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

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ABSTRACT

Repeated measures analysis was used to evaluate the effect of long-term CO_2 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 2×2 factorial experimental design of nutrition and water. A significant interaction effect of CO_2 and nutrition on mean A_{sat} was observed for juvenile foliage. Also, juvenile foliage exposed to +350 μmol mol^{-1} CO_2 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 CO_2, water and nutrient treatments in two ways. First, A_{sat} was significantly increased as a result of elevated CO_2 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} CO_2 treatments. This accelerated response resulted in a greater potential for photosynthate 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 $\times$ CO_2 interaction was observed for the mature foliage, suggesting that g_{sat} increased with increasing CO_2 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 CO_2 concentrations.

Key-words: *Pinus taeda* L.; elevated CO_2; net photosynthesis, repeated measures analysis; stomatal conductance.

INTRODUCTION

Current atmospheric carbon dioxide (CO_2) concentration limits photosynthetic rates in C_3 plants. Predicted increases in atmospheric CO_2 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 CO_2 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 CO_2 (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 CO_2 concentration, the effects of elevated CO_2 must be considered in conjunction with other environmental resources such as water and nutrients.

Most studies which have evaluated the effects of CO_2 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 CO_2 under irrigated conditions. Ellsworth *et al.* (1995) examined physiological responses at the leaf and canopy scales after exposing entire loblolly pine trees to elevated CO_2 using the free-air CO_2 enrichment (FACE) technique. Murthy *et al.* (1996) used branch chamber technology to examine the effect of CO_2 on gas exchange of foliage at various nutrient and moisture levels.

Statistical analyses of CO_2 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 CO_2, nutrition and water on seasonal light-saturated stomatal conductance to water

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vapour (g_{sat}) and net photosynthetic rates (A_{sat}) for branches of loblolly pine trees in the second year of CO_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 × 50 m) in each of four blocks. The subplot treatment was CO_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 μmol mol^{-1} CO_2 24 h d^{-1} from March 1993 until November 1994 using the branch chamber technology developed by Teskey, Dougherty & Wiselogel (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_2 environment, it was exposed to the imposed CO_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_2 treatments. Bud-break also occurred under the imposed CO_2 treatments. Thus, the developmental and physiological effects enhanced CO_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_2 treatments for 9 months.

A portable infrared gas analyser (ADC-LCA3, Analytical Development Corporation, Hoddesdon, UK[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 μmol m^{-2} 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.

[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_{sat} and g_{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_{sat} and g_{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_{sat} and g_{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_{sat} and g_{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

