New Phytologist

Research

Interactive effects of ozone and climate on tree growth and water use in a southern Appalachian forest in the USA

S. B. McLaughlin[1,2], M. Nosal[3], S. D. Wullschleger[2] and G. Sun[4]

[1]Department of Ecology and Evolutionary biology, University of Tennessee, Knoxville, TN, USA; [2]Oak Ridge National Laboratory, Environmental Sciences Division, Oak Ridge, TN, USA; [3]Department of Mathematics and Statistics, University of Calgary, Calgary Alberta, Canada; [4]US Forest Service, Southern Global Change Program, Raleigh, NC, USA

Author for correspondence:
S. B. McLaughlin
Tel: +01 540 348 4342
Fax: +1 540 348 4342
Email: lochlainnsbp@earthlink.net

Received: *7 December 2006*
Accepted: *4 January 2007*

Summary

- A lack of data on responses of mature tree growth and water use to ambient ozone (O_3) concentrations has been a major limitation in efforts to understand and model responses of forests to current and future changes in climate.
- Here, hourly to seasonal patterns of stem growth and sap flow velocity were examined in mature trees from a mixed deciduous forest in eastern Tennessee (USA) to evaluate the effects of variations in ambient O_3 exposure and climate on patterns of stem growth and water use.
- Ambient O_3 caused a periodic slowdown in seasonal growth patterns that was attributable in part to amplification of diurnal patterns of water loss in tree stems. This response was mediated by statistically significant increases in O_3-induced daily sap flow and led to seasonal losses in stem growth of 30–50% for most species in a high-O_3 year.
- Decreased growth and increased water use of mature forest trees under episodically high ambient O_3 concentrations suggest that O_3 will amplify the adverse effects of increasing temperatures on forest growth and forest hydrology.

Key words: climate, forests, growth, models, ozone (O_3), water use.

New Phytologist (2007) **174**: 109–124

doi: 10.1111/j.1469-8137.2007.02018.x

Introduction

As the role of fossil fuel combustion in global climate change has been more clearly defined in recent decades (Thompson, 1995), there has been increasing evidence that there will be direct, indirect, and interactive effects of the pollutants responsible for climate change on biological systems (IPCC, 2001). Changes in carbon fixation and allocation to roots and shoots, alterations in nutrient supply, and changes in sensitivity to water stress have been demonstrated at ambient concentrations of air pollutants for a few intensively studied tree species from diverse forest systems, and such changes have important implications for how forests can be expected to respond to climatic stresses (Skarby *et al.*, 1998; McLaughlin & Percy, 1999). Among the air pollutants, ozone (O_3), which can adversely affect both human and ecological systems (Finlayson-Pitts & Pitts, 1993), is perhaps the most significant. Ozone occurs in the troposphere at toxic concentrations over widespread areas of the industrialized world (Chameides *et al.*, 1994). In this respect, O_3 is already an important part of the chemical climate with potentially significant implications for both the rate and the direction of the response of ecological systems to changes in temperature and precipitation patterns.

Direct effects of O_3 in reducing forest productivity have generally been estimated in the 1–10% range for forests in both Europe (Broadmeadow, 1998) and the USA (Chappelka & Samuelson, 1998). Yield loss estimates that have been incorporated into biogeochemical models suggest that O_3 may offset much of the gains in productivity projected with increasing atmospheric CO_2 (Ollinger *et al.*, 2002) and nitrogen deposition (Felzer *et al.*, 2004). These models provide new insights into important biogeochemical feedbacks in global change biology, but they still suffer from uncertainties in the current understanding of basic physiological mechanisms underlying responses of forests to ambient air pollutants. These uncertainties are both scalar and conceptual. The scalar uncertainties are those inherent in transferring results derived from small seedlings or saplings in controlled environments to mature forests (Samuelson & Kelly, 2001), while the conceptual uncertainties are a result of the lack of consensus on the direction and rate of responses of tree water use patterns to ambient O_3 exposures (Mansfield, 1998; Robinson *et al.*, 1998).

Efforts to scale results of studies with seedlings to responses of large trees to air pollutants have to date been largely unsatisfactory (Samuelson & Kelly, 2001). There are many reasons for this, including differences between small and mature trees in energy budgets, canopy:root balance and architecture, and carbon allocation patterns. Scaling efforts have highlighted the need for improved data on physiological responses of mature trees in natural settings (Fuhrer *et al.*, 1997; Samuelson & Kelly, 2001). The additional problem of defining appropriate responses of plant stomata to O_3 exposure (Maier-Maercker, 1998; Mansfield, 1998) is critical to understanding how forest water use is affected by O_3. Stomatal responses to O_3 are clearly complex, with variations in degree and direction of response being associated with species, exposure level, and length of exposure (McAinish *et al.*, 2002).

It is apparent that generalized water use patterns derived from small plants in small containers artificially exposed to high O_3 concentrations can significantly misrepresent responses of mature trees in native soils. In chamber environments at high O_3 exposure levels, stomatal closure and protection of plants from drought and additional O_3 exposure can occur (Tingey & Hogsett, 1985). By contrast, there is abundant evidence from studies with seedlings and saplings in controlled exposures (Keller & Hasler, 1984; Reich & Lassoi, 1984; Skarby *et al.*, 1987; Lee *et al.*, 1990; Wallin & Skarby, 1992; Pearson & Mansfield, 1993; Matyssek *et al.*, 1995) that stomatal control of transpiration may be reduced following O_3 exposure. This can lead to greater stomatal apertures, increased transpiration, and/or delayed stomatal closure at night. In addition, empirical evidence from larger trees including both *in situ* physiological measurements of foliage (Maier-Maercker & Koch, 1992; Maier-Maercker, 1997; Grulke *et al.*, 2002a) and tree- and stand-level growth responses (McLaughlin & Downing, 1995, 1996; Zahner *et al.*, 1989; Peterson *et al.*, 1995) in natural settings suggests that drought effects may be enhanced, not reduced, by concurrent stresses posed by O_3 and reduced water availability.

New high-resolution measurement techniques offer great promise for identifying and evaluating individual and combined effects of multiple stresses, including O_3, on forest growth (McLaughlin *et al.*, 2002). These techniques include measurements of the diurnal and seasonal patterns of stem shrinkage and expansion which reflect the dynamics of daily and seasonal stem growth and water use patterns (Downes *et al.*, 1999; Zweifel *et al.*, 2001), as well as direct measures of sap flow and patterns of whole-tree transpiration (Wullschleger *et al.*, 2001). Diurnal fluctuations in the stem radius of large trees are a part of a rapid recharge system that supplies active meristematic growing centers with water during periods of maximum water demand. Thus, these fluctuations reflect daily patterns of potentially growth-limiting degrees of water stress (McLaughlin *et al.*, 2003). Diurnal stem shrinkage and expansion patterns are strongly influenced by water use and movement through tree stems; and a close linkage between transpiration of upper crown foliage, sap velocity, stem shrinkage, root radius, and the diurnal course of stem radius fluctuations has been demonstrated for subalpine Norway spruce (*Pices abies*; Zweifel & Hasler, 2001). Diurnal stem increment patterns have recently been used to explore both the dynamics of bark beetle attacks and their influence on water supply in boles of loblolly pine (*Pinus taeda*) in Tennessee in the USA (Wullschleger *et al.*, 2004) as well as the influences on growth of drought episodes for Norway spruce, pubescent oak (*Quercus pubescens*), and Scots pine (*Pinus sylvestris*) in Europe (Zweifel *et al.*, 2005).

The increasing evidence that O_3 can alter the efficiency of water use by forest trees has particularly important implications for predicting forest growth patterns in a future climate in which water demand will be increased by increasing temperatures, and water supply will, at least occasionally, be more limited by severe droughts (Hanson & Weltzin, 2000; IPCC, 2001). The high sensitivity of growth to small increases in water stress (Hsaio *et al.*, 1976) suggests that increased water use associated with higher O_3 exposure would increase the frequency and degree of O_3-induced limitations on forest growth. The studies upon which we report here have been directed at testing the hypothesis that ambient O_3 concentrations increase water stress in forested ecosystems in agroindustrial regions. This has required obtaining continuous (hourly) measurements of concurrent changes in growth and water use by mature forest trees under ambient O_3 exposures in the field. In this process we have used high-resolution measurements of diurnal cycles of growth and water use of mature forest trees together with climatic data to evaluate the relative importance of variations in O_3 and environmental stress on forest growth processes. We report here on patterns of growth and water use of individual forest trees and in a following paper (McLaughlin *et al.*, 2007) on effects of increased water use on soil moisture and late-season streamflow of associated watersheds.

Materials and Methods

Site characterization and monitoring

These experiments were designed to characterize variations in tree growth and tree water use in response to natural variations in O_3 and meteorological variables at diverse forested sites in eastern Tennessee, USA. We have linked measurements of stem growth of 86 forest trees distributed across three sites over

2–3 yr, with high-resolution measurements of stem growth, sap flow, and soil moisture for a subset of six trees at a single site. The three forested sites, Look Rock (LR), Twin Creeks (TC), and Oak Ridge (OR), represent a wide range of elevations (from 250 m (OR) to 750 m (LR)), productivity (a poorer quality xeric higher elevation ridge site (LR) to a more mesic cove hardwood site (TC)), and stand histories (severely disturbed in 1999 (OR) to undisturbed for > 65 yr (TC)). Data on air temperature, solar radiation, relative humidity, rainfall and O_3 concentrations were provided by monitoring stations maintained by the National Park Service (http://www2.nature.nps.gov/air/data/index.htm). The Look Rock site was monitored by a station located approx. 500 m from that site, while the Twin Creeks site was represented by data from Cades Cove, a location within the Smoky Mountains National Park and *c.* 33 km south-west of Twin Creeks. This station was shown to provide comparable O_3 concentrations to those at Twin Creeks in earlier years when parallel measurements were conducted at both sites (Jim Renfro, Air Quality Specialist with the National Park Service, conducted parallel monitoring of ozone at Twin Creeks and Cades Cove in the Great Smoky Mountains National Park and advised us (pers. comm.) of the comparable data from the two sites). Ozone data for the Oak Ridge site were provided by a dedicated on-site Dasibi ozone monitor (Model 108-AH; Dasibi Environmental, Glendale, CA, USA) located 200 m from the yellow poplar (*Liriodendron tulipifera*) stand.

