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Margaret M. O'Connell-Payne

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of the requirements for

Ph.D. degree in Forestry

Daniel E. Keathley

Dr. Daniel E. Keathley
Major professor

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PHYSIOLOGICAL STUDIES OF BLUE AND ENGELMANN SPRUCE: LIGHT AND WATER RELATIONS

By

Margaret M. O'Connell-Payne

A DISSERTATION

Submitted to
Michigan State University
in partial fulfillment of the requirements
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DOCTOR OF PHILOSOPHY

Department of Forestry

1997

ABSTRACT

PHYSIOLOGICAL STUDIES OF BLUE AND ENGELMANN SPRUCE: LIGHT AND WATER RELATIONS

By

Margaret M. O'Connell-Payne

The cause of widespread Engelmann spruce mortality documented in natural and artificially-regenerated stands on high elevation sites has been attributed to either desiccation or solarization (inhibition of photosynthesis by high light intensities). To study solarization, blue spruce, Engelmann spruce and their F_1 hybrid were grown in a common garden at 3200 m elevation in the San Juan National Forest, southwestern Colorado. Gas exchange rates, total chlorophyll, chlorophyll fluorescence and growth variables were measured. No significant differences between species or hybrids were detected for any trait examined, and there was no evidence of solarization. Physiological response to soil moisture deficits was examined in Engelmann and blue spruce to determine the effect of decreased soil moisture on survival. Forty-eight, three-year old trees were arranged in a split-plot design in the greenhouse and subjected to three water levels. Increased survival, water potential and rates of photosynthesis in blue spruce paralleled increased partitioning of carbon to the roots, which increased the root mass available for water absorption. That blue spruce had a higher survival rate than Engelmann spruce when subjected to the low, or no water treatments suggests that drought stress, a confounding factor of high light, could be involved in Engelmann spruce mortality on high elevation sites. Two additional studies focused on the response of gas exchange of blue and Engelmann spruce to different light regimes. Stomatal conductance was found to be significantly higher in Engelmann spruce than in blue spruce over the course of a day, indicating higher rates of water loss through stomata (relative to blue spruce), and suggesting a trait through which Engelmann and blue spruce adapted to their respective habitats in southwestern Colorado. Photosynthetic light response studies were inconclusive due to the condition of the plant material used in the studies.

DEDICATION

To Mary T. Hoff.
She provided more than half my genetics
and created a great environment,
despite an inordinate amount of stress.

Her confidence in me made this possible.

ACKNOWLEDGMENTS

The list of people that helped contribute to this dissertation and helped contribute to the author's well-being is extensive. I am extremely grateful to the late Jim Hanover for accepting me as his student and am equally grateful to Dan Keathley and Don Dickmann for their willingness to advise me upon Jim's untimely death.

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This project would have surely flopped if it were not for the help and advice of Paul Bloese, Randy Klevickas and Roy Prentice, (all of Tree Research Center fame), advising on how to keep trees alive...long enough to kill them. Although he'd probably admit it to be a scary thought, I have Paul to thank for everything I know about SAS and SYSTAT. Paul was very generous with his time, humor and sarcasm; I will miss the latter two a great deal.

I would also like to acknowledge all of the help I received with handling the bureaucracy of graduate school from Barb Anderson, Joyce Beadle, Jean Ecker, Juli Kerr, Sue Plesko, and Connie Miller. Whether I forgot to register for credits, locked myself out of the lab, or just needed a smiling face, these friends would always come through for me.

The final group of Michigan State Department of Forestry employees to acknowledge are the students. I would like to express thanks to Andy David for numerous things: 1) discussions about spruce genetics and physiology, 2) musical compositions about my chlorophyll extractions, 3) field assistance, and 4) pie-baking. However, this quantity of gratitude is small compared to (but confounded with) how

much I appreciate his friendship. Thanks to the many folks who came to my spruce re-potting parties: Bob Morikawa, Joe Zeleznik, Rick Close, Eric Stafne, Dave Lemmien, Sarah Synowiec, Mark Hare, Jill Fisher and Mike Powers. Thanks to Mark Hare, Bob Morikawa, Jill Fisher and Mike Powers for discussions and advice about statistical analysis over many cappuccino excursions. Kudos to Rick "Downtown" Close for the many hours of tree phys talk and for showing me the TDR ropes, or cables, as the case may be. I am grateful to Tesfai Mebrahtu for teaching me how to use the Licor and for his frequent demonstrations of gas exchange (it's not what you think....no, it's not that either). And all of my gratitude for the help of the aforementioned people pales in comparison to how much I value their friendship. In additional, there are many other faculty and a slough of graduate and undergraduate students that I would like to acknowledge for contributing to the tremendous atmosphere of camaraderie in the Department of Forestry. My lunch hours will never be as fun or enlightening as the lunch hours experienced during my Michigan State years. Thanks and go green!

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PREFACE

The trees are drawing me near
I've got to find out why
Those distant voices I hear
explain it all with a sigh

The Moody Blues

To everything
Turn turn turn
There is a season
Turn turn turn

The Byrds

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LIST OF SYMBOLS AND ABBREVIATIONS

A	net CO_2 assimilation rate (μmols*$m^{-2}s^{-1}$)
D_1	one of the protein components of the photosystem II reaction center
D_2	one of the protein components of the photosystem II reaction center
E	transpiration rate (mmols*$m^{-2}s^{-1}$)
E X B	Engelmann x blue spruce hybrid
Engel	Engelmann spruce
Engelm	Engelmann spruce
F_v/F_m	photochemical efficiency
g_s	stomatal conductance rate (μmols*$m^{-2}s^{-1}$)
P680	chlorophyll a reaction center of photosystem II
PAR	photosynthetically active radiation (400-700 nm)
PPFD	photosynthetic photon flux density (μmols*$m^{-2}s^{-1}$)
Q_B	secondary quinone acceptor
SOD	superoxide dismutase (an anti-oxidizing enzyme)
TDR	time domain reflectometry
WUE	water use efficiency (μmols CO_2/mmol H_2O)

INTRODUCTION

Blue spruce (*Picea pungens* Engelm.) and Engelmann spruce (*Picea engelmannii* Parry ex Engelm.) are closely related trees with ranges that overlap several places in the Rocky Mountains (Fowler and Roche 1975; Fechner 1985). Blue spruce occurs in the central and southern Rocky Mountains (Fechner 1990). Engelmann spruce is distributed from British Columbia and Alberta, Canada, south through all western states to New Mexico and Arizona (Alexander and Shepperd 1990). The two species co-occur in elevational bands representing the warmer third of Engelmann spruce habitat, which is the cooler half of the blue spruce habitat (Jones and Bernard 1977). Blue spruce is found from 1830 meters to 2740 meters in its northern range and between 2130-3050 meters in its southern range (Fechner 1990). In the Rocky Mountains of Utah, Wyoming and Colorado, the elevational range of Engelmann spruce is between 2438-3505 meters (Alexander and Shepperd 1990).

In the southern part of the range of blue spruce (southwest Colorado, Arizona and New Mexico), blue spruce dominates in habitats that are too warm and dry for Engelmann spruce (Fechner 1985). It has been reported that blue spruce, in comparison to Engelmann spruce, can: a) withstand drought better (Jones and Bernard 1977; Fechner 1990), b) better adapt to high temperatures (Jones and Bernard 1977) and c) survive full exposure to sunlight at the seedling stage (Jones and Bernard 1977).

Attempts at planting Engelmann spruce on high elevation sites in the central and southern Rocky Mountains in the 1950's-1970's were problematic. Mortality was high in Engelmann spruce plantations, particularly on sites with southern exposure. Needles on open-grown trees became chlorotic due to "solarization," which is defined as "reduced photosynthesis due to high intensity light" (Ronco 1970). Seedlings died during the first winter as a result of the irreversible injury sustained from solarization during the summer (Ronco 1972). Ronco's finding led to planting and natural seeding guidelines that recommended protection from insolation either by making use of protective slash or planting seedlings near natural features that provided shade (Ronco 1972; Noble and Alexander 1977; Noble and Ronco 1978; Alexander 1984; 1987). Successful natural regeneration on south aspects was not to be expected (Noble and Alexander 1977).

In forestry literature, Ronco's documentation of solarization in Engelmann spruce (*Picea engelmannii* Parry) is frequently cited as an example of photoinhibition in trees. Photoinhibition, i.e., reduced photosynthetic efficiency in response to high photon flux densities of visible light (400-700 nm) (Powles 1984), is a plant stress condition first noted by Ewart in 1896 (Barber and Andersson 1992). Other terms used to describe the phenomenon of reduction of capacity for photosynthesis induced by visible light include: photooxidation, photoinactivation, photolability, solarization and photo-dynamic reactions (Powles 1984). Technically, though, these terms are not completely synonymous with photoinhibition and relate to other photo-damaging processes (see Powles 1984, p. 16 for review).

Photoinhibition is caused by damage to photosystem II and is common to all photosynthetic organisms which evolve oxygen (Barber and Andersson 1992). The reaction center of photosystem II contains the D_1 and D_2 polypeptides, pheophytin, quinones and P680. On the molecular level, photoinhibition occurs when the D_1 protein is damaged or resynthesized slowly or not at all (Barber and Andersson 1992). The D_1 polypeptide is a 32 kilodalton protein encoded by the psbA gene in the chloroplast (Barber and Andersson 1992). It contains important binding sites for the water-splitting/oxygen generating reactions of photosynthesis (Barber and Andersson 1992). D_1 binds Q_B (a secondary quinone acceptor), P680, (a chlorophyll a complex and primary electron donor), pheophytin, (a primary electron acceptor), and the manganese cluster (involved in water oxidation and oxygen generation) (Barber and Andersson 1992).

Photoinhibition can be also be induced by drought stress, and, in fact, many examples of drought induced photoinhibition exist in the literature in *Lycopersicon* (Havaux 1992), *Solanum* (Havaux 1992), *Nerium* (Bjorkman and Powles 1984), and *Picea* (Toivonen and Vidaver 1988; Eastmann and Camm 1995). In addition to death from solarization, another hypothesis to explain Engelmann spruce mortality on high elevation, exposed sites was proposed by Noble and Alexander (1977): drought. High light intensity was important insofar as it increased temperature and vapor pressure deficits resulting in drought stress.

As a species native to subalpine habitats, it is logical to assume that Engelmann spruce has adapted to the radiation present at high elevations. For instance, it is well known that ultra-violet wavelengths can cause damage to photosynthetic apparatus

(Powles 1984). However, of ten species of conifer seedlings, Engelmann spruce was one of three species that actually increased in growth in response to increasing ultra-violet b radiation (Sullivan and Teramura 1988). Hence it seems reasonable to hypothesize that Engelmann spruce must be adapted to high levels of photosynthetically active radiation that are found at high elevations. As a seedling, Engelmann spruce usually occurs in the understory beneath lodgepole pine (*Pinus contorta* Dougl. ex Loud.) and trembling aspen (*Populus tremuloides* Michx.). However, like other shade-tolerant understory trees, it gradually becomes a dominant canopy member, slowly becoming exposed to higher photosynthetic photon flux density (PPFD) as it extends beyond its nursing overstory species. For Engelmann spruce, becoming a dominant necessitates adaptation to the high PPFD found at high elevations. Likewise, the high elevation light regime is probably involved in Engelmann spruce cone formation.

The focus of this dissertation was to examine the relative importance of light and water as variables in the solarization of Engelmann spruce. The first chapter examines whether the high light regimes present at a high elevation site in southwestern Colorado induce photoinhibition in Engelmann spruce, blue spruce and the Engelmann X blue spruce hybrid. The second chapter examines the hypothesis that blue spruce and Engelmann spruce would be equally affected by water deficits. The third and final chapter describes the response of gas exchange rates of blue and Engelmann spruce to increasing photosynthetic photon flux densities and to a diurnal light regime.

Chapter One: Investigation of solarization in Engelmann spruce, blue spruce and Engelmann X blue spruce hybrids in a high elevation common garden in southwestern Colorado

Abstract

To study the phenomenon of solarization (inhibition of photosynthesis by high light intensities), blue spruce (*Picea pungens* Engelm.), Engelmann spruce (*Picea engelmannii* Parry ex Engelm.) and their F_1 hybrid were grown in a common garden at 3200 m elevation with northeastern exposure above the Scotch Creek drainage, in the San Juan National Forest, southwestern Colorado. Gas exchange rates (photosynthesis, stomatal conductance and transpiration) and total chlorophyll were measured in years 1 and 2 after planting. Length of current year's growth and height were measured in years 1, 2, 5 and 6 after planting. Chlorophyll fluorescence (F_v/F_m, the ratio of variable fluorescence to maximal fluorescence) was measured in year 5 after planting. None of the blue spruce or F_1 hybrids suffered any mortality despite growing at 3200 meters elevation. One Engelmann spruce died (out of 16 planted) from an undetermined tip dieback (i.e., only the bottom branches had foliage); death of this individual was not from solarization. No significant differences between species or hybrids were detected for any trait examined and there was no evidence of solarization. Comparisons of gas exchange rates from trees at the test site in Colorado were made to pooled means of gas exchange from rangewide representatives grown in a provenance-progeny test at Kellogg Experimental Forest, Kalamazoo County, MI, to test whether the lack of variability seen in gas exchange rates was due to seed source; however, no significant differences were detected among provenances.

Introduction

Silvicultural guidelines for planting and natural seeding of Engelmann spruce (*Picea engelmannii* Parry ex Engelm.) recommend protection from direct light either by providing protective slash or planting seedlings near debris that provide shade (Ronco 1972; Noble and Alexander 1977; Noble and Ronco 1978; Alexander 1984; 1987). It has been postulated that enhanced overwintering success can be obtained on open sites by protecting seedlings from long duration exposure to high intensity light, which can cause foliage solarization. Holman (1930) defined solarization as the failure of leaves to produce starch due to the direct inhibitory effect of excess light energy on photosynthesis (photoinhibition).

Solarization in Engelmann spruce was first reported by Ronco (1970a) who described yellow or yellowish-green foliage which appeared first on older needles and became evident in current growth by the end of the growing season. Subsequent studies have revealed that solarization includes two separate phenomena, "photoinhibition" and "photooxidation." Photoinhibition is defined as a decrease of photosynthetic capacity which, independent of changes in pigment concentration, is induced by exposure to high intensities of visible light (400-700 nm) (Powles 1984). Photoinhibition is not caused by stomatal limitation to CO_2 diffusion (Powles 1984); photoinhibition is caused by a disruption of electron transport in photosystem II (Barber and Andersson 1992). "Photooxidation" refers to photodestruction of photosynthetic pigments after long-term exposure to strong light (Powles 1984). Chlorophyll bleaching due to photooxidation is not regarded as a primary effect but may occur upon prolonged irradiation probably as a secondary process following severe inactivation or destruction of reaction centers (Krause 1988). Thus, photoinhibition precedes photooxidation; bleaching occurs after a certain degree of photoinhibition has occurred (Powles 1984).

