

## PHYSIOLOGICAL AND DEVELOPMENTAL EFFECTS OF O<sub>3</sub> ON COTTONWOOD GROWTH IN URBAN AND RURAL SITES

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**Abstract.** Previously we found that cloned cottonwood saplings (*Populus deltoides*) grew twice as large in New York, New York, USA, compared to surrounding rural environments and that soils, temperature, CO<sub>2</sub>, nutrient deposition, and microclimatic variables could not account for the greater urban plant biomass. Correlations between final season biomass and cumulative O<sub>3</sub> exposures, combined with twofold growth reductions in an open-top chamber experiment provided strong evidence that higher cumulative O<sub>3</sub> exposures in rural sites reduced growth in the country. Here, we assess the field gas exchange, growth and development, and allocation responses underlying the observed growth differences and compare them with isolated O<sub>3</sub> responses documented in the open-top chamber experiment. Cottonwoods showed no visible foliar injury, reduced photosynthesis of recently expanded foliage, early leaf senescence, protective reduction in stomatal conductance, or compensatory allocation to shoot relative to root biomass for either the chamber or field experiment. Instead, O<sub>3</sub>-impacted chamber plants had significantly higher conductance and reduced photosynthesis of older foliage that led to reduced leaf area production and a twofold biomass reduction in the absence of visible injury. Rural-grown field plants showed the same pattern of significantly higher conductance in the absence of concomitant increases in photosynthesis that was indicative of a loss of stomatal control. Incremental changes in foliar production were also significantly inversely related to fluctuations in ambient O<sub>3</sub> exposures. The similarity in biomass, gas exchange, phenological, and allocation responses between chamber and field experiments indicate that mechanisms accounting for reduced growth at rural sites were consistent with those in the open-top chamber O<sub>3</sub> experiment. This study shows the limitation of visible symptoms as a sole diagnostic factor for documenting detrimental O<sub>3</sub> impacts and points toward a new approach to show O<sub>3</sub> impacts when visible injury is not present. Namely, O<sub>3</sub>-impacted vegetation showed an unusual inverse relationship of increased conductance with lower photosynthesis of older foliage that was indicative of a loss of stomatal control. This increased stomatal conductance of O<sub>3</sub>-impacted vegetation accentuates pollutant flux into affected foliage and has important implications for system water balance during warm, dry portions of the growing season when O<sub>3</sub> concentrations are highest.

**Key words:** foliar production; open-top chamber; ozone; photosynthesis; *Populus deltoides*; premature leaf senescence; root:shoot ratios; rural; stomatal conductance; stomatal control; urban.

### INTRODUCTION

We previously showed that cloned cottonwood saplings (*Populus deltoides* Marsh., eastern cottonwood, clone ST109) attained twice the biomass in New York City (NYC), New York, USA, compared to surrounding rural environments (Gregg et al. 2003). Extensive experimentation was performed to determine potential beneficial effects of the urban environment. However, soil transplants, nutrient budgets, chamber experiments, and multiple regression analyses showed that soil attributes, microenvironment, and elevated urban tem-

perature, CO<sub>2</sub>, and nutrient deposition (Bornstein 1968, Peterson 1969, Lovett et al. 2000, Idso et al. 2001) could not account for the greater urban plant biomass. Rather, the secondary nature of the reactions of ozone (O<sub>3</sub>) formation and NO<sub>x</sub> titration reactions within the city center (Isaksen et al. 1978, Cleveland and Graedel 1979) resulted in significantly higher cumulative O<sub>3</sub> exposures at rural sites. Final season biomass was significantly inversely related to ambient O<sub>3</sub> exposures, and an open-top chamber experiment showed a twofold biomass reduction in response to elevated-O<sub>3</sub> treatments (Gregg et al. 2003). There was no visible foliar injury to confirm detrimental rural O<sub>3</sub> impacts, but visible symptoms are neither universal nor entirely diagnostic (Reich and Amundson 1985, Reich 1987, Saxe 1991, Karnosky et al. 1992) and were not present with the twofold growth reductions in the open-top chamber O<sub>3</sub> experiment. In this paper, we assess the gas exchange, growth and

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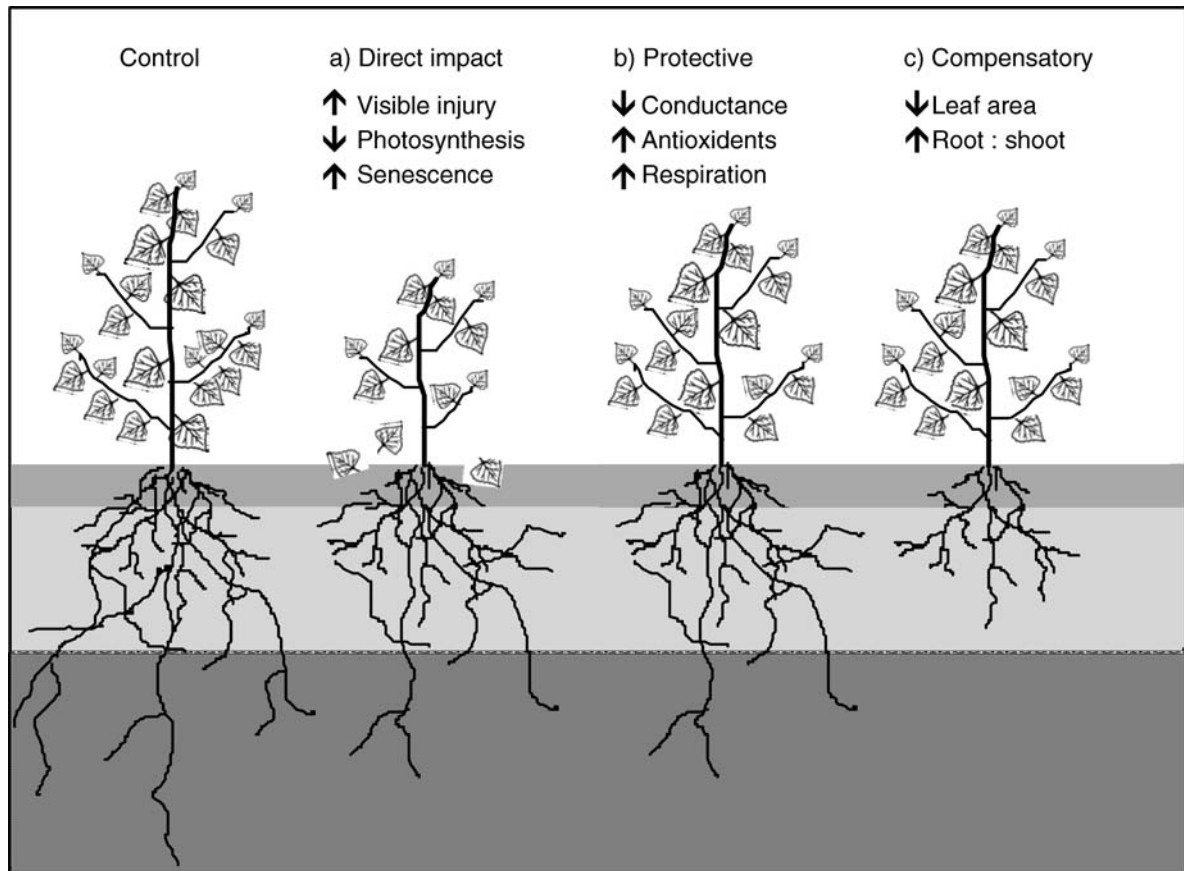


FIG. 1. Physiological and biomass responses to elevated O<sub>3</sub> exposures. Mechanisms of O<sub>3</sub>-induced growth reduction range from direct impacts of visible foliar injury, reduced photosynthesis, and premature leaf senescence to protective and compensatory responses that minimize direct impacts but reduce net C assimilation.

development, and allocation responses underlying the rural growth reductions and compare these with well-documented O<sub>3</sub> impacts and isolated O<sub>3</sub> responses shown in the open-top chamber experiment. The direct chamber vs. field comparisons provide insight to mechanisms responsible for observed growth differences between urban and rural field sites and suggest additional field measures to document O<sub>3</sub> impacts when visible injury is not present.

