Temple and others 1988, Temple 1990, Tingey and others 1994). However, production WUE was lowered by O_3 in these studies primarily by reducing carbon assimilation and harvest biomass more than water loss. Increased leaf construction cost with O_3 suggested that the efficiency of both carbon and water use declined as well (Tingey and others 1994). However, these few experiments, only one of which directly measured plant water use (Tingey and others 1994), provide a limited assessment of how O_3 affects seasonal whole-plant water use, especially in combination with elevated CO_2 concentrations.

The combined effects of elevated CO_2 and O_3 on plant water use have received almost no attention. To our knowledge, no study has reported on the combined effects of elevated CO_2 and O_3 on cumulative transpiration or production WUE. In radish (*Raphanus sativus* L.) and spring wheat (*Triticum aestivum* L.), photosynthetic WUE was increased by elevated CO_2 and either not affected by O_3 until late in the experiment (Barnes and Pfirrman 1992, Donnelly and others 2000) or had values intermediate between the O_3-ambient CO_2 and elevated CO_2 treatments (Mulholland and others 1997). The decline in photosynthetic WUE in the O_3-CO_2 treatments was possibly caused by injury by O_3 (Barnes and Pfirrman 1992). However, these measurements indicate WUE at the leaf level, not the whole-plant level.

Therefore, this study was undertaken to test the effects of elevated atmospheric CO_2 and O_3 on seasonal whole-plant water loss and production WUE of soybean [*Glycine max* (L.) Merr.] treated under field conditions in open-top chambers.

Methods and Materials

The experiments were performed at a site 5 km south of Raleigh, NC, USA (36°N, 79°W). Soybean (cv. Essex) was planted on 31 May 2000 and 2001 and grown in pots containing 21 L of a 2:1:1 mixture of sandy loam soil:sand:Metro Mix 220 (Scotts Sierra Horticultural Products Co., Marysville, OH, USA; 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) (pH 6.2). Pot temperature fluctuation was moderated by a sleeve of 0.6-cm-thick bubble wrap coated on both sides with aluminum (Reflectix; Reflectix, Inc., Markleville, IN, USA) fit tightly around each pot. Plants were irrigated at 2200 h with

Table 1.   Seasonal 12-h (0800–2000 EST) daily average CO_2 and O_3 concentrations in the 2-year experiment (2000 and 2001)[a]

Growing season	Treatment	$[CO_2]$ (μmol mol^{-1})	$[O_3]$ (nmol mol^{-1})
2000	CF-A	368	25
	CF-CO$_2$	726	21
	O$_3$-A	365	72
	O$_3$-CO$_2$	715	70
2001	CF-A	371	13
	CF-CO$_2$	714	15
	O$_3$-A	371	73
	O$_3$-CO$_2$	715	72

[a]Plants were treated with charcoal-filtered air (CF) or CF air plus O_3 (O_3) in combination with ambient (A) or elevated CO_2 (CO_2).

drip tubes as needed to prevent visible signs of water stress during the day. Plants were fertilized biweekly with an aqueous solution containing 2.5 g of soluble fertilizer (10-30-20, N-P-K) (Peters Professional, Scotts-Sierra Horticultural Products Co.). The initial fertilization included 0.31 g L^{-1} of a micronutrient formulation (STEM, Scotts-Sierra Horticultural Products Co.). Insects and mites were controlled with applications of acephate (Valent USA Corp., Walnut Creek, CA, USA), bifenthrin (Whitmire Micro-Gen Research Laboratories, Inc., St. Louis, MO, USA), and abamectin (Syngenta Crop Protection, Inc., Greensboro, NC, USA). Plants were also treated with the fungicide mefenoxam (Syngenta Crop Protection, Inc.).

