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Photoprotection, not increased growth, characterizes the response of Engelmann spruce (*Picea engelmannii*) seedlings to high light, even when resources are plentiful

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Summary

• To investigate the effects of resource limitation on the use of light by Engelmann spruce seedlings (*Picea engelmannii*), we examined the effects of nitrogen (N) supply on growth and physiological acclimation.

• Seedlings were grown under a factorial combination of two levels of light (100%, 33% full light) and two levels of N-supply (100 mg l^{-1} and 10 mg l^{-1}). Biomass, foliage physiology, and pigment composition were measured.

• No significant differences were found in growth or photosynthetic capacity between seedlings grown under high and low light, regardless of whether seedlings were grown under conditions of high or low N-supply. Both a decrease in the capacity for light capture and an increase in the capacity for thermal dissipation of excess absorbed light occurred with growth at high relative to low light as well as at low relative to high N-supply.

• Damage to foliage from excess light appeared to be avoided through a combination of downward adjustments in chlorophyll and upward adjustments in photoprotective xanthophyll cycle carotenoids.

Key words: *Picea engelmannii*, photoinhibition, photosynthesis, nutrition, biomass, chlorophyll, carotenoids, xanthophylls.

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Introduction

Poor regeneration of Engelmann spruce (*Picea engelmannii* Parry *ex* Engelm.) after forest harvesting has led to questions about the cause of growth limitations (Dobbs, 1972; Vyse, 1981; Butt, 1990). Observations that the establishment of naturally regenerated (Alexander & Shepperd, 1990; Klinka *et al.*, 2000) and planted (Ronco, 1970b) Engelmann spruce seedlings may be improved by the provision of partial shade suggest that light in open sites may be well in excess of that which can be used by spruce seedlings. Studies with other temperate tree species have shown that in the juvenile stage full sun conditions may not be required for maximum biomass production (Shirley, 1945; Marquis, 1966; Brix, 1970; Eis, 1970; Loach, 1970; Drew, 1983; Mitchell & Arnott, 1995), particularly under conditions of water or nutrient stress (Canham *et al.*, 1996). Where growth does not increase with increasing light availability, mechanisms for avoiding damage to the foliage by excess light can become of principal importance in the response of tree species to high irradiance.

Pigments such as chlorophylls and carotenoids may have an important role in regulating the balance between light absorption and light use, and thus for avoiding damage to foliage under high light conditions. For example, a marked reduction in chlorophyll can occur as a mechanism for balancing light absorption and light use (e.g. Khamis *et al.*, 1990; Bungard *et al.*, 1997; Verhoeven *et al.*, 1997, see also Adams *et al.*, 1995). That balance can also be regulated through increases in carotenoids of the xanthophyll cycle functioning to thermally dissipate light energy when it is absorbed in excess of what can be used in photochemistry (for reviews, see Demmig-Adams & Adams, 1992b; Björkman & Demmig-Adams, 1994). The maximum capacity for thermal energy dissipation is considered to be set by the total pool size of xanthophyll cycle pigments (V + A + Z; Thayer & Björkman, 1990), which consists of

the three carotenoids, antheraxanthin (A), zeaxanthin (Z), and violaxanthin (V). Antheraxanthin and zeaxanthin are the components essential for thermal energy dissipation and are formed from violaxanthin in response to excess absorbed light (Björkman & Demmig-Adams, 1994; Demmig-Adams & Adams, 1996; Demmig-Adams *et al.*, 1996).

We chose to study the effects of nitrogen (N) limitation on light use by Engelmann spruce because N can be especially important for physiological acclimation to high irradiance (Ferrar & Osmond, 1986; Seeman *et al.*, 1987), and because the interactive effects of light and N-supply on the xanthophyll cycle and other carotenoids have been little explored in higher plants, and contrasting results have been obtained (compare Bungard *et al.*, 1997 with Verhoeven *et al.*, 1997). Although our primary interest was in the physiological acclimation of spruce to high light and N-stress, we took a whole-plant approach to understanding growth responses by examining biomass allocation in addition to physiological acclimation. Although the effects of light on the growth of angiosperm tree species have frequently been studied in relation to both biomass allocation and physiological responses (e.g. Walters *et al.*, 1993a, 1993b; Kitajima, 1994; Walters & Reich, 1996; Poorter, 1999), there are few such studies of conifer species (Grassi & Minotta, 2000).

The objectives of this study were to determine: whether the constraint to light use by Engelmann spruce seedlings can be alleviated by the provision of adequate N; and to what extent changes in chlorophyll and the xanthophyll cycle carotenoids may have a potentially important role in the protection of spruce from damage by high irradiance.

Materials and Methods

Growth conditions

Engelmann spruce seed was obtained from a 1585-m elevation source in the Rocky Mountains of interior British Columbia, Canada (51°0′ N, 118°49′ W; seedlot #8136, B.C. Min. For., Victoria, BC, Canada). On 18 March 1998, the seed was stratified and sown in PBS 415B styro-blocks (Beaver Plastics, Edmonton, AB, Canada) filled with a 2 : 1 v : v peat : vermiculite mixture to which 1800 g^{-1} m^{-3} coarse dolomite lime had been added. Sytroblocks of seedlings were then placed in a glasshouse at the Pacific Forestry Centre, Victoria, BC, Canada. During establishment (for 40 d following germination), all seedlings were fertilized weekly at the same low application rate of 10 mg l^{-1} N (20 : 20 : 20 N-P-K fertilizer with micronutrients, Plant-Prod, Plant Products Co. Ltd, Brampton, ON, Canada).

