

Convergence of leaf display and photosynthetic characteristics of understory *Abies amabilis* and *Tsuga heterophylla* in an old-growth forest in southwestern Washington State, USA

HIROAKI ISHII,^{1,2} KEN-ICHI YOSHIMURA¹ and AKIRA MORI^{3,*}

¹ Graduate School of Agricultural Science, Kobe University, Kobe 657-8501, Japan

² Corresponding author (hishii@alumni.washington.edu)

³ School of Resource and Environmental Management, Simon Fraser University, Burnaby, BC V5A 1S6, Canada

Received January 8, 2009; accepted May 21, 2009; published online June 12, 2009

Summary We compared the morphological and physiological characteristics of understory trees of *Abies amabilis* (Dougl. ex Loud.) Dougl. ex J. Forbes and *Tsuga heterophylla* (Raf.) Sarg. growing adjacent to each other in an old-growth forest in southwestern Washington State, USA. We hypothesized that, despite contrasting branching patterns and crown architectures, the two species should exhibit convergence in leaf display and photosynthetic gain per light intercepting area, because these are important properties determining their survival in the light-limited understory. The branching pattern of *A. amabilis* was regular (normal shoot-length distribution, less variable branching angle and bifurcation ratio), whereas that of *T. heterophylla* was more plastic (positively skewed shoot-length distribution, more variable branching angle and bifurcation ratio). The two species had similar shoot morphologies: number of leaves per unit shoot length and leaf to axis dry mass ratio. Leaf morphology, in contrast, was significantly different. Leaves of *A. amabilis* were larger and heavier than those of *T. heterophylla*, which resulted in lower mass-based photosynthetic rate for *A. amabilis*. Despite these differences, the two species had similar levels of leaf overlap and area-based photosynthetic characteristics. Needle longevity of *A. amabilis* was nearly twice that of *T. heterophylla*. The leaf N contents of current and 1-year-old leaves were lower for *A. amabilis* than for *T. heterophylla*. However, the leaf N content of *A. amabilis* did not change from current leaves to 6-year-old leaves, whereas that of *T. heterophylla* decreased with increasing leaf age. *Abies amabilis* had deeper crowns than *T. heterophylla* and retained branches with low relative growth rates. Longer branch retention may compensate for the lower branch-level assimilation rate of *A. amabilis*.

We inferred that the convergence of leaf display and photosynthetic characteristics between *A. amabilis* and *T. heterophylla* may contribute to the persistence of both species in the understory of this forest.

Keywords: carbon economy, photosynthesis, shade tolerance.

Introduction

An important factor determining the survival of plants in the forest understory is light interception efficiency. Minimizing leaf overlap and maximizing photosynthetic gain per light intercepting area are critical for survival in the light-limited understory (Percy and Valladares 1999, Valladares and Niinemets 2007). Shade-tolerant understory species, in common have several morphological characteristics such as greater leaf area per plant mass (leaf area ratio, LAR), less root mass per plant mass (root mass fraction) and monolayer crown architecture (Kohyama 1980, O'Connell and Kelly 1994, Ishii and Takeda 1997, Duchesneau et al. 2001, Valladares et al. 2002, Mori and Takeda 2004). In addition, physiological characteristics that increase carbon-use efficiency such as minimizing maintenance respiration, dark respiration rate of leaves and maximizing photosynthetic rate in low light are considered important for survival in the shaded understory (Percy 2007, Valladares and Niinemets 2008).

In the mid-elevation, old-growth coniferous forests of the Pacific northwest coast of North America, saplings and small trees of *Abies amabilis* (Dougl. ex Loud.) Dougl. ex J. Forbes and *Tsuga heterophylla* (Raf.) Sarg. often occur adjacent to each other in the understory (Franklin and Dyrness 1973). Both species are able to regenerate and persist in the understory forming populations with stable size structures (Franklin and DeBell 1988, Hannon and Franklin 1989, Parish and Antos 2006). When canopy gaps

* Present address: Department of Environment and Information Sciences, Yokohama National University, Yokohama 240-8501, Japan.

are formed, understory trees of both species are released, resulting in advance regeneration (Gray 1997, VanPelt and Franklin 1999). Persistence and advance regeneration play an important role in maintaining the populations of shade-tolerant species in old-growth coniferous forests of this region (Messier et al. 1999, Antos et al. 2005, Parish and Antos 2006).

The two species have contrasting rules of architectural development (i.e., shoot elongation and branching pattern). *Abies amabilis* has deterministic shoot elongation (Kozłowski 1964) and regular branching pattern (Ishii et al. 2003, Mori et al. 2008). In contrast, *T. heterophylla* has indeterminate shoot elongation and less regular branching pattern (O'reilly et al. 1994, Ishii et al. 2003). Despite these differences, understory trees of the two species often occur adjacent to each other and exhibit similar architectural characteristics, such that leaves are arranged with minimal overlap within shoots and branches (Figure 1).

In the forestry literature, *A. amabilis* is considered to be the more shade tolerant than *T. heterophylla* (Minore 1979). However, recent reevaluation has indicated that the two species may be equally shade tolerant (Niinemets and Valladares 2006). For canopy trees and saplings growing in open environments, maximum photosynthetic rates are lower for *A. amabilis* than for *T. heterophylla* (Bond et al. 1999, Lewis et al. 2000, Mitchell 2001, Renninger et al. 2007). This difference may be due to the differences in leaf morphology and longevity. *Abies amabilis* has more leaf mass per area (LMA) and longer leaf longevity than *T. heterophylla* (Ishii et al. 2003). When compared across species, these characteristics are associated with lower leaf photosynthetic rate (Kloeppel et al. 2000, Wright et al. 2004).

In this study, we compared the morphological and physiological characteristics between adjacent understory

trees of *A. amabilis* and *T. heterophylla* in an old-growth forest in southwestern Washington State, USA. We postulated that, despite having inherently different rules of architectural development and maximum photosynthetic rate, understory trees of the two species should exhibit convergence in leaf display and photosynthetic gain per light intercepting area, because these are important properties determining their survival in the light-limited understory. We compared shoot and leaf morphology, leaf display, mass- and area-based photosynthetic rates, leaf longevity, leaf N content and growth rates between adjacent trees of the two species to infer mechanisms allowing persistence of the two species in the understory of this forest.

