

Dynamic Phenotypic Plasticity in Photosynthesis and Biomass Patterns in Douglas-Fir Seedlings

Anita C. Koehn
Gerald I. McDonald
David L. Turner
David L. Adams



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Abstract

As climate changes, understanding the mechanisms long-lived conifers use to adapt becomes more important. Light gradients within a forest stand vary constantly with the changes in climate, and the minimum light required for survival plays a major role in plant community dynamics. This study focuses on the dynamic plasticity of Douglas-fir (*Pseudotsuga menziesii* var. *glauca* [Beissn.] Franco) seedlings grown in contrasting light environments. Plasticity in Douglas-fir seedlings was primarily achieved by a combination of the physiological processes: maximum photosynthesis, quantum yield, F_v/F_m , K_m (the light constant), light compensation point, and the ratio of needle area to needle weight (specific leaf area). Specific leaf area was the most plastic of the biomass parameters measured.

Keywords: *Pseudotsuga menziesii* var. *glauca* (Beissn.) Franco, dynamic phenotypic plasticity, photosynthesis, Douglas-fir, biomass allocation, light constant

Authors

Anita C. Koehn is a Soil Scientist with the USDA-ARS Northwest Soils and Irrigation Laboratory in Kimberly, ID.

Geral I. McDonald (retired) is a Research Plant Pathologist with the USDA Forest Service, Rocky Mountain Research Station in Moscow, ID.

David L. Turner is a Mathematical Statistician with the Rocky Mountain Research Station in Logan, UT.

David L. Adams is a Professor Emeritus with the College of Natural Resources at the University of Idaho in Moscow, ID.

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Introduction

As climate changes, it becomes more important to understand its impact on long-lived conifers and their adaptation mechanisms. Changes in climate alter many aspects of plant growth and leaf area production simultaneously, which, in turn, alter the availability of light in plant communities (Valladares and Niinemets 2008). The light gradient in forest stands is a highly heterogeneous factor that influences a tree's ability to survive and compete in the plant community. In addition to light gradients in a forest stand, there are also light gradients within the crown of a forest tree; therefore, all trees are exposed to some degree of shading in their lifetime (Valladares and Niinemets 2008). Although light is necessary for photosynthesis, both low and high sunlight can limit plant growth and survival. Plants can experience high heat, desiccation, excessive irradiance, and ultraviolet radiation stresses in high light (Demmig-Adams and Adams 2006). Trees must invest in protective and adaptive mechanisms in order to adjust to changing light gradients.

Adaptive mechanisms can be expressed as species' plasticity, or the ability for species to adapt by changing biomass allocation patterns, leaf structure, and physiology (Ackerly and others 2000). Plasticity in the allocation of biomass to functionally distinct tissues such as roots, leaves, stems, and reproductive structures allows plants to enhance access to a specific resource that may be limited. For example, in response to low light availability, spotted lady's thumb (*Polygonum persicaria*) increased the proportion of their tissue allocated to leaves, effectively maximizing leaf surface area for increased photon capture. Although the plants produced less biomass, the developmental shift raised the photosynthetic effectiveness of each gram of biomass so as to promote successful growth and reproduction despite limited light (Sultan 2003). This type of allocational plasticity may contribute to the species' ability to inhabit a broad range of light habitats (Sultan 2003 and sources therein). An intriguing and important aspect of plastic response to environment that can describe acclimation or adaptation to changing environments over time is dynamic plasticity, which has become meaningful in evaluating diversity and how plants develop and function in changing environments (Sultan 2004).

Conifers normally adjust their physiology repeatedly in nature to survive extremely heterogeneous environments. In the northern Rocky Mountains, the mean temperatures in January, the coldest winter month, range from -5.2 °C to 1.6 °C with an extreme low of -34.4 °C. In August, the warmest summer month, the mean low and high temperatures range from 9.8 °C to 28.1 °C, respectively, and temperatures have been measured as high as 42.8 °C (Hermann and Lavender 1990; <http://snow.ag.uidaho.edu/>; climate means from 1893 to 2006). In the winter, conifers initially adjust to colder temperatures through frost-hardening at temperatures slightly above 0 °C. The second phase is winter stress, which occurs at subzero temperatures and includes changes in energy absorption and photochemical transformation through energy partitioning and accompanying changes in chloroplast metabolism, allocation, and partitioning. These plastic changes are reflected in decreased quantum yield of photosynthesis and a reduction in F_v/F_m , the ratio of variable chlorophyll fluorescence to maximum fluorescence, for exposed needles (Öquist and Huner 2003). The effects of temperature fluctuations on photosynthesis and carbon gain eventually determine productivity, growth, and distribution of conifers on various spatial

scales, and the fluctuations may act as global sensors to environmental change (Ensminger and others 2006).

Douglas-fir, a major timber species with broad ecological amplitude, is native to the inland northwest region of the United States. It is physiologically attuned to its environment, and adaptive variation among populations is closely related to geographic, physiographic, and climatic variables (Rehfeldt 1983). Flexibility within the populations of Douglas-fir allows each population to produce progenies preadapted to a range of environmental conditions (Rehfeldt 1978). Douglas-fir ranks intermediate in shade tolerance among its associates, being more tolerant than western larch, ponderosa pine, lodgepole pine, southwestern pine, and aspen. First-year seedlings grow best under light shade, and older seedlings require full sunlight (Hermann and Lavender 1990).

