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Seasonal variation in photosynthetic capacity of montane conifers

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Summary

1. The capacity to perform photosynthesis, given appropriate environmental conditions, is reflected by measurements of the maximum quantum yield of photosystem II (F_v/F_m). The seasonal course of F_v/F_m may help define the length of the annual photosynthetic period in temperate evergreen forests.

2. F_v/F_m was measured 31 times from September to May on six conifer species located along an altitudinal gradient between 400 and 1400 m a.s.l. in northern Idaho, USA. The species were Western Redcedar (*Thuja plicata* Donn ex D. Donn), Douglas Fir (*Pseudotsuga menziesii* (Beissn.) Franco), Engelmann Spruce (*Picea engelmannii* Parry), Grand Fir (*Abies grandis* (Dougl.) Lindl.), Ponderosa Pine (*Pinus ponderosa* Laws.) and Lodgepole Pine (*Pinus contorta* Dougl.).

3. Species differed in mean F_v/F_m, in magnitude of response to maximum and minimum temperature, and in altitude effects other than temperature. For two species F_v/F_m in spring differed from that in autumn, even when measured at the same air temperature. Regardless of temperature, most species maintained some photosynthetic capacity all winter.

4. A lag of several days to 2 months was observed in the response of F_v/F_m to ambient temperature. The lag differed among species. It was not observed in Grand Fir, and was as long as 2 months in Western Redcedar. Over all species combined the best overall correlation was with a moving average of maximum temperature over the previous 30 days.

5. The correlation between F_v/F_m and maximum temperature was generally stronger than that with minimum temperature (average root mean-squared error was reduced by 10%), presumably because maximum temperatures better reflect daytime photoinhibitory conditions.

6. A reduced model predicted F_v/F_m based on species and maximum temperature; this model can be used to parameterize models describing the annual cycle of photosynthetic capacity for the six conifer species included in this study.

Key-words: chlorophyll fluorescence, F_v/F_m, model parameterization, temperature

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Introduction

The seasonal duration of photosynthesis remains relatively uncertain in the evergreen forests of western North America. The duration of the photosynthetic season is particularly difficult to predict if species differ in the rate at which photosynthetic capacity is lost with the onset of winter and regained in the spring. Genotypic differences among evergreen species may allow cold-tolerant species to have increased annual productivity as a result of longer photosynthetic seasons (Marshall, Rehfeldt & Monserud 2001). Although several conifer species can maintain some photosynthetic capacity following exposure to low winter temperatures (Leverenz & Öquist 1987; Weger, Silim & Guy 1993), other species undergo a distinct photochemical inactivation during the winter months (Westin, Sundblad & Hällgren 1995; Rose & Haase 2002). Genotypes tolerant of cold temperatures may be better adapted to utilize warmer temperatures for photosynthesis following prolonged exposure to low temperatures (Fracheboud *et al.* 1999). An assessment of species-specific and seasonal differences in photosynthetic capacity would

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increase our ability to predict annual carbon fixation and define a theoretical upper limit for production at a given site (Leverenz & Öquist 1987). Accurate description of the change in photosynthetic capacity throughout the season will improve modelled estimates of ecosystem carbon exchange (Bergh, McMurtrie & Linder 1998; Wilson, Baldocchi & Hanson 2001; Hari & Mäkelä 2003).

Chlorophyll fluorescence has become a standard method for measuring photosynthetic capacity in intact leaves (van Kooten & Snel 1990; Bolhár-Nordenkampf & Öquist 1993; Maxwell & Johnson 2000). It provides a quick and simple method for determining the photochemical capacity of photosystem II. The ratio of variable to maximal fluorescence (F_v/F_m) measures the proportion of open photosystem II centres and quantifies the efficiency of energy capture, generally termed the maximum quantum yield of photosystem II (Genty, Briantais & Baker 1989). Changes in F_v/F_m are proportional to the quantum yield of CO_2 fixation (mol CO_2 mol photon^{-1}) for a wide range of species under saturating CO_2 and low O_2 (Genty *et al.* 1989). In support of this theoretical relationship, Leverenz & Öquist (1987) found a significant correlation between quantum yield and F_v/F_m ($r^2 = 0{\cdot}91$) in Scots Pine (*Pinus sylvestris*). Generally, quantum yield decreases with temperature in the vicinity of 0 °C (Leverenz & Öquist 1987; Cannell & Thornley 1998). The correlation between F_v/F_m and the quantum yield of photosynthesis allows for quick, meaningful measurements even during winter.

Fluorescence measurements can be used in evergreen forests to detect physiological changes resulting from environmental stress (Ball *et al.* 1994). Environmental conditions such as excess light, extreme temperature or drought can cause photooxidation or photoinhibitory damage, changing the efficiency of non-photochemical quenching and decreasing F_v/F_m (Krause 1988; Genty *et al.* 1989; Ball *et al.* 1994; Westin *et al.* 1995; Maxwell & Johnson 2000). In this manner, exposure to cold temperatures and high light during the winter months decreases electron transport, photon yield and photosynthetic capacity (Du, Nose & Wasano 1999). Decreases in F_v/F_m indicate photoinhibition (Krause 1988), a protective mechanism for dissipating excess energy (Ball *et al.* 1994). The increased quenching of fluorescence during winter largely results from downregulation and increased zeaxanthin concentrations within the thylakoid membrane (Oberhuber & Bauer 1991). Genotypes able to tolerate cold temperatures show rapid photosynthetic recovery when temperatures rise in spring (Fracheboud *et al.* 1999). Differences in photosynthetic capacity, measured by chlorophyll fluorescence, describe species- and genotype-specific differences in photosynthetic reduction during winter. F_v/F_m is also correlated with other effects of winter freezing, such as tissue damage (Rose & Haase 2002), seed death (Binder *et al.* 1996) and cold hardening (Öquist & Huner 1991).

