

Effects of elevated atmospheric CO₂ on phenology, growth and crown structure of Scots pine (*Pinus sylvestris*) seedlings after two years of exposure in the field

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Summary Three-year-old Scots pine (*Pinus sylvestris* L.) seedlings were grown for two years in the ground in open-top chambers supplied with either an ambient or elevated (ambient + 400 $\mu\text{mol mol}^{-1}$) CO₂ concentration. Phenological observations and measurements of height and stem diameter growth, absolute and relative growth rates, starch and soluble carbohydrate concentrations of the needles, and crown structure and needle properties were made at frequent intervals throughout the two growing seasons. Elevated CO₂ significantly advanced the date of bud burst in both years. The increase in total needle area in response to elevated CO₂ was accounted for by longer shoots and an increase in individual needle area in the first year, and by an increase in the number and length of shoots in the second year. Stem diameter and tree height were enhanced more by the elevated CO₂ treatment in the first year than in the second, indicating a decreased effect of CO₂ on growth over time. This was confirmed by a study of absolute and relative growth rates of leader shoots. During the first growing season of CO₂ enrichment, mean weekly relative growth rates over the growing season (RGR_m) were significantly enhanced. During the second year, RGR_m in ambient CO₂ closely matched that in elevated CO₂.

Keywords: canopy structure, carbohydrates, elevated carbon dioxide, growth, open-top chambers, phenology, relative growth rate.

Introduction

Enhancement of plant growth and yield in elevated atmospheric CO₂ concentration is well documented (Kimball 1983, Idso and Idso 1994, Curtis and Wang 1998). The effect is the result of increased photosynthetic carbon acquisition and changes in carbon allocation (Acock and Allen 1985, Eamus and Jarvis 1989).

However, several studies have demonstrated that, following an initial enhancement of carbon assimilation, a reduction in growth rate and assimilation occurs, referred to as acclimation (Surano et al. 1986, Brown 1991). This decline in relative growth response to elevated CO₂ over time has been attributed to modification in several plant properties, including phenol-

ogy, morphology, carbon allocation and photosynthesis (Ceulemans and Mousseau 1994, Lee and Jarvis 1995). It has been suggested that species with indeterminate growth and therefore large sink capacity are less likely to exhibit acclimation than species with determinate growth (Kaushal et al. 1989).

In trees, elevated CO₂ can increase total leaf area (Koch et al. 1986), leaf weight (Brown and Higginbotham 1986, Norby and O'Neill 1989), leaf weight to area ratio (Conroy et al. 1986, Berryman et al. 1993, Pettersson et al. 1993), and branching frequency (Sionit et al. 1985, Samuelson and Seiler 1993). Root biomass, root length, root branching and lateral root production are also reported to increase in response to elevated CO₂ (Rogers et al. 1994, Day et al. 1996, Janssens et al. 1998).

Several studies have shown that elevated CO₂ affects the growth rhythm of forest trees by altering the timing of bud burst and growth cessation (Cannell and Smith 1986, Murray et al. 1994, Ceulemans et al. 1995). In the boreal region, early bud burst results in the early onset of growth thereby prolonging the short growing season and potentially increasing wood production (Beuker 1994). Earlier bud burst, however, may increase the risk of frost damage from late spring frosts (Murray et al. 1989). Increased atmospheric CO₂ concentrations may also affect bud phenology directly through changes in tree biochemistry and physiology. For example, changes in starch or hormonal concentrations may alter dormancy status and growth patterns, by shifting the timing and duration of the vegetative season (Cannell 1990).

Although there have been many studies on the effects of elevated CO₂ on growth (Norby and O'Neill 1986, Bazzaz et al. 1990, Miao et al. 1992, Murray et al. 1996, Tissue et al. 1997) and phenology (Murray et al. 1994, Ceulemans et al. 1995) of tree species, there have been few investigations of the effects of CO₂ on crown structure (Wang 1996, Chen et al. 1997, Kubiske et al. 1997, Rey and Jarvis 1997). Our objective was to characterize the seasonal evolution of growth, crown structure and phenology of Scots pine seedlings exposed to elevated CO₂ for two growing seasons.

Materials and methods

Plant material and growth conditions

Dormant, 3-year-old, pot-grown Scots pine (*Pinus sylvestris* L.) seedlings obtained from the Institute for Forestry and Game Management in Groenendaal (Belgium), were planted in the ground in four open-top fumigation chambers (OTC) situated on the campus of the University of Antwerp (UIA), on March 21, 1996. Mean annual temperature and rainfall at the site are 11.98 °C and 769 mm, respectively. All seedlings were from the same Belgian provenance (south from Samber and Maas) and were about 0.4 m tall at the time of planting. Eleven trees per chamber were planted in a circle, 70 cm apart from each other and from the walls. To reduce the boundary effect, each OTC was surrounded by seedlings of the same seed lot. The original heavy loam soil was excavated to a depth of 0.5 m and replaced by forest soil (about 0.12% N on a dry mass basis). No nutrients or water were applied during the experiment.

Each decagonal OTC (diameter 3 m, height 4 m) was made of 1-m wide Perspex (Plexiglas) acrylic sheets, and had a usable ground area of 7.1 m². Incoming air was supplied at a rate of about 5000 m³ h⁻¹, or nearly two air volume changes per minute. Air distribution within each OTC was effected through a flexible duct supplying a perforated, fiber-reinforced polythene annulus positioned 1 m above the ground. Two OTCs were supplied with air at ambient CO₂ concentration (about 350 μmol mol⁻¹) and two were supplied with air at an elevated CO₂ concentration (ambient + 400 μmol mol⁻¹). Carbon dioxide input was regulated by a mass flowmeter/controller. The CO₂ treatments started on April 1, 1996 and have been applied continuously since then on a 24-h basis, including during the winter. Results of the first two growing seasons in the OTCs (1996 and 1997) are reported.

Phenology

In spring, bud phenology and flushing were assessed two to three times a week based on a discrete class system with: 0 = closed buds, 1 = slight swelling, 2 = swollen bud, 3 = bud elongation, 4 = bud burst, 5 = needle elongation (Figure 1). Buds were assumed to have burst when the separation of needles was visible. For all phenological observations, mean values (22 plants per treatment) and standard errors are presented. In November 1997, the amount of litterfall was determined by measuring the dry mass (DW) of the fallen needles under each tree in each OTC.

Carbohydrate and starch analysis

Near the end of the first growing season (i.e., end of September 1996), current-year needles (30 needles from each of 11 trees in each OTC) were collected from the third whorl laterals for carbohydrate analysis and placed in a freeze dryer within 30 min of collection. The dried needles were ground and shipped to the University of Edinburgh, Scotland, where the samples were analyzed for sugars (inositol, sorbitol, galactose, glucose, fructose, sucrose) with a Dionex DX-500 HPLC (Dionex Corp., Sunnyvale, CA) fitted with a GP40 gradient pump, a

PA-1 CarboPac and an ED40 electrochemical detector. On the remaining needle samples, starch was measured colorimetrically following solubilization with perchloric acid and hydrolysis with anthrone and sulfuric acid (Fales 1951, Rose et al. 1991).

Tree growth

Growth of all plants in each OTC was measured monthly. Tree height was measured with a ruler from the ground to the shoot apex. Stem diameter at the base of the crown (15 cm above ground level) was measured with a digital micrometer in two separate orientations. Absolute (elevated – ambient) and relative ((elevated – ambient)/ambient) differences in height and diameter were calculated to assess changes due to elevated CO₂.

Absolute and relative growth

Leader shoot growth of all plants was measured weekly throughout the 1996 and 1997 growing seasons. Initially, the length of the current-year terminal shoot on the main stem was measured from the base of the lowest brown scale to the tip of the bud, and later on, it was measured from the base of the shoot to the base of the whorl of buds developing at its apex.

Weekly relative stem extension growth rates (RGR_w) were calculated as described by Evans (1972) and weekly absolute growth rates (AGR_w) after Květ et al. (1971) as:

$$\text{RGR}_w = (\ln l_2 - \ln l_1)/(t_2 - t_1), \quad (1)$$

$$\text{AGR}_w = (l_2 - l_1)/(t_2 - t_1), \quad (2)$$

where l_1 and l_2 are leader lengths (mm) at times t_1 and t_2 (days). At the end of each growing season (1996 and 1997), seasonal mean weekly relative (RGR_m) and absolute (AGR_m) leader growth rates were calculated.

