Seasonal trends of light-saturated net photosynthesis and stomatal conductance of loblolly pine trees grown in contrasting environments of nutrition, water and carbon dioxide

R. MURTHY, 1 S. J. ZARNOCH 2 & P. M. DOUGHERTY 3

1 Department of Forestry, College of Forest Resources, Box 8002, North Carolina State University, Raleigh, NC 27695, USA, 2 Southern Research Station, USDA Forest Service, Asheville, NC 28802, USA, and 3 Westvaco, PO Box 1950, Summerville, SC 29484, USA

ABSTRACT

Repeated measures analysis was used to evaluate the effect of long-term CO2 enhancement on seasonal trends of light-saturated rates of net photosynthesis (A sat) and stomatal conductance to water vapour (g sat) of 9-year-old loblolly pine (Pinus taeda L.) trees grown in a 2 x 2 factorial experimental design of nutrition and water. A significant interaction effect of CO2 and nutrition on mean A sat was observed for juvenile foliage. Also, juvenile foliage exposed to +350 μmol mol –1 CO2 had a higher rate of increase of A sat between late summer and early autumn. This would lead to a greater potential for recharging carbohydrate reserves for winter. Mature foliage was affected by CO2, water and nutrient treatments in two ways. First, A sat was significantly increased as a result of elevated CO2 in January, a period when stomatal conductance was only 47% of the maximum observed rate. Secondly, the rate of increase of A sat from winter to early spring was accelerated as a result of both nutrient + water and +350 μmol mol –1 CO2 treatments. This accelerated response resulted in a greater potential for photosynthetic production during the period when growth initiation occurred. Nutrient, water or carbon dioxide treatments did not significantly alter trends in g sat for mature or juvenile foliage. A significant nutrition x CO2 interaction was observed for the mature foliage, suggesting that g sat increased with increasing CO2 and nutrition. These results may have important consequences for the determination of the water use efficiency of loblolly pine. In spite of low g sat in the winter to early spring period, there was a substantial gain in A sat attributable to elevated CO2 concentrations.

Key-words: Pinus taeda L.; elevated CO2; net photosynthesis, repeated measures analysis; stomatal conductance.

INTRODUCTION

Current atmospheric carbon dioxide (CO2) concentration limits photosynthetic rates in C3 plants. Predicted increases in atmospheric CO2 concentration should therefore increase photosynthesis. Since forests cover one-third of the Earth’s land area (Kramer 1981) and are estimated to account for ~70% of the terrestrial atmospheric carbon exchange (Waring & Schlesinger 1985), an increase in photosynthesis could have important consequences for the determination of the future role of forests in the global carbon cycle.

The response of woody species to elevated CO2 concentration is generally characterized by an increase in photosynthetic rate (Norby & O’Neill 1991; Samuelson & Seiler 1992; Lee, Barton & Jarvis 1993; Stewart & Hoddinott 1993). Stomatal conductance has either been unaffected by elevated CO2 (Bunce 1992; Murthy et al. 1996) or has decreased (Tolley & Strain 1984, 1985; Surano et al. 1986; Tyree & Alexander 1993). The magnitude of these responses can be expected to vary with nutrient supply (Conroy, Barlow & Bevege 1986; Tissue, Thomas & Strain 1993; Thomas, Lewis & Strain 1994) and available soil moisture (Miao, Wayne & Bazzaz 1992). Therefore, to assess the potential response of forest trees to increasing atmospheric CO2 concentration, the effects of elevated CO2 must be considered in conjunction with other environmental resources such as water and nutrients.

Most studies which have evaluated the effects of CO2 on carbon and water exchange of loblolly pine (Pinus taeda L.) have involved seedlings or saplings. Only three studies have been made on field-grown trees. Teskey (1995) examined physiological responses of branches exposed to elevated CO2 under irrigated conditions. Ellsworth et al. (1995) examined physiological responses at the leaf and canopy scales after exposing entire loblolly pine trees to elevated CO2 using the free-air CO2 enrichment (FACE) technique. Murthy et al. (1996) used branch chamber technology to examine the effect of CO2 on gas exchange of foliage at various nutrient and moisture levels.

Statistical analyses of CO2 exposure studies have usually been based on data obtained at the end of the studies or at discrete intervals during the study. Additional information can be gained by analysing entire response patterns of physiological parameters over the seasons. In this paper, we report the effects of elevated CO2, nutrition and water on seasonal light-saturated stomatal conductance to water...
vapour (g_{sat}) and net photosynthetic rates (A_{sat}) for branches of loblolly pine trees in the second year of CO\textsubscript{2} exposure.