The evidence implicating light intensity as the cause of Engelmann spruce mortality in Ronco's (1970a) study is compelling. Whether the reduction in Engelmann spruce mortality under direct light was associated with photooxidation or photoinhibition is not clear from these studies. A light response curve was plotted using well-watered, shaded and unshaded three year old Engelmann spruce and two year old lodgepole pine (*Pinus contorta* Dougl. ex Loud.). The shaded Engelmann spruce photosynthesized at a higher rate than the open-grown trees, although trees in both treatments became light-saturated at the same intensity. The light response curve for lodgepole pine did not differ significantly with shading (Ronco 1970a). In addition, shade during the growing season had no effect on lodgepole pine but lowered mortality of Engelmann spruce. In another study, branches of shaded Engelmann spruce that grew beyond a protective shingle became chlorotic once exposed to direct sunlight (Ronco 1970a). The bleaching of the needle pigments suggests photooxidation.

However, several observations suggest that solarization is not the cause of the mortality commonly observed in open grown Engelmann spruce seedlings. First, solarization is not a problem over the entire range of Engelmann spruce, but is mainly a problem in Engelmann spruce-subalpine fir (*Abies lasiocarpa* (Hook.) Nutt.) stands in the central and southern Rocky Mountains (Ronco 1975). Second, potted seedlings of Engelmann spruce become chlorotic at lower elevations where the intensity of light is lower (Ronco 1970b). Third, in a study comparing seedling survival on northern versus southern aspects, few seedlings died from solarization on either aspect but instead succumbed to drought (although no description is given as to how cause of death was determined) (Noble and Alexander 1977; Alexander 1984; 1987). Ronco (1972) acknowledged that decreased soil moisture, an environmental factor confounded with high light, may play a role in Engelmann spruce solarization. However in his experiments he was unable to detect differences in water deficits between shaded and unshaded trees. Shade alters a broad array of growth conditions in addition to improved soil water status, such as lower air and soil temperatures, lower vapor pressure deficits and lower evapotranspiration (Noble and Alexander 1977; Alexander 1984; 1987). Hence, the actual mechanism by which shade increases the survival of Engelmann spruce has not been identified and the interaction of water stress and light has never been tested.

This paper reports the results of a study of the causes of solarization of Engelmann spruce through a common garden experiment with Engelmann spruce, blue spruce (*Picea pungens* Engelm.) and their F_1 hybrid (*Picea engelmannii* X *P. pungens*) above Scotch Creek in southwestern Colorado. In this drainage, Engelmann spruce exists in large pure stands at high elevations (2550+ m) just above and only occasionally contacting the relatively small, island populations of blue spruce. Blue spruce was selected for this study due to its ability to tolerate high temperature and water stress, (two environmental factors confounded with high light intensity) (Jones and Bernard 1977; Fechner 1990) and its ability to hybridize with Engelmann spruce (Ernst et al. 1990). Photoinhibition can result from either 1) exposure to high photosynthetic photon flux densities without any additional stress, 2) an interaction between light and other environmental stress factors (e.g., low temperature, low soil moisture) (Powles 1984) or 3) these other environmental stress factors alone. We sought to test whether solarization always occurs in Engelmann spruce seedlings when exposed to high PPFD. If solarization is the cause of a decreased photosynthetic rate in Engelmann spruce, then the hybrids would have a photosynthetic rate intermediate between blue spruce and Engelmann spruce. By planting the three spruce taxa in the southern part of Engelmann spruce range and at an elevation of 3200 m, it ensured foliage exposure to the light levels reported to result in the solarization response. Light intensities at 3000 meters may climb up to 50% above light intensities on cloudless days at low elevations (Tranquillini 1979).

Materials and methods

Five-year old plants from forty seedlots from a partial diallel mating design done by Ernst et al. (1990) were hand-planted in the San Juan National Forest at an elevation of 3200 m. The plantation is located above Scotch Creek, a drainage that empties into the Dolores River *ca.* 5 km south of Rico, CO, along state highway 145. Elevation in the drainage runs from greater than 3050 m at the upper end to 2590 m where Scotch Creek empties into the Dolores River. For a more detailed description of the Scotch Creek drainage, see Schaefer and Hanover (1985, 1990).

The experimental design was a randomized complete block. Each of four blocks contained four blue spruce, four Engelmann spruce and two Engelmann X blue spruce hybrids (2 X 2 m spacing). Taxa were not replicated equally due to the small number of hybrid plants.

Seeds were sown in August of 1984 and grown using accelerated optimal growth (Hanover et al. 1976) from 1984 until spring of 1985, when they were moved into a shadehouse at Michigan State University's Tree Research Center, Ingham County, MI, USA. Trees remained in the shadehouse until they were planted on September 7, 1989.

The five year old trees were planted on a cutover Engelmann spruce site that had a 60% slope, northeastern aspect and deep, sandy loam soil. The site contained debris, (that covered the lowermost branches of the planted trees) including charred logs, from a prescribed burns that occurred in 1979 and 1980 (Robert Vermillion, United States Forest Service-Dolores Ranger District, personal communication). No site preparation treatments other than fire were used.

Growth measurements included length of current year's growth (determined by measuring the leader in cm) and total height in m. Measurements were made in early September of 1990, 1991, 1994 and 1995.

Gas exchange measurements were made on clear mornings (09:00-12:00) with a LiCor 6200 portable photosynthetic analyzer (LiCor Inc., Lincoln NE) using the one liter leaf chamber, the default K constant and 15 second measurement span (1 measurement per second). Gas exchange was measured at ambient CO_2 concentrations on September 07, 1990 and September 02, 1991 when the trees were six and seven years old, respectively. Two measurements were made per individual on current year's growth of an east-facing, uppermost lateral branch. Twigs were prepared by removing needles at the twig area over which the leaf chamber would clamp. Twigs were detached, kept on ice and returned to Michigan for leaf area determination. Leaf areas were calculated using the methods of Seiler and Cazell (1990).

After leaf areas were determined, needles were removed for chlorophyll analysis. Chlorophyll was extracted from current year's needles for 7 hours in dimethyl-sulfoxide according to the methodologies of Hiscox and Israelstam (1979) and absorbance was analyzed using a Perkin-Elmer Lambda 4B uv-vis spectrophotometer. Chlorophyll amount was calculated using the equations of Arnon (1949).

Chlorophyll fluorescence (variable fluorescence to maximal fluorescence ratio or F_v/F_m) was measured in the field using a Morgan CF-1000 chlorophyll fluorescence meter at the following settings: 1,000 μmols of quanta/m^2*s and a 60 s measurement period. Eight to ten needles were enclosed in the dark-acclimation cuvette for 15-20 minutes before measuring.

Gas exchange parameters were also collected between 10:00 and 12:00 on July 14, 1994 from rangewide representatives (see Table 5) of both blue and Engelmann spruce in a provenance-progeny test established at Michigan State University's Kellogg Experimental Forest, Kalamazoo County, Michigan in 1970 and 1971. Seven individuals of each taxa were measured twice using methodology and instrumentation identical to that used in Colorado.

Data collected were analyzed using SYSTAT (Systat, Inc., Evanston, IL) to assess whether the assumptions of ANOVA were met. None of the variables tested significant for the Bartlett's test for homogeneity of variances. All data were then statistically analyzed using ANOVA (and LSD for current year's growth) and the general linear model procedure of SAS (SAS Institute Inc., Cary, N.C.).

Results

Mean plant height per species at the time of planting is reported in Table 1 and was not significantly different between species (F=2.08, P=0.141), $\alpha = .05$). There was no mortality in the Colorado plantation during 1990 and 1991, however two Engelmann spruce experienced severe tip dieback i.e., only the lower branches of these trees had foliage. Data were not collected from those two individuals. The cause of the dieback remains undetermined. None of the blue spruce or hybrid spruce showed these symptoms. The first sign of mortality was observed in 1995, when one of the Engelmann spruce showing the dieback symptoms died.

There were no significant differences ($\alpha = .05$) among taxa for length of current year's growth (Table 2), although there were differences between 1990 and the other three years. Length of current year's growth in 1990 was about twice as much as growth in 1991, 1994 and 1995. Differences in tree height (Table 3) among blue spruce, Engelmann spruce and the Engelmann X blue spruce hybrid were not statistically significant ($\alpha = .05$) in any of the four years measured. Tree height averaged 0.61 m (in 1990) and 0.67 m (in 1991) for Engelmann spruce. Tree heights are similar to other reports for 5-8 year old planted Engelmann spruce in Utah (0.51-0.61 m) (Alexander and Shepperd 1990). Growing in the southern part of its range, blue spruce averaged between 0.48-0.59 m after five growing seasons (Jones 1975). Five year old blue spruce height in the current study averaged 0.61 m.

Gas exchange measurements (Table 4) also failed to show significant differences among the three taxa ($\alpha = .05$). Average photosynthetic rates ranged from 3.7 μmols

TABLE 1: Mean values and standard errors for height of blue spruce (*Picea pungens* Engelm.), Engelmann spruce (*Picea engelmannii* Parry ex Engelm.) and the artificial hybrid, Engelmann X blue spruce at time of planting in 1989

	Mean Height (M)	**Standard error**
Blue spruce (N = 16)	0.58	.03
Engelmann spruce (N = 14)	0.48	.03
Engelmann X blue spruce (N = 8)	0.51	.04

TABLE 2: Mean values and standard errors for length of current year's growth of blue spruce (*Picea pungens* Engelm.), Engelmann spruce (*Picea engelmannii* Parry ex Engelm.) and the artificial hybrid, Engelmann X blue spruce in 1990, 1991, 1994 and 1995

	1990	1991	1994	1995
		BLUE (n = 16)		
Length of current year's growth (cm)	14.0	6.1	6.2	7.5
Standard error	0.8	0.9	0.8	0.9
		ENGELMANN (n = 14)		
Length of current year's growth (cm)	14.7	7.2	5.8	6.7
Standard error	0.9	0.7	0.7	0.9
		E X B HYBRIDS (N = 8)		
Length of current year's growth (cm)	17.4	6.2	5.6	5.7
Standard error	1.7	0.8	1.1	1.4
		F values		
	1.8	0.8	0.2	0.7
		$P > F_{.05}$		
	.18	.45	.85	.51

TABLE 3: Mean values and standard errors for height of blue spruce (*Picea pungens* Engelm.), Engelmann spruce (*Picea engelmannii* Parry ex Engelm.) and the artificial hybrid, Engelmann X blue spruce in 1990, 1991, 1994 and 1995

	1990	**1991**	**1994**	**1995**
		BLUE **(n = 16)**		
Total height (M)	0.61	0.66	0.90	0.97
Standard error	.02	.03	.05	.04
		ENGELMANN **(n = 14)**		
Total height (M)	0.61	0.67	0.79	0.92
Standard error	.03	.04	.05	.05
		E X B HYBRIDS **(N = 8)**		
Total height (M)	0.70	0.76	0.90	0.97
Standard error	.05	.06	.07	.07
		F values		
	0.88	0.70	1.44	0.32
		$P > F_{.05}$		
	.43	.50	.254	.728

TABLE 4: Mean values and standard errors (in parentheses) of gas exchange and total chlorophyll of blue spruce (*Picea pungens* Engelm.), Engelmann spruce (*Picea engelmannii* Parry ex Engelm.) and the artificial hybrid, Engelmann X blue spruce, in 1990 and 1991

	BLUE (n=16)		ENGELM (n=14)		E X B (n=8)		F value Pr > $F_{.05}$	F value Pr > $F_{.05}$
	1990	**1991**	**1990**	**1991**	**1990**	**1991**	**1990**	**1991**
Photosynthesis (mmol CO_2*$m^{-2}s^{-1}$)	3.7	3.7	5.0	4.7	4.5	4.4	1.4	1.3
Standard error	(.5)	(.4)	(.4)	(.4)	(.3)	(.8)	.27	.29
Stomatal conductance (mmol *$m^{-2}s^{-1}$)	0.17	0.09	0.20	0.09	0.15	0.08	0.78	0.63
Standard error	(.04)	(.01)	(.04)	(.01)	(.03)	(.01)	.47	.54
Transpiration (mmol H_2O*$m^{-2}s^{-1}$)	3.3	2.9	4.0	3.1	4.4	2.9	1.1	0.5
Standard error	(.4)	(.2)	(.4)	(.3)	(.6)	(.3)	.35	.62
Total chlorophyll (mg chl/g fr wt)	0.9	0.9	1.0	1.0	1.0	0.9	1.2	1.3
Standard error	(.07)	(.05)	(.05)	(.07)	(.2)	(.1)	.30	.29

$CO_2/m^{-2}s^{-1}$ (1990) for blue spruce to 5.0 µmols $CO_2/m^{-2}s^{-1}$ (1990) for Engelmann spruce. Average rates for stomatal conductance were significantly different (α = .05) between 1990 and 1991.

Total chlorophyll content was not significantly different among blue spruce, Engelmann spruce and the hybrid spruce (α = .05) (Table 4). The needles tested in 1990 (one year after planting) did not have a significantly different level of chlorophyll from needles taken in 1991.

F_v/F_m ratios ranged from 0.76 for the hybrid to 0.78 for Engelmann spruce (Table 5) and were not significantly different among taxa (α = .05). The overall magnitude of those values does not suggest damage to photosystem II; for a normal, healthy leaf the ratio should be near 0.8 (Bjorkman and Demmig 1987).

Because all of the trees in the Colorado plantation were derived from parents found in the Dolores River drainage, gas exchange measurements of other seed sources were undertaken to eliminate geographic location as a possible explanation for the homogeneity of results. Table 5 lists the seed sources and elevations for the blue and Engelmann spruce measured at Kellogg Experimental Forest, MI, USA. Table 7 shows gas exchange results from measurements taken from these trees. There were also no significant differences between blue and Engelmann spruce for any of these gas exchange measurements (α = .05).

Discussion

Despite growing at 3200 m elevation in southwest Colorado, none of the trees in the plantation died until year six (post-planting) when one Engelmann spruce (out of

TABLE 5: Mean values and standard errors (in parentheses) of variable fluorescence to maximal fluorescence ratios (F_v/F_m) of blue spruce (*Picea pungens* Engelm.), Engelmann spruce (*Picea engelmannii* Parry ex Engelm.) and the Engelmann X blue spruce hybrid in 1994.