Materials and methods

Study site and species

This study was conducted at the Wind River Canopy Crane Research Facility (WRCCRF), which is located in the Thornton T. Munger Research Natural Area (TTM-RNA), Gifford Pinchot National Forest, southwestern Washington State, USA (45°49' N and 121°57' W; 371 m elevation). The TTM-RNA is located in the transient snow zone of the Cascade Mountains. Average annual temperature is 8.7 °C, with average monthly temperatures ranging from 0.1 °C in January to 17.7 °C in July. Annual precipitation is 2223 mm, with much of it falling as rain and snow in winter. The snow depth during December through March is over 1 m (Shaw et al. 2004). The TTM-RNA is a 500-year-old old-growth forest dominated by *Pseudotsuga menziesii* (Mirb.) Franco var. *menziesii* and *T. heterophylla*. Other tree species in the stand include (in order of basal area) *Thuja plicata* Donn ex D. Don, *Taxus brevifolia* Nutt., *A. amabilis* and *Abies grandis* (Dougl. ex D. Don) Lindl. Shaw et al. (2004) gave a detailed description of the vegetation and ecological setting.

The two study species, *A. amabilis* and *T. heterophylla*, are the dominant species in the understory, contributing 27% and 41%, respectively, of the total number and 14% and 29% of the total basal area of trees shorter than 5 m (Ishii et al. 2000). Both species are represented in all size classes from understory to canopy trees and regenerate well in the understory (Shaw et al. 2004).

We chose six pairs of adjacent, similar-sized trees of *A. amabilis* and *T. heterophylla* for this study (Table 1). We chose pairs of trees growing in dark, closed canopy, understory conditions within a 1-ha area south of the 10-ha canopy crane plot (Chen et al. 2004). We took hemispherical photographs above each tree to quantify the light environment and it was determined that there was no difference among the sample trees [Two-way analysis of variance (ANOVA), location $\times$ species, $F = 6.244$, $P = 0.067$]. One sample branch section (ca. 30–50 cm long, hereafter sample branch) was chosen from a primary branch in the

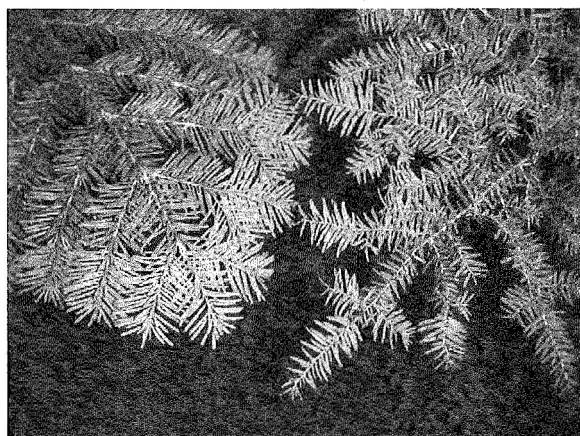


Figure 1. Plagiotropic branches of understory trees of *A. amabilis* (left) and *T. heterophylla* (right) growing adjacent to each other in the old-growth *Pseudotsuga-Tsuga* forest at WRCCRF, southwestern Washington, USA.

Table 1. Structural attributes and light environment of the six pairs of understory trees of *A. amabilis* and *T. heterophylla* in the old-growth *Pseudotsuga-Tsuga* forest at WRCCRF. Trees with the same number were located adjacent to each other.

Tree species	Tree height (cm)	Stem length (cm)	Basal diameter (mm)	Canopy openness (%)
<i>A. amabilis</i>				
1	111.3	112.3	24.3	8.84
2	132.4	133.1	38.2	8.14
3	137.3	138.5	27.2	8.73
4	68.8	76.7	17.8	7.97
5	62.1	69.8	20.9	6.90
6	68.1	73.3	13.8	8.20
Mean $\pm$ SE	96.7 $\pm$ 5.7	100.6 $\pm$ 5.2	23.7 $\pm$ 1.4	8.14 $\pm$ 0.20
<i>T. heterophylla</i>				
1	94.5	91.4	22.4	6.79
2	97.8	102.6	21.8	6.84
3	128.5	135.8	33.2	8.17
4	123.2	75.5	26.1	7.43
5	78.2	81.9	21.3	6.90
6	81.0	72.8	12.2	7.20
Mean $\pm$ SE	100.5 $\pm$ 3.5	93.3 $\pm$ 3.9	22.8 $\pm$ 1.1	7.20 $\pm$ 0.13

For trees 1, 4 and 6 of *T. heterophylla*, stem length was shorter than tree height because the highest branch pointed upward.

middle crown of each tree where the longest branches tended to occur. We chose branches that were not over-topped by other branches. Each sample branch was cut from the tree, immediately recut under water and transported to the laboratory for measurement.

Branching pattern and shoot morphology

All sample branches were plagiotropic. We drew two-dimensional branch maps to analyse the branching pattern. We measured the length of each annual increment and the horizontal branching angle and bifurcation ratio (Steingraeber et al. 1979) at each junction.

We used image analysis to quantify leaf overlap within the branches. Each sample branch was laid on a slide viewer, illuminated from below and photographed with high contrast to obtain the silhouette image for measurement of branch silhouette area (SA_B). All leaves were then removed from the sample branch, mixed thoroughly and a subsample was taken for the measurement of leaf area. All leaves in the subsample were laid without overlap on a slide viewer, flattened with a piece of transparent acrylic board, illuminated from below and photographed at high contrast to obtain the projected image for the measurement of leaf area. All leaves in the subsample were then oven-dried to constant weight at 65°C to obtain the leaf area to dry mass ratio. This ratio was used to estimate the total leaf area of the sample branch (LA_B) from the total leaf dry mass.

Five to seven 1-year-old growth increments (hereafter shoots) at the distal end of the secondary axes (considering the branch main axis as the primary axis) were cut from each sample branch for measurement of shoot and leaf morphology. We measured the length of each shoot (L_s) using digital calipers to the nearest 0.1 cm. The shoot was then laid on a slide viewer, illuminated from below and

photographed with high contrast to obtain the silhouette image for measurement of shoot silhouette area (SA_s). All leaves on the shoot were removed, laid flat on the slide viewer without overlap, flattened with a piece of transparent acrylic board, illuminated from below and photographed at high contrast to obtain the projected image for measurement of leaf area (LA_s).

All photographs were taken using a digital camera (Nikon Coolpix 4500, Nikon Corp., Tokyo, Japan) at 4.5 Megapixel resolution, following methods described in the work by Niinemets (2005). All images were analysed using image analysis software (ImageJ 1.34, National Institute of Health, <http://rsb.info.nih.gov/ij/>). We also used the images of the shoots to obtain the total number of leaves per shoot (n_L). All leaves and shoot axes were then oven-dried to constant weight at 65°C to obtain the total dry mass (M_s , including both leaves and shoot axis) and leaf dry mass (M_L) of each shoot.

