Elevated CO_2 reduces O_3 flux and O_3-induced yield losses in soybeans: possible implications for elevated CO_2 studies[1]

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Abstract

Soybeans were grown for three seasons in open-top field chambers to determine (1) whether elevated CO_2 (360 versus 700 μmol mol^{-1}) alleviates some of the yield loss due to pollutant O_3, (2) whether the partial stomatal closure resulting from chronic O_3 exposure (charcoal-filtered air versus 1.5 × ambient concentrations) is a cause or result of decreased photosynthesis, and (3) possible implications of CO_2/O_3 interactions to climate change studies using elevated CO_2. Leaf conductance was reduced by elevated CO_2, regardless of O_3 level, or by exposure to O_3 alone. As a result of these effects on conductance, high CO_2 reduced estimated midday O_3 flux into the leaf by an average of 50% in charcoal-filtered air and 35% in the high O_3 treatment. However, while exposure to O_3 reduced seed yields by 41% at ambient CO_2 levels, the yield reduction was completely ameliorated by elevated CO_2. The threshold midday O_3 flux for yield loss appears to be 20–30 nmol m^{-2} s^{-1} in this study. Although elevated CO_2 increased total biomass production, it did not increase seed yields. A/C_i curves show a large reduction in the stomatal limitation to photosynthesis due to elevated CO_2, but no effect of O_3. These data demonstrate that (1) reduced conductance due to O_3 is the result, and not the cause, of reduced photosynthesis, (2) 700 μmol mol^{-1} CO_2 can completely ameliorate yield losses due to O_3 within the limits of these experiments, and (3) some reports of increased yields under elevated CO_2 treatments may, at least in part, reflect the amelioration of unrecognized suppression of yield by O_3 or other stresses.

Key words: Stomatal limitation, elevated CO_2, O_3 flux, *Glycine max*, yield suppression.

Introduction

Plant-related interactions are likely between pollutant gases and the anthropogenically generated increases in atmospheric CO_2 concentrations. Of particular interest are possible interactions between CO_2 and tropospheric ozone, which is among the most phytotoxic of air pollutants (Heck, 1989). Elevated CO_2 can increase net photosynthesis while decreasing leaf conductance (reviewed in Cure and Acock, 1986; Allen, 1990) leading to increased water use efficiency (Kimball and Idso, 1983). In this case, conductance is decreased because of increased internal $[CO_2]$ (C_i) (Mott, 1988) as a result of increased ambient concentrations. In contrast, O_3 exposure decreases leaf conductance, but it also decreases net photosynthesis and water use efficiency (Reich *et al.*, 1985; Vozzo *et al.*, 1995). These observations suggested that O_3-induced decreases in conductance were an indirect consequence of damage to the photosynthetic apparatus leading to increased C_i and subsequent stomatal closure. Direct evidence supporting this view was lacking until the

recent study of McKee *et al.* (1995) in which they manipulated C_i in wheat leaves and showed that, in their system, the stomatal limitation was unaffected by O_3 exposure. They concluded that increased C_i, leading to reduced conductance, resulted from decreased photosynthesis and that reduced photosynthesis was not the result of some direct effect of O_3 on stomata. It is important for understanding the mechanisms of O_3-induced yield suppression in field situations to establish whether or not there is a similar lack of effect of O_3 on the stomatal limitation to photosynthesis for soybean grown in open-top chambers under natural irradiance conditions.

In addition to increasing net photosynthesis and *WUE*, elevated CO_2 might also play a role in protecting crops from damaging tropospheric O_3. It has been reported (Miller *et al.*, 1988, 1995) that reductions in stomatal conductance due to water stress might moderate the effects of high levels of tropospheric O_3, presumably by limiting access of the O_3 to the mesophyll. Similarly, elevated CO_2 might also restrict entry of damaging O_3 via reductions in stomatal conductance without the adverse effects of the water stress. Information on the interactive effects of O_3 and CO_2 on conductance and yield is limited. Allen (1990) combined a model for the effects of atmospheric CO_2 on stomatal conductance in soybean with a model for the effects of O_3 on soybean yield. The combined models suggested a 15% increase in yield with elevated CO_2 in O_3-fumigated plants because of a reduction in pollutant entry into the leaf. A field study by Kramer *et al.* (1991) supported the notion that elevated CO_2 could provide protection against O_3 damage in soybean as characterized by elevated polyamine levels. Also, Mulchi *et al.* (1992) suggested that a 150 μmol mol^{-1} addition of CO_2 had 'a protective role against adverse effects of O_3 exposure' on seed yield. Three questions arise from the aforementioned observations. (1) Is the partial stomatal closure resulting from chronic O_3 exposure a result of reduced photosynthesis under conditions approaching a field situation? (2) Can elevated CO_2 protect soybean yields from ozone damage through its effect on leaf conductance? (3) What are the implications of the interactions between elevated CO_2 and O_3 for experimental protocols and interpretation of results of climate change studies using elevated CO_2? These questions are addressed in this paper.