MATERIALS AND METHODS

Study site characteristics and study design

The study site is located in Scotland county, North Carolina, USA (latitude 32°55’ N and longitude 81°47’ W). The study was a split-plot design with main-effect treatments of nutrition and water. The four whole-plot treatment combinations of control (C: no water or nutrients), water only (W), nutrients only (N), and nutrients + water (N + W) were randomly assigned to one of four treatment plots (50 x 50 m) in each of four blocks. The subplot treatment was CO\textsubscript{2} concentration.

The nutrient plots were initially treated with macro- and micro-nutrients in March 1992. Foliar nitrogen concentrations were monitored monthly and appropriate nutrients were applied to maintain foliar N concentrations at 1-4% of dry mass. Other nutrients were maintained in balance with N. Additional details on site conditions and history and fertilization schedule are given by Murthy et al. (1996).

The irrigated plot received water treatment from May 1993 until November 1994. Available soil water was calculated as the volumetric water content between field capacity and permanent wilting point (–1.5 MPa) in the upper 50 cm of the soil profile. The soil water content was monitored 2–3 times per week using time domain reflectometry (Topp & Davis 1985). Water was applied when 40% of the available soil water was depleted from the upper 50 cm of the soil profile. The threshold of 40% was chosen because tree diameter growth is limited when soil water drops below this level (Bassett 1964). Irrigation was not applied from November 1993 to March 1994 because available soil water was above this threshold level.

Three subplot treatments of carbon dioxide concentrations were randomly assigned to three branches of a single tree in each of the 16 whole-plot treatment plots. Three branches were randomly selected from the mid-crown (1989 or 1990 whorl) of each designated tree and exposed to ambient, ambient + 175 and ambient + 350 $\text{mol mol}^{-1}$ CO\textsubscript{2} 24 h d\textsuperscript{-1} from March 1993 until November 1994 using the branch chamber technology developed by Teskey, Dougherty & Wiselgel (1991). Photosynthetic photon flux density (PPFD) and chamber air temperature were measured at 4 min intervals in each chamber during the study period and averaged to hourly values. Standard weather data were collected from a weather station located on the site.

Plant material

The average lifespan of loblolly pine needles is 18–20 months. Thus, a given flush or cohort of needles persists over two growing seasons, starting from bud-break in March, maturing in December the same year and senescing in October to November of the following year. In this study, foliage in its first growing season (March to December) was identified as juvenile, and foliage in its second growing season (January to November) as mature. Important aspects of these two cohorts of foliage with respect to this study are outlined below.

(1) Mature 1994 Cohort (M94). This foliage was considered juvenile in 1993 and mature in 1994 (January 1994 to September 1994). The important attribute of this cohort was that, although it developed from a bud initially formed in ambient CO\textsubscript{2} environment, it was exposed to the imposed CO\textsubscript{2} treatments from bud-break (March 1993) until senescence (November 1994). Data for this cohort of foliage were collected from January to September 1994.

(2) Juvenile 1994 Cohort (J94). This foliage was in its juvenile phase in 1994 (March to December). It developed from buds that were initiated under the imposed CO\textsubscript{2} treatments. Bud-break also occurred under the imposed CO\textsubscript{2} treatments. Thus, the developmental and physiological effects enhanced CO\textsubscript{2} might have on $A_{sat}$ or $g_{sat}$ would be observable by studying this cohort of foliage. Data for this cohort of foliage were collected from May 1994 to September 1994.

Physiological measurements

Light-saturated net photosynthesis and stomatal conductance to water vapour were measured once a month from January 1994 to September 1994 on the M94 cohort and from May to September 1994 on the J94 cohort. At the initiation of this study, branches had already experienced the CO\textsubscript{2} treatments for 9 months.

A portable infrared gas analyser (ADC-LCA3, Analytical Development Corporation, Hoddesdon, UK\textsuperscript{1}) equipped with a Parkinson leaf cuvette (PLC-3) was used for all $A_{sat}$ and $g_{sat}$ measurements. Each measurement was made by enclosing three fascicles (nine needles) of first-flush foliage in the leaf cuvette. Leaf cuvette conditions during each measurement were maintained at saturating photosynthetic photon flux density (PPFD $> 1600 \text{ mol m}^{-2} \text{ s}^{-1}$), 40% relative humidity, and temperatures of 25–30 °C in summer and autumn and 15–20 °C in winter and early spring. Temperatures at which measurements were taken were in the range of optimum temperatures established for net photosynthesis of loblolly pine for summer and winter periods (Strain, Higginbotham & Mulroy 1976). The carbon dioxide concentration in the cuvette was held at the same concentration to which the branches were exposed in the branch chamber. All $A_{sat}$ and $g_{sat}$ measurements were made between 0300 and 0900 h in the morning to minimize water stress and to maintain cuvette temperatures at the above-mentioned levels. After $A_{sat}$ and $g_{sat}$ measurements were obtained, needles used for the gas exchange measurement were collected and their total leaf surface area calculated.

\textsuperscript{1}The use of trade or firm names is for reader information and does not imply endorsement by the U.S. Department of Agriculture of any product or service.

