

Growth responses of yellow-poplar (*Liriodendron tulipifera* L.) exposed to 5 years of O₃ alone or combined with elevated CO₂

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ABSTRACT

Field-grown yellow-poplar (*Liriodendron tulipifera* L.) were fumigated from May to October in 1992–96 within open-top chambers to determine the impact of ozone (O₃) alone or combined with elevated carbon dioxide (CO₂) on sapling growth. Treatments were replicated three times and included: charcoal-filtered air (CF); 1 × ambient ozone (1 × O₃); 1.5 × ambient ozone (1.5 × O₃); 1.5 × ambient ozone plus 350 p.p.m. carbon dioxide (1.5 × O₃ + CO₂) (target of 700 p.p.m. CO₂); and open-air chamberless plot (OA). After five seasons, the total cumulative O₃ exposure (SUM₀₀ = sum of hourly O₃ concentrations during the study) ranged from 145 (CF) to 861 (1.5 × O₃) p.p.m. × h (parts per million hour). Ozone had no statistically significant effect on yellow-poplar growth or biomass, even though total root biomass was reduced by 13% in the 1.5 × O₃-exposed saplings relative to CF controls. Although exposure to 1.5 × O₃ + CO₂ had a stimulatory effect on yearly basal area growth increment after two seasons, significant increases in shoot and root biomass (~60% increase relative to all others) were not detected until the fifth season. After five seasons, the yearly basal area growth increment of saplings exposed to 1.5 × O₃ + CO₂-air increased by 41% relative to all others. Based on this multi-year study, it appears that chronic O₃ effects on yellow-poplar growth are limited and slow to manifest, and are consistent with previous studies that show yellow-poplar growth is not highly responsive to O₃ exposure. In addition, these results show that enriched CO₂ may ameliorate the negative effects of elevated O₃ on yellow-poplar shoot growth and root biomass under field conditions.

Key-words: elevated O₃ + elevated CO₂; hardwood trees; scaling O₃ responses.

INTRODUCTION

Current projections suggest that forests within the eastern United States will continue to be exposed to increasing levels of tropospheric O₃ (Chameides *et al.* 1994) and ambient CO₂ (Watson *et al.* 1990). Rural regions of eastern USA typically average summer daytime levels in excess of 0.05 p.p.m. O₃ (Lefohn, Edwards & Adams 1994) with occasional O₃ episodes above 0.100 p.p.m. Tropospheric O₃ has long been known to be phytotoxic (Middleton 1956) and causes the greatest amount of damage on vegetation of any gaseous pollutant by reducing growth and productivity of many plant species through reductions in photosynthesis, accelerated leaf senescence and decreased root growth (Bortier, Ceulemans & de Temmerman 2000; Rudorff, Mulchi & Lee 2000). Elevated CO₂ concentrations generally increase plant growth through increased carbon assimilation, biomass and leaf area (Ceulemans & Mousseau 1994; Sax, Ellsworth & Heath 1998). It is hypothesized that plants growing in elevated CO₂ may be protected from O₃ damage by reducing O₃ uptake because elevated CO₂ often reduces stomatal conductance (Eamus & Jarvis 1989; Mousseau & Saugier 1992). Elevated CO₂ may also provide substrates for detoxification and repair processes against O₃ damage (Rudorff *et al.* 2000).

Empirical information on the combined effects of the O₃ and CO₂ on woody plants is limited (Allen 1990; Olszyk *et al.* 2000). In some studies, elevated CO₂ offsets the negative effects of O₃ in deciduous species (Volin, Reich & Givnish 1998; Broadmeadow & Jackson 2000), whereas in others no ameliorative effects are detected (Kull *et al.* 1996). Multi-year field fumigations are needed to accurately assess the chronic response of tree species to the combination of elevated CO₂ and O₃. This can be achieved by exposing the same population of trees to gaseous pollutants during their development from seedlings to saplings or mature trees (Lee & Jarvis 1995; Retzlaff, Williams & DeJong 1997; Rey & Jarvis 1997). These studies are very costly to run and only a few have been undertaken, resulting in a paucity of empirical data on the response of saplings and mature trees to gaseous pollutants (Karnosky *et al.* 1999; Isebrands *et al.* 2001). These types of studies can serve as an important linkage to extrapolate seedling pol-

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lutant response data to larger and more structurally complex older trees.

In the current study, we investigated how field-planted yellow-poplar (*Liriodendron tulipifera* L.) seedlings grown under less than optimal conditions (unmanipulated soil moisture and fertility) responded to chronic levels of elevated O_3 either alone or combined with elevated CO_2 over five seasons. The effects of elevated O_3 on yellow-poplar, an ecologically and economically important hardwood species in eastern USA forests, have been well studied in potted seedlings under environmentally controlled conditions showing both inhibitions and stimulations in growth and physiological processes (Chappelka, Chevone & Seiler 1988; Jensen & Patton 1990; Roberts 1990; Cannon, Roberts & Bargar 1993; Rebbeck & Loats 1997; Loats & Rebbeck 1999). Long considered an O_3 -sensitive species, yellow-poplar is used as a bioindicator of O_3 in eastern US forests (Davis & Skelly 1992). Our objectives were to determine: (1) if the responses of field-grown saplings to multi-year O_3 exposures were comparable with those of seedlings in shorter-term O_3 exposures under more controlled conditions; and (2) if doubling ambient CO_2 concentrations would ameliorate negative O_3 growth effects.

MATERIALS AND METHODS

Seedling culture and site characteristics

In 1991, a field plantation of yellow-poplar was established at the Northeastern Research Station's Forestry Sciences Laboratory in Delaware, Ohio, USA (latitude 40°21' N longitude 83°04' W), by clearing a 26 m by 73 m area in a 20-year-old-abandoned American elm (*Ulmus americana* L.) plantation. The glacial till soil was primarily of the Blunt Series with pockets of the Morley Series. Soil textures were determined to be primarily clay loams. Soils were chemically analysed prior to planting to a depth of 25 cm and were considered adequate in total N ($24.7 \mu\text{g g}^{-1} \text{NH}_4\text{-N}$ and $8.2 \mu\text{g g}^{-1} \text{NO}_3\text{-N}$). Mean soil pH was 6.39 and CEC was 13.9 meq/100 g dry soil. Extractable ion concentrations were $7.4 \mu\text{g g}^{-1} \text{P}$; $103.5 \mu\text{g g}^{-1} \text{K}$; $1909 \mu\text{g g}^{-1} \text{Ca}$; and $217.6 \mu\text{g g}^{-1} \text{Mg}$. Soil chemical properties were measured annually; no major changes in concentrations were observed throughout the study.

