Growth and Yield Responses of Snap Bean to Mixtures of Carbon Dioxide and Ozone

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ABSTRACT

Elevated CO_2 concentrations expected in the 21st century can stimulate plant growth and yield, whereas tropospheric O_3 suppresses plant growth and yield in many areas of the world. Recent experiments showed that elevated CO_2 often protects plants from O_3 stress, but this has not been tested for many important crop species including snap bean (*Phaseolus vulgaris* L.). The objective of this study was to determine if elevated CO_2 protects snap bean from O_3 stress. An O_3–tolerant cultivar (Tenderette) and an O_3–sensitive selection (S156) were exposed from shortly after emergence to maturity to mixtures of CO_2 and O_3 in open-top field chambers. The two CO_2 treatments were ambient and ambient with CO_2 added for 24 h d^{-1} resulting in seasonal 12 h d^{-1} (0800–2000 h EST) mean concentrations of 366 and 697 $\mu L\ L^{-1}$, respectively. The two O_3 treatments were charcoal-filtered air and nonfiltered air with O_3 added for 12 h d^{-1} to achieve seasonal 12 h d^{-1} (0800–2000 h EST) mean concentrations of 23 and 72 nL L^{-1}, respectively. Elevated CO_2 significantly stimulated growth and pod weight of Tenderette and S156, whereas elevated O_3 significantly suppressed growth and pod weight of S156 but not of Tenderette. The suppressive effect of elevated O_3 on pod dry weight of S156 was approximately 75% at ambient CO_2 and approximately 60% at elevated CO_2 (harvests combined). This amount of protection from O_3 stress afforded by elevated CO_2 was much less than reported for other crop species. Extreme sensitivity to O_3 may be the reason elevated CO_2 failed to significantly protect S156 from O_3 stress.

Carbon dioxide (CO_2) concentrations in the troposphere are expected to continue rising to levels that significantly increase plant growth and yield (Allen, 1990; Cure and Acock, 1986; Kimball et al., 1993; Watson et al., 1990). Conversely, ozone (O_3) concentrations in the troposphere are high enough to suppress plant growth and yield in many areas of the world (Heck et al., 1984; USEPA, 1996).

Because O_3 and CO_2 cause opposite plant responses, numerous studies considering effects of O_3–CO_2 mixtures have been performed over the past 10 yr. Most of these studies revealed that the apparent stimulation caused by CO_2 enrichment is much greater when O_3 concentrations are also high (Barnes and Pfirrmann, 1992; Heagle et al., 1993, 1998, 1999b; Idso and Idso, 1994; Miller et al., 1998; Mortensen, 1992; Mulchi et al., 1992; Rao et al., 1995; Reinert et al., 1998). Apparently, CO_2 protects plants from O_3 stress, causing steeper CO_2 response curves for plants exposed to stressful O_3 levels than for plants exposed to lower O_3 levels. Moreover, the level of the interaction seems to be dictated by the relative amount of O_3 stress and CO_2 enrichment. At a given O_3 level, O_3–sensitive plants may be more responsive to CO_2 enrichment than O_3–tolerant plants.

Bean is more sensitive to O_3 than many other plant species. Ozone can injure leaves and suppress yield of some popular bean cultivars (Heggestad et al., 1980; Schenone et al., 1992), although some are very tolerant (Meiners and Heggestad, 1979; Davis and Kress, 1974; Tonneijck, 1983). Carbon dioxide enrichment increased net carbon assimilation rate and growth, and decreased stomatal conductance of snap bean (Mjwara et al., 1996; Radoglou and Jarvis, 1992; Radoglou et al., 1992; Tognoni et al., 1967). However, effects of CO_2 enrichment on pod weight of snap bean have not been reported. Although seasonal exposure to O_3–CO_2 mixtures usually shows that CO_2 enrichment protects plants from O_3 stress, an exception was shown for snap bean in a short-term experiment (Heck and Dunning, 1967). Carbon dioxide at approximately 850 $\mu L\ L^{-1}$ for 90 min before and during a 30-min exposure to 300 nL L^{-1} of O_3 resulted in significant protection of tobacco (*Nicotiana tabacum* L. 'Bel-W3') but not 'Pinto' snap bean from foliar injury (Heck and Dunning, 1967). Effects of long-term seasonal exposure to CO_2 enrichment or to mixtures of O_3 and CO_2 on foliar injury, growth, and pod weight of snap bean have not been reported.