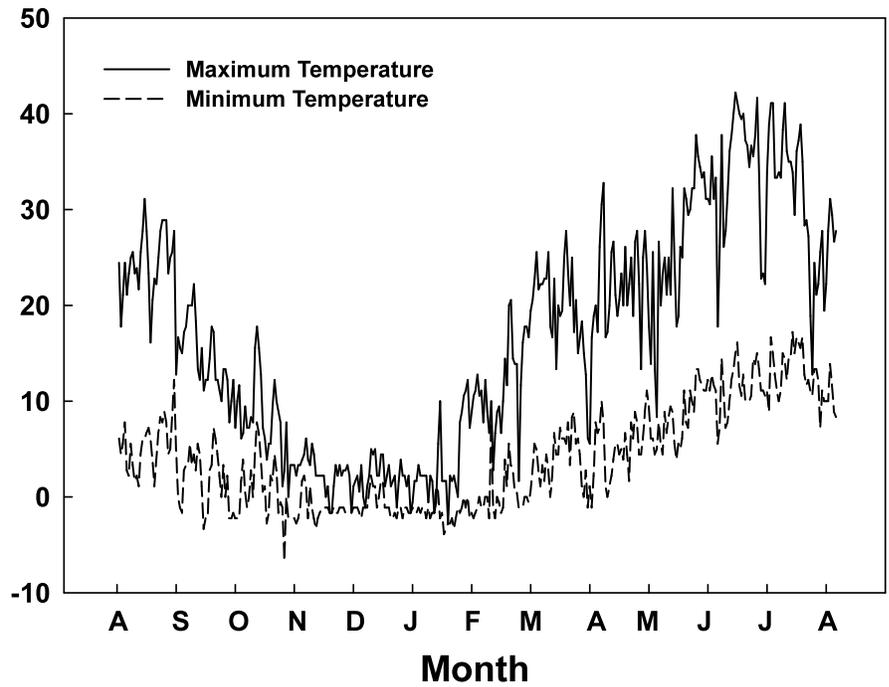
Few studies examine dynamic phenotypic plasticity in conifers. Douglas-fir is used in this study to observe natural processes of dynamic phenotypic plasticity in a conifer species with changing light environments. It was chosen for this purpose because it occupies a diverse range of habitats, retains its needles for extended periods of time, and must adjust to seasonal changes in temperature. This paper focuses on how contrasting light environments and seasonal changes affect the plasticity in photosynthetic physiology and biomass allocation patterns in Douglas-fir.

Materials and Methods

Plant Material

The seed source was a single population of Douglas-fir (*Pseudotsuga menziesii* var. *glauca* [Beissn.] Franco) collected from the Clearwater National Forest near Elk River, Idaho (116°07' W, 45°50' N; 884 m elevation). Seedlings were sown May 1988 in plastic containers filled with 650 cm³ of potting soil and were grown in ambient light supplemented with 24-hour, 40-watt indoor fluorescent grow lights. Seedlings were moved outdoors to a shade house the first week of June 1988 and were given adequate water and fertilizer. During September and October 1988, the seedlings were redistributed in trays to every other cell to lessen shading of adjacent plants. The seedlings overwintered outdoors on the ground shaded with lath and mulched with wood chips to avoid root damage from freezing winter temperatures. Seedlings were brought into a fiberglass greenhouse April 10, 1989, and remained there throughout the measurement period. Variations in light intensity were provided for the seedlings in the greenhouse by combinations of shade cloth and cheese cloth to achieve light treatments of 24, 14, and 7 percent of full sunlight. A fourth treatment, 34 percent of full sunlight, did not include shade cloth; the greenhouse structure reduced full sunlight by 66 percent. Maximum ambient light intensities in Moscow, ID, in the summer are as high as 2000 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Light measurements were taken with a ceptometer (Decagon Devices, Pullman, WA) and quantum sensor (Omnidata Model ES-240, Omnidata International, Inc., Logan, UT). Minimum and maximum air temperatures (fig. 1) in the greenhouse were recorded using a hydrothermograph (Oakton Instruments, Vernon Hills, IL). Samples for O₂ evolution, chlorophyll fluorescence, and biomass were taken over a two-week period once a month from September 1989 through August 1990.

Figure 1. Daily minimum and maximum temperatures (°C) for August 1989 through August 1990 recorded by a hydrothermograph placed in an open area in the greenhouse within the study treatments.



Growth and Biomass Analysis

Seedlings that were sampled for O₂ evolution and chlorophyll fluorescence were harvested at the end of each month and were dried at 65 °C for 24 hours. The dried seedlings were stored and dried again at 65 °C for at least two hours and were then weighed immediately. The dried seedlings were partitioned into 1990 stem and needles, 1989 stem and needles, 1988 shoot (included 1988 needles and 1988 stems), and roots; each biomass unit was weighed individually. Needle area was measured on a sample of 10 needles using a high-resolution monochrome TV camera, a PCVISION^{plus} Framegrabber, and Java video analysis software (Jandel Scientific, Corte Medera, CA). The total needle area for 1989 was calculated from the sample used for O₂ evolution as follows:

$$\frac{1989 \text{ sample area (mm}^2\text{)}}{1989 \text{ sample weight (gr)}} * \text{total 1989 needle weight (gr)} * \frac{1 \text{ m}^2}{1,000,000 \text{ mm}^2}.$$

Ten needles from the 1990 growth portion of the seedling were sampled to determine needle area and weight, and the total area was calculated using the same method for the 1989 needles. Total weight, needle weight ratio, stem weight ratio, root weight ratio, and specific leaf area (SLA) were calculated for each seedling. Needle, stem, and root weight ratios were the weights of the respective parts divided by the total seedling weight. SLA was calculated as:

$$\frac{\text{leaf area (1989 + 1990 needles)}}{\text{total needle weight (1989 + 1990 needles)}}.$$

Chlorophyll Fluorescence

A Hansatech fluorescence probe was inserted at a 40° angle into the side of the upper jacket of the LD-2 leaf disc electrode system (Hansatech Limited, UK). The probe was fitted with a 740-nm interference filter. The excitation light, Hansatech LS1, contained an array of 36 LEDs (light emitting diodes) and was positioned over the plexiglass window of the upper jacket. Needle samples were taken from the seedling's terminal shoot between 0830 and 1400 and were immediately placed on moist filter paper in petri dishes and kept at room temperature. The needles were attached to tape and the ends were cut to fit into the LD-2 chamber. Needles were dark-adapted for one hour on moist filter paper in a petri dish covered with foil and were then transferred to the LD-2 chamber under weak, green light. Chlorophyll fluorescence measurements were taken in ambient air at 20 °C. Readings began 20 to 45 msec before the light was turned on, were recorded at one-msec intervals, and continued for a total of approximately two sec. Immediately after chlorophyll fluorescence measurements were completed, O₂ evolution measurements were taken on the same needle sample. All needles measured for O₂ evolution and chlorophyll fluorescence developed in the light treatments during the 1989 growing season.

