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RESEARCH

Elevated Carbon Dioxide and Ozone Effects on Peanut: I. Gas-Exchange, Biomass, and Leaf Chemistry

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ABSTRACT

The effects of elevated CO_2 and ozone (O_3) on net photosynthetic rate (*A*) and growth are generally antagonistic although plant responses are highly dependent on crop sensitivity to the individual gases and their concentrations. In this experiment, we evaluated the effects of various CO_2 and O_3 mixtures on leaf gas-exchange, harvest biomass, and leaf chemistry in peanut (*Arachis hypogaea* L.), an O_3–sensitive species, using open-top field chambers. Treatments included ambient CO_2 (about 375 μmol mol^{-1}) and CO_2 enrichment of approximately 173 and 355 μmol mol^{-1} in combination with charcoal-filtered air (22 nmol O_3 mol^{-1}), nonfiltered air (46 nmol O_3 mol^{-1}), and nonfiltered air plus O_3 (75 nmol O_3 mol^{-1}). Twice-ambient CO_2 in charcoal-filtered air increased *A* by 23% while decreasing seasonal stomatal conductance (g_s) by 42%. Harvest biomass was increased 12 to 15% by elevated CO_2. In ambient CO_2, nonfiltered air and added O_3 lowered *A* by 21% and 48%, respectively, while added O_3 reduced g_s by 18%. Biomass was not significantly affected by nonfiltered air, but was 40% lower in the added O_3 treatment. Elevated CO_2 generally suppressed inhibitory effects of O_3 on *A* and harvest biomass. Leaf starch concentration was increased by elevated CO_2 and decreased by O_3. Treatment effects on foliar N and total phenolic concentrations were minor. Increasing atmospheric CO_2 concentrations should attenuate detrimental effects of ambient O_3 and promote growth in peanut but its effectiveness declines with increasing O_3 concentrations.

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Abbreviations: *A*, net photosynthetic rate; AA, ambient air; CF, charcoal-filtered; g_s, stomatal conductance; LMPA, leaf mass per unit leaf area; NF, nonfiltered; OZ, 1.56 × ambient O_3; PPFD, photosynthetic photon flux density; WAP, weeks after planting; WUE, water use efficiency.

Atmospheric concentrations of CO_2 have risen from preindustrial levels of approximately 280 μmol mol^{-1} in 1750 to about 375 μmol mol^{-1} currently (Keeling and Whorf, 2005). The expected continued rise in atmospheric CO_2 concentration, apart from possible influences of increasing temperature and other changes in environmental conditions, is anticipated to stimulate biomass production and perhaps yield of many C_3 crops (Ainsworth and Long, 2005; Jablonski et al., 2002; Kimball et al., 1993; Prasad et al., 2005). Elevated CO_2 typically improves net photosynthetic rates (*A*), plant–water relations, and photosynthetic water use efficiency (Ainsworth and Long, 2005; Polley, 2002; Prasad et al., 2005). It also lessens O_3–induced stress in some crop species (Fiscus et al., 2002; Olszyk et al., 2000; Poorter and Pérez-Soba, 2001). Conversely, stimulation of plant growth at elevated CO_2 can be curtailed by O_3 (Barnes and Wellburn, 1998; Olszyk et al., 2000; Poorter and Pérez-Soba, 2001).

Tropospheric O_3 levels since the 1960s have been sufficiently high to suppress growth and yield of many C_3 crops in a number

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of industrialized countries worldwide (Treshow and Bell, 2002; Mauzerall and Wang, 2001). In addition, emissions of O_3 precursors and areas affected by O_3 pollution continue to grow (Dentener et al., 2005; Prather et al., 2003). Ozone impairs growth primarily by inhibiting *A* and perhaps translocation processes, which limit availability of photosynthate needed for biomass production (Fiscus et al., 2005; Heath and Taylor, 1997; Long and Naidu, 2002). Root production appears particularly susceptible to O_3 exposure (Cooley and Manning, 1987; Grantz et al., 2006). Allocation of C and energy resources to detoxification and repair processes in O_3–stressed plants likely detracts from growth as well (Heath and Taylor, 1997). Experiments with *Arabidopsis thaliana* mutants and pairs of O_3–sensitive and –tolerant plant lines suggest that many detrimental effects of O_3 are initiated and mediated by increased reactive oxygen species formation along with changes in plant hormone levels, antioxidant metabolism, cellular ion fluxes, and gene expression (Heath and Taylor, 1997; Kangasjarvi et al., 2005).

Ozone toxicity is likely reduced at elevated CO_2 by lowered O_3 uptake due to CO_2–induced partial stomatal closure (Allen, 1990; Barnes and Wellburn, 1998; Booker and Fiscus, 2005; Fiscus et al., 1997; McKee et al., 1997; Olszyk et al., 2000; Polle and Pell, 1999; Poorter and Pérez-Soba, 2001). It has also been suggested that increased availability of photosynthate and energy equivalents for growth and detoxification processes at elevated CO_2 aid in ameliorating O_3 damage (Allen, 1990; Barnes and Wellburn, 1998; Booker and Fiscus, 2005; McKee et al., 1997; Polle and Pell, 1999; Poorter and Pérez-Soba, 2001). Nevertheless, plant responses to elevated CO_2 and O_3 depend in part on the gas concentrations, crop sensitivity, developmental stage, cumulative exposure, and other experimental conditions. For example, multiple studies with wheat (*Triticum aestivum* L.) found few statistically significant interactions between elevated CO_2 and O_3 on biomass production because O_3 levels or crop cultivar sensitivity were too low to result in significant O_3 effects (Bender et al., 1999). Other studies with highly O_3–susceptible lines of clover (*Trifolium repens* L.), potato (*Solanum tuberosum* L.), and snap bean (*Phaseolus vulgaris* L.) found that growth inhibition by O_3 was altered little by CO_2 enrichment (Heagle et al., 1993, 2002, 2003). In the main, however, most studies determined that elevated CO_2 partially or completely ameliorated the damaging effects of O_3 on *A* and biomass production. This was observed in cotton (*Gossypium hirsutum* L.), soybean [*Glycine max* (L.) Merr.], wheat, and other crop species (Barnes and Pfirrman, 1992; Booker and Fiscus, 2005; Booker et al., 2005; Cardoso-Vilhena et al., 2004; Heagle et al., 1999, 2000; Miller et al., 1998; Olszyk et al., 2000; Plessl et al., 2005; Poorter and Pérez-Soba, 2001; Reid and Fiscus, 1998). Cumulative exposure and ontogeny were significant factors in several experiments in which amelioration of O_3 effects on *A* by elevated CO_2 declined as development progressed and O_3 injury accumulated (Barnes and Pfirrman, 1992; Mulchi et al., 1992; Reid and Fiscus, 1998). It is unclear how peanut (*Arachis hypogaea* L.), an agronomically important and O_3–sensitive species, would respond to increasing concentrations of atmospheric CO_2 and O_3.

Previous controlled environment and field experiments indicated that ambient O_3 concentrations in the southeastern United States caused foliar injury and suppressed growth in peanut (Ensing et al., 1985; Heagle et al., 1983). Previous studies also found that peanut was quite responsive to increasing levels of atmospheric CO_2 (Prasad et al., 2005). The objective of our study was to compare the effects of season-long exposures to various concentrations of CO_2 and O_3, administered singly and in mixtures, on foliar injury, leaf gas-exchange, harvest biomass production, and leaf chemistry in peanut. It was hypothesized that elevated CO_2 and O_3 would have concentration-dependent, counteracting effects on *A* and biomass production, which would be reflected by foliar injury assessments and some leaf chemistry components, such as chlorophyll and nonstructural carbohydrate concentrations. In addition, plant responses to nonfiltered (NF) air in open-top chambers were compared with plant responses to ambient air to assess chamber effects at ambient levels of CO_2 and O_3.

