AN ABSTRACT OF THE THESIS OF

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Title: Effects of Shade on Performance and Chlorophyll Fluorescence of Four Pacific Northwest Seedling Conifer Species

Signature redacted for privacy.

Abstract approved: _____

Robert William Rose, Jr.

The influence of shading intensity on performance, chlorophyll fluorescence emissions, the slope of the fluorescence induction curve, chlorophyll contents, and stomatal conductance of four Pacific Northwest conifer species [ponderosa pine (*Pinus ponderosa* Laws.), Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco), western redcedar (*Thuja plicata* Donn.), and western hemlock (*Tsuga heterophylla* (Raf.) Sarg.)] was investigated. Seedlings were grown under shade-cloth tents (supported by PVC pipe frames) using a repeated measures design for approximately 29 weeks in 1993 at Forest Research Laboratory, Oregon State University, Corvallis, Oregon. Four shade treatments (0, 30, 50, and 70% of full sunlight) with four replications each were used. Shading significantly influenced morphology, chlorophyll fluorescence, and chlorophyll contents

of all four species.

Although seedlings elongated most under 70% shade and least in full sunlight, biomass production significantly decreased with increasing shade. Overall, best performance was achieved with 30% shade.

Chlorophyll fluorescence (F_{max}, F_v, F_v / F_{max}), measured with an integrating fluorometer, significantly decreased over time with increasing shade for all four species. There was a significant decrease in final F_{max}, F_v, F_v / F_{max} of 27, 48, and 29%, respectively, from 0% to 70% shade. The slope of the fluorescence induction curve between the 60 and 120 second portion of the curve, regardless of shade treatment, had a significant linear relationship with time and species.

Chlorophyll contents of old and new needles were strongly negatively correlated with all chlorophyll fluorescence variables and directly related with increasing shade. Chlorophyll *b* and total chlorophyll in old needles substantially increased by 188 and 158% from 0% to 70% shade. Stomatal conductance of old needles, regardless of shade treatment, had a linear relationship with time and species while in new needles, this relationship was quadratic.

Findings of the study show that further evaluation of fluorescence emissions and the slope of the fluorescence curve is warranted. To make it

more meaningful in context with seedling's physiological status, measurements should be made concomitantly with CO_2 assimilation.

Effects of Shade on Performance and Chlorophyll Fluorescence of Four Pacific Northwest Seedling Conifer Species

By

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DEDICATION

This dissertation is dedicated to the loving and inestimable caring, prayers, and memories of my parents, Mr/Mrs Sardar Abdul Rehman Khan, deceased father-in-law, Sardar Mohammad Ashraf khan, and Late Sheikh Allah Yar Khan (May Allah be pleased with both of them).

Effects of Shade on Performance and Chlorophyll Fluorescence of Four Pacific Northwest Seedling Conifer Species

1. INTRODUCTION

1.1. Rationale

The purpose of this research was to investigate the effects of shade on the morphology and physiology of four Pacific Northwest conifer species (ponderosa pine, Douglas-fir, western red cedar, and western hemlock) under differing levels of shade (0%, 30%, 50%, and 70% of full sunlight). This has particular significance to ecosystem management because in some cases silvicultural systems that use small openings for regeneration, may contribute to overall management objectives particularly on federal lands (Tappeiner 1995, personal communication).

This new approach to cutting requires a new strategy to planting and that we revisit the morphological and physiological requirements of seedlings planted in other than full sunlight. While the shade "tolerance" of different species has been understood for decades, it has never been quantified on a "whole plant" seedling basis using chlorophyll fluorescence.

Can chlorophyll emissions discern differences in seedling responses at different shade levels and, thereby, be related to changes in seedling adaptability? To date there is no conclusive evidence to show that fluorescence instrumentation is sensitive enough to pick out shade response differences by species. This is the first of what is hoped will be a series of experiments aimed at using fluorescence to measure subtle physiological shifts leading to morphological changes in seedlings planted in small forest openings.

1.2. Shade/Low Light and Seedling Adaptability

Plants require optimal levels of light intensity for efficient photosynthesis which is species-specific. As light intensity increases, the rate of photosynthesis continues to increase to a point. However, when light saturation is reached, photosynthesis does not increase even with further increases in light intensity (Cleary et al. 1988). Conversely, decreasing light intensity reduces photosynthetic activity. Growth of red oak (*Quercus rubra* L.) and yellow-poplar (*Liriodendron tulipiferal* L.) was significantly reduced at low levels of light intensity (20% of full sunlight) and soil

moisture content (Kolb et al. 1990). Low light levels are accompanied by increases in chlorophyll *a* and chlorophyll *b* contents. Hawkins and Lister (1985) found higher chlorophyll *a* and *b* contents in Douglas-fir seedlings grown under a 30% light regime than those in full sunlight. Similarly, when grown in low light conditions, significantly higher concentrations of chlorophyll were reported in shade-tolerant blitz (*Impatients hybrida*) (Armitage and Vines 1982), *Aucuba japonica* 'variegata' (Andersen et al. 1991), and *Abies amabilis* (Dougl.) Forbes) and western hemlock (Mitchell and Arnott 1995).

Light accompanied by temperature changes also plays an important role in regulating stomatal opening and closing and hence stomatal water loss. Water loss through stomata is largely a function of the vapor pressure deficit at the leaf-air interface. The deficit in vapor pressures is simply the difference between vapor pressure in the leaf stomata (usually considered to be saturated) and that of surrounding air (Hobbs 1992). In the morning when transpirational demands are low due to mild temperature, stomatal conductance of the seedlings is increased. At the same time, the demand for CO_2 inside the leaf is high enough to decrease stomatal resistance to allow CO_2 diffusion, thus resulting in more efficient photosynthetic activities. But,

with increasing temperature and possibly decreasing water supply in the latter part of the day, vapor pressure deficit increases as a result of the soil-plant-air-continuum (SPAC) of water movement that exerts a strong transpirational pull at the stomata. This results in augmented transpirational demands and water loss thus putting sun-grown plants in danger of lethal dehydration. Under such conditions they protect themselves by conserving water through stomatal closure. But this is accompanied by decreased CO_2 diffusion resulting into reduced photosynthesis (Salisbury and Ross 1969, Bidwell 1974, Taiz and Zeiger 1991). It has been reported that water stress results in increased abscisic acid production in mesophyll cells which regulates stomatal opening and closing by changing osmotic potential of the guard cells (Bidwell 1974, Taiz and Zeiger 1991). Abscisic acid sharply increases in water-stressed plants which causes the guard cells to lose potassium ion concentration and turgor resulting in stomatal closure (Moore 1989).

1.3. Light and Photoinhibition

It is well known that high light intensities above the light saturation point of photosynthesis cause two important phenomena in many plants: photoinhibition (an inhibition of photosynthesis) and photooxidation (bleaching or solarization). Of the light absorbing leaf pigments, damage to chlorophyll causes the greatest extent of quality reduction (Powles 1984, Krause 1988, Binder et al. 1988, Tyystjarvi et al. 1991, DeLucia et al. 1991). The phenomenon of photoinhibition occurs due to a biochemical change in the 32KD D1 protein (herbicide binding protein, HBP-32 associated with quinone B) which is involved in the electron transport chain of photosystem II (Powles 1984, Hader and Tevini 1987, Taiz and Zeiger 1991). Seedlings grown in full sunlight have a more rapid and maximal repair capacity from photoinhibition than those which are shade acclimated (Samuelsson et al. 1987). This is attributed to the presence of high levels of messenger ribonucleic acid (mRNA) responsible for quinone B (Q_B) protein synthesis in the chloroplast of sun-grown seedlings (Anderson 1986). Q_B is continuously re-synthesized from mRNA in light and plays an important role in electron transfer in the Z-scheme of photosynthesis. Photoinhibition

becomes evident only in cases where the rate of Q_B inactivation or degradation exceeds the rate of its repair or synthesis (Anderson 1986, Hader and Tevini 1987, Krause 1988). Photoinhibition also occurs when shade-grown plants are exposed to high light levels which are greater than their photosynthetic system can handle (Davies et al. 1984 in Vidaver et al. 1991).

1.4. Assessing Seedling Performance

Increased attention has been given to evaluating the adverse effects of specific environmental conditions on plants due to either natural or human cause (Bilger and Schreiber 1990). Evaluating seedling performance is an important step to successful nursery management programs. Prior to the last decade, criteria for judging and defining seedling quality were based primarily on morphological characteristics or physical appearance (Sutton 1982, Hobbs 1984, Brissettee and Carlson 1987). Although, simple to use, morphological correlations with reforestation planting success are inconsistent and often do not reflect the vigor or non-visible damage of seedlings (Sutton 1982, Hobbs 1984, Mexal and Landis 1990). Instead,

physiological characterization has increasingly gained greater importance within the last decade for stock quality assessment (Zaerr and Lavender 1980). Although form and structure are still considered significant factors in seedling quality to tolerate environmental stress, physiological vigor is recognized more and more as a precondition for good survival and growth. However, physiological assessment of seedling quality and vigor should never be the sole quality determinant because there is no one effective method of measuring seedling quality (Lavender 1989). To meet this combined approach of seedling quality assessment, the Nursery Technology Cooperative (NTC) at Oregon State University (OSU) has developed the "Target Seedling Concept"; as defined as "a target seedling embodies those structural and physiological traits that can be quantitatively linked to reforestation success" (Rose et al. 1990a).

There are a number of physiological tests to evaluate seedling physiological status but, unfortunately, most of them are too slow, and because they are destructive, plants can not be retested either at a later time or when outplanted. Thus, their efficiency, at operational levels, is minimal at best (Zaerr 1985, Hawkins and Binder 1990, Vidaver et al. 1991).

Non-intrusive techniques play an important role in the detection of stress effects on photosynthetic activity of plants. An emerging technique is the measurement of chlorophyll fluorescence using an integrating fluorometer (Lichtenthaler et al. 1986). Because chlorophyll fluorescence is inversely related to the rate of photosynthesis, more severely affected and photosynthetically inactive plants yield higher fluorescence emissions. Thus, it has potential as a good measure of a plant's physiological status (Krause and Weis 1984). Measuring chlorophyll fluorescence emissions is an ideal physiological approach because it is non-invasive, non-destructive, and fast (i.e. the technique takes only a few minutes to accomplish) (Bolhar-Nordenkampf et al. 1988b, Vidaver et al. 1989, Vidaver et al. 1991).

1.4.1. Chlorophyll Fluorescence

1.4.1.1. *A Brief History*

Not all light energy absorbed by green pigments of plants is utilized in photochemical light reactions of photosynthesis. Part of the absorbed light is given off as heat or emitted in the form of chlorophyll fluorescence.

As early as 1874, Muller (1874) in Schreiber (1983) using a suitable combination of colored glasses observed chlorophyll fluorescence with his naked eye, and recognized a correlation between chlorophyll fluorescence and photosynthetic activity. However, the systematic investigation of this correlation was not possible for more than 50 years until light sensitive devices and signal recorders were developed. In 1931, H. Kautsky, a German scientist, was the first one to detail and study this phenomenon. The resulting fluorescence induction curve has since been then referred to as "the Kautsky Curve" (Figure 1).

1.4.1.2. *Definition*

Chlorophyll fluorescence is defined as the electromagnetic radiation emitted when a chlorophyll molecule goes from an excited singlet state [$S^{a}_{(D, D^{*})}$] reached by absorption of red light (680 nm) to a singlet ground state [$S_{(D, D)}$] (Nobel 1991). Fluorescence emissions originate from chlorophyll *a* molecules embedded in the thylakoid membranes settling in the stroma matrix of a chloroplast (Hoober 1984, Hader and Tevini 1987). In the next section, I discuss this further.

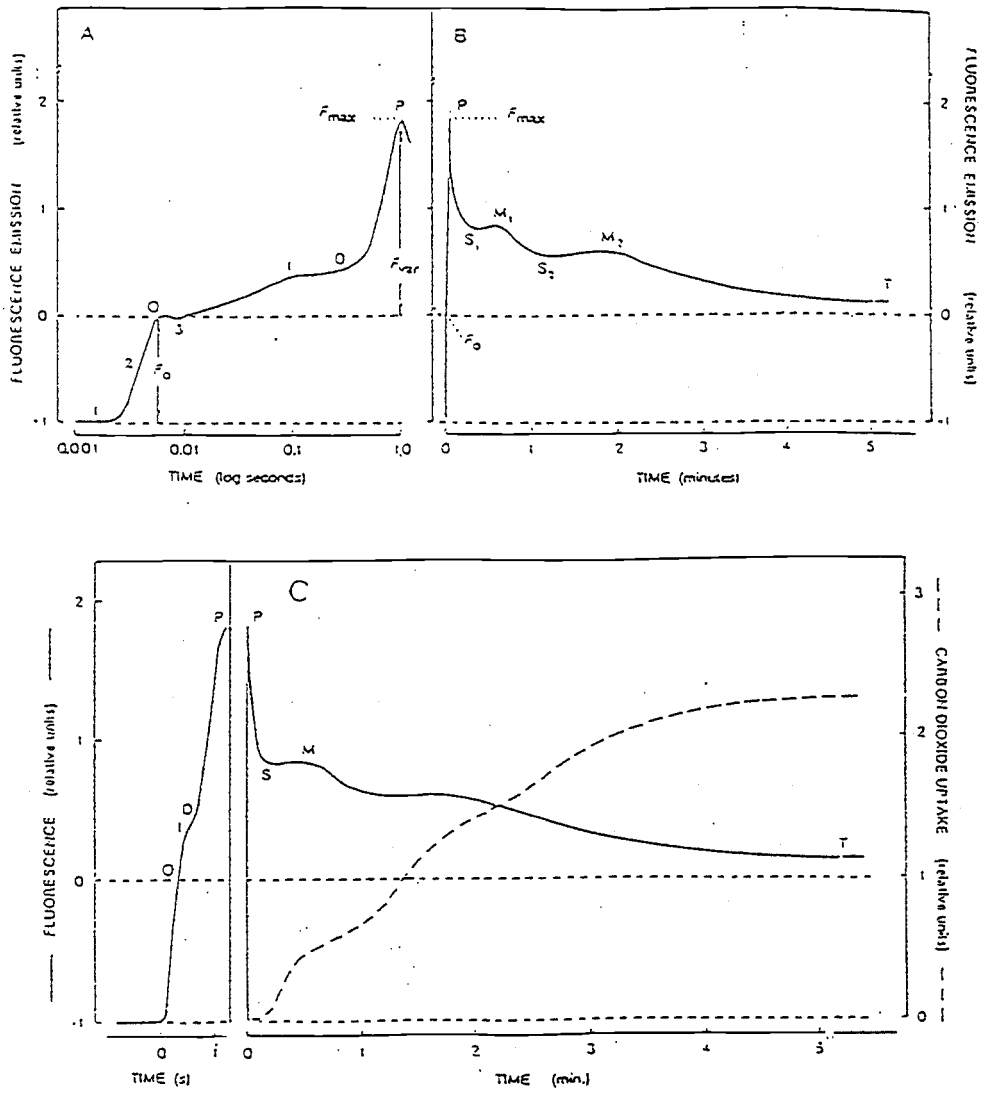

Figure 1. The Kautsky or fluorescence induction curve: A) the fast phase ("O" to "P"); B) the slow phase ("P" to "T"); C) time course for CO_2 uptake and fluorescence emissions to steady state for pretreated maize leaves on sudden illumination (modified after Vidaver et al. 1991).

1.4.1.3. *Chlorophyll and Chlorophyll Fluorescence*

Chlorophyll *a* and chlorophyll *b* are important components of the photosynthetic system. These pigments are cyclic tetrapyrrole compounds with porphyrin ring structures (consisting of 4 rings: A-D and an alcohol phytol esterified to a carboxyl-group substituent in ring D). Chlorophyll *a* and chlorophyll *b* differ from each other only in that the methyl group (CH_3) on pyrrole ring B of chlorophyll *a* is replaced by a formyl group (CHO) in chlorophyll *b* at carbon atom number seven. This results in differing polarity and absorption spectra of the molecules (Lehninger 1982, Hoober 1984, Hader and Tevini 1987, Taiz and Zeiger 1991).

The photosynthetic process involves two photosystems, photosystem II (PSII) and photosystem I (PSI). The reaction center II of PSII consists of one chlorophyll *a* molecule with its absorption maxima at 680 nm, hence known as chlorophyll P680. P680 accompanied with proteins and pheophytin is surrounded by 40-60 additional chlorophyll *a* molecules which comprise the core complex II of photosystem II. This system is in turn surrounded by approximately 70 chlorophyll *a* molecules, 70 chlorophyll *b* molecules and some carotenoid molecules. This

constitutes the light harvesting complex II of PSII. The reaction center I of PSI is believed to consist of two chlorophyll *a* molecules because there are two absorption maxima at 682 nm and 700 nm and generally known as chlorophyll P700. It is surrounded by about 40-120 additional chlorophyll *a* molecules and proteins which comprise the core complex I of PSI. Light harvesting complex I of PSI consists of approximately 40 chlorophyll *a* and 40 chlorophyll *b* molecules and other carotenoids (Hader and Tevini 1987).

Thus, the chlorophyll *a* molecule forms the reaction centers of both photosystems and is exclusively responsible for primary photochemical processes of photosynthesis. The bulk of the remaining chlorophyll *a* and *b* molecules, carotenoids, and phycobilins (found in algae) harvest light energy from sunlight and funnel it to the active chlorophyll P680 and P700 molecules embedded in reaction centers II and I of PSII and PSI, respectively.

1.4.1.4. *Mechanism of Chlorophyll Fluorescence*

Of the photosynthetically active part (PhAR, 400-700 nm) of incident radiation, which amounts to about 40-50% of the total solar

radiation (1.3 kW. m^{-2}) (Larcher 1983, Taiz and Zeiger 1991), peak absorption by chlorophyll *a* and *b* occurs in the blue and red regions of the spectrum (Hoober 1984). When a photon strikes a chlorophyll molecule it becomes excited and the electron is raised from a ground state in the molecular orbital to an excited state. Blue photons (of short wavelength and higher energy) raise electrons to the excited singlet state 2 [$S^{b}_{(D, D^{*})}$], while red photons (of long wavelength and lower energy) raise electrons to the excited singlet state 1 [$S^{a}_{(D, D^{*})}$] (Walker 1988). The upper singlet state of chlorophyll excited by blue light [$S^{b}_{(D, D^{*})}$] is extremely unstable and goes to the lower excited singlet state [$S^{a}_{(D, D^{*})}$] in about 10^{-12} second, that is, before any appreciable blue fluorescence can take place. Because of such rapid energy degradation by a radiationless de-excitation, none of this energy is available for photosynthesis. It is the red, more long-lived excited state which is the starting point of photosynthesis and, therefore, of most of the biological energy transduction (Walker 1988, Nobel 1991).

Light energy absorbed by chlorophyll *a* and other accessory pigments of the plants is funneled to the reaction center II of PSII, where some (0-20%) is utilized for photosynthesis and is immediately converted into chemically bound energy in the form of NADPH and ATP. However,

not all absorbed light energy is utilized in photochemical reactions. The majority of it may be dissipated and lost as heat (75-97%) and a minor part (3-5% of total absorbance) is re-emitted as chlorophyll *a* fluorescence (Larcher 1983, Nobel 1983, 1991). Thus, the fluorescence emission is essentially all in the lower energy red region and is solely emitted by PSII (Lichtenthaler et al. 1986, Lichtenthaler and Rinderle 1988, Krause and Weis 1988, Bolhar-Nordenkampf et al. 1989, Taiz and Zeiger 1991). Photosynthetic electron transport is initiated by the absorption of photons by P680 from sunlight. P680 is excited to donate electron to pheophytin present in reaction center II of PSII, thus leaving itself in an oxidized state* ($P680^+$). The holes created by light in $P680^+$ in reaction center II of PSII are filled by the electrons provided by water photolysis using the oxygen-evolving system. Water photolysis is catalyzed by manganese (Mn) containing an enzyme complex "Y". An electron donor "Z" is involved in electron transfer from water to $P680^+$. Upon receiving electrons from "Z" $P680^+$ becomes reduced ($P680^-$) to again donate its electron to pheophytin which is an intermediate electron donor to quinone A, the primary electron acceptor in photosynthetic electron transport chain. Quinone A transfers its

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* Oxidation = loss or removal of electrons; Reduction = gain or addition of electrons

electrons to quinone B via a redox system of quinone pool, cytochrome *f*, plastocyanin (PC), iron-sulfur protein complexes (Fe-S_A and Fe-S_B), and ferredoxin (Fd) to the terminal acceptor NADP reductase which catalyzes electrons to NADP. NADP is reduced to NADPH under simultaneous uptake of protons from the stroma (Bidwell 1974, Lehninger 1982, Hader and Tevini 1987, Taiz and Zeiger 1991).

Fluorescence intensity depends on the above mentioned redox state of the electron transport chain in Z-scheme of photosynthesis and on whether the energy absorbed by the chlorophyll can be utilized photochemically. There would be no fluorescence if the total absorbed and transferred photochemical energy could be converted into chemical energy without losses (i.e. 100% quantum efficiency, which does not exist in nature). When reaction center II of PSII is closed, or all primary electron acceptors of PSII are reduced, fluorescence increases. Quinone A, the primary electron acceptor of PSII effectively quenches fluorescence as it transfers its energy to reduce quinone B and itself becomes oxidized (Hader and Tevini 1987). But, when the electron transport between quinone A and quinone B is blocked by the application of herbicides (such as DCMU) or adverse environmental factors, fluorescence increases rapidly to a maximal

value and remains there. This happens because quinone B is biochemically changed and is no longer able to accept electrons from quinone A and transfer to PSI. Thus the electron transport chain is interrupted and photosynthetic efficiency is inhibited (Taiz and Zeiger 1991).

