An In Vivo Analysis of the Effect of Season-Long Open-Air Elevation of Ozone to Anticipated 2050 Levels on Photosynthesis in Soybean[1]

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Rising atmospheric carbon dioxide concentration ($[CO_2]$) is widely recognized, but less appreciated is a concomitant rise in tropospheric ozone concentration ($[O_3]$). In industrialized countries, $[O_3]$ has risen by 0.5% to 2.5% per year. Tropospheric $[O_3]$ is predicted to reach a global mean of >60 nL L^{-1} by 2050 with greater averages locally. Previous studies in enclosures suggest that this level of $[O_3]$ will decrease leaf photosynthesis, thereby limiting growth and yield of *Glycine max* L. Merr. SoyFACE (Soybean Free Air gas Concentration Enrichment) is the first facility to elevate atmospheric $[O_3]$ (approximately 1.2× current) in replicated plots under completely open-air conditions within an agricultural field. Measurements of gas exchange (assimilation versus light and assimilation versus intercellular $[CO_2]$) were made on excised leaves from control and treatment plots ($n = 4$). In contrast to expectations from previous chamber studies, elevated $[O_3]$ did not alter light-saturated photosynthesis (A_{sat}, $P = 0.09$), carboxylation capacity ($V_{c,max}$, $P = 0.82$), or maximum electron transport (J_{max}, $P = 0.66$) for the topmost most recently fully expanded leaf at any stage of crop development. Leaves formed during the vegetative growth stage did not show a significant ozone-induced loss of photosynthetic capacity as they aged. Leaves formed during flowering did show a more rapid loss of photosynthetic capacity as they aged in elevated $[O_3]$. A_{sat}, $V_{c,max}$, and J_{max} ($P = 0.04$, 0.004, and 0.002, respectively) were decreased 20% to 30% by treatment with ozone. This is noteworthy since these leaves provide photosynthate to the developing grain. In conclusion, a small (approximately 20%) increase in tropospheric $[O_3]$ did not significantly alter photosynthetic capacity of newly expanded leaves, but as these leaves aged, losses in photosynthetic carbon assimilation occurred.

In the northern mid-latitudes (36°–59°N), surface ozone has risen from an estimated preindustrial 10 nL L^{-1} to an average regional concentration of almost 60 nL L^{-1} today (1%–2% per year; Chameides et al., 1994; Prather et al., 2001). Although once considered a problem of conurbations, long-distance and even intercontinental ozone transport has resulted in a steady increase in rural areas hundreds and thousands of kilometers from the original sources of pollution (Prather et al., 2001; Gregg et al., 2003; Prather et al., 2003). Nearly one-quarter of the Earth's surface is currently at risk from mean tropospheric ozone in excess of 60 nL L^{-1} during midsummer with even greater local concentrations occurring (Fowler et al., 1999a, 1999b). A compilation of 10 different models projects that July surface ozone concentration ($[O_3]$) in the northern mid-latitudes is likely to rise by a further 20% over the next 50 years (Prather et al., 2001, 2003). The current mean global concentration of 60 nL L^{-1} is well above the mean concentration of 40 nL L^{-1} that has been determined as a threshold for damage to sensitive plant species (Fuhrer et al., 1997; Mills et al., 2000). For plants grown at chronic background $[O_3]$ concentrations (40–80 nL L^{-1}), substantial invisible damage, i.e. decreased photosynthetic rates, occurs in many plants before visible symptoms typically manifest at higher levels of ozone (>200 nL L^{-1}; for review, see Ashmore, 2002; Long and Naidu, 2002). The world's most widely planted dicotyledonous crop (FAO-UN, 2002), soybean (*Glycine max* L. Merr.), is among the more ozone sensitive species (for review, see Ashmore, 2002). Mean July ozone levels are projected to increase over this century by 30 nL L^{-1} in the midwest U.S. and by 50 nL L^{-1} in eastern China (Prather et al., 2003), two of the largest soybean production areas of the globe (FAO-UN, 2002).

In a meta-analysis of 53 peer-reviewed studies of the effects of elevated $[O_3]$ (approximately 70 nL L^{-1} compared to ozone-free air) on soybean photosynthesis and production, elevated $[O_3]$ was found to significantly decrease leaf net carbon assimilation (*A*) by 23% in soybean (Morgan et al., 2003). Parallel large decreases in leaf total nonstructural carbohydrates were observed with decreased photosynthesis, suggesting that production was limited by photoassimilate supply (Morgan et al., 2003). Therefore, decreased photosynthesis appears to be the key change driving yield losses
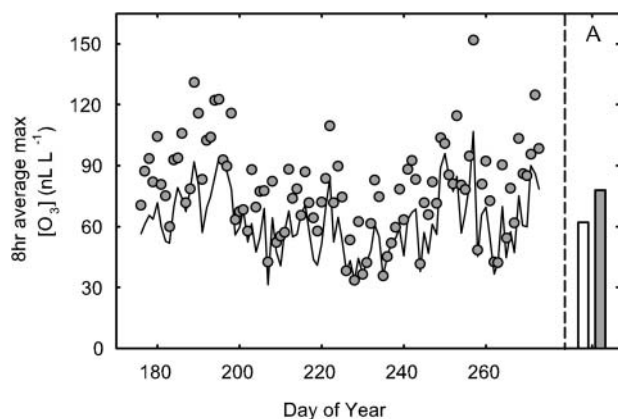
Figure 1. Maximum 8-h average $[O_3]$ for ambient (black line) and elevated $[O_3]$ treatments (gray symbol) and seasonal average $[O_3]$ in control (62 nL L^{-1}; white bar) and elevated (75 nL L^{-1}; gray bar) treatments. On some dates, 225 and following, some ambient symbols are occluded by elevated treatment symbols due to shutdown of the treatment system (see "Materials and Methods").