MATERIALS AND METHODS

Plant Culture Conditions and Gas Treatments

The experiment was conducted with peanut cuiltivar NC-V11, during 2002 and 2003 at a site 5 km south of Raleigh, NC (35°44′ N, 78°41′ W). The original topsoil in the field was excavated in 1984 and replaced with about 30 cm of Norfolk sandy loam (fine-loamy, kaolinitic, thermic Typic Kandiudult) to improve soil uniformity (Miller et al., 1988). The Norfolk sandy loam overlies an Appling sandy loam (fine, kaolinitic, thermic Typic Kanhapludult). Since 1985, the field has been cultivated periodically with cotton and soybean using conventional tillage and soil fertilization practices.

In preparation for the peanut experiment, the field was chisel-plowed and disked. The field was then limed and fertilized according to soil test recommendations with 94 kg K ha^{-1} on 15 Apr. 2002 and 13 Mar. 2003. Granular gypsum (86% $CaSO_4$) was applied to the field at a rate of 897 and 1344 kg ha^{-1} on 1 July 2002 and 3 July 2003. Before planting, seeds were treated with a commercial *Bradyrhizobium* preparation (RhizoFlo, Becker Underwood Inc., Ames, IA) and then planted on 15 May 2002 and 13 May 2003. Plants were sown in rows with 1-m spacing and with plant spacing of 9 cm (11 plants m^{-2}). Plants were irrigated with soaker hoses in 2002 and with emitters (Spot Spitters, Roberts Irrigation, San Marcos, CA) in 2003 installed parallel to each row at a distance of approximately 10 cm. Total irrigation in 2002 and 2003 was 35 cm and 17 cm, respectively. Plots were sprayed to control insects with acephate (*O,S*-dimethyl acetylphosphoramidothioate) (Whitmire Micro-Gen

Research Laboratories, Inc., St. Louis, MO) on 31 May and 17 June 2002, and 6 June 2003; bifenthrin [(2-methyl-1,1-biphenyl-3-y1)-methyl-3-(2-chloro-3,3,3-trifluoro-1-propenyl)-2,2-dimethylcyclopropanecarboxylate] (Whitmire Micro-Gen Research Laboratories, Inc.) on 6 Aug. 2002, and 22 July and 6 Sept. 2003; and, imidacloprid (1-[(6-chloro-3-pyridinyl)methyl]-*N*-nitro-2-imidazolidinimine) (Bayer Corporation, Kansas City, MO) on 6 Sept. 2003. None of these insecticides has been reported to affect crop responses to elevated CO_2 or O_3.

Plants were exposed to mixtures of CO_2 and O_3 in cylindrical open-top chambers, 3 m in diameter by 2.4 m tall beginning on 30 May 2002 and 3 June 2003. Gas dispensing and monitoring were conducted as described for CO_2 (Rogers et al., 1983) and O_3 (Heagle et al., 1979). Supplementary O_3 was generated by electrostatic discharge in dry O_2 (model GTC-1A, Ozonia North America, Elmwood Park, NJ) and dispensed 12 h daily (0800–2000 h EST) in proportion to ambient O_3 concentrations. Ozone concentration in the chambers was monitored at canopy height using UV photometric O_3 analyzers (model 49, Thermo Environmental Instruments Co., Franklin, MA). The O_3 analyzers were calibrated once every 2 wk (model 49 PS calibrator, Thermo Environmental Instruments Co.). Carbon dioxide was dispensed from a 12.7-Mg liquid receiver 24 h daily and was monitored at canopy height with infrared CO_2 analyzers (model 6252, Li-Cor, Inc., Lincoln, NE). The CO_2 monitors were calibrated once every 2 wk with CO_2 standards. Dispensing of CO_2 at night (1900–0700 h EST) was decreased by half to prevent concentrations from exceeding daytime target levels by more than 50%. Ground-level CO_2 concentrations typically increase at night, and levels in the control treatment reached 500 μmol mol^{-1} at times.

The experiment consisted of all combinations of three CO_2 treatments and three O_3 treatments (Tables 1, 2). The CO_2 treatments were ambient CO_2, ambient plus 173 μmol CO_2 mol^{-1}, and ambient plus 355 μmol CO_2 mol^{-1}. The O_3 treatments were charcoal-filtered (CF) air, NF air, and NF air plus 1.56 times ambient O_3. Air filtration by activated charcoal lowered ambient O_3 concentrations to levels considered nonphytotoxic for peanut (Heagle et al., 1983). An additional treatment of approximately 634 μmol CO_2 mol^{-1} added to NF air was included to test the effects of a higher CO_2 concentration. Plants were also grown in ambient air within chamber frames lacking plastic panels to assess chamber effects. All CO_2 and O_3 treatments were administered 7 d wk^{-1}, and continued until 30 Sept. 2002 and 5 Oct. 2003, when plants were harvested.

Leaf and Plant Biomass Sampling

Once a month from late June through late September (21 June, 19 July, 26 August, and 19 September in 2002; 7 July, 28 July, 25 August, and 25 September in 2003), the third leaf down from the apex of a branch on four plants was obtained from each chamber. One plant in each quadrant of the chamber was sampled. Leaf petioles were removed and leaflet samples were pooled by chamber at each sampling date, frozen in liquid N_2, and stored at −80°C for later analysis of leaf chemistry. Ten leaf disks (1.6-cm diameter) were also obtained from each sample of leaflets, freeze-dried, and weighed for determination of leaf

Table 1. Elevated CO_2 and O_3 treatments, treatment abbreviations, and number of replicate chambers per treatment in each year of the 2-yr experiment.

O_3 treatment	CO_2 treatment	Treatment abbreviation	Number of replicate chambers per year
Charcoal-filtered air	Ambient	CF-375	3
Charcoal-filtered air	+173 μmol CO_2 mol^{-1}	CF-548	2
Charcoal-filtered air	+355 μmol CO_2 mol^{-1}	CF-730	3
Nonfiltered air	Ambient	NF-375	2
Nonfiltered air	+173 μmol CO_2 mol^{-1}	NF-548	2
Nonfiltered air	+355 μmol CO_2 mol^{-1}	NF-730	2
Nonfiltered air	+634 μmol CO_2 mol^{-1}	NF-1009	2
1.5 × ambient O_3	Ambient	OZ-375	3
1.5 × ambient O_3	+173 μmol CO_2 mol^{-1}	OZ-548	2
1.5 × ambient O_3	+355 μmol CO_2 mol^{-1}	OZ-730	3
Ambient air	Ambient	AA	3

Table 2. Average monthly and seasonal meteorological conditions, and CO_2 and O_3 treatment concentrations in the 2-yr experiment.†

Parameter	Year	May‡	June	July	Aug.	Sept.	Season
Temperature, °C	2002	19.5	25.6	26.5	25.3	22.9	25.1
	2003	18.0	23.2	25.2	25.3	21.2	23.7
Relative humidity, %	2002	50	53	62	62	70	59
	2003	73	66	73	75	68	71
PPFD, mol m^{-2} d^{-1}	2002	52.4	49.4	43.3	36.0	32.8	42.8
	2003	31.5	42.0	41.8	37.2	33.9	37.3
Rain, cm§	2002	1.3	5.7	10.5	15.8	5.4	
	2003	8.4	14.8	18.6	22.3	8.4	
[CO_2], μmol mol^{-1}		¶					
Ambient	2002		372	372	375	385	376
+173			539	566	545	551	550
+355			744	759	705	712	730
+634			1058	1099	950	963	1018
Ambient	2003		376	370	373	378	374
+173			542	533	552	551	545
+355			754	709	733	720	729
+634			1026	967	1030	972	999
[O_3], nmol mol^{-1}		¶					
Charcoal-filtered air	2002		27	20	20	19	21
Nonfiltered air			56	52	46	41	49
1.56 × ambient O_3			86	89	73	63	79
Ambient			57	51	46	42	49
Charcoal-filtered air	2003		24	22	20	20	22
Nonfiltered air			49	45	43	41	44
1.56 × ambient O_3			74	72	72	66	71
Ambient			51	47	45	43	46

†Treatments are shown in Table 1. Relative humidity values are for the daytime period (photosynthetic photon flux density [PPFD] > 50 μmol mol^{-1}). CO_2 and O_3 concentrations are 12 h d^{-1} (0800 to 2000 h EST) averages.