1.4.1.5. *Fluorescence Induction Kinetics*

The fluorescence emissions of a pre-darkened leaf rise via the ground fluorescence (F_0) to a maximum level (F_{max}) then decreases to a steady state (F_s) value as photochemistry increases in response to electron transfer away from quinone A (Lichtenthaler et al. 1977, Lichtenthaler and Rinderle 1988). The Kautsky curve consists of two phases: 1) the fast phase (Figure 1A) in which fluorescence rises very rapidly from F_0 to F_{max} in 0.1 to 1 second; and 2) the slow phase (Figure 1B) in which fluorescence decreases from F_{max} to F_s within three to five minutes (Vidaver et al. 1991). The components of the fast phase of the Kautsky curve are termed O, I, D, P and those of the slow phase are termed P, S, M, T (Figure 1). The "O" level is the initial, minimum, dead, or ground fluorescence (F_0) and occurs when all electron acceptors in the electron transport chain are fully oxidized (i.e.

the electron carriers are open to receive and pass electrons through electron transport chain). F_0 is independent of photochemistry. The "I" level (intermediate stage) represents the stage of fluorescence during which there is a rapid reduction of quinone A, a primary electron acceptor associated with water photolysis (splitting) and charge accumulation. The "D" level (dip) represents reduction of quinone B, a secondary electron acceptor, by electron transfer from quinone A, thus quinone A is oxidized. The "P" level refers to maximum fluorescence (F_{max}) which occurs when quinone A and other plastoquinones are highly reduced (electron acceptors are blocked and are unable to accept and transfer electrons). At this point, photochemical quenching approaches zero. The "S and M" levels represent declines in fluorescence from the "P" level and occur as photochemistry and net photosynthetic CO_2 assimilation increase in response to electron transfer and CO_2 uptake (Figure 1C). The "T" level (terminal state) represents the steady state of the fluorescence, F_s (Papageorgious 1975, Krause and Weis 1984, Krause and Weis 1991, Vidaver et al. 1991). Variable fluorescence (F_v) refers to the difference between F_{max} and F_0 (i.e. $F_v=F_{max} - F_0$) (Lichtenthaler 1988, DiMarco et al. 1988, Bolhar-Nordenkampf et al. 1989).

1.4.1.6. *Fluorescence Quenching*

In a general sense, quenching denotes all processes that decrease fluorescence yield below its potential peak. Quenching is also reflected by decreases in F_v / F_{max} (Krause and Weis 1991). There are two types: 1) photochemical quenching, which occurs through energy transformations via the above process and predominantly reflects photochemistry, and 2) non-photochemical quenching, which occurs due to biochemistry (i.e. CO_2 assimilation), changes in intrathylakoid pH, phosphorylation of light harvesting complexes, and magnesium depletion in thylakoids (Bolhar-Nordenkampf et al. 1989, Krause and Weis 1991). Normally a polyphasic quenching is observed, sometimes interspersed by one or several secondary peaks, until F_s is reached in a few minutes (Krause and Weis 1991). The rate of fluorescence quenching from F_{max} to F_s is a direct measure of the photosynthetic conversion of the absorbed light quanta (Lichtenthaler et al. 1986). This quenching rate can be estimated by measuring the slope of the slow phase of the Kautsky curve. For a detailed explanation and mechanism of quenching, see Krause and Weis (1984 and 1991) and Demmig and Winter (1988).

1.4.1.7. *Fluorescence Measuring Devices*

There are two main kinds of fluorometer systems: 1) those which use fiber optics to guide low to high excitation light intensities to a relatively small leaf area (Bolhar-Nordenkampf et al. 1989) and 2) those which enclose an intact seedling shoot in an integrating sphere with low to moderately high excitation light intensities (Dube and Vidaver 1990). A few commercially available fluorometers like Pulse-Amplitude Modulation (PAM) /101, 102, 103 (Heinz Walz of Federal Republic of Germany); Pulse-Amplitude Modulation Fluorometer, a prototype of PAM 101, 102, 103; Brancker SF-30 (R. Brancker Ltd, Ottawa, Canada); Modulated Fluorescence Measurement System (MFMS) (Hansatech Ltd of UK); Morgan CF-1000 (the USA); Pocket Computer Fluorometer (Larcher and Cernusca 1985, Austria), and Plant Stress Meter (PSM) (BioMonitor of Sweden) are included in the first category of fluorescence measuring devices. These systems are all useful for detecting chlorophyll fluorescence but they sample only a small portion of a needle per probe. They can not assess large samples or entire seedling shoots in conifer applications (Hawkins and Binder 1990). However, most of these fluorometers can be

used for quenching analyses. For a detailed description on these and some other instruments, see Bolhar-Nordenkampf et al. (1989) and Mohammed et al. (1995).

Conversely, an integrating fluorometer system developed by Pacific Fluorotec Corporation of Simon Fraser University, Burnaby, British Columbia, Canada has the capability of measuring whole or a part of an intact seedling (Toivonen and Vidaver 1984). Various components of this system (Figure 2) tungsten lamp, an integrating sphere, light source, electronic shutter, optical fibers, light dispersion cone, the fluorescence (F) detector, and the sphere excitation light (I) detector are uniquely incorporated and arranged in an appropriate housing. These are interfaced to a microcomputer which triggers the shutter opening and acquires and stores the fluorescence emission data on the onset of shutter opening. The system is completely controlled by software through the A/D board installed in the PC (Pacific Fluorotec Corporation 1991). The software package includes

two separate programs. "FluoroView" is the main data acquisition and analysis program. The second smaller program "Panel" is used to manually operate and test the instrument.

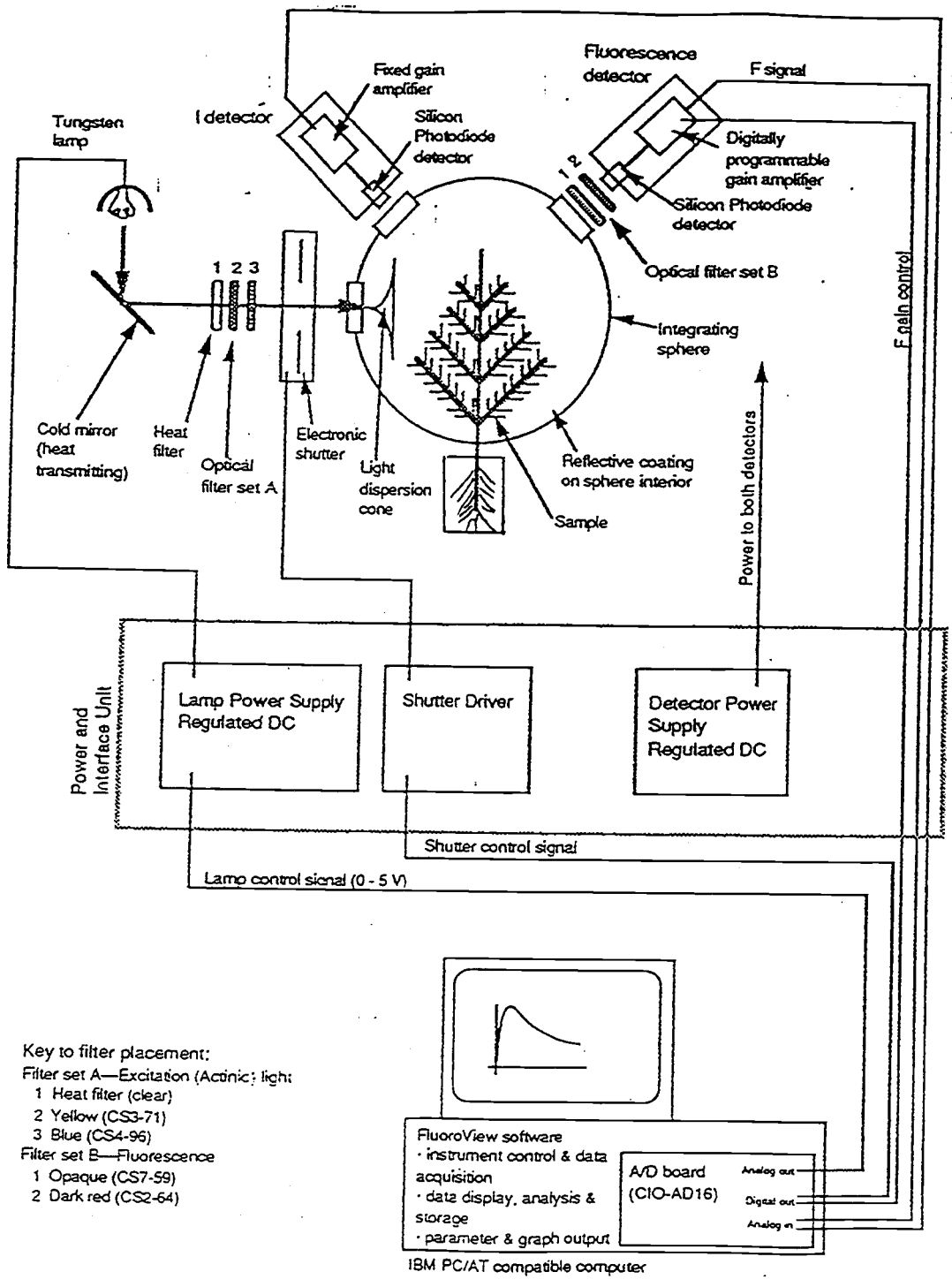

Figure 2. Schematic of the integrating fluorometer system and its components (modified after Pacific Fluorotec Corporation 1991).

In comparison to other systems, the integrating fluorometer system has some distinct advantages:

1) detects the integrated or representative sample fluorescence induction response from the entire sample;

2) monitors the averaged fluorescence response of the whole seedling rather than the few square millimeters of leaf surface, thus avoiding the sampling problems by minimizing the response extremes from older or younger, base, mid-height, or tip of needles, damaged or dead needles, and therefore is a good indicator of the photochemical status of the whole shoot. (Toivonen and Vidaver 1984, Vidaver et al. 1989, Vidaver et al. 1991);

3) normalizes the data. Thus removing the effect of sample size (fluorescence emission amplitude) when comparing data from different samples or when averaging the responses of more then one sample (Vidaver et al. 1991). The normalization also compensates for differences in chlorophyll contents (Vidaver et al. 1989);

4) has a variety of configurations for ease of use from a completely portable, battery-powered version for field use, a coupled microprocessor data-acquisition and control system for laboratory use, and an adjustable sphere diameter and excitation light output to accommodate small tissue-cultured material up to plants or shoots over 10 cm tall (Toivonen and Vidaver 1984);

5) easily removed and replaced components with possible future upgraded versions or with user-customized units;

6) easy to use software, reporting a range of parameters10 and recording fast and slow fluorescence kinetics.

Nevertheless, this system has some disadvantages in comparison to other systems. Most significant is that the integrating fluorometer used in the current study has an approximately 25 cm diameter sphere and cannot measure seedlings' shoots larger than 20 cm. Also, the system is not designed for quenching analyses.

1.4.2. Chlorophyll Fluorescence and a Seedling's Physiological Status

Chlorophyll fluorescence emissions provide useful information about the physiological status of seedlings. Hawkins and Lister (1985) reported that chlorophyll fluorescence analyses can be used as a rapid and inexpensive means of screening for Douglas-fir's dormancy status. The experiments conducted by Vidaver et al. (1989) on white spruce seedlings suggested that integrated non-destructive F_v assessment can rapidly provide information about the physiological status of nursery-grown seedlings. The potential operational uses of F_v are in areas such as, determination of lifting dates and in assessing post-storage physiological recovery.

Chlorophyll fluorescence has a wide range of applications. It can be used to analyze partial reactions of the electron transport chain and the

site of inhibitor activity of herbicides, such as urea, triazine, and diuron (Hader and Tevini 1987, Falk and Samuelsson 1992). It has been used to detect effects of frost hardening and dehardening on the photosynthetic electron transport chain in isolated chloroplasts of *Pinus sylvestris* (Martin et al. 1978). It has also been successfully used to assess the functioning of the photosynthetic apparatus (Krause and Weis 1988, Rinderle and Lichtenthaler 1988) and the effects of heat, light, and drought stress (Ögren and Öquist 1985, Strand and Lundmark 1987, Ögren 1990), cold acclimation, freezing stress and photoinhibition (Krause and Somersalo 1989), chilling injury and recovery in attached and detached bean and maize leaves (Smillie et al. 1987), low temperature hardiness in blackcurrant (*Ribies nigrum* L.) (Brennan and Jefferies 1990), cold tolerance in Picea species (Adams and Perkins 1993), and salinity tolerance in barley (*Hordeum vulgare* L.) (Belkhodja et al. 1994).

Chlorophyll fluorescence has been increasingly used to understand both the mechanism of photosynthesis and the factors affecting it. Applications range simply from rapidly identifying injuries to leaves in the absence of visible symptoms to a detailed analysis of causes of change in photosynthetic capacity (Bolhar-Nordenkampf et al. 1989). Chlorophyll

fluorescence is not only used as a probe of the photosynthetic process but is also sensitive to other physiological effects which influence photosynthesis. For example, distinctive changes in chlorophyll fluorescence are observed within 30 minutes of application of the herbicide glyphosate and days before any visual symptoms of damage may be apparent. Glyphosate does not inhibit photosynthetic reaction rather impairs metabolic processes outside the chloroplast (Ireland et al. 1986 in Bolhar-Nordenkampf et al. 1989). Thus, measurement of chlorophyll fluorescence can be an important tool in photosynthetic research. Chlorophyll fluorescence has great potential as a field technique, both for the analysis of photosynthetic processes and as a rapid screening for invisible injuries to leaves (Bolhar-Nordenkampf et al. 1989, Krause and Weis 1991).

1.4.2.1. *F_v / F_{max} and Photochemical Efficiency of PSII*

Of the measured chlorophyll fluorescence variables, F_v / F_{max} has been used as a good indicator of the photochemical and quantum efficiency of the PSII reaction center (Bohlar-Nordenkampf and Lechner 1988b, Adams et al. 1990, Ottander and Öquist 1991, Öquist and

Hunner 1991, Camm et al. 1991, Camm et al. 1993) and photoinhibition (Samuelsson and Richardsson 1982, Demmig and Bjorkman 1987, Bolhar-Nordenkampf and Lechner 1988a, Bolhar-Nordenkampf et al. 1989, Krause and Somersalo 1989, Krause and Weis 1991, Ögren 1991, DeLucia et a l. 1991). The decrease in F_v / F_{max} is characterized by a decline in F_v (Krause and Somersalo 1989, Giersch and Krause 1991). This shows the usefulness of F_v / F_{max} as an indicator of the overall photosynthetic activities of seedlings.

1.4.2.2. *Past and Current Research*

Undoubtedly, a great deal of work has been carried out on chlorophyll fluorescence emissions and their role in understanding the physiological status of seedlings. But generally this research has mostly used non-integrating fluorometer systems and has been limited to a few countries; e.g. Austria for freezing stress, Canada for frost and cold hardiness, England, and Germany for photoinhibition and acid rain, Sweden for cold tolerance, and the United States for cold hardiness. Most of the research has focused on the molecular aspects of fluorescence emissions

(electron transport chain, quinone systems, water photolysis, light harvesting systems, reaction center of photosystems II, chloroplast membrane, CO_2 assimilation, O_2 evolution, and etc), cold acclimation, dormancy induction, freezing stress, photoinhibition, etc. under certain, highly controlled conditions.

The research on chlorophyll fluorescence emissions of whole plants became possible a decade before the integrating fluorometer was developed by Canadian researchers in 1984. A review of chlorophyll fluorescence literature showed that very little work has been done on shading in relation to fluorescence emissions and chlorophyll contents in conifer seedlings. Some of the studies were conducted on white pine (*Pinus monticola* Dougl), red pine (*P. resinosa*), hemlock (*Thuja spp.*), and ailanthus (*Ailanthus altissima* (Mill.) (Bourdeau and Laverick 1958), sitka spruce (Lewandoska and Jarvis 1977), *Brassaia actinophylla* Endl, *Philidendron oxycardium* (Schott) Bunt, and *Aphelandra squarrosa* Nees 'Dania', non-woody plants (Poole and Conover 1979), Sprinter Salmon, sun-tolerant and Blitz, shade-tolerant (Armitage and Vines 1982), Douglas-fir (Hawkins and Lister 1985), *Aucuba japonica*, a shrub (Andersen et al. 1991), and *amabilis fir (Abies amabilis* (Dougl.) Forbes) and western hemlock (Mitchell and Arnott 1995).

The synthesized conclusion from these studies is that chlorophyll contents increased with increasing shade levels for all species studied.

Renowned researchers in Austria, Canada, England, Germany, Sweden, and the United States were contacted to find out if someone had actually established controlled shade treatments to determine chlorophyll fluorescence emissions in conifer seedlings using an integrating fluorometer system. The response showed that very little work has been conducted on the topic to date.

Studies, conducted by Hawkins and Lister (1985), Falk and Samuelsson (1992), Camm and Lavender (1993), and Mitchell and Arnott (1995), used PAM systems to measure chlorophyll fluorescence emissions under different light or shade levels.

Hawkins and Lister (1985) used a prototype of plant productivity fluorometer, model SF-10 to investigate chlorophyll fluorescence emissions in two-year old Douglas-fir seedlings grown under full ambient light, 30%, and ≥ 93% transmission of ambient light. This system measured only up to 2-3 cm^2 needle area (Lister 1995, personal communication). It is not clear whether these light treatments were replicated. They used current-year needles from terminal and lateral branches in mid to upper-crown region.

They concluded that chlorophyll fluorescence emissions can be used as a rapid and inexpensive means of screening Douglas-fir's dormancy status. They also found increased chlorophyll *a* and chlorophyll *b* contents under 30% light regime compared to open conditions.

Falk and Samuelsson (1992) used PSM to monitor recovery from photoinhibitory effects on cells of the green alga (*Chlamydomonas reinhardtii*) using three photon flux densities of 500, 1400, and 2200 μmol $m^{-2} S^{-1}$ to. The treatments do not appear to be replicated. They concluded that recovery was both rapid and complete after moderate photoinhibition (1400 μmol $m^{-2} S^{-1}$).

In the study conducted by Camm and Lavender (1993), lodgepole pine (*Pinus contorta* Dougl. var. latifolia Englem) and white spruce (*Picea glauca* [Moench] Voss) seedlings were grown under short daylength (8-hour photoperiod, achieved by covering seedlings with opaque plastic) and natural daylength (15 hour, 34 minutes photoperiod) and then stored in cold storage at 2 ^{0}C under different light intensities. Seedlings were measured for chlorophyll fluorescence using PAM fluorometer (Heinz Walz, Effeltrich). They concluded that both species responded differently to nursery and cold-storage photoperiods and had different F_v / F_{max} values.

Mitchell and Arnott (1995) used a Morgan CF-1000 fluorometer to evaluate morphology, chlorophyll fluorescence emissions, chlorophyll content, and gas exchange of one-year old amabilis fir and western hemlock seedlings grown under 0%, 30%, 60% shade of full sunlight using shade cloth and 30% shade using lath slats. The shade levels were replicated two times. Because the authors used the Morgan CF-1000 fluorometer (PAM system), fluorescence measurements on a whole-plant basis were not possible because only a small portion of one needle per probe were measured. The fluorescence emissions were measured only once at the end of the study. The authors found increased shoot height and decreased biomass production under 60% shade as compared to full sunlight. But there were no significant differences among shade treatments for any of the chlorophyll fluorescence variables and chlorophyll contents. However, shaded seedlings tended to have higher chlorophyll *a* and chlorophyll *b* concentrations than those grown in full sunlight.

There is another important aspect of fluorescence measurements that needs thorough exploration and further scrutiny. The slope of the fluorescence curve has not been quantified and investigated to date. However, some researchers such as Lichtenthaler et al. (1986),

Lichtenthaler (1988), Lichtenthaler and Rinderle (1988), and Haitz and Lichtenthaler (1988) have described a ratio of fluorescence decrease (F_d) to the steady state (F_s). This is called the Rfd ratio and has been used as a vitality index for seedlings. Vitality index as defined by Lichtenthaler, Rinderle, and Haitz is "a measure or indicator of the potential photosynthetic capacity of a leaf". Values greater than 2.5 depict better photosynthetic conversion of the absorbed light quanta. This cannot be compared to the slope of the fluorescence curve determined in the current study for certain reasons. The above mentioned researchers have used a completely different approach, by measuring F_d at 690 and 730 nm as compared to integrating system measuring at approximately 650 nm. It is also not clear which points on the fluorescence curve were used for the model. The methodology for modeling this ratio or any statistical information was not provided.

Though the current study seems to be related to a few of the above mentioned studies, in fact only one of these (Mitchell and Arnott 1995) is closely related. However, Mitchell and Arnott did not address some important aspects of the chlorophyll fluorescence measurements such as measuring fluorescence over time on a whole seedling basis to evaluate the trend during plant establishment, calculating the slope on any portion of the

fluorescence induction curve to know something about fluorescence quenching, correlating chlorophyll content with chlorophyll fluorescence emissions as affected by a variety of shade levels, or evaluating the effects of shade on a variety of species.