Impacts of elevated O<sub>3</sub> exposures can occur as direct impacts on carbon (C) assimilation or by protective and compensatory responses that offset O<sub>3</sub> impacts but reduce the total C available for growth (Fig. 1). Visible injury is the primary diagnostic indicative of detrimental O<sub>3</sub> impacts under field conditions and is typically detected as mild chlorosis or adaxial leaf surface chlorotic or necrotic stippling (Skelly et al. 1987, 1999, U.S. Environmental Protection Agency 1996, Flagler 1998, Innes et al. 2001, USDA Forest Service 2002, Bussotti et al. 2003) (Fig. 1). However, vegetation that does not exhibit visible injury may still experience reduced photosynthesis (Reich and Amundson 1985, Wang et al. 1986a, Pell 1987, Coleman et al. 1995b) or

premature leaf senescence (Tingey and Taylor 1982, Heck et al. 1988, Ballach et al. 1992), which act individually or in combination to reduce the total C assimilated. In the absence of direct impacts on C assimilation vegetation may also exhibit protective and repair responses that reduce the total available C. Protective reduction in stomatal conductance minimizes pollutant flux into affected foliage but limits the C available for assimilation (Temple 1986, Reich 1987, Saxe 1991, Mansfield and Pearson 1996). Antioxidant production detoxifies free radicals before damage occurs but requires allocation of fixed C to the synthesis of detoxifying enzymes (Gupta et al. 1991, Lee 1991). Substantial growth reductions can also result from increased respiratory costs associated with the repair of non-visible damage (Black 1984, Amthor 1988). Protective and repair mechanisms can also lead to compensatory reallocation of resources to various plant tissues which further conceal the O<sub>3</sub> impact. Plants with access to fewer resources can produce less leaf area but produce leaves of similar life span and photosynthetic capacity (Pye 1988). And those that are more C- than nitrogen- or water-limited often allocate more resources

aboveground to enhance C assimilation, leading to inhibited root growth and reduced carbohydrate storage due to compensatory repair and growth of new photosynthetic tissues (Coleman et al. 1995a, Rennenberg et al. 1996, Andersen 2003). Vegetation exhibiting O<sub>3</sub>-induced growth reductions in the absence of visible foliar injury could therefore exhibit direct impacts of reduced photosynthesis or premature leaf senescence, protective reduction in stomatal conductance, antioxidant production or increased respiratory costs, compensatory reallocation of resources to reduced foliar production or lower root:shoot ratios, or some combination of the above.

Responses used to detect mechanisms of O<sub>3</sub>-related growth reductions in this study included a range of gas exchange, phenology, and allocation measures. Gas exchange measures tested for differences in photosynthesis or stomatal conductance, phenology comparisons determined whether differences in total leaf area were due to reduced foliar production or early leaf senescence, and allocation responses determined whether there was compensatory reallocation of resources to shoot relative to root tissues. Comparisons across this range of variables determined whether growth reductions that could not be explained by direct impacts were due to protective and compensatory responses. Mechanisms of reduced growth in field exposures were compared to those in the open-top chamber O<sub>3</sub> experiment, and multiple regression analyses were performed to determine whether incremental changes in foliar production were related to the ambient O<sub>3</sub> exposures.

## MATERIALS AND METHODS

### Field experiments

Urban and rural sites were located along known steep pollution gradients to the north of New York City in the Hudson Valley (HV) and to the east of the city on Long Island (LI, Fig. 2). Sites were located in (1) Millbrook (at the Institute of Ecosystem Studies; 41°47.67' N, 73°45.64' W), (2) Carmel (at the New York State Department of Environmental Conservation air monitoring station; 41°27.11' N, 73°42.46' W), (3) the Bronx (at the New York Botanical Garden; 40°51.67' N, 73°52.60' W), (4) Hunts Point (at the Hunts Point sewage treatment plant; 40°48.24' N, 73°53.16' W), (5) Austoria (at the Con Edison Fuel Depot; 40°47.16' N, 73°53.97' W), (6) Hempsted (at Eisenhower Park; 40°43.97' N, 73°34.30' W), (7) Upton (at the Brookhaven National Laboratory; 40°52.20' N, 72°52.66' W), and (8) Riverhead, New York (at Cornell University's Long Island Horticultural Research Laboratory; 40°57.67' N, 72°42.83' W). Studies were conducted in sites 1, 3, and 8 in year 1, sites 1, 3, 4, 5, 6, and 8 in year 2, and sites 6–8 for the final growing season.

Rooted cottonwood cuttings (Dirr and Heuser 1987) were transplanted into the field in the first week of July when they were 10–25 cm in height with 5–12 leaves and

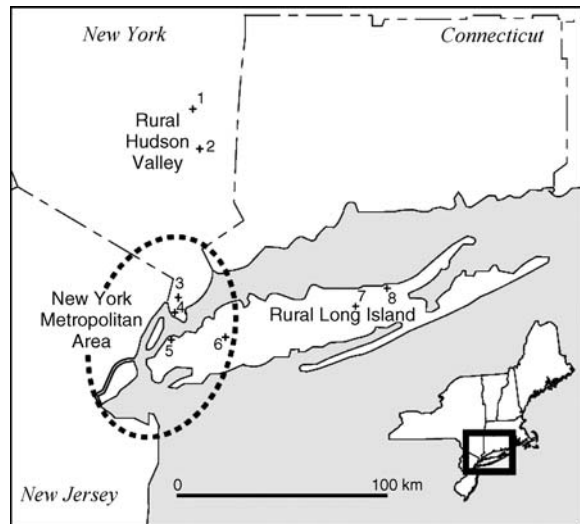


FIG. 2. Urban and rural sites in the vicinity of New York City: (1) Millbrook, (2) Carmel, (3) the Bronx, (4) Hunts Point, (5) Austoria, (6) Hempsted, (7) Upton, and (8) Riverhead, New York, USA.

attained a maximum height of 171 cm by final harvest in the second week of September. Within each site, cottonwoods were grown in soils transplanted from urban and rural primary forest stands previously shown to vary in pollutant concentrations (Pouyat et al. 1991, 1995, Gregg 1999). One urban and one rural forest soil were transplanted to all sites in year 1 ( $N = 5$  plants/soil origin); two urban and rural forest soils were transplanted to all sites within each transect in year 2 (4 forest soils/site, 8 forest soils total,  $N = 10$  plants/soil origin). Maximum growth responses to urban and rural sites were also documented by growing the cottonwoods in fertilized potting soils (perlite:peat:topsoil, 1:2:1, volume/volume; Osmocote 14-14-14 fertilizer, Scotts, Marysville, Ohio, USA, 113.4 g/pot;  $N = 10$  plants/site for all sites and years of the experiment). All soils were placed in 19-L pots buried into the ground to provide realistic soil temperatures, with holes dug 50% beyond the pot depth and back-filled with gravel and sand to provide drainage. Soils for the urban/rural contrast for the HV comparison were collected from Pelham Bay and Van Cortland Parks in the Bronx and Housenonic and Mohawk State Forests in Litchfield County, Connecticut. Soils for the urban/rural contrast on LI were collected from Alley Pond and Cunningham Parks in Queens, the Nature Conservancy's David Weld Preserve in Nissequogue, and Mr. Edward Stevenson's woods in Mt. Sinai, New York.

Local variation in light and precipitation was minimized by growing the plants in open fields with drip irrigation (3.8 L/d in four intervals at 06:00, 10:00, 14:00, and 18:00). Temperature effects on season length were controlled by synchronizing transplant and harvest dates within each growing season. Remaining microenvironmental (air temperature at 1.5 m, precipitation,

TABLE 1. O<sub>3</sub> exposures for urban and rural sites near New York, New York, USA, for the three years of field experiments and for the open-top chamber experiment.

Measurement type	Mean O <sub>3</sub> (ppb)		Maximum O <sub>3</sub> (ppb)		AOT40 O <sub>3</sub> (ppm-h)	
	Urban	Rural (×)	Urban	Rural	Urban	Rural
Field study						
Year 1						
HV	20.2	36.3 (1.8)	106	112	1.6	5.1
LI	23.4	39.7 (1.7)	109	129	3.5	6.4
Year 2						
HV	33.2	47.4 (1.4)	126	150	5.6	9.9
LI	36.5	45.6 (1.3)	129	172	6.6	9.3
Year 3						
HV	29.4	41.3 (1.4)	103	134	3.9	8.1
LI	26	46.2 (1.8)	131	121	6.4	10.2
Open-top chamber						
1× ambient		33		84		5.1
1.8× ambient		59 (1.8)		179		29.6
3× ambient		98 (3)		314		70.4

Notes: Urban/rural comparisons are shown for the Hudson Valley (HV, site 1 vs. site 3) and Long Island (LI, site 5 vs. site 8) contrasts (site locations shown in Fig. 2). Comparisons include the mean and maximum concentrations (ppb) and the area over threshold 40 ppb (AOT40, ppm-h, Sofiev and Tuovinen 2001). All values are for 12-h daytime exposures (08:00–20:00 hours). Values in parentheses show the 1.3- to 3.0-fold greater mean O<sub>3</sub> exposures for rural compared to urban field sites and elevated compared to ambient O<sub>3</sub> chambers.

relative humidity, and photosynthetically active radiation) and air quality (O<sub>3</sub>, NO, NO<sub>2</sub>, SO<sub>2</sub>, particulates, Pb, and wet deposition constituents) attributes were monitored at adjacent environmental monitoring stations. Temperature differences between sites were tested using growing degree-days over a 10°C base temperature (Jones 1996), and O<sub>3</sub> exposures were summarized as the mean, maximum, and AOT40 (cumulative values over 40 parts per billion [ppb]; Sofiev and Tuovinen 2001) for 12 h/d.