Because plant species and cultivars vary in response to elevated O_3 and CO_2 singly, research to measure interactive effects of O_3 and CO_2 is needed with additional species. In this study, effects of season-long exposure to mixtures of O_3 and CO_2 were examined for an O_3–tolerant cultivar and an O_3–sensitive selection of snap bean in open-top field chambers.

MATERIALS AND METHODS

Plant Culture

The experiment was performed with snap bean at our field site 5 km south of Raleigh, NC. A commercial snap bean cultivar (Tenderette) and a snap bean selection (S156) derived from a cross between the O_3–sensitive cultivar (Oregon 91) and the O_3–tolerant cultivar (Wade Bush) (Reinert and Eason, 2000) were used. Both of these cultigens (Cg) exhibit determinate growth. Tenderette is very resistant to foliar injury caused by O_3 (Meiners and Heggestad, 1979), whereas S156 is very sensitive (Burkey and Eason, 2002). Seeds were planted 4 cm apart in pots containing 20 L of Metro-Mix 200 and 45 g of Osmocote (14–14–14, N–P–K) slow release fertilizer (Scotts-

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Published in J. Environ. Qual. 31:2008–2014 (2002).

Abbreviations: CF, open-top field chamber receiving charcoal-filtered air; Cg, cultigen, cultivar, or selection of snap bean; NCER, net carbon exchange rate; OZ, open-top field chamber receiving nonfiltered air with O_3 added for 12 h d^{-1}; SC, stomatal conductance.

Table 1. Meteorological conditions and ozone and carbon dioxide concentrations during studies to determine snap bean response to mixtures of carbon dioxide and ozone.

	23–31 May	1–30 June	1–11 July	12–31 July	1–31 July	1–7 August	1–20 August	Seasonal means
Mean temperature, °C	21	25	26	24	25	25	25	24
Mean relative humidity, %	69	70	67	78	74	81	75	73
Mean total PAR†, mol m^2 d^{-1}	43	49	50	40	43	44	47	46
Rainfall, mm	4	168	14	79	93	76	101	366 (total)
Ozone, nL L^{-1}‡								
Ambient air	44	56	59	49	53	38	50	53
CF§	21	25	23	20	21	15	21	23
OZ	65¶	73	87	76	80	47	68	72
Carbon dioxide, μL L^{-1}‡								
Ambient	367	369	359	363	361	366	366	366
Approximately double ambient	642	685	718	713	715	709	712	697

† Photosynthetically active radiation.
‡ Ozone was added to the open-top field chambers receiving nonfiltered air with O_3 added for 12 h d^{-1} (0800–2000 h EST) (OZ). Carbon dioxide was added to double-ambient chambers for 24 h d^{-1}. Ozone and carbon dioxide concentrations are 12 h d^{-1} (0800–2000 h EST) means.
§ *Open-top field chamber receiving charcoal-filtered air.*
¶ Ozone concentrations for 30–31 May.

