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Acclimation responses of mature *Abies amabilis* sun foliage to shading

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Abstract This paper addresses two main questions. First, can evergreen foliage that has been structurally determined as sun foliage acclimate physiologically when it is shaded? Second, is this acclimation independent of the foliage ageing process and source-sink relations? To investigate these questions, a shading and debudding experiment was established using paired branches on open-grown *Abies amabilis* trees. For each tree, one branch was either shaded, debudded, or both, from before budbreak until the end of summer, while the other branch functioned as a control. Foliage samples were measured both prior to and during treatment for photosynthesis at light saturation (A_{max}), dark respiration, nitrogen content, chlorophyll content, chlorophyll-to-nitrogen ratio and chlorophyll *a:b* ratio. All age classes of foliage responded similarly during the treatment, although pre-treatment values differed between age classes. Within 1 month after the treatment began, A_{max} was lower in shaded foliage and remained lower throughout the treatment period. For debudded branches, A_{max} was lower than the controls only during active shoot elongation. At the end of the treatments in September, A_{max} in shade-treated sun foliage matched the rates in the true shade-formed foliage, but nitrogen remained significantly higher. By 1.5 months after treatment, chlorophyll content in shaded foliage was higher than in controls, and the chlorophyll *a:b* ratio was lower for the shaded foliage. On debudded branches, chlorophyll content and chlorophyll *a:b* ratio were similar to the values in control samples. Shading lowered the rate of nitrogen accumulation within a branch, while removing debudding decreased the amount of sequestered N that was exported from the older foliage to supply new growth. By September, chlorophyll content in shade-treated foliage was higher than that in the control sun foliage or in true shade foliage. The chlorophyll increase as a result of shading was unexpected. However, the chlorophyll-to-nitrogen ratio was identical for the shade-treated sun foliage and the true shade foliage while being significantly lower than the control sun foliage. It appears that acclimation to shading in mature foliage involves a reallocation of nitrogen within the leaf into thylakoid proteins. A redistribution of resources (nitrogen) among leaves is secondary and appears to function on a slower time scale than reallocation within the leaf. Thus, *A. amabilis* foliage that is structurally determined as sun foliage can acclimate to shade within a few months; this process is most likely independent of ageing and is only slightly affected by source-sink relations within a branch.

Key words Pacific silver fir · Light acclimation
Leaf nitrogen · Chlorophyll · Resource allocation

Introduction

As leaves form, they develop morphological and physiological characteristics that appear to be adaptive for the light conditions under which they developed (Boardman 1977; Björkman 1981; Givnish 1988). However, light conditions within plant canopies change as plants, especially trees, grow. This is particularly important in evergreen trees, where long leaf lifespans and rapid height growth mean that many leaves spend much of their lives under light conditions very different from those under which they formed. Yet remarkably little is known about the degree to which evergreen leaves can acclimate to changes in their light environment once they are fully formed. There have been many studies of changes in leaf characteristics with age (e.g., Field and Mooney 1983; Hom and Oechel 1983; Teskey et al. 1984; Sheriff et al. 1986; Kazda and Weilgony 1988), but most of these studies described the changes simply as results of ageing; the observed changes were not considered in the context of acclimation from sun to shade. Osmond and

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Chow (1988) stated "We know very little of the capacity for a fully expanded leaf formed in the sun or shade to acclimate to a change in light environment."

Changes in photosynthetic characteristics after leaf growth has been completed have generally been considered to be solely related to ageing. However, since these changes usually shift the photosynthetic characteristics of sun foliage closer to those of shade foliage, some of these changes might be related to acclimation rather than ageing. It has long been known that A_{max} and dark respiration typically decrease with increasing leaf age (e.g., Clark 1961; Hom and Oechel 1983; Teskey et al. 1984); however, A_{max} and dark respiration are also typically lower in shade foliage. Sutinen (1987) noted that chloroplasts in older *Picea abies* needles tended to have more thylakoid membranes and less stroma than younger needles. Since shade-formed foliage also tends to have more thylakoid membranes (Anderson et al. 1988), the changes observed by Sutinen are consistent with a post-expansion acclimation to a reduced-light environment. Foliar nitrogen content also declines in older leaves in a wide variety of species (Aslam et al. 1977; van den Driessche 1984; Teskey et al. 1984; Tyrrell and Boerner 1987; Nilsen et al. 1988). Again, this has traditionally been considered as an ageing process, but Field (1983, 1987) has suggested that this decline with age might be adaptive rather than an inevitable consequence of ageing.

It seems unlikely that light acclimation would be tied solely to leaf age in forest trees, since under natural conditions the rate of change in the light environment with time can be extremely variable. Evergreen leaves on the south side of an isolated tree may remain in nearly full sunlight for over a decade, while those on trees in deep forest may go from full sunlight to deep shade in a few years. Thus evergreen trees, in particular, would gain a significant advantage from a mechanism that matched photosynthetic characteristics to the light environment rather than a strictly programmed decline in photosynthetic capacity and nitrogen with age.

Besides changes in age and light, seasonal changes in shoot growth patterns can also cause changes in photosynthetic activity of mature leaves. Expanding shoots are very strong sinks, second only to developing cones (Dickmann and Kozlowski 1970), so for trees that are not sexually mature, local sink strength is probably strongest during the shoot elongation period. Teskey et al. (1984) found that photosynthetic capacity of 1-year-old *Abies amabilis* foliage reached a maximum in the late spring when shoots were elongating. Local sink strength and the relationship of sinks to sources would also be spatially variable during the shoot elongation period, since both the amount of new shoot growth per branch and the ratio of new growth to old growth vary sharply with branch position in the canopy and exposure (Brooks 1987; D.G. Sprugel, unpublished data). It is very likely, then, that variations in local sink strength might play an important role in determining the variation of photosynthetic characteristics in a tree canopy, especially during periods of rapid growth.