	Blue (n = 16)	**Engel (n = 14)**	**E X B (n = 8)**	**F val** **$P > F_{.05}$**
F_v/F_m	0.76	0.78	0.76	2.54
Standard error	(.01)	(.01)	(.01)	0.10

TABLE 6: Seedlots measured from blue spruce/Engelmann spruce provenance-progeny test (compartments 29/30) at Kellogg Experimental Forest, MI, USA, and their corresponding elevations.

Accession	National Forest Seed source	State	Elevation (m)
Engelmann spruce			
3246	Roosevelt	Colorado	2700
3250	Sante Fe	New Mexico	2900
3252	Coconino	Arizona	2900
3253	Payette	Idaho	1900
3256	Dixie	Utah	3000
3259	Wenatchee	Washington	2500
3630	Gila	New Mexico	2500
Blue spruce			
8001	Fishlake	Utah	2700
8051	White River	Colorado	2700
8069	Manti-La Sal	Utah	2300
8081	Bridger-Teton	Wyoming	1800
8163	San Juan	Colorado	2500
8181	White River	Colorado	2400
8235	Carson	New Mexico	2600
8248	White River	Colorado	2500

TABLE 7: Mean values and standard errors (in parentheses) of gas exchange rates of blue spruce (*Picea pungens* Engelm.) and Engelmann spruce (*Picea engelmannii* Parry ex Engelm.) from random seed sources from a provenance-progeny test at Kellogg Experimental Forest, MI, USA

	BLUE	**ENGELMANN**	**F value**
	(n = 7)	**(n = 7)**	**Pr > $F_{.05}$**
Photosynthesis	5.2	4.2	0.88
(mmol CO_2*$m^{-2}s^{-1}$)	(0.7)	(0.8)	0.37
Stomatal conductance	0.18	0.18	0.99
(mmol *$m^{-2}s^{-1}$)	(0.40)	(0.02)	0.00
Transpiration	3.20	3.50	0.23
(mmol H_2O*$m^{-2}s^{-1}$)	(0.55)	(0.32)	0.64

sixteen) died of unknown causes. One year after planting, this individual lost all needles on its uppermost remaining branches; however needles on the lower branches were still living until 1995. The 39 trees in the plantation not only survived, but showed no sign of photoinhibition. All three species performed equally well at this site as indicated by survival, growth and physiological data. Tree age may have also factored into the low mortality seen in Engelmann spruce in this study. Alexander (1984) found that Engelmann spruce seedlings that survived through the fifth growing season on either north or south aspects generally were still alive at year ten. The trees in the current study were planted at age five.

Length of current year's growth (Table 2) was not different among taxa; however, growth in 1990 was significantly different than in the three other years (1991, 1994, 1995). Shoot length in spruce may be determined primarily by 1) the amount of stored metabolites that are available at bud-set (Bongarten 1986) and 2) temperature during bud formation (Deal et al. 1990; Junttila and Nilson 1993). Because spruce exhibit fixed or determinate growth, buds that emerged in 1990 were set in the season prior to transplant, while the trees were in a Michigan shadehouse. Favorable conditions in the controlled environment of the shadehouse may have caused more metabolites to be allocated to shoot primordia. Warmer temperatures could have resulted in increased mitosis, thus increased stem unit formation, resulting in larger buds and subsequent 1990 growth.

That there were no growth differences among taxa is of particular interest when considering Rehfeldt's (1994) common garden and greenhouse study of Engelmann and blue spruce. In comparison to 16 provenances of Engelmann spruce, Rehfeldt (1994) found that Engelmann spruce populations from the San Juan Mountains exhibited growth characteristics similar to blue spruce. In the greenhouse, populations from the San Juan Mountains exhibited indeterminate growth following determinant growth (Rehfeldt 1994), a phenomenon documented in blue spruce (Hanover et al. 1976, Bongarten 1986). Whether Engelmann spruce from this geographic location has more growth potential in the field relative to Engelmann spruce from other provenances should be tested further. All of the trees in our common garden were derived from controlled crosses using parental trees from one drainage in the San Juan Mountains and there were no significant differences among Engelmann, blue and their hybrid for any growth or physiological parameter measured.

David (1996) has found natural hybrids between Engelmann and blue spruce in the Scotch Creek drainage and has molecular evidence to suggest introgression, thus phenological similarities in flowering exist between blue and Engelmann spruce which permit gene flow, however viable seed is only produced if Engelmann spruce is the female parent (Ernst et al. 1990). Because chloroplast DNA (cpDNA) is inherited paternally in the Pinaceae, all of the E X B hybrids contain the cpDNA of blue spruce. Our results demonstrate physiological and growth similarities as well between the two species and show that, at least at an early age, hybrids are viable in the natural environment. However, physiological similarities between blue and Engelmann are not restricted to trees from the Scotch Creek drainage. Gas exchange rates of blue and Engelmann spruce from various geographic areas (Tables 6 and 7) were measured at a provenance-progeny test at Kellogg Experimental Forest and gas exchange values were of the same magnitude as those seen in the Colorado common garden and were not significantly different between blue and Engelmann spruce. These results contrast with some other spruce species. Manley and Ledig (1979) were able to use photosynthetic rate as a segregating trait for red and black spruce hybrids and their backcrosses.

In Scotch Creek, as in many areas sympatric between blue and Engelmann spruce, blue spruce is found at lower elevations relative to Engelmann spruce. As such, both taxa exhibit physiological adaptations related to their elevational distribution. The location of the common garden was in Engelmann spruce habitat, outside of blue spruce's elevational range. Planting blue spruce at 3200 m elevation subjected it to a shorter growing season (fewer frost free days). The data in this study suggest that blue spruce was able to adapt and that blue spruce dormancy is strongly regulated by environment and is physiologically plastic in traits relating to dormancy induction. Similarly, Jones (1975) found that three-year old blue spruce seemed well-adapted to full sun on a clear-cut site at 2,760 meters in the Apache National Forest, Arizona; however, it fared much better on northern slopes than on southern slopes because of more available soil moisture. It is worth noting the silvicultural significance of blue spruce's acclimation to our high elevation common garden. Whatever environmental factors preclude blue spruce from inhabiting the subalpine zone in nature have not affected its viability when planted in the area of this study. It may be plausible to include blue spruce in high elevation reforestation plans on some sites.

It is not known why no solarization effects were observed on Engelmann spruce, in this study even though Ronco (1972) observed this phenomenon. However, it is difficult to separate a number of confounding factors when considering the consequences of high light intensity on a tree seedling. Ronco (1970a) witnessed solarization on three-year-old Engelmann spruce seedlings that was especially pronounced on southern-exposed slopes; seedlings provided with shade had higher photosynthetic and survival rates. Ronco (1970a, 1975) concluded that shade provision was necessary for Engelmann spruce survival. Alexander's experiments (1984) showed that shade was important, on both north and south aspects, at reducing temperature, which resulted in increased soil moisture and reduced vapor pressure deficit. Because our unshaded trees at 3200 m elevation remained healthy, it is likely that survival of exposed Engelmann spruce seedlings depends on a balance of temperature, moisture and light thresholds. Light of high irradiance without stressful levels of these predisposing factors does not always result in solarization. The unshaded trees in this study did not show symptoms of solarization; there was no reduction in gas exchange or chlorophyll amount in Engelmann spruce, blue spruce or their hybrid. Equally important, F_v/F_m ratios, a diagnostic variable for photoinhibition (Ögren 1991), were not significantly different and were of high enough magnitude to indicate optimal photochemical efficiency.

The common garden was located in the southern part of the Engelmann spruce range and photosynthetic photon flux densities typically measured 2400 mmols of quanta/m^2*s on clear days, two factors that should have increased the likelihood of the solarization response, yet, no symptoms were seen. Unless tree age is a factor (Alexander 1984), our results suggest that solarization in Engelmann spruce, as described by Ronco (1972), is not caused solely from exposure to a high photosynthetic flux density, but instead is induced by an interaction between high photon flux densities and other environmental stress factors that are confounded with high light (e.g., Moisture deficits, high temperature and high vapor pressure deficits). Further work will investigate water use properties and light response to determine the thresholds that might predispose Engelmann spruce to solarize.

Acknowledgments

The common-garden experiment at 3200 m in the San Juan National Forest was designed and planted by the late Dr. James W. Hanover who likewise made the arrangements with the USDA National Forest Service in Dolores, CO for its implementation. Many thanks to 1) Paul Bloese for statistical advice, 2) Dr. Andrew David for suggestions and assistance in data collection and 3) Dr. James Flore for the use of his fluorescence meter.

Chapter Two: The effect of decreased soil moisture on survival of Engelmann spruce

Abstract

Physiologic response to soil moisture deficits was examined in Engelmann spruce and blue spruce to determine the effect of decreased soil moisture on survival. Forty-eight, three-year-old, trees from southwestern Colorado provenances were arranged in a split-plot design, in the greenhouse, using species as the main factor and water level (well-watered control, 25% of control and no water) as the sub-factor. The 25% water and the no water treatments resulted in higher mortality, lower stem water potential, lower root biomass, and lower rates of photosynthesis for Engelmann spruce in comparison to blue spruce. Increased survival, water potential and rates of photosynthesis in blue spruce paralleled increased root mass at the experiment's end. The lesser ability of Engelmann spruce to survive the low water treatments could mean that drought stress, a confounding factor of high light, may be involved in the solarization response previously reported for this species.

Introduction

Protective adaptations dealing with photosynthetic acclimation to water deficits are beginning to be understood in spruce. In interior spruce (*Picea glauca* (Moench) Voss X *Picea engelmannii* Parry ex. Engelm.), water deficits led to a decrease in photochemical efficiency in photosystem II before a reduction in gas exchange was observed, suggesting that down-regulation of electron transport is a drought response (Eastmann and Camm 1995). In white spruce (*Picea glauca* (Moench) Voss), inactivation of the oxygen-evolving complex is a protective mechanism for tolerance of the combination of water stress and high light intensity (Toivonen and Vidaver 1988); inactivating the oxygen evolving complex results in fewer toxic oxygen species being formed. Removing toxic oxygen species via anti-oxidant enzymes is another photoprotective mechanism, important during times of water deficit (Gillies and Vidaver 1990). In red spruce (*Picea rubens* Sarg.) needles, superoxide dismutase (SOD) has been extracted (Tandy et al. 1989). SOD is the cell's primary defense against damage by O_2^-. SOD and other anti-oxidant enzymes could be more prevalent in species of spruce that tolerate extremes in light and water.

Susceptibility to solarization has been suggested as the cause of mortality in regeneration of Engelmann spruce on exposed, high elevation sites (Ronco 1970). Solarization is the failure of leaves to produce starch due to the direct inhibitory effect of excess light energy on photosynthesis (photoinhibition) (Holman 1930). Others (Noble and Alexander 1977; Alexander 1984; 1987) suggest temperature and water stress as a more probable cause of Engelmann spruce mortality on these sites. Planting and seedling guidelines for successful Engelmann spruce regeneration (Ronco 1970, 1972, 1975) recommend planting or seeding in shaded spots. Shade alters a broad array of growth conditions such as higher soil water status, moderated air and soil temperatures, and lower vapor pressure deficits and evapotranspiration rates (Noble and Alexander 1977; Alexander 1984; 1987). Partial stomatal closure in the shade could be partially responsible for reduced mortality of shaded seedlings compared to those transplanted in clearcuts with no protection (Kaufmann 1976). The actual mechanism by which shade increases the survival of Engelmann spruce has not been identified and the interaction of water and light has never been tested.

Previous work (Chapter 1) suggested that solarization is induced by an interaction between high light intensity and other environmental stresses confounded with high light (e.g., moisture deficits, high temperature, high vapor pressure deficits). This conclusion was based on the results of a common garden experiment using Engelmann spruce, blue spruce (*P. pungens*) and their hybrid planted at 3200 m elevation, which subjected the trees to high photosynthetic photon flux densities, but did not result in any detectable physiological differences. Given that exposure to the high light conditions of a high elevation environment did not cause solarization or a differing physiological response between blue and Engelmann spruce, the relative effects of soil moisture status on the survival of blue and Engelmann spruce were tested, with the aim of determining whether water deficits could be a confounding factor involved in mortality associated with solarization.

Materials and Methods

Forty-eight, three-year old spruce trees were arranged as four blocks in a split-plot design using species (blue or Engelmann) as the main factor and water treatment as

a sub-factor. The watering treatments were based on spruce daily water usage. Water usage of ten blue spruce and ten Engelmann spruce was determined by watering once and weighing daily through two, three-day drying cycles. The average daily water use, designated the control, was measured to be 80 ml per seedling. One quarter of that amount, 20 ml, was designated the 25% water treatment. The third water treatment consisted of no water. The control and the 25% water groups were watered daily from August 10, 1993-November 11, 1993.

Trees were propagated from seed of southwestern Colorado provenances and were grown in five gallon pots containing three parts peat moss to one part vermiculite. Temperature and relative humidity were not regulated; photoperiod was set at 18 hours light, 6 hours dark.

To ensure watering treatments reached the roots, each five gallon pot contained a one-inch diameter pvc irrigation tube, through which watering took place. Six quarter-inch holes were drilled into the base of each watering tube, which was then inserted into the soil at a 45 degree angle from the base of the pot.

Average soil moisture for the vicinity of the watering tube was determined weekly from weeks one through eleven using time domain reflectometry (TDR) (Tektronix TDR 1053C) (Topp and Davis 1985; Baker and Allmaras 1990) and 30 cm by .6 cm diameter stainless steel rods. Two rods per pot were inserted parallel to each other at 10 cm apart.

Tree mortality was determined using three factors: 1) needles would abscise upon touching, 2) needle color had a gray caste and 3) no gas exchange took place.

Height was measured to the nearest 0.1 cm at the beginning of the experiment after the trees had set a terminal bud.

Gas exchange parameters were measured weekly from with a LiCor 6200 portable photosynthetic analyzer (LiCor Inc., Lincoln, NE) using the one liter leaf chamber, the default K constant and 15 second measurement span (1 measurement per second). Gas exchange was measured at greenhouse CO_2 concentrations (between 700-800 ppm). One measurement was made per individual on the current year's needles on an east-facing, uppermost lateral branch. The experiment began after the buds had fully expanded thus there was no additional foliar growth through the duration of the experiment. Twigs were prepared by removing needles from the area over which the leaf chamber would clamp. Twigs were detached at the end of the experiment and brought into the lab for leaf area determination using the methods of Seiler and Cazell (1990). In data analysis, a value of zero was entered for gas exchange parameters upon the tree's death to indicate that physiologically, no gas exchange was taking place.

Stem predawn water potential was measured (destructively) once every week at 0400 hours using a PMS pressure chamber (PMS Instrument Co., Corvallis, Oregon, USA). Due to destructive sampling, only one block of 12 trees was measured each week. The last three measuring dates were dropped from the analysis because on these dates the selected block contained dead trees.