1.4.2.3. *Objectives of the Current Study*

The current study investigated the effects of shade on the morphology and physiology of four Pacific Northwest conifer species [ponderosa pine (2+0), Douglas-fir, western redcedar, and western hemlock (P+1)] grown under four shade levels (0%, 30%, 50%, and 70% of full sunlight, using shade cloth supported by PVC pipe frames) at Forest Research Laboratory, Oregon State University, Corvallis, Oregon. The specific objective of the study was to evaluate and quantify:

1) morphological and physiological (chlorophyll fluorescence, slope of chlorophyll fluorescence curve, chlorophyll content, and stomatal conductance) responses of the seedlings to different shade environments

To meet the objective, following five null hypotheses were

tested: (to clarify why these hypotheses are stated as "null hypotheses", refer to the Appendix 1)

1) $H_{0:}$ the morphological development of the four species is not different under four shade levels;

2) $H_{0:}$ chlorophyll fluorescence emissions are not different among species and shade levels;

3) $H_{0:}$ the slope of the fluorescence curve between the 60 and 120 second portion is not different among species and shade levels;

4) $H_{0:}$ chlorophyll content is not different among species and shade levels;

5) $H_{0:}$ there is no correlation between chlorophyll content and chlorophyll fluorescence emissions

2. MATERIALS AND METHODS

2.1. Plant Material

Four species of seedlings (ponderosa pine, Douglas-fir, western redcedar, and western hemlock) were used in this study. All seedlings were plug+1 except ponderosa pine, which was 2+0. Ponderosa pine and Douglas-fir seedlings were provided by International Paper Company's Kellogg Nursery, Lebanon District, OR. Western redcedar and western hemlock seedlings were supplied by the Oregon State Department of Forestry, D. L. Phipps Nursery, Salem District, OR. Seedlings were bare-rooted, cold-stored at 2-3 ^{0}C, and planted in February 1993 in individual peat-pots (40 cm x 17.5 cm x 10 cm) containing a 1:2:1:1 sterilized mixture of peat:pumice:soil:sand. The pots had three holes at the bottom for drainage. A total of 320 seedlings were planted.

2.2. Treatments

The potted seedlings were placed on two raised beds (21.89 m x 1.85 m) and divided into four sections at the Forest Research Laboratory,

Oregon State University, Corvallis. Seedlings were grown under four levels of shade: 0% (full sunlight), 30%, 50%, and 70% of full sunlight (ratings listed by the shade cloth manufacturer, Lumite Industries, P.O. Box 977, Gaimesville Georgia, 30503. Telephone: 404-532-9758) for a period of approximately 29 weeks, (February 8, 1993 to September 14, 1993). Each shade treatment was replicated four times and was accomplished by using sewn shade cloth of the specified shade level supported by a PVC pipe frame. These treatments, which resembled small tents (1.83 m x 1.37 m x 1.22 m), were placed over the seedlings. The PVC pipe frames were firmly fixed to the ground so they would not blow away (Figure 3).

Light intensity in the shade treatments was measured on a sunny day, July 8, 1993, at 1100h with a LiCOR Model LI-1600 quantum sensor registering in the 400 to 700 nanometer (nm) waveband. Actual photosynthetic photon flux in full sunlight (0% shade) was 1703 to 1957 μmol m^{-2} s^{-1}. Photon fluxes for 30%, 50%, and 70% shade were 1250 to 1363, 759 to 903, and 390 to 440 μmol m^{-2} s^{-1} respectively of full sunlight.

The shade treatments were randomly assigned to the experimental material. Each shade treatment consisted of a total of 20 seedlings (five seedlings per species). The seedlings were completely randomized and

A

B

Figure 3. Shade tents, supported by PVC pipe frames, used to impose shade treatments to ponderosa pine, Douglas-fir, western redcedar, and western hemlock seedlings: A) overall view of shade treatments; B) side view of 70% shade.

placed pot to pot within each shade treatment. Seedlings were watered when needed. To avoid confounding effects, there were no other treatments, such as fertilizers or pesticides, applied to the seedlings. The plots and pots were weeded by hand to minimize additional effects on seedling development.

2.3. Measurements

Table 1 shows a list of various morphological and physiological variables measured at different times during the course of the study.

2.3.1. Morphology

2.3.1.1. *Initial Measurements*

Before planting, each seedling was non-destructively measured for shoot height, stem diameter, total fresh weight, root volume, and shoot volume. Shoot height was measured from the root collar (just above the cotyledon scar) to the base of the terminal bud. Stem diameter was

Table 1. List of morphological and physiological variables measured during the course of the study.

Variables Measured	Measurement Times
A) Morphology	
Shoot height	Initial, Final
Stem diameter	Initial, Final
Total fresh weight	Initial, Final
Root volume	Initial, Final
Shoot volume	Initial, Final
Total dry weight	Final
Shoot:root ratio	Final
Terminal height	Initial, Periodic (over time), Final
Relative growth rate	Final
Sturdiness quotient	Final
Dickson quality index	Final
B) Physiology	
F_{max}	Initial, Periodic (over time), Final
F_v	Initial, Periodic (over time), Final
F_v / F_{max}	Initial, Periodic (over time), Final
Slope of fluorescence	Initial, Periodic (over time), Final
Chlorophyll *a* (old/new needles)	Initial, Final
Chlorophyll *b* (old/new needles)	Initial, Final
Total chlorophyll (old/new needles)	Initial, Final
Stomatal conductance (old/new needles)	Final (but measured three times in one day)

measured with a digital caliper at the root collar. Fresh weight of whole seedlings was measured on a digital balance before root/shoot volume measurements. Root and shoot volumes were measured in cm^3 using the water displacement method (Burdett 1979).

2.3.1.2. *Periodic Measurements*

Terminal height growth of 192 seedlings was determined at two-week intervals from the day seedlings initiated budbreak and continued throughout the study.

2.3.1.3. *Final Measurements*

At the end of the study (Sept 15, 1993) the same seedlings were destructively harvested and the same morphological characteristics mentioned above were measured. In addition, dry weight of shoots and roots were obtained on the same seedlings by partitioning them into shoots and roots and oven-drying at 65-70 ^{0}C for 48 hours (Feret 1982, Rose et al.1992). Total dry weight of the seedlings was then calculated by adding shoot and root dry weights.

Seedling quality indices such as mean relative growth rate of total fresh weight (RGR), sturdiness quotient (SQ), and Dickson quality index (DQI) were calculated using the following formulae:

1) $RGR = (\log_e W_2 - \log_e W_1)/(T_2 - T_1)$ (Hunt 1982, Harper 1982)

Where

W_1 = initial total fresh weight
W_2 = final total fresh weight
T_1 = date of budbreak
T_2 = date of growth cessation
(note: $\log_e \equiv \ln$)

2) $SQ = H/D$ (Thompson 1985)

where

H = final shoot height (cm)
D = final stem diameter (mm)

3) $DQI = TDW/((H/D) + (SDW/RDW))$ (Dickson et al. 1960 in Thompson 1985)

where

TDW = final total seedling dry weight (g)
SDW = final shoot dry weight (g)
RDW = final root dry weight (g)

RGR is a measure of the growth rate of the whole plant per unit time with respect to the initial weight of the plant and may be expressed in such terms as g day^{-1} or g $week^{-1}$ (Hunt 1982, Harper 1982). It is the most important index of productivity. It is an overall growth index for comparing the rate of growth of different species at different times or different environments (Radosevich and Osteryoung 1987). SQ is a shoot height: stem diameter ratio and reflects the stocky or spindly nature of the seedlings (Thompson 1985). DQI was devised by evaluating how well a number of possible combinations of morphological characteristics can be used to predict field performance of white spruce and white pine seedlings (Dickson et al. 1960 in Thompson 1985). The index was successfully used by Roller (1977) in Thompson (1985) and Ritchie (1984).

2.3.2. Physiology

2.3.2.1. *Chlorophyll Fluorescence*

A total of 128 randomly selected seedlings were non-destructively measured for initial, periodic (once a month), and final chlorophyll fluorescence emissions (F_{max}, F_v, and F_v / F_{max}). The same potted seedlings were repeatedly measured over time from April through September, 1993 with an integrating fluorometer developed by Pacific Fluorotec Corporation of Simon Fraser University, Burnaby, British Columbia, Canada (Toivonen and Vidaver 1984). The fluorometer system software used was FluoroView version 0.5d. The initial testing, calibration, diagnosis, and operation of the instrument were carried out using the procedure detailed in the user's manual (Pacific Fluorotec Corporation 1991).

Before the seedlings were scanned for fluorescence emissions, they were preconditioned according to the recommendations of Binder and Fielder (1991). Seedlings were removed from shade treatments between 1630h and 1730h the day before measurement and watered to field capacity. They were then placed in a preconditioning box equipped with 200 to 250

μmol m^{-2} sec^{-1} of light (12 Northwestern F40T12/Artic 5000 40 watt fluorescent tubes) for light adaptation for approximately 1.5 hours. The lights were then turned off for 11 hours. The lights were turned on the next morning at 600h for approximately two and a half hours. Following this light-dark-light exposure, seedlings were removed from the preconditioning box at 0830h and brought to the laboratory for fluorescence measurements. Windows, doors, and other sources of incoming light in the laboratory were fully covered with black plastic to meet the complete dark conditions. Before putting the seedlings in the sphere, they were dark-adapted for about 30 minutes. A portion of the branches and terminal shoot of each seedling was scanned for fluorescence emissions for 180 seconds. A preliminary trial indicated that keeping the seedlings in the dark more than 15 minutes did not affect their fluorescence emissions. All measurements were taken between 0900 and 1500h at the beginning of each month for six months, April to September, 1993.

To observe a fully developed Kautsky fluorescence curve, seedlings must be placed in complete darkness so that most, if not all, of the electron carriers in the electron transport chain or Z-scheme of photosynthesis are in the oxidized state and levels of proton gradient and hence adenosine

triphosphate (ATP) formation are minimal (Vidaver et al. 1991). There should be no photosynthetic activity immediately prior to the time of fluorescence testing.

Dark adaptation is thus essential for dissipation of excited energy within the chloroplast membrane, oxidation of primary electron acceptors, and change in light-activated enzymes. Some dark time is required for these changes to occur. If these changes are not complete before illuminating plants for fluorescence measurements, they may alter the shape of the Kautsky curve and the resulting F_v / F_{max} ratio. Thus, for an accurate assessment a minimum dark adaptation period of 15-30 minutes is normally considered sufficient (Larcher and Cernusca 1985 in Bolhar-Nordenkampf et al. 1989).

2.3.2.2. *Slope of Chlorophyll Fluorescence*

Sabin (1994) developed a program to determine the slope of the 60-120 seconds portion of the slow phase of fluorescence induction curve (Figure 4). The area of the curve is between F_{max} and F_s and is indicative of the rate of fluorescence quenching (reduction in fluorescence).

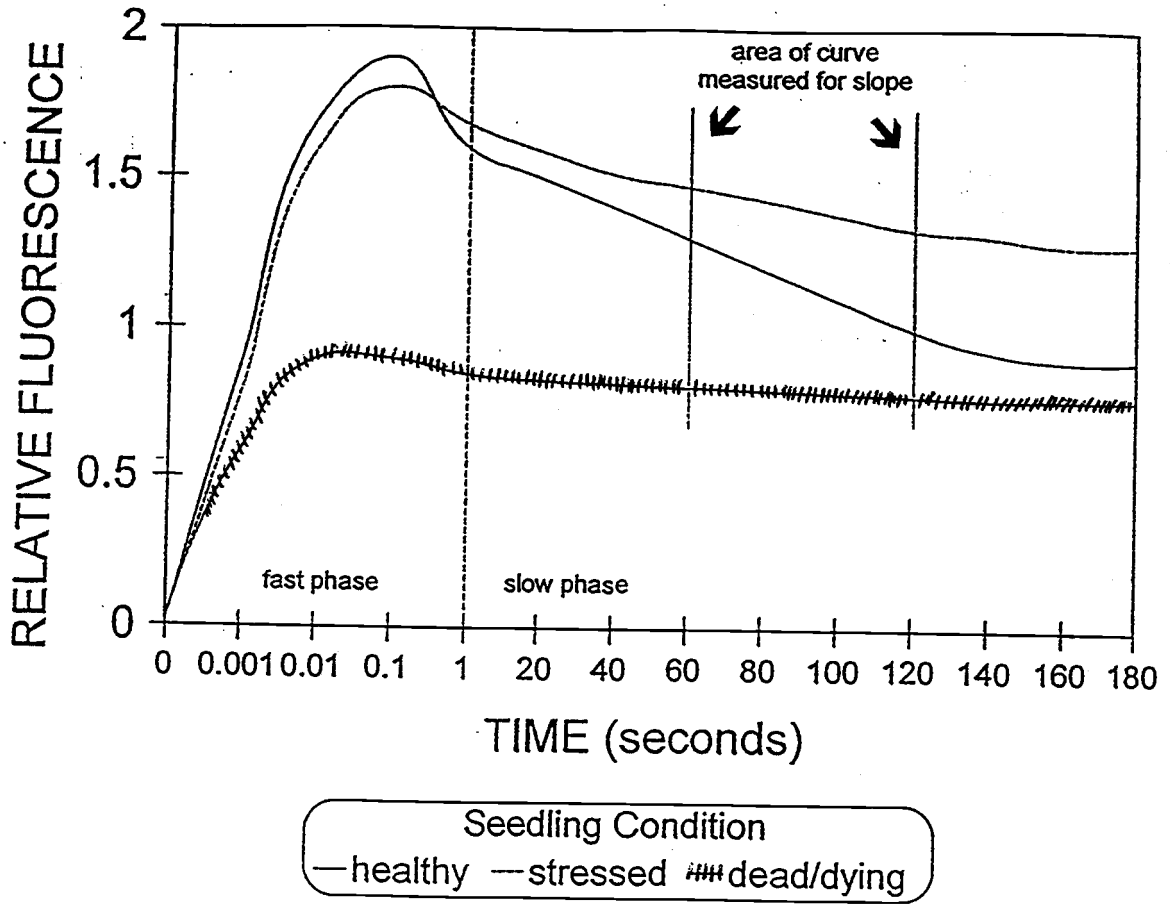

Figure 4. The schematic of fluorescence induction curve showing fluorescence emissions of a healthy, stressed, and dead/dying seedling scanned for 180 seconds. Area of curve for slope in the current study was between the 60 and 120 second portion of the curve.

2.3.2.3. *Chlorophyll Content (old and new needles)*

Initial chlorophyll *a*, chlorophyll *b*, and total chlorophyll contents were measured on previous season's needles of 10 trees per species before shade treatments were applied. These seedlings were not the same as those used for the study. However, they were randomly selected from the same group of seedlings used in the study.

Final chlorophyll *a*, chlorophyll *b*, and total chlorophyll contents were measured on the old and new needles of the same set of 128 seedlings selected for chlorophyll fluorescence measurements. These measurements were made at the end of the study before destructively harvesting the seedlings. Chlorophyll content extraction was accomplished using N, N-Dimethylformamide (DMF) as a chlorophyll extractant following the methods of Moran and Porath (1980) and Inskeep and Bloom (1985).

2.3.2.3.1. ***Sample Collection***

Approximately 150 mg of randomly selected needles were removed from the seedlings. These samples were immediately placed in labeled paper envelopes. The needle samples were covered with black opaque

shield them from any natural or artificial light in order to minimize the risks of chlorophyll deterioration. When the samples were collected from one block, they were cold-stored at 2-3 ^{0}C until collection from the other blocks was completed and then brought to the laboratory for further processing.

2.3.2.3.2. ***Sample Preparation***

Needle samples were weighed in near complete darkness to minimize risk of chlorophyll degradation in bright light in the laboratory. Exactly 100 mg of needles from each sample were weighed using a digital balance and transferred to labeled 15 ml test tubes. Then, five ml of DMF was added to each sample with a pipette. All the sample tubes were stacked into properly labeled tube racks and completely covered first with aluminum foil and then with black opaque plastic sheet. Tube racks were placed in cold storage at 2-3 ^{0}C for incubation.

2.3.2.3.3. ***Sample Incubation***

Chlorophyll content extraction was completed by incubating samples in cold storage at 2-3 ^{0}C. Ponderosa pine, Douglas-fir, and western

hemlock samples were incubated for five days while western redcedar samples were incubated for 15 days. This period of incubation was determined by running a preliminary trial which indicated that western redcedar took considerably longer than the other three species for complete chlorophyll extraction.

2.3.2.3.4. *Spectrophotometric Measurements*

Approximately one ml of DMF extract (chlorophyll dissolved in DMF solvent) was analyzed spectrophotometrically using a Beckman model DU 40 recording spectrophotometer to measure absorbance values in the visible region of light spectrum at wavelengths of 647 and 667 nm. Chlorophyll *a* and *b* contents were then calculated by substituting the absorbance values into the following equations (Moran 1982) and were presented in mg/g fresh weight of needles:

$$\text{chlorophyll } a = (12.64\ A_{664}) - (2.99\ A_{647})$$
$$\text{chlorophyll } b = (-5.60\ A_{664}) - (23.26\ A_{647})$$

Total chlorophyll content was then calculated by adding chlorophyll *a* and *b* values.

2.3.2.4. *Stomatal Conductance (old and new needles)*

Stomatal conductance was measured on intact needles of old and new foliage at the end of the study before destructively harvesting the seedlings. The measurements were made on the same set of 128 seedlings randomly selected for chlorophyll content and chlorophyll fluorescence measurements. The same seedlings were repeatedly measured three times a day: morning (0900h to 1030h); afternoon (1130h to 1300h) and evening (1515h to 1630h). Four continuous days were needed to complete the measurements.

Stomatal conductance was measured using a steady state porometer, model LI-1600 (LiCOR, Inc., Lincoln, Nebraska, 68504, USA). Seedlings were kept in their respective shade treatments for stomatal conductance measurements. Three fascicles consisting of nine needles were used for ponderosa pine seedlings whereas, approximately three to four cm long foliage of a small lateral branch was selected for Douglas-fir, western redcedar, and western hemlock seedlings.

After completing the measurements, the selected needles were removed from the seedlings and measured for their needle area. Total needle area (cm^2) of ponderosa pine was calculated by multiplying the average needle width (cm) with a porometer cuvette diameter (5.0 cm), giving an estimate of 5.787 cm^2. Needle area (1-side) of the other three species was determined using a leaf area meter (LiCOR, Model 3100), equipped with "AgVision System" hardware and "AgImage Plus version 1.08" software (Decan Devices, Inc., Pullman, Washington, 99163). This system was attached to a video camera and a 12 inch black and white video monitor. Total needle area (cm^2) of a particular sample was measured digitally. This needle area was used to calculate actual stomatal conductance of the needles. Thus stomatal conductance measured with a porometer was divided by the needle area of the respective sample and was expressed in units of mmol m^{-2} S^{-1}.

2.4. Statistics

2.4.1. Experimental Design

The study was conducted using a repeated measures design with four blocks, four shade treatments, four species, and five seedlings in each block/shade/species, for a total of 320 seedlings in the study. Repeated measurements were made on the same set of seedlings over time. Shade treatments (whole-plots) were replicated four times while five seedlings from each of the four species (sub-plots) were completely randomized within each shade treatment (Figure 5). Thus, a group of five seedlings of each species within each shade treatment was an experimental unit and each of these five seedlings was a sampling unit. There were a total of 16 treatment plots (four block x four shade treatments) in the study (Figure 5).

