Temperature response of parameters of a biochemically based model of photosynthesis. I. Seasonal changes in mature maritime pine (*Pinus pinaster* Ait.)

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ABSTRACT

Responses of plant processes to temperature may vary according to the time scale on which they are measured. In this study, both short-term and seasonal responses of photosynthesis to temperature were examined. A field study of seasonal changes in the temperature response of photosynthesis was conducted on two provenances, French and Moroccan, of mature maritime pine (*Pinus pinaster* Ait.). Measurements were made every 2 months over a 1-year period and used to parameterize a mechanistic model of photosynthesis. Temperature responses of maximum Rubisco activity, V_{cmax}, and potential electron transport rate, J_{max}, were obtained for each measurement period, as was the response of stomatal conductance, g_s, to water vapour pressure deficit (VPD). Absolute values of V_{cmax} and J_{max} at 25 °C were related to needle nitrogen content, N_{area}. N_{area}, and thus V_{cmax} and J_{max}, were negatively correlated with the mean minimum temperature in the month preceding measurements. The ratio of $J_{max} : V_{cmax}$ at 25 °C varied between 1 and 1·7 but did not show any seasonal trend. Nor was there any seasonal trend in the relative temperature response of V_{cmax}, which had an activation energy H_a of approximately 57 kJ mol⁻¹ throughout the experiment. The activation energy of J_{max} was also close to constant throughout the experiment, averaging 39 kJ mol⁻¹. For the French provenance, the optimal temperature of J_{max} was positively correlated with the maximum temperature of the previous day, but no such correlation was found for the Moroccan provenance. The response of g_s to VPD also varied seasonally, with much stronger stomatal closure in winter months. Taken together, these results implied a translational shift downwards of the photosynthetic temperature response curve with increasing T_{prev}, and a shift in the temperature optimum of photosynthesis of 5–10 °C between summer and winter. These results illustrate that the short-term temperature response of photosynthesis varies significantly on a seasonal basis.

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INTRODUCTION

With the predicted greenhouse-induced rise in global surface temperature possibly already under way (IPCC 2001), the effects of increasing temperature on plant growth and ecosystem function have become a major area of concern (e.g. Gunderson, Norby & Wullschleger 2000; Rustad *et al*. 2001). Temperature effects on individual plant processes have been extensively studied (e.g. Long & Woodward 1988). The results of these individual-process studies may be integrated using process-based computer models to predict effects of increasing temperature on overall forest ecosystem function (e.g. McMurtrie & Wang 1993; Kellomäki, Vaisanen & Kolstrom 1997; Grant & Nalder 2000; Medlyn *et al*. 2000). For most such models, photosynthesis is the driving process, being the mechanism by which plants take up carbon and thus a key determinant of the rate of plant growth. Photosynthetic response to temperature is therefore an important part of the models.

Unfortunately, many current models of the response of plant growth to temperature effectively ignore much of what is known about effects of temperature on photosynthesis. This odd state of affairs arises because many models use the biochemically based photosynthesis model of Farquhar, von Caemmerer & Berry (1980), a mechanistic model that can realistically describe photosynthetic responses to environmental variables. However, this model is not straightforward to parameterize, and there have been very few studies that have fully parameterized the temperature responses of the model (Leuning 1997). Hence, although it is well-known that photosynthetic temperature responses can (a) change in response to environmental conditions and (b) differ among species (Berry & Björkman 1980), the lack of parameters means that these differences are commonly overlooked in modelling studies. In this work we aimed to rectify this problem by studying seasonal variation of the temperature dependence of model param-

eters. The study was carried out on mature maritime pine trees growing near Bordeaux, SW France. Several other studies have shown seasonal shifts in the photosynthetic temperature response in field-grown evergreen species (Strain, Higginbotham & Mulroy 1976; Slatyer & Morrow 1977; Guehl 1985), so we anticipated a similar seasonal shift in mature maritime pine. In the companion article (Medlyn *et al.* 2002) these results are included in a review of existing experimental data, which addresses the problem of species differences in the temperature dependence of model parameters.

Our primary aim was to quantify the seasonal variation in parameters of the Farquhar *et al.* (1980) photosynthesis model. A second aim was to use these parameters to identify the processes driving any seasonal variation in the temperature response of photosynthesis. Several alternative hypotheses have been proposed for the mechanisms causing changes in this temperature response. The Farquhar *et al.* (1980) model is mechanistic, i.e. it describes explicitly the biochemical processes in the leaf. Alternative hypotheses can thus be framed in terms of the model, and model parameters used to test these hypotheses.

Hypotheses proposed for the seasonal variation in the photosynthetic temperature response are generally couched in terms of acclimation to temperature: that the temperature response shifts to reflect the ambient seasonal temperature (e.g. Strain *et al.* 1976; Slatyer & Morrow 1977; Battaglia, Beadle & Loughhead 1996). Other factors could potentially influence the seasonal variation, such as changes in incident radiation or foliar phenology, but for consistency with previous reports we focus here on mechanisms related to temperature acclimation. Berry & Björkman (1980), in their review of the temperature dependence of photosynthesis, suggested that acclimation to cold and warm temperatures involved different mechanisms. At low temperatures, the activity of temperature-limited enzymes such as ribulose-1,5-*bis*phosphate carboxylase-oxygenase (Rubisco) and Fru-P2 phosphatase may limit photosynthesis (Berry & Björkman 1980), so acclimation to low temperature may involve an increase in the capacity of these enzymes. At high temperature, by contrast, acclimation may involve changes in the heat stability of the electron transport apparatus (Armond, Schreiber & Björkman 1978). Acclimation of the response of stomatal conductance to temperature would also affect photosynthesis through changes in intercellular CO_2 concentration. An alternative hypothesis was proposed by Hikosaka (1997), who suggested that acclimatory responses represent an optimal allocation of nitrogen such that the two major limiting process to photosynthesis, Rubisco activity and ribulose-1,5-*bis*phosphate (RuBP) regeneration, are colimiting at the growth temperature. A further hypothesis, put forward by Makino, Nakano & Mae (1994), is that the cell-wall conductance to CO_2 increases with growth temperature.