(l) was not affected (Fiscus et al., 1997; Zheng et al., 2002) or did not decrease photosynthesis (Farage and Long, 1999). These findings, however, are based primarily on controlled environment experiments, which can substantially modify the plant responses to environmental conditions (McLeod and Long, 1999; Ainsworth et al., 2002; Jablonski et al., 2002).

Projected ozone vulnerability of soybean and other crops is based on extensive and detailed studies conducted in controlled environments and in open-top chambers (for review, see Ashmore, 2002; Morgan et al., 2003). Lacking is confirmation that effects observed in these modified environments will also be evident in plants grown in the open-air field conditions. In open-top chambers, air is continuously stirred and forced through the plant canopy, thereby possibly increasing the coupling of lower leaves to the bulk atmosphere and exposing leaves to higher $[O_3]$ than they normally receive in undisturbed canopies. Similarly, in the natural environment when the air is
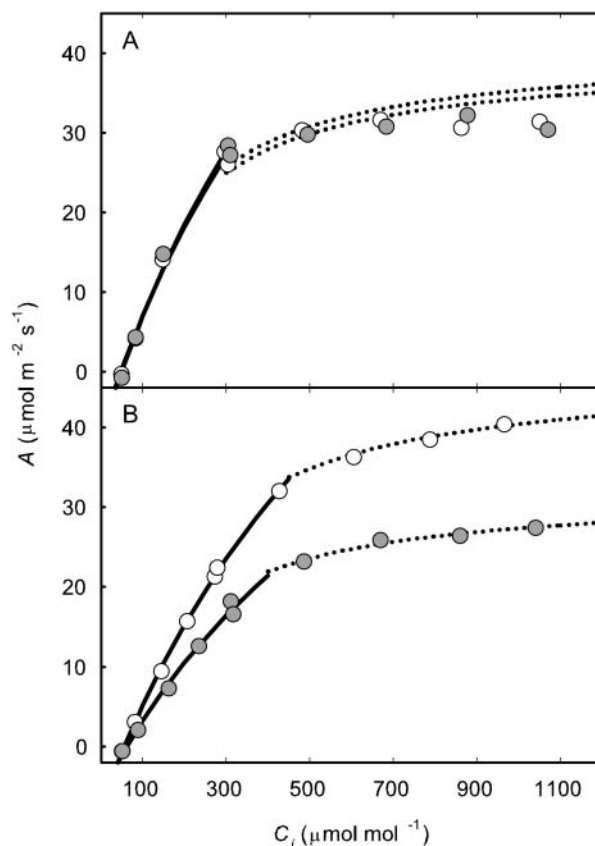
in soybean subjected to elevated $[O_3]$. For soybean grown in open-top chambers, elevated $[O_3]$ decreased A in newly expanded leaves over the growing season, with the greatest decreases occurring during pod filling (Mulchi et al., 1992; Reid and Fiscus, 1998). Additionally, previous studies with soybean (Mulchi et al., 1992; Booker et al., 1997; Fiscus et al., 1997) and other crops (McKee et al., 1995) have shown that as leaves age, cumulative exposure to ozone impairs photosynthesis.

Typically, in field-grown plants, photosynthetic assimilation in saturating light (A_{sat}) is limited by the maximum rate of carboxylation ($V_{c,max}$), which reflects the in vivo activity of Rubisco (Sage, 1994; Rogers and Humphries, 2000). Decreases in the activity and amount of Rubisco, rather than in electron transport and capacity for ribulose 1,5-bisphosphate (RuBP) regeneration, are primarily responsible for the deleterious effects of ozone on A (Farage et al., 1991; McKee et al., 1995; Pell et al., 1997; Farage and Long, 1999; Noormets et al., 2001; Zheng et al., 2002). Elevated $[O_3]$ has been shown to decrease $V_{c,max}$ in soybean, and these decreases become progressively larger over the growing season (Reid and Fiscus, 1998). The maximum rate of electron transport (J_{max}) for RuBP regeneration is the other major photosynthetic rate-limiting process (Sage, 1990). Ozone-induced declines in $V_{c,max}$ were not reflected in concomitant losses in J_{max}, suggesting that the thylakoid membrane capacity for electron transport was not impaired enough to detect via gas-exchange methods (Farage and Long, 1999).

In addition to effects on Rubisco and RuBP regeneration, other factors that could potentially affect A are decreased stomatal aperture (Zheng et al., 2002) and conductance (McKee et al., 1995; Fiscus et al., 1997; Farage and Long, 1999; McKee et al., 2000). Although stomatal aperture and conductance were decreased by elevated $[O_3]$ treatments, the stomatal limitation to A