Materials and methods

In 1993, 1994 and 1995, soybeans (*Glycine max* (L.) Merr. cv. Essex) were grown in 21 000 cm^3 pots in a 2:1:1 (by vol.) mixture of soil, sand and Metro-Mix 220 (WR Grace and Co., Cambridge, MA)[1] in open-top field chambers (Heagle *et al.*, 1979) at Raleigh, NC, USA. Plants were watered daily and fertilized biweekly with 'Peters Blossom Booster' (10–30–20:N-P-K) (Grace-Sierra Horticultural Products Co., Milpitas, CA)[1] and three times during the season with 'Peters STEM' soluble

trace elements and micronutrient mix (Grace-Sierra Horticultural Products Co., Milpitas, CA)[1]. Further details of plant cultural conditions may be found in Miller *et al.* (1994).

During all three years when weather and lighting conditions (no precipitation after sundown on the previous day and *PAR* >1000 μmol m^{-2} s^{-1}) permitted, midday leaf conductances (g_W) were measured on the abaxial and adaxial surfaces of the fourth or fifth main-stem node from the apex with an LI-1600 Steady State Porometer (LI-COR, Lincoln, NE)[1]. Over the course of these experiments, the porometer was factory calibrated several times and subject to periodic operational checks including measurements of wet filter paper to ensure that the measured conductance conformed adequately to the manufacturer's specified boundary layer conductance. In 1993, it was not possible to measure all the treatments on the same day. In 1995 leaf conductances were not measured with a frequency adequate to provide meaningful ontogenetic patterns so only the means are reported. Thus temporally defined statistical treatment comparisons were performed only for 1994.

Midday O_3 flux into the leaf was estimated as

$$J_{O_3} = g_{O_3}([O_3]_a - [O_3]_l) \tag{1}$$

where the subscripts *a* and *l* indicate the ambient air and leaf interior; $[O_3]_a$ is taken as the bulk air concentration at the time that water vapour conductance was measured; and $[O_3]_l$ is assumed to be zero (Laisk *et al.*, 1989). The conductance to O_3 (g_{O_3}) was estimated by dividing the conductance for water vapour by the ratio of the binary diffusivities for water vapour in air to O_3 in air ($D_{H2O/Air}/D_{O3/Air}$). The binary diffusivity of O_3 in air was calculated for an average midday temperature of 305 K using the procedure of Fuller *et al.* (1966). This procedure relies heavily on knowledge of diffusion volumes, which were estimated by linear regression of known diffusion volumes for H_2, D_2, N_2, O_2, CO, NH_3, CO_2, H_2O, and air. Previous ozone flux calculations (Laisk *et al.*, 1989; Wang *et al.*, 1995) have used the algorithm developed by Chen and Othmer (1962). The algorithm of Fuller *et al.* (1966) yields values for the binary diffusivities of CO_2 in air and H_2O in air that agree to within 5% of handbook (Weast and Astle, 1981) values, while the calculations of Chen and Othmer (1962) underestimate the handbook value for H_2O in air by 14%. However, because the binary diffusivity of O_3 in air as calculated by Chen and Othmer (1962) is also thought to be underestimated by about 10%, the ratio of 1.67 obtained by Laisk *et al.* (1989) is still within 2% of the ratio of 1.64 obtained from Fuller *et al.* (1966) calculations. Even though the difference between these ratios is small, the latter value was used, which was considered to be slightly more accurate. Seasonal mean midday O_3 fluxes for each treatment were calculated from only those days on which g_W was measured.

