

4 SoyFACE: the Effects and Interactions of Elevated [CO₂] and [O₃] on Soybean

D.R. ORT, E.A. AINSWORTH, M. ALDEA, D.J. ALLEN, C.J. BERNACCHI, M.R. BERENBAUM, G.A. BOLLERO, G. CORNIC, P.A. DAVEY, O. DERMODY, F.G. DOHLEMAN, J.G. HAMILTON, E.A. HEATON, A.D.B. LEAKEY, J. MAHONEY, T.A. MIES, P.B. MORGAN, R.L. NELSON, B. O'NEIL, A. ROGERS, A.R. ZANGERL, X.-G. ZHU, E.H. DeLUCIA, and S.P. LONG

4.1 Introduction

SoyFACE is the first FACE experiment to focus on a seed legume and on corn and the first to explore the interactions of both elevated (e)[CO₂] and e[O₃] on the growth and development of an arable crop. The intent of the SoyFACE experiment is to orchestrate a coordinated and comprehensive investigation of the impact of atmospheric change on this expansive agroecosystem, addressing questions ranging from rhizosphere biology through to seed composition and employing techniques from genomics to micrometeorology. SoyFACE completed its fourth season of operation in 2004. This chapter provides a description of the SoyFACE facility and its operation and overviews the published results from the 2001–2003 growing seasons.

4.2 Site Description

The SoyFACE facility is located in Champaign, IL, USA (40°02' N, 88°14' W, 228 m above sea level; <http://www.soyface.uiuc.edu>) situated on 32 ha of farmland within the Experimental Research Station of the University of Illinois. The soil is a deep (up to 1.25 m), organically rich Flanagan/Drummer series typical of northern and central Illinois "prairie soils" (fine-silty, mixed, mesic Typic Endoaquoll). Highly detailed information on the physical and chemical characteristics of Champaign County Illinois soils from the USDA Natural

Resources Conservation Service can be found at: http://soils.usda.gov/survey/online_surveys/illinois/. The field is tile-drained and has been in continuous cultivation to arable crops for over 100 years. Agronomic practices in use at the site are typical for this region of Illinois. No nitrogen fertilizer is added to the soybean crop, whereas the corn (*Zea mays*) crop receives 202 kg N ha⁻¹ (157 kg ha⁻¹ as 28% 1:1 urea:ammonium nitrate liquid pre-plant and 45 kg ha⁻¹ credit from previous soybean N₂ fixation). Soybean (*Glycine max* L. Merr. cv Pana in 2001; cv Pioneer 93B15 in 2002 and thereafter) and corn (Pioneer cv 34B43) each occupy one-half of the field and follow an annual rotation. In 2001, it was found that cv. Pana grew about 1.5 m at this site and was vulnerable to lodging, leading to its replacement by the shorter but related cv 93B15 for subsequent years. Both soybean cultivars were similar indeterminate lines of maturity group III which formed 20 000–30 000 nodules m⁻², amounting to 25 g m⁻² in mass in the control and 32 g m⁻² in the e[CO₂] treatment plots. Soybean was seeded using a mechanical seed planter to a field density of about 200,000 plants ha⁻¹ at row spacing of 0.38 m (15 in); and the corn row spacing was 0.76 m (30 in at a seed density of 74 100 ha⁻¹). The experimental plots were oversown by hand on the day of planting and thinned after emergence to ensure uniform plant density.

An on-site weather station (MetData 1-type; Cambell Scientific, Logan, Utah) measured air temperature (T_{air} ; for an explanation of abbreviations, see end of chapter) and relative humidity at a height of 3 m. A quantum sensor (model QSO; Apogee Instruments, Logan, Utah) measured incident photosynthetic photon irradiance (PPI) at a height of 3 m. Data were averaged and logged at 10 min intervals throughout the growing season. Tipping bucket rain gauges (model 52202; R.M. Young, Traverse City, Mich.) were distributed throughout the field and recorded rainfall events in 0.0001 m increments throughout the season. Weather data is posted on the SoyFACE website (<http://www.soyface.uiuc.edu/weather.htm>). The Illinois State Water Survey weather station (<http://www.sws.uiuc.edu/data/climatedb/>) in Urbana, Ill. (40°05' N, 88°14' W) is situated 3 km north of the SoyFACE site and at the same altitude.

4.3 Experimental Treatments

4.3.1 Field Layout and Blocking

To control for topographic (<1 m) and soil variation, each 16-ha half of the field was divided into 16 blocks, each able to accommodate two 20-m diameter octagonal plots. One plot in each block was untreated but otherwise outfitted with treatment equipment. The atmosphere in the second plot was

amended during daylight hours, from crop emergence until harvest, using a FACE system (Miglietta et al. 2001; see Chapter 2).

4.3.2 CO_2 Treatment

The target $e[\text{CO}_2]$ was 550 ppm, as projected for the year 2050 by the Intergovernmental Panel on Climate Change (Prentice et al. 2001). The average $[\text{CO}_2]$ over the 2001–2003 seasons was 372 ppm in four ambient and 549 ppm in four elevated soybean plots. One-minute-average CO_2 concentrations were within $\pm 10\%$ of the 550 mmol mol^{-1} target for more than 85% of the time. On those rare instances when wind speeds dropped below 0.2 m s^{-1} , CO_2 fumigation cycled around the octagon to maintain the $[\text{CO}_2]$ within the plot as close to the 550 mmol mol^{-1} set point as possible. Air temperature, PPI, and precipitation were recorded at 15-min intervals throughout the growing season. In alternate years (i.e., 2002, 2004) there were four $e[\text{CO}_2]$ treatment plots and four control plots in corn.