One-year-old bareroot stock originating from seeds collected in south-eastern Tennessee was obtained from a private nursery in western Pennsylvania, USA. Approximately 250 uniform bare-rooted seedlings were planted 2.1 m by 2.1 m apart in July 1991 to serve as buffer trees between the plots. In May 1992 within each chamber or plot, 12 randomly chosen seedlings were planted 47 cm apart in a circular pattern approximately 1.8 m in diameter and 0.6 m from the inside edge of a standard 3-m-diameter open-top chamber (OTC) (Heagle, Body & Heck 1973) or equivalent chamberless plot. During the early establishment of seedlings in 1992 and 1993, ambient rainfall was occasionally supplemented in order to minimize severe water stress.

In 1992, methyl [1-[(butylamino)carbonyl]-1*H*-benzimidazol-2-yl] carbamate [Benomyl 50E® (14.7 mg active ingredient L^{-1}); DuPont, Wilmington, DE, USA] was applied biweekly from mid-August to mid-September as a prophylactic to control powdery mildew, a serious foliar disease of yellow-poplar in central Ohio that can kill young seedlings. As observed in previous studies, Benomyl did not protect the seedlings from oxidant injury (Rebbeck 1996a; Loats & Rebbeck 1999). No other applications of pesticides were made during the study. In 1993 and 1994, sporadic outbreaks of aphids were observed with a fairly uniform distribution in all plots. Insecticidal soap [Safer® (39 mL L^{-1}); Gardens Alive, Lawrenceburg, IN, USA] was applied biweekly to suppress aphid populations. Biological control measures were subsequently adopted and commercially reared ladybugs (*Hippodamia* sp.) were released biweekly from mid-July until late August during each season.

Pollutant exposure and experimental design

The experiment was a randomized block design with three replicates of the following treatments: (1) charcoal-filtered air (CF); (2) $1 \times$ ambient O_3 ($1 \times O_3$); (3) $1.5 \times$ ambient O_3 ($1.5 \times O_3$); (4) $1.5 \times$ ambient O_3 plus 350 p.p.m. CO_2 above ambient (target CO_2 concentration was 700 p.p.m) ($1.5 \times O_3 + CO_2$); and (5) open-air chamberless plot (OA). Due to the prohibitive costs associated with supplying twice ambient CO_2 to three additional open-top chambers, we did not characterize the effects of elevated CO_2 alone on yellow-poplar growth. Gases were dispensed 24 h per day from mid-May until mid-October in 1992–96. The polyvinyl-chloride film panels were removed each season when gas exposures were terminated. In spring 1994, standard OTCs (3 m in diameter, 2.4 m high) were extended to a height of 4.6 m. The mean volumetric airflow within chambers was $47 \text{ m}^3 \text{ min}^{-1} \pm 10\%$ and was provided by a single blower. In spring 1995, standard OTCs were replaced with larger open-top chambers (4.6 m in diameter, 9.2 m high) similar to those developed by Heagle *et al.* (1989), but doubled in height. Air was blown into the bottom of each chamber via two 2HP, 91.4-cm-diameter fans (Penn Ventilator Inc., Philadelphia, PA, USA), each housed in a galvanized sheet-metal box (123 cm wide, 123 cm high, 123 cm long). Unfiltered ambient air entered each chamber after passing through particulate filters and connecting plastic ducts at approximately $6.8 \text{ m}^3 \text{ s}^{-1}$.

Ozone was generated from vaporized liquid oxygen with an electric spark discharge O_3 generator (OREC Model 03V10-0; OREC, Glendale, AZ, USA) and was dispensed into the $1 \times O_3$ and $1.5 \times O_3$ OTC's through Teflon tubing when ambient levels exceeded 0.03 p.p.m. (Rebbeck 1996a; Rebbeck & Loats 1997). Gaseous CO_2 was vaporized from liquid CO_2 (14 000 kg reservoir, MG Industries, Malvern, PA, USA) and dispensed through Teflon tubing into the OTCs and adjusted manually with needle valves (Part 8513D-2-E-4E-1 A, Brooks Instrument Division, Emerson Electric, Hatfield, PA, USA) to maintain target levels. The

O₃ concentration was regulated with mass-flow control valves (Model FC260, Tylan General, San Diego, CA, USA) through a microcomputer to match a set-point value based on the most recent ambient O₃ reading. Each chamber was monitored for O₃ and CO₂, and air temperature (subset of chambers) every 2 min. Hourly means were automatically calculated and stored on a personal computer. Seasonal average O₃ and CO₂ concentrations were calculated for each chamber/plot. However, seasonal average concentration often does not correlate with plant injury because it does not include important exposure factors such as episodic peaks and the cumulative effects of low O₃ concentrations. To better relate plant responses to O₃ treatments, cumulative O₃ exposures for each chamber/plot were estimated (Bortier *et al.* 2000). Two cumulative exposure indices were calculated each growing season: SUM₀₀ (p.p.m. × h) that is equal to the sum of all hourly average O₃ concentrations; and SUM₀₆ (p.p.m. × h) is equal to the sum of all hourly concentrations above 0.059 p.p.m. Daily single-point and weekly multi-point calibrations of the O₃ monitor (Model 49PS; Thermo Electron Instruments, Hopkinton, MA, USA) were made with a multi-gas calibrator (Model 8500; Monitor Laboratories, San Diego, CA, USA). CO₂ levels were monitored with a Li-Cor Model 6251 infrared gas analyser (Li-Cor Inc., Lincoln, NE, USA) with daily single-point calibrations and zero span checks, and weekly multi-point calibrations using certified CO₂ span gases (AGA Industries, Cleveland, OH, USA). Ambient photosynthetic photon fluence rate (PPFR) over the waveband 400–700 nm (Li-Cor quantum sensor), relative humidity (%RH) and air temperature [Model XN217 (Hydrometrix RH sensor and Fenwal Electronics UUT51J1 thermistor) Campbell Scientific, Logan, UT, USA] were monitored with a Campbell 21X datalogger.