Crown structure, crown profile and needle properties

Nondestructive measurements of crown structure were conducted in November 1996 and 1997 and included: length and number of shoots in each tree whorl, needle density in each whorl, and whorl distance from the tree top. The number of additional short shoots (proleptic branches) formed at the base of the highest whorl was quantified. Needle characteristics assessed included: length and radius of the needle (mm), and individual needle fresh (FW) and dry mass (DW). Individual needle surface area, specific leaf area (SLA), leaf water content (WC) and tissue dry to fresh weight ratio (DW/FW) were calculated. The surface area of an individual needle (all-sided area) was determined by measuring the radius and length and assuming that needles are sectors of a half cylinder. Needle density was determined as the number of needles on a 5-cm long section from the middle of the shoot from the south side of the canopy. Five fascicles (needle pairs, with two needles per pair) were taken for determination of needle surface area, FW, DW and SLA, and the results were expressed on an individual needle area basis. After dimension measurements, the needles were weighed and oven-dried at 50 °C to constant

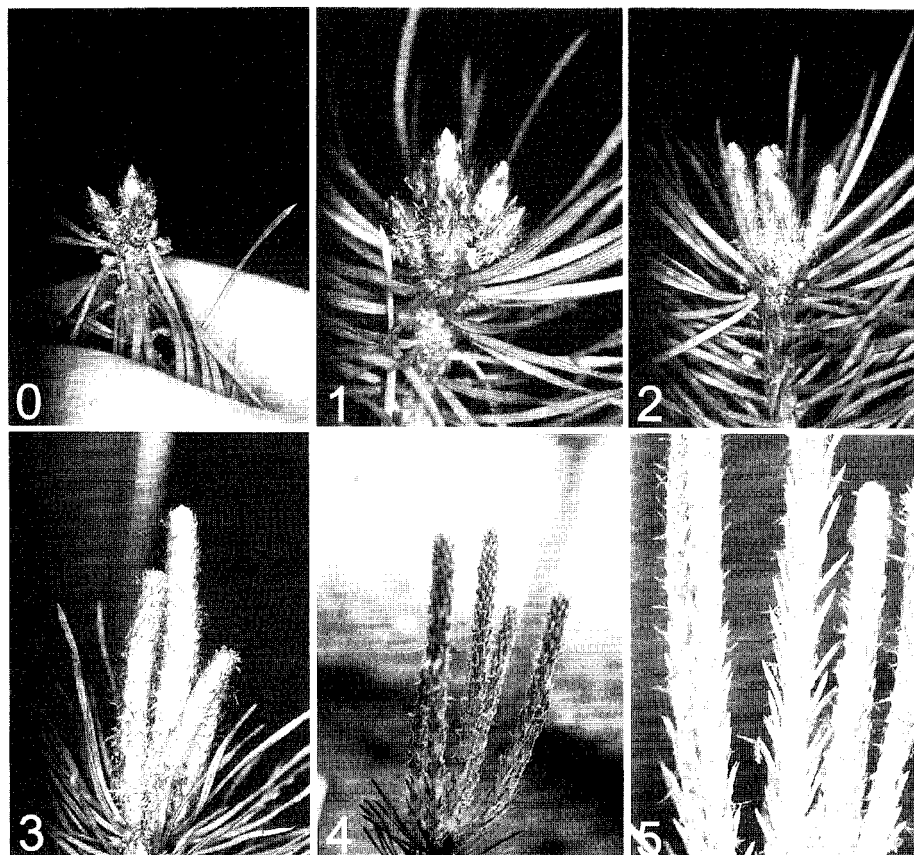


Figure 1. Spring bud phenology of Scots pine (*Pinus sylvestris* L.). Five arbitrary classes were used to quantify phenological development. Class: 0 = bud closed, 1 = bud shows a slight swelling, 2 = swollen bud, 3 = start of bud elongation, 4 = bud burst, 5 = needle elongation.

weight (5 days). Current-year foliage surface area, FW and DW of needles in each whorl and for the whole tree were computed from measurements of individual shoots. Mean values for 12 trees per treatment in 1996 and eight trees in 1997 were used. Leaf area index (LAI) was calculated as the ratio of total projected leaf area of all trees in the OTC divided by the ground area of the OTC (7.1 m²).

Statistical analysis

Because there were only two OTCs (replicates) per treatment, subreplicates (trees) were used rather than real replicates (chambers), in the statistical analysis. So, all trees from two chambers belonging to the same treatment were pooled, giving 22 subreplicates. Initial soil characteristics, air temperature and photon flux density (PPFD) at plant level were similar in all four OTCs. Therefore, differences in plant characteristics between OTCs were most probably attributable to differences in CO₂ concentration of the air. Nevertheless, one-way analysis of variance (ANOVA) and Tukey's test were applied for all OTCs separately, to determine if there were differences between chambers from the same treatment. Subsequently, for the overall comparison between treatments, data were subjected to ANOVA, with the exception of canopy structural and individual needle characteristics, where a two-way ANOVA with repeated measures was used to test the main effect of CO₂ concentration (treatment) and position within the canopy. The

ANOVAs and Tukey's test were performed with Statmost 2.5 (Data Most Corporation, Salt Lake City, UT) software.

Results

Phenology

During both the first (1996) and second (1997) growing seasons in the OTCs, spring bud phenology of plants in elevated CO₂ differed significantly from that of plants in ambient CO₂. The elevated CO₂ treatment hastened bud burst by 6 days in 1996 (May 11 versus May 17; $P < 0.05$) and by 9 days in 1997 (April 26 versus May 5; $P < 0.001$) (Figure 2).

Autumn bud phenology was not followed because bud set was difficult to quantify without destructive dissection of the buds. However, it was observed that elevated CO₂ treatment hastened bud development in the autumn of the first growing season, and buds overwintered in Stage 1 (slightly swollen bud; Figure 1). After the dormancy period, buds in the elevated CO₂ treatment developed faster than buds in the ambient CO₂ treatment.

By the end of the second growing season in the OTCs, a marked increase in needle abscission was observed in the elevated CO₂ treatment. In November 1997, a few two-year-old needles and some one-year-old needles were present on plants in the ambient CO₂ treatment, whereas in the elevated CO₂ treatment, no one-year-old needles were retained on the

plants. Dry mass of litterfall was significantly higher ($P < 0.01$) in the elevated CO_2 treatment than in the ambient CO_2 treatment (53.0 ± 2.9 versus 33.3 ± 7.2 g_{DW} per tree).

Carbohydrate and starch analysis

Elevated CO_2 concentration increased total foliar sugar concentration by 10%, but the effect was not significant. Concentrations of inositol, sorbitol and fructose increased significantly in response to elevated CO_2 (Table 1). The elevated CO_2 treatment resulted in a significant increase (25%) in foliar starch concentration (Table 1).

Growth

Carbon dioxide enrichment significantly enhanced both stem height (Figure 3) and diameter growth (Figure 4) at the end of the first and second growing seasons. Compared with the beginning of each growing season, stem height increased by 43 and 60% by the end of the first year in the ambient and elevated CO_2 treatments, respectively, and the corresponding values at the end of the second year were 78 and 97% (Figure 3). The absolute difference in mean stem height between treatments was 4.1 cm after one growing season and 21.8 cm after two growing seasons (Figure 3). Compared with the beginning of the growing season, stem diameter increased by 130 and 190% in the ambient and elevated CO_2 treatments by the end of the first year, respectively, and the corresponding values for the end of the second year were 91 and 80% (Figure 4). The absolute difference in mean stem diameter between treatments was 4.0 mm after one growing season and 7.2 mm after two

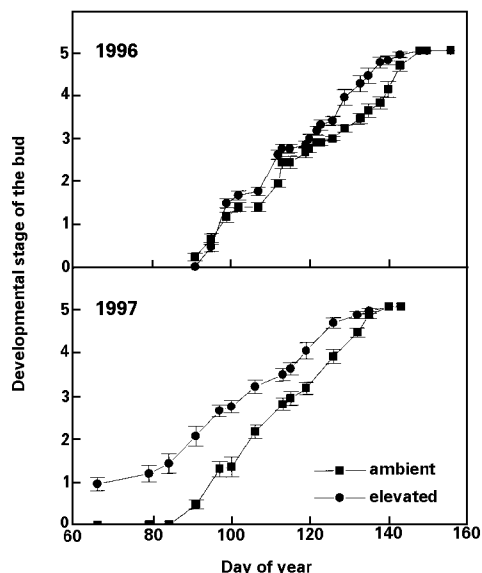


Figure 2. Phenological bud development of young Scots pine seedlings during the first (1996) and the second (1997) year in open top chambers supplied with air containing an ambient (■) or elevated (●) CO_2 concentration. The phenological development was quantified through an arbitrary "class" system. Five classes were used as defined in Figure 1 and range from 0 (closed bud) to 5 (needle elongation). Mean values per treatment \pm SE of 22 plants in 1996 and 22 plants in 1997 are shown.