These hypotheses can be framed in terms of the Farquhar *et al.* (1980) model of photosynthesis as follows. The model calculates the photosynthetic rate as the minimum of two possible limitations, Rubisco activity (represented by the parameter V_{cmax}) and electron transport or RuBP regeneration (represented by the parameter J_{max}). Both limitations depend on the intercellular CO_2 concentration, which is determined by stomatal conductance, g_s. Both parameters J_{max} and V_{cmax} are generally strongly correlated with foliar nitrogen content (e.g. Field 1983). Thus, acclimation to low temperature by an increase in enzyme capacity would be evidenced by an increase in the absolute value, but not the relative temperature response, of Rubisco activity V_{cmax}. Acclimation to high temperature involving a change in the thermal stability of the electron transport machinery would be indicated by a change in the temperature response, and particularly the temperature optimum of the potential electron transport rate J_{max}. If acclimation of stomatal conductance occurred, a change in the temperature dependence of g_s but not that of V_{cmax} or J_{max}, would be seen. If Hikosaka's (1997) proposal that acclimation involved a shift in the balance between Rubisco activity and electron transport were true, one would expect to see a change in the ratio of J_{max} to V_{cmax}. Finally, because an increase in cell wall conductance would result in an increase in the rate of CO_2-limited photosynthesis for no increased investment in Rubisco, the hypothesis of Makino *et al.* (1994) could be evidenced by a change in the rate of Rubisco activity (and hence, V_{cmax}) per unit leaf nitrogen content.

We fitted the Farquhar *et al.* (1980) model to photosynthetic measurements made every 2 months over the course of a year, and used the above outline to investigate each of the above hypotheses of the mechanism of seasonal variation in the photosynthetic temperature response. We studied two provenances, one local and one from Morocco. In line with the idea that species that are exposed to the widest range of growth conditions in their native environment acclimate most strongly to changes in environmental conditions (Berry & Björkman 1980), we predicted that the Moroccan provenance would show more seasonal variation in the temperature response than the local provenance.

MATERIALS AND METHODS

Site

The study was carried out on mature maritime pine trees (*Pinus pinaster* Ait.) growing near Bordeaux, south-west France (56°37′N, 3°38′E). Measurements were made approximately every 2 months between July 1999 and October 2000. Details of measurements are given in Table 1. Measurements commenced in 'Le Pavillon', a stand of 29-year-old trees. Unfortunately, this stand was destroyed in the December 1999 windstorm, so measurements were continued in a neighbouring stand 'St Albans' of 18-year-old trees. Both sites are flat and have a sandy, well-drained substrate. Both stands were genetic trials. The first stand was established to study the phenotypical expression of various characters (Guyon & Kremer 1982), whereas the second consists of hybrid trees resulting from

Table 1. Details of measurements made. T_{av30} is the mean temperature in the 30 d prior to measurements

Measurement	Date	T_{av30} (°C)	Stand	Needle age (months)
July 1999	29/6–8/7/99	18·3	Pavillon	9
September 1999	24/8–1/9/99	21·0	Pavillon	11
November 1999	16/11–25/11/99	13·6	Pavillon	2
January 2000	25/1–1/2/00	6·7	St Albans	4
March 2000	27/3–3/4/00	10·1	St Albans	6
June 2000	7/6–14/6/00	18·9	St Albans	8
October 2000	17/10–26/10/00	13·3	St Albans	0·5

the first, established with the aim of comparing the performances of the hybrids (Harfouche & Kremer 2000). Only non-hybridized trees were used for this study. The two stands had similar leaf area indices of 2–3 m² m⁻² (B.E.M., personal observation) and comparable needle nitrogen concentrations (see later).

The two provenances chosen for study were Landes, originating locally, and Tamjout, originating in the mountains of Morocco. The local climate has mean winter minimum temperatures of 1 °C, with regular frosts, and mean summer maxima of 25 °C, with extremes up to 38 °C. The climate in the region of origin of the Moroccan provenance is drier, with similar winter temperatures, but higher summer temperatures.

Gas exchange measurements

At each measurement date, branches were taken from three trees of each of the two provenances. All trees used were in the interior of the stand. Branches with a southerly orientation were cut from the third whorl just after sunrise, and were brought back to the laboratory to be re-cut under water. One branch was taken each day; the measurement period thus lasted up to 10 d. Measurements were made on needles from the youngest fully expanded age class at each time point. Needle expansion is completed at the end of September (Bosc 1999) so that October measurements were made on newly expanded needles, whereas the August measurements were made on needles that were almost one year old.

Measurements were made with an open path gas analyser (Walz Compact Minicuvette system; Walz, Effeltrich, Germany). Six needles (three fascicles) were placed across the measurement cuvette. Measurements were made at five temperature/dewpoint combinations as follows: 15/1, 20/10, 25/14, 30/17, 35/20 °C. Measurements were made in random order. It would have been desirable to maintain a constant vapour pressure deficit (VPD) across temperatures, but this was not possible owing to the problem of condensation at high temperatures. Variations in VPD were minimized by regulating the temperature of the laboratory between approximately 15 and 25 °C in order to avoid condensation.

At each temperature, photosynthesis was measured at the following CO_2 concentrations: 1500, 1100, 350, 200, 100, 50, 0 µmol mol⁻¹. On changing temperature, between 30 and

60 min were required for the system to equilibrate. During this time the CO_2 concentration was maintained at 350 µmol mol⁻¹ to avoid stomatal closure. During measurements, the needles were illuminated from both sides with light sources (Fiber illuminator FL-400, Special Fiberoptics 400-F; Walz, Germany) providing 1400 µmol m⁻² s⁻¹. Needle temperature was measured with a thermocouple held against the needle with wire. A typical set of A–C_i curves thus obtained is shown in Fig. 1.

After completion of the measurements, the measured needles were removed. Needle length (l) and diameter (d) were measured in order to estimate the total photosynthetic surface area, calculated as $(1 + \pi/2)ld$. Note that all rates given in this paper are expressed per unit total needle area because, with bidirectional illumination, all needle surfaces were light-saturated. One needle from each fascicle was frozen and later assayed for chlorophyll a and b content, determined colorimetrically using a spectrophotometer (Inskeep & Bloom 1985). The second needle from each fascicle was dried at 70 °C for 72 h, mineralized with hot sulphuric acid and assayed colorimetrically for concentrations of N and P using the Technicon auto-analyser (O'Neill & Webb 1970).

