Interactive Effects of Ambient Ozone and Climate Measured on
Growth of Mature Loblolly Pine Trees

S. B. McLaughlin*

Environmental Sciences Division

Oak Ridge National Laboratory¹

P.O. Box 2008

Oak Ridge, TN 37831-6034

Telephone: 615-574-7358/Fax: 615-576-9939

and

D. J. Downing

Engineering Physics and Mathematics Division

Oak Ridge National Laboratory

P.O. Box 2008

Oak Ridge, TN 37831-6367

Telephone: 615-574-6325/Fax: 615-574-0680

MASTER

*Corresponding author.

¹Oak Ridge National Laboratory is managed by Martin Marietta Energy Systems Inc. for the U.S.,
Department of Energy under contract DE-ACO5-84OR21400.

ABSTRACT

Analysis of the seasonal growth patterns of mature loblolly pine trees over the interval 1988-1993 has provided the first direct measurement of reductions of stem growth of large forest trees by ambient ozone. Patterns of stem expansion and contraction of 34 trees were examined in eastern Tennessee using serial measurements with sensitive dendrometer band systems. Study sites varied in soil moisture, soil fertility, and stand density. Levels of ozone, rainfall, and temperature varied widely over the six year study interval. Regression analysis identified statistically and biologically significant influences of ozone on stem growth. Acting either individually or in interaction with high temperature and moisture stress, higher levels of ozone were associated with reduced stem expansion of individual trees within and across years. Observed responses to ozone were relatively rapid, differed widely among trees, and across years, and were significantly amplified by low soil moisture and high air temperatures. Both short term responses, clearly tied to changing stem water status, and longer term cumulative responses were identified. These data indicate that relatively low levels of ambient ozone can significantly reduce growth of mature forest trees and that interactions between ambient ozone and climate are likely to be important modifiers of future forest growth and function. Additional studies of mechanisms of short term response and inter species comparisons are clearly needed.

DISCLAIMER

This report was prepared as an account of work sponsored by an agency of the United States Government. Neither the United States Government nor any agency thereof, nor any of their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by trade name, trademark, manufacturer, or otherwise does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or any agency thereof. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or any agency thereof.

DISCLAIMER

Portions of this document may be illegible in electronic image products. Images are produced from the best available original document.

INTRODUCTION

The health of southeastern pine forests has been a subject of great interest and substantial controversy since significant growth declines were detected in unmanaged pine stands by U.S. Forest Service surveys in the mid 1980s (Sheffield et al., 1985 and Sheffield and Cost, 1987). Modeling studies focusing on the possible role of stand competition and climate on observed changes found that most of the growth reduction occurring in recent decades was not explained by natural factors (Zahner et al., 1990 and Bechtold et al., 1991). Air pollution, particularly ozone has been suggested as a possible causative factor, based on regional patterns of response of large trees and the known sensitivity of tree seedlings to ozone exposure (Zahner et al., 1989; McLaughlin, 1985; Shriner et al., 1990; and McLaughlin, 1994). Dissenting opinions on the significance and possible causes of observed growth trends have been registered based on instability of growth trends of some individual trees (Van Deusen, 1992) and the mensurational techniques (Zeide, 1992) used to establish pre-decline reference growth rates.

Diagnosis of the causes of the apparent growth decline has been difficult because of the lack of obvious visual changes in regional forest condition (Barnard et al., 1990). Gradients in visual injury of foliage along strong ozone exposure gradients aided in the classical diagnosis of ozone as a primary contributor to the decline of pines in the San Bernardino Mountains of California in the 1970s (Miller and McBride, 1975). Such gradients have not been apparent on southern pines under the generally lower ozone concentrations (Lefohn and Lucier, 1991) of the Southeast.

The approximately 25 million hectares of southern pine forests, with annual stemwood sales of \$6 billion (USDA, 1988), have been the basis for a substantial research effort aimed at quantifying and understanding the causes of apparent growth losses within the region. The National Acid Precipitation Assessment Program (Hertel and McKinney-McNeal, 1991) sponsored much of this

1 research to evaluate the possible roles of tropospheric ozone and acid deposition. The resultant
2 series of controlled studies established that elevated levels of ozone can significantly alter the
3 physiology and growth of seedling and sapling trees of three regionally important timber species:
4 loblolly, slash, and shortleaf pine (McLaughlin et al., 1994; Shelburne et al., 1990; and Dean and
5 Johnson, in press).

6 A major concern with most controlled studies to date has been the significant uncertainty
7 involved in extrapolating data derived from seedling and sapling exposures to elevated ozone in
8 chambers to expected responses of mature trees under ambient ozone levels in the field. Principal
9 points of concern include differences in metabolism between juvenile and mature trees, the use of
10 ozone exposure levels significantly above current ambient exposure regimes, and chamber-induced
11 modifications in plant growing environment, which may modify plant responses. In this study, we
12 report results of six years of field research in which repeated measures and statistical analyses of stem
13 expansion patterns have been used to identify growth responses of mature trees to ambient levels of
14 ozone in an undisturbed forest environment.

16 METHODS

17
18 We have evaluated ozone impacts on mature forest trees by measuring the influence of
19 fluctuations in levels of ambient ozone and other environmental variables on the short and
20 intermediate term dynamics of stem growth of individual trees. In this study we distinguish between
21 stem expansion, which includes both positive and negative changes measured over short time intervals
22 (typically 1-7 day intervals) and stem growth measured over longer time intervals. Stem expansion
23 (and contraction) is clearly related to longer term growth, and it was for that reason that we have
24 evaluated this parameter as a growth index. To characterize seasonal growth patterns, short term

1 changes in stem circumference of 28-34 mature trees were initially evaluated over five growing
2 seasons (May-October, 1988-1992) using a sensitive and inexpensive dendrometer band system.

