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# Interrelationships among light, photosynthesis and nitrogen in the crown of mature *Pinus contorta* ssp. *latifolia*

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**Summary** Scaling leaf-level measurements to estimate carbon gain of entire leaf crowns or canopies requires an understanding of the distribution of photosynthetic capacity and corresponding light microenvironments within a crown. We have compared changes in the photosynthetic light response and nitrogen (N) content (per unit leaf area) of *Pinus contorta* Dougl. ssp. *latifolia* Engelm. (lodgepole pine) leaves in relation to their age and light microenvironment. The vertical gradient in integrated daily photosynthetic photon flux density (PPFD) from the upper to the lower crown of lodgepole pine was similar in magnitude to the horizontal gradient in daily PPFD along shoots from young to old leaves. The relationship between light-saturated net photosynthesis ($A_{max}$) and daily PPFD was significant for both young and old leaves. However, old leaves had a lower $A_{max}$ than young leaves in a similar daily irradiance regime. For leaves of all ages from throughout the crown, $A_{max}$ was linearly related to the estimated daily net carbon gain that leaves could achieve in their natural PPFD environment (estimated $A_{day}$) ($r^2 = 0.84$, $P < 0.001$, $n = 39$), indicating that estimated $A_{day}$ may be dominated by carbon fixed when leaves are light-saturated and operating at $A_{max}$. Comparison of the PPFD required to achieve $A_{max}$ and the PPFD available to the leaves showed that all of the measured leaves ($n = 39$), regardless of their position in the crown or age, were in light environments that could light-saturate photosynthesis for a similar proportion of the day. For all data pooled, foliar N was weakly correlated with daily PPFD. Analyzing each leaf age class separately showed that foliar N was significantly related to daily PPFD, $A_{max}$, and estimated $A_{day}$ for the youngest leaves but not for middle-aged or old leaves. Therefore, the general theory that foliar N is allocated within a crown according to total daily light availability was supported only for young (1–4 years old) leaves in this study.

*Keywords: conifers, crown architecture, evergreens, leaf age, light acclimation, light gradients, light microenvironments, lodgepole pine, nitrogen partitioning.*

## Introduction

Scaling leaf-level measurements to estimate carbon gain of leaf canopies requires knowledge of the physiological response to the microenvironments within crowns, as well as the corresponding distribution of resources (Kull and Jarvis 1995). Several studies have shown a relationship between photosynthesis and light availability in the canopies of both herbaceous (Schimel et al. 1991, Werger and Hirose 1991, Schieving et al. 1992, Hirose and Werger 1994) and deciduous tree species (Bongi et al. 1987, DeJong et al. 1989, Ellsworth and Reich 1993). The observed nonuniform distribution of photosynthesis within the canopy may be evidence for photosynthetic acclimation of leaves during development to their respective light microenvironments. Assuming a tight coupling of leaf photosynthesis to the light environment, estimates of canopy photosynthesis could be made using a simple "big-leaf" modelling approach (Kull and Jarvis 1995). However, the assumption of complete photosynthetic acclimation in canopies to incident irradiance has not been tested extensively.

Complete photosynthetic acclimation within the canopy of evergreen species requires initial light acclimation of new leaves, as well as an ongoing capability for acclimation by aging leaves as they become more distal from the shoot tip and, thus, more shaded over time (Schoettle and Smith 1991, Brooks et al. 1996). Light acclimation with leaf age is especially necessary for evergreen conifers with long leaf life spans, because over 70% of the leaf canopy can be old leaves (Wood 1973, Schulze et al. 1977, Schoettle 1994). Photosynthetic acclimation of aging leaves to the prevailing light environment has been shown in the shrub *Lepechinia calycina* (Benth.) Epl. (Field 1981), the conifer *Abies amabilis* Dougl. *ex* J. Forbes (Brooks et al. 1996) and in crops (Pearce et al. 1968).

Foliar nitrogen (N) concentration has been used to estimate net photosynthesis because of the strong coupling between the two (e.g., Field and Mooney 1986). The distribution of foliar N is also positively associated with light availability in the canopies of herbaceous species (Schimel et al. 1991, Werger and Hirose 1991, Schieving et al. 1992, Hirose and Werger 1994) and deciduous woody species (DeJong and Doyle 1985, Bongi et al. 1987, DeJong et al. 1989, Ellsworth and Reich 1993, Niinemets 1997). Foliar N, light availability and leaf aging are also related in vines (Ackerly 1992, Hikosaka et al.

1994) and a shrub (Field 1983). An association of foliar nutrient concentration with light-saturated photosynthesis has been found in deciduous and herbaceous species (Field and Mooney 1986, Reich et al. 1995). In evergreen conifers, the relationship between foliar N and light-saturated photosynthesis is strong for young leaves (Reich et al. 1995), but it is much weaker when conifer leaves of all ages are considered (Sheriff et al. 1986, Brooks et al. 1996), as might be the association of foliar N and light availability.

Leaves of *Pinus contorta* Dougl. ssp. *latifolia* Engelm. (lodgepole pine) can live for up to 22 years (Schoettle 1990*a*), and more than 75% of the foliage of the crown is more than one year old (Schoettle 1994). In addition, the daily irradiance available to leaves decreases substantially with leaf age, position on the stem and depth in the canopy (Schoettle and Smith 1991). We compared the light environment, physiological characteristics and daytime net $CO_2$ uptake of leaves from throughout the crown of *Pinus contorta* ssp. *latifolia*. The specific objectives were: (1) to examine the relationship between photosynthetic light response of leaves of all ages and measured light microenvironments in the canopy; (2) to assess the use of foliar N as a measure of light-saturated net photosynthesis and daytime carbon gain; and (3) to examine the occurrence of foliar nitrogen with respect to the corresponding light microenvironment in the crown of mature lodgepole pine.