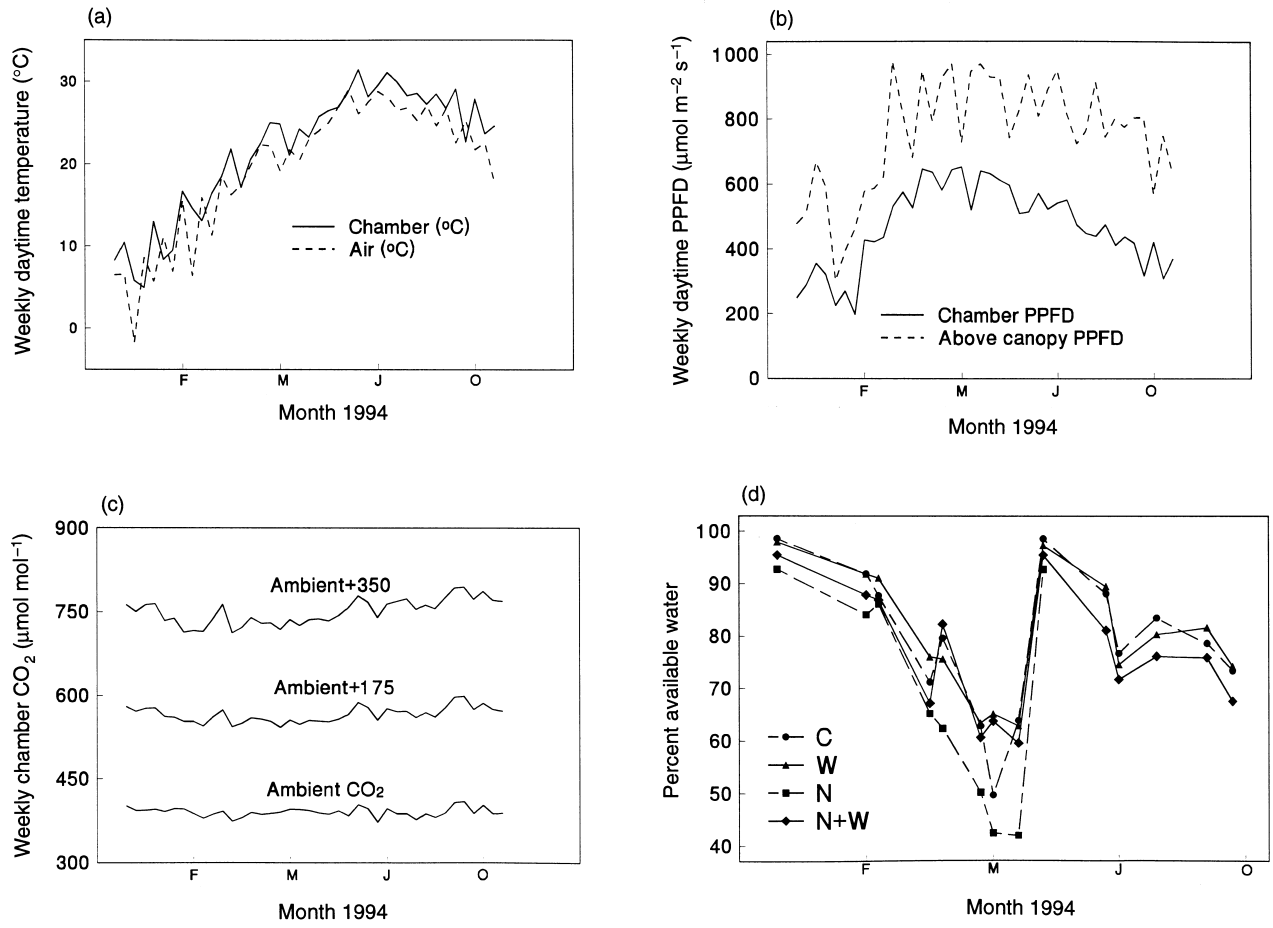

Figure 1. Average weekly daytime (a) temperature, (b) photosynthetic photon flux density, (c) CO_2 concentration during the photoperiod (average of 48 branch chambers), and (d) percentage available soil water in the upper 50 cm of the soil profile determined for control, water, nutrients, and nutrients + water plots.

temperature exceeded ambient temperature by ≤ 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 μmol m^{-2} 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 CO_2 concentrations (PPFD > 40 μmol m^{-2} s^{-1}) within the chambers for the three CO_2 treatments were 385, 559 and 741 μmol mol^{-1} with standard deviations of 14·2, 25 and 25, respectively (Fig. 1c). Average CO_2 concentrations in the ambient CO_2 chambers were slightly above current global ambient CO_2 concentrations (360 μmol 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_{sat})

M94 Cohort (January to September 1994)

Mean light-saturated net photosynthetic rates (A_{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 CO_2 treatments on A_{sat} (Table 1). RMA also detected a significant time × CO_2 interaction, indicating different response curves for the 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 CO_2 treatments had significant effects on mean A_{sat}. These analyses also revealed that significant nutrition (N) × CO_2 and water (W) × CO_2 interactions occurred during the June to September period (Table 2). Interactions of time × N were significant for all three time periods, while interactions of