Photosynthesis

We measured the photosynthetic light response of 1-year-old shoots on sample branches from trees 1, 2 and 3 of both species. From each sample branch, we chose three shoots at the distal end of secondary axes for measurement. The shoots were cut from the sample branch under water and the base of the shoot was sealed in a silicone tube attached to a reservoir of water placed 50 cm above the shoot. We measured photosynthetic rates of the cut shoots using the LI-6400 portable gas exchange system fitted with a conifer chamber (LI-6400-05, Li-Cor Inc., Lincoln, NE). Air temperature and CO₂ concentration in the cuvette were maintained at 25°C and 370 mmol mol⁻¹, respectively. An external halogen-type light source (MHF-M1001, Moritex Inc., Japan) was used to provide up to

1500 $\mu\text{mol m}^{-2} \text{s}^{-1}$ of photosynthetic photon flux density (PPFD). We varied the light intensity from maximum to zero to obtain the photosynthetic light curve and dark respiration rate of each shoot. After measurement of photosynthesis, SA_s , LA_s , M_s and M_L were measured for each shoot following the procedures described above.

Leaf longevity, N content and growth rate

To obtain the age structure of leaves, we aged and counted all foliated annual increments in the sample branch, divided them into age classes and measured the dry mass of leaves in each age class after oven-drying at 65°C to constant weight. Mean leaf age was calculated by weighting the age of each cohort by their relative contribution to total leaf mass of the sample branch. A static life table of the leaf population was created based on the unit leaf dry mass per centimeter of axis length, and life expectancy (e_x) was calculated for each age class following calculations described in the work of Begon and Mortimer (1981).

A subsample of the dried leaves in each age class was used to measure the N content of the leaves. We measured the N content of current leaves to 13-year-old leaves of *A. amabilis* and current leaves to 4-year-old leaves of *T. heterophylla*. Two replicate samples (ca. 5–7 mg) were ground to fine powder and the N content was measured using the NC analyser (Sumigraph NC-900, Sumika Chemical Analysis Service Ltd., Tokyo, Japan). Values obtained from the two replicate samples were averaged to obtain the N content for each leaf age class.

After all measurements of morphology and photosynthesis were completed, we numbered each primary branch attached to the main stem and measured its distance from the ground along the main stem (branch position). Then, we removed all the branches from the main stem and partitioned them into current-year growth increments and older branch sections. The dry mass of each partition was determined after oven-drying to constant weight at 65°C.

Data analysis

We calculated the number of leaves per unit shoot length (n_L/L_s) as a measure of leaf packing along the shoot axis. We used the dry mass fraction of leaves relative to the shoot (LMF_s) as a measure of relative dry matter allocation to photosynthetic versus respiratory biomass within shoots:

$$\text{LMF}_s = M_L/M_s. \quad (1)$$

We also calculated mean area and mass of individual leaves (A_l and M_l , respectively) as measures of leaf size:

$$A_l = \text{LA}_s/n_L, \quad (2)$$

$$M_l = M_L/n_L. \quad (3)$$

To quantify the leaf overlap at the branch and shoot levels, we calculated the silhouette to leaf area ratio of the sample branches and shoots (SPAR_B and SPAR_s , respectively):

$$\text{SPAR}_B = \text{SA}_B/\text{LA}_B, \quad (4)$$

$$\text{SPAR}_s = \text{SA}_s/\text{LA}_s. \quad (5)$$

The silhouette to leaf area ratio has been interpreted as measures of the efficiency of leaf display and light interception (Oker-Blom and Smolander 1988, Leverenz et al. 2000).

To quantify the dry mass investment relative to light capturing area of shoots and leaves, we calculated shoot mass per area (SMA) and LMA (Niinemets and Kull 1995a, 1995b, Ishii et al. 2007):

$$\text{SMA} = M_s/\text{SA}_s, \quad (6)$$

$$\text{LMA} = M_L/\text{LA}_s. \quad (7)$$

Photosynthesis measurements were converted to mass- and area-based rates by dividing the net photosynthetic rates of each shoot by M_L , SA_s and LA_s . Photosynthetic light response curves were obtained by fitting the following equation to the relationship between light intensity and net photosynthetic rate (P) for each shoot (Johnson and Thornley 1984):

$$P = \frac{\phi I + P_{\max} - \sqrt{(\phi I + P_{\max})^2 - (4\theta\phi I P_{\max})}}{2\theta} - R, \quad (8)$$

where I is the light intensity (PPFD, $\mu\text{mol m}^{-2} \text{s}^{-1}$) and R is the observed dark respiration rate ($\mu\text{mol CO}_2 \text{m}^{-2} \text{s}^{-1}$). Parameters ϕ (quantum yield, mol mol^{-1}), $P_{\max}$ (maximum rate of net photosynthesis, $\mu\text{mol CO}_2 \text{m}^{-2} \text{s}^{-1}$) and θ (convexity of the photosynthetic response curve) were estimated using nonlinear least squares regression in Systat 9.0 (SPSS, Chicago, IL).

We calculated the following measures of branch-level dry mass allocation and growth rate. For each sample branch, we calculated the leaf mass and leaf area per branch mass (LMF_B and LAR_B , respectively). The relative growth rate of each branch (RGR_B) was calculated as the ratio of the dry mass of current-year growth increments to that of older branch sections. Although RGR_B does not take into account the diameter growth of branches, previous studies have indicated that there is very little diameter growth in suppressed understory trees of conifer species (Wright et al. 1998, Parent et al. 2002, Antos et al. 2005, Kobe 2007, Mori et al. 2008).

We compared branch-level assimilation rate between the two species by calculating the maximum rate of assimilation ($A_{\max,B}$). We assumed that $P_{\max}$ decreased with increasing leaf age in proportion with leaf N content. We calculated the mass-based $P_{\max}$ for each leaf age class from the measured $P_{\max}$ of 1-year-old leaves and N content of each age class. The calculated $P_{\max}$ for each age class was multiplied by the total leaf dry mass of the age class for each branch. This was summed for each branch and divided

by total branch dry mass to obtain $A_{max,B}$. We used this value to represent the potential maximum rate of assimilation for all leaves on the branch, considering that it does not take into account the branch maintenance respiration, which was not measured in this study.

We compared branching pattern, morphology, photosynthetic characteristics, leaf longevity, leaf N content and measures of biomass allocation and growth rate between the two species in an ANOVA with a randomized block design, where species was considered as the main effect and pairs of trees (location) as blocks. Shoots were considered as replicates within each tree.