Table 1. Soluble sugars and starch concentrations (mg cm^{-2}) in current-year needles of Scots pine seedlings in September 1996, after one growing season under ambient and elevated CO_2 conditions. Mean values of 22 plants per treatment \pm SE are shown. Within each row different letters indicate a significant difference ($P < 0.05$).

Sugar	Ambient	Elevated
Inositol	0.078 (0.009) a	0.103 (0.015) b
Sorbitol	0.007 (0.001) a	0.010 (0.004) b
Galactose	0.006 (0.004) a	0.004 (0.005) a
Glucose	0.244 (0.067) a	0.216 (0.065) a
Fructose	0.194 (0.033) b	0.215 (0.032) a
Sucrose	0.009 (0.022) a	0.043 (0.080) a
Total	0.538 (0.107) a	0.591 (0.108) a
Starch	0.32 (0.12) a	0.40 (0.12) b

growing seasons (Figure 4). The maximum difference in relative height and stem diameter ((elevated CO_2 - ambient CO_2)/ambient CO_2) occurred after 13 months of CO_2 exposure (on May 15), when the height and diameter of trees grown in elevated CO_2 were 22 and 41% greater, respectively, than those of trees grown in ambient CO_2 . At the end of the first growing season, differences in relative height and diameter between treatments were 6 and 29%, respectively; and the corresponding values after two growing seasons were 20 and 27%.

Absolute and relative growth

In both the 1996 and 1997 growing seasons, the high RGR_w at the beginning of the growing season was followed by a sharp and continuous decrease in RGR_w until the end of the growing season. This rapid decline in RGR_w after April accounted for the loss of growth enhancement in the elevated CO_2 treatment between May and June (Figure 5). Terminal shoot lengths of seedlings in elevated CO_2 were 21 and 36% larger at the end of the 1996 and 1997 growing seasons, respectively, than the corresponding values for seedlings in ambient CO_2 (Figure 5).

The AGR_m of trees grown in elevated CO_2 remained higher compared with that of the ambient-grown trees in both years (Table 2). In contrast, the RGR_m of trees grown in elevated CO_2 was significantly higher than that of ambient-grown trees in the first year, but not the second (Table 2).

Crown structure, crown profile and needle properties

For all branch and crown characteristics considered, significant differences were observed between CO_2 treatments (with the exception of number of shoots) and locations within the crown (different whorls) (Table 3). During the first year, the main effect of elevated CO_2 on branch characteristics was a 28% increase in total shoot length (Figure 6). Within the crown, the longest shoots were in the uppermost layer. Because the elevated CO_2 treatment had no impact on bud formation during the year before the experiment, the number of shoots did not differ between treatments during the first year, and was highest in the lowest layer of the crown.

During the first year, LAIs in the elevated and ambient CO_2 treatments were 0.56 ± 0.04 and 0.42 ± 0.02 , respectively.

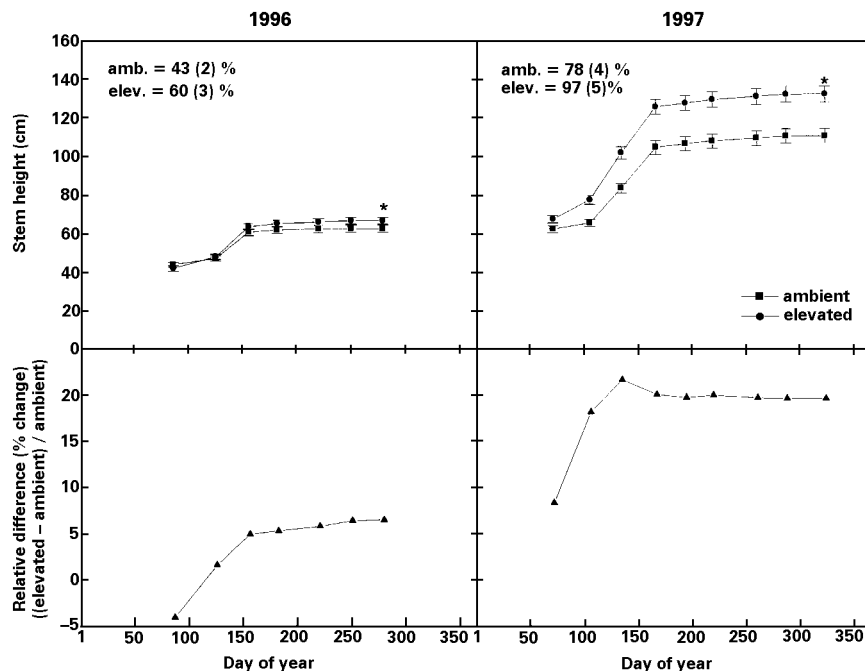


Figure 3. Time course of stem height and relative difference (% change) in height of young Scots pine seedlings during their first (1996, left panels) and second year (1997, right panels) of growth in different open-top chambers. The open-top chambers were ventilated with air at ambient or elevated (ambient + 400 $\mu\text{mol mol}^{-1}$) atmospheric CO₂ concentration. Numbers in the figure in the upper left hand corner of each panel represent percentage increase of initial height in relation to tree height values at the end of the 1996 and 1997 growing season, respectively. Mean values per treatment \pm SE of 22 plants in each year are shown. Statistical differences between the treatments at the end of the growing season were assessed using ANOVA (* = $P \leq 0.05$).

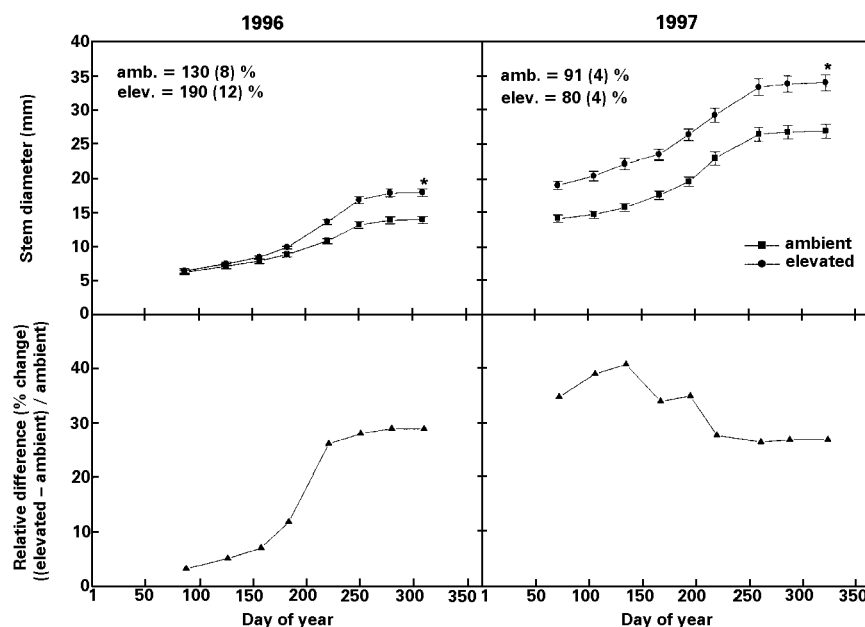


Figure 4. Time course of stem diameter and relative difference (% change) in stem diameter of young Scots pine seedlings during their first (1996, left panels) and second (1997, right panels) year of growth at ambient and elevated CO₂ concentration. Numbers in the figure in the upper left hand corner of each panel represent percentage increase of initial height in relation to tree height at the end of the 1996 and 1997 growing season, respectively. Mean values per treatment \pm SE of 22 plants in each year are shown. Statistical differences between the treatments at the end of the growing season were assessed using ANOVA (* = $P \leq 0.05$).