## Methods

To sample the full range of potential variation in the crown, measurements were made of young (1–4 years old), middle-aged (5–8 years old) and old (9–15 years old) leaf age classes from shoots in the upper, middle and lower thirds of the crown. The mean age of the young, middle-aged and old leaf age classes was 2.1, 7.0 and 11.6 years, respectively. Because current-year leaves were not fully developed at the time of the measurements, they were not included in the physiological measurements.

### *Site characteristics*

Measurements were made in an even-aged stand of *P. contorta* near Fox Park (41°21′ N, 106°19′ W), WY. The site is typical of closed-canopy mature lodgepole pine stands in the central Rocky Mountains, and is in the mid-elevation range (2800 m) for the species in the region. Average tree age was approximately 90 years, stand density was 2200 stems $ha^{-1}$, and the soil was a well-drained typic cryoboralf (Fahey et al. 1985). The site appeared free of extensive pathogen or pest infestation. The region is characterized by short cool summers (mean July temperature of 13 °C) and long cold winters (mean January temperature of –11 °C) (Alexander et al. 1985). Mean annual precipitation is 60 cm, with about two-thirds coming in the form of snow from October to May (Fahey et al. 1985).

### *Tree and shoot selection*

Scaffolding was erected with minimal disturbance to the canopy to provide access to the crown of one tree. The tree was typical of those in the stand based on height, diameter at breast height (DBH), sapwood cross-sectional area and foliar N concentration. All experimental shoots originated from main branch axes and had actively growing terminals with at least 4 cm of leaf-free stem behind the oldest leaves. These criteria enabled an accurate assessment of leaf age to be made using budscale scars on the axis of the shoot, and ensured that the oldest leaves were the leaves that the shoot could support (Schoettle and Smith 1991). Five shoots from the upper crown and four shoots from each of the middle and lower crown thirds were sampled.

### *Photosynthetic photon flux density (PPFD) measurements*

Photosynthetic photon flux density (PPFD) (400–700 nm) was measured from 0800 to 1600 h each day for each shoot sampled. Measurements were taken immediately above the youngest leaves at the shoot tip, the middle-aged leaves in the center of the foliated length and the oldest leaves at the proximal end of the foliated length (Schoettle and Smith 1991). The PPFD was measured with a level quantum sensor (Model 190SA, Li-Cor Inc., Lincoln, NE) and recorded with a Li-Cor data logger (Model LI-1000) every 30 min. Each shoot was measured for 3 or 4 days from June through early July 1989. Daily PPFD was estimated by integrating below the curve of PPFD versus time of day for each of the three positions along the shoot and for each day the shoot was measured (Schoettle and Smith 1991).

To ensure that all leaf age classes and shoots from all canopy positions were measured on days that had comparable above-canopy PPFDs, a shoot from each of the lower, middle and upper crown thirds was selected for each day of measurements and PPFDs available to all three leaf age classes on each shoot were measured. The daily PPFD above the canopy for the 13 measurement days was 35.6 mol $m^{-2}$ $day^{-1}$ (± 1.4 SE, $n = 13$) and 34.8 mol $m^{-2}$ $day^{-1}$ (± 1.2 SE, $n = 36$) for the entire study period of June through early July, indicating typical measurement days. To check that PPFD measured at a 30-min interval was adequate to characterize the daily PPFD and the quantitative distribution of PPFD, we recorded PPFD at 5-min intervals over 7 days at a fixed location in the canopy with a data logger. The integrated daily PPFD was similar when data from a 5- and 30-min interval were compared for each day (*t*-test, $P > 0.20$), as were the frequency distributions of PPFD. In addition, the relative magnitude among days for daily PPFD was the same when the data from throughout the daylight period, or from 0800 to 1600 h, were used to make the estimate.

### *Net photosynthesis*

The photosynthetic light response was determined for each of the three leaf age classes (young, middle-aged and old leaves) on each shoot for which incident PPFD was measured. Measurements were made with a Li-Cor LI-6200 portable gas exchange system with a specially designed cuvette that allowed for a natural needle orientation and needle arrangement on the shoot during the photosynthesis measurements of each age class (Figure 1).

Shoots for photosynthetic measurements were excised from

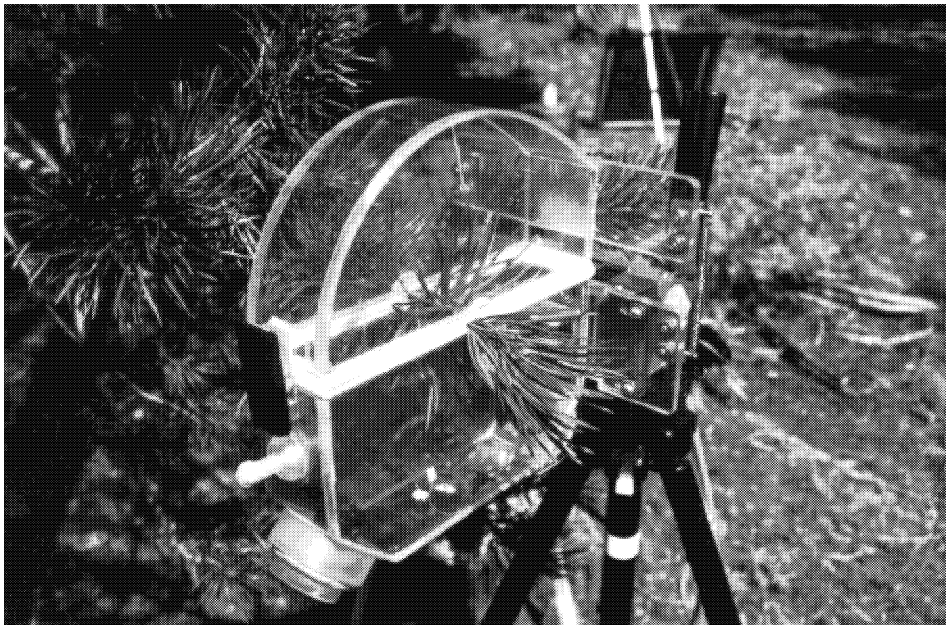

Figure 1. Gas-exchange cuvette used with the Li-Cor LI-6200 portable photosynthesis system for measurement of leaf cohorts along an intact lodgepole pine shoot. A 2-year-old leaf cohort is enclosed in the cuvette.