Sierra Horticultural Products Co., Marysville, OH). Seeds were planted on 15 May and seedlings emerged on 22 May. They were thinned to two per pot on 25 May and to one per pot on 31 May. Plants were irrigated with drip tubes as needed to prevent visible symptoms of water stress. Pot temperatures were moderated with an insulating cylinder composed of 0.6-cm-thick bubble wrap coated on both sides with aluminum (Reflectix [Markleville, IN] TM) fit tightly around each pot. This method of temperature moderation has proven more effective than grain straw as a mulch (Heagle et al., 1999a). Thrips were controlled with acephate (Orthene 75S at 3.9 mL L^{-1}; Valent USA Corporation, Walnut Creek, CA) on 26 May and 6 June. Twospotted spider mites were controlled with bifenthrin (Talstar F at 5.2 mL L^{-1}; FMC, Philadelphia, PA) and abamectin (Avid 0.15 EC at 0.3 mL L^{-1}; Merck & Co., Rahway, NJ) on 22 July.

Treatments

Plants were exposed to O_3 and CO_2 in open-top field chambers, 3 m in diameter × 2.4 m tall (Heagle et al., 1973). The treatment design was a factorial with two O_3 and two CO_2 treatments and two snap-bean cultigens. The whole plot (chamber) treatments were the O_3 × CO_2 combinations arranged in a randomized complete block design with four blocks in 16 chambers. The O_3 treatments were charcoal-filtered (CF) air and nonfiltered air with O_3 added proportionally to the ambient O_3 concentration (OZ). The CO_2 treatments were ambient and approximately double ambient. The two cultigens (Tenderette and S156) were the subplots. Plants were placed in a 2 × 2 Latin square arrangement in each of the four chamber quadrants with the convention that two pots of a given cultigen could not be adjacent within a given row or column.

General dispensing and monitoring protocols have been described for O_3 (Heagle et al., 1979) and for CO_2 (Rogers et al., 1983). Carbon dioxide enrichment began on 23 May and O_3 exposures began on 30 May. Exposures continued through 23 August. Ozone was dispensed for 12 h d^{-1} (0800–2000 h EST) and CO_2 for 24 h d^{-1}. Both gases were monitored for 24 h d^{-1} at canopy height in the center of each chamber. Ozone was monitored with UV analyzers (TECO Model 49; Thermo Environmental Instruments, Franklin, MA) calibrated biweekly with a TECO Model 49 PS calibrator. Carbon dioxide was monitored with infrared analyzers (LI 6252; LI-COR, Lincoln, NE) calibrated biweekly with pressurized tank CO_2 over the range of concentrations used in these experiments. Mean concentrations of O_3 and CO_2 and meteorological conditions during the experiment are shown in Table 1. The seasonal mean 12 h d^{-1} O_3 concentration in the CF treatment was 23 nL L^{-1} (0.43 times ambient), and in the OZ treatment was 72 nL L^{-1} (1.36 times ambient) (Table 1). The seasonal mean 12 h d^{-1} CO_2 concentrations were 366 μL L^{-1} (ambient) and 697 μL L^{-1} for the elevated CO_2 treatment.

Measurements

Foliar net carbon exchange rates (NCER) and stomatal conductance (SC) were measured with a portable photosynthesis system (LI-6200; LI-COR). Measures were made at 28, 29, 39, and 43 d after planting (DAP) between 1030 and 1330 h EST at chamber conditions of relative humidity, temperature,

Table 2. Net carbon exchange rate and stomatal conductance of two snap bean cultigens on four days during exposure to mixtures of ozone and carbon dioxide.

