

Variability of the photosynthetic characteristics of mature needles within the crown of a 25-year-old *Pinus pinaster*

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Summary Photosynthetic characteristics of 1- and 2-year-old needles were determined in excised shoots of maritime pine (*Pinus pinaster* Ait.) with an open gas exchange system. We used the nonlinear least mean squares method to derive values for quantum yield of electron transport (α), maximum carboxylation velocity (V_{cmax}), and maximum electron transport rate (J_{max}), from photosynthetic response curves to light and CO_2 . Crown height had no significant effect on any of the parameters; however, V_{cmax} and J_{max} , as well as α were 43, 26 and 35% higher, respectively, in 1-year-old needles than in 2-year-old needles. The main effect of irradiance on needles was a small decline in leaf concentrations of nitrogen and phosphorus from the top to the bottom of the canopy. Only J_{max} demonstrated a linear relationship with both nitrogen content ($R^2 = 0.42$) and irradiance at the shoot level. Because needle age accounted for most of the variability in photosynthesis, we incorporated needle age into the photosynthesis model of Farquhar et al. (1980). The modified model underestimated the daily assimilation rate of 1-year-old needles in the field, especially when assimilation rates were high.

Keywords: maritime pine, model, needle age, nitrogen, phosphorus, photosynthesis.

Introduction

There has been renewed interest in photosynthesis because of the need to define its role with respect to the sensitivity of forest canopies to environmental changes and predicted global climate change. Photosynthesis has been studied at scales ranging from the leaf (Farquhar et al. 1980, Field 1983, Harley et al. 1985, Leuning 1990) to the canopy (Jarvis 1989, McMurtrie et al. 1992, Harley and Baldocchi 1995, Terashima and Hikosaka 1995). At the leaf level, the biochemical model developed by Farquhar et al. (1980), modified to take into account an eventual phosphate limitation (Sharkey 1985), has been used to characterize photosynthesis of C_3 plants (Harley et al. 1985, Leuning 1990, Sage 1990, Leuning et al. 1991, Harley et al. 1992, Wullschlegel 1993, Lewis et al. 1994, Epron et al. 1995). Although there have been several attempts to model photosynthesis in deciduous tree species (Leuning 1990, Leuning et al. 1991, Epron et al. 1995), the Farquhar model has rarely been parameterized for coniferous species

(McMurtrie et al. 1992 on *Pinus radiata* D. Don, Wang and Jarvis 1993 on *P. sylvestris* L. shoots, Lewis et al. 1994 on *P. taeda* L. seedlings, and Ben Brahim et al. 1996 on *P. pinaster* Ait. seedlings).

Both nitrogen distribution within the tree crown and the relationship between leaf nitrogen content and maximum rates of carboxylation and electron transport have been investigated in several tree species (Field 1983, Leuning et al. 1991, Harley et al. 1992, Medlyn 1996). It has been hypothesized that N distribution within the canopy is related to acclimation of the photosynthetic apparatus to the light environment (Leverenz and Jarvis 1979, Field 1983). However, little is known about this phenomenon in sparse leaf crowns (LAI ranging from 2.5 to 3.5 depending on season) such as that of maritime pine (*Pinus pinaster*), an Atlantic coniferous tree species whose natural range extends from Northern Morocco to central France, including Algeria, Portugal, Spain and Italy.

A detailed understanding of canopy photosynthetic processes requires that both the heterogeneity of canopy microenvironment and leaf photosynthetic characteristics be known. Therefore, we examined variability in the main photosynthetic parameters of a biochemical model in needles of adult *P. pinaster*. Specifically, we studied 1- and 2-year-old needles originating from several different layers within the tree crown, both to assess heterogeneity in needle characteristics, including light status, nitrogen and phosphorus contents, and to determine whether differences in needle characteristics corresponded to differences in photosynthetic capacity. We also compared measurements of daily assimilation with model calculations.

Material and methods

Site

The Bray field site (44°42' N, 0°46' W), which has been used as a study site since 1987 (Berbigier et al. 1991, Diawara et al. 1991, Loustau et al. 1992, Granier and Loustau 1994, Berbigier and Bonnefond 1995), is located 20 km southwest of Bordeaux, France. The site has a mean annual temperature of 12.5 °C and receives a mean annual rainfall of 930 mm (1951–1990). The stand is a homogenous artificial forest composed of 25-year-old maritime pines with an understory consisting

mainly of *Molinia coerulea* (L.) Moench. In 1995, stocking density was 630 stems ha⁻¹ and LAI varied between 2.5 in winter to 3.5 in summer. Tree mean height and breast height diameter were 18 m and 83 cm, respectively. Stand characteristics have been described in detail in previous papers (e.g., Granier and Loustau 1994).

Photosynthesis measurements

In May 1995, leaf gas exchange measurements were made in the laboratory on shoots cut from a single tree, made accessible by scaffolding. Branches were selected at three levels inside the crown (top, middle and bottom), each branch included a 1-year-old (needles 1994) shoot and a 2-year-old (needles 1993) shoot. There were three replications for each crown level \times needle age combination. Early in the morning, the branches were cut, placed immediately in water, taken to the laboratory and recut under preboiled water to avoid cavitation (Roberts 1978). Preliminary tests indicated that this procedure did not affect stomatal functioning during the experiment.