Plants were treated in 2.4-m tall $\times$ 3-m-diameter open-top field chambers from germination to physiological maturity (126 d) with reciprocal treatments of CO_2 and O_3. Plants were treated with charcoal-filtered air (CF) or CF air plus O_3 (O_3) in combination with ambient (A) or elevated CO_2 (CO_2) (Table 1). Ozone was produced by electrostatic discharge in dry O_2 (model GTC-1A, Ozonia North America, Elmwood Park, NJ, USA) and monitored using UV photometric O_3 analyzers (model 49, Thermo Environmental Instruments Co., Franklin, MA, USA). The O_3 analyzers were calibrated biweekly (model 49 PS calibrator, Thermo Environmental Instruments Co.). Ozone was added in a prescribed function based on historic measurements of ambient O_3 at our location to provide approximately 1.5 times ambient air O_3 concentrations 12 hours daily (0800–2000 h EST). Carbon dioxide was dispensed from a 12-ton liquid receiver 24 hours daily to provide approximately twice the ambient air concentration and was monitored with infrared CO_2 analyzers (model 6252, Li-Cor, Inc. Lincoln, NE, USA). The CO_2 monitors were calibrated biweekly with CO_2 standards. The air 10 cm below the top of the plant canopy was sam-

Table 2. Meteorological data for the 2-year experiment summarized by growing season and month[a]

Growing season	Month	Temperature (°C)	RH (%)	PPFD (mol m^{-2} d^{-1})	Rainfall (cm)
2000	June	28 ± 1	64 ± 3	44 ± 2	17
	July	28 ± 1	70 ± 2	38 ± 2	6
	August	28 ± 1	68 ± 2	40 ± 2	14
	September	24 ± 1	73 ± 2	29 ± 2	23
2001	June	27 ± 1	68 ± 2	45 ± 2	24
	July	27 ± 1	71 ± 3	41 ± 3	22
	August	29 ± 1	71 ± 2	42 ± 2	11
	September	24 ± 1	67 ± 3	28 ± 2	4

[a]Temperature and relative humidity (RH) values are ambient, daytime averages (sunrise to sunset). Photosynthetic photon flux density (PPFD) values are ambient daily integrals. Values are means ± s.e. Total monthly rainfall is also shown.

pled in each chamber through Teflon tubing, and the concentration of CO_2 and O_3 in it was measured every 30 minutes throughout the experiment. Ambient air temperature, relative humidity, daily total photosynthetic photon flux density (PPFD) and rainfall were monitored at a meteorological station at our location.

Whole-plant water loss was determined by measuring the mass change of a potted plant placed on an electronic balance (model B50AS20, Ohaus Corporation, Florham Park, NJ, USA) in each chamber. Evaporation from the pots was estimated by measuring mass changes of a soil-filled pot placed on an additional balance in one of the chambers. Soil in all pots was covered with 2 to 3 cm of small pine nugget mulch to reduce evaporation. Daily plant water loss was calculated as the difference in mass between 0600 and 2000 h of a potted plant, minus the mass difference of the soil-filled pot over the same period. Increases in pot mass caused by precipitation events were deleted from the data sets as practicable. Measurements of daily plant water loss began at 7 weeks after planting in both years of the experiment. Observations from a total of 50 days were included in the data sets from each year of the experiment. Plants on the balances were destructively sampled weekly to determine leaf area and were replaced with another plant previously exposed to similar treatment conditions in a separate chamber. The plant on the balance was surrounded by 12 border plants. The projected ground area occupied per plant in the experimental array was approximately 0.25 m² at maximum leaf area. Upon maturity, five plants in each chamber were harvested for yield. Average seed yield from the five plants was used as the chamber replicate value. Production WUE was calculated as grams of seed yield per plant per kilogram average daily water loss per plant (average value for weeks 8 to 17 after planting).