We monitored F_v/F_m of six conifer species growing in northern Idaho from the end of the growing season, throughout the winter, and into the early summer when the maximum photosynthetic potential returned. We hypothesized that: (1) there is a difference in F_v/F_m among species; (2) F_v/F_m responds to temperature and species vary in their temperature response; and (3) F_v/F_m of individuals within a given species, but growing at different altitudes, will respond differently to temperature.

Materials and methods

STUDY SPECIES AND SITES

F_v/F_m was monitored on 39 conifer trees distributed along an altitudinal gradient in northern Idaho, USA. The species monitored were Western Redcedar (*Thuja plicata* Donn ex D. Don), Douglas Fir (*Pseudotsuga menziesii* (Beissn.) Franco), Engelmann Spruce (*Picea engelmannii* Parry), Grand Fir (*Abies grandis* (Dougl.) Lindl.), Ponderosa Pine (*Pinus ponderosa* Laws.) and Lodgepole Pine (*Pinus contorta* Dougl.). Measurements were performed 31 times, from 7 September 2001 until 14 May 2002, at eight sites. The sites were chosen between Kendrick, ID (46°61′ N, 116°65′ W) and the summit of Moscow Mountain (46°49′ N, 116°53′ W), approximately 12 km north-east of Moscow, ID. Sites were selected based on accessibility and altitude, and varied in age and stand structure. Each site was established with an approximate change in elevation of 150 m between 381 and 1403 m above sea level. At each location we sampled foliage from two 1-year-old shoots from two individuals representing each tree species present. At each subsequent sampling date we returned to the same trees per species and location. Foliage was sampled between 8 : 00 am and 12 : 00 pm for each sampling period. In general, foliage was collected first at the lowest elevation, and samples were subsequently collected up the mountain. However, this sampling strategy was not constant as times occurred when samples were collected at the top of the mountain first, or in the middle of the altitudinal transect. However, sample collection was never randomized between sites because sites were up to 20 km apart. Diurnal variation in F_v/F_m has been reported for some conifers (Ball *et al.* 1994); however this variation is marginal if the measurements are carried out over the same time period for each successive sampling period (Vidaver *et al.* 1991), especially when compared with the pronounced seasonal changes reported in the current study.

FLUORESCENCE MEASUREMENTS

Needles were harvested from the sun-exposed portion of whorls accessible from the ground. Following collection, needles were dark-adapted for at least 20 min before fluorescence measurements were performed. This amount of time is sufficient to minimize the electron gradient and oxidize electron carriers within the chloroplast (Vidaver *et al.* 1991). Fluorescence was measured after a saturating photon flux density of

1000 μmol m^{-2} s^{-1} for 2 s using a chlorophyll fluorometer (Morgan CF-1000 Opti-Sciences, Tyngsboro, MA, USA). Needles were arranged for dark adaptation with the adaxial side up, so that individual needles did not overlap one another, yet completely filled the clip. Tape was used to secure the needles within the clips, to ensure a light-tight seal for the period of dark acclimation.

TEMPERATURE MEASUREMENTS

Temperatures were estimated at each site along the transect using a calibrated extrapolation protocol. Thermometers were placed at each site to record the minimum and maximum air temperature since the previous visit. However, missing values resulted from lost and stolen thermometers, and no temperature data were available at the first visit. Complicating the problem, maximum–minimum thermometers record temperatures since the last visit, which varied in length. To overcome these problems, weather station data collected from a SNOTEL (SNOpack TELemetry) site at the top of Moscow Mountain were used to calibrate our temperature measurements (the US Department of Agriculture's Natural Resources Conservation Service operates this automated real-time hydrometeorological data network in the western USA: www.wcc.nrcs.usda.gov/snotel). First, minimum and maximum temperature ($T_{\min}$ and $T_{\max}$) were calculated from the SNOTEL data for the same measurement period as our max–min thermometers, making a direct comparison possible. We then fitted our $T_{\min}$ and $T_{\max}$ measurements against the SNOTEL data separately for each site. This yielded site-specific calibration equations for the SNOTEL data. We then used these site-specific calibration equations to derive daily $T_{\min}$ and $T_{\max}$ per site. We also computed the moving average of $T_{\min}$ and $T_{\max}$ over time windows ranging from 1 to 60 days. For example, the moving average with a window width of 2 would be $(T_{t=-1} + T_{t=-2})/2$.

DATA ANALYSIS

Missing values

The fluorometer used in the field analysis did not report values if a minimum number of fluorescence events was not exceeded. This resulted in an effective threshold of F_v/F_m that varied by species. In the data collected, these minimum values were estimated as 0·52 (Grand Fir), 0·40 (Lodgepole Pine), 0·48 (Engelmann Spruce), 0·57 (Ponderosa Pine), 0·54 (Douglas Fir), and 0·32 (Western Redcedar). Our data were thus left-censored, which complicated the analysis. We used a tobit model for left-censored data, which accounts for the bias due to the missing data (Breen 1996), as follows. A simple linear model was fitted to each tree, relating the cube-transformed F_v/F_m to $T_{\max}$ as:

$$(F_v/F_m)^3 = \beta_0 + \beta_1 \log(T_{\max} + 10) + \varepsilon \qquad \text{eqn 1}$$