We used an open gas exchange system, with a controlled environment minicuvette (Compact Minicuvette System, Walz, Effeltrich, Germany). Temperature was controlled with a Peltier element, air humidity was controlled with a dewpoint generator, air composition (O₂, CO₂, N₂) was controlled by several mass flow meters (Gas Mixing Unit GMA-2, Walz), and light was supplied to both the top and bottom of the chamber through a double-sided unit (Fiber Illuminator FL-400, Special Fiberoptics 400-F, Walz, and a metal halide lamp). The required range of irradiances was obtained by means of the electronic regulator of the fiber illuminator and neutral filters. Incident PAR was calibrated with a PAR sensor (Captor LI-190, Li-Cor, Inc., Lincoln, NE) and uniformity of PAR inside the chamber was checked. Irradiances of upward and downward PAR were maintained equal at each step of the A–PAR curves. Differential CO₂ and H₂O concentrations were measured between the measuring circuit and a reference circuit. Environmental parameters that were continuously measured in the chamber included: air temperature (T_a) (Temperature Sensor VCT 1000, Walz), relative humidity (RH) (humidity sensor), and air CO₂ concentration (C_a) (Analyser IR, Binos 100, Leybold Heraeus, Hanau, Germany). Leaf temperature was measured with a nickel-chrome thermocouple. From the total leaf area, assimilation rate (A , $\mu\text{mol m}^{-2} \text{s}^{-1}$), transpiration rate (E , $\text{mmol m}^{-2} \text{s}^{-1}$), stomatal conductance (g_w , $\text{mmol m}^{-2} \text{s}^{-1}$) and internal CO₂ concentration (C_i , $\mu\text{mol mol}^{-1}$) were calculated according to Farquhar and von Caemmerer (1982). During measurements, T_a was maintained at 25 °C, RH at 81% and O₂ concentration at 20%. To obtain A– C_i curves, 10 needles were exposed to high irradiance (1400 $\mu\text{mol m}^{-2} \text{s}^{-1}$) and increasing CO₂ concentration (C_a = 0 to 2000 $\mu\text{mol mol}^{-1}$). For the A–PAR curves, the same 10 needles were exposed to ambient CO₂ concentration (360 $\mu\text{mol mol}^{-1}$) and increasing irradiance (0 to 1400 $\mu\text{mol m}^{-2} \text{s}^{-1}$).

The same system was used to measure daily assimilation rates of needles of a 1-year-old shoot from the top of the crown for 3 days in July. Temperature and air CO₂ concentration were monitored outside the chamber and reproduced inside the

cuvette, downward radiation (PAR_d) was measured at the needle level with a light sensor (LI-190, Li-Cor, Inc.) and humidity was controlled with a dewpoint generator (7 °C in the morning and 12 °C in the afternoon).

All measurements were expressed on a total leaf area basis. Because the needles were randomly positioned in the assimilation chamber, there was a 50% probability that the flat side of each needle received PAR_d. Therefore, we assumed that the assimilation of all the needles in the chamber was equivalent to the sum of the assimilation of half of the total needle area illuminated at upward radiation (PAR_u) and the assimilation of half of the total needle area illuminated at PAR_d.

Specific leaf area (SLA)

The ten needle pairs sampled for gas exchange measurements were collected and their length and middle diameter measured to calculate their area, assuming needles to be semi-cylinders. Needles were oven-dried at 65 °C for 48 h and weighed. Specific leaf area ($\text{m}^2 \text{kg}^{-1}$) was calculated as the ratio of needle area to needle dry weight.

Mineral concentrations

Needle contents of nitrogen and phosphorus were measured with a Technicon auto-analyser (Technicon, Tarrytown, NY) (O'Neill and Webb 1970), from the central parts of the needles used for the gas exchange measurements (18 shoots). Nitrogen and phosphorus concentrations are expressed on either a total leaf area basis (g m^{-2} ; N_a , P_a) or a dry weight basis (%; N_m , P_m).

Light intensity within the crown

In July, 45 light sensors, constructed from amorphous silicon cells (SOLEMS SA, Palaiseau, France) and calibrated against a PAR sensor (LI-190, Li-Cor, Inc.), were placed on bars located at the positions of the nine 1-year-old shoots. At each location, four sensors faced upward to measure downward radiation (PAR_d), and one sensor faced downward to measure upward radiation (PAR_u). The light ratio, which is an integrated value of several days of cumulative PAR_d at the shoot level divided by cumulative PAR_d above the tree, was used to characterize light status at the shoot level.

From August 1995 to November 1995, 72 LI-190 sensors were used to measure PAR_d and PAR_u within the crown of a nearby tree. Measurements taken from the middle of the crown were used to fit a linear multiple regression of upward radiation versus downward radiation and time. We used this regression to estimate upward radiation in the field in July, knowing the time of the day and the downward radiation above the needles. The estimate of upward radiation was then used to recalculate the assimilation rates of the needles.

Statistical analysis

Effects of height and needle age on quantum yield of electron transport (α), maximum rate of carboxylation (V_{cmax}), maximum rate of electron transport (J_{max}), dark respiration rate (R_d), needle mineral concentrations and SLA were evaluated by linear ANOVA, using the SAS software package (1986,

SAS Institute, Inc., Cary, NC). Multiple range tests were used to compare mean values (Student Newman-Keuls). For Tables 2 and 3, means with the same letters are considered not to be significantly different at the 5% tolerance level.

Theory

*Estimation of the assimilation model parameters V_{cmax} , R_d , J_{max} , α , θ and Γ^**

We used the biochemical leaf model developed by Farquhar et al. (1980) to estimate maximum carboxylation velocity, V_{cmax} , dark respiration, R_d , maximum electron transport rate, J_{max} , apparent quantum efficiency of electron transport, α , and CO_2 compensation point, Γ^* . The constants and reference values employed in the calculations are given in Table 1. We also determined the potential electron transport rate, J , in terms of incident PAR, as described by Farquhar and Wong (1984, stated in Leuning 1990).

The value of θ , corresponding to the shape of the non-rectangular hyperbola, was determined to be 0.95 and assumed to be constant. Leuning (1990) concluded that θ was constant for leaves of all C_3 plants.

Values of V_{cmax} , R_d , and α were estimated from leaf gas exchange measurements by nonlinear least mean squares methods using the SAS software package (1986; SAS Institute Inc.). Parameter α was fitted to the A -PAR curves for irradiances ranging from 50 to $150 \mu\text{mol m}^{-2} \text{s}^{-1}$ (see Figure 1). This range has been determined by Kirschbaum and Farquhar (1987) to avoid the overestimation resulting from the Kok effect. Parameters V_{cmax} and R_d were fitted to the A - C_i curves for C_i values of $< 600 \mu\text{mol mol}^{-1}$ where assimilation is supposed to be limited by Rubisco (see Figure 2). The maximum electron transport rate (J_{max}) was estimated by solving the general equation of the Farquhar model for maximum assimilation, A_{max} (measured at $2000 \mu\text{mol mol}^{-1} \text{CO}_2$ and $1400 \mu\text{mol m}^{-2} \text{s}^{-1}$ PAR), when assimilation is limited by RuP_2 regeneration.

