

SPECTRAL REFLECTANCE OF *PICEA RUBENS* (PINACEAE) AND *ABIES BALSAMEA* (PINACEAE) NEEDLES ALONG AN ELEVATIONAL GRADIENT, MT. MOOSILAUKE, NEW HAMPSHIRE, USA¹

ANDREW D. RICHARDSON,² GRAEME P. BERLYN, AND
TIMOTHY G. GREGOIRE

Yale University, School of Forestry and Environmental Studies, 370 Prospect Street, New Haven, Connecticut 06511 USA

Relationships among elevation, foliar morphology, spectral reflectance, and chlorophyll fluorescence of two co-occurring montane conifers, red spruce (*Picea rubens* Sarg.) and balsam fir (*Abies balsamea* [L.] Mill.), were investigated along two transects from 460 to 1460 m on Mt. Moosilauke in the White Mountains of New Hampshire, USA. Spectral reflectance (300–1100 nm wavelengths) and the chlorophyll fluorescence F/F_m ratio were measured on dark-adapted needles. Foliar morphology (needle size, shape, and mass) and nitrogen concentrations were measured in the laboratory. Reflectance spectra varied between species and with elevation. Two chlorophyll measures, red edge position and a chlorophyll-based difference index ($\text{Chl NDI} = R750 - R705/R750 + R705$), indicated more chlorophyll in fir than in spruce and decreasing chlorophyll with increasing elevation in both species. The structure-independent pigment index ($\text{SIPI} = R800 - R445/R800 - R680$) increased with elevation, indicating an increasing carotenoid : chlorophyll ratio. The photochemical reflectance index ($\text{PRI} = R531 - R570/R531 + R570$), a measure of photosynthetic radiation use efficiency, decreased with increasing elevation up to 1370 m. In the highest elevation site, within the stunted alpine krummholz at 1460 m, PRI was higher than at 1370 m, but still lower than at 1070 m. This same pattern was evident in the chlorophyll fluorescence F/F_m measurements. These independent indices indicate higher stress in spruce than fir, which may be related to the “spruce decline” reported in the northeastern USA. Results also indicate progressively increasing stress with increasing elevation up to 1370 m. Stress appears to be lower at 1460 m than at 1370 m, despite the harsher conditions at the very summit of Mt. Moosilauke. This may be a consequence of stress-tolerant physiology and/or prostrate architecture.

Key words: chlorophyll fluorescence; elevational gradient; foliar morphology; krummholz; leaf photosynthetic efficiency; spectral reflectance; spruce-fir; stress.

Across a wide range of scales, from stand-level studies using remote sensing to leaf-level studies using portable spectrometers, leaf reflectance spectra have been used to probe the physiology, structure, and nutrient content of plant foliage (Peterson et al., 1988; Martin and Aber, 1997; Gamon and Qiu, 1999; Gamon and Surfus, 1999; Ourcival, Joffre, and Rambal, 1999). Because these measurements can be made nonobtrusively and, in some cases, from great distances, the application of leaf reflectance to the quantification of a wide variety of leaf traits and properties shows great promise.

Characteristics of leaf reflectance spectra are determined by the surface properties of the leaf, as well as internal structure and biochemical components. One example of this is the distinctive “red edge,” which occurs as a sharp increase in reflectance around 700 nm. The red edge exists because of the strong chlorophyll *a* absorption band centered around 670–680 nm, coupled with scattering of near-infrared reflectance within the leaf, which causes large reflectance above 700 nm (Curran, Dungan, and Gholz, 1990; Gitelson and Merzlyak,

1996). The red edge shifts to shorter wavelengths under stress or senescence as a product of decreases in chlorophyll (Curran, Dungan, and Gholz, 1990).

Foliar health and photosynthetic efficiency can be assessed with reflectance; the photochemical reflectance index, PRI, is correlated with photosynthetic radiation use efficiency, i.e., $\text{PRUE} = \text{net CO}_2 \text{ assimilation/incident photosynthetic photon flux density}$ (Peñuelas, Filella, and Gamon, 1995; Gamon, Serrano, and Surfus, 1997; Peñuelas et al., 1997; Gamon and Qiu, 1999). To prevent photodamage, plants can dissipate excess radiation either through chlorophyll fluorescence or the xanthophyll de-epoxidation cycle (Filella et al., 1996; Peñuelas and Filella, 1998; Gamon and Qiu, 1999). In the xanthophyll cycle, the antenna pigment violaxanthin is converted to zeaxanthin, a photoprotective pigment with an energy level below that of chlorophyll *a*; this provides a sink for excess energy (Demmig-Adams and Adams, 1996). Xanthophyll cycle pigments can be detected at 531 nm using fine-resolution spectral reflectance measurements (Gamon and Qiu, 1999). Parallel changes in PRI and the maximum quantum yield of PS II, estimated by fluorescence techniques, have been noted under dark-light-dark transitions, and both measures have been correlated with instantaneous gas-exchange based measures of PRUE (Peñuelas, Filella, and Gamon, 1995).

Although leaf anatomy, morphology, and physiology are known to change along elevational and latitudinal gradients, little research has been conducted to understand the associated changes in reflectance (Tranquillini, 1979; Körner, 1999). In a recent study, Filella and Peñuelas (1999) studied *Quercus ilex* at 200 and 1200 m and *Rhododendron ferrugineum* at 2200

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² Author for correspondence (tel.: 203 432-5153, fax: 203 432-3929, e-mail: andrew.richardson@yale.edu).

m and found a higher carotenoid:chlorophyll *a* ratio in *Q. ilex* foliage at 1200 m than at 200 m. Furthermore, based on PRI, PRUE was shown to decrease with increasing elevation: PRI of *Q. ilex* was lower at 1200 m than at 200 m, and PRI of *R. ferrugineum* at 2200 m was lower still. However, to our knowledge, no studies have been conducted along an entire elevational gradient.

To investigate relationships between reflectance and elevation, we studied needles from mature red spruce (*Picea rubens* Sarg.) and balsam fir (*Abies balsamea* [L.] Mill.) trees along two transects from 460 to 1460 m in the White Mountains of New Hampshire. These two species, both shade-tolerant Pinaceae, are the dominant montane tree species at the northern end of the Appalachian range. Previous research has demonstrated changes in the temperature optimum for photosynthesis, cuticle thickness and epicuticular wax properties, and levels of pollution stress, for either one or both of these species along similar elevational gradients in the northeastern United States (Fryer and Ledig, 1972; DeLucia and Berlyn, 1984; Boyce and Berlyn, 1988; Boyce, McCune, and Berlyn, 1991; Berlyn et al., 1993). Moss and Rock (1991) studied reflectance from red spruce branches cut at two elevations, 790 and 960 m, and found that the red edge was generally at a lower wavelength (indicating lower chlorophyll concentrations) at 960 m than at 760 m.