4.3.3 O_3 Treatment

The seasonal target of 1.2 times the current ozone concentration was based on projected future mean global tropospheric concentrations, which suggest a 20% increase by 2050 (Prather et al. 2001). Because of the reactivity of O_3 with water, fumigation was held in abeyance during periods when leaf surfaces were damp (e.g., most mornings). Thus to achieve the target concentration over the entire growing season, the set point was 1.5 times the continuously monitored ambient level. Elevation of $[\text{O}_3]$ was based on the method of Miglietta et al. (2001; see also Chapter 2), but in this instance using compressed air enriched in ozone instead of compressed CO_2 . 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 $[\text{O}_3]$ to the target $[\text{O}_3]$ of 1.5% current with a gas concentration monitor (model 49 O_3 analyzer; Thermo Environmental Instruments, Franklin, MA; calibration by US EPA Equivalent Method EQQA-0880-047; ranges 0–0.05–1.0 ppm), anemometer, and wind direction vane 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 fumigation began 20 days after seeding and continued for the remainder of the growing season until harvest. Fumigation operated during daylight hours and stopped to prevent damage to leaves when the crop was wet with dew or rain or when wind speeds dropped below 0.2 m s^{-1} ,

when control would be inadequate to avoid accumulation of high $[O_3]$ (>300 ppb) near the edges of the plots (similar protocols were followed at Aspen FACE; see Chapter 12). In 2002 and 2003, the 8-h average $[O_3]$ was 62 ppb and 50 ppb, respectively, under current conditions (control) and 75 ppb and 63 ppb in the $e[O_3]$ treatment. The effective treatment over the season was 121 % in 2002 and 125 % in 2003 of ambient $[O_3]$ and fumigation control was maintained at ± 10 % of the set point concentration for 77 % of the time, and at ± 20 % for 93 % of the time. Sampling plots were located at a minimum of 2 m internal to the octagon of horizontal pipes, to minimize any residual effect of the injection system.

4.3.4 $CO_2 \times O_3$ Treatment

The combined $CO_2 \times O_3$ treatment, begun in the 2003 growing season, was achieved by combining the treatments described above for the individual gases within the same experimental plots. The technology and the performance are as described above. No results have yet been published on the combined treatment.

4.4 Resource Acquisition

4.4.1 Effects of $[CO_2]$ Treatment on Photosynthesis

In the short term, an increase in $[CO_2]$ stimulates net photosynthesis in C_3 plants because the current $[CO_2]$ is insufficient to saturate Rubisco activity and because CO_2 inhibits the competing process of photorespiration. Therefore, an increase in net photosynthesis in $e[CO_2]$ is anticipated, regardless of whether Rubisco activity or regeneration of ribulose-1,5-bisphosphate (RuBP) is limiting assimilation, and regardless of whether light is saturating or not (Long and Bernacchi 2003). The seasonal profile of stimulation of soybean leaf photosynthesis by an increase in $[CO_2]$ to $552 \text{ mmol mol}^{-1}$ was examined under open-field conditions (Rogers et al. 2004). Diurnal measurements of net leaf CO_2 uptake (A) were supported by simultaneous measurements of leaf carbohydrate dynamics, water vapor flux, modulated chlorophyll fluorescence, and microclimate conditions. Measurements were made from pre-dawn to post-dusk on 7 days, covering different developmental stages from the first node formation through complete seed fill. Across the 2001 growing season, the daily integrals of leaf photosynthetic CO_2 uptake (A') increased by nearly 25 % in $e[CO_2]$ even as the average mid-day stomatal conductance (g_s) decreased by 22 % (Table 4.1). However, while theory

Table 4.1 The effects of growth at elevated carbon dioxide on resource allocation in FACE-grown soybean. Values *in italics* indicate significance at $\alpha = 0.1$ or better. *Dashes* indicate missing data. See list of abbreviations for parameter definitions

Parameter	Percentage change e[CO ₂]		Reference
	2001	2002	
<i>A'</i>	24.6	–	Rogers et al. (2004)
<i>g_s</i> (mid-day)	–29.9	–	Rogers et al. (2004)
<i>g_s</i> (diurnal)	–10	–10	Bernacchi et al. (2005)
<i>l</i>	–8	–4.5	Bernacchi et al. (2005)
<i>J_{PSII}'</i>	4.5	–	Rogers et al. (2004)
<i>A_{sat}</i> (season-long)	20	16	Bernacchi et al. (2005)
<i>V_{c,max}</i> (season-long)	–4	–6	Bernacchi et al. (2005)
<i>g_m</i>	–	No change	Bernacchi et al. (2005)
<i>J_{max}</i> (season-long)	No change	No change	Bernacchi et al. (2005)
<i>V_{c,max}/J_{max}</i> (season-long)	–5	–5	Bernacchi et al. (2005)

predicts that stimulation of *A* should be seen at all light levels, [CO₂] enhancement of *A* was apparent only when PPI was above 1000 $\mu\text{mol m}^{-2} \text{s}^{-1}$. The greatest stimulation of *A* was observed during the early- to mid-grain filling stages (Bernacchi et al. 2005). There was no evidence of any loss of stimulation toward the end of the growing season; in fact the largest stimulation of *A* occurred during late-seed filling (Rogers et al. 2004; Bernacchi et al. 2005). In contrast to *A'*, daily integrals of PSII electron transport (*J_{PSII}'*), measured by modulated chlorophyll fluorescence, were not significantly increased by e[CO₂] (Table 4.1). Although the results show sustained increase in *A* by soybean in response to growth in e[CO₂], it is only approximately half of the maximum stimulation predicted from theory (Rogers et al. 2004).