We used the temperature dependence that McMurtrie et al. (1992) proposed for *Pinus radiata* (cf. Field 1983, Kirschbaum and Farquhar 1984) to adjust the parameters to the range of leaf temperatures encountered in the field in July (12 to 27°C).

$$V_{\text{cmax}} = V_{\text{cmax0}} (1 - 0.0025 (T - T_0)^2), \quad (1)$$

$$J_{\text{max}} = J_{\text{max0}} (1 - 0.0025 (T - T_0)^2), \quad (2)$$

where V_{cmax0} and J_{max0} are the photosynthetic values at reference temperature T_0 . We used an Arrhenius equation to describe the temperature dependence of R_d (Leuning 1990). We related Γ^* to temperature through the temperature dependence of Rubisco kinetic characteristics (Farquhar et al. 1980).

$$\Gamma^* = \frac{K_c k_o}{2K_o k_c} \text{O}_2, \quad (3)$$

Table 1. Constants and reference values used for temperature dependence calculations (Farquhar et al. 1980, Leuning 1990).

	Parameter	Value	Units
Reference temperature	T_0	298.2	K
Michaelis constant for carboxylation	K_{c0}	460	$\mu\text{mol mol}^{-1}$
Michaelis constant for oxygenation	K_{o0}	330	mmol mol^{-1}
Activation energy for carboxylation	E_{Kc}	59356	J mol^{-1}
Activation energy for oxygenation	E_{Ko}	36000	J mol^{-1}
Gas constant	R	8.31	$\text{J K}^{-1} \text{mol}^{-1}$
Activation energy for respiration	E_{KRd0}	53000	J mol^{-1}

where k_c and k_o are the turnover constants of the Rubisco carboxylation and oxygenation sites, respectively. The k_o/k_c ratio is constant within the temperature range of 20 to 30°C (Harley et al. 1985) and equals 0.21 (Farquhar et al. 1980, Harley et al. 1985). Both the Michaelis constant for CO_2 , K_c , and the competitive inhibition constant for O_2 with respect to CO_2 in the Rubisco reaction, K_o , increase with temperature (Jordan and Ogren 1984, Brooks and Farquhar 1985, Harley et al. 1985). Their dependence can be described by an Arrhenius equation, with the reference parameter values given by Farquhar et al. (1980).

Results

The response curves of assimilation to increasing CO_2 concentration and PAR were classical in form (Sharkey 1985, Sage 1990, Harley et al. 1992). All light response curves were hyperbolic (Figures 1A and 1B) and showed a weak concavity and a threshold. The CO_2 response curves could be classified into two types (Figures 2A and 2B): (1) truncated hyperbolas, with a saturating threshold at high C_i (Figure 2A); and (2) more regular shaped hyperbolas, without any well-marked threshold (Figure 2B). The saturated CO_2 response curves corresponded mainly to 1-year-old needles (seven out of nine 1-year-old sampled shoots), whereas the more regularly shaped CO_2 response curves were characteristic of 2-year-old needles (eight out of nine 2-year-old sampled shoots).

Estimation of V_{cmax} , α , J_{max} and R_d

Neither needle nor crown height had a significant effect on R_d (Table 2). The calculated R_d values ranged from 0 to $1 \mu\text{mol m}^{-2} \text{s}^{-1}$, with a mean of $0.37 \mu\text{mol m}^{-2} \text{s}^{-1}$ ($\text{SD} = 0.27 \mu\text{mol m}^{-2} \text{s}^{-1}$). Values of V_{cmax} , J_{max} and α were 43, 26 and 35% higher, respectively, in 1-year-old needles than in 2-year-old needles. Because crown height had no significant effect on these parameters (Table 2), we conclude that the observed

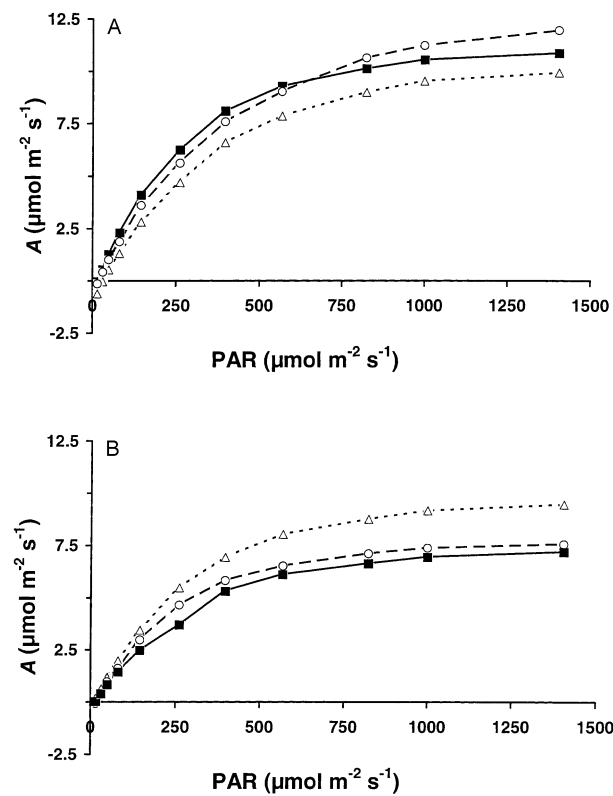


Figure 1. (A) A–PAR curves for 1-year-old needles. (B) A–PAR curves for 2-year-old needles. Measurements were made at 25 °C, 210 mmol mol⁻¹ O₂ and constant air CO₂ concentration of 360 μmol mol⁻¹. Each graph corresponds to one particular shoot with (■) corresponding to top, (○) middle, and (△) bottom shoots.

variability in these three parameters was mainly associated with needle age ($R^2 = 0.52$ for V_{cmax} , $R^2 = 0.30$ for J_{max} , and $R^2 = 0.41$ for α , respectively).