Growth and biomass measurements

Date of budbreak and length of terminal stem dieback at the start of each growing season was recorded for each seedling beginning in 1993 (12 plants × 3 chambers × 5 treatments = 180 plants). Stem height and diameter (permanently marked at approximately 2.5 cm above soil surface) were measured in July and September 1992. In 1993–96, monthly stem height and diameter measurements were made throughout each exposure season (May–September). Stem height measurements included total height (measured from tree base at the ground to tree apex) and yearly height growth increment (length of current year's stem). In late September 1993, the biomass of six yellow-poplar from each chamber/plot (6 plants × 3 chambers × 5 treatments = 90 plants, 90 plants were left in the ground) was determined by destructive harvest. The root systems were excavated from a fixed volume (25 cm × 25 cm × 25 cm) of soil immediately surrounding the decapitated seedling and were separated into taproot and first-order laterals. The soil that had been removed was returned to its original location within

each plot. In September 1994, three saplings were harvested (3 plants × 3 chambers × 5 treatments = 45 plants, 45 plants were left in the ground) from each plot. The roots were not excavated to minimize damage to the three remaining trees within each chamber. In late September 1996, above-ground biomass of the three remaining trees from each plot/chamber was harvested (45 trees). At this final harvest, the stem diameter was measured on the portion of the main stem that was initiated at the start of the study (1992) and at a height of 50 cm from the ground. Stem basal area at 50 cm from the ground was calculated. In late winter 1997, the roots were excavated from a fixed volume of soil (91 cm top width, 71 cm long, 46 cm bottom width) using a commercial tree spade. Roots were separated into tap, first- and second-order laterals. In all harvests, the total plant height, length of current terminal, length and diameter of each year's height growth increment, basal stem diameter, branch lengths and numbers were measured. Leaf area was estimated using a calibrated LI-3100 area meter (Li-Cor, Inc.) at every harvest. Following growth measurements, the tissue of each tree was separated into leaves, stem and branches, oven-dried at 70 °C to constant mass [g dry weight (DW)] and weighed. For each sapling, the root : shoot ratio, specific leaf area (cm² g⁻¹), the ratio of total leaf area to lateral roots, and leaf area ratio [LAR = total leaf area/sapling mass (shoot + root DW)] was calculated.

Statistical analyses

Treatment effects were tested using only the chambered plots and comparisons were made between OA- and 1 × O₃-exposed seedlings to assess the effect of chambers on seedling growth. Diameter and height growth for each year (1992–96) were analysed to test for overall treatment effects (CF, 1 × O₃, 1.5 × O₃ and 1.5 × O₃ + CO₂ plots) with a randomized block design using repeated measures analysis (RMA) (SAS System for Mixed Models; Littell *et al.* 1996). Repeated measures analysis was used to compare treatment means over the five growing seasons. The covariance structure of the growth response data (height, basal diameter and basal area increments) was determined to be autoregressive, in that measurements taken at adjacent time points were more highly correlated than measurements taken several time points apart. A mixed model with random [block and time (year)] and fixed (treatment) effects with an autoregressive covariance structure was used. The three chamber replicates per treatment were used in the analyses. Least square means ± 1 SE are presented. *A priori* single degree of freedom contrasts were used to ascertain differences among treatments over time. In addition, multiple degree of freedom contrasts were used to determine treatment differences at specific time points. Biomass data were analysed using General Linear Model to test for significant treatment effects using chamber means (*n* = 3) (SAS 1999). Effects were considered significant if *P* < 0.10 unless otherwise stated.

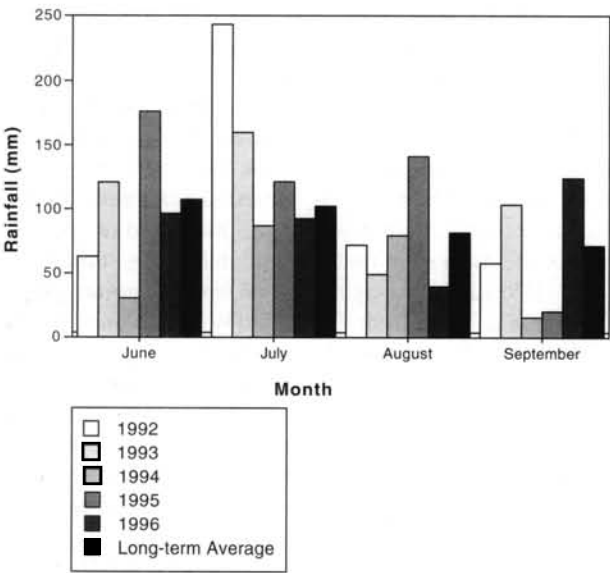


Figure 1. Monthly rainfall (mm) patterns during each growing season (1992–96) compared with the 50-year average.

RESULTS

Pollutant and environmental conditions

Mean monthly ambient air temperatures for June to September 1992–96 ranged from 14.1 to 24.7 °C. Air tempera-

tures within OTCs were approximately 1 °C above ambient. During the winter months (December–March), minimum air temperature ranged from –32.6 to 10.7 °C and maximum air temperature ranged from –19.8 to 25.1 °C. The coldest temperatures occurred in January 1994 during two days of record-breaking low temperatures. From June to September PPFR ranged from 21.8 to 43 mol m⁻² d⁻¹. Monthly rainfall during each growing season varied year to year from the long-term 50-year average for the site (Fig. 1). Annual precipitation was 914, 1024, 677, 1143 and 1125 mm in 1992–96, respectively, in comparison with the long-term average of 993 mm.

Actual O₃ levels were as follows: CF ranged from 0.15 × to 0.30 × ambient O₃; 1 × O₃ ranged from 0.98 × to 1.03 × ambient O₃; 1.5 × O₃ ranged from 1.31.5 × to 1.68 × ambient O₃; and 1.5 × O₃ + CO₂ ranged from 1.20 × to 1.58 × ambient O₃. Cumulative O₃ exposure (p.p.m. × h) was calculated as both SUM₀₀ (sum of every hourly mean) and SUM₀₆ (sum of all hourly O₃ values exceeding 0.059 p.p.m) for each season (Table 1). In 1992–96, 51, 46, 7, 84 and 89%, respectively, of the exposure days had daily hourly maximum ambient O₃ concentrations which exceeded 0.050 p.p.m. Daily levels of hourly maximum ambient O₃ exceeded 0.080 p.p.m. for 6.3% of the exposure days in both 1992 and 1993, compared with 0, 30 and 36% in 1994, 1995 and 1996, respectively. Seasonal 24 h mean ambient O₃ concentration ranged from 0.032 to 0.046 p.p.m.