Experimental treatments

Styro-blocks of seedlings were then randomly assigned to one of two contrasting nutrition treatments (20 : 20 : 20 N-P-K fertilizer, Plant-Prod). The treatments, hereafter referred to 'high N' (HN) or 'low N' (LN) supply treatments, were applied once per week as 100 mg l^{-1} N and 10 mg l^{-1} N, respectively.

Styroblocks of seedlings within each N treatment were then randomly assigned to two groups and transferred to an open nursery compound into one of two contrasting light treatments: (1) 'high light' (HL) 100% full light (no shade) and (2) 'low light' (LL) 33% full light (66% shade as provided by tents of spectrally neutral shade cloth (Westgrow, Victoria, BC, Canada) suspended 2.5 m above the seedlings), respectively, as measured under clear-sky conditions at midday in midsummer by means of quantum sensor (LI-190SA, Li-Cor, Lincoln, NB, USA). Absolute light in the high light treatment was approximately 1750 μmol m^{-2} s^{-1} over the waveband 400–700 nm.

The four treatment combinations comprising the experiment were thus: high light, high N-supply (HL-HN); high light, low N-supply (HL-LN); low light, high N-supply (LL-HN); and low light, low N-supply (LL-LN). Treatments were applied from 8 July until 21 August 1998 during which time seedlings exhibited free growth. Seedlings were watered as required throughout to maintain a well-watered state.

Experimental design, sampling, and statistical analysis

A completely randomized design was used in which two replicates of the 2 × 2 factorial treatment combination of light (L) and N-supply were applied to multiple seedlings per experimental unit. Disregarding seedlings around the edges of styro-blocks, subsamples of seedlings were selected randomly from each experimental unit for measurement of various aspects of growth, carbon allocation or allometry, and physiology. One group of seedlings was used for measurements of gas exchange, biomass, carbon allocation, specific leaf area, and foliar N concentration (n = 6 per experimental unit, but with two composite samples for N). Additional random samples of seedlings (n = 6 per experimental unit) were used for the analysis of foliar pigment composition and *in situ* chlorophyll fluorescence. All statistical analyses were carried-out using the GLM procedure of SAS (SAS Institute Inc., Cary, NC, USA), with light levels and N supply levels considered fixed, and replicates and observations random.

Gas exchange

Seedlings used for gas exchange measurements at the end of the experiment were removed from the out-door treatments and held at low light (approximately 5–10 μmol m^{-2} s^{-1}) for approximately 2 h before measurement. Light-response curves of CO_2 assimilation were then constructed at ambient CO_2 concentration for the entire above-ground portion (shoot) of each plant using an open-flow gas exchange system (LCA-4, Analytical Development Company, Hertfordshire,

UK) fitted with a conifer cuvette. Photosynthetic photon flux density (PPFD, μmol photons m^{-2} s^{-1}) was supplied by a high-intensity water-jacketed metal halide lamp (H400R8; Agri-Cool Lighting Ltd, New Westminister, BC, Canada) and varied by interposing neutral density shade screens between the light source and the leaf chamber, in nine steps from dark to beyond light saturation. Mutual shading among needles was minimized by using aluminium foil to reflect and scatter incident light penetrating to the bottom of the cuvette. Air temperature varied in the range 18–24°C and leaf-to-air vapour pressure deficit (VPD) in the range 1.0–1.6 KPa.

Gas exchange parameters were computed from the raw data after Field *et al.* (1991). An iterative nonlinear least square curve-fitting package (TableCurve, v 1.0, Jandel Scientific, San Rafael, CA, USA) was then used to fit the light response of the CO_2 assimilation rate of individual seedlings to the function given by Leverenz (1987), for determination of whole shoot dark respiration rate (Rd), photosynthetic light compensation point (LCP), apparent quantum yield (ϕ_i), and maximum photosynthetic rate (A_{max}). Gas exchange results were qualitatively similar whether expressed on leaf dry mass (nmol g^{-1} s^{-1} CO_2) or a leaf area (μmol m^{-2} s^{-1} CO_2) basis. For simplicity, only mass-based results are presented (gas exchange was primarily of interest from the perspective of carbon economy and growth).

Biomass, carbon allocation, and specific leaf area

For destructive sampling of biomass, carbon allocation, and specific leaf area, roots (R) were separated from shoots (S) by severing the plant at the root collar, roots washed free of growth media, and the shoot partitioned into leaves and woody material. Total projected leaf area of each plant was measured on the freshly detached needles (LI-3100, Li-Cor), plant fractions were oven-dried separately for 48 h at 65°C, and the dry mass of each fraction determined. The following measures of carbon allocation or allometry were then computed from the component biomass measures: shoot to root biomass ratio (S/R), leaf weight ratio (LWR, g leaves/g plant), and leaf area ratio (LAR, total projected leaf area/g plant). Specific leaf area (SLA, cm^2 g^{-1}) was computed as the mean of three projected area measurements for all needles from a given plant divided by the corresponding oven-dry mass of needles.