All 320 seedlings were measured for initial and final morphological characteristics (n=80). Three seedlings per block, shade treatment, and species (192 seedlings total, n=48) were randomly selected for periodic terminal height growth measurements. Two seedlings per block, shade, and species (128 seedlings total, n=32) were randomly assigned for chlorophyll

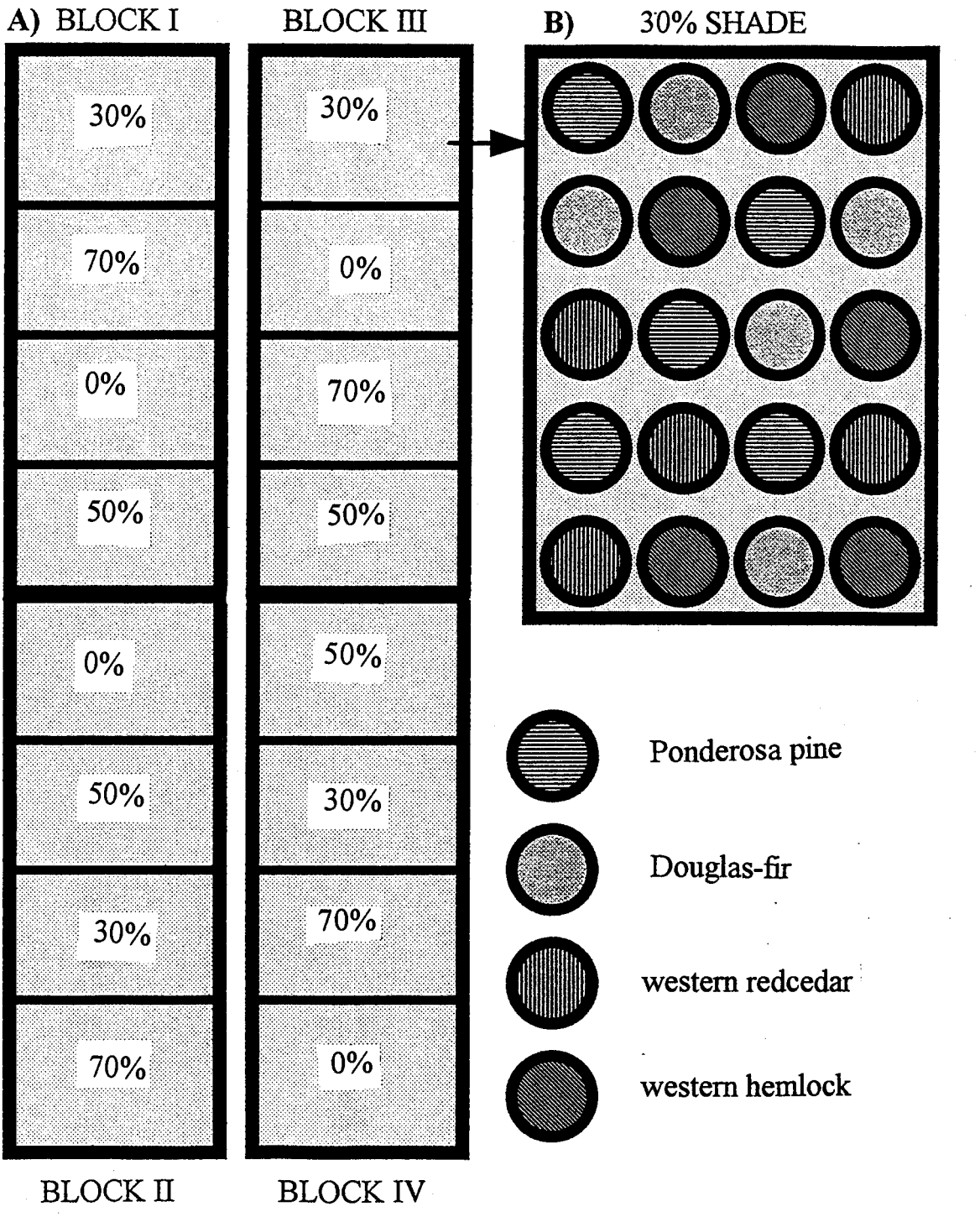

Figure 5. Experimental design used in the study: A) Two raised planting beds divided into four sections "blocks", each block consisting of four shade treatments; B) enlargement of a 30% shade treatment showing five plants of each of the four species.

fluorescence, chlorophyll content (old and new needles), and stomatal conductance (old and new needles) measurements.

2.4.2. Constant Variance Assumptions

Violations of one or more of the assumptions made in analysis of variance (ANOVA) or linear regression analysis may lead to erroneous results. Often data will not conform to the assumptions implicit in the analysis, but transforming the data to a different scale may lead to an appropriate model. Before determining what transformation should be used, an investigator must first determine if the assumptions are valid, and if they are not valid, how they are being violated (Sabin and Stafford 1990).

All data were tested for normality, linearity, and constant variance to ensure the validity of these necessary assumptions. The following transformations were necessary to achieve a normal and homogenous distribution of the variance:

1) log transformation for initial total fresh weight, root and shoot volumes, final total fresh weight, root volume, total dry weight, and shoot:root ratio, final F_{max}, F_v, and F_v / F_{max}, repeatedly measured over time F_{max} and F_v, final chlorophyll *a*, chlorophyll *b*, and total chlorophyll contents (old and new needles); and

2) square root transformation for final stomatal conductance measurements (old and new needles)

Values used in graphical or tabulated presentations in the thesis are medians (back-transformed means from log and square root transformations).

2.4.3. Error Terms

The statistical design is shown in Table 2. In a split-plot design, sub-plot units are grouped together and are closer to each other within each whole-plot unit. Therefore, whole-plot variance (σ^2) is expected to be greater than sub-plot σ^2. When whole-plot σ^2 is occasionally equal or less than sub-plot σ^2, it means that variation between sub-units is maximized at the expense of variation among whole-plots. This is contrary to the purpose of a split-plot design (Stafford and Sabin 1992 unpubl). This only can happen by random chance. In this situation, the error terms should be combined to form a more appropriate error term (Steel and Torrie 1980). Computing Expected Mean Squares [E(MS)] can be helpful understanding this situation (Stafford and Sabin 1992). Table 2A shows E(MS) of a split-plot design while E(MS) of a repeated measures design are presented in

Table 2. Expected Mean Squares, E(MS): A) split-plot design of chlorophyll *a* (old/new needles); B) repeated measures design of slope of the chlorophyll fluorescence [b=block; sh=shade; sp=species; t=time; σ^2=variance (random effects); and Q=fixed effects]. Note: Standard statistical notations would be $\alpha \equiv$ b*sh, $\beta \equiv$ b*sh*sp, $\gamma \equiv$ b*t, $\delta \equiv$ b*sh*t, and $\epsilon \equiv$ b*sh*sp*t. For a commom reader's ease, I chose to use b, sh, sp, and t as defined above.

A) Split-Plot Design

Source	DF	Expected Mean Squares, E(MS)
Block	3	$\sigma^2 + 2\sigma^2_{b*sh*sp} + 8\sigma^2_{b*sh} + 32\sigma^2_{b}$
Shade	3	$\sigma^2 + 2\sigma^2_{b*sh*sp} + 8\sigma^2_{b*sh} + Q$ (sh, sh*sp)
Whole-Plot Error	9	$\sigma^2 + 2\sigma^2_{b*sh*sp} + 8\sigma^2_{b*sh}$
Species	3	$\sigma^2 + 2\sigma^2_{b*sh*sp} + 8\sigma^2_{b*sh} + Q$ (sp, sh*sp)
Shade*Species	9	$\sigma^2 + 2\sigma^2_{b*sh*sp} + Q$ (sh*sp)
Sub-Plot Error	36	$\sigma^2 + 2\sigma^2_{b*sh*sp}$
Plant to Plant Error	64	σ^2

Table 2. Continued

B) Repeated Measures Design

Block	3	$\sigma^2 + 2\sigma^2_{b*sh*sp*t} + 8\sigma^2_{b*sh*t} + 32\sigma^2_{b*t} + 6\sigma^2_{b*plant(sh*sp)} + 12\sigma^2_{b*sh*sp} + 48\sigma^2_{b*sh} + 192\sigma^2_{b}$
Shade	3	$\sigma^2 + 2\sigma^2_{b*sh*sp*t} + 8\sigma^2_{b*sh*t} + 32\sigma^2_{b*t} + 6\sigma^2_{b*plant(sh*sp)} + 12\sigma 2_{b*sh*sp} + 48\sigma^2_{b*sh} + Q$ (sh, sh*sp, sh*t, sh*sp*t)
Error (A)*	9	$\sigma^2 + 2\sigma^2_{b*sh*sp*t} + 8\sigma^2_{b*sh*t} + 32\sigma^2_{b*t} + 6\sigma^2_{b*plant(sh*sp)} + 12\sigma^2_{b*sh*sp} + 48\sigma^2_{b*sh}$
Species	3	$\sigma^2 + 2\sigma^2_{b*sh*sp*t} + 8\sigma^2_{b*sh*t} + 32\sigma^2_{b*t} + 6\sigma^2_{b*plant(sh*sp)} + 12\sigma^2_{b*sh*sp} + Q$ (sp, sh*sp, sp*t, sh*sp*t)
Shade*Species	9	$\sigma^2 + 2\sigma^2_{b*sh*sp*t} + 8\sigma^2_{b*sh*t} + 32\sigma^2_{b*t} + 6\sigma^2_{b*plant(sh*sp)} + 12\sigma^2_{b*sh*sp} + Q$ (sh*sp, sh*sp*t)
Error (B)	36	$\sigma^2 + 2\sigma^2_{b*sh*sp*t} + 8\sigma^2_{b*sh*t} + 32\sigma^2_{b*t} + 6\sigma^2_{b*plant(sh*sp)} + 12\sigma^2_{b*sh*sp}$
Error (C)	64	$\sigma 2 + 2\sigma^2_{b*sh*sp*t} + 8\sigma^2_{b*sh*t} + 32\sigma^2_{b*t} + 6\sigma^2_{b*plant(sh*sp)}$
Time	5	$\sigma^2 + 2\sigma^2_{b*sh*sp*t} + 8\sigma^2_{b*sh*t} + 32\sigma^2_{b*t} + Q$ (t, sh*t, sp*t, sh*sp*t)
Error (D)	15	$\sigma^2 + 2\sigma^2_{b*sh*sp*t} + 8\sigma^2_{b*sh*t} + 32\sigma^2_{b*t}$
Time*shade	15	$\sigma^2 + 2\sigma^2_{b*sh*sp*t} + 8\sigma^2_{b*sh*t} + Q$ (sh*t, sh*sp*t)
Error (E)	45	$\sigma^2 + 2\sigma^2_{b*sh*sp*t} + 8\sigma^2_{b*sh*t}$
Time*species	15	$\sigma^2 + 2\sigma^2_{b*sh*sp*t} + Q$ (sp*t, sh*sp*t)
Time*shade*species	45	$\sigma^2 + 2\sigma^2_{b*sh*sp*t} + Q$ (sh*sp*t)
Error (F)	180	$\sigma^2 + 2\sigma^2_{b*sh*sp*t}$
Error (G) Time	320	σ^2

* Error (A)=block*time; Error (B)=block*shade*species;
Error (C)=block*plant(shade*species); Error (D)=block*time;
Error (E)=block*shade*time; Error (F)=block*shade*species*time;
Error (G)=times

Table 2B. In a split-plot design seedling to seedling variation is given by σ^2 (Table 2A). The sub-plot σ^2 is composed of two parts, σ^2 plus $\sigma^2_{b*sh*sp}$, where σ^2 is the variation between seedling to seedling and $\sigma^2_{b*sh*sp}$ is the variance of the two seedlings of the same species in the same shade treatment from block to block. The whole-plot σ^2 has three components: σ^2, $\sigma^2_{b*sh*sp}$, and σ^2_{b*sh} where the new term reflects the variation of the eight seedlings receiving the same shade treatment from block to block (Table 2A).

The individual components are called "variance components". Each variance component in a sub-sample is included in those in the sample above it. If the third component in whole-plot σ^2, by random chance, is zero, whole-plot σ^2 and sub-plot σ^2 will be equal, which should be combined to make a more appropriate error term. In this situation, whole-plot hypotheses should be tested with the same error term used for testing sub-plot hypotheses. The same principle may be applied to understand the σ^2 in Table 2B. For more details, see Steel and Torrie (1980) and Stafford and Sabin (1992 unpubl).

The primary objectives of combining error terms were:

1) to provide a better estimate of error variances for testing hypotheses;

2) to report more appropriate P-values and ultimately the research findings; and

3) to provide a complete picture of the variation in the experiment to a reader.

NOTE: ANOVA tables presented in the thesis are constructed after the error terms have been combined in various analyses where it was deemed necessary in the light of the above explanation.

2.4.4. Statistical Analyses

All the data were analyzed using Statistical Analysis System (SAS 1994).

2.4.4.1. *Analyses of Initial Measurements*

Initial morphological data (shoot height, stem diameter, total fresh weight, and root and shoot volumes) were analyzed to see if the species differ in their initial sizes. Whole-plot error was less than the sub-plot error

for shoot height and sub-plot error was less than the plant to plant variation for stem diameter, therefore error terms were combined (Appendix 2).

Initial chlorophyll *a*, chlorophyll *b*, and total chlorophyll contents were analyzed to see what levels of chlorophyll contents the species had before shade treatments were imposed.

2.4.4.2. *Analyses of Final Measurements*

Data from final shoot height, stem diameter, total fresh weight, root and shoot volumes, total dry weight, shoot:root ratio, relative growth rate, sturdiness quotient, and Dickson quality index; final F_v, F_{max}, and F_v / F_{max}; and final chlorophyll *a*, chlorophyll *b*, and total chlorophyll contents (old and new needles) measurements were analyzed as a split-plot design with shade as the whole-plot treatment and species as the sub-plot treatment.

Whole-plot error was less than the sub-plot error for Dickson quality index and final F_{max}, therefore, both error terms were combined to form a more appropriate error term (Appendices 3C and 4A respectively). For final chlorophyll *a*, chlorophyll *b*, and total chlorophyll contents (new needles), sub-plot error was less than or equal to plant to plant variation, therefore,

both error terms were combined to form a more appropriate error term (Appendix 5). The Mixed Procedure (SAS 1994) was used to compute correct standard errors for any significant shade by species interactions (Appendices 5 and 6A) .

2.4.4.3. *Analyses of Repeated Measurements over Time*

Data from total terminal height growth, F_v, F_{max}, and F_v / F_{max}, the slope of the chlorophyll fluorescence curve, and stomatal conductance (old and new needles) measured over time was subjected to repeated measures analyses. All the data were analyzed using the following steps in SAS:

1) original data were plotted to examine the general trend of the data and used as a benchmark for comparison with final graphs generated by fitting regression models;

2) data were checked for normality and constant variance and transformed where needed (see section 2.4.3.);

3) ANOVA tables were checked for validity of different error terms (see section 2.4.4. and Table 2B):

    a) for periodic terminal height growth and slope of chlorophyll fluorescence data, Error (E) ≤ Error (F), therefore, error terms were combined (Appendices 7 and 8 respectively).

b) Error (A) ≤ Error (B) ≤ Error (C) and Error (F) ≤ Error (G) for stomatal conductance of old needles therefore, error terms were combined (Appendix 9).

c) Error (A) ≤ Error (B) ≤ Error (C) for stomatal conductance of new needles, therefore error terms were combined (Appendix 10).

4) repeated measures analyses of variance were conducted (Appendices 7 to 13) to test hypotheses for :

a) between-subjects effects (shade, species, and shade by species effects of split-plot portion of the analysis).

b) within-subject effects (time, time by shade, time by species, and time by shade by species effects of repeated measures up to cubic polynomials).

c) sphericity:

(1) tests the hypothesis that the Huynh-Feldt conditions are met (Huynh and Feldt 1970), when it is not significant (α=0.05) it is reasonable to assume that conditions are met and analysis can be run as a split-split-plot design instead of a repeated measures analyses of variance (i.e. do not use adjusted P-values).

(2) if the Huynh-Feldt Epsilon is > 1, run analysis as a split-split-plot design.

5) regression models were fitted with time (linear, quadratic, and cubic polynomials) and checked for significance at the $\alpha \leq$ 0.05 level.

6) results were plotted:

a) compute polynomial regression coefficients (linear, quadratic, or cubic, whatever was significant in step # 5) to model the trend with time. The regression models used for different variables were as follows:

(i) periodic terminal height growth (Table 3):

$$\text{Growth}=\mu + \beta_i + \alpha_j + \beta_{1i}*\text{Time} + \alpha_{1j}*\text{Time} + \beta_{2i}*\text{Time}^2 + \alpha_{2j}*\text{Time}^2 + \beta_{3i}*\text{Time}^3 + \alpha_{3j}*\text{Time}^3$$

(ii) F_{max} (Table 4):

$$F_{max}=\mu + \beta_i + \alpha_j + \beta_{1i}*\text{Time} + \alpha_{1j}*\text{Time} + \beta_{2i}*\text{Time}^2 + \alpha_{2j}*\text{Time}^2 + \beta_{3i}*\text{Time}^3 + \alpha_{3j}*\text{Time}^3$$

(iii) F_v and F_v / F_{max} (Tables 5 and 6):

$$F_v \text{ and } F_v / F_{max}=\mu + \beta_i + \alpha_j + \beta_{1i}*\text{Time} + \alpha_{1j}*\text{Time} + \beta_{2i}*\text{Time}^2 + \beta_{3i}*\text{Time}^3$$

(iv) slope of chlorophyll fluorescence (Table 7):

$$\text{slope}=\mu + \beta_i + \beta_{1i}*\text{Time}$$

(v) stomatal conductance of old needles (Table 8A):

$$\text{conductance old}=\mu + \beta_i + \beta_{1i}*\text{Time}$$

(vi) stomatal conductance of new needles (Table 8B):

$$\text{conductance new}=\mu + \beta_i + \beta_{1i}*\text{Time} + \beta_{2i}*\text{Time}^2$$

Table 3. Polynomial regression coefficients for total terminal height growth. (Growth=$\mu + \beta_i + \alpha_j + \beta_{1i}$*Time + α_{1j}*Time + β_{2i}*Time^2 + α_{2j}*Time^2 + β_{3i}*Time^3 + α_{3j}*Time^3)

Spp/Shade	Intercept	Linear	Quadratic	Cubic
ponderosa pine				
0%	0.086	-0.617	0.393	-0.028
30%	0. 226	-0.827	0.466	-0.033
50%	0.154	-0.928	0.537	-0.037
70%	0.838	-1.530	0.662	-0.044
Douglas-fir				
0%	-6.003	7.389	-1.147	0.055
30%	-5.863	7.179	-1.074	0.050
50%	-5.935	7.078	-1.003	0.046
70%	-5.251	6.476	-0.878	0.039
western redcedar				
0%	-1.365	0.703	0.293	-0.024
30%	-1.225	0.493	0.366	-0.029
50%	-1.297	0.392	0.437	-0.033
70%	-0.613	-0.210	0.562	-0.040
western hemlock				
0%	-3.837	3.846	-0.468	0.018
30%	-3.697	3.636	-0.395	0.013
50%	-3.769	3.535	-0.324	0.009
70%	-3.085	2.933	-0.199	0.002

Table 4. Polynomial regression coefficients (log transformed) for F_{max}. ($F_{max}=\mu + \beta_i + \alpha_j + \beta_{1i}$*Time + α_{1j}*Time + β_{2i}*Time^2 + α_{2j}*Time^2 + β_{3i}*Time^3 + α_{3j}*Time^3)

Spp/Shade	Intercept	Linear	Quadratic	Cubic
ponderosa pine				
0%	0.009	0.847	-0.210	0.016
30%	-0.140	1.004	-0.269	0.021
50%	-0.175	1.130	-0.331	0.028
70%	-0.157	1.126	-0.341	0.029
Douglas-fir				
0%	1.374	-1.043	0.337	-0.030
30%	1.225	-0.886	0.278	-0.025
50%	1.190	-0.760	0.216	-0.018
70%	1.208	-0.764	0.206	-0.017
western redcedar				
0%	0.274	0.669	-0.192	0.017
30%	0.125	0.826	-0.251	0.022
50%	0.090	0.952	-0.313	0.029
70%	0.108	0.948	-0.323	0.030
western hemlock				
0%	0.632	0.053	0.023	-0.003
30%	0.483	0.210	-0.036	0.002
50%	0.798	0.336	-0.098	0.009
70%	0.466	0.332	-0.108	0.010

Table 5. Polynomial regression coefficients (log transformed) for F_v. ($F_v=\mu + \beta_i + \alpha_j + \beta_{1i}$*Time + α_{1j}*Time + β_{2i}*Time^2 + β_{3i}*Time^3)

Spp/Shade	Intercept	Linear	Quadratic	Cubic
ponderosa pine				
0%	-1.518	1.941	-0.531	0.044
30%	-1.571	1.906	-0.531	0.044
50%	-1.418	1.832	-0.531	0.044
70%	-1.360	1.782	-0.531	0.044
Douglas-fir				
0%	1.600	-2.459	0.773	-0.068
30%	1.547	-2.294	0.773	-0.068
50%	1.700	-2.568	0.773	-0.068
70%	1.758	-2.618	0.773	-0.068
western redcedar				
0%	-1.068	1.646	-0.502	0.044
30%	-1.121	1.611	-0.502	0.044
50%	-0.968	1.537	-0.502	0.044
70%	-0.910	1.487	-0.502	0.044
western hemlock				
0%	-0.374	0.458	-0.090	0.007
30%	-0.427	0.423	-0.090	0.007
50%	-0.274	0.349	-0.090	0.007
70%	-0.260	0.299	-0.090	0.007

Table 6. Polynomial regression coefficients for F_v/F_{max}.
($F_v / F_{max} = \mu + \beta_i + \alpha_j + \beta_{1i}*Time + \alpha_{1j}*Time + \beta_{2i}*Time^2 + \beta_{3i}*Time^3$)

Spp/Shade	Intercept	Linear	Quadratic	Cubic
ponderosa pine				
0%	0.135	0.466	-0.128	0.011
30%	0.126	0.457	-0.128	0.011
50%	0.162	0.439	-0.128	0.011
70%	0.173	0.428	-0.128	0.011
Douglas-fir				
0%	0.839	-0.526	0.165	-0.014
30%	0.830	-0.535	0.165	-0.014
50%	0.866	-0.553	0.165	-0.014
70%	0.877	-0.564	0.165	-0.014
western redcedar				
0%	0.244	0.394	-0.120	0.011
30%	0.235	0.385	-0.120	0.011
50%	0.271	0.418	-0.120	0.011
70%	0.282	0.356	-0.120	0.011
western hemlock				
0%	0.404	0.117	-0.024	0.002
30%	0.395	0.108	-0.024	0.002
50%	0.431	0.090	-0.024	0.002

Table 7. Polynomial regression coefficients for the slope of the chlorophyll fluorescence curve between the 60 and 120 second portion of the curve. (Slope=$\mu + \beta_i + \beta_{1i}$*Time)

Species	Intercept	Linear (β_{1i})	P-values H0: $\beta_{1i}=0$
ponderosa pine	-6.660×10^{-4}bc	3.23910^{-4}	0.0001
Douglas-fir	-2.16710^{-5}ac	2.27010^{-4}	0.0001
western redcedar	-1.45610^{-4}a	2.75910^{-4}	0.0001
western hemlock	-2.17710^{-4}a	1.25210^{-4}	0.0042

Table 8. Polynomial regression coefficients (square root transformed) for stomatal conductance: A) old needles; B) new needles. (Old needles=$\mu + \beta_i + \beta_{1i}$*Time; New needles=$\mu + \beta_i + \beta_{1i}$*Time + β_{2i}*Time^2)

A) Old Needles

Species	Intercept	Linear
ponderosa pine	9.488	-1.175
Douglas-fir	6.196	-0.785
western redcedar	11.512	-1.457
western hemlock	11.343	-1.857

B) New Needles

Species	Intercept	Linear	Quadratic
ponderosa pine	3.059	5.284	-1.364
Douglas-fir	10.756	-3.003	0.285
western redcedar	10.271	-1.969	0.25
western hemlock	6.364	2.548	-0.941

Where:

μ=overall mean
β_i=effect of species i
α_j=effect of shade treatment j
β_{1i}=linear effect of species i
α_{1j}=linear effect of shade treatment j
β_{2i}=quadratic effect of species i
α_{2j}=quadratic effect of shade treatment j
β_{3i}=cubic effect of species i
α_{3j}=cubic effect of shade treatment j

b) final results were plotted using coefficients in step # 6a (i to vi). X- axis and Y-axis of graphs represented time and variable of interest respectively. Each graph had four lines representing four different shade treatments for each individual species for periodic terminal height growth and F_v / F_{max}. Where, for slope of chlorophyll fluorescence and stomatal conductance (old and new needles), each of four lines on the graphs represented each of the four species (time by shade was not significant).