Figure 2. Example responses of A/C_i from soybean grown in current (62 nL L^{-1} 8-h daily average; white symbol) or elevated (approximately 1.2 × current; gray symbol) $[O_3]$. Assimilation rates are for the topmost fully expanded trifoliate on DOY 225 (A) and leaves from that same cohort after 26 d of elevated $[O_3]$ treatment (DOY 251; B). The $V_{c,max}$ (solid lines) was fit to the initial portion of the response and J_{max} (dotted lines) was fit using the photosynthetic model of Farquhar et al. (1980) with temperature corrections by Bernacchi et al. (2001, 2003) as described previously (Long and Bernacchi, 2003).
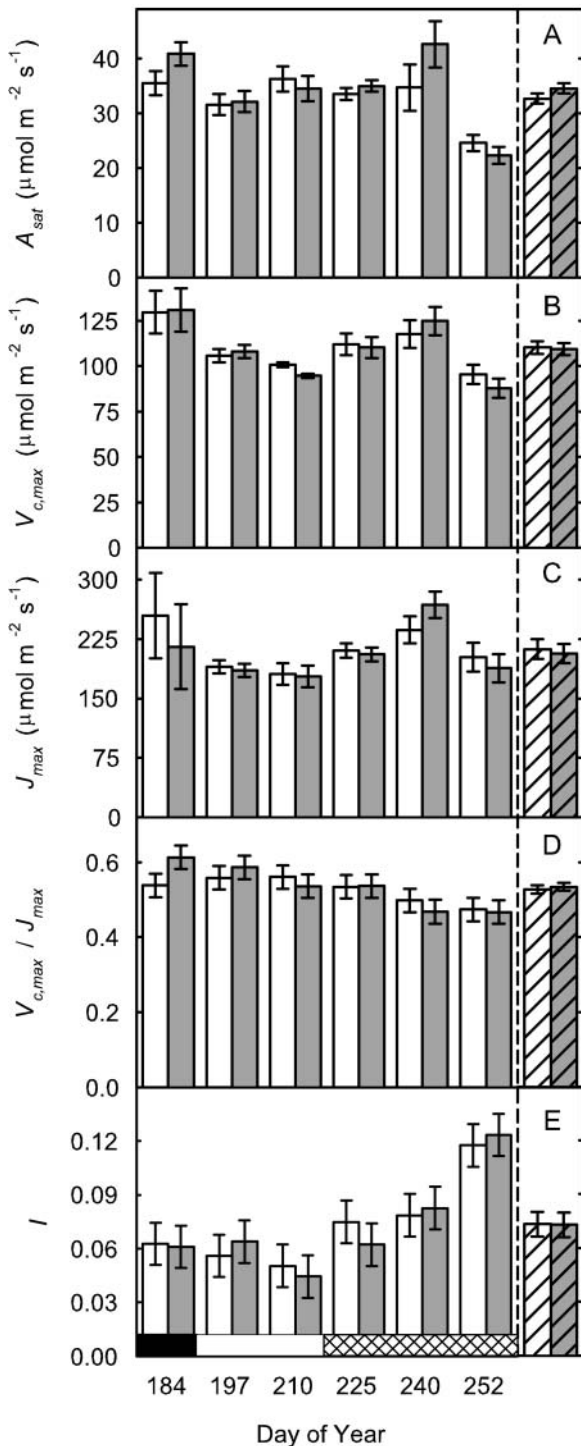
Figure 3. Photosynthetic parameters from the topmost fully expanded trifoliate from soybean grown in current (62 nL L^{-1} 8-h daily average; white bar) or elevated (approximately 1.2 × current; gray bars) [O_3] over the growing season. Parameters are A_{sat} (A), $V_{c,max}$ (B), J_{max} (C), $V_{c,max}/J_{max}$ (D), and l (which was calculated as percentage difference between potential photosynthesis at growth [CO_2] and actual photosynthesis; E). Season averages are reported as the striped pair of bars at right of each figure. Each bar is the least squared mean ($n = 4$), and the error bars are ± the SE of the difference in least square means. Significant differences (P value ≤ 0.05) are starred. Developmental

still, [O_3] within or near the canopy is likely depleted; a condition that could not develop in the stirred air of a chamber. While enclosure studies have provided important insights into mechanisms, meaningful extrapolation of experimental results to real-world agriculture may require validation in systems where perturbations of the soil-plant-atmosphere continuum are minimal, such as Free-Air gas Concentration Enrichment (FACE) systems (McLeod, 1995; McLeod and Long, 1999).

We have used such an open-air treatment facility (Ainsworth et al., 2004; Rogers et al., 2004; www.soyface.uiuc.edu) to determine how photosynthetic capacity in soybean responded to elevated [O_3] exposure in the field and to further expand on previous chamber studies. Specifically, we predicted that (1) with open-air [O_3] elevation, photosynthetic capacity losses would be less severe than observed in prior open-top and controlled environment chamber studies, (2) decrease in A_{sat} would result primarily from decreased $V_{c,max}$, (3) ozone-induced decrease in photosynthesis would increase with leaf age due to cumulative exposure, and (4) elevated [O_3] would not alter l. Over the 2002 growing season, leaf photosynthetic parameters were measured to assess these potential photosynthetic alterations caused by elevated [O_3].

RESULTS

The ozone fumigation system provided a consistent treatment across the growing season. The season mean of the maximum daily 8-h average [O_3] was 62 nL L^{-1} for the control plots and 75 nL L^{-1} for the elevated [O_3]. Daily 8-h average concentrations for both the ambient and elevated [O_3] treatments are shown in Figure 1. The effective treatment over the season was 1.2× ambient [O_3]. Based on 1-min average concentrations, the achieved elevation was within ±10% of the set point concentration 74% of the time and within ±20% of the set point 90% of the time.

Photosynthesis Was Not Inhibited by Ozone in Newly Expanded Leaves

On day 225 (early pod-filling stage), there was no difference in the A to intercellular carbon dioxide concentration ([CO_2]) ratio (A/C_i) response curves of a recently expanded leaf grown in current ambient compared with elevated [O_3] (Fig. 2A). Photosynthesis of newly expanded leaves was measured on six dates across the growing season from vegetative growth through flowering to the late stages of pod filling. At all stages, except final pod filling, photosynthetic rates (A_{sat}) were high and photosynthetic capacities, as in-

stages are represented by the horizontal bar: vegetative (black), flowering (white), and pod filling (cross-hatched).

dicated by $V_{c,max}$ and J_{max} values, were at the upper end of reported ranges for these variables (Wullschleger, 1993). However, there was no evidence of any effect of elevated $[O_3]$ on A_{sat}, $V_{c,max}$, or J_{max} on any of these six dates covering all major growth stages of the crop (Fig. 3; Table I). This confirms the inference from the single A/C_i responses illustrated (Fig. 2A). Although l rose on the final sampling date, during late pod filling, there was again no effect of growth in elevated ozone (Fig. 3E; Table I). A very small but significant decrease in photochemical quenching (qP) was observed (Fig. 4D; Table I) but was not reflected in A_{sat}. The maximum apparent quantum efficiency ($\phi_{CO2,max}$) averaged approximately 0.06 throughout, which is close to the maximal value expected in normal air (Long et al., 1993), and was completely unaffected by elevated $[O_3]$ (Fig. 4A; Table I). The maximum light-adapted apparent quantum efficiency of PSII ($\phi_{PSII,max,l}$), electron transport at growth $[CO_2]$ and saturating light (J_{sat}), and probability of an absorbed photon reaching an open PSII reaction center (F_v'/F_m') were all similarly unaffected by growth in elevated $[O_3]$ (Fig. 4; Table I).