The goal of this study was to identify the factors responsible for changes in photosynthetic characteristics after leaf formation is complete. Our specific objectives were to determine whether sun-formed *A. amabilis* (Pacific silver fir) foliage has the capacity to change its photosynthetic apparatus as light decreases in such a way that it more closely matches true shade foliage in response, and whether this process is independent of ageing and source-sink effects.

Materials and methods

Study site

The research was conducted in a 35-year-old *A. amabilis* stand (elevation: 1150 m) about 75 km south-east of Seattle, in the Findley Lake research area on the City of Seattle's Cedar River watershed. The stand was clearcut in 1955 and regenerated naturally. Mean stem density is still high (c. 8.5 stems m^{-2}). However, disturbance during the logging operation eliminated both advanced regeneration and seeds in many areas, so the trees are not evenly distributed; very dense clumps (up to 30 stems m^{-2}) covering 5–30 m^2 are interspersed with areas of up to 50 m^2 containing only a few trees, so in spite of the overall high density of the stand, open-grown trees were easily found. Tree heights range from 0.5 to 8 m. The study trees were all open-grown trees ranging in height from 4 to 6 m. In this *A. amabilis* stand, needles persist on the trees for a maximum of around 13–15 years.

Study design

A manipulative experiment using paired branches was used to separate the effect of age and source-sink relations from potential acclimation responses. Fifteen branch pairs were used, each consisting of two branches from the same whorl of the tree and similar in diameter, length, exposure, and length of last year's leader growth. Only branches from the upper third of the canopy of open-grown trees were used so that all of the foliage was formed under generally open conditions. Each branch pair was on a different tree. Within each pair, one (randomly chosen) branch was the control and the other was randomly assigned to one of three treatments: shading (S), where light was about 80% that of controls, debudding (B), where all the buds on the treatment branch were removed, and shading+debudding (SB). On each treated and control branch, 1-, 2-, and 4-year-old foliage was monitored for changes in selected physiological indicator variables through the treatment period. Thus all comparisons reported here are for foliage of the same genotype, age, thickness, and initial light conditions, differing only in the treatment applied during the experiment.

Treatments were applied on 12 May 1992, and were continued throughout the summer until early September. Bud break occurred at the beginning of June and shoot elongation was largely completed by mid-July. In the shading treatment, branches were shaded to reduce ambient light levels by 80–90% (measured with a LI-COR quantum sensor) while simultaneously simulating the light quality of *A. amabilis* shade (which was measured with a Spectron CE-590 spectroradiometer). The structures used to shade branches were designed to permit relatively free air movement, so that temperatures did not increase over ambient temperatures. These structures were composed of an internal lightweight frame that was suspended over the branch, an open mesh (1-cm pore size) nylon bag that surrounded the branch and had an opening for branch access, and a dark green pure cotton cloth, which was perforated by small holes and was stitched to the top of the open mesh bag. For the debudding treatment, all buds on the selected branches were removed following the procedures of Weikert et al. (1989).

To monitor changes in the photosynthetic apparatus, five physiological indicators of sun and shade acclimation were selected

based upon the literature (Björkman 1981; Seemann et al. 1987; Anderson et al. 1988; Givnish 1988; Nilsen et al. 1988; Evans 1989). These were: (1) A_{max}, (2) dark respiration, (3) leaf nitrogen, (4) the chlorophyll-to-nitrogen ratio, and (5) the chlorophyll *a:b* ratio. In addition to these five physiological indicators, leaf thickness and leaf mass per unit area were also measured.

Prior to treatment, all the parameters mentioned above were measured on each foliage sample. A small sample of needles was removed for measurements of leaf thickness, leaf mass per unit area, nitrogen content, and chlorophyll content, while gas-exchange measurements were measured in situ on prepared sites on each branch. After the treatments were applied to the branches, A_{max}, respiration, and chlorophyll were measured approximately every other week until September. In early September, branches were harvested and all the other physiological and morphological variables were measured again. For the shading treatment and controls, morphological characteristics and nitrogen content were also measured for current foliage. On a subset of branch pairs, branches were harvested, and transported to the laboratory in plastic bags to measure photosynthetic light curves under controlled conditions.

At the end of the field season, true shade-formed foliage was collected from trees that were near the study trees. These samples were used as a baseline for *A. amabilis* shade foliage, and were measured for all the above physiological and morphological parameters.

Physiological measurements

Photosynthesis at light saturation (A_{max}) was measured in the field using a portable photosynthesis system (LI-COR Model 6200 with 0.25-l chamber). Since this instrument is not climate-controlled, ambient temperature conditions were used, and sampling was carried out so that foliage samples of the same age on paired branches were measured sequentially to minimize temperature variation between pairs selected for comparison. *A. amabilis* has a broad, flat photosynthetic temperature optimum between 10 and 20°C (Teskey et al. 1984), so minor temperature changes between measurements did not pose a problem. Teskey et al. (1984) found that all ages of *A. amabilis* sun foliage reach light saturation at or below 700 μmol m^{-2} s^{-1}, so a supplementary quartz-halogen lamp producing 850 μmol m^{-2} s^{-1} was used. Foliage samples were shaded from solar radiation so that light levels were the same for all measurements. Measurements took less than 1 min so chamber heating resulting from the lamp was negligible.