Longer shoots together with greater individual needle areas (Tables 3 and 4) accounted for a 33% increase in total needle area in response to elevated CO₂. In both treatments there was a trend for bigger needles in the upper part of the crown. Although needle length did not differ within the crown profile (Tables 3 and 4), needle dry mass (DW) was highest in the uppermost crown layer, and was 54% higher in the elevated CO₂ treatment than in the ambient CO₂ treatment. Specific leaf area (SLA) decreased with height in the crown in both treatments and was lower in the elevated CO₂ treatment than in the ambient CO₂ treatment. Water content (WC) of needles in the

elevated CO₂ treatment was significantly lower than that of needles in the ambient CO₂ treatment, and increased with crown height in both treatments (Tables 3 and 4).

During the second growing season in elevated CO₂, the Scots pine trees showed distinct changes in growth pattern compared with the first year. This is characteristic of the determinate growth pattern of *Pinus* species, where current-year elongation is determined by the previous year's stored reserves. The elevated CO₂ treatment significantly increased the number of shoots by 57%, but it had no effect on needle density. More shoots were produced in the lower part of the

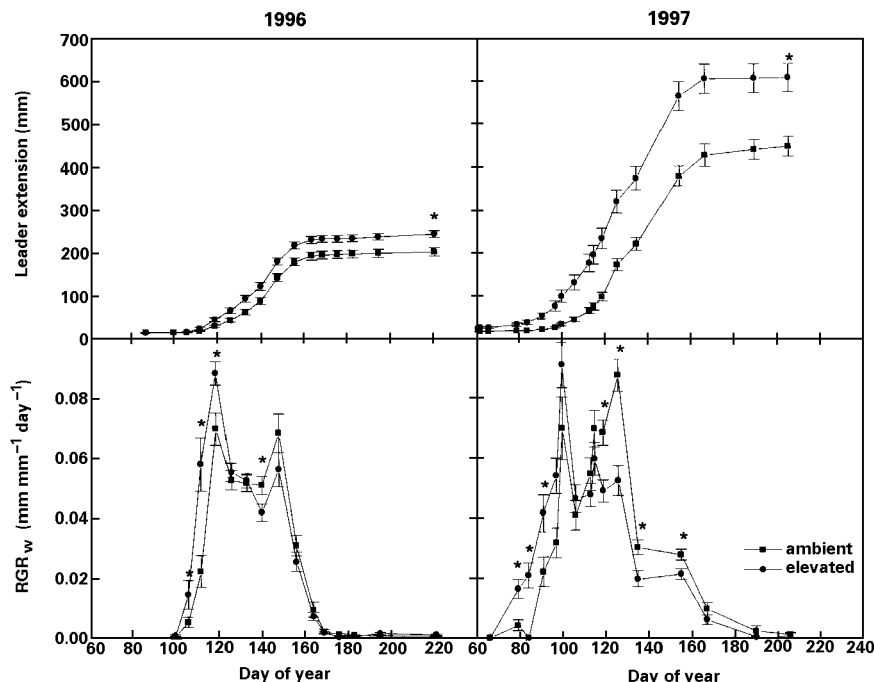


Figure 5. Time course of leader extension (upper panels) and weekly relative growth rate (RGR_w) of the leader (lower panels) of young Scots pine seedlings during their first (1996, left panels) and second (1997, right panels) growing seasons at ambient and elevated atmospheric CO_2 concentration. Mean values per treatment \pm SE of 22 plants in each year are shown. Statistical differences between the treatments at a specific date were assessed using ANOVA, (* = $P \leq 0.05$). For the upper panels (leader extension) only the differences at the end of the growing season were analyzed, whereas for the lower panels all sampling data were compared.

Table 2. Seasonal mean weekly absolute (AGR_m , $mm\ day^{-1}$) and relative (RGR_m , $mm\ mm^{-1}\ day^{-1}$) leader elongation rates for Scots pine seedlings growing in ambient or elevated (ambient + $400\ \mu mol\ mol^{-1}$) atmospheric CO_2 concentrations. Mean values of 22 plants per treatment \pm SE are shown after the first (1996) and second (1997) growing season. Values followed by the same letter within the same column are not significantly different at $P \leq 0.05$.

	Treatment	AGR_m	RGR_m
1996	Ambient	1.404 (0.067) a	0.0199 (0.0004) a
	Elevated	1.720 (0.056) b	0.0215 (0.0002) b
1997	Ambient	2.984 (0.161) c	0.0227 (0.0004) c
	Elevated	4.046 (0.220) d	0.0222 (0.0004) cb

crown in elevated CO_2 than in ambient CO_2 (Figure 6, Table 3). Also, significantly ($P = 0.04$) more short shoots (proleptic branches) were formed from the base of the highest whorl in elevated CO_2 than in ambient CO_2 . Total needle area per tree increased in response to elevated CO_2 , mainly as a result of increases in total shoot length and total number of shoots, but also because of a slight increase in individual needle area. The relative difference in total needle area and total shoot length between trees in the ambient and elevated CO_2 treatments was 125 and 127%, respectively.

During the second year, estimated LAI was 3.66 ± 0.26 and 1.62 ± 0.24 in the elevated and ambient CO_2 treatments, respectively. Needle length and radius significantly differed with height in the crown, with the longest and thickest needles in the upper part of the crown (Tables 3 and 4). Specific leaf area was always lower in the lower crown than in the upper crown and generally higher in the second year of treatment than the first (Tables 3 and 4).

Discussion

Bud burst was advanced by elevated CO_2 during both the first and the second year of the experiment. Also, because overwintering buds in the elevated CO_2 treatment were already slightly advanced in phenological stage compared with buds in the ambient CO_2 treatment, it was inferred that elevated CO_2 enhanced bud development in the autumn of 1996. Repo et al. (1996) reported that dehardening in Scots pine seedlings grown at elevated temperatures proceeded significantly faster if the seedlings were exposed to elevated CO_2 . Hänenen et al. (1993) observed that premature bud burst increases the risk of frost damage in Scots pine. However, premature growth onset in trees exposed to elevated CO_2 may be compensated by increased frost hardiness as a result of increased soluble sugar concentration. With increased atmospheric CO_2 concentrations, cells may accumulate greater quantities of sugars and other energy storage compounds. Soluble sugars, which make up nearly all the nonstructural carbohydrates of coniferous shoots during the winter (Ericsson 1979), play an important role in cellular cryoprotection (Crowe et al. 1991) and are essential to the development of frost hardiness (Repo et al. 1996).

In some studies with conifers, elevated CO_2 had no effect on carbohydrate concentration (Campagna and Margolis 1989, Chomba et al. 1993), whereas other studies showed increased accumulation (Barnes et al. 1995). In our experiment, the concentrations of soluble carbohydrates did not change significantly in response to elevated CO_2 .

We observed enhanced and earlier needle fall in autumn in elevated CO_2 than in ambient CO_2 . Premature needle abscission in the elevated CO_2 treatment may have resulted from changes in transpiration rate (Surano et al. 1986), or the remobilization of nutrients.

Table 3. Statistical significance (two-way ANOVA) of the effects of crown level (location within the crown) and treatment (CO₂ concentration) on different individual needle characteristics (SLA = specific leaf area, WC = water content, DW = dry mass of individual needle, FW = fresh weight of individual needle) and canopy structural parameters at the end of the first (1996) and second (1997) growing season in open-top chambers (** = $P \leq 0.01$, * = $P \leq 0.05$, and ns = $P \geq 0.05$).