Results

Branching pattern and morphology

The two species had contrasting branching patterns (Figure 2). The frequency distribution of shoot lengths for *A. amabilis* was normal (mean = 2.63 cm). In contrast, the frequency distribution of shoot lengths for *T. heterophylla* was positively skewed such that 37.5% of the shoots were shorter than 1 cm. Branching angle and bifurcation ratio were both smaller ($F = 37.76$, $P < 0.01$ and $F = 19.71$, $P < 0.01$, respectively) and less variable for *A. amabilis* than for *T. heterophylla*.

The two species had similar shoot morphologies (Table 2), with similar values for n_L/L_s and LMF_s . Leaf morphology, in contrast, was significantly different. *Abies amabilis* had larger and heavier leaves than *T. heterophylla*.

The two species had similar $SPAR_B$ and $SPAR_s$ values indicating that they had similar levels of leaf overlap within branches and shoots. However, because leaves of *A. amabilis* had more mass than those of *T. heterophylla*, SMA and LMA were both greater for *A. amabilis* indicating that *A. amabilis* invested more mass per leaf area than *T. heterophylla*.

Photosynthesis

Mass-based rates of net photosynthesis were lower for *A. amabilis* than *T. heterophylla* at all light levels and mass-based R_d and P_{max} of *A. amabilis* were lower than those of *T. heterophylla* indicating that *A. amabilis* had lower dark respiration rate, but also less photosynthetic gain per dry mass invested in foliage (Table 3). However, both leaf-area- and shoot-silhouette-area-based photosynthetic characteristics of the two species were similar.

Leaf longevity, N content and growth rate

Leaf longevity was longer for *A. amabilis* than for *T. heterophylla* (Figure 3). Maximum observed leaf ages were 13 and 7 years for *A. amabilis* and *T. heterophylla*, respectively. Mean leaf age and life expectancy of current-year leaves (e_0) were about three times longer for *A. amabilis* than for *T. heterophylla*.

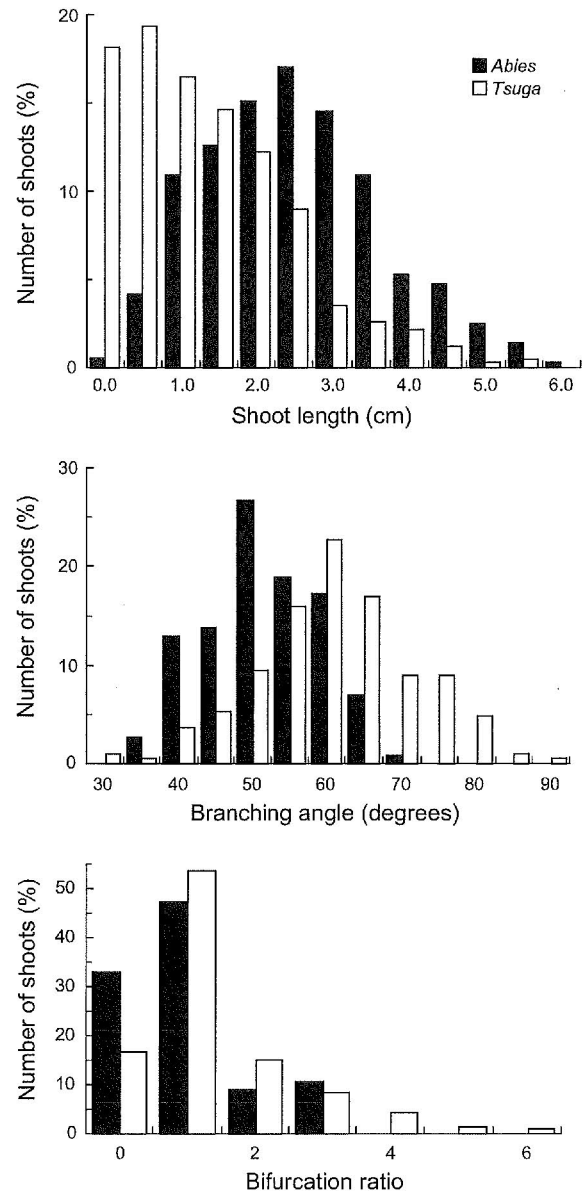


Figure 2. Relative frequency distributions of shoot length, branching angle and bifurcation ratio, of the understory trees of *A. amabilis* (filled bars) and *T. heterophylla* (open bars) in the old-growth *Pseudotsuga-Tsuga* forest at WRCCRF, southwestern Washington, USA. The data for all sample branches were pooled to obtain the overall distributions for each species.

The leaf N content of current leaves and 1-year-old leaves was lower for *A. amabilis* than for *T. heterophylla* (Figure 4). Leaf N content of *A. amabilis* did not change from current leaves to 6-year-old leaves, whereas that of *T. heterophylla* decreased with increasing leaf age.

Abies amabilis had deeper crowns with more numerous primary branches than *T. heterophylla* (Figure 5). The crown ratio of *A. amabilis* was as much as 0.6 of tree height,

Table 2. Morphological characteristics of understory trees of *A. amabilis* and *T. heterophylla* in the old-growth *Pseudotsuga*–*Tsuga* forest at WRCCRF. Values are the mean $\pm$ SE for the six study trees of each species.

	<i>A. amabilis</i>	<i>T. heterophylla</i>
<i>Shoot morphology</i>		
n_L/L_S	12.33 $\pm$ 0.417	11.48 $\pm$ 0.180
LMF _S	0.883 $\pm$ 0.002	0.881 $\pm$ 0.003
<i>Leaf morphology</i>		
A_1	0.23* $\pm$ 0.02	0.13 $\pm$ 0.02
M_1	2.45* $\pm$ 0.16	0.92 $\pm$ 0.02
<i>Leaf display</i>		
SMA	120.1 $\pm$ 3.2	78.0* $\pm$ 3.0
LMA	107.2* $\pm$ 3.2	68.6 $\pm$ 1.7
SPAR _B	0.871 $\pm$ 0.015	0.894 $\pm$ 0.033
SPAR _S	0.893 $\pm$ 0.007	0.898 $\pm$ 0.010

ANOVA, * $P < 0.01$.

Table 3. Mass- ($\mu\text{mol CO}_2 \text{ g}^{-1} \text{ s}^{-1}$) and area-based ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) photosynthetic characteristics of 1-year-old shoots of understory *A. amabilis* and *T. heterophylla* trees in the old-growth *Pseudotsuga*–*Tsuga* forest at WRCCRF. Values are the mean $\pm$ SE of the six study trees of each species; $n = 9$ shoots for each species.