Since high-elevation ecosystems are carbon limited and hence highly susceptible to stress, they can be considered "indicator ecosystems" (Berlyn et al., 1993). Thus, elucidation of the physiological differences between high- and low-elevation populations may help us to better understand the effects of abiotic stressors such as acid precipitation or other pollutants. Leaf reflectance should prove invaluable in this regard, because plant responses to stress may have spectral signatures that can be detected using remote sensing technology (Rock et al., 1986). Furthermore, elevational gradients provide researchers with convenient "natural experiments" from which we may be able to learn about plant response to global climate change (Körner, 1999).

The primary objective of the present study was to expand on previous studies by investigating elevation-related changes in reflectance measurements. We hypothesized that differences in photosynthetic capacity, pigmentation (chlorophylls for photosynthesis vs. carotenoids for photoprotection), light quality, and photoprotective mechanisms, such as increased epicuticular wax, could result in differences in reflectance characteristics between species along the elevational gradient.

MATERIALS AND METHODS

Study site—Mature red spruce (*Picea rubens*) and balsam fir (*Abies balsamea*) trees were sampled along two elevational gradients on Mt. Moosilauke (elevation 1463 m). This peak is the farthest west of the 48 four-thousand-foot (1220 m) summits in New Hampshire's White Mountains. In the mountains of the northeastern United States, spruce-fir forests extend from ~700 to 1400 m, and these two species generally represent the two most common tree species found between the lowland deciduous forests and the high-elevation alpine tundra (Cogbill and White, 1991). Although not as common at low elevation as they are higher up, spruce and fir are often found even below 460 m in this area.

Field sampling—Two transects were established on the mountain, one on the east side, generally following the Gorge Brook Trail, and one on the south side, generally following the Carriage Road Trail. Sampling was conducted in late July (east transect) and early August (south transect) of 1999. Three

trees of each species were sampled at 460, 760, 1070, 1370, and 1460 m along each transect. Treeline was taken to be the limit of closed forest, where trees still had a vertical growth habit, and was located at 1370 m. Above this was a zone of prostrate, stunted krummholz, or "elfin wood," extending to 1460 m.

In total, 60 individuals were studied. Trees were selected to be representative of mature, healthy trees at each elevation. All sampled trees were growing along the edge of the trail or adjacent to a gap, and only needles on the southeast or south-facing part of the crown were selected: in this way, only "sun needles" were obtained. At the three lowest elevation sites, an 8 m pole pruner was used to collect samples; at the two highest sites, samples could be clipped directly off the small trees using hand pruners. Both current-year (1999 flush) and previous-year (1998 flush) needles were collected. Samples were kept cool and dark and carried back down the mountain for chlorophyll fluorescence and spectral reflectance measurements.

Chlorophyll fluorescence F_v/F_m was measured on 25 different needle bunches (3–5 needles) from each needle age class using an OS-500 Modulated Fluorometer (Opti-Sciences, Tyngsboro, Massachusetts, USA). F_v/F_m was re-measured 24 h later on two sets of branches (2 elevations \times 3 trees at each elevation \times 2 needle age classes), but the difference between the measurements was not significant ($P = 0.55$). This suggests that in the time between when the branches were cut and when the measurements were completed (~2–3 h), it is unlikely that any major changes in F_v/F_m occurred.

Spectral reflectance over 306–1138 nm was measured using a UniSpec Spectral Analysis System with a 0.5 mm diameter mini-foreoptic and an internal 6.8 W halogen lamp (PP Systems, Haverhill, Massachusetts, USA). Individual needles were held in a black plastic PVC (polyvinyl chloride) leaf clip at a 60° angle relative to the foreoptic. A Spectralon reflectance standard was scanned before each needle age class. Nine different needles were scanned (each scan representing the average of four passes) for each needle age class, and scans were averaged to produce a percentage reflectance spectrum ($R_\lambda =$ leaf radiance at wavelength λ /reflectance standard radiance at wavelength λ) for each tree.

Fluorescence and reflectance parameters were measured in a cool, darkened room on dark-adapted foliage. Chlorophyll fluorescence F_v/F_m is generally measured on dark-adapted leaves because this ensures that PS II reaction centers are open and the potential efficiency of PS II can be assessed. Additionally, since certain spectral characteristics are known to change rapidly with irradiance (Gamon, Serrano, and Surfus, 1997), reflectance was measured on dark-adapted leaves in order to standardize the measurements across different field sampling days.

Leaf morphology—Branches with needles were oven-dried at 70°C so that the needles could be easily picked off each branch. Different needle age classes were not kept separate. Twenty-five needles were arranged in a 5 \times 5 grid on the glass of a flatbed scanner (model Expression 636, Epson America, Torrance, California, USA), and needles were scanned as black and white images at 59 pixels/cm (150 dpi), using a threshold setting selected to minimize edge shadows and glare. Image analysis using particle recognition routines (NIH Image, in the public domain and available free over the Internet at <http://rsb.info.nih.gov/nih-image/>) was conducted to measure the projected area and length of each individual needle. The 25 needles were weighed to 0.0001 g using an electronic balance (model ER 182 A, A+D Company, Tokyo, Japan). A total of 50 needles were measured from each tree.

Nitrogen concentrations—Oven-dried needle samples were ground to a fine powder in a small coffee grinder. Foliar nitrogen content (%N, grams nitrogen per gram oven-dry leaf tissue) was determined using a Leco CHN 600 combustion analyzer (Leco, St. Joseph, Michigan, USA). Two replicate samples were analyzed from each tree. Rye flour standards (Alpha Resources, Stevensville, Michigan, USA) were used to monitor quality control.

Reflectance indices—A variety of indices were used to characterize complex spectra and make comparisons possible between species and elevations. These indices have been derived by other authors based on knowledge of the reflectance properties of pigments and biochemical components.

TABLE 1. *P* values for effects included in analysis of variance (ANOVA) conducted on various leaf traits of balsam fir and red spruce. In a split-plot design, three trees of each species were sampled at each of five elevations along two separate transects, Mt. Moosilauke, New Hampshire, USA (see text for further details). Data were not separated by year of needle flush for either morphological traits or nitrogen status. A repeated-measures split-plot analysis was used for reflectance indices ANOVAs to test for differences between current-year and previous-year needle flushes. The transect effect (and associated interactions) were included in the model as blocking factors but *P* values are not reported below. “Linear elevation contrast” and “quadratic elevation contrast” are, respectively, hypothesis tests of the mutually orthogonal linear and quadratic contrasts obtained by treating the “elevation” effect as a continuous variable. Abbreviations: LMA = leaf mass to area ratio, %N = foliar nitrogen, percentage basis by dry mass; N g/cm² = foliar nitrogen, mass per unit leaf area basis; PRI = photochemical reflectance index; Chl NDI = chlorophyll normalized difference index; Red edge λ = wavelength λ (in nanometres) of the maximum slope of the reflectance spectrum at wavelengths between 690 and 740 nm; SIPI = structure-independent pigment index; F_v/F_m = ratio of variable chlorophyll fluorescence to maximal chlorophyll fluorescence.