The treatments consisted of all factorial combinations of two CO_2 levels and two O_3 levels, and the treatments were assigned to chambers in a completely randomized design. In the 2000 growing season, one chamber was used for each treatment. In the 2001 growing season, there were two replicate chambers per treatment. The 2 years were combined for the statistical analysis. In each year, plant growth and water use variables were summarized on a weekly and seasonal basis. Data were checked for homogeneity of variance and normality of distribution. A ln transformation was applied to the data before statistical analysis. Treatment effects and means for weekly plant leaf area and water use variables were estimated using a repeated measures model in which chambers constituted the whole plots and week was the repeated factor (SAS Proc Mixed) (Littell and others 1996). The model included main effects and two-factor interactions of year, CO_2, and O_3 at the whole plot level, and interactions between the whole plot factors and the effect of weeks. Several models for the within-chamber correlation over time were examined, and a compound symmetric covariance structure was found to fit best. The seasonal average daily water loss, yield, and WUE were analyzed using a three-factor analysis of variance for the effects of year, CO_2, and O_3 (SAS Institute 1985). Seasonal averages were calculated as the average of the weekly data.

Results

Average seasonal gas treatment concentrations and average monthly daytime air temperatures, relative humidity, and PPFD were similar between the 2000 and 2001 growing seasons (Tables 1, 2). Rainfall was higher in July and lower in September in 2001 compared with 2000, although plants were irrigated daily. However, main effects of year in the 2-year experiment were significant for all growth and water use variables [Table 3 (weekly comparisons), Table 4 (seasonal comparisons)]. The significant year effect was attributed to consequences of greater plant size in 2001 compared with 2000. For example, average daily water loss per

Table 3.   Statistical analysis summary for treatment effects by week[a]

Source	df	DWL	DWLA	Leaf area
Year	1	0.001	0.051	0.001
CO_2	1	0.112	0.003	0.001
O_3	1	0.001	0.416	0.001
Year $\times$ CO_2	1	0.049	0.171	0.477
Year $\times$ O_3	1	0.026	0.487	0.100
CO_2 $\times$ O_3	1	0.003	0.469	0.006
Year $\times$ CO_2 $\times$ O_3	1	0.284	0.387	0.843
Week	9	0.001	0.001	0.001
Week $\times$ year	9	0.001	0.004	0.574
Week $\times$ CO_2	9	0.101	0.983	0.919
Week $\times$ O_3	9	0.179	0.666	0.502
Week $\times$ CO_2 $\times$ O_3	9	0.262	0.615	0.110

[a]Significance (probability) of effects of CO_2, O_3, and the combined gases on daily water loss per plant (DWL), daily water loss per unit leaf area (DWLA), and leaf area from 8 to 17 weeks after planting. Data combined for the 2-year experiment.

plant (DWL), leaf area, and yield per plant was 41, 28, and 72% higher, respectively, in 2001 compared with 2000. Also, year $\times$ gas treatment interactions for DWL and daily water loss per unit leaf area (DWLA) were statistically significant (Tables 3, 4). Significant year $\times$ CO_2 and year $\times$ O_3 interactions for DWL and DWLA were attributed to relatively larger treatment effects in 2000 compared with 2001. In 2000, for example, average DWL in the O_3-A treatment was 64% of that in the CF-A treatment, whereas in 2001 it was 77% of that in the CF-A treatment. Otherwise, treatment responses differed by 5% or less between years of the experiment. Significant year $\times$ week interactions for the water use variables reflected the fact that responses differed between years of the experiment as the growing season progressed (Table 3).

Average DWL on a weekly and seasonal basis was lower in the elevated CO_2 and O_3 treatments compared with the control treatment (Figures 1, 2). While the main effect of O_3 was statistically significant, the CO_2 treatment effect was not, although the CO_2 $\times$ O_3 interaction was significant (Tables 3, 4). The significant interaction and pattern of treatment responses indicated that elevated CO_2 effects were significantly different from those in the CF-A treatment, but not in the O_3-A treatment (see segregated treatment comparisons in Table 4). The treatment interaction is more easily visualized in Figure 2A, where average DWL for the entire experimental period is shown. Seasonal average DWL was lower by 17% and 28% in plants treated with elevated CO_2 and O_3, respectively, compared with the control. Water loss from plants treated with both elevated CO_2 and O_3 was 22% lower than the control.