3 The dendrometer bands used in these studies were 25 mm wide and 0.12 mm thick. They
4 were marked at two reference points near adjoining ends, and placed around the stem at a height of
5 approximately 1.5 m. Spring tension was approximately 2 kg. Bands were measured manually with
6 an electronic caliper approximately twice weekly during 1988, 1989, 1991, and 1992, and once every
7 1-2 weeks during 1990. Measurement precision on duplicate measurements was typically ± 0.02 mm.
8 Band measurements were initiated on 28 trees in 1988 and 6 trees along the forest edge were added
9 in 1989. The summed band readings over the initial 5 year study interval were on the average within
10 0.75 cm of the calculated change in circumference estimated with a standard diameter tape for the
11 five year remeasurement. During 1993, an exceptionally dry year in which less frequent
12 measurements were made, general growth trends were characterized for annual scale comparisons,
13 but these data were used only for annual scale analyses.

14 Daily rainfall and hourly temperature data collected from the nearby Walker Branch
15 Watershed (2-4 km from the study sites) were provided by the National Oceanic and Atmospheric
16 Administration. Hourly ozone data collected from a rural station approximately 30 km from the study
17 site were provided by the Environmental Protection Agency. Weekly values of a moisture supply
18 index derived for eastern Tennessee were obtained from the National Weather Service.

19 The moisture supply index (MSI) used in these studies is merely a weekly derivation of the
20 Palmer Drought Severity Index (Palmer, 1965), a monthly indicator used for studies of forest growth
21 responses to climate. MSI is a calculated index of the balance between water supply, water storage
22 in soil, and water demand due to evaporation and runoff. Values between +1 and -1 are considered
23 normal, while index values less than -2.0, -3.0, or -4.0 are indicative of moderate, severe, and extreme,

1 drought, respectively, on this log scale index. Soil moisture data were collected at soil depths of
2 15 cm and 30 cm from each stand using Time Domain Reflectometry (Topp, 1987).

3 Sampled trees represented a wide variety of stand conditions including stand density, soil
4 fertility, and soil moisture as shown in Table 1. All trees were in the 50-90 year age range and were
5 a part of the dominant overstory canopy. Regression and correlation analyses were used to evaluate
6 growth response to inter and intra-annual variations in ozone, rainfall, air temperature, and the
7 weekly index of moisture stress.

8 Our analyses of these data were directed toward determining which environmental variables
9 most significantly influenced short term fluctuations in stem circumference increment and to explore
10 interactions among these factors across yearly time scales. Visual examination of temporal patterns
11 of stem expansion in relationship to fluctuating environmental variables was used to evaluate
12 candidate variables and time scales for analyzing the influence of environmental variables on the
13 growth process. Subsequent statistical analyses were applied at three levels. First, short-term
14 fluctuations in stem expansion of each tree were examined in response to a wide range of predictor
15 variables describing rainfall, temperature, and exposure dose for each of 5 years for which short term
16 (≤ 3 day) data were available. Second, analyses of weekly fluctuations in stem expansion were
17 conducted using selected indicator variables expressed on a weekly scale. A weekly indicator of soil
18 moisture stress was added at this stage and stepwise regression analysis was performed to identify key
19 interactions and their interannual variability. Finally, a growth response model was developed around
20 the entire 1988-1992 data set to quantify influences of interacting environmental variables on stem
21 growth. Annual growth rates were then evaluated in relationship to ozone exposure dose across six
22 years to test the significance of previously selective indicator variables on annual stem growth.

23 Initial screening analyses were performed at the individual tree level by first removing the
24 linear time-based trends from each seasonal growth curve and then evaluating the strength and

1 consistency of partial correlation coefficients (Johnson and Wichern, 1988) of 27 variables describing
2 ozone exposure dose, rainfall, and temperature. Variables included combinations of daily 7 h maxima,
3 as well as threshold doses accumulating exposures (part per billion hours (ppbh) at and above 40 ppb,
4 60 ppb, and 80 ppb. An additional variable, W126, a sigmoidal weighing function was used. The
5 W126 function uses 0.04 ppm as a lower threshold for significant exposure and increases the weights
6 of higher concentrations exposures, up to a maximum weightings at 100 ppb. It was described and
7 evaluated by Lefohn et al. (1992) for loblolly pine seedling responses to ozone in controlled studies.
8 Delayed responses were considered by including 3 and 7 day lagged responses to temperature, rainfall,
9 and ozone as well as 23 different formulations of ozone dose.

11 RESULTS

13 The 1988-1992 study interval provided an excellent range of test conditions with respect to
14 both environmental variables and associated stem growth (Table 2). In 1988, ozone exposure was
15 the highest of the past 15 years (Lefohn and Lucier, 1991) and was coupled with warm, dry
16 conditions. By contrast, 1989 provided the lowest ozone exposure of recent years with relatively
17 moist, cool conditions. The remaining years were well distributed between these extremes with
18 respect to ozone, temperature, and moisture availability. Large differences in mean levels of stem
19 growth occurred between years, trees and sites. Differences among wet and dry sites ranged from
20 a minimum of 27% in 1989 to a maximum of 50% in 1988. Mean circumference growth across sites
21 varied by 75% across years from a minimum of 8.8 mm in 1988 to a maximum of 15.4 mm in 1989.

22 Examination of the consistency of responses across stands indicated a high degree of
23 consistency among trees in response to fluctuating environmental conditions. This was particularly
24 true in 1992, a relatively low stress year when growth slowed significantly twice during the early

1 season (days 130-145 and days 175-185, Figure 1). Trees on poorer quality sites (Stands A and B)
2 had a lower overall growth rate, but similar within-season growth dynamics. Similarly, the twofold
3 difference in stand stocking levels between Stands A and B, made no difference in growth rates or
4 patterns. The temporal overlay of fluctuating ozone levels in 1992 indicated that high ozone levels
5 occurred in association with both periods of slowed growth.