Theory

Data were fitted to the Farquhar *et al.* (1980) model as described in the companion paper (Medlyn *et al.* 2002), giving responses of the model parameters J_{max} and V_{cmax} to leaf temperature. The temperature response of V_{cmax} was fitted using an Arrhenius equation:

$$V_{cmax}(T_k) = V_{cmax}(25) \exp\left[\frac{E_a(T_k - 25)}{298RT_k}\right] \qquad (1)$$

where $V_{cmax}(25)$ is the value of V_{cmax} at 25 °C, E_a is the activation energy of V_{cmax}, R is the gas constant (8·314 J mol⁻¹ K⁻¹) and T_k is leaf temperature in K. The temperature response of J_{max} was fitted using a peaked function:

$$J_{max}(T_k) = J_{max}(T_{opt}) \frac{H_d \exp\left[\frac{H_a(T_k - T_{opt})}{(T_k R T_{opt})}\right]}{H_d - H_a\left(1 - \exp\left(\frac{H_d(T_k - T_{opt})}{(T_k R T_{opt})}\right)\right)}, \qquad (2)$$

which has parameters $J_{max}(T_{opt})$, H_a, H_d, and T_{opt}. The full four-parameter equation was over-parameterized and sig-
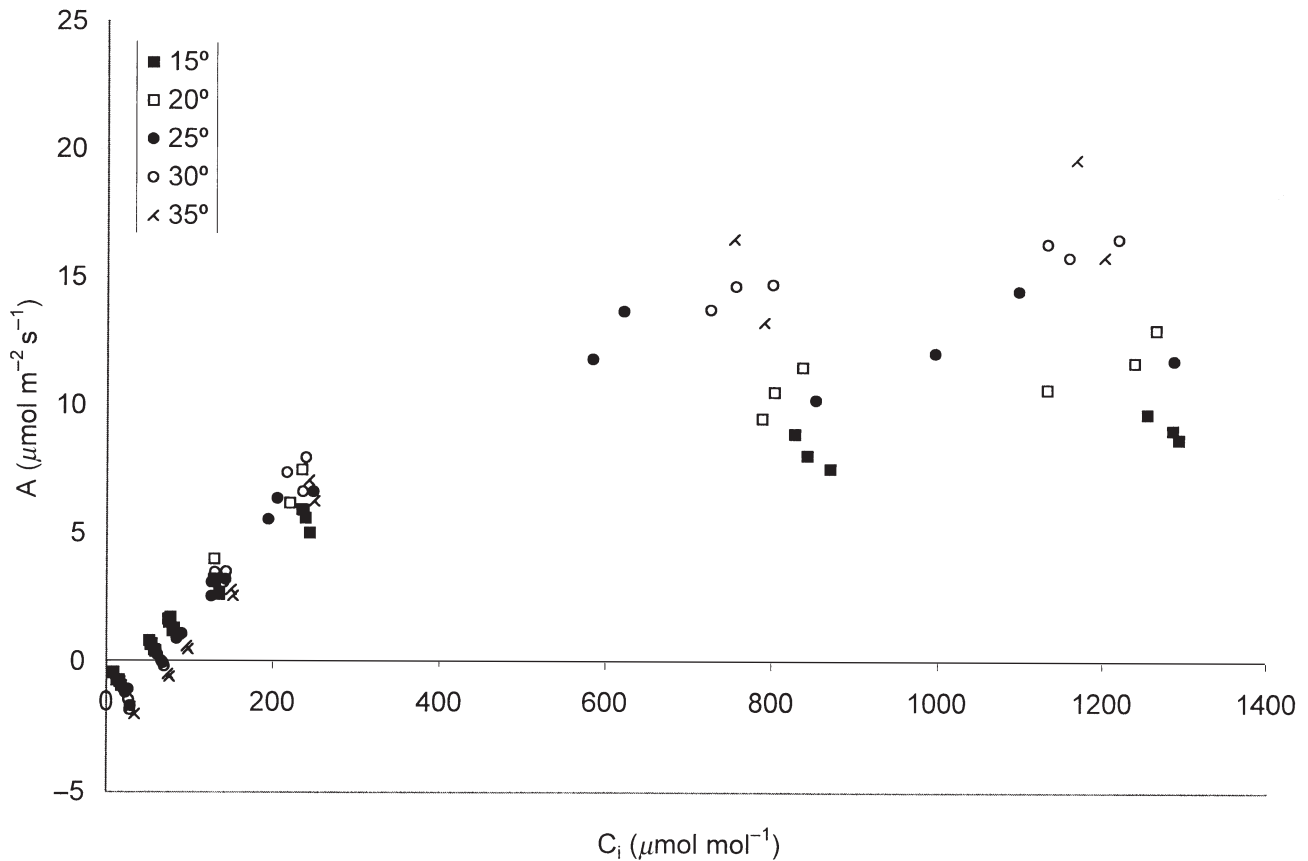
Figure 1. Typical set of A–C_i curves obtained at five different temperatures from three replicate branches. Measurements made on local provenance in August 1999.

nificant values of H_d could not be obtained. Hence, following Dreyer *et al.* (2001), the parameter H_d was assumed constant and equal to $200 \, kJ \, mol^{-1}$.

Additionally, changes in stomatal conductance were quantified as follows. Stomatal conductance has been shown to respond more slowly to environmental conditions than photosynthesis (Jones 1992), and time constraints meant that it was not possible to measure the true equilibrium value of g_s for each temperature. Thus, as an indicator of the equilibrium value for each temperature, we took the value of g_s obtained at the last point on each CO_2 response curve. In general, stomatal conductance had stabilized by this point. For each month and for each provenance we then fitted a straight line regression to the response curve of g_s to VPD. Although temperature and VPD were highly correlated, g_s was more strongly related to VPD, as has been found elsewhere (Jones 1992).

Meteorological data were obtained from the 'Le Bray' field site (Berbigier, Bonnefond & Mellmann 2001), about 1 km distant. The mean temperature during the 30 d prior to each measurement set (T_{av30}) was calculated and is shown in Table 1. Mean daily minimum and maximum temperatures over the 30, 14, 7, 3 and 1 d prior to each measurement were also calculated and tested as indices of growth temperature.

RESULTS

Leaf chemistry

In the local provenance, there was a considerable variation in needle nitrogen content (N_{area}) over the course of the study. N_{area} increased from the time of needle expansion (October) over winter and then declined from March onwards. This pattern resulted in a negative correlation between N_{area} and several measures of ambient temperature. The strongest correlation was found with the mean minimum temperature of the 30 d prior to measurements (T_{mn30}) (Fig. 2). In the Moroccan provenance, however, the decline in needle nitrogen content did not begin until August, resulting in a weak and non-significant correlation between N_{area} and ambient temperature. Needle nitrogen per unit mass was significantly higher in the Moroccan provenance throughout the study, but when nitrogen was expressed on a surface area basis, there was only a significant difference between provenances in June 2000 ($P < 0.05$, Student's *t*-test).