Oxygen Evolution

Oxygen evolution was measured with a Hansatech leaf disc electrode system (Hansatech Limited, UK) following the procedures of Delieu and Walker (1981, 1983). A Polycorder 700 (Omnidata International, Inc., Logan, UT), a 12-volt power supply, and an electrical circuit were used in this study to regulate the excitation voltage at 700 mvolts for the Clark-type oxygen electrode. The Polycorder 700 was programmed to record and display data and to regulate the lights for chlorophyll fluorescence measurements. Oxygen evolution was measured at 20 ± 1 °C and 5 percent CO₂. Temperature was controlled using water that circulated from a controlled-temperature water bath through the Hansatech cells. The CO₂ source was a sodium carbonate/bicarbonate/borate buffer solution that wet capillary matting at the bottom of the chamber. The system was calibrated by injecting 1 cm³ of air into the chamber, recording the change in voltage, and correcting for standard temperature and pressure. Oxygen evolution measurements were taken at eight photon flux densities (PFD): 30, 50, 95, 170, 230, 430, 820, and 1500 μmol m⁻² s⁻¹ (from September 1989 to January 1990, O₂ evolution was measured at 170, 430, 820, and 1500 μmol m⁻² s⁻¹). Dark respiration was measured with all lights off at the beginning of the sampling period. Light was supplied by the Hansatech LS-2 light unit and intensities were varied using neutral density filters; photon flux density was measured with a quantum sensor. Twelve O₂ evolution readings from each needle sample were obtained over a three-minute period at one PFD. Oxygen evolution rate for each PFD was calculated as the slope from linear regression of O₂ evolution versus time (R² for the linear regression analyses ranged from 0.83 to 0.99; Koehn and McDonald 1991). These values were used to obtain the light response curve used in data analysis.

Data Calculations and Analysis

The light response curve was analyzed using Enzfitter (Leatherbarrow 1987). Enzfitter uses Michaelis-Menton enzyme kinetics (a nonlinear regression analysis) to calculate maximum photosynthetic capacity (Pmax) and the light constant, K_m (the light intensity at one-half Pmax). Quantum yield was calculated as the slope from linear regression using gross O_2 evolution rates from the first three PFD (30, 50, and 95 $\mu\text{mol m}^{-2} \text{s}^{-1}$) where the O_2 evolution rate was linear (dark respiration rates were added to O_2 evolution rates for each sample). Quantum yield for September through January was estimated using linear regression on the values measured at 0 and 170 $\mu\text{mol m}^{-2} \text{s}^{-1}$. The light compensation point (the photon flux density where oxygen evolution equals respiration) was calculated as the x-intercept from linear regression on oxygen evolution rate at the first three PFD and the dark respiration rate versus PFD for each measurement.

The ratio of variable chlorophyll fluorescence to maximum fluorescence, F_v/F_m , was derived from the Kautsky curve (Kautsky and Hirsch 1931). Initial chlorophyll fluorescence, F_o , was the first mvolt reading recorded by the polycorder after the light was turned on. Maximum chlorophyll fluorescence, F_m , was the maximum mvolt value measured by the polycorder, and variable fluorescence was calculated as $F_v = F_m - F_o$. Using this method to estimate chlorophyll fluorescence, we were able to obtain F_v/F_m values of 0.80 to 0.83 during periods of no winter stress; this data is comparable to data published by Björkman and Demmig (1987).

Similar to methods described by Valladares and others (2000), an index of plasticity for each treatment was calculated as the difference between the minimum and maximum of the mean values (the mean value for each replication in each month was used to calculate the minimum and maximum overall mean values) calculated for each light treatment divided by the maximum mean value for the specific light treatment. The light compensation point and dark respiration sometimes had zero values, so the plasticity index had little meaning for these variables and was not reported here.

The experimental design was a randomized complete block design with one species (Douglas-fir), four replications, and four light treatments. A total of 960 seedlings were included in the study. Each month for 12 months, five seedlings were randomly selected from each treatment in each replication. Data were analyzed using Proc Mixed (SAS) where treatment was a fixed effect, replication was the random effect, and month was a repeated measure. Because of the significance of the month by treatment interaction, treatments for each month were analyzed separately. The Tukey-Kramer adjustment from Proc Mixed was used to determine significant differences between treatments with adjusted $P \leq 0.05$.

Results

O_2 Evolution and Chlorophyll Fluorescence

Each physiological variable we measured graphically displayed a different rhythmic pattern in the adjustment of the Douglas-fir seedlings to seasonal environment and light treatment. Significant fluctuations in Pmax occurred in December, February, and May when the seedlings were undergoing physiological adjustments to cold hardiness, deep winter dormancy, and active growth,

respectively (fig. 2A). In the wintertime (December, January, and February), there was a 36 percent reduction (from $12.02 \mu\text{mol m}^{-2} \text{s}^{-1}$ to $7.69 \mu\text{mol m}^{-2} \text{s}^{-1}$) in Pmax for the seedlings grown in the 34 percent light treatment. F_v/F_m responded to treatments and seasonal changes with significant differences primarily in the winter months of December, January, February, and March when the sun needles experienced photoinhibition (fig. 2B). There was a F_v/F_m reduction of 20 percent (from 0.80 to 0.60) in the winter for the 34 percent treatment seedlings compared to the summer, whereas F_v/F_m remained near 0.80 for the seedlings in the 7 percent treatment (fig. 2B). Quantum yield, an indication of the efficiency of O_2 evolved per $\mu\text{mol m}^{-2} \text{s}^{-1}$ of light, generally followed a similar pattern to Pmax with the exception of the apparent adjustment in Pmax that occurred between October and November (fig. 2C). There was a distinctive pattern of respiration for all treatments during the year, with the highest dark respiration rates measured in February for 7 and 14 percent treatments and in March for seedlings in the 24 and 34 percent light treatments (fig. 2D). Significant differences occurred between treatments in dark respiration in April and September. Overall, respiration rates in the winter were 2.2 to 2.9 times higher than the average annual respiration rates for seedlings in each light treatment (fig. 2D). K_m , the light constant that reflects the affinity of the enzyme complexes of photosynthesis for