the crown. The cut-end of each shoot was immediately immersed in water, recut and inserted, while under water, into a water-filled plastic tube. Tests conducted at weekly intervals throughout the measurement period indicated that the light-saturated rate of net photosynthesis ($A_{max}$) of leaves remained statistically unchanged from the rate before excision for at least 3 h after being excised (*t*-test, $P > 0.05$). Neutral-density screens and natural sunlight were used to generate nine PPFDs ranging from 5 to 1400 μmol $m^{-2}$ $s^{-1}$. Shoots were exposed to each PPFD for 10 min before measurement of net photosynthesis (*A*) (see Figure 2). All measurements were taken in the morning between 0800 and 1100 h to minimize vapor pressure deficit (VPD) influences and avoid the frequent intermittent cloud cover that occurs in the region in the afternoon (Knapp and Smith 1988).

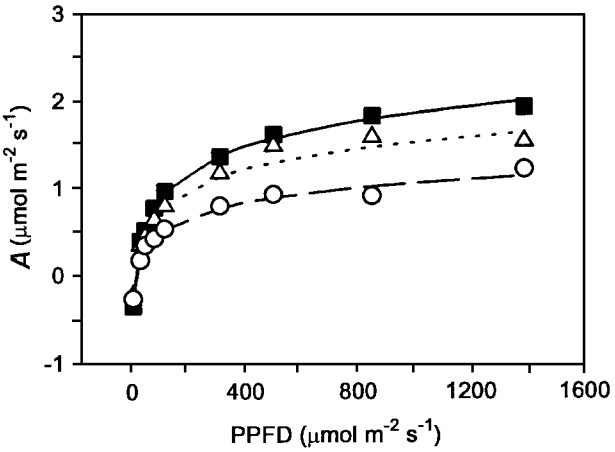


Figure 2. Photosynthetic light response of young, middle-aged and old leaves from one shoot (Shoot 12 from Table 1). The lines are the curves fit with a log-linear model (see Methods and Table 1). Symbols: ■ = young leaves; △ = middle-aged leaves; and ○ = old leaves.

Net photosynthesis of each needle age class on the shoot was calculated on the basis of total leaf surface area, projected leaf area of detached leaves, silhouette leaf area on the shoot and dry weight, as recommended by Smith et al. (1991). Because we were interested in physiological changes with leaf age, the gas exchange measurements reported here are expressed only on a total leaf surface area basis (glass-bead method, Thompson and Leyton 1971). (Leaf area per mass of leaves decreases with leaf age as a result of an increase in mass of non-photosynthetic tissue—lignin and vascular tissue—over time, whereas leaf surface area does not change with leaf age.) The photosynthetic rates reported here appear low because we expressed *A* on a total leaf area basis and maintained the natural needle orientation during measurement of *A*. When expressed on a silhouette leaf area basis, *A* was similar to the values obtained when individual leaves of lodgepole pine are oriented perpendicular to the irradiance during measurement (see Yoder et al. 1994). Ratios for the conversion of our values to other leaf area bases are available in Schoettle (1990*b*, Table A. 1).

Apparent quantum use efficiency was calculated as the slope of the linear regression of the four points of the photosynthetic light response curves for PPFDs between 5 and 130 μmol $m^{-2}$ $s^{-1}$.

### *Foliar N*

The same leaves used for photosynthetic and PPFD measurements were analyzed for total nitrogen by micro-Kjeldahl analysis (Parkinson and Allen 1975). Foliar N was expressed on a total leaf surface area basis.

### *Estimating daytime net $CO_2$ uptake*

We fit the photosynthetic light response data to the log-linear regression equation (Table 1). Through the day (from 0800 to

Table 1. Regression equations ($A$ = slope(logPPFD) + intercept) and coefficients ($n$ = 9) for the photosynthetic light response relationships for young, middle-aged and old leaves from the upper, middle and lower crown third of lodgepole pine.