Dark respiration was measured following the technique described by Brooks et al. (1991). The foliage was shaded for 30 min prior to measurement to avoid the peak in dark respiration following active photosynthesis (Heichel 1970). Respiration were measured with the same infra-red gas analyzer as photosynthesis at light saturation, with a chamber modified to block all light.

Photosynthetic light curves were measured on two branch pairs from each treatment at the end of the treatment period. Branches were harvested and transported to the laboratory in plastic bags. Once in the laboratory, branch ends were recut under water, and allowed to hydrate overnight at 4°C. Prior to measurements, the branch ends were recut under water again and attached to a water source. Light curves were measured using the LI-COR 6200 with a 0.25-l chamber modified with a peltier module; temperature in the cuvette was controlled at 15°C, the photosynthetic optimum for *A. amabilis* (Teskey et al. 1984). A sodium vapor discharge lamp was suspended over near-infrared reflecting glass and light levels were altered using layers of neutral density shade cloth and measured with the LI-COR quantum sensor.

Nitrogen content of the foliage was measured using a modified Kjeldahl technique described by Parkinson and Allen (1975). Foliage samples on which leaf area and dry weight had been measured were ground to pass a 20-mesh screen. A subsample of 100 mg was digested and analyzed with a Technicon autoanalyzer using industrial method no. 108–71w.

Chlorophyll was analyzed spectrophotometrically as described by Sesták (1971) using a Bausch and Lomb Spectronic 1001 spectrophotometer. Five to ten needles were collected in the field from foliage of the same age near each of the gas exchange sample sites. Chlorophyll content was calculated using equations developed by Arnon (1949) and modified by Ziegler and Egle (1965).

We measured leaf thickness, leaf area, fresh and dry weight of all foliage samples both before and after the treatment period. The pre-treatment values were measured on subsamples of needles trimmed from the shoots around the gas exchange sites, while the post-treatment values were measured on shoots where gas exchange had been measured throughout the season. Leaf thickness was determined using a dial indicator micrometer accurate to 0.01 mm. Projected leaf area was determined by using an Optimas image analysis system. Needles were removed from the shoot and spread out on a back-light table for high contrast before the image was taken by the system. Leaf dry weight was determined after drying overnight at 70°C. These samples were then used to measure nitrogen content.

Statistical analysis

Because the study was designed around a paired-sample design, each treatment sample was compared with a control sample of the same age from the same whorl on the same tree, thus blocking for potential genetic, branch age, and foliage-age-related variation. Pre-treatment differences were accounted for by examining the change in the values over the treatment period (post-treatment –pre-treatment). For most statistical analyses, a difference value was used that combined the two blockings stated above [(post-treatment –pre-treatment) –(postcontrol –precontrol)]. These values were compared with zero using paired *t*-tests and a two-way ANOVA across foliage age and treatment. In most cases, a one-tailed test was used because the direction of change for each indicator variable had been specified. When foliage from the treatments was compared with true shade foliage from other trees, the paired sampling design could not be used. For these cases, values from shade-treated foliage (S and SB) were averaged together and compared with averages of their controls and true shade-foliage samples. The two shade treatments were combined for this analysis since the results were similar, and graphical presentation would be clearer.

Results

Gas exchange measurements

The shading (S) and shading and debudding (SB) treatments significantly reduced photosynthesis at light saturation (A_{max}, μmol^{-2} s^{-1}) compared to their controls (Table 1) whereas there was no difference between the debudding (B) treatment and its control at the end of the treatment period. In the shading treatments (S and SB), A_{max} was about half the value of the controls. These differences were the same even if A_{max} was expressed on a unit weight basis (data not shown). All pre-treatment values of A_{max} were substantially lower than the final values in late August, perhaps because pre-treatment values were measured in early May prior to budbreak when nighttime temperatures were still close to 0°C, while final values were measured under warm late-summer conditions. At the end of the season, A_{max} in shade-treated sun foliage (S, SB, 1-year-old only) was similar to A_{max} in true shade foliage, and significantly lower than A_{max} of the control sun foliage (Fig. 1). Respiration rates

Table 1 Pre-treatment means (*Pre-treat*±SE, n=15) and post-treatment means (*Post-treat*±SE, n=15) are presented for all the physiological indicator variables. The age classes of foliage are combined in this table. Significance values are based on paired t-tests for the differences between a treated and control pair over the treatment period as described in the text under Materials and methods/Statistical analysis. Pre-treatment values were taken 7 days before treatment began while post-treatment values were collected 100 days after treatment began. *Mass/area* indicates leaf mass per unit area