	Morphological traits and nitrogen status					
	Needle area	Needle length	Needle mass	LMA	%N	Ng/cm ²
Elevation	0.0382	0.2780	0.0416	0.2869	0.3060	0.5217
Species	0.0001	0.0001	0.0002	0.0044	0.0001	0.0007
Elevation × Species	0.2345	0.3006	0.2376	0.7259	0.5875	0.0508
Linear elevation contrast	0.0065	0.0635	0.0064	0.0547	0.3924	0.2581
Quadratic elevation contrast	0.2948	0.3743	0.4236	0.8732	0.3395	0.6916
	Reflectance and fluorescence indices					
	PRI	Chl NDI	Red edge λ	SIPI	F_v/F_m	
Elevation	0.0001	0.0001	0.0001	0.0001	0.0001	
Species	0.0512	0.0001	0.0001	0.0001	0.2647	
Elevation × Species	0.5220	0.5817	0.2345	0.9204	0.3286	
Year	0.0222	0.0001	0.0001	0.0001	0.0001	
Year × Elevation	0.1857	0.0016	0.8633	0.0414	0.0179	
Year × Species	0.0416	0.0517	0.0018	0.0001	0.0012	
Year × Elevation × Species	0.3192	0.0937	0.0359	0.3410	0.6604	
Linear elevation contrast 1998	0.0255	0.0138	0.0009	0.1548	0.0055	
Linear elevation contrast 1999	0.0049	0.0067	0.0022	0.2091	0.0004	

The photochemical reflectance index, which is correlated with photosynthesis (see above), was calculated as $PRI = (R531 - R570)/(R531 + R570)$ (Gamon, Serrano, and Surfus, 1997).

A revised version of the normalized difference vegetation index, which is well correlated with and sensitive to a wide range of chlorophyll *a* concentrations, was calculated as $chl\ NDI = (R750 - R705)/(R750 + R705)$ (Gitelson and Merzlyak, 1994). This index was used because many of the other indices used for estimating chlorophyll *a* have been shown to saturate at moderate to high levels of chlorophyll; saturation does not occur with this index because R750 is insensitive to chlorophyll content (Gitelson and Merzlyak, 1994; Gitelson, Merzlyak, and Lichtenthaler, 1996). The chl NDI is similar to Carter’s (1994) stress ratio R695/R760, which is known to be sensitive to a wide variety of stress agents, except that Carter’s ratio does not have difference normalization. Through difference normalization, chl NDI can range from -1.0 to +1.0.

Total chlorophyll content is correlated with the red edge position (Curran, Dungan, and Gholz, 1990), which is the wavelength λ (in nanometres) of the maximum slope of the reflectance spectrum at wavelengths between 690 and 740 nm. Red edge λ, measured in nanometres, was determined using the first-difference spectrum, calculated as $(R_n - R_{n-1})/(\lambda_n - \lambda_{n-1})$, over this range. The first-difference spectrum measures how much reflectance changes from one wavelength to the next: it is an approximation of the slope, or first derivative, of the raw reflectance spectrum.

The structure-independent pigment index, SIPI, which is correlated with the carotenoids : chlorophyll *a* ratio (Peñuelas and Filella, 1998), was calculated as $(R800 - R445)/(R800 - R680)$.

Analysis of variance—For each of the morphological measures, foliar nutrient measures, and spectral reflectance indices, analysis of variance (ANOVA) was conducted using the generalized linear model (GLM) procedures of SAS 6.12 (SAS Institute, Cary, North Carolina, USA). A split-plot design was used to take into account the fact that both species were sampled at the same site, and hence the elevation × transect × species treatments were not applied independently (Kuehl, 2000). The two transects were taken to be independent replicates of the elevation treatment. The three trees of each species sampled

at each site were taken to be subsamples of each experimental unit. Transect was used as a blocking factor, and both transect × elevation and transect × elevation × species were specified as random effects in the model. The mean square error of transect × elevation was used to test hypotheses about the elevation factor. The mean square error of transect × elevation × species was used to test hypotheses about the species and elevation × species factors. Certain data could be separated into current-year and previous-year foliage, and for these series a repeated-measures ANOVA was used, and both the main effect of year and interaction effects with year were included in the model.

Stepwise regression—Stepwise regression procedures of Statview 5.0 (SAS Institute, Cary, North Carolina, USA) were used to look for relationships between %N, F_v/F_m , leaf mass to area ratio (LMA), and reflectance spectra. The threshold values for the partial *F*-ratio test to add (*F*-to-enter) or delete (*F*-to-remove) independent variables from the stepwise regression were set at 4.000 and 3.996, respectively (Abacus Concepts, 1992). Different regressions were run using both the original reflectance spectra and the first-difference spectra. Two-thirds of the data (two of the three trees of each species at each site) were used to calibrate the stepwise regression; predicted values for the remaining one-third of the data were used to assess the fit of each regression by the root MSE (mean squared error).

RESULTS

Needle morphology—The ANOVAs (Table 1) on the morphological traits needle area, needle length, needle mass, and leaf mass to area ratio (LMA) were all significant at *P* < 0.0001. The species was significant at *P* < 0.01 in each ANOVA. The elevation effect was significant at *P* < 0.05 in the needle area and needle mass ANOVAs. The interaction of these two factors was not significant in any of the four ANOVAs.

Across all elevations, fir needles were about twice as large

TABLE 2. Means by species and elevation for different traits of balsam fir and red spruce foliage sampled at five elevations on Mt. Moosilauke, New Hampshire, USA. Means and standard errors (SE) are least square marginal means from split-plot ANOVAs (see Table 1). For reflectance indices, 1999 designates current-year foliage, 1998 designates previous-year foliage. Abbreviations: LMA = leaf mass to area ratio; %N = foliar nitrogen, percentage basis by dry mass; N g/cm² = foliar nitrogen, mass per unit leaf area basis; Chl NDI = chlorophyll normalized difference index; Red edge λ = wavelength λ (in nanometres) of the maximum slope of the reflectance spectrum at wavelengths between 690 and 740 nm; SIPI = structure-independent pigment index.