Cultigen	Carbon dioxide†	Ozone†	Net carbon exchange rate‡				Stomatal conductance‡			
			28 DAP	29 DAP	39 DAP	43 DAP	28 DAP	29 DAP	39 DAP	43 DAP
	μL L^{-1}	nL L^{-1}	μmol m^{-2} s^{-1}				mol m^{-2} s^{-1}			
S156	366	23	21.5 (1.0)	23.1 (0.7)	16.4 (4.0)	16.4 (1.8)	1.53 (0.20)	1.96 (0.12)	0.54 (0.27)	0.53 (0.11)
		72	21.4 (2.2)	21.6 (1.0)	11.0 (3.7)	6.8 (1.6)	1.17 (0.07)	1.28 (0.15)	0.77 (0.33)	0.42 (0.06)
	697	23	32.3 (0.0)	37.2 (0.4)	31.7 (2.5)	26.1 (1.5)	1.15 (0.02)	1.38 (0.12)	0.51 (0.01)	0.59 (0.05)
		72	31.9 (1.7)	35.1 (2.2)	27.0 (4.5)	24.2 (1.0)	1.00 (0.08)	1.36 (0.09)	0.60 (0.27)	0.52 (0.06)
Tenderette	366	23	23.5 (0.4)	23.2 (0.5)	12.3 (5.4)	11.5 (3.6)	1.83 (0.03)	1.90 (0.23)	0.44 (0.28)	0.46 (0.23)
		72	21.9 (2.2)	23.8 (0.2)	11.3 (0.8)	13.7 (2.7)	1.32 (0.04)	1.74 (0.04)	0.25 (0.05)	0.53 (0.16)
	697	23	37.9 (0.1)	37.4 (0.2)	19.7 (2.8)	23.1 (1.4)	1.27 (0.56)	1.14 (0.23)	0.20 (0.05)	0.45 (0.10)
		72	34.4 (1.0)	35.4 (2.4)	29.9 (2.5)	21.5 (2.5)	1.11 (0.05)	1.56 (0.20)	0.50 (0.12)	0.36 (0.01)

† Ozone was added to the 72 nL L^{-1} chambers for 12 h d^{-1} (0800–2000 h EST). Carbon dioxide was added to 697 μL L^{-1} chambers for 24 h d^{-1}. Ozone and carbon dioxide concentrations are seasonal 12 h d^{-1} (0800–2000 h EST) means.
‡ DAP, days after planting. Each value is the mean (standard error) for six leaves (one leaf on each of two plants in each of three replicate chambers) at 29 and 43 DAP and four leaves (one leaf on each of two plants in each of two replicate chambers) at 28 and 39 DAP.

Table 3. Vegetative and reproductive responses of S156 and Tenderette snap bean plants to mixtures of ozone and carbon dioxide measured at the midseason harvest.

Cultigen	Carbon dioxide†	Ozone†	Vegetative measures					Reproductive measures							
			Foliar injury	Leaf area	Leaf dry wt.	Stem dry wt.	Root dry wt.	Filled pods			Immature pods			Total shoot wt. (leaf, stem, pod)	Root wt./ shoot wt.	Pod dry wt./ stem dry wt.
								Number	Fresh wt.	Dry wt.	Number	Fresh wt.	Dry wt.			
	μL L^{-1}	nL L^{-1}	%	cm^2	g				g			g			g g^{-1}	
S156	366	23	21	5 320	17.1	25.6	8.7	58	227.1	33.5	21	8.2	0.8	77.6	0.12	1.46
		72	90	1 682	5.5	16.9	3.9	21	60.6	7.7	33	9.7	1.0	31.6	0.12	0.60
	697	23	49	7 136	25.8	41.5	14.5	77	334.1	41.6	31	10.2	1.1	110.6	0.13	1.09
		72	82	3 795	14.0	30.2	8.0	34	122.2	13.8	39	10.9	1.1	60.1	0.13	0.54
Tenderette	366	23	14	10 173	39.1	45.7	20.0	42	165.6	16.6	40	11.4	1.3	103.1	0.20	0.38
		72	16	9 757	35.0	44.9	21.2	34	126.6	13.0	41	12.4	1.4	94.6	0.23	0.30
	697	23	33	12 406	70.0	66.2	29.2	28	102.9	9.9	47	10.7	1.3	148.0	0.21	0.16
		72	30	11 145	63.2	69.4	25.5	42	159.3	15.7	44	11.0	1.4	150.5	0.18	0.22

† Ozone and carbon dioxide concentrations shown are seasonal 12 h d^{-1} means. Concentrations for specific periods are shown in Table 1. Each response value is the mean of 16 plants (four plants in each of four chambers).