| Crown position and shoot ID | | Leaf age | Slope | Intercept | $r^2$ |
|---|---|---|---|---|---|
| Lower | 1 | Young | 0.5833 | −0.5318 | 0.896 |
| | | Middle-aged | 0.8970 | −0.8669 | 0.961 |
| | | Old | 0.5597 | −0.3877 | 0.927 |
| | 2 | Young | 0.7439 | −1.0577 | 0.933 |
| | | Middle-aged | 0.6828 | −0.6463 | 0.956 |
| | | Old | 0.5003 | −0.6429 | 0.911 |
| | 3 | Young | 0.4527 | −0.6023 | 0.935 |
| | | Middle-aged | 0.6295 | −0.6589 | 0.915 |
| | | Old | 0.4344 | −0.4268 | 0.926 |
| | 4 | Young | 0.7558 | −0.7014 | 0.948 |
| | | Middle-aged | 0.6601 | −0.5559 | 0.964 |
| | | Old | 0.5651 | −0.5048 | 0.942 |
| Middle | 5 | Young | 0.6198 | −0.8223 | 0.960 |
| | | Middle-aged | 0.6514 | −1.1045 | 0.981 |
| | | Old | 0.4708 | −0.6962 | 0.991 |
| | 6 | Young | 0.8210 | −1.1731 | 0.857 |
| | | Middle-aged | 0.7126 | −0.8028 | 0.974 |
| | | Old | 0.6512 | −0.8231 | 0.994 |
| | 7 | Young | 0.8496 | −1.2453 | 0.930 |
| | | Middle-aged | 0.5729 | −0.8373 | 0.929 |
| | | Old | 0.5406 | −0.9134 | 0.957 |
| | 8 | Young | 0.5597 | −0.6229 | 0.966 |
| | | Middle-aged | 0.6299 | −0.6221 | 0.950 |
| | | Old | 0.4666 | −0.7354 | 0.900 |
| Upper | 9 | Young | 0.6740 | −0.9676 | 0.981 |
| | | Middle-aged | 0.5349 | −0.6233 | 0.984 |
| | | Old | 0.4851 | −0.6775 | 0.967 |
| | 10 | Young | 1.1929 | −1.4987 | 0.960 |
| | | Middle-aged | 0.8941 | −0.9749 | 0.948 |
| | | Old | 0.5834 | −0.6428 | 0.969 |
| | 11 | Young | 0.8808 | −1.1036 | 0.967 |
| | | Middle-aged | 0.7473 | −0.7911 | 0.941 |
| | | Old | 0.6234 | −0.6756 | 0.958 |
| | 12 | Young | 0.9718 | −1.0573 | 0.959 |
| | | Middle-aged | 0.7862 | −0.8355 | 0.964 |
| | | Old | 0.5711 | −0.6635 | 0.965 |
| | 13 | Young | 0.8900 | −1.0507 | 0.866 |
| | | Middle-aged | 0.7767 | −0.8187 | 0.881 |
| | | Old | 0.8419 | −1.0364 | 0.927 |

1600 h), $A$ was estimated by applying the regression equation for the photosynthetic light response curve derived for measured leaves to each PPFD measurement taken above those needles. The maximum daily net carbon gain that a leaf age class could achieve in its natural light environment (estimated $A_{day}$) was calculated by integrating estimated $A$ versus time of day for each leaf age class, on each shoot, and for each day PPFD was measured. The mean estimated $A_{day}$ for each age class on each shoot was calculated as the mean for the 3 to 4 days of light measurements. The possibility of lowered $A$ caused by environmental factors other than light was not included in the calculation of estimated $A_{day}$. As a result, estimated $A_{day}$ reflects the maximum possible daily net carbon gain of a leaf according to its natural light environment, rather than the actual net carbon gain of a leaf during any given day.

In addition to fitting the photosynthetic light response data to the log-linear equation (Table 1), we also fit the data to the quadratic equation of Prioul and Chartier (1977) and Parsons et al. (1997), as well as to a non-rectangular hyperbola equation (data not shown). Estimated $A_{day}$ varied less than 10% depending on the model used. Statistical analyses for crown position and leaf age effects on estimated $A_{day}$ did not vary among the models.

To test how well total daytime PPFD and the light response of photosynthesis were matched, we also calculated a hypothetical estimated $A_{day}$, assuming that the young leaves were in the natural light environment measured for old leaves of the same shoot, and vice versa.

### *Statistical analysis*

A two-way analysis of variance (position in the crown by leaf

age) was used to detect differences among the individual physiological traits (SAS Institute Inc., Cary, NC). The interrelationships among physiological factors or leaf age were determined by linear regression analyses (GANOVA 4, University of Calfornia–Los Angeles, Los Angeles, CA). Where specified, *t*-tests were conducted. An $\alpha$-value of 0.05 was used for all tests.

## Results

### *Distribution of sunlight in the crown*

Mean daily PPFD incident on leaves within the crown varied from 4.4 to 24.6 mol $m^{-2}$ $day^{-1}$ corresponding to approximately 12% to 66% of the daily irradiance measured above-canopy. Generally, leaves in the upper crown tended to be in higher light environments than leaves in the lower crown, although the differences were not statistically significant (Table 2). Young leaves were in daily PPFD environments ranging from 5.8 to 24.6 mol $m^{-2}$ $day^{-1}$, whereas the daily PPFD environments of old leaves ranged from 4.6 to 20.8 mol $m^{-2}$ $day^{-1}$; however, young leaves of a given shoot were consistently in a higher irradiance than older leaves (Table 2).

### *Photosynthetic light response in the crown*

The photosynthetic light response of leaves varied within the crown (Tables 1 and 2). The light-saturated photosynthetic rate ($A_{max}$ on a total leaf area basis) was greater for leaves in the upper crown than for leaves in either the middle or lower crown locations. Leaf age class was negatively associated with $A_{max}$, decreasing from 1.34 μmol $CO_2$ $m^{-2}$ $s^{-1}$ for young leaves to 0.92 μmol $CO_2$ $m^{-2}$ $s^{-1}$ for old leaves (Table 2). Expressed on silhouette leaf area, $A_{max}$ decreased from 7.45 μmol $m^{-2}$ $s^{-1}$ for young leaves to 4.81 μmol $m^{-2}$ $s^{-1}$ for old leaves. The PPFD at which leaves achieved photosynthetic light saturation (PPFD to achieve 90% of $A_{max}$) decreased with leaf age and increasing depth in the crown (Table 2). There were no differences in the apparent quantum yield (the slope of the linear regressions for PPFD versus *A* at four PPFDs between 5 and 130 μmol $m^{-2}$ $s^{-1}$) for leaves from throughout the crown (data not shown, $P > 0.05$).