Ambient CO₂ concentrations varied diurnally, with a

Table 1. Seasonal 24-h mean O₃ concentration and seasonal cumulative O₃ exposure^a (SUM₀₀ and SUM₀₆) during five seasons of exposure in Delaware, Ohio, USA 1992–96

Treatment		Seasonal 24 h mean ozone concentration (p.p.m)									
Target	Actual	1992		1993		1994		1995		1996	
OA ^b	—	0.035		0.032		0.035		0.042		0.046	
CF (control)	0.24×	0.010		0.005		0.011		0.012		0.011	
1 × O ₃	1.00×	0.035		0.032		0.035		0.042		0.048	
1.5 × O ₃	1.48×	0.046		0.051		0.049		0.059		0.078	
1.5 × O ₃ + CO ₂	1.42×	0.042		0.050		0.047		0.061		0.073	

Treatment	Seasonal cumulative ozone exposure (p.p.m. × h)										Total (1992–96)	
	1992		1993		1994		1995		1996			
	SUM ₀₀	SUM ₀₆	SUM ₀₀	SUM ₀₆	SUM ₀₀	SUM ₀₆	SUM ₀₀	SUM ₀₆	SUM ₀₀	SUM ₀₆	SUM ₀₀	SUM ₀₆
OA	103	22.0	105	17.3	128	33.9	120	48.8	126	60.0	582	182.0
CF	27	0.3	16	0.0	39	0.0	36	0.0	27	0.0	145	0.3
1 × O ₃	104	34.9	107	23.6	125	42.6	117	54.6	130	73.0	583	228.7
1.5 × O ₃	136	81.2	171	135.4	176	121.7	166	131.3	212	192.2	861	661.8
1.5 × O ₃ + CO ₂	124	67.6	166	129.4	170	114.1	172	136.5	198	176.7	830	624

^aSeasonal cumulative ozone exposure: SUM₀₀ equals the sum of each daily 24 h total exposure for the entire exposure season. SUM₀₆ equals the sum of each daily hourly mean that exceeded 0.059 p.p.m. O₃ during the entire exposure season.

^bOA, open-ambient air chamberless treatment; CF, charcoal-filtered air; 1×O₃, 1 times ambient ozone; 1.5 × O₃, 1.5 times ambient ozone; 1.5 × O₃ + CO₂, 1.5 times ambient ozone plus 2 times ambient carbon dioxide (target CO₂ concentration was 700 p.p.m). Ozone was added to all 1×O₃, 1.5 × O₃ and 1.5 × O₃ + CO₂ chambers.

daily mean of 355 p.p.m., ranging from 330 p.p.m. (early to mid-afternoon) to 455 p.p.m. (pre-dawn). In the $1.5 \times O_3 + CO_2$ plots, seasonal mean CO_2 concentrations were 653, 727, 740, 688 and 728 p.p.m. in 1992–96, respectively. CO_2 concentrations within all other chambered plots did not vary significantly from the open-air plots. Diurnal patterns of both O_3 and CO_2 in these plots were similar to those found under ambient conditions.

Growth response

In general, trees broke bud in late April each year. Mean date of bud burst was 20 April, 23 April, 17 April and 23 April in 1993–96, respectively. Date of bud burst was not affected by exposure to $1.5 \times O_3$ - or $1.5 \times O_3 + CO_2$ -air throughout the study. On average, all trees broke bud

within a week of one another each season. In spring 1993, dieback of the main terminal stem averaged 10 cm in CF-, $1 \times O_3$ - and $1.5 \times O_3$ -air compared to 23 cm in $1.5 \times O_3 + CO_2$ -air ($P = 0.012$). No significant difference in the amount of stem dieback was observed in spring 1994. In spring 1995 and 1996, visual assessments of stem dieback appeared to be uniform across treatments.

Figure 2a–c summarizes total stem height, stem diameter and basal area measured each October during the study. By the end of the fifth growing season, mean sapling stem height was 720 cm, diameter was 94 mm and basal area was 73 cm^2 . The chronic effects of O_3 and $1.5 \times O_3 + CO_2$ were determined over the 5 year study by comparing the yearly growth increments of yellow-poplar for each season using RMA (Fig. 2d–f, Table 2). Yearly height growth increment was not affected by exposure to $1.5 \times O_3$ or $1.5 \times O_3 + CO_2$.