Parameter	Location within crown		Treatment	
	1996	1997	1996	1997
Surface area of individual needle (cm ²)	**	**	**	ns
Needle length (mm)	ns	*	*	**
Needle radius (mm)	**	**	**	**
SLA (cm ² g ⁻¹)	**	**	**	ns
WC (g g ⁻¹)	**	ns	ns	ns
DW/FW (%)	**	ns	ns	ns
Surface area (m ² stem ⁻¹)	**	**	**	**
Needle DW (g stem ⁻¹)	**	**	**	**
Number of shoots (g stem ⁻¹)	**	**	ns	**
Mean shoot length (cm stem ⁻¹)	**	**	**	**
Total shoot length (cm stem ⁻¹)	**	**	**	*

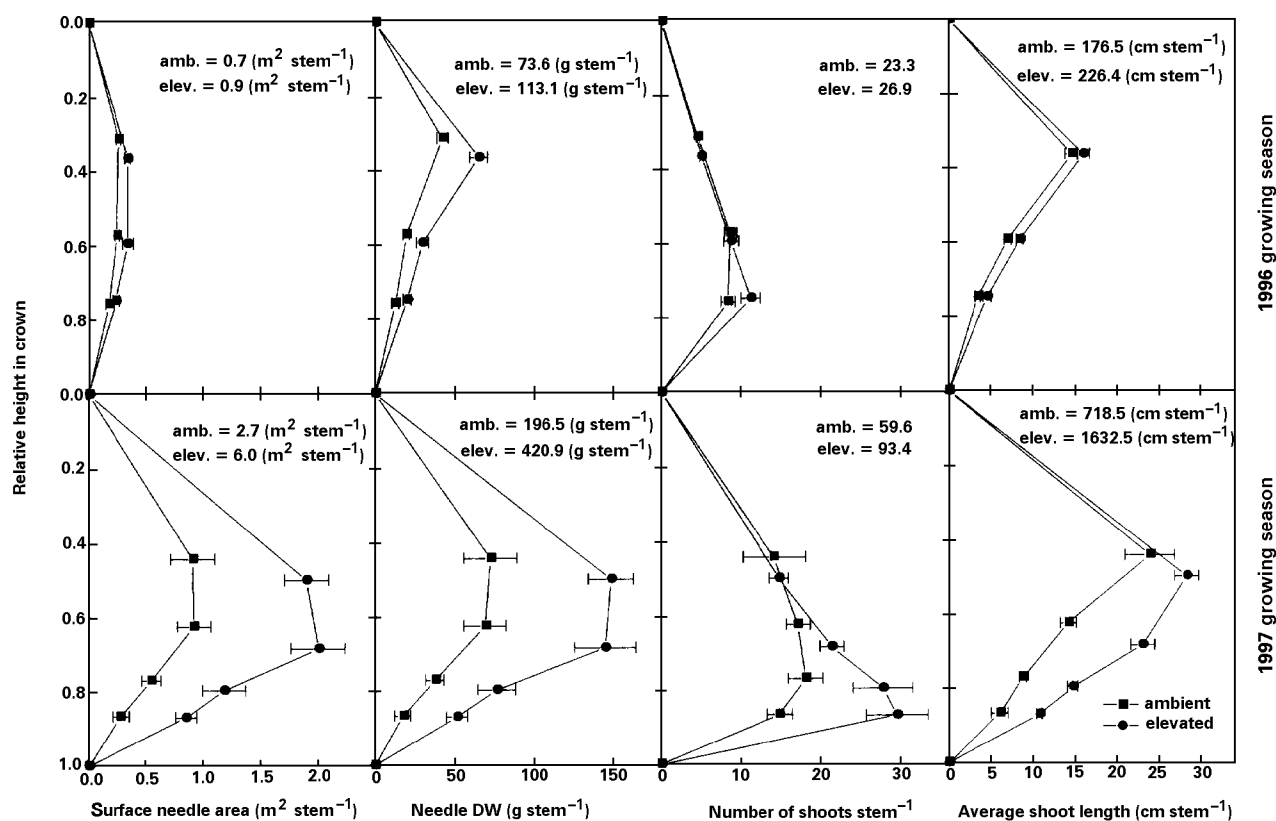


Figure 6. Needle surface area, needle dry mass (DW), shoot number and average shoot length (abscissa) as a function of the relative height within the crown (ordinate, where 1.0 represents ground level and 0.0 represents the top of the tree) for Scots pine grown in open-top chambers in ambient or elevated (ambient + 400 $\mu\text{mol mol}^{-1}$) CO₂ during the first (1996) and second (1997) years of treatment. Numbers in the figure represent total needle area, total needle DW, total shoot number or total shoot length for a given treatment. Mean values per treatment \pm SE of 12 plants in 1996 and 8 plants in 1997 are shown.

In agreement with other published reports (Conroy et al. 1986, Koch et al. 1986, Conroy et al. 1988, Norby and O'Neill 1991, El Kohen and Mousseau 1994, Wilkins et al. 1994,

Curtis et al. 1995, Wang 1996, Tissue et al. 1997), we observed a tendency toward a larger total leaf area in response to elevated CO₂. This increase in total needle area in response to

Table 4. Individual needle characteristics (SLA = specific leaf area, WC = water content ((FW – DW)/DW), DW/FW = dry mass/fresh mass ratio) as a function of location within the crown, and crown averages for current-year foliage in the ambient and elevated (ambient + 400 $\mu\text{mol mol}^{-1}$) CO₂ treatments in the first (1996) and second (1997) growing season of the treatment. Numbers 1–4 indicate the whorl number, where 1 is the lowest and 3 or 4 the highest layer in the crown. Values are means \pm SE for individual plants (12 plants in 1996 and eight plants in 1997) in two open-top chambers for each CO₂ treatment.

Parameter	1996					1997				
	CO ₂ Treatment	1	2	3	Canopy average	1	2	3	4	Canopy average
Surface area (cm ²)	Ambient	2.54 (0.26)	2.75 (0.15)	3.17 (0.12)	2.82 (0.18)	1.79 (0.14)	2.19 (0.14)	2.14 (0.19)	2.66 (0.16)	2.20 (0.16)
	Elevated	3.05 (0.19)	3.30 (0.22)	4.04 (0.25)	3.45 (0.22)	1.99 (0.15)	1.90 (0.12)	2.54 (0.19)	2.81 (0.19)	2.31 (0.16)
Needle length (mm)	Ambient	95.82 (8.31)	92.91 (3.94)	99.68 (2.87)	96.13 (5.04)	75.44 (3.58)	83.63 (3.61)	82.63 (4.79)	90.42 (1.36)	83.03 (4.08)
	Elevated	101.25 (4.44)	101.82 (4.68)	113.22 (5.69)	105.43 (4.94)	69.75 (4.13)	66.14 (2.88)	79.02 (4.25)	80.46 (4.41)	73.84 (3.92)
Needle radius (mm)	Ambient	1.02 (0.03)	1.14 (0.03)	1.24 (0.03)	1.13 (0.02)	0.92 (0.03)	1.01 (0.03)	1.00 (0.04)	1.14 (0.02)	1.02 (0.10)
	Elevated	1.16 (0.03)	1.25 (0.04)	1.37 (0.03)	1.26 (0.04)	1.10 (0.03)	1.11 (0.04)	1.25 (0.05)	1.36 (0.05)	1.20 (0.04)
SLA (cm ² g ⁻¹)	Ambient	141.32 (10.58)	128.45 (2.68)	63.41 (1.58)	111.06 (4.95)	160.07 (2.52)	145.67 (7.52)	138.54 (5.61)	124.24 (4.21)	142.13 (7.47)
	Elevated	120.68 (3.58)	116.35 (2.34)	54.29 (1.65)	97.11 (2.52)	168.47 (8.81)	154.50 (2.89)	140.32 (4.19)	132.00 (3.01)	148.82 (4.72)
WC (g g ⁻¹)	Ambient	1.15 (0.05)	1.11 (0.05)	1.69 (0.03)	1.32 (0.04)	1.37 (0.13)	1.41 (0.17)	1.40 (0.09)	1.34 (0.09)	1.38 (0.12)
	Elevated	1.10 (0.07)	1.15 (0.06)	1.62 (0.05)	1.29 (0.06)	1.56 (0.10)	1.55 (0.10)	1.33 (0.08)	1.36 (0.05)	1.45 (0.08)
DW/FW (%)	Ambient	46.83 (1.02)	47.56 (1.08)	37.20 (0.41)	43.86 (0.84)	43.03 (2.12)	42.91 (2.81)	41.99 (1.49)	43.07 (1.52)	42.75 (1.98)
	Elevated	48.14 (1.67)	46.94 (1.35)	38.38 (0.75)	44.49 (1.26)	39.50 (1.49)	39.66 (1.49)	43.29 (1.49)	42.46 (0.92)	41.23 (1.35)

elevated CO₂ treatment was accounted for by longer shoots and an increase in individual needle area in the first year, and by an increase in the number and length of shoots in the second year. Increased secondary branching has also been reported by Tinus (1972), Sionit et al. (1985), Idso et al. (1991), Mortenson (1994) and Wilkins et al. (1994).