Down-regulation of light-saturated photosynthesis (*A_{sat}*) at e[CO₂], which typically involves a decrease in the amount and/or activity of Rubisco, has been demonstrated for many C₃ species (Long et al. 2004; Chapter 14, however see Chapter 6). But, did down-regulation occur in the SoyFACE experiment and can it account for the smaller than predicted stimulation of *A* that was observed? Potential Rubisco carboxylation (*V_{c,max}*) and electron transport through photosystem II (*J_{max}*) were determined from the responses of *A_{sat}* to intercellular [CO₂] (*C_i*) at biweekly intervals over the 2001 and 2002 growing seasons (Bernacchi et al. 2005). Measurements were made under controlled conditions on leaves harvested at predawn to ensure that determination of *A_{sat}* was not obscured by factors such as transient water stress or mid-day photoinhibition. Elevated [CO₂] increased *A_{sat}* by 15–20 %, even though stomatal conductance was reduced (Table 4.1). There was a small, yet statistically significant decrease in *V_{c,max}* which in turn drove a decrease in *V_{c,max}/J_{max}* (Table 4.1), inferring a shift in resource investment away from Rubisco. The decrease in *V_{c,max}/J_{max}* was not an illusion caused by a decrease in mesophyll

conductance (g_m), which was unchanged by $e[\text{CO}_2]$. While g_s was significantly decreased across both growing seasons, the limitation imposed on photosynthesis by the stomata (l) was lower (Table 4.1), implying that stomata represented less of a limitation to photosynthesis for plants growing at $e[\text{CO}_2]$ (see also Chapter 14). Although there was no progressive decline in A_{sat} during either season, analyses of the A versus C_i responses showed that, even in an N-fixing species grown without rooting restriction and under open-field conditions, down-regulation of photosynthesis occurred. This down-regulation, small yet statistically significant, is in effect an optimization of photosynthesis to $e[\text{CO}_2]$ in that the decrease in $V_{c,\text{max}}$ would result in lower rates of A only when measured at lower $[\text{CO}_2]$ with little or no loss at $e[\text{CO}_2]$.

Experiments were also conducted at SoyFACE to test the “source-sink” hypothesis of down-regulation by examining acclimation of photosynthesis in lines of soybean differing by single genes that altered sink capacity either by an ability to nodulate or by switching between determinate and indeterminate growth habits (Ainsworth et al. 2003). By restricting vegetative growth after flowering, the stem termination associated with determinate growth would be expected to limit the size of the carbon sink. Because the respiratory rate of a nodulated root system can be an order of magnitude greater than its non-nodulated counterpart (Vessey et al. 1988), root nodules are strong sinks for carbohydrates (see Chapter 18). Soybean isolines, in which single-locus gene substitutions changed indeterminate growth to determinate and nodulated roots to non-nodulated, resulted in enhanced down-regulation of photosynthesis at $e[\text{CO}_2]$. Whereas down-regulation in the nodulating indeterminate varieties Pana and Pioneer 93B15 discussed above (Bernacchi et al. 2005) was driven solely by decreases in $V_{c,\text{max}}$, both $V_{c,\text{max}}$ and J_{max} decreased when sink strength was reduced by genetically limiting nodulation and vegetative stem growth (Ainsworth et al. 2003). Increases in the total non-structural carbohydrate (i.e., starch plus ethanol-extractable carbohydrates), which frequently portend photosynthetic down-regulation in response to $e[\text{CO}_2]$ (see Chapter 16), were twice as great when sink capacity was reduced by genetically controlled stem termination. These sink-manipulation experiments strongly support the premise that genetic capacity for the utilization of photosynthate is critical for the ability of plants to sustain enhanced photosynthesis when grown at $e[\text{CO}_2]$.

Corn is the third most important food crop globally in terms of production; and demand is predicted to increase by 45 % from 1997 to 2020 (Pingali 2001). Although our FACE experiment has focused primarily on soybean, corn has also been grown within the experiment, such that the ecosystem in this agricultural rotation is continuously treated with $e[\text{CO}_2]$. Previous laboratory studies suggest that, under favorable growing conditions, C_4 photosynthesis is not typically enhanced by $e[\text{CO}_2]$, yet stomatal conductance and transpiration are decreased, which can indirectly increase photosynthesis in dry climates. Given the deep soils and relatively high rainfall of the United States

Corn Belt, it was predicted that photosynthesis would not be enhanced by $e[\text{CO}_2]$. The diurnal course of gas exchange of upper canopy leaves was measured in situ across the growing season of 2002 (Leakey et al. 2004). Contrary to the prediction, growth at $e[\text{CO}_2]$ significantly increased A by up to 41 % and by 10 % on average. Greater A was associated with greater intercellular $[\text{CO}_2]$, lower stomatal conductance, and lower transpiration. In two of four cultivars grown, significant increases in production were observed. Summer rainfall during 2002 was very close to the 50-year average for this site, indicating that the year was neither atypical nor a drought year. However, stimulation of photosynthesis was limited to periods of mild drought, as following rainfall there was no effect of $e[\text{CO}_2]$ on photosynthesis (Leakey et al. 2004). The results suggest that, even in the wetter areas of the Corn Belt, photosynthesis and yield increase if there are any periods of water stress (see Chapter 3, concerning related results with sorghum).