6 By contrast, in 1988, high ozone exposure accompanied moderate drought during the spring
7 and early summer. While still responsive to short term environmental variables, trees on the drier
8 upland sites (stands A and B) experienced stem shrinkage during this unfavorable period while those
9 on wetter sites grew steadily, albeit slowly (Figure 2). Differences between faster and slower growing
10 trees developed earliest on the poor quality site and at the same time as ozone exposure increased
11 significantly. Differences were at maximum approximately two weeks before the most negative values
12 of a weekly Moisture Stress Index (MSI) indicated that drought had peaked. Differences decreased
13 with decreasing ozone around Day 190 even though MSI was at its most negative value.

14 Slowdown in stem expansion was consistently associated with periods of higher ozone
15 exposure throughout the five year study. These slowdowns were sometimes accompanied by stem
16 shrinkage, as shown in Figures 1 and 2. Our analyses of statistical relationships among short term
17 fluctuations in stem circumference increment and environmental variables are presented here more
18 in an exploratory sense than a confirmatory one since normal time series criteria were not strictly met.
19 A frequent concern in sequential measurements of biological processes is the influence of serial
20 correlations among interdependent measurements. Because measurements were not always equally
21 spaced through time, time series analyses to test serial correlations could not be applied. In the
22 present application, measurements were treated as if they were independent. The least squares
23 procedures used here still provided unbiased estimates of regression parameters; however, our

1 capabilities to detect significant responses may have been somewhat reduced by serial correlations
2 among measurements over time.

3 As a result of statistical screening among variables for their strength and consistency as
4 predictors of stem expansion rate, the 3 day moving averages of temperature, rainfall, and ozone
5 exposure dose ≥ 40 ppb were selected as strongest predictor variables for growth during successive
6 3-day growth periods. Ozone or ozone temperature interactions were significantly ($p < 0.05$) and
7 inversely correlated with stem growth of many trees over most years.

8 Stepwise regression analyses (SAS, and Draper and Smith, 1981) were next applied including
9 a weekly time scale and the weekly-based moisture stress index (MSI) to determine the relative
10 importance of ozone, MSI, temperature, and rainfall across each tree for each of the 5 years. These
11 analyses highlighted the variations in relative importance of environmental variables across the five
12 year study period (Table 3). The number of trees for which direct or interactive ozone influences
13 on growth were detected was directly related to the seasonal ozone exposure, reaching a maximum
14 of 27 of 28 trees in 1988. The number of trees responding to ozone was significantly reduced during
15 the two years with lowest ozone exposure, 1989 and 1992, and temperature and moisture stress were
16 the dominant variables. Interactions between ozone and either MSI or temperature were noted on
17 4 of the 5 years.

18 The five years of data on weekly responses of stem growth to environmental variables were
19 then examined collectively to generalize responses across years. The model was developed in two
20 ways: First a generalized five year version was developed based on with modeled multiyear
21 environmental influences on high frequency departures from the 5-year average growth trend. The
22 single slope regression model had an R^2 of 0.95 and produced generally higher estimates of ozone
23 impact; however, we have presented the more conservative, and likely more precise, annual scale

model here. This version coupled modeled multiyear environmental influences with annual growth trends determined for each year as shown below, where:

$$\text{Annual Stem Growth} = S \cdot \text{Time} + 0.0036 \cdot \text{OZ} \cdot \text{MOIST} + 0.0380 \cdot \text{RAIN} + 0.0515 \cdot \text{TEMP} - 0.0000533 \cdot \text{OZ} \cdot \text{TEMP}$$

where: S = slope of the annual growth trend by year. The values ($\pm S_e$) for 1988-1992 were 0.0529 (0.005), 0.117 (0.005), 0.104(0.004), 0.098 (0.004), and 0.143 (0.004), respectively.

T = time from the beginning of the annual measurement cycle (approximately mid-May) in days

OZ = average of 3-day mean ozone exposure for all hours at or above 40 parts per billion

MOIST = Moisture Stress Index, calculated weekly. The $\text{OZ} \cdot \text{MOIST}$ coefficient had a S_e of 0.0006

TEMP = Average 3-day air temperature during the hours 0900 to 0800. The $\text{OZ} \cdot \text{TEMP}$ coefficient had a S_e of 0.000026

RAIN = Average 3-day rainfall in mm d^{-1} . The RAIN coefficient had a S_e of 0.014

Diagnostic tests of collinearity indicated that multicollinearity among variables included in this model was very low. The largest index of collinearity for included terms was 8.6, substantially below the critical value of 30 considered significant by most researchers (Belsky et al., 1980). Regression terms included were significant at the 0.05 level and the model form was consistent across the 28 trees examined in the 5-year data set. The model was highly significant ($F_{8,82} = 906$, $P = 0.0001$) and explained 99% of the variation in weekly stem expansion across the five years (see Table 4). However, as should be expected with the inclusion of time as an independent variables, nearly 98% of the total variation explained by the model was due to time trends and 1% was associated with the

1 high frequency responses which we measured at a weekly scale. Approximately 51% of the high
2 frequency variability, which remained after time trends were removed, was explained by the
3 environmental terms. Interactions between ozone and moisture stress accounted for 58% of this
4 total, while rain, temperature, and temperature * ozone interactions accounted for 25%, 12%, and
5 5% respectively.

6 Significant ozone effects were included in the model as interaction terms involving both soil
7 moisture stress and high temperatures. Estimated impact of these terms on total seasonal growth
8 were made using environmental data from each year and ranged from a maximum of 15% in 1988
9 to near zero in 1989 and 1992, as shown in Table 4. To illustrate the importance of ozone
10 interactions with climate, estimates of ozone effects under the actual conditions occurring during each
11 year as described in Table 4 were accompanied by model derived estimates of growth impacts at two
12 additional moisture stress levels: no moisture stress ($MSI = 0$) and mild stress ($MSI = -1$). In 1988,
13 the driest year, the model predicted a reduced response to a relative decrease in moisture stress at
14 $MSI = 0$. Responses increased in all other years as $MSI = 0$ represented an increase stress level
15 relative to base conditions in those relatively more moist years. At $MSI = -1$, responses were
16 typically magnified by a factor of three in all years compared to a no stress simulation.