#### Open-top chamber experiment

To isolate O<sub>3</sub> effects, cottonwoods were exposed to nonfiltered ambient air (A), 1.8 times ambient (1.8×), and 3.0 times ambient (3.0×) O<sub>3</sub> concentrations in open-top chambers (2.6 × 7.7 × 2.6 m, described in Mandl et al. [1989]; *N* = 5 plants/chamber) at a distant rural site outside Ithaca, New York, at the Boyce Thompson Institute field facility (~270 km northwest of NYC; 74.5° W, 42.5° N). These treatments were a subsidiary component of a large, nested design that randomly allocated the three O<sub>3</sub> levels to four chambers per treatment for a concurrent study on aspen (*P. tremuloides*; Yun and Laurence 1999). The range of exposures spanned lower urban to extreme high mean and AOT40 exposures and show the pattern and mechanisms of reduced growth with increasing O<sub>3</sub> exposures.

Cottonwoods were again grown from rooted cuttings and transplanted into 19-L pots with perlite:peat:topsoil and slow-release fertilizer so conditions matched those of the field experiments as closely as possible. Plants were moved into the chambers on 7 July when they were 30–38 cm with 10–15 leaves, watered twice per week to

field capacity, and attained a maximum height of 81–117 cm by final harvest on 21 September.

Ozone exposures were conducted for 9 h/d (0900 to 1800 hours) using standard production, delivery, and monitoring systems (Heagle et al. 1979). Ozone was generated using pure oxygen passed through electrostatic discharge plates of an O<sub>3</sub> generator (Griffin Model 1A; Technics, Lodi, New Jersey, USA) and was delivered to each chamber via Teflon tubing. The O<sub>3</sub> concentrations of each chamber were monitored with a time-sharing system in which each observation lasted 1.5 min in duration using a scanning valve (Scanivalve model CTLR2P/S2-S6; Scanivalve, San Diego, California, USA) and a UV-photometric O<sub>3</sub> analyzer (Model 49; Thermo Electron, Hopkinton, Massachusetts, USA). An automated IBM-PC based data logging system calculated the hourly mean concentration for each chamber, and chamber O<sub>3</sub> concentrations were controlled manually by adjusting the flow to maintain the O<sub>3</sub> treatment levels at 1.8× and 3.0× the ambient concentrations. Fumigations were terminated when ambient concentrations were <20 ppb due to reduced accuracy of fumigation treatments. Monthly determinations of line efficiencies to O<sub>3</sub> depletion were taken into account in determining chamber O<sub>3</sub> concentrations, and the O<sub>3</sub> monitor was subjected to zero and span checks using a calibrated O<sub>3</sub> source twice per week.

#### Mechanisms underlying growth responses

*Gas exchange.*—Leaf-level gas exchange was measured with a portable “closed” infrared gas analyzer with a 0.25-L cuvette (LI-COR 6200; LI-COR, Lincoln, Nebraska, USA) following methods described in Dawson and Ehleringer (1993). For the field experiments,



measurements were collected in full sun ( $>1000 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ; light saturation =  $700 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  for this species; Gregg 1999) between 10:00 and 15:00 on the most recently fully expanded foliage in years 2 and 3. The five largest plants in each soil type were measured at all sites in mid-September in year 2, and all plants were measured at all sites in the third week of August in year 3. Confounding age vs. pollutant effects were avoided for plants with substantially different growth rates in urban and rural sites by focusing gas exchange comparisons on recently expanded foliage. Measurements for the chamber experiments followed the same procedures as for the field experiment but were collected for both the first and fourth most recently expanded leaves of each plant between 10:00 and 12:00 in the third week of July. Diurnal time-course measures showed no significant difference in gas exchange parameters for repeat measures of the same foliage at the start and end of the chamber or field measurement intervals ( $P > 0.25$ ).

*Growth and development.*—Patterns of cottonwood growth and development were assessed by monitoring total leaf area and the new area produced and senesced throughout the growing season. Area was assessed by measuring the length of each fully expanded leaf at each measurement interval, and the most recently fully expanded leaves were marked to distinguish the new area produced from that senesced for the following interval. Measurements were taken twice per week in year 1, every three weeks in year 2, and each month for the open-top chamber experiment. The relationship between length and area did not vary between sites, soils, and years of the experiments ( $F_{5,48} = 1.0$ ,  $P = 0.41$ ), so a single regression was used for the area conversions for all treatments (area =  $3.772 - 1.611(\text{length}) + 0.745(\text{length}^2)$ ,  $r^2 = 0.976$ ,  $P < 0.001$ ). Foliage was also examined for herbivory (classified as 0–1%, 2–25%, 26–50%, 51–75%, and 76–100% for each leaf), but  $\ll 1\%$  of total leaf area was damaged for the field experiment, and there was no damage in the chamber study (Gregg 1999). However, loss of some apical meristems in the field experiment due to spittle-bug infestation (site 6 in year 1), deer browsing (sites 2 and 7 in year 2), and irrigation theft (site 5 in year 3) prevented inclusion of data from these sites in our final analyses.

*Root:shoot allocation.*—At the end of the season plants were clipped at the soil surface to divide biomass between root and shoot tissues, and roots were washed carefully to retain fine-root biomass. Final season above- and belowground dry masses were then used to calculate percentage of root vs. total plant biomass for both field and chamber experiments.

#### Statistical analyses

*Field.*—ANOVAs (Sokal and Rohlf 1981) were used to test for urban vs. rural site and soil effects on plant attributes (gas exchange, total foliage produced or senesced, and root:shoot ratios) for each year of experiments. Linear contrasts were then performed to

determine whether significant site or soil effects were due to urban vs. rural sites or urban vs. rural soil origin. A nested design was used in year 2 to account for soil transplants within the northern and eastern comparisons. Multiple regression analyses were also performed to determine whether incremental changes in leaf area production were related to cumulative  $\text{O}_3$  exposure (area over threshold 40 ppb, AOT40; U.S. Environmental Protection Agency 1996) independent of effects due to site, soil, growing degree-days (base  $15^\circ\text{C}$ ), or measurement interval. Sites were included in the analyses if  $\text{O}_3$  was monitored and there was no damage to the apical meristems (sites 1, 3, and 8 in year 1, sites 1, 3, 5, and 8 in year 2). Durbin-Watson tests showed that the data were not autocorrelated and collinearity was eliminated by removing factors with variance inflation factors greater than 10 (Sokal and Rohlf 1981). The Mauchly criterion (SAS Institute 1998) showed that the residuals were spherical, so more robust univariate  $F$  tests were used (Huynh and Feldt 1970).

*Chamber.*—Univariate  $F$  tests were used to test for treatment differences in photosynthesis, stomatal conductance, total area produced or senesced, and root:shoot ratios using treatment and chamber within treatment as the main effects ( $N = 5$  plants/chamber). Because the mean square errors for the chamber-within-treatment effect were less than half those of the within-chamber variation, the most conservative test of treatment effects was to test against within-chamber variation but use a conservative compromise of 4 df (J. W. Gregg and E. H. Lee, unpublished manuscript). Dunnett's test (Sokal and Rohlf 1981) was used to test for differences between ambient and  $\text{O}_3$ -treated plants, and linear contrasts tested for differences between the  $3.0\times$  and  $1.8\times$  ambient  $\text{O}_3$  exposures.

Levene's test for homoscedasticity and Shapiro-Wilk's  $W$  test for normality were performed on residuals from all analyses to check for violations in the model assumptions (Sokal and Rohlf 1981). Residual analyses identified 1–3 outliers for measurements in year 2 that were greater than three standard deviations from zero and were excluded from final analyses. All analyses were performed using JMP, a Macintosh version of the SAS statistical package (SAS Institute 1998).