For DWL, differential treatment responses became evident 9 weeks after planting and persisted through the remainder of the experiment (Figure 1A). Consistent treatment effects were indicated by the lack of significant week $\times$ gas treatment interactions (Table 3). Plants entered reproductive phase at 9 weeks after planting, and maximum DWL occurred 1 to 2 weeks later (Figure 1A). Daily water loss per plant declined thereafter in all treatments until the end of the experiment.

DWLA on a weekly and seasonal basis was significantly lower in the elevated CO_2 treatments compared with the ambient CO_2 treatments (Figures 1B and 2B; Tables 3, 4). Elevated CO_2 treatments suppressed seasonal average DWLA by 28%, whereas it was not significantly affected by the O_3 treatments (Figure 2B, Table 4). These responses were due in part to treatment effects on leaf area (Figure 1C). Main effects of CO_2 and O_3 on leaf area as well as the CO_2 $\times$ O_3 interaction were statistically significant (Tables 3, 4). Average treatment responses and segregated treatment comparisons (Table 4) indicated that leaf areas in the CO_2 treatments were 9% greater than in the CF-A treatment, whereas leaf area per plant in the O_3-A treatment was suppressed 28%. Both effects were statistically significant. Leaf area in the O_3-CO_2 treatment was not significantly different from that in the CF-CO_2 treatment. This indicated that the lower DWL in the O_3-A treatment was caused by lower leaf area, with the result that DWLA was about equal in the O_3-A and CF-A treatments (Figures 1A and 1B, 2A and 2B). Lower DWL and DWLA in the CO_2 treatments occurred despite a 9% increase in leaf area, presumably because of reduced stomatal conductance.

Consistent treatment effects on DWLA and leaf area per plant during the experimental period were indicated by the lack of significant week $\times$ gas treatment interactions (Table 3). Maximum DWLA occurred between 8 and 11 weeks after planting, followed by a decline to a lower level thereafter in all treatments until the end of the experiment. Maximum leaf area occurred between 12 and 13 weeks after planting.

Seed yield per plant was about 16% higher in the elevated CO_2 treatments compared with the control treatment (Figure 2C, Table 4). In contrast, seed yield was 34% lower in the O_3-A treatment compared with the CF-A treatment. The significant main effects and interaction between CO_2 and O_3 indicated that there was no significant effect of O_3 in the CO_2-O_3 treatment (Table 4). Average WUE was 45% higher in the elevated CO_2 treatments, but neither the main O_3 effect nor CO_2 $\times$ O_3 interaction was statistically significant (Figure 2D, Table 4).

Table 4.   Statistical analysis summary for treatment effects by season[a]

Source	df	DWL	DWLA	Leaf area	Yield	WUE
Year	1	0.001	0.006	0.001	0.001	0.003
CO$_2$	1	0.186	0.001	0.001	0.001	0.001
O$_3$	1	0.001	0.142	0.001	0.002	0.699
Year $\times$ CO$_2$	1	0.185	0.046	0.104	0.262	0.833
Year $\times$ O$_3$	1	0.056	0.186	0.414	0.432	0.438
CO$_2$ $\times$ O$_3$	1	0.004	0.320	0.001	0.004	0.148
Error	5					
A vs. CO$_2$ in CF	1	0.006	0.001	0.016	0.023	0.001
A vs. CO$_2$ in O$_3$	1	0.066	0.001	0.001	0.001	0.001
CF vs. O$_3$ in A	1	0.001	0.099	0.001	0.001	0.202
CF vs. O$_3$ in CO$_2$	1	0.075	0.632	0.158	0.534	0.422

[a]Significance (probability) of effects of CO$_2$ O$_3$, and the combined gases on daily water loss per plant (DWL), daily water loss per unit leaf area (DWLA), leaf area, seed mass per plant (Yield), and production water use efficiency (WUE). Values for DWL, DWLA, and leaf area indicate significance of comparisons among seasonal treatment averages calculated from weeks 8 though 17 after planting. Significance of effects of CO$_2$ in charcoal-filtered (CF) and CF plus O$_3$ (O$_3$) treatments as well as significance of effects of O$_3$ in ambient (A) and elevated CO$_2$ (CO$_2$) treatments are also shown.