17 These analyses, which are based on empirically derived relationships between ozone exposure
18 and climatic variables, demonstrate the potential importance of climatic interactions on ozone induced
19 limitations on annual stem growth. However, analyses of the time dependent growth trends for each
20 year identified more persistent differences apparently related to cumulative effects that were specific
21 to each year. These differences, which were not included in model estimates of short-term
22 environmental influences on growth, potentially represented the accumulative effects of the same
23 variables operating over time periods longer than the weekly time intervals used in those analyses.

1 To test the influence of ozone on the longer term trends, annual growth increments for each
2 year were evaluated in a linear regression involving annual circumference growth, MSI, and two
3 indices of ozone exposure dose (mean daily exposure hours of $O_3 > = 40$ ppb (equivalent to D340)
4 and $> = 80$ ppbh). For these analyses, annual growth and environmental data were included from
5 1993, an extremely dry year in which seasonally averaged MSI (-1.57) was comparable to the driest
6 weekly interval in 1988. Results shown in Figure 3 indicate that annual variations in ozone exposure
7 were negatively associated with annual variations in growth rate. This relationship was stronger on
8 the wet site ($R^2 = 0.71$, $P < 0.035$) than on the dry site ($R^2 = 0.40$, $P < 0.17$). Expressions of ozone
9 exposure dose based on 40 ppb and 80 ppb thresholds were equally powerful as predictors of annual
10 stem growth increment. By contrast, seasonal moisture stress index (average MSI) explained 44%
11 of the annual variance in growth on wet sites ($P < 0.15$) and even less ($R^2 = 0.17$, $P = 0.41$) on drier
12 sites.

13 Despite the higher significance of ozone dose compared to MSI in contributing to variations
14 in annual growth, it is clear that the two variables were inversely correlated and that the degree of
15 correlation was related to the time frame utilized. Weekly correlations between ozone exposure and
16 MSI were not significant for any single year; however, the correlation was highly significant when five
17 years of measurement data were combined ($R^2 = 0.46$, $P < 0.01$). Monthly data derived from all six
18 years were highly correlated ($R^2 = 0.41$, $P < 0.01$). Mean annual MSI was very highly
19 correlated with chronic exposure to ozone at concentrations > 40 ppb ($R^2 = 0.88$, $p < 0.01$) but less
20 highly correlated ($R^2 = 0.55$, ns) with higher level exposures (> 80 ppb).

21 Our statistical analyses, particularly the short term evaluations (Table 3) have used
22 responsiveness and consistency of associations of MSI, ozone, and other variables to statistically
23 examine individual and interactive effects of these variables. Consistency can also be evaluated by
24 examining relative trends in key variables during specific episodes. Such an opportunity was provided

1 during 1993 (Figure 4), when extremely dry conditions were coupled with high ozone exposure early
2 in the growing season, and rather low ozone levels late in the season. Contrasts in growth among
3 these periods and a similar dry period during 1988 (see Figure 2), are shown in Table 5. They
4 provide strong evidence that high ozone levels were the critical component of slow growth during the
5 drought episodes observed in this study. The relatively high growth rates observed in September of
6 1993, when ozone was reduced and drought was high, are an important statistic supporting attribution
7 of reduced growth of these mature loblolly pine trees to high ozone levels.

8 9 DISCUSSION

10
11 Collectively our data indicate that ambient levels of tropospheric ozone occurring in the
12 southeastern U.S. act individually and interactively with climate to significantly influence stem
13 expansion of mature loblolly pine trees. The observed responses demonstrate the cardinal requisites
14 for attributing cause and effect (Moesteller and Tukey, 1977): consistency of response to ozone
15 across trees and stands and responsiveness to fluctuating levels of ozone within and between years.
16 The remaining criterion, existence of a mechanism for observed response to ozone, is also easily
17 inferred from a variety of previous physiological and growth studies (McLaughlin, 1994).

18 Many physiological insights into growth effects of ozone on loblolly pine have been identified
19 from controlled laboratory and field studies (Sestak et al., 1991; Richardson et al., 1992; Edwards et
20 al., 1992; McLaughlin, 1994; and Taylor, 1994). Much of the evidence supports alteration of
21 carbohydrate economy as a principal mechanism of effect (McLaughlin, 1994). Among the symptoms
22 of carbohydrate alteration are reduced carbon assimilation, increased carbon loss in respiration, and
23 reduced transport of photoassimilates to root systems, all of which affect the availability of
24 carbohydrates for growth. Ozone has also been shown to reduce both the amount (Kress et al., 1992)

1 and functional efficiency of loblolly pine foliage retained during subsequent growing seasons following
2 exposure (Richardson et al., 1992 and Sestak et al., 1991), thereby providing a ready mechanism for
3 chronic effects on growth. While these studies have been conducted on seedling and saplings and
4 not mature trees, they have likely significance for mature trees as well. Recent comparisons of leaf
5 physiological responses among seedling and mature red oak trees (Hanson et. al., 1994), for example,
6 indicated that mature trees was more sensitive to ozone than seedlings.

7 The rapidity of changes in stem expansion following short-term increases in ozone exposure
8 noted in the present study suggests that ozone is altering stem water balance as well as slowing stem
9 growth. Stem shrinkage is an expected consequence of differential rates of water supply by roots and
10 water demand by foliage. Stem shrinkage as well as slowed growth is known to occur in trees under
11 water stress (Kramer and Kozlowski, 1979) as stored water is moved to areas of greatest water
12 demand. Though not well documented, water stress can apparently be increased by ozone exposure,
13 based on altered root growth or altered control of foliar water loss.