Trait	Species	Site elevation (m)					± SE
		460	760	1070	1370	1460	
Morphology							
Needle area (cm ²)	Balsam fir	0.245	0.201	0.186	0.181	0.176	± 0.011
	Red spruce	0.098	0.085	0.094	0.067	0.076	
Needle length (cm)	Balsam fir	1.64	1.43	1.27	1.34	1.26	± 0.08
	Red spruce	0.91	0.88	0.86	0.78	0.85	
Needle mass (g)	Balsam fir	0.00543	0.00458	0.00370	0.00352	0.00343	± 0.00032
	Red spruce	0.00267	0.00213	0.00243	0.00172	0.00192	
LMA (g/cm ²)	Balsam fir	0.0223	0.0228	0.0198	0.0192	0.0195	± 0.0016
	Red spruce	0.0270	0.0248	0.0260	0.0255	0.0247	
Nitrogen status							
%N	Balsam fir	1.481	1.612	1.655	1.551	1.584	± 0.067
	Red spruce	1.121	1.175	1.103	1.074	1.230	
N g/cm ²	Balsam fir	0.0331	0.0366	0.0328	0.0297	0.0308	± 0.0008
	Red spruce	0.0299	0.0291	0.0285	0.0275	0.0303	
Reflectance indices							
Chl NDI	Balsam fir 1998	0.436	0.442	0.435	0.389	0.376	± 0.020
	Red spruce 1998	0.352	0.355	0.321	0.297	0.333	
	Balsam fir 1999	0.377	0.356	0.346	0.288	0.293	
	Red spruce 1999	0.307	0.295	0.275	0.207	0.229	
Red edge λ (nm)	Balsam fir 1998	709.5	710.6	710.0	703.5	704.6	± 0.8
	Red spruce 1998	703.5	704.0	701.3	700.2	702.9	
	Balsam fir 1999	704.6	705.1	704.0	700.8	701.3	
	Red spruce 1999	701.3	701.8	700.8	697.5	699.1	
SIPI	Balsam fir 1998	0.978	0.991	0.971	1.015	1.044	± 0.009
	Red spruce 1998	1.013	1.018	0.996	1.039	1.066	
	Balsam fir 1999	0.954	0.931	0.912	0.973	1.017	
	Red spruce 1999	1.002	1.009	0.978	1.044	1.069	

(mean ± 1 SD = 0.198 ± 0.031 cm²/needle) as spruce (0.084 ± 0.013 cm²/needle) in projected area. Fir needles from 460 m were 39% larger in projected area than those from 1460 m; spruce needles were 28% larger (Table 2). Similar patterns were evident for needle length and needle mass (Table 2). Thus fir needle morphology was consistently more plastic in response to elevation than spruce needle morphology.

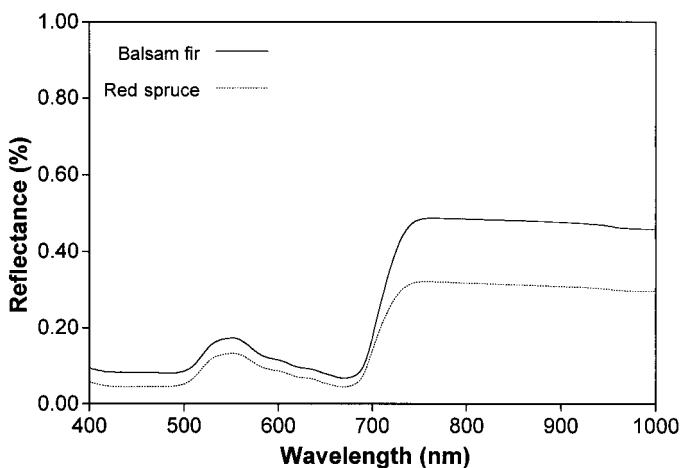


Fig. 1. Reflectance spectra of red spruce and balsam fir needles sampled at five elevations on Mt. Moosilauke, New Hampshire, USA. Spectra represent the average of all 30 trees sampled for each species.

Leaf mass to area ratio was the least plastic morphological measure, but still exhibited a decreasing trend with increasing elevation for both species. At 460 m, fir LMA was 14% higher than at 1460 m; the figure was 9% for spruce. Across all elevations, spruce LMA was higher (mean ± 1 SD = 0.026 ± 0.002 g/cm²) than fir (0.021 ± 0.002 g/cm²) (Table 2).

Foliar nitrogen—On a percentage basis by mass (%N), foliar nitrogen varied significantly between the two species ($P < 0.0001$, Table 1). On a mass per unit leaf area basis (N g/cm²), foliar nitrogen varied between the two species ($P < 0.0007$), and differences between species also appeared to vary with elevation ($P = 0.05$ for elevation × species effect, Table 1). Both nitrogen measures were higher in fir than in spruce (Table 2). However, based on the P values for both the linear and quadratic orthogonal elevation contrasts, neither %N nor N g/cm² showed any significant trends with regard to elevation (Table 2).

Across all 60 trees studied, %N was negatively correlated ($r = -0.73$) with LMA, but N g/cm² was almost perfectly uncorrelated with LMA ($r = 0.01$). LMA is an indicator of needle thickness, and thus the total amount of nitrogen per unit needle area may be independent of leaf morphology.

Reflectance spectra—Reflectance spectra varied between species (Fig. 1) and with elevation, but spectra for both species were characterized by a broad peak at 550 nm, a trough at 670 nm, a sharp increase through the red edge around 700 nm,

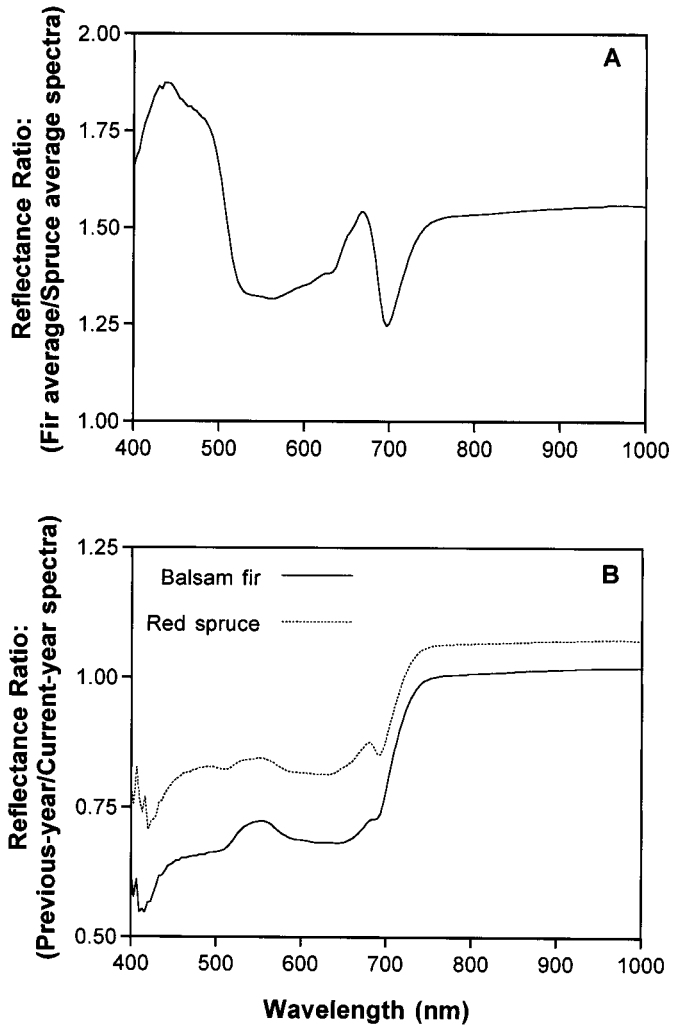


Fig. 2. Reflectance ratio spectra for red spruce and balsam fir sampled at different elevations on Mt. Moosilauke. (A) Ratio spectrum was derived by dividing the average balsam fir reflectance spectrum at each site by the corresponding red spruce reflectance spectrum and then averaging across all sites. (B) Ratio spectra were derived by dividing the previous-year reflectance spectrum for each tree by the corresponding current-year spectrum. Spectra represent the average across all 30 trees sampled of each species.