Photosynthetic parameters at 25 °C

Values of the parameters V_{cmax} and J_{max} at 25 °C are given in Tables 2 and 3. There were no significant differences

Table 2. Parameters of temperature response of V_{cmax} (Eqn 1). The parameter $V_{cmax}(25)$ gives the estimated value of V_{cmax} at 25 °C, expressed per unit total needle surface area. The standard deviation of $V_{cmax}(25)$ and the standard error of E_a are given in parentheses. n = number of data points used in regression

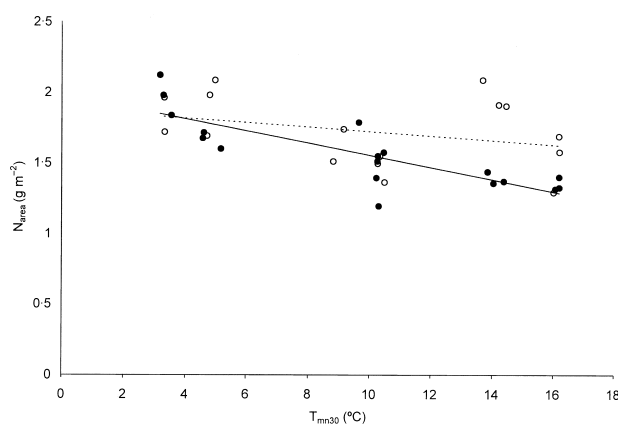|	$V_{cmax}(25)$ $(\mu mol\,m^{-2}\,s^{-1})$	E_a $(kJ\,mol^{-1})$	r^2	n
Local				
July 1999	51·2 (9·5)	47·2 (11·2)	0·80	10
September 1999	34·7 (2·0)	62·2 (2·8)	0·99	14
November 1999	61·3 (3·8)	61·0 (4·8)	0·96	14
January 2000	60·0 (14·8)	68·2 (5·9)	0·96	16
March 2000	55·8 (8·0)	63·7 (3·0)	0·98	15
June 2000	59·9 (0·7)	48·4 (3·4)	0·96	15
October 2000	41·4 (5·7)	47·0 (5·9)	0·89	15
Moroccan				
July 1999	35·1 (3·9)	93·8 (15·1)	0·93	10
September 1999	39·4 (2·9)	55·5 (3·7)	0·98	13
November 1999	59·7 (3·5)	55·2 (3·0)	0·98	15
January 2000	58·5 (5·5)	54·4 (4·7)	0·95	13
March 2000	60·4 (15·7)	76·6 (4·3)	0·98	16
June 2000	78·5 (12·6)	51·6 (3·4)	0·97	15
October 2000	50·6 (7·4)	58·6 (2·2)	0·99	10

Figure 2. Needle nitrogen content per unit area, N_{area}, as a function of the mean minimum temperature over the 30 d prior to measurement, T_{mn30}. Fitted regression lines are shown. Open symbols and broken line: Moroccan provenance. Closed symbols and solid line: local provenance. Equations for regression lines: Moroccan provenance: $N_{area} = 1\cdot91 - 0\cdot016 T_{mn30}$, $r^2 = 0\cdot10$, $P > 0\cdot1$. Local provenance: $N_{area} = 1\cdot99 - 0\cdot043 T_{mn30}$, $r^2 = 0\cdot67$, $P < 0\cdot001$.

between the provenances for any month ($P > 0\cdot05$, Student's t-test). Values of both $V_{cmax}(25)$ and $J_{max}(25)$ varied by a factor of two throughout the year. For the local provenance, both parameters were correlated with ambient temperature. The index of ambient temperature giving the strongest correlation was T_{mn30}, the mean daily minimum temperature over the 30 d prior to the measurement (Fig. 3), although strong correlations were also found with the average and mean maximum temperatures over the 30 and 3 d prior to measurement. The correlation between the parameters and ambient temperature may have been medi-

ated by changes in nitrogen content, as both $V_{cmax}(25)$ and $J_{max}(25)$ were correlated with needle nitrogen (Fig. 4). The relationships between $V_{cmax}(25)$ and $J_{max}(25)$ and needle nitrogen content were not significantly different between the Moroccan provenance and the local provenance ($P > 0\cdot1$, F-test). However, for the Moroccan provenance, only $J_{max}(25)$ was significantly correlated with ambient temperature (Fig. 3).

In Fig. 5, the relationships between Rubisco activity and needle nitrogen content are compared for warm and cool times of year (average temperature above or below 18°, respectively). Regression lines for the two times of year are not significantly different ($P > 0\cdot1$, F-test).

Table 3. Parameters of temperature response of J_{max} (Eqn 2). The parameters $J_{max}(25)$ and $J_{max}(T_{opt})$ give the estimated values of J_{max} at 25 °C and at the optimal temperature T_{opt}, respectively, expressed per unit total needle surface area. Standard deviations of parameters $J_{max}(25)$ and $J_{max}(T_{opt})$, and standard errors of parameters H_a and T_{opt}, are given in parentheses. n = number of data points used in regression