14 Reduced root growth has been the most consistent growth response of trees to ozone in
15 controlled exposure studies (McLaughlin, 1994) and would reduce the trees' capacity to resupply
16 water lost in evapotranspiration. Altered water balance is also an expected response to changes in
17 stomatal function. Increased rates of water loss in transpiration have been reported on loblolly pine
18 seedlings exposed to ozone and drought (Lee et al., 1992). In addition, large field grown spruce in
19 Europe have been found to have diminished capacity to control stomatal water loss in ambient air
20 (Maier-Marker and Koch, 1993) or ozonated air (Wallin and Skarby, 1992) compared to air purified
21 by charcoal filtration.

22 Previous modeling studies based on longer term radial growth trends provide additional
23 support for the potential significance of ozone to growth of mature forests. Zahner et al. (1989)
24 estimated that approximately 12% of the 30-50% radial growth decline observed in even-aged stands

1 of loblolly pine between 1955 and 1980 was due to climate (drought), and that nonclimatic factors
2 that were not associated with stand competition, had contributed about 2/3 of the approximately 1%
3 per year average decrease since 1950. Grissino-Mayer (1992) also found that growth of southern
4 pines was well explained by temperature and rainfall until the 1960s, when other unidentified factors
5 began to exert strong external control. In both of the above studies, regional air pollution was
6 suggested as a likely contributor to the unexplained growth reductions.

7 The use of serial measurements with dendrobands provides a sensitive tool for addressing the
8 role of multiple environmental stresses on tree growth. While our application of this technique in
9 a multiple regression approach to evaluating pollutant effects is a new approach, the concepts of
10 multivariate analysis of intraannual tree growth patterns involving the use of dendrographs were
11 introduced long ago (Fritts, 1960; 1962). Our empirical model derived from in situ data on large
12 trees, indicates that ozone can strongly influence tree growth, particularly during periods of water
13 stress and/or high temperatures. Failure to consider these interactions may lead to significant errors
14 in identifying and estimating the effects of current and future physical climate and air pollution on
15 tree growth.

16 We have also attempted to define an ozone exposure threshold for adverse effects on growth.
17 This approach evaluated the relative strengths of many different indices of exposure as predictors of
18 changes in stem expansion. A major issue in the process of quantifying the phytotoxicity of chronic
19 ozone exposure has been the relative importance of episodic high level exposures in the overall
20 exposure history (Hogsett et al., 1988; Lefohn and Foley, 1993). Some analyses of results from
21 controlled exposure studies have suggested that plant growth is influenced more by ozone
22 concentration than by total dose (EPA, 1986). Our results and those of others (Lefohn and Foley,
23 1993; Lefohn et al., 1992) suggest that total exposure dose at and above a threshold level is a
24 valuable index for evaluating growth responses of forest trees in the field. We did not exhaustively

1 test various concentration weighing functions in these analyses, but did find that the 3 day average
2 of ozone exposure hours at and above 40 ppb (D340) provided somewhat stronger relationships to
3 growth than the concentration weighted function (W126) with a similar threshold as described by
4 LeFohn et al., 1992).

5 The exposure response threshold level for mature loblolly pine identified in this study is
6 similar to the response threshold of 45 ppb (12h mean) suggested by Taylor (1994) based on
7 controlled exposures with loblolly pine seedlings. For sensitive individual trees, an even lower
8 threshold (25 ppb) was indicated, a value only 30% above the natural background level of 19 ppb
9 (Taylor, 1994). Recent studies in the Netherlands (Steingrover, 1994) found that 40 ppb was the
10 threshold for adverse physiological effects on foliage of mature Douglas fir. Responses included both
11 reduced photosynthetic rate and incomplete stomatal closure in response to moisture stress.

12 In summary, these results document for the first time growth inhibition of mature forest trees
13 that is significantly influenced by their exposure to ambient levels of ozone. The negative effects of
14 ozone were exerted most strongly under soil moisture stress (when MSI becomes negative) and
15 through negative interactions with high air temperature. These measurements on large trees
16 represent a first step in documenting the influence of ozone on water relations and growth processes
17 at the whole tree level. Documenting the specific mechanisms involved was not the focus of this
18 study, but should be addressed in future research.

19 Our measurements have demonstrated both episodic and chronic components of reduced stem
20 growth. Both aspects were influenced by ozone exposure interacting with climatic stresses. These
21 results are supported by inferential evidence from both controlled ozone exposure studies and from
22 modeling studies that have indicated that factors other than stand dynamics and climate have
23 contributed significantly to decreases in radial growth of some natural stands of southern pines during
24 recent decades.

1 This study has focused on one, commercially important species, within one region. Our
2 sampling scheme has broadened the applicability of these results by inclusion of widely varying site
3 factors and highly variable climatic conditions. However, we suggest that similar analyses of loblolly
4 and other species in other regions would be desirable in developing more refined predictive
5 capabilities for assessing regional influences of ozone on forest growth.

6 The ambient environmental conditions during the five years of this study encompassed annual
7 shifts in temperature and precipitation such as have been predicted as more normal in the decades
8 ahead (Joyce et al., 1990). In addition ozone exposure dose varied by a factor of approximately three
9 fold (1988 vs 1989) over the course of this study. It is anticipated that high regional levels of ozone
10 exposure will become more frequent in coming decades based on projected increases in NO_x
11 emissions in large agro-industrial regions of the world (Chameides et al., 1994).

12 Our research suggests that ozone is already an important part of the current chemical
13 climate. Possible increases in temperature as predicted by global scale models, particularly coupled
14 with reduced rainfall, and increased NO_x emissions will likely increase the ecological and economic
15 importance of tropospheric ozone. Increased rainfall on the other hand, could reduce ozone impacts.
16 Because of the ozone - climate interactions demonstrated in these data, we suggest that stronger
17 consideration of such interactions will be necessary in predicting the effects of both atmospheric
18 pollution and future climate change on forest growth and forest ecosystem function.