## RESULTS

### Gas exchange

*Field experiment.*—There was no reduction in photosynthesis for recently expanded foliage of cottonwoods grown in rural compared to urban field sites. Photosynthesis was higher for the smaller, thicker leaves of the stunted rural-grown cottonwoods when compared on an area basis (Fig. 3A). However, conversion to a more appropriate comparison per unit leaf mass showed no significant difference in photosynthesis between urban-grown and rural-grown cottonwoods (Fig. 3B) or for the range of forest and fertilized potting soil treatments (area,  $F_{8,100} = 0.8$ ,  $P = 0.57$ ; mass,  $F_{8,47} = 0.5$ ,  $P = 0.83$ ), and there was no site-by-soil interaction (mass,  $F_{16,47} = 1.7$ ,  $P$

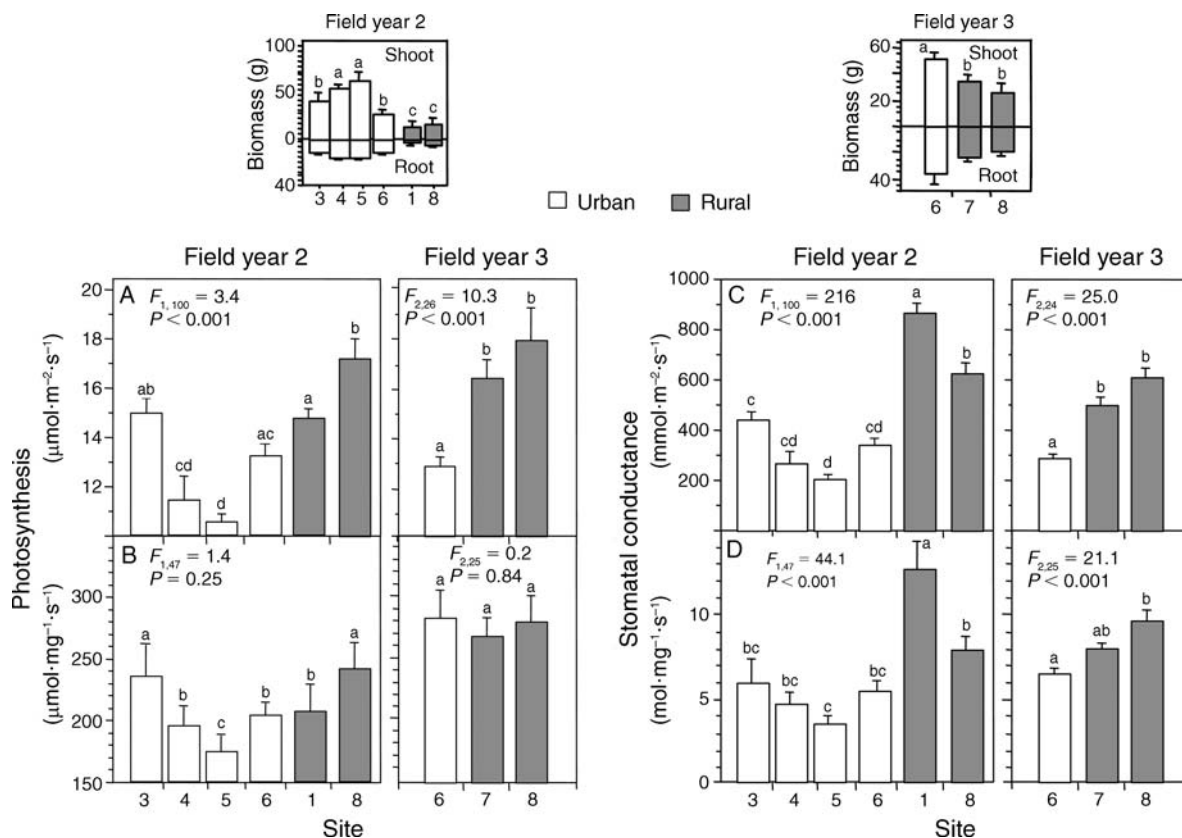


FIG. 3. (A, B) Photosynthesis and (C, D) stomatal conductance (mean  $\pm$  SE) of recently expanded cottonwood (*Populus deltoides*) foliage grown in urban (open) and rural (shaded) sites in the vicinity of New York City for two years of field experiments (site locations shown in Fig. 2). Measurements are presented per unit leaf area (A, C) and per unit leaf mass (B, D).  $F$  and  $P$  statistics indicate results of the linear contrasts from ANOVAs comparing urban and rural field sites. Lowercase letters indicate sites that were significantly different in Tukey-Kramer hsd comparisons of all pairs. Small graphs at the top of the figure show above- and belowground biomass for the same plants (adapted from Gregg et al. [2003]).

= 0.099). Photosynthetic comparisons on a mass basis remained higher for rural LI plants compared to those in several urban sites in year 2, but this pattern did not occur for rural HV plants or for the following growing season and was not reflected in the biomass results.

Stomatal conductance was significantly higher for cottonwoods grown in rural sites (Fig. 3C, D) whether considered on a mass or an area basis. This response persisted across the range of forest and fertilized potting soil treatments (area,  $F_{8,100} = 1.6$ ,  $P = 0.13$ ; mass,  $F_{8,47} = 1.5$ ,  $P = 0.18$ ) with no significant site-by-soil interactions (area,  $F_{16,100} = 0.9$ ,  $P = 0.53$ ). There was no consistent difference in precipitation between urban and rural sites ( $F_{1,10} = 0.85$ ,  $P = 0.36$ ). And irrigation ( $3.8 \text{ L} [6.2 \text{ cm}]\cdot\text{plant}^{-1}\cdot\text{d}^{-1}$ ) far exceeded precipitation (maximum  $2.4 \text{ cm/d}$ ) and the maximum water transpired by the larger urban plants by the end of the growing season ( $2.7 \text{ L}\cdot\text{plant}^{-1}\cdot\text{d}^{-1}$ ;  $[0.44 \text{ mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}] \times [0.025 \mu\text{mol}/\mu\text{mol}] \times [0.32 \text{ m}^2] \times [43\,200 \text{ s/d}^{-1}] \times [18 \text{ g/mol}] \times [0.001 \text{ L/g}]$ ), so variation in soil moisture does not appear to account for conductance differences.

**Chamber experiment.**—Cottonwoods in the  $1.8\times$  ambient  $\text{O}_3$  chambers also showed no difference in

photosynthesis ( $F_{1,4} = 0.003$ ,  $P = 0.95$ ) for recently expanded foliage. By contrast, foliage exposed to  $3.0\times$  ambient  $\text{O}_3$  ( $F_{1,4} = 6.9$ ,  $P = 0.058$ ) and older foliage for both the  $1.8\times$  and  $3.0\times$  ambient treatments showed the expected photosynthetic reductions (Fig. 4A). Stomatal conductance did not vary for recently expanded foliage of cottonwoods exposed to the  $1.8\times$  ( $F_{1,4} = 0.18$ ,  $P = 0.69$ ) or  $3.0\times$  ambient treatments ( $F_{1,4} = 0.06$ ,  $P = 0.81$ ), but older foliage exhibited the same increased conductance for the elevated  $\text{O}_3$  treatments as shown for the rural field sites (Fig. 4B). There were no differences in specific leaf mass between  $\text{O}_3$  exposure levels, so all gas exchange comparisons were made on an area basis.