$A_{\text{sat}}$ and $g_{\text{sat}}$ were expressed on a total leaf surface area basis. Further details of the physiological measurements and leaf area determination are provided by Murthy et al. (1996).

**Statistical analysis**

The basic study design was a split-plot with four replications where the main-effect treatments were nutrition and water and the subplot factor was CO$_2$ concentration. However, because $A_{\text{sat}}$ and $g_{\text{sat}}$ measurements were taken monthly, the study consisted of another factor, time, over which responses were measured on each experimental unit. Traditionally, these types of study have been analysed as individual split-plots at each measurement time or as split-split-plots. However, repeated measures analysis (RMA) is more appropriate because the repeated measures factor (time) cannot be assigned at random and the observations are serially correlated.

In this study, the multivariate method and an analysis of contrasts, as described by Gumpertz & Brownie (1993), was applied to $A_{\text{sat}}$ and $g_{\text{sat}}$ data obtained for M94 and J94 cohorts. In addition, data from the M94 cohort of foliage were divided into three time periods (January to March, March to June and June to September) which corresponded to biologically significant time periods in the ontogeny of a cohort of foliage. The response of $A_{\text{sat}}$ and $g_{\text{sat}}$ observed for each time period was analysed separately using RMA. This allowed us to investigate treatment effects on responses at smaller time intervals. Response functions were fitted to the data using linear, quadratic and cubic terms for each period, but only the intercepts and slopes for the linear term have been reported in this paper.

The Bonferroni approach was used for pairwise comparisons to assure an experimentwise error of 0.05 for a particular set of tests. This resulted in an individual pairwise comparison error of 0.05/$s$, where $s$ is the number of comparisons in the set.

**RESULTS**

**Environmental characteristics**

The average daytime branch chamber temperature during summer 1994 was 27.9 °C (Fig. 1a). The daytime chamber

![Figure 1](image-url)

*Figure 1.* Average weekly daytime (a) temperature, (b) photosynthetic photon flux density, (c) CO$_2$ concentration during the photoperiod (average of 48 branch chambers), and (d) percentage available soil water in the upper 50 cm of the soil profile determined for control, water, nutrients, and nutrients + water plots.
temperature exceeded ambient temperature by \( \leq 1, 2, 3, 4 \) and \( 5 \) °C, respectively, for \( 49.6, 31.4, 14.4, 3.1 \) and \( 1.5 \)% of the total readings. The daily average (photoperiod) PPFD within the branch chamber was \( 422 \, \mu \text{mol m}^{-2} \text{s}^{-1} \) (Fig. 1b). The noon PPFD within the chamber was \( 50 \)% less than the PPFD above the canopy as a result of mid-crown branch position and reduced light transmission through the plastic covering of the branch chamber. Daily average \( \text{CO}_2 \) concentrations (PPFD \( \geq 40 \, \mu \text{mol m}^{-2} \text{s}^{-1} \)) within the chambers for the three \( \text{CO}_2 \) treatments were \( 385, 559 \) and \( 741 \, \mu \text{mol mol}^{-1} \) with standard deviations of \( 14.2, 25 \) and \( 25 \), respectively (Fig. 1c). Average \( \text{CO}_2 \) concentrations in the ambient \( \text{CO}_2 \) chambers were slightly above current global ambient \( \text{CO}_2 \) concentrations (\( 360 \, \mu \text{mol mol}^{-1} \)).

In the irrigated plots, available soil water in the upper \( 50 \) cm of the soil profile was maintained at more than \( 60 \)% of total available soil water throughout the study period except in April and May when it dropped to \( 58 \)%.

Available soil water in non-irrigated plots dropped to \( 42 \)% during April and May (Fig. 1d).

### Trends in light-saturated net photosynthesis (\( A_{\text{sat}} \))

**M94 Cohort (January to September 1994)**

Mean light-saturated net photosynthetic rates (\( A_{\text{sat}} \)) for the M94 cohort in all treatments increased sharply from a minimum in January 1994 to a maximum in March to April 1994, remained high through May and then declined steadily towards senescence (Figs 2a–d). Repeated measures analysis over the entire period indicated significant effects of nutrition and \( \text{CO}_2 \) treatments on \( A_{\text{sat}} \) (Table 1). RMA also detected a significant time \( \times \text{CO}_2 \) interaction, indicating different response curves for the \( \text{CO}_2 \) treatments. None of the other sources were significantly different.