During 1994, the gas exchange characteristics of fully expanded canopy leaves (nodes 4 or 5 from the apex) were determined in the laboratory. Assimilation/internal CO_2 concentration (A/C_i) curves were measured using an open gas exchange system consisting of an LI-6262 infrared gas analyser (LI-COR, Lincoln, NE) and a 5.5 l temperature controlled Lexan cuvette. Leaf temperature was controlled at a nominal 28 °C. The incoming gas was brought to the desired CO_2 concentration by mixing CO_2-free air with 5% CO_2 in air. The air was then humidified to create a vapour pressure deficit (*VPD*) of 1.5–1.6 kPa (about 55% relative humidity). The flow rate in the cuvette ranged from 5 to 9.5 l min^{-1} depending on the leaf photosynthetic activity. Irradiance was set to 1300 μmol m^{-2} s^{-1} *PAR* using four 500 W quartz-halogen lamps (Regent Lighting Corp., Burlington, NC). Light response curves showed this level of *PAR* to be above saturation for photosynthesis.

The light was filtered through a 5 cm layer of water to reduce the heat load on the cuvette. Stomatal limitation was estimated from the A/C_i data for the actual growth [CO_2] by the method of Farquhar and Sharkey (1982). Seed yield was determined at physiological maturity. Plants were air-dried, the pods mechanically threshed and the seed weight per plant determined. In 1993 and 1994, shoots were severed at ground level, the roots separated from the soil and both separately oven-dried at 60 °C to constant weight. In 1995, biomass other than seed weight, was not measured.

In each year, the experiment was a completely randomized design of two replicates with two levels of O_3 (charcoal-filtered air (CF) and a nominal 1.5 × ambient concentration (OZ)) and two levels of CO_2 (ambient (nominal 360 μmol mol^{-1}) and a nominal 700 μmol mol^{-1}) for a total of eight open-top chambers. During all three years, the supplemental CO_2 was administered for 24 h d^{-1} while the O_3 addition was confined to the 12 h period centred around noon. Seasonal concentrations of the gases are given in Table 1.

Data for each year were combined across the season and subjected to a two-way ANOVA. Pairwise comparisons were performed using a t-test to determine individual treatment differences. During 1994 and 1995, all treatments were measured on the same day; thus data for each day could be analysed by ANOVA. The yield data for all three years were subjected separately to pairwise comparison using a t-test. Differences were deemed not significant if $P > 0.05$.

Results and discussion

Conductance

There is considerable ontogenetic and temporal variability in leaf conductance (Fig. 1), even when measurements were confined to relatively high levels of *PAR*. The relationships in the raw data are difficult to interpret, but the clarity of the visual presentation may be enhanced simply by drawing a boundary line connecting the highest values of each data set and removing all data below that line from the figure (Fig. 2). All available data were still included in the statistical analyses (Table 2).

The conductance changed most rapidly during vegetative growth (Fig. 2) and reached a peak at 45–60 d after planting (DAP), about the time of flowering. The shift of this peak forward in time, possibly due to the accelerated development caused by O_3, can easily confuse the relationship between treatment effects. Thus, depending on the exact timing of the measurements during the
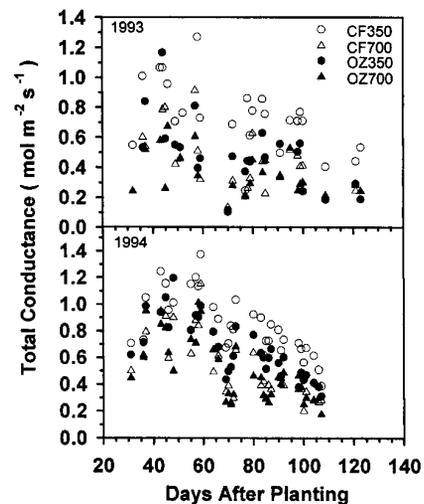

Fig. 1. Mean midday conductance for 1993 and 1994. Each point is the combined abaxial and adaxial conductance for eight leaves from each treatment.
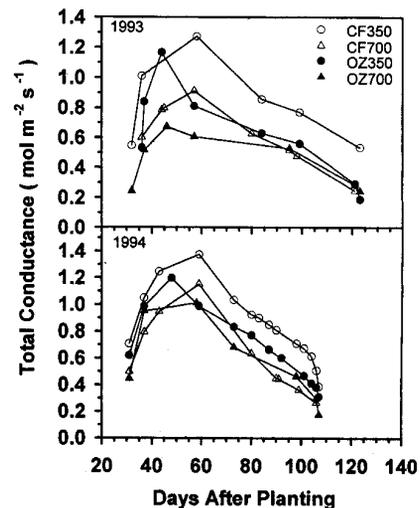

Fig. 2. Upper boundary line for leaf conductance effects.