Foliar N

Foliar N concentration was determined using two composite samples of oven-dried foliage from three plants each per experimental unit. Composite samples were ground to pass a 40 mm-mesh sieve, then analysed for total organic N concentration (%, dry mass basis) by an autoanalyser (FP-228, Leco Corporation, St. Joseph, MI, USA).

Chlorophylls and carotenoids

Foliage samples for analysis of chlorophyll and carotenoid content were collected *in situ* at midday under clear-sky conditions, quick-killed in liquid N, and stored at −70°C until analysis by high performance liquid chromatography (HPLC). For HPLC, frozen subsamples of approximately 150 mg foliage per plant were homogenized (Virtis Virtishear, The Virtis Company, Inc., Gardiner, NY, USA) in near darkness in 2 ml ice-cold 100% acetone to which approximately 25 mg $NaHCO_3$ had been added to prevent acidification. The sample was centrifuged at 1500 *g* for 2 min, the pellet re-extracted on ice for 5 min with 1 ml of 100% acetone, the extracts combined, and the total volume of the sample made-up to 5 ml. A 1-ml aliquot of the extraction was then centrifuged at 12 000 *g* for 5 min at 4°C, and the supernatant filtered through a 0.45-μm syringe-end filter into an amber vial before injection into the HPLC (Series 1100, Hewlett Packard, Waldbronn, Germany). Pigment separation was achieved on a 0.5-μm C18 250 mm × 4.6 mm I.D. column (Spherisorb ODS-1, Alltech, Deerfield, IL, USA) preceded by a 0.5-μm C18 guard-column (ODS-1, Alltech). The HPLC method of Gilmore & Yamamoto (1991) was used for all analyses but with modifications identical to Königer *et al.* (1995). All samples were analysed immediately following extraction. Chlorophylls were expressed per unit leaf area and carotenoids were expressed per unit total chlorophyll (Chl *a* + *b*).

Chlorophyll fluorescence

Chlorophyll fluorescence was measured *in situ* out-doors in the experimental treatments at midday on two separate dates. In both cases, foliage was dark-adapted with leaf clips for 30 min before measurement with a chlorophyll fluorescence meter (CF-1000, Morgan Scientific, Andover, MA, USA) at a photon flux density of 700 μmol m^{-2} s^{-1} (Mitchell & Arnott, 1995). The ratio of variable fluorescence to maximum fluorescence (Fv/Fm), a measure of the photochemical efficiency of photosystem II (Kitajima & Butler, 1975), was determined at midday on a clear-sky day, and then again on the same plants at midday on an overcast day following 3 d of completely overcast-sky conditions. A depression in Fv/Fm from which seedlings did not recover during this extended period in shade was considered symptomatic of 'chronic' (e.g. Greer & Laing, 1992; Skillman & Osmond, 1998) or prolonged, stress-dependent photoinhibition (Long *et al.*, 1994).

Results

Biomass, carbon allocation, and specific leaf area

Irrespective of N-supply, seedlings grown at 100% full light ('high light', HL) did not differ significantly from those

Table 1 Biomass, carbon allocation, and specific leaf area of Engelmann spruce (*Picea engelmannii*) seedlings grown under two levels of light and two levels of nitrogen supply

	LL		HL		ANOVA		
Seedling trait	LN	HN	LN	HN	Source	*F*-ratio	*P*-value
Total biomass, mg	161 (0.5)	391 (4.2)	166 (7.7)	497 (75)	L	2.19	0.213
					N	55.63	**0.002**
					L × N	1.82	0.248
Leaf biomass, mg	55 (0.8)	118 (2.6)	60 (3.4)	139 (10)	L	5.74	0.075
					N	166.87	**0.000**
					L × N	2.28	0.205
Stem biomass, mg	17 (0.6)	61 (2.6)	17 (0.8)	75 (9.8)	L	1.86	0.244
					N	99.14	**0.001**
					L × N	2.11	0.220
Root biomass, mg	88 (0.7)	212 (1.1)	89 (5.2)	283 (55)	L	1.67	0.266
					N	33.30	**0.005**
					L × N	1.62	0.272
S/R biomass Ratio	0.85 (0.026)	0.84 (0.026)	0.88 (0.014)	0.77 (0.081)	L	0.14	0.726
					N	1.47	0.292
					L × N	1.15	0.345
LAR, g leaves/g plant	21.2 (0.7)	21.3 (0.4)	21.0 (0.1)	18.9 (1.5)	L	2.46	0.192
					N	1.61	0.274
					L × N	1.85	0.245
LWR, cm^2 leaves/g plant	0.35 (0.011)	0.30 (0.002)	0.36 (0.005)	0.28 (0.023)	L	0.00	0.971
					N	25.91	**0.007**
					L × N	2.03	0.227
SLA, $cm^2\ g^{-1}$	61.9 (0.9)	70.5 (0.9)	58.1 (1.2)	66.6 (0.01)	L	20.49	**0.011**
					N	101.23	**0.001**
					L × N	0.01	0.928

Values are means with standard errors in parentheses (n = 2 replicates of the four treatment combinations of light and nitrogen). Abbreviations: LL, low light treatment; HL, high light treatment; LN, low nitrogen-supply treatment; HN, high nitrogen-supply treatment; S/R, shoot to root biomass ratio; LAR, leaf area ratio; LWR, leaf weight ratio; SLA, specific leaf area. ANOVA results for seedling height and diameter were qualitatively similar to those for total biomass (data not shown).