and a gently decreasing plateau above 750 nm. Averaged across all samples, fir reflectance was higher than spruce for all wavelengths.

Reflectance in the UV range (≤ 400 nm) was higher from fresh fir tissue than spruce ($P = 0.0007$) and for both species UV reflectance generally increased with increasing elevation. However, for both current-year and previous-year needles of each species, UV reflectance was lower at 1460 m than at 1370 m.

To illustrate differences between species, the ratio of fir reflectance to spruce reflectance was calculated for each sampling site using species averages. Averaged across elevations, the fir/spruce ratio spectrum exhibited peaks around 450 and 680 nm, and a plateau above 750 nm (Fig. 2A). Across the entire measured spectrum, fir needles reflected between 25 and 85% more at a given wavelength than did spruce needles.

Absorption by water at 970 nm was higher in spruce than in fir ($P < 0.0001$). There were significant differences between elevations ($P < 0.0001$), with a weak overall trend towards

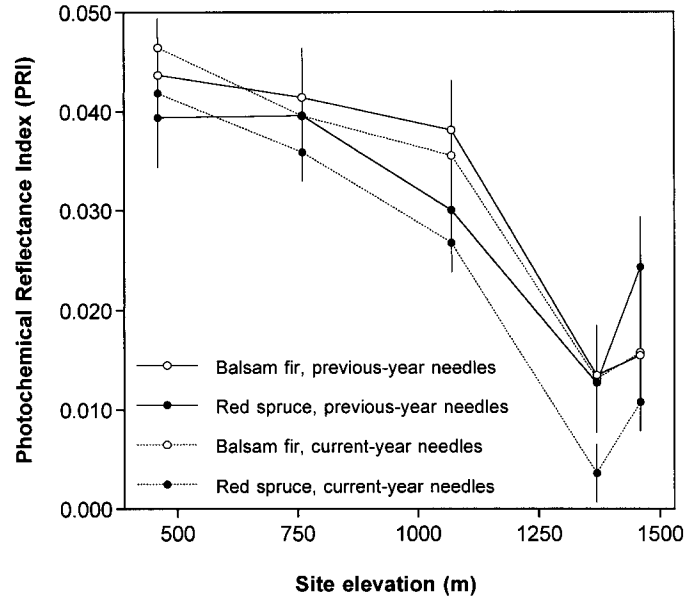


Fig. 3. Change in the photochemical reflectance index (PRI) of balsam fir and red spruce trees sampled at five different elevations on Mt. Moosilauke, New Hampshire, USA. Each point represents the least-square marginal mean calculated by repeated measures split-plot ANOVA (see text for further details). Vertical bars indicate ± 1 SE.

decreasing reflectance at 970 nm with increasing elevation. This trend was more significant for current-year foliage ($P = 0.05$) than for previous-year foliage ($P = 0.17$).

The relationship between current- and previous-year needle reflectances are best illustrated by the spectra obtained by dividing previous-year foliage reflectance by current-year foliage reflectance for each tree sampled (Fig. 2B). A previous-year/current-year ratio of 1.0 indicates that reflectance was the same for both needle age classes; a ratio > 1.0 indicates that previous-year needles had higher reflectance than current-year needles. For both species (averaged across all elevations), previous-year foliage reflected less in the visible wavelengths than current-year foliage. The difference between years was larger for fir (average 68% relative reflectance) than spruce (average 82% relative reflectance) for wavelengths ≤ 700 nm. Above 750 nm, previous-year and current-year foliage reflected similar amounts.

Reflectance indices—All ANOVAs were significant at $P < 0.0001$. The elevation effect was significant at $P < 0.0001$ in all models (Table 1). The species effect was significant at $P < 0.0001$ in three models, with PRI (species effect $P = 0.05$) the exception. The species \times elevation interaction effect was not significant in any of the four models, indicating that the response to elevation was similar for both spruce and fir. Differences between current-year and previous-year flushes were generally significant ($P < 0.05$) and varied with species for all indices.

The PRI was higher in fir than in spruce and varied significantly both with elevation and year of needle formation (Table 1). PRI decreased with increasing elevation from 460 to 1370 m. However, for both spruce and fir, and for both current- and previous-year foliage, PRI was higher at the 1460 m sites than at the 1370 m sites (Fig. 3). This overall trend is captured by

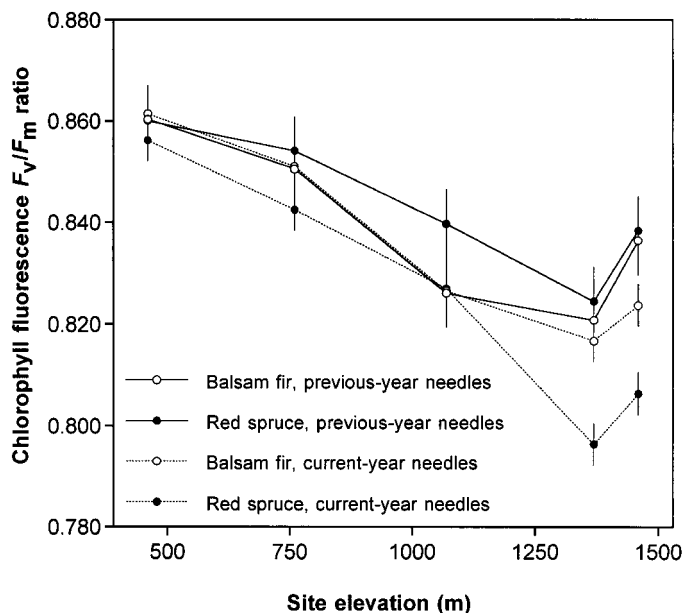


Fig. 4. Change in chlorophyll fluorescence F_v/F_m ratios of balsam fir and red spruce trees sampled at five different elevations on Mt. Moosilauke, New Hampshire, USA. Each point represents the least-square marginal mean calculated by repeated measures split-plot ANOVA (see text for further details). Vertical bars indicate ± 1 SE.

the significant ($P < 0.05$) linear orthogonal contrasts on elevation for both current-year and previous-year needle data.