Roots of all trees were harvested, oven-dried (for ten days) and weighed for root biomass determination. Measurement occurred either at the end of the experiment or when the tree died, depending on its treatment group assignment.

Stem diameter at the end of the experiment was measured at ten centimeters from the soil using a digital caliper. To calculate stem volume, stem diameter was squared and multiplied by the tree height. Because spruce exhibit determinate growth, growth does not occur after a terminal bud is set. These trees had set a terminal bud before the experiment began.

Statistical analysis was done using Multivariate General Linear Hypothesis ANOVA and MANOVA programs of Systat (Evanston, IL). Root dry weight and stem volume were analyzed using ANOVA. Pre-dawn water potential was analyzed using repeated measures ANOVA. Fisher's test for least significant difference was used to detect differences among treatment combinations. Gas exchange data were analyzed using the fully factorial MANOVA procedure. For all analyses, a 10% probability level was used as the test criterion for significant differences.

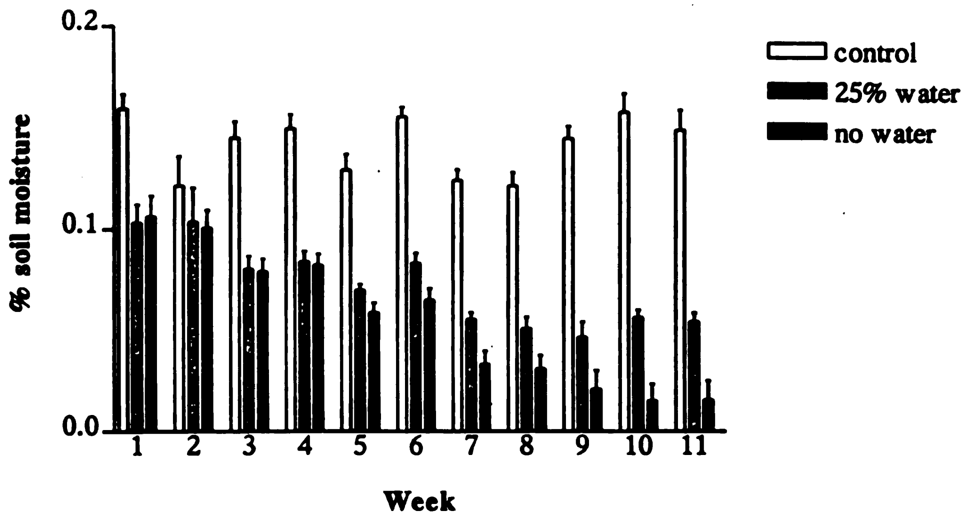
Results

Initial tree height: The blue spruce trees used in this experiment averaged 0.68 m (standard error = 0.02) in height and the Engelmann spruce averaged .55 m (standard error = 0.02). These means are statistically significant at $\mu = .05$ (F = 2.73 P = 0.261).

Soil moisture: The three water treatments, (control, 25%, 0) did not immediately correlate to relative percent soil water (Figure 1), but by week six differences were well established.

Soil moisture for the control groups of both species fluctuated between 11-16%. The soil moisture data were not analyzed statistically, but are included as indication for

Engelmann spruce



Blue spruce

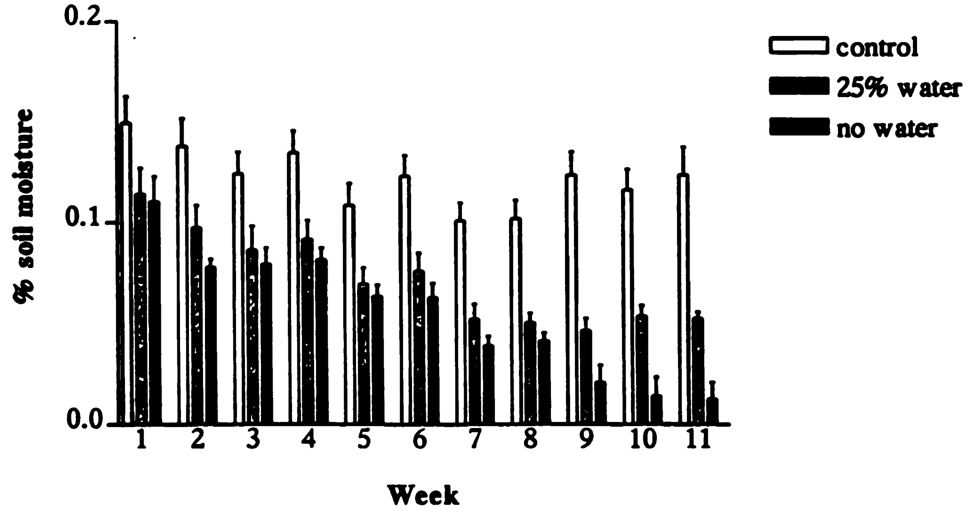
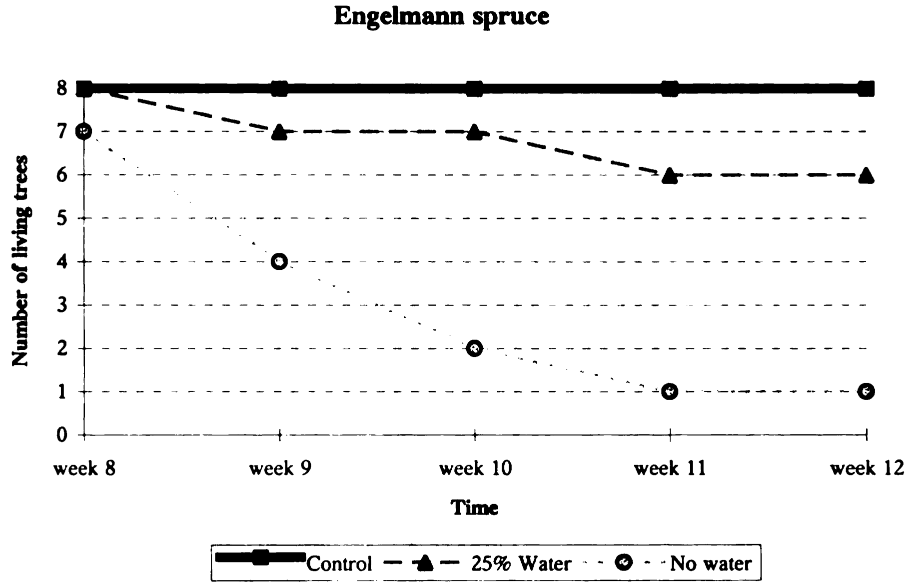


Figure 1. Soil moisture percentages of pots containing Engelmann spruce and blue spruce over twelve weeks at three water levels (control, 25% water and no water)



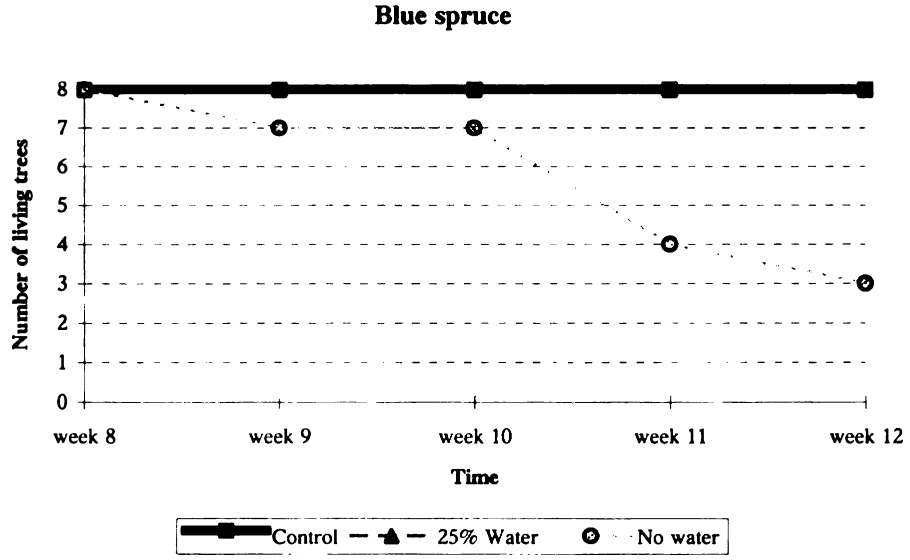


Figure 2. Mortality of Engelmann spruce and blue spruce during weeks 8-12 of water stress treatments (control, 25% water and no water)

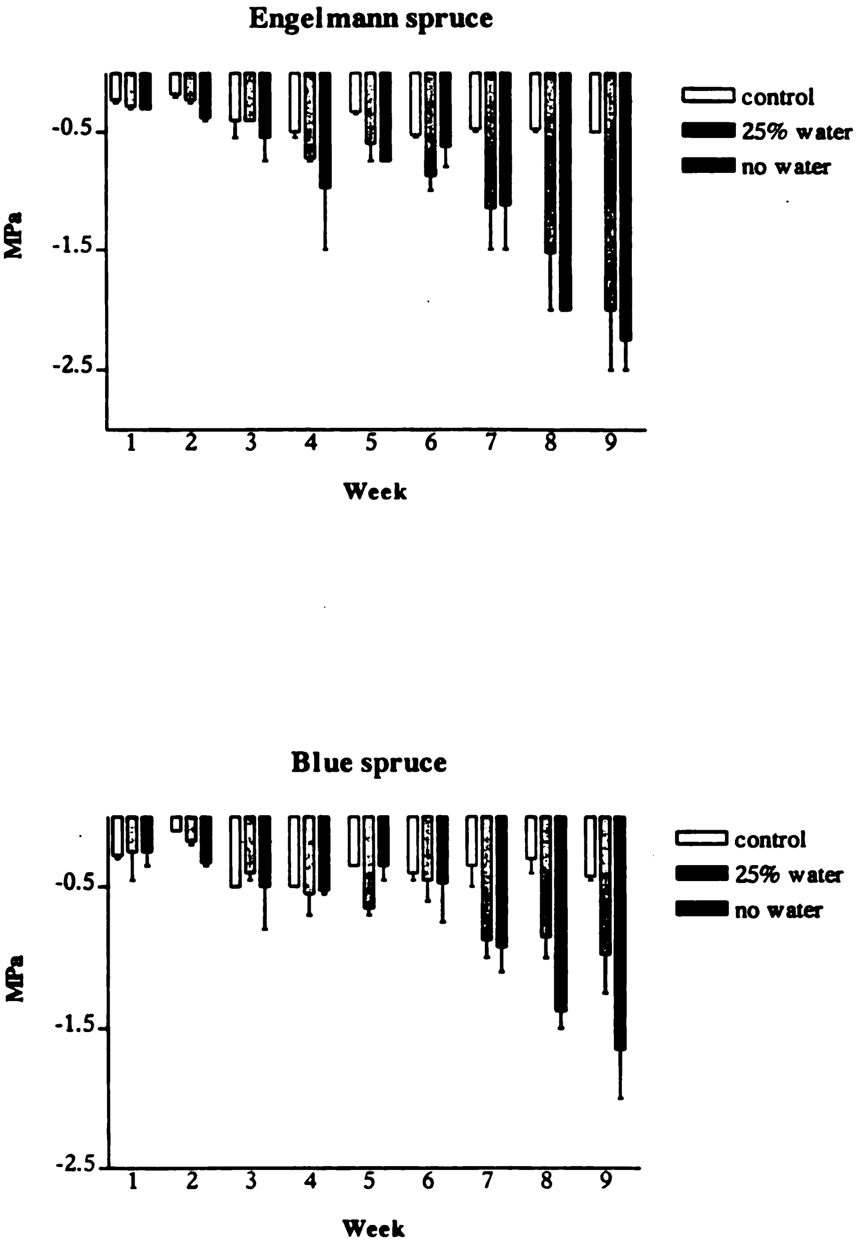


Figure 3. Pre-dawn water potential of Engelmann spruce and blue spruce over twelve weeks at three levels of water treatment (control, 25% water and no water)

whether the water treatments correlated with percent soil moisture levels. For each treatment there was a substantial difference between Engelmann and blue spruce.

Mortality: No trees died in either blue or Engelmann spruce control group. The first tree to die occurred among the Engelmann spruce in the “no water” treatment (Figure 2) during week 8. Three additional trees died the following week resulting in 50% (4 dead/4living) mortality by week 9. At the experiment's end, only one tree in this treatment combination was alive. In contrast, the “blue spruce-no water” treatment experienced 38% mortality by the end of the experiment.

All of the trees in the "blue spruce-25% water" treatment were alive at the end of the experiment. However, trees in the “Engelmann spruce 25% water” treatment experienced a 25% mortality. Not only did more Engelmann spruce die overall, they also began to die first.

Pre-dawn water potential: The water level treatments resulted in water potentials (Figure 3) that differed significantly between species (F = 4.6; P = 0.08). There were no significant interactions between block, species or treatment. Mean water potentials for the trees in the two control groups were not significantly different, however mean water potentials differed between species for the 25% and no water treatment groups. Each week’s data point represents the average of two individual trees.

Blue spruce had a higher water potential than Engelmann spruce in the low water (25% and no water) treatments. The low water treatments resulted in a lower water potential, relative to the controls, by week five (Figure 3). By week 6, the water potential of the Engelmann-spruce-no water treatment group and the Engelmann spruce-25% water treatment group began to decrease and they were more negative than the blue spruce no water treatment. The blue spruce-25% treatment combination (Figure 3) began to decrease between weeks six and seven and equilibrated at about -1 MPa; notably, the water potential of trees in the blue spruce 25% treatment group began to decrease towards week seven of the experiment. Whereas, water potential of trees in the Engelmann spruce 25% water group decreased at a greater rate than Engelmann spruce from week seven onward.

Gas exchange: Photosynthetic rates (Figure 4) were significantly different between species (F= 2.27; P=.078), with blue spruce showing higher photosynthetic rates than Engelmann spruce with the exception that photosynthetic rates of trees in the "blue spruce no water" treatment generally were lower than the "Engelmann spruce no water" treatment onward from week 5. Response to the treatment combinations began to vary about six weeks into the experiment. From six weeks to the experiment's end, both control groups had the highest photosynthetic rate and the no-water treatments had the lowest photosynthetic rate. The 25% water treatment groups had photosynthetic rates intermediate to the other two water levels, except at week 12, where the Engelmann spruce low water treatments converge.

Similarly, water use efficiency (Figure 5) (photosynthetic rate/transpiration rate) differed significantly between the two species (F=2.73; P=.08), with blue spruce having higher water use efficiency than Engelmann spruce for all low water treatments during the final third of the experiment. There were no significant interactions between block, species or treatment.

Stomatal conductance (Figure 6) was not significantly different between species (F=0.52; P=0.85), but was significantly different between blocks (F=4.32, P=.005).