grown at 33% full light ('low light', LL) with regard to total biomass, component biomass (leaf, stem, root biomass), allocation of biomass into above- and below-ground components (S/R), the proportion of biomass in leaves (LWR, g leaves/g plant), or the proportion of biomass displayed as leaf area (LAR, cm^2 leaves/g plant) (Table 1). By contrast, at a given light level, growth of seedlings at high N supply (HN, 100 mg l^{-1} N) relative to low N supply (LN, 10 mg l^{-1} N) significantly increased total biomass and all components of total biomass (leaf, stem, and root biomass). As with light, N-supply did not affect S/R and although LWR was significantly reduced in the high N-supply treatment relative to the low N-supply treatment, leaf area ratio (LAR, cm^2 leaves/g plant) did not differ between HN and LN plants because of opposing changes in specific leaf area (LAR = LWR * SLA). Significant interactions between light and N (L × N) were absent for all measures of seedling biomass and carbon allocation (Table 1).

N and gas exchange

Light had no significant effect on foliar N concentration (%N), whereas N-supply was associated with a > 3-fold increase in %N, from 0.82 to 0.90% at low N-supply to 2.81% at high N-supply (Table 2). As with foliar %N, photosynthetic capacity (A_{max}) and dark respiration rate (Rd) also did not vary significantly with the light environment during growth, but within a given light treatment both parameters were significantly greater at high N-supply than at low N-supply (Table 2). Light and N-supply treatments had no significant effect on the apparent quantum yield of CO_2 assimilation (ϕ_i). The photosynthetic light compensation point (LCP) was significantly higher for seedlings grown at high light relative to low light and at high N-supply relative to low N-supply (the higher LCP of high light relative to low light plants appeared attributable to the combination of marginally nonsignificant reductions in ϕ_i and increases in Rd).

Table 2 Foliar nitrogen (N) and whole-shoot gas exchange parameters for Engelmann spruce (*Picea engelmannii*)

	LL		HL		ANOVA		
Seedling trait	LN	HN	LN	HN	Source	*F*-ratio	*P*-value
Foliar N Concentration, % dry mass	0.82 (0.013)	2.81 (0.15)	0.90 (0.003)	2.81 (0.10)	L	0.18	0.697
					N	441.61	**0.000**
					L × N	0.23	0.658
A_{max}, nmol CO_2 g^{-1} s^{-1}	47.09 (1.77)	93.77 (8.14)	47.25 (0.20)	81.73 (1.95)	L	1.93	0.238
					N	89.94	**0.001**
					L × N	2.04	0.227
Rd, nmol CO_2 g^{-1} s^{-1}	1.67 (0.21)	4.18 (0.26)	2.79 (0.16)	4.75 (0.85)	L	3.35	0.141
					N	23.25	**0.009**
					L × N	0.34	0.589
ϕ_i, mol CO_2/mol photons	0.041 (0.006)	0.045 (0.002)	0.032 (0.002)	0.037 (0.003)	L	6.63	0.062
					N	1.88	0.242
					L × N	0.04	0.857
LCP, μmol photons m^{-2} s^{-1}	6.4 (0.04)	13.1 (0.19)	15.9 (0.05)	18.9 (2.57)	L	35.05	**0.004**
					N	13.87	**0.020**
					L × N	1.98	0.232

Values are means with standard errors in parentheses (n = 2 replicates of the four treatment combinations of light and N). Abbreviations: LL, low light treatment; HL, high light treatment; LN, low nitrogen-supply treatment; HN, high nitrogen-supply treatment; Amax, light-saturated photosynthetic rate; ϕ_i, apparent quantum yield (based on incident light); LCP, photosynthetic light compensation point; Rd, dark respiration rate. ANOVA results for all parameters were not qualitatively different when expressed on a leaf area basis (data not shown).

Chlorophylls and carotenoids

Treatment effects on total chlorophyll (Chl *a* + *b*) were characterized by an interaction between light and N (Table 3). Under high N-supply, growth at high light relative to low light resulted in a 64% reduction in Chl *a* + *b*. Under low N-supply, where Chl *a* + *b* was already low, the light-dependent reduction in Chl *a* + *b* was not observed. However, the ratio Chl *a*/*b* increased at high light relative to low light irrespective of N-supply.

Expressed per unit Chl *a* + *b*, the xanthophyll cycle pigment pool size (V + A + Z), lutein, β-carotene, and the total pool size of all carotenoids all increased in response to an increase in light availability and a decrease in N-supply (Table 3). For α-carotene, a significant interaction between light and N indicated that, similar to the situation for Chl *a* + *b*, the reduction in this carotenoid at high light relative to low light was observed only for plants grown at high N-supply (Table 3), an effect mirrored also in the ratio α/β-carotene (not shown). Neoxanthin was slightly but significantly reduced at high light relative to low light, but N-supply had no significant effect on this carotenoid (Table 3). The contributions of V + A + Z and individual carotenoids to the total carotenoid pool size (per unit Chl *a* + *b*) were not maintained constant as the pool size varied in response to differences in light and N (Fig. 1); as with pool sizes, differences in composition were most marked between treatments representing the extremes in potential light stress (HL-LN vs LL-HN). Differences in the percent conversion of the xanthophyll cycle to its photoprotectively active components (antheraxanthin and zeaxanthin) were similarly most marked between HL-LN and LL-HN treatments (Fig. 2). Therefore, HL-LN seedlings not only had larger xanthophyll cycle pool sizes (V + A + Z) than seedlings in the other L × N treatment combinations, but HL-LN plants also converted a greater percentage of the V + A + Z pool to components active in thermal energy dissipation (83% conversion vs 8% conversion in LL-HN plants; Fig. 2).