Measurements of stem increment

Measurements of stem increment were of two types. Less intensive manual measurements were designed to describe broader patterns of response in terms of magnitude and consistency across species, size classes, and sites. These were accompanied by high-resolution electromechanical measurements designed to describe diurnal increment patterns associated with hourly changes in water use and stem increment for a few intensively monitored trees. The 86 trees for which manual measurements were obtained represented 10 species.

Manual measures of circumference changes at approximately 2-wk intervals were recorded for all trees at all sites and were linked to electromechanical measurements of radius changes at 30-min intervals for six trees at the high-elevation LR site. The manual measures were of gap changes on tensioned metal bands and were obtained with digital calipers to an average precision of 0.02 mm. High-resolution measurements of radius changes were obtained with electromechanical dendrometers (Agroelectronics Inc., Tucson, AZ, USA) mounted at approx. 1.5 m above ground. Electronic sensors (sensitivity = 1 uv = 0.006 mm radius change) were calibrated to manual circumference measurements over the season by bands on each intensively monitored tree.

The 14 trees selected for electronic growth analysis in this mixed deciduous natural stand were chosen to allow replication (two or three trees per species) of the dominant species at each site. More emphasis was placed on red oak (*Quercus rubra* L.), chestnut oak (*Quercus prinus* L.), and yellow poplar (*Liriodendron tulipifera* L.) than on other species. Choices of canopy dominant and codominant individuals were made within a radius circumscribed by the approx. 80 m wired extension from the central data collection center.

Diurnal radius measurements were linked to measurements of sap flow on a subset of six trees at the LR site at 30-min intervals using sap flow sensors. For sap flow measurements, the power demands of the sensors restricted sampling to six trees, measurements of which could be sustained by available solar-powered recharge of marine storage batteries. These were colocated on trees with electronic dendrometers to provide concurrent measures of stem expansion and water use. These in turn were linked to measurements of soil water content at 10- and 22-cm depths 1 m from the base of individual sampled trees and based on rates of temperature conductance through buried ceramic probes. Radial increment, sap flow, and soil moisture data were automatically logged on to on-site data storage and retrieval systems for downloading at 2–3-wk intervals.

Measurements of sap flow velocity

Six trees were selected for hourly measurements of sap flux in 2001 and 2002. Species included red oak, hickory (*Carya* sp.), yellow poplar (*L. tulipifera*) and pitch pine (*Pinus rigida* Mill.). These trees were largely canopy dominants or codominants based on height and crown area. Diameter at breast height (d.b.h.) ranged from 27.7 to 45.2 cm. Our objective with these measurements was to describe environmental influences on patterns of sap flow velocity, not to estimate total canopy water use nor to define the stomatal mechanisms involved. Instantaneous xylem sap flux (J_S) was measured with continuously heated thermal dissipation probes (TDP-30; Dynamax, Houston, TX, USA). These devices operated on the constant power principle (Granier, 1987) and were comprised of two cylindrical probes, each 2.0 mm in diameter, which were inserted 30 mm into the sapwood of a tree. The probes were inserted one above the other, *c.* 4 cm apart. The upper probe was installed at a height of 1.3 m. Each probe contained a copper–constantan thermocouple and the temperature difference between the two probes was influenced by sap velocity in the vicinity of the heated probe. Thermal dissipation probes were installed in late March of each year and removed after visible signs of canopy senescence were apparent (usually after 1 November). All probes were installed on the north side of trees to avoid direct solar heating and insulated with Styrofoam and shielded with aluminum foil to minimize temperature fluctuations in the sapwood. Sapwood thickness was determined for each tree by removing 5-mm-diameter cores of wood with an increment borer. In cases where sapwood thickness was less than the 30-mm probe length, the measured temperature differentials were corrected according to Clearwater *et al.* (1999).

Data analysis strategies

Because both the temporal patterns and the biological effects of environmental variables, such as temperature, radiation, and O_3 exposure level, are interrelated, it was important in our analyses to address both an appropriate range of environmental variables that could influence the dependent variables examined at each scale (tree growth, water use, soil moisture status, and streamflow hydrology) and to match appropriate time scales for relating responses to predictor variables at each organizational level considered. Thus we conducted exploratory analyses with a wide range of the driving climatic variables (temperature, radiation, rainfall, vapor pressure deficit (VPD), and O_3 exposure) to identify appropriate parameters for inclusion in multivariate models designed to predict responses of tree growth and water use. In the Supplementary Material (available online) we describe the principal variables examined in developing empirical models of stem increment and sap flow as well as model performance indicators developed for each of the six trees on which both processes were measured. Also included are measures of cross-correlation among the most important variables. Partial correlation coefficients for the influence of each independent variable on the stem increment and sap flow are also noted. Only variables that were identified as statistically significant (typically at $P \leq 0.05$) were included in final models developed at each analytical scale, and these also are identified.

Modeling stem increment patterns Responses of stem increment to environmental variables were examined as hourly averages, as portions of the diurnal shrink/recovery cycle (McLaughlin *et al.*, 2003), and as daily averages. We present here hourly scale analyses using two approaches, linear regression analysis and the development of a generalized additive model (GAM), which allowed curvilinear curve fitting to observed response surfaces. Before these analyses were begun, however, we did extensive analysis of the autocorrelation and cross-correlation of the time series of both independent and dependent variables explored. These included determination of cross-correlation and autocorrelation functions as well as spectral analyses and coherence analyses to identify dominant patterns of variation and response within the data time series. Not surprisingly, the strong diurnal pattern of most variables driven by solar radiation was apparent. As the resultant autocorrelation and cross-correlation with respect to time were statistically highly significant, we investigated all time series of monitored data with various degrees of time lag up to 24 h. Because of the dose-dependent nature of the phytotoxic effects of O_3, we also examined a wide range of parametrizations of O_3 exposure in hourly time series including running averages of up to 3 h and cumulative dose over threshold values (the accumulated O_3 exposure dose over a 60 ppb threshold (AOT_{60}) and that over a 40 ppb threshold (AOT_{40})) as well as squares and cubes of O_3 concentrations. Other meteorological predictors included in the best models were hourly values of VPD, temperature, solar radiation and rainfall.

Detailed time series analysis revealed that differenced time series of original observations had the greatest potential for building predictive regression models of the influence of O_3 and climate variables on tree growth. This is very logical from the biological point of view, because the differenced time series of dendrometer readings, which represents a rate of change in stem radius, best describes tree growth and hence tree response to changing environmental variables. Since the differenced time series contains information about lagged data, it captures to some degree autocorrelations within the series. Thus all regression analyses and growth model building were performed in terms of differenced time series of dendrometer readings. In addition, we used partial correlation analyses to identify those independent variables that were individually most important in explaining variability in stem increment over time.

Ultimately, the best regression algorithm was applied in order to find the best subsets of possible predictors of the effect of O_3 and meteorological variables on tree growth. The optimal conditions in this algorithm were based on the highest R^2-adjusted value as well as on the Cp-Mallows value (Rawlings, 1988). The best regression algorithm optimizes the process of selection of predictors by eliminating the subsets of variables with high cross-correlations. Many thousands of possible models with different combinations of predictors were evaluated from the point of view of how well they captured the effect of O_3 and meteorological variables and how well they represented the biological and ecological considerations. Furthermore, the goodness of fit of these various models was evaluated by applying all considered models to different observed data sets, predicting the tree response from such synthetic models, and comparing simulations with actual observed tree growth. The predictor variables and the final models derived from them were selected on the basis of statistical goodness of fit as well as on the compliance with biological and ecological considerations.

In analyzing responses of hourly stem increment to environmental variables, we considered only models that were highly significant statistically ($P \leq 0.05$). Furthermore, we considered only model formats that were significant for all species tested, so that our results would be more broadly applicable to regional forest types. The final model, which provided the best fit between the observed tree radial growth data and model-predicted values, was a GAM. It consisted of two parts: the first part included a multiple linear regression, and the second (additive) part incorporated additive nonlinear smoothers, which captured the essentially nonlinear relationship between the predictors and response variables. This model had a general form as follows:

$$\eta(Y) = \alpha + \sum_{j=1}^{p} \beta_j X_j + \sum_{j=1}^{p} f_j(X_j) + \varepsilon$$

(Y, the response variable, given by the differentiated time series of the monitored hourly tree radii; X_j, the predictors: cumulative AOT_{60}, VPD, an interaction term $AOT_{60} \times$ VPD, temperature, solar radiation, and rainfall; f_j, cubic splines of the above-named predictors, representing a nonlinear relationship between the response and the predictors; α, the regression intercept; β_j, the regression coefficient corresponding to predictor X_j; η, the identity link function.)

For the prediction of the actual tree growth, the time series of predicted changes in tree radius obtained from the above model was integrated (using time series cumulative sum procedures) to obtain a prediction of the actual seasonal radial tree growth. Computational procedures for model fitting and assessment of goodness of fit were performed using S-Plus statistical computing language (Chambers & Hastie, 1992; Venables & Ripley, 1999) and the GAM procedure (Hastie & Tibshirani, 1990). An analogue to the coefficient of determination in GAM was computed (Nosal, 2007) as follows:

$$SS_{GAM} = \text{Null.Deviance} - \text{Residual.Deviance}$$

$$R^2_{GAM} = SS_{GAM}/\text{Null.Deviance}$$

Modeling daily sap flow patterns Sap flow velocity data were analyzed as daily averaged rates of sap movement up the stems of each individual tree. A wide range of potential predictor variables were considered in initial exploratory analyses using stepwise linear regression to identify best descriptors of daily sap flow patterns. We tested our working hypothesis regarding O_3-induced amplification of water stress in both 2001 and 2002, which represented low and high years of O_3 exposure, respectively. As a dependent variable, sap flow velocity was evaluated as a maximum hourly average, a 12-h average, and a 24-h average. The 24-h average sap flow velocity was ultimately selected as it was both highly sensitive to O_3 exposure and would allow us to capture O_3 influences on total daily water use, including any delay in stomatal closure. Comparison of peak hourly, 6-h, and 8-h average O_3 concentrations identified the daily maximum hourly O_3 concentration as most influential in affecting sap flow. We also used lagged responses at times ranging from 1 to 4 d before the sap-flow measurement day. Ultimately, a stepwise regression model was run for each tree with the potential predictor variables describing preceding temperature, rainfall, solar radiation, vapor pressure, and O_3 exposure.