The maximum daily net $CO_2$ gain that leaves could achieve in their measured light environment (estimated $A_{day}$) decreased with crown depth and leaf age class (Table 2). Estimated $A_{day}$ was linearly correlated with $A_{max}$ irrespective of leaf age class or position of the shoot in the crown (Figure 3).

### *Relationship of photosynthetic characteristics with light*

For all data pooled, $A_{max}$ and estimated $A_{day}$ were linearly related with daily PPFD ($r^2 = 0.31$ and $r^2 = 0.48$, respectively; $n = 39$; Figure 4). A positive slope occurred for all three leaf age classes, but the intercept was greater for young and middle-aged leaves than for old leaves. Therefore, in a similar daily irradiance, old leaves had a lower $A_{max}$ and estimated $A_{day}$ than younger leaves. There was no correlation between daily PPFD and the PPFD required to saturate net photosynthesis ($r^2 = 0.10$) or apparent quantum yield ($r^2 = 0.08$).

When the light environment of particular leaves was characterized by the quantitative variability in irradiance throughout the day and compared to the photosynthetic light response of the same leaves, we found that the percentage of time between 0800 and 1600 h when leaves were exposed to PPFDs above photosynthetic light saturation was similar (range of 25% to 41%) for leaves of all ages in all crown positions (Table 2).

### *Distribution of N in the crown*

Foliar N varied throughout the crown from 0.64 to 1.31 g N $m^{-2}$ and was significantly greater in the top third than in the bottom third of the crown for young leaves. Foliar N of older leaves was low throughout the crown (Table 3). Foliar N was lower for old leaves than for young leaves, and the decrease with age was greatest in the upper portion of the crown.

### *Relationship of foliar N with photosynthetic characteristics and light*

Foliar N was correlated with $A_{max}$ when all data were considered ($r^2 = 0.36$, $P = 0.05$, $n = 39$, Figure 5A). However, when

Table 2. Summary of photosynthetic traits[1] of leaves from different leaf age classes and crown locations expressed on a total leaf surface area basis. Values within the columns for crown location or leaf age class followed by different letters are significantly different ($\alpha = 0.05$). Values are means (± SE).

| Leaf age and Location | Daily PPFD (mol $m^{-2}$ $day^{-1}$) | $A_{max}$ (μmol $CO_2$ $m^{-2}$ $s^{-1}$) | Sat. PPFD (μmol $m^{-2}$ $s^{-1}$) | Estimated $A_{day}$ (mmol $CO_2$ $m^{-2}$ $day^{-1}$) | % Day above sat. PPFD |
|---|---|---|---|---|---|
| *Crown third* | | | | | |
| Upper | 14.7 (1.6) a | 1.41 (0.11) a | 313 (28) a | 28.1 (2.3) a | 40.8 (4.1) a |
| Middle | 10.4 (1.3) a | 1.04 (0.10) b | 447 (37) b | 16.2 (2.0) b | 24.9 (2.2) a |
| Lower | 9.8 (1.6) a | 1.03 (0.08) b | 198 (18) c | 20.6 (2.4) b | 31.6 (4.2) a |
| *Leaf age class* | | | | | |
| Young | 13.9 (1.8) a | 1.34 (0.10) a | 370 (38) a | 25.2 (2.7) a | 35.4 (3.6) a |
| Middle-aged | 11.8 (1.5) b | 1.27 (0.08) a | 332 (38) ab | 24.3 (2.5) a | 29.9 (3.2) a |
| Old | 9.9 (1.5) c | 0.92 (0.09) b | 254 (36) b | 16.8 (2.2) b | 36.0 (4.5) a |

[1] Abbreviations: Daily PPFD = integrated daily PPFD; $A_{max}$ = light-saturated net photosynthetic rate; Sat. PPFD = PPFD required to attain 90% $A_{max}$; Estimated $A_{day}$ = daytime net photosynthetic $CO_2$ gain (see text for details); % Day above sat. PPFD = percentage of the day between 0800 and 1600 h when incident radiation above the leaf exceeded the sat. PPFD.

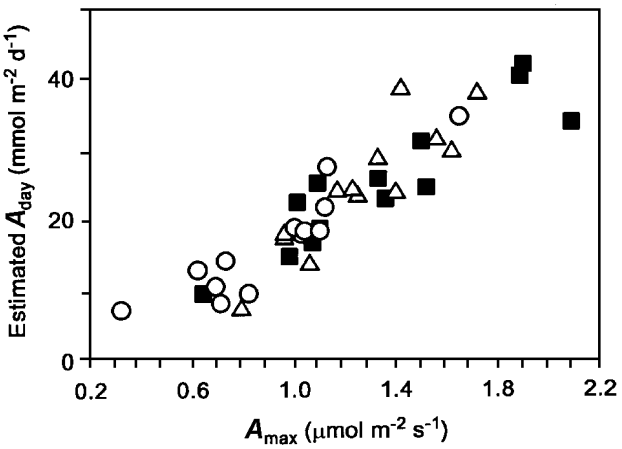


Figure 3. Linear relationship between $A_{max}$ and estimated $A_{day}$ for leaves of all ages and from all crown positions. Estimated $A_{day}$ = 22.81($A_{max}$) – 4.02; $r^2$ = 0.84; $n$ = 39. Symbols are as in Figure 2.

the data were analyzed by leaf age class, the relationship was significant for young leaves ($r^2$ = 0.50, $P$ < 0.01, $n$ = 13), but not for middle-aged ($r^2$ = 0.01) or old leaves ($r^2$ = 0.0003). Foliar N was correlated with estimated $A_{day}$ in the same manner (Figure 5B).