In response to CO₂ enrichment, Scots pine seedlings grew taller and produced thicker stems, during both the first and the second growing seasons. Similar results on height growth have been reported for other conifers (Tinus 1972, Higginbotham et al. 1985, Sionit et al. 1985, Guehl et al. 1994, Pushnik et al. 1995). We observed 6.5 (1996) and 20% (1997) increases in stem height in response to elevated CO₂. The increase in the second year is comparable to the 19 and 22% increases reported by Guehl et al. (1994) and Teskey (1995), respectively. In several studies on conifers, no effects of elevated CO₂ on stem diameter were observed (Pushnik et al. 1995), but in other studies elevated CO₂ resulted in a significant increase in stem diameter (Tinus 1972, Sionit et al. 1985, Surano et al. 1986, Guehl et al. 1994). In our study, elevated CO₂ increased stem diameter by 29% in 1996 and 27% in 1997 compared with ambient-grown trees. Guehl et al. (1994) reported an increase in stem diameter of 14% in response to elevated CO₂. The major

increase in diameter growth occurred after the completion of height growth. The relative difference in stem diameter between ambient and elevated CO₂ treatments tended to decrease during the second year, after an initial increase during the first year. A similar relative decrease over time in CO₂-stimulation of stem diameter growth was found by Hättenschwiler et al. (1997) for two Mediterranean forest tree species growing for 30 years near natural CO₂ springs.

Although elevated CO₂ may increase tree growth (see reviews by Eamus and Jarvis 1989, Idso and Idso 1994) and consequently mean RGR, many studies have demonstrated that the enhancement is reduced if the treatment is prolonged (e.g., Ceulemans and Mousseau 1994). Furthermore, evidence for a faster decline in mean RGR with tree age has been reported in elevated CO₂ than in ambient CO₂ (Bazzaz 1990, Poorter 1993).

In our study, Scots pine grown at an elevated CO₂ concentration had significantly higher RGR_w at the beginning of the growing season, but later in the season RGR_w declined rapidly, becoming lower than that of plants exposed to the ambient CO₂ concentration. Similarly, Murray et al. (1996) found a stimulation of leader shoot growth by elevated CO₂ only at the beginning of the season. The greater length of the leading shoots of

seedlings in elevated CO₂ relative to those in ambient CO₂ was mainly the result of an initial stimulation in relative growth rate (higher AGR_w and RGR_w), rather than a longer period of growth. Elevated CO₂ treatment also hastened bud burst in both years.

The effect of CO₂ treatment on leader shoot extension (RGR_m) varied between years. During the first year, seasonal mean weekly relative growth (RGR_m) of plants in elevated CO₂ was significantly increased relative to that of control plants, but no such effect was apparent in the second year. Similarly, Norby et al. (1996) and Rey and Jarvis (1997) found that trees grew at the same RGR after a few growing seasons regardless of CO₂ treatment. These results are consistent with other studies reporting that elevated CO₂ treatment stimulates growth only in the early stages of the experiments (Brown and Higginbotham 1986, Brown 1991, Coleman et al. 1993, DeLucia et al. 1994, Norby et al. 1995, Tissue et al. 1996, Tissue et al. 1997).

Although we do not know what caused the effect of elevated CO₂ on relative growth rate to diminish, down-regulation of photosynthesis in the elevated CO₂ treatment (measured at 350 μmol mol⁻¹ CO₂) was observed in 1997 (authors' unpublished data) and could account for the decrease in RGR. A reduction in relative growth rate in the elevated CO₂ treatment mediated by limitations on photosynthesis might be mediated by impacts on: (i) nutrient supply, (ii) sink strength, and (iii) LAI, as discussed in the following paragraphs.

(i) Nutrient supply limitation As a result of the initially accelerated growth and subsequent increased demand for nutrients, the growth of plants in elevated CO₂ may become limited by the supply of nutrients, particularly nitrogen (Brown and Higginbotham 1986). In our experiment, nutrient concentrations in the soil were similar in both treatments in April 1996. However, soil analysis at the end of 1997, revealed that concentrations of N and nitrate-N were lower in the elevated CO₂ treatment than in the ambient CO₂ treatment (N: 12.50 ± 1.48 versus 20.33 ± 2.35 mg N l⁻¹; and nitrate-N: < 5.00 versus 9.17 ± 2.20 mg nitrate-N l⁻¹), indicating that plants in the elevated CO₂ treatment used the available soil nutrient pool faster than the trees grown in the ambient treatment. A reduction in N content of plant tissues has been attributed to physiological changes in plant nitrogen-use efficiency (e.g., Norby et al. 1986). In September 1996, the N concentration of needles formed in 1996 was higher in the elevated CO₂ treatment than in the ambient CO₂ treatment (1.63 ± 0.04 versus 1.35 ± 0.05) (g m⁻²); however, the N concentration of needles in the elevated CO₂ treatment decreased throughout the 1997 growing season reaching a value of 0.66 ± 0.05 g m⁻² in September 1997 compared with a value of 1.28 ± 0.09 g m⁻² for needles in the ambient CO₂ treatment. The decline in leaf N presumably led to a decline in photosynthesis and, hence, growth in 1997, because photosynthesis is closely correlated with leaf nitrogen concentration (Evans 1989, Pettersson et al. 1993, Kellomäki and Wang 1997).

(ii) Sink limitation According to the sink limitation hypothesis, high photosynthetic rates can only be maintained while the demand for photosynthates is high. Because Scots pine has a

predetermined growth pattern, both shoot number and shoot length are to a large extent determined by the number of primordia initiated during the previous year (Bollman and Sweet 1976, Stenberg et al. 1994). It is possible, therefore, that at some point during the second year of our experiment, sink capacity fell below the potential for photosynthetic production in elevated CO₂, thereby causing photosynthetic down-regulation and a decline in RGR_w.

(iii) Leaf area index According to Hsiao's (1982) model, seedlings in the exponential growth phase are generally source limited before canopy closure, and biomass production is generally determined by leaf RGR. However after canopy closure, biomass production becomes proportional to canopy assimilation rate. Higher leaf area results in a higher biomass but this response can be maintained only as long as leaf area increases.

We found that RGR_m of the terminal leader was higher in the elevated CO₂ treatment than in the ambient CO₂ treatment during the first year of our experiment, when the canopy was open. During the second year, canopy density increased, and LAI of plants in the elevated CO₂ treatment was 125% higher than that of plants in the ambient CO₂ treatment, indicating that elevated CO₂ led to more rapid canopy closure (Hättenschwiler et al. 1997). However, if reduced whole-plant photosynthesis resulting from increased needle self-shading (Poorter 1993) limits growth, then tree height and diameter growth of Scots pine could be limited by LAI after exposure to elevated CO₂ for two growing seasons. If this is true, growth of Scots pine is unlikely to be stimulated by elevated CO₂ treatment after canopy closure, when trees attain a relatively constant leaf area.

Photosynthesis mirrored all or some of the above mentioned limitations and resulted in decreased RGR. Differences in photosynthetic rates between treatments were not significant at the beginning of the 1997 growing season (8% lower in the elevated CO₂ treatment), when sink demand was high, because the trees were actively growing in height and canopy closure had not occurred. In midsummer, when height growth was completed, photosynthetic rates declined significantly (36%) in the elevated CO₂ treatment compared with the ambient CO₂ treatment as a result of increased self-shading and source-sink limitations. In September, the decline in photosynthetic rate was slightly less (23%) than in midsummer, probably because of stem diameter and root growth and therefore higher sink demand and increased nutrient acquisition.

Growth-related processes provide another possible explanation for the observed lowering of relative growth rate in response to elevated CO₂. For ontogenetic reasons, RGR declines as plants get larger and this is particularly evident in young woody plants (Jarvis and Jarvis 1964, Evans 1972). Thus, if growth of young trees is enhanced by elevated CO₂ the size-related decline in RGR will be accelerated.