The censoring threshold was unknown, but we set this to the minimum value observed for each tree separately. This is a biased but consistent (bias diminishes with increasing sample size) estimate of the censoring level (Zuehlke 2003). We compared the fits from the tobit model to two simple linear models, both of which were of the form of equation 1. The first model used F_v/F_m where the 'low' value was replaced with zero, and for the second model 'low' was replaced with the minimum observed for the tree. The tobit model and the second simple linear model (where censored values were imputed) were similar in terms of their estimated coefficients. The first simple model gave substantially lower predictions and gave poor fits at higher temperatures. From this analysis we concluded that the second model, which used minimum observed F_v/F_m for the 'low' values, was unlikely to bias inferences based on predictor variables. Therefore in all subsequent analyses we used this imputed data set. However, it should be noted that even with this adjustment, some bias may remain in the predicted values of F_v/F_m, especially when many 'low' readings were recorded. The majority of 'low' values occurred between February and March. Over the study period, Grand Fir had 10% 'low' values, Ponderosa Pine 15%, Douglas Fir 30% and Western Redcedar 21%. The minor species, Lodgepole Pine and Engelmann Spruce, of which only two trees each were monitored, had 39 and 55% missing values, respectively.

Mixed-effects models

We hypothesized that F_v/F_m declines with temperature. However, it was unclear which measure of temperature should be used. In a simple preliminary mixed-effects model, F_v/F_m was related to a complete model with $T_{\min}$, $T_{\max}$ and their interaction; terms were then deleted from the model. All terms were significant in the full model; however, the root mean-squared error (RMSE) of the model that included only $T_{\max}$ was only 1·6% higher than that of the complete model. The model with only $T_{\min}$ had an RMSE 13·1% higher than the complete model. These results show that most of the information about F_v/F_m is incorporated in $T_{\max}$, and for reasons of parsimony we used only $T_{\max}$ in subsequent analysis of the relationship between F_v/F_m and temperature.

To test the difference between autumn and spring in F_v/F_m, and the response to temperature, we constructed a class variable as follows. We fitted a parabola to $T_{\min}$ between September and January, and from this fit computed the time when temperatures transitioned from falling to rising. The class variable was 0 before this date ('cooling down') and 1 otherwise ('warming up'). If this variable was not significant, it would indicate no difference in F_v/F_m between autumn and spring, except that already explained by temperature.

To investigate the dynamics of the relationship between F_v/F_m and temperature, we tested different periods over which temperature is averaged. If F_v/F_m correlated better to temperature of the last day than to that of the last 30 days, this would be evidence to suggest that F_v/F_m responds rapidly to temperature. We hypothesized that the process would be slow, and F_v/F_m would correlate better to the moving average of temperature over the last several days.

We next analysed species differences, altitude effects and seasonality within a mixed model. The two readings per tree were averaged for simplicity. Two of the measured species occurred only at one site: Engelmann Spruce and Lodgepole Pine. We excluded these species from the comprehensive mixed-effects model analysis, although we report the temperature response. The inferences we have made on altitude are minimized, as it was not replicated across multiple transects. We included altitude as a continuous variable to explain variation across our sites. Altitude is a good proxy for temperature, but other variables correlated with altitude include soil type, soil moisture, nitrogen status and barometric pressure (Woodward 1987).

The covariance structure used in model fitting depends on the sampling design (Gregoire, Schabenberger & Barrett 1995; Schabenberger & Pierce 2002). In this experiment the design is nested: observations within trees within sites. The random effects of the mixed-effects model accounted for this structure. As we remeasured the same trees during the season, we expected F_v/F_m to be correlated within trees. To accommodate the repeated-measures data structure we used a continuous autoregression (CAR) model. This model (CAR1) estimates and corrects for correlation among residuals within trees (Pinheiro & Bates 2000). We used likelihood ratio tests to assess whether this correlation structure was necessary. The CAR1 model was used because the variable period between measurements excluded other autoregression models (Pinheiro & Bates 2000).

The main assumptions underlying mixed models were tested as outlined by Pinheiro & Bates (2000). To decide between competing models, we used the goodness-of-fit criteria AIC and RMSE (the standard deviation of the residuals). Each site or tree was allowed to have a unique variance, at the expense of a proliferation of the number of parameters. We tested the efficacy of this procedure with likelihood ratio tests (Pinheiro & Bates 2000). All analyses were performed using the open source statistical computing package R (Ihaka & Gentleman 1996; www.r-project.org).

After testing all fixed effects as part of the hypotheses, we built a simplified model of F_v/F_m. Fixed effects were dropped one by one from the full model, and the increase in RMSE was taken as an indication of the relative importance of the variable. These results were used to justify the use of a simple function, relating F_v/F_m only to T_{max}, for each species. The simple equation was of the form of equation 1.

Results

Daily maximum and minimum temperatures decreased immediately following initiation of the study in early September 2001 (day of year 250). Seven weeks later, in late October, minimum temperatures dropped below 0 °C for the first time (Fig. 1a). About 5 weeks later, at the end of November (DOY 334), temperatures fell into a range of similar maxima and minima, with averages slightly below 0 °C. Temperatures remained fairly constant in this range for more than 100 days, until around mid-March (DOY 440). They then increased until the end of the study in May 2002 (DOY 500). On any given day, minimum daily temperature was not influenced by altitude (0·04 °C per 1000 m, SE = 0·40), but maximum daily temperature declined strongly with altitude (−11·7 °C per 1000 m, SE = 0·81).