4.4.2 Effects of $[\text{O}_3]$ Treatment on Photosynthesis

A number of prior enclosure studies with soybean (Mulchi et al. 1992) and other plants (McKee et al. 1995, 2000; Zheng et al. 2002) suggest that the effects of $e[\text{O}_3]$ on photosynthesis accumulate with leaf age, reflecting the cumulative uptake of ozone. In the 2002 SoyFACE experiment, two cohorts of leaves were followed from completion of expansion through senescence, a period of approximately 35 days (Morgan et al. 2004b). 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 $e[\text{O}_3]$ -treated leaves showed a high A_{sat} which declined over their lifetime, but there was no evidence of any $e[\text{O}_3]$ effect relative to the controls. The later leaf cohort completed expansion near the beginning of pod-filling and persisted throughout pod-filling. At full leaf expansion, A_{sat} was high but, in contrast to the earlier cohort, there was a significant $e[\text{O}_3]$ treatment effect from the accumulation of damage, resulting in the treated leaves reaching an average A_{sat} of 0 (leaf senescence) while control leaves still maintained >30 % of the original A_{sat} . This accelerated decline of A_{sat} in the $e[\text{O}_3]$ treated leaves was accompanied by accelerated losses in $V_{\text{c,max}}$ and a lesser but significant loss in J_{max} . Unlike the first cohort, which moved deeper into the canopy as more nodes and leaves were added above, the second cohort developed near the completion of node formation and remained close to the top of the canopy throughout its life. This difference in canopy position likely explains the response differences as the two cohorts aged, given that under open-air conditions ozone only reaches the lower canopy by diffusion down through the upper canopy, resulting in a rapid decline of $[\text{O}_3]$ with canopy depth. It should be noted that the significant effect of $e[\text{O}_3]$ on the second cohort is of particular agronomic importance, since these are the leaves most responsible for

providing photoassimilate during seed filling.

4.4.3 Effects of [CO₂] and [O₃] on Canopy Development

Any changes in canopy structure and duration caused by growth in $e[\text{CO}_2]$ and $e[\text{O}_3]$ would be expected to impact the productivity of agro-ecosystems (Drake et al. 1997; Long et al. 2004). By improving carbon assimilation and efficiency of water use, $e[\text{CO}_2]$ may increase the leaf area index (LAI). Additionally, by raising the maximum quantum yield of photosynthesis ($\phi_{\text{CO}_2, \text{max}}$), $e[\text{CO}_2]$ may decrease the light compensation point, increasing the carbon gain in deeper-shaded leaves, which may in turn maintain a positive carbon balance thereby driving a further increase in LAI by delaying leaf abscission. In contrast, $e[\text{O}_3]$ accelerates senescence and may thereby reduce LAI. These predictions were tested at SoyFACE during the 2001 and 2002 growing seasons (Dermody et al. 2005). Maximum LAI increased from 6.7 ± 0.2 in ambient air to 7.4 ± 0.2 in $e[\text{CO}_2]$. The $\phi_{\text{CO}_2, \text{max}}$ of shade leaves in $e[\text{CO}_2]$ increased from 0.059 ± 0.002 in ambient air to 0.067 ± 0.002 . Elevated [CO₂] also extended the growing season: for example, the average LAI of soybeans growing in $e[\text{CO}_2]$ on 23 September (1.6 ± 0.1) was ~33 % greater than in ambient air (1.1 ± 0.1). This was not delayed senescence, as there was no enhanced retention of shade leaves but rather a sustained addition of new nodes and leaves at the top of canopy later in the growing season. Although $e[\text{O}_3]$ did not affect the maximum LAI, it shortened the growing season by as much as 40 %, reducing LAI from 3.5 ± 0.2 in ambient air to 2.1 ± 0.2 near the end of the growing season. No effect of $e[\text{O}_3]$ on $\phi_{\text{CO}_2, \text{max}}$ was detected.

4.4.4 Effects of [CO₂] and [O₃] on Insect Herbivory

A common feature of growth at $e[\text{CO}_2]$ and $e[\text{O}_3]$ is an alteration of leaf chemical composition that can influence the palatability and nutritional quality of foliage for herbivorous insects. For example, plants grown at $e[\text{CO}_2]$ and $e[\text{O}_3]$ often produce leaves with a lower nitrogen and soluble protein content (Mulchi et al. 1992; Cotrufo et al. 1998) and plants grown at $e[\text{CO}_2]$ commonly accumulate sugars and starch in their foliage, also affecting palatability by altering C:N (Cotrufo et al. 1998; Long et al. 2004). To meet their nutritional requirements, some herbivores exhibit “compensatory feeding” by increasing their consumption of foliage with lower N content (Bezemer and Jones 1998; Whittaker 1999). To test these predictions, levels of herbivory were measured in soybean grown in ambient air and air enriched with CO₂ or O₃ at SoyFACE (Hamilton et al. 2005). FACE is unique among facilities for elevating either [CO₂] or [O₃], in allowing the free movement of insect pests and predators. Exposure to $e[\text{O}_3]$ appeared to have no effect on insect herbivory. Growth at

e[CO₂] significantly increased the susceptibility of soybeans to herbivory early in the season, with the amount of leaf area removed increasing from 5 % in controls to more than 11 %. There was no evidence of compensatory feeding in that leaf nitrogen content and C:N ratio were unaltered in those leaves experiencing increased herbivory. Rather than feeding in an effort to compensate for poor nutritional value, it appears that elevated sugar concentrations stimulated Japanese beetles (*Popillia japonica* Newman) to increase consumption of leaves grown at e[CO₂]. Levels of soluble leaf sugars were increased by >30 % at e[CO₂] (Chapter 16) and coincided with a significant increase in the density of Japanese beetle. In two-choice feeding trials, Japanese beetles and Mexican bean beetles (*Epilachna varivestis* Mulsant.) preferred foliage grown at e[CO₂] to foliage grown at ambient [CO₂] (see Chapter 6 for potato herbivory). These results imply that growth at e[CO₂] has the potential to increase crop susceptibility to pests, particularly those insects stimulated to feed by sugar availability, and thus possibly increasing the need for insect pest management.