Results

Environmental conditions during the study interval

All three study sites were within a 50-km radius, so meteorological conditions were generally similar across sites for any year. Average conditions at the intensively studied LR site for the 2001–2003 growing seasons are summarized in Table 1, while seasonal patterns of cumulative O_3 exposure for 2001–2003 are shown in Fig. 1(a). Daily peak O_3 concentrations are also contrasted among the three study sites in 2002, a year of high ozone exposure (Fig. 1b). The study years 2001–2003 provided a wide range in patterns of O_3 exposure, as noted in Fig. 1(a). Cumulative seasonal O_3 exposures > 60 ppb (= nl l^{-1}) were near average in 2001 and 2003, and 50% above average in 2002. Doses in 2002, in turn, were approx. 50% below the maximum exposure dose over the past 23 yr. Rainfall patterns were similar between 2001 and 2002, particularly during the first half of the growing season, while O_3 exposure levels, as noted above, were much higher in 2002. By contrast, O_3 exposure levels in

Table 1 Summary of meteorological data for the three years at the Look Rock site[a]

Variable[b]	DOY	2001	2002	2003
Rainfall	121–180	0.14	0.16	0.22
(mm h^{-1})	181–228	0.22	0.17	0.29
Temperature	121–180	18.6	18.8	18.0
(°C)	181–228	21.9	23.4	21.3
VPD	121–180	0.85	0.78	0.47
(g H_2O g^{-1} air)	181–228	0.81	0.98	0.45
Radiation	121–180	218	229	205
(W m^{-2} h^{-1})	181–228	206	227	192
O_3 AOT_{60}	121–180	5.2	7.4	3.8
(ppm h)	181–228	7.7	18.5	6.3
	91–304	11.5	24.0	11.7
O_3 MaxHD	121–180	68.1	69.2	64.0
(ppb)	181–228	64.9	82.9	65.9
Palmer drought	121–180	0.22	−0.88	4.26
severity index[c]	181–228	1.12	−1.54	5.11
	90–300	0.23	0.80	4.56

[a] Data are summarized for the time period before and after the end of June (day of year (DOY) 180) and averages are provided over each time interval.

[b] Data on rainfall, temperature, vapor pressure deficit (VPD) and radiation are 24-h averages. Other variables include AOT_{60}, accumulated O_3 exposure dose over a 60 ppb threshold and MaxHD, daily maximum O_3 exposure levels. These data were obtained from a National Park Service monitoring site at Look Rock approx. 500 m from the study site (National Park Service web address: http://www2.nature.nps.gov/air/data/index.htm).

[c] The Palmer drought severity index (PDSI) measures cumulative meteorological drought derived from measures of moisture supply in rainfall, moisture storage in representative soils of the region, and drying potential of the atmosphere (see Palmer, 1965). The PDSI scale of relative drought severity ranges from ≤ −4.00 (extreme drought) to ≥ +4.00 (extremely moist). Values in the range −1.99 to 1.99 are considered midrange. Moderate values of moisture (+) or drought (–) are ± (2.00–2.99) and severe values are ± (3.00–3.99). Data used in these studies were obtained from the National Oceanic and Atmospheric Administration, National Climate Data Center (http://ncdc.noaa.gov/oa/climate/prelim/drought/palmer.html).

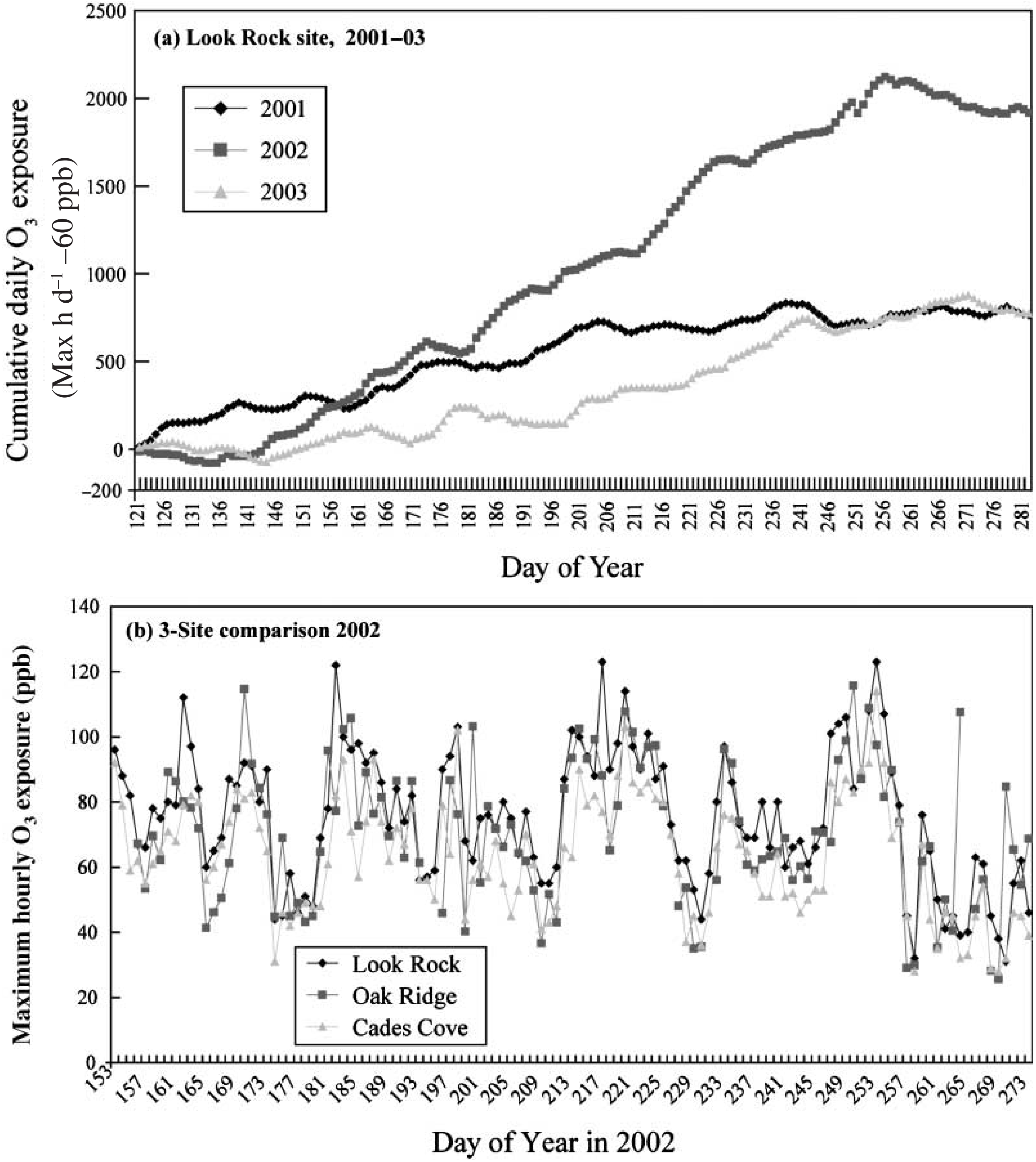

Fig. 1 (a) Cumulative ozone (O_3) exposure during three growing seasons indicated that rates of increase in O_3 exposure at Look Rock, TN, USA differed widely both within and among years. We have used here a daily running sum of maximum hourly O_3 concentration per day minus 60 ppb. This metric, which leads to decreasing accumulative values during lower exposure days (maximum hourly value < 60 ppb), helps one to identify periods of potential recovery of vegetation from pollution-induced stress (year 2002 > day of year (DOY) 245 as well as periods within each year when potential O_3 stress was increasing rapidly (year 2002 > DOY 145, DOY > 205). (b) A comparison of the daily maximum O_3 exposure levels (MaxHD) at the three study sites shows that the seasonal patterns of occurrences of higher multi-day peak O_3 exposures were much more comparable among sites than were seasonally averaged exposure levels. Data for the Oak Ridge site were obtained from an on-site monitor. Data for Cades Cove were obtained from the National Park Service (http://www2.nature.nps.gov/air/data/index.htm) and are considered highly representative of the Twin Creeks site, approximately 33 km to the north-east.

2001 and 2003 were similar, but rainfall was higher and more evenly distributed in 2003.

The comparative patterns in peak hourly O_3 concentration per day (O_3MaxHD) for 2002 were selected for contrast between years (Fig. 1a) and sites (Fig. 1b), because O_3MaxHD was found to be a very significant predictor of water use at tree, soil, and watershed scales and occurred in well-defined patterns that were synchronized by synoptic weather patterns. Seasonally averaged values of O_3MaxHD were also found to be much more similar among sites in 2002 than were seasonally averaged daily mean exposure levels or accumulated levels above a threshold. The range in values of seasonally averaged O_3MaxHD across sites was only 19% of the minimum value among the three sites in 2002, while variations in accumulated dose over the 40 and 60 ppb thresholds, AOT_{40} and AOT_{60}, were much larger, 183% and 293% of the minimum, respectively, across the three study sites.

Responses of tree growth to ozone and climatic variables

Seasonal growth patterns derived from manual dendrometers The three years and three sites included in this study provided a broad and fortuitous range of conditions, and combinations of conditions, under which to evaluate the role of component climate variables on tree growth. Notable among these conditions were the doubling of O_3 exposure levels in 2002 at comparable amounts of rainfall to 2001. Associated with increased O_3 exposures in 2002 were decreases in growth that were concurrent across sites, ranging from −14% to −63% across species and averaging −40% in the nine of 10 species that responded negatively in 2002 (Table 2). The single species that did not appear to respond negatively to 2002 O_3 levels was chestnut oak (*Q. prinus*), a drought-resistant species that had shown high resistance to O_3 in previous controlled exposures of seedling trees from the area. (H. Neufeld, pers. comm.; in experiments in open-top chambers conducted at the National Park Service, Uplands Research Laboratory, near Gatlinburg, TN, USA in 1991, no growth effects of visible injury to chestnut oak saplings were detected at O_3 levels that exceeded those at Look Rock in 2002. Chestnut oak was considered one of the most resistant species examined.) These results were supported by our modeling studies with this species, which indicated that hourly stem increments in chestnut oak responded positively to highest levels of VPD and radiation during the 2002 growing season, whereas other species typically responded negatively to these conditions (data not shown).