**Conductance/photosynthesis.**—Cottonwoods in the ambient  $\text{O}_3$  chambers and the urban field sites exhibited the typical positive relationship between stomatal conductance and photosynthesis as that expected for  $\text{C}_3$  vegetation (Fig. 5; Nobel 1991). However, this relationship was reversed for plants in the  $3.0\times$  ambient  $\text{O}_3$  exposures (Fig. 5A), and plants in the  $1.8\times$  ambient  $\text{O}_3$  treatment showed a split response, with younger foliage following the positive relationship of the ambient treatment ( $F_{1,12} < 0.001$ ,  $P = 0.99$ ) and older foliage

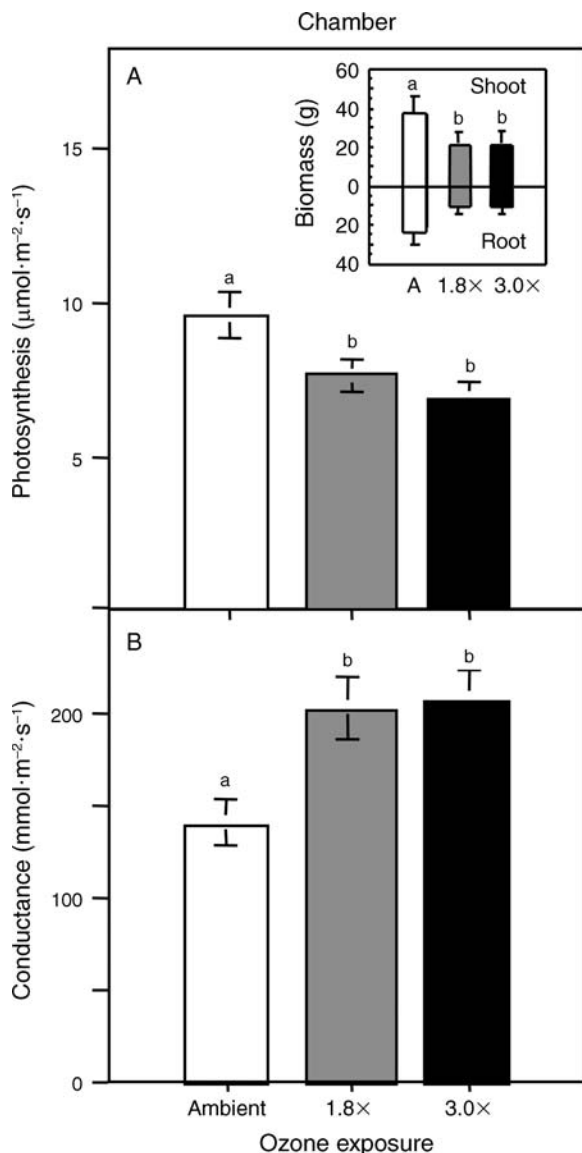


FIG. 4. (A) Photosynthesis and (B) stomatal conductance (mean  $\pm$  SE) of the fourth most recently expanded leaves of cottonwood clones (*Populus deltoides*) grown in open-top chambers exposed to ambient, 1.8 $\times$  ambient, and 3.0 $\times$  ambient  $\text{O}_3$  concentrations (33, 59, and 98 ppb 12-h mean concentrations, respectively). Lowercase letters indicate treatments that were significantly different from controls (Dunnett's test). Fill shades correspond to the three  $\text{O}_3$  treatments. The inset shows above- and belowground biomass for the same plants.

exhibiting the inverse relationship of the  $\text{O}_3$ -impacted foliage ( $F_{1,10} = 0.8$ ,  $P = 0.39$ ).

Gas exchange measures for field experiments were collected only for recently expanded foliage and therefore did not show the inverse  $g/A$  relationship seen for the older foliage. However, conductance values at rural sites exceeded the maximum recorded for this species (Bassman and Zwier 1991) and were substantially higher for a given photosynthetic rate when

compared to urban sites or that expected for  $\text{C}_3$  vegetation (Fig. 5B). A positive conductance/photosynthesis relationship remained for the northern HV field site, but there was no relationship between these variables for the eastern rural site on LI.

#### Growth and development

Similar to biomass results (Figs. 3 and 4, insets) total leaf area and the rate of foliar production were

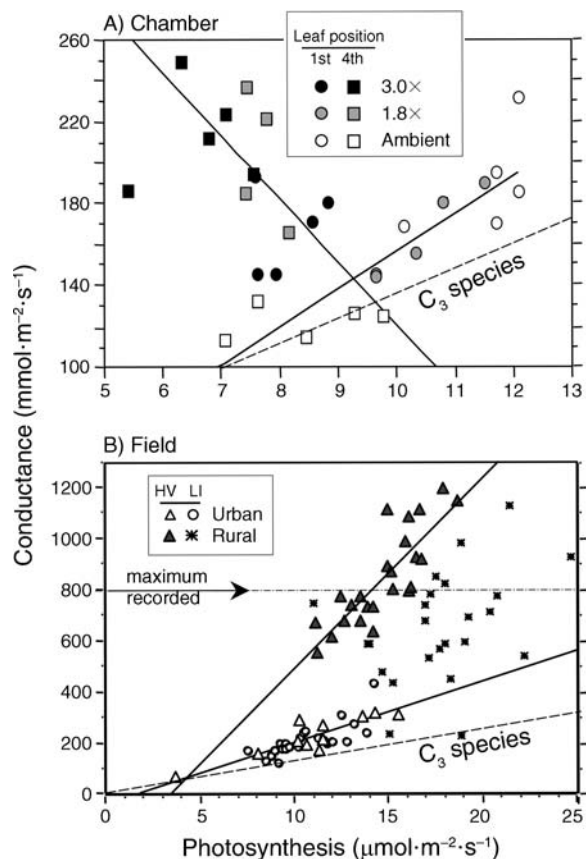


FIG. 5. (A) Stomatal conductance ( $g$ ) vs. photosynthesis ( $A$ ) for cottonwood clones (*Populus deltoides*) grown in open-top chambers exposed to ambient, 1.8 $\times$ , and 3.0 $\times$  ambient treatments. Circles and squares represent the first and fourth most recently expanded foliage, respectively. The positive linear relationship for the ambient  $\text{O}_3$  treatment ( $y = 0.01862A - 0.03$ ,  $R^2 = 0.75$ ) is contrasted with the negative relationship for foliage in the 3.0 $\times$  elevated  $\text{O}_3$  chambers ( $y = -0.03A + 0.42$ ,  $R^2 = 0.52$ ). The dashed line shows the  $g/A$  relationship for  $\text{C}_3$  species under optimal conditions (Nobel 1991). (B) Conductance vs. photosynthesis for recently expanded foliage of cottonwoods grown in the urban and rural field sites from the Hudson Valley (HV) and Long Island (LI) contrasts (solid triangles, open triangles, open circles, and asterisks represent sites 1, 3, 5, and 8, respectively). Linear relationships for the urban sites ( $y = 0.075A - 0.26$ ,  $R^2 = 0.69$ ) and rural site 1 ( $y = 0.024A - 0.04$ ,  $R^2 = 0.66$ ) are shown. The dashed line again shows the  $g/A$  relationship for  $\text{C}_3$  species under optimal conditions (Nobel 1991). The dot-dashed line shows the maximum conductance recorded for well-watered, fertilized plants of this species (Bassman and Zwier 1991).