When all of the data were considered, foliar N was weakly correlated with daily PPFD ($r^2$ = 0.23, $n$ = 39; Figure 6). When the data for each leaf age class were tested separately, only foliar N of young leaves was significantly related to daily PPFD ($r^2$ = 0.67, $P$ < 0.01, $n$ = 13).

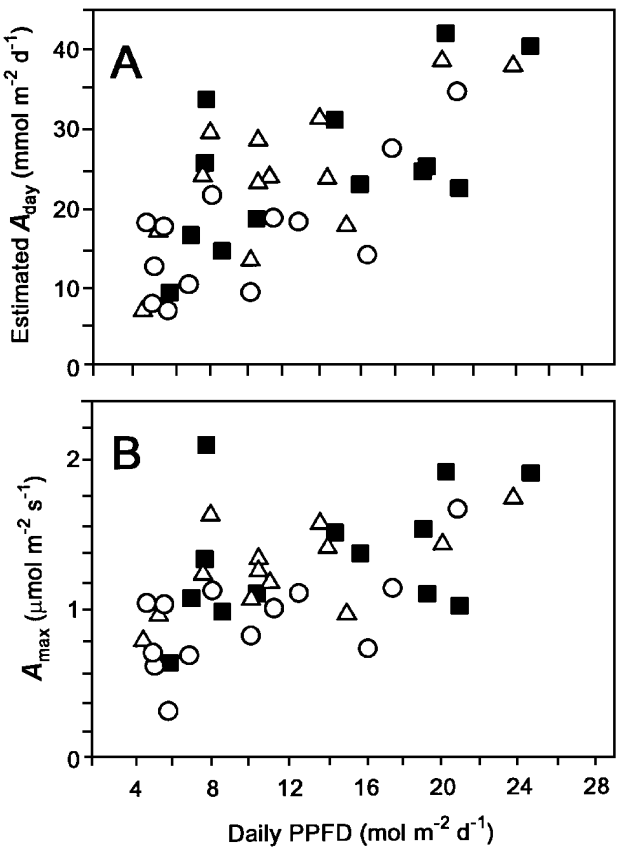


Figure 4. A. Linear relationship between daily PPFD and estimated $A_{day}$. The general relationship for all data points was: estimated $A_{day}$ = 1.11(daily PPFD) + 8.93, $r^2$ = 0.48, $n$ = 39. The relationship was significant ($P$ < 0.01) for young ($r^2$ = 0.55, $n$ = 13), middle-aged ($r^2$ = 0.53, $n$ = 13) and old leaves ($r^2$ = 0.50, $n$ = 13). The slope of the relationship was the same for all leaf ages, but the intercept differed significantly ($P$ < 0.05) between old leaves and young or middle-aged leaves (young and middle-aged leaves: estimated $A_{day}$ = 1.11(daily PPFD) + 10.09, $r^2$ = 0.52, $n$ = 26; old leaves: estimated $A_{day}$ = 1.11(daily PPFD) + 6.58, $r^2$ = 0.51, $n$ = 13). B. Linear relationship between daily PPFD and $A_{max}$. The general association was $A_{max}$ = 0.036(daily PPFD) + 0.75, $r^2$ = 0.31, $n$ = 39. The relationship was significant for young ($r^2$ = 0.44, $P$ < 0.01, $n$ = 13), middle-aged ($r^2$ = 0.36, $P$ > 0.05, $n$ = 13) and old leaves ($r^2$ = 0.38, $P$ > 0.05, $n$ = 13). The slope of the relationship was the same for all leaf ages, but the intercept differed significantly ($P$ < 0.05) between old leaves and young or middle-aged leaves (young and middle-aged leaves: $A_{max}$ = 0.036(daily PPFD) + 0.84, $r^2$ = 0.39, $n$ = 26; old leaves: $A_{max}$ = 0.036(daily PPFD) + 0.56, $r^2$ = 0.39, $n$ = 13). Symbols are as in Figure 2.

## Discussion

Differences in the photosynthetic light response of leaves that develop in different light environments provide a potential mechanism for maximizing plant carbon gain (Björkman 1981, Givnish 1988). As a herbaceous plant grows in height, new leaves overtop older leaves, and gradients in light environment and leaf age from the top to the bottom of the crown are generated. In canopies of species with long-lived leaves, there is a second potentially important light gradient along shoots from the young to the old leaves that is nested within the vertical light gradient (Field 1981, Schoettle and Smith 1991, Brooks et al. 1996). Consequently, there are leaves forming and aging throughout the canopy volume. Therefore, to maximize carbon gain in these crowns, photosynthetic acclimation to the light environment could be advantageous not only during leaf development, but also over time as leaves age and become more shaded.

Data and analytical models have been used to generate estimates of the distribution of photosynthetic capability as a function of the vertical gradient in light environments in both herbaceous and deciduous species (Bongi et al. 1987, DeJong et al. 1989, Schimel et al. 1991, Werger and Hirose 1991, Schieving et al. 1992, Hirose and Werger 1994, Ellsworth and Reich 1993), as well as along the horizontal light gradient with leaf aging (Field 1981, Brooks et al. 1996). However, we are not aware of any study that has examined the photosynthetic variation of leaves within a canopy along both light gradients.

In the crown of lodgepole pine, the gradient in mean daily PPFD from the upper to the lower crown (vertical gradient) was similar in magnitude to the mean daily PPFD gradient along shoots from the youngest to the oldest leaves (horizontal gradient). The photosynthetic characteristics of leaves also varied within the canopy. The relationship between light-saturated net photosynthesis ($A_{max}$) and daily PPFD was significant, but differed between young and old leaves (Figure 4).