Table 2 Annual changes in circumference growth among tree species at three forested sites in eastern Tennessee, USA, over 2–3 yr

				Percent change in annual circumference increment[a]	
Site	Elevation (m)	Species[b]	*n*	2002	2003
Look Rock	750	Yellow poplar	21	−26 (3.4)	−38 (3.5)
		Red oak	8	−42 (6.8)	+1 (8.2)
		Pine sp.	6	−62.5 (5.2)	−2.9 (15.6)
		Hickory sp.	2	−14	+30
		Chestnut oak	3	+44	+55
		All trees	40	−29 (5.1)	−17.1 (5.2)
		All except chestnut oak[c]	37	−35 (3.4)	−20.6 (4.7)
Oak Ridge	250	Yellow poplar	11	−49.6 (3.6)	7.5 (26.7)
Twin Creeks	700	Yellow poplar	9	−62 (5.8)	
		Black cherry	8	−75 (10.6)	
		Shortleaf pine	5	−16.8 (17.7)	
		Hemlock	3	−21.9	
		Red maple	4	−59.6	
		Red oak	3	−43.5	
		Sugar maple	3	−63.8	
		All trees	35	−53.4 (5.5)	

[a] Data at both the Look Rock (LR) and Oak Ridge sites are calculated as comparisons to growth recorded in 2001. For Twin Creeks, plots were established in 2002 and comparisons are to growth experienced in 2003. Ozone (O_3) exposure levels in 2001 and 2003 were very similar (see Table 1). Values are means with standard errors indicated where $n \geq 5$.

[b] Taxonomic names of the listed species are as follows: yellow poplar, *Liriodendron tulipiferea*; red oak, *Quercus rubrum*; pine sp. at Look Rock included six pitch pine (*Pinus rigida*), one Virginia pine (*Pinus virginiana*) and one white pine (*Pinus strobus*); hickory, *Carya* spp.; chestnut oak, *Quercus prinus*; black cherry, *Prunus serotina*; shortleaf pine, *Pinus echinata*; hemlock, *Tsuga canadensis*; red maple, *Acer rubrum*; sugar maple, *Acer saccharum*.

[c] Data are shown with chestnut oak excluded.

Seasonal patterns of circumference growth across sites and years for yellow poplar (Fig. 3) and red oak (Supplementary Material, Fig. S2) represented well the differences in seasonal levels and patterns of stem growth observed across many species at the three sites over the 3-yr study period. These included, most notably, a significant growth slow-down in 2002 beginning in late May (after day of year (DOY) 165) compared with growth in either 2001 or 2003. The slow-down in 2002 was not apparently related to temperature or precipitation differences among the years (Table 1). Intraseasonal averages for these parameters shown in Table 1 reflect very similar conditions between 2001 and 2002 through late June. Major differences in O_3 exposure levels between 2002 and other years (Fig. 1) had developed immediately in advance of observed growth differences (Fig. 2).

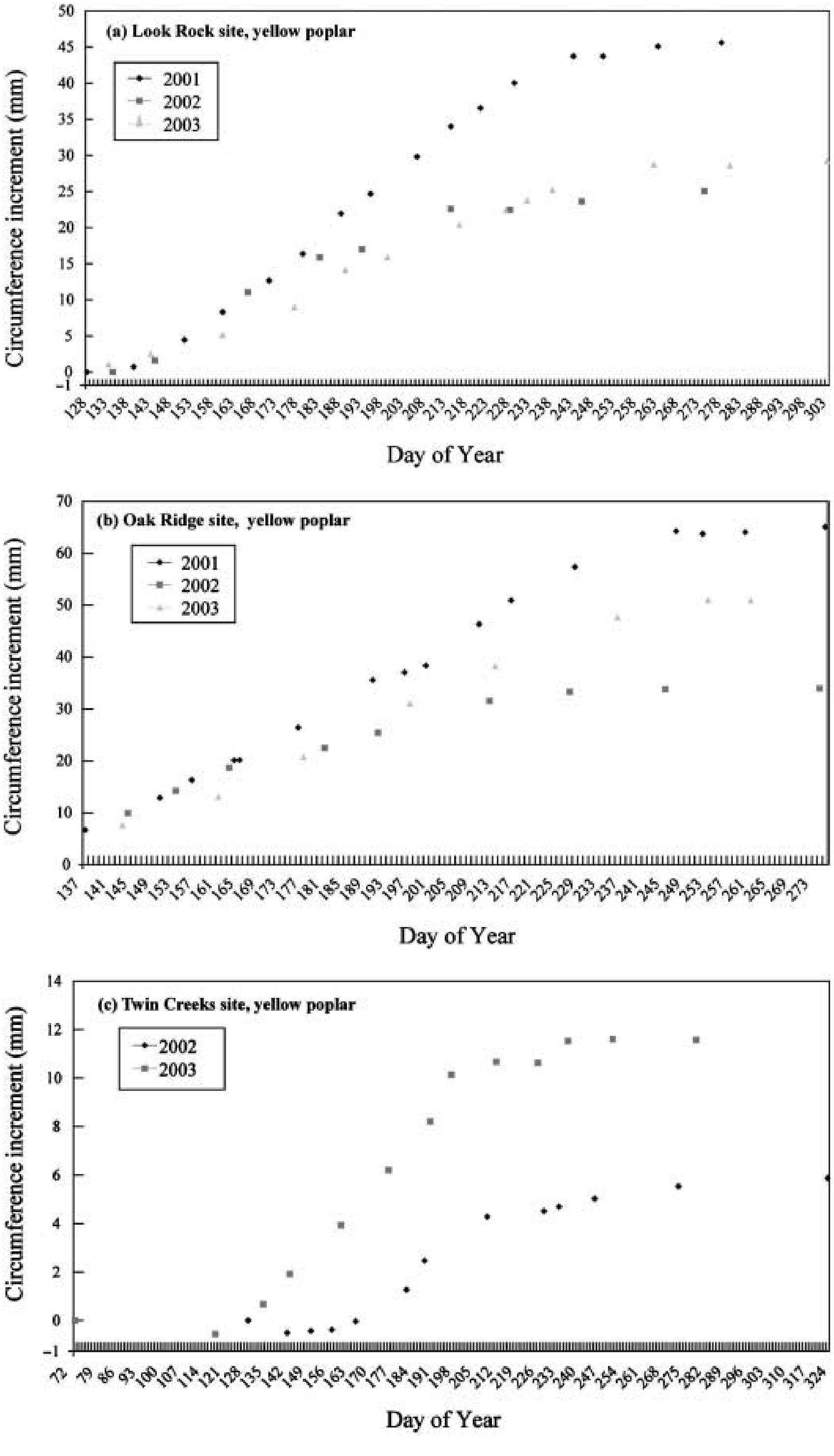

Fig. 2 Comparative seasonal patterns of stem growth of yellow poplar (*Liriodendron tulipiferea*) were measured with manual dendrometers in 2001–2003 at Look Rock (a) and Oak Ridge (b) and in 2002 and 2003 at Twin Creeks (c). Data points represent averages of measurements for the same three trees at each site and date.

Clearly, stem growth was not linear over the first 100 d of the growing season and was characterized by episodic response and recovery patterns to seasonally variable patterns of environmental stress, including, in particular, O_3 exposure. Recovery to 2001 growth levels occurred in 2003 at the OR site (Table 2 and Fig. 2b), but was delayed at the higher elevation LR site (Fig. 2a) where 2002 O_3 exposure levels were higher.

Diurnal growth patterns Closer examination of seasonal growth dynamics, including the hourly and daily increment patterns that underlie those dynamics, are apparent in the high-resolution dendrometer data for six trees representing four species (Fig. 3). The 2002 data revealed a pattern of episodic amplification of the diurnal patterns of stem shrinkage and swelling by which trees in this region respond to daily levels of water stress and accumulate daily increments in stem growth (McLaughlin *et al.*, 2003). Observed patterns indicate that trees were often experiencing reduced hydration and net loss of daily increment for several days following conditions temporally associated with the highest O_3 exposures in 2002. This was most apparent in the most sensitive trees, pitch pine and red oak, which experienced the most pronounced episodic growth loss and eventual stem shrinkage after a modest mid-July drought accompanied by a series of high O_3 exposures (Fig. 3).

Analyses at both hourly and daily (not shown) time scales indicated that O_3 was having a detectable negative effect on stem expansion that was statistically significant for all trees. Significant variables identified with the empirical growth models are described in the next section.

Models of stem growth dynamics To evaluate the comparative roles of environmental variables in contributing to the patterns shown in Fig. 3, a time series of approx. 2500 hourly measurements of tree radius during the 2002 growing season was investigated for six individual trees representing four species. Influences of environmental variables on the hourly radius increment of individual trees as well as the averaged increments across these six trees were examined. In addition to O_3, other meteorological predictors included in the best models were hourly values of VPD, temperature, solar radiation, rainfall, and a product interaction term to capture $O_3 \times$ VPD interactions. Exploratory analyses on the influences of the 17 independent variables selected to characterize climatic influences on hourly stem growth of individual trees were implemented