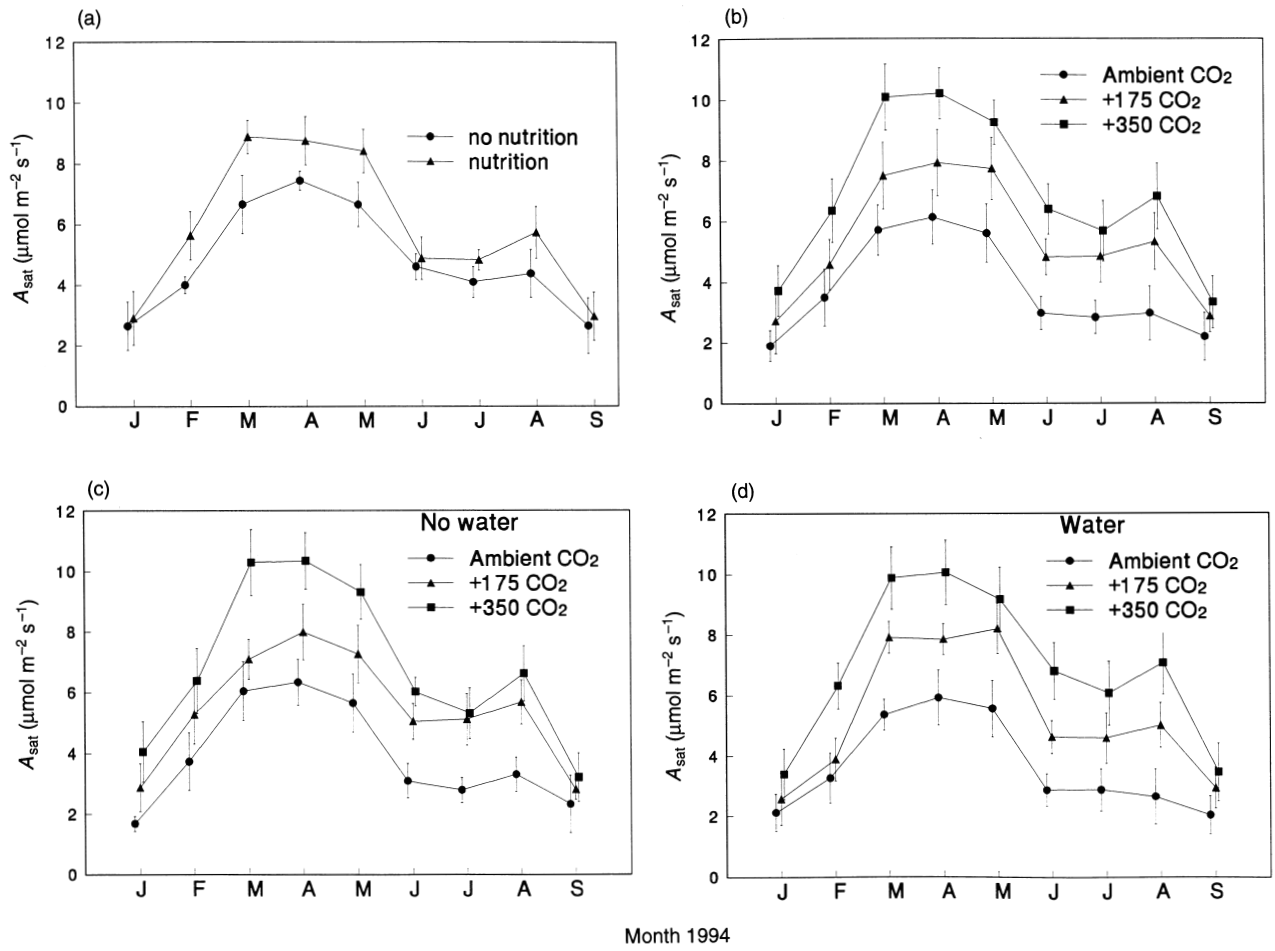

Figure 2. Monthly trends in A_{sat} rates of the M94 (January to September 1994) cohort of foliage for (a) the nutrition and no nutrition treatments (each point is an average of 24 values), (b) the CO_2 treatments, (c) CO_2 treatments that received no water treatments and (d) CO_2 treatments that received water treatment. Each point in (b), (c) and (d) is an average of 16 values.