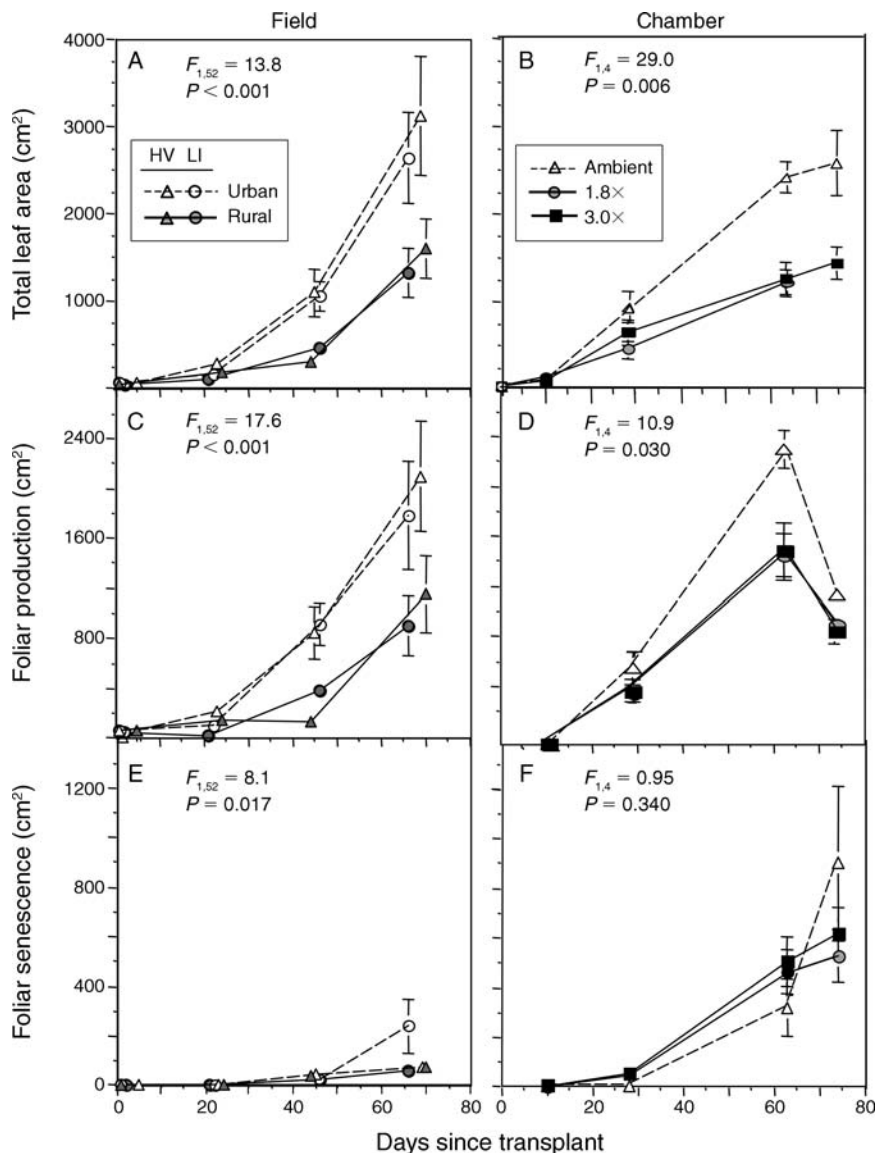


FIG. 6. (A, B) Total leaf area, (C, D) foliar production, and (E, F) foliar senescence (mean  $\pm$  SE) vs. time through the season for cottonwoods (*Populus deltoides*) grown in urban and rural field sites and in chambers with ambient vs. elevated O<sub>3</sub>. For field experiments, solid triangles, open triangles, open circles, and solid circles represent sites 1, 3, 5, and 8, respectively (locations provided in Fig. 1; HV, Hudson Valley; LI, Long Island).  $F$  and  $P$  statistics indicate results of linear contrasts from ANOVAs comparing urban and rural sites and Dunnett's tests comparing ambient and 1.8 $\times$  ambient O<sub>3</sub> exposures (chamber trials).

significantly lower for plants at rural sites and in the elevated-O<sub>3</sub> exposures (Fig. 6A–D). Area was smallest for plants grown in soils transplanted from one urban forest to all sites in year 1 ( $F_{1,51} = 3.8$ ,  $P = 0.057$ ), but there was no consistent urban/rural soil effect ( $F_{1,53} = 0.02$ ,  $P = 0.86$ ) for the more robust comparison across four urban and four rural forest soils in year 2. Significant site-by-soil interactions (year 1,  $F_{4,51} = 3.7$ ,  $P = 0.010$ ; year 2,  $F_{16,53} = 6.5$ ,  $P < 0.001$ ) showed that the greatest difference in leaf area between urban and rural sites was for faster growing plants in fertilized potting soils.

Substantial reductions in leaf area were not related to early leaf senescence for either the field or chamber experiment (Fig. 6). Instead, senescence was significantly higher at urban site 3 in year 1 ( $F_{1,51} = 18.2$ ,  $P < 0.001$ ) and site 5 in year 2 (Fig. 6). However, the peak in urban senescence was concurrent with branch proliferation and elongation, so appeared to be due to canopy thinning in response to self-shading. Increased foliar turnover for larger plants would account for greater senescence in year 1 when plants grew the largest (Gregg et al. 2003) and for the greater senescence for cottonwoods grown in fertilized potting soil treatments ( $F_{5,10} = 6.9$ ,  $P = 0.005$ ).



TABLE 2. ANOVA tables for analyses of total leaf area ( $\text{cm}^2$ ) vs. site, soil, cumulative  $\text{O}_3$  exposure (area over threshold 40 ppb [AOT40], ppm-hours; see Table 1), and time through the season for cottonwood clones (*Populus deltoides*) grown in urban and rural sites in the vicinity of New York, New York, USA.

ANOVA	df	MS	F	P
Year 1				
Site	2	958 034	4.2	0.015
Urban/rural contrast	(1)	905 338	4.0	0.046
Soil	2	701 634	3.1	0.046
Urban/rural contrast	(1)	5240 189	23.2	<0.001
Site $\times$ Soil	4	2767 273	12.2	<0.001
Ozone	1	13 658 397	60.4	<0.001
Ozone $\times$ Soil	2	4 337 382	19.2	<0.001
Time	6	20 652 009	91.4	<0.001
Error	401	226 061		
Year 2				
Transect	1	7797	<0.0	0.829
Site (Transect)	2	10 191	<0.0	0.941
Soil (Transect)	8	1 150 383	6.9	<0.001
Urban/rural contrast	(1)	5231	<0.0	0.860
Site $\times$ Soil (Transect)	8	583 530	3.5	<0.001
Ozone	1	8 959 639	53.4	<0.001
Time	3	17 247 886	102.9	<0.001
Error	383	601 063		

with no differences for smaller trees grown in the forest soils ( $F_{4,40} = 1.2$ ,  $P = 0.318$ ). Chamber results showed no significant differences in senescence for independent comparisons at each measurement interval ( $P > 0.25$ ) or for cumulative senescence by final harvest ( $P = 0.34$ ).

Changes in total leaf area between measurement intervals were inversely related to fluctuations in cumulative  $\text{O}_3$  exposure independent of effects due to site, soil, or time through the season (Table 2, Fig. 7). Inclusion of  $\text{O}_3$  in the regression model reduced the significance of the original between-site variation in year 1 and accounted for all of the variation in year 2. Soil results again showed that significant differences for individual urban and rural soils in year 1 did not hold for more robust comparisons the following year. The significant site-by-soil interactions also show that the greatest growth differences between sites was for faster growing plants in the fertilized potting soil treatments. The significant  $\text{O}_3$ -by-soil interaction shows that differences between soil treatments were lowest for sites with higher  $\text{O}_3$  exposures. This result was consistent for within-transect comparisons in year 2 (HV,  $F_{1,179} = 6.0$ ,  $P < 0.001$ ; LI,  $F_{1,190} = 31.2$ ,  $P < 0.001$ ) when separate soil transplants among northern and eastern transects prevented inclusion of the  $\text{O}_3$ -by-soil interaction in the full regression model.

Colinearity with time forced removal of the nonsignificant temperature effect from the multiple regression analyses (year 1,  $F_{1,400} = 1.5$ ,  $P = 0.222$ ; year 2,  $F_{1,382} < 0.0$ ,  $P = 0.963$ ). These results were consistent with those of Gregg et al. (2003) that showed no relationship between final season biomass and temperature whether summarized as the mean, maximum, minimum, or growing degree-days (Fig. 8). By contrast, the inverse relationship between foliar production and ambient  $\text{O}_3$