Prolonged Ozone Exposure Resulted in Significant Inhibition of Photosynthesis in Older Leaves during Pod Filling

Two leaf cohorts were tracked from completion of expansion to senescence; the first was formed during vegetative development (V5, day of year [DOY] 185) and the second during pod filling (R4, DOY 225). In the vegetative leaf cohort, there was no significant effect of ozone on any photosynthetic parameter, except l (Fig. 5; Table II). But significant losses due to elevated ozone were apparent in A_{sat}, $V_{c,max}$, and J_{max} as this second cohort of leaves aged (Figs. 2B and 5, A–C), and this was confirmed by significant treatment by age interactions (Table II). There was also a small but significant decrease in $V_{c,max}/J_{max}$ (Fig. 5D), implying

a slightly greater effect of the treatment on in vivo Rubisco activity relative to capacity for RuBP regeneration. There was no significant effect of ozone on l in this cohort, but the significant decrease in A_{sat} with age was paralleled by significant losses in J_{sat} and qP (Fig. 6, B and D; Table II). Capacity for light-limited photosynthesis, as indicated by $\phi_{CO2,max}$, was decreased significantly by elevated ozone only on the final date on which this cohort was examined (Fig. 6A), suggesting that impairment of light-limited photosynthesis occurred later than impairment of processes determining light-saturated photosynthetic rate.

DISCUSSION

To our knowledge, this is the first study to examine the effects of elevated $[O_3]$ on photosynthesis of soybean, or of any arable crop, under fully open-air conditions. A statistical review of 53 independent published reports from enclosure studies of elevated $[O_3]$ effects on A_{sat} of newly expanded leaves suggested that growth at approximately 70 nL L^{-1} ozone caused a 20% decrease in A_{sat} relative to photosynthetic rates in plants grown under ozone-free air (Morgan et al., 2003). A practical disadvantage of FACE is that the control is the current $[O_3]$, and, unlike chamber studies, it is not possible to deplete the gas concentration in the control, making direct comparisons with these prior studies impossible. Therefore, FACE is useful in providing realistic field comparisons between current and future projected $[O_3]$, but cannot reveal the effects of an ozone-free atmosphere. However, given that 40 nL L^{-1} is the threshold for any ozone effects on plants (Fuhrer et al., 1997) and that above this threshold, damage increases linearly with concentration (Mills et al., 2000; Ashmore, 2002), we would expect that physiological effects would follow these whole-plant responses. Therefore, the $[O_3]$ in-

Table I. *Analysis of variance of fixed effects on photosynthetic and gas-exchange parameters of topmost fully expanded leaves taken from field-grown soybean in current and elevated $[O_3]$*

The F statistic and associated P values were calculated from a mixed model of a repeated measure analysis in a randomized complete block design ($n = 4$).

|	Day of Year		Treatment		Day of Year by Treatment	
	F	P Value	F	P Value	F	P Value
A_{sat}	56.42	<0.0001	3.96	0.0881	3.20	0.0531
$V_{c,max}$	22.77	<0.0001	0.06	0.8151	2.06	0.0959
J_{max}	14.32	<0.0001	0.21	0.6593	1.27	0.3257
C_i inflection	26.01	<0.0001	2.26	0.1832	1.94	0.1164
$V_{c,max}/J_{max}$	10.79	<0.0001	0.47	0.4972	1.84	0.1323
l	25.13	<0.0001	0.00	0.9530	0.57	0.7219
$\phi_{CO2,max}$	3.26	0.0382	0.65	0.4341	1.19	0.3632
θ	7.56	0.0106	1.74	0.2256	2.07	0.1847
J_{sat}	5.67	0.0039	0.07	0.8028	13.07	<0.0001
$\phi_{PSII,max,l}$	1.02	0.4422	2.52	0.2106	0.74	0.6034
qP	8.74	0.0005	8.15	0.0290	9.83	0.0003
F_v'/F_m'	4.00	0.0177	0.56	0.4882	0.47	0.7928
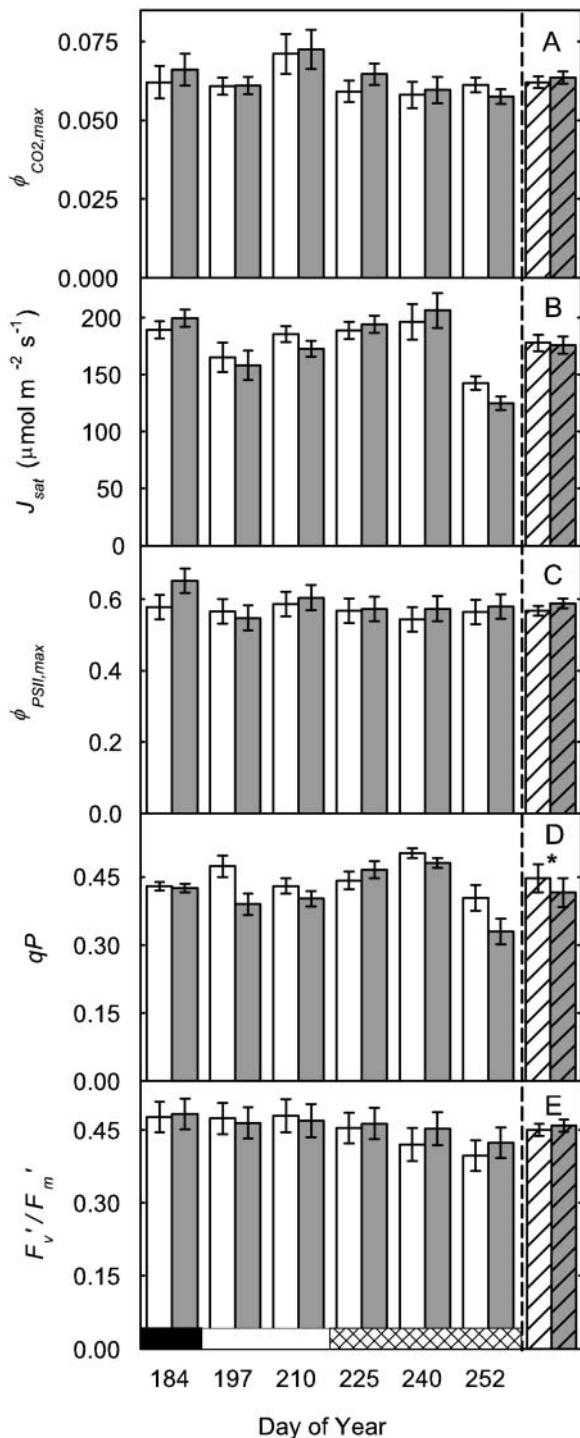
Figure 4. Electron transport and fluorescence parameters of the leaves described in Figure 3. Parameters are $\phi_{CO2,max}$ (calculated as the initial slope of the photosynthetic carbon assimilation response to light; A), J_{sat} (B), $\phi_{PSII,max,l}$ (C), qP (D), and F_v'/F_m' at 2,000 μmol m^{-2} s^{-1} (E). All parameters were calculated from fluorescence measurements of electron transport rates in increasing light. Data presented as in Figure 3.