4.5 Resource Transformation

4.5.1 Effects of e[CO₂] Treatment on Crop Production and Yield

To date, only two large-scale and fully replicated FACE experiments have examined effects of e[CO₂] on yields of C₃ grain crops: wheat and rice. Over 3 years of growth, rice seed yield was increased by 7–15 % (Kim et al. 2003) and wheat yield increased by 8 % in two growing seasons (Kimball et al. 1995; Chapter 3) at e[CO₂]. Modern soybean cultivars grown in the mid-west United States include many indeterminate cultivars that fix nitrogen, thereby creating and sustaining additional carbon sinks. Indeterminate nodulated soybeans provide a good test of the maximum response to the e[CO₂] of the future atmosphere that can be anticipated under actual field conditions. The effect of growth in e[CO₂] on above-ground net primary production (ANPP) and yield was investigated at SoyFACE over three growing seasons. Additionally, via sequential harvests at 2-week intervals, a study investigated how the patterns of production and partitioning were differentially affected with time and developmental stage across the growing season (Morgan et al. 2005a). Although a different cultivar was used in 2001 and a hailstorm defoliated the crop mid-season in 2003, the relative enhancement of seed yield due to e[CO₂] was remarkably similar (~15 %) across the 3 years (Table 4.2). For cv Pana grown in 2001, e[CO₂] increased seed yield by greater number of seeds per pod. The increased seed yield in e[CO₂] for cv Pioneer 93B15 grown during 2002 and 2003 was due to an increase in the number of pods per plant, with no

Table 4.2 The effects of growth at $e[\text{CO}_2]$ or $[\text{O}_3]$ on resource transformation in FACE-grown soybean. Values *in italics* indicate significance at $\alpha = 0.1$ or better. *Dashes* indicate missing data

Parameter	Treatment	Percentage change at $e[\text{CO}_2]$ or $[\text{O}_3]$			Reference
		2001	2002	2003	
Seed yield	$[\text{CO}_2]$	16	15	15	Morgan et al. (2005a)
	$[\text{O}_3]$	-	-15	-25	Morgan et al. (2005b)
Harvest index	$[\text{CO}_2]$	-3	-2.0	-2	Morgan et al. (2005a)
	$[\text{O}_3]$	-	-2.5	-3	Morgan et al. (2005b)
Litterfall	$[\text{CO}_2]$	-	38	16	Morgan et al. (2005a)
	$[\text{O}_3]$	-	No change	-39	Morgan et al. (2005b)
ANPP	$[\text{CO}_2]$	-	15	17	Morgan et al. (2005a)
	$[\text{O}_3]$	-	-11	-23	Morgan et al. (2005b)
Nodes	$[\text{CO}_2]$	21	22	8	Morgan et al. (2005a)
	$[\text{O}_3]$	-	-	-	

increase in the number of seeds per pod. There was a consistent and significant, albeit small, decline in harvest index in all 3 years (Table 4.2). During much of the season, the portion of the assimilated carbon that accumulated in leaves as non-structural carbohydrate was small (<10%) for both cultivars and was independent of growth $[\text{CO}_2]$, implying enhanced export at $e[\text{CO}_2]$. However, towards the end of the season, export of photosynthate slowed and there was a significant, $[\text{CO}_2]$ -dependent daytime accumulation of non-structural carbohydrates in source leaves (Rogers et al. 2004, Chapter 16). Biweekly litter production was not measured in 2001, but was significantly increased by $e[\text{CO}_2]$ in 2002, although not in 2003 (Table 4.2), probably due to the removal of much of the canopy by the July hailstorm. Above-ground net primary production, the sum of the dry mass and cumulative litter production, was significantly increased by 2002 and 2003 (Table 4.2), but the difference was only evident in the later part of the growing season and was the result of the prolonged growing season under $e[\text{CO}_2]$. Continued addition of nodes in $e[\text{CO}_2]$ likely explains the increased stem dry mass and height; and the additional leaves associated with these nodes may explain the significant extension of the growing season by 2–7 days across the 3 years.

4.5.2 Effects of O₃ Treatment on Crop Production and Yield

Among the major crops, soybean is one of the most susceptible to ozone, with adverse effects apparent at concentrations as low as 40 ppb (Ashmore 2002; Fuhrer et al. 1997). Nearly one-quarter of the earth's surface is currently at risk from tropospheric ozone in excess of 60 ppb during mid-summer, with even greater concentrations occurring in isolated regions (Fowler et al. 1999a, b), with Western Europe, the mid-west and eastern United States, and eastern China being exposed to some of the highest background levels (Prather et al. 2001). The SoyFACE ozone experiment is the first on soybean or any other row crop using free air fumigation. While the effects of ozone on soybean photosynthesis at SoyFACE are subtle (see Section 4.4.2; Morgan et al. 2004b), the effects on biomass and seed yield are robust (Morgan et al. 2005b). Seed yield decreased by 15 % in 2002 and 25 % in 2003 for soybean grown in e[O₃] (Table 4.2). The larger yield losses in 2003 likely are the consequence of the July hail-storm that severely damaged the crop and from which the e[O₃] plants recovered more slowly. In 2002, yield reduction was entirely due to a 14 % decrease in individual seed weight. While yield losses in 2003 resulted from both 7 % decrease in seed weight and a 16 % decrease in pods per plant (i.e., four fewer pods per plant). Elevated [O₃] had similar impacts on both yield and shoot dry mass at maturity but there was a trend, albeit not statistically significant, to a slightly reduced harvest index (Table 4.2). Decreases in the shoot dry mass of e[O₃] grown plants relative to controls appeared late in the 2002 growing season; and leaf, stem, and pod dry mass all reflected this late season difference. However, in 2003 decreases were apparent earlier, notably in stem dry mass, possibly reflecting a weakened ability to recover following the mid-July hail-storm. The decreased production in e[O₃] grown plants in 2003 following the hail may also explain the lower biweekly litterfall (Table 4.2). The depression in cumulative ANPP caused by e[O₃] increased as the growing season progressed. In 2002, significant differences developed late in the growing season and persisted throughout the remainder of the soybean lifecycle. The cumulative effect of e[O₃] over the 2002 season decreased ANPP by 11 % compared to controls (Table 4.2). In 2003, ANPP of the control was 50 % lower than in 2002 and the impact of e[O₃] was greater, decreasing ANPP by 23 % relative to controls.