19
20

REFERENCES

- Barnard, J., Lucier, A.A., Johnson, A.H., Brooks, R.T., Karnosky, D.F., and Dunn, P.H. 1990. Chapter 16 in State of Science Changes in Forest Health and Productivity in the United States of America Technology. National Acid Precipitation Assessment Program.
- Bechtold, W.A., Ruark, G.A., and Lloyd, F.T. 1991. Changing stand structure and regional growth reductions in Georgia's natural pine stands. *For. Sci.* 37: 703-717.
- Belsky, D.A., Kuh, E., and Welsch, R.E. 1980. Regression Diagnostics. P. 105.
- Chameides, W.L., Kasibhatla, P.S., Yienger, J., and Levy, H., II. 1994. Growth of continental-scale metro-agro-plexes regional ozone pollution, and world food production. *Science* 264: 74-77.
- Dean, T.J., and Johnson, J.D. 1992. Growth response of young slash pine trees to simulated acid rain and ozone stress. *Can. J. For. Res.* 22: 839-848.
- Draper, N.R., and Smith, H. 1981. Applied Regression Analysis, 2nd Ed., Wiley.
- Edwards, N.T. 1992. Three year growth responses of *Pinus taeda* L. to simulated rain chemistry, soil magnesium status, and ozone. *Water, Air, and Soil Pollution* 63: 105-118.
- Fritts, H.C. 1960. Multiple regression analysis of radial growth in individual trees. *Forest Sci.* 6(4): 334-349.
- Fritts, H.C. 1962. The relevance of dendrographic studies to tree ring research. *Tree Ring Bulletin* 24: 9-11.
- Fritts, H.C. 1962. An approach to dendroclimatology: Screening by means of multiple regression techniques. *J. Geophys. Res.* 67(4): 1413-1420.
- Grissino-Mayer, H.D., and Butler, D.R. 1993. Effects of climate on growth of shortleaf pine (*Pinus echinata* Mill.) in Northern Georgia: A dendroclimatic study. *Southeastern Geographer* 1: 65-81.

- 1 Hanson, P.J., Samuelson, L.J., Wullschleger, S.D., Tabberer, T.A., and Edwards, G.S. 1994. Seasonal
2 patterns of light-saturated photosynthesis and leaf conductance for mature and seedling
3 Quercus rubra L. foliage: differential sensitivity to ozone expoasure. Tree Physiology (in
4 press).
- 5 Hertel, G.D., and McKinney-McNeal. 1991. The Forest Response Program. USDA Forest Service
6 Ag. Info. Bull. 622: 19.
- 7 Hogsett, W.E., Tingey, D.T., and Lee, E.H. 1988. Exposure indices: Concepts for development and
8 evaluation of their use. In Assessment of Crop Loss from Air Pollutants, W.W. Heck, O.C.
9 Taylor, D.T. Tingey (eds.), Elsevier Applied Science Publishing, London.
- 10 Johnson, R.A., and Wichern, D.W. 1988. Applied Multivariate Statistical Analysis, 2nd Ed., D.C.
11 (1965), A monthly indicator used for studies of forest growth. Prentice Hall.
- 12 Joyce, L.A., Fosberg, M.A., and Comanor, J.M. 1990. Climate Change and America's Forests.
13 USDA Gen. Tech. Rept. RM-187, 12 pp.
- 14 Kramer, P.J., and Kozlowski, T.T. 1979. Physiology of Woody Plants, Academic Press, 811 p.
- 15 Kress, L.W, Allen, H.L., Mudano, J.E., and Stow, T.K. 1992. Impact of ozone on loblolly pine
16 seedling foliage production and retention. Env. Tox. Chem. 11: 1115-1128.
- 17 Lee, W.S., Chevone, B.I., and Seiler, J.R. 1990. Growth and gas exchange of loblolly pine seedlings
18 as influenced by drought and air pollutants. Water Air and Soil Poll. 51: 105-116.
- 19 Lefohn, A.S., Shadwick, D.S., Somerville, M.C., Chappelka, A.H., Graeme Lockaby, B., and Meldahl,
20 R.S. 1992. The characterization and comparison of ozone exposure indices used in assessing
21 the response of loblolly pine to ozone. Atm. Env. 26: 287-298.
- 22 Lefohn, A.S., and Foley, J.K. 1993. Establishing relevant ozone standards to protect vegetation and
23 human health: Exposure/dose-response considerations. Air & Waste 43: 106-112.

- 1 Lefohn, A.S., and Lucier, A.A. 1991. Spatial and temporal variability of ozone exposure in forested
2 areas of the United States and Canada: 1978-1988. *J. Air Waste Mgt.* **41**: 694-701.
- 3 Maier-Maercker, U., and Koch, W. 1992. The effect of air pollution on the mechanism of stomatal
4 control. *Trees* **7**: 12-25.
- 5 McLaughlin, S.B. 1985. Effects of air pollution on forests: A critical review. *J. Air Poll. Contr.*
6 *Assoc.* **35**: 516-534.
- 7 McLaughlin, S.B. 1994. Forest declines: Some perspectives on linking processes and patterns.
8 pp. 315-338 in *Plant Responses to the Gaseous Environment*. R.G. Alscher and A.R.
9 Welburn, (eds.), Chapman and Hall.
- 10 McLaughlin, S.B., Layton, P.A., Adams, M.B., Edwards, N.T., Hanson, P.J., O'Neill, E.G., and
11 Roy, W.K. 1994. Growth responses of 53 open-pollinated loblolly pine families to ozone and
12 acid rain. *J. Env. Qual.* **23**: 247-257.
- 13 Miller, P.R., and McBride, J.R. 1975. Effects of Air Pollutants on Forests. Pp. 196-236 in
14 J.B. Mudd and T.T. Kozlowski (eds.), Academic Press.
- 15 Mosteller, F., and Tukey, J. 1977. *Data Analysis and Regression: A second Course in Statistics*,
16 Reading, MA, Addison-Wesley.
- 17 Palmer, W.C. 1965. *Meteorological Drought*, U.S. Weather Bureau, Washington.
- 18 Richardson, C.T., Sasek, T.W., and Fendick, E.A. 1992. Implications of physiological responses to
19 air pollution for forest decline in the southeastern USA. *Env. Toxic. and Chem.* **11**: 1105.
- 20 SAS/STAT User's Guide, Version 8, Fourth Edition, N12, SAS Institute, Inc.
- 21 Sasek, T.W., Richardson, C.J., Fendick, E.A., Bevington, S.R., and Kress, L.W. 1991. Carry-over
22 effects of acid rain and ozone on the physiology of multiple flushes of loblolly pine seedlings.
23 *For. Sci.* **37**: 1078-1098.
- 24 Sheffield, R.M. et al. 1985. *USDA Bull.* SE-83, 112 p.