Variable	Treatment					
	Shading		Shading and debudding		Debudding	
	Pre-treat	Post-treat	Pre-treat	Post-treat	Pre-treat	Post-treat
A_{max} (μmol m^{-2} s^{-1})						
Treated	2.76 ± 0.47	3.27 ± 0.43	2.99 ± 0.66	3.46 ± 0.27	3.21 ± 0.58	5.77 ± 0.53
Control	2.70 ± 0.58	6.45 ± 0.31 ***	2.89 ± 0.48	6.19 ± 0.30 ***	3.33 ± 0.55	6.04 ± 0.38
Resp. (μmol m^{-2} s^{-1})						
Treated	1.02 ± 0.22	0.44 ± 0.09	0.53 ± 0.14	0.95 ± 0.16	0.70 ± 0.22	1.01 ± 0.15
Control	1.01 ± 0.25	0.82 ± 0.14 *	0.47 ± 0.20	0.89 ± 0.14	0.60 ± 0.23	0.91 ± 0.15
Nitrogen (g m^{-2})						
Treated	3.95 ± 0.20	4.00 ± 0.23	3.46 ± 0.14	3.71 ± 0.15	3.63 ± 0.18	3.82 ± 0.16
Control	3.98 ± 0.22	4.25 ± 0.27 *	3.71 ± 0.10	4.06 ± 0.15	3.47 ± 0.16	3.64 ± 0.15
Chl (g m^{-2})						
Treated	0.54 ± 0.04	0.87 ± 0.04	0.45 ± 0.04	0.81 ± 0.03	0.44 ± 0.03	0.62 ± 0.03
Control	0.51 ± 0.04	0.71 ± 0.05 ***	0.47 ± 0.04	0.68 ± 0.04 ***	0.43 ± 0.04	0.61 ± 0.03
Chl *a*:*b* ratio						
Treated	2.85 ± 0.05	2.71 ± 0.04	2.93 ± 0.06	2.74 ± 0.04	2.65 ± 0.10	3.07 ± 0.04
Control	2.73 ± 0.07	2.93 ± 0.06 ***	2.79 ± 0.07	2.98 ± 0.06 ***	2.65 ± 0.09	3.00 ± 0.05
Chl:N (x100)						
Treated	12.3 ± 1.0	22.0 ± 0.6	13.4 ± 0.9	22.1 ± 0.5	12.0 ± 0.7	16.3 ± 0.8
Control	12.8 ± 0.7	17.0 ± 0.7 ***	13.3 ± 0.9	16.7 ± 0.6 **	12.5 ± 0.7	16.5 ± 0.7
Mass/area (g m^{-2})						
Treated	426 ± 12	367 ± 8	407 ± 12	355 ± 10	418 ± 12	375 ± 7
Control	424 ± 10	386 ± 9 **	417 ± 10	391 ± 9 ***	414 ± 13	378 ± 8
Thickness (mm)						
Treated	0.82 ± 0.02	0.82 ± 0.02	0.80 ± 0.02	0.79 ± 0.02	0.79 ± 0.02	0.80 ± 0.01
Control	0.82 ± 0.02	0.82 ± 0.01	0.81 ± 0.02	0.81 ± 0.02	0.79 ± 0.02	0.79 ± 0.02

*P≤0.05, **P≤0.01, ***P≤0.001

were significantly reduced compared to their controls in the shaded-only foliage (S). Respiration rates in the other two treatments were not significantly different from their controls (P=0.30 for SB, P=0.44 for B, paired t-test).

Gas exchange measured under controlled environmental conditions at the end of the treatment period illustrated trends similar to the field data (Fig. 2). A_{max} (CO_2 flux at light saturation) and respiration (CO_2 flux at 0 photosynthetic photon flux density, PPFD) in shaded samples (S,SB) were lower than the controls but debudding (B) had no significant effect. As expected, older foliage had lower light-saturation values than younger foliage in both treated and control samples.

Photosynthesis changed rapidly after shading; shade-treated sun foliage and their controls were significantly different within a month of shading (Table 2). For the shading treatment (S), differences in A_{max} between shading and controls increased until early July and remained relatively constant after that. For the debudding treatments (SB and B) the largest difference in A_{max} between treated and control branches occurred during rapid shoot elongation (early June). This period was the only time that a significant difference was found in A_{max} for the debudding treatment (B). The pattern in A_{max} for the SB treatment was intermediate between that noted for the S and B treatments.

Leaf nitrogen

Nitrogen content per unit leaf area averaged 3.7 g m^{-2} prior to treatment; however, contrary to expectations, nitrogen content prior to treatment was higher in older than in younger age classes (Table 3). In general, nitrogen content increased over the season for all foliage samples except in the shade-treated sun foliage (S). The gain in nitrogen over the treatment period was not influenced by the age of the foliage. The leaf nitrogen content for shade-treated sun foliage (S, SB) was significantly higher than the true shade foliage, and only slightly, but significantly lower than the control sun foliage (Fig. 1, Table 1).

Chlorophyll

Prior to treatment, chlorophyll content was significantly greater in older foliage than in younger foliage for all 15 trees (Table 3). Chlorophyll content increased over the season for all foliage samples (Fig. 3, Table 1). In the two shading treatments (S, SB), chlorophyll content increased considerably more than in their controls, while for debudding alone, there was no significant difference between foliage on the treated and control branches at the end of the growing season (Table 1). Chlorophyll

Table 2 Mean differences of photosynthesis at light saturation (A_{max},μmol m^{-2} s^{-1}) between treated and control samples (treated –control, ±SE, n=15) over the season for all three treatments. T days is the number of days since the treatment began. Significance values are based on paired t-tests and levels of significance are indicated by *stars* as stated in Table 1. The June 2 sample was taken at time of active bud elongation

Date	T days	Shading	Shading & Debudding	Debudding
May 5	–7	–0.36 ± 0.39	0.19 ± 0.32	0.07 ± 0.50
June 2	21	–1.17 ± 0.48 *	–3.34 ± 0.54 ***	–2.66 ± 0.31 ***
June 20	39	–1.94 ± 0.48 **	–1.42 ± 0.47 *	–0.76 ± 0.67
July 9	58	–3.68 ± 0.73 **	–2.16 ± 0.52 **	0.99 ± 0.39
July 28	77	–3.72 ± 0.52 ***	–2.14 ± 0.43 **	–0.95 ± 1.08
Aug. 20	100	–3.15 ± 0.78 **	–1.96 ± 0.32 ***	–0.74 ± 0.41