crease from 62 nL L^{-1} in the control plots to 75 nL L^{-1} in the treated plots here should have decreased A_{sat} by 12% (derived from Ashmore, 2002). Based on the expected 12% loss, our experiment would have detected a significant ($\alpha = 0.05$) effect over the growing season 99.6 times out of 100 (power = 0.9962, see "Materials and Methods" for description). However, there was no evidence of any effect on A_{sat} of recently expanded leaves at any growth stage in this crop; in fact, a nonsignificant, slight increase was observed, contrasting sharply to expectations from previous chamber studies (e.g. Miller et al., 1991; Mulchi et al., 1992; Vozzo et al., 1995; Booker et al., 1997; Reid and Fiscus, 1998).

Further A_{sat}, stomatal conductance (g_s), $V_{c,max}$, and J_{max} of the control and treated plants equaled or exceeded the highest rates of the prior chamber studies (for review, see Morgan et al., 2003), suggesting other differences between open-air and enclosure-grown plants. Another possibility is that the cultivar used in this study is exceptionally resistant to ozone, but by comparison to 22 different lines representing a broad range of soybean germplasm that were scored for accelerated senescence, this cultivar appeared among the more sensitive lines (R.L. Nelson, personal communication). Therefore, these results suggest a substantial difference between the effects of elevated ozone on the photosynthesis of recently expanded leaves when examined in open-air treatment conditions versus chambers. This might be attributed either to the substantially altered environment of chambers and/or to the continuous delivery of ozone to the leaf caused by continual stirring and pumping of air within chambers.

A number of prior studies with soybean (Mulchi et al., 1992) and other plants (McKee et al., 1995, 2000; Zheng et al., 2002) have suggested that the effects of elevated [O$_3$] on photosynthesis accumulate with leaf age and that this reflects cumulative uptake of ozone. Two cohorts of leaves were followed from completion of expansion through senescence, a period of approximately 35 d. The first cohort of leaves was formed during the vegetative stage of growth and remained green until about halfway through the flowering phase. At complete leaf expansion, both control and elevated [O$_3$] treated leaves showed a high A_{sat} (approximately 40 μmol m^{-2} s^{-1}), which subsequently declined to 15 μmol m^{-2} s^{-1} over their lifetime. However, at no point in the lifetime of these leaves was there evidence of any elevated ozone effect relative to the controls (Fig. 5; Table II). The second cohort completed expansion near the beginning of pod filling and persisted throughout the pod-filling developmental stage. Again, at completion of expansion, A_{sat} was high (35 μmol m^{-2} s^{-1}). In contrast to the previous cohort, there was a highly significant treatment effect resulting from the accumulation of damage such that by the time the treated leaves reached an average A_{sat} of 0 (leaf senescence), control leaves still maintained an A_{sat} of 11 μmol m^{-2} s^{-1} (Fig. 5; Table II).
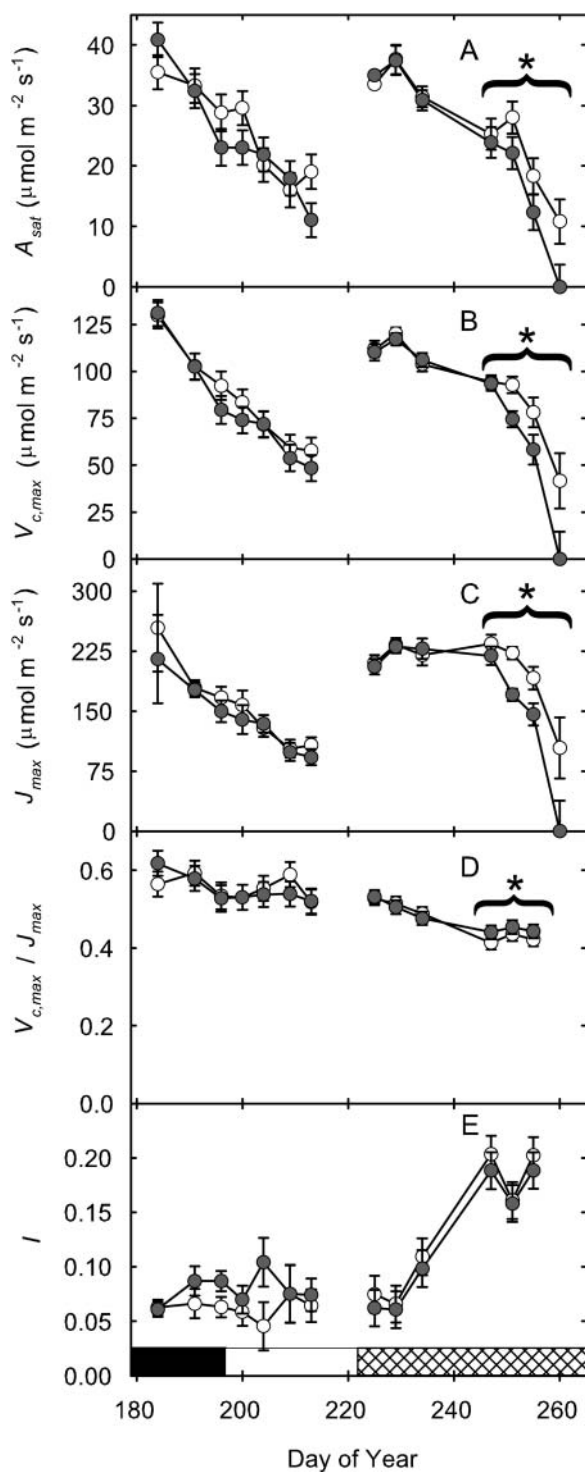
Figure 5. Photosynthetic parameters of two leaf cohorts grown in current (62 nL L^{-1} 8-h daily average; white symbol) or elevated (approximately 1.2 $\times$; gray symbol) [O$_3$] over the growing season. The first cohort was initiated in the vegetative phase of growth (V5), the second during flowering (R4) and persisted to late pod filling (R7). Parameters are A_{sat} (A), $V_{c,max}$ (B), J_{max} (C), $V_{c,max}/J_{max}$ (D), and I (E). Each point is the mean ($n = 4$), and the error bars are $\pm$ the SE of the difference in least square means. Significant linear contrasts over the last 3 or 4 d are noted with a starred bracket. Values of 0 indicate the absence of leaves at that time point. Vegetative (black), flowering