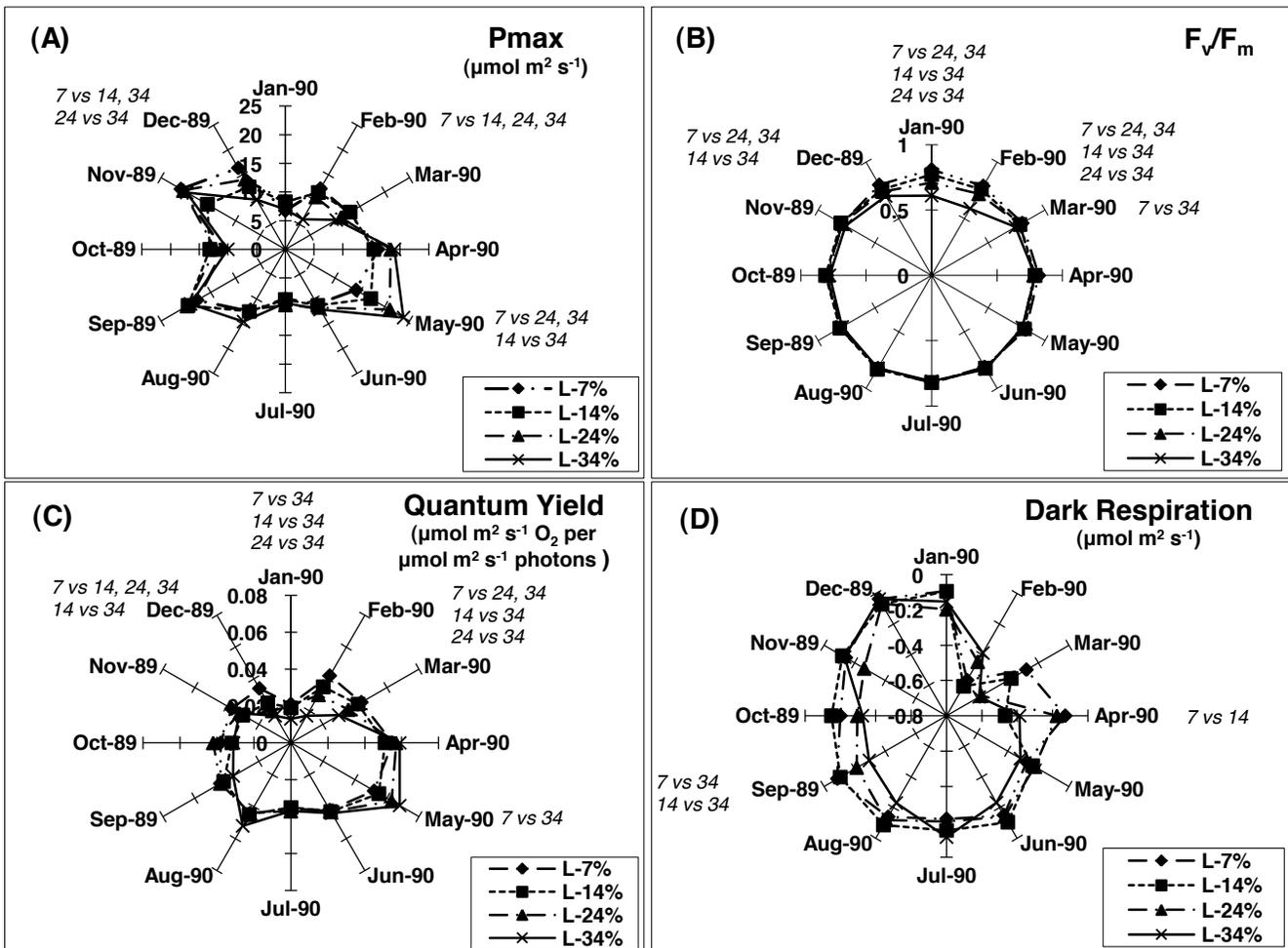
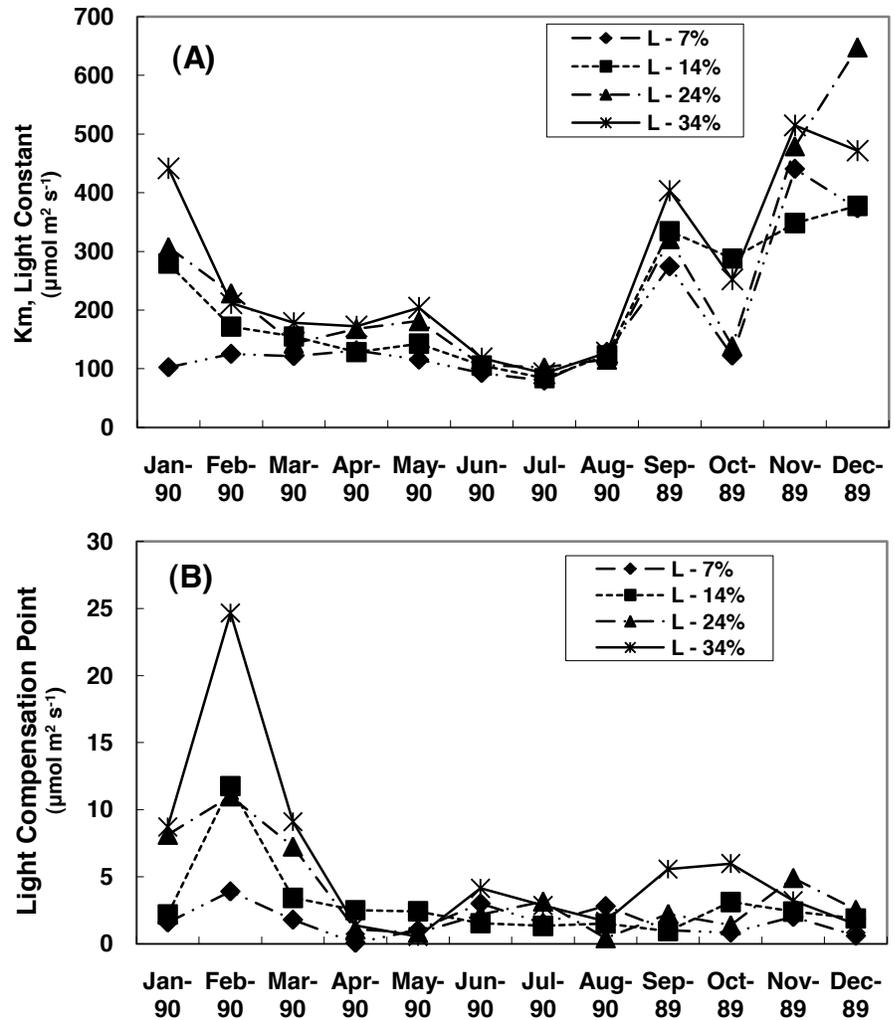


Figure 2. Maximum photosynthetic capacity (Pmax) for oxygen evolution (A), F_v/F_m (B), quantum yield (C), and dark respiration (D) for Douglas-fir seedlings from September 1989 through August 1990. The units on the radial arms for F_v/F_m (B) are relative units. The y-values are represented as months with the significant differences between treatments listed adjacent to each month ($\alpha = 0.05$, $n = 20$).

the substrate light, varied significantly between the 7 and 34 percent light treatments in January, February, May, and September. There was also a difference in K_m between the 7 and 24 percent treatments in February (fig. 3A). The light compensation point, or the light intensity where O_2 evolution equals respiration, was similar for all treatments for the entire year except in February when the light compensation point for the seedlings in the 34 percent treatment was 4.3 times higher than the average for the 12-month period (fig. 3B). Although there are differences in January and March, they are insignificant because of the variation in the measurements as the physiology of the enzyme complexes adjusted to the changing temperatures and seasons (Laisk and Eichelmann 1989).