Chlorophyll fluorescence

When measured out-of-doors *in situ* under clear-sky conditions at midday (30 min dark acclimation), the photochemical efficiency of photosystem II (Fv/Fm) was lower for seedlings grown at high light than at low light, and was lower for seedlings grown at low N-supply than those grown at high N-supply (Table 4). When Fv/Fm was remeasured at midday under completely overcast skies following a 3 d period of overcast-sky conditions, the apparent effect of the growth light environment had been lost, while that of N-supply remained.

Discussion

Whole-plant biomass of Engelmann spruce seedlings grown at high light (HL, 100% full light) did not differ significantly

Table 3 Chlorophyll content, chlorophyll a/b ratio, and carotenoid composition of Engelmann spruce (*Picea engelmannii*) foliage as determined by HPLC

Pigment parameter	LL		HL		ANOVA		
	LN	HN	LN	HN	Source	*F*-ratio	*P*-value
Chl *a* + *b*, μmol m^{-2} leaf area	230.0 (27.6)	1053.9 (82.1)	240.4 (35.9)	383.7 (45.9)	L	39.96	0.003
					N	85.88	0.001
					L × N	42.52	**0.003**
Chl *a*/*b* ratio	2.86 (0.12)	2.74 (0.01)	3.22 (0.01)	2.90 (0.02)	L	18.63	**0.013**
					N	13.11	**0.022**
					L × N	2.89	0.165
V + A + Z, Mmol mol $(Chl\ a + b)^{-1}$	110.8 (8.6)	59.9 (2.9)	195.5 (17.1)	114.8 (3.5)	L	50.37	**0.002**
					N	44.89	**0.003**
					L × N	2.31	0.204
Lutein, mmol mol $(Chl\ a + b)^{-1}$	260.9 (8.6)	134.1 (4.7)	331.5 (18.9)	227.3 (9.6)	L	49.15	**0.002**
					N	97.72	**0.001**
					L × N	0.94	0.387
β-Carotene, mmol mol $(Chl\ a + b)^{-1}$	77.8 (3.6)	46.9 (1.8)	95.6 (4.1)	72.0 (3.0)	L	43.18	**0.003**
					N	69.82	**0.001**
					L × N	1.28	0.322
α-Carotene, mmol mol $(Chl\ a + b)^{-1}$	5.1 (0.2)	46.9 (2.7)	5.8 (1.3)	13.1 (1.7)	L	94.87	0.001
					N	207.60	0.000
					L × N	102.60	**0.001**
Neoxanthin, mmol mol $(Chl\ a + b)^{-1}$	45.9 (1.8)	44.7 (0.3)	40.2 (0.5)	43.2 (0.1)	L	14.31	**0.019**
					N	0.88	0.402
					L × N	5.10	0.087
Total Carotenoids, Mmol mol $(Chl\ a + b)^{-1}$	500.7 (18.8)	332.5 (1.2)	668.5 (38.3)	470.4 (14.3)	L	46.11	**0.003**
					N	66.18	**0.001**
					L × N	0.44	0.542
V + A + Z/total Carotenoids, Mmol mol $(carotenoid)^{-1}$	220.3 (8.3)	180.2 (9.3)	291.2 (9.0)	243.5 (0.1)	L	76.53	**0.001**
					N	32.67	**0.005**
					L × N	0.24	0.647

Values are means with standard errors in parentheses (n = 2 replicates of the four treatment combinations of light and N). Abbreviations: LL, low light treatment; HL, high light treatment; LN, low nitrogen-supply treatment; HN, high nitrogen-supply treatment; Chl, chlorophyll; V, violaxanthin; A, antheraxanthin; Z, zeaxanthin, V + A + Z, xanthophyll cycle pigment pool size. ANOVA results for chlorophyll were qualitatively similar when expressed per unit leaf fresh or dry mass rather than per unit leaf area, and those for foliar carotenoid content were qualitatively similar when computed per unit Chl *a* rather than per Chl *a* + *b* (data not shown).

Table 4 Ratio of variable fluorescence to maximum fluorescence (Fv/Fm) of Engelmann spruce (*Picea engelmannii*) foliage at midday on a clear-sky day and at midday on a completely overcast day following a 3-d period of overcast sky conditions

Sky conditions	LL		HL		ANOVA		
	LN	HN	LN	HN	Source	*F*-ratio	*P*-value
Clear	0.656 (0.019)	0.772 (0.028)	0.630 (0.014)	0.697 (0.004)	L	7.55	**0.052**
					N	25.15	**0.007**
					L × N	1.76	0.255
Overcast	0.678 (0.017)	0.805 (0.031)	0.715 (0.011)	0.777 (0.010)	L	0.04	0.845
					N	24.82	**0.008**
					L × N	2.91	0.163

Values are means with standard errors in parentheses (n = 2 replicates of the four treatment combinations of light and N). Abbreviations: LL, low light treatment; HL, high light treatment; LN, low nitrogen-supply treatment; HN, high nitrogen-supply treatment.