Table 1. *Gas concentrations for the experiments*

CO_2 concentrations are 24 h means throughout the growing season in all cases. O_3 concentrations are 12 h daily means. Two values for the supplemental [O_3] are given: the * indicates the 12 h [O_3] averaged only over those days on which conductance measurements were made; the other column is the season-long 12 h average.

Year	[O_3] CF (nmol mol^{-1})	[O_3]* Supplement (nmol mol^{-1})	[O_3] Supplement (nmol mol^{-1})	[CO_2] Ambient (μmol mol^{-1})	[CO_2] Elevated (μmol mol^{-1})
1993	22	102	92	369	713
1994	25	95	70	372	703
1995	21	105	83	370	696

Table 2. *Seasonal mean midday leaf conductance to water vapour* (g_W), *estimated midday* O_3 *flux into the leaf, seed yield at physiological maturity and peak seasonal biomass*

CO_2 and O_3 treatments are indicated as nominal values. Numbers followed by the same letter in each column for each year are not significantly different at $P < 0.05$. Total biomass was not measured in 1995.

Year	O_3 Treatment	[CO_2] (μmol mol^{-1})	g_W (mol m^{-2} s^{-1})	O_3 flux (nmol m^{-2} s^{-1})	Seed yield (g plant^{-1})	Total biomass (g plant^{-1})
1993	CF	360	0.707 a	11.5 a	147.0 a	287.5 ac
		700	0.441 b	5.8 b	154.4 a	427.4 b
	1.5×	360	0.498 b	32.9 c	92.1 b	213.0 c
		700	0.369 c	25.2 d	163.6 a	373.7 ab
1994	CF	360	0.846 a	12.8 a	185.7 a	337.3 a
		700	0.520 b	7.8 b	190.7 a	470.6 b
	1.5×	360	0.665 c	35.8 c	120.3 b	243.1 c
		700	0.452 b	23.6 d	190.6 a	447.5 b
1995	CF	360	0.629 a	10.3 a	186.3 a	
		700	0.378 bc	6.1 b	173.3 a	
	1.5×	360	0.462 b	28.2 c	92.7 b	
		700	0.324 c	20.3 d	179.3 a	

season, one might see an increase, decrease, or no change in conductance due to O_3, especially in short-duration experiments. Most of the $O_3 \times CO_2$ interactions detected by ANOVA were attributable to the shift in developmental timing. In 1993, elevated CO_2 ameliorated many of the effects of O_3 pollution, but it did not restore the normal developmental timing of the conductance peak. The data for 1994 were ambiguous on this particular point.

Conductance during all three years was significantly reduced by CO_2 regardless of the level of O_3 present (Table 2). Consistent reductions in conductance due to O_3, however, occurred only at ambient CO_2 levels. Conductance consistently tended to be reduced by the supplemental O_3 treatment under elevated CO_2, but, although the differences were similar in magnitude during all three years (−16%, −13% and −14% for 1993, 1994 and 1995, respectively), they were significant only during 1993. A critical assessment of the significance of effects during the 1994 season (Fig. 3) shows there were significant CO_2 effects for 29 of the 30d on which measurements were made, but there were significant effects due to O_3 on only 13d and significant interactions between CO_2 and O_3 on only 12d. In every instance of a statistically significant difference in conductance due either to elevated O_3 or elevated CO_2, conductance was reduced below that of the controls. The data are less clear when there were interactions. Prior to 55 DAP significant interactions occurred on only 2d. In the first of these interactions, 37 DAP, O_3 had no effect on conductance at 360 μmol mol^{-1} of CO_2, but increased conductance with O_3 at 700 μmol mol^{-1}. In the other instance at 48 DAP, O_3 increased conductance at ambient CO_2 and decreased it at elevated CO_2. Starting at 55 DAP, which is about the time of flowering, and continuing throughout the rest of the season, the interaction was always the same when it
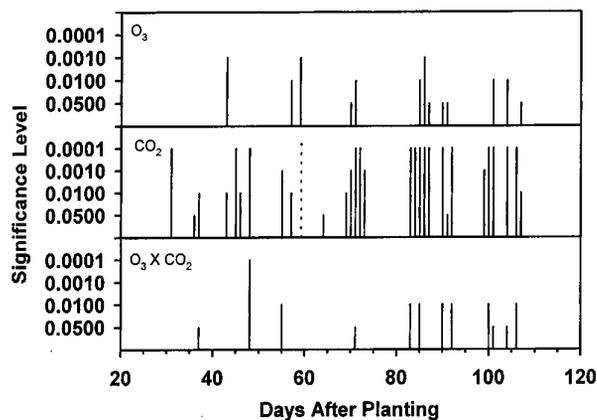