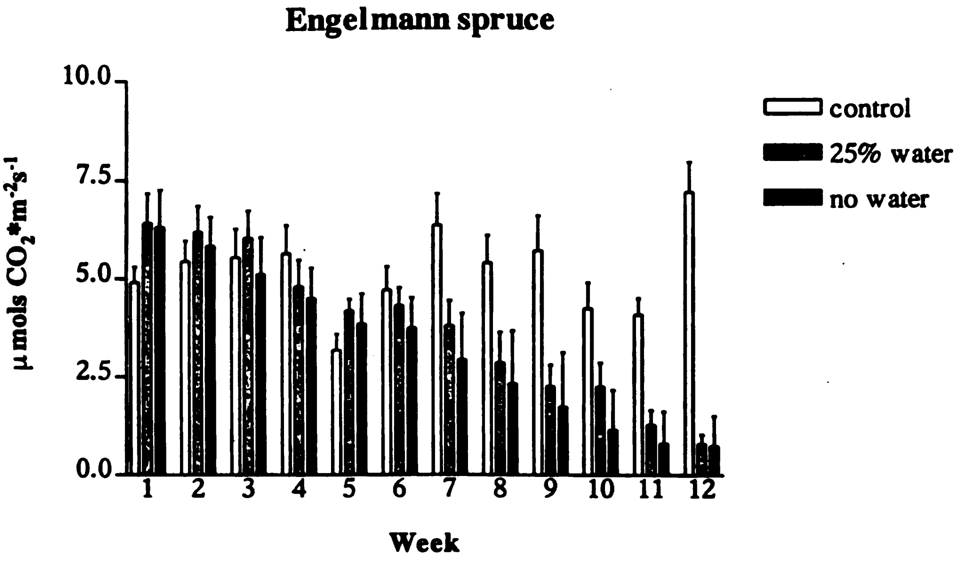
Stomatal conductance of all treatment combinations tended to decrease over the course of the experiment.

Biomass

Root dry weight: When pots were disassembled for determination of root dry weight, it was observed that the unsuberized roots in the blue spruce low water treatments tended to congregate around the watering holes. In some trees, roots actually grew into the watering tubes.

Root dry weight (Figure 7A) was significantly different between species (F = 2.8; P = .10), with blue spruce having more root biomass than Engelmann spruce in all low water treatments. Fisher's least significant difference test detected significant differences among treatments for root dry weight. The blue spruce control was not significantly different from the Engelmann spruce control, but was significantly different ($\alpha = 0.05$) from all other treatment combinations. Blue spruce 25% water treatment is not significantly different from the Engelmann spruce control or the Engelmann 25% water treatment, but was significantly different from the blue no water treatment. The blue no water treatment was not significantly different from the Engelmann spruce 25% water or the Engelmann spruce no water treatment.

Average stem volume: Stem volume (Figure 7B) was significantly different between species (F = 2.9; P = .03). Fisher's least significant difference test detected significant differences among treatments for stem volume. The blue spruce control was not significantly different than the Engelmann spruce control or the blue spruce 25%water treatment, but was significantly different ($\alpha = 0.05$) from the blue spruce no water, and both Engelmann spruce low water treatments.



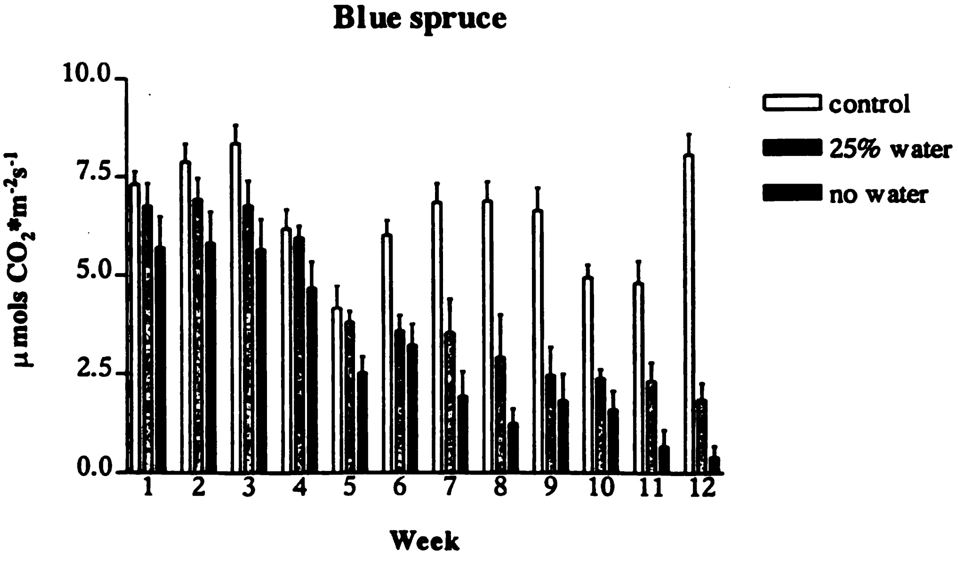
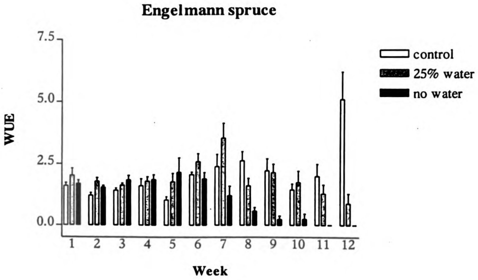


Figure 4: Photosynthetic rates of Engelmann spruce and blue spruce over twelve weeks at three water levels (control, 25% water and no water)



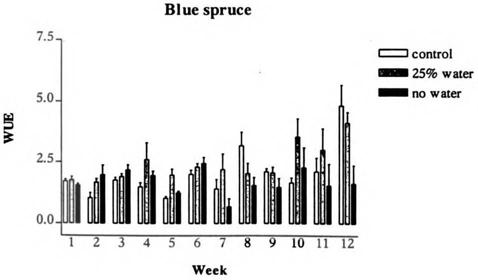


Figure 5. Water use efficiency (A/E) of Engelmann spruce and blue spruce over twelve weeks at three water levels (control, 25% water and no water)

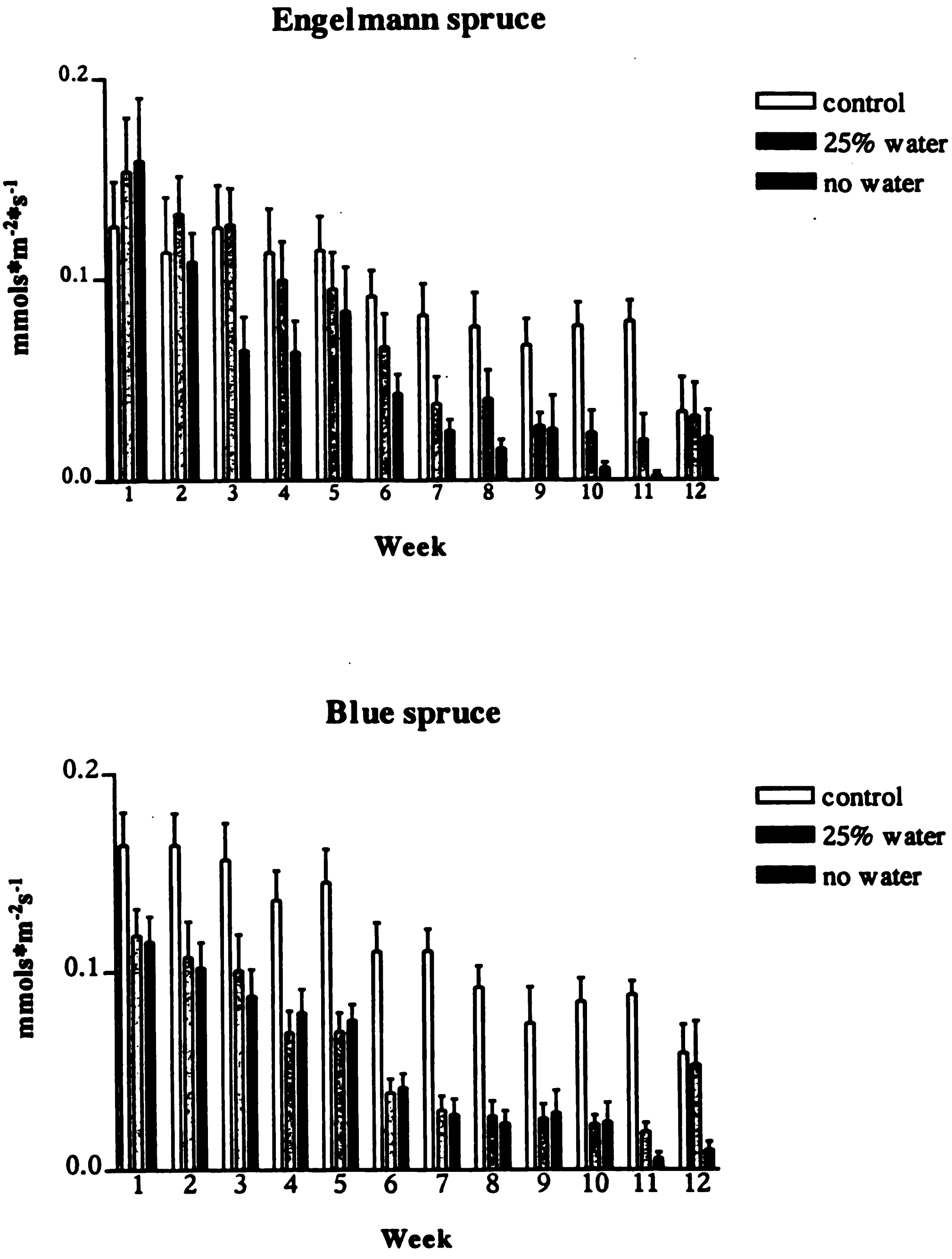


Figure 6. Stomatal conductance of Engelmann spruce and blue spruce over twelve weeks at three water levels (control, 25% water and no water)

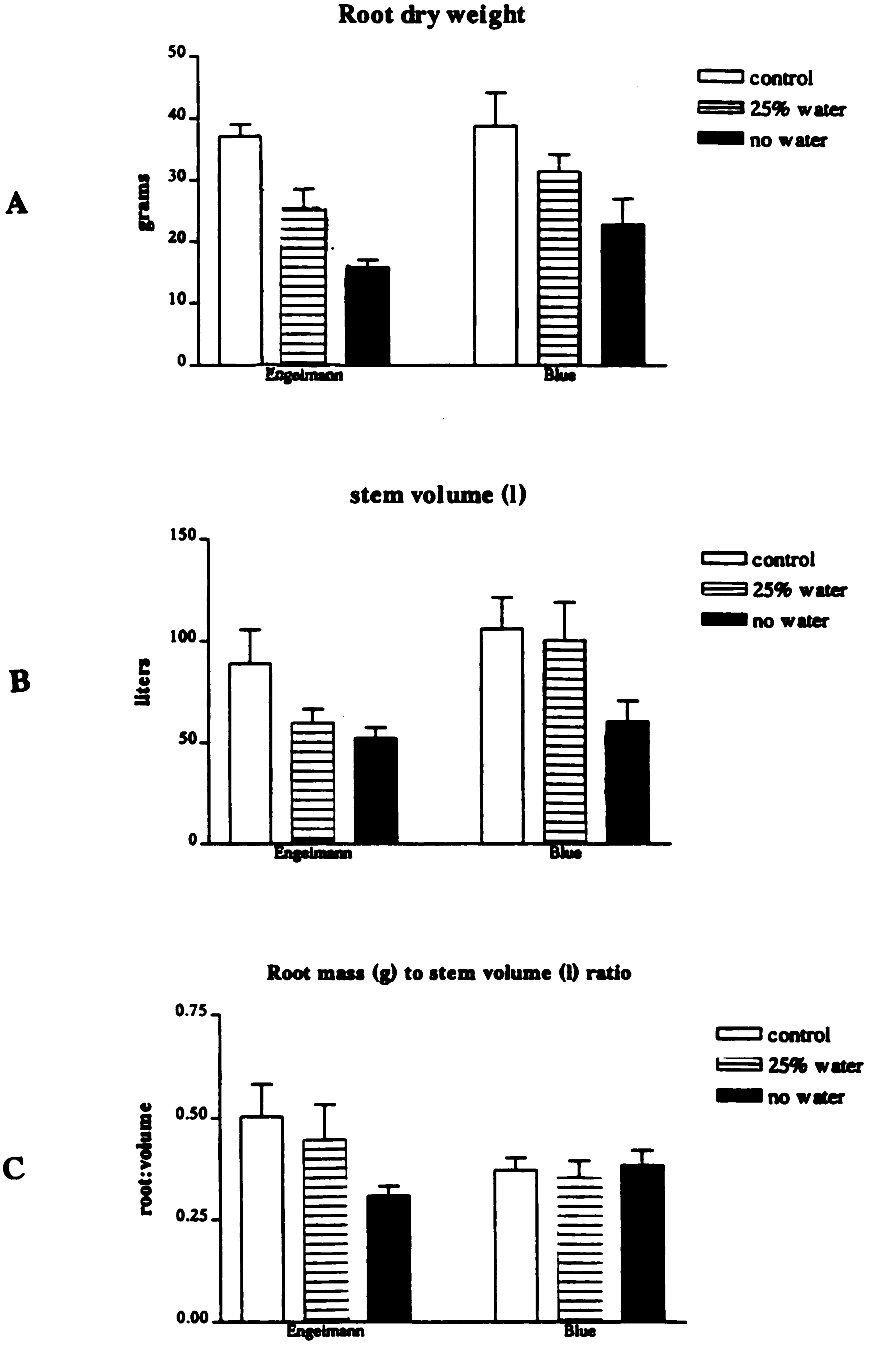


Figure 7. Biomass comparisons between Engelmann spruce and blue spruce at the end of twelve weeks at the three water levels (control, 25% water and no water). A: root dry weight (grams) B: Average stem volume (liters) C: Root mass:stem volume

The blue spruce 25% water treatment was significantly different ($\alpha = 0.05$) from the blue spruce no water, and both Engelmann spruce low water treatments. The blue spruce no water treatment was not significantly different from any of the blue spruce treatment groups.