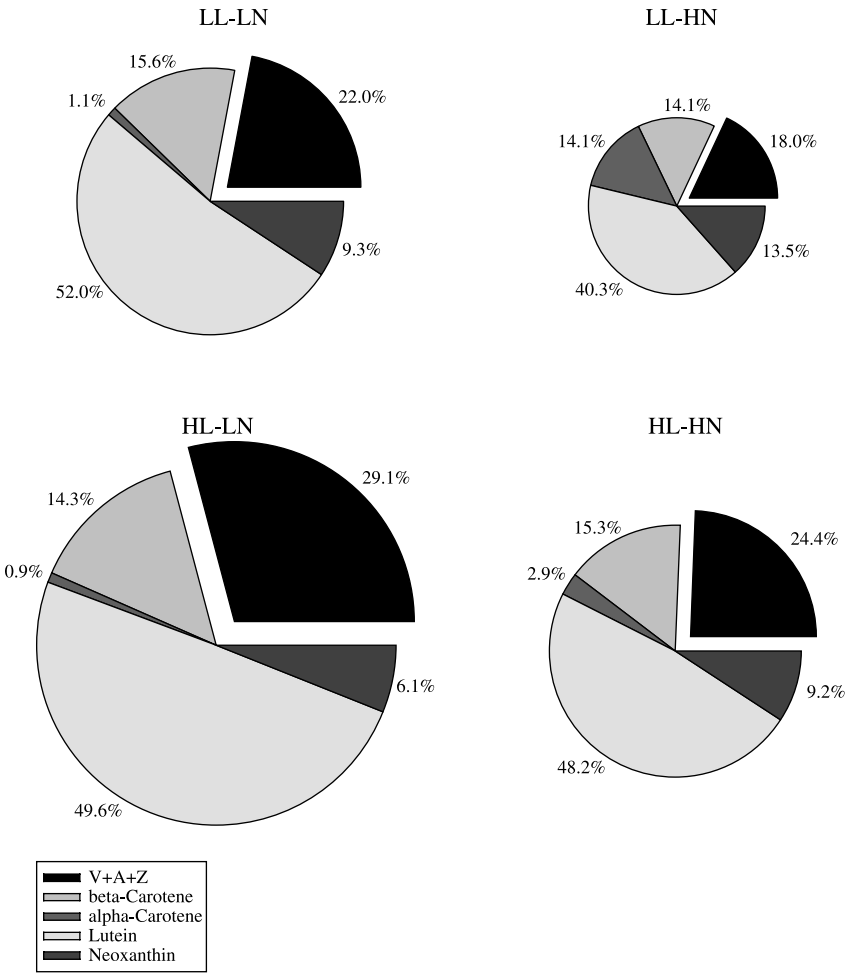

Fig. 1 Foliar carotenoid content and composition of Engelmann spruce (*Picea engelmannii*) seedlings in response to a 2 × 2 factorial combination of light (L) and nitrogen (N) supply. The relative size of each pie is representative of the mean total carotenoid pool size in each treatment, expressed per unit Chl *a* + *b*. Relative contributions of component carotenoids are then scored as a percentage of the total pool. The L–N interaction is significant for %β-carotene, %α-carotene, and %lutein. Light and nitrogen treatments are significant for %V + A + Z (xanthophyll cycle pigment pool size) and %neoxanthin (L × N nonsignificant). Treatment abbreviations: LL, low light (33% full light); HL, high light (100% full light); LN, low N-supply (10 mg l^{-1} N); HN, high N-supply (100 mg l^{-1} N).

from those grown at low light (LL, 33% full light), regardless of whether seedlings were grown at high or low N-supply. This is in apparent contrast to the situation observed for some other forest tree species in which light requirements for seedling growth increase with increasing N-supply (Canham *et al.*, 1996). Although in the present study some trend was evident for biomass of high light plants to be greater than that of low light plants under conditions of high N-supply, the apparent improvement was slight given the 3-fold difference in irradiance.

If Engelmann spruce seedlings in the high light treatment were using the additional light available in that treatment, then for seedling growth to be similar in high and low light environments, seedlings in the low light treatment would be expected to exhibit morphological and physiological traits associated with enhanced growth efficiency. However, for the same whole-plant biomass achieved in high light and low light environments, allocation to above-ground vs below-ground biomass (S/R), allocation to leaves (LWR) and leaf area (LAR) were similar for seedlings in both light treatments. In addition, although light compensation points differed between high and low light plants, whole-shoot dark respiration rates and apparent quantum yields were only marginally different (nonsignificant) and high and low light plants did not differ in terms of whole-shoot photosynthetic capacity (A_{max}) or foliar N concentration (%N). Thus, the similar growth responses of seedlings reared at high and low light appeared to be related instead to a lack of capacity of seedlings in the high light treatment to use the additional light available. Given that A_{max} is in general linearly dependent on foliar N (Field & Mooney, 1986; Evans, 1989), the lack of response to light of whole-shoot photosynthetic capacity (A_{max}) and growth of spruce appeared related in particular to an inability to increase foliar %N in response to the increase in light availability. Indeed, that photosynthesis and growth of spruce were N-limited at high light is evidenced by the marked and concomitant increases in foliar %N, A_{max}, and growth observed for this species when grown at high N-supply in comparison to low N-supply at a given level of irradiance.