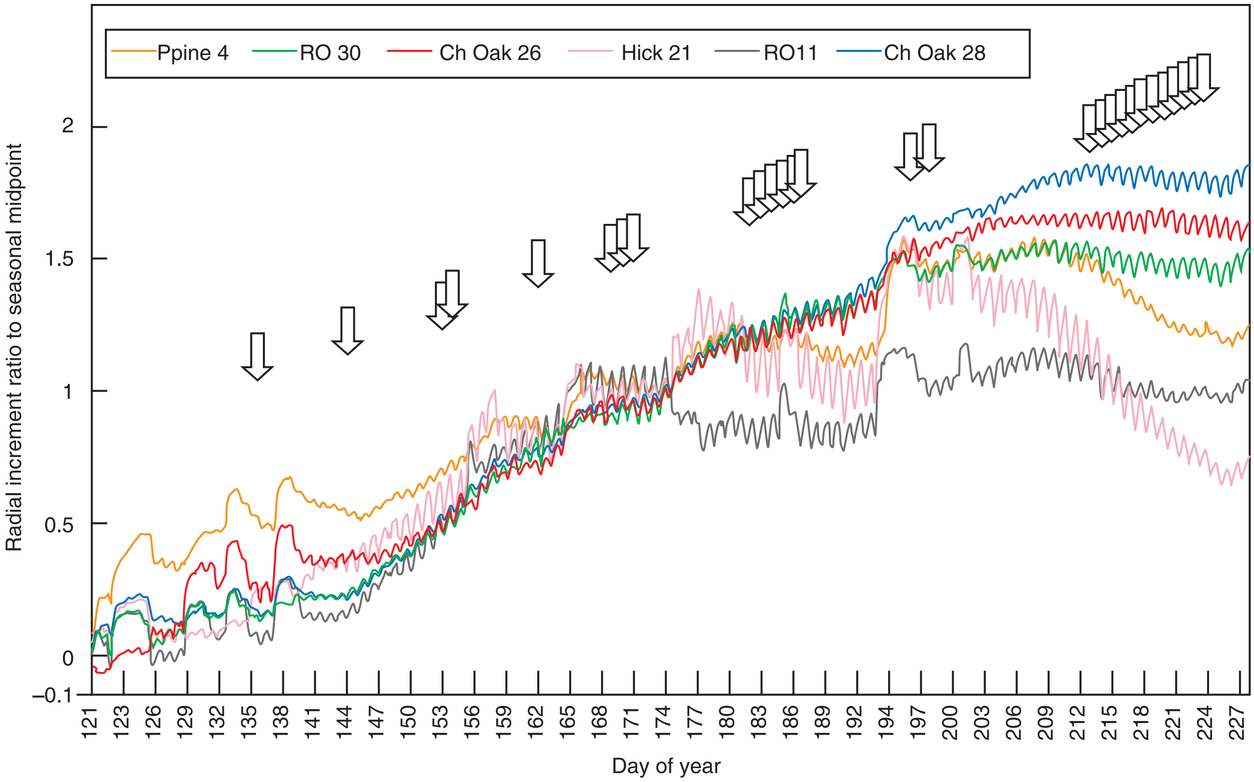

Fig. 3 Hourly average stem increment data for six individual trees during the 2002 growing season reflect the daily patterns of water loss and recovery that are closely tied to net daily growth rates. Distinct periods of amplification of the daily increment cycles that led ultimately to loss of growth rates were consistently associated with multi-day periods of highest ozone (O_3) exposure during the growing season. Peaks of ozone exposure (daily maximum hour ≥ 100 ppb for 1 d or ≥ 85 ppb for two consecutive days) are indicated by arrows.

using desktop statistical screening (STATISTIX 2000, version 7.0; Analytical Software, Talahassee, FL, USA).

Ultimately, time series linear regression models (Chambers & Hastie, 1992; Venables & Ripley, 1999) were developed using candidate variables identified in the exploratory stepwise regression analyses. Variation in the hourly time series was, as expected, strongly influenced by diurnal changes in solar radiation and VPD. Model performance was improved (R^2 = 0.50, $P < 0.001$) by using the average growth data across six trees expressing a range in seasonal growth dynamics and apparent sensitivity to O_3. Both total and relative contributions of significant variables to the six-tree model are shown in Table 3. Ozone exerted its dominant effects (19% of observed variability) in this model as a product interaction term with VPD.

However, as we suspected that many of the modeled relationships were curvilinear, we developed for the same six trees a GAM (Hastie & Tibshirani, 1990; Grulke *et al.*, 2002b) of seasonal growth dynamics which included two components, multiple linear regression combined with additive nonlinear smoothers, as described in 'Data analyses strategies' above. The GAM, using the same variables identified as significant in the best regression (REG) analysis, provided an improved fit ($R^2 = 0.59$), and was highly significant statistically ($P < 0.001$).

In Fig. 4, we have used the most advanced model (GAM) to compare observed growth patterns against model predictions for 2002. Simulations included predicted growth responses both at ambient O_3 and in two reduced O_3 scenarios: a 50% reduction in total O_3 exposure, and a 50% reduction in AOT_{60}. The first O_3 reduction produced a calculated AOT_{60} of near zero, a value close to the lowest AOT_{60} of the past 23 yr (0.6 ppm h recorded in 1989) near the Walker Branch watershed. The second produced an AOT_{60} of 12 ppm h, a value very similar to AOT_{60} values in 2001 and 2003 (11.5 and 11.7 ppm h, respectively) at the LR site.

Table 3 Characteristics and performance of a multiple regression model of average hourly stem increment of six mature trees (four species) at Look Rock, TN, USA during the 2002 growing season

	Contribution to R^2		
Independent variable	Relative percentage	Total percentage	Influence
$O_3 \times$ VPDd	19.58	9.71	–
CumlAOT$_{60}$	0.38	0.19	–
Temperature (°C)	3.78	1.88	+
Radiation (W m^{-2} s^{-1})	74.31	36.85	+
Rainfall (mm h^{-1})	1.94	0.96	+

Model type: multivariate best regression using time series analyses.
Dependent variable: mean hourly radial stem increment.
Model R^2 (adjusted): 0.495; $P > F \leq 0.001$; standard error = 5.27.
Degrees of freedom (d.f.): regression = 5 d.f.; total = 2567 d.f.
$O_3 \times$ VPDd, product of ozone level (accumulated O_3 exposure dose over a 60 ppb threshold (AOT_{60})) and hourly change in vapor pressure deficit (VPDd); CumlAOT$_{60}$, cumulative AOT_{60} (O_3 > 60 ppb) exposure.

The GAM results shown in Fig. 4 provided very good reproduction of the actual seasonal growth pattern and level

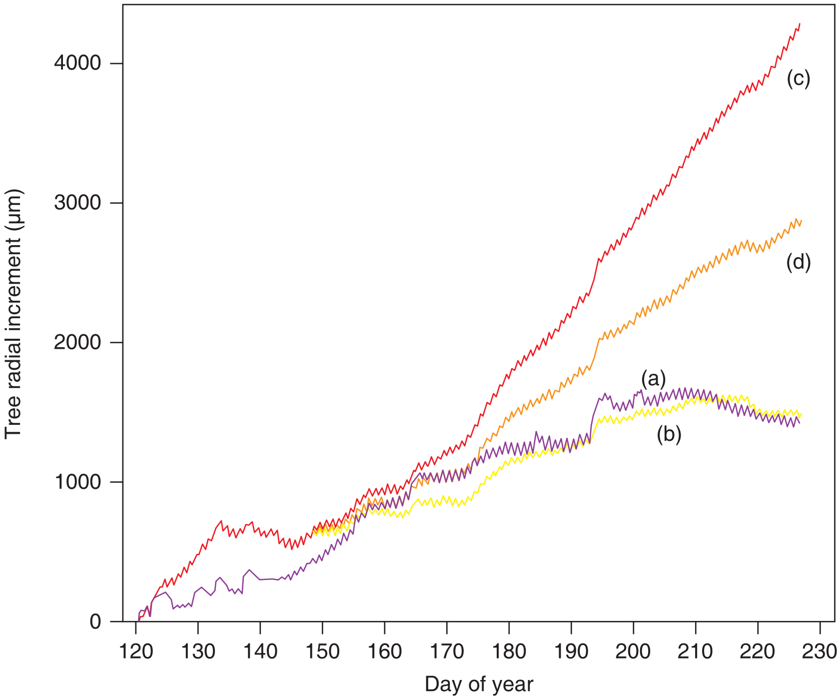

Fig. 4 A generalized additive model was developed around the observed patterns of response of stem increment to a wide array of environmental variables. Plots show: (a) average radius accumulation at the Look Rock site in 2002; (b) simulated increment in 2002 using the generalized additive model (GAM); (c) simulated increment under 2002 conditions but with a 50% reduction in 2002 ozone (O_3) levels (accumulated O_3 exposure dose over a 60 ppb threshold (AOT_{60}) near 0); and (d) simulated increment as in (b), but with a 50% reduction in AOT_{60}. The reduction of AOT_{60} by 50% produced changes in seasonal growth dynamics that agreed well with growth patterns observed in 2001, as seen in Fig. 2.

achieved by the average of six subject trees in 2002. The upper curve in Fig. 4 represents expected growth under 'control' conditions (AOT near 0). With an increase in AOT_{60} from the 'control' level to approximate average exposures in 2001/2003 ($AOT_{60} = 12$ ppm h), simulated seasonal growth decreased by *c.* 33%. The 2002 ambient simulation at higher AOT_{60} (24 ppm h) represented an approximate doubling of the 2001/2003 ambient O_3 levels and resulted in a further growth reduction of 48% compared with these more average years.

As a model validation exercise, simulated differences in growth resulting from reducing O_3 levels in the 2002 model shown in Fig. 4 can be evaluated by comparing them with field data. Those data included measured differences that occurred across the three sites at variable O_3 levels. By reducing simulated O_3 levels by 50%, we approximated actual O_3 levels in 2001. Simulated growth of the six-tree average was 48% lower at higher O_3 levels in 2002 than in 2001. By comparison, dendrometer data from 40 trees representing five species at LR in 2002 indicated that actual growth was reduced in the same range, by 37%, compared with 2001. For all 86 trees across the three sites in the study, the average reduction in 2002 compared with adjacent years with lower O_3 was 42%. The timing of growth pattern divergence between 2001 and 2002 shown in Fig. 4 (a reduction in growth rate originating in the DOY 165 to 18O range in 2002) also agreed well with observed patterns for yellow poplar (Fig. 2) and other species (Table 2). Thus, model simulations and field results were in general agreement regarding the level and timing of O_3 effects.

Our experience with the GAM procedure indicates that we were able to achieve a significantly better goodness of fit with the curvilinear GAM than with linear regression (REG) techniques. Model performance for REG analyses was also improved by using the average growth data across six trees, with $R^2 = 0.50$ ($P < 0.001$), compared with typical R^2 values of 0.32–0.40 for individual tree models, which reflected variability in both seasonal growth dynamics and apparent sensitivity to O_3 among trees. Both total and relative contributions of significant variables to the six-tree REG model are shown in Table 3. Predictor variables in all models included seasonal cumulative O_3 AOT_{60}, a VPD × O_3 interaction term, temperature, rainfall, and solar radiation. It should be noted that, while we identified and used AOT_{60} in our model simulations, comparable results were obtained with AOT_{40} as the descriptor of O_3 exposure. All models were based on 2567 total degrees of freedom, which contributed to a very high statistical power and a highly significant goodness of fit to selected variables.