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 CO_2. df refers to degrees of freedom

	Mature 1994		Juvenile 1994	
Source	df	$P > F$	df	$P > F$
N	1	0·00*	1	0·00*
W	1	0·68	1	0·26
N×W	2	0·13	1	0·06
CO_2	2	0·00*	2	0·00*
N×CO_2	2	0·44	2	0·01*
W×CO_2	2	0·48	2	0·75
N×W×CO_2	2	0·22	2	0·55
Time[1]	8	—	4	—
Time×N	8	0·14	4	0·07
Time×W	8	0·56	4	0·02*
Time×N×W	8	0·47	4	0·25
Time×CO_2	16	0·00*	8	0·00*
Time×N×CO_2	16	0·58	8	0·00*
Time×W×CO_2	16	0·17	8	0·60
Time×N×W×CO_2	16	0·10	8	0·91

* Significant at $P \leq 0{\cdot}05$.
[1] The test for Time could not be performed because the number of time periods exceeded the number of blocks (4) in the experiment.

time × CO_2 were significant only for the January to March and June to September time periods. Significant time × W × CO_2 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), CO_2 (Fig. 2b) and W × CO_2 (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 CO_2 treatments during each of the three time periods and had the following ranking: + 350 μmol mol^{-1} CO_2 > + 175 μmol mol^{-1} > ambient CO_2 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} CO_2 treatment than that observed for foliage in the + 175 μmol mol^{-1} and ambient CO_2 treatments (Table 3). Slope of the A_{sat} trend decreased in all three CO_2 treatments during the March to June period, but the rate of decline of A_{sat} for foliage in the + 175 μmol mol^{-1} CO_2 and ambient CO_2 treatments was not significantly different. However, A_{sat} of foliage in the + 350 CO_2 treatment declined at a faster rate than that observed for the other two CO_2 treatments. During June to September, A_{sat} continued to decline, with the rate of decline being significantly different between the three CO_2 treatments. A_{sat} declined at a significantly faster rate in the + 350 μmol mol^{-1} CO_2 treatment, followed by the + 175 μmol mol^{-1} and ambient CO_2 treatments (Table 3).

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 CO_2 treatments from May 1994 to a seasonal maximum in September 1994 (Fig. 3). Repeated measures analyses indicated significant effects of nutrition and CO_2 treatments and a significant N × CO_2 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 CO_2 for the time periods January to March, March to June and June to September for the M94 cohort

Source	Jan–Mar $P > F$	Mar–Jun $P > F$	Jun–Sep $P > F$
N	0·00*	0·00*	0·00*
W	0·35	0·95	0·99
N×W	0·21	0·14	0·07
CO_2	0·00*	0·00*	0·00*
N×CO_2	0·73	0·44	0·03*
W×CO_2	0·96	0·15	0·00
N×W×CO_2	0·49	0·51	0·39
Time	—	—	—
Time×N	0·02*	0·00*	0·01*
Time×W	0·30	0·86	0·32
Time×N×W	0·79	0·93	0·06
Time×CO_2	0·00*	0·08	0·00*
Time×N×CO_2	0·44	0·15	0·16
Time×W×CO_2	0·01*	0·01*	0·58
Time×N×W×CO_2	0·91	0·20	0·05*

* Significant at $P \leq 0{\cdot}05$.

Table 3. Intercepts and slopes of the linear component of the RMA of A_{sat} for the time periods January to March, March to June and June to September for the M94 cohort of foliage and from May to September for the J94 cohort of foliage

	Mature 1994				Juvenile 1994			
	Jan–Mar		Mar–Jun		Jun–Sep		May–Sep	
Source	Intercept	Slope	Intercept	Slope	Intercept	Slope	Intercept	Slope
CO_2 treatments								
Ambient CO_2	1·80 a[1]	1·90 a	6·42 a	–0·88 a	3·07 a	–0·22 a	0·04 a	0·76 a
+175 μmol mol^{-1} CO_2	2·54 b	2·39 a	8·24 b	–0·82 a	5·28 b	–0·54 b	1·18 b	0·70 a
+350 μmol mol^{-1} CO_2	3·54 c	3·19 b	10·79 c	–1·20 b	6·77 c	–0·81 c	2·20 c	0·97 b
Nutrient and water treatments								
Control	2·71 ab	2·08 a	7·77 a	–0·79 a	4·95 a	–0·57 a	2·92 a	0·53 a
Water	2·15 a	1·93 a	6·99 a	–0·61 a	4·56 a	–0·53 a	0·23 b	0·84 b
Nutrient	2·89 b	2·86 b	9·35 b	–1·23 b	5·10 ab	–0·44 a	1·27 b	0·90 b
N + W	2·76 b	3·10 b	9·82 b	–1·24 b	5·54 b	–0·53 a	0·93 b	0·97 b

[1] Numbers with same letters in a column for a given treatment are not significantly different at $P \leq 0{\cdot}05$.