4.6 Consequences for Future Soybean Crop Management and Plant Breeding

The soybean/corn rotation occupied about 62×10^6 ha in the United States during 2003, more than that of any other crop system, making it among the largest ecosystems in the contiguous 48 States (USDA 2004). Soybean is glob-

ally the most important dicotyledonous seed crop in terms of area planted and is also a major source of food protein worldwide (FAO-UN 2002). When grown in the field under FACE fumigation, stimulation of ANPP and yield by $e[\text{CO}_2]$ were smaller than predicted from open-top chamber studies (reviewed by Ainsworth et al. 2002). Elevated $[\text{CO}_2]$ increased the ANPP and seed yield of field-grown soybeans by about 15%, even while the harvest index decreased by about 3%. From the statistical summary of published reports of the $e[\text{CO}_2]$ response of soybean in enclosure studies, the yield was 24% greater in plants grown in 450–550 ppm $[\text{CO}_2]$ than in current $[\text{CO}_2]$, with a 9% decrease in harvest index (Ainsworth et al. 2002). Although the response found at SoyFACE for soybean is only 60% of that predicted from the meta-analysis of soybean enclosure studies, the overall yield stimulation by $e[\text{CO}_2]$ is greater than in FACE grown rice (7–15%; Kim et al. 2003) and spring wheat (8%; Kimball et al. 1995). This difference among FACE experiments with C_3 crops may reflect the indeterminate nature of the soybean cultivars grown and/or the nitrogen-fixing capacity of soybean. The effects of $e[\text{CO}_2]$ are apparent in soybean during early vegetative growth in enclosure experiments and are sustained through the duration of the experiment (Ziska and Bunce 1995; Miller et al. 1998). The greatest stimulation of dry mass due to $e[\text{CO}_2]$ was found to occur during flowering, declining through pod-filling (Ainsworth et al. 2002). In contrast to these findings, no significant increase in any growth parameter was observed until pod-filling in SoyFACE in any of the 3 years. Furthermore, the subsequent relative stimulation by $e[\text{CO}_2]$ remained constant throughout pod-filling to maturity. The extended growing season of the SoyFACE soybean crop was also counter to the previous reports for soybean from enclosure studies where there was either a lack of effect or a shortened growing cycle. Taken together, the comparison of results between FACE and enclosure experiments suggest that our current projections of future food supply, based largely on chamber studies, are overly optimistic. Resolving this potential overestimation of global food supply will require more studies with major food crops and comparisons of the different technologies for examining the effects of $e[\text{CO}_2]$ on crops (see Chapter 24).

In contrast to $e[\text{CO}_2]$, the effect of ozone on soybean yield at SoyFACE was close to that predicted from chamber studies, although this was perhaps by happenstance since the cause for the decrease in yield appears to be different. The meta-analysis of prior chamber studies suggests that decreased net photosynthesis alone was responsible for decreased production at the moderate elevations of $[\text{O}_3]$ used in the SoyFACE experiment (Morgan et al. 2004a). However, photosynthetic analyses of soybean grown under FACE fumigation showed decreases in leaf photosynthesis only as leaves entered senescence, which was accelerated in $e[\text{O}_3]$ (Morgan et al. 2004b). Accelerated senescence induced by $e[\text{O}_3]$ would limit season-long canopy photosynthesis and account for the dry mass decreases and yield losses, despite no response in leaf photosynthesis. Thus projection of crop yield should focus on the relationship of

ozone deposition during the reproductive developmental stage. In addition to the effects of ozone on the normal developmental program of soybean, the defoliation resulting from the 2003 hailstorm provided a unique, albeit unplanned, illustration of the effect of $e[\text{O}_3]$ on a field crop's compromised ability to recover from an extreme event. This might include outbreaks of defoliating insects, high winds, as well as hail; all of which could increase with global climate change and further exacerbate the impact on soybean production. Based on calculations from published linear responses, every 1 ppb increase in tropospheric $[\text{O}_3]$ potentially results in up to a 0.6 % yield reduction calculated from soybean yield response in the unstressed year, based on a 40-ppb threshold for damage and an assumed linear response (Mills et al. 2000; Ashmore 2002). With global $[\text{O}_3]$ increasing by 10 ppb over the next half century (Prather et al. 2001), this potentially will have significant impact on global agriculture, especially in two of the major soybean growing areas of the globe, which are projected to see large $[\text{O}_3]$ increases, i.e., China and the United States mid-west (Fowler et al. 1999a; Prather et al. 2003).

4.7 Conclusions

The SoyFACE experiment is the first to focus on the affects of $e[\text{CO}_2]$ and $e[\text{O}_3]$ on a seed legume under fully open-air conditions. The experiment mimicked $e[\text{CO}_2]$ and $e[\text{O}_3]$ predicted for the middle of this century and was conducted in one of the world's major production areas for corn and soybean under cultivation and management techniques standard for the industry in the United States corn-belt region. Growth of soybean at $e[\text{CO}_2]$ resulted in an approximately 25 % increase in the daily integral of net leaf CO_2 uptake, a 20 % increase in the rate of light saturated CO_2 uptake, a 15 % increase in seed yield, a 15 % increase in above ground primary productivity, and a 20 % increase in node number. Growth of soybean at $e[\text{CO}_2]$ also resulted in approximately a 30 % decrease in mid-day stomatal conductance, a 10 % decrease in stomatal conductance averaged over the day, an 8 % decrease in the limitation of photosynthesis by stomatal conductance, and a 2–3 % decrease in harvest index.