By contrast to what was observed here for Engelmann spruce, studies of the response of *Picea* species to 'release' from suppression (overstory removal) suggest that acclimation of

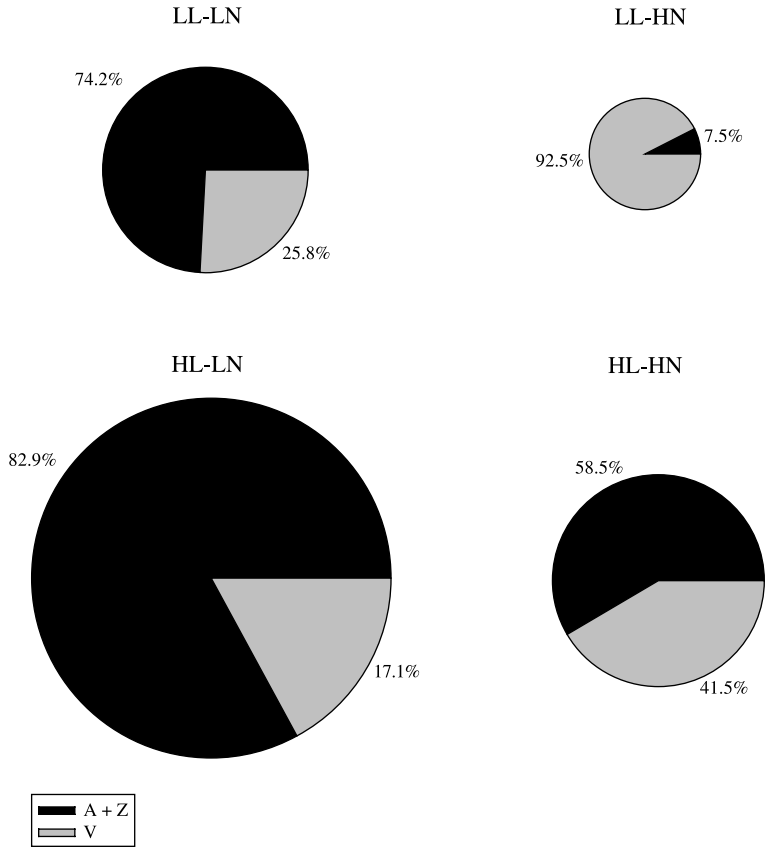

Fig. 2 Relative size and conversion state of the xanthophyll cycle pigment pool (V + A + Z) in Engelmann spruce (*Picea engelmannii*), as measured at mid-day under clear-sky conditions in summer. The relative size of each pie is representative of the mean total V + A + Z pool size in each light (L) × nitrogen (N) treatment combination (from Table 3). The percentage conversion of each xanthophyll cycle pool to its photoprotectively active components is then given as (A + Z)/(V + A + Z) * 100%, where A is antheraxanthin, Z is zeaxanthin, and V is violaxanthin. The *P*-values are $P = 0.050$ for L × N; $P = 0.017$ for L; $P = 0.004$ for N. Treatment abbreviations are as in Figure 1.

spruce to high irradiance may involve increases in both foliar N concentration and shoot-level photosynthetic capacity (Lieffers *et al.*, 1993). However, increases in foliar N concentration (%N) and photosynthetic capacity reported in such studies may be dependent on a concomitant increase in N-availability associated with the removal of competing vegetation, and the situation may also be different for plants not suddenly exposed to light levels much higher than the growth irradiance. Some studies have examined the physiological acclimation of spruce grown continuously in sun vs shade environments, but the contrast which has often been made is that between the extremes of the natural light gradient where, as expected, photosynthetic capacity is higher for seedlings or saplings in open sites than in deep understory shade (Carter & Smith, 1985, 1988; Man & Lieffers, 1997). On the other hand, studies comparing the physiological performance of spruce grown at high light and partial shade suggest that photosynthesis may be comparable or higher in partially cut areas than in clear-cut areas (Ronco, 1970a; Man & Lieffers, 1999, but see Carter & Smith, 1988).

Although in this study Engelmann spruce was apparently unable to use the additional light available in the high light treatment for increased photosynthesis and growth, damage to seedlings by excess light appeared to be avoided through adjustments in foliar pigments important to the regulation of the balance between light absorption and light use. In addition to a light-dependent increase in the chlorophyll *a*/*b* ratio (Boardman, 1977; Björkman, 1981), at high N-supply Engelmann spruce seedlings exhibited two biochemical adjustments which may reduce the potential for damage by light in excess of that which could be used in photochemistry (Björkman & Demmig-Adams, 1994): a reduction in chlorophyll content (capacity for light absorption); and an increase in the xanthophyll cycle pigment pool size (capacity for thermal dissipation of excess absorbed light energy).

At low N-supply, high irradiance acclimation of Engelmann spruce appeared less complete. Where Chl $a + b$ was already low as a result of growth at low N-supply, the light-dependent reduction in Chl $a + b$ was not observed and although V + A + Z increased, the increase in V + A + Z was proportionately less than that observed at high N-supply (76% vs 92%). Engelmann spruce therefore exemplifies a third pattern of response of Chl $a + b$ and V + A + Z to high light and N-stress (low N-supply) to that which has previously been reported. In *Spinacia oleracea* (Verhoeven *et al.*, 1997) both a reduction in Chl $a + b$ and an increase in V + A + Z occurred in response to high light under conditions of N-stress, while in *Clematis vitalba* (Bungard *et al.*, 1997), Chl $a + b$ was reduced without changes in V + A + Z or other carotenoids. By contrast, at low N-supply foliar acclimation of

Engelmann spruce to high light (HL-LN) involved only adjustments in V + A + Z, because light-dependent reductions in Chl *a* + *b* were inhibited.