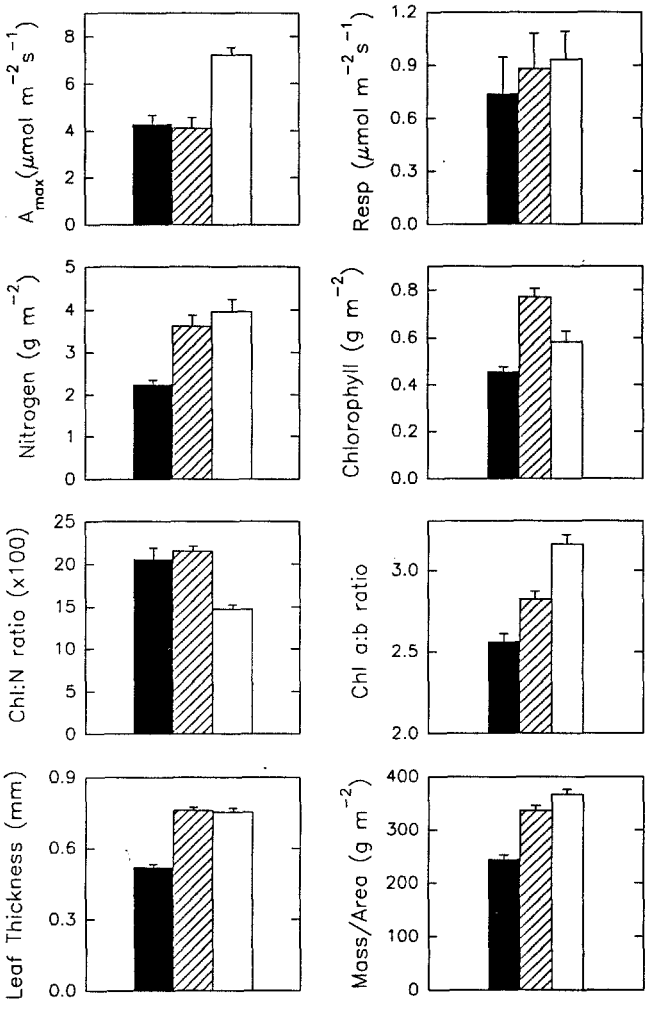

Fig. 1 Physiological and morphological characteristics of 1-year-old true-shade foliage (*solid bars*), shade-treated sun foliage (*shaded bars*), and sun foliage remaining in the sun (*open bars*) measured in September. *Error bars* indicate the SEM (n=10). The data used for the shade-treated sun foliage are the average for the shading (S) and shading+debudding (SB) treatments. The data for the sun foliage remaining in the open are the controls for both shading treatments

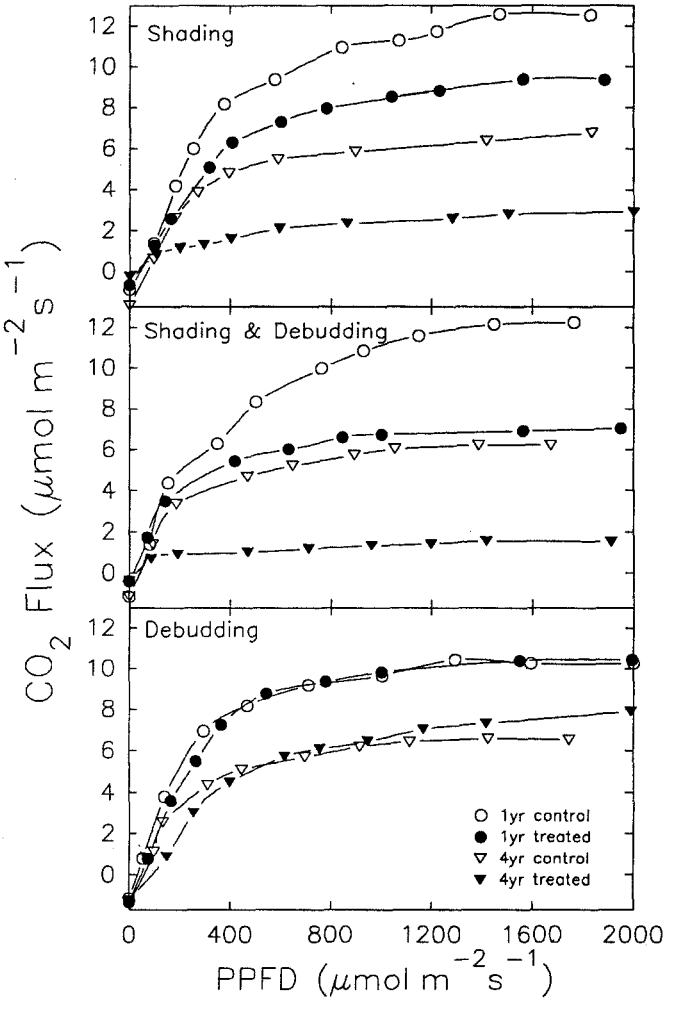

Fig. 2 Photosynthetic light response curves measured on representative branches at the end of the growing season for the three main treatments (*PPFD* photosynthetic photon flux density)

content responded relatively rapidly to an increase in shading (Fig. 3). After 17 days of shading, some of the trees showed significant differences between the treated and control samples. After 70 days of shading, chlorophyll contents were significantly higher than in unshaded controls in all of the trees. The change in chlorophyll over the treatment period (S, SB) was greater in younger foliage than in older foliage (ANOVA, P<0.05, F=5.4). As a result, differences in chlorophyll between age classes at the end of the treatment period were smaller for the shade-treated foliage than for control foliage. The levels of chlorophyll in the shade-treated sun foliage (S, SB) were significantly higher than in both true shade foliage and control sun foliage (Fig. 1).