Changes in rates of water use by trees

The changes in the amplitude of daily and seasonal stem increment patterns noted in Fig. 3 suggested that, despite rather low moisture stress levels indicated by the Palmer drought severity index (Palmer, 1965 and Table 1), study trees were episodically under water stress in 2002. Increases in both VPD and O_3 levels (Table 1) were potential contributors to observed increases in sap flow rates. Both daily maximum and 24-h average sap flow velocities were very sensitive to peak O_3 levels, and increasing sap flow rates were also reflected in decreased soil moisture in the rooting zone of sample trees (McLaughlin *et al.*, 2007). Sensitivity analyses indicated that increases in sap flow rates were most closely associated with the maximum hourly O_3 concentration on the measurement day.

Models of daily averaged sap flow velocity The influence of environmental variables describing solar radiation, VPD, temperature, rainfall, O_3, and soil moisture were evaluated as influences on daily sap flow velocity on each of six intensively monitored trees in both 2001 and 2002. As VPD also increases with increasing O_3, we developed stepwise multiple regressions to consider concurrent influences of these and other variables and to identify individual and combined variables that most significant influenced daily sap flow. Stepwise regression analysis identified maximum hourly O_3 concentrations per day (MaxHD), temperature, and soil moisture status as the most consistent and statistically significant variables affecting sap flow velocity of the six trees examined in 2002. Interestingly, solar radiation was significant for only two of six trees in stepwise regressions for 2002. By contrast, analyses with the same set of independent variables identified radiation and VPD as the most significant contributors to sap flow under the lower O_3 levels that occurred in 2001. The shift in the relative importance of environmental variables identified as significantly influencing sap flow as O_3 exposure increased from 2001 to 2002 is summarized in Table 4.

A typical stepwise regression model using the tree 'Red Oak 11' (RO11) as an example is shown in Fig. 5. This model, based on 2002 data, included terms describing temperature (24-h average), soil moisture (06:00 h value at 22 cm for RO11), and maximum hourly O_3 on the measurement day (O_3MaxHD) and took the form:

Table 4 Analyses of variables significantly affecting sap flow of six trees at the Look Rock site, suggesting increased importance of ozone (O_3) and air temperature during the 2002 season

	Significant responses			
	2001		2002	
Variable	$P \leq 0.05$	$P \leq 0.25$	$P \leq 0.05$	$P \leq 0.25$
Rain	6	0	1	1
Temperature	2	1	6	0
O_3	0	3	4	1
VPD	3	2	1	1
Radiation	5	0	2	0
Day of year	2	1	2	1

Data are for six trees in both 2001 and 2002.
VPD, vapor pressure deficit.

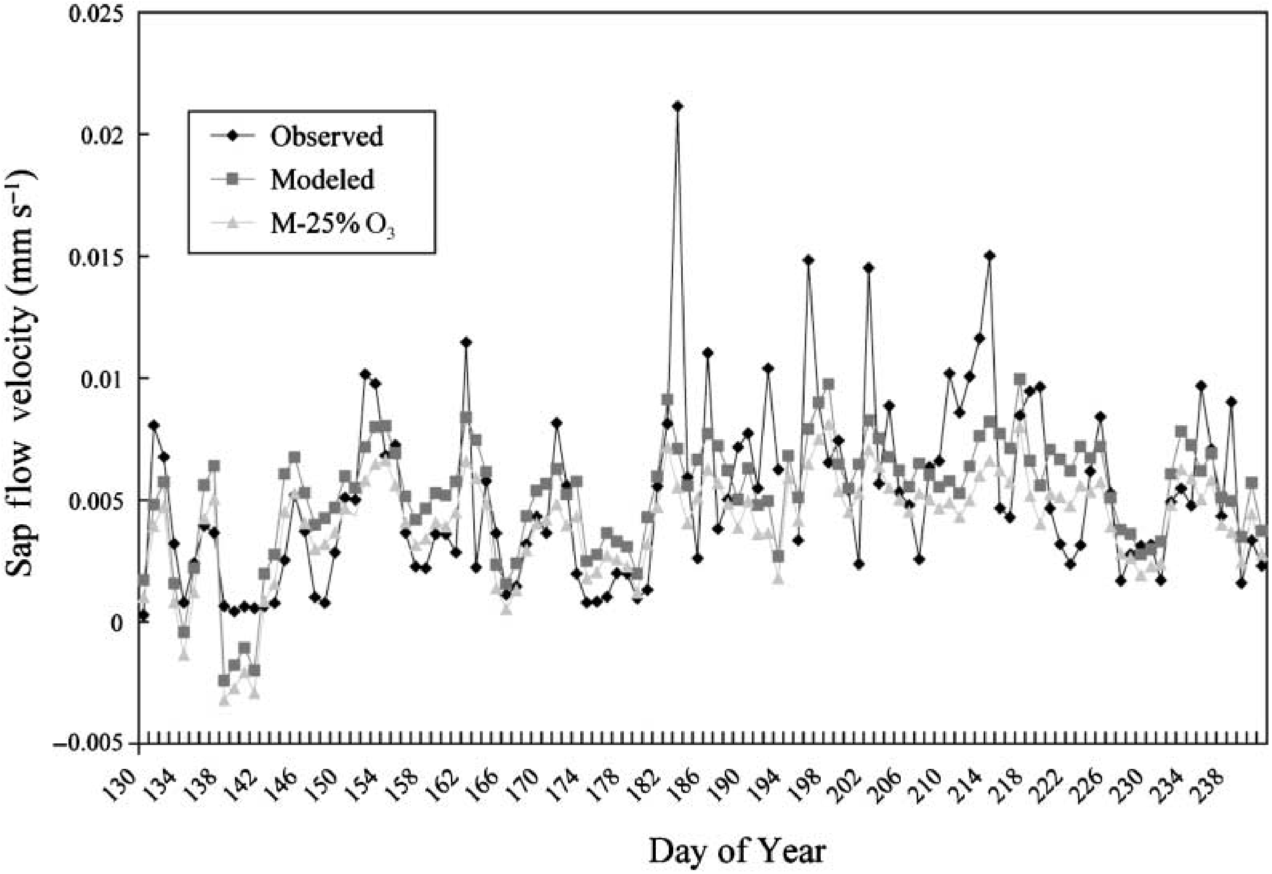

Fig. 5 An empirical model of the effects of environmental variables on daily averaged sap flow velocity for the tree 'Red Oak 11' at Look Rock was developed for the 2002 growing season. Maximum hourly ozone (O_3) exposure level, average daily temperature, and soil moisture status at 22 cm (SMRO11) were identified as significant contributors to daily variability in sap flow. This figure compares actual data, a model simulation of those data, and a model simulation with the O_3 level (maximum hourly) reduced by 25% each day.

$$\text{Sap flow velocity} = -0.0067 + 0.0000639\ (\text{O}_3\text{MaxHD}) + 0.000527\ (\text{temp}) - 0.00146\ (\text{SMRO11})$$

Model R^2 was 0.36 ($P < 0.001$ for $n = 110$) and the seasonal variations in model performance vs actual sap flow for this tree are shown in Fig. 5. The seasonally averaged model estimate exceeded measured sap flow for RO11 by 3%. The coefficient of variation was lower for modeled (44%) than for measured (72%) sap flow velocity for the 2002 seasonal analysis interval. The O_3 predictor was significant at the $P \leq 0.01$ level. To estimate the potential influence of a change in O_3 exposure levels on tree water use, we reduced maximum daily ozone exposure (O_3MaxHD) levels in the model by 25% below actual 2002 levels. The average reduction in sap flow estimated by this 25% reduction in O_3 levels was 21% (Fig. 5). Model performance data and the identity of significant variables influencing sap flow for both stepwise and best regression analyses of each of the six trees examined were determined (Supplementary Material, Table S7). These analyses confirmed the strong and specific influence of O_3 exposure as a component of environmental variables that influenced the sap flow velocity of the subject trees.

Discussion

Implications of changes in tree growth

Our data from three different forested sites indicate that growth effects associated with high ambient O_3 exposures of mature forest trees can be large (30–50%) on average across multiple species for individual sites. They can also be expected to vary significantly among species and between years. Growth reductions were also found to be accompanied by changes in patterns of water use that would likely amplify the growth effects in drier years. Measurements with a network of manual bands revealed patterns of growth rate reductions that were closely associated with the timing of high O_3 exposures within years. These patterns also related well to the differences in seasonal exposure among years. Time series analyses for intensively monitored trees at one of the three sites identified O_3 exposure (AOT_{60}) both alone and in combination with VPD as a significant contributor to the loss in stem increment. As expected, the daily stem increment patterns were highly influenced by diurnal patterns of radiation, VPD and precipitation. Smaller effects of O_3 on diurnal patterns became increasingly significant as an accumulative reduction in growth levels occurred as the season progressed. The cumulative negative effects of O_3 on growth at our study sites in 2002 significantly exceeded generalized estimates of O_3-induced growth limitations previously reported for predominantly saplings and seedlings in the USA and Europe (Broadmeadow, 1998; Chappelka & Samuelson, 1998). It is important to note that our reference to cumulative effects does not infer that we detected increasing sensitivity of growth to O_3 exposure over time, rather that the hourly and daily effects detected with the time series analyses were additive and were integrated over time to produce larger seasonal growth effects. Differential sensitivity over time was not evaluated in these studies, but can be examined through analysis of sequential intervals with time series techniques.