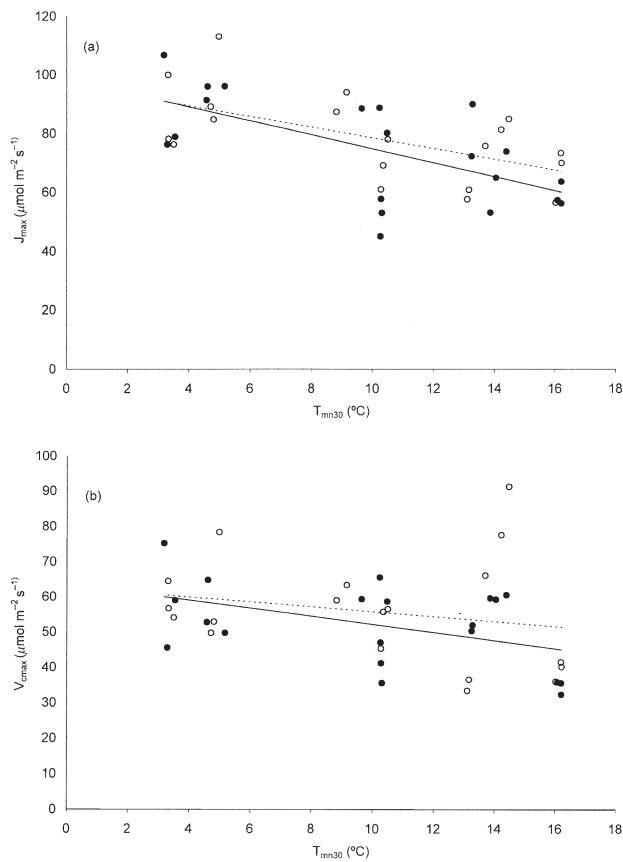| Provenance	Date	$J_{max}(25)$ $(\mu mol\,m^{-2}\,s^{-1})$	$J_{max}(T_{opt})$ $(\mu mol\,m^{-2}\,s^{-1})$	$H_a(kJ\,mol^{-1})$	T_{opt} (°C)	r^2	n
Local	July 1999	81·2 (12·5)	113·5 (17·5)	43·0 (29·8)	34·9 (6·5)	0·92	10
	September 1999	59·2 (4·0)	83·7 (5·6)	34·5 (9·1)	36·7 (8·5)	0·97	14
	November 1999	85·9 (4·9)	107·2 (6·1)	31·4 (16·8)	34·1 (2·6)	0·87	14
	January 2000	87·3 (16·8)	112·0 (21·6)	40·0 (6·2)	33·5 (0·4)	0·99	16
	March 2000	94·5 (2·7)	131·9 (3·7)	44·5 (18·7)	34·7 (2·6)	0·90	15
	June 2000	64·1 (10·4)	95·1 (15·5)	44·5 (17·2)	35·9 (6·4)	0·95	15
	October 2000	52·0 (6·4)	62·6 (7·7)	34·0 (17·3)	32·7 (1·5)	0·83	15
Moroccan	July 1999	59·3 (2·3)	126·5 (4·8)	56·8 (11·1)	39·8 (14·0)	1·00	10
	September 1999	66·8 (8·9)	89·8 (12·0)	32·5 (12·9)	35·9 (6·4)	0·97	13
	November 1999	86·6 (8·0)	119·3 (11·0)	34·1 (11·6)	36·2 (7·8)	0·96	15
	January 2000	84·8 (13·1)	108·3 (16·7)	35·9 (18·3)	33·9 (2·4)	0·91	13
	March 2000	95·7 (15·2)	139·5 (22·1)	51·8 (14·5)	34·6 (1·6)	0·96	16
	June 2000	80·8 (4·6)	107·3 (6·1)	39·4 (10·7)	34·3 (2·3)	0·97	15
	October 2000	65·2 (5·8)	93·4 (8·3)	40·8 (13·7)	35·8 (4·0)	0·98	10

Figure 3. (a) The potential electron transport rate J_{max} and (b) the maximum rate of Rubisco activity V_{cmax}, at 25 °C, as a function of the mean minimum temperature over the 30 d prior to measurement. Symbols as for Fig. 2. Equations for regression lines: local provenance: $J_{max} = 98 \cdot 6 - 2 \cdot 37 T_{mn30}$, $r^2 = 0 \cdot 39$, $P = 0 \cdot 003$; $V_{cmax} = 63 \cdot 7 - 1 \cdot 14 T_{mn30}$, $r^2 = 0 \cdot 20$, $P = 0 \cdot 047$. Moroccan provenance: $J_{max} = 96 \cdot 8 - 1 \cdot 82 T_{mn30}$, $r^2 = 0 \cdot 34$, $P = 0 \cdot 009$; $V_{cmax} = 62 \cdot 8 - 0 \cdot 69 T_{mn30}$, $r^2 = 0 \cdot 04$, $P > 0 \cdot 1$.

The ratio of the two parameters $(J_{max}(25) : V_{cmax}(25))$ varied from 1 to 1·7 over the course of the experiment (Fig. 6). This variation could not be explained by ambient temperature, nitrogen content or needle age.

Temperature response of J_{max} and V_{cmax}

For V_{cmax}, an Arrhenius relationship described the temperature response well, with model r^2 ranging from 0·92 to 0·99. The estimated parameters of this response are given in Table 2. The activation energy parameter E_a ranged from 47 to 75 kJ mol^{-1}, with similar values for both provenances. There were weak and non-significant correlations of E_a with all measures of ambient temperature (r^2 ranging from 0·2 to 0·4 for the local provenance and less than 0·1 for the Moroccan provenance). This limited variability in E_a suggests that, although the absolute amount of enzyme present may have varied throughout the year, the underlying temperature response of the enzyme activity did not change.

To describe the temperature response of J_{max}, a peaked function was necessary. The estimated parameters of the function are given in Table 3. The function explained more than 90% of variability in most measurement periods, except in November 1999 and October 2000, when the optimal temperatures appeared to differ among the newly expanded needles. As for V_{cmax}, the activation energy, H_a, did not differ between provenances. There was no seasonal trend. Values ranged from 31 to 51 kJ mol^{-1} with an average value of 39 kJ mol^{-1}. The optimum temperature for J_{max}, T_{opt}, varied between 32·7 and 39·8 °C, with no clear difference between the two provenances. For the local provenance, T_{opt} was correlated with most measures of ambient temperature; the strongest correlation was with T_{mx1}, the maximum temperature on the previous day (Fig. 7a). However, for the Moroccan provenance, there was only a weak and non-significant correlation between T_{opt} and ambient temperature (Fig. 7b). For both provenances, the slope of the regression equations was low (0·1–0·2), indicating that the optimal temperature of photosynthesis increased much more slowly than the ambient temperature.
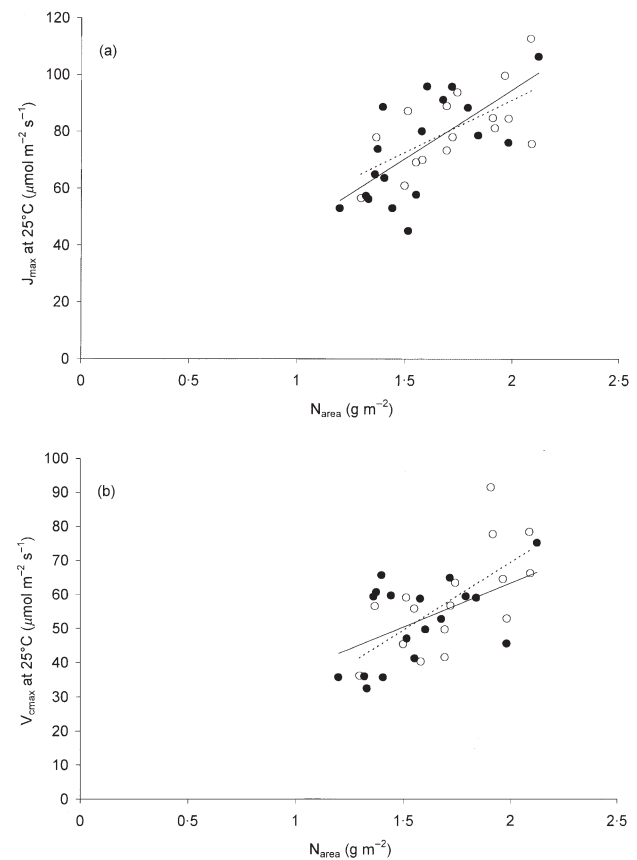