This accelerated decline in the elevated [O$_3$] treated leaves was underlain by accelerated losses in $V_{c,max}$ and J_{max} but with a greater decline in $V_{c,max}$. In a study of elevated [O$_3$] on *Plantago major*, $V_{c,max}$ and J_{max} were also found to decrease as leaves aged (Zheng et al., 2002). These results are consistent with prior observations that Rubisco activity appears one of the most vulnerable parts of the photosynthesis apparatus (Farage et al., 1991; Pell et al., 1994; Long and Naidu, 2002). Beside decreased capacity for light-saturated photosynthesis, light-limited photosynthesis, as indicated by $\phi_{CO2,max}$, was also significantly decreased but only on the final measurement date (Fig. 6; Table II). This later emergence of an effect on light-limited photosynthesis is consistent with earlier studies of the kinetics of ozone damage to the photosynthetic apparatus in vivo, which demonstrated that a decline in Rubisco activity precedes any effect on the photosynthetic membrane (for review, see Pell et al., 1994). Decrease in $\phi_{CO2,max}$ probably represents photoinhibition resulting from decreased capacity for carbon metabolism, causing decreased J_{PSII} and qP (Farage et al., 1991).

What might underlie the striking difference between these two cohorts of leaves? Unlike the first cohort, which was overlain by new layers of leaves as more nodes were added to the stem, the second cohort was formed close to completion of node formation and therefore remained close to the top of the canopy throughout its life. This could explain the differences in response as the two cohorts aged since under open-air conditions ozone reaches the lower canopy only by diffusion down through the upper canopy. Since leaves are an infinite sink for ozone, the [O$_3$] is likely to decline rapidly with canopy depth. This contrasts to increasing [O$_3$] in chambers where stirring is likely to deliver a similar [O$_3$] to all leaves. Thus, the first cohort of leaves may have been protected from ozone exposure as they aged, in contrast to the second cohort. However, the significant effect of elevated [O$_3$] on the second cohort is of particular importance, since these are the leaves that provide photoassimilate to the filling pods. A similar effect was observed in wheat, where damage was most pronounced with aging in the flag leaf, which provides most photoassimilate to the developing grain (McKee et al., 1995).

In summary, this first investigation (to our knowledge) of elevation of [O$_3$] to anticipated 2050 levels under fully open-air field conditions suggests that the effects on photosynthesis in a sensitive plant, such as soybean, are substantially less than those predicted by prior chamber studies—particularly during vegetative growth. This raises doubts about the efficacy of screens for resistance germplasm based on growth stages prior to grain filling. However, the results do confirm that elevated [O$_3$] accelerates loss of light-saturated photo-

(white), and pod-filling (cross-hatched) developmental stages are represented by the horizontal bar.

Table II. *Analysis of variance of fixed effects on photosynthetic and gas-exchange parameters of two leaf cohorts taken from field-grown soybean in current and elevated [O_3]*

The *F* statistic and associated *P* values were calculated from a repeated measures, mixed model analysis of a randomized complete block design (*n* = 4).