2.4.4.4. *Correlation Analyses*

Pearson correlation (r) analysis was performed to see linear association between various final morphological and physiological response variables. The variables included were: shoot height, F_{max}, F_v, F_v / F_{max}, chlorophyll *a*, chlorophyll *b*, and total chlorophyll contents (old and new needles).

3. RESULTS

3.1. Most Significant Findings (Table 9)

Though seedlings elongated most under 70% shade and least in full sunlight, biomass production significantly decreased with increasing shade. Relative growth rate and Dickson quality index of all species significantly decreased with increasing shade (0% to 70%). Sturdiness quotient substantially increased with increasing shade. All four species also differed significantly among each other for all morphological variables. This indicated that morphological development of the species differed under different shade regimes which led to the rejection of the first hypothesis which stated that morphological development of four species is not different under four shade levels.

Repeated measurements of chlorophyll fluorescence emissions and at the end of the study significantly decreased with increasing shade among all species. Similarly, all four species significantly differed from one another for all the chlorophyll fluorescence variables. Thus, the second hypothesis was rejected that chlorophyll fluorescence emissions are not

Table 9. P-values of morphological and physiological variables measured in the study: A) initial morphology; B) final morphology; C) final physiology; D) total terminal height growth and chlorophyll fluorescence; E) the slope of the fluorescence curve and stomatal conductance.

Variables Measured	Shade	Species	Shade*Species
A) Initial Morphology			
Shoot height	0.7275	0.0001	0.9619
Stem diameter	0.5076	0.0001	0.5866
Total fresh weight*	0.6230	0.0001	0.8108
Root volume*	0.5438	0.0001	0.6541
Shoot volume*	0.5458	0.0001	0.9141
B) Final Morphology			
Shoot height	0.8864	0.0001	0.4176
Stem diameter	0.0486	0.0001	0.4598
Total fresh weight*	0.029	0.0001	0.6269
Root volume*	0.0016	0.0001	0.2742
Shoot volume	0.798	0.0001	0.5133
Total dry weight*	0.0328	0.0001	0.5579
Shoot:root ratio*	0.0001	0.0001	0.0946
Relative growth rate	0.0458	0.0001	0.6956
Sturdiness quotient	0.0496	0.0001	0.5858
Dickson quality index	0.0001	0.0001	0.414

* Data were log-transformed

Table 9. Continued

C) Final Physiology (Chlorophyll Fluorescence and Chlorophyll Contents)

Variables Measured	Shade	Species	Shade*Species
F_{max}*	0.0001	0.0014	0.2296
F_v*	0.0006	0.0008	0.2489
F_v/F_{max}*	0.0006	0.0006	0.2318
Chlorophyll *a* (new needles)*	0.0001	0.0001	0.0001
Chlorophyll *b* (new needles)*	0.0001	0.0001	0.0001
Total chlorophyll (new needles)*	0.0001	0.0001	0.0001
Chlorophyll *a* (old needles)*	0.0001	0.0001	0.048
Chlorophyll *b* (old needles)*	0.0001	0.0001	0.2155
Total chlorophyll (old needles)*	0.0001	0.0001	0.0812

* Data were log-transformed

Table 9. Continued

D) Total Terminal Height Growth and Chlorophyll Fluorescence Repeatedly Measured over Time

Variables Measured→ **Effects ↓**	**Total Terminal Height Growth**	**F_{max}***	**F_v***	**F_v/F_{max}***
Shade	0.2103	0.0057	0.0021	0.0019
Species	0.0001	0.0001	0.0001	0.0001
Shade*Species	0.9088	0.3196	0.2448	0.3196
Time	0.0004	0.0002	0.0025	0.0017
Linear	0.0476	0.2003	0.2600	0.1743
Quadratic	0.0329	0.0324	0.1082	0.0856
Cubic	0.1146	0.0229	0.0450	0.0210
Time*Shade	0.0004	0.0001	0.0001	0.0001
Linear	0.0521	0.0007	0.0006	0.0005
Quadratic	0.3685	0.6451	0.5882	0.6015
Cubic	0.0231	0.0192	0.0941	0.0643
Time*Species	0.0001	0.0001	0.0001	0.0001
Linear	0.0001	0.0001	0.0001	0.0001
Quadratic	0.0001	0.0001	0.0001	0.0001
Cubic	0.0001	0.0001	0.0001	0.0001
Time*Shade*Species	0.4795	0.5233	0.3908	0.4549
Linear	0.5079	0.2533	0.3047	0.2670
Quadratic	0.2629	0.6038	0.7133	0.7490
Cubic	0.0708	0.8285	0.5841	0.6822

* Data were log-transformed

Table 9. Continued

E) Slope of the Fluorescence Curve and Stomatal Conductance Repeatedly Measured over Time.

Variables Measured→ **Effects ↓**	**The Slope of the Fluorescence Curve bewtween the 60 and 120 Second Portion**	**Stomatal* Conductance (old needles)**	**Stomatal* Conductance (new needles)**
Shade	0.0788	0.7943	0.9543
Species	0.0001	0.0001	0.0009
Shade*Species	0.6377	0.2626	0.3653
Time Linear Quadratic Cubic	0.0003 0.0194 0.0778 0.2259	0.0006 0.0040 0.2641	0.0001 0.0015 0.1123
Time*Shade Linear Quadratic Cubic	0.5930 0.8609 0.1027 0.9234	0.1593 0.0826 0.7526	0.1407 0.1656 0.1975
Time*Species Linear Quadratic Cubic	0.0001 0.0097 0.1624 0.0671	0.0001 0.0007 0.0528	0.0001 0.0001 0.0001
Time*Shade*Species Linear Quadratic Cubic	0.2854 0.3854 0.2987 0.4974	0.1689 0.0563 0.8213	0.5289 0.7382 0.1476

* Data were square root transformed

different among species.

The slope between the 60 and 120 second portion of the fluorescence induction curve had a significant linear relationship with time and species regardless of shade regimes. This led to accepting the third hypothesis that the slope of the fluorescence curve between the 60 and 120 second portion is not different among species and shade levels.

Chlorophyll contents in old and new needles substantially increased with increasing shade in all species. Similarly, there were highly significant differences among all four species for chlorophyll contents. Thus, the fourth hypothesis was rejected that chlorophyll contents are not different among species under different shade regimes.

Chlorophyll contents of old and new needles were strongly negatively correlated with all chlorophyll fluorescence variables. Increased chlorophyll contents with increasing shade resulted in reduced fluorescence emissions. These findings led to rejecting the fifth hypothesis that chlorophyll contents and chlorophyll fluorescence are not correlated.

3.2. Detailed Description of the Results

3.2.1. Initial Morphology

There were highly significant differences in morphological characteristics (shoot height, stem diameter, total fresh weight, and root and shoot volumes) between species before shade treatments were applied (Appendix 2: P=0.0001 for all). With the exception of stem diameter, western redcedar had the highest values for all morphological characteristics and ponderosa pine had the lowest values (Table 10). This finding led to relative growth analyses.

3.2.2. Relative Growth Rate

Relative growth rate of total fresh weight significantly decreased with increasing shade (Appendix 3: P=0.0443). There were little differences in values of seedlings grown in 0%, 30%, and 50% shade. However, seedlings grown in full sunlight (0% shade) had significantly larger values than in 70% shade (Table 11). Similarly, species differed significantly from one another

Table 10. Initial morphological measurements. Within species means for each morphological parameter followed by the different letters are significantly different at the $\alpha \leq 0.05$ level (Fisher's Protected Least Significant Difference). Means of total fresh weight, and root and shoot volumes are back-transformed from log transformations and are presented as medians.

Variables → **Species ↓**	**Shoot Hight** **(cm)**	**Stem Dia-meter** **(mm)**	**Total Fresh Weight** **(g)**	**Root Volume** **(cm^3)**	**Shoot Volume** **(cm^3)**
ponderosa pine	27.24d	7.25b	28.92c	8.27d	23.24c
Douglas-fir	46.76b	7.86a	57.27a	23.79b	36.28b
western redcedar	53.64a	7.11b	63.27a	26.95a	45.92a
western hemlock	39.35c	6.30c	40.40b	16.08c	25.35c

for relative growth rate (Appendix 3: P=0.0001). Western redcedar had the highest relative growth rate while ponderosa pine the lowest (Table 11).

Sturdiness quotient significantly increased with increasing shade (Appendix 3: P=0.0496). Seedlings grown under 0% shade had 11% lower values as compared to 70% shade (Table 11). Conversely, the Dickson quality index decreased with increasing shade (Appendix 3: 0.0001). There were little differences in values of seedlings grown in 0%, 30%, and 50% shade. However, seedlings grown in full sunlight (0% shade) had approximately 54% larger values than in 70% shade (Table 11). Species also differed significantly from one another for above mentioned characteristics (Appendices 3: P=0.0001 for both). Western redcedar had the largest values for sturdiness quotient and Dickson quality index while ponderosa pine had the smallest values (Table 11).

3.2.3. Periodic Terminal Height Growth

There were highly significant time by shade and time by species interactions for periodic terminal height growth (Appendix 7: adjusted P=0.0004 and 0.0001, respectively). Time by shade interaction had a

Table 11. Growth analyses[1]. Within shade (averaged over species) and within species (averaged over shade), means for each parameter followed by the different letters are significantly different at the $\alpha \leq 0.05$ level (Fisher's Protected Least Significant Difference).

Variables → **Shade/** **Species ↓**	**Relative Growth Rate of Total Fresh Weight** **g day^{-1}**	**Sturdiness Quotient**	**Dickson Quality Index**
Shade			
0%	4.49a	5.44b	5.19a
30%	4.47a	5.36b	5.09a
50%	4.43a	5.64ab	4.47a
70%	4.21b	6.09a	3.37b
Species			
ponderosa pine	3.86c	4.22d	2.59c
Douglas-fir	4.41b	4.17b	3.96b
western redcedar	4.92a	6.59a	6.62a
western hemlock	4.41b	5.26c	4.58b

[1] Calculations are based on final meausrements

significant cubic relationship (P=0.0231) while linear, quadratic, and cubic relationships were found for time by species interactions (P=0.0001 for all).

Figure 6 indicates that terminal height growth in all four species increased progressively over time with increasing levels of shade: 70% > 50% > 30% > 0% shade (apparent decreases, noticeable in graphs, are an artifact and consequence of fitting cubic polynomial and are not reflected in the data). All species elongated most under 70% shade and least in full sunlight (0% shade) (Figure 6). Western redcedar and ponderosa pine followed nearly the same trend of growth response to the four different shade levels while Douglas-fir and western hemlock responded differently (Figure 6). Data indicated that species' response to different shade levels was the same in the beginning but as time progressed the shade effect was more pronounced.

3.2.4. Final Morphology

Final stem diameter, total fresh weight, root volume, and total dry weight significantly decreased (Appendix 14: P= 0.0486, 0.0290, 0.0016, and 0.0328 respectively) by 9%, 26%, 48%, and 26%, respectively from 0%

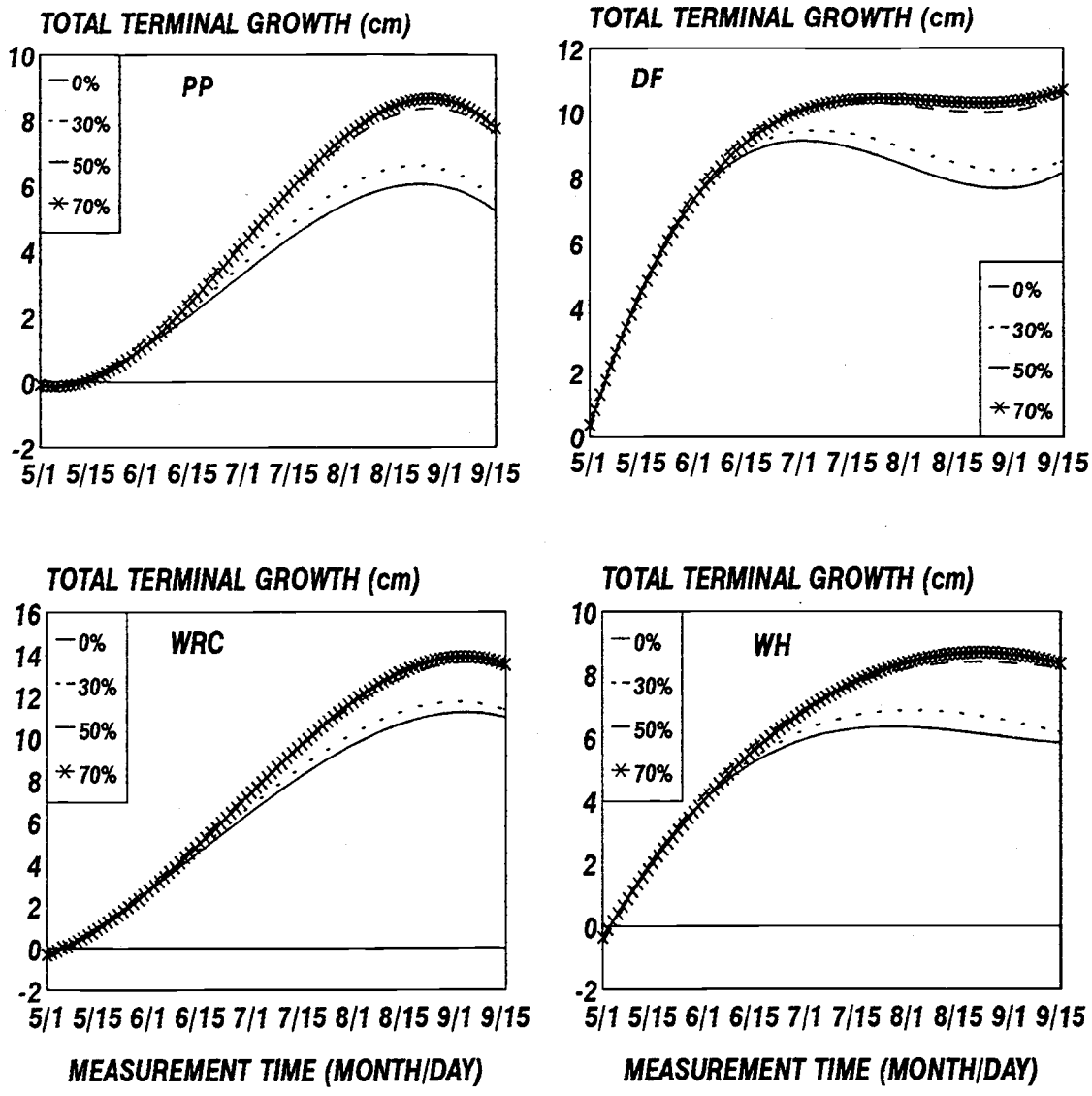

Figure 6. Effect of shade on seedling periodic terminal height growth, measured over time. Each line of each graph represents a regression equation for time by shade interaction fitting a cubic model. [Abbreviations used for species are: PP=ponderosa pine, DF=Douglas-fir, WRC=western redcedar, and WH=western hemlock]

to 70% shade (Table 12). Shoot height, though not statistically different under all shade levels, increased by 3% with increasing shade (Appendix 13, Table 12). Shoot:root ratio substantially increased (P=0.0001) by 44% from 0% to 70% shade (Table 12). Species also differed significantly from one another for all above mentioned characteristics (Appendix 14: P=0.0001 for all). Ponderosa pine had the smallest values while western redcedar had the largest (Table 12). There were no significant shade by species interactions for final morphological variables (Appendix 14).

Similarly, actual growth (final-initial) of stem diameter, root volume, shoot volume, and total fresh weight, significant decreased (P=0.0162, 0.0001, 0.5244, and 0.0023 respectively) by 36, 53, 20, and 42% respectively, with increasing shade (Appendix 15).

The results presented under sections 3.1.2 to 3.1.4 indicated that morphological development of seedlings differed under differing shade levels, thus the first hypothesis was rejected.

Table 12. Final morphological measurements. Within shade (averaged over species) and within species (averaged over shade), means for each morphological parameter followed by the different letters are significantly different at the $\alpha \leq 0.05$ level (Fisher's Protected Least Significant Difference). Means of total fresh weight, root volume, total dry weight, and shoot:root ratio were back-transformed from log transformations and are presented as medians.

Variables → Shade/ Species ↓	Shoot Height (cm)	Stem Diameter (mm)	Total Fresh Weight (g)	Root Volume (cm^3)	Shoot Volume (cm^3)	Total Dry Weight (g)	Shoot:Root Ratio (dry weight)
Shade							
0%	50.64a	9.33a	86.08a	40.75a	59.84a	29.51a	1.27c
30%	51.10a	9.45a	89.06a	40.76a	62.23a	30.43a	1.34c
50%	50.98a	9.09ab	80.92ab	36.34a	60.91a	27.87ab	1.45b
70%	52.01a	8.53b	68.44b	27.49b	57.67a	23.41b	1.83a
Species							
ponderosa pine	34.13d	8.20c	32.49d	17.27c	34.90d	32.49d	1.86a
Douglas-fir	58.32b	9.53b	88.69b	35.97b	69.81b	30.46b	1.94a
western redcedar	65.84a	10.20a	132.37a	69.82a	91.43a	46.01a	1.19b
western hemlock	46.45c	8.49c	77.65c	38.46b	44.59c	25.91c	1.05c

3.2.5. Chlorophyll Fluorescence Measurements over Time

There were highly significant time by shade and time by species interactions for F_{max}, F_v, and F_v / F_{max} (Appendices 10 to 12: P=0.0001 for all). Time by shade interaction for F_{max} had significant linear and cubic relationships (Appendix 11: P=0.0007 and 0.0192 respectively). Whereas, for F_v and F_v / F_{max} the interaction had a linear relationship (Appendices 12 and 13: P=0.0006 and 0.0005 respectively). On the other hand, species showed significant linear, quadratic, and cubic effects with time for all three chlorophyll fluorescence variables (Appendices 11 to 13: P=0.0001 for all).