Detailed RMA analyses of the M94 cohort data for the three time periods revealed that both nutrition and \( \text{CO}_2 \) treatments had significant effects on mean \( A_{\text{sat}} \). These analyses also revealed that significant nutrition (N) \( \times \text{CO}_2 \) and water (W) \( \times \text{CO}_2 \) interactions occurred during the June to September period (Table 2). Interactions of time \( \times \text{N} \) were significant for all three time periods, while interactions of

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**Figure 2.** Monthly trends in \( A_{\text{sat}} \) rates of the M94 (January to September 1994) cohort of foliage for (a) the nutrition and no nutrition treatments (each point is an average of 24 values), (b) the \( \text{CO}_2 \) treatments, (c) \( \text{CO}_2 \) treatments that received no water treatments and (d) \( \text{CO}_2 \) treatments that received water treatment. Each point in (b), (c) and (d) is an average of 16 values.
time × CO₂ were significant only for the January to March and June to September time periods. Significant time × W × CO₂ treatment effects were also observed for the January to March and March to June period. The nature of these higher order interactions with time are illustrated for N (Fig. 2a), CO₂ (Fig. 2b) and W × CO₂ (Figs 2c & d).

Response function analyses conducted on observed A_sat trends for the three time periods revealed the following results. Average A_sat (intercept) was significantly different between all three CO₂ treatments during each of the three time periods and had the following ranking: +350 μmol mol⁻¹ CO₂ > +175 μmol mol⁻¹ > ambient CO₂ treatment. However, the rates of increase or decrease of A_sat within each of the three time periods did not maintain the same ranking as observed for average A_sat. The rate of increase of A_sat (slope) during the January to March period was significantly higher for foliage in the +350 μmol mol⁻¹ CO₂ treatment than that observed for foliage in the +175 μmol mol⁻¹ and ambient CO₂ treatments (Table 3). Slope of the A_sat trend decreased in all three CO₂ treatments during the March to June period, but the rate of decline of A_sat was not significantly different. However, A_sat rates of increase and decrease of A_sat within each of the three time periods exceeded the number of blocks (4) in the experiment.

Table 1. Statistical significances (P > F) for repeated measures analyses on A_sat for foliage grown with or without nutrition (N) or water (W) and in three concentrations of CO₂. df refers to degrees of freedom.

<table>
<thead>
<tr>
<th>Source</th>
<th>Mature 1994</th>
<th>Juvenile 1994</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>df</td>
<td>P &gt; F</td>
</tr>
<tr>
<td>N</td>
<td>1</td>
<td>0.00*</td>
</tr>
<tr>
<td>W</td>
<td>1</td>
<td>0.68</td>
</tr>
<tr>
<td>N × W</td>
<td>2</td>
<td>0.17</td>
</tr>
<tr>
<td>CO₂</td>
<td>2</td>
<td>0.00*</td>
</tr>
<tr>
<td>N × CO₂</td>
<td>2</td>
<td>0.17</td>
</tr>
<tr>
<td>W × CO₂</td>
<td>2</td>
<td>0.48</td>
</tr>
<tr>
<td>N × W × CO₂</td>
<td>2</td>
<td>0.22</td>
</tr>
<tr>
<td>Time¹</td>
<td>8</td>
<td>—</td>
</tr>
<tr>
<td>Time × N</td>
<td>8</td>
<td>0.07</td>
</tr>
<tr>
<td>Time × W</td>
<td>8</td>
<td>0.56</td>
</tr>
<tr>
<td>Time × N × W</td>
<td>8</td>
<td>0.47</td>
</tr>
<tr>
<td>Time × CO₂</td>
<td>16</td>
<td>0.00*</td>
</tr>
<tr>
<td>Time × N × CO₂</td>
<td>16</td>
<td>0.58</td>
</tr>
<tr>
<td>Time × W × CO₂</td>
<td>16</td>
<td>0.17</td>
</tr>
<tr>
<td>Time × N × W × CO₂</td>
<td>16</td>
<td>0.10</td>
</tr>
</tbody>
</table>

* Significant at P ≤ 0.05.
¹ The test for Time could not be performed because the number of time periods exceeded the number of blocks (4) in the experiment.

Response function analyses to determine the effect of nutrient and water treatments on A_sat trends revealed that, for the January to March period, average A_sat (intercept) was significantly higher for foliage from plots that received nutrition compared to A_sat of foliage from plots that received water only, but was not different from that obtained for foliage in the control plots (Table 3). For the March to June period, average A_sat of foliage from plots that received nutrition were significantly higher than A_sat of foliage from the control and watered-only plots. During the June to September period, foliage from plots that received both nutrition and water had significantly higher A_sat rates than foliage from the other three whole-plot treatments.

The rates of increase of A_sat during the January to March period were significantly higher for foliage from plots that received nutrition than for those that did not (Table 3). A_sat decreased in all four whole-plot treatments during the March to June period, but the rates of A_sat decline for foliage from plots that received nutrition were significantly higher than those observed for foliage from either the control or plots receiving water only. No significant differences were observed in A_sat trend (slopes) between any of the whole-plot treatments during the June to September period.

