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Seasonal Photoprotective Responses in Needles of Korean Fir (*Abies koreana*) over an Altitudinal Gradient on Mount Halla, Jeju Island, Korea

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Abstract

Chloroplast pigments and chlorophyll fluorescence were characterized in needles of Korean fir (*Abies koreana*) in summer, winter, and spring at three altitudes on Jeju Island, Korea. High light-harvesting efficiency (intrinsic photosystem II efficiency) and indirect evidence for high photosynthetic rates (high levels of β -carotene and chlorophyll *b*) during the growing season contrasted with mid-winter downregulation of light-harvesting efficiency involving retention of high zeaxanthin levels and locked-in photoprotective thermal dissipation (from low chlorophyll fluorescence emission). Neoxanthin levels were inversely correlated with sustained photoprotection in the winter, and lutein to xanthophyll cycle carotenoid levels decreased from summer to winter, suggesting that zeaxanthin plays the prominent role in winter photoprotection of Korean fir needles. Summer was apparently most conducive to photosynthesis, consistent with high levels of summer precipitation on Jeju Island, and in contrast to fir and other conifers in a climate with dry summers at high altitudes in Colorado, U.S.A., where studies have shown that the wet spring is the season most favorable for photosynthesis. Lastly, despite there being only 300 m difference in altitude among the three sites, there were discernible differences in (i) accumulation of zeaxanthin in winter (as an indicator for the severity of conditions, with highest levels at the highest altitude), (ii) apparent photosynthesis rates in summer (from β -carotene levels, with highest levels at the highest altitude), and (iii) transition to increased photosynthesis in spring (from fluorescence emission levels, slowest at the highest altitude).

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Introduction

Plants at high altitude are exposed to severe environmental conditions, including large diurnal temperature fluctuations, low temperatures, and high irradiance levels, all of which can impact growth and photosynthesis. Given the evergreen nature of their needles, conifers must employ mechanisms to protect their photosynthetic apparatus during severe winters and recover their capacity for photosynthesis during the subsequent spring (Yamazaki et al., 2003; Zarter et al., 2006a, 2006b). Several photoprotective mechanisms have been invoked as preventing photodamage of the photosynthetic apparatus in overwintering evergreens, including (i) enhanced cyclic electron transport around photosystem I (Huner et al., 1988; Ivanov et al., 2001; Öquist and Huner, 2003), (ii) degradation of key photosystem II proteins (D1 and the oxygen evolving complex) to inhibit superoxide formation (Ottander et al., 1995; Adams et al., 2004, 2006; Zarter et al., 2006a, 2006b, 2006c), and (iii) sustained engagement of the carotenoids zeaxanthin and antheraxanthin in continuously high levels of photoprotective energy dissipation, resulting in sustained low PSII efficiency, during the winter (Adams and Demmig-Adams, 1994, 1995; Adams et al., 1995, 2002, 2004, 2006; Verhoeven et al., 1996, 1998; Adams and Barker, 1998). It has also been reported that, in zeaxanthin-free isolated major light-harvesting complexes (LHCII), neoxanthin (Dall'Osto et al., 2007; Iliaia et al., 2011; Zubik et al., 2011) and lutein (Iliaia et al., 2011; Jahns and Holzwarth, 2012; Wahadoszamen et al., 2012) are able to quench fluorescence and thus facilitate

thermal energy dissipation (Iliaia et al., 2011; Ruban et al., 2012). For lutein, further support for a possible involvement in thermal energy dissipation has been provided under physiologically relevant conditions in species containing lutein epoxide that is de-epoxidized to lutein in response to excess light (Matsubara et al., 2008; Esteban et al., 2008, 2010; Förster et al., 2011). Some authors have concluded that neoxanthin and lutein are the dissipators of excess energy in LHCII, but others have cautioned that neoxanthin and lutein might only act as dissipators when zeaxanthin is not present (Liao et al., 2010). While field studies are not able to dissect molecular mechanisms, the correlations and natural acclimation trends found in the present evaluation can nevertheless provide some useful direction for molecular studies.