Root mass to stem volume ratio: The root mass to stem volume ratios (Figure 7C) were not significantly different among treatment groups ($F = 1.8$; $P = 0.13$)

Discussion

Blue spruce has been observed to be more drought tolerant than Engelmann spruce (Jones and Bernard 1977); but this has never been tested under controlled conditions. The higher mortality and lower water potential (Figures 2 and 3) of Engelmann spruce suggests that Engelmann spruce lacks the physiological traits of blue spruce for surviving and adapting to low water regimes. In both 25% water treatment conditions, suberized and unsuberized roots were located predominantly around watering tubes, especially in blue spruce. And there was more total root biomass in the blue spruce pots (Figure 7A) relative to Engelmann spruce pots at each of the two low water levels. However, non-significant differences ($P = 0.13$) between treatment groups of root mass to stem volume ratios refute any hypothesis about increased carbon partitioning in blue spruce (as compared to Engelmann spruce) when subjected to drought stress, unless a Type II error occurred.

Clearly, if there is a higher root mass in a pot, there is added mass that can function in water absorption. What remains the outstanding unanswered question is whether this higher root mass was present at the beginning of the experiment.

There are two ways to interpret the results of this experiment. The first interpretation is that blue spruce had a larger root system at the outset. More root mass sustained the higher water potential for blue spruce relative to Engelmann spruce. Higher water potential means more water available for carbon fixation, thus the significant differences seen in photosynthesis, water use efficiency and ultimately, mortality.

The second interpretation is based on the probability of a Type II error occurring in the statistical analyses of the root mass to stem volume data. Visually, the graph (Figure 7C) depicts blue spruce maintaining its root:stem ratio at low water levels. In contrast, Engelmann spruce has a lower root:stem at low water treatments. It could be hypothesized that blue spruce continues root growth (and the same ratio of carbon partitioning) during water stress conditions while Engelmann spruce does not.

Future studies should address carbon partitioning changes in these two taxa in order to understand the relationship between root growth and water stress.

If blue spruce is able to maintain root growth under drought stress conditions, this could explain why its water potential and survival was higher than Engelmann spruce, and why blue spruce is more drought tolerant than Engelmann spruce.

The gas exchange data (Figure 4) support the differing levels of drought tolerance in blue and Engelmann spruce. Water use efficiency and rates of net photosynthesis were lower for Engelmann spruce than for blue spruce. Photosynthetic acclimation to water deficit is known to vary in some spruce species (Eastmann and Camm 1995). In interior spruce, (*Picea glauca* (Moench.) Voss. X *Picea engelmannii* Parry ex. Engelm.), stomatal restriction of CO_2 availability is the initial limitation on their physiological response to water stress (Seiler and Cazell 1990); they do not undergo osmotic adjustment or show photosynthetic or stomatal acclimation to water stress. Well-watered seedlings were as drought hardy as water-stressed seedlings and maintained photosynthesis at equally low water potential (Seiler and Cazell 1990).

Stomatal conductance (Figure 6) was unresponsive to the treatment combinations in this study. This may indicate a similarity with red spruce or could be a result of elevated carbon dioxide levels in the greenhouse during the study. There was a significant difference for stomatal conductance between blocks, suggesting a CO_2 gradient that ran from east to west in the greenhouse. The CO_2 generated by the propane heater used in the greenhouse from September onward was not factored into the original experimental design. CO_2 levels increased coincident with decreasing soil moisture levels (Figure 1). Hence, one month into the experiment, carbon dioxide levels were measured to be twice that of ambient which may be why the stomatal conductance of all of the treatments, including controls, decreased with time. Stomata could close, minimizing water loss, and the trees would not be limited by CO_2 because CO_2 levels were elevated. The elevated CO_2 levels minimized the trade-off between water loss and CO_2 acquisition, and should also be considered in the interpretation of the significant water use efficiency results. The significant water use efficiency result was likely due to the photosynthetic differences between treatment groups, not any difference in transpiration. Analysis of the stomatal conductance data do not suggest that Engelmann spruce is losing more water through its stomata than blue spruce. However, it could be that under field conditions, water stress would lead to stomatal limitations in Engelmann spruce; this remains to be tested.

Similarly, because of the confounding nature of the elevated CO_2 and the CO_2 gradient in this experiment, it is impossible to determine which of the gas exchange variables lends itself as the most critical indicator or threshold value for spruce demise or survival when subjected to water stress.

The marked differences in survival and physiological responses between blue and Engelmann spruce following exposure to moisture stress provides some evidence that drought stress could be a significant factor in the reported solarization of Engelmann spruce, particularly if species differences in photosynthesis, water potential and mortality are due to root mass to stem volume differences. Previous work (Chapter 1) showed that solarization in Engelmann spruce is not always caused by exposure to high photosynthetic flux densities. An interaction between high light intensity and other environmental stress factors that are confounded with high light (e.g., moisture deficits, high temperature, high vapor pressure deficits) may lead to the reported solarization response.

There is precedent for water stress being a predisposing factor to photoinhibition in plants such as *Lycopersicon* (Havaux 1992), *Solanum* (Havaux 1992), *Nerium* (Bjorkman and Powles 1984), and *Picea* (Toivonen and Vidaver 1988; Eastmann and Camm 1995). Given the higher mortality of Engelmann spruce in this study in response to exposure to drought, it is likely that a lessening of drought stress in the shade is a major factor in the increased survival of Engelmann spruce seedlings at high elevations.

Engelmann spruce may lack the photoprotective mechanisms documented in white spruce (Toivonen and Vidaver 1988; Eastmann and Camm 1995) or it has a slower rate of synthesis of SOD in comparison to red spruce (Tandy et al. 1988. Engelmann spruce seedlings probably have not evolved mechanisms to survive the combination of water stress and high light; as a shade tolerant species, naturally nursed under aspen (*Populus tremuloides* Michx.) and lodgepole pine (*Pinus contorta* Dougl. ex Loud.) it usually is not subjected to the combination of high light and water deficits. On exposed sites, its inability to tolerate the high light-low water interaction through photoprotective mechanisms or its inability to access available soil moisture, may be one mechanism through which selection acts in Engelmann spruce.

The significance of moisture level on survival of Engelmann spruce seedlings shown in this study highlights the importance of a balance of temperature, moisture and light thresholds in establishing Engelmann spruce seedlings at high elevations. Although the chemical mechanism of solarization is not yet known, water stress is likely involved as a predisposing factor. Without this predisposing factor, light of high irradiance did not result in solarization in Engelmann spruce, blue spruce or their hybrid in a common garden test in southwestern Colorado (Chapter 1).

This study's results, in combination with the studies of Ronco (1970) and Noble and Alexander (1977) suggest that shade is important in regeneration of Engelmann spruce because it 1) lowers temperature and improves tree water status by decreasing vapor pressure deficit (thus lowering transpiration) and 2) increases soil moisture levels (and thus plant water potential).

Acknowledgments

We would like to thank Dr. Donald Dickmann and Dr. James Flore for assistance in experimental design. We are grateful to Dr. Carl Ramm and Dr. Ivan Mao for statistical advice. Also, we would like to thank Dr. Robert Schutzkie and Dr. James Flore for the use of their time domain reflectometer. And finally we appreciate the time and thoughts of Dr. Donald Dickmann and Dr. James Hancock for data discussion.

Chapter Three: Light response and diurnal gas exchange patterns of blue and Engelmann spruce

Abstract

The results of two studies evaluating the response of blue and Engelmann spruce to different light regimes are presented. The first study compared rates of photosynthesis and stomatal conductance of blue and Engelmann spruce over the changing light regime of a diurnal time course. Rates of photosynthesis and stomatal conductance for Engelmann spruce were higher than that of blue spruce over the course of the diurnal light regime. Results of photosynthetic light response studies showed no significant differences between photosynthetic light compensation points, quantum efficiencies or light saturation points between blue and Engelmann spruce. However, these experiments were performed on plant material of low vigor. Shade-preconditioned Engelmann spruce demonstrated photoinhibition when subjected to light of increasing PPFD, an observation inconsistent with a previous report, however, this result is most likely tainted by the low-vigor condition of the plant material.

Introduction

In the southern half its range, blue spruce dominates in habitats that are too warm and dry for Engelmann spruce (Jones and Bernard 1977). That the two species are found in habitats that differ in moisture leads to the hypothesis that Engelmann spruce and blue spruce differ in their ability to tolerate moisture deficits. In fact, soil moisture deficits (under controlled conditions), resulted in a significant difference between the two taxa in photosynthetic rate, water use efficiency, mortality and root mass at the experiment's end (Chapter 2). In general, soil moisture deficits were more detrimental for Engelmann spruce than for blue spruce.

Soil moisture deficits may cause Engelmann spruce mortality when it is planted on high elevation, exposed sites in its natural range. A debate over the relative importance of water and light extremes as the cause of Engelmann spruce mortality has led to three hypotheses. Hypothesis 1: Ronco (1970) believed high light intensity to be the primary cause of Engelmann spruce mortality via solarization; water deficits were a secondary environmental factor involved in solarization. Hypothesis 2: Kaufmann (1976) argued that the role of water deficits and high light be considered equally in further investigations of Engelmann spruce mortality. Hypothesis 3: Noble and Alexander (1977) believed that water deficits led to Engelmann spruce death; high light intensity was important only insofar as it increased temperature and vapor pressure deficits, thus increasing stomatal conductance and transpiration. Engelmann spruce opens its stomata widely in full sun (Kaufmann 1976) which results in more water loss than if stomata were partially- or fully-closed. Stomatal sensitivity (or the lack thereof) in response to the diurnal light regime is likely involved in the relative roles that water and light play in Engelmann spruce mortality.

This chapter includes results of experiments with diurnal light and increasing photosynthetic photon flux density (PPFD). The first experiment tested whether the rates of photosynthesis and stomatal conductance over a diurnal time course were the same between blue and Engelmann spruce. If the rate of stomatal conductance was significantly higher for Engelmann spruce than for blue spruce, it would further implicate water deficits as the cause of Engelmann spruce mortality.

A second experiment, generation of photosynthetic light response curves, tested whether blue and Engelmann spruce respond similarly to increasing photosynthetic photon flux density (PPFD) to assess whether photoinhibition occurred at a higher PPFD.

Materials and Methods

Diurnal study: Seeds of Engelmann spruce and blue spruce from southwestern Colorado provenances were sown on August 10, 1990 and grown using accelerated optimal growth (Hanover et al. 1976) from 1990 until spring of 1991, when they were moved into a shadehouse at Michigan State University's Tree Research Center, Ingham County, MI, USA. Trees remained in the shadehouse until they were measured on July 27, 1993.

Gas exchange rates of sixteen individuals each of blue spruce and Engelmann spruce were measured using a LiCor 6200 portable photosynthetic analyzer (LiCor Inc., Lincoln, NE) using the one liter leaf chamber, the default K constant and a 15 second measurement span (one measurement per second). Rates of gas exchange were measured at ambient CO2 concentrations, on a cloudless day from 08:00 to 16:00.

Measurements on the three-year old trees were made in the shadehouse where photosynthetic photon flux densities averaged around 900 μmols of quanta/m-2s-1 at mid-day.

The experimental design for the diurnal study was a randomized complete block, using species as treatments. There were four blocks containing eight trees (four blue spruce and four Engelmann spruce). There were four replicates per two treatments within one block.

Twigs were prepared for gas exchange measurements by removing needles at the twig area over which the leaf chamber would clamp. At the end of the day, twigs were detached and returned to the lab for leaf area determination. Leaf areas were calculated using the methods of Seiler and Cazell (1990).

Data collected were analyzed using SYSTAT (Systat, Inc., Evanston, IL) to assess whether the assumptions of ANOVA were met. None of the variables tested significant for the Bartlett's test for homogeniety of variances. All data were statistically analyzed using ANOVA and the general linear model procedureof SYSTAT.

Light _Response_ _Curve_ (150-800 PPFD): On May 11, 1995, four nine-year-old Engelmann spruce were dug up from a plantation established in 1990 at the Sandhill Research Area, Tree Research Center, Ingham County, MI, USA. On July 26, 1995, four nine year old blue spruce were removed from the same plantation. Trees were placed in five gallon pots with a soil mixture of three parts peat to one part vermiculite. The species were not dug up at the same time because the original blue spruce trees to be used in the experiment became severely mite infested and had chlorotic foliage. The decision to not use them in the experiment came two and a half months after the Engelmann spruce had been removed. Both species were acclimated in a lath house prior to their transport into a Conviron PGV 36 walk-in plant growth chamber on August 8, 1995.

The experimental design for the light response study was a randomized complete block with four blocks. In each block, there were two trees, one of each treatment, (i.e., one blue spruce, one Engelmann spruce).

The growth chamber was programmed to a 18 h photoperiod at the following settings (06:00: air temperature = 20°C, relative humidity 50%, PAR = 150 μmols m^{-2} s^{-1}; 09:15: same as 06:00, except PAR = 300 μmols m^{-2} s^{-1}; 11:00, same as 06:00, except PAR = 500 μmols m^{-2} s^{-1}; 12:30: same as 06:00, except PAR = 700 μmols m^{-2} s^{-1}). Gas exchange was measured on August 11 and August 15, 1995 using lateral twigs and an open-gas exchange system described by Sams and Flore (1982) and modified as follows: (a) an ADC 225 MK3 Infrared Gas Analyzer (Analytical Development Company, Hoddeson, UK) was used to measure differential CO2 concentrations at the inlet and outlet of the leaf chambers; (b) air flow entering the chambers was regulated using the following Matheson equipment (Matheson Instruments, Horsham, PA): 8100 series flow meters and 8200 series mass flow controllers connected to a model 8219 multi-channel Dyna-Blender. Gas exchange measurements were conducted at an air temperature of 20°C, 50% relative humidity and the following PAR: 150, 300, 400, 600 and 800 μmol m^{-2} s^{-1}.

Gas exchange parameters were calculated using the BASIC program of Moon and Flore (1986). Response of photosynthesis to PPFD were analyzed by nonlinear regression. Curve fitting was done using the Marquardt compromise method of successive approximations. The best fit curve, evaluated by analysis of residuals and r2 was the monomolecular asymptotic function (Hunt 1980) of the type:

$Y = B(1) * \{1.0 - B(2)\ e{-}B(3) * X\}$, where B(1), B(2) and B(3) are the asymptotic value, minimum value and rate constant, respectively (Layne 1992). Light saturation point was determined by the corresponding light level to the maximum rate of photosynthesis (B1).

Leaf areas were calculated using the methods of Seiler and Cazell (1990). Data were statistically analyzed using SYSTAT's general linear model procedure for ANOVA. Linear regression coefficients were calculated using GraphPad Prism.

Light Response Curve (0-100 PPFD): A light response curve at low light intensities was constructed using twigs of blue and Engelmann spruce. DeLucia and Smith (1987) determined that gas exchange measurements were not significantly different in cut and attached shoots of Engelmann spruce after 24 hours of detachment. Twigs of nine year old blue and Engelmann spruce from the plantation described above were clipped at 07:00, immediately placed upright in a bucket of water, and brought into the Conviron programmable walk-in growth chamber (also described above).