Figure 4. (a) The potential electron transport rate J_{max} and (b) the maximum rate of Rubisco activity V_{cmax}, as a function of needle nitrogen content. Symbols as for Fig. 2. Equations for regression lines: local provenance: $J_{max} = 48 \cdot 7 N_{area} - 2 \cdot 5$, $r^2 = 0 \cdot 45$, $P = 0 \cdot 002$; $V_{cmax} = 25 \cdot 8 N_{area} + 11 \cdot 7$, $r^2 = 0 \cdot 27$, $P = 0 \cdot 027$. Moroccan provenance: $J_{max} = 37 \cdot 4 N_{area} + 16 \cdot 5$, $r^2 = 0 \cdot 43$, $P = 0 \cdot 006$; $V_{cmax} = 40 \cdot 1 N_{area} - 10 \cdot 6$, $r^2 = 0 \cdot 44$, $P = 0 \cdot 005$.
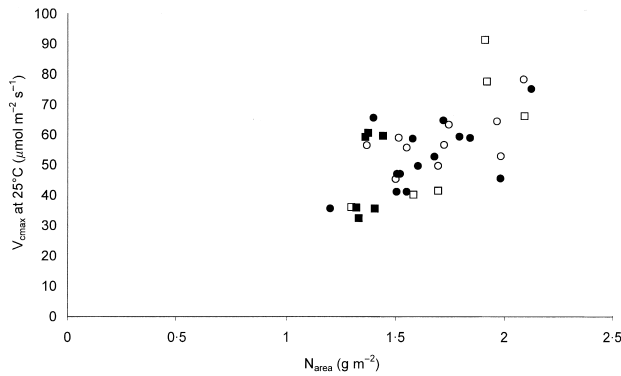
Figure 5. The maximum rate of Rubisco activity V_{cmax} as a function of needle nitrogen content. Different symbols indicate different seasons and provenances. Open symbols: Moroccan provenance. Filled symbols: local provenance. Circles: warmer months ($T_{av} > 18\,°C$). Squares: cooler months ($T_{av} < 18\,°C$).

Stomatal conductance

There was a clear change in the response of stomatal conductance to vapour pressure deficit over the course of the year. Table 4 shows the regression equations and correlation coefficient (r) between g_s and VPD. In summer (July 1999 and June 2000) r is close to zero or positive, indicating no stomatal closure, whereas in winter there was a strong negative response of g_s to VPD.

Implied temperature response of photosynthesis

To summarize the impact on photosynthesis of all the changes described above, the Farquhar *et al.* (1980) model

was implemented in MathCAD (MathSoft Engineering & Education, Inc., Cambridge, MA, USA), using the equations given by Medlyn *et al.* (2002) and incorporating the parameters in Tables 2–4. The model was used to generate temperature responses of needle photosynthesis at an atmospheric CO_2 concentration of $350\,\mu mol\,mol^{-1}$. Responses were also generated under the assumption that g_s did not vary with temperature, assuming values of 60 and $70\,mmol\,m^{-2}\,s^{-1}$ for local and Moroccan provenances, respectively.

In both provenances, the optimal temperature of light-saturated photosynthesis (incident radiation = $1000\,\mu mol\,m^{-2}\,s^{-1}$) increased by 7–8 °C between the winter and summer months. This shift in T_{opt} was caused by the altered g_s response, because modelled temperature responses with g_s held constant showed no change in T_{opt} between seasons. At light saturation, photosynthesis was limited by Rubisco activity at most temperatures, and there was no seasonal change in the optimal temperature of Rubisco-limited photosynthesis.

Modelled temperature response curves for photosynthesis at an incident radiation of $500\,\mu mol\,m^{-2}\,s^{-1}$ (A_{500}) are illustrated in Fig. 8. At this radiation level, photosynthesis is generally limited by RuBP-regeneration. As with the higher radiation level, the optimal temperature of photosynthesis increased between the winter and summer months in both provenances. The shift was more pronounced in the local provenance (increase in T_{opt} of 8 °C) than in the Moroccan provenance (increase of 4 °C). In the Moroccan provenance, the shift in T_{opt} was largely caused by the altered g_s response. In the local provenance, however, even with g_s held constant, there was an increase of 4 °C in T_{opt} between winter and summer months. This increase was caused by the increase in the optimal temperature for J_{max} (Fig. 7).
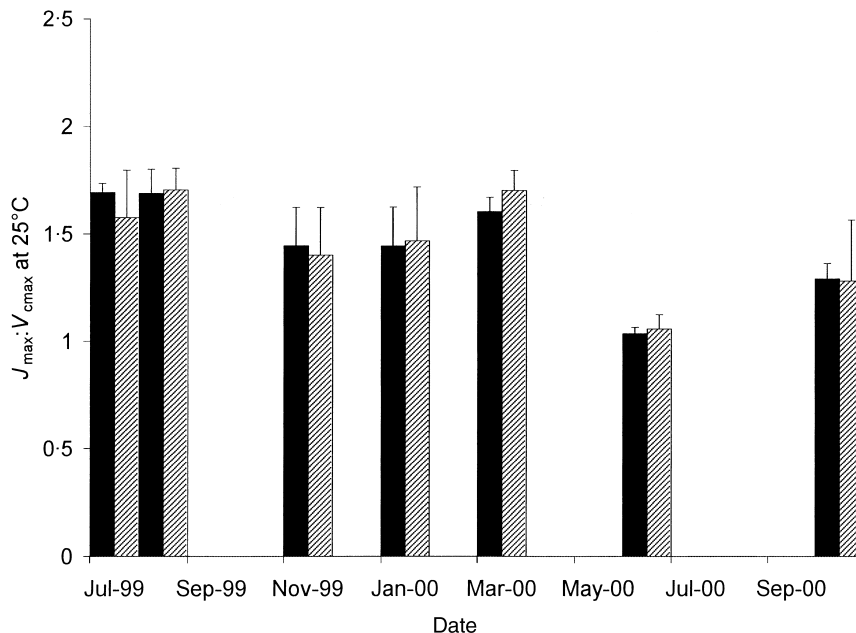