F_v/F_m declined with falling temperatures, beginning with the steep temperature decline in late November (DOY 325). Values remained low until the spring warming began in late March 2002 (DOY 450) (Fig. 1b). During this period values were more closely related to

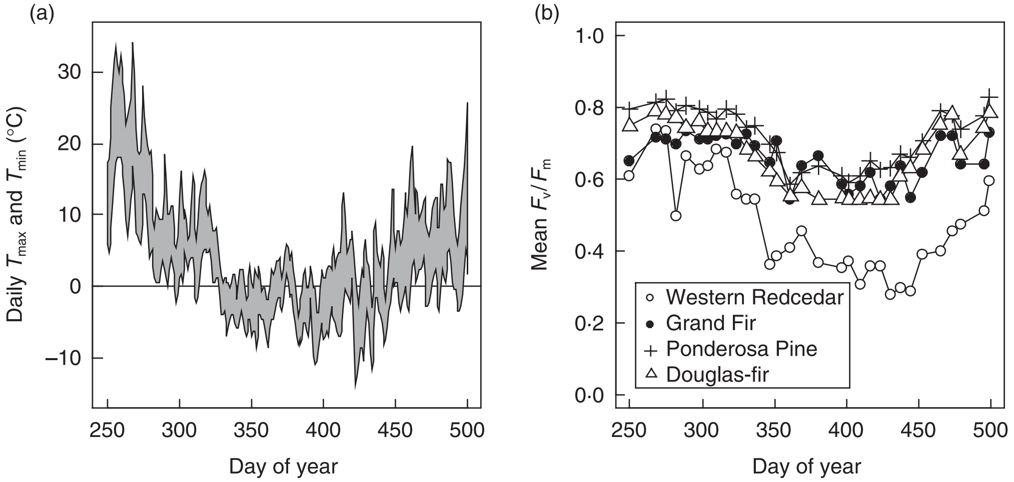

Fig. 1. Seasonal course of temperature and F_v/F_m. (a) Daily T_{max} and T_{min} values estimated by averaging along our altitude transect. The shaded area indicates the range between T_{max} and T_{min}. (b) F_v/F_m was averaged by species over all trees and sites per visit. Lines show the aggregate F_v/F_m values, excluding the 'low' readings. DOY 250 was 7 September 2002.

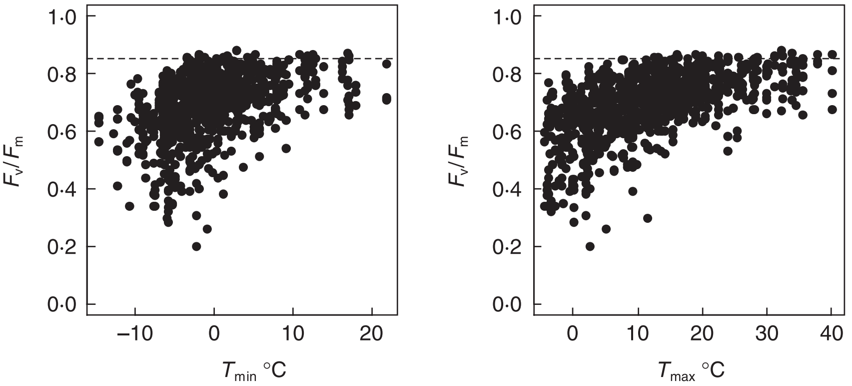

Fig. 2. Raw data for all species pooled as a function of T_{min} and T_{max} from the day preceding measurement. Dashed line, 'maximum' value of F_v/F_m (0·83) for most plant species in unstressed conditions (Björkman & Demmig 1987). This graph shows that F_v/F_m is a more consistent function of T_{max} than T_{min}, which was shown comparing goodness of fit between different models (see text).

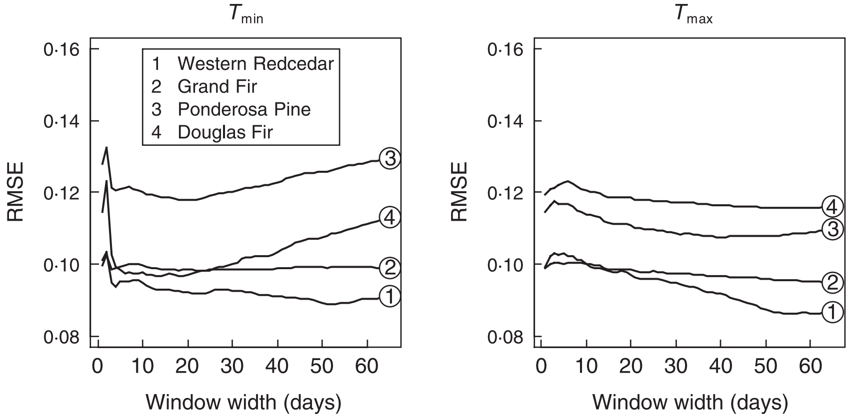

Fig. 3. Variation in the residual mean square error (RMSE) of the simple model (equation 1) fitted to temperature minima (T_{min}) and maxima (T_{max}) and averaged over windows ranging from 1 to 65 days. Better predictions have lower RMSE values.

T_{max} than T_{min} for three of the four species (Figs 2 and 3). We fitted a set of simple models (equation 1) to the data, using a moving average of T_{max} or T_{min} as the independent variable. The model was fitted by species, excluding Lodgepole Pine and Engelmann Spruce because only two trees were measured throughout the sampling period. The moving average was calculated over windows of varying width to describe the dynamics of the relationship between F_v/F_m and temperature. The results, which are presented in terms of the RMSE of the fitted model, show worse fits as higher values. The results therefore indicate that significant differences in lag time exist among species (Fig. 3). For three of the four species fitted, T_{max} predicted F_v/F_m better than T_{min}, as indicated by its lower RMSE (Fig. 3). Douglas Fir was the only species for which T_{min} explained more variation than T_{max} (Fig. 3). Three of the four species were relatively insensitive to window width for T_{max}. The exception was Western Redcedar, which had the lowest overall RMSE for T_{max}, but the best averaging time was 52 days. For T_{min} a window of 5–10 days before measurement gave the best fit for all species.