Jeju Island is located about 100 km from the southernmost tip of the Korean Peninsula and is biogeographically regarded as a subtropical or temperate rain forest area. However, the presence of Mount Halla (33°22'29"N, 126°31'53"E; a peak of 1950 m), in the center of the island, provides a wide range of climatic conditions, especially with regard to temperature. Consequently, the vegetation of Jeju Island is vertically categorized from low to high altitude into warm-temperate evergreen forest zone, temperate deciduous forest zone, and subalpine coniferous forest zone, depending on altitude, aspect, and distance from the coast. Within the subalpine coniferous forest, distributed from 1300 m to the summit of Mount Halla, Korean fir (*Abies koreana* Wilson) colonizes intensively on the northern and eastern slopes of the mountain and also on the northern and eastern slopes of the valleys, oreums (volcanic cones unique to Jeju Island), and other geographical features on

the southern and/or western slopes of the mountain. Korean fir is an endemic species in Korea (Lee, 1986).

It has been proposed that the upper part of the subalpine coniferous forest zone, from 1800 m to the summit of Mount Halla, is similar to an alpine zone climatologically (Kong, 2007). Therefore, near the summit of the mountain, trees might be exposed to harsher winter conditions, such as low temperatures, high irradiance, and strong winds. Tree height/crown width ratio and needle length/needle width ratio of Korean fir decreased with increasing altitude on Mount Halla (Kang et al., 1990). The latter report suggests that needles of Korean fir might be exposed to harsher conditions with increasing altitude where Korean fir grows, particularly during the winter season. Although many studies have been conducted on photoinhibition, or sustained photoprotection, in evergreen leaves or needles resulting from an interaction of high irradiance and low temperature (Adams and Demmig-Adams, 1994, 1995; Adams and Barker, 1998; Adams et al., 1995, 2001, 2002; Verhoeven et al., 1996, 1998, 1999; Ebbert et al., 2005; Zarter et al., 2006c), few studies have examined the effects of high altitude on conifer needles during harsh winter conditions (Yamazaki et al., 2003; Zarter et al., 2006a, 2006b; Koh et al., 2009).

Materials and Methods

STUDY SITES

This study was conducted on trees from three sites on Mount Halla of Jeju Island, off of the southern tip of the Korean Peninsula. All study sites were located on the eastern slope of Mount Halla: Site A (33°21'58.3"N, 126°33'29.5"E; 1500 m) in Jindallae near the lower limit of the distribution of Korean fir, Site B (33°21'42.7"N, 126°32'58.0"E; 1671 m) in the center of Korean fir vegetation in a subalpine forest, and Site C (33°21'32.8"N, 126°32'30.4"E; 1800 m) near the summit of Mount Halla.

PLANT MATERIAL

The three populations of Korean fir (*Abies koreana* Wilson) were studied at monthly intervals from June 2010 to May 2011. The needles and shoots were collected at midday (13:00 to 15:00) from the south-facing 1.3- to 1.5-m-height branches of the trees that received direct solar light during most of the day. For immediate *in situ* measurement of chlorophyll fluorescence, needles that had developed in spring 2010 were sampled from 5 trees at each site. For pigment analysis in the laboratory, the shoots were collected from 5 trees at each site, frozen under dry ice in the dark, and brought to the laboratory. The needles were detached from the shoots and stored at -80°C until pigment analysis.

ENVIRONMENTAL PARAMETERS

Temperature and precipitation data collected by the Jindallae Weather Station (33°21'58.3"N, 126°33'29.5"E; 1500 m), which is located near Site A, were obtained from the Jeju Regional Meteorological Administration.

CHLOROPHYLL FLUORESCENCE MEASUREMENTS

Chlorophyll fluorescence was measured immediately after collection at the study sites. The needles were collected at midday

(13:00 to 15:00) and aligned closely side-by-side on gas-permeable tape. Subsequently, the needles were dark adapted for at least 15 min using leaf clips. Following dark adaptation, the fluorescence induction kinetics was measured with a portable fluorometer, Plant Efficiency Analyzer (PEA; Hansatech Ltd., King's Lynn, Norfolk, England), in a fast kinetics mode with $1500\ \mu\text{mol photons m}^{-2}\text{s}^{-1}$ of actinic light. The fluorescence data and chlorophyll fluorescence induction kinetics (OJIP transient; Strasser et al., 2000) recorded by the PEA were processed by the software Biolyzer 3.0 (R. M. Rodriguez, Bioenergetics Lab., Geneva, Switzerland). Measurement of the dark-adapted PSII efficiency F_v/F_m (as opposed to F_v'/F_m' in the light) allows for an assessment of the level of dark-sustained photoprotective energy dissipation that may be locked-in under conditions of stress (Adams and Demmig-Adams, 2004; Adams et al., 2006).