Fig. 3. Statistical significance of leaf conductance effects for the 1994 season. Maximum P values determined from ANOVA performed on data from each sampling day are indicated on the ordinate. There were 30 sampling days throughout the season. The dashed line indicates the only sampling day on which there was no significant CO_2 effect.

occurred; that is O_3 reduced conductance at ambient CO_2, but had no effect at elevated CO_2 levels.

Stomatal limitation

A statistically significant reduction in stomatal limitation due to CO_2 occurred throughout the entire season (Table 3), with the magnitude of the effect declining through the season. ANOVA indicated significant ($P = 0.022$) CO_2 and O_3 ($P = 0.048$) effects during the last sampling period, but pairwise comparisons showed no direct CO_2 or O_3 effects. Thus, O_3 alone had no significant effect at any time during the season.

Consistent with previous reports, net photosynthesis, measured at C_a equal to the [CO_2] at which the plants were grown, was increased by elevated CO_2 (Cure and Acock, 1986; Long and Drake, 1992). As expected, even

Table 3. *Per cent stomatal limitation during four sampling periods throughout the 1994 season*

Days after planting (DAP) are means for the sampling periods and stomatal limitation values are averages of six plants. Numbers followed by the same letter in each column are not significantly different at $P < 0.05$.

DAP		41	58	78	98
O_3 Treatment	$[CO_2]$ (μmol mol^{-1})	(%)			
CF	360	32.5 a	33.6 a	42.6 a	33.7 a
	700	13.5 b	21.2 b	31.2 b	26.9 ab
OZ	360	30.1 a	35.3 a	38.6 a	27.8 ab
	700	12.3 b	19.2 b	29.3 b	23.5 b

though leaf conductance was consistently reduced, the stomatal limitation to photosynthesis was substantially lower as a result of the elevated CO_2 treatment. Conversely, although net photosynthesis and conductance declined in response to O_3, the stomatal limitation to photosynthesis was unaffected by O_3 (see also Sasek and Richardson, 1989). This observation is consistent with, and necessary to, the hypothesis that reductions in leaf conductance due to chronic O_3 exposure are an indirect result of damage to the photosynthetic machinery. Such damage, by reducing the rate of carbon fixation, would lead to elevated C_i and, consequently, to stomatal closure (Mott, 1988). Thus, these data confirm the conclusions of McKee *et al.* (1995) for growth chamber-grown wheat. However, the conditions of measurement of the A/C_i curves were sufficiently different from the open-top chambers that some caution may be warranted regarding this conclusion. Specifically, although *PAR* levels were saturating in the laboratory gas exchange cuvette, they were frequently much lower than in the open-top chambers; the spectral distribution of the light was not the same in the chambers as in the cuvette; the *VPD* in the chambers during 1994 ranged from 1.4 to 2.8 kPa (mean $\pm$ sd = 2.2 $\pm$ 0.4) in the chambers, while it was controlled to 1.5–1.6 kPa in the cuvette; boundary layer conditions were different in the two systems and, finally, in the open-top chambers the entire upper canopy was exposed to ambient lighting, while in the cuvette, only the leaf under study was receiving saturating light. Thus the certainty of these conclusions with regard to the effects of O_3 and CO_2 on the stomatal limitation to photosynthesis must await the repetition of the A/C_i curves under actual open-top chamber conditions.