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References

- Acock, B. and L.H. Allen. 1985. Crop responses to elevated carbon dioxide concentration. *In* Direct Effects of Increasing Carbon Dioxide on Vegetation. Eds. B. Strain and A. Cure. U.S. Department of Energy, Washington, D.C., pp 53–97.
- Barnes, J.D., T. Pfirrmann, K. Steiner, C. Lutz, U. Busch, H. Kuchenhoff and H.D. Payer. 1995. Effects of elevated CO₂, elevated O₃ and potassium deficiency on Norway spruce (*Picea abies* (L.) Karst.): seasonal changes in photosynthesis and non-structural carbohydrate content. *Plant Cell Environ.* 18:1345–1357.
- Bazzaz, F.A. 1990. The response of natural ecosystems to rising global CO₂ levels. *Annu. Rev. Ecol. Syst.* 21:167–196.
- Berryman, C.A., D. Eamus and G.A. Duff. 1993. The influence of CO₂ enrichment on growth, nutrient content and biomass allocation of *Marantes corymbosa*. *Aust. J. Bot.* 41:195–209.
- Beuker, E. 1994. Adaptation to climatic changes of the timing of bud burst in populations of *Pinus sylvestris* L. and *Picea abies* (L.) Karst. *Tree Physiol.* 14:961–970.
- Bollman, M.P. and G.B. Sweet. 1976. Bud initiation of *Pinus radiata* in New Zealand. 1. The initiation and extension of the leading shoot of one clone at two sites. *N.Z. J. For. Sci.* 6:376–392.
- Brown, K.R. 1991. Carbon dioxide enrichment accelerates the decline in nutrient status and relative growth rate of *Populus tremuloides* Michx. seedlings. *Tree Physiol.* 8:161–173.
- Brown, K. and K.O. Higginbotham. 1986. Effects of carbon dioxide enrichment and nitrogen supply on growth of boreal tree seedlings. *Tree Physiol.* 2:223–232.
- Campagna, M.A. and H.A. Margolis. 1989. Influence of short-term atmospheric CO₂ enrichment on growth, allocation patterns, and biochemistry of black spruce seedlings at different stages of development. *Can. J. For. Res.* 19:773–782.
- Cannell, M.G.R. 1990. Modelling the phenology of trees. *Silva Carelica* 15:11–27.
- Cannell, M.G.R. and R.I. Smith. 1986. Climatic warming, spring bud burst and frost damage on trees. *J. Appl. Ecol.* 23:177–191.
- Ceulemans, R. and M. Mousseau. 1994. Effects of elevated atmospheric CO₂ on woody plants. *New Phytol.* 127:425–446.
- Ceulemans, R., X.N. Jiang and B.Y. Shao. 1995. Effects of elevated atmospheric CO₂ on growth, biomass production and nitrogen allocation of two *Populus* clones. *J. Biogeogr.* 22:261–268.
- Chen, S.G., I. Impens and R. Ceulemans. 1997. Modelling the effects of elevated atmospheric CO₂ on crown development, light interception and photosynthesis of poplar in open-top chambers. *Global Change Biol.* 3:97–106.
- Chomba, B.M., R.D. Guy and H.G. Weger. 1993. Carbohydrate reserve accumulation and depletion in Engelmann spruce (*Picea engelmannii* Parry): Effects of cold storage and prestorage CO₂ enrichment. *Tree Physiol.* 13:351–364.
- Coleman, J.S., K.D.M. McConnaughay and F.A. Bazzaz. 1993. Elevated CO₂ and plant nitrogen-use: is the tissue nitrogen concentration size-dependent? *Oecologia* 93:195–200.
- Conroy, J.P., E.W.R. Barlow and D.I. Bevege. 1986. Response of *Pinus radiata* seedlings to carbon dioxide enrichment at different levels of water and phosphorus: growth, morphology and anatomy. *Ann. Bot.* 7:165–177.
- Conroy, J.P., M. Koppers, B. Koppers, J. Virgona and E.W.R. Barlow. 1988. The influence of CO₂ enrichment, phosphorus deficiency and water stress on the growth, conductance and water use of *Pinus radiata* D. Don. *Plant Cell Environ.* 11:91–98.
- Crowe, J.H., J.F. Carpenter, L.M. Crowe and T.J. Anchordoguy. 1991. Are freezing and dehydration similar stress vectors? A comparison of modes of interaction of stabilizing solutes with biomolecules. *Cryobiology* 27:219–231.
- Curtis, P.S., C.S. Vogel, K.S. Pregitzer, D.R. Zak and J.A. Teeri. 1995. Interacting effects of soil fertility and atmospheric CO₂ on leaf growth and carbon gain physiology in *Populus euramericana* (Dode) Guinier. *New Phytol.* 129:253–263.
- Curtis, P.S. and X. Wang. 1998. A meta-analysis of elevated CO₂ effects on woody plant mass, form, and physiology. *Oecologia* 113:299–313.
- Day, F.P., E.P. Weber, C.R. Hinckle and B.G. Drake. 1996. Effects of elevated atmospheric CO₂ on fine root length and distribution in an oak-palmetto scrub ecosystem in central Florida. *Global Change Biol.* 2:143–148.
- DeLucia, E.H., R.M. Callaway and W.H. Schlesinger. 1994. Offsetting changes in biomass allocation and photosynthesis in ponderosa pine (*Pinus ponderosa*) in response to climate change. *Tree Physiol.* 14:669–667.
- Eamus, D. and P.G. Jarvis. 1989. The direct effect of increase in the global atmospheric CO₂ concentration on natural and commercial temperate forests. *Adv. Ecol. Res.* 19:1–55.
- El Kohen, A. and M. Mousseau. 1994. Interactive effects of elevated CO₂ and mineral nutrition on growth and CO₂ exchange of sweet chestnut seedlings (*Castanea sativa*). *Tree Physiol.* 14:679–690.
- Ericsson, A. 1979. Effects of fertilization and irrigation on the seasonal changes of carbohydrate reserves in different age-classes of needles on 20-year-old Scots pine trees (*Pinus sylvestris*). *Physiol. Plant.* 45:270–280.
- Evans, G.C. 1972. Relative growth rate. *In* The Quantitative Analysis of Plant Growth. Eds. D.J. Anderson, P. Greigh-Smith and F.A. Pitelka. Blackwell Scientific Publications, Oxford, U.K., pp 246–254.
- Evans, J.R. 1989. Photosynthesis and nitrogen relationships in leaves of C₃ plants. *Oecologia* 78:9–19.
- Fales, F.W. 1951. The assimilation and degradation of carbohydrates by yeast cells. *J. Biol. Chem.* 193:113–124.
- Guehl, J.M., C. Picon, G. Aussenac and P. Gross. 1994. Interactive effects of elevated CO₂ and soil drought on growth and transpiration efficiency and its determinants in two European forest tree species. *Tree Physiol.* 14:707–724.
- Häninen, H., S. Kellomäki, K. Laitinen, B. Pajari and T. Repo. 1993. Effect of increased winter temperature on the onset of height growth of Scots pine: a field test of a phenological model. *Silva Fenn.* 4:251–257.
- Hättenschwiler, S., F. Miglietta, A. Raschi and C. Körner. 1997. Thirty years of *in situ* tree growth under elevated CO₂: a model for future forest response? *Global Change Biol.* 3:463–471.
- Higginbotham, K.O., J.M. Mayo, S. L'Hirondelle and D.K. Krystofiak. 1985. Physiological ecology of lodgepole pine (*Pinus contorta*) in an enriched CO₂ environment. *Can. J. For. Res.* 15:417–421.
- Hsiao, T.C. 1982. The soil-plant-atmosphere continuum in relation to drought and crop production. *In* Drought Resistance in Crops, with Emphasis on Rice. Eds. International Rice Research Institute, Los Banos, Philippines, pp 39–52.