Table 3 Physiological parameters for different age classes of foliage prior to treatment. The *P* values were calculated using a one-way ANOVA on control samples only. Each age class had 15 samples, one from each tree

Variable	p	1-year-old	2-year-old	4-year-old
A_{max} (μmol m^{-2} s^{-1})	0.015	3.4 ± 0.4	3.2 ± 0.6	2.8 ± 0.4
Resp. (μmol m^{-2} s^{-1})	NS	0.70 ± 0.12	0.69 ± 0.13	0.66 ± 0.22
N (g m^{-2})	0.024	3.34 ± 0.16	3.69 ± 0.20	3.94 ± 0.17
Chl (g m^{-2})	< 0.001	0.37 ± 0.03	0.48 ± 0.04	0.58 ± 0.04
Chl *a:b* ratio	< 0.001	2.83 ± 0.12	2.76 ± 0.10	2.60 ± 0.05
Chl:N (×100)	< 0.001	10.6 ± 0.5	12.9 ± 0.6	14.9 ± 0.6
Mass/area (g m^{-2})	< 0.001	381 ± 4	406 ± 7	453 ± 7
Thickness (mm)	< 0.001	0.75 ± 0.01	0.78 ± 0.01	0.87 ± 0.02

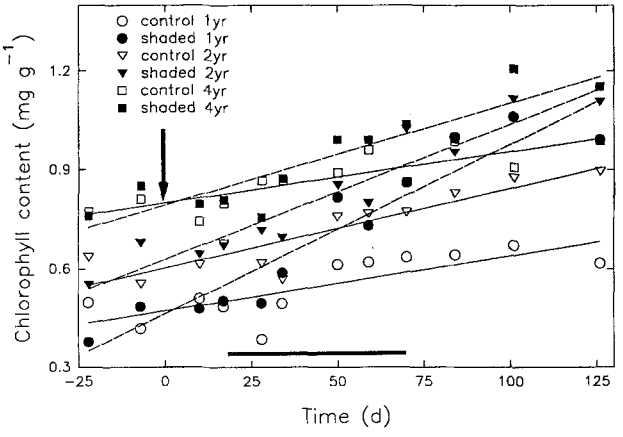

Fig. 3 Changes in total chlorophyll over the season for a shade-treated branch and its control. The data are representative of the pattern seen in all S and SB trees. *Time* is days of treatment with zero being the day treatments were installed, 12 May, indicated by the *arrow*. The *dashed lines* indicate the general seasonal trends of chlorophyll content in shaded foliage, while *solid lines* were used for control samples. The *thick solid line* at the bottom represents the acclimation transitional period for chlorophyll. Prior to the line no significant differences were observed between treated branches and controls; after that time all shade-treated branches were significantly different

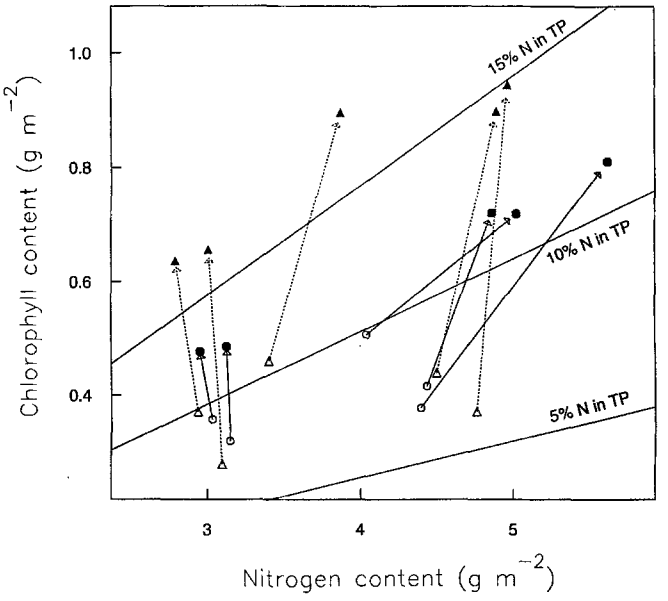

Fig. 4 The change in chlorophyll and nitrogen content over the treatment period for 1-year-old foliage (S only for graphical clarity, but SB shows a similar pattern). *Open symbols* are pre-treatment values and *closed symbols* are post-treatment values with *arrows* connecting the sample from its pre-treatment value to its post-treatment value (each pair of points connected by an arrow represents one foliage sample). *Triangles* represent shade-treated samples and *circles* represent control samples. The *lines* represent percentages of total nitrogen associated with thylakoid proteins and chlorophyll (*TP*) (based on 50 mol N mol^{-1} chlorophyll, Evans 1989)

The chlorophyll-to-nitrogen ratios in both shading treatments (S and SB) were significantly higher than the ratios in their controls at the end of the season, whereas prior to treatment, the (pre)treated and controls had been equivalent (Table 1). For the debudding treatment, there was no significant change observed in response to the treatment. The chlorophyll-to-nitrogen ratio increased over the treatment period regardless of treatments (Table 1), mainly a result of the increase in chlorophyll content (Fig. 4). Chlorophyll content increased more in foliage with relatively higher nitrogen contents (c. 3.5 g N m^{-2}), but the chlorophyll-to-nitrogen ratio remained higher in foliage with lower nitrogen contents (c. 2 g N m^{-2}) over the treatment period (Fig. 4). At the end of the season, the chlorophyll-to-nitrogen ratio in shade-treated sun foliage (S and SB) was as the same as in true shade foliage, even though, when compared separately, values for both nitrogen and chlorophyll differed significantly from those in true shade foliage (Fig. 2). The chlorophyll-to-nitrogen ratio for shade-treated and true shade foliage were significantly higher than the control sun foliage.