	<i>A. amabilis</i>	<i>T. heterophylla</i>
<i>Leaf mass based</i>		
R	3.10* $\pm$ 0.11	5.18 $\pm$ 0.06
ϕ	0.47* $\pm$ 0.09	1.05 $\pm$ 0.11
P_{max}	32.1* $\pm$ 3.7	51.9 $\pm$ 3.8
<i>Leaf area based</i>		
R	0.34 $\pm$ 0.02	0.38 $\pm$ 0.02
ϕ	0.094 $\pm$ 0.005	0.076 $\pm$ 0.008
P_{max}	3.58 $\pm$ 0.47	3.78 $\pm$ 0.21
<i>Shoot silhouette area based</i>		
R	0.40 $\pm$ 0.02	0.43 $\pm$ 0.01
ϕ	0.080 $\pm$ 0.004	0.085 $\pm$ 0.008
P_{max}	4.07 $\pm$ 0.46	4.36 $\pm$ 0.33

ANOVA, * $P < 0.01$.

whereas that of *T. heterophylla* was less. For all trees of *A. amabilis*, RGR_B decreased with decreasing branch position and there were several branches with no current-year growth ($\text{RGR}_B = 0$). In contrast, for *T. heterophylla*, RGR_B varied less with branch position, with the exception of tree 1, where it decreased with decreasing branch position. There were no branches of *T. heterophylla* lacking current-year growth.

Branch biomass allocation and potential carbon gain differed between the two species. The LMF_B and LAR of branches were lower for *A. amabilis* than for *T. heterophylla* (Table 4). Calculated values of $\text{A}_{\text{max},B}$ were lower for *A. amabilis* than for *T. heterophylla*.

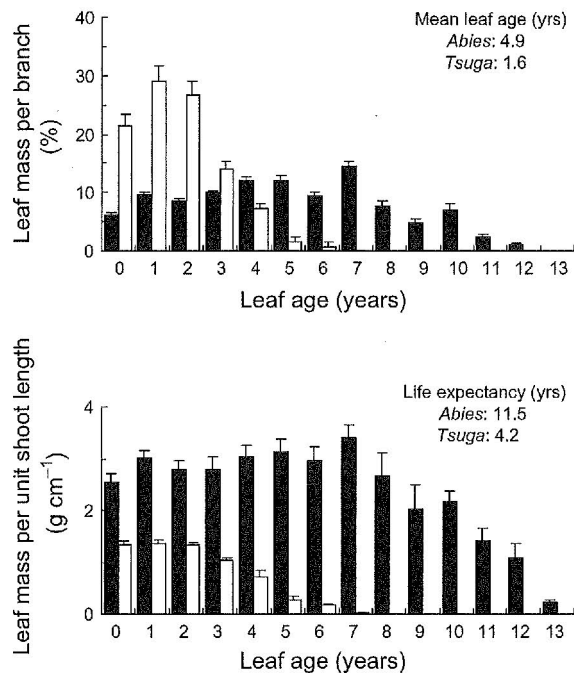


Figure 3. Dry mass distribution across leaf age classes for branches of the understory trees of *A. amabilis* (filled bars) and *T. heterophylla* (open bars) in the old-growth *Pseudotsuga*–*Tsuga* forest at WRCCRF, southwestern Washington, USA. Bars indicate mean values per sample branch. Error bars indicate one standard error.

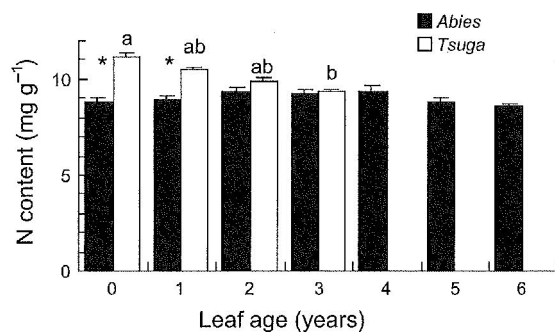


Figure 4. Nitrogen content of leaves of the understory trees of *A. amabilis* (filled bars) and *T. heterophylla* (open bars) in the old-growth *Pseudotsuga*–*Tsuga* forest at WRCCRF, southwestern Washington, USA. Error bars indicate one standard error. Asterisk indicates significant difference between *A. amabilis* and *T. heterophylla* ($P < 0.05$). Open bars labelled with the same letter are not significantly different ($P > 0.05$).

Discussion

We found that, despite having different inherent branching patterns, understory trees of *A. amabilis* and *T. heterophylla*

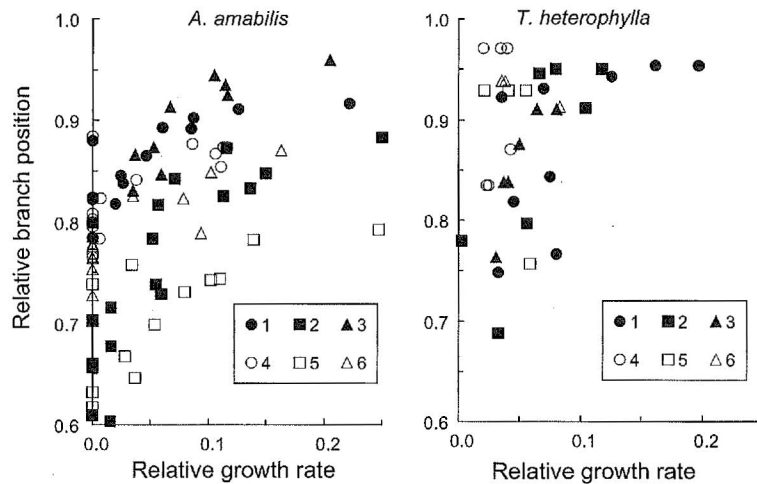


Figure 5. Relative growth rate of branches in relation to relative branch position along the main stem (distance from ground/stem length) for the understory trees of *A. amabilis* and *T. heterophylla* in the old-growth *Pseudotsuga*–*Tsuga* forest at WRCCRF, southwestern Washington, USA. Paired trees located adjacent to each other have common symbols across species.

Table 4. Branch- and tree-level biomass allocation and relative growth rate of understory trees of *A. amabilis* and *T. heterophylla* in the old-growth *Pseudotsuga*–*Tsuga* forest at WRCCRF. Values are the mean $\pm$ SE for the six study trees of each species. The relative growth rates of branches are shown in Figure 5.

Species	<i>A. amabilis</i>	<i>T. heterophylla</i>
<i>Branch level</i>		
LMF _B	0.59* $\pm$ 0.01	0.70 $\pm$ 0.01
LAR	47.2* $\pm$ 1.3	93.8 $\pm$ 1.8
A _{max,B}	0.78** $\pm$ 0.01	1.45 $\pm$ 0.02

ANOVA, * $P < 0.05$, ** $P < 0.01$.

show convergence in characteristics that determine light interception efficiency. *Abies amabilis* avoids mutual shading among leaves by laying out shoots in a regular branching pattern (i.e., normal shoot-length distribution, less variable branching angle and bifurcation ratio). In contrast, the branching pattern of *T. heterophylla* was more plastic (i.e., positively skewed shoot-length distribution, more variable branching angle and bifurcation ratio) and numerous small shoots ($L_S < 1$ cm) filled in spaces between fewer large shoots. However, as indicated by similar values of SPAR_B, there was no quantitative difference in the degree of leaf area overlap between the two species, suggesting convergence in leaf display.