Figure 3. K_m , the light constant, (A) and the light compensation point (B) for Douglas-fir seedlings from September 1989 through August 1990. For K_m , significant differences between the 7% and 34% light treatments occur for months Feb-90, May-90, and Sep-90, and treatments 7% and 24% light are significantly different in the month of Feb-90 ($\alpha = 0.05$, $n = 20$). For the light compensation point, treatments 7% and 34% differed significantly in Feb-90.



Biomass Allocation Patterns

Biomass allocation patterns were generally more concentric circles with less crossing-over between treatments than was found in the physiological parameters. Total weight of the seedlings followed the expected pattern: seedlings receiving the most light energy (34 percent treatment) had the greatest biomass accumulation and it decreased with decreasing light (fig. 4A). The shade seedlings (7 percent light treatment) at the end of the study accumulated an

average of 44 percent of the total weight (or had a 56 percent reduction in total weight) of the sun seedlings (34 percent light treatment) with only a 27 percent reduction in light energy. The seedlings in the 14 percent treatment weighed 81 percent of the sun seedlings, and the 24 percent treatment seedlings weighed 96 percent of the sun seedlings. Because the weights of the seedlings in the 7 percent treatment were reduced at a higher percent than the percent reduction in light quantity, our interpretation is that Douglas-fir may be maladapted at the lower light intensity. The root/shoot ratio appears to be one of the most plastic of the biomass parameters because it fluctuates more with season (especially for seedlings in the 34 percent light treatment) than the other biomass parameters except specific leaf area (SLA) (fig. 4B). The root weight ratio, stem weight ratio, and needle weight ratio parameters changed depending on the light treatment (figs. 4C, 4D, and 5A, respectively). Sun seedlings allocated more to roots than stems or needles, whereas shade seedlings allocated more to the supporting stem tissues and slightly more to needles.

SLA, the ratio of needle area to needle weight, is a key trait in the maintenance of a species' growth rate under low light conditions. There are two distinctive phases in the annual SLA pattern in seedlings grown in the low light

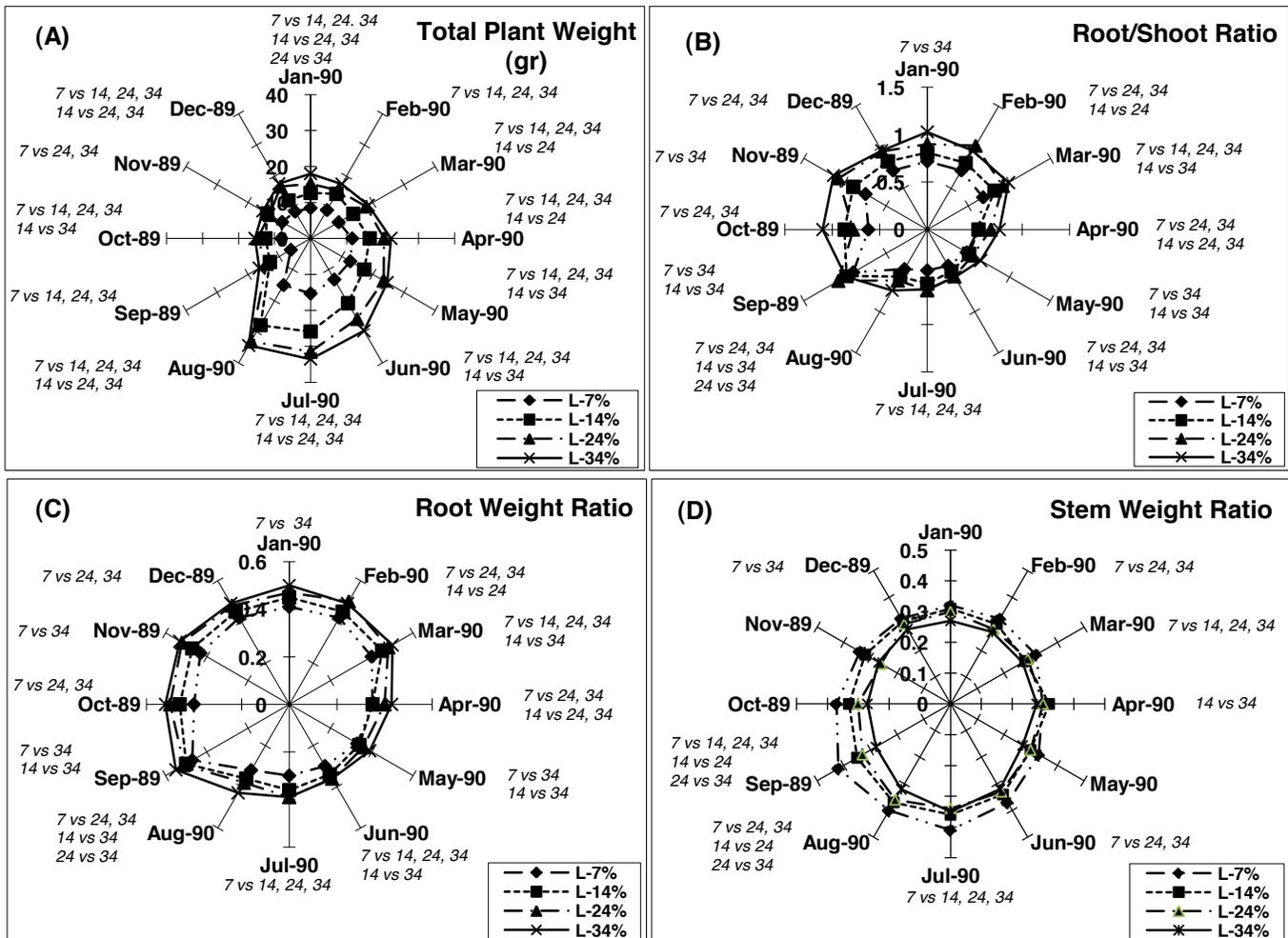


Figure 4. Total plant dry weight (A), root/shoot ratio (B), root weight ratio (C), and stem weight ratio (D) for Douglas-fir seedlings from September 1989 through August 1990. The y-values are represented as months with the significant differences between treatments listed adjacent to each month (n = 20, except for November 1989: $\alpha = 0.05$, n = 8).