‡15–31 May 2002 and 13–31 May 2003.

§Seasonal total irrigation was equivalent to approximately 35 and 17 cm of rain in 2002 and 2003, respectively.

¶Gas treatments began on 30 May 2002 and 3 June 2003.

mass per unit leaf area (LMPA). At harvest, two 2-m-long rows of plants measured from the center of each chamber were subdivided into 1-m segments. Twelve plants (three plants from one, randomly selected end of each 1-m-row segment) per chamber were sampled for biomass. Harvested plants were air-dried to a constant weight, and dry mass of leaves, stems, roots, pods, and culls was measured. Culls were defined as immature pods less than 1 cm in diameter and pods of any size exhibiting symptoms of disease.

Visible injury (percentage of chlorosis and necrosis) on the upper 13 leaves on the main stem of two plants per chamber was evaluated on 5 Aug. 2002 and 8 Aug. 2003. Visible injury estimates among leaves were averaged to yield a per chamber value.

Gas-Exchange Measurements

Net photosynthesis was measured at growth CO_2 and O_3 conditions in the chambers using a portable photosynthesis system (Model 6200, Li-Cor Inc.) and a 1-L cuvette. Measurements were made on the second leaf down from the apex of a branch on three plants per chamber between 1000 and 1300 h when ambient photosynthetic photon flux density (PPFD) > 1000 μmol m^{-2} s^{-1}. Net photosynthesis was measured in all the treatments except the +173 μmol CO_2 mol^{-1} and ambient air treatments due to time limitations for making the measurements. Average PPFD, relative humidity, and leaf temperature was 1746 μmol m^{-2} s^{-1}, 46%, and 36°C, respectively, during the measurements.

In addition, midday leaf conductance was measured with a steady-state porometer (Model 1600M, Li-Cor, Inc.) on the abaxial and adaxial surfaces of a leaflet on the third leaf down from the apex of a branch between 1100 and 1300 h when ambient PPFD > 1000 μmol m^{-2} s^{-1}. One leaf on each of four plants was measured in each of two replicate chambers per treatment. Average PPFD, relative humidity, and leaf temperature was 1387 μmol m^{-2} s^{-1}, 45%, and 32°C, respectively, during the measurements. Leaf conductance measurements were corrected for the standard boundary layer conductance imposed by the instrument (2.7 mol m^{-2} s^{-1}, Li-Cor 1600M Instruction Manual, Revision 6, 1989), and reported as stomatal conductance (g_s).

Gas-exchange measurements were made during each week from July through September in 2002 and 2003 when weather conditions permitted (A and g_s were measured on 34 and 42 occasions, respectively). Measurements were averaged on a weekly basis for subsequent data analysis.

Leaf Chemistry Assays

Five leaf disks (0.85 cm diameter) were obtained for determination of chlorophyll concentration. Leaf disks were extracted with 3 mL of 95% ethanol (2×) overnight at 4°C, and chlorophyll concentration was determined spectrophotometrically by the following equation:

$$\mu g \text{ chlorophyll } mL^{-1} = \left(13.7A_{665} - 5.76A_{649}\right) + \left(25.8A_{649} - 7.6A_{665}\right)$$

(Lichtenthaler and Wellburn, 1983). The chlorophyll concentration, expressed as micrograms of chlorophyll per square centimeter leaf area, was then obtained.

For assays of nonstructural carbohydrates and total soluble phenolics, freeze-dried leaf tissue samples were ground to pass a 0.5-mm mesh screen. Starch and soluble sugars were determined enzymatically by the UV absorbance method (R-Biopharm, Inc., Marshall, MI). To solubilize starch, tissue samples (25 mg) were each mixed with 2.4 mL of dimethylsulfoxide and 600 μL of 8 *M* HCl in sealed polypropylene tubes for 1 h at 60°C. Samples were then neutralized with 600 μL of 8 *M* NaOH and diluted to 15 mL with 112 m*M* citrate buffer (pH 4). Solutions were filtered, and 50-μL aliquots were assayed according to kit instructions. Starch was hydrolyzed to D-glucose by amyloglucosidase. D-glucose was determined indirectly by first forming D-glucose-6-phosphate with ATP and hexokinase followed by the formation of D-gluconate-6-phosphate by glucose-6-phosphate dehydrogenase and $NADP^+$. The amount of NADPH formed in the second reaction is stoichiometric to the amount of D-glucose formed by the hydrolysis of starch. The concentration of NADPH was determined by measuring the absorbance of the reaction solution at 340 nm. Results were expressed as D-glucose equivalents.

To determine total soluble phenolic concentrations, tissue samples (25 mg) were extracted with 1 mL of freshly prepared 250 m*M* sodium citrate containing 2% (w/v) sodium bisulfite (pH 7) (3×) for 5 min at room temperature with periodic mixing, centrifuged (16 000 × *g*), and the supernatants pooled by sample (Blum, 1997). A 50-μL aliquot of each sample was mixed with 475 μL of 0.25 *M* Folin-Ciocalteu reagent (Sigma-Aldrich Chemical Co., St. Louis, MO) and 475 μL of 1 *M* Na_2CO_3, incubated at room temperature for 1 h, and absorbance of the solutions was measured at 724 nm. Results were expressed as 4-coumaric acid equivalents using a standard curve.

Statistical Analysis

In each year of the experiment, the treatments consisted of all factorial combinations of three CO_2 levels and three O_3 levels along with an additional very high CO_2–NF air treatment combination. The treatments were assigned to chambers in a completely randomized design. Chamber treatments were randomly reassigned in the second year of the experiment. There were three replicate chambers for each of the high and low CO_2 × O_3 combinations (n = 12) (Table 1). In addition, there were two replicate chambers for each of the +173 μmol CO_2 mol^{-1} and NF air treatment combinations (n = 10) (Table 1). Two chambers were used for the very high CO_2 (+634 μmol mol^{-1})–NF air treatment combination. Assay results from plant tissue samples obtained within a chamber were averaged for use as a chamber replicate value. Results from the 2-yr experiment were combined for the statistical analyses, and the effect of year was treated as a fixed variable. Data were checked for homogeneity of variance, and a ln transformation was applied to the gas-exchange data before analysis. Treatment effects and least-squared means for harvest biomass measurements in the 3 CO_2 × 3 O_3 × 2 yr factorial experiment were determined using analysis of variance techniques (SAS Proc Mixed, SAS System for Windows, Ver. 8.2; Littell et al., 1996). Treatment effects and least-squared means for periodically measured gas-exchange processes (A and g_s), LMPA, and leaf chemical components were estimated using a repeated measures model in which chambers constituted the whole plots and sampling period was the repeated factor (SAS Proc Mixed; Littell et al., 1996). The model included interactions between the whole plot factors and the effect of sampling period. Effects of the very

high CO_2–NF air treatment as well as the ambient air treatment were evaluated in separate analysis of variance tests.

RESULTS

Environmental Conditions

The 2002 field season was slightly hotter and drier from mid-May through September compared with the 2003 field season (Table 2). Mean daytime ambient CO_2 concentration during the experiment was 375 μmol mol^{-1}, and the elevated CO_2 treatments concentrations averaged 548 (1.46 × ambient CO_2) and 730 (1.95 × ambient CO_2) μmol mol^{-1} (12 h average, 0800–2000 h EST) (Table 2). The additional high CO_2 treatment concentration averaged 1009 μmol mol^{-1} (2.69 × ambient CO_2). Average daytime ambient O_3 concentration (AA) for the 2002 and 2003 growing seasons was 48 nmol mol^{-1} (12 h average), which was typical for the area. The average O_3 concentration in the CF air, NF air, and added O_3 (OZ) treatments was 22 (0.48 × ambient O_3), 46 (0.96 × ambient O_3), and 75 (1.56 × ambient O_3) nmol mol^{-1} (12 h average) (Table 2).