These measured values of V_{cmax} , J_{max} and α and R_d were subsequently used to calculate assimilation (A_{mod}) of the needles for each shoot. Figure 3 shows the fits of the model together with the measured values for six A–PAR curves. At low irradiance (under 400–600 μmol m⁻² s⁻¹ PAR), assimilation was

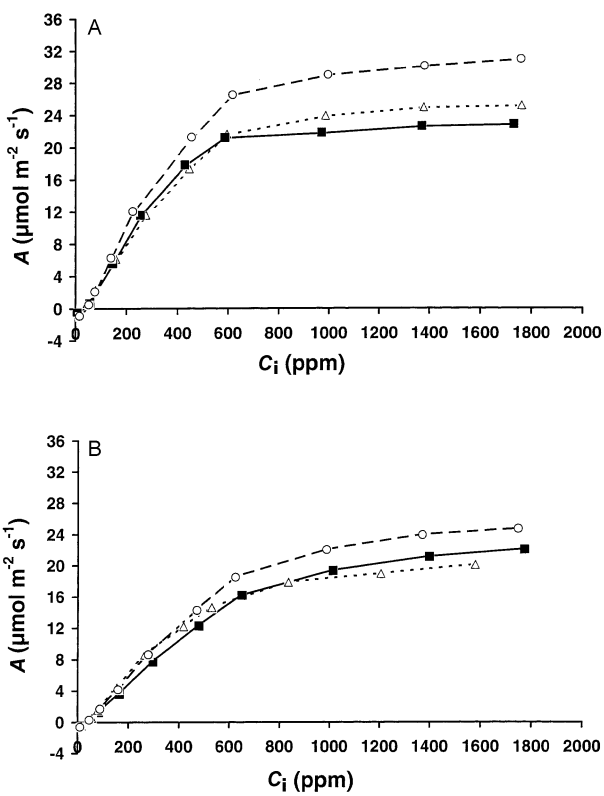


Figure 2. (A) A–C_i curves for 1-year-old needles. (B) A–C_i curves for 2-year-old needles. Measurements were made at 25 °C, 210 mmol mol⁻¹ O₂ and constant light intensity of 1400 μmol m⁻² s⁻¹. Each graph corresponds to one particular shoot with (■) corresponding to top, (○) middle, and (△) bottom shoots.

limited by RuP₂ regeneration, whereas at irradiances of > 600 μmol m⁻² s⁻¹ assimilation was limited by Rubisco activity. Co-limitation occurred when both Rubisco and RuP₂ were limiting. The co-limitation irradiance, PAR_{co}, was calculated for each shoot. Neither crown height nor needle age had a significant effect on PAR_{co} (Table 3), and there was no significant relationship between PAR_{co} and the light ratio (data not shown).

Table 2. Values of the photosynthetic parameters V_{cmax} , α , J_{max} and R_d as a function of needle age (1- and 2-year-old needles) and height within the crown. For each parameter, means with the same letter are not significantly different at $P = 0.05$. Values in parenthesis are standard deviations. Because there were differences with needle age but not with crown height, we have indicated the mean values by age, irrespective of crown height.

	V_{cmax} (μmol m ⁻² s ⁻¹)		α		J_{max} (μmol m ⁻² s ⁻¹)		R_d (μmol m ⁻² s ⁻¹)	
	1 year	2 year	1 year	2 year	1 year	2 year	1 year	2 year
Bottom	50.1 ^a (2.5)	37.8 ^b (4.2)	0.150 ^a (0.012)	0.113 ^b (0.016)	98.9 ^a (8.2)	97.8 ^b (8.3)	0.25 ^a (0.11)	0.25 ^a (0.24)
Middle	59.8 ^a (2.3)	35.6 ^b (6.1)	0.139 ^a (0.029)	0.101 ^b (0.022)	122.5 ^a (1.3)	94.1 ^b (10.8)	0.44 ^a (0.46)	0.49 ^a (0.31)
Top	49.3 ^a (8.1)	37.9 ^b (17.0)	0.136 ^a (0.030)	0.103 ^b (0.035)	119.7 ^a (21.9)	78.2 ^b (37.6)	0.44 ^a (0.13)	0.33 ^a (0.36)
Total mean by age	53.1 ^a (6.7)	37.1 ^b (9.3)	0.142 ^a (0.023)	0.106 ^b (0.023)	113.7 ^a (16.2)	90.0 ^b (22.0)	0.38 ^a (0.26)	0.36 ^a (0.28)