When the combined data set was analysed, we found that a model based on T_{max} with a moving-average window of 52 days gave the best fit. However, this long window resulted in a narrower range of temperatures, making model fitting more problematic. We therefore narrowed the window to 30 days, which increased the RMSE by only 1% relative to that at 52 days. Moreover, because many models run on a monthly time step, the 30 day window provided a better match to the temporal scale of other model parameters.

Next we tested whether species, altitude and season (autumn or spring) explained variation in F_v/F_m after the temperature effect had been accounted for (Table 1). The following predictor variables were significant: species; T_{max}; $\log(T_{max} + 10)$; all two-way interactions with species; the interaction of $\log(T_{max} + 10)$ and altitude; and the three-way interaction between species, $\log(T_{max} + 10)$ and altitude (Table 1). To test the relative contributions of each of these variables, we dropped each of the model terms one by one and estimated the resulting increase in RMSE (Table 2). $\log(T_{max} + 10)$ explained the most variation (RMSE increased by 48% when it was dropped from the model). The species effect explained almost as much (RMSE increased by 40% when it was dropped). Season and the linear term of T_{max} contributed little to the fit, although both were

Table 1. Results of fixed-effects ANOVA

Parameter	$\mathrm{num}_{\mathrm{d.f.}}$	$\mathrm{den}_{\mathrm{d.f.}}$	F value	$P\ (F > F_{\mathrm{obs}})$
Intercept	1	982	1569·5	<0·0001
Species	3	21	43·3	<0·0001
$\log(T_{\max} + 10)$	1	982	62·9	<0·0001
$T_{\max}$	1	982	44·1	<0·0001
Season	1	982	2·3	0·13
Altitude	1	6	1·6	0·25
Species × $\log(T_{\max} + 10)$	3	982	11·8	<0·0001
Species × $T_{\max}$	3	982	7·6	<0·0001
Species × season	3	982	6·0	0·0004
Species × altitude	3	21	6·4	0·003
$\log(T_{\max} + 10)$ × altitude	1	982	18·4	<0·0001
Species × $\log(T_{\max} + 10)$ × altitude	3	982	7·0	<0·0001

Degrees of freedom are given for numerator ($\mathrm{num}_{\mathrm{d.f.}}$) and denominator ($\mathrm{den}_{\mathrm{d.f.}}$) for the F statistic. $T_{\max}$ refers to the maximum daily air temperature (°C); season is a class variable denoting autumn or spring (see Materials and methods).

Table 2. Percentage increase in RMSE (standard deviation of residuals) when each predictor is dropped from the full model, e.g. when species is dropped from the model, both species and all its interactions are dropped

Fixed effect	% RMSE increase
Species	40·6
$T_{\max}$ and $\log(T_{\max} + 10)$	33·8
$\log(T_{\max} + 10)$	48·3
$T_{\max}$	3·1
Altitude	22·7
Season	1·5

significant. Altitude was also an important variable, but only for Douglas Fir and Ponderosa Pine (Table 3). These analyses resulted in individual curves relating F_v/F_m to $T_{\max}$ for each species (Fig. 4). Finally, the full mixed-effects model was used to predict F_v/F_m during the season (Fig. 5b). The predictions mimic the measurements well (Fig. 1b), except perhaps at the lower modelled values in Douglas Fir from DOY 360 to 440 (Fig. 1b). Western Redcedar had the lowest maximum F_v/F_m measurements and the sharpest decline following the seasonal onset of cold temperatures (Fig. 5b); the springtime return of high F_v/F_m was delayed relative to what one might predict from temperature alone (Fig. 5a). It is interesting that $T_{\max}$ was not significant for Western Redcedar in the full model (Table 3); we speculate that the season effect, which was correlated with temperature, explained this variation. When other variables in the full model were dropped, $T_{\max}$ was highly significant for Western Redcedar. Grand Fir behaved similarly, although the seasonal effect was much less pronounced than in Western Redcedar (Fig. 5a). We also fitted a reduced model of F_v/F_m as a function of $T_{\max}$, ignoring the separate effects of altitude and season. Coefficients of the reduced model appear in Table 4. This simplest of all models may be most suitable for modelling the loss of photosynthetic capacity by the mixed-species canopy in winter.

Table 3. Estimated coefficients for the full model (standard error of estimate in parentheses)

Species	β_{0k}	$P(t > t_{\mathrm{obs}})$	β_{1k}	$P(t > t_{\mathrm{obs}})$	β_{2k}	$P(t > t_{\mathrm{obs}})$	β_{3k}	$P(t > t_{\mathrm{obs}})$	β_{4k}	$P(t > t_{\mathrm{obs}})$	β_{5k}	$P(t > t_{\mathrm{obs}})$
Grand Fir	−0·783 (0·68)	0·26	0·439 (0·22)	0·051	−0·0186 (0·0043)	<0·0001	−0·0529 (0·0167)	0·0016	−0·000063 (0·000541)	0·91	0·000030 (0·000167)	0·86
Ponderosa Pine	−4·31 (0·59)	<0·0001	1·59 (0·19)	<0·0001	−0·0289 (0·0042)	<0·0001	0·00451 (0·0121)	0·71	0·00243 (0·000507)	0·0001	−0·000723 (0·000153)	<0·0001
Douglas Fir	−5·44 (0·69)	<0·0001	1·87 (0·23)	<0·0001	−0·0313 (0·0053)	<0·0001	0·00967 (0·0132)	0·47	0·00375 (0·000625)	<0·0001	−0·00104 (0·000189)	<0·0001
Western Redcedar	−0·962 (0·85)	0·27	0·34 (0·29)	0·23	−0·00262 (0·00425)	0·54	−0·0586 (0·0194)	0·0025	0·000375 (0·000705)	0·60	−0·000074 (0·000231)	0·75