The likely mechanisms of reduced growth rates at daily and seasonal time scales examined here include both chronic alterations in photosynthetic production and carbon allocation and increased levels of water stress. Cumulative reductions in gross photosynthetic rates (P_g) of 40-yr-old ponderosa pine caused by increases in O_3 exposure, including amplification of drought effects, have been documented at ambient exposure levels in California using GAM procedures (Grulke *et al.*, 2002b). In that study, differences in P_g were apparent at exposure levels ranging from 120 to 200 ppm h (24hSumO60; defined as the sum of all ozone exposures at concentrations of $\geq$ 60 ppb) among sites. By comparison, the LR site, using the same O_3 exposure metric, experienced 234 ppm h (ul l^{-1} h) of O_3 exposure over the 15 April–15 October interval compared with 288 ppm h at the most polluted California site over the same time interval. Weinstein *et al.* (2001) predicted a 42% decline in yellow poplar growth in the Great Smoky Mountains at a seasonal O_3 exposure level (24hSumO60) of 105 ppm h. (A 42.4% growth reduction was projected at a 50% increase in O_3 exposure above 1992 levels measured at 70 ppm h at nearby Cove Mountain in the Great Smoky Mountains.) Their estimates were based on scaling up leaf-level photosynthesis measurements at the TC site, one of our study locations, using a tree growth model parametrized mainly from seedling data.

In 2002, trees at LR had experienced 113 ppm h of exposure (24hSumO60) at around DOY 180, the time at which growth appeared to most consistently differentiate from 2001 levels (Figs 3, 4). Growth patterns observed in this study (Fig. 3) were indicative of responses to episodic stress associated with identifiable exposure events. These episodes occurred relatively early in the growing season and were followed by resumption of near-normal growth rates for some species and diminished growth capacity for others. A slower recovery of growth rates to 2001 levels was particularly apparent in 2003 at the LR site (see Fig. 2a and Table 2), which experienced the highest O_3 exposures among sites in 2002.

Implications of changes in sap flow velocity

The large interannual changes in growth rates observed in this study in 2002 are likely attributable in part to inherently greater sensitivity of mature trees to O_3 and in part to interacting effects of O_3 and moisture stress under field conditions, particularly in 2002. Enhancement of the amplitude of the daily cycles of daily water loss and recovery observed in this study following the highest O_3 exposures suggested interference by O_3 in whole-tree water balance. Cell division and expansion are among the most sensitive physiological processes to water stress and are affected at much lower stress levels than is photosynthesis (Hsaio *et al.*, 1976). Thus, water stress effects under these conditions may in fact be more important in limiting growth than reductions in photosynthetic production (Luxmoore *et al.*, 1997).

Potential enhancement of daily drought cycles for foliage of Norway spruce trees by O_3 exposure has been reported as a consequence of reduced stomatal control of water loss (Maier-Maercker, 1997). In addition to increased daytime transpiration following O_3 exposure, lack of stomatal closure at night may also have increased total water loss rates as well as O_3 uptake. Night-time stomatal conductance in response to increasing O_3 levels has been found to range from 10–20% of daytime values in mature ponderosa pine (Grulke *et al.*, 2004) to 50% in birch (*Betula pendula*) cuttings (Matyssek *et al.*, 1995). The increases in loss of soil moisture from soils at this site in 2002, as discussed in the following study (McLaughlin *et al.*, 2007), support the significance of increasing canopy conductance to levels of available soil moisture at this site during 2002.

The uncertainties imposed by inadequate understanding of the nature and direction of stomatal responses to ambient O_3 can lead to significant uncertainties in efforts to model and predict forest responses to both O_3 and climate (Grulke *et al.*, 2002a; Paoletti & Grulke, 2005). Ollinger *et al.* (1997), for example, assumed that O_3 induced stomatal closure in their simulations of regional changes in forest productivity, but noted that increased rather than decreased stomatal conductance would have increased the projected negative effects of O_3 on forest productivity by over 50% (from −7 to −11% annual loss). Hanson *et al.* (2005), by contrast, projected slightly decreased transpiration of deciduous forests based on mechanistic estimates of the combined effects of increasing CO_2, temperature, and O_3. The results upon which we have reported here indicate that increased rather than decreased water use should be expected in a future climate that combines a warmer temperature with either stable or increasing O_3 exposure conditions. Observed responses of both growth and water use varied within and between species and were significantly influenced by the differences in O_3 exposure levels within and between years.

Despite interspecific and interannual variations in the magnitude of impacts in individual years, related findings of another study (McLaughlin *et al.*, 2007) indicated that changes in patterns of water use were detectable at the forest landscape level in observed changes in stream-flow within the study region over longer time frames. Thus, generalizations about mechanisms of response derived from the study of a relatively few individual forest trees have been supported by the observed importance of similar independent variable subsets identified in independent analyses of responses measured at larger scales. These larger scale measurements integrate responses across a broader cross-section of species and larger spatial scales.

Implications for integrated forest responses

Modeling studies based on physiological data should ultimately provide additional insights in determining critical O_3 exposure levels and key physiological changes that will influence plant responses under future climatic conditions (Fuhrer *et al.*, 1997). Because O_3 exposure may cause changes in stomatal relationships to light, VPD, and internal CO_2 concentrations (Grulke *et al.*, 2002a) as well as changes in internal metabolic pathways that affect carboxylation efficiency (Wallin & Skarby, 1992), it is very important that such data be obtained in the field under realistic environmental conditions and under O_3 exposure conditions (both level and variability) that reflect ambient and near-future O_3 concentrations. Clearly, very high levels of VPD, soil moisture stress, and O_3 in various combinations can cause severe plant stress responses. However, understanding and predicting ambient O_3 effects on forest growth and water use at low to moderate levels of stress will likely provide more valid estimates and understanding of the real potential of O_3 to amplify longer term effects of climate change on regional forest systems.

In conclusion, our results indicate that O_3 episodically increases the rate of water use and limits growth of mature forest trees within the study region. Measurements of stem increment at scales ranging from hourly to weekly indicate that disruptions in growth processes were related to apparent increases in water stress and were occurring in conjunction with episodic increases in O_3 concentration during the growing season. Relatively small effects on stem increment exerted at the hourly scale had a much larger accumulative effect on total stem growth over the entire growing season. Our measurements indicate that peak hourly O_3 exposures play a dominant role in influencing rates of water loss through transpiration and can delay the recovery of stem expansion following periods of high moisture demand, which frequently accompany higher O_3 exposures. Such empirically derived relationships point to the need for more detailed process studies aimed at improved understanding of the mechanisms underlying these responses. Our measurements and models in these necessarily smaller scale intensive measurement systems have highlighted some significant relationships between O_3 concentrations, climatic variables, and tree growth and water use patterns. However, it will ultimately be important to test these relationships across a broader range of sites and a wider range of environmental conditions.

The responses measured here have significant implications for forest ecosystem processes and function in a warming global climate. Of primary concern are alterations in competitive relationships among species and size classes of vegetation. In addition, alterations of soil moisture and chemistry resulting from higher rates of transpiration and increased water removal by mature forest trees may also influence the hydrology of forest watersheds, as suggested by the analyses of McLaughlin *et al.* (2007). At this stage, we believe that we have provided evidence and some useful methodology that may prove helpful in beginning to evaluate these important relationships more thoroughly.

Acknowledgements

This research was supported primarily by funding from the US Forest Service Southern Global Change Research Program under a Collaborative Agreement with the University of Tennessee and additionally through a research grant from the National Park Service with Auburn University. The professional and administrative support of Steve McNulty (USFS), Jim Renfro and Tammie Maniero (NPS), and Art Chappelka (Auburn University) and the technical support of Jeff Riggs (Oak Ridge National Laboratory) are gratefully acknowledged by the authors. Oak Ridge National Laboratory is managed by UT-Battelle, LLC, for the DOE under contract DE-AC05-00OR22725.

References

Broadmeadow M. 1998. Ozone and forest trees. *New Phytologist* **139**: 123–125.

Chambers JM, Hastie TJ. 1992. *Statistical models in S.* Pacific Grove, CA, USA: Wadsworth & Brooks.

Chameides WL, Kasibhata Yienger J, Levy Y. 1994. The growth of continental-scale metro-agroplexes, regional ozone pollution, and world food production. *Science* **264**: 74–77.

Chappelka AH, Samuelson L. 1998. Ambient ozone effects on forest trees of the eastern United States: a review. *New Phytologist* **139**: 91–108.

Clearwater MJ, Meinzer FC, Andrade JL, Goldstein G, Holbrook NM. 1999. Potential errors in measurement of nonuniform sap flow using heat dissipation probes. *Tree Physiology* **19**: 681–687.

Downes G, Beadle C, Worledge D. 1999. Daily stem growth patterns in irrigated *Eucalyptus globules* and *E. nitrens* in relation to climate. *Trees* **14**: 102–111.

Felzer B, Kicklighter D, Melillo J, Wang C, Zhuang Q, Prinn R. 2004. Effects of ozone on net primary production and carbon sequestration in the conterminous United States using a biogeochemistry model. *Tellus* **56B**: 230–248.

Finlayson-Pitts BJ, Pitts JN Jr. 1993. Atmospheric chemistry of tropospheric ozone formation: Scientific and regulatory implications. *Air and Waste* **43**: 1091–1100.

Fuhrer J, Skarby L, Ashmore MR. 1997. Critical levels for ozone effects on vegetation in Europe. *Environmental Pollution* **97**: 91–106.

Granier A. 1987. Evaluation of transpiration in a Douglas-fir stand by means of sap flow measurements. *Tree Physiology* **3**: 309–320.

Grulke NE, Alonso R, Nguyen T, Cascio C, Dobrowolski W. 2004. Stomata open at night in pole-sized mature ponderosa pine: implications for O_3 exposure metrics. *Tree Physiology* **24**: 1001–1010.

Grulke NE, Preisler HK, Fan CC, Retzlaff WA. 2002a. A statistical approach to estimate O_3 uptake of ponderosa pine in a Mediterranean climate. *Environmental Pollution* **119**: 163–175.

Grulke NE, Preisler HK, Rose C, Kirsch J, Balduman L. 2002b. O_3 uptake and drought stress effects on carbon acquisition of ponderosa pine in natural stands. *New Phytologist* **154**: 621–631.

Hanson PJ, Weltzin JF. 2000. Drought disturbance from climatic change: response of United States forests. *Science of the Total Environment* **262**: 205–220.

Hanson PJ, Wullschleger SD, Norby RJ, Tschaplinski TJ, Gunderson CA. 2005. Importance of changing CO_2, temperature, precipitation, and ozone on carbon and water cycles of an upland-oak forest: incorporating experimental results into model simulations. *Global Change Biology* **11**: 1402–1423.