Figure 6. Ratio of $J_{max}:V_{cmax}$ at 25 °C during each measurement period (geometric average). Solid bars, local provenance; striped bars, Moroccan provenance. Error bars show standard deviations.
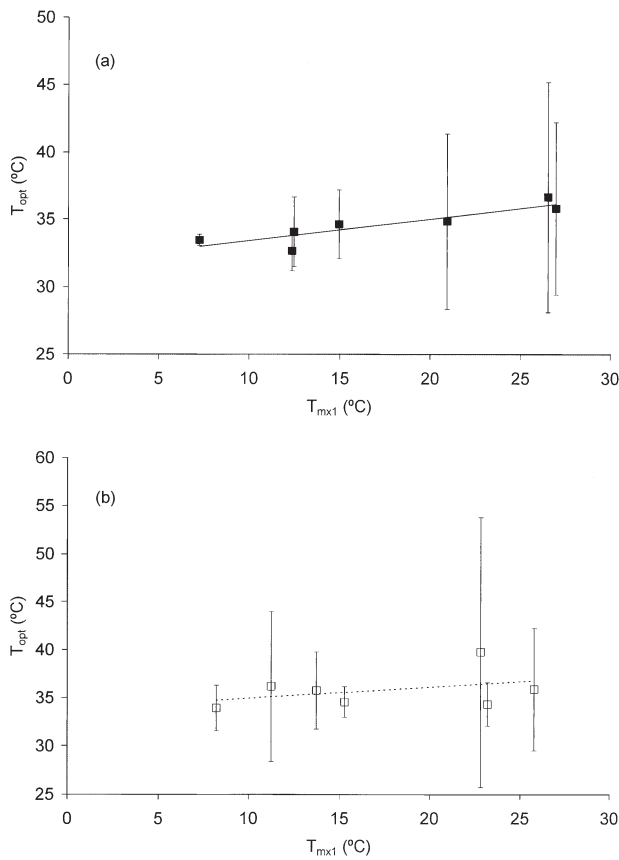
Figure 7. Relationship between the optimal temperature of J_{max} (T_{opt}) and the maximum temperature on the day prior to measurement (T_{mx1}) for (a) local provenance and (b) Moroccan provenance. Error bars show parameter standard errors. Regression equations: local provenance: $T_{opt} = 31 \cdot 8 + 0 \cdot 16 T_{mx1}$, $r^2 = 0 \cdot 79$. Moroccan provenance: $T_{opt} = 33 \cdot 8 + 0 \cdot 11 T_{mx1}$, $r^2 = 0 \cdot 16$.

Figure 8. Modelled temperature response of photosynthesis at an atmospheric CO_2 concentration of $350\,\mu$mol mol^{-1} and incident radiation of $500\,\mu$mol m^{-2} s^{-1}. (a) Local provenance; (b) Moroccan provenance. Thin lines: August (summer); Heavy lines: January (winter). Solid lines assume measured g_s response to VPD and dashed lines assume constant g_s.

Figure 8 also illustrates that, for any given temperature, needle photosynthesis was higher in the winter months than in summer months. This pattern was observed in both provenances.

DISCUSSION

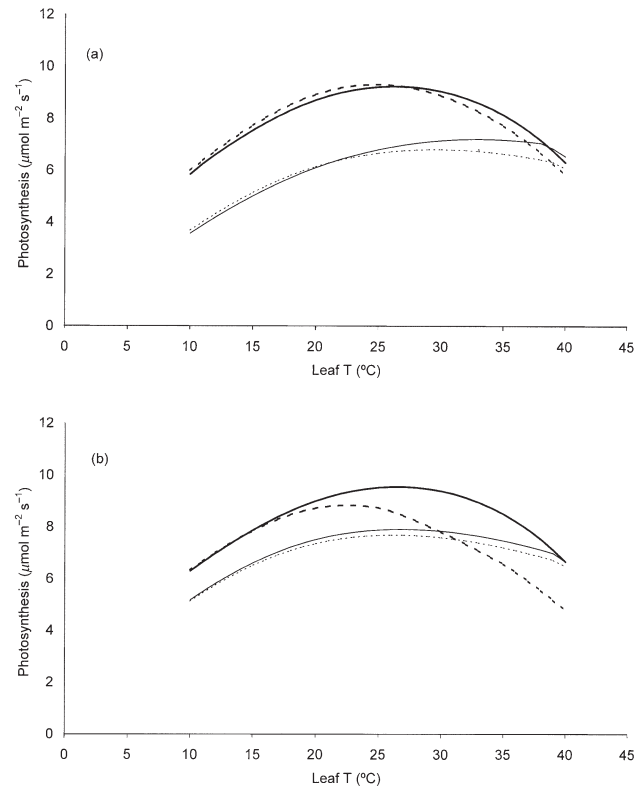Many current models of forest CO_2 exchange, particularly those employing the Farquhar *et al.* (1980) photosynthesis model, assume that the temperature response of photosynthesis is constant (e.g. Thornley & Cannell 1996; Williams *et al.* 1996; Kellomäki *et al.* 1997; Baldocchi & Meyers 1998; Grant & Nalder 2000; Medlyn *et al.* 2000). This study shows that this assumption is unwarranted. For two provenances of maritime pine, a clear seasonal change in the temperature response curve of photosynthesis was observed (Fig. 8). There was both a shift in temperature optimum and a translational (vertical) shift of the curve. Several changes in the underlying processes combined to bring about this seasonal variation.

One mechanism appeared to be a shift in the temperature optimum of electron transport rate (Fig. 7). Such a shift

Month	Local	r	Moroccan	r
July 1999	$g_s = 53 + 1 \cdot 37D$	0·15	$g_s = 54 + 0 \cdot 66D$	0·37
September 1999	$g_s = 50 + 0 \cdot 88D$	0·46	$g_s = 75 + 0 \cdot 11D$	0·04
November 1999	$g_s = 58 - 0 \cdot 32D$	−0·28	$g_s = 70 + 0 \cdot 07D$	0·08
January 2000	$g_s = 74 - 0 \cdot 76D$	−0·62	$g_s = 73 - 1 \cdot 23D$	−0·72
March 2000	$g_s = 82 - 0 \cdot 91D$	−0·49	$g_s = 100 - 0 \cdot 94D$	−0·50
June 2000	$g_s = 61 - 0 \cdot 05D$	−0·05	$g_s = 73 - 0 \cdot 05D$	−0·03
October 2000	$g_s = 87 - 1 \cdot 39D$	−0·61	$g_s = 102 - 1 \cdot 27D$	−0·53

Table 4. Equations between stomatal conductance (mmol m^{-2} s^{-1}) and VPD (kPa) for each measurement month, for local and Moroccan provenances. r denotes the correlation coefficient of the equation. The stomatal conductance values used were the final points on each A–C_i curve (see text for further details)

is generally ascribed to changing heat stability of the photosynthetic apparatus and has been clearly demonstrated for desert shrubs using *in vitro* methods (Armond *et al.* 1978). The shift found here for the local provenance was not, however, statistically significant. Gas exchange measurements were not possible above 35 °C owing to stomatal closure in response to high VPD, resulting in large standard errors on the parameter T_{opt}. Other methods of measuring J_{max} that do not suffer from this problem, such as chlorophyll fluorescence (Niinemets, Oja & Kull 1999) would be required to confirm the trend identified here.