Further, the interactions between time × W, time × CO_2 and time × N × CO_2 were all significant (Table 1). These higher order interactions of nutrient, water and CO_2 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 CO_2 treatments with the following ranking: + 350 μmol mol^{-1} CO_2 > + 175 μmol mol^{-1} > ambient CO_2 treatment. However, the rate of increase of A_{sat} of foliage in the + 350 μmol mol^{-1} CO_2 treatment was significantly higher than A_{sat} of foliage in the other two CO_2 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 CO_2 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 CO_2 revealed that A_{sat} was increased by elevated CO_2 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} CO_2 treatments, compared to A_{sat} in the ambient CO_2 treatment (Fig. 4). For the J94 cohort, the + 350 and + 175 μmol mol^{-1} CO_2 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 CO_2 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 CO_2 treatments (Fig. 5a). However, g_{sat} for the ambient CO_2 treatment was lower than that observed for the remaining CO_2 treatments for most of the study period (May to August 1994).

RMA detected no significant nutrient, water or CO_2 treatment effects on mean g_{sat} for the M94 cohort (Table 4). The only significant interaction detected was a N × CO_2 interaction. It appears that addition of nutrients in conjunction with elevated CO_2 increased g_{sat}, while in the no added nutrient treatment g_{sat} increased slightly in the + 175 μmol mol^{-1} CO_2 treatment and then decreased in the + 350 μmol mol^{-1} CO_2 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 CO_2 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 CO_2 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