Hastie TJ, Tibshirani RJ. 1990. *Generalized additive models.* London, UK: Chapman & Hall.

Hsaio TC, Acevedo E, Fereres E, Henderson DW. 1976. Stress metabolism. Water stress, growth, and osmotic adjustment. *Philosophical Transactions of the Royal Society of London* **273**: 479–500.

International Panel on Global Climate Change. 2001. *Technical summary.* Report of the Panel on Climate Change. Geneva, Switzerland: IPCC Secretariat.

Keller T, Hasler R. 1984. The influence of a fall fumigation with ozone on stomatal behavior of spruce and fir. *Oecologia* **64**: 284–286.

Lee WS, Chevone BI, Seiler JR. 1990. Growth and gas exchange of loblolly pine seedlings as influenced by drought and air pollutants. *Water, Air, and Soil Pollution* **51**: 105–116.

Luxmoore RJ. Pearson SM, Tharp ML, McLaughlin SB. 1997. Scaling up physiological responses of loblolly pine to variation in ozone and rainfall. In: Mickler RA, Fox S, eds. *The productivity and sustainability of southern forest ecosystems in a changing environment.* New York, NY, USA: Springer, 407–428.

Maier-Maercker U. 1997. Experiments on the water balance of individual attached twigs of *Picea abies* (L.) Karst. in pure and ozone-enriched air. *Trees* **11**: 229–239.

Maier-Maercker U. 1998. Predisposition of trees to drought stress by ozone. *Tree Physiology* **19**: 71–78.

Maier-Maercker U, Koch W. 1992. The effect of air pollution on the mechanism of stomatal control. *Trees* **7**: 12–25.

Mansfield T. 1998. Stomata and plant water relations: does air pollution create problems? *Environmental Pollution* **101**: 1–11.

Matyssek R, Gunthardt-Goerg M, Maurer S, Keller T. 1995. Nighttime exposure to ozone reduces whole-plant production in *Betula pendula. Tree Physiology* **15**: 159–165.

McAinish MR, Evans NH, Montgomery LT, North KA. 2002. Calcium signaling in stomatal responses to pollutants. *New Phytologist* **153**: 441–447.

McLaughlin SB, Downing DJ. 1995. Interactive effects of ambient ozone measured on mature forest trees. *Nature* **374**: 252–257.

McLaughlin SB, Downing DJ. 1996. Interactive effects of ambient ozone and climate measured on mature loblolly pine trees. *Canadian Journal of Forest Research* **26**: 670–681.

McLaughlin SB, Wullschleger SD, Sun G, Nosal M. 2007. Interactive effects of ozone and climate on water use, soil moisture content and streamflow in a southern Appalachian forest in the USA. *New Phytologist* **174**: 125–136.

McLaughlin SB, Percy K. 1999. Forest health in North America: some perspectives on actual and potential roles of climate and air pollution. *Water, Air, and Soil Pollution* **116**: 151–197.

McLaughlin SB, Shortle WC, Smith KT. 2002. Dendroecological applications in air pollution research and environmental chemistry: Research needs. *Dendrochronologia* **20**: 133–157.

McLaughlin SB, Wullschleger SD, Nosal M. 2003. Diurnal and seasonal changes in stem increment and water use by yellow poplar trees in response to environmental stress. *Tree Physiology* **23**: 1125–1136.

Ollinger SV, Aber JD, Reich PB. 1997. Simulating ozone effects on forest productivity: interactions among leaf and stand-level processes. *Ecological Applications* **7**: 1237–1251.

Ollinger SV, Aber JD, Reich PB, Freuder RA. 2002. Interactive effects of nitrogen deposition, tropospheric ozone, elevated CO_2, and land use history on the carbon dynamics of northern hardwood forests. *Global Change Biology* **8**: 545–562.

Nosal M. 2007. Classification of sums of squares in Generalized Additive Models (GAM). *Proceedings of the 6th Annual Hawaii International Conference on Statistics, Mathematics and Related Fields, Honolulu, HA, USA*, 961–967.

Palmer WC. 1965. *Meteorological drought.* Washington, DC, USA: US Weather Bureau.

Paoletti E, Grulke NE. 2005. Does living in elevated CO_2 ameliorate tree responses to ozone? A review on stomatal processes. *Environmental Pollution* **137**: 483–493.

Pearson M, Mansfield TA. 1993. Interacting effects of ozone and water stress on the stomatal resistance of beech (*Fagus sylvatica* L.). *New Phytologist* **123**: 351–358.

Peterson DL, Silsbee DG, Poth M, Arbough J, Biles FE. 1995. Growth responses of big-cone Douglas fir (*Pseudotsuga macrocarpa*) to long term ozone exposure in southern California. *Journal of Waste and Air Management Association* **45**: 36–45.

Rawlings JO. 1988. *Applied Regression Analysis.* Pacific Grove, CA, USA: Wadsworth & Brooks/Cole, 183.

Reich PB, Lassoi JP. 1984. Effects of low level ozone exposure on leaf diffusive conductance and water use efficiency caused be air pollutants. *Plant, Cell & Environment* **7**: 661–668.

Robinson MF, Heath J, Mansfield TA. 1998. Disturbances in stomatal behavior caused by air pollutants. *Journal of Experimental Botany* **49**: 461–469.

Samuelson L, Kelly JM. 2001. Scaling ozone effects from seedlings to forest trees. Tansley Review 21. *New Phytologist* **149**: 21–41.

Skarby L, Ro-Poulsen H, Wellburn FAM, Sheppard LJ. 1998. Impacts of ozone on forests: a European perspective. *New Phytologist* **139**: 109–122.

Skarby L, Troeng E, Bostrom C-A. 1987. Ozone uptake and effects on transpiration, net photosynthesis, and dark respiration. *Forest Science* **33**: 801–808.

Thompson DJ. 1995. The seasons, global temperature, and precession. *Science* **268**: 59–68.

Tingey DT, Hogsett WE. 1985. Water stress reduces ozone injury via a stomatal mechanism. *Plant Physiologist* **77**: 944–947.

Venables WN, Ripley BD. 1999. *Modern applied statistics with S-Plus*, 3rd edn. New York, NY, USA: Springer Verlag.

Wallin G, Skarby L. 1992. The influence of ozone on the stomatal and non-stomatal limitation of photosynthesis in Norway spruce, *Picea abies* (L.) Karst exposed to soil moisture deficit. *Trees* **6**: 128–136.

Weinstein DA, Gollands B, Retzlaff WA. 2001. The effects of ozone on lower slope forest of the Great Smoky National Park: Simulations linking individual tree model to a stand model. *Forest Science* **47**: 29–42.

Wullschleger SD, Hanson PJ, Todd DE. 2001. Transpiration from a multispecies forest as estimated by xylem sap flow techniques. *Forest Ecology and Management* **143**: 205–213.

Wullschleger SD, McLaughlin SB, Ayers MP. 2004. High resolution analysis of stem increment and sap flow for loblolly pine trees attacked by southern pine beetle. *Canadian Journal of Forest Research* **24**: 2387–2393.

Zahner R, Saucier JR, Myers RK. 1989. Tree-ring model interprets growth decline in natural stands of loblolly pine in the southeastern United States. *Canadian Journal of Forest Research* **19**: 612–621.

Zweifel R, Hasler R. 2001. Dynamics of water storage in mature subalpine *Picea abies*: temporal and spatial patterns of change in stem radius. *Tree Physiology* **21**: 561–569.

Zweifel R, Item H, Hasler R. 2001. Link between diurnal stem radius changes and tree water relations. *Tree Physiology.* **21**: 869–877.

Zweifel R, Zimmermann L, Newbery DM. 2005. Modeling tree water deficit from microclimate: an approach to quantifying drought stress. *Tree Physiology* **25**: 147–156.

Supplementary Material

We have provided supplementary discussion of analytical approaches in Tables S1–S10 and Figs S1 and S2 in support of the analyses presented in this manuscript. This material identifies the variables examined in development of empirical models of growth increment and sap flow and provides additional documentation of the cross-correlations and serial correlation among data streams examined. In addition, model parameters and metrics of performance for growth increment and sap flow of each of the six intensively monitored trees are presented.

The following supplementary material is available for this article online:

Fig. S1 Rainfall totals for all three years were similar, but intraseasonal signals, such as a mild drought that began around day of year (DOY) 200 in 2002, were readily apparent. LR, Look Rock, TN, USA.

Fig. S2 Comparative seasonal patterns of stem growth of red oak (*Quercus rubra*) were measured with manual dendrometers in (a) 2001–2003 at Look Rock, TN, USA and in (b) 2002 and 2003 at Twin Creeks. Data points represent averages of measurements for three trees at each date.

Table S1 Definition of variables used in analyses of hourly stem increment data for six trees from Look Rock, TN, USA, during the 2002 growing season

Table S2 Comparison of model performance characteristics and variables identified as statistically significant in contributing to hourly stem increment values for six individual trees at Look Rock, TN, USA in 2002

Table S3 Pearson correlation coefficients determined for over 2500 hourly values of variables identified in the six-variable best regressions for six trees for which hourly increments were measured at Look Rock, TN, USA in 2002

Table S4 Partial correlation coefficients for each of the six best regression subsets identified as significantly influencing hourly radial increment of the tree 'Red Oak 30' at Look Rock, TN, USA during the 2002 growing season

Table S5 Summary of best multiple regressions for hourly radial increments of each of five mature forest trees at Look Rock, TN, USA during the 2002 growing season

Table S6 Definitions of variables used in analysis of environmental influences on daily sap flow rates of six intensively monitored forest trees at Look Rock, TN, USA in 2002

Table S7 Stepwise model performance and variables included in six-variable best regression models of sap flow of six individual trees at Look Rock, TN, USA in 2002

Table S8 Pearson correlation coefficients associated with sap flow velocity in the tree 'Red Oak 11' and the predominant independent variables identified in Table S7

Table S9 Partial correlation coefficients (*R*) of independent variables influencing daily mean sap flow velocity of the tree 'Red Oak 11' (see Fig. 5 and Table S7)

Table S10 Serial autocorrelation within time-lagged data series used in sap flow velocity analyses

This material is available as part of the online article from: http://www.blackwell-synergy.com/doi/abs/10.1111/j.1469-8137.2007.02018.x
(This link will take you to the article abstract.)