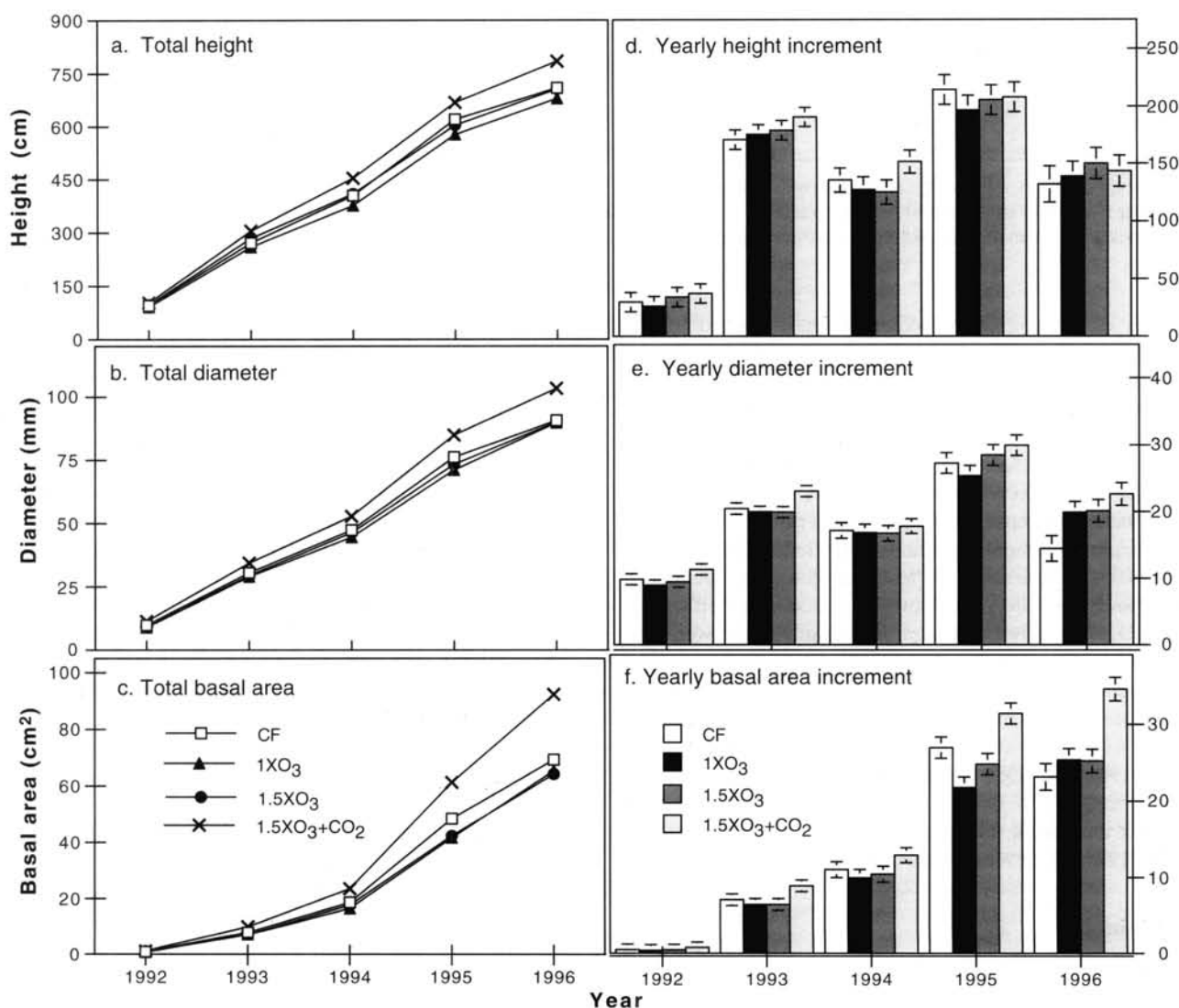


Figure 2. (a) Yearly total stem height (cm); (b) total stem diameter (mm); (c) total basal area (cm^2); (d) yearly height growth increment (cm); (e) yearly diameter growth increment (mm); and (f) yearly basal area growth increment (cm^2) of yellow-poplar seedlings exposed to O_3 alone or O_3 plus 700 p.p.m. CO_2 , measured each September from 1992 to 1996. Least square means ± 1 SE are represented ($n = 3$ chamber replicates per treatment). Significant within-year treatment effects on basal area growth increment were detected in years 2–5 (see Table 2).

Table 2. Summary of repeated measures analysis and tests of effects on yearly stem growth of yellow-poplar to subambient, ambient O₃, elevated O₃ alone or combined with elevated CO₂ for 5 years (1992–96)

Source	Numerator d.f.	Denominator d.f.	P-value			
			Yearly growth			
			Diameter increment	Height increment	Basal area increment	
(a) Overall effects						
Treatment	3	6	0.086	0.393	0.009	
Year	4	31	< 0.001	< 0.001	< 0.001	
Treatment by year	12	31	0.319	0.949	0.004	
(b) Contrasts across years ^a						
CF versus 1.5 × O ₃	1	6	0.339	0.770	0.803	
CF versus 1 × O ₃ and 1.5 × O ₃	1	6	0.131	0.670	0.306	
All versus 1.5 × O ₃ + CO ₂	1	6	0.021	0.141	0.002	
1.5 × O ₃ versus 1.5 × O ₃ + CO ₂	1	6	0.099	0.351	0.005	
Year 3 versus all other years	1	32	–	–	< 0.001	
Year 4 versus all other years	1	32	–	–	< 0.001	
Year 5 versus all other years	1	32	–	–	< 0.001	
(c) Basal area increment – within year contrasts			Year 2	Year 3	Year 4	Year 5
CF versus 1.5 × O ₃	1	32	0.567	0.684	0.278	0.379
All versus 1.5 × O ₃ + CO ₂	1	32	0.029	0.042	< 0.001	< 0.001
1.5 × O ₃ versus 1.5 × O ₃ + CO ₂	1	32	0.015	0.091	0.002	< 0.001

^aSingle d.f. contrasts were made across years unless noted otherwise. Year 1 = 1992; Year 2 = 1993; Year 3 = 1994; Year 4 = 1995; Year 5 = 1996. Because significant treatment by year interactions were only detected for basal area growth in years 2, 3, 4 and 5, single d.f. treatment contrasts are presented in (c). Refer to Fig. 2 for treatment means.

Yearly diameter growth increment was impacted by the treatments ($P = 0.086$) but with no significant treatment by year interactions. Elevated O₃ (1.5 × O₃) alone had no significant effect on the yearly stem growth diameter increment but when combined with 700 p.p.m. CO₂ (1.5 × O₃ + CO₂), the yearly diameter growth increment increased 14% relative to all others ($P = 0.021$). Yearly basal area growth increment was impacted by the treatments ($P = 0.009$) and significant treatment by year interactions were detected ($P = 0.004$). Contrasts were tested to determine treatment differences within each growing season. The effects of 1.5 × O₃ on yearly basal area growth increment were not detected. The effects of 1.5 × O₃ + CO₂ air were first detected after two seasons of exposure. The yellow-poplar basal area growth increment was 33, 24, 28 and 41% greater in 1.5 × O₃ + CO₂-air in comparison with all others in 1993, 1994, 1995 and 1996, respectively. Yearly basal area growth increment of 1.5 × O₃ + CO₂-trees was 37, 24, 27 and 37% greater than those trees exposed to 1.5 × O₃-alone in 1993, 1994, 1995 and 1996, respectively (Table 2).