Discussion

The potential benefit of atmospheric CO$_2$ enrichment on crop water use might be the most important consequence of elevated CO$_2$ concentrations for agriculture (Morison 1993, Unsworth and Hogsett 1996). Plant water relations appear to be improved by elevated CO$_2$ concentrations because of small increases in leaf water potential combined with larger decreases in the turgor loss potential, which together serve to buffer the plant against water stress (Allen and others 1998). In addition, elevated CO$_2$ concentrations consistently increase production WUE, a critical measure of the realized benefit of increased CO$_2$, although the magnitude of this increase is difficult to predict (Polley 2002). There might also be a decrease in whole-plant water use with elevated CO$_2$.

A review by Cure and Acock (1986) showed that for C3 crops a doubling of atmospheric CO$_2$ concentrations decreased transpiration more than 20%, although they cautioned that results based on single plant experiments might overestimate the effect of elevated CO$_2$ on transpiration of field crops. Although the results from our study indicated that whole-plant water use was lowered by elevated CO$_2$, it remains uncertain whether increasing concentrations of CO$_2$ will lower water use of crops in the field. Available field measurements of crop transpiration indicate a pattern of little effect of elevated CO$_2$ on total water use (Polley 2002). In our study, because the plants were grown in large pots in order to measure water loss, water losses caused by deep percolation and runoff were not considered, but would have to be evaluated when effects on a field scale were assessed. Evaporation from the pots was accounted for by measuring mass loss of a soil-filled pot, but evapora-

tion from a field would be more complex as growth and canopy closure occurred. With canopy closure, there is a tendency for whole crop energy balance to govern area-wide evapotranspiration. In addition, growth and canopy closure could be accelerated by elevated CO$_2$ concentrations and thus lower evaporative water losses (Polley 2002). Effects and feedbacks between leaf and air temperatures, vapor pressure gradients, and aerodynamic conductances also would apply (Unsworth and Hogsett 1996, Polley 2002). A soil/vegetation/atmosphere (energy) transfer model indicated negligible changes in simulated transpiration for soybean with a doubling of atmospheric CO$_2$ concentration (Carlson and Bunce 1996).

However, using a model that also included soil evaporation as a parameter, Morison (1993) calculated that water use by a crop such as soybean would decrease 10 to 15% with twice-ambient CO$_2$ concentration. Using the CROPGRO-soybean model with full energy balance and control of stomatal conductance, the predicted seasonal transpiration decreased 7 to 16% and WUE increased 53 to 61% with a doubled CO$_2$ concentration for soybean grown in three different years in Florida and Ohio with both irrigation and natural precipitation conditions (Boote and others 1997). Our estimate of 17% lower plant water loss with elevated CO$_2$ is generally consistent with these model calculations.