Year, species, elevation, and year \times elevation were significant factors in the chl NDI ANOVA, but other interactions were not significant (Table 1). The Chl NDI was higher in fir than in spruce for both 1999 and 1998 foliage and decreased consistently with elevation up to the treeline at 1370 m (Table 2).

For both years' foliage, the red edge λ was at a longer wavelength in fir than in spruce, and for both species the red edge λ was at a longer wavelength in previous-year foliage compared to current-year foliage (Table 2). The species \times year interaction factor was significant ($P = 0.0018$, Table 1) because the difference between spruce and fir was smaller in 1999 foliage than 1998 foliage (Table 2). The red edge λ was generally located at a shorter wavelength with increasing elevation, but occurred at a slightly longer wavelength at 1460 m compared to 1370 m. This difference was significant at $P = 0.10$ (by Fisher's PLSD) for previous-year foliage but not current-year foliage. Across all 60 trees studied, the red edge λ was well correlated with %N ($r = 0.73$).

The main effects of species, year, and elevation, plus the species \times year and year \times elevation interactions, were all significant in the SIPI ANOVA (Table 1). The SIPI was higher in spruce than in fir and was higher in previous-year foliage than current-year foliage, although the difference between species was larger in current-year foliage than previous-year foliage (Table 2). The SIPI generally increased with increasing elevation for both species (Table 2), although the linear orthogonal contrasts were not significant at $P < 0.05$ for either current-year or previous-year foliage.

Chlorophyll fluorescence—The F_v/F_m ratio varied significantly between current- and previous-year foliage and with elevation, however the species effect was significant only in

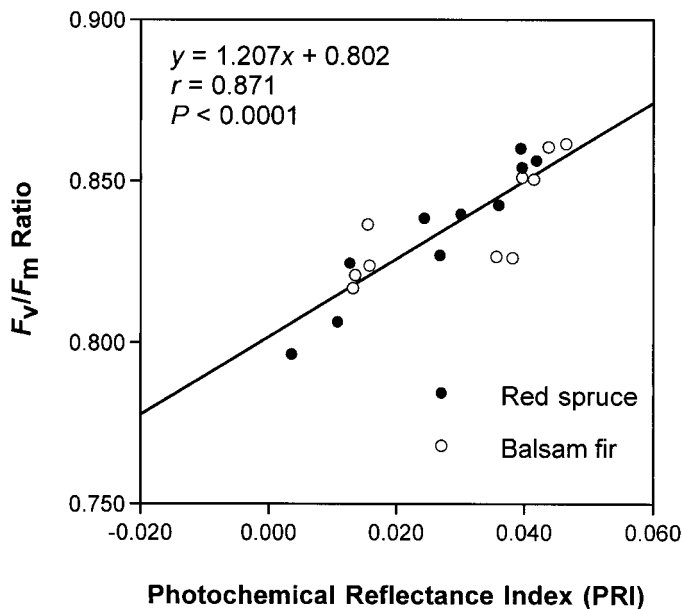


Fig. 5. Correlation between photochemical reflectance index (PRI) and chlorophyll fluorescence F_v/F_m ratio for red spruce and balsam fir foliage. Data are shown for both current-year and previous-year needles. For each species, data have been pooled by elevation.

its interaction with the year effect (Table 1). Current-year foliage had lower F_v/F_m than previous-year foliage, although the difference between years was larger for spruce than for fir and was more pronounced at higher elevations (Fig. 4, Table 1). For both spruce and fir, and for both 1999 and 1998 foliage, F_v/F_m showed a steady decrease with increasing elevation from 460 to 1370 m. In all cases, F_v/F_m was higher at 1460 m than at 1370 m (Fig. 4). F_v/F_m was well correlated with PRI ($r = 0.61$, $P < 0.0001$) when measurements for each tree and needle age class were compared. The relationship was even stronger ($r = 0.87$, $P < 0.0001$) when trees were pooled by elevation and species (Fig. 5).

Relationships between needle structure and reflectance—LMA was well correlated with reflectance at several particular wavelengths, as well as with a number of reflectance indices. The strongest correlation was with the slope of the reflectance spectrum (fresh tissue) at 755 nm: the flatter the slope at the upper "shoulder" of the red edge, the greater the mass of tissue per unit leaf area. This relationship held across species, but only weakly within species ($P = 0.20$ for spruce, $P = 0.63$ for fir). The same was true for all other correlations: within each species, the correlation was never highly significant. For these data, variation in needle structure could not be detected reliably using reflectance data.

Relationships among reflectance, %N, F_v/F_m , and LMA—Correlograms (Fig. 6) indicate that reflectance at certain wavelengths, of both the original spectra and the first-difference spectra (described in Methods section), were weakly correlated with %N. However, those wavelengths with the highest correlation to the dependent variable were not necessarily those selected into the stepwise regression, and although the correlation coefficients were higher for first-differenced data, the original spectra performed better in predicting %N. Wavelengths 651, 732, 956, 979, and 982 nm were included (five

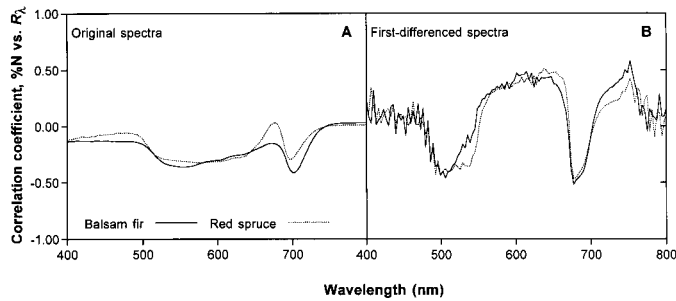


Fig. 6. Correlograms between foliar nitrogen (%N) and percentage reflectance (R_λ) of red spruce and balsam fir, for wavelengths $400 \leq \lambda \leq 800$ nm. (A) Untransformed reflectance spectra; (B) first-differenced reflectance spectra.

steps, $r = 0.930$, root MSE fitted = 0.094) in the fitted calibration equation for foliar %N (root MSE predicted = 0.163) (Fig. 7). The wavelengths selected do not correspond directly to chlorophyll or carotenoid absorption peaks. The first-differenced spectra performed almost as well (root MSE predicted = 0.165), although an entirely different set of wavelengths was selected (410, 755, 778, 794, and 868 nm). Stepwise regression did not produce a good predictive equation for F_v/F_m or LMA.