Visible Injury

Both elevated CO_2 and O_3 increased visible foliar injury (Table 3), although symptoms differed among the treatments. Elevated CO_2 caused chlorotic mottle and irregular patches of white necrotic areas while O_3 produced diffuse chlorosis and brownish stipple. Visible injury was highest in the OZ-375 treatment compared with the control (CF-375). Elevated CO_2 suppressed O_3–induced visible injury, although symptoms of injury from both gases were apparent.

Table 3. Foliar visible injury at midseason for peanut exposed to mixtures of CO_2 and O_3.†

Treatment	Visible injury (% chlorosis and necrosis)
CF-375	4.7 ± 2.6
CF-548	13.8 ± 3.2
CF-730	17.9 ± 2.6
NF-375	29.3 ± 3.2
NF-548	22.3 ± 3.2
NF-730	20.2 ± 3.2
NF-1009	26.4 ± 3.2
OZ-375	54.0 ± 2.6
OZ-548	31.8 ± 3.2
OZ-730	32.2 ± 2.6
Source‡	
Year	**
CO_2	*
O_3	***
Year × CO_2	NS§
Year × O_3	NS
CO_2 × O_3	***
Year × CO_2 × O_3	NS

*Significant treatment effects and interactions $P \le 0.05$.

**Significant treatment effects and interactions $P \le 0.01$.

***Significant treatment effects and interactions $P \le 0.001$.

†Treatments are described in Table 1. Values are means ± SE of each treatment combination for both years of the experiment.

‡NF-1009 treatment not included.

§NS, nonsignificant.

Photosynthesis and Stomatal Conductance

Net photosynthesis in upper canopy leaves in the control treatment (CF-375) was fairly steady at 30 to 38 μmol CO_2 m^{-2} s^{-1} from 8 through 19 wk after planting (WAP) in this indeterminate-growth plant (Fig. 1). Average *A* was 23% higher in the CF-730 treatment than in the control (Table 4, Fig. 1). In contrast, NF air (NF-375) and added O_3 (OZ-375) suppressed *A* by 21 and 48%, respectively. The NF-375 effect on *A* was large in 2002, but marginal in 2003 (data not shown), leading to the significant year × O_3 interaction reported in Table 4. Inhibitory effects of O_3 on *A* were generally attenuated by elevated CO_2, although the CO_2 effect tended to decline toward the end of the growing season. Averaged over the season, *A* in the NF-730 and OZ-730 treatments was 19 and 10% higher, respectively, than in the control treatment. Average *A* was 30% higher in the NF-1009 treatment compared with the control, but the rate was not significantly different from the NF-730 treatment ($P \ge 0.05$).

Increased CO_2 concentration in the CF-548 and CF-730 treatments reduced average g_s by 16 and 42%, respectively, compared with the control from 11 through 19 WAP (Table 4, Fig. 2). Average g_s in the NF-375 treatment was not significantly different from the control. Likewise, gas treatment effects on g_s in the NF-548 and NF-730 treatments were not significantly different from those in the respective CF air-elevated CO_2 treatments (CF-548 and CF-730) ($P \ge 0.05$). In the OZ-375 treatment, g_s was suppressed by 18% relative to the control. The combined effect of elevated CO_2 and O_3 on g_s in the OZ-548 and OZ-730 treatments was additive; g_s was lower in these treatments than in the respective CF-548 and CF-730 treatments ($P \le 0.05$). In the OZ-548 and OZ-730 treatments, g_s was reduced by 39 and 50%, respectively, compared with the control. Average g_s in the NF-1009 treatment was 59% lower than the control.

Seasonal average water use efficiency (WUE) (*A*/transpiration) was approximately doubled in the twice-ambient CO_2 treatments (Table 5). The higher CO_2 level in the NF-1009 treatment resulted in the highest WUE value. In contrast, WUE was 18 and 40% lower in the NF-375 and OZ-375 treatments, respectively, compared with the control. Elevated CO_2 fully compensated for O_3 effects on WUE.

Harvest Biomass

Main treatment effects of elevated CO_2 and O_3 were statistically significant for all harvest biomass components measured except cull dry mass (Table 6). Elevated concentrations of CO_2 increased biomass while O_3 suppressed it. Among the

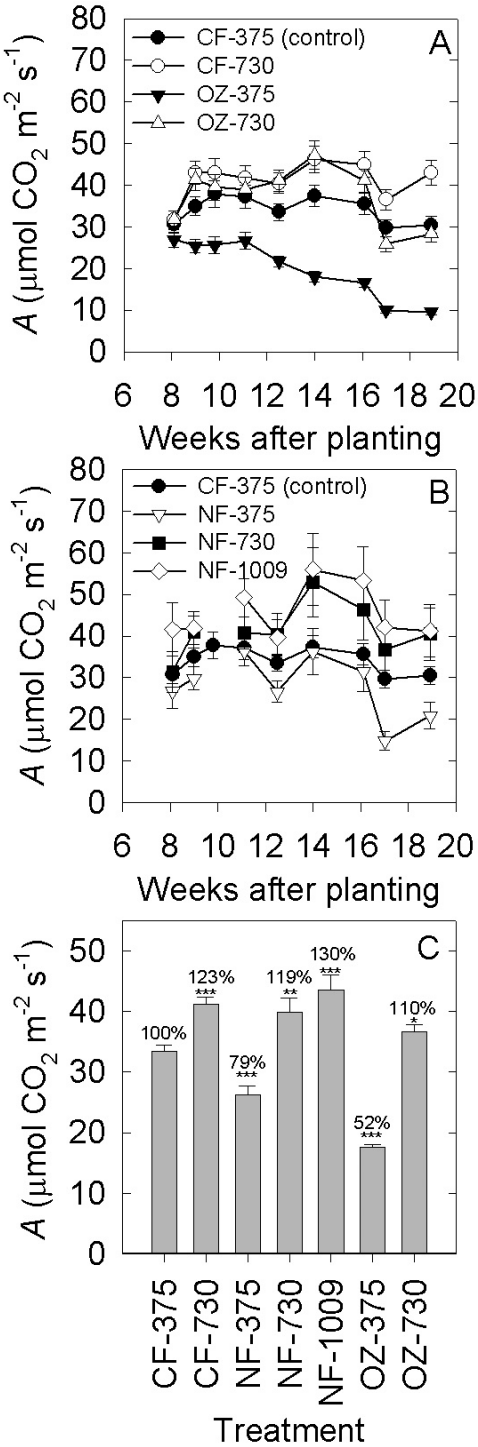

Figure 1. Effects of CO_2 and O_3 on average net photosynthesis (*A*) of upper canopy leaves of peanut from 8 through 19 wk after planting in the 2-yr experiment (A, B). Seasonal average *A* is also shown (C). Treatments included charcoal-filtered air (CF)–ambient CO_2 (CF-375) (control), CF air plus 355 μmol CO_2 mol^{-1} (CF-730), nonfiltered air (NF)–ambient CO_2 (NF-375), NF air plus 355 μmol CO_2 mol^{-1} (NF-730), NF air plus 634 μmol CO_2 mol^{-1} (NF-1009), 1.5 × ambient O_3–ambient CO_2 (OZ-375), and 1.5 × ambient O_3 plus 355 μmol CO_2 mol^{-1} (OZ-730). Values are means ± SE from two or three replicate chambers per treatment in each year of the experiment (see Table 1). Values above the bars in panel C indicate percentage of the control treatment. Significant treatment effects are indicated as $P \leq 0.05$ (*), $P \leq 0.01$ (**), and $P \leq 0.001$ (***).