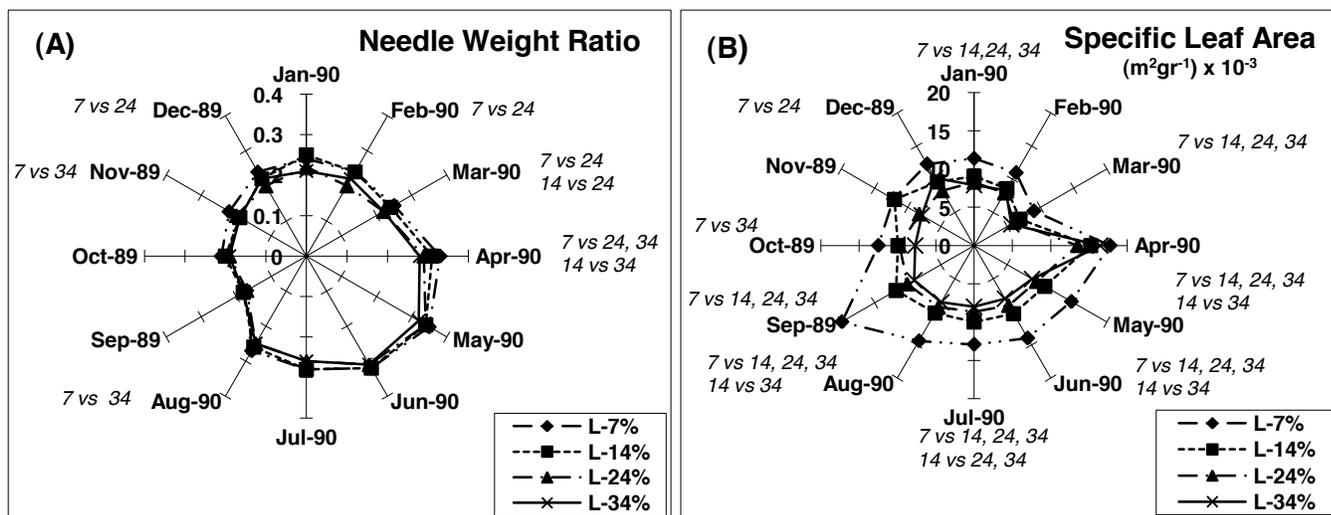


Figure 5. Needle weight ratio (A) and specific leaf area (SLA) (B) for Douglas-fir seedlings from 1989 through August 1990. The y-values are represented as months with the significant differences between treatments listed adjacent to each month ($n = 20$, except for November 1989: $\alpha = 0.05$, $n = 8$).

treatment in this study. The first phase occurred in the fall with a decrease in SLA from September to October; the second phase occurred in the spring with an increase in SLA with budburst from March to April (fig. 5B). Generally, seedlings in the higher light treatment showed the increase in SLA at budburst but did not show the decrease in the fall.

Plasticity Index

The plasticity index values were highest for physiological variables compared to biomass parameters. The exception was F_v/F_m , which had a very low plasticity index (0.08 to 0.39) but had the greatest difference in plasticity between treatments (table 1). Pmax and quantum yield plasticity indices ranged from 0.72 to 0.85, K_m had the highest index of 0.90 to 0.94, and the biomass ratios had indices of 0.61 or below. One interpretation of the plasticity index is that some parameters varied with season (Pmax and K_m) while other factors were influenced more by light treatment (F_v/F_m).

Table 1. The plasticity index is calculated as the maximum value minus the minimum value divided by the maximum value. The maximum and minimum values are calculated from the average for each replication for each month (4 replications * 12 months; $n = 48$).

Light treatment	Annual plasticity index									
	Pmax	Quantum yield	F_v/F_m	K_m	Total plant weight	Root/shoot ratio	Stem weight ratio	Needle weight ratio	Root weight ratio	SLA
7%	0.82	0.74	0.08	0.90	0.74	0.61	0.49	0.61	0.45	0.70
14%	0.82	0.75	0.14	0.94	0.65	0.55	0.33	0.54	0.37	0.71
24%	0.72	0.80	0.25	0.93	0.70	0.64	0.44	0.54	0.43	0.61
34%	0.85	0.85	0.39	0.94	0.65	0.64	0.39	0.50	0.42	0.82
Difference	0.13	0.11	0.31	0.04	0.09	0.09	0.16	0.11	0.08	0.21

Discussion

Physiology and Plasticity

The dynamic plasticity we described in this study is represented primarily by Douglas-fir's physiological response to the combination of light acclimation and season. For instance, the changes in P_{max} , F_v/F_m , quantum yield, and dark respiration (fig. 2) indicate physiological rearrangements that take place with seasonal changes in temperature, and these changes vary with light environment. This ability to make different physiological adjustments in response to environmental changes over time is important in allowing forest trees to cope with variations in light conditions within a forest canopy. Conifers use strategies that involve down-regulating photosynthesis by inactivating photosystem II reaction centers and by re-organizing pigment-protein complexes that harvest light during the growing season to complexes that quench light energy during autumn and winter (Öquist and Huner 2003; Ensminger and others 2006). Recent research by Verhoeven and others (2009) in balsam fir compares the changes in light harvesting protein complexes (LPC) between sun and shade needles in the winter. The shade needles maintained most of their LPC in the winter, whereas sun needles had reduced amounts, indicating that the reorganization of LHC during acclimation to winter stress in balsam fir varied depending on the light environment to which the needles were exposed. The consequences of these strategies are reflected in the reduction of F_v/F_m , the maximum yield of primary photochemistry (Kitajima and Butler 1975). The sun-grown seedlings in our study demonstrated an adjustment to winter temperatures measured by changes in F_v/F_m ; however, there was little change in the F_v/F_m amplitude with chilling temperatures in shade-grown seedlings (fig. 2B). A reduction in quantum yield (the efficiency of O_2 produced per photons of light absorbed) occurred in February and March in shade seedlings, but it was less than with sun needles (0.039 versus 0.023 for the 7 percent and 34 percent treatments, respectively; fig. 2C). Quantum yield fully recovered in early summer in sun needles to a maximum of 0.057, whereas the shade needles remained at a lower efficiency (fig. 2C). There are physiological costs in the re-organization of pigment-protein complexes, and shade seedlings in our study evidently did not need to expend the energy in the process of reorganizing complexes (Öquist and Huner 2003). Ecologically, Douglas-fir from the Intermountain West of the United States ranks intermediate in shade tolerance and is considered both a seral and climax species depending on the moisture and temperature regime of the forest site (Hermann and Lavender 1990). It has evolved to be very plastic in the reorganization of the pigment-protein complexes in response to differences in light environment and temperature changes.