J94 Cohort (May to September 1994)

Irrespective of the nutrient or water treatment, A_sat of the J94 cohort gradually increased for all three CO₂ treatments from May 1994 to a seasonal maximum in September 1994 (Fig. 3). Repeated measures analyses indicated significant effects of nutrition and CO₂ treatments and a significant N × CO₂ interaction effect on A_sat (Table 1).

Table 2. Statistical significances (P > F) for repeated measures analyses on A_sat for foliage grown with or without nutrition (N) or water (W) and in three concentrations of CO₂ for the time periods January to March, March to June and June to September for the M94 cohort.

<table>
<thead>
<tr>
<th>Source</th>
<th>Jan–Mar P &gt; F</th>
<th>Mar–Jun P &gt; F</th>
<th>Jun–Sep P &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>0.00*</td>
<td>0.00*</td>
<td>0.00*</td>
</tr>
<tr>
<td>W</td>
<td>0.35</td>
<td>0.95</td>
<td>0.99</td>
</tr>
<tr>
<td>N × W</td>
<td>0.21</td>
<td>0.14</td>
<td>0.07</td>
</tr>
<tr>
<td>CO₂</td>
<td>0.00*</td>
<td>0.00*</td>
<td>0.00*</td>
</tr>
<tr>
<td>N × CO₂</td>
<td>0.73</td>
<td>0.44</td>
<td>0.03*</td>
</tr>
<tr>
<td>W × CO₂</td>
<td>0.96</td>
<td>0.15</td>
<td>0.00</td>
</tr>
<tr>
<td>N × W × CO₂</td>
<td>0.49</td>
<td>0.51</td>
<td>0.39</td>
</tr>
<tr>
<td>Time</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Time × N</td>
<td>0.02*</td>
<td>0.00*</td>
<td>0.01*</td>
</tr>
<tr>
<td>Time × W</td>
<td>0.30</td>
<td>0.86</td>
<td>0.32</td>
</tr>
<tr>
<td>Time × N × W</td>
<td>0.79</td>
<td>0.93</td>
<td>0.06</td>
</tr>
<tr>
<td>Time × CO₂</td>
<td>0.00*</td>
<td>0.08</td>
<td>0.00*</td>
</tr>
<tr>
<td>Time × N × CO₂</td>
<td>0.44</td>
<td>0.15</td>
<td>0.16</td>
</tr>
<tr>
<td>Time × W × CO₂</td>
<td>0.01*</td>
<td>0.01*</td>
<td>0.58</td>
</tr>
<tr>
<td>Time × N × W × CO₂</td>
<td>0.91</td>
<td>0.20</td>
<td>0.05*</td>
</tr>
</tbody>
</table>

* Significant at P ≤ 0.05.
Further, the interactions between time × W, time × CO₂ and time × N × CO₂ were all significant (Table 1). These higher order interactions of nutrient, water and CO₂ treatments with time suggest that separate A_sat response curves are needed to describe these effects (Fig. 3).

Response function analyses for the J94 foliage revealed that average A_sat (intercept) was significantly different between all three CO₂ treatments with the following ranking: +350 μmol·mol⁻¹ CO₂ > +175 μmol·mol⁻¹ > ambient CO₂ treatment. However, the rate of increase of A_sat of foliage in the +350 μmol·mol⁻¹ CO₂ treatment was significantly higher than A_sat of foliage in the other two CO₂ treatments (Table 3). No significant differences were observed between the rates of A_sat increase for foliage in the +175 μmol·mol⁻¹ and ambient CO₂ treatments. Average A_sat (intercept) of foliage in the control plot was significantly higher than A_sat of foliage from the other three whole-plot treatments, while the rate of increase (slope) of A_sat for foliage from the control treatment was significantly lower than that of A_sat of foliage from the other three whole-plot treatments.

Trend in percent gains in A_sat

An examination of the percentage increase of A_sat that can be attributed to elevated concentrations of CO₂ revealed that A_sat was increased by elevated CO₂ over the entire study period. A_sat of the M94 cohort of foliage increased by an average of 83 and 38% for the +350 and +175 μmol·mol⁻¹ CO₂ treatments, compared to A_sat in the ambient CO₂ treatment (Fig. 4). For the J94 cohort, the +350 and +175 μmol·mol⁻¹ CO₂ treatments increased A_sat an average of 69 and 23%, respectively. Percentage increases in A_sat varied from month to month for both cohorts of foliage, but significant responses to CO₂ treatments were maintained for all months.

Trends in light-saturated stomatal conductance to water vapour (g_sat)

M94 Cohort (January to September 1994)

The general trend in g_sat did not show clear separation between the three CO₂ treatments (Fig. 5a). However, g_sat for the ambient CO₂ treatment was lower than that observed for the remaining CO₂ treatments for most of the study period (May to August 1994).