Table 4. Probabilities of elevated CO_2 and O_3 treatment effects on net photosynthesis (*A*) and stomatal conductance (g_s) in the 2-yr experiment. Net photosynthesis was measured from 8 through 19 wk after planting (WAP) in 2002 and 2003. Stomatal conductance was measured from 10 through 19 WAP in 2002 and 2003.

Effect	*A*†	g_s‡
Year	**	***
CO_2	***	***
O_3	***	***
Year × CO_2	NS§	NS
Year × O_3	*	NS
CO_2 × O_3	***	NS
Year × CO_2 × O_3	NS	NS
WAP	***	***
WAP × CO_2	***	***
WAP × O_3	***	NS
WAP × CO_2 × O_3	*	NS

*Significant treatment effects and interactions $P \leq 0.05$.

**Significant treatment effects and interactions $P \leq 0.01$.

***Significant treatment effects and interactions $P \leq 0.001$.

†Does not include the 175 μmol CO_2 mol^{-1} (CF-548, NF-548, and OZ-548), NF-1009, and AA treatments. Treatments are described in Table 1.

‡Does not include the NF-1009 and AA treatments.

§NS, nonsignificant.

various biomass components, stem biomass on an absolute mass basis was most affected by the gas treatments.

Total biomass of plants in the CF-548 and CF-730 treatments was 12 to 15% higher than in the control treatment (Table 6). Stem dry mass was increased about 24% in these two elevated CO_2 treatments, while root dry mass was increased 33% in the CF-730 treatment. Leaf, pod, and cull biomass in the CF-548 and CF-730 treatments was not significantly different from the control.

Average dry mass of harvest components in the NF-375 and NF-548 treatments was not significantly different from the control treatment (Table 6). Elevated CO_2 stimulated biomass production in the NF-730 and NF-1009 treatments, but the difference in biomass between the two treatments was not statistically significant ($P \geq 0.05$). Component and total biomass were reduced 34 to 44% (excluding culls) in the OZ-375 treatment relative to the control. An increase in the CO_2 concentration by 50% partially compensated for the added O_3 effect on biomass (compare OZ-375 vs. OZ-548). However, the biomass stimulation attributable to a 50% increase in CO_2 in CF air was diminished by added O_3 (compare CF-548 vs. OZ-548). Twice-ambient concentrations of CO_2 completely ameliorated the effects of added O_3 on biomass components, and CO_2 × O_3 interactions were statistically significant except for roots and culls (Table 6). Year × CO_2 interactions for leaf and stem biomass were caused by larger increases in biomass between the +173 and +355 treatments in 2002 than in 2003.

Elevated CO_2 concentrations slightly increased partitioning of biomass to stems at the expense of pods (Table 7). In addition, there were small differences between the 2002 and 2003 experiments for pod biomass partitioning ratio (significant year × CO_2 interaction). Main effects of the O_3 treatments on biomass partitioning were not statistically significant. However, pod mass ratio was 10% higher in the OZ-375 treatment compared with the control ($P \leq 0.05$).

Leaf Mass Per Area and Leaf Chemistry

Seasonal average LMPA increased 15 to 20% in all the elevated CO_2 treatments and decreased 11% in the OZ-375 treatment compared with the control (Table 8). However, LMPA declined in the second half of the field season in all treatments, and the decline at elevated CO_2 was greater in the NF and OZ treatments than in the CF treatments (data not shown). The increase in LMPA with increasing CO_2 levels was greater in the 2002 experiment than in 2003, especially between the +173 and +355 CO_2 treatments (significant year × CO_2 interaction). The NF-375 treatment had no statistically significant effect on LMPA. The CO_2 × O_3 interaction for LMPA was not statistically significant.

The most noteworthy treatment effects of elevated CO_2 and O_3 on leaf chemistry were on chlorophyll and starch concentrations (Table 8, Fig. 3). Both elevated CO_2 and added O_3 suppressed average seasonal leaf chlorophyll concentrations by 17 to 28%, although elevated CO_2 reduced chlorophyll concentration during vegetative growth while added O_3 lowered it during reproductive stages. Starch concentrations were higher at elevated CO_2 and lower in the NF-375 and OZ-375 treatments compared with the control. Stimulatory effects of elevated CO_2 on starch mass were diminished by added O_3 and vice versa. Elevated CO_2 increased soluble sugar concentrations while O_3 treatment effects were not statistically significant. Gas treatment effects on N and total phenolics concentrations were relatively small, although some differences were statistically significant.

Open-Top Chamber Effects

A comparison between plants grown in the NF-375 and AA treatments indicated that harvest biomass components, leaf soluble sugars, N, and total phenolics were not significantly different (Table 9). Visible foliar injury at midseason was lower in the AA treatment, as were seasonal average g_s and chlorophyll concentration, compared with the NF-375 treatment. Leaf mass per area and starch concentrations were 8 and 33% higher, respectively, for plants grown in the AA treatment compared with the NF-375 treatment.

DISCUSSION

Amelioration of O_3 damage at elevated CO_2 has been found in a number of experiments with C_3 crop plants (Booker and Fiscus, 2005; Booker et al., 2005; Craigon et al., 2002; Fiscus et al., 2002; Heagle et al., 1999, 2000; Olszyk et al.,

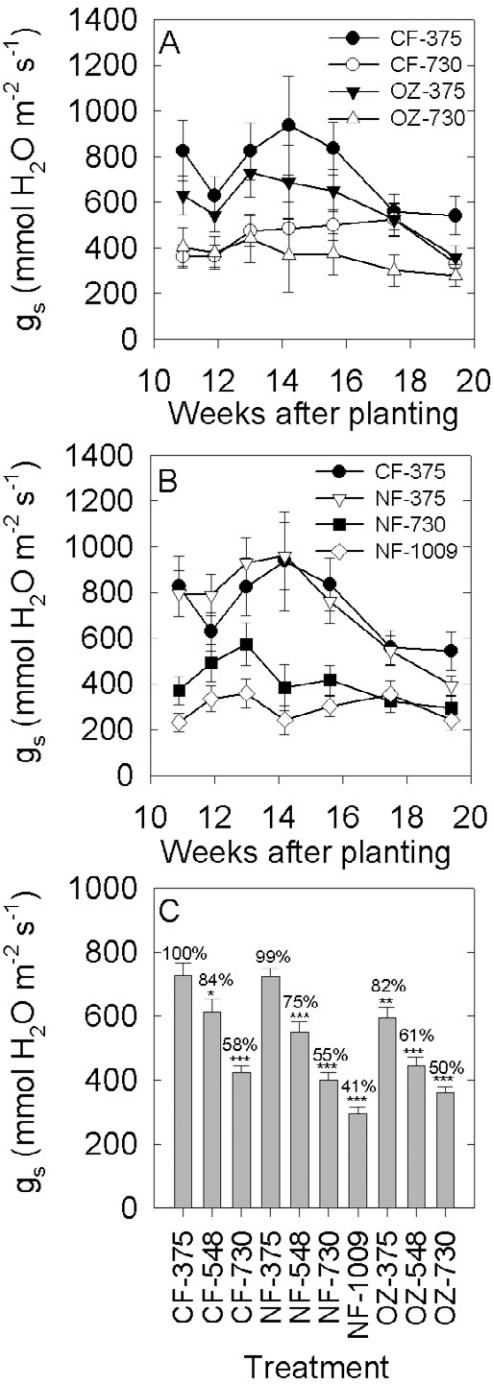

Figure 2. Effects of CO_2 and O_3 on average stomatal conductance (g_s) of upper canopy leaves of peanut from 10 through 19 wk after planting in the 2-yr experiment (A, B). Seasonal average g_s is also shown (C). Treatments were charcoal-filtered air (CF)–ambient CO_2 (CF-375) (control), CF air plus 173 μmol CO_2 mol^{-1} (CF-548), CF air plus 355 μmol CO_2 mol^{-1} (CF-730), nonfiltered air (NF)–ambient CO_2 (NF-375), NF air plus 173 μmol CO_2 mol^{-1} (NF-548), NF air plus 355 μmol CO_2 mol^{-1} (NF-730), NF air plus 634 μmol CO_2 mol^{-1} (NF-1009), 1.5 × ambient O_3–ambient CO_2 (OZ-375), 1.5 × ambient O_3 plus 173 μmol CO_2 mol^{-1} (OZ-548), and 1.5 × ambient O_3 plus 355 μmol CO_2 mol^{-1} (OZ-730). Values are means ± SE from two or three replicate chambers per treatment in each year of the experiment (see Table 1). Values above the bars in panel C indicate percentage of the control treatment. Significant treatment effects are indicated as $P \leq 0.05$ (*), $P \leq 0.01$ (**), and $P \leq 0.001$ (***).