- 1 Sheffield, R.M. and Cost, N.D. 1987. Behind the decline. *J. For.* 87: 29-33.
- 2 Shelburne, V. B., Reardon, J.C., and Paynter, V.A. 1990. The effect of acid rain and ozone exposure
3 on growth parameters on shortleaf pine. *Proc. 6th Biennial Silv. Res. Conf.*
- 4 Shriner, D.S., Heck, W.W., McLaughlin, S.B., Johnson, D.W., and Peterson, C.E. 1990. Responses
5 of vegetation to atmospheric deposition and air pollution, Volume 18 of National Acidic
6 Precipitation Assessment Program. 350 p.
- 7 Steingrover, E.G. 1994. Effect of ozone and drought on forest-grown Douglas fir. In proceedings
8 of IUFRO Conference, Air Pollution and Multiple Stresses, Sept. 7-9, 1994, New Brunswick,
9 CA.
- 10 Taylor, G.E. 1994. Role of genotype in the response of loblolly pine to tropospheric ozone: Effects
11 at the whole-tree, stand, and regional level. *J. Env. Qual.* 23: 63-82.
- 12 Topp, G.C. 1987. The application of time-domain reflectometry (TDR) to soil water content
13 measurement. *International Conference on Measurement of Soil and Plant Water Status*, July
14 1987.
- 15 USDA. 1988. The South's Fourth Forest: Alternatives for the Future. U.S. Forest Service Report
16 No. 24.512.
- 17 Van Deusen, P.C. 1992. Growth trends and stand dynamics in natural loblolly pine in the
18 southeastern United States. *Can. J. For. Res.* 21: 660.
- 19 Wallin, G., and Skarby, L. 1992. The influence of ozone on the stomatal and non-stomatal limitation
20 of photosynthesis in Norway spruce, *Picea abies* (L.) Karst, exposed to soil moisture deficit.
21 *Trees* 6: 128-136.
- 22 Zahner, R., Saucier, J.R., and Meyers, R.K. 1990. Tree-ring model interprets growth decline in
23 natural stands of loblolly pine in the Southeastern United States. *Can. J. For. Res.* 19: 612-
24 621.

- 1 Zeide, B. 1992. Reevaluation of forest inventory data from loblolly pine stands in the Georgia
- 2 Piedmont and mountain areas. p. 17 in R.B. Flagler, The Response of Southern Commercial
- 3 Forests to Air Pollution, Air and Waste Manage. Assoc., Pittsburgh, PA.
- 4

ACKNOWLEDGEMENTS

We wish to acknowledge the assistance of J. D. Joslin, W. K. Roy and A. Stone, and B. L. Jackson in data collection and compilation, Drs. D. Marx (U.S. Forest Service), and Drs. T. J. Blasing, S. E. Lindberg, R. J. Luxmoore, D. S. Shriner, S. D. Wulschleger and, J. J. Beauchamp for advanced technical review of this manuscript. This research was sponsored by the U.S. Forest Service Global Change Research Program through Interagency Agreement No. 1824-F036-A1 with the U.S. Department of Energy.

FIGURES

1. Mean seasonal growth curves for five stands during the 1992 growing season reflected a high degree of consistency among stands in responses to environmental variables. The three day moving average of ozone exposure (≥ 40 ppb) shows the variability in ozone levels over the same time period. High ozone episodes were registered at the beginning of two obvious periods of slowing growth (days 139-145 and days 175-185) during an otherwise relatively low ozone exposure year.
2. Comparison plots of the differences in the seasonal dynamics in circumference-growth of the fastest and slowest growing trees were examined to evaluate the timing and cumulative significance of growth differences of trees in poor and better quality sites during 1988. Note both episodic departures in growth rates between tree categories early in the season followed by chronic differences in growth rate that developed over time. Overlays of the weekly average of 3-day avg. Ozone exposure dose (≥ 40 ppb) and weekly values of moisture stress (MSI) are indicated for comparison with emerging trends.
3. Regressions of annual growth during six years in relation to annual average daily ozone exposure dose (concentrations ≥ 40 ppbh per day) showed the strongest statistical relationship to circumference growth on the wet sites. Data were more variable when related to average growth of the 12 trees on drier sites or when Moisture Stress Index was used as the predictor variable.
4. Mean seasonal growth trends of trees on wet and dry sites, and tree 3 (the fastest growing tree on dry sites) are shown in relationship to two indices of ozone exposure dose and Moisture Stress Index (MSI). Note the recovery of growth during the very dry September when ozone exposure levels were relatively low.

1992 Growth vs. Ozone Exposure

Loblolly Pine Stem Growth VS Annual Ozone Exposure

Loblolly Pine Stem Growth VS Annual Moisture Stress

Loblolly Pine 1993

Dendrometer vs O₃ vs MSI

Table 1. Characteristics of loblolly pine stands used in a study of factors influencing seasonal dynamics of growth.