RMA detected no significant nutrient, water or CO₂ treatment effects on mean g_sat for the M94 cohort (Table 4). The only significant interaction detected was a N × CO₂ interaction. It appears that addition of nutrients in conjunction with elevated CO₂ increased g_sat, while in the no added nutrient treatment g_sat increased slightly in the +175 μmol·mol⁻¹ CO₂ treatment and then decreased in the +350 μmol·mol⁻¹ CO₂ treatment (Fig. 6).

J94 Cohort (May to September 1994)

Light-saturated stomatal conductance gradually increased from May to August 1994 and then decreased during September 1994 (Fig. 5b). RMA detected no significant nutrient, water or CO₂ treatment effects nor interactions for g_sat for the J94 cohort (Table 4).

DISCUSSION

Generally, the results from repeated measures analyses of this study indicated that both nutrition and CO₂ had a significant effect on A_sat when averaged over time. This agrees with other reports where analyses were conducted at the end of the study (see review by Eamus & Jarvis 1989).

Nutrition had a strong positive effect on mean A_sat of the M94 cohort during the entire 9 months of the study period. Although the time × N interaction was not significant over the entire study period (January to September), it was significant when the data were analysed for each of the individual smaller time periods. This was a result of lower variability in the observations within the smaller time periods.
Evans (1989) concluded that addition of nutrients, especially nitrogen, enhances photosynthetic rates. Similarly, Thomas, Lewis & Strain (1994) showed an increase in net photosynthesis of loblolly pine seedlings after the addition of nitrogen and phosphorus. An enhancement of photosynthetic capacity as a result of improved nutrition is supported by our results.

Examination of the slopes and intercepts of the trends of $A_{sat}$ of the M94 cohort provides a clearer explanation of the effects of nutrients on the seasonal response of $A_{sat}$. Addition of nutrients, with or without water, accelerated the rate of $A_{sat}$ increase from winter to early spring (January to March) and, consequently, resulted in a more rapid decline in the following months (March to June). One could speculate that the rapid rate of increase of $A_{sat}$ early in the season was due to a greater availability of nutrients for the M94 cohort. An increase in $A_{sat}$ could also occur because of reallocation of nutrients such as nitrogen from other tissue components back to the mature leaves during a period when sink competition for nutrients is low. In the January to March period, only one cohort of foliage, M94, is present and growth of other tissue components is minimal. Later in the season, competition for nutrients by actively growing J94 foliage may have caused a decline in $A_{sat}$ of the M94 cohort. Again, during the final stages of the M94 cohort the addition of nutrients resulted in a more rapid decline of $A_{sat}$. Irrespective of the cause of increased or decreased availability of nutrients, a higher rate of $A_{sat}$ increase with improved nutrition at the beginning of the year would allow foliage to enhance its carbon fixation potential rapidly during winter to early spring and to attain higher $A_{sat}$ by the time of bud-break as illustrated in Fig. 2a.

Similar to the M94 cohort, nutrition significantly increased $A_{sat}$ of the J94 foliage when averaged over the study period. Although the J94 foliage did not exhibit a significant time $\times$ N interaction there were strong indications that it may exist ($P = 0.07$). This was further substantiated by the highly significant time $\times$ CO$_2$ and time $\times$ N $\times$ CO$_2$ interactions. These observations suggest a synergistic influence of CO$_2$ and nutrients on $A_{sat}$ during late summer for the J94 foliage in the elevated CO$_2$ and nutrient treatments. The implications of a higher rate of increase of $A_{sat}$ in late summer could translate to higher carbohydrate availability for the foliage in the +350 μmol mol$^{-1}$CO$_2$ + nutrient treatment during the autumn and winter period. Based on the work of D. A. Sampson & P. M. Dougherty (unpublished results) carbon gain during autumn and winter was likely to be important in restoring carbohydrates depleted during the late summer period. During the developmental stage, the J94 foliage metabolic activity related to growth was higher (Murthy 1995). This could have contributed to the observed interaction between the effects of time, N and CO$_2$. These results agree with reports by Tissue, Thomas & Strain (1993) who found that an increase in photosynthetic rate in elevated high CO$_2$ was achieved only when supplemental nitrogen was added.

Water alone did not appear to have any significant effect on $A_{sat}$ of the M94 cohort averaged over the study period. However, it is difficult to isolate the effects of water on the slopes of the M94 $A_{sat}$ response curves because there were significant time $\times$ water $\times$ CO$_2$ interactions during the first two time periods (Figs 2b & d).

Although water did not have a significant effect on $A_{sat}$ of J94 foliage when averaged over the study period, there...
was a significant time × water interaction, indicating that the slopes were different and that two separate response functions with different slopes were needed, as illustrated in Fig. 3c. Addition of water appears to have influenced $A_{sat}$ only in June and July. During this period, soil moisture was sufficient but chamber temperatures were at their seasonal high. High temperature resulted in sufficient vapour pressure deficits to cause water stress problems. Our results also indicated that addition of water was beneficial in enhancing the rate of increase of $A_{sat}$ of J94 foliage during the June to September period over that of the control plots.