P value reports the test that the coefficient is not zero. Western Redcedar does not show a significant relationship with temperature; this effect is incorporated in β_{3k}, the seasonal effect. These coefficients should not be interpreted individually, but only as part of the full model. The coefficients are β_{0k}, species intercept; β_{1k}, slope of $\log(T_{\max} + 10)$; β_{2k}, slope of $T_{\max}$; β_{3k}, season effect; β_{4k}, altitude effect; β_{5k}, altitude × $\log(T_{\max} + 10)$ interaction. Estimated random effects were intercept and slope of $\log(T_{\max} + 10)$ for trees within sites. The between-site standard deviation for the intercept was estimated at 0·32, and the slope 0·09. The random effect of tree within site had an estimated intercept standard deviation of 0·09 and a slope standard deviation of 0·04.

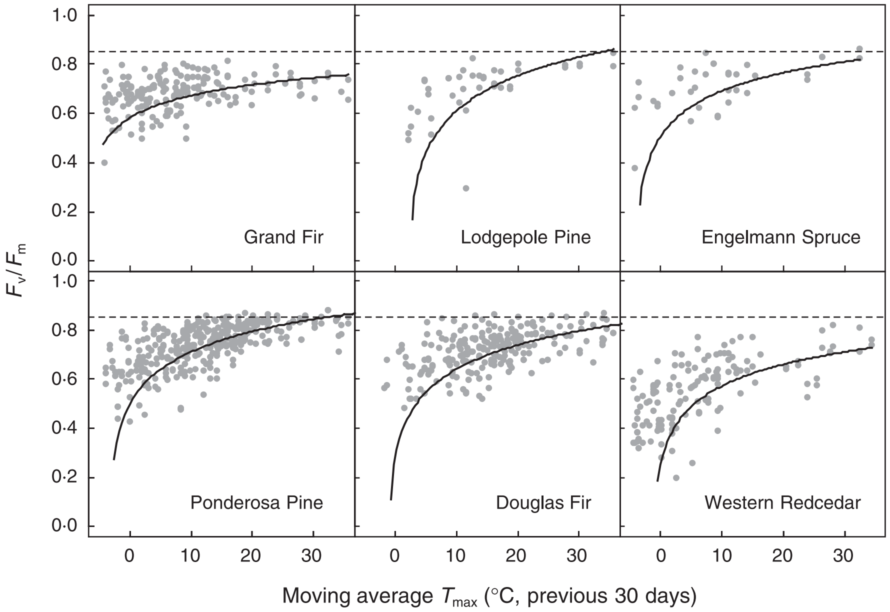

Fig. 4. Measured and fitted F_v/F_m as a function of T_{max} (averaged over the last 30 days) for all six species. Each line is the prediction for each species separately, from a mixed model of the form in equation 1. For all coefficients see Table 4.

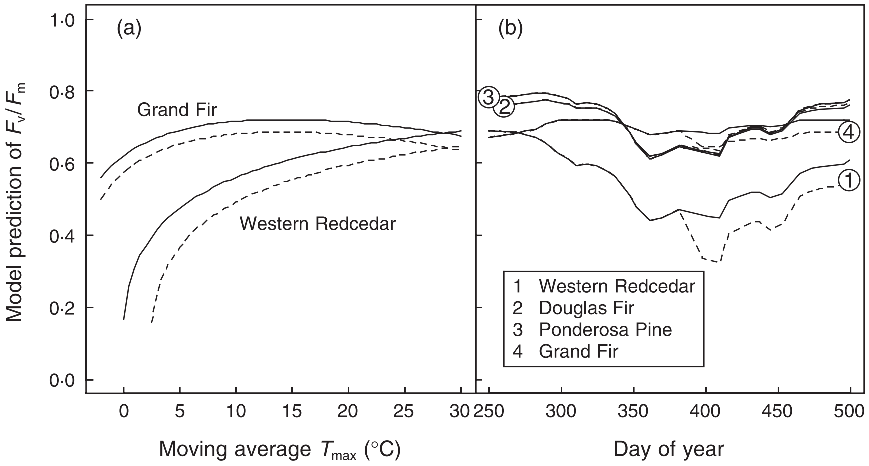

Fig. 5. Full mixed-effects model predictions of F_v/F_m for a moving average T_{max} and day of year. (a) Predictions for Grand Fir and Western Redcedar during autumn (solid line) and spring (dashed line). Only Grand Fir and Western Redcedar had a significant 'season' class variable on top of temperature. The difference between autumn and spring F_v/F_m is constant because the interaction between season and T_{max} was not significant. (b) Seasonal course of F_v/F_m from model predictions for the four main species. Predictions were obtained for a site of mean altitude (850 m), and T_{max} was averaged over the eight sites for each visit. F_v/F_m was also predicted with the season class variable set to 'autumn', and is shown with dashed lines. The difference between the dashed and solid line is the effect of season apart from that already explained by temperature. DOY 250 was 7 September 2002.

Discussion

Daily maximum temperature, averaged over multiple days, was the best predictor of winter reduction in F_v/F_m for three of the four species measured, both seasonally and along the altitude gradient. Wintertime reductions in photosynthetic capacity have been reported for many conifer species, including White Spruce (*Picea glauca*; Binder & Fielder 1996), Norway Spruce (*Picea abies*; Westin *et al.* 1995), Red Spruce (*Picea rubens*; Lawson, Perkins & Adams 2000), Black Spruce (*Picea mariana*; Gaumont-Guay *et al.* 2003), coastal Douglas Fir (Rose & Haase 2002) and Western Redcedar (Weger *et al.* 1993). However, this study provides new information related to the lag times, the relative importance of T_{max} and T_{min}, and differences among co-occurring species. We discuss below the probable mechanisms underlying these results, the significance of the species differences, and the utility of the results for parameterizing models of seasonal photosynthesis.