DISCUSSION

Patterns related to elevation—Trees sampled in the present study displayed similar trends with regard to elevation for both PRI and F_v/F_m . Both of these measures of radiation use efficiency progressively decreased with increasing elevation up to the treeline, but then increased in the highest-elevation krummholz site. The reversal of the declining trend above treeline in this study occurred for both species and for both current-year and previous-year foliage. This suggests that the highest-elevation krummholz trees are under less stress than those at the treeline, in agreement with chlorophyll-based indices such as chl NDI and red edge λ . DeLucia and Berlyn (1984) found an almost identical pattern in the thickness of the total cuticular layer for balsam fir on the same mountain; a similar pattern was shown for red spruce on Whiteface Mt. in the nearby Adirondacks of New York (Berlyn et al., 1993). This suggests that through some combination of adaptations (architectural, morphological, anatomical, or physiological) to the extremely harsh climate above treeline, the trees that manage to survive in the krummholz zone are actually better adapted to their environment than those trees in the presumably less-harsh climate at treeline (DeLucia and Berlyn, 1984). This could be related to the prostrate krummholz crown architecture, which might result in a more favorable microenvironment than the vertical growth form found at treeline (Körner, 1999). During the summer, wind speed is known to be greatly reduced within clumps of alpine plants (Bliss, 1962). In dwarf alpine plants, leaf/air temperature gradients of $>10^\circ\text{C}$ are not uncommon, as plants growing close to the ground effectively decouple their microenvironment from the ambient environment (Körner, 1999). Furthermore, the prostrate krummholz form greatly increases the ratio of leaf area to aboveground biomass (leaf area ratio, LAR), so the amount of photosynthetic tissue per unit of supporting tissue (branches and stem) is much higher. The krummholz form is thus a very efficient architecture when carbon is limited.

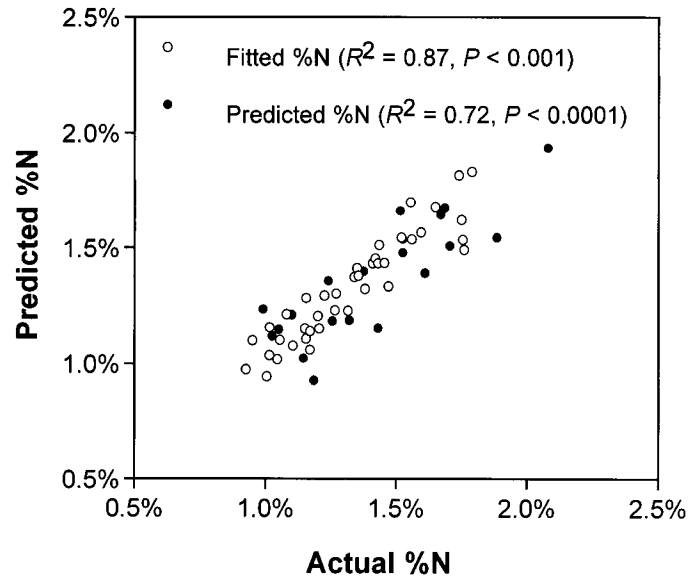


Fig. 7. Comparison of fitted and predicted values for foliar nitrogen (%N) using stepwise regression on spectral reflectance data. Data pooled for spruce and fir. Twenty individuals of each species were used to develop the predictive equation (fitted values); ten individuals of each species were used to test the equation (predicted values).

The concentration of xanthophyll cycle carotenoids relative to chlorophylls is known to increase under environmental stress (Demmig-Adams and Adams, 1996), and in the present study SIPI indicated an increase with elevation in the carotenoid:chlorophyll ratio. Long-lived foliage generally has a greater investment in photoprotective xanthophyll cycle pigments (Gamon, Serrano, and Surfus, 1997; Filella and Peñuelas, 1999), and this is probably the case with the spruce and fir studied on Mt. Moosilauke. The low PRI values at high elevations may be associated with large xanthophyll cycle pigment pools and a larger proportion of these pigments in the protective zeaxanthin and antheraxanthin states, as shown for *Rhododendron ferrugineum* (Peñuelas, Filella, and Gamon, 1995; Filella et al., 1996; Gamon, Serrano, and Surfus, 1997; Filella and Peñuelas, 1999).

Chlorophyll fluorescence is related to PS II electron transfer, and low F_v/F_m is an indicator of damage to the photosynthetic apparatus and the overall physiological health of the plant, since changes in F_v/F_m cause parallel changes in the rate of photosynthesis (Bolhar-Nordenkamp et al., 1989; Krause and Weis, 1991). Measured on dark-adapted leaves, the F_v/F_m parameter is an indicator of the potential photochemical efficiency of PS II and the quantum yield (Ball et al., 1994). There are, therefore, direct relationships between xanthophyll cycle dissipation of excess radiation, chlorophyll fluorescence, and photosynthetic efficiency. As with chlorophyll fluorescence, the excess radiation that is dissipated by the xanthophyll cycle is not available for photosynthesis, and hence photosynthetic efficiency is low when PRI is low (Demmig-Adams and Adams, 1996). Declines in F_m have been observed concurrently with increases in the concentration of zeaxanthin (Demmig-Adams et al., 1989), so although measured in very different ways, the parallel changes in PRI and F_v/F_m are to be expected because the energy dissipation processes are complementary.

Therefore, stress-related reductions in PS II efficiency may be smaller in the stunted alpine krummholz at 1460 m than at

the 1360 m treeline. Although climatic conditions increase steadily in severity with increasing elevation, there appears to be a physiological inflection point at 1360 m such that those trees that are able to survive past the limit of continuous forest are under less stress than those at the treeline, some 90 m lower. Alpine plants have maximal rates of photosynthesis at least equal to lowland plants (Körner, 1999), and so the generally decreasing rates of efficiency with increasing elevation suggested by the present study and Filella and Peñuelas (1999) are somewhat surprising. A possible explanation is that herbaceous obligate alpine plants have high rates of photosynthesis, whereas high-elevation ecotypes of woody plants generally do not share this adaptation.

Chlorophyll indices—Moss and Rock (1991), also studying red spruce on Mt. Moosilauke, found that there was an excellent correlation ($R^2 = 0.87$) between total chlorophyll content and red edge λ ; this has also been reported for other species (Curran, Dungan, and Gholz, 1990; Vogelmann, Rock, and Moss, 1993; Gitelson, Merzlyak, and Lichtenthaler, 1996). However, the values Moss and Rock (1991) present for red spruce red edge λ (generally in the range 710–725 nm) are higher than those found in the present study. This shift to shorter wavelengths of the red edge could be due to atmospheric pollution, heavy metals, or other stress agents and probably indicates abnormally low chlorophyll levels (Rock et al., 1986, 1994). Moss and Rock (1991) reported that a single acidic cloud event (pH 2.7) caused a significant decrease in the red edge λ at their high-elevation site; other authors have demonstrated decreases in red edge λ under a variety of stress factors (Carter, 1993; Vogelmann, Rock, and Moss, 1993). Both red edge λ and chl NDI indicated similar trends, i.e., decreasing chlorophyll (indicative of increasing stress) with increasing elevation up to 1370 m. Furthermore, these indices indicate that the spruce on Mt. Moosilauke may be experiencing more stress (very broadly defined) than fir. This is in agreement with the recent studies of spruce decline across the northeastern United States (Eagar and Adams, 1992). However, because the physiological response to stress appears to be similar for numerous stress agents, it is therefore impossible to identify any one stressor (e.g., pollution, acid precipitation, or climatic stress) as the cause without further research (Carter, 1993).