This study shows that CO$_2$ treatment significantly affected trends in $A_{sat}$, irrespective of age of the foliage (mature or juvenile), or the length of time the foliage had been exposed to CO$_2$ treatment. In this study, mature foliage was exposed to CO$_2$ treatment for a total period of 17 months and the juvenile foliage for a period of 5 months. Although some studies measured seasonal trends of $A_{sat}$ (Gunderson et al. 1993; El Kohen & Mousseau 1994; Curtis et al. 1995), none statistically analysed trends over time. For example, Gunderson et al. (1993) and Curtis et al. (1995) periodically measured photosynthesis over time at growth CO$_2$ concentrations and found a significant increase in leaf-level photosynthesis attributable to elevated CO$_2$ for all measurement periods. The authors, however, did not analyse their data for time × CO$_2$ interactions.

In addition to the overall effect of elevated CO$_2$ on $A_{sat}$, interactions of time × CO$_2$ and time × W × CO$_2$ were significant at different time periods in the ontogeny of the M94 cohort. These time × treatment interactions may have been caused by external environmental variables that change with time or by internal changes within the foliage such that the responses of $A_{sat}$ to CO$_2$ or CO$_2$ and water varied with time. For instance, although CO$_2$ treatment significantly affected ($P = 0.0001$) overall $A_{sat}$ when averaged over the late spring to early summer months (March to June), rates of decrease (slopes) were not significantly different between the CO$_2$ treatments ($P = 0.08$ for time × CO$_2$). This lack of interaction could be because this is a period when other environmental variables are often least limiting.

Exposure of the M94 cohort to elevated CO$_2$ (+ 350 μmol mol$^{-1}$) resulted in: maintenance of higher winter $A_{sat}$, an accelerated rate of increase of $A_{sat}$ during the winter to early spring period, attainment of a higher maximum $A_{sat}$ in May, and a faster rate of decline of $A_{sat}$ in the June to September period as compared to the $A_{sat}$ trends observed for the ambient CO$_2$ treatment. Undoubtedly the major effect of elevated CO$_2$ was to increase $A_{sat}$ in all months of the year. However, the accelerated rate of increase in winter and early spring is also biologically significant. This response allows foliage recovering from low winter $A_{sat}$ to enhance its carbon fixation potential rapidly to attain a higher $A_{sat}$ by the time of bud-break.

Comparable to plots that received nutrition, the + 350 μmol mol$^{-1}$ CO$_2$ treatment accelerated the rate of $A_{sat}$ increase of the J94 cohort in the May to September period. This suggests that elevated CO$_2$ will permit the J94 foliage to contribute more to replenishing carbohydrate reserves in the autumn–winter period.
The M94 and J94 cohorts differed in their response to the applied treatments in two ways. First, the lack of a significant time × N × CO₂ interaction in the M94 cohort suggests that, for mature foliage, nutrition and CO₂ effects on A_sat are additive. The response to CO₂ and nutrition was different for the juvenile developing foliage. For this foliage there was a strong time × N × CO₂ interaction. Secondly, water appeared to interact with CO₂ to affect A_sat trends in the M94 cohort, while in the J94 cohort addition of water tended to change A_sat trends over time but did not interact with CO₂ treatment.

Unlike the response of A_sat, the responses of g_sat of the M94 and J94 cohorts to the applied treatments were similar. The trends in g_sat were based on measurements made in the early morning hours under light-saturated conditions and minimum water stress. Our results from the RMA indicate that, within the range of CO₂ administered and for the conditions under which g_sat was measured, nutrient, water or CO₂ treatment did not significantly affect the overall average study period g_sat for either M94 or J94 foliage. The N × CO₂ interaction effect on g_sat of the M94 cohort was significant over the entire study period. Contrary to a number of reports in which g_sat decreased in response to elevated CO₂ concentrations (Surano et al. 1986; Hollinger 1987; Fetcher et al. 1988; Grulke, Hom & Roberts 1993; Thomas, Lewis & Strain 1994), in this study the observed interaction of N × CO₂ suggests that, with increasing nutrition and CO₂, g_sat may actually increase. The data also suggest that in the absence of added nutrition elevated CO₂ may not significantly increase g_sat. The lack of g_sat response to elevated CO₂ and the possible interaction effect of CO₂ and nutrition on g_sat have important implications for the water use efficiency of loblolly pine under predicted future conditions. Several studies have also reported a lack of stomatal response to elevated CO₂ in trees (Norby & O’Neill 1991; Bunce 1992; Gunderson et al. 1993; Johnsen 1993; Teskey 1995; Liu & Teskey 1995 & Murthy et al. 1996). However, exactly how elevated CO₂ influences stomatal response is still unclear.