The pattern of respiration rates in the Douglas-fir seedlings in this study indicated higher rates of O_2 uptake (higher dark respiration) in February in the shade needles and in March in the sun needles. The increase in respiration rates in sun needles in March coincides with a greater demand for photosynthates as sun needles begin the re-organization and repair of the pigment-protein complexes of photosynthesis. Because the capacity for photosynthesis in the growing season was greater than with dark respiration in the winter, spring maintenance energy costs were not at an overall deficit for the Douglas-fir seedlings and

growth was positive. Photosynthesis and respiration in conifers can continue throughout the winter, especially when temperatures rise above 5 °C (Perry 1971). In Siberian Scots pine, there was photometabolic activity measured by variable amounts of reaction center D1 proteins or changes in F_v/F_m , which were linked to the variable climate during the cold season (Ensminger and others 2004). In this respect, cold hardiness in conifers allows for dynamic physiological processes that balance light absorption, dissipation of excess light energy, and light utilization during winter months (Ensminger and others 2004).

An interesting oscillation of P_{max} and K_m was observed in the fall. In studies on oscillations in photosynthesis, Laisk and Eichelmann (1989) observed that minimums in photosynthesis that represent a reduction in the stability of the photosynthetic apparatus occur, followed by an increase in photosynthesis rates because of regulation processes in the photosynthetic apparatus. The adjustments in P_{max} from October to November coincided with changes in K_m . In enzyme systems, K_m indicates changes in enzyme affinity for substrates (Mohr and Schopfer 1995). In this study, light is the substrate for the enzyme-complex reactions of photosynthesis; therefore, the affinity of these enzyme complexes must have readjusted between October and November (figs. 2A and 3A). These adjustments allowed the seedlings to continue photosynthesis at higher levels longer into the fall season despite a decrease in optimum temperatures for photosynthesis.

Biomass Allocation and Physiology

SLA, or the increase in leaf surface area relative to leaf weight that will capture light energy under low photon flux densities, is known to enhance light-harvesting efficiency (Björkman 1981) and is a key trait in the maintenance of species growth rate under low irradiance levels (Poorter 2001). The Douglas-fir seedlings varied SLA with season and treatment, but seedlings in the low light treatment typically had a higher SLA (fig. 5B). These results agree with a study by Chen (1997) that compared the plasticity of allocation patterns of Douglas-fir, Engelmann spruce, and ponderosa pine. Chen (1997) found Douglas-fir had the highest SLA plasticity and the highest ratio of aboveground to belowground biomass of the three species. Considering that Douglas-fir is a shade-tolerant species, especially in the early seedling stage, it seems a beneficial trait for Douglas-fir to be able to dynamically change SLA as the tree matures and canopy light conditions change.

The sun seedlings allocated more growth to roots than shoots compared to the shade seedlings, but the shade seedlings allocated more growth to the stem portion of the plant (fig. 4). This is true in other sun/shade studies of forest species such as Pacific silver fir (*Abies amabilis*), in which shade seedlings allocated more biomass to stems. The increased investment in the trunk of the saplings helped support branches that were restricted to the top of a flat crown (Mori and others 2008). Mori and others (2008) also found slower foliage turnover in shade saplings, which would compensate for the increased investment in support organs or stems. The increased needle weight ratio of shade seedlings in our study agrees with Chan and others (2003) on growth allocation of interior Douglas-fir in contrasting environments. In their study, Douglas-fir also adapted to reduced light by producing more needles per unit branch and stem.

Conclusion

Our study with Douglas-fir seedlings demonstrates there are different patterns in plasticity between physiological variables and biomass allocation patterns related a seedling's light environment. Some of the variables we measured changed with environment and the change was constant with time (for example, needle weight ratio), while others were more plastic (for example, P_{max} , F_v/F_m , SLA, and root/shoot ratio) and changed with environment and time. Seasonal photosynthesis patterns such as the hardening and dehardening processes and their modulation by abiotic factors are becoming important issues in predicting tree responses to anticipated climate change (Saxe and others 2001). The concern for forest tree species with respect to climate change is that the natural processes of evolution in forest ecosystems will not respond quickly enough to the predicted rate of climate change (Rehfeldt and others 2006). Therefore, understanding the magnitude and timing of plastic responses to environmental changes may be helpful in managing long-lived forest tree species in the future (Sultan 2003). Also, if models are to be used in a dynamic sense over long periods of time, a description of carbon allocation patterns with changing environments is important (Landsberg and Gower 1997).

This study on individual morphological and physiological development of Douglas-fir in different light environments is an initial probe into understanding developmental mechanisms that are important to the processes of evolutionary change. Important cues of rapid response to abiotic factors identified in this study are P_{max} , SLA, F_v/F_m , and root/shoot ratio. Shifts in seasonal physiology and biomass allocation patterns will likely become more important to the survival of the long-lived Douglas-fir species in response to forecasted rapid changes in the environment (Sultan 2004). This study provides forest managers with information on the resiliency of Douglas-fir, and results are important in future forest management strategies.

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References

- Ackerly, D.D., Dudley, S.A., Sultan, S.E., Schmitt, J., Coleman, J.S., Linder, R.C., Sandquist, D.R., Geber, M.A., Evans, A.S., Dawson, T.E., Lechowicz, M.J. 2000. The evolution of plant ecophysiological traits: Recent advances and future directions. *Bioscience*. 50(11): 979-994.
- Björkman, O. 1981. Responses to different quantum flux densities. In: Lange, O.L., Nobel, P.S., Osmond, C.B., Zielgler, H., eds. *Encyclopedia of plant physiology*, vol. 12A: Interactions with the physical environment. Berlin, Heidelberg: Springer-Verlag: 57-107.