Over the treatment period, both chlorophyll *a* and *b* increased in concentration over time ($P<0.01$); although for the shade-treated foliage (S and SB), chlorophyll *b* increased relatively more than chlorophyll *a*, which significantly reduced the chlorophyll *a:b* ratio relative to the controls. In the debudding treatment and all control foliage, the chlorophyll *a:b* ratio increased over the treatment period (Table 1), indicating that for sunlight foliage chlorophyll *a* increased relatively more than chlorophyll *b*. Similarly to chlorophyll content, older foliage had lower *a:b* ratios than younger foliage prior to treatment (Table 3), but age did not affect the degree of change over the treatment period, so at the end of the treatment period, older shade-treated foliage still had lower chlorophyll *a:b* ratios. After the onset of the shading treatment, changes in the chlorophyll *a:b* ratio were detected at the

same time as changes in chlorophyll content. By the end of the treatment period all shade-treated samples had lower ratios than their respective controls. The chlorophyll *a:b* ratio at the end of the season was still not quite as low as the ratio in true shade foliage, but the ratio was closer to the true shade than to the control sun foliage (Fig. 2).

Morphology

Prior to treatment, needles ranged in leaf thickness from 0.64 to 0.98 mm and older needles were significantly thicker than younger needles (Table 3). Leaf thickness remained stable over the treatment period, so none of the treatments affected the thickness of mature foliage, and leaf thickness did not change over the season (Table 1). The pattern of decreasing needle thickness with younger age on these branches was maintained in the newly expanded foliage on the controls (data not shown).

Whereas leaf thickness did not change with time or treatment, leaf mass per unit area decreased over the treatment period (Table 1). Prior to treatment, leaf mass per unit area ranged from 328 to 505 g m^{-2}, and like leaf thickness, leaf mass per unit area increased with leaf age (Table 3). During the treatment period, all foliage samples lost weight but shade-treated samples (S and SB) decreased more than their respective controls. Since leaf dimensions did not change over the treatment period, leaf density must have, presumably through changes in starch storage. Even with the change in leaf mass per unit area, shade-treated foliage at the end of the season weighed significantly more than true shade foliage (Fig. 1).

Discussion

Acclimation response

Traditional studies of sun-shade acclimation have focused on characterizing either foliage produced in sunny or shady environments or foliage following a dramatic shift in light. This study used a different approach in which fully expanded sun foliage was shaded and then its responses were physiologically and morphologically characterized. For evergreen conifers, acclimation after leaves have fully matured is particularly important since leaf lifespans can be long and height growth of the tree can be rapid. In shade-tolerant conifers such as *A. amabilis,* many leaves spend much of their lives under light conditions very different from those under which they formed.

A. amabilis foliage produced in the sun can acclimate physiologically to decreases in light even after the foliage is mature and morphologically determined as sun foliage. This acclimation response seems to have two components: a rapid response, strongly regulated by the current light environment of the foliage, and seemingly independent of source-sink and age effects, and a slower response that is influenced by source-sink interactions and potentially by the ageing process. In the first component, photosynthesis changed relatively rapidly in response to changing light, and in association, resources within the foliage were reallocated internally. After 100 days of shading, sun foliage had similar rates of photosynthesis at light saturation (A_{max}) and similar values for both the chlorophyll-to-nitrogen ratio and the chlorophyll *a:b* ratio as foliage formed in the shade. The second component of acclimation, the reallocation of resources among the foliage in different parts of the tree, appears to be much slower than reallocation of resources within the needles. At the end of the treatment period, nitrogen content was lower in shade-treated sun foliage, but still much higher than true shade foliage. Thus, the rapid change in photosynthetic rates is not related to a change in bulk leaf nitrogen, but instead may be due to reallocation of nitrogen within the needles.

The physiological and biochemical indicators of sun-shade acclimation suggest that much of the rapid response to shading involves reallocation of nitrogen among pools within the leaf. Chlorophyll content, the chlorophyll *a:b* ratio, and A_{max} in shade-treated sun foliage began changing from the controls within 1 month of treatment, and differences continued to increase until the last measurement. Although true shade foliage had lower chlorophyll content than sun foliage, the chlorophyll content consistently and significantly increased in all shade-treated foliage (S, SB).