CO_2 concentrations, and O_3 concentrations when PAR was greater than 1000 μmol m^2 s^{-1}. At each date, one fully expanded upper-canopy leaf (usually the second youngest) from each of two plants per cultigen was sampled for each mixture treatment in each of two plots on 28 and 39 DAP and in each of three plots on 29 and 43 DAP.

At 57 DAP, foliar injury (chlorosis and necrosis) of the upper canopy was estimated in 5% increments (0–100%) on four plants of each cultigen in all plots. Beginning at 57 DAP, plants in the south half of each chamber were harvested on four consecutive days. Plants were cut at the stem base and separated into stems, leaves, filled pods (pods with obvious seed expansion), and immature pods (tiny pods with no obvious seed expansion). Leaf areas were measured with a LI-3100 area meter (LI-COR). Numbers and fresh weights of filled and immature pods were recorded and roots were washed. Stems, leaves, pods, and roots were dried to constant weight at 55°C and weighed.

The remaining eight plants per plot were harvested when most pods were brown and growth was judged to be minimal. The S156 matured sooner than Tenderette, and leaves and pods of S156 plants in the OZ plots turned brown sooner than S156 plants in CF plots. Therefore, S156 plants were harvested between 84 and 86 DAP in the OZ plots and at 98 DAP in the CF plots. Tenderette plants in all plots were harvested between 98 and 101 DAP. Filled and immature pods were counted. Pods and stems were dried to constant weight at 55°C and weighed.

Statistical Analyses

Data were analyzed with the plot (chamber) mean for each cultigen by treatment combination from each block. Because of the large cultigen difference in sensitivity to O_3 and exposure duration, data were analyzed for each cultigen separately and for the cultigens combined. Residual plots were examined for nonnormality, outliers, and heterogeneous variances. All variables were analyzed without transformation except for leaf dry weight, which was analyzed with the square-root transformation.

RESULTS

Net Carbon Exchange Rate and Conductance

Elevated CO_2 increased net carbon exchange rate (NCER) and generally suppressed stomatal conductance (SC) of both cultigens (Table 2). Effects of O_3 on NCER and SC varied with the CO_2 treatment and was different for the two cultigens. On the last two measurement days, O_3 suppressed NCER of S156 in ambient CO_2, but less O_3 effect was noted with plants at elevated CO_2. Little effect of O_3 on NCER was noted for Tenderette. Effects of O_3 on SC for both cultigens were variable across measurement dates at both CO_2 treatment concentrations.

Midseason Harvest

Symptoms of O_3 injury included chlorosis, bronzing, and early senescence of middle-aged and older leaves, whereas the prominent symptom at elevated CO_2 was chlorosis of newly expanded canopy leaves. Ozone caused severe foliar injury of S156 but not of Tenderette, and elevated CO_2 significantly injured both cultigens (Tables 3 and 4). For S156, elevated CO_2 caused chloro-

Table 4. Mean squares and significance levels from combined analyses of variance for growth and yield responses measured at midseason and final harvest of two snap bean cultigens (Cg) exposed to mixtures of ozone and carbon dioxide.

Harvest	Source	df	Foliar injury	Leaf area × 10^{-5}	Leaf dry wt.†	Stem dry wt.	Root dry wt.	Total shoot wt. (leaf, stem, pod)	Root/shoot dry wt. × 10^4	Pod/stem dry wt. × 100
Midseason	block	3	77.0 *	2.5	0.1	46.6	9.6	262.2	12.1	0.3
	O_3	1	5 031.3**	374.6**	7.5**	154.0	96.1**	5 244.4**	0.0	101.3**
	CO_2	1	1 361.8**	284.9**	20.9**	2 757.6**	275.7**	13 176.9**	1.5	27.1**
	$O_3 \times CO_2$	1	812.5*	1.5	0.1	0.9	21.8	20.9	19.7	10.2*
	Error a	9	96.7	13.8	0.2	36.0	7.5	226.0	7.0	1.7
	Cg	1	11 109.8**	3 263.7**	87.0**	6 276.6**	1 860.2**	23 392.3**	476.9**	345.7**
	Cg × O_3	1	5 157.5**	140.5**	2.8**	249.6	38.6*	4 091.0**	0.2	97.6**
	Cg × CO_2	1	82.1	0.5	1.7**	127.9	6.4	777.8	23.5	1.0
	Cg × O_3 × CO_2	1	498.1**	6.5	0.1	22.2	5.1	118.8	10.3	1.5
	Error b	12	19.2	13.6	0.2	60.5	5.2	274.3	6.9	1.2