Table 3. Distribution of mean foliar N (± SE; total leaf surface area basis) by leaf age class within each crown third of lodgepole pine. The vertical gradient in foliar nitrogen was statistically significant for young leaves ($P < 0.001$, $n = 13$) and not for middle-aged ($P = 0.288$, $n = 13$) or old ($P = 0.112$, $n = 13$) leaves. The effect of leaf age class on foliar N was significant in the top ($P < 0.001$, $n = 15$), middle ($P = 0.030$, $n = 12$), and bottom ($P = 0.003$, $n = 12$) of the crown. There was significant crown position by leaf age class interaction; $P < 0.001$. Within a crown position, values followed by different letters are statistically different.

| Crown position | Leaf age class | Foliar N (g N $m^{-2}$) | Sample size |
|---|---|---|---|
| Upper | Young | 1.28 (0.02) a | 5 |
| | Middle-aged | 0.95 (0.03) a | 5 |
| | Old | 0.76 (0.04) b | 5 |
| Middle | Young | 1.00 (0.06) a | 4 |
| | Middle-aged | 1.01 (0.02) a | 4 |
| | Old | 0.85 (0.04) b | 4 |
| Lower | Young | 0.90 (0.06) a | 4 |
| | Middle-aged | 0.94 (0.03) a | 4 |
| | Old | 0.71 (0.02) b | 4 |

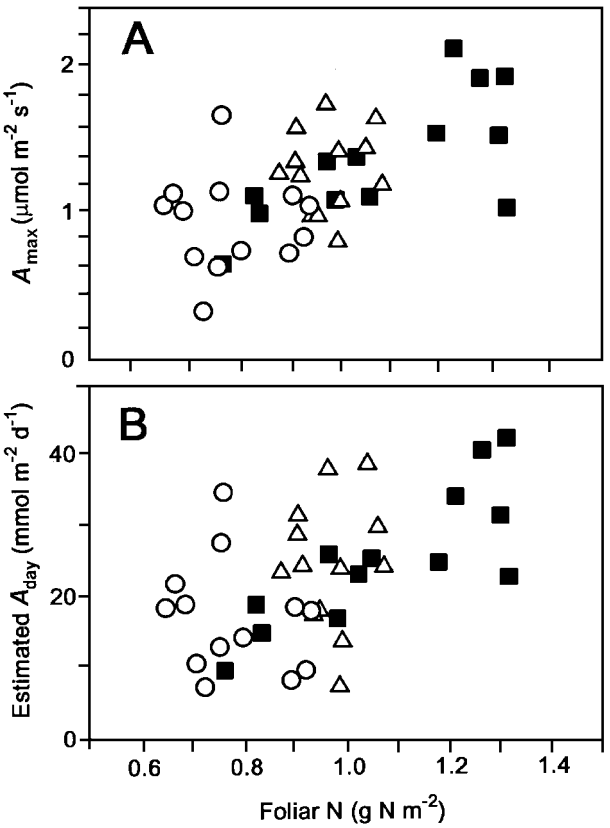


Figure 5. A. Relationship between foliar N and $A_{max}$ for leaves from all leaf age classes and crown positions ($A_{max} = 1.29N - 0.03$, $r^2 = 0.36$, $n = 39$). The relationship was significant for young leaves ($A_{max} = 1.50N - 0.27$, $r^2 = 0.50$, $P < 0.01$, $n = 13$), but not significant for middle-aged ($r^2 = 0.01$, $P > 0.05$, $n = 13$) and old leaves ($r^2 = 0.00$, $P > 0.05$, $n = 13$). B. Relationship between foliar N and estimated $A_{day}$ for leaves from all leaf age classes and crown positions (estimated $A_{day} = 27.59N - 3.79$, $r^2 = 0.28$, $n = 39$). The relationship was significant for young leaves (estimated $A_{day} = 38.92N - 16.73$, $r^2 = 0.66$, $P < 0.01$, $n = 13$), but not significant for middle-aged ($r^2 = 0.01$, $n = 13$) and old leaves ($r^2 = 0.05$, $n = 13$). Symbols are as in Figure 2.

Older leaves had a lower $A_{max}$ than younger leaves in a similar daily irradiance. For all leaves throughout the crown, there was a strong linear relationship between $A_{max}$ and estimated $A_{day}$ ($r^2 = 0.84$, $P < 0.001$, $n = 39$), suggesting that estimated $A_{day}$ was dominated by carbon fixed when the leaves were light-saturated and operating at $A_{max}$. Comparison of the PPFD required to light-saturate net photosynthesis and the quantitative variability of PPFD available to the leaves through the day showed that all of the measured leaves ($n = 39$), regardless of their position in the crown or age, were in light environments that could saturate photosynthesis for a similar proportion of the day. These data provide empirical support for the theory presented by Takenaka (1989) stating that the optimal $A_{max}$ for maximizing long-term carbon gain is determined by the frequency of high photon irradiance (sunflecks and patches) rather than the average, or daily sum, of PPFD.

The classic definition of shade acclimation suggests that an acclimated leaf should be more productive in the shade than a non-acclimated leaf (Björkman 1981, Givnish 1988). Our data provide no evidence for this type of acclimation in *P. contorta* with leaf age. For example, if old leaves retained the photosynthetic light response characteristics of young leaves on the same shoot, our results indicate that they would fix more carbon during the day, even in their more shaded light environment, than they do with their natural physiology (Table 4). In addition, leaves with the physiology of old leaves would fix more carbon during the day if they were in the light environment of the younger leaves on the same shoot. In other words, the photosynthetic changes that occurred with leaf age in lodgepole pine did not enhance daytime carbon gain of the leaf, according to our estimates based on changes in light regimes and the light response of photosynthesis. However, we did not quantify nighttime respiration with leaf aging, or the influence of other possible environmental factors other than light regime.