O_3 flux and seed yield

As expected, the season-long mean for the estimated O_3 flux into the leaf increased with increased ambient O_3 concentrations, but the increase was moderated by elevated CO_2 levels (Table 2). For example, for all three years, doubling the $[CO_2]$ reduced the estimated midday

O_3 flux by an average of 44% in CF air. In the supplemental O_3 treatment the flux was reduced by an average of 29%. All the treatment responses were significantly different.

During the three years of the experiment, seed weight per plant was significantly reduced by an average 41% in response to the supplemental O_3 treatment at ambient levels of CO_2. While pairwise comparison showed no response of seed yield to elevated CO_2 alone, the high CO_2 treatment completely ameliorated the yield suppression caused by O_3 at ambient CO_2 levels. This $CO_2 \times O_3$ interaction term from ANOVA was significant at $P = 0.0012$ in 1993 and at $P < 0.0001$ in the other two years.

The relationship between the estimated mean midday O_3 flux and seed yield for the 3 years (Fig. 4) suggests a possible threshold value in the range of about 20–30 nmol m^{-2} s^{-1}, above which seed yield may be decreased. Of course, interpretation of Fig. 4 may not be as simple as it appears since the elevated CO_2, which restricts entry of O_3, may also have some additional beneficial effect in counteracting the damage resulting from the O_3 that does enter. In addition, the reader is well advised to bear in mind the limitations of the estimated O_3 flux calculations. These fluxes were based on measurements of water vapour conductance which were restricted to leaves in the upper canopy and to the 2 h period surrounding solar noon. The porometer itself imposes an artificial boundary layer condition which might considerably alter calculation of the O_3 flux into the leaf if the undisturbed boundary layer conditions were known. Then too, scaling from individual leaves to the canopy level in order to estimate whole plant exposure has always been problematical.

Lack of a significant effect of elevated CO_2 on yield is contrary to many previous reports (Kimball, 1983; Cure and Acock, 1986). Prior and Rogers (1995), however, also reported a lack of effect of CO_2 enrichment in well-watered soybean grown in open-top chambers, which
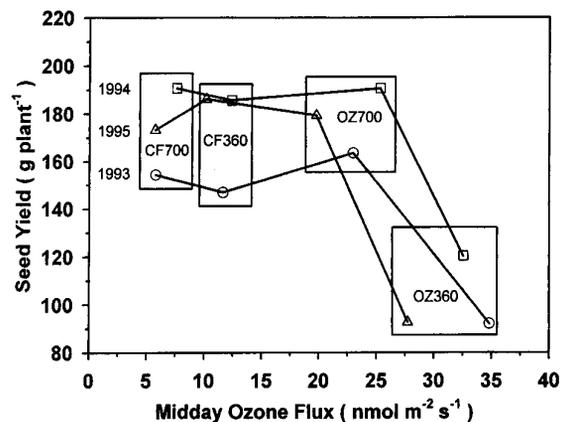

Fig. 4. Seed yield per plant as related to estimated midday O_3 flux. Years are indicated by different symbols and treatments are indicated inside the appropriate boxes of symbols.