			Filled pods			Immature pods		
Midseason			Number	Fresh wt.	Dry wt.	Number	Fresh wt.	Dry wt. × 100
	block	3	30.3	1 108.8	38.5	41.5	9.3	11.3
	O_3	1	2 728.8**	65 140.2**	1 321.7**	170.0	6.0	10.4
	CO_2	1	357.8	9 597.2	51.9	323.5	0.4	6.8
	$O_3 \times CO_2$	1	114.4	1 246.6	26.8	28.6	1.1	2.0
	Error a	9	147.4	2 762.3	38.6	100.8	14.6	17.8
	Cg	1	979.0*	17 961.0*	859.4**	1 185.2**	21.2	93.0*
	Cg × O_3	1	3 795.4**	78 370.9**	1 551.4**	232.5	0.6	0.0
	Cg × CO_2	1	693.8	19 702.4**	163.2*	18.4	14.0	6.4
	Cg × O_3 × CO_2	1	431.4	9 892.1	64.5	0.1	0.0	0.3
	Error b	12	152.2	2 350.4	26.4	145.0	9.7	13.4

Final			Filled pods number	Immature pods number	All pods dry wt.	Stem dry wt.	Pod wt./ stem wt.
	block	3	150	106	257	118.8	0.3
	O_3	1	5 513**	1 219**	10 945**	1 766.0**	5.6**
	CO_2	1	4 198**	12	2 056**	3 090.0**	0.4
	$O_3 \times CO_2$	1	61	5	158	83.2	0.1
	Error a	9	122	38	181	118.0	0.2
	Cg	1	2 628**	30	15 012**	15 721.0**	3.5**
	Cg × O_3	1	9 783**	15	7 584**	0.4	10.5**
	Cg × CO_2	1	713	61	62	972.0**	1.0**
	Cg × O_3 × CO_2	1	198	5	127	43.3	0.7**
	Error b	12	169	16	190	118.1	0.1

* Significant at the 0.05 probability level.
** Significant at the 0.01 probability level.
† Leaf dry weight data were transformed by square root before statistical analysis.

sis in the CF but not in the OZ treatments, and O_3 caused more injury at ambient than at double-ambient CO_2. Either of these differences may have caused the significant $O_3 \times CO_2$ interaction for S156 (Table 4).

For S156, O_3 significantly suppressed all vegetative growth and filled pod measures at both CO_2 levels. For example, compared with the CF treatment, the OZ treatment suppressed S156 filled pod fresh weight by 73% in ambient CO_2 and by 63% in elevated CO_2. For Tenderette, however, O_3 did not significantly affect any measured growth or reproductive component (Table 3) and the Cg $\times$ O_3 interaction was significant for most response measures (Table 4).

Carbon dioxide enrichment significantly increased vegetative growth of both cultigens and these effects were generally independent of the O_3 treatment (Tables 3 and 4). For the O_3 treatments combined, total shoot weight (leaves, stems, and pods) of S156 and Tenderette was 56 and 51% greater, respectively, at elevated than at ambient CO_2 (Table 3). Carbon dioxide enrichment also increased the number and weight of filled pods of S156, but an opposite trend occurred for Tenderette in the CF chambers (Table 3) so that the Cg $\times$ CO_2 effect was significant for filled pod weight (Table 4). For example, in CF air, filled pod dry weight of Tenderette was 40% less in elevated than in ambient CO_2. This trend for lower filled pod weight at elevated than at ambient CO_2 did not occur for Tenderette in the OZ chambers (Table 3).