Cohort 1 (V5, DOY 185)	Day of Year		Treatment		Day of Year by Treatment	
	F	P Value	F	P Value	F	P Value
A_{sat}	23.81	<0.0001	1.20	0.3175	3.98	0.0268
$V_{c,max}$	65.46	<0.0001	4.21	0.0566	0.63	0.7022
J_{max}	52.28	<0.0001	1.29	0.2852	1.08	0.4947
C_i inflection	33.93	<0.0001	1.71	0.2288	1.76	0.2185
$V_{c,max}/J_{max}$	4.54	0.0044	0.24	0.6269	1.10	0.3946
l	1.29	0.3377	12.65	0.0034	1.76	0.1952
$\phi_{CO2,max}$	1.57	0.3001	2.80	0.2969	0.76	0.6334
θ	0.54	0.7676	0.78	0.4060	0.45	0.8222
Cohort 2 (R4, DOY 225)						
A_{sat}[a]	66.93	<0.0001	6.14	0.0412	2.88	0.0293
$V_{c,max}$[a]	76.45	<0.0001	4.65	0.0037	22.07	<0.0001
J_{max}[a]	24.95	<0.0001	27.36	0.0020	9.11	0.0001
C_i inflection[b]	51.68	<0.0001	0.01	0.9196	0.77	0.5876
$V_{c,max}/J_{max}$[b]	13.52	<0.0001	1.25	0.3056	0.88	0.5163
l[b]	13.23	<0.0001	1.69	0.2419	0.11	0.9884
$\phi_{CO2,max}$[a]	29.25	<0.0001	2.61	0.2221	8.62	0.0005
θ[b]	2.71	0.0606	0.05	0.8279	2.04	0.1303
J_{sat}[b]	19.73	<0.0001	2.94	0.1370	1.15	0.3541
$\phi_{PSII,max,l}$[b]	6.38	0.0014	0.17	0.6854	0.25	0.9332
qP[b]	7.94	<0.0001	1.62	0.2114	2.36	0.0613
F_v'/F_m'[b]	5.13	0.0028	0.00	0.9777	0.42	0.8270

[a] Value of 0 used for elevated [O_3] treatment on DOY = 260, when leaves had senesced. [b] Calculations through day 255 since plants grown in elevated [O_3] did not have leaves.

synthetic capacity in the late-season cohort of leaves, which are critical in providing assimilate to the developing seeds, and that the most significant loss is in apparent in vivo Rubisco activity.

MATERIALS AND METHODS

Site Description

The soybean FACE (SoyFACE) facility is contained within a 32 ha field (80 acre; South Farms, University of Illinois at Urbana-Champaign; 40°03′21.3″N, 88°12′3.4″W, 230 m elevation). The soil is a Drummer-Flanagan soil series (fine-silty, mixed, mesic Typic Endoaquoll) typically very deep and formed from loess and silt parent material deposited on the till and outwash plains. This site has been in continuous cultivation of arable crops for more than 100 years. Soybean (*Glycine max* L. Merr. cv Pana, 2001, and cv Pioneer 93B15, 2002 and 2003) and corn each occupied one-half of the field and follow an annual rotation. After the fall corn harvest, the stubble was chopped with a mower followed by tillage with a rip chisel, and conventional single-pass cultivator tillage was used in the spring. Soybean row spacing was 0.38 m (15 in), and cultivation followed typical Illinois agricultural practices (Ainsworth et al., 2004; Rogers et al., 2004).

Within the SoyFACE site, four blocks, each containing one control and one elevated [O_3] treatment plot, were nested within the 16 ha planted with soybean. The plots were separated by at least 100 m to avoid cross-contamination. Treatments were control (current [O_3]) and elevated (current [O_3] × 1.2) arranged in a randomized complete block design (*n* = 4) to control for topographic and soil variation across the field. The target of 1.2× current concentration was based on projected future mean global tropospheric concentrations, which suggest a 20% increase by 2050 (Prather et al., 2001, 2003).

Elevation of [O_3] was based on the method of Miglietta et al. (2001), using compressed air enriched in ozone replacing compressed CO_2 in the earlier

design. In this system, each plot is defined by an octagon (21 m diameter) of horizontal pipes that release ozone to provide a constant elevation above the current level. Ozone release was maintained at approximately 10 cm above the top of the crop canopy throughout the growing season by raising the horizontal pipes as the crop grew taller. As described previously for CO_2 by Miglietta et al. (2001), the quantity and duration of the ozone release was controlled by a proportional integral derivative algorithm for computer feedback that compares achieved [O_3] to the desired target [O_3] of 1.2× current with a gas concentration monitor (model 49C O_3 analyzer; Thermo Environmental Instruments, Franklin, MA; calibration USA EPA Equivalent Method EQQA-0880-047, range 0–0.05–1.0 ppm), anemometer, and wind direction vane (12005; R.M. Young, Traverse City, MI) mounted in the center of each ring. Ozone was generated by passing pure oxygen through a high-voltage electrical field generating a composite gas consisting of approximately 10% ozone and 90% oxygen (GSO-40; Wedeco Environmental Technologies, Herford, Germany). Using a mass flow controller, the ozone/oxygen mixture was added to a compressed air stream through a Venturi bypass system. Ozone-enriched air was injected through supersonic velocity jets into the wind, thus entraining surrounding air in a turbulent motion that diluted the ozone before it was carried back across the plot by the wind, as described previously for CO_2 enrichment (Miglietta et al., 2001). Fumigation began 20 d after planting in 2002 and continued throughout the daylight hours until harvest. Sampling plots were located a minimum of 2 m internal to the octagon of horizontal pipes to minimize any residual effect of the injection system.