DETERMINATION OF PIGMENT COMPOSITION BY HPLC

To extract the pigments, 0.1 g of needles was frozen in liquid nitrogen and ground with a mortar and pestle in ice-cold 1 mL DMF. The homogenate was centrifuged at $10,000\times g$ for 10 min, and the supernatant was then passed through a $0.2\ \mu\text{m}$ membrane filter. Pigments were determined according to the method of Thayer and Björkman (1990), with some modifications. Twenty-five μL of extract were injected into the HPLC system (Flexar FX-10; Perkin Elmer, Inc., Waltham, Massachusetts, U.S.A.) with a Waters Spherisorb ODS-1 column ($4.0 \times 250\text{ mm}$). A mobile solvent mixture of acetonitrile:methanol:0.2 M Tris-HCl (pH 8.0) (72:12:7, v/v/v) was eluted for 3 min to allow the column to equilibrate. A linear gradient from mobile solvent to gradient solvent containing methanol:hexane (4:1, v/v) was used for 10 min, followed by isocratic elution with gradient solvent for 7 min. This was followed by a 2-min linear gradient from gradient solvent to mobile solvent and isocratic elution with mobile solvent for 3 min to allow the column to re-equilibrate with mobile solvent prior to the next injection. All pigments were eluted from the column within about 15 min at a flow rate of 2 mL/min. The eluted pigments were monitored at 440 nm. Pigment concentrations were estimated by using factors for converting peak area to nanomoles, as determined for this solvent mixture by Thayer and Björkman (1990).

Needle pigment contents are only shown for July (summer), February (winter), and April (spring transition) as key months where in-depth analysis revealed significant differences among the dates and three field sites. Pigment contents are expressed on a fresh weight basis as well as relative to each other. Needles in February had a significantly lower water content compared to needles in July (fresh weight to dry weight ratio of 2.56 ± 0.07 versus 2.91 ± 0.05 [mean \pm standard deviation, $n = 3$], respectively), whereas the fresh to dry weight ratio of needles in April (2.76 ± 0.11) was not significantly different from those in July and February. Thus, pigment comparisons on a fresh weight basis would have differed by no more than 10% among the different dates due to difference in needle water content. β -carotene is associated with the core proteins of the photosystems (Yamamoto and Bassi, 1996) and is therefore used as a proxy for photosynthetic rates.

Statistical analysis was performed using the SPSS statistical package version 7.5 (SPSS Inc., Chicago, Illinois, U.S.A.). One- and two-way analyses of variance (ANOVA), with/without subsequent Duncan's multiple range tests, were used to test for study site effects on chlorophyll fluorescence and for the effects and interactions of growth season and study site on pigment contents, respectively. Linear or non-linear regression analysis was performed by a process of trial and error for fitting the relationships between chlorophyll *b* level and the levels of the various xanthophylls and β -carotene.

Results

SEASONAL TEMPERATURE AND PRECIPITATION PATTERNS

Total annual precipitation between June 2010 and May 2011 was 5488 mm, with 4793 mm falling between March and September (Fig. 1, part A). These latter months also encompassed the active growth period for Korean fir (personal observation). The three months with the greatest precipitation were June, July, and August (Fig. 1, part A), during which the highest temperatures of the year, consistently in the low to mid-20s °C range, were also recorded (Fig. 1, part B). In contrast, between October 2010 and March 2011, temperatures were lower and precipitation accounted for no more than 13% of total annual precipitation (Fig. 1, part A). From late December 2010 until early February 2011, maximum air temperature remained below 0 °C and minimum air temperature remained continuously above 0 °C beginning in early May (Fig. 1, part B).

SEASONAL CHANGES IN PSII CHLOROPHYLL FLUORESCENCE CHARACTERISTICS

Intrinsic (dark-adapted) PSII efficiency (F_v/F_m) was at maximal levels during the summer months, then decreased in the fall and remained below 0.4 from January until March (Fig. 1, part C). As temperatures increased in spring, F_v/F_m rose and reached high values by May (Fig. 1, part C). The seasonal changes in intrinsic F_v/F_m were paralleled by changes in absolute chlorophyll fluorescence yield at both the F_m (Fig. 1, part D) and F_o (Fig. 1, part E) level. The low levels of intrinsic PSII efficiencies in the winter were accompanied by the strongest quenching of F_m and F_o (Fig. 1).