The effect of foliage age on g_sat was much greater than the influence of the applied treatments. The average g_sat of J94 foliage in its first growing season was 0.14 mol m⁻² s⁻¹ while that of M94 was 0.09 mol m⁻² s⁻¹ in its second season, a 55% decline. This ageing effect occurred in all treatments and would have significant effects on canopy gas exchange since mature foliage accounts for more than 50% of canopy foliage from January to October. The observed decline in g_sat of foliage from its first growing season to the second was probably the result of continued foliage ageing and, perhaps, environmental conditions. Low g_sat observed in the M94 cohort during winter to early spring may have been caused by low temperature. Several studies have reported significant reductions in g_sat as a result of low air or soil temperatures (Kozlowski 1943; DeLucia 1986; Day, DeLucia & Smith 1989; Day, 1995).

![Figure 5. Monthly trends in g_sat for (a) the M94 (January to September 1994) cohort of foliage and (b) the J94 (May to September 1994) cohort of foliage. Each point is an average of 16 values.](image)

| Table 4. Statistical significances (P > F) for repeated measures analyses on g_sat for foliage grown with or without nutrition (N) or water (W) and in three concentrations of CO₂ |
|-----------------------------------------------|-----------------|-----------------|
| Source                                       | Mature 1994     | Juvenile 1994   |
| N                                            | df              | P > F           | df              | P > F           |
| W                                            | 1               | 0.41            | 1               | 0.82            |
| N × W                                        | 1               | 0.60            | 1               | 0.43            |
| CO₂                                          | 2               | 0.16            | 2               | 0.17            |
| N × CO₂                                      | 2               | 0.04*           | 2               | 0.37            |
| W × CO₂                                      | 2               | 0.29            | 2               | 0.41            |
| N × W × CO₂                                  | 2               | 0.08            | 2               | 0.18            |
| Time¹                                        | 8               | —               | 4               | —               |
| Time × N                                     | 8               | 0.54            | 4               | 0.64            |
| Time × W                                     | 8               | 0.30            | 4               | 0.23            |
| Time × N × W                                 | 8               | 0.19            | 4               | 0.36            |
| Time × CO₂                                   | 16              | 0.12            | 8               | 0.09            |
| Time × N × CO₂                               | 16              | 0.36            | 8               | 0.40            |
| Time × W × CO₂                               | 16              | 0.46            | 8               | 0.92            |
| Time × N × W × CO₂                           | 16              | 0.84            | 8               | 0.52            |

* Significant at P ≤ 0.05.

¹The test for Time could not be performed because the number of time periods exceeded the number of blocks (4) in the experiment.
Heckathorn & DeLucia 1991). Future studies should quantify the relationship of winter $g_{sat}$ to soil and air temperatures because winter temperatures are expected to increase as the global climate changes. If temperature is the major factor controlling $g_{sat}$ during winter, it could have substantial effects on winter CO$_2$ and water exchange. In spite of low winter to early spring $g_{sat}$ observed in this study, an increase in $A_{sat}$ in elevated CO$_2$ was observed throughout this period, indicating a strong positive effect of high CO$_2$ concentrations on carbon gain.

Branch chamber technology has been used by several workers (Dufrêne, Pontailler & Saugier 1993; Teskey 1995; Murthy et al. 1996) to study the effects of differential atmospheric gas treatments, and is based on the assumption that branch gas exchange is autonomous to a large degree (Sprugel, Hinckley & Schaap 1991). Branch chambers are particularly useful for exposing mature parts of a tree to ozone or CO$_2$ treatments for long time periods and represent a low-cost method, and perhaps the only method, for studying the physiological responses of large mature trees to CO$_2$. The advantages and disadvantages of branch chambers have been outlined in detail by Teskey, Dougherty & Wiselogel (1991) and Barton, Lee & Jarvis (1993) and will not be dealt with here.

This study reveals the following important points. (1) Elevated CO$_2$ concentration greatly increases and alters the seasonal pattern of $A_{sat}$. Both responses are important in determining the potential amount of photosynthate that could be fixed annually by loblolly pine. (2) Both nutrition and elevated CO$_2$ increase the rate of $A_{sat}$ in mature and juvenile foliage. This probably contributes to an enhanced amount of carbohydrates for both bud-break and growth in spring and for storage and recharge of carbohydrate pools in late autumn. (3) The age of the foliage is important in determining whether interactions of CO$_2$, nutrition and water occur. (4) The $g_{sat}$ of loblolly pine is not greatly affected by elevated CO$_2$ concentrations, but is probably a function of age and environmental limitations such as water stress in summer and possibly low temperatures in the winter. (5) Nutrition and CO$_2$ may interact to increase $g_{sat}$ in mature foliage. The continued response of $A_{sat}$ and $g_{sat}$ to the applied treatments and their changes with age have important consequences for the water use efficiency of loblolly pine.

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