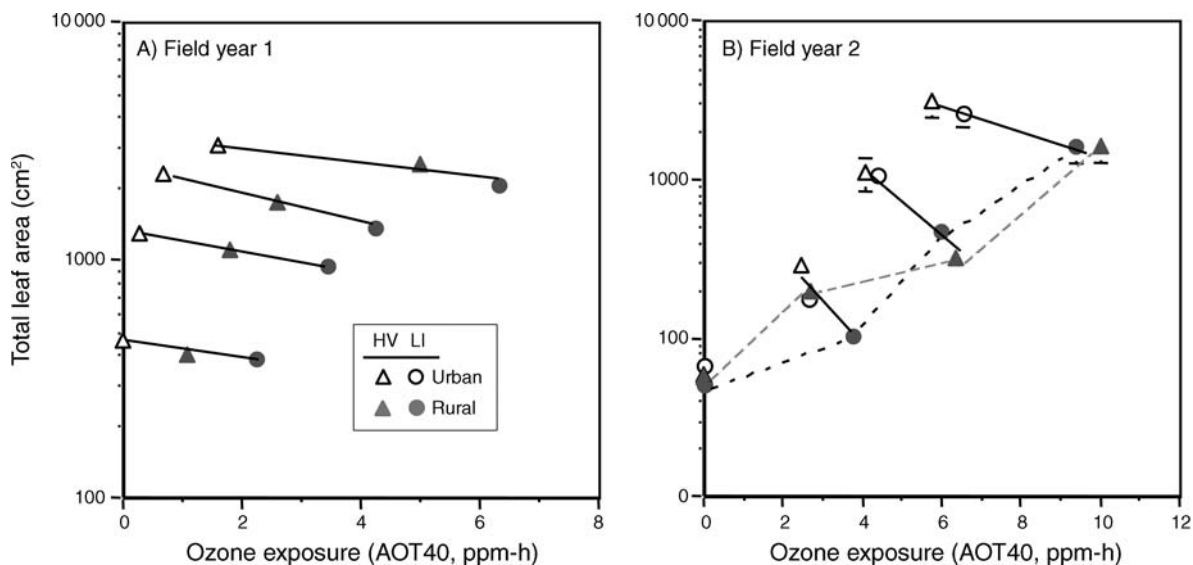


FIG. 7. Total leaf area (mean  $\pm$  SE; errors not shown were smaller than the symbol) vs. cumulative  $\text{O}_3$  exposure (area over threshold 40 ppb [AOT40], ppm-hours; see Table 1) for cottonwood clones (*Populus deltoides*) grown in urban (open symbols) and rural (closed symbols) sites from the Hudson Valley (HV; triangles) and Long Island (LI; circles) contrasts in the first two years of field experiments (solid triangles, open triangles, open circles, and solid circles represent sites 1, 3, 5, and 8, respectively; site locations are provided in Fig. 1). Solid lines show the inverse relationship between leaf area and  $\text{O}_3$  at each measurement interval. The dashed lines in panel (B) show changes in total leaf area over time at rural sites in the Hudson Valley (site 1, gray dashes) and on Long Island (site 8, black dashes). Note that the site with the lowest leaf area switched in accordance with alterations in cumulative  $\text{O}_3$  exposures. These same patterns held whether  $\text{O}_3$  was summarized as the mean concentrations or AOT40.

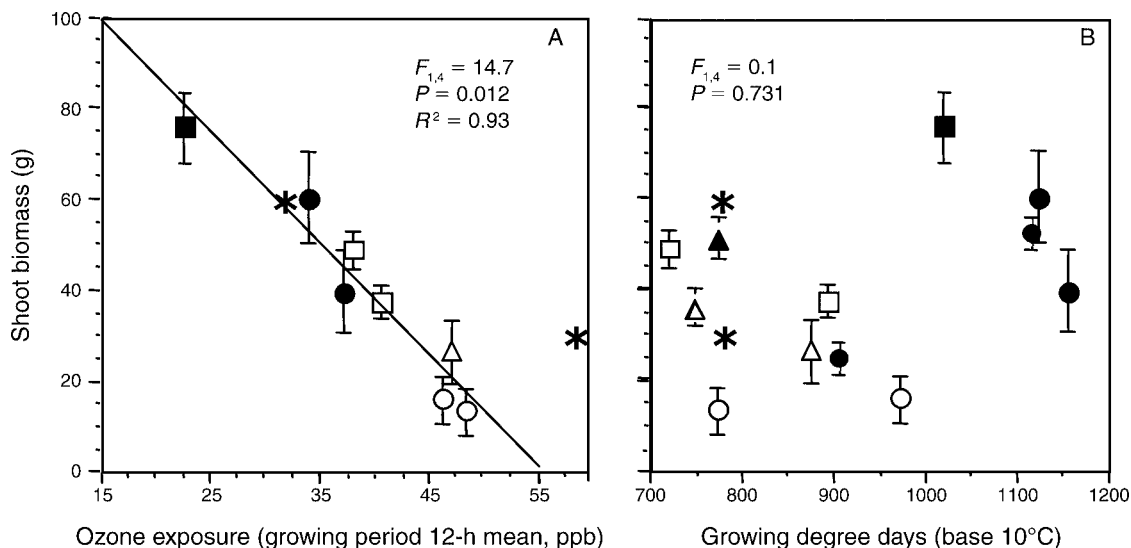


FIG. 8. Final season biomass (mean  $\pm$  SE) vs. (A) O<sub>3</sub> exposure (growing period 12-h mean) and growing degree-days (base 10°C) for cottonwoods grown in potting soils for the three years (squares, circles, and triangles indicate years 1, 2, and 3, respectively; solid and open symbols represent urban and rural sites, respectively; and asterisks indicate biomass from the open-top chamber experiment).  $F$  and  $P$  statistics indicate results from a forward stepwise multiple regression analysis (probability to remain in the model  $<$  0.25; adapted from Gregg et al. [2003]). Results were consistent for mean or area over threshold 40 ppb (AOT40) comparisons, with means presented to enable comparison with chamber exposures. The temperature graph includes results from field sites where O<sub>3</sub> data were not available.

exposures held despite the switch in sites with the highest O<sub>3</sub> exposures between measurement intervals (Fig. 7). The rural-most LI site had the smallest leaf area in year 1 and experienced the highest O<sub>3</sub> exposures but not the coolest temperatures. This same site had the least foliage and highest O<sub>3</sub> by the second measurement interval of year 2, but the rural HV site accumulated the highest O<sub>3</sub> exposure by the next measurement interval by which time it also exhibited the smallest leaf area. This switch in the sites with the least leaf area in accordance with alterations in cumulative O<sub>3</sub> exposures shows no relationship with the consistently warmer temperatures of eastern LI compared to northern, rural HV sites ( $t_{69} = 34.1$ ,  $P < 0.001$ ).

#### Root : shoot allocation

There was no consistent difference in percentage of root biomass for urban vs. rural sites or for the ambient vs. elevated O<sub>3</sub> exposures (Figs. 3 and 4). The proportion of root biomass was lower for the potting soil treatments in the urban sites in year 1 when the cottonwoods were largest and pot capacity could have inhibited root growth (year 1,  $F_{1,50} = 16.2$ ,  $P < 0.001$ ; Barrett and Gifford 1995). However, there was no significant difference in biomass allocation for the following two growing seasons (year 2,  $F_{1,190} = 1.5$ ,  $P = 0.22$ ; year 3,  $F_{1,27} = 0.9$ ,  $P = 0.34$ ), the forest soil treatments (year 1,  $F_{1,24} = 2.5$ ,  $P = 0.104$ ; year 2,  $F_{1,190} = 1.9$ ,  $P = 0.171$ ), the site-by-soil interaction (year 1,  $F_{4,51} = 0.9$ ,  $P = 0.47$ ; year 2,  $F_{20,268} = 1.1$ ,  $P = 0.39$ ), or the open-top chamber experiment (chambers,  $F_{1,4} = 2.15$ ,  $P = 0.216$ ).

#### DISCUSSION

Cottonwoods that grew half as large at rural compared to urban field sites also attained half the leaf area, but showed none of the symptoms that often accompany detrimental O<sub>3</sub> impacts. There was no visible foliar injury, reduced photosynthesis for recently expanded foliage, early leaf senescence, protective reduction in stomatal conductance, or compensatory allocation to shoot vs. root tissues. However, plants that grew half as large in the elevated O<sub>3</sub> chambers also failed to show any of these responses. Instead, O<sub>3</sub>-impacted chamber plants exhibited increased conductance and reduced photosynthesis of older foliage that led to reduced leaf area production and an overall reduction in biomass accumulation. Rural-grown field plants showed the same pattern of significantly higher conductance in the absence of concomitant increases in photosynthesis that was indicative of a loss of stomatal control. Incremental changes in foliar production were also significantly inversely related to fluctuations in ambient O<sub>3</sub> exposures.

It is perhaps not surprising to find such substantial O<sub>3</sub> impacts in the absence of protective reduction in stomatal conductance or compensatory allocation to shoot relative to root tissues. Nevertheless, we were surprised to find twofold growth reductions in the absence of visible foliar injury or premature leaf senescence, especially since these are well-documented responses for a number of *Populus* species (Gupta et al. 1991, Ballach et al. 1992). However, our results are in agreement with Lee et al. (2001), who showed that some

cottonwood clones exhibited no visible injury until prolonged exposure to 150 ppb 8 h/d for 21 d, exposures far greater than the seasonal mean 98 ppb from the 3.0× ambient exposures in our chamber experiment.