Initially F_v / F_{max} was approximately in the range of 0.47 and 0.53 for all species at all four shade levels (Figure 7). As time progressed, F_v / F_{max} significantly changed (between April and September) for all species grown under all four shade treatments (Figure 7). For western redcedar, western hemlock, and ponderosa pine seedlings, F_v / F_{max} significantly increased shortly after the start of the experiment. This increase was greatest at full sunlight (0% shade) and least at 70% shade. In contrast, during the same period of time F_v / F_{max} decreased in Douglas-fir seedlings. This decrease was most at 70% shade and least at 0% shade. However, the overall effect

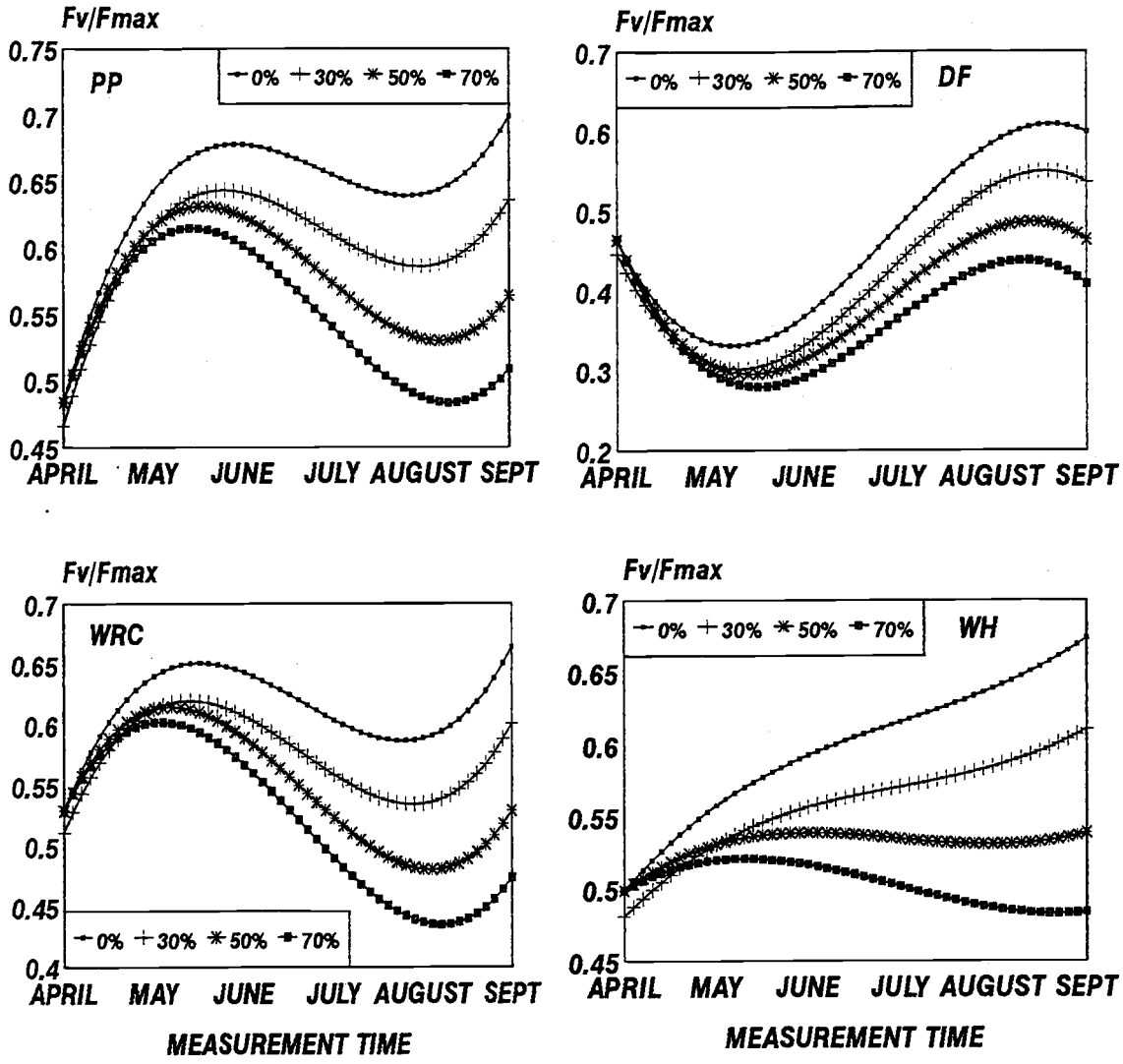

Figure 7. Effect of shade on F_v / F_{max} measured over time. Each line of each graph represents a regression equation for time by shade interaction fitting a cubic model. [Abbreviations used for species are: PP=ponderosa pine, DF=Douglas-fir, WRC=western redcedar, and WH=western hemlock]

of shade on F_v / F_{max} in all four species was ranked as: 0% > 30% > 50% > 70% (Figure 7).

3.2.6. Final Chlorophyll Fluorescence

All three chlorophyll fluorescence variables (F_{max}, F_v, and F_v / F_{max}) were significantly affected by shade (Appendix 3: P=0.0001, 0.0006, and 0.0006 respectively). There was a considerable decrease in F_{max}, F_v, and F_v / F_{max} of 27%, 48%, and 29% (respectively) from 0% to 70% shade (Figure 8A). Similarly, all four species differed significantly from each other for all three variables (Appendix 4: P=0.0014, 0.0008, and 0.0006, respectively) and were ranked as follows: western hemlock > ponderosa pine > western redcedar > Douglas-fir (Figure 8B). There were no significant shade by species interactions for final chlorophyll fluorescence measurements (Appendix 4).

The findings reported under sections 3.2.5 and 3.2.6. led to reject the second hypothesis that fluorescence emissions are not different among species under all shade regimes.

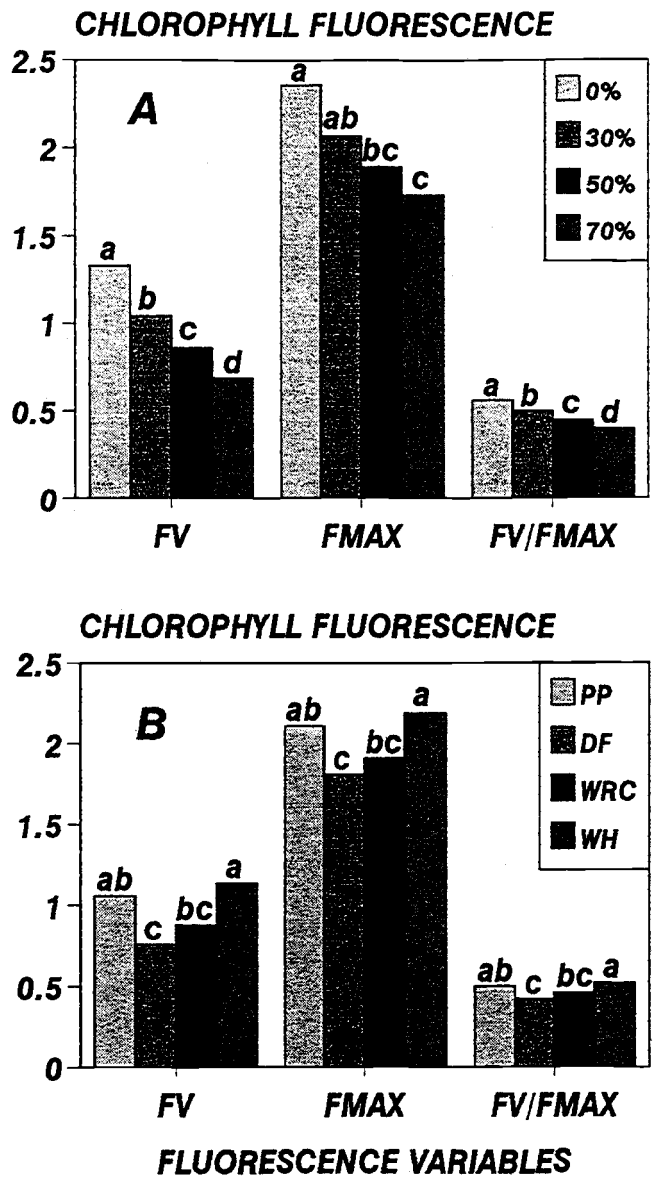

Figure 8. Effect of shade on final chlorophyll fluorescence emissions: A) within shade (averaged over species); B) within species (averaged over shade). For each parameter, bars with different letters are significantly different at the $\alpha \leq 0.05$ level (Fisher's Protected Least Significant Difference). Means are back-transformed from log transformations and are presented as medians.

3.2.7. Slope of Chlorophyll Fluorescence over Time

Species had a significant interaction with time for mathematically computed chlorophyll fluorescence slope between the 60 and 120 second portion of the curve (Appendix 8: P=0.0001). There was a significant linear change (P=0.0097) in chlorophyll fluorescence slope over time in all four species (Figure 9). Douglas-fir and western redcedar showed a positive slope throughout the growth period. In contrast, western hemlock and ponderosa pine seedlings had a negative slope at the start of the study when the seedlings were not in growth phase (Figure 9). There was neither a significant time by shade interaction nor main effect of shade on chlorophyll fluorescence slope (Appendix 8). Thus, hypothesis number three is accepted that slope of chlorophyll fluorescence between the 60 and 120 second portion of the curve is not different among species and shade levels.

3.2.8. Initial Chlorophyll Contents (old needles)

There were no statistical differences for initial chlorophyll *a*, chlorophyll *b*, and total chlorophyll contents among species. However, chlorophyll *a* and total chlorophyll contents were slightly higher in

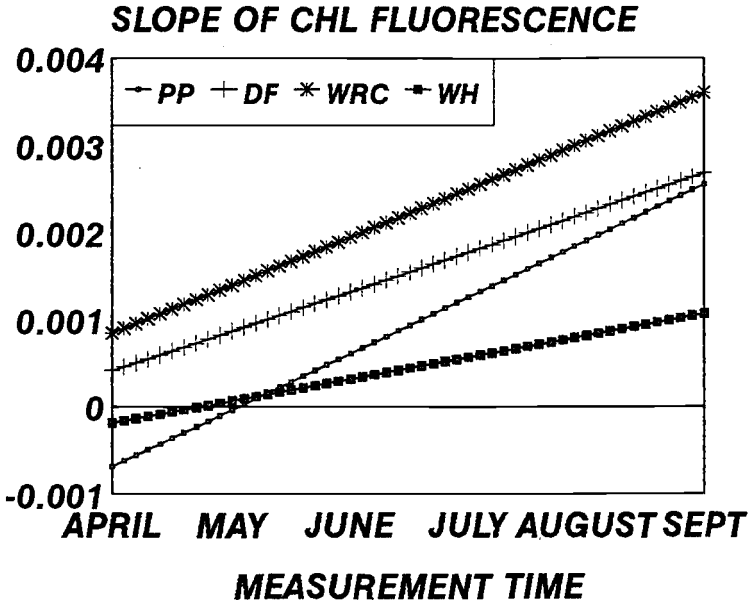

Figure 9. Effect of shade on slope curve between the 60 and 120 second portion of the fluorescence induction curve. Each line of the graph represents a regression equation for time by species interaction fitting a linear model.

Douglas-fir, followed by ponderosa pine, western redcedar, and western hemlock. Conversely, ponderosa pine had slightly higher chlorophyll *b* content followed by western redcedar, Douglas-fir, and western hemlock. Means and standard errors are presented in Table (13).

3.2.9. Final Chlorophyll Contents (old and new needles)

There was a significant shade by species interaction for final chlorophyll *a* content in old needles (Appendix 6: 0.0480). Chlorophyll *a* content significantly increased with increasing shade level (0 to 70%) in all four species (Table 14). Western hemlock had the highest chlorophyll *a* content while ponderosa pine the lowest (Table 14). On the other hand, chlorophyll *b* and total chlorophyll contents were significantly affected by main effects of shade (i.e. there were no significant interactions between shade and species (Appendix 6: P=0.0001 for both). There was a substantial increase in chlorophyll *b* and total chlorophyll contents of 188 and 158% (respectively) from 0% to 70% shade (Table 14).

There were highly significant shade by species interactions for chlorophyll *a*, chlorophyll *b*, and total chlorophyll contents in new needles

Table 13. Initial chlorophyll contents for ponderosa pine, Douglas-fir, western redcedar, and western hemlock. Means are presented in mg/g of fresh weight of the needles.

Species	Variables	Means (mg/g)	Standard Errors
ponderosa pine	chlorophyll *a*	1.044	0.057
Douglas-fir		1.161	0.088
western redcedar		1.007	0.054
western hemlock		0.875	0.036
ponderosa pine	chlorophyll *b*	0.319	0.024
Douglas-fir		0.272	0.114
western redcedar		0.303	0.020
western hemlock		0.242	0.011
ponderosa pine	total chlorophyll	1.363	0.080
Douglas-fir		1.433	0.135
western redcedar		1.310	0.073
western hemlock		1.118	0.046

Table 14. Final chlorophyll contents of old needles: A) chlorophyll *a*; B) chlorophyll *b*; C) total chlorophyll. In table A, within each column and row, medians with different letters are significantly different at the $\alpha \leq 0.05$. Lowercase bold letter (s) with medians is for column-wise median comparison while uppercase is for row-wise mean comparison. In Tables B and C, within shade (averaged over species) and within species (averaged over shade) medians with different letters are significantly different at the $\alpha \leq 0.05$. Values presented are medians in mg/g fresh weight of needles (back-transformed means from log transformation).

A) Chlorophyll *a*

Species ⟶ Shade ↓	Ponderosa pine	Douglas-fir	western redcedar	western hemlock
0%	0.292**a** A	0.365**a** A	0.278**a** A	0.711**a** B
30%	0.370**a** A	0.598**b** BC	0.396**a** A	0.802**ab** C
50%	0.742**b** A	0.694**bc** A	0.735**b** A	1.061**bc** B
70%	0.707**bc** A	0.862**cd** AB	0.911**c** BC	1.293**c** C

	B) Chlorophyll *b*	C) Total Chlorophyll
Shade		
0%	0.108b	0.492c
30%	0.144b	0.660b
50%	0.238a	1.035a
70%	0.311a	1.276a
Species		
ponderosa pine	0.144b	0.633c
Douglas-fir	0.166b	0.768b
western redcedar	0.173b	0.722bc
western hemlock	0.280a	1.222a

(Appendix 4: P=0.0001 for all). All three variables significantly increased with increasing shade in all four species (Table 15). Western hemlock had the largest amount of chlorophyll *a*, chlorophyll *b* and total chlorophyll contents while ponderosa pine the lowest (Table 15). This led to rejecting the fourth hypothesis that chlorophyll content is not different in all four species subjected to four different shade regimes.

3.2.10. Pearson Correlation Coefficients (r)

Chlorophyll *a*, chlorophyll *b*, and total chlorophyll contents of ponderosa pine, Douglas-fir, and western redcedar (old and new needles) were strongly negatively correlated with F_{max}, F_v, F_v / F_{max} (Tables 16 and 17). This led to rejecting the fifth hypothesis that there is no correlation between chlorophyll content and chlorophyll fluorescence emissions.

In western hemlock no correlation was found among these variables (Table 17). However, shoot height of western hemlock was negatively correlated with all three chlorophyll fluorescence variables (Table 17) while that of Douglas-fir was negatively correlated with F_v, F_v / F_{max} (Table 16).

Table 15. Final chlorophyll contents of new needles: A) chlorophyll *a*; B) chlorophyll *b*; C) total chlorophyll. In each table, within each column and row, medians with different letters are significantly different at the $\alpha \leq 0.05$. Lowercase bold letter (s) with medians is for column-wise median comparison while uppercase letter (s) is for row-wise median comparison. Values presented are medians in mg/g fresh weight of needles (back-transformed means from log transformation).

A) Chlorophyll *a*

Species → / Shade ↓	Ponderosa pine	Douglas-fir	western redcedar	western hemlock
0%	0.186**a** A	0.302**a** B	0.386**a** C	0.935**a** D
30%	0.236**a** A	0.400**b** B	0.428**a** B	0.955**a** C
50%	0.488**b** A	0.460**bc** A	0.564**b** A	1.235**b** B
70%	0.767**c** A	0.945**d** A	0.806**c** A	1.415**bc** B

B) Chlorophyll *b*

0%	0.043**a** A	0.074**a** B	0.108**a** C	0.208**a** D
30%	0.058**a** A	0.111**b** B	0.115**a** B	0.191**a** D
50%	0.136**b** A	0.135**bc** A	0.148**a** A	0.355**b** B
70%	0.217**c** A	0.300**d** B	0.220**c** A	0.510**c** D

C) Total Chlorophyll

0%	0.229**a** A	0.376**a** B	0.494**a** C	1.143**a** D
30%	0.294**a** A	0.511**b** B	0.543**a** B	1.146**a** C
50%	0.624**b** A	0.595**b** A	0.712**b** A	1.590**b** B
70%	0.984**c** B	1.245**c** C	1.026**c** BC	1.925**c** A

Table 16. Pearson correlation coefficients for chlorophyll contents of old and new needles, total shoot height, total shoot volume, and final chlorophyll fluorescence emissions: A) ponderosa pine; B) Douglas-fir. In each column and row, values in the first line represent Pearson correlation coefficients (strength of linear association between two response variables) while values in parentheses in the second line are P-values.

	A) ponderosa pine				B) Douglas-fir			
	F_{max}	F_v	F_v/F_{max}	**shoot height**	F_{max}	F_v	F_v/F_{max}	**shoot height**
chl*a* (old)	-0.55 (0.0010)	-0.55 (0.0010)	-0.55 (0.0012)	-0.12 (0.5070)	-0.68 (0.0001)	-0.68 (0.0001)	-0.65 (0.0001)	0.16 (0.3940)
chl*b* (old)	-0.53 (0.0017)	-0.53 (0.0017)	-0.53 (0.0019)	-0.13 (0.4713)	-0.68 (0.0001)	-0.68 (0.0001)	-0.66 (0.0001)	0.15 (0.4164)
tot chl (old)	-0.55 (0.0011)	-0.55 (0.0011)	-0.54 (0.0013)	-0.12 (0.4972)	-0.68 (0.0001)	-0.68 (0.0001)	-0.66 (0.0001)	0.15 (0.3991)
chl*a* (new)	-0.64 (0.0001)	-0.64 (0.0001)	-0.72 (0.0001)	-0.36 (0.0403)	-0.60 (0.0003)	-0.60 (0.0003)	-0.61 (0.0002)	-0.02 (0.8977)
chl*b* (new)	-0.64 (0.0001)	-0.64 (0.0001)	-0.73 (0.0001)	-0.34 (0.0533)	-0.59 (0.0004)	-0.59 (0.0004)	-0.61 (0.0002)	-0.06 (0.7621)
tot chl (new)	-0.64 (0.0001)	-0.64 (0.0001)	-0.72 (0.0001)	-0.36 (0.0428)	-0.60 (0.0003)	-0.60 (0.0003)	-0.61 (0.0002)	-0.03 (0.8592)
shoot height	-0.08 (0.6612)	-0.08 (0.6608)	0.01 (0.9738)		0.02 (0.9116)	-0.48 (0.0050)	-0.43 (0.0133)	

Table 17. Pearson correlation coefficients for chlorophyll contents of old and new needles, total shoot height, total shoot volume, and final chlorophyll fluorescence emissions: A) western redcedar; B) western hemlock. In each column and row, values in the first line represent Pearson correlation coefficients (strength of linear association between two response variables) while values in parentheses in the second line are P-values.

	A) western redcedar				B) western hemlock			
	F_{max}	F_v	F_v/F_{max}	**shoot height**	F_{max}	F_v	F_v/F_{max}	**shoot height**
chl*a* (old)	-0.52 (0.0025)	-0.52 (0.0025)	-0.58 (0.0005)	0.03 (0.8639)	0.09 (0.6293)	0.09 (0.6296)	-0.01 (0.9819)	-0.05 (0.7895)
chl*b* (old)	-0.50 (0.0032)	-0.50 (0.0032)	-0.57 (0.006)	-0.03 (0.8495)	0.004 (0.9811)	0.004 (0.9814)	-0.09 (0.6415)	-0.001 (0.9973)
tot chl (old)	-0.52 (0.0025)	-0.52 (0.0025)	-0.58 (0.0005)	0.01 (0.9359)	0.06 (0.7550)	0.06 (0.7553)	-0.04 (0.8463)	-0.03 (0.8814)
chl*a* (new)	-0.45 (0.0093)	-0.45 (0.0093)	-0.47 (0.0006)	0.12 (0.5171)	0.09 (0.6160)	0.09 (0.6162)	-0.02 (0.9251)	-0.05 (0.7711)
chl*b* (new)	-0.42 (0.0161)	-0.42 (0.0162)	-0.44 (0.0122)	0.10 (0.5737)	0.05 (0.7669)	0.05 (0.7673)	-0.04 (0.8218)	0.02 (0.9263)
tot chl (new)	-0.45 (0.0104)	-0.45 (0.0104)	-0.46 (0.0075)	0.12 (0.5286)	0.08 (0.6682)	0.08 (0.6684)	-0.03 (0.8821)	-0.03 (0.8868)
shoot height	0.04 (0.8261)	0.04 (0.8264)	0.07 (0.7036)		-0.48 (0.0050)	-0.48 (0.0050)	-0.43 (0.0133)	

3.2.11. Stomatal Conductance (old and new needles)

There were significant time by species interactions for stomatal conductance in both old and new needles (Appendices 8 and 9: P=0.0001 for both). Species had a significant linear trend with time for old needles (Appendix 8: P=0.0007). Whereas, new needles species had both linear and quadratic trends with time (Appendix 9: P=0.0001 for both).

Figures 10A and B indicated a significant decrease in conductance from morning to afternoon in all four species. In both old and new needles, western redcedar had the highest stomatal conductance while Douglas-fir the lowest (Figures 10A and B). In new needles, stomatal conductance in ponderosa pine seedlings increased from morning to noon and then declined in the afternoon (Figure 10B). There was neither a time by shade significant interaction nor main effect of shade on stomatal conductance of both old and new needles.