Twigs from three individuals of each species were collected. Using species as treatments, two twigs (one blue spruce and one Engelmann spruce) were assigned to one block in a randomized complete block design with three blocks. Hence, there were a total of three blocks, with one blue spruce and one Engelmann spruce per block.

The methods used to construct the low light response curve were identical to above except for (a) the PARs at which gas exchange was measured were 0, 50, 75 and 100 μmols m^{-2} s^{-1} and (b) the measurements were made on detached twigs. The base of each cut branch was re-cut after measurements at each light level. All of the measurements were completed within four hours after twig detachment.

Quantum efficiency was determined by calculating the linear regression coefficient for each species when photosynthetic rate was plotted versus increasing photosynthetic photon flux density. Data were statistically analyzed using the ANOVA procedure of SYSTAT, using the general linear model. Linear regression coefficients and x-intercepts were calculated using GraphPad Prism.

Results

Diurnal gas exchange: Diurnal photosynthetic response differed for Engelmann and blue spruce (Figure 1), with Engelmann spruce photosynthesizing at a higher rate ($F=3.91$ $P=.06$; block*species interaction not significant). Photosynthetic rates of both species peaked between 10:00 and 12:00. Blue spruce photosynthetic rate was highest at 10:00 when it was measured at 6.2 μmols*$m^{-2}s^{-1}$ and Engelmann spruce photosynthetic rate was highest at 12:00 with a rate of 7.0 μmols*$m^{-2}s^{-1}$. Photosynthetic rates of both species declined during successive afternoon hours; response curves of both species were similar in shape.

Diurnal patterns of stomatal conductance also differed for Engelmann and blue spruce (Figure 2), with Engelmann spruce having higher rates of stomatal conductance than blue spruce ($F=9.28$ $P=0.005$). In contrast to diurnal photosynthetic curves, the curves, the shapes of the stomatal conductance curves were different for the two species. From 10:00-12:00, stomatal conductance increased for Engelmann spruce but did not change for blue spruce. By 16:00, stomatal conductance rates increased for Engelmann spruce but decreased for blue spruce (.1550-.1117 μmols*$m^{-2}s^{-1}$).

Light Response Curve (150-800 PPFD): Photosynthetic response to increasing PPFD (between 150 and 800 μmols m^{-2} s^{-1}) was different for blue and Engelmann spruce (F=45.5 P=0.00) (Figure 3). At all levels of PPFD, Engelmann spruce had a higher photosynthetic rate relative to blue spruce. Light saturation occurred at about 400 μmols m^{-2} s^{-1} for both species. Both species also showed evidence of photoinhibition (decreased photosynthetic rate with increasing light intensity) beyond 400 μmols m^{-2} s^{-1}. Linear regression analysis assigned a r^2 value of 0.09 for Engelmann spruce and 0.01 for blue spruce.

Response of stomatal conductance to increasing PPFD (between 150 and 800 μmols m-2 s-1) (Figure 4) was different for blue and Engelmann spruce (F=8.11 P=0.08) (Figure 4). At three light levels, Engelmann spruce had a higher rate of stomatal conductance relative to blue spruce. Stomatal conductance rates for both taxa were highest at the lowest levels of light, 150 μmols m^{-2} s^{-1} for Engelmann spruce and 300 μmols m^{-2} s^{-1} for blue spruce. Rates of stomatal conductance sharply decreased at the light saturation point (400 μmols m^{-2} s^{-1}) for both taxa.

Light Response Curve (0-100 PPFD*)*: Photosynthetic response at increasing low light intensities differed for Engelmann and blue spruce (F=5.88 P=.03) (Figure 5) however there was a significant light*species interaction. Engelmann spruce had a

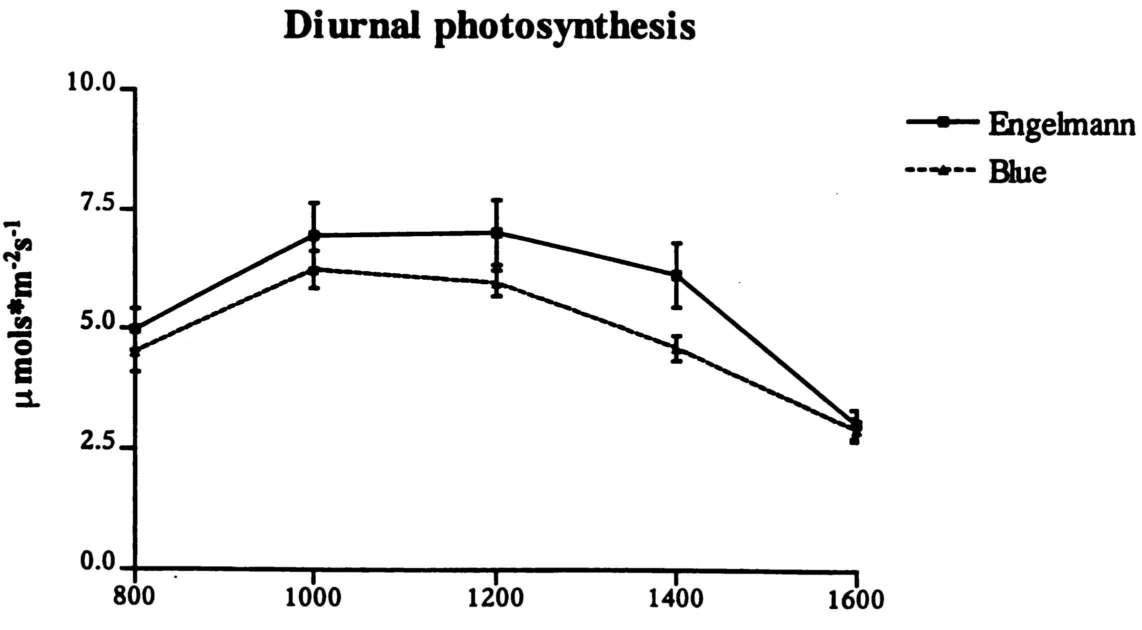


Figure 1: Diurnal photosynthetic rate of Engelmann spruce (*Picea engelmannii* Parry ex. Engelm.) and blue spruce (*Picea pungens* Engelm.) measured on July 27, 1993

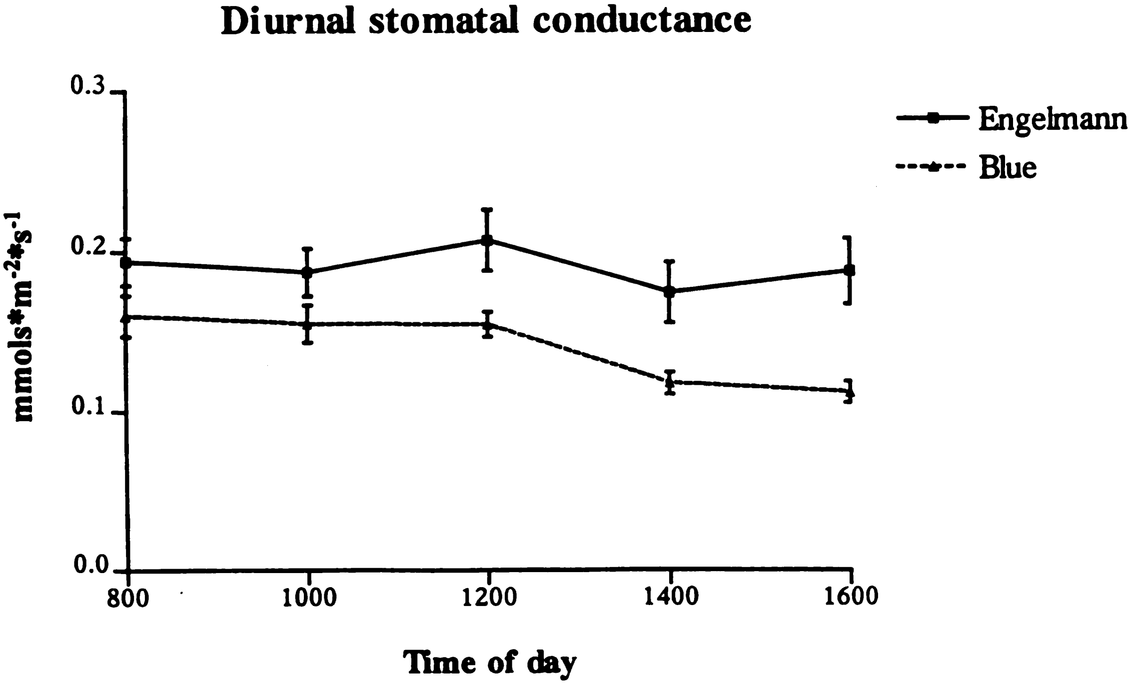


Figure 2: Diurnal stomatal conductance rate of Engelmann spruce (*Picea engelmannii* Parry ex. Engelm.) and blue spruce (*Picea pungens* Engelm.) measured on July 27, 1993.

higher quantum efficiency (r^2 = 0.88) and a lower light compensation point (31.1 μmols m^{-2} s^{-1}) than blue spruce (r^2 = 0.80; 34.2 μmols m^{-2} s^{-1}). These differences were not found to be significantly different (α = 0.05).

Discussion

Diurnal photosynthesis patterns of blue and Engelmann spruce are similar to other conifers, (Hodges 1967); photosynthetic rate increases in the morning hours, peaks at mid-day and decreases towards dusk. There is one difference between the curves reported for other conifers (Hodges 1967), in that a mid-day photosynthesis depression (defined as decreased photosynthesis shortly after 12:00 due to increased vapor pressure deficits and the lag time between transpiration and absorption) was not detected for either blue or Engelmann spruce. Stomatal conductance (Figure 2) did decrease for both species between 12:00 and 14:00, indicating that stomata were closing in the early afternoon hours, most likely in response to more water being lost through transpiration than water gained through root absorption.

Stomatal conductance rates for Engelmann spruce were significantly higher than for blue spruce (Figure 2), suggesting that relative to blue spruce, Engelmann spruce loses more water over the course of a day. In comparison to Engelmann spruce, blue spruce dominates in habitats that are too warm and dry for Engelmann spruce (Jones and Bernard 1977) in the southern part of blue spruce range, i.e., Engelmann spruce is found on cooler, moister sites. These stomatal conductance data, in conjunction with the higher mortality of Engelmann spruce when grown under extreme water deficits (Chapter 2), provide evidence that desiccation would be a more

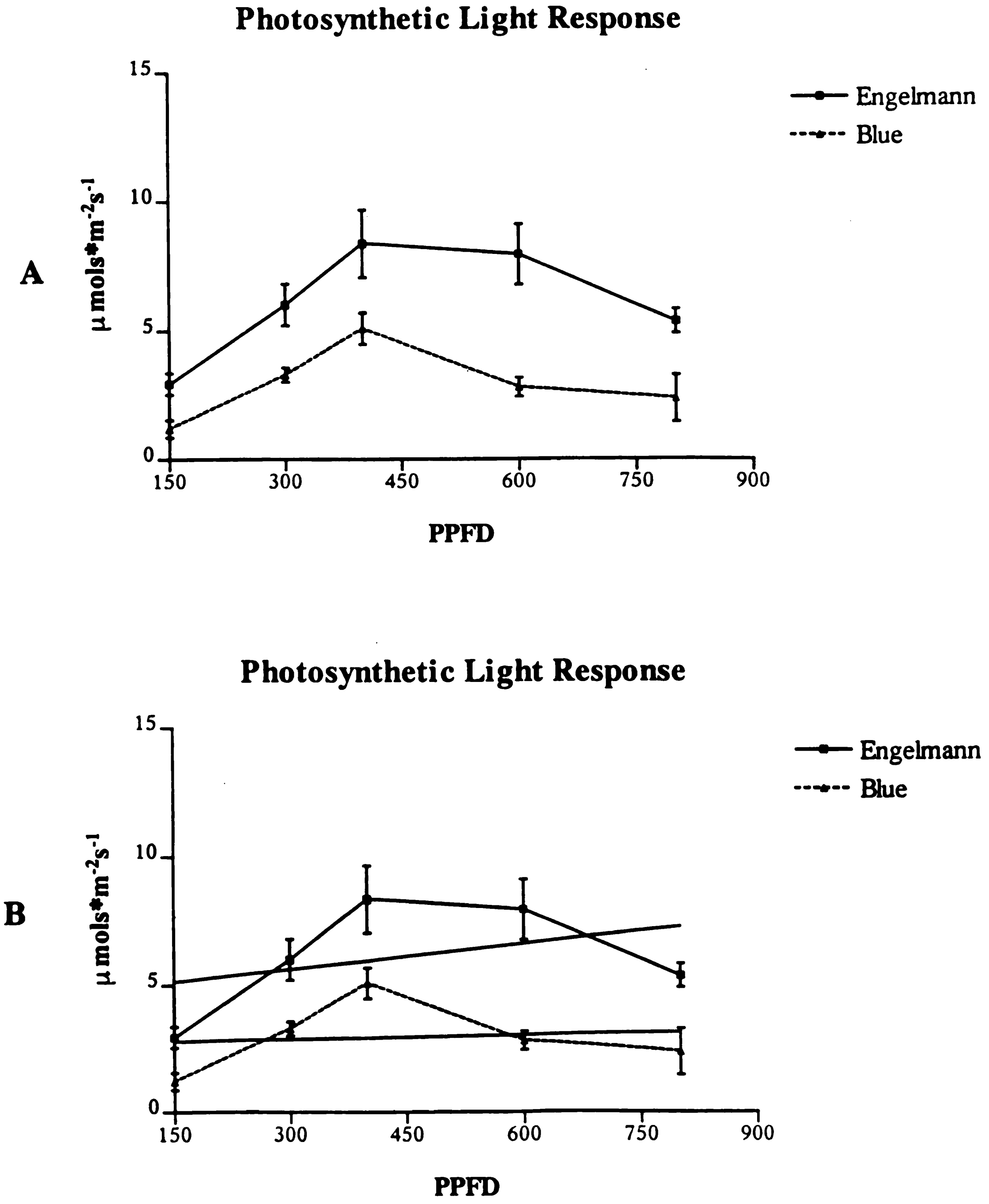


Figure 3: **A.** Response of Engelmann spruce and blue spruce photosynthetic rate to increasing photosynthetic photon flux density (PPFD) and **B.** corresponding linear regression lines