Ratios of pod weight to stem weight were larger for S156 than for Tenderette in all treatments (Table 3). Elevated CO_2 significantly decreased the ratio of pod weight to stem weight for both cultigens (Table 3). Elevated O_3 decreased pod weight to stem weight for S156 but not for Tenderette, and the Cg $\times$ O_3 interaction was significant (Table 4).

The ratios of root to shoot weight responses of the cultigens were significantly different (Tables 3 and 4). For S156, ratios of root to shoot weight were not affected by O_3 or CO_2 (Table 3). However, for Tenderette, the ratio of root to shoot weight was higher in OZ than in CF at ambient CO_2, but was lower in OZ than in CF at elevated CO_2, and the $O_3 \times CO_2$ interaction was significant for Tenderette (Table 3). The high Tenderette root to shoot ratio in OZ + ambient CO_2 was probably related to the comparatively low filled pod weight in that treatment (Table 3).

Final Harvest

Ozone significantly suppressed filled pod number and pod weight of S156 but not of Tenderette (Table 5), resulting in a significant Cg $\times$ O_3 interaction for these variables (Table 4). Elevated CO_2 generally increased pod number, pod weight, and stem weight of both cultigens (Tables 4 and 5). The CO_2 effect was significant for these measures in the analysis for the cultigens combined (Table 4) and significant or nearly so for all measures except number of immature pods in the analysis for the cultigens separately (Table 5). The Cg $\times$ O_3, Cg $\times$ CO_2, and Cg $\times$ O_3 $\times$ CO_2 interactions were significant for the ratio of pod weight to stem weight (Table 4). Ozone dramatically decreased the ratio of pod weight to stem weight for S156 but increased the ratio for Tenderette (Table 5). Elevated CO_2 decreased the ratio of pod weight to stem weight of Tenderette but not of S156. For S156 in the OZ treatment, the ratio of pod weight to stem weight was higher at elevated than at ambient CO_2 (Table 5).

DISCUSSION

The number and weight of filled pods generally increased between the midseason and final harvest, except for S156 at ambient CO_2 and high O_3. In this treatment, S156 was severely stressed by O_3 with estimated visible injury at 90% at the midseason harvest. This high level of stress apparently caused abscission of immature pods and no increase in number and weight of filled pods between the midseason and final harvest. The decline

Table 5. Growth and reproductive responses of S156 and Tenderette snap bean to mixtures of ozone and carbon dioxide at final harvest.†

			Number per plant		Dry weight per plant		
Cultigen	Carbon dioxide‡	Ozone‡	Filled pods	Immature pods	All pods	Stems	Pod wt./stem wt.
	μL L^{-1}	nL L^{-1}			g		
S156	366	23	79	15	75.7	22.7	3.47
		72	20	0	7.5	7.2	1.05
	697	23	114	12	94.0	30.5	3.17
		72	51	0	26.7	16.7	1.62
Tenderette	366	23	77	12	95.0	58.6	1.70
		72	78	1	80.4	38.0	2.19
	697	23	83	16	99.8	83.7	1.31
		72	99	5	102.0	74.2	1.45
	Source	df	Probability of a value > *F* from analysis of variance				
S156	block	3	0.84	0.30	0.59	0.08	0.40
	O_3	1	0.00	0.00	0.00	0.00	0.00
	CO_2	1	0.00	0.63	0.02	0.00	0.51
	$O_3 \times CO_2$	1	0.72	0.63	0.94	0.65	0.05
Tenderette	block	3	0.16	0.10	0.07	0.79	0.12
	O_3	1	0.18	0.00	0.33	0.09	0.04
	CO_2	1	0.05	0.11	0.06	0.00	0.00
	$O_3 \times CO_2$	1	0.23	0.99	0.20	0.50	0.21

† Each response value is the mean of 16 plants (four plants in each of four chambers).
‡ Ozone and carbon dioxide concentrations shown are seasonal 12 h d^{-1} means. Concentrations for specific periods are shown in Table 1.

in stem weight between the midseason and final harvest, especially for S156 at high O_3, can be explained by translocation of assimilate and respiration, which usually accompanies plant senescence.