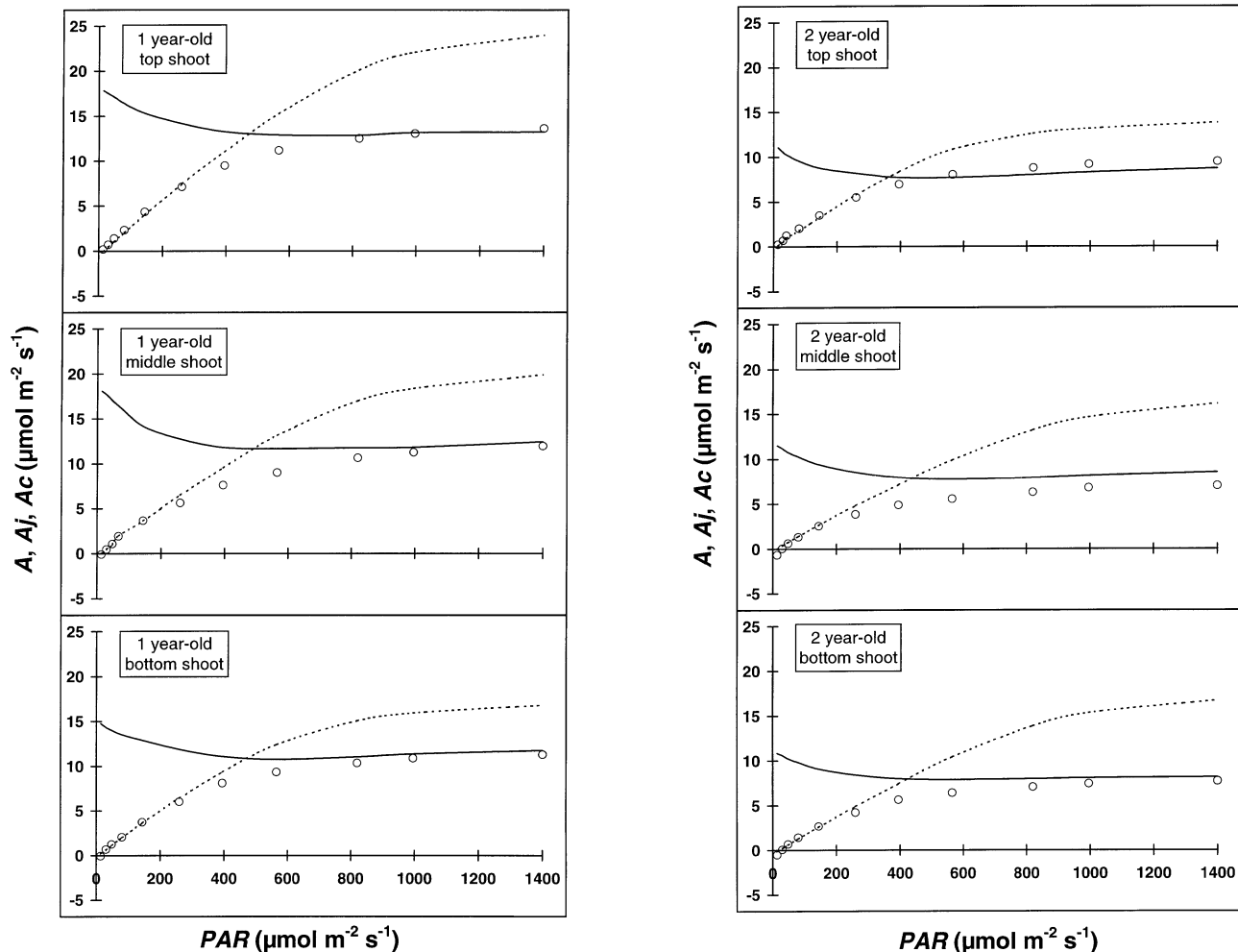


Figure 3. A–PAR curves for 1- and 2-year-old shoots: photosynthesis is limited first by RuP₂ regeneration, then by Rubisco activity. Measurements were made at 25 °C, 210 mmol mol⁻¹ O₂ and constant air CO₂ concentration of 360 μmol mol⁻¹. Each graph corresponds to one particular shoot: top graphs correspond to top shoots, middle graphs to middle shoots, bottom graphs to bottom shoots. A, Experimental points (○); A_j, RuP₂ regeneration limitation curve (---); A_c, Rubisco limitation curve (—). Note: the apparent variation in A_c with light resulted from variation in C_i, because ambient carbon dioxide concentration, C_a, not C_i, was controlled during the experiment.

Needle mineral concentrations and SLA and relationships with the light ratio and photosynthetic characteristics

When expressed on a needle dry weight basis, nitrogen and phosphorus concentrations were similar throughout the crown. Among the needle age × canopy height combinations, only the mineral concentrations of the 1-year-old needles in the top and middle of the crown were significantly higher. Nitrogen and phosphorus concentrations expressed on a leaf area basis were higher in the top two-thirds of the crown than in the lower third of the crown (Table 3). Nitrogen and phosphorus concentrations of 1-year-old needles from the top and middle of the crown were higher than in the corresponding 2-year needles. Correlation analysis demonstrated a strong relationship between N and P concentrations on leaf area (N_a, P_a (g m⁻²)), and on dry weight basis (N_m, P_m (% DW)) and the light ratio at the shoot level ($R^2 = 0.84, 0.71$ and $0.82, 0.55$, respectively).

Specific leaf area was significantly higher (21.3%) in the lower third than in the upper two-thirds of the crown, and was negatively correlated with light ratio ($R^2 = 0.67$).

Although V_{\max} , α and R_d showed no significant relationship with either light ratio or needle mineral concentration (data not shown), J_{\max} increased linearly with shoot light regime and needle N concentration (Figure 4, $R^2 = 0.47$ and 0.33 , respectively).

Parameterization of the Farquhar photosynthesis model for adult maritime pine

Because most of the observed variability in photosynthesis was associated with needle age, we introduced needle age into the Farquhar model:

$$A = (1 - \frac{\Gamma^*}{C_i}) \min(W_c(\text{age}); W_j(\text{age})) - R_d, \quad (4)$$

Table 3. Mean values of needle nitrogen and phosphorus concentrations on a dry weight (N_m , P_m , %) and a leaf area (N_a , P_a , $g\ m^{-2}$) basis, mean values of specific leaf area (SLA, $m^2\ kg^{-1}$) and mean values of light intensity at the co-limitation point (PAR_{co} , $\mu mol\ m^{-2}\ s^{-1}$) as a function of needle age and height within the crown. Also presented are light ratios for 1-year-old shoots as a function of crown height. For each parameter, means with the same letter are not significantly different at $P = 0.05$. Values in parenthesis are standard deviations.

	N_m % dry weight	P_m	N_a $g\ m^{-2}$ leaf area	P_a	SLA $m^2\ kg^{-1}$	PAR_{co} $\mu mol\ m^{-2}\ s^{-1}$	Light ratio
<i>1-Year-old needles</i>							
Bottom	1.13 ^a (0.03)	0.072 ^a (0.001)	1.48 ^a (0.13)	0.094 ^a (0.008)	7.66 ^a (0.78)	456 ^a (68)	0.53 ^a (0.15)
Middle	1.23 ^{ab} (0.09)	0.075 ^{ab} (0.006)	2.00 ^{cd} (0.29)	0.121 ^b (0.018)	6.20 ^b (0.47)	597 ^a (134)	0.83 ^b (0.14)
Top	1.36 ^b (0.10)	0.081 ^b (0.006)	2.27 ^d (0.12)	0.135 ^b (0.007)	6.00 ^b (0.21)	478 ^a (33)	0.95 ^c (0.08)
<i>2-Year-old needles</i>							
Bottom	1.10 ^a (0.10)	0.070 ^a (0.006)	1.53 ^{ab} (0.10)	0.098 ^a (0.005)	7.16 ^a (0.21)	436 ^a (14)	–
Middle	1.13 ^a (0.05)	0.068 ^a (0.002)	1.79 ^{bc} (0.11)	0.108 ^{ab} (0.003)	6.31 ^b (0.24)	460 ^a (23)	–
Top	1.17 ^a (0.06)	0.072 ^a (0.005)	1.97 ^c (0.12)	0.122 ^b (0.009)	5.91 ^b (0.16)	496 ^a (170)	–

with W_c , the rate of carboxylation limited by Rubisco activity, and W_j , the rate of carboxylation limited by electron transport, and where Γ^* only depends on temperature ($\Gamma^*(25\ ^\circ C) =$