At the leaf level, efficient carbon gain in low light can be realized by minimizing LMA to maximize light intercepting area per biomass investment and minimizing dark respiration rate to lower the photosynthetic light compensation point (Craine and Reich 2005, Pearcy 2007). In this study, the pattern of biomass allocation at the shoot level was similar between the two species as indicated by similar values of n_L/L_S and LMF_S. There were, however, significant differences in leaf morphology and leaf longevity. *Abies amabilis*

produced larger leaves with higher LMA and longer leaf longevity than *T. heterophylla*. As a result, 1-year-old leaves of *A. amabilis* had lower mass-based photosynthetic rate, as well as dark respiration rate. However, there was no difference in area-based photosynthetic characteristics between the two species, suggesting convergence in photosynthetic gain per light intercepting area.

The negative correlation between photosynthetic capacity and leaf longevity has been observed for various species across a wide range of biomes (Reich et al. 1999, Wright et al. 2004). Theoretical cost-benefit analysis (Kikuzawa 1991) and empirical evidence (Kloeppel et al. 2000) indicate that initial mass investment and the rate of functional decline determine leaf longevity such that leaves with greater mass show slower decline in photosynthetic capacity and are retained longer. Although we did not measure photosynthetic rates of older leaves, higher N concentration in older leaves of *A. amabilis* suggested that photosynthetic rate declines more slowly with leaf age for *A. amabilis* than for *T. heterophylla*. Hence, we inferred that, despite lower mass-based photosynthetic rate, *A. amabilis* obtains sufficient time-integrated carbon gain for higher mass investment in leaves by maintaining leaves longer. On the other hand, *T. heterophylla* produces leaves with less mass and faster functional decline. The mass-based photosynthetic rate of new leaves of *T. heterophylla* is high, suggesting that there is sufficient photosynthetic gain despite short leaf longevity.

The pattern of biomass allocation and growth at the branch level supported trends observed at the leaf level. Branches of *A. amabilis* were retained longer than those of *T. heterophylla*, as indicated by higher crown ratio. *Abies amabilis* maintained numerous branches with no current-year growth ($RGR_B = 0$). Branches of *A. amabilis* also had lower LMF_B and LAR than those of *T. heterophylla*, which indicated that *A. amabilis* invests more biomass in

support tissue than *T. heterophylla*. These results imply greater maintenance respiration and lower net carbon gain at the branch level for *A. amabilis*. Increasing leaf longevity may compensate for greater maintenance respiration if leaf photosynthetic functions are maintained longer (Kikuzawa 1991, Lusk 2004). Our calculations suggested that at the branch level, the maximum rate of assimilation ($A_{max,B}$) for *A. amabilis* was about half that of *T. heterophylla*. Longer branch retention may compensate for the lower branch-level assimilation rate of *A. amabilis*. More detailed measurements of the branch- and tree-level carbon budget are needed to clarify whether net carbon gain at the tree level is similar between the two species.

Shade tolerance is defined as 'the minimum light under which a plant can survive' and depends on the efficiency of carbon gain in low light (Valladares and Niinemets 2008). In terms of tree architecture, this involves maximizing leaf area per plant mass and minimizing leaf overlap (Valladares and Niinemets 2007). Adaptive responses to the shaded environment of the understory lead to convergence in light interception characteristics, such as crown architecture and leaf display, among coexisting species (Sterck et al. 2003, Pearcy et al. 2004). For example, despite contrasting crown architectures and leaf habits, tropical understory plants have similar efficiency of leaf display and light absorption (Poorter and Werger 1999, Valladares et al. 2002). Shade-tolerant conifers are characterized by high LAR and monolayer crown architecture consisting of plagiotropic branches and shoots with high SPAR (Kohyama 1980, O'Connell and Kelty 1994, Leverenz 1996). Although species vary widely in morphological and architectural characteristics, functional convergence (*sensu* Meinzer 2003) may be realized through different mechanisms such as coordination between crown architecture and phyllotaxy to maximize light interception per leaf area (Valladares et al. 2002) or among LMA, LMF and net assimilation rate to maintain positive RGR (Reich et al. 1998).

Despite inherent differences in branching pattern, the small understory trees of *A. amabilis* and *T. heterophylla* in this study showed convergence in leaf display and area-based photosynthetic rates. In addition, the trade-offs between mass-based photosynthetic rate and leaf longevity, and between branch-level assimilation rate and branch retention suggest that time-integrated net carbon gain at the tree level may be similar for both species. For shade-tolerant species, persistence of saplings and small trees in the understory is important for maintaining stable populations as well as for advanced regeneration after disturbance (Antos et al. 2005, Parish and Antos 2006, Woods 2008). Functional convergence of leaf display and photosynthetic characteristics between small understory trees of *A. amabilis* and *T. heterophylla* found in this study may contribute to the long-term persistence and coexistence of the two species in the understory of this forest.

Acknowledgments

This study was conducted at the Wind River Canopy Crane Research Facility, a cooperative scientific venture among the University of Washington, the US Forest Service Pacific Northwest Research Station and the US Forest Service Gifford Pinchot National Forest. We thank the WRCCRF staff, E.D. Ford and members of the Canopy Dynamics Laboratory, CFR, UW for facilitating the study. We also thank Drs. T. Saito and E. Nabeshima for assistance with field work and Drs. T. Mizoguchi and N. Makita of FFPRI Kansai Branch for help with N analysis. This research was funded by grants from the Nissan Science Foundation, Hyogo Science and Technology Association, Asahi Beer Foundation, The Interdisciplinary Research Institute of Environmental Science and the Japanese Ministry of Education, Culture, Science, Sports and Technology (Grant-in-Aid for Young Scientists (B) 20780118).