This unexpected pattern of chlorophyll dynamics can be more readily understood if chlorophyll is used as an indicator of thylakoid proteins and is considered as part of the total nitrogen pool in a leaf (Evans 1989). The chlorophyll-to-nitrogen ratio can then be expressed as the percentage of total nitrogen that is invested in the thylakoid membranes (assuming 50 mol N mol^{-1} chlorophyll, Evans 1989). Using that conversion in this study, about 16–17% of the total nitrogen in both true shade and shade-treated sun foliage (S, SB) was allocated to the thylakoid proteins, while in the sun-formed controls only around 11% was allocated to these proteins. The increase in the chlorophyll-to-nitrogen ratio observed in this study under shading reflected an increase in chlorophyll content while nitrogen remained relatively constant (Table 1). In addition, lower A_{max} in shade-treated foliage (Table 1, Fig. 2) indicates that soluble photosynthetic proteins may also be lower than in sun controls even though total nitrogen was relatively similar (Fig. 1). Based on Evans' work, these changes indicate that the internal allocation of nitrogen to the thylakoid membranes increased under shading such that it was similar to the nitrogen allocation pattern in true shade foliage. A mechanism reallocating nitrogen within the leaf in response to a change in light environment would be a logical consequence if plants function according to the optimization theory in resource allocation (Bloom et al. 1985; Field et al. 1992).

Although the chlorophyll-to-nitrogen ratio can change relatively quickly in response to changes in light, and

shifts seasonally, other studies have shown that it appears to be stable over a range of water and nutritional levels in foliage. Thompson and Wheeler (1992) found that the relationship between chlorophyll and nitrogen was stable for sun foliage (all with similar light exposure) of *Pinus radiata* over a range of nitrogen and irrigation treatments that resulted in a large range of nitrogen and chlorophyll levels. The proportion of nitrogen in thylakoid proteins for *A. amabilis* is generally lower than for other plants; Evans (1989) found the mean value for a range of sun plants to be 19%, whereas shade plants allocated approximately 38% of the nitrogen to thylakoid proteins. This difference may be due to the longevity of *A. amabilis* foliage and high-elevation stresses; more nitrogen may be associated with structural material or compounds not involved with photosynthesis.

The chlorophyll *a:b* ratio was also very responsive to changes in light. Increases in chlorophyll *b* relative to *a* could indicate an increase in antenna size since chlorophyll *b* is found mainly in the light-harvesting antenna, while chlorophyll *a* is found in the light-harvesting complex and the primary antenna of both photosystems (Baker and Markwell 1985). A decrease in the chlorophyll *a:b* ratio is correlated with a decrease in the activity of the photosystems, and an increased amount of secondary antenna associated with the photosystems (Anderson et al. 1988). Such changes in the chlorophyll *a:b* ratio could indicate that changes in the chlorophyll-to-nitrogen ratio were not completely due to shifts in nitrogen allocation within the leaf but were also related to an increase in antenna size of the photosynthetic unit.

The decrease in A_{max} and shifting of internal ratios appears to be the primary component of light acclimation in *A. amabilis* foliage and they occur relatively rapidly in response to shading. However, there is also a second component that involves the distribution of nitrogen within the whole plant, and occurs much more slowly than changes in the internal ratios. Field and Mooney (1986) have hypothesized that the optimal distribution for nitrogen within the plant would be for leaves in the highest-light environments to have the highest nitrogen content and leaves in low light to have low concentrations of nitrogen. *A. amabilis* foliage from high- and low-light environments shows such a pattern (Brooks 1993). However, in this experiment the nitrogen content of the shade-treated sun foliage (S only) was only slightly lower than the controls, and not nearly as low as in true shade foliage.

It seems likely that the formation of such gradients in nitrogen occurs over a longer period than that used in this study. Although nitrogen flows passively with either carbohydrates or water, there is no clear mechanism for rapid nitrogen movement along its own source-sink gradient. In the early spring, nutrients in the xylem are carried in the transpiration stream and accumulate in the transpiring leaves; then, when bud break occurs, these nutrients are retranslocated from the mature leaves to the phloem along with carbon (Pate 1980). Later in the growing season, additional nutrients from the transpiration stream accumulate in the transpiring leaves, replacing those that were lost during budbreak. Thus foliar P and N concentrations fluctuate annually; increasing in the spring, declining during new shoot expansion and increasing again after shoot growth has stopped. This cycling of nutrients in evergreen conifers may significantly alter the relationship of nitrogen and photosynthesis, and the degree to which changes in nitrogen content reflect acclimation. This is particularly true in this study since photosynthesis changed rapidly in response to shading. One summer of shading after 5–8 years of growth probably did not significantly change the relative distribution of nitrogen to the foliage. As a result, a mechanism regulating total nitrogen allocated to a leaf may be slower to respond to changes in light than a mechanism regulating how that nitrogen is allocated within the leaf.

Acclimation versus the ageing process

One interpretation for the changes observed here in response to shading could be that shading accelerates the ageing process so that the foliage is not acclimating but ageing faster. If the shade-related changes observed in this study resulted from an increased rate of ageing, then all of the changes should indicate a decline and slow degradation of leaf function typical of the ageing process (Chabot and Hicks 1982). Since *A. amabilis* is an evergreen, seasonal changes in leaf function may confound the ageing process. Photosynthesis and nitrogen content did not decline in shade-treated foliage, but they did not increase through the season as they did in the controls. One could argue that this could be accelerated ageing influenced by a seasonal response. However, chlorophyll increased in shade-treated foliage; a decrease in chlorophyll content would be expected in an ageing leaf. Another difference between ageing and acclimation is that age-related changes are irreversible (Leopold 1975), but not changes associated with acclimation. Although we did not try to reverse the process, other studies have observed that older foliage can reverse seemingly age-related changes when returned to a brightly illuminated environment (Burkey and Wells 1991; Gauhl 1976). Burkey and Wells (1991) found a reversal in a recent study with soybeans; they noted that older sun-formed foliage, shaded by the developing canopy could increase photosynthesis and other parameters in response to an increase in light. Thus, we attribute the changes in response to shading to a mechanism of acclimation and not to accelerated ageing.

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