Spectral differences between species and needle age classes—The generally increasing trend in UV reflectance with elevation suggests that these species may be adapted to reduce the damaging effects of UV radiation at high elevations. Higher UV reflectance by fir may also increase its future competitiveness relative to spruce: increased UV levels due to thinning of the ozone layer could have especially negative consequences for spruce. However, Filella and Peñuelas (1999) suggested that increases in UV-B absorbing compounds (flavonoids and anthocyanins) located in the epidermal cells, as well as increased leaf thickness, may be the most important modes of protection against UV radiation, rather than increased reflectance. Although thickness was not measured directly for the spruce and fir needles in this study, the negative correlation between LMA and elevation might indicate that needles are actually thinner at high elevations. This contradicts the general pattern of leaf thickness increasing along elevational gradients (Stover, 1944; Filella and Peñuelas, 1999) and thick leaves in obligate alpine plants (Körner, 1999), but sug-

gests that these conifers are not using increased thickness as a strategy to reduce UV damage.

Absorption by water is the main determinant of spectral characteristics in the 700–1300 nm range, although internal structure may also be important, and differences in reflectance spectra between species have been attributed to foliar anatomy (Carter, 1991). The amount of intercellular air space, the spacing and arrangement of mesophyll parenchyma, and the degree of lignification of epidermal cell walls are thought to be especially significant anatomical factors (Peñuelas et al., 1993; Rock et al., 1994). Changes in these structural features occur with needle development, which may explain some of the differences shown by this study with regard to previous-year (fully mature) vs. current-year (developing) needles. In addition, cellular damage associated with the “spruce decline” is known to increase with needle age, and this damage will have effects on needle reflectance (Rock et al., 1986; Vogelmann and Rock, 1988; Moss et al., 1998). Rock et al. (1986) attributed some stress-related near-infrared reflectance changes to cellular damage that affects the refractions occurring at cell wall–water–air interfaces. Somewhat higher reflectance above 700 nm in previous-year spruce needles (Fig. 2B) may be the result of increased cellular damage in older needles.

The previous-year/current-year ratio spectra (Fig. 2B) are shaped differently from those presented by Rock et al. (1994). Most noticeably, the ratio spectra from the present study fail to exhibit a reflectance ratio >1.0 around 660–690 nm, because previous-year reflectance in the “chlorophyll well” was in fact some 20–30% lower than current-year reflectance. In contrast to our results, Rock et al. (1994) found that reflectance in the chlorophyll well was higher in previous-year needles, resulting in a narrow but definite peak in the ratio spectra from 660–690 nm. Despite this difference in the ratio spectra, the two chlorophyll-based indices, chl NDI and red edge λ , indicate that the previous-year needles we studied had higher chlorophyll contents than current-year needles. This is actually in agreement with Rock et al. (1994), who used pigment extraction techniques and found that total chlorophyll content increased from current-year to previous-year needles. The maximum depth of the chlorophyll well appears, therefore, to convey little information about leaf chlorophyll contents.

Rock et al.’s data indicated that reflectance in the blue–green wavelengths (450–510 nm) is higher from previous-year needles than current-year needles, and they suggest this may be due to increases in the amount of epicuticular wax with increasing needle age. The data from the present study show that reflectance in these wavelengths is lower for previous-year needles than for current-year needles. This may be due to pigmentation differences (carotenoids and chl) or abrasion and consequent removal of surface waxes by snow and ice crystals during the first winter following leaf expansion (Boyce and Berlyn, 1988; Hadley and Smith, 1989). Previous-year needles appeared to have a less prominent glaucous bloom than the current-year needles. Wax abrasion can result in increased rates of cuticular water loss or increased foliar wettability, although cuticle thickness may increase slightly in the second year (DeLucia and Berlyn, 1984; Boyce and Berlyn, 1988).

Stepwise regression—Other studies have used either partial least squares regression (PLS) or stepwise regression to fit equations relating spectral data (which may be transformed or differenced) with biochemical and anatomical data (Card et al., 1988; Peterson et al., 1988; Wessman et al., 1988; Curran,

Dungan, and Gholz, 1990; Lacaze and Joffre, 1994; Yoder and Pettigrew-Crosby, 1995; Bolster, Martin, and Aber, 1996; Ourcival, Joffre, and Rambal, 1999). In some cases, results can be as accurate as those obtainable by traditional wet chemical methods, although dried and ground plant material has generally been used for such analyses.

The stepwise regressions developed in this paper were able to estimate foliar %N using reflectance from fresh tissue but performed poorly with regard to F_v/F_m and LMA. Other researchers have found it necessary to include near-infrared wavelengths to obtain satisfactory regressions: visible wavelengths alone are normally insufficient (Card et al., 1988; Lacaze and Joffre, 1994; Ourcival, Joffre, and Rambal, 1999). This is probably because the organic bonds in leaf biochemical components exhibit vibrational stretching modes that absorb radiation at frequencies in the middle-infrared wavelengths (Card et al., 1988; Peterson et al., 1988). Without near-infrared data, the weaker harmonics of these bonds (in the visible wavelengths) must be relied upon. The present study spanned wavelengths from 300 to 1100 nm, and therefore accuracy might have been improved if longer wavelengths were included. Another factor is that individual wavelengths in the present study were not nearly as well correlated with %N as in other studies (Lacaze and Joffre, 1994; Ourcival, Joffre, and Rambal, 1999). However, the stepwise regression procedure selected similar wavelengths (730 and 980 nm) as reported by other authors for nitrogen estimation (Yoder and Pettigrew-Crosby, 1995).

Conclusions—Gamon and Qiu (1999) point out that one reason that ecologists are hesitant to make greater use of remote sensing is the “fuzzy” nature of data measured from great distances. Therefore, understanding the leaf-level differences in reflectance spectra between different ecotypes or phenotypes of the same species is vital if remotely sensed canopy level data are to be used to understand process and function at scales from leaf level to ecosystem level. First we must understand these differences at the leaf level, using data obtained on the ground. The data presented in this paper give convincing evidence that differences between species and among populations of a species along an elevational gradient can be detected using spectral reflectance data and that these differences can be interpreted in relation to altitudinal plant biology.

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