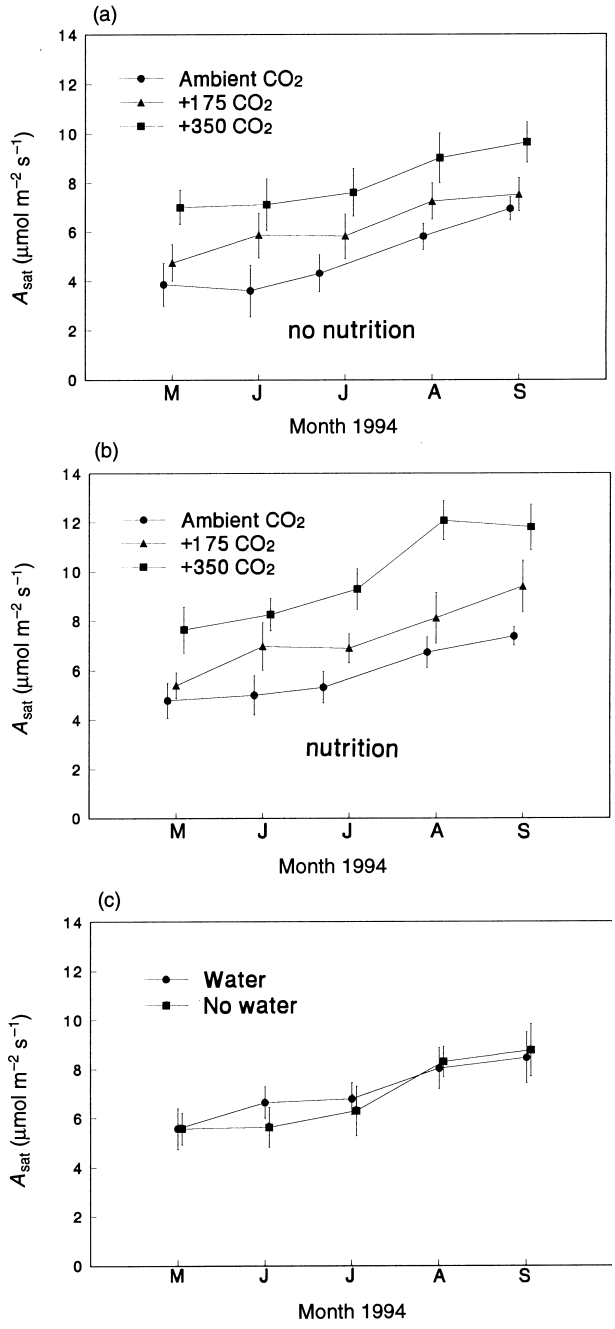

Figure 3. Monthly trends in A_{sat} of the J94 (May to September 1994) cohort of foliage for (a) CO_2 treatments that did not receive nutrition treatment (b) CO_2 treatments that received nutrition treatment (each point is an average of 16 values), and (c) no water and water treatments (where each point is an average of 24 values).

period relative to that of the entire study period. 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 × N interaction there were strong indications that it may exist ($P = 0{\cdot}07$). This was further substantiated by the highly significant time × CO_2 and time × N × 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 × water × 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

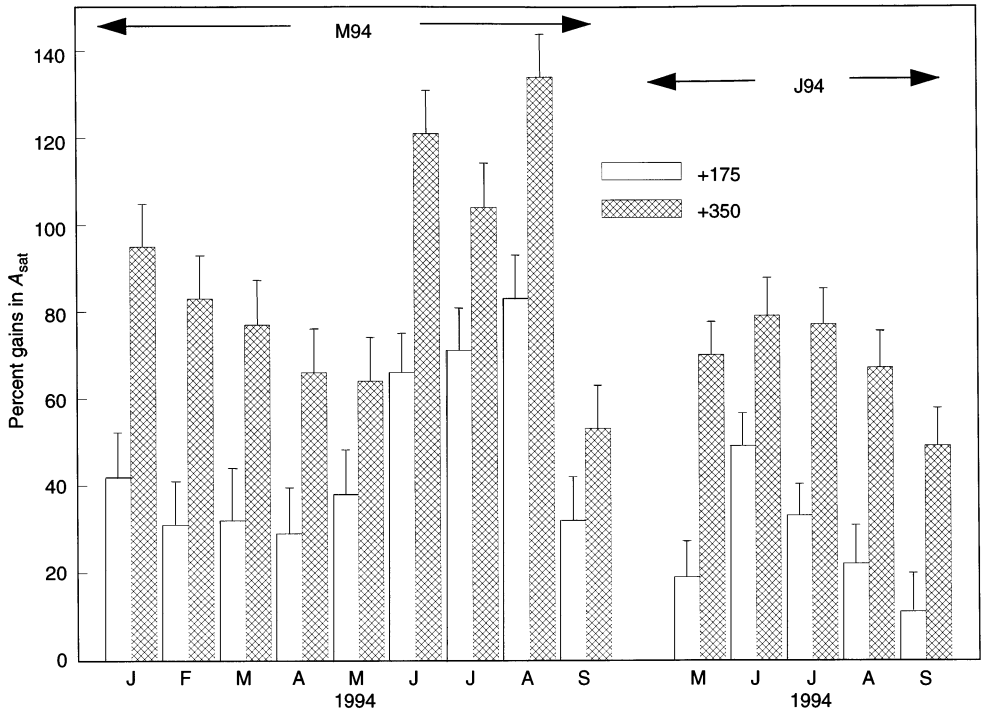

Figure 4. Monthly trends in percentage gains in A_{sat} for the + 350 μmol mol^{-1} (crossed) and + 175 μmol mol^{-1} (empty) CO_2 treatments over that of the ambient CO_2 treatment for M94 (January to September 1994) and J94 (May to September 1994) cohorts of foliage.

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.