Measurements on Newly Expanded Leaves

Predawn every 2 weeks from crop emergence until senescence, two uppermost fully expanded soybean leaves per plot were randomly selected, and the petioles were cut under water. The petioles of the excised leaves were placed in deionized water throughout the duration of the measurements. Gas exchange and fluorescence measurements were made on two leaves per replicate plot. Based on previous studies, cutting leaves predawn ensured that
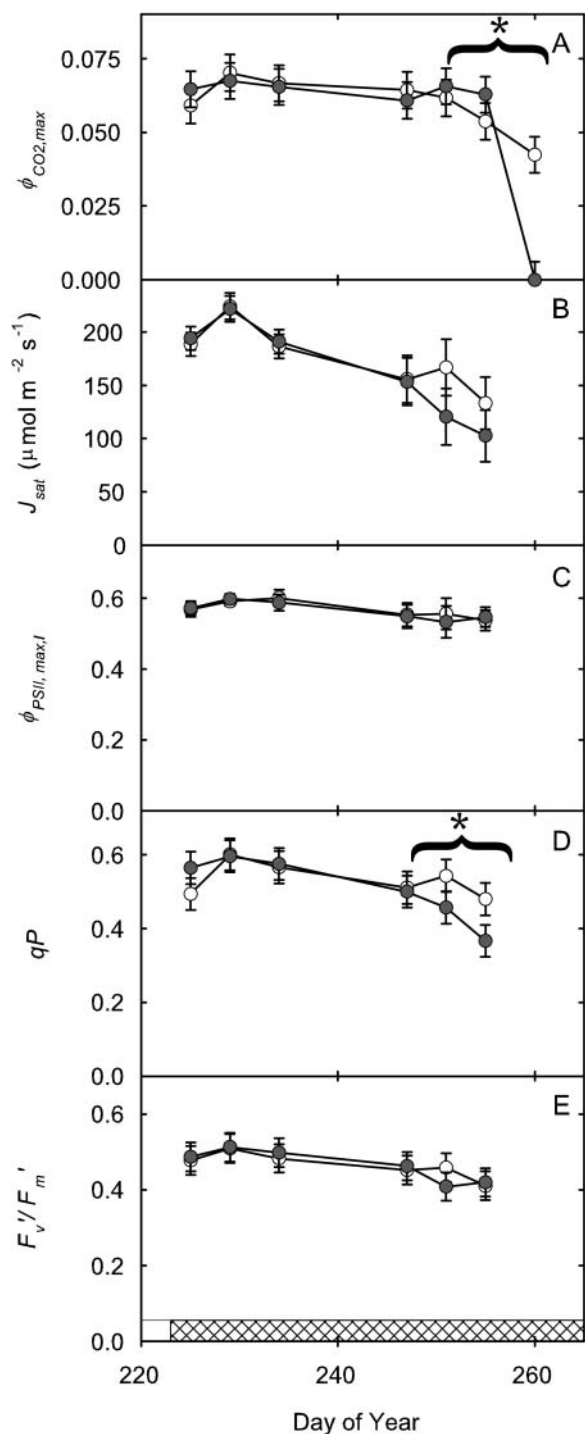
Figure 6. Electron transport and fluorescence parameters of the leaves described in Figure 5. $\phi_{CO2,max}$ (A), J_{sat} (B), $\phi_{PSII,max,I}$ (C), qP (D), and F_v'/F_m' at 1,500 μmol m^{-2} s^{-1} (E). Data are presented as in Figure 5.

leaves were not water stressed, photoinhibited, or triose-phosphate limited (Ainsworth et al., 2004); this allowed measurement of potential rates of the tissue, independent of transient diurnal changes that could depress apparent photosynthetic capacity. Furthermore, photosynthetic rates achieved previously with leaves cut from the site in this way (Ainsworth et al., 2004) equaled or exceeded maximum rates of attached leaves measured in the field (Rogers et al., 2004). The excised leaves were kept in low light (<10 μmol m^{-2} s^{-1}) until

15 min prior to when measurements were to be taken, at which time they were light acclimated (approximately 1,000 μmol m^{-2} s^{-1} white light) outside the gas exchange chamber. Leaf gas-exchange measurements were coupled with measurements of chlorophyll fluorescence using an open gas-exchange system (LI-6400; LI-COR, Lincoln, NE) with an integrated fluorescence chamber head (LI-6400-40 leaf chamber fluorometer; LI-COR). Responses of A to C_i were measured and $V_{c,max}$ and J_{max} calculated as described previously (Bernacchi et al., 2001, 2003; Long and Bernacchi, 2003). The two highest C_i values were not used to calculate J_{max}, thereby avoiding photosynthetic assimilation rates that could possibly be triose-phosphate limited. Stomatal limitation to photosynthesis (l) was determined following the method of Farquhar and Sharkey (1982), as described previously (Long and Bernacchi, 2003).

To determine the response of A to light (Q), chamber [CO_2] was set to 370 μmol mol^{-1} and leaf temperature to 25°C. The initial slope of the A versus Q curves was fit with a linear function to estimate $\phi_{CO2,max}$. In addition, entire curves were fit to a nonrectangular empirical function to estimate A_{sat} and the convexity of the light response (θ, as described previously [Long and Drake, 1991; Long and Hällgren, 1993]). Simultaneous measurements of modulated chlorophyll fluorescence were made with the A versus Q response. The rate of electron transport (J), qP, and F_v'/F_m' were determined as described previously (Bernacchi et al., 2002).

Effects of Leaf Age

Measurements of photosynthesis were made on leaf cohorts from their initial expansion until they were visibly senescenced (i.e. 75% yellow). Twice during the growing season (DOY 184 and 225), newly expanded leaves were marked with plastic flagging to aid in identification. For each leaf cohort, one leaf from two different plants was excised predawn, as described above, every 3 to 5 d from emergence until senescence. Measurements of the A versus C_i, A versus Q, and A versus J were made, and analysis of the curves were as described above except that qP and F_v'/F_m' were calculated from measurements at 1,500 μmol m^{-2} s^{-1} to compensate for lower light saturation as the leaves aged.

Statistical Analysis

For all parameter comparisons, a repeated measures, mixed model ANOVA (PROC MIXED version 8.02; SAS Institute, Cary, NC) was used with DOY, treatment, and the DOY by treatment interaction as fixed effects. The best fit variance/covariance matrices were chosen for each variable using Akaike's information criterion (Keselman et al., 1998; Littell et al., 1998, 2000). A priori pairwise linear comparisons were made between treatments across days and between treatments within days ($\alpha = 0.05$). The sensitivity of the experiment was tested by calculation of the power of the test (power = $1 - \beta$), where β is the probability of committing a Type II error (Kuehl, 2000). Calculation of β was based on the experimental replication ($r = 4$), Type I error rate of 5% ($\alpha = 0.05$), estimated experimental variance from our measurements, and an expected 12% difference between the estimated means (derived from Ashmore, 2002).

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