- Björkman, O., Demmig, B. 1987. Photon yield of O₂ evolution and chlorophyll fluorescence characteristics at 77K among vascular plants of diverse origins. *Planta*. 170: 489-504.
- Chan, S.S, Radosevich, S.R., Grotta, A.T. 2003. Effects of contrasting light and soil moisture availability on the growth and biomass allocation of Douglas-fir and red alder. *Canadian Journal of Forest Research*. 33: 106-117.
- Chen, H.Y.H. 1997. Interspecific responses of planted seedlings to light availability in interior British Columbia: Survival, growth, allometric patterns, and specific leaf area. *Canadian Journal of Forest Research*. 27: 1383-1393.
- Delieu, T., Walker, D.A. 1981. Polarographic measurement of photosynthetic oxygen evolution by leaf discs. *New Phytologist*. 89: 165-175.
- Delieu, T., Walker, D.A. 1983. Simultaneous measurement of photosynthetic oxygen evolution and chlorophyll fluorescence from leaf pieces. *Plant Physiology*. 73: 534-541.
- Demmig-Adams, B., Adams, W.W., III. 2006. Photoprotection in an ecological context: The remarkable complexity of thermal energy dissipation. *New Phytologist*. 172: 11-21.
- Ensminger, I., Busch, F., Huner, N.P.A. 2006. Photostasis and cold acclimation: Sensing low temperature through photosynthesis. *Physiologia Plantarum*. 126: 28-44.
- Ensminger, I., Sveshnikiv, D., Campbell, D.A., Funks, C., Jansson, S., Lloyd, J., Shibistova, O., Öquist, G. 2004. Intermittent low temperatures constrain spring recovery of photosynthesis in boreal Scots pine forests. *Global Change Biology*. 10: 995-1008.
- Hermann, R.K., Lavender, D.P. 1990. *Pseudotsuga menziesii* (Mirb.) Franco. Douglas-fir. In: Burns, R.M., Honkala, B., tech. coords. *Silvics of North America* vol. 1: Conifers. Agric. Handb. 654. Washington, DC: U.S. Department of Agriculture, Forest Service: 527-540.
- Kautsky, H., Hirsch, A. 1931. Neue versuche zur kohlenstoff-assimilation. *Naturwissenschaften* 19: 964.
- Kitajima, M., Butler, W.L. 1975. Quenching of chlorophyll fluorescence and primary photochemistry in chloroplasts by dibromothymoquinone. *Biochimica et Biophysica Acta*. 376: 105-115.
- Koehn, A., McDonald, G. 1991. A method for photosynthetic calibration of process models for conifer ecosystems. In: Harvey, Alan E., Neuenschwander, Leon F., comps. *Proceedings—Management and productivity of western-montane forest soils; 1990 April 10-12; Boise, ID. Gen. Tech. Rep. INT-280. Ogden, UT: U.S. Department of Agriculture, Forest Service, Intermountain Research Station: 129-142.*
- Laisk, A., Eichelmann, H. 1989. Towards understanding oscillations: A mathematical model of the biochemistry of photosynthesis. *Philosophical Transactions of the Royal Society of London B*. 323: 369-384.
- Landsberg, J.J., Gower, S.T. 1997. Ecosystem process models. In: *Applications of physiological ecology to forest management*. San Diego, CA: Academic Press, Inc.: 247-276.
- Leatherbarrow, R.J. 1987. *Enzfitter*. New York: Elsevier Science Publishers BV. 89 p.
- Mohr, H., Schopfer, P. 1995. *Plant physiology*. Berlin, Heidelberg: Springer-Verlag. 629 p.
- Mori, A.S., Mizumachi, E., Sprugel, D.G. 2008. Morphological acclimation to understory environments in *Abies amabilis*, a shade- and snow-tolerant conifer species of the Cascade Mountains, Washington, USA. *Tree Physiology*. 28: 815-824.
- Öquist, G., Huner, N.P.A. 2003. Photosynthesis of overwintering evergreen plants. *Annual Review of Plant Biology*. 54: 329-355.
- Perry, T.O. 1971. Dormancy of trees in winter. *Science*. 171: 29-36.
- Poorter, L. 2001. Light-dependent changes in biomass allocation and their importance for growth of rain forest tree species. *Functional Ecology*. 15: 113-123.
- Rehfeldt, G.E. 1978. Genetic differentiation of Douglas-fir populations from the northern Rocky Mountains. *Ecology*. 59(6): 1264-1270.

- Rehfeldt, G.E. 1983. Ecological adaptations in Douglas-fir (*Pseudotsuga menziesii* var. *glauca*) populations. III. Central Idaho. Canadian Journal of Forest Research. 13: 626-632.
- Rehfeldt, G.E., Crookston, N.L., Warwell, M.V., Evans, J.S. 2006. Empirical analyses of plant-climate relationships for the western United States. International Journal of Plant Sciences. 167(6): 1123-1130.
- Saxe, H., Cannell, M.G.R., Johnsen, Ø., Ryan, M.G., Vourlitis, G. 2001. Tree and forest functioning in response to global warming. New Phytologist. 149: 369-400.
- Sultan, S.E. 2003. Phenotypic plasticity in plants: A case study in ecological development. Evolution and Development. 5(1): 25-33.
- Sultan, S.E. 2004. Promising directions in plant phenotypic plasticity. Perspectives in Plant Ecology, Evolution, and Systematics: 227-233.
- Valladares, F., Martinez-Ferri, E., Balaguer, L., Perez-Corona, E., Manrique, E. 2000. Low leaf-level response to light and nutrients in Mediterranean evergreen oaks: A conservative resource-use strategy? New Phytologist. 148: 79-91.
- Valladares, F., Niinemets, Ü. 2008. Shade tolerance, a key plant feature of complex nature and consequences. Annual Review of Ecology, Evolution, and Systematics. 39: 237-257.
- Verhoeven, A., Osmolak, A., Moreales, P., Crow, J. 2009. Seasonal changes in abundance and phosphorylation status of photosynthetic proteins in eastern white pine and balsam fir. Tree Physiology. 29: 361-374.



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