Previous studies have reported that antecedent weather data were necessary to predict current photosynthetic capacity of evergreens in the wintertime. Schaberg *et al.* (1995) reported the highest correlation between winter photosynthetic rates and maximum daily temperature occurring 4–6 days before measurement.

Table 4. Coefficients for the simple model relating F_v/F_m^3 to $\log(T_{max} + 10)$: this model predicts the cube-transformed F_v/F_m for each species separately, and the average response (fit to all data) is also provided (standard error of estimate in parentheses)

Species	Intercept	Slope
Grand Fir	−0·057 (0·10)	0·12 (0·030)
Lodgepole Pine	−0·93 (0·17)	0·39 (0·052)
Engelmann Spruce	−0·26 (0·14)	0·19 (0·044)
Ponderosa Pine	−0·48 (0·092)	0·29 (0·027)
Douglas Fir	−0·62 (0·10)	0·30 (0·030)
Western Redcedar	−0·38 (0·11)	0·19 (0·031)
Average response	−0·45 (0·12)	0·25 (0·037)

Lawson *et al.* (2000) found the patterns of winter F_v/F_m measurements of Red Spruce to be significantly correlated with current air temperatures over the preceding 72 h. However, these authors did not present formal comparisons using other windows to calculate the moving average of temperature. Tanja *et al.* (2003) similarly found that photosynthetic rates estimated from eddy flux data were best correlated with a 5 day running-average temperature. Medlyn, Loustau & Delzon (2002) reported the strongest correlation between the maximum rate of carboxylation and the potential rate of electron transport to mean daily minimum temperature occurring over the previous 30 days. We found similar delays in the response of photosynthetic capacity to changing ambient temperature, but the nature of the relationship and length of the delay differed among species. This delay suggests that the mechanisms controlling winter photosynthesis are slow to damage, slow to repair, or both. The observed species differences suggest that the mechanisms may vary among species.

Four major mechanisms have been hypothesized to control wintertime photosynthesis. These are: photoinhibition (Öquist & Huner 2003); desiccation due to extracellular freezing; xylem blockage due to freezing of the stem, roots or soils; and inhibition of the dark reactions of photosynthesis. These mechanisms are not necessarily independent, and may act as a correlated set of acclimatory responses (Öquist & Huner 2003). Each would have different controls and different lag times, and each would have different effects on F_v/F_m. Therefore empirical descriptions of the temperature–response curves may offer clues as to which are most important in a given system.

Photoinhibition is defined as oxidative damage to the light-harvesting apparatus in leaves; it occurs most often when bright light and low temperatures occur simultaneously (Strand & Lundmark 1987). Previously, photoinhibition was identified as a probable cause of wintertime depression of photosynthesis in boreal Black Spruce (Gaumont-Guay *et al.* 2003). Photoinhibition may occur at temperatures well above the lethal minimum temperature (Öquist & Huner 2003). Because the combination of bright light and low temperatures would occur only during the daytime, one might expect photoinhibitory control to be associated with T_{max}. The effect would likely be more pronounced in spring than autumn due to stronger irradiance in spring (Westin *et al.* 1995). The reversal of photoinhibition is relatively slow, especially at low temperatures (Öquist 1983; Lamontagne, Margolis & Bigras 1998), leading to long lag times. Both these characteristics of photoinhibition (seasonality and lag times associated with T_{max}) were observed in the current study.

A second potential mechanism is dehydration due to extracellular freezing. Although this certainly occurs in some species, and occurs in conifers during the growing season, it is unlikely to be the major control in native vegetation (Lamontagne *et al.* 1998; Strand *et al.* 2002). Most conifers tolerate much colder temperatures than the night-time minima reported along this transect (cf. Strand & Lundmark 1987). Although cytoplasmic freezing would be rapid (Schwarz *et al.* 1997), recovery from freezing damage might be rather slow. However, if extracellular freezing were the mechanism of loss, then one might think that T_{min} would better predict F_v/F_m than T_{max}, because the freezing would be unaffected by light and would proceed furthest at the lowest temperatures reached. Still, because the initial response would be rapid and the recovery would probably be slow, we might be unable to distinguish between photoinhibition and extracellular freezing as potential mechanisms leading to the loss of photosynthetic capacity.

A third mechanism potentially controlling photosynthesis is xylem freezing. Trees cannot sustain water losses due to transpiration if their stems or roots (or soils) are frozen. Therefore some have argued that stem (and soil) freezing may be a major control over winter photosynthesis (Schwarz *et al.* 1997; Jarvis & Linder 2000). Although we cannot address this question directly with our data, one could assume that T_{min} would correlate better with photosynthesis than T_{max} if this mechanism were important. Regardless, a direct correlation between xylem freezing and changes in fluorescence has yet to be documented. A fourth possibility is that the dark reactions of photosynthesis control photosynthetic rates during the wintertime. The rates of these reactions are strongly dependent on temperature, especially as temperatures approach 0 °C (Strand *et al.* 2002; Gaumont-Guay *et al.* 2003). Dark-acclimated chlorophyll fluorescence measures a photochemical trait, but it is possible that we could detect this effect if it was induced by feedback inhibition (Öquist & Huner 2003). While these other mechanisms have the potential to govern wintertime photosynthesis, our results suggest that photoinhibition, and perhaps extracellular freezing, are the most probable candidates.