In addition to steady state F_o and F_m levels, chlorophyll *a* fluorescence OJIP transients (following the entire transition of fluorescence emission increases from a state of open to largely closed PSII reaction centers) were also measured in July, February, and April for trees from all three altitudes (Fig. 2, parts A–C). OJIP transients of needles exhibited changes depending on growth season. The O levels and P plateaus exhibited high fluorescence emission in July, very low fluorescence emission in February, and intermediate levels in April. In April, needles from the three different altitudes exhibited differences in OJIP transients (corresponding to similar significant trends for differences in F_o and F_m levels; Fig. 1), with the highest P plateaus for the trees at the lowest altitude (1500 m) and the lowest P plateaus for trees from the highest altitude (1800 m) (Fig. 2, part C). The transitions from the intermediate phases O–J (thought to reflect reduction of the PSII reaction center's electron acceptor Q_A) as well as those from J–I (thought

to reflect reduction of the subsequent electron acceptor Q_B) were altered in proportion with each other and concomitant to the depressions in P plateaus for all seasons and all altitudes.

SEASONAL CHANGES IN PIGMENT COMPOSITION

In summer (July), total chlorophyll (Fig. 3, part A) and chlorophyll *b* levels (Table 1) were significantly higher on a fresh weight basis compared to either winter (February) or the spring transition month of April (with no significant differences between the latter two months). Chlorophyll *a/b* ratios were lowest in the summer, intermediate in the spring, and highest in the winter, consistent with the preferential enrichment in chlorophyll *b* in the summer (Table 1). There were no differences in chlorophyll characteristics among the three altitudes on any of the dates examined.

Compared to the summer (July), the winter (February) and spring (April) levels of β -carotene and neoxanthin were lower, the pools of the xanthophyll cycle carotenoids ($V + A + Z$) were higher, and the level of lutein was not, for the most part, significantly different (Table 2). Table 3 shows the levels of β -carotene and all xanthophylls relative to total chlorophyll levels (on a chlorophyll *a + b* basis), revealing similar trends as seen on a fresh weight basis, with neoxanthin and β -carotene levels being lowest, $V + A + Z$ pools being highest, and lutein exhibiting little difference in winter compared to the summer. In particular, the level of zeaxanthin increased strongly in the winter on both a fresh weight and on a $V + A + Z$ basis (Table 2), as well as on a chlorophyll *a + b* basis (Table 3). No zeaxanthin was present in these leaves (after some dark adaptation) in the summer, and retained zeaxanthin levels were quite low in spring compared to winter. During winter, there were also significant differences in zeaxanthin content across the three altitudes, with lower zeaxanthin levels at 1500 m compared to the higher altitudes (Fig. 4; see also Tables 2 and 3). Somewhat contrasting trends to that seen in zeaxanthin were exhibited by β -carotene levels: β -carotene levels were (i) highest in the summer and lowest in the winter, and (ii) higher in the summer for the trees at the highest altitude versus one or both of the lower altitudes (Tables 2 and 3).

Figure 3 offers, in addition to the comparison of total chlorophyll levels as discussed above, comparisons of the ratios of $Z + A$, neoxanthin, and lutein to the total $V + A + Z$ pool across seasons and altitudes. The level of $(Z + A)/(V + A + Z)$ exhibited a markedly different pattern from the other three parameters shown in Figure 3. The conversion state of the xanthophyll cycle pigments, expressed as $(Z + A)/(V + A + Z)$, was above 0.9 in winter (February) and below 0.4 in both summer (July) and during the spring transition month of April (Fig. 3). In contrast, the relative levels of neoxanthin and lutein to $(V + A + Z)$ exhibited overall trends similar to that in chlorophyll *a + b* on a fresh weight basis, with higher levels in the summer and significantly lower levels in both winter and spring, and no significant trends with altitude.