Table 5. Seasonal average photosynthetic water use efficiency (WUE) of upper canopy peanut leaves exposed to mixtures of CO_2 and O_3.†

Treatment	WUE‡ (μmol CO_2 m^{-2} s^{-1}/mmol H_2O m^{-2} s^{-1})
CF-375	1.49 ± 0.05 (100)
CF-730	3.37 ± 0.12 (226***)
NF-375	1.23 ± 0.08 (82**)
NF-730	2.95 ± 0.19 (198***)
NF-1009	4.25 ± 0.28 (285***)
OZ-375	0.89 ± 0.03 (60***)
OZ-730	3.07 ± 0.11 (206***)
Source§	
Year	NS¶
CO_2	***
O_3	***
Year × CO_2	NS
Year × O_3	NS
CO_2 × O_3	***
Year × CO_2 × O_3	NS

*Significant treatment effects and interactions $P \leq 0.05$.

**Significant treatment effects and interactions $P \leq 0.01$.

***Significant treatment effects and interactions $P \leq 0.001$.

†WUE values were calculated from measurements of *A* and transpiration made from 8 though 19 wk after planting in 2002 and 2003. Treatments are described in Table 1.

‡Values are means ± SE of each treatment combination for all sampling dates in both years of the experiment. Values in parentheses indicate percentage of the control treatment and statistical significance of the difference from the control treatment (CF-375).

§NF-1009 treatment not included.

¶NS, nonsignificant.

2000; Plessl et al., 2005; Poorter and Pérez-Soba, 2001). Results of our study also showed that elevated CO_2 generally counteracted the O_3 stress we applied to NC-V11 peanut in terms of biomass production and yield (Table 6) (also see Burkey et al., 2007). This response was accompanied by significant increases in *A* and decreases in g_s (Fig. 1, 2), suggesting likely causative relationships among treatment effects on g_s, *A*, growth, and yield. However, elevated CO_2 did not completely abate the detrimental effects of added O_3. For example, *A* in the NF-730 and OZ-730 treatments declined in advance of that in the CF-730 treatment near the end of the experiment. This has been observed in previous CO_2 × O_3 experiments with radish (*Raphanus sativus* L.) and soybean (Barnes and Pfirrman, 1992; Mulchi et al., 1992; Reid and Fiscus, 1998). Also, biomass and yield in the OZ-548 treatment were about 20% lower than in the CF-548 and NF-548 treatments (Table 6) (also see Burkey et al., 2007). Thus, biomass production responses observed in this experiment indicated that CO_2 concentrations expected by the year 2050 (approximately 550 μmol mol^{-1}) (Prather et al., 2003) will not fully protect peanut against possibly higher tropospheric O_3 levels. This scenario is particularly relevant for some major peanut-producing regions of the world, such as eastern China, central India, and central Africa (Rhoades and Nazarea, 2003), where ground-level O_3 concentrations are predicted to increase dramatically in the next 50 yr (Dentener et al., 2005; Prather et al., 2003; Wang and Mauzerall, 2004).

Table 6. Harvest biomass of peanut exposed to mixtures of CO_2 and O_3 in the 2-yr experiment.†

Treatment	Leaf	Stem	Root	Pod	Cull	Total biomass
			g $plant^{-1}$			
CF-375	35.2 ± 1.9 (100)	46.5 ± 3.1 (100)	2.7 ± 0.3 (100)	54.5 ± 2.9 (100)	1.8 ± 0.3 (100)	140.8 ± 6.6 (100)
CF-548	38.8 ± 2.3 (110)	57.8 ± 3.8 (124*)	2.9 ± 0.3 (107)	61.5 ± 3.5 (113)	1.4 ± 0.3 (78)	162.4 ± 8.1 (115*)
CF-730	38.5 ± 2.1 (109)	57.4 ± 3.4 (123*)	3.6 ± 0.3 (133*)	56.9 ± 3.2 (104)	1.8 ± 0.3 (100)	158.2 ± 7.4 (112)
NF-375	32.9 ± 2.3 (93)	38.8 ± 3.8 (83)	2.2 ± 0.3 (81)	51.8 ± 3.5 (95)	1.3 ± 0.3 (72)	126.9 ± 8.1 (90)
NF-548	38.5 ± 2.3 (109)	53.9 ± 3.8 (116)	2.8 ± 0.3 (104)	60.6 ± 3.5 (111)	1.5 ± 0.3 (83)	157.2 ± 8.1 (112)
NF-730	43.6 ± 2.3 (124**)	61.6 ± 3.8 (132**)	3.2 ± 0.3 (118)	65.2 ± 3.5 (120*)	1.6 ± 0.3 (89)	175.2 ± 8.1 (124**)
NF-1009	41.4 ± 2.3 (118*)	67.7 ± 3.8 (146***)	3.8 ± 0.3 (140**)	64.0 ± 3.5 (117*)	1.8 ± 0.3 (100)	178.8 ± 8.1 (127**)
OZ-375	20.7 ± 1.9 (59***)	26.4 ± 3.1 (57***)	1.5 ± 0.3 (56**)	35.8 ± 2.9 (66***)	0.6 ± 0.3 (33**)	85.1 ± 6.6 (60***)
OZ-548	30.8 ± 2.3 (88)	43.0 ± 3.8 (92)	2.3 ± 0.3 (85)	50.0 ± 3.5 (92)	0.9 ± 0.3 (50*)	127.0 ± 8.1 (90)
OZ-730	40.5 ± 1.9 (115)	62.8 ± 3.1 (135***)	3.2 ± 0.3 (118)	59.8 ± 2.9 (110)	1.4 ± 0.3 (78)	167.7 ± 6.6 (119**)
Source‡						
Year	***	***	***	***	***	***
CO_2	***	***	***	***	NS§	***
O_3	***	**	*	***	*	***
Year × CO_2	***	*	NS	NS	NS	NS
Year × O_3	NS	NS	NS	NS	*	NS
CO_2 × O_3	**	**	NS	*	NS	**
Year × CO_2 × O_3	*	NS	NS	NS	NS	NS

*Significant treatment effects and interactions $P \leq 0.05$.

**Significant treatment effects and interactions $P \leq 0.01$.

***Significant treatment effects and interactions $P \leq 0.001$.

†See Table 1 for treatment descriptions. Values are means ± SE of each treatment combination for both years of the experiment. Values in parentheses indicate percentage of the control treatment and statistical significance of difference from the control treatment (CF-375).

‡NF-1009 treatment not included.

§NS, nonsignificant.