Biomass

1993 Harvest

Exposure to either 1.5 × O₃- or 1.5 × O₃ + CO₂-air did not significantly impact leaf area, shoot or root biomass after two seasons of treatment. Sapling biomass averaged 310.3 g DW for stems, 142.6 g DW for branches, 237.9 g DW for leaves, 53.9 g DW for lateral roots and

76.5 g DW for tap roots. Specific leaf area averaged 169 cm² g⁻¹ and total leaf area averaged 3.96 m² per sapling. The mass of upper canopy terminal leaves for saplings exposed to 1.5 × O₃ + CO₂-air was 1.25 times greater than all other treated saplings. The ratio of first-order lateral to tap root biomass was 12–20% higher in saplings exposed to 1.5 × O₃ + CO₂-air compared with all other treated saplings ($P = 0.024$). Saplings exposed to 1.5 × O₃-air had 11% higher leaf area ratios (LAR) than CF-exposed saplings, whereas those grown in 1.5 × O₃ + CO₂-air had 6–18% lower LAR compared with all other saplings ($P = 0.020$) (Table 3). The ratio of total leaf area per sapling to first-order lateral roots was 27% lower in saplings exposed to 1.5 × O₃ + CO₂-air compared with saplings exposed to 1.5 × O₃ alone ($P = 0.013$).

1994 Harvest

After three seasons, no significant treatment effects on leaf area or above-ground biomass (branches, stem, or leaves) were detected. Stem dry weight averaged 770 g, branch dry weight averaged 466 g, leaf dry weight averaged 465 g and total leaf area per sapling averaged 8 m². Roots were not excavated in this harvest.

1996 Harvest

A 16-fold increase in standing live plant (shoot + root) biomass was observed from 1993 to 1996. After five seasons of

Table 3. The leaf area ratio^a and ratio of total leaf area to lateral roots of yellow-poplar saplings exposed to subambient O₃, ambient O₃, elevated O₃ alone or combined with elevated CO₂ after two (1993) and five (1996) seasons

Treatment	Leaf area ratio (cm ² g ⁻¹)	Leaf area : lateral root ratio (cm ² g ⁻¹)
1993		
CF	47.6 ± 2.3	733.5 ± 66.1
1 × O ₃	52.6 ± 2.4	845.4 ± 66.9
1.5 × O ₃	53.2 ± 2.2	869.9 ± 63.5
1.5 × O ₃ + CO ₂	44.7 ± 2.3	635.1 ± 64.4
1996		
CF	28.7 ± 1.1	317.5 ± 29.6
1 × O ₃	32.4 ± 1.8	342.7 ± 18.9
1.5 × O ₃	29.9 ± 1.8	362.8 ± 18.9
1.5 × O ₃ + CO ₂	21.4 ± 1.6	236.7 ± 16.3
Statistical significance (<i>P</i> -value)		
1993	0.033	0.049
1996	0.002	0.001

^a Leaf Area Ratio (LAR) = Total leaf area/tree dry mass.

Trees were sampled in mid-September. Each value represents the least square mean ± 1 SE (*n* = 3 chamber replicates per treatment: six trees per chamber in 1993; and three trees per chamber in 1996).

Table 4. A summary of the tests of significance for the effects of subambient O₃, ambient O₃, elevated O₃ alone or combined with elevated CO₂ on yellow-poplar growth and biomass measured at the final destructive harvest in September 1996

Response variable	Treatment effect ^a <i>P</i> -value
Growth measurements at 1996 harvest	
Total stem height	0.181
Diameter of stem initiated in 1992	0.109
Stem diameter at 50 cm from ground	0.026
Basal area at 50 cm from ground	0.028
Above-ground biomass	
Stem	0.021
Branches	0.633
Leaves	0.733
Shoot	0.153
Stem + branches	0.086
Root biomass	
Laterals	0.059
Tap	0.051
Total (tap + laterals)	0.049
Plant (shoot + root)	0.129
Root : shoot ratio	0.505

n = 3 chamber replicates per treatment. Three trees per chamber were harvested in September 1996.

^a Treatments included in tests of significance for 'treatment effect' included CF, 1 × O₃, 1.5 × O₃ and 1.5 × O₃ + CO₂ plots. Single d.f. contrasts comparing specific treatments, e.g. CF versus 1.5 × O₃, 1.5 × O₃ versus 1.5 × O₃ + CO₂, etc. were used. Please refer to Fig. 3 for more details.

exposure, significant treatment effects on shoot and root biomass were detected (Table 4). Both shoot and root biomass were approximately 60% greater in saplings exposed to 1.5 × O₃ + CO₂-air in comparison with all other saplings (Fig. 3). Although statistically significant O₃ effects on above- and below-ground biomass were not detected, root (first-order laterals, tap and total) biomass was reduced 13% in saplings exposed to 1.5 × O₃-air relative to CF controls. Root biomass (first- and second-order laterals, tap and total root system) of saplings exposed to 1.5 × O₃ + CO₂-air was on average 1.7 times greater than saplings exposed to 1.5 × O₃-air (*P*-values ranged from 0.02 to 0.04). Stem biomass of saplings exposed to 1.5 × O₃ + CO₂ increased 53 and 60% in comparison with CF- and 1.5 × O₃-exposed saplings, respectively. No treatment effects on leaf and branch biomass or root : shoot ratios were detected.

Treatment effects on total leaf area (average 36.4 m² per sapling) and specific leaf area (average 187.8 cm² g⁻¹) were not observed. Treatment effects on LAR and the ratio of total leaf area to lateral root dry weight were similar to