Elevated CO_2 was much less protective against O_3 stress in the highly O_3–sensitive snap bean cultigen S156 than for any of the other crops studied. For example, in one year with soybean, O_3 yield suppression was 37% at ambient CO_2, but was negligible at double-ambient CO_2 (Heagle et al., 1998). In a second season, O_3 decreased soybean yield by 40% at ambient CO_2 and by 16% at double-ambient CO_2 (Heagle et al., 1998). Similar results were found with cotton (Heagle et al., 1999b) and with an O_3–sensitive cultivar of wheat (Heagle et al., 2000). In the present study, however, doubled CO_2 provided very little protection against severe O_3 suppression of pod yield for S156, even though exposure to elevated CO_2 alone stimulated pod yield by 24%. For Tenderette, however, doubled CO_2 completely prevented the already less severe pod yield suppression (15%) due to O_3. It appears that the extreme sensitivity to O_3 in S156 overwhelmed what protection elevated CO_2 might have provided.

The present results do not adequately show whether differences in effects of elevated CO_2 on stomatal conductance account for differences in the protective effects of elevated CO_2 among species. The maximum decrease in stomatal conductance of S156 caused by elevated CO_2 was approximately 30%, whereas elevated CO_2 decreased stomatal conductance of soybean by approximately 40% (J.E. Miller, personal communication, 2001). Further research is needed to determine the degree to which differences in CO_2 effects on stomatal conductance are related to differential levels of CO_2 protection from O_3 stress.

Our results confirmed the difference in O_3 sensitivity of the two cultigens. Under the exposure conditions employed, elevated O_3 suppressed the bean yield of sensitive S156 by 80 to 90%, but did not significantly affect the yield of tolerant Tenderette. The basis for this difference in O_3 response does not appear to involve O_3 exclusion. A cultigen comparison of midday SC for each date × treatment combination (Table 2) revealed very few cases where S156 and Tenderette were different, and where differences were observed there was no trend to suggest that O_3 uptake was greater in S156. Overall, cultigen differences in SC were small compared with the large difference in O_3 effect on yield. Studies are planned to determine whether a subtle difference in leaf gas exchange (e.g., diurnal pattern, stomata response to environmental factors) might explain the observed differences in O_3 sensitivity. Differences in cultigen detoxification of O_3 in the leaf interior may explain the differential sensitivity. This hypothesis is supported by recent observations that extracellular ascorbic acid content is significantly higher in tolerant Tenderette than in sensitive S156 (Burkey and Eason, 2002).

Prior to the present study, experimental evidence strongly suggested that elevated CO_2 protects O_3–sensitive plants from O_3 stress. Such protection generally resulted in greater growth and yield enhancement for O_3–sensitive than for O_3–tolerant plants. The present study shows that protection from O_3 stress is not necessarily controlled by relative sensitivity to O_3, by the O_3 concentration, or by relative response to CO_2 enrichment. The degree of $O_3 \times CO_2$ interaction for a given species or cultivar cannot be predicted from response to the individual gases. These results emphasize the need to understand interactive effects between O_3 and CO_2 on yield of major food crops to improve estimates of crop yield at CO_2 concentrations expected in the future.

ACKNOWLEDGMENTS

We thank Bob Philbeck, Fred Mowry, and Jeff Barton for dispensing and monitoring support and Bianca Bradford, Julie Clingerman, Josh Collins, James Jackson, Robin Randall, Aminah Thompson, and Renee Tucker for technical support and Barbara Shew and Steve Shafer for manuscript review.

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