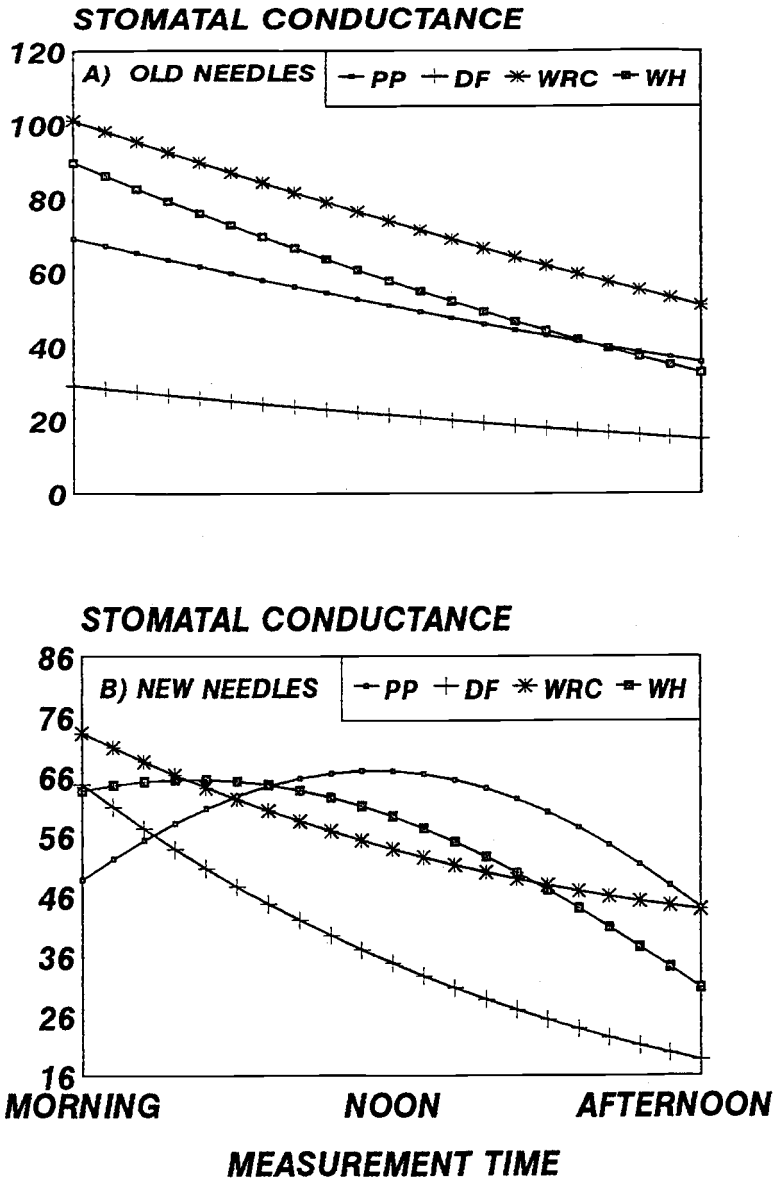

Figure 10. Effect of shade on stomatal conductance: A) old needles; B) new needles. Each line of both graphs represents a regression equation for time by species interaction fitting a linear model for old needles and quadratic model for new needles. Means are back-transformed from square root transformation and are presented as medians.

4. DISCUSSION

The results of this study support earlier findings that light intensity impacts seedling growth (Hodges and Scott 1968, Minore 1971, Emmingham and Waring 1973, Dunlap and Helms 1983, Hinsely 1986, Walstad and Kuch 1987, Wiebel et al. 1994, Elliot and White 1994, Gottschalk 1994, Tognetti et al. 1994, Mitchel and Arnott 1995). Plants grown under very low light intensities usually have thinner leaves with larger leaf surface areas than those grown in full sunlight. They develop huge grana stacks in the chloroplast oriented in all directions in order to effectively utilize low light levels (Hader and Tevini 1987, Lichtenthaler 1988). In the current study, significantly higher shoot:root ratios, final shoot height, and total terminal height growth in seedlings under 70% shade indicated that seedlings primarily invested their photosynthates in the build-up of a larger pigment antenna (Lichtenthaler et al. 1981, Kolb et al. 1990). They changed their allocation pattern to favor shoot elongation in limited light environments.

But, all of this occurred at the expense of the seedlings' overall performance, biomass production, and sturdiness quotient. Higher shade

environments resulted in substantially reduced relative growth rate, stem diameter, total fresh and dry weights, and root volume as compared to seedlings grown in full sun, indicating more cell wall material in sun shoots (Lewandowska and Jarvis 1977). Similar reductions in biomass have also been found in western hemlock and Douglas-fir (Brix 1970), Fraser fir (*Abies fraseri* [(Pursh) Poir.] (Hinseley 1986), Douglas-fir and *incense-cedar (Calocedrus decurrens* [(Torr.) Florin] (Minore 1988), long leaf and loblolly pine (Barnett 1989), *Aucuba japonica* (Andersen et al. 1991), mangosteen (*Garcinia mangostana* L.) (Wiebel et al. 1994), *Quercus rubra Quercus velutina*, *Prunus serotina*, and *Acer rubrum* (Gottschalk 1994), red pine (Elliott and White 1994), and western hemlock and amabilis fir seedlings (Michell and Arnott 1995) grown under higher shade levels.

In the current study, though, seedlings grown in full sunlight had larger morphological values and relative growth rate as compared to 70% shade, those under 30% shade showed over all better performance. Seedling performance, as determined by the Dickson quality index and sturdiness quotient, was greatly affected by increasing shade levels. Increased values of the sturdiness quotient were found in seedlings under

70% shade which are not considered good for seedling performance and survival when outplanted (Roller 1977 in Thompson 1985).

Seedling development in response to shading also varied greatly by species. Ponderosa pine had the most reduced relative growth rate and biomass production with increased shading than the other three species, proving its lower shade tolerance. Western redcedar and western hemlock had higher relative growth rates and biomass production than Douglas-fir, indicating their greater shade tolerance. Western hemlock, which had lower initial stem diameter and total fresh weight than Douglas-fir, had equal relative growth rate to that of Douglas-fir after 29 weeks.

Though, actual growth (final-initial morphological variables) of all four species is shown in Appendices 15, seedlings' performance under varied shade regimes is evaluated and discussed based on relative growth rate. Actual growth or absolute growth rate are not easily interpreted because they are more a function of current size than efficiency. For example, a large bare-root seedling may show greater growth than a containerized seedling despite poor physiological condition (Blake and Sutton 1987). Because actual growth depends, in part, on the amount of

growing material, it would be meaningless to compare it among species of different initial size

(Ledig 1974). Therefore, to compensate for differences in initial seedling size, growth rate should be expressed relative to the amount of growing material already present (Harper 1982, Hunt 1982, Radosevich and Osteryoung 1987).

All three chlorophyll fluorescence variables (F_{max}, F_v, F_v / F_{max}) measured in the current study had a strong inverse relationship with shade. All four species (stored in cold room before planting under similar conditions) started out with almost the same photochemical efficiencies, F_v / F_{max} ratios, (approximately ranging from 0.47 to 0.53 relative units) at the start of the experiment (April). As time progressed, their photochemical efficiencies significantly changed, reaching maximum values of F_v / F_{max} 0.76, 0.61, 0.72, and 0.69 (relative units) in ponderosa pine, Douglas-fir, western redcedar, and western hemlock, respectively (Appendix 14). There are no published F_v/F_{max} values for these species which can be used as reference to evaluate their photochemical efficiency. However, values in the range of 0.83-0.85 (Bjorkman 1987) or 0.75-0.85 (Butler and Kitajima 1975 in Bolhar-Nordenkampf et al. 1989) have been shown to be highly

correlated with the quantum yield of intact leaves exposed to various levels of photoinhibition (Demmig and Bjorkman 1987). The F_v / F_{max} values obtained for different conifer species under different shade conditions in the current study are comparable to those previously reported.

The fact that ponderosa pine and western redcedar are quite different in shade-endurance, the former being "intolerant" (light demanding) and the latter being "very tolerant" (Baker 1950), their similar F_v / F_{max} pattern needs further investigation. Data suggested that both these species were more efficient at collecting and utilizing light energy (indicated by higher F_v / F_{max} values) followed by western hemlock and Douglas-fir (very tolerant and intermediate, respectively).

As mentioned in the introduction, there are very few studies on shading and chlorophyll fluorescence. Falk and Samuelsson (1992) applied differing light intensities (500, 1400, and 2200 μmol m^{-2} s^{-1}) to evaluate the photoinhibitory effect in unicellular green algal cells (*Chlamydomonas reinhardtii*). They reported that recovery was both complete and rapid after treating the cells with 1400 μmol m^{-2} s^{-1} light intensity (moderate photoinhibition). The results of this study conducted on unicellular green algal cells can not be compared with the present study because of

differences in objectives, species, and technology. There were no statistics in the experiment.

The study conducted by Mitchell and Arnott (1995) is related with the present study (for details see section 1.4.2.2). Various fluorescence variables were measured using a non-integrating fluorometer system in one-year old amabilis fir and western hemlock seedlings grown under different shade environments. It is not indicated whether the samples were dark-adapted before measuring fluorescence, which makes it difficult to compare with this study. It is surprising that there were no significant shade effects on any of the chlorophyll fluorescence variables measured in the study. Further, no attempt was made to find correlations between chlorophyll fluorescence and chlorophyll content, which are undisputedly inversely related to each other. In the present study, chlorophyll fluorescence and chlorophyll content were clearly affected by shade and were strongly inversely correlated to each other.

The higher chlorophyll fluorescence emissions observed in this study could be explained by the fact that seedlings grown in full sunlight may have a damaged photosynthetic system due to either inhibited chlorophyll synthesis or accelerated photo-oxidative bleaching of chlorophyll

(photooxidation or solarization) resulting in reduced chlorophyll content (Armitage and Vines 1982, Powless 1984, Lichtenthaler and Rinderle 1988, Binder et al. 1988). This would affect photosynthetic apparatus of the plants. Because, a portion of the re-emitted light in the form of fluorescence (Krause and Weis 1991) will not be re-absorbed by low chlorophyll molecules found in seedlings in full sunlight (Lichtenthaler 1988) resulting in increased fluorescence emissions (Krause and Weis 1991).

As seedlings were not fertilized during the course of the study, it is likely that the plants were deficient in nutrients. Nutrients like nitrogen, magnesium, manganese, copper, zinc, and iron play important roles in chlorophyll synthesis, photosynthesis, water photolysis, and electron transport chain of photosynthetic process. The deficiency of these nutrients might have affected over all physiological efficiency of plants growing in full sunlight. The noticeable chlorosis of the plants in full sunlight was probably due to deficiency of some of these nutrients (Salisbury and Ross 1978, Landis 1985, Cleary et al. 1988, van den Driessche 1989).

There is the possibility that plants may have undergone transplant shock which could be more severe in plants grown in full sunlight than those under shade. In the newly planted young seedlings in full sunlight, the rate

of transpiration presumably exceeds that of absorption. Plants can be stressed under these conditions and their photosynthetic functioning can be affected, resulting in increased fluorescence.

Also, at high light intensities activation of photosynthetic (Calvin-Benson or C_3 Cycle) enzymes, including ribulose 1,5-bisphosphate carboxylase/oxygenase (RuBisCO) may be suppressed and light harvesting complex proteins are denatured. This affects overall photosynthetic carbon reduction (PCR) of the photosynthetic process (Bidwell 1974, Lehinger 1982) resulting in low re-oxidation capacity of quinone A. This phenomenon was observed in damaged spruce needles with lower chlorophyll contents resulting in increased fluorescence emissions (Lichtenthaler and Rinderle 1988).

Conversely, in the current study under 70% shade seedlings had reduced fluorescence emissions due to increased chlorophyll molecules per unit area (Lichtenthaler et al. 1986). The reduction in fluorescence with increased chlorophyll molecules was due to reabsorption of the emitted fluorescence by chlorophyll molecules (Krause and Weis 1988, Lichtenthaler et al. 1986, Lichtenthaler and Rinderle 1988, Buschmann and Lichtenthaler 1988, Lichtenthaler 1988). This was also evident by the data

that seedlings having similar chlorophyll contents at the start of the current study had similar fluorescence emissions. As the time progressed increasing shade resulted in increased chlorophyll contents followed by decreased fluorescence emissions. Thus, the data indicated that chlorophyll content was inversely proportional to chlorophyll fluorescence and directly related to increasing shade.

The strong negative correlation between chlorophyll content and fluorescence emissions found in the current study is in agreement with previous reports [damaged spruce needles (*Picea abies* Karst.) (Lichtenthaler and Rinderle 1988, Rinderle and Lichtenthaler 1988, Buschmann and Lichtenthaler 1988), cherrylaurel (*Prunus laurocerasus* L.), etiolated bean (*Phaseolus vulgaris* L.), and tobacco (*Nicotiana tabacum* L.) (Buschmann and Lichtenthaler 1988)].

For years F_v / F_{max} has been used to indicate photosynthetic and quantum efficiency of PSII (see introduction section 1.4.2.1 for details). The slope of the Kautsky curve is another worthwhile parameter which can be used to check the vitality (the power to live or grow) of plants and document stress effects on photosynthetic apparatus. It can also be used to

assess the rate of fluorescence quenching and photosynthetic functioning and efficiency of the seedlings.

Measuring and quantifying the slope of the fluorescence induction curve has not been reported previously. Some researchers (Lichtenthaler et al. 1986, Lichtenthaler and Rinderle 1988, Lichtenthaler 1988) have computed a mathematical ratio by measuring fluorescence decrease (F_d) from F_{max} to F_s and correlated it with photosynthetic efficiency of spruce and fir seedlings. This is called the Rfd ratio ($Rfd=F_d/F_s$), values which are higher than 2.5 indicate that the photosynthetic apparatus is fully functional.

The above papers using the Rfd ratio are general research papers without any statistics and experimental designs and do not explain how the ratio was modeled. No specific treatments were tested nor replicated. These papers appear to be merely case-histories: unreplicated experiments with limited applicability, or scope of inference. There is no way of knowing if the results are significant or if they can be reproduced (Stafford and Sabin 1992, unpubl). It is possible that this is the main reason that these models were not exercised or reproduced by other researchers since they were first presented in 1986. On the other hand, the slope of the fluorescence curve calculated between the 60 and 120 second portion of the curve in the current

study is supported by strong statistics, experimental design, properly replicated controlled treatments, and methodology.

Since measuring the slope of the fluorescence curve is a new idea, the significance of the positive slopes shown in this study is not known. Theoretically, the lower the CO_2 assimilation, the lower the fluorescence quenching and the more positive slope. This indicates that quinone A is not fully oxidized. That is, some of the electron carriers of the PSII reaction center are not fully open to accept electrons and pass onward through the system (Vidaver et al. 1991).

The slope of the fluorescence curve determined between the 60 and 120 second of the scan period may not be representative of the whole photosynthetic system of the plant. This is the period when the fluorescence curve is almost approaching to steady state. Other portions of the curve also need evaluation to get an overall picture of the photosynthetic functioning of the seedlings.

Stomatal conductance decreased significantly in all four species from morning to afternoon and was not affected by shade. Higher rate of stomatal conductance in the morning indicated that the stomata were fully open in the seedlings allowing more CO_2, thus resulting in increased

photosynthesis. Conversely, decreased stomatal conductance in the afternoon, indicated that plants were probably stressed, resulting in stomatal closure and reduced photosynthesis. Generally, increasing stomatal conductance increases photosynthesis (via optimal photosynthetic biochemical components). This should reduce fluorescence emissions as photosynthesis and chlorophyll fluorescence are inversely related to each other (Krause and Weis 1984).

The purpose of the foregoing discussion is not to disregard other peoples' work. I am simply trying to put the results of this study in context with those of others and in perspective with the available resources, technology, methodology, and physical limitations. There has been an improvement in instrumentation and software in chlorophyll fluorescence which can improve fluorescence research and data interpretation.

Measuring fluorescence emissions with a non-integrating system involves a small portion of a needle and the results obtained may or may not be normalized. This may cause additional variation in the data when the results are interpreted from individual needles to whole plants. Differences in sampling protocols and lack of standardization of methodology may lead to potentially large experimental variation and subsequent errors in data

interpretation (Mohammed et al. 1995). This is especially important when fluorescence is measured in outdoor trials, where environmental conditions, particularly light, are quite variable. Dark-adapting samples to make sure electron carriers are fully oxidized will present special challenges. Clearly, standardization of sample collection, particularly when small individual needles are being used, is essential to accomplish accurate and repeatable results. The integrating fluorometer will help smooth out confounding effects from one or more of these factors. Compared to other instruments, it is unique in that it takes into account the whole shoot, provides both normalized and un-normalized Kautsky data and follows a well established dark-adaptation protocol (Mohammed et al. 1995).

Like any other study, the current study was also confronted with some limitations. The sample size for physiological measurements should have been larger. This would have minimized variability in the data, specifically in slope of the fluorescence curve and stomatal conductance. Stomatal conductance is highly variable and requires a large amount of data for proper analyses and interpretation (Livingston and Black 1987). Therefore, measurements should be made on a large sample size to minimize the variation. That is probably the main reason no significant shade effects on

these characteristics were found in this study. But, this itself was constrained by other limitations. The physical aspect of moving seedlings and the great amount of time involved in measuring seedlings made it difficult to have a large sample size. It may be the reason so many studies do not use rigorous statistical analyses.

Although, the uniform shade environments created with shade-cloth in the current study are not representative of actual light conditions in forests, the data indicate that seedlings of all four species grow poorly and have low photochemical efficiency in deep shade (70%). These findings have some ecological and silvicultural implications. The species used significantly differed in relative growth rate, chlorophyll contents, photochemical efficiency, and stomatal conductance in response to widely different light intensities. This was because of differences in their "degree of tolerance" which is well documented and established in the literature (Baker 1934, 1950). Tolerance is defined as "the ability of the plant to survive under deep shade" (Baker 1950) or "the ability of a plant to survive and grow under the shade of a forest canopy" (Bourdeau and Laverick 1958). Species used in the current study have been given tolerance ratings from "very tolerant" for western redcedar and western hemlock to

"intermediate" for Douglas-fir, and to "intolerant" for ponderosa pine (Baker 1950).

Seedlings grown under intense shade (70%) had substantially higher shoot growth and chlorophyll contents than those in full sunlight and 30%. This raises an interesting question. Do we need to raise seedlings under deeper shade to promote their shoot height and chlorophyll contents and what are the implications in context with out-planting in small openings under natural conditions? The data shows that seedlings grown under deeper shade perform poorly which is in strong agreement with previously reported findings. A higher chlorophyll content does not guarantee better photosynthetic efficiency in terms of increased biomass production and sturdiness (see earlier part of this discussion). As stated earlier, seedlings having higher chlorophyll content and larger shoots under 70% shade had significantly reduced relative growth rates, biomass production, and photochemical efficiency. Though shade-grown seedlings are considered more efficient in utilizing low levels of light due to far lower compensation point (about 5% of normal daylight, 2000 $\mu E\ m^{-2}\ s^{-1}$) than sun-grown seedlings, they contain less RuBisCO and have a low carboxylation rate (Hader and Tevini 1987, Taiz and Zieger 1991). As a result, shade-grown

plants have reduced carbon uptake compared to nitrogen (Margolis et al. 1988) as well as reduced carbon storage components like sugar and starch (Matson and Waring 1984) and carbon-rich compounds like lignin and phenolics (Waring et al. 1985).

To successfully grow and survive under harsh environmental conditions in the field, a target seedling has to have some minimum standards of reserve carbohydrates (Marshall 1985), shoot:root ratio (Thompson 1985, Mexal and Landis 1990), root system (Carlson and Miller 1990), and physiological vigor (Zaerr 1985, Hawkins and Binder 1990). For comprehensive details on these and many other characteristics, refer to Duryea and Brown (eds.) (1984), Duryea (ed.) (1985), and Rose et al. (eds.) (1990b).

Shade-grown seedlings with reduced morphological and physiological vigor as seen in the current study, when exposed to high light intensity can suffer from photoinhibition (reduced photosynthesis) as a result of chlorophyll bleaching (Kozlowski 1957, Powless 1984, Kamaluddin and Grace 1992). Therefore, shade-grown seedlings in the nursery or planted under dense canopies in forests, are not likely to grow well compared to those in exposed areas. However, all seedlings benefit from moderate shade

for the first year or two to avoid increasing water and light stress caused by competing vegetation, thus ensuring successful establishment of seedlings (Hobbs 1992, Tesch and Helms 1992). Once they become fully established, both tolerant and intolerant species increase in growth as light intensity increases to full sunlight (Tesch and Helms 1992).

Concluding the discussion it can be reiterated that based on the findings of the current and previous studies, a moderate amount of shade (30%) is beneficial for seedlings to increase their photochemical efficiency and biomass production.

5. CONCLUSIONS

At both shade extremes (i.e. 0 and 70% shade) the growth of all four species was poor both morphologically and physiologically. Best growth occurred under 30% shade. Among species, western redcedar and western hemlock had greater biomass production and relative growth rate followed by Douglas-fir and ponderosa pine, proving their greater shade tolerance. Ponderosa pine proved to be intolerant to high shade levels.

Chlorophyll fluorescence was inversely related to shade regimes. There was a substantial amount of decrease in all fluorescence variables with increasing shade intensity. Data indicated that ponderosa pine and western redcedar were more efficient at collecting and utilizing light intensity (i.e. higher photosynthetic efficiency of PSII indicated by higher F_v / F_{max} values) followed by western hemlock and Douglas-fir. In general, photochemical efficiency of all four species decreaed with increasing shade indicating that seedlings' morphological responses to increasing shade levels were menifested in their physiological responses.

The slope of the fluorescence curve between the 60 and 120 second portion of the scan period and stomatal conductance, regardless of shade levels, were linearly related with time in all four species.

Chlorophyll content was directly related to increasing shade in all four species. Western hemlock, which had the smallest amount of chlorophyll a, b, and total chlorophyll at the begining of the experiment, had significantly higher amounts than the other three species after 29 weeks of growth. Chlorophyll content was strongly negatively related to fluorescence emissions.

In general, this study showed that seedlings of all four species performed better under 30% shade. Based on previous studies, all seedlings benefit from moderate shade for the first year or two to avoid increasing water and light stress caused by competing vegetation, thus ensuring successful establishment of seedlings. Once they become fully established, both tolerant and intolerant species increase in growth as light intensity increases to full sunlight. Over a long period of time, probably tolerant species will outgrow intolerant species, thus leading to an unevenaged stand structure.

Based on the findings of this study, it seems obvious that further evaluation of fluorescence emissions and the slope of the fluorescence curve is warranted. To make it more meaningful and useful in context with seedling's physiological status, it should be measured concomitantly with CO_2 assimilation under controlled conditions.