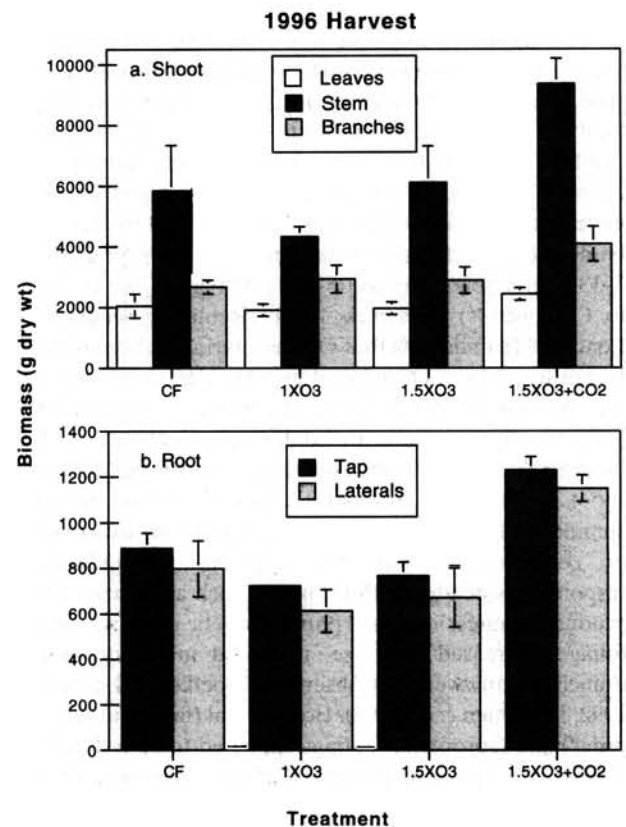


Figure 3. Yellow-poplar biomass (g dry weight) after five seasons (1996) of exposure to O₃ and elevated O₃ + CO₂-air: (a) shoot – leaves, stem and branches, and (b) root – tap root and lateral root. (*n* = 3 chamber replicates per treatment with three trees per chamber). Significant treatment effects on biomass were detected (see Table 4). Single degrees of freedom contrasts comparing 1.5 × O₃ versus 1.5 × O₃ + CO₂ treatments were significant for the following parameters: stem (*P* = 0.04), stem + branches (*P* = 0.07), lateral roots (*P* = 0.03), tap roots (*P* = 0.02) and total roots (*P* = 0.02).

those observed in 1993 (Table 3). Ozone ($1 \times O_3$ or $1.5 \times O_3$) did not affect LAR, but saplings grown in $1.5 \times O_3 + CO_2$ -air had 25–34% lower LAR in comparison with all other saplings ($P = 0.002$). The ratio of total leaf area to first-order lateral roots of saplings exposed to $1.5 \times O_3 + CO_2$ -air was reduced by 35% in comparison with those exposed to $1.5 \times O_3$ -air ($P = 0.001$).

Chamber effects

Stem growth of saplings exposed to ambient O_3 within open-top chambers ($1 \times O_3$) was 21% greater than that of saplings grown in open-air chamberless plots ($P = 0.06$). No differences in diameter growth, plant biomass or spring terminal dieback were detected between the two groups throughout the study.

DISCUSSION

O_3 effects

This study is one of a few to investigate the cumulative effects of multiple seasons of O_3 exposure on tree seedlings as they mature (some trees were flowering and producing seed) and increase in structural complexity. Over five seasons of exposure, O_3 had no apparent effect on growth or biomass. However, by the fifth season $1.5 \times O_3$ saplings exhibited reductions of 13% in both lateral and tap root mass relative to CF-air controls even though decreases were not statistically significant. Growth reductions were limited despite declines in seasonal net photosynthesis of 3–13% in ambient air and 5–26% in $1.5 \times O_3$ -air (relative to CF-controls) (Rebbeck 1996b; Rebbeck, Scherzer & Loats 1998), indicating that O_3 was altering carbon fixation. Because apparent O_3 -induced growth reductions were not initiated until the fifth season of exposure, it appears that yellow-poplar may have the ability to compensate for O_3 -induced decreases in photosynthesis and sustain root and shoot growth for a considerable period of time before a cumulative threshold is reached. However, the exact mode of compensation was not apparent. Compensatory responses associated with O_3 injury, such as increased leaf production rates, increased photosynthetic rates of younger foliage, increased leaf size, increased internode and/or branch lengths were not observed (Tjoelker & Luxmoore 1992; Pääkkönen *et al.* 1996). Both lateral (first- and second-order) and taproots of saplings appeared to be reduced by elevated O_3 . It is quite possible that the magnitude of those reductions in root mass would have amplified had exposures continued. With many woody species, root biomass is often reduced more than shoots by O_3 (Laurence *et al.* 1994). Reduced root systems due to O_3 may make trees more susceptible to drought and nutrient stress (Yun & Laurence 1999) and increase susceptibility to pathogens and insects. The susceptibility to O_3 can also be altered by nitrogen supply (Noormets *et al.* 2001). However, both soil and foliar nitrogen levels were well within the normal limits for yellow-poplar (Scherzer, Rebbeck & Boerner 1998);

and no decreases in foliar chlorophyll were observed after the first exposure season (Rebbeck *et al.* 1998).

Our previous work with yellow-poplar supports the findings of the current study. In a two-season open-top chamber study, the first season growth of potted 1-year-old bareroot yellow-poplar seedlings was stimulated in the presence of elevated O_3 ($1.5 \times$ ambient, 107 p.p.m. $\times$ h) despite a 12% reduction in leaf photosynthetic rate (Rebbeck 1996a; Rebbeck & Loats 1997), and after two seasons, few growth effects were observed despite reductions of 21–42% in leaf photosynthetic rates.

We observed no changes in leaf area, shoot or leaf biomass or stem heights attributable to ambient or elevated O_3 . It seems plausible to suggest that both pot-grown seedlings and field-grown saplings of yellow-poplar do not immediately alter allocation patterns in response to chronic exposures to elevated O_3 despite significant reductions in leaf carbon fixation. Changes in root : shoot ratios associated with O_3 exposures were not observed in our current study. However, the ratio of total leaf area to first-order lateral roots increased for saplings exposed to $1.5 \times O_3$ when measured after the second and fifth seasons. These increases suggest that more carbon may have been retained in the leaves for increased repair of O_3 -impacted foliage.

Based on the results of our present study, the response of field-grown saplings exposed to O_3 over 5 years appear to be very similar to seedling responses observed in shorter-term exposures under more controlled conditions (Chappelka *et al.* 1988; Tjoelker & Luxmoore 1992; Cannon *et al.* 1993; Rebbeck 1996a; Rebbeck & Loats 1997; Loats & Rebbeck 1999). Isebrands *et al.* (2001) also reported similarities in the sensitivity of *Populus tremuloides* to O_3 between those exposed for one season in open-top chambers and those exposed longer-term in a free air carbon exchange (FACE) system. Demonstrating these similarities in O_3 responses between seedlings and saplings should greatly enhance the predictive modelling effort to scale the impacts of O_3 from juvenile to mature trees (Kolb & Matyssek 2001).