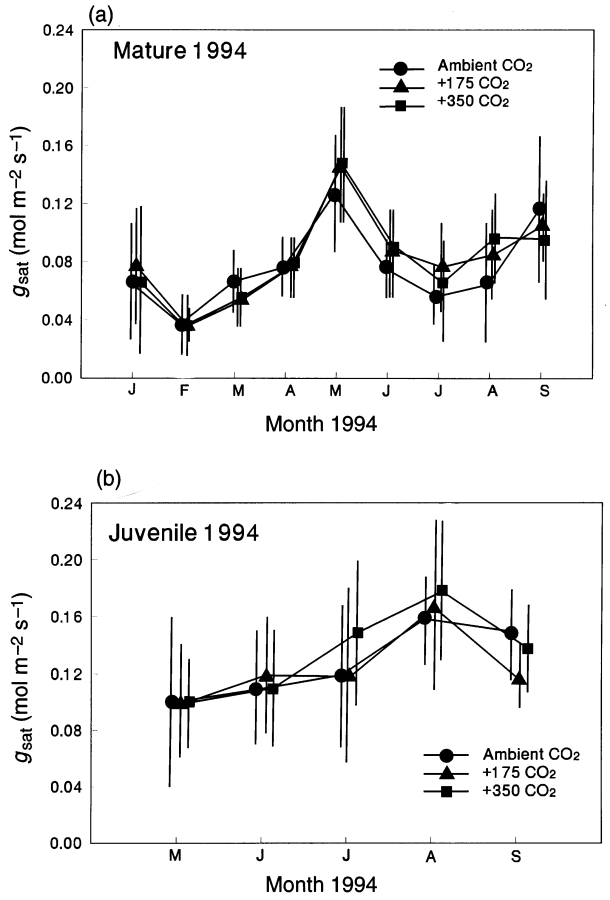

Figure 5. Monthly trends in g_{sat} for (a) the M94 (January to September 1994) cohort of foliage and (b) the J94 (May to September 1994) cohort of foliage. Each point is an average of 16 values.

The M94 and J94 cohorts differed in their response to the applied treatments in two ways. First, the lack of a significant time × N × CO_2 interaction in the M94 cohort suggests that, for mature foliage, nutrition and CO_2 effects on A_{sat} are additive. The response to CO_2 and nutrition was different for the juvenile developing foliage. For this foliage there was a strong time × N × CO_2 interaction. Secondly, water appeared to interact with CO_2 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 CO_2 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 CO_2 administered and for the conditions under which g_{sat} was measured, nutrient, water or CO_2 treatment did not significantly affect the overall average study period g_{sat} for either M94 or J94 foliage. The N × CO_2 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 CO_2 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 × CO_2 suggests that, with increasing nutrition and CO_2, g_{sat} may actually increase. The data also suggest that in the absence of added nutrition elevated CO_2 may not significantly increase g_{sat}. The lack of g_{sat} response to elevated CO_2 and the possible interaction effect of CO_2 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 CO_2 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 CO_2 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,

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 CO_2

	Mature 1994		Juvenile 1994	
Source	df	$P > F$	df	$P > F$
N	1	0·41	1	0·82
W	1	0·60	1	0·43
N×W	1	0·31	1	0·33
CO_2	2	0·16	2	0·17
N×CO_2	2	0·04*	2	0·37
W×CO_2	2	0·29	2	0·41
N×W×CO_2	2	0·08	2	0·18
Time[1]	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×CO_2	16	0·12	8	0·09
Time×N×CO_2	16	0·36	8	0·40
Time×W×CO_2	16	0·46	8	0·92
Time×N×W×CO_2	16	0·84	8	0·52

* Significant at $P \leq 0·05$.
[1] The test for Time could not be performed because the number of time periods exceeded the number of blocks (4) in the experiment.

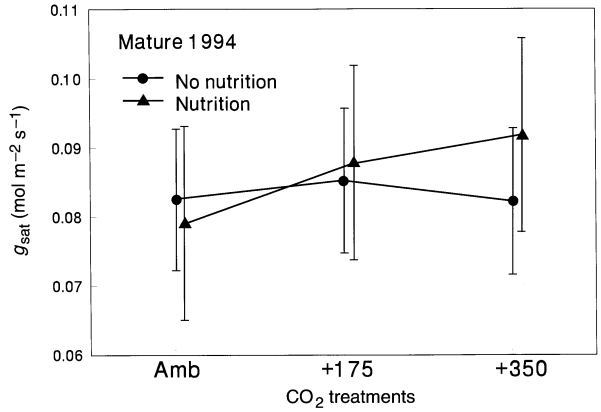

Figure 6. Effect of interaction between addition of nutrients, no added nutrients and CO_2 treatments on g_{sat} of M94 foliage.

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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