In a greenhouse experiment, average daily water loss of spaced, pot-grown, 4-week-old soybean plants was reduced 25% by 700 μmol CO$_2$ mol^{-1} compared with ambient CO$_2$ over a 17-day treatment period (Serraj and others 1999). Results from a controlled environment experiment with pot-grown wheat, maize (*Zea mays* L.) and cotton treated with ambient and 700 μmol
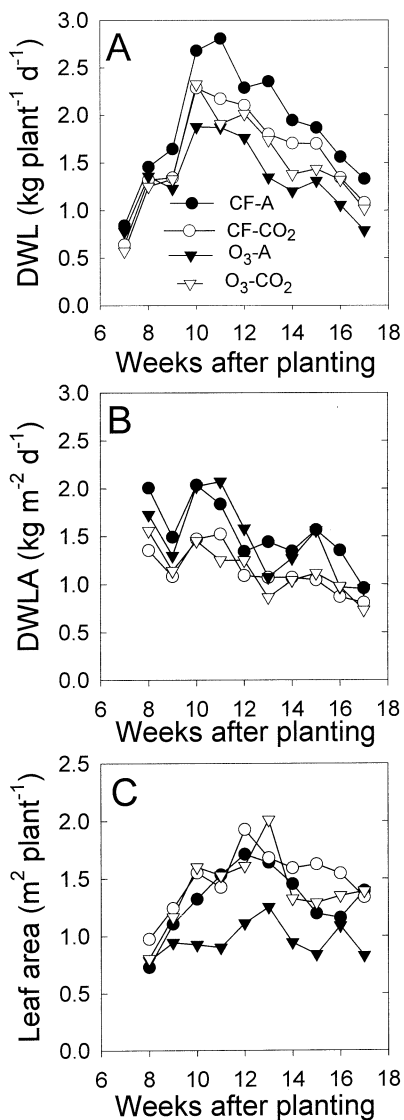
Figure 1. Weekly mean daily water loss per plant (DWL) **(A)**, daily water loss per unit leaf area (DWLA) **(B)**, and leaf area per plant **(C)** between 8 and 17 weeks after planting. Combined data for the 2-year experiment.

Figure 2. Daily water loss per plant (DWL) **(A)** and daily water loss per unit leaf area (DWLA) **(B)** averaged over the period between 8 and 17 weeks after planting. Seed mass per plant (Yield) **(C)** and production water use efficiency (WUE) **(D)** are also shown. Combined data for the 2-year experiment. Values are means ± 95% confidence intervals.

CO_2 mol^{-1} for up to 101 days indicated that total evapotranspiration of well-watered plants was reduced as a result of CO_2 enrichment by 17, 22, and 6% for wheat, maize, and cotton, respectively (Kang and others 2002). For well-watered cotton grown in open-top chambers, Kimball and others (1993) found that seasonal water use was decreased less than 10% by elevated CO_2 concentrations based on lysimeter data. Average daily transpiration per plant or per unit leaf area from reproductive-phase soybean plants treated with twice-ambient CO_2 concentrations in open-top chambers was reduced about 43% compared with plants treated with
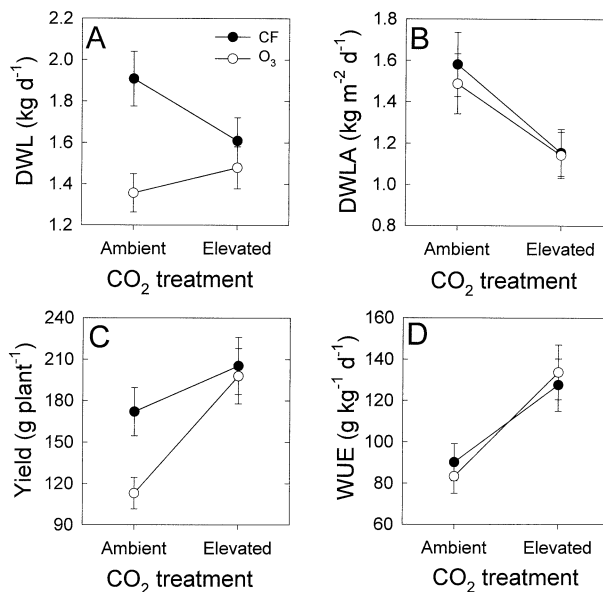
ambient CO_2 concentrations, based on sap flow measurements (Dugas and others 1997).