References

- Antos, J.A., H.J. Guest and R. Parish. 2005. The tree seedling bank in an ancient montane forest: stress tolerators in a productive habitat. *J. Ecol.* 93:536–543.
- Begon, M. and M. Mortimer. 1981. *Population ecology: a unified study of animals and plants*. Blackwell Scientific Publications, London.
- Bond, B.J., B.T. Farnsworth, R.A. Coulombe and W.E. Winner. 1999. Foliage physiology and biochemistry in response to light gradients in conifers with varying shade tolerance. *Oecologia* 120:183–192.
- Chen, J., K.T. Paw U, S.L. Ustin, T.H. Suchanek, B.J. Bond, K.D. Brosofske and M. Falk. 2004. Net ecosystem exchanges of carbon, water, and energy in young and old-growth Douglas-fir forests. *Ecosystems* 7:534–544.
- Craine, J.M. and P.B. Reich. 2005. Leaf-level light compensation points in shade-tolerant woody seedlings. *New Phytol.* 166:710–713.
- Duchesneau, R., I. Lesage, C. Messier and H. Morin. 2001. Effects of light and intraspecific competition on growth and crown morphology of two size classes of understory balsam fir saplings. *For. Ecol. Manag.* 140:215–225.
- Franklin, J.F. and D.S. DeBell. 1988. Thirty-six years of tree population change in an old-growth *Pseudotsuga-Tsuga* forest. *Can. J. For. Res.* 18:633–639.
- Franklin, J.F. and C.T. Dyrness. 1973. *Natural vegetation of Oregon and Washington*. Oregon State University Press, Corvallis.
- Gray, A.N. 1997. Microsite controls on tree seedling establishment in conifer forest canopy gaps. *Ecology* 78:2458–2473.
- Hannon, M.E. and J.F. Franklin. 1989. Tree seedlings on logs in *Picea-Tsuga* forests of Oregon and Washington. *Ecology* 70:48–59.
- Ishii, H. and H. Takeda. 1997. Effects of the spatial arrangement of aerial stems and current-year shoots on the demography and growth of *Hydrangea hirta* in a light-limited environment. *New Phytol.* 136:443–453.
- Ishii, H., J.H. Reynolds, E.D. Ford and D.C. Shaw. 2000. Height growth and vertical development of an old-growth *Pseudotsuga-Tsuga* forest in southwestern Washington State, USA. *Can. J. For. Res.* 30:17–24.

- Ishii, H., E.D. Ford and D.G. Sprugel. 2003. Comparative crown form and branching pattern of four coexisting tree species in an old-growth *Pseudotsuga-Tsuga* forest. *Eurasian J. For. Res.* 6:99-109.
- Ishii, H., S. Kitaoka, T. Fujisaki, Y. Maruyama and T. Koike. 2007. Plasticity of shoot and needle morphology and photosynthesis of two *Picea* species with different site preferences in northern Japan. *Tree Physiol.* 27:1595-1605.
- Johnson, I.R. and J.H.M. Thornley. 1984. A model of instantaneous and daily canopy photosynthesis. *J. Theor. Biol.* 107:531-545.
- Kikuzawa, K. 1991. A cost-benefit analysis of leaf habit and leaf longevity of trees and their geographical pattern. *Am. Nat.* 138:1250-1263.
- Kloppel, B.D., B.D. Gower, J.G. Vogel and P.B. Reich. 2000. Leaf-level resource use for evergreen and deciduous conifers along a resource availability gradient. *Funct. Ecol.* 14: 281-292.
- Kobe, R.K. 2007. Sapling growth as a function of light and landscape-level variation in soil water and foliar nitrogen in northern Michigan. *Oecologia* 147:119-133.
- Kohyama, T. 1980. Growth pattern of *Abies mariesii* saplings under conditions of open-growth and suppression. *Botanical Magazine, Tokyo*, 93:13-24.
- Kozlowski, T.T. 1964. Shoot growth in woody plants. *Bot. Rev.* 30:335-392.
- Leverenz, J.W. 1996. Shade-shoot structure, photosynthetic performance in the field, and photosynthetic capacity of evergreen conifers. *Tree Physiol.* 16: 109-114.
- Leverenz, J.W., D. Whitehead and G.H. Stewart. 2000. Quantitative analyses of shade-shoot architecture of conifers native to New Zealand. *Trees* 15:4249.
- Lewis, J.D., R.B. McKane, D.T. Tingley and P.A. Beedlow. 2000. Vertical gradients in photosynthetic light response within an old-growth Douglas-fir and western hemlock canopy. *Tree Physiol.* 20:447-456.
- Lusk, C.H. 2004. Leaf area and growth of juvenile temperate evergreens in low light: species of contrasting shade tolerance change rank during ontogeny. *Funct. Ecol.* 18:820-828.
- Meinzer, F.C. 2003. Functional convergence in plant responses to the environment. *Oecologia* 134:1-11.
- Messier, C., R. Doucet, J.-C. Ruel, Y. Claveau, C. Kelly and M.J. Lechowicz. 1999. Functional ecology of advance regeneration in relation to light in boreal forests. *Can. J. For. Res.* 29:812-823.
- Minore, D. 1979. Comparative autecological characteristics of northwestern tree species - a literature review. USDA Forest Service Pacific Northwest Forest and Range Experiment Station General Technical Report, PNW-87.
- Mitchell, A.K. 2001. Growth limitations for conifer regeneration under alternative silvicultural systems in a coastal montane forest in British Columbia, Canada. *For. Eco! Manag.* 145: 129-136.
- Mori, A. and S.F. Hasegawa. 2007. Structural characteristics of *Abies mariesii* saplings in a snowy subalpine parkland in central Japan 27: 141-148.
- Mori, A. and H. Takeda. 2004. Functional relationships between crown morphology and within-crown characteristics of understory saplings of three codominant conifers in a subalpine forest in central Japan. *Tree Physiol.* 24: 661-670.
- Mori, A., E. Mizumachi and D.G. Sprugel. 2008. Morphological acclimation to understory environments in *Abies amabilis*, a shade- and snow-tolerant conifer species of the Cascade Mountains, Washington, USA. *Tree Physiol.* 28:815-824.
- Niinemets, U. 2005. Light capture efficiency decreases with increasing tree age and size in the southern hemisphere gymnosperm *Agathis australis*. *Trees* 19:177-190.
- Niinemets, U. and O. Kull. 1995a. Effects of light availability and tree size on the architecture of assimilative surface in the canopy of *Picea abies*: variation in needle morphology. *Tree Physiol.* 15:307-315.
- Niinemets, U. and O. Kull. 1995b. Effects of light availability and tree size on the architecture of assimilative surface in the canopy of *Picea abies*: variation in shoot structure. *Tree Physiol.* 15:791-798.
- Niinemets, U. and F. Valladares. 2006. Tolerance to shade, drought, and waterlogging of temperate northern hemisphere trees and shrubs. *Ecol. Monogr.* 76:521-547.
- O'Connell, B.M. and M.J. Kely. 1994. Crown architecture of understory and open-growth white pine (*Pinus strobus* L.) saplings. *Tree Physiol.* 14:89-102.
- Oker-Blom, P. and H. Smolander. 1988. The ratio of shoot silhouette area to total needle area in Scots pine. *For. Sci.* 34:894-906.
- O'reilly, C., J.N. Owens, J.T. Arnott and B.G. Dunsworth. 1994. Effects of nursery culture on morphological development of western hemlock seedlings during field establishment. 2. Survival, shoot length components, and needle length. *Can. J. For. Res.* 24:61-70.
- Parent, S., H. Morin and C. Messier. 2002. Missing growth rings at the trunk base in suppressed balsam fir saplings. *Can. J. For. Res.* 32:1776-1783.
- Parish, R. and J.A. Antos. 2006. Slow growth, long-lived trees, and minimal disturbance characterize the dynamics of an ancient, montane forest in coastal British Columbia. *Can. J. For. Res.* 36:2826-2838.
- Pearcy, R.W. 2007. Responses of plants to heterogeneous light environments. In *Functional Plant Ecology*. Eds. F. Pugnaire and F. Valladares. Taylor and Francis, New York, pp 213-257.
- Pearcy, R.W. and F. Valladares. 1999. Resource acquisition by plants: the role of crown architecture. In *Physiological Plant Ecology*. Eds. M.C. Press, J.D. Scholes and J.D. Barker. Blackwell Science, Oxford, pp 45-66.
- Pearcy, R.W., F. Valladares, U. Wright and E.L. de Paulis. 2004. A functional analysis of the crown architecture of tropical forest *Psychotria* species: do species vary in light capture efficiency and consequently in carbon gain? *Oecologia* 139:163-177.
- Poorter, L. and M.J.A. Werger. 1999. Light environment, sapling architecture, and leaf display in six rain forest tree species. *Am. J. Bot.* 86: 1464-1473.
- Reich, P.B., M.G. Tjoelker, M.B. Walters, D.W. Vanderklein and C. Buschena. 1998. Close association of RGR, leaf and root morphology, seed mass and shade tolerance in seedlings of nine boreal tree species grown in high and low light. *Funct. Ecol.* 12:327-338.
- Reich, P.B., D.S. Ellsworth, M.B. Walters, J.M. Vose, C. Gresham, J.C. Volin and W.D. Bowman. 1999. Generality of leaf trait relationships: a test across six biomes. *Ecology* 80:1955-1969.