Lower midday values of Fv/Fm for Engelmann spruce in all treatments under clear-sky conditions than under prolonged overcast-sky conditions suggested that all seedlings exhibited some amount of dynamic (readily reversible) photoinhibition of photosynthesis as a result of exposure to excess light on clear days. Photoinhibition of photosynthesis during the midday period on clear days has been widely reported for plants of diverse taxa and life-forms in open sites and in forest gaps (e.g. Long *et al.*, 1994; Lovelock *et al.*, 1994; Krause & Winter, 1996). Under clear-sky conditions, Fv/Fm was lowest for Engelmann spruce grown at high light and low N-supply (HL-LN), and these same seedlings also had the greatest percentage of the xanthophyll cycle pigment pool in the photoprotectively active state (cf. Bungard *et al.*, 1997). A higher conversion state of the xanthophyll cycle pool has been shown to be associated with a lower percentage use of absorbed light in photochemistry and a higher rate of xanthophyll cycle-dependent thermal energy dissipation in the antennae (Verhoeven *et al.*, 1997, see also Khamis *et al.*, 1990). Thus, these results suggested, in agreement with results for V + A + Z and Fv/Fm, that seedlings grown at high light or low N-supply were making less efficient use of absorbed light than seedlings grown at low light or high N-supply and were dissipating excess light by means of xanthophyll-cycle dependent thermal energy dissipation. The rank-order of treatments in terms of the efficiency of use of absorbed light therefore was: LL-HN > HL-HN > LL-LN > HL-LN. Differences between the two intermediately ranked treatments (HL-HN and LL-LN) were however minor, suggesting that in spruce similar levels of light stress can be achieved by growing seedlings at low light (33% full light) and low N-supply (10 mg N l^{-1}) as by growing seedlings at high light (100% full light) and high N-supply (100 mg N l^{-1}) (see Huner *et al.*, 1996).

That Fv/Fm of Engelmann spruce seedlings grown at low N-supply did not recover following 3-d of overcast-sky conditions (cf. control Fv/Fm value of 0.805 for LL-HN plants) suggested also a level of 'chronic' or prolonged, stress-dependent photoinhibition (e.g. Greer & Laing, 1992; Skillman & Osmond, 1998) for spruce seedlings under conditions of N-stress. Thus, in the absence of an increase in photosynthetic capacity in response to growth at high light, changes in foliar pigments (Chl *a* + *b*, V + A + Z) appeared sufficient to protect the photosynthetic apparatus of spruce against damage by excess light when N-supply was ample, but the response may possibly have been less than sufficient when N-supply was low.

The light-dependent changes in V + A + Z and other carotenoids observed in Engelmann spruce in this study were in accord with those reported previously (e.g. Thayer & Björkman, 1990; Demmig-Adams & Adams, 1992a; Königer *et al.*, 1995; Logan *et al.*, 1996), but with the exception of neoxanthin. Although neoxanthin does not typically respond to light (Thayer & Björkman, 1990; Demmig-Adams & Adams, 1992a; Königer *et al.*, 1995; Logan *et al.*, 1996), a slight but significant decrease in this pigment at high light was observed in this work. This response, however, appeared largely attributable to that of plants grown at low N-supply (L × N interaction marginally nonsignificant, $P = 0.087$).

Reported effects of N-supply on the xanthophyll cycle pigments and other individual carotenoids per unit chlorophyll are more variable than the effects of light. In one study V + A + Z, lutein, β-carotene, and neoxanthin (per Chl *a* + *b*) all increased under N-stress (Verhoeven *et al.*, 1997, see also Solberg *et al.*, 1998) similar to the present study, but elsewhere N-supply was observed to have no significant effect on these carotenoids (Bungard *et al.*, 1997). Information on α-carotene is less available, but our results for Engelmann spruce are in agreement with the association reported between N-deficiency and low α-carotene concentrations and contents in open-grown individuals of the congeneric species *Picea abies* (Solberg *et al.*, 1998). Our results show that in Engelmann spruce the decrease in α-carotene at high light relative to low light can be inhibited at low N-supply, similar to what we observed for Chl *a* + *b* in this species. This might suggest that the light- or season-dependent changes in α-carotene commonly reported for conifers (Adams & Demmig-Adams, 1994; Siefermann-Harms, 1994; Ottander *et al.*, 1995) and other species in which this taxonomically restricted pigment is known to occur (Thayer & Björkman, 1990; Demmig-Adams & Adams, 1992a; Logan *et al.*, 1996) may not necessarily be observed under conditions of N-stress.

In summary, the lack of growth response to light observed for young Engelmann spruce seedlings in this study appeared related to an inability of this species to increase foliar N concentration and photosynthetic capacity in response to high irradiance. Improved N-availability did not alleviate the constraint to the use of high light. Foliage damage (sunscald, leaf necrosis) from excess light appeared to be avoided through a combination of downward adjustments in chlorophyll and upward adjustments in photoprotective xanthophyll cycle carotenoids. An interaction between light and N-supply was described for α-carotene as well as for Chl *a* + *b* in this species.

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