The major factor in common for gas exchange comparisons between chamber and field experiments was the increased stomatal conductance in the absence of concomitantly higher photosynthesis for both rural field sites and the elevated O<sub>3</sub> chambers. While lower urban humidity (−10%), higher CO<sub>2</sub> concentrations (+50 ppm), and high concentration of multiple urban pollutants could also have reduced conductance in urban sites, thereby helping to offset detrimental pollutant effects (e.g., Peterson 1969, Volin et al. 1998, Idso et al. 2001), rural conductance exceeded the maximum values measured for this species (800 mmol·m<sup>−2</sup>·s<sup>−1</sup>; Bassman and Zweir 1991, Nobel 1991) with no concomitant increase in photosynthesis. Thus, the negative feedback of reduced stomatal conductance with higher internal CO<sub>2</sub> concentrations (Paoletti and Grulke 2005) does not appear to have been operating, resulting in an increased conductance/photosynthesis relationship that fell outside the range of that expected for C<sub>3</sub> vegetation (Nobel 1991). This response was indicative of a loss of stomatal control that has been documented for a number of species in response to elevated O<sub>3</sub> exposures (Hassen et al. 1994, Mairer-Maercker 1989, 1997, Robinson et al. 1998). Mairer-Maercker (1989) showed the degree of lignification of subsidiary and guard cell walls of Norway spruce saplings resulted in loss of stomatal control in response to elevated O<sub>3</sub> exposures. Hassen et al. (1994) showed that electron microscope images of the radish seedlings exposed to elevated O<sub>3</sub> had considerable loss of turgor and collapse of epidermal tissues not evident in control plants. Greater stomatal conductance in the absence of visible injury suggests that the primary O<sub>3</sub> impact may have been incurred via cuticular and epidermal damage rather than direct impact to the mesophyll. Increased conductance would in turn compound detrimental O<sub>3</sub> impacts by increasing pollutant flux into affected foliage (Coyne and Bingham 1982).

The lack of difference in photosynthesis for recently expanded foliage for either the chamber or field experiment was likely due to measurement of younger foliage not yet affected by the elevated O<sub>3</sub> exposures (Reich et al. 1985, Coleman et al. 1995b, Grulke et al. 2002). Note that foliage exposed to 3.0× ambient O<sub>3</sub> and older foliage for both the 1.8× and 3.0× ambient treatments did show the expected photosynthetic reductions. Reduced photosynthesis of older foliage would be consistent with field measurements showing the loss of stomatal control and the tight inverse relationship between foliar production and ambient O<sub>3</sub> exposures.

Though the rate of plant development is generally related to growing degree-days (Jones 1996) the temperature/growth relationship was altered by the cumulative O<sub>3</sub> exposures. Elevated temperatures accelerate

the reactions of O<sub>3</sub> formation (Butler 1979, Olszyna et al. 1997), so it is often considered not possible to determine the relative importance of temperature and O<sub>3</sub> in regression analyses. However, there was no relationship between temperature and O<sub>3</sub> in urban and rural sites in the vicinity of NYC ( $F_{1,7} = 0.17$ ,  $P < 0.69$ ,  $r^2 = 0.03$ ). The urban sites were warmer but did not have higher O<sub>3</sub> concentrations, and the eastern rural sites were warmer than the northern sites but O<sub>3</sub> was not consistently higher. These analyses demonstrate the utility of performing multiple comparisons in space and time to provide sufficient variation between factors to determine their relative importance in multiple regression analyses. Comparisons in time were important both within and between growing seasons.

Substantial reductions in foliar production in the absence of visible injury for both chamber and field experiments was likely due to increased respiratory costs due to antioxidant production or the repair of nonvisible damage. Protective and repair mechanisms could have prevented visible injury while maintaining a net positive carbon balance, thereby preventing the onset of premature leaf senescence. Although increased respiratory costs often lead to reduced allocation to root tissues (Andersen 2003), the increased transpirational demand could have offset this reallocation of resources due to the need to maintain water balance. This mechanism may help to explain why reduced allocation to root tissues does not occur for all species (Landolt et al. 2000) and can be delayed to the following growing season (Andersen et al. 1991).

In contrast to the nearly twofold growth reductions for the 1.8× ambient O<sub>3</sub> exposures, there was no further growth reduction and no gas exchange, phenological, or allocation differences between the 1.8× and 3.0× ambient O<sub>3</sub> exposures. These results contrast with those of an adjacent aspen congener in the same chambers that showed less impact for the 1.8× ambient O<sub>3</sub> exposures but continued growth reduction, greater foliar symptoms, and increased foliar senescence in response to the 3.0× ambient O<sub>3</sub> exposures (Yun and Laurence 1999). Greater stomatal conductance for cottonwoods in the 1.8× ambient O<sub>3</sub> treatments would have enhanced O<sub>3</sub> flux into affected foliage, thereby helping to account for the substantial growth reductions for this treatment. However, conductance did not vary between the 1.8× and 3.0× ambient O<sub>3</sub> treatments, so protective reduction in stomatal conductance could not have offset the expected greater impact of the 3.0× O<sub>3</sub> exposures. Instead, continuous production of new foliage not yet affected by the elevated O<sub>3</sub> exposures (Reich et al. 1985, Coleman et al. 1995b, Grulke et al. 2002) likely accounts for maintenance of growth despite the inclement conditions. These results show a case in which the usefulness of dose calculations for predicting detrimental O<sub>3</sub> impacts (Reich 1987) could be enhanced by including age structure to account for variable stomatal

conductance and altered exposure periods for foliage of different ages within an individual canopy.

Remaining factors that could potentially account for observed growth differences between urban and rural environments include increased N deposition (+28.1 mg) and the elevated temperatures (+1.8°C) and CO<sub>2</sub> concentrations (+50 ppm) in the urban environment (Gregg et al. 2003). However, soil transplants, nutrient budgets, and results showing the greatest growth differences for the fertilized potting soil treatments that had three orders of magnitude more N than that available from deposition indicated that N deposition could not account for increased growth in the urban environment. Chamber experiments simulating the urban and rural thermal and CO<sub>2</sub> environments also showed no effect of the elevated urban temperatures and CO<sub>2</sub> concentrations. These results are in agreement with Wait et al. (1999) and G. Barron-Gafford, K. Grieve, T. Paige, L. Patterson, R. Murthy, and K. Griffin (*unpublished manuscript*) who have shown the unresponsiveness of cottonwood to 550 and 800 ppm CO<sub>2</sub> concentrations, respectively. They also agree with results from asymmetric warming studies that have shown the importance of increased respiratory costs with warmer night temperatures for offsetting potential growth increases with warmer daytime temperatures (Manunta and Kirkham 1996, Griffin et al. 2002, Turnbull et al. 2002).

Overall, chamber results showing twofold growth reductions for 1.8× and 3.0× ambient O<sub>3</sub> exposures indicated that the maximum growth reduction occurred somewhere below the 1.8× ambient exposures in the range of those at rural sites. Documentation of similar growth reduction together with consistent mechanisms accounting for reduced growth at rural sites and in the elevated O<sub>3</sub> chambers indicates that higher rural O<sub>3</sub> exposures could account for observed growth differences between urban and rural sites. These results are consistent with extensive research documenting detrimental impacts of ambient O<sub>3</sub> exposures for a variety of herbaceous, tree, and crop species for rural/remote sites throughout the northeastern United States (Reich and Amundson 1985, Sandermann et al. 1997), including the rural Hudson Valley and Long Island sites from this study in which detrimental impacts of ambient O<sub>3</sub> exposures have been shown for trembling aspen, hybrid poplar, cottonwood, black locust, and pumpkin (Wang et al. 1986a, b, McGrath 1995).

Documentation of substantial O<sub>3</sub> impacts in the absence of visible injury highlights the difficulty using visible injury as the sole diagnostic for documenting detrimental O<sub>3</sub> impacts under field conditions (U.S. Environmental Protection Agency 1996, USDA Forest Service 2002, Bussotti et al. 2003). Instead, gas exchange, growth and development, and allocation comparisons between chamber and field experiments helped reveal factors of potential value in documenting detrimental O<sub>3</sub> impacts when visible injury is not

present. Gas exchange parameters and leaf area production often vary in response to elevated O<sub>3</sub> exposures, but responses are not O<sub>3</sub>-specific, so these parameters are generally not recommended for use in documenting detrimental O<sub>3</sub> impacts (Saxe 1991). Therefore, it was not until we examined the combined conductance vs. photosynthetic relationship that we were able to show the loss of stomatal control. Multiple regression analyses also showed that although the rate of plant development is generally related to growing degree-days (Jones 1996), the temperature/growth relationship was altered by the cumulative O<sub>3</sub> exposures. Chamber results showing the inverse conductance/photosynthesis relationship within an individual canopy indicate that future field comparisons showing increased conductance with lower photosynthesis for older foliage would be indicative of a loss of stomatal control resulting from detrimental oxidant impacts. Whereas reduced stomatal conductance in response to drought conditions generally minimizes pollutant flux to exposed foliage (Reich et al. 1985), the loss of stomatal control would accentuate pollutant uptake and expedite water loss in response to elevated O<sub>3</sub> exposures. Results showing increased stomatal conductance with detrimental impacts could have important implications for system water balance during warm, dry portions of the growing season when O<sub>3</sub> concentrations are highest.

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