- Renninger, H.J., F.C. Meinzer and B.L. Gartner. 2007. Hydraulic architecture and photosynthetic capacity as constraints on release from suppression in Douglas-fir and western hemlock. *Tree Physiol.* 27:33–42.
- Shaw, D.C., J.F. Franklin, K. Bible, J. Klopatek, E. Freeman, S. Grenne and G.G. Parker. 2004. Ecological setting of the Wind River old-growth forest. *Ecosystems* 7:427–439.
- Steingraeber, D.A., L.J. Kascht and D.H. Franck. 1979. Variation of shoot morphology and bifurcation ratio in sugar maple (*Acer' saccharum*) saplings. *Am. J. Bot.* 66:441–445.
- Storck, F., M. Martinez-Ramos, G. Dyer-Leal, J. Rodriguez-Velazquez and L. Poorler. 2003. The consequences of crown trails for the growth and survival of tree saplings in a Mexican lowland rainforest. *Funct. Eco!* 17:194–200.
- Valladares, F. and U. Niinemets. 2007. The architecture of plant crowns: from design rules to light capture and performance. In *Functional Plant Ecology*. Eds. F. Pugnaire and F. Valladares. Taylor and Francis, New York, pp 101–149.
- Valladares, F. and U. Niinemets. 2008. Shade tolerance, a key plant feature of complex nature and consequences. *Annu. Rev. Eco! Syst.* 39:237–257.
- Valladares, F., J.B. Skillman and R.W. Pearcy. 2002. Convergence in light capture efficiencies among tropical forest understory plants with contrasting crown architectures: a case of morphological compensation. *Am. J. Bot.* 89:1275–1284.
- VanPelt, R. and I.F. Franklin. 1999. Response of understory trees to experimental gaps in old-growth Douglas-fir forests. *Eco! Appl.* 9:504512.
- Woods, K.D. 2008. Living long by staying small: stem layering as an adaptive life-history trait in shade-tolerant tree seedlings. *Can. J. For. Res.* 38:480–487.
- Wright, E.F., rCD. Coates, C.D. Canham and P. Bartemucci. 1998. Species variability in growth response to light across climatic regions in northwestern British Columbia. *Can. J. For. Res.* 28:871–886.
- Wright, U., P.B. Reich, M. Westoby et al. 2004. The worldwide leaf economics spectrum. *Nature* 428:821–827.

Appendix

Abbreviations

<i>AI</i>	area of individual leaves (cm ²)
<i>A_{max,B}</i>	maximum rate of assimilation of branches (g C g ⁻¹ h ⁻¹)
<i>CIF</i>	dry mass ratio of shoot axis to leaves
<i>LAB</i>	total leaf area of branch (cm ²)
<i>LAs</i>	leaf area of 1-year-old shoot (cm ²)
<i>ILL/ Ls</i>	number of leaves per cm shoot length
<i>LAR</i>	leaf area ratio of branch (cm ² g ⁻¹)
<i>LMA</i>	leaf mass per area (g m ⁻²)
<i>LMFB</i>	leaf mass fraction of branches (g g ⁻¹)
<i>LMFs</i>	leaf mass fraction of shoots (g g ⁻¹)
<i>Ls</i>	length of shoot axis (cm)
<i>LL</i>	leaf length (mm)
<i>Ms</i>	dry mass of shoot (g)
<i>M.L</i>	dry mass of leaves on shoot (g)
<i>Mr</i>	dry mass of individual leaves (mg)
<i>ILL</i>	number of leaves per shoot
<i>P_{max}</i>	maximum rate of net photosynthesis (mmol CO ₂ m ⁻² s ⁻¹ or mmol CO ₂ g ⁻¹ s ⁻¹)
<i>R</i>	dark respiration rate (mmol CO ₂ m ⁻² s ⁻¹ or mmol CO ₂ g ⁻¹ s ⁻¹)
<i>RGR_B</i>	relative growth rate of branch
<i>SA_B</i>	silhouette area of branch (cm ²)
<i>SAs</i>	silhouette area of shoot (cm ²)
<i>SMA</i>	shoot mass per area (g m ⁻²)
<i>SPAR_B</i>	silhouette to leaf area ratio of branch
<i>SPARs</i>	silhouette to leaf area ratio of shoot
<i>()</i>	convexity of the photosynthetic light curve
<i>▷</i>	quantum yield (mol mol ⁻¹)