- Idso, K.E. and S.B. Idso. 1994. Plant responses to atmospheric CO₂ enrichment in the face of environmental constraints: a review of the past 10 years' research. *Agric. For. Meteorol.* 69:153–203.
- Idso, S.B., B.A. Kimball and S.G. Allen. 1991. CO₂ enrichment of sour orange trees: 2.5 years into a long-term experiment. *Plant Cell Environ.* 14:351–353.
- Janssens, I.A., M. Crookshanks, G. Taylor and R. Ceulemans. 1998. Elevated CO₂ increases fine root production, respiration, rhizosphere respiration and soil CO₂ efflux in Scots pine seedlings. *Global Change Biol.* 4:871–878.
- Jarvis, P.G. and M.S. Jarvis. 1964. Growth rates of woody plants. *Physiol. Plant.* 17:654–666.
- Kaushal, P., J.M. Guehl and G. Aussenac. 1989. Differential growth response to atmospheric carbon dioxide enrichment in seedlings of *Cedrus atlantica* and *Pinus nigra* ssp. *laricio* var. *Corsicana*. *Can. J. For. Res.* 19:1351–1358.
- Kellomäki, S. and K.J. Wang. 1997. Effects of long-term CO₂ and temperature elevation on crown nitrogen distribution and daily photosynthetic performance of Scots pine. *For. Ecol. Manag.* 99:309–326.
- Kimball, B.A. 1983. Carbon dioxide and agricultural yield: an assemblage and analysis of 430 prior observations. *Agron. J.* 75:779–788.
- Koch, K.E., P.H. Jones, W.T. Avigne, L.H. Allen. 1986. Growth and dry matter partitioning and diurnal activities of RuBP carboxylase in citrus seedlings maintained at two levels of CO₂. *Physiol. Plant.* 67:477–484.
- Kubiske, M.E., K.S. Pregitzer, C.J. Mikan, D.R. Zak, J.L. Maziasz and J.A. Teeri. 1997. *Populus tremuloides* photosynthesis and crown architecture in response to elevated CO₂ and soil N availability. *Oecologia* 110:328–336.
- Kvêt, J., J.P. Ondok, J. Necas and P.J. Jarvis. 1971. Methods of growth analysis. In *Plant Photosynthetic Production: Manual of Methods*. Eds. Z. Šesták, Z. Ěatsky and P.G. Jarvis. Dr. W. Junk N.V. Publishers, The Hague, pp 343–391.
- Lee, H.S.J. and P.G. Jarvis. 1995. Trees differ from crops and from each other in their responses to increases in CO₂. *J. Biogeogr.* 22:323–330.
- Miao, S.L., P.M. Wayne and F.A. Bazzaz. 1992. Elevated CO₂ differentially alters the responses of co-occurring birch and maple seedlings to a moisture gradient. *Oecologia* 90:300–304.
- Murray, M.B., M.G.R. Cannell and R.I. Smith. 1989. Date of bud burst of fifteen tree species in Britain following climatic warming. *J. Appl. Ecol.* 26:693–700.
- Murray, M.B., R.I. Smith, I.D. Leith, D. Fowler, H.S.J. Lee, A.D. Friend and P.J. Jarvis. 1994. Effects of elevated CO₂, nutrition and climatic warming on bud phenology in Sitka spruce (*Picea sitchensis*) and their impact on the risk of frost damage. *Tree Physiol.* 14:691–706.
- Murray, M.B., I.D. Leith and P.G. Jarvis. 1996. The effect of long term CO₂ enrichment on the growth, biomass partitioning and mineral nutrition of Sitka spruce (*Picea sitchensis* (Bong.) Carr.). *Trees* 10:393–402.
- Mortenson, L.M. 1994. The influence of carbon dioxide or ozone concentration on growth and assimilate partitioning in seedlings of nine conifers. *Acta Agric. Scand.* 44:157–163.
- Norby, R.J. and E.G. O'Neill. 1986. Effects of atmospheric CO₂ enrichment on the growth and mineral nutrition of *Quercus alba* seedlings in nutrient poor soil. *Plant Physiol.* 82:83–89.
- Norby, R.J. and E.G. O'Neill. 1989. Growth dynamics and water use of seedlings of *Quercus alba* L. in CO₂-enriched atmospheres. *New Phytol.* 111:491–500.
- Norby, R.J. and E.G. O'Neill. 1991. Leaf area compensation and nutrient interactions in CO₂-enriched seedlings of yellow-poplar trees (*Liriodendron tulipifera* L.). *New Phytol.* 117:515–528.
- Norby, R.J., E.G. O'Neill and R.J. Luxmoore. 1986. Effects of atmospheric CO₂ enrichments on the growth and mineral nutrition of *Quercus alba* seedling in nutrient-poor soil. *Plant Physiol.* 82:83–89.
- Norby, R.J., S.D. Wullschlegel, C.A. Gunderson and C.T. Nietch. 1995. Increased growth efficiency of *Quercus alba* trees in a CO₂-enriched atmosphere. *New Phytol.* 131:91–97.
- Norby, R.J., S.D. Wullschlegel and C.A. Gunderson. 1996. Tree responses to elevated CO₂ and implications for forests. In *Carbon Dioxide and Terrestrial Ecosystems*. Eds. G.W. Koch and H.A. Mooney. Academic Press, New York, pp 1–19.
- Pettersson, R., A.J.S. McDonald and I. Stadenberg. 1993. Response of small birch plants (*Betula pendula* Roth.) to elevated CO₂ and nitrogen supply. *Plant Cell Environ.* 16:1115–1121.
- Poorter, H. 1993. Interspecific variation in the growth response of plants to an elevated ambient CO₂ concentration. *Vegetatio* 104/105:77–97.
- Pushnik, J.C., R.S. Demaree, J.L. Houppis, W.B. Flory, S.M. Bauer and P.D. Anderson. 1995. The effect of elevated carbon dioxide on a Sierra-Nevadan dominant species: *Pinus ponderosa*. *J. Biogeogr.* 22:249–254.
- Repo, T., H. Hänninen and S. Kellomäki. 1996. The effects of long-term elevation of air temperature and CO₂ on the frost hardiness of Scots pine. *Plant Cell Environ.* 19:209–216.
- Rey, A. and P.G. Jarvis. 1997. Growth response of young birch trees (*Betula pendula* Roth.) after four and a half years of CO₂ exposure. *Ann. Bot.* 80:809–816.
- Rogers, H.H., G.B. Runion and S.V. Krupa. 1994. Plant responses to atmospheric CO₂ enrichment with emphasis on roots and the rhizosphere. *Environ. Pollut.* 83:155–189.
- Rose, R., C.L. Rose, S.K. Omi, K.R. Forry, D.M. Durall and W.L. Bigg. 1991. Starch determination by perchloric acid vs. enzymes: Evaluating the accuracy and precision of six colorimetric methods. *J. Agric. Food Chem.* 39:2–11.
- Samuelson, L.J. and J.R. Seiler. 1993. Interactive role of elevated CO₂, nutrient limitations, and water stress in the growth responses of red spruce seedlings. *For. Sci.* 39:348–358.
- Sionit, N., B.R. Strain, H. Hellmers, G.H. Riechers and C.H. Jaeger. 1985. Long-term atmospheric CO₂ enrichment affects the growth and development of *Liquidambar styraciflua* and *Pinus taeda* seedlings. *Can. J. For. Res.* 15:468–471.
- Stenberg, P., T. Kuuluvainen, S. Kellomäki, J. Grace, E.J. Jokela and H.L. Gholz. 1994. Crown structure, light interception and productivity of pine trees and stands. *Ecol. Bull.* 43:20–34.
- Surano, K.A., P.F. Daley, J.L.J. Houppis, J.H. Shinn, J.A. Helms, R.J. Palassou and M.P. Costella. 1986. Growth and physiological responses of *Pinus ponderosa* Dougl. ex P. Laws. to long-term CO₂ concentrations. *Tree Physiol.* 2:243–259.
- Teskey, R.O. 1995. A field study of the effects of elevated CO₂ on carbon assimilation, stomatal conductance and leaf and branch growth of *Pinus taeda* trees. *Plant Cell Environ.* 18:565–573.
- Tinus, R. 1972. CO₂ enriched atmosphere speeds growth of ponderosa pine and blue spruce seedlings. *Tree Planters' Notes* 23:12–15.
- Tissue, D.T., R.B. Thomas and B.R. Strain. 1996. Growth and photosynthesis of loblolly pine (*Pinus taeda*) after exposure to elevated CO₂ for 19 months in the field. *Tree Physiol.* 16:49–59.
- Tissue, D.T., R.B. Thomas and B.R. Strain. 1997. Atmospheric CO₂ enrichment increases growth and photosynthesis of *Pinus taeda*: a 4 year experiment in the field. *Plant Cell Environ.* 20:1123–1134.

Wang, K. 1996. Canopy CO₂ exchange of Scots pine and its seasonal variation after four-year exposure to elevated CO₂ and temperature. *Agric. For. Meteorol.* 82:1–27.

Wilkins, D, J.-J. Van Oosten and R.T. Besford. 1994. Effects of elevated CO₂ on growth and chloroplast proteins in *Prunus avium*. *Tree Physiol.* 14:769–779.