Foliar N was not a good predictor of $A_{max}$ or estimated $A_{day}$ when data for all leaves in the crown were pooled. However, when leaf age classes were examined separately, the foliar N–$A_{max}$ association was significant in young leaves (Figure 5A), but was not significant in older leaves, which comprised over 50% of the foliar canopy (Schoettle 1989). Our data are consistent with those of Reich et al. (1995) who reported a significant linear relationship between foliar N and $A_{max}$ for young leaves among nine conifer species; their study did not examine the relationship for old leaves. Although the biochemical basis for the foliar N–$A_{max}$ relationship is known (Evans 1989), this relationship appears less obvious when all needle age classes are included (Brooks et al. 1996, this study). Without a better understanding of N partitioning among photosynthetic and non-photosynthetic pools within a conifer leaf, it is not clear if foliar N is limiting the photosynthetic capabili-

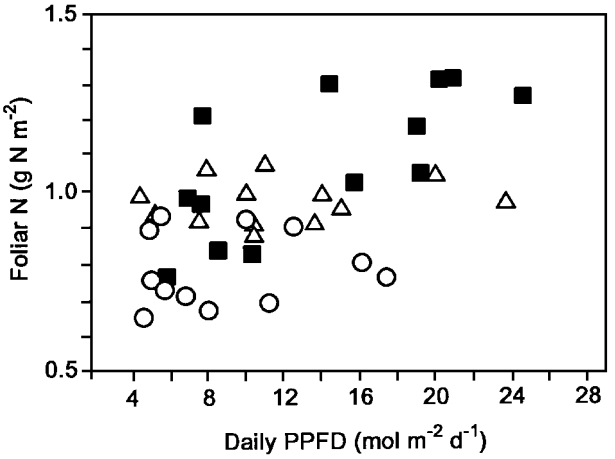


Figure 6. Relationship between daily PPFD and foliar N for leaves of all age classes and crown positions (N = 0.015(daily PPFD) + 0.76, $r^2$ = 0.23, $n$ = 39). The relationship was significant for young leaves (N = 0.026(daily PPFD) + 0.67, $r^2$ = 0.67, $P$ < 0.001, $n$ = 13), but was not significant for middle-aged ($r^2$ = 0.01, $P$ > 0.05, $n$ = 13) and old leaves ($r^2$ = 0.00, $P$ > 0.05, $n$ = 13). Symbols are as in Figure 2.

ties of old leaves in this study.

Nitrogen allocation theory predicts that foliar N is distributed within the canopy according to daily PPFD (Mooney and Gulmond 1982, Field 1983, Hirose and Werger 1987, Chen et al. 1993). When all data were pooled, there was a weak relationship between foliar N and daily PPFD; however, the relationship was significant only for the youngest leaves (Figure 6). Therefore, if we consider only that portion of the lodgepole pine canopy that is similar to that of a deciduous tree (i.e., young leaves along the vertical light gradient), our data are in agreement with the nitrogen allocation theory. In contrast, when leaf aging is considered, daily integrated PPFD is correlated with the occurrence of foliar N in the crown for young leaves but not for old leaves. Similarly, the foliar N–$A_{max}$ relationship was significant only for the young leaves. Therefore, the assumption that foliar N is a good predictor of $A_{max}$ may not be universal among leaf ages for all plant types.

The observed distribution of foliar N in the crown of *P. contorta* suggests that the controls for N allocation to young leaves may differ from those in aging leaves. One might speculate that old leaves serve as a partial nutrient source in support of new growth (Monk 1966, Lange et al. 1987, Nambiar and Fife 1987, Weikert et al. 1989). If so, the foliar N of aging leaves would be a function of the initial foliar N at maturation and the magnitude of N translocation over time (Fife and Nambiar 1984). Thus, although the initial foliar N may be a function of the daily PPFD for young leaves, the translocation of N from old leaves may be a function of the sink strength of local growing points (Lange et al. 1987, Weikert et al. 1989), rather than the daily PPFD of the aging leaves. This may contribute to the weaker relationship found here between foliar N and daily PPFD in older leaves than in young leaves.

Table 4. Estimated $A_{day}$ (mmol $m^{-2}$ $day^{-1}$) of *Pinus contorta* leaves based on the measured photosynthetic light response of young and old leaves and computed for the light environment of both young and old leaves. Means (± SE, $n$ = 13) were calculated from measurements on 13 shoots from throughout the crown. Means followed by different letters are significantly different ($P$ < 0.01).

| Light environment | Photosynthetic light response | Estimated $A_{day}$ (mmol $m^{-2}$ $day^{-1}$) |
|---|---|---|
| Young leaves | Young leaves | 25.2 (2.6) a |
| Young leaves | Old leaves | 22.5 (2.2) a |
| Old leaves | Young leaves | 18.3 (2.3) c |
| Old leaves | Old leaves | 16.8 (2.2) d |

## Conclusions

The vertical gradient in integrated daily PPFD from the upper to the lower crown of lodgepole pine was similar in magnitude to the horizontal gradient in integrated daily PPFD along shoots from the young to the old leaves. For all leaves throughout the crown, $A_{max}$ was a good predictor of estimated $A_{day}$. This relationship suggests that estimated $A_{day}$ is dominated by carbon fixed when the leaves are photosynthetically light-saturated and operating at $A_{max}$. This may be a consequence of the adjustment of the photosynthetic light response to the quantitative variability of irradiance through the day. Foliar N was a good predictor of $A_{max}$ for young leaves but not for older leaves. The light microenvironment corresponded only to a part of the foliar N distribution within the canopy of lodgepole pine. Nitrogen allocation theory, which suggests that N is allocated in relation to light availability, was consistent with our data for young leaves, but was not supported by our data for older leaves.

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