Temperature sensitivity and the lag times associated with predictions of F_v/F_m differed among species. These species differences may complicate the parameterization of models in mixed-species stands, yet acknowledging these differences may result in improved accuracy of model predictions. For the majority of species measured, more variation was explained when predictions of F_v/F_m were based on T_{max} than on T_{min}. Douglas Fir was the only species that had a higher correlation with T_{min} when values were averaged over the previous 14 days. Of the six species measured in this study, Douglas Fir is the only one that occurs from the upper to lower timberline (Cooper, Neiman & Roberts 1991). Western Redcedar was unique in that predictions of F_v/F_m were extremely sensitive to T_{max}, and improved as we increased the average number of days included in the averaging window up to 52 days. This response may result from the tendency of Western Redcedar to have lower F_v/F_m values in spring than in autumn (Table 3). This species maintains photosynthetic capacity into the winter, but it is one of the last to recover in spring (Fig. 5b). A pronounced loss of greenness is frequently observed in this species during the coldest part of winter, especially at high attitudes. Grand Fir and Ponderosa Pine showed little response to either measure of temperature (T_{min} or T_{max}) and little evidence of a seasonal reduction in photosynthetic capacity. The tendency of Ponderosa Pine to retain photosynthetic capacity has been noted before (Marshall, Rehfeldt & Monserud 2001). The most consistent lag across the four species was a lag time of around 5 days associated with T_{min}. Although we have not emphasized it, because it was not the best predictor of F_v/F_m, this lag probably indicates a consistent recovery mechanism after physiological damage resulting from several cold nights.

The photosynthetic algorithms in forest process models often include some combination of variables related to quantum yield and A_{max}, the maximum light-saturated photosynthetic rate. As noted earlier, there is a direct mechanistic relationship between F_v/F_m and quantum yield. The correlation with A_{max} is less direct, but nonetheless frequently observed (Öquist & Strand 1986; Strand & Lundmark 1995; Binder & Fielder 1996). Because photosynthetic algorithms differ, their potential for parameterization using fluorescence data will also differ. Implementing such a parameterization for individual models was beyond the scope of this study.

The observed interaction between altitude and temperature (Table 1) is consistent with the expectation that populations within a species differ in temperature sensitivity of F_v/F_m along a transect. Such differences might be genetic. For example, Schaberg *et al.* (1995) observed population differences in F_v/F_m in Red Spruce. Likewise, many of the species measured here show pronounced differences in growth rate and frost-hardiness with elevation (Rehfeldt 1982, 1986, 1989). For example, it is not recommended to move Douglas Fir seed more than 140 m from its altitude of origin (Rehfeldt 1979); this distance was substantially exceeded along our transect. Although our statistical analysis found that altitude explained relatively little variation after temperature had been accounted for, it is worth remembering that we studied only a single transect at constant latitude. Many of the conifer species studied are distributed along extensive latitudinal ranges and can display substantial population differences (Zhang & Marshall 1994, 1995). Had we sampled a latitudinal gradient, it is likely that previously observed variation among populations would have contributed to a stronger altitude effect. Finally, the altitude effect might also have resulted from differential acclimation of a uniform genotype to a particular microclimate.

The duration of seasonal growth influences the annual estimates of coniferous forest productivity (Bergh *et al.* 1998). To reflect annual production accurately, a more detailed understanding of seasonal growth and temperature response is needed (Leverenz & Öquist 1987; Medlyn *et al.* 2002). Leverenz & Öquist (1987) reported seasonal differences in the response of quantum yield to varying temperature. Such differences make it difficult to define maximum quantum yields based on temperature alone. Estimates of winter photosynthesis by forest process models could be improved with a greater incorporation of climatic variability in northern latitudes (McMurtrie *et al.* 1994; Gaumont-Guay *et al.* 2003). Specifically, predictions may be improved with accounts of cold acclimation and winter differences in frost-induced photoinhibition (Gaumont-Guay *et al.* 2003); direct reduction in photosynthetic rates caused by low temperatures (Linder & Flower-Ellis 1992; McMurtrie *et al.* 1994; Bergh *et al.* 1998); incorporation of soil thawing and soil temperatures (Bergh & Linder 1999); and seasonal differences in photosynthetic recovery following temperature change (Linder & Flower-Ellis 1992; McMurtrie *et al.* 1994; Bergh *et al.* 1998; Lundmark *et al.* 1998; Bergh & Linder 1999; Medlyn *et al.* 2002). The species differences between temperature (T_{max} or T_{min}) and the moving average window widths describing the best relationship illustrate the complexity of this system even during the wintertime, and increase the difficulty of modelling the system. However, fluorescence measurements provide a quick and reliable method to describe this complication for future analyses and model predictions.

A detailed analysis of these issues has been made using the BIOMASS model in boreal coniferous forests (Bergh *et al.* 1998). This analysis found that the largest discrepancy between actual and potential photosynthesis occurs when the effects of temperature changes are not included in estimates of annual production. We have shown here that measurements of chlorophyll fluorescence can aid in determining seasonal differences in photosynthetic capacity that result from changes in daily minimum and maximum temperatures, altitude, species and season. These measurements can now be used to improve estimates of net primary production in cold-temperate conifer forests varying in species composition and climate (Lundmark *et al.* 1998).

Conclusions

We found species and altitude differences in the response of photosynthetic capacity to seasonal temperature changes. These responses were significantly lagged relative to the temperatures that caused them; the lags also varied among species. We developed a set of species-specific models allowing predictions of F_v/F_m to be made based on simple meteorological variables, which are recorded daily at most weather stations (Table 4). These models are applicable for other sites within the interior north-west of North America and may provide a template for similar studies of other temperate evergreen species.

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