they suggested was due to limiting phosphorous availability. There is no reason to believe that phosphorous availability was limiting in our experiment since the inorganic phosphate content of leaf samples was highest in the elevated CO_2 treatments (Booker, unpublished data). The experiment of Mulchi *et al.* (1992) provides the only other data involving combined effects of elevated O_3 and CO_2 on soybean yield. There are several differences between their experiment and ours that may cause significant differences in interpretation. The concentrations of O_3 and CO_2 employed in their study were generally lower than ours. Their seasonal 7 h mean $[O_3]$s were 23 and 66 nmol mol^{-1} for the CF and high O_3 treatment and their $[CO_2]$ levels were a nominal 350, 400 and 500 μmol mol^{-1}. Also, their CO_2 supplements were applied for only 12 h d^{-1}, which might have allowed for a certain degree of physiological reacclimation during the remaining 12 h of each day. Enhancement of photosynthesis is well documented during short-term exposures to high CO_2 (Cure and Acock, 1986) while continuous long-term exposure to high CO_2 often results in a reduction of photosynthesis below this enhanced rate (De Lucia *et al.*, 1985; Socias, *et al.*, 1993). Twelve hour pulses of CO_2 enrichment are also a concern since respiration may increase with increasing CO_2 (Thomas *et al.*, 1993; Thomas and Griffin, 1994), or, it has been shown to decrease in both short- and long-term continuous exposures to elevated CO_2 (Bunce, 1995). Nonetheless, even though yields were lower under elevated O_3 conditions in the experiment of Mulchi *et al.* (1992), the response to CO_2 was much more pronounced. The only comparable O_3 treatments between the present experiment and Mulchi *et al.* (1992) were the CF controls, which in their study did not show a continually increasing trend at all CO_2 levels. Another similarity between the present study and that of Mulchi *et al.* (1992) is that the greatest enhancement in yield with increased CO_2 was observed at the highest level of O_3. In fact, examination of their data shows that in the 150 μmol mol^{-1} CO_2 addition the fractional yield response to CO_2 steadily increased over the range of O_3 levels employed. The yield increases over the controls (CF-350 μmol mol^{-1} CO_2) were about 11%, 18% and 23% for the CF, NF (non-filtered air) and NF + O_3 (non-filtered air plus supplemental O_3) treatments. It should be noted, however, that the 11% increase was very near the limit of detectability for their data. Considering site and exposure protocol differences between these two experiments that make precise data comparisons difficult, the data of Mulchi *et al.* (1992) may not be entirely contradictory to our conclusion that some reports of yield increase in response to elevated CO_2 may result from elevated CO_2 ameliorating the detrimental effects of ambient O_3. Indeed, when the seed yields in the high O_3 treatment are averaged over the 3 years of our experiment, an apparent CO_2-induced yield

increase of 75% is seen. Under more normal O_3 loads for this location, yield increases in response to doubled $[CO_2]$ in the range of 30–40% would be possible. Clearly, this draws attention to the need to monitor ambient levels of air pollutants in CO_2-enrichment studies.

Biomass production

During 1993 and 1994, the only years for which complete data sets are available, total biomass was increased by elevated CO_2 regardless of the O_3 level (Table 2). At ambient CO_2 levels, O_3 significantly suppressed total biomass production during 1994 but the reduction was not significant in 1993. At elevated CO_2 levels, O_3 had no effect on total biomass in either 1993 or 1994.

The effect of chronic O_3 exposure on total biomass production is already well documented (Krupa and Kickert, 1989; Miller *et al.*, 1994). However, these data show that the negative impact of O_3 on biomass production is completely eliminated at high CO_2 levels. Although suppression of biomass production due to O_3 is reflected in a substantial suppression of yield at ambient CO_2 levels, there is neither an O_3 effect at high CO_2 nor a direct effect of CO_2 on seed yield.

Conclusions

In this paper it has been shown that stomatal responses to O_3 must be interpreted within the context of normal ontogenetic variability. Accelerated development due to O_3 poses a major interpretational obstacle which, if not considered, may lead to erroneous conclusions regarding effects of O_3 on leaf conductance. This is especially true of both short-term experiments and experiments in which temporal sampling is limited.

Suppression of seed yield by O_3 was completely ameliorated by the double-ambient CO_2 treatment. A major part of this amelioration probably was due to CO_2-induced decreases in conductance that limited access of O_3 to the leaf interior. The data also suggest the possibility of a threshold midday O_3 flux in the range of 20–30 nmol m^{-2} s^{-1}, below which O_3 damage is not reflected in seed yield. Although O_3 also reduced conductance, it had no effect on the stomatal limitation to photosynthesis under the conditions of these experiments. This result, which is consistent with the conclusion of McKee *et al.* (1995) for growth chamber-grown wheat, demonstrates that stomatal closure in response to O_3 exposure is also the result, rather than the cause, of reduced photosynthesis in plants grown in open-top chambers under high irradiance conditions.

Finally, enhanced CO_2 had no effect on yield in the CF treatment but, because of amelioration of damage could give the appearance of greatly increasing yield in the presence of damaging levels of O_3. These observations

suggest the possibility that some of the reports of CO$_2$-induced yield increases might reflect the amelioration of yield suppression by unmonitored background O$_3$ or other environmental stresses. Workers need to be aware of the potential for such interactions in elevated CO$_2$ studies, especially when using Free Air Carbon Enrichment (FACE) systems where it is generally not possible to filter out damaging pollutants.

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