In our experiment, the initial decrease in DWL in the elevated CO_2 treatments occurred at 9 weeks after planting (Figure 1A). Thereafter, DWL in the elevated CO_2 treatments was less than that in the control treatment. These responses might be related to a decline in carboxylation efficiency and an ontogenetic shift forward in development because of elevated concentrations of CO_2 (Reid and Fiscus 1998) that resulted in lower plant water use. Stomatal conductance of soybean leaves tends to decline as fully expanded leaves age (Reich and others 1985, Fiscus and others 1997). Therefore, season-long estimates of elevated CO_2 effects on crop water loss might not fully reflect physiological and ontogenetic changes that occur during the growing season.

In our study, atmospheric CO_2 enrichment increased leaf area, seed yield, and WUE, which has been observed in previous studies with soybean (Boote and others 1997, Fiscus and others 2001, Ainsworth and others 2002) and a number of other crop species (Kimball and others 1993, Unsworth and Hogsett 1996, Olszyk and others 2000, Amthor 2001). Ozone suppressed leaf area and yield at ambient CO_2 concentrations, which also has been observed in previous studies with soybean and other crop species (Miller 1988, Heagle

1989, Heagle and others 1998, Miller and others 1998, Olszyk and others 2000, Fiscus and others 2001, 2002).

The suppression by O_3 of leaf area was likely responsible for the lower DWL. Our finding that DWLA was not significantly different in the CF-A and O_3-A treatments suggested that O_3 effects on leaf conductance and leaf-to-air vapor pressure deficits were insufficient to affect average transpiration. Tingey and others (1994) found that leaf area of bush bean plants was reduced by O_3, but effects on cumulative transpiration were inconsistent. The inconsistency was attributed to variability in the response of daily transpiration to O_3. In contrast, results from our study showed a very consistent and close relationship between average weekly DWLA in the CF-A and O_3-A treatments (Figure 1B).

Suppressive effects of O_3 on plant water loss at the field scale, however, might be complicated by indirect effects of reduced plant growth and consequently increased soil evaporation. Reduced growth will probably reduce soil water extraction, but this could be partially offset by increased evaporation. For example, O_3 had no apparent effect on cumulative evapotranspiration of an alfalfa crop despite a reduction in leaf transpiration (Temple and Benoit 1988). Possibly, evaporation increased in O_3-treated plots because of exposed soil as a result of premature leaf abscission. Production harvest mass declined more than lower transpiration reduced evapotranspiration so WUE was lower in O_3 treatments (Temple and Benoit 1988). In our study, however, production WUE in the O_3-A treatment was not significantly different from the control treatment. Production WUE was reduced from 12 to 25% by O_3 in other crops such as Alfalfa, cotton and bush bean (Temple and Benoit 1988, Temple 1990, and others 1994).

In combination, elevated CO_2 concentration generally counteracted the deleterious effects of O_3 on soybean leeaf area and yield. This response has been observed in previous studies with soybean and other crop species (Unsworth and Hogsett 1996, Heagle and others 1998, Miller and others 1998, Olszyk and others 2000, Rudorff and others 2000, Fiscus and others 2001, 2002). Water loss per plant and per leaf area in the O_3-CO_2 treatments generally mirrored responses in the CF-CO_2 treatments (Figures 1, 2). The ameliorating effects of elevated CO_2 concentration on O_3 injury thus inclde effects on whole-plant water use.

In conclusion, this experiment indicated the twice-ambient CO_2 concentrations resulted in an average 17% decrease in water loss per plant even though leaf area was slightly stimulated by elevated CO_2. Our results suggest that decreased water use would prmarily occur during reproductive growth. However, application of these results to field situations requires addi-

tional experimentation with in-ground grown plants. Overall, the impact of O_3 was suppressed soybean growth and yield with a commensurate decrease in plant water use. The deleterious effects of elevated O_3 were generally negated by elevated CO_2. conversely, effects of elevated CO_2 on plant responses were not significantly affected by elevated O_3.

Acknowledgements

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