Stand	Topography	Trees Measured	Fertility ¹	Stand Characteristics		
				Basal Area m ² ha ⁻¹	% Soil Moisture 1992 ²	Diameter Growth Range cm stem ⁻¹ yr ⁻¹
A	Upland	8	Low	28.1	14 - 24	0.15 - 0.84
B	Upland (unthinned)	8	Low	40.9	18 - 26	0.39 - 0.78
C	Terrace	6	High	29.2	32 - 38	0.62 - 1.12
D	Bottom	6	High	32.9	24 - 33	0.73 - 1.16
F	Bottom-edge	6	High	22.1	27 - 34	0.53 - 1.18

¹Soil analyses representing A horizon soils of low vs. high fertility sites were nitrogen (0.089% vs. 0.133%), calcium (632 vs. 3034 mg kg⁻¹), and P (10.05 vs. 11.10 mg kg⁻¹), respectively.

²Data represent ranges in soil moisture by volume occurring between 5/29/92 and 10/6/92. Approximately one measurement per week was made by time domain reflectometry.

Table 2. Variations in rainfall, temperature, ozone exposure dose, and Moisture Stress Index contributed to the significant variations in stem growth during the five years of this study.

Year	Mean environmental conditions ¹				Tree growth (mm) ²	
	Rainfall (mm)	Temperature (°C)	MSI	Ozone (ppb•h)	Wet	Dry
1988	3.95	31.1	-0.12 (-1.62)	451	11.54	6.05
1989	6.93	27.7	1.17 (0.19)	162	18.75	13.72
1990	6.92	28.0	0.15 (-0.63)	381	14.89	11.50
1991	5.45	29.0	0.25 (-0.04)	373	13.68	7.51
1992	0.95	26.6	0.53 (-0.02)	306	16.65	11.88

¹Environmental data include averages of daily rainfall (mm), daytime (0900-2000) temperature, weekly Moisture Supply Index (MSI) with the seasonal minimum in parenthesis, and the sum of three day ozone dosage included in hours with ≥ 40 ppb ozone levels (ppb•h).

²Mean annual circumference growth rates are shown separately for the 12 trees in wetter, better quality sites (Stands C and D, Table 1) and the 16 trees on drier sites (Stands A and B). The number of measurements per year was 37, 23, 12, 31, and 35 during the 1988-1992 interval.

Table 3. Stepwise multiple regression was used to determine the number of trees for which statistically significant responses ($P \leq 0.05$) to environmental variables occurred during each year. Both individual and interactive influences of ozone with temperature and soil moisture are included.

Variables	Numbers of trees responding significantly (0.05)				
	1988	1989	1990	1991	1992
Soil moisture	6	25	12	6	14
Rainfall	10	0	0	32	3
Temperature	8	24	19	28	35
Ozone	27	0	11	7	5

Table 4. Analysis of variance of terms included in a multiyear empirical model of environmental influences on annual stem circumference growth rate.

Source	Degrees of Freedom	Sum of squares	Mean square	F-value	P-value
Regression	9	8765.6	973.9	906.1	0.0001
* Annual slopes	9	8672.3	1734.5	822.1	0.0001
Ozone x SMI	1	54.4	54.4	36.4	0.0001
Rainfall	1	23.1	23.1	18.6	0.0001
Temperature	1	11.3	11.3	10.1	0.0021
Ozone x temperature	1	4.6	4.6	4.3	0.0423
Error	82	88.1	1.1		

* The sum of squares reported here is the sequential sum of squares due to that term being added to the model which includes all terms above it. Thus, the sum of squares due to Ozone x SMI is the increase in sum of squares due to Regression by adding the Ozone x SMI term to the model where the model includes annual slopes. The error term for calculating the MSE and testing the factors is obtained by subtracting the sequential sum of squares from the total sum of squares. For example, the sum of squares for error corresponding to the Rainfall factor is $8853.7 - 8672.3 - 23.1 = 103.9$, with degrees of freedom equal to $91 - 5 - 1 - 1 = 84$. Therefore, the $MSE = 103.9/84 = 1.24$ and the F-value associated with Rainfall is $F = 23.1/1.24 = 18.6$.

Table 5. An empirically derived model of environmental influences on tree growth over the 5 year interval was used to estimate changes in annual stem growth that were attributable to ambient ozone exposure during each year. Ambient conditions of each year were used in the base case while MSI was reduced to 0 and then -1 in successive simulations.

	<u>Ozone induced growth change (%)¹</u>			
	Moisture Stress Index			
	Base case ²	0	-1	Predicted annual circumference ³ Gain (mm)
1988	-15	-10	-41	7.16
1989	+2	-2	-6	14.82
1990	-5	-5	-17	12.55
1991	-2	-5	-18	12.27
1992	+1	-3	-10	17.17

¹Ozone impacts were estimated as a percentage change in growth attributable to sum of terms involving ozone interactions. Only short term effects are considered in model estimates.

²Base case MSI values ranged from -0.12 in 1988 to 1.17 in 1989 (see Table 2).

³For the actual conditions experienced during each test year.

Table 6. Comparative analysis of circumference growth rate across time periods varying significantly in soil moisture stress and ozone exposure. Note particularly the increased growth rate on the dry site in 1993 during a very dry period with low ozone exposure.

COMPARATIVE TRENDS IN O₃, MSI, AND GROWTH

Year	Interval	Monthly mean			
		O ₃ exposure (≥ 40 ppbh/d)	Drought MSI	Growth (mm/30d)	
1988	June	624	-0.50	0.48	Dry site
				2.70	Wet site
1993	June	616	-1.40	-0.86	Dry site
				2.47	Wet site
1993	September	345	-2.0	3.68	Dry site
				2.34	Wet site