Growth of soybean at $e[\text{CO}_2]$ caused about a 5 % decrease in the ratio of maximum carboxylation capacity compared to maximum electron transport capacity, indicative of acclimation to optimize photosynthetic performance to the higher $[\text{CO}_2]$ conditions. Growth of soybean at $e[\text{CO}_2]$ extended the growing season and resulted in increased herbivory by Japanese beetles.

Growth of soybean at $e[\text{O}_3]$ was largely deleterious to soybean although the effects developed slowly over the course of the growing season. $e[\text{O}_3]$ resulted in decreases in seed yield (15–25 %), above-ground primary productivity (11–23 %), and harvest index (2–3 %). Growth at $e[\text{O}_3]$ caused accelerated senescence of the crop.

Abbreviations

- A = Net leaf CO_2 uptake
 A' = Daily integral of net leaf CO_2 uptake
 A_{sat} = Light-saturated CO_2 uptake
 ANPP = Above-ground primary productivity
 C_i = Intercellular CO_2 concentration
 g_m = Leaf mesophyll conductance
 g_s = Stomatal conductance
 J_{max} = Maximum rate of electron transport
 J_{PSII} = Daily integral of photosystem II electron transport
 l = Stomatal limitation to photosynthesis
 LAI = Leaf area index
 PPI = Photosynthetic photon irradiance
 Rubisco = Ribulose-1,5-bisphosphate carboxylase/oxygenase
 RuBP = Ribulose-1,5-bisphosphate
 T_{air} = Air temperature
 $V_{c,\text{max}}$ = Maximum RuBP saturated rate of carboxylation
 $\phi_{\text{CO}_2,\text{max}}$ = Maximum apparent quantum efficiency of CO_2 uptake

References

- Ainsworth EA, Davey PA, Bernacchi CJ, Dermody OC, Heaton EA, Moore DJ, Morgan PB, Naidu SL, Ra HY, Zhu X, Curtis PS, Long SP (2002) A meta-analysis of elevated $[\text{CO}_2]$ effects on soybean (*Glycine max*) physiology, growth and yield. *Global Change Biol* 8:1–15
- Ainsworth EA, Rogers A, Nelson R, Long SP (2003) Testing the “source-sink” hypothesis of down-regulation of photosynthesis in elevated $[\text{CO}_2]$ in the field with single gene substitutions in *Glycine max*. *Agric For Meteorol* 122:85–94
- Ashmore MR (2002) Effects of oxidants at the whole plant and community level. In: Bell JNB, Treshow M (eds) *Air pollution and plants*. Wiley, London
- Bernacchi CJ, Morgan PB, Ort DR, Long SP (2005) The growth of soybean under free air $[\text{CO}_2]$ enrichment (FACE) stimulates photosynthesis while decreasing in vivo Rubisco activity. *Planta* 220:434–446
- Bezemer TM, Jones TH (1998) Plant–insect herbivore interactions in elevated atmospheric CO_2 : quantitative analyses and guild effects. *Oikos* 82:212–222
- Cotrufo M, Ineson P, Scott A (1998) Elevated CO_2 reduces the nitrogen concentration of plant tissues. *Global Change Biol* 4:43–54
- Dermody O, Long SP, DeLucia EH (2005) How does elevated CO_2 or ozone affect the leaf-area index of soybean when applied independently? *New Phytol* doi:10.1111/j.1469-8137.2005.01565.x
- Drake BG, Gonzalez-Meler MA, Long SP (1997) More efficient plants: a consequence of rising CO_2 . *Annu Rev Plant Physiol* 48:609–639
- FAO–UN (2002) *FAO trade yearbook* (vol 165). FAO, Rome