$30\ \mu mol\ mol^{-1}$) and $\theta = 0.95$. We fixed $R_d(25\ ^\circ C)$ to the mean value of our fittings (i.e., $R_d(25\ ^\circ C) = 0.37\ \mu mol\ m^{-2}\ s^{-1}$), and $V_{cmax}(25\ ^\circ C)$, $J_{max}(25\ ^\circ C)$ and α varied according to needle age (Table 2). The temperature dependencies of V_{cmax} and J_{max} have been described previously (see Equations 1 and 2 and Table 1) and an Arrhenius equation was used to describe the temperature dependency of R_d (Leuning 1990). Values for leaf temperature, air CO_2 and O_2 concentrations, and downward radiation (PAR_d) are based on field measurements. Upward radiation (PAR_u) was estimated from the following regression adjusted for our set of radiation data ($R^2 = 0.82$):

$$PAR_u = 38.92 + 0.064 PAR_d + 2.90 \cos\left(\frac{TU - 0.5}{0.5} \pi\right), \quad (5)$$

Figure 5A represents the daily course of measured PAR_d in the assimilation chamber during the 3-day measurement period in July. The daily course of estimated PAR_u in the chamber, based on measurements made in October, ranged from 0 to $180\ \mu mol\ m^{-2}\ s^{-1}$. Figure 5B shows daily assimilation rates measured during the same 3-day period in July on a 1-year-old shoot of an independent tree, along with estimated daily assimilation rates for the same period. Although the values of measured and estimated assimilation were of the same magnitude, the model underestimated assimilation, especially at high assimilation rates where half of total needle area was limited by the carboxylation rate and half of total needle area was limited by the electron transport rate (Figures 5B and 6).

Discussion

Methodological limitations

Three points about the methodological limitations of the study are relevant for discussion. First, we used a constant value of

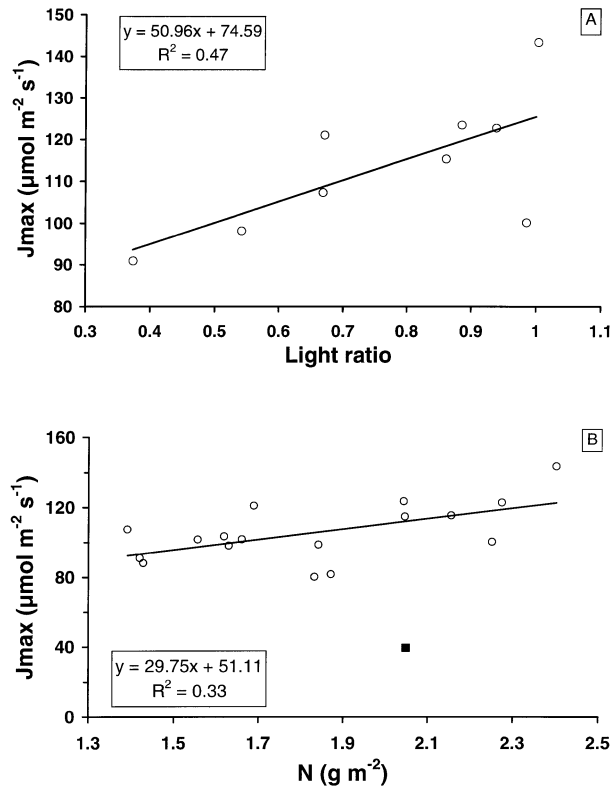


Figure 4. Maximum electron transport rate (J_{max}) versus ratio of received light at the shoot level (A) and leaf N concentration (B). Open circles (○) represent experimental data. The line represents best linear fit. The closed square represents a point not included in the regression.

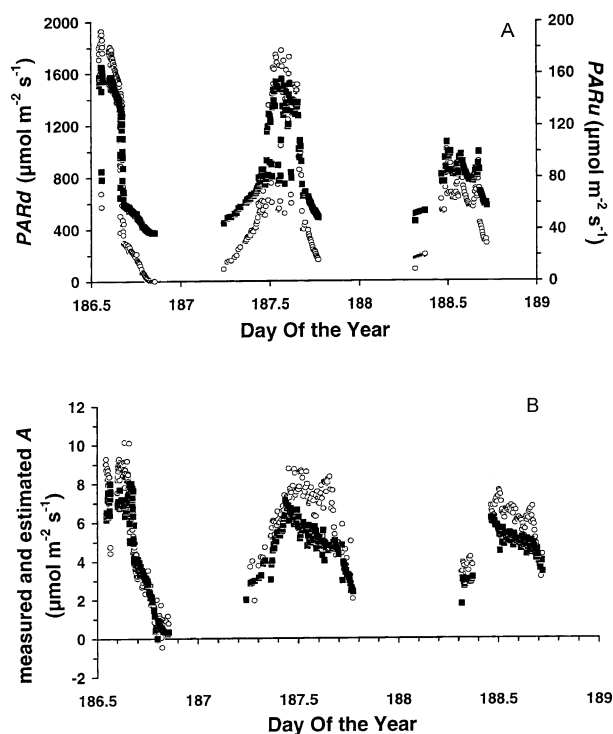


Figure 5. (A) Daily course of downward radiation (PAR_d , \circ) as measured inside the assimilation chamber in July, in the field. Daily course of upward radiation (PAR_u , \blacksquare) as estimated from measurements made in October, in the crown of a 25-year-old maritime pine, following Equation 5. (B) Daily assimilation rates of measured assimilation (\circ) and estimated assimilation (\blacksquare) on days of the year 186, 187, 188. Measurements were made in the field on a 1-year-old shoot of an independent tree.

0.95 for θ . We did not fit θ on the experimental data because the A - PAR curve at ambient CO_2 is limited by Rubisco activity at high PAR . However, setting θ to 0.95 influenced the fit of α . To avoid this bias, future studies would better fit θ on CO_2 -saturated ($C_a > 2000 \mu\text{mol mol}^{-1}$) A - PAR curves to ensure conditions under which the curvature depends only on light attenuation.

Second, we used Γ^* instead of the specificity factor (S) for Rubisco. The specificity factor and Γ^* are directly correlated (Jordan and Ogren 1984, Brooks and Farquhar 1985) and both have been extensively used to study photosynthesis (Γ^* by Farquhar et al. 1980, Harley et al. 1985, Kirschbaum and Farquhar 1987, Leuning 1990, Sage 1990, Leuning et al. 1991, McMurtrie et al. 1992, Wang and Jarvis 1993; and S by Harley et al. 1992, Epron et al. 1995). We selected Γ^* to provide continuity with our previous studies on maritime pine seedlings (Ben Brahim et al. 1996).