O_3 + CO_2 effects

Although direct inferences as to the effects of enriched CO_2 -air alone on the growth of yellow-poplar cannot be made from this study, we first observed significant increases ($\sim 33\%$) in basal area growth increment in saplings exposed to $1.5 \times O_3 + CO_2$ after two seasons of exposure. After five seasons, the basal area growth increment increased by $\sim 41\%$, whereas the stem and root biomass increased by $\sim 60\%$ in these saplings relative to all others. During the first three seasons of this study, seasonal net photosynthesis of saplings grown in $1.5 \times O_3 + CO_2$ -air was 41–80% higher than trees exposed to $1.5 \times O_3$ alone (Rebbeck *et al.* 1998). These results suggest that elevated CO_2 ameliorated the O_3 -induced reductions in carbon fixation and growth.

Stem mass and basal area growth increment was enhanced in $1.5 \times O_3 + CO_2$ -air without any detectable changes in total leaf area, suggesting that alterations in

allocation patterns had occurred. We found that the ratio of total leaf area to first-order lateral roots decreased for those exposed to elevated O₃ + elevated CO₂ relative to CF controls, which was opposite to the response observed in saplings exposed to elevated O₃-alone. This decrease in the ratio of leaf area to first-order lateral roots suggests that the addition of elevated CO₂ to elevated O₃ shifted carbon below-ground. A similar allocation shift to fine roots in saplings exposed to enriched CO₂ alone has been reported for yellow-poplar (Norby *et al.* 1992) and *Betula pendula* (Rey & Jarvis 1997). We saw no changes in root : shoot ratios in 1.5 × O₃ + CO₂-exposed saplings. Dickson *et al.* (2001) also reported limited effects of elevated O₃ alone or when combined with elevated CO₂ on root : shoot ratios. Yellow-poplar has a rapidly growing and extensive root system, and during its juvenile stage has a flexible rooting habit that is not exhibited in most other species (Renshaw & Doolittle 1958; Beck 1990). Further study is needed to elucidate the long-term root and allocation responses to the combined effects of elevated CO₂ and O₃ on field-grown tree species.

It has been suggested that bud break, leaf phenology, frost hardiness and other developmental processes of woody species may be altered by exposure to gaseous pollutants, but few reports are available (Mousseau & Saugier 1992; Lee & Jarvis 1995). El Kohen & Mousseau (1994) reported later bud burst and earlier bud set in sweet chestnut exposed to elevated CO₂, resulting in a shorter growing season, and Rebbeck (1996a) reported that exposure to twice ambient O₃ delayed bud burst of yellow-poplar and black cherry seedlings. Isebrands *et al.* (2001) attributed an increased shoot dieback of *Populus tremuloides* exposed in elevated CO₂ to a continuation of growth into the autumn frost period, but saw no combined effect of elevated CO₂ and O₃. We did observe a significant increase in shoot dieback in 1.5 × O₃ + CO₂-air, but only in the 1992–93 dormant season. It is possible that the buds and stem tissue had not adequately hardened. That effect however, was short-lived, and no subsequent treatment effects were detected. We saw no significant change in bud burst associated with elevated O₃ alone or combined with elevated CO₂, in fact, all trees broke bud between 17 and 23 April each spring.

The stimulation of basal area growth and root mass that we observed on yellow-poplar support the hypothesis that enriched CO₂ can ameliorate the negative effects of elevated O₃. Dickson *et al.* (2001) reported that the addition of elevated CO₂ (+ 150 p.p.m. above ambient) to elevated O₃ (~ 97 p.p.m. × h cumulative dose) counteracted the negative O₃ response of an O₃-tolerant *Populus tremuloides* clone but had no effect on an O₃-sensitive clone. In a FACE exposure study of these same two aspen clones, Noormets *et al.* (2001) reported similar clonal responses to elevated CO₂ alone or when combined with elevated O₃. Isebrands *et al.* (2001) reported that after three seasons of exposure, *Populus tremuloides* height, diameter and volume increased in elevated CO₂, decreased in elevated O₃, and in elevated CO₂ + O₃ did not differ from ambient controls. Significant clonal differences in response were noted. The growth

responses of yellow-poplar in our current study were most like that of the O₃-tolerant aspen clone (Dickson *et al.* 2001; Isebrands *et al.* 2001; Noormets *et al.* 2001). Although others have also observed stimulations in growth and biomass of seedlings and young saplings grown in the mixture of elevated O₃ and CO₂, this has not been consistently observed in all woody species (Barnes *et al.* 1995; Kull *et al.* 1996; Lippert *et al.* 1996; Palomäki *et al.* 1996; Kellomäki & Wang 1997; Dickson *et al.* 1998, 2001). Because there is considerable variation in the magnitude of response of deciduous species to simultaneous exposures to elevated O₃ and CO₂ (Eamus & Jarvis 1989; Mousseau & Saugier 1992; Ceulemans, Jiang & Shao 1995a, b; Kull *et al.* 1996; Dickson *et al.* 1998, 2001; Karnosky *et al.* 1999; Isebrands *et al.* 2001), caution in the interpretation and comparison of studies must be used. Pollutant exposure levels (e.g. how do responses at 500 p.p.m. CO₂ compare with responses at 700 p.p.m. CO₂), genetic homogeneity and cultural conditions can vary considerably from study to study. The ability of enriched CO₂ to counteract O₃-induced stress is determined by the CO₂ concentration, the plant response to the CO₂ enrichment, and the magnitude of the O₃ stress (Rudorff *et al.* 2000). The year-to-year variations in responses observed in our current study reinforces the importance of environmental factors such as temperature, rainfall and light on modifying tree responses to pollutants and further demonstrates the need to study the integrated response of long-lived woody species to gaseous pollutants.

Summary

Our current study represents one of the first to examine the response of field-grown hardwoods to chronic O₃ alone or combined with elevated CO₂ (~ 700 p.p.m) with minimal irrigation and no fertilization over multiple years. This work addresses the critical need to better understand if seedlings and older trees express the same susceptibility or tolerance to gaseous pollutants. We have demonstrated similarities in the pollutant response between field-grown yellow-poplar saplings and pot-grown seedlings (exposed either in open-top chambers or controlled environmental conditions), and that it can take several years before detectable biomass impacts are expressed, even for a fast-growing species such as yellow-poplar. The chronic O₃ and CO₂ response of individual species needs to be well characterized under field conditions before meaningful predictions of the impacts of future climates on forested ecosystems can be made.

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