- Fowler D, Cape JN, Coyle M, Flechard C, Kuylenstierna J, Hicks K, Derwent D, Johnson C, Stevenson D (1999a) The global exposure of forests to air pollutants. *Water Air Soil Pollut* 116:5–32
- Fowler D, Cape JN, Coyle M, Smith RI, Hjellbrekke AG, Simpson D, Derwent RG, Johnson CE (1999b) Modeling photochemical oxidant formation, transport, deposition and exposure of terrestrial ecosystems. *Environ Pollut* 100:43–55
- Fuhrer J, Skarby L, Ashmore MR (1997) Critical levels for ozone effects on vegetation. *Eur Environ Pollut* 97:91–106
- Hamilton JG, Dermody O, Aldea M, Zangerl AR, Rogers A, Berenbaum MR, DeLucia EH (2005) Anthropogenic changes in tropospheric composition increase susceptibility of soybean to insect herbivory. *Environ Entomol* 34:479–485
- Kim HY, Lieffering M, Kobayashi K, Okada M, Miura S (2003) Seasonal changes in the effects of elevated CO₂ on rice at three levels of nitrogen supply: A free air CO₂ enrichment (FACE) experiment. *Global Change Biol* 9:826–837
- Kimball BA, Pinter PJ, Garcia RL, Lamorte RL, Wall GW, Hunsaker DJ, Wechsung G, Wechsung F, Kartschall T (1995) Productivity and water use of wheat under free-air CO₂ enrichment. *Global Change Biol* 1:429–442
- Leakey ADB, Bernacchi CJ, Ort DR, Long SP (2004) Will photosynthesis of maize (*Zea mays*) in the US cornbelt increase in future [CO₂] rich atmospheres? An analysis of diurnal courses of CO₂ uptake under free-air enrichment. *Global Change Biol* 10:951–962
- Long SP, Bernacchi CJ (2003) Gas exchange measurements, what can they tell us about the underlying limitations of photosynthesis? Procedures and sources of error. *J Exp Bot* 54:2393–2401
- Long SP, Ainsworth EA, Rogers A, Ort DR (2004) Rising atmospheric carbon dioxide: Plants FACE the future. *Annu Rev Plant Biol* 55:591–628
- McKee IF, Farage PK, Long SP (1995) The interactive effects of elevated CO₂ and O₃ concentration on photosynthesis in spring wheat. *Photosynth Res* 45:111–119
- McKee IF, Mulholland BJ, Craigon J, Black CR, Long SP (2000) Elevated concentrations of atmospheric CO₂ protect against and compensate for O₃ damage to photosynthetic tissues of field-grown wheat. *New Phytol* 146:427–435
- Miglietta F, Peressotti A, Vaccari FP, Zaldei A, deAngelis P, Scarascia-Mugnozza G (2001) Free-air CO₂ enrichment (FACE) of a poplar plantation: the POPFACE fumigation system. *New Phytol* 150:465–476
- Miller JE, Heagle AS, Pursley WA (1998) Influence of ozone stress on soybean response to carbon dioxide enrichment II. Biomass and development. *Crop Sci* 38:122–128
- Mills G, Hayes F, Buse A, Reynolds B (2000) Air pollution and vegetation. In: UN/ECE IPC (ed) Annual report 1999/2000 of UN/ECE IPC vegetation. Center for Ecology and Hydrology, Bangor
- Morgan PB, Ainsworth EA, Long SP (2004a) Elevated O₃ impact on soybeans, a meta-analysis of photosynthetic, biomass, and yield responses. *Plant Cell Environ* 26:1317–1328
- Morgan PB, Bernacchi CJ, Ort DR, Long SP (2004b) An in vivo analysis of the effect of season-long open-air elevation of ozone to anticipated 2050 levels on photosynthesis in soybean. *Plant Physiol* 135:2348–2357
- Morgan PB, Bollero GA, Nelson RL, Dohleman FG, Long SP (2005a) Smaller than predicted increase in aboveground net primary production and yield of field-grown soybean under fully open-air [CO₂] elevation. *Global Change Biol* 11:1856–1865
- Morgan PB, Bollero GA, Nelson RL, Long SP (2005b) Season-long elevation of ozone concentration by 20 % under fully open-air conditions decreases the growth and production of Midwest soybean crops by ca. 20 %. *Environ Pollut* (in press)

- Mulchi CL, Slaughter L, Saleem M, Lee EH, Pausch R, Rowland R (1992) Growth and physiological-characteristics of soybean in open-top chambers in response to ozone and increased atmospheric CO₂. *Agric Ecosyst Environ* 38:107–118
- Pingali PL (2001) Meeting world maize needs: technological opportunities and priorities for the public sector. In: CIMMYT (ed) 1999–2000 World maize facts and trends. CIMMYT, Mexico City
- Prather M, Ehhalt D, Dentener F, Derwent R, Dlugokencky E, Holland E, Isaksen I, Katima J, Kirchhoff V, Matson P, Midgley P, Wang M (2001) Atmospheric chemistry and greenhouse gases. In: Houghton JT, Ding Y, Griggs DJ, Noguer M, Linder PJ van der, Dai X, Maskell K, Johnson CA (eds) *Climate change 2001: the scientific basis. Contribution of working group I to the third assessment report of the intergovernmental panel on climate change*. Cambridge University Press, Cambridge, pp 239–280
- Prather M, Gauss M, Berntsen T, Isaksen I, Sundet J, Bey I, Brasseur G, Dentener F, Derwent R, Stevenson D, Grenfell L, Hauglustaine D, Horowitz L, Jacob D, Mickley L, Lawrence M, Von Kuhlmann R, Muller J-F, Pitari G, Rogers H, Johnson M, Pyle J, Law K, Van Weele M, Wild O (2003) Fresh air in the 21st century? *Geophys Res Lett* 30:1100, doi: 10.1029/2002GL016285
- Prentice IC, Farquahar GD, Fasham MJR, Goulden ML, Heimann M, Jaramillo VJ, Khashgi HS, Le Quere C, Scholes RJ, Wallace DWR (2001) The carbon cycle and atmospheric carbon dioxide. In: Houghton JT, Ding Y, Griggs DJ, Noguer M, van der Linder PJ, Dai X, Maskell K, Johnson CA (eds) *Climate change 2001: the scientific basis. Contribution of working group I to the third assessment report of the intergovernmental panel on climate change*. Cambridge University Press, Cambridge, pp 183–230
- Rogers A, Allen DJ, Davey PA, Morgan PB, Ainsworth EA, Bernacchi CJ, Cornic G, Dermody O, Dohleman FG, Heaton EA, Mahoney J, Zhu X-G, DeLucia EH, Ort DR, Long SP (2004) Leaf photosynthesis and carbohydrate dynamics of soybeans grown throughout their life-cycle under free-air carbon dioxide enrichment. *Plant Cell Environ* 27:449–458
- USDA (2004) *Crop production 2003 summary*. Agricultural Statistics Board, National Agricultural Statistics Service, Washington, D.C.
- Vessey JK, Walsh KB, Layzell DB (1988) Oxygen limitation of N₂ fixation in stem-girdled and nitrate-treated soybean. *Physiol Plant* 73:113–121
- Whittaker JB (1999) Impacts and responses at population level of herbivorous insects to elevated CO₂. *Eur J Entomol* 96:149–156
- Zheng Y, Shimizu H, Barnes JD (2002) Limitations to CO₂ assimilation in ozone-exposed leaves of *Plantago major*. *New Phytol* 155:67–78
- Ziska LH, Bunce JA (1995) Growth and photosynthetic response of 3 soybean cultivars to simultaneous increases in growth temperature and CO₂. *Physiol Plant* 94:575–584