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APPENDICES

NOTE: ANOVA tables (2-15) in the thesis/appendices are constructed after the error terms have been combined in various analyses where it was deemed necessary in the light of the explanation given in sections 2.4.3. through 2.4.4.3.

Appendix 1. I am using the standard statistical approach of stating the null hypotheses which is actually the converse of my "research hypotheses" (Mendenhall 1975) or that which I truly hope to be able to support. Using the first hypothesis as an example, I would like to be able to reject the hypothesis as stated and support the hypothesis that "the morphological development of the four species are not different under four shade regimes". However, by starting from the null hypotheses, statistically I am able to directly evaluate the probability of incorrect decisions. If we reject the null hypotheses, we immediately know the probability of making an incorrect decision (i.e. α). Otherwise, if we started from the alternative hypotheses (the converse of null hypotheses), we would have to compute β which, in most instances, is more difficult. Therefore, I have elected to use the approach of "proof by contradiction" and have developed null hypotheses which I hope to be able to reject which are the converse of my actual research hypotheses. By doing so, if the test leads to rejection of the null hypotheses, I will immediately have a measure of the confidence in these conclusions.

Appendix 2. Analysis of variance table for initial morphological measurements: A) shoot height; B) stem diameter; C) total fresh weight; D) root volume; and E) shoot volume. Data for total fresh weight, root and shoot volumes were log-transformed.

A) Shoot Height

SOURCE	DF	SS	MS	P
Block	3	59.706	19.902	0.6705
Shade	3	127.443	42.481	0.7275
Species	3	30630.967	10210.322	0.0001
Shade*Species	9	285.235	31.693	0.9619
Error (A)	45	4373.03	97.178	
Error (B)	256	9841.7	38.444	

B) Stem Diameter

Block	3	6.748	2.249	0.0752
Shade	3	2.748	0.916	0.5076
Error (A)	9	9.867	1.096	
Species	3	98.387	32.796	0.0001
Shade*Species	9	7.255	0.806	0.5866
Error (B)	292	282.746	0.968	

Appendix 2. Continued

C) Total Fresh Weight

SOURCE	DF	SS	MS	P
Block	3	0.287	0.096	0.3707
Shade	3	0.288	0.096	0.623
Error (A)	9	1.408	0.156	
Species	3	30.491	10.164	0.0001
Shade*Species	9	0.575	0.064	0.8108
Error (B)	36	4.024	0.112	
Error (C)	256	37.074	0.091	

D) Root Volume

Block	3	0.367	0.122	0.3906
Shade	3	0.505	0.168	0.5438
Error (A)	9	2.015	0.224	
Species	3	67.742	22.581	0.0001
Shade*Species	9	1.036	0.115	0.6541
Error (B)	36	5.465	0.152	
Error (C)	256	77.130	0.122	

E) Shoot Volume

Block	3	0.172	0.057	0.6542
Shade	3	0.315	0.105	0.5458
Error (A)	9	1.249	0.139	
Species	3	24.146	8.049	0.0001
Shade*Species	9	0.522	0.058	0.9141
Error (B)	36	4.933	0.137	
Error (C)	256	27.151	0.106	

Appendix 3. Analysis of variance table: A) relative growth rate of total fresh weight; B) sturdiness quotient; and C) Dickson quality index.

A) Relative Growth Rate

SOURCE	DF	SS	MS	P
Block	3	0.264	0.088	0.3740
Shade	3	2.362	0.787	0.0443
Error (A)	9	1.745	0.194	
Species	3	26.855	8.952	0.0001
Shade*Species	9	0.841	0.093	0.7116
Error (B)	36	4.862	0.135	
Error (C)	128	10.766	0.084	

B) Sturdiness Quotient

Block	3	1.634	0.545	0.4949
Shade	3	25.382	8.461	0.0496
Error (A)	9	19.649	2.183	
Species	3	256.607	85.536	0.0001
Shade*Species	9	10.099	1.122	0.5858
Error (B)	36	48.154	1.338	
Error (C)	256	174.282	0.681	

C) Dickson Quality Index

Block	3	29.220	9.740	0.0373
Shade	3	167.813	55.938	0.0001
Species	3	578.174	192.725	0.0001
Shade*Species	9	60.041	6.671	0.4140
Error (A)	45	284.736	6.327	
Error (B)	256	870.745	3.401	

Appendix 4. Analysis of variance table for final chlorophyll fluorescence measurements: A) F_{max}; B) F_v; C) F_v / F_{max}. Data were log transformed.

A) F_{max}

SOURCE	DF	SS	MS	P
Block	3	0.234	0.078	0.0112
Shade	3	1.702	0.567	0.0001
Species	3	0.751	0.250	0.0014
Shade*Species	9	0.504	0.056	0.2296
Error (A)	45	1.837	0.041	
Error (B)	64	1.245	0.019	

B) F_v

Block	3	1.302	0.434	0.0013
Shade	3	7.592	2.531	0.0006
Error (A)	9	1.432	0.159	
Species	3	3.213	1.071	0.0008
Shade*Species	9	1.867	0.207	0.2489
Error (B)	36	5.551	0.154	
Error (C)	256	4.733	0.074	

C) F_v/F_{max}

Block	3	0.434	0.145	0.0003
Shade	3	2.124	0.708	0.0006
Error (A)	9	0.403	0.045	
Species	3	0.867	0.289	0.0006
Shade*Species	9	0.492	0.055	0.2318
Error (B)	45	1.422	0.040	
Error (C)	256	1.264	0.020	

Appendix 5. Analysis of Variance table for final chlorophyll contents of new needles: A) chlorophyll *a*; B) chlorophyll *b*; C) total chlorophyll. Data were log transformed.

A) Chlorophyll *a*

SOURCE	DF	SS	MS	P
Block	3	0.196	0.065	0.2756
Shade	3	16.241	5.414	0.0001
Error (A)	9	0.926	0.103	
Species	3	22.612	7.538	0.0001
Shade*Species	9	3.257	0.362	0.0001
Error (B)	100	4.979	0.050	

B) Chlorophyll *b*

Block	3	0.328	0.109	0.2888
Shade	3	25.921	8.640	0.0001
Error (A)	9	1.985	0.221	
Species	3	21.994	7.331	0.0001
Shade*Species	9	3.418	0.380	0.0001
Error (B)	100	5.599	0.086	

C) Total Chlorophyll

Block	3	0.223	0.074	0.2554
Shade	3	18.099	6.033	0.0001
Error (A)	9	1.092	0.121	
Species	3	22.780	7.593	0.0001
Shade*Species	9	3.099	0.344	0.0001
Error (B)	100	5.417	0.054	

Appendix 6. Analysis of variance table for final chlorophyll contents of old needles: A) chlorophyll *a*; B) chlorophyll *b*; C) total chlorophyll. Data were log transformed.

A) Chlorophyll *a*

SOURCE	DF	SS	MS	P
Block	3	0.105	0.035	0.7388
Shade	3	16.918	5.639	0.0001
Error (A)	9	1.446	0.161	
Species	3	7.927	2.642	0.0001
Shade*Species	9	2.322	0.258	0.0480
Error (B)	36	4.274	0.119	
Error (C)	64	5.337	0.083	

B) Chlorophyll *b*

Block	3	0.137	0.046	0.7744
Shade	3	21.780	7.260	0.0001
Error (A)	9	2.642	0.294	
Species	3	7.937	2.646	0.0001
Shade*Species	9	1.851	0.206	0.2155
Error (B)	36	5.206	0.145	
Error (C)	64	7.891	0.123	

C) Total Chlorophyll

Block	3	0.109	0.036	0.7481
Shade	3	17.808	5.936	0.0001
Error (A)	9	1.733	0.193	
Species	3	7.864	2.621	0.0001
Shade*Species	9	2.081	0.231	0.0812
Error (B)	36	4.347	0.121	
Error (C)	64	5.724	0.089	

Appendix 7. Analysis of variance table for Repeated Measures Design for total terminal height growth. Huynh-Feldt Epsilon=0.2549, adjusted P-values were used for repeated measures part of the ANOVA.

SOURCE	DF	SS	MS	P
Block	3	770.825	256.975	0.0992
Shade	3	497.853	165.951	0.2130
Error (A)	9	818.974	90.997	
Species	3	4017.911	1339.304	0.0001
Shade*Species	9	351.930	39.103	0.9088
Error (B)	36	3259.362	90.538	
Error (C)	128	7358.331	57.487	**ADJ. P ↓**
Time	9	18947.946	18947.946	0.0004
Linear	1	16673.895	16673.895	0.0476
Quadratic	1	2172.891	2172.891	0.0329
Cubic	1	2.772	2.772	0.1146
Error (D)	27	204.300	7.567	
Time*Shade	27	374.450	13.869	0.0004
Linear	3	336.520	112.173	0.0521
Quadratic	3	7.485	2.495	0.3685
Cubic	3	20.424	20.424	0.0231
Time*Species	27	2644.459	97.943	0.0001
Linear	3	1456.152	485.384	0.0001
Quadratic	3	406.855	406.855	0.0001
Cubic	3	688.331	229.444	0.0001
Time*Shade*Species	81	251.426	3.104	0.4795
Linear	9	151.101	16.789	0.5079
Quadratic	9	45.248	5.028	0.2629
Cubic	9	26.223	2.914	0.0708
Error (E)	405	1267.782	3.130	
Error (F)	1152	2672.462	2.320	

Appendix 8. Analysis of variance table for Repeated Measures Design for the slope of the chlorophyll fluorescence between the 60 and 120 second portion of the curve. Huynh-Feldt Epsilon=1.6156, adjusted P-values were not used .

SOURCE	DF	SS	MS	P
Block	3	0.00000932	0.00000311	0.4612
Shade	3	0.00003023	0.00001008	0.0788
Error (A)	9	0.00002933	0.00000326	
Species	3	0.00008553	0.00002851	0.0001
Shade*Species	9	0.00001245	0.00000138	0.6377
Error (B)	36	0.00006528	0.00000181	
Error (C)	64	0.00005744	0.0000009	
Time	5	0.00017376	0.00003475	0.0003
Linear	1	0.00012687	0.00012687	0.0194
Quadratic	1	0.00003443	0.00003443	0.0778
Cubic	1	0.00000947	0.00000977	0.2259
Error (D)	15	0.00005505	0.00000369	
Time*Shade	15	0.00001191	0.00000079	0.5930
Linear	3	0.00000148	0.00000049	0.8414
Quadratic	3	0.00050672	0.00016891	0.0710
Cubic	3	0.00007536	0.00002512	0.9221
Time*Species	15	0.00005582	0.00000372	0.0001
Linear	3	0.00001213	0.00000404	0.0108
Quadratic	3	0.00042157	0.00014052	0.1155
Cubic	3	0.00120140	0.00040047	0.0664
Time*Shade*Species	45	0.00004106	0.00000091	0.2854
Linear	9	0.00000935	0.00000104	0.3829
Quadratic	9	0.00077226	0.00008581	0.2776
Cubic	9	0.00133439	0.00014827	0.4932
Error (E)	225	0.00017686	0.00000079	
Error (F)	320	0.00015838	0.00000049	

Apendix 9. Analysis of variance table for Repeated Measures Design for stomatal conductance of old needles. Huynh-Feldt Epsilon=1.3887, adjusted P-values were not used. Data was square root transformed.

SOURCE	DF	SS	MS	P
Block	3	29.766	9.922	0.2651
Shade	3	7.616	2.539	0.7943
Species	3	826.049	275.35	0.0001
Shade*Species	9	84.487	9.384	0.2626
Error (A)	109	806.811	7.402	
Time	2	453.542	226.771	0.0006
Linear	1	444.945	453.945	0.0040
Quadratic	1	8.597	8.597	0.2641
Error (B)	6	41.034	6.839	
Time*Shade	6	29.455	4.909	0.1593
Linear	3	26.195	8.732	0.0826
Quadratic	3	3.260	1.087	0.7526
Error (C)	18	49.545	2.753	
Time*Species	6	53.510	8.918	0.0001
Linear	3	39.353	13.118	0.0007
Quadratic	3	14.157	4.719	0.0528
Time*Shade*Species	18	43.972	2.443	0.1689
Linear	9	35.550	3.950	0.0653
Quadratic	9	8.422	0.936	0.8213
Error (D)	200	365.887	1.829	

Appendix 10. Analysis of variance table for Repeated Measures Design for stomatal conductance of new needles. Huynh-Feldt Epsilon=1.9639, adjusted P-values were not used. Data was square root transformed.

SOURCE	DF	SS	MS	P
Block	3	37.029	12.343	0.1233
Shade	3	2.066	0.689	0.9543
Species	3	111.734	37.245	0.0009
Shade*Species	9	62.433	6.937	0.3653
Error (A)	109	684.207	6.277	
Time	2	301.621	150.810	0.0001
Linear	1	284.913	284.913	0.0015
Quadratic	1	16.708	16.708	0.1123
Error (B)	6	16.751	2.792	
Time*Shade	6	21.872	3.645	0.1407
Linear	3	11.697	3.899	0.1656
Quadratic	3	10.175	3.392	0.1975
Error (C)	18	34.989	1.944	
Time*Species	6	138.490	23.082	0.0001
Linear	3	93.547	31.182	0.0001
Quadratic	3	44.943	14.981	0.0001
Time*Shade*Species	18	27.510	1.528	0.5289
Linear	9	13.090	1.454	0.7382
Quadratic	9	14.420	1.602	0.1476
Error (D)	72	116.327	1.616	
Error (E)	128	170.761	1.334	

Appendix 11. Analysis of variance table for Repeated Measures Design for F_{max}. Huynh-Feldt Epsilon=1.3605, adjusted P-values were not used. Data was log transformed.

SOURCE	DF	SS	MS	P
Block	3	0.507	0.169	0.1409
Shade	3	1.807	0.602	0.0057
Error (A)	9	0.648	0.072	
Species	3	5.804	1.935	0.0001
Shade*Species	9	0.471	0.052	0.3196
Error (B)	36	1.844	0.051	
Time	5	0.955	0.191	0.0002
Linear	1	0.069	0.069	0.2003
Quadratic	1	0.427	0.427	0.0324
Cubic	1	0.210	0.210	0.0229
Error (C)	15	0.29	0.019	
Time*Shade	15	1.147	0.076	0.0001
Linear	3	0.935	0.312	0.0007
Quadratic	3	0.048	0.016	0.6451
Cubic	3	0.120	0.040	0.0192
Error (D)	45	0.607	0.013	
Time*Species	15	4.352	0.290	0.0001
Linear	3	0.812	0.271	0.0001
Quadratic	3	1.366	0.455	0.0001
Cubic	3	1.475	0.492	0.0001
Time*Shade*Species	45	0.443	0.010	0.5233
Linear	9	0.190	0.021	0.2533
Quadratic	9	0.082	0.009	0.6038
Cubic	9	0.054	0.006	0.8285
Error (E)	180	1.816	0.010	

Appendix 12. Analysis of variance table for Repeated Measures Design for F_v. Huynh-Feldt Epsilon=1.3432, adjusted P-values were not used. Data was log transformed.

SOURCE	DF	SS	MS	P
Block	3	1.796	0.599	0.1270
Shade	3	8.134	2.711	0.0021
Error (A)	9	2.169	0.241	
Species	3	28.432	9.477	0.0001
Shade*Species	9	2.06	0.229	0.2448
Error (B)	36	6.082	0.169	
Time	5	2.845	0.569	0.0025
Linear	1	0.235	0.235	0.2600
Quadratic	1	0.762	0.762	0.1082
Cubic	1	0.182	0.182	0.0450
Error (C)	15	1.362	0.091	
Time*Shade	15	5.107	0.340	0.0001
Linear	3	4.290	1.430	0.0006
Quadratic	3	0.174	0.058	0.5882
Cubic	3	0.511	0.170	0.0941
Error (D)	45	2.417	0.054	
Time*Species	15	21.038	1.403	0.0001
Linear	3	3.578	1.193	0.0001
Quadratic	3	5.834	1.944	0.0001
Cubic	3	8.624	2.875	0.0001
Time*Shade*Species	45	2.017	0.045	0.3908
Linear	9	0.662	0.074	0.3047
Quadratic	9	0.278	0.031	0.7133
Cubic	9	0.340	0.038	0.5841
Error (E)	180	7.579	0.042	

Appendix 13. Analysis of variance table for Repeated Measures Design for F_v / F_{max}. Huynh-Feldt Epsilon=1.3887, adjusted P-values were not used.

SOURCE	DF	SS	MS	P
Block	3	0.097	0.033	0.1286
Shade	3	0.462	0.154	0.0019
Error (A)	9	0.119	0.013	
Species	3	1.513	0.504	0.0001
Shade*Species	9	0.105	0.012	0.3196
Error (B)	36	0.347	0.01	
Time	5	0.160	0.032	0.0017
Linear	1	0.019	0.019	0.1743
Quadratic	1	0.047	0.047	0.0856
Cubic	1	0.019	0.019	0.0210
Error (C)	15	0.071	0.005	
Time*Shade	15	0.287	0.019	0.0001
Linear	3	0.243	0.081	0.0005
Quadratic	3	0.010	0.003	0.6015
Cubic	3	0.029	0.010	0.0643
Error (D)	45	0.131	0.003	
Time*Species	15	1.083	0.072	0.0001
Linear	3	0.193	0.064	0.0001
Quadratic	3	0.315	0.105	0.0001
Cubic	3	0.429	0.143	0.0001
Time*Shade*Species	45	0.096	0.002	0.4549
Linear	9	0.037	0.004	0.2670
Quadratic	9	0.014	0.002	0.7490
Cubic	9	0.014	0.002	0.6822
Error (E)	180	0.379	0.002	

Appendix 14. Analysis of variance table for final morphological measurements: A) shoot height; B) stem diameter; C) total fresh weight; D) Root volume; E) shoot volume; and F) total dry weight. Data for total fresh weight, root volume, and total dry weight were log-transformed.

A) Shoot Height / **B) Stem Diameter**

SOURCE	DF	SS	MS	P	SS	MS	P
Block	3	357.516	119.17	0.1172	9.423	3.141	0.1753
Shade	3	82.281	27.427	0.8864	40.417	13.472	0.0486
Error (A)	9	1171.074	130.119		31.006	3.445	
Species	3	46309.871	15436.624	0.0001	207.484	69.161	0.0001
Shade*Species	9	600.418	66.413	0.4176	19.808	2.201	0.4598
Error (B)	36	2272.470	63.124		79.455	2.207	
Error (C)	256	15396.464	60.142		483.379	1.888	

C) Total Fresh Weight / **D) Root Volume**

Block	3	0.879	0.293	0.0511	1.047	0.349	0.0740
Shade	3	3.282	1.094	0.0290	8.282	2.761	0.0016
Error (A)	9	2.050	0.228		2.051	0.228	
Species	3	44.599	14.886	0.0001	78.638	26.213	0.0001
Shade*Species	9	1.170	0.130	0.6269	2.147	0.239	0.2742
Error (B)	36	5.923	0.165		6.638	0.184	
Error (C)	256	28.596	0.112		38.179	0.149	

E) Shoot Volume / **F) Total Dry Weight**

Block	3	4663.269	1554.423	0.0157	1.253	0.418	0.0354
Shade	3	856.106	285.369	0.7980	3.292	1.097	0.0328
Error (A)	9	7582.938	842.549		2.155	0.239	
Species	3	156120.78	52040.260	0.0001	45.025	15.008	0.0001
Shade*Species	9	5331.475	592.386	0.5133	1.282	0.142	0.5579
Error (B)	36	22990.219	638.617		5.880	0.163	
Error (C)	256	113005.20	441.427		36.839	0.144	

Appendix 15. Actual growth (final-initial) of morphological variables. Within shade (averaged over species) and within species (averaged over shade), means for each morphological parameter followed by the different letters are significantly different at the $\alpha \leq 0.05$ level (Fisher's Protected Least Significant Difference). Means were back-transformed from log transformations and are presented as medians.

Variables → Shade/ Species ↓	Shoot Height (cm)	Stem Diameter (mm)	Total Fresh Weight (g)	Root Volume (cm^3)	Shoot Volume (cm^3)
Shade					
0%	6.94a	1.59a	31.58a	18.00a	16.72a
30%	7.35b	1.60a	30.12a	17.48a	17.10a
50%	7.56b	1.27ab	29.83a	16.02a	17.20a
70%	9.49b	1.02b	18.37b	8.52b	13.55a
Species					
ponderosa pine	6.28b	0.67c	14.99c	8.12c	7.34c
Douglas-fir	9.96a	1.05b	19.06c	7.65c	22.66a
western redcedar	9.95a	2.53a	60.35a	38.51a	31.63a
western hemlock	5.87b	1.86a	30.22b	17.97b	12.67b

Appendix 16. Mean and maximum F_v/F_{max} values (relative units) averaged over shade, for four conifer species used in the study.

Species	Month	Mean	Maximum
ponderosa pine	April	0.491	0.554
	May	0.576	0.621
	June	0.669	0.763
	July	0.561	0.732
	August	0.491	0.723
	September	0.514	0.710
Douglas-fir	April	0.476	0.519
	May	0.260	0.364
	June	0.345	0.433
	July	0.434	0.573
	August	0.403	0.576
	September	0.437	0.605
western redcedar	April	0.527	0.618
	May	0.604	0.717
	June	0.592	0.720
	July	0.525	0.623
	August	0.438	0.558
	September	0.470	0.617
western hemlock	April	0.488	0.553
	May	0.554	0.677
	June	0.523	0.648
	July	0.549	0.692
	August	0.541	0.681
	September	0.531	0.660