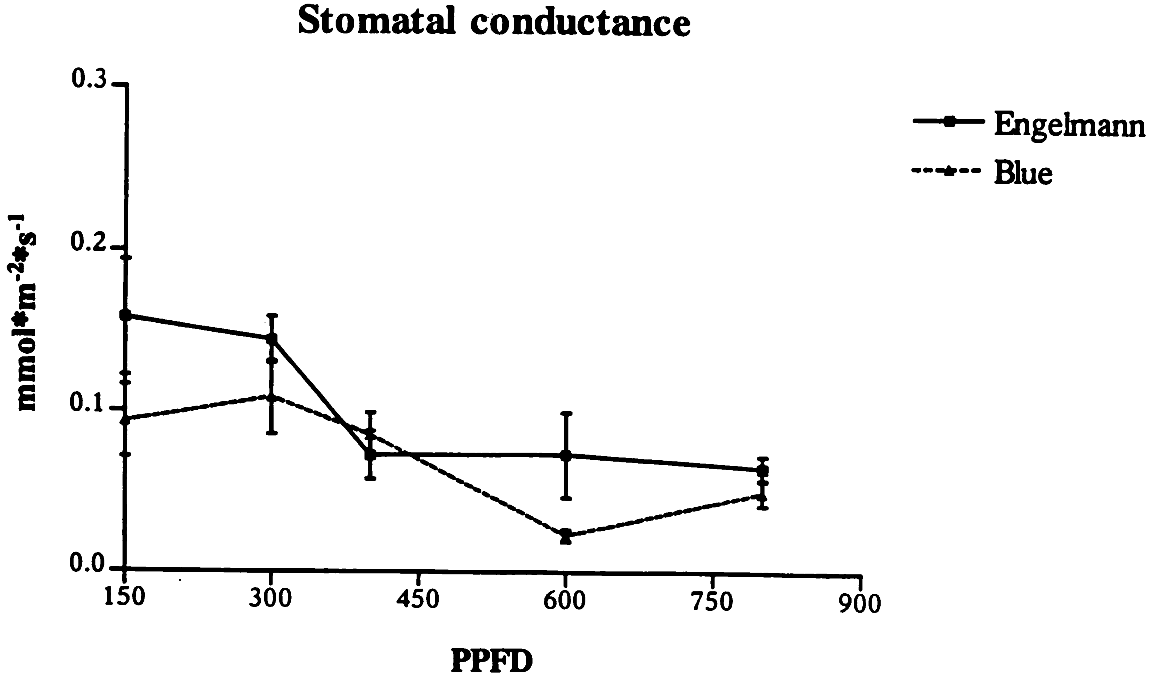
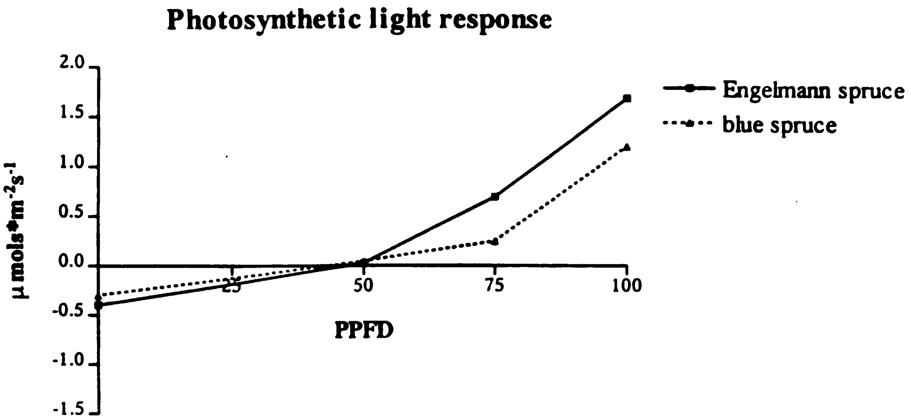


Figure 4: Response of Engelmann spruce and blue spruce stomatal conductance rate to increasing photosynthetic photon flux density (PPFD)

A



B

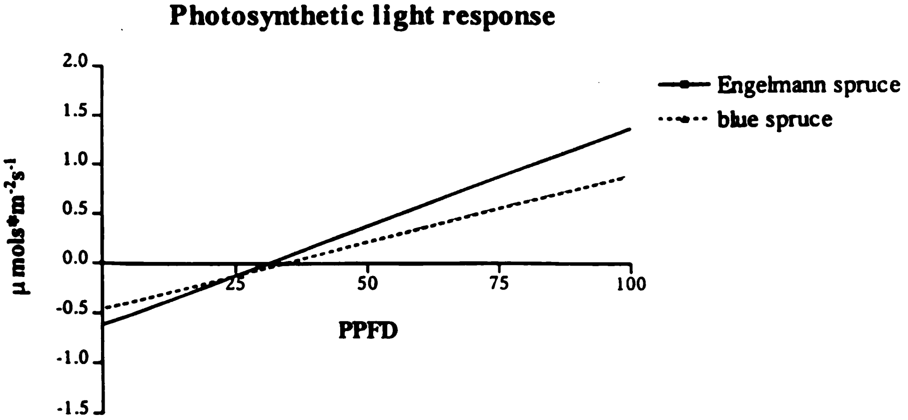


Figure 5: **A**: Response of Engelmann spruce and blue spruce photosynthetic rate to low photosynthetic photon flux densities (PPFD) and **B**. corresponding linear regression lines

serious threat to Engelmann spruce than to blue spruce if Engelmann spruce were planted on exposed sites.

Kaufmann (1976) found that Engelmann spruce stomata tend to open widely in full sun. As a seedling, Engelmann spruce usually occurs in the understory beneath lodgepole pine (*Pinus contorta* Dougl. ex. Loud.), and trembling aspen (*Populus tremuloides* Michx.) (Alexander 1987). Therefore if Engelmann spruce seedlings were transplanted on exposed sites, stomata may remain open until sunset. It is thus plausible that they can lose more water than can be replaced by root absorption, particularly if stomata remain open in the sun and are insensitive to changing soil moisture status. In Sitka spruce, the critical leaf water potential for stomatal closure is -2 MPa; significant closure occurs only when reduction in soil water potential results in much lower leaf water potential (Jarvis 1980). If a similar threshold applies to Engelmann spruce, it could be too late to prevent desiccation on an exposed site.

The results depicted in Figures 3 and 4 should be viewed with skepticism due to the condition of the plant material. These trees were dug up from the field when they were not dormant. Worse, the two species were dug up at two different times; there was a two month difference in their excavation dates. The ensuing discussion on light saturation points and curve shapes is included as an exercise, despite the aforementioned confounding factors.

The low light response curve (Figure 5) showed both Engelmann and blue spruce responding as a shade tolerant species. Shade tolerant plants generally have lower light compensation points and a higher quantum efficiency than shade intolerants

(Kozlowski et al. 1991). Engelmann spruce, classified as shade-tolerant, and blue spruce, classified as intermediate in shade tolerance, exhibited similar light compensation points and quantum efficiencies (Figure 5). Figure 3 shows both species reaching light saturation at ca. 400 μmols m^{-2} s^{-1}, which is about 22% of full sun. Light saturation for other spruce range from 17.5% of full sun (70 W m^{-2} s^{-1}) (Szaniawski and Wierzbicki 1978) to 75% of full sun (1500 μE m^{-2} s^{-1}) for Norway spruce (*Picea abies* (L.) Karst) (Lange et al. 1986), with the slope of the latter's light response curve decreasing at 500 μmols m^{-2} s^{-1}. Similar results have been reported for Sitka spruce (Leverenz and Jarvis 1979; Morison and Jarvis 1983;) and for shade-adapted foliage of black spruce (*Picea mariana* (Mill.) B.S.P.) (Leverenz 1987). In red spruce (*Picea rubens* Sarg.), net photosynthetic rates began to level off at 250 μmols m^{-2} s^{-1} (Alexander et al. 1995). Ronco's (1970) light response curve for Engelmann spruce showed light saturation occurring at 4,000 foot-candles, or about 40% of full sun, (assuming that full sun is 1,800 μmols m^{-2} s^{-1} or 10,000 foot candles), nearly twice that determined in the current study.

The rate of stomatal conductance decreased coincident with light saturation, at 400 μmols m^{-2} s^{-1} (Figure 4). Consistent with the diurnal study, Engelmann spruce had a significantly higher rate of stomatal conductance than blue spruce, thus providing additional evidence that Engelmann spruce may be prone to higher rates of water loss than blue spruce. Tracking photosynthetic and stomatal conductance rates simultaneously show as photosynthesis increases with increasing PPFD in Engelmann spruce, stomatal conductance rates decrease, hitting their lowest rate at light saturation then leveling off when Engelmann spruce photosynthetic rates begin to decrease. This suggests that as stomata begin to close, photosynthetic rates decrease, implying a stomatal limitation on photosynthesis. Blue spruce stomatal conductance rates parallel photosynthetic rates a bit more closely; both rates are lowest at 600 μmols*m^{-2}s^{-1}. Stomatal conductance also begins to decrease at light saturation. Similar to Engelmann spruce, tracking of the two gas exchange rates suggests that there are stomatal limitations on carbon fixation because after 400 μmols*m^{-2}s^{-1} , photosynthetic rates decrease.

The shape of the light response curve for Engelmann and blue spruce (Figure 3) is not the typical, hyperbolic curve seen for most plants, including the Engelmann spruce in Ronco's (1970) study. In the current study, at the higher photosynthetic photon flux densities, photosynthesis of both blue and Engelmann spruce decreased, suggesting photoinhibition. There is precedent for this shape of a light response curve in spruce. A light response curve constructed for Norway spruce also showed evidence of photoinhibition at high light intensities (140 Wm^{-2}) (Szaniawski and Wierzbicki 1978). In contrast, photosynthetic light response of both shade-preconditioned and sun-preconditioned Engelmann spruce in Ronco's (1970) study was hyperbolic in shape.

At this juncture, it is important to define photoinhibition and solarization because different authors use the same words to describe different phenomena. Ronco (1970) defines solarization as "a phenomenon in which photosynthesis is inhibited by high light intensities" (p. 332). However, using this definition, Ronco (1970) did not provide evidence for solarization in Engelmann spruce because neither shaded or unshaded trees showed inhibition of photosynthesis at high light intensities. The unshaded trees photosynthesized at a lower rate at all light intensities relative to the shaded trees. Ronco does provide evidence for photoinhibition as it is defined by Powles (1984), "a time- and light-dependent decline of photosynthesis" (p. 16), as Ronco's unshaded trees did photosynthesize at a lower rate (relative to shaded trees) after being exposed to high intensity light for a long time. The Engelmann spruce in the current study showed a "light dependent decline" in photosynthesis as opposed to the "time-dependent decline" reported for Engelmann spruce in Ronco's (1970) work. Unfortunately, the confounding created by low plant vigor in the current study can not provide evidence for concluding that shade-preconditioning in Engelmann spruce leads to decreased photosynthesis at high light intensity. Were it not for the confounding, the photoinhibition observed in the current study's light response curve for blue and Engelmann spruce could be attributable to the months of shade preconditioning in the lath house, prior to the transport of the trees to the growth chamber. Yet, shade preconditioning of Engelmann spruce in Ronco's (1970) study lead to an increased rate of photosynthesis. However, photoinhibition does occur when leaves developed in low light are suddenly exposed to bright light or when obligate shade species are kept under strong light (Bjorkman 1981). Again, the discrepancy is most likely due to the low vigor exhibited by the plant material in the current study. Repeating the light response curve using healthy three year old seedlings of both species would resolve whether photoinhibition occurs in Engelmann spruce at higher light intensities.

This study demonstrated that Engelmann spruce has higher rates of photosynthesis than blue spruce in response to diurnal light regimes and to increasing photosynthetic photon flux density. Most notably, rates of stomatal conductance over a diurnal time course were higher for Engelmann spruce than they were for blue spruce. This result complements their respective habitats (blue on drier soils, Engelmann on moister soils) and parallels the increased mortality and decreased water use efficiency (relative to blue) measured in Engelmann spruce subjected to water stress treatments (see Chapter 2). The light response studies showed that the lower light compensation point and increased quantum efficiency of Engelmann spruce (relative to blue spruce) supports its classification as a shade tolerant species. Both Engelmann spruce and blue spruce exhibited decreased photosynthetic rates as PPFD increased, but the curve represented by these results are suspect. Low vigor of the plant material and its shade-preconditioning may have influenced the shape of the curve. On the other hand, that Engelmann spruce was not more susceptible than blue spruce to a photoinhibitory response provides additional evidence to support the precept that high light per se may not be as lethal as once thought.

Acknowledgments

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Conclusions

In a common garden at 3200 m elevation in southwestern Colorado, the gas exchange and growth parameters of blue spruce and Engelmann spruce were not significantly different. Photosystem II of either species did not show any evidence of inefficiency as determined by analysis of the variable fluorescence to maximal fluorescence ratio (F_v/F_m). However, when stressed for water, blue and Engelmann spruce showed a differential response. Blue spruce had a lower mortality rate than Engelmann spruce, and a higher root mass at the end of twelve weeks of water stress treatment. Engelmann spruce had lower rates of gas exchange relative to blue spruce at the end of the experiment. Per unit of water absorbed, blue spruce had a higher CO_2 fixation rate. Throughout the ambient light of a diurnal time course, the stomatal conductance rate of blue spruce was lower than that of Engelmann spruce. The higher rate of stomatal conductance measured in Engelmann spruce suggests that Engelmann spruce loses water through its needles at a faster rate than blue spruce. It is therefore likely, that when planted on a northern, exposed, high elevation site, the threat of desiccation is greater than the threat of light of high intensity for Engelmann spruce seedlings.

Recommendations

The relative importance of water and light extremes as factors resulting in the reported mortality of Engelmann spruce on high elevations sites is still not clearly resolved. Due to the confounding nature of light with temperature and the effect temperature has on enzyme kinetics and physical properties, it is difficult to isolate "the cause" of a phenomenon like solarization. What follows are some recommended experiments that could help elucidate the cause or at least identify physiological genetic differences and similarities between blue and Engelmann spruce.

1. Repeat the common garden experiment on a southwest Colorado slope with southern exposure to see whether critical thresholds would be reached that would elicit the photoinhibitory response.
2. To test the hypothesis of whether blue spruce roots have a greater surface area per unit volume of soil (in comparison to Engelmann spruce), soil cores from the root zone of each species could be taken from the common garden plantation and mass of root tissue per soil volume could be determined. This could be done at various depths.
3. Increasing temperature is a confounding factor of increasing light intensity. A temperature response curve should be performed in order to determine whether high temperature leads to a differential photosynthetic and stomatal conductive response between blue and Engelmann spruce.
4. Repeat the photosynthetic light response curve (and include lower light intensities) using healthy three year blue and Engelmann spruce seedlings.

5. Acclimate blue and Engelmann spruce to (at least) 1,000 PPFD and repeat a photosynthetic light response curve to see if a similar curve results to the one generated after trees were preconditioned in the lathhouse.
6. Under controlled growth chamber conditions, determine the thresholds of light, water and temperature for Engelmann spruce at which photoinhibition and photooxidation occur.

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