In accordance with the hypothesis that acclimation to low temperatures involves an increase in the capacity of temperature-limited enzymes such as Rubisco (Berry & Björkman 1980), we found that Rubisco activity V_{cmax} decreased significantly with increasing ambient temperature. The Rubisco activity was also strongly correlated with foliar nitrogen content, which varied with ambient temperature. It was not clear therefore whether the change in Rubisco activity was part of a process of temperature acclimation (Berry & Björkman 1980), or a phenological phenomenon, whereby nitrogen is stored in needles over winter and retranslocated over summer to build new needles. Manipulative experimental studies would be required to distinguish between phenological and acclimatory processes.

Importantly, we also found a distinct seasonal change in the stomatal response to VPD, which explained a considerable proportion of the seasonal change in photosynthetic temperature response. Many previous studies have considered only the overall response of photosynthesis, making it difficult to assess the role of stomatal conductance (Berry & Björkman 1980). However, those studies in which the contribution of stomatal conductance was calculated have generally agreed that it can be a major factor in seasonal variation (e.g. Ferrar, Slatyer & Vranjic 1989; Ellsworth 2000).

Two other proposed hypotheses related to temperature acclimation were not supported by the results of this study. Hikosaka (1997) put forward the hypothesis that temperature acclimation is a result of a re-allocation of nitrogen between electron transport and carboxylation processes in order to ensure both sets of processes are colimiting at ambient conditions. Under this hypothesis, it is predicted that the ratio of J_{max} to V_{cmax} at a given temperature should be lower for plants experiencing low temperatures. In this study, no seasonal shift in this ratio was observed. Other studies investigating acclimation to growth temperature have similarly failed to find any evidence of such a shift (Ferrar *et al.* 1989; Bunce 2000).

A change in cell-wall conductance to CO_2 has also been implicated in plant acclimation to ambient temperature. Makino *et al.* (1994) formulated this hypothesis after finding that CO_2-limited photosynthesis per unit Rubisco varied with growth temperature. Leaf Rubisco content as a fraction of leaf nitrogen was unchanged with growth temperature. In contrast, we found in the current study that Rubisco activity (estimated from CO_2-limited photosynthe-

sis) as a function of leaf nitrogen content was not affected by the ambient temperature (Fig. 5). Thus, although we did not directly measure cell-wall conductance, we find it unlikely that this conductance was involved in the seasonal changes we observed.

Differences between the two provenances were found. We had hypothesized that the Moroccan provenance, adapted to more extreme temperatures, would show more seasonal variation than the local provenance. The results of the study do not appear to support this hypothesis; smaller seasonal shifts in the photosynthetic temperature response curves were found for the Moroccan than the local provenance. It was interesting that, at most times of year, photosynthetic rates in the Moroccan provenance were higher than in the local provenance. In the Bordeaux area, growth rates of the Moroccan provenance are well below those of the local provenance. The physiological reasons for this difference are not well known but may be related to drought avoidance behaviour in the Moroccan provenance (Guyon & Kremer 1982).

The shift in the optimal temperature of photosynthesis, $T_{opt,A}$, observed in this study has also been observed in most other studies of seasonal temperature acclimation in field-grown evergreen trees (Neilson, Ludlow & Jarvis 1972; Strain *et al.* 1976; Slatyer & Morrow 1977; Guehl 1985; Battaglia *et al.* 1996). Some found more dramatic shifts in $T_{opt,A}$ than that observed here (e.g. Strain *et al.* 1976) but most observed that the change in $T_{opt,A}$ was less than the seasonal change in mean daily temperature (Battaglia *et al.* 1996). In most cases there was a good correlation between $T_{opt,A}$ and mean daily temperature, although Neilson *et al.* (1972) found $T_{opt,A}$ of *Picea sitchensis* in Scotland was lowest in May and highest in October In contrast to the shift in $T_{opt,A}$, the translational shift in the photosynthesis–temperature response observed here does not correspond with results from other studies. Strain *et al.* (1976) and Slatyer & Morrow (1977) found that the maximal rate of photosynthesis was higher in summer than winter, in direct contrast to the current study, whereas others found no change in the maximal rate of photosynthesis (Guehl 1985; Battaglia *et al.* 1996). Here, seasonal differences in the maximal rate of photosynthesis could be explained by changes in foliar nitrogen content (Figs 2 & 4). Unfortunately, previous studies give no information on seasonal changes in foliar nutrition, making it difficult to assess why they differ from our results.

Our work differs from these previous studies in that we analysed photosynthetic temperature responses in terms of the parameters J_{max} and V_{cmax} of the Farquhar *et al.* (1980) photosynthesis model, giving insights into the mechanisms underlying photosynthetic temperature acclimation. To our knowledge, only one other study has examined seasonal changes in the temperature response of J_{max} and V_{cmax} (Ellsworth & Klimas, submitted). Their study, on *Pinus taeda*, confirms the results presented here: absolute values of V_{cmax} and J_{max} were lower in summer than winter, and the optimum temperature of J_{max} increased with ambient temperature.

Recommendations for modelling

The study has two major implications for the modelling of the photosynthetic temperature response in forest canopy models. Firstly, it is clear that this response varies seasonally. The seasonal variation could be captured in models as follows: (i) values of J_{max} and V_{cmax} at 25 °C should vary seasonally, either as a function of ambient temperature (Fig. 3) or of foliar nitrogen content, where seasonal values are known (Fig. 4); (ii) the temperature optimum of J_{max} should be a function of ambient temperature (Fig. 7); (iii) the response of stomatal conductance to VPD should vary as a function of time of year (Table 4).

A second implication is that modelling of forest responses to increasing temperatures should take potential acclimation of the photosynthetic temperature response into account. Although the results of this study do not unequivocally show that temperature acclimation occurs in mature trees, because a number of other factors such as phenological development or acclimation to changes in incident radiation may have been involved, acclimation to ambient temperature is the most often proposed explanation for the observed seasonal variation in the photosynthetic temperature response (Strain *et al.* 1976; Slatyer & Morrow 1977; Battaglia *et al.* 1996). Based on this study, acclimation to temperature in field-grown trees may involve changes in values of J_{max} and V_{cmax} at 25 °C, the optimum temperature of J_{max}, and stomatal sensitivity to VPD. In the companion article (Medlyn *et al.* 2002) we address this question further by examining photosynthetic temperature responses from trees growing in different climates.

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