Third, the use of the bi-directional light system to irradiate the whole needle area was not completely satisfactory, because the semi-cylindrical shape of the needle prevented a uniform distribution of light to the whole needle area. Jordan and Smith (1993) reported that only 35% of the needle area of a semi-cylindrical needle of *Pinus sylvestris* received direct light,

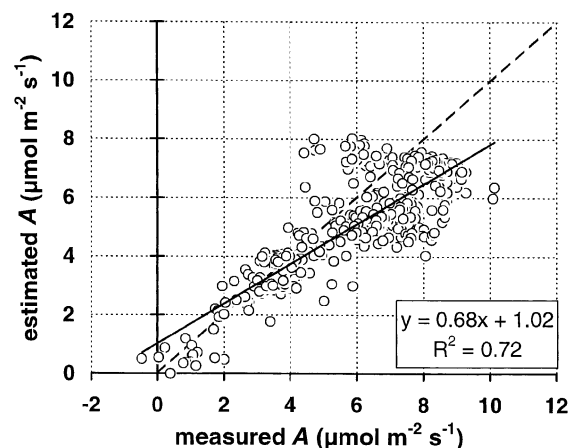


Figure 6. Estimated assimilation versus measured assimilation. Measurements were made in the field on one shoot from an independent tree, independently of gas exchange measurements made in May. They were conducted on three consecutive days (DOY 186, 187, 188). The solid line (—) represents the best fitting on the points and the dotted line (---) represents the equation $y = x$.

when illuminated on its round side. We conclude that it would have been better to use an integrating sphere than a bi-directional light source because the integrating sphere scatters light around the whole needle and so provides a uniform diffuse radiation to the whole needle area (Öquist et al. 1978).

Photosynthetic characteristics

Our measured values of the main photosynthetic parameters of the biochemical model seem low when compared with those of other species and may be associated with our methodology. Values of R_d showed large variability (mean $0.37 \mu\text{mol m}^{-2} \text{s}^{-1}$, $\text{SD} = 0.27 \mu\text{mol m}^{-2} \text{s}^{-1}$). Maximum quantum efficiency (mean 0.031) was at the low end of the range for C_3 plants (Farquhar et al. 1980, Ehleringer and Pearcy 1983, Harley et al. 1985, Kirschbaum and Farquhar 1987, Leuning et al. 1991, Harley et al. 1992, Wang et al. 1995, Ben Brahim et al. 1996), but was similar to values obtained for *Eucalyptus grandis* W. Hill ex Maiden (0.032, Leuning 1990) and *Pinus sylvestris* (0.017 to 0.057 depending on time of the year, Leverenz and Öquist 1987). Our V_{cmax} and J_{max} values corresponded with the lower range of values collected for C_3 plants (Farquhar et al. 1980, Harley et al. 1985, Leuning 1990, Leuning et al. 1991, Harley et al. 1992, Lloyd et al. 1992, McMurtrie et al. 1992, Wullschlegel 1993, Lewis et al. 1994, Epron et al. 1995, Ben Brahim et al. 1996), and were similar to values obtained for *Macadamia integrifolia* Maiden & Betche and *Citrus paradisi* Macfady, tree species with thick leaves (Lloyd et al. 1992). Values of V_{cmax} and J_{max} of woody plants are usually lower than values found in herbaceous species; moreover, among trees, conifers tend to have lower values than deciduous trees (Wullschlegel 1993). Nevertheless, because not all of our A - C_i curves reached a threshold, we may have consistently underestimated J_{max} by using the Rubisco-limited value of A_{max} . Moreover, we ignored the internal resistance to

CO₂ diffusion, which presumably led to an underestimation of the photosynthetic characteristics studied. Epron et al. (1995) concluded that the observed differences between values of V_{cmax} and J_{max} of woody and herbaceous plants are the result of errors in the estimation of CO₂ concentration at the carboxylation sites, C_c , caused by not taking into account the internal resistance to CO₂ diffusion.

The N and P concentrations of maritime pine needles (Table 3) were also at the low end of the range found in woody plants (*Pinus pinaster*, Keay et al. 1968; *Pinus radiata*, Donald and Glen 1974, Leuning et al. 1991, Lloyd et al. 1992). However, the declines in N and P concentrations with needle age were consistent with observations by Lemoine et al. (1988) on *Pinus pinaster* and Wells and Metz (1963) on *Pinus taeda*.

Variability of photosynthetic characteristics within the crown

The main factor determining variability in photosynthetic characteristics was needle age. The effects of needle age were presumably associated with physiological or physical changes, or both. For example, the finding that, in the upper part of the crown, N concentration was lower in 2-year-old needles than in 1-year-old needles (Table 3) is consistent with reallocation to growing shoots (Nambiar 1990, Rook 1991). Because much of the needle N is located in Rubisco (28%, Evans 1989; 20%, Friend 1991) and the proportion of total N in Rubisco increases with leaf N content (Evans 1989), we can assume that the Rubisco pool declines with needle age. Consequently, V_{cmax} will be lower in 2-year-old needles than in 1-year-old needles. Leaf N is also located in the thylakoid membranes constituting the light harvesting pigment-protein complexes (cytochrome *b/f*) of the electron transport chain. It has been shown that relatively small increases in needle N content can enhance electron transport capacity through an increase in the pigment-protein complexes (Evans 1989). Therefore, the observed difference in N concentrations between 1- and 2-year-old needles could partly explain the decrease in J_{max} with needle age.

Membrane electron carriers or chlorophyll, or both, may have been partly destroyed because of senescence or photo-oxidation occurring in summer, resulting in a decrease in the apparent values of J_{max} and α with needle age. We attributed the decrease in α with needle age—which contrasts with the pattern observed in *Pinus sylvestris* (Wang et al. 1995) where α increased with needle age—to changes in needle structure, such as thickening of the cuticle and partial lignification of the internal tissues, because these changes alter the optical properties of the needle and hence the determined values of J_{max} and α . Such anatomical modifications have been observed in thin sections of maritime pine needles (Loustau, unpublished data).