Table 7. Partitioning of harvest biomass among organs (organ mass/total mass) of peanut as influenced by CO_2 and O_3 in the 2-yr experiment.†

Treatment	Leaf mass/total mass	Stem mass/total mass	Root mass/total mass	Pod mass/total mass
CF-375	0.247 ± 0.006 (100)	0.330 ± 0.011 (100)	0.020 ± 0.002 (100)	0.389 ± 0.010 (100)
CF-548	0.238 ± 0.008 (96)	0.356 ± 0.014 (108)	0.018 ± 0.003 (90)	0.378 ± 0.012 (97)
CF-730	0.242 ± 0.007 (98)	0.362 ± 0.013 (110)	0.022 ± 0.002 (110)	0.361 ± 0.011 (93)
NF-375	0.258 ± 0.008 (104)	0.304 ± 0.014 (92)	0.018 ± 0.003 (90)	0.409 ± 0.012 (105)
NF-548	0.243 ± 0.008 (98)	0.344 ± 0.014 (104)	0.018 ± 0.003 (90)	0.386 ± 0.012 (99)
NF-730	0.242 ± 0.008 (98)	0.353 ± 0.014 (107)	0.020 ± 0.003 (100)	0.375 ± 0.012 (96)
NF-1009	0.220 ± 0.008 (89**)	0.379 ± 0.014 (115**)	0.025 ± 0.003 (125)	0.365 ± 0.012 (94)
OZ-375	0.241 ± 0.006 (98)	0.310 ± 0.011 (94)	0.018 ± 0.002 (90)	0.423 ± 0.010 (109*)
OZ-548	0.242 ± 0.008 (98)	0.336 ± 0.014 (100)	0.018 ± 0.003 (90)	0.396 ± 0.012 (102)
OZ-730	0.235 ± 0.006 (95)	0.373 ± 0.011 (113**)	0.022 ± 0.002 (110)	0.361 ± 0.010 (93)
Source‡				
Year	***	NS§	***	*
CO_2	NS	***	NS	***
O_3	NS	NS	NS	NS
Year × CO_2	NS	NS	NS	*
Year × O_3	NS	NS	NS	NS
CO_2 × O_3	NS	NS	NS	NS
Year × CO_2 × O_3	*	NS	NS	NS

*Significant treatment effects and interactions $P \leq 0.05$.

**Significant treatment effects and interactions $P \leq 0.01$.

***Significant treatment effects and interactions $P \leq 0.001$.

†Treatments are described in Table 1. Values are means ± SE of each treatment combination for both years of the experiment. Values in parentheses indicate percent of the control treatment and statistical significance of difference from the control treatment (CF-375).

‡NF-1009 treatment not included.

§NS, nonsignificant.

Similarly, modeled yield responses of an O_3–sensitive wheat cultivar to various future scenarios of atmospheric CO_2 and O_3 concentrations indicated that potential yield increases due to elevated CO_2 c oncentrations could be halved or more by increasing concentrations of tropospheric O_3 (Hertstein et al., 1995). Therefore, evaluations of crop responses to elevated CO_2 need to consider possible influences of ambient O_3 in their assessments.

There were limitations in the stimulatory effect of elevated CO_2 on peanut growth as well. The NF-1009 treatment indicated that average *A* and biomass components were not significantly different from the NF-730 treatment (Table 6). Seed yield was also not further increased in the NF-1009 treatment compared with the NF-730 treatment (Burkey et al., 2007). Growth constraints at high CO_2 levels could be related to increased plant competition, limitations in water and mineral nutrient availability, and genetic potential of the plant. Stanciel et al. (2000) found that dry mass production of hydroponically grown 'Georgia Red' peanut declined at 1200 μmol CO_2 mol^{-1} compared with 800 μmol CO_2 mol^{-1}. Our results indicate that there is a maximum potential for biomass and yield stimulation by elevated CO_2 in NC-VII peanut.

A shift in biomass allocation toward pods was observed in the OZ-375 treatment but not in the NF-375 and elevated CO_2 treatments (Table 7). Similar changes in pod mass ratio have been found in previous studies with soybean and other crop species (Booker and Fiscus, 2005; Cooley and Manning, 1987; Miller et al., 1998). Evidently, pods become strong sinks for photosynthate in O_3–treated plants. Nonetheless, the net effect of O_3 on pod biomass is typically not positive because of the overriding suppression of total plant biomass by O_3 (Miller et al., 1998).

Concentrations of total soluble phenolics were not significantly affected by the gas treatments in our study (Table 8). However, changes in LMPA with elevated CO_2 and O_3 can make assays of total phenolics difficult to interpret unless treatment effects on specific compounds and nonstructural carbohydrate concentrations are taken into account. By expressing total phenolic concentrations on a leaf area basis, we eliminated the influence of gas treatments effects based on leaf dry mass, but a more comprehensive analysis of phenolic compound biosynthesis will be required to determine whether there are specific CO_2 and O_3 effects in peanut. Ozone-induced increases in insoluble, polymeric phenolic–iron–protein complexes are more commonly observed in injured leaves and are thought to be responsible for the brown-colored lesions (stipple) in leaves of many plant species exposed to O_3 (Booker and Miller, 1998; Howell and Kremer, 1973). Peanut leaves exhibited chlorosis and stipple in response to O_3 in our study (Table 3), suggesting that loss of cellular integrity

Table 8. Seasonal average leaf mass per unit leaf area and leaf chemistry of peanut exposed to mixtures of CO_2 and O_3 in the 2-yr experiment.†

Treatment	Leaf mass per area	Chlorophyll	Starch	Soluble sugars	N	Total phenolics
	mg cm^{-2}		μg cm^{-2}			
CF-375	7.3 ± 0.1 (100)	38.4 ± 1.1 (100)	1.5 ± 0.1 (100)	0.09 ± 0.01 (100)	0.21 ± 0.01 (100)	0.22 ± 0.01 (100)
CF-548	8.4 ± 0.1 (115***)	34.9 ± 1.4 (91*)	2.1 ± 0.1 (140***)	0.13 ± 0.01 (144***)	0.20 ± 0.01 (95)	0.22 ± 0.01 (100)
CF-730	8.8 ± 0.1 (120***)	29.4 ± 1.1 (76***)	2.3 ± 0.1 (153***)	0.12 ± 0.01 (133**)	0.20 ± 0.01 (95)	0.23 ± 0.01 (104)
NF-375	7.1 ± 0.1 (97)	37.6 ± 1.3 (98)	1.2 ± 0.1 (80**)	0.10 ± 0.01 (111)	0.22 ± 0.01 (105)	0.22 ± 0.01 (100)
NF-548	8.3 ± 0.1 (114***)	34.3 ± 1.3 (89*)	2.0 ± 0.1 (133***)	0.13 ± 0.01(144***)	0.21 ± 0.01 (100)	0.22 ± 0.01 (100)
NF-730	8.9 ± 0.1 (122***)	31.2 ± 1.3 (81***)	2.4 ± 0.1 (160***)	0.12 ± 0.01 (133**)	0.20 ± 0.01 (95)	0.25 ± 0.01 (114)
NF-1009	8.7 ± 0.1 (119***)	27.5 ± 1.3 (72***)	2.5 ± 0.1 (167***)	0.12 ± 0.01 (133**)	0.19 ± 0.01 (90*)	0.24 ± 0.01 (109)
OZ-375	6.5 ± 0.1 (89***)	27.7 ± 1.1 (72***)	1.0 ± 0.1 (67***)	0.10 ± 0.01 (111)	0.19 ± 0.01 (90**)	0.21 ± 0.01 (95)
OZ-548	7.7 ± 0.1 (105*)	31.8 ± 1.3 (83***)	1.7 ± 0.1 (113)	0.12 ± 0.01 (133**)	0.20 ± 0.01 (95)	0.23 ± 0.01 (104)
OZ-730	8.3 ± 0.1 (114***)	30.7 ± 1.1 (80***)	2.0 ± 0.1 (133***)	0.12 ± 0.01 (133**)	0.20 ± 0.01 (95)	0.25 ± 0.01 (114)
Source‡						
Year	*	**	**	***	***	***
CO_2	***	***	***	***	NS	***
O_3	***	***	**	NS	**	NS
Year × CO_2	*	NS§	NS	NS	NS	NS
Year × O_3	NS	*	NS	NS	*	NS
CO_2 × O_3	NS	***	NS	NS	NS	NS
Year × CO_2 × O_3	NS	NS	NS	*	NS	NS
WAP	***	***	***	***	***	***
WAP × CO_2	***	***	*	NS	*	NS
WAP × O_3	***	***	**	NS	***	NS
WAP × CO_2 × O_3	NS	NS	NS	NS	NS	NS

*Significant treatment effects and interactions $P \leq 0.05$.

**Significant treatment effects and interactions $P \leq 0.01$.

***Significant treatment effects and interactions $P \leq 0.001$.

†Leaf chemistry components are expressed on a leaf area basis. See Table 1 for treatment descriptions. Values are means ± SE of each treatment combination for all sampling dates in both years of the experiment. Values in parentheses indicate percent of the control treatment and statistical significance of difference from the control treatment (CF-375). WAP, weeks after planting.

‡NF-1009 treatment not included.

§NS, not significant at $P > 0.05$.

and polymerization of preexisting phenolics occurred in response to O_3.

It was somewhat surprising that the inhibitory effects of NF air on *A*, along with the increase in visible foliar injury, were not translated into statistically significant decreases in biomass and yield (also see Burkey et al., 2007). However, inhibition of *A* and starch accumulation in upper canopy leaves did not become apparent in the NF-375 treatment until late in the growing season, which suggests that growth and reproductive processes might have been only mildly affected in this treatment until the later developmental stages when cumulative exposure effects were sufficient to impair photoassimilation.

Effects of the open-top field chambers on visible injury, biomass production, g_s, LMPA, and leaf chemistry were compared in the AA and NF-375 treatments. Few statistically significant differences between the treatments were indicated (Table 9). Stomatal conductance was 12% lower in the AA treatment compared with the NF-375 treatment, while LMPA and starch concentration values were higher. In a previous study (Heagle et al., 1983), peanut biomass and yield were 8 to 25% higher in an AA treatment compared with the NF treatment, although a statistical analysis of the results was not reported. It is known that open-top chambers impose higher daytime air temperatures, lower PPFD, continuous air movement, and other changes in environmental factors that differ from ambient conditions (Heagle et al., 1979; Kimball et al., 1997). Therefore, it is not unexpected that plant growth per se in ambient air is often different from that in chambers. However, a number of comparisons between AA and NF treatments indicated that relative plant responses to O_3 were not significantly influenced by the chamber environment (Heagle, 1989). We recognize that plant responses to elevated CO_2 and O_3 in our experiment have the potential to be confounded by the use of CF and NF air in the treatments. This protocol was adopted from the USDA National Crop Loss Assessment Network program (Heagle, 1989). The use of CF and NF air was deemed acceptable because levels of other air pollutants such as NO, NO_2, and SO_2 were

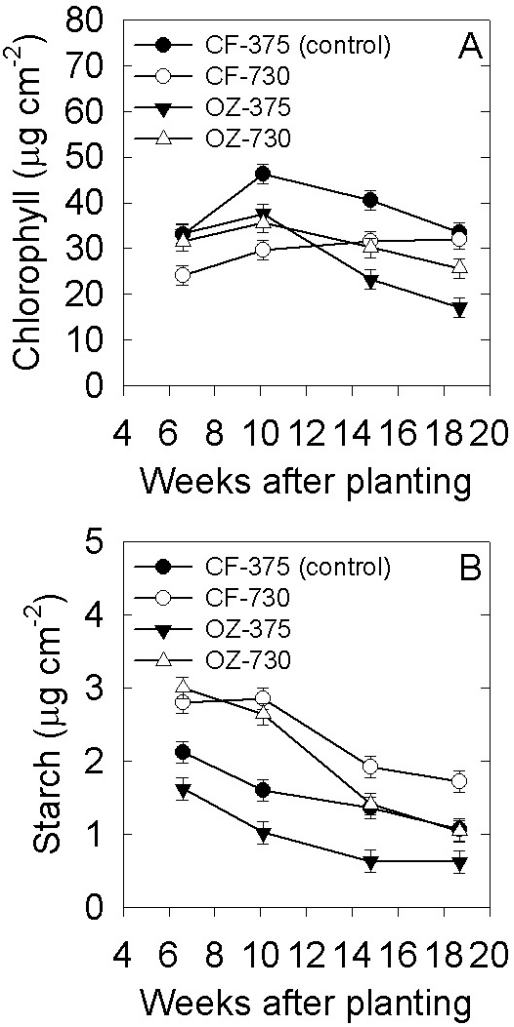

Figure 3. Effects of CO_2 and O_3 on chlorophyll (A) and starch (B) concentrations in upper canopy leaves of peanut from 6 through 19 wk after planting in the 2-yr experiment. Treatments shown are charcoal-filtered air (CF)–ambient CO_2 (CF-375) (control), CF air plus 355 μmol CO_2 mol^{-1} (CF-730), 1.5 × ambient O_3–ambient CO_2 (OZ-375), and 1.5 × ambient O_3 plus 355 μmol CO_2 mol^{-1} (OZ-730). Values are means ± SE from two or three replicate chambers per treatment in each year of the experiment (see Table 1).

below phytotoxic levels at our location. Ambient air is also entrained in all chambers during the course of the experiment. Thus, we have no reason to presume that the use of CF and NF air would lead to unrecognized interactions in our experiment.

In conclusion, results of our experiment indicated that *A*, starch biosynthesis, and biomass production in NC-V11 peanut was suppressed by ambient and higher levels of O_3. Increasing concentrations of CO_2 should ameliorate these responses, although its effectiveness will likely depend on concurrent O_3 concentrations and other changes in environmental conditions.

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Table 9. Open-top chamber effects on visible foliar injury at midseason, harvest biomass, stomatal conductance (g_s), leaf mass per unit leaf area (LMPA), and leaf chemistry of peanut. Plants were exposed to nonfiltered air (NF-375) and ambient air (AA; chamber frames without side panels).†

Parameter	Treatment	
	NF-375	AA
Visible injury (% chlorosis and necrosis)	29.3 ± 1.8	20.6 ± 1.5 **
Biomass		
Leaf, g $plant^{-1}$‡	32.9 ± 2.5	34.2 ± 3.6 (104)
Stem, g $plant^{-1}$	38.8 ± 2.5	34.8 ± 2.1 (90)
Roots, g $plant^{-1}$	2.2 ± 0.2	2.0 ± 0.2 (91)
Pods, g $plant^{-1}$	51.8 ± 3.8	61.0 ± 3.1 (118)
Culls, g $plant^{-1}$	1.3 ± 0.3	1.1 ± 0.2 (85)
Total, g $plant^{-1}$	127.0	133.1 (105)
g_s, mmol H_2O m^{-2} s^{-1}	711 ± 19	627 ± 18 (88***)
LMPA, mg cm^{-2}	7.1 ± 0.2	7.7 ± 0.2 (108**)
Leaf chemistry		
Chlorophyll, μg cm^{-2}	37.6 ± 1.2	33.8 ± 1.0 (90*)
Starch, μg cm^{-2}	1.2 ± 0.1	1.6 ± 0.1 (133*)
Soluble sugars, μg cm^{-2}	0.10 ± 0.01	0.12 ± 0.01 (120)
N, μg cm^{-2}	0.22 ± 0.01	0.23 ± 0.01 (104)
Total phenolics, μg cm^{-2}	0.22 ± 0.01	0.24 ± 0.01 (109)

*Significant treatment effects and interactions $P \leq 0.05$.

**Significant treatment effects and interactions $P \leq 0.01$.

***Significant treatment effects and interactions $P \leq 0.001$.

†Values are means ± SE of two (NF-375) or three (AA) replicate chambers for all sampling occasions in both years of the experiment. Values in parentheses indicate percentage of the NF-375 treatment. Effect of year was statistically significant ($P \leq 0.05$) for all parameters, but the treatment × year interactions were not significant.

‡Values and statistics for leaf mass per plant in the AA treatment are for 2002 only because pathogen-related defoliation occurred in this treatment during the last 2 wk of the study in 2003. Leaf mass in 2003 was only 3.6 ± 2.9 g $plant^{-1}$, and was not included in the analysis. Total biomass value for the AA treatment includes this adjustment as well.

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