The different shapes of the $A-C_i$ curves for the 1- and 2-year-old needles suggest that either internal resistance to CO₂ diffusion was higher for 2-year-old needles than for 1-year-old needles or the rate of utilization of triose phosphates limited assimilation at high CO₂ concentrations in the 1-year-old needles (Sharkey 1985, Sage 1990). The effects of needle age on V_{cmax} and J_{max} may be related to CO₂ diffusion resistance, which was ignored in our calculations (Epron et al. 1995, Lloyd et al. 1995). Use of $A-C_c$ curves rather than $A-C_i$

curves might have reduced the observed effects of needle age. However, the finding that 1-year-old needles had similar P concentrations to 2-year-old needles but higher values of the photosynthetic parameters, suggests that triose phosphates may have limited photosynthesis of the 1-year-old needles at elevated light and CO₂.

Because PAR decreases from the top and outside to the bottom and inside of the crown, the differences in N and P concentrations with canopy height and needle age could partly result from acclimation of the needles to their environment. The SLA measurements support this hypothesis (Table 3). Stenberg et al. (1995) concluded that differences in SLA are associated with the development of mesophyll tissues, which, in turn, is dependent on the light environment of the needles.

The absence of a significant effect of the light regime at the shoot level on photosynthetic characteristics was unexpected and contrasts with results obtained for 14-year-old *Picea sitchensis* (Bong.) Carrière (Leverenz and Jarvis 1979). The large variability in R_d may explain the absence of a relationship with PAR. Alternatively, the lack of an effect of shoot light regime on photosynthetic parameters could be attributed to the low extinction of light within the sparse crown of maritime pine (light ratio ranging from 0.95 to 0.53) compared with denser broad-leaved tree species that exhibit an optimum PAR for photosynthetic functioning (Field 1983, Lloyd et al. 1995). Only J_{max} showed some relationship with needle N concentration, whereas both J_{max} and V_{cmax} have been related to needle N concentration in other species (Field 1983, Sage 1990, Leuning et al. 1991). The absence of a significant relationship between V_{cmax} and N was unexpected, because there was a linear relationship between J_{max} and V_{cmax} (Figure 7: $J_{\text{max}} = 1.62V_{\text{cmax}} + 28.87$, $R^2 = 0.68$; cf. Wullschlegel (1993): $J_{\text{max}} = 1.64V_{\text{cmax}} + 29.10$). It may be that the narrow range of needle N concentrations of our samples (Table 3) explains the absence of a significant relationship between V_{cmax} and N. We conclude, therefore, that even if the needles acclimated to their light environment, as indicated by the vertical gradient of SLA, and the N and P concentrations, our results do not support the hypothesis of an optimization of the distribution of nitrogen and photosynthetic capacities within the canopy. This conclu-

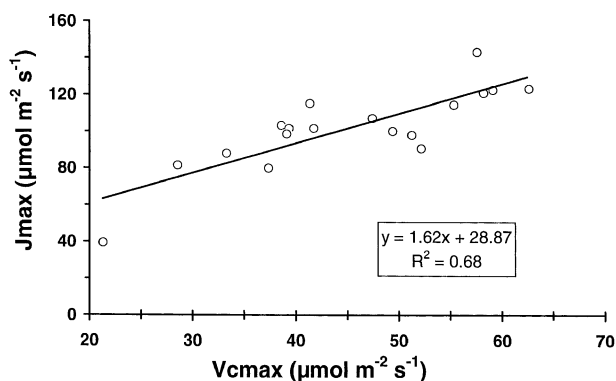


Figure 7. Maximum electron transport rate (J_{max}) versus maximum carboxylation rate (V_{cmax}). Open circles (○) represent the experimental data. The line represents the best linear fit.

sion is supported by the experiments on PAR_{co} . If leaf photosynthesis is optimal at the co-limitation point, we should have observed a decrease in PAR_{co} with a decrease in canopy height and light ratio in an optimized crown. However, we only considered the principal shoots of the branches, which are in the outer shell of the crown, and vertical light attenuation. Horizontal attenuation of light and the functioning characteristics of needles situated in the inner shell of the crown (on the small shoots held by the branches) were not considered.

A comparison of the measured assimilation data with the model predictions showed that the model was consistent. We were able to use the estimated values of V_{cmax} , J_{max} , α and R_d to calculate daily assimilation rates of 1-year-old needles without major error either in the range or in the daily fluctuations; however, the model underestimated assimilation, especially at high assimilation rates. The observed differences between the calculated and measured assimilation values may be related to: (i) the estimation of upward irradiance (PAR_u); (ii) the distribution of light on the needle area; or (iii) the temperature dependency used to adjust the photosynthetic parameters.

With respect to the first explanation, we found that model estimates were significantly changed when PAR_u was increased (data not shown). We conclude that the estimation of PAR_u , based on Equation 5, led to an underestimation of assimilation because the radiation data were obtained from a different tree than the assimilation measurements. The parameters of Equation 5 would be different with another set of radiation data (different season, differences in crown structure, etc.). Therefore, simultaneous measurements of downward irradiance (PAR_d), PAR_u and gas exchange under similar conditions are necessary to provide an accurate data set.

With respect to the second explanation, we considered that the needle comprised independent needle areas, receiving either PAR_d or PAR_u ; however, because of the semi-cylindrical shape of the needle, light was not distributed evenly on the total needle area (Wang and Jarvis 1993, Stenberg et al. 1995). Consequently, our approximation biased the estimation of both intercepted light and photosynthesis.

With respect to the third explanation, we chose the model that McMurtrie et al. (1992) used for *Pinus radiata*, because preliminary tests indicated that several other temperature dependencies (Farquhar et al. 1980, Harley et al. 1992, McMurtrie et al. 1992, Harley and Baldocchi 1995, Leuning et al. 1995, Lloyd et al. 1995) of the photosynthetic parameters were not satisfactory for maritime pine. Clearly, the collection of accurate data is needed to model precisely the photosynthetic response of maritime pine to temperature.

We have demonstrated that needle age underlies much of the variability in photosynthetic characteristics of adult maritime pine trees and shown that the major photosynthetic parameters of the model do not exhibit a vertical pattern within the tree crown. Our attempt to model needle photosynthesis in the field has emphasized the sensitivity of the model to light and temperature. Integration of a radiation distribution model with the photosynthetic model is needed for accurate simulations of photosynthesis at the needle and shoot levels.

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