Lastly, Figure 5 presents relationships between chlorophyll *b* levels, as the chlorophyll parameter showing the greatest change over the seasons, and levels of the various xanthophylls and β -carotene. These relationships clearly establish that neoxanthin content is positively and linearly correlated with chlorophyll *b* content (Fig. 5, part A), as is β -carotene (Fig. 5, part B). In contrast, lutein shows little change with chlorophyll *b* content, i.e. as chloro-

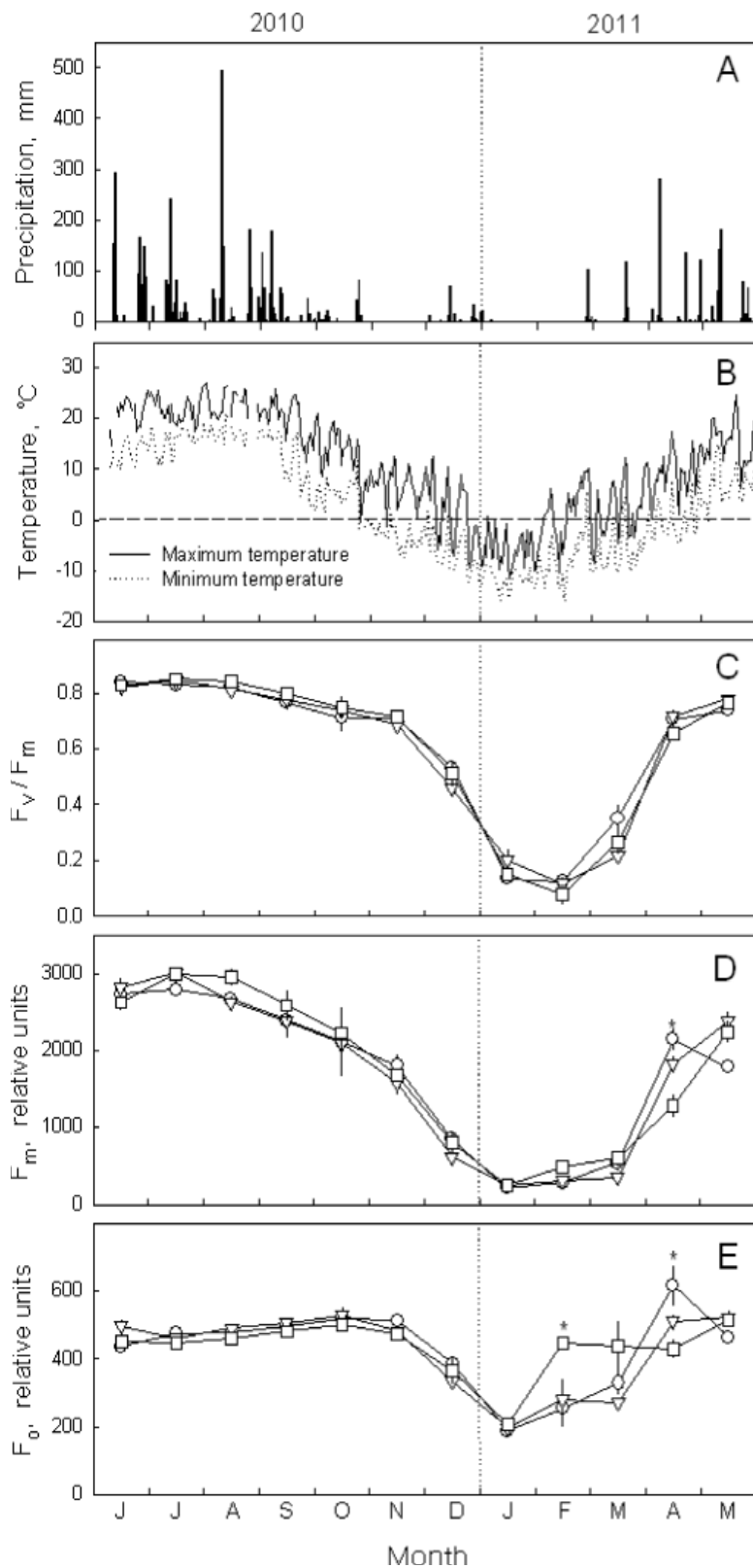


FIGURE 1. (A) Precipitation and (B) maximum and minimum air temperature at the Jindallaebat Weather Station, Mount Halla, which is located near Site A (at 1500 m), and midday characterization of chlorophyll fluorescence (F_v/F_m in C, F_m in D, F_o in E, dark-adapted for a minimum of 15 min) from needles of Korean fir growing at different altitudes (1500 m, \circ ; 1671 m, ∇ ; 1800 m, \square) from June 2010 through May 2011. The vertical dotted line denotes the transition from 2010 to 2011. Each value in C, D, and E represents the mean \pm standard deviation of 5 different trees. Significantly different values (evaluated by one-way ANOVA test, $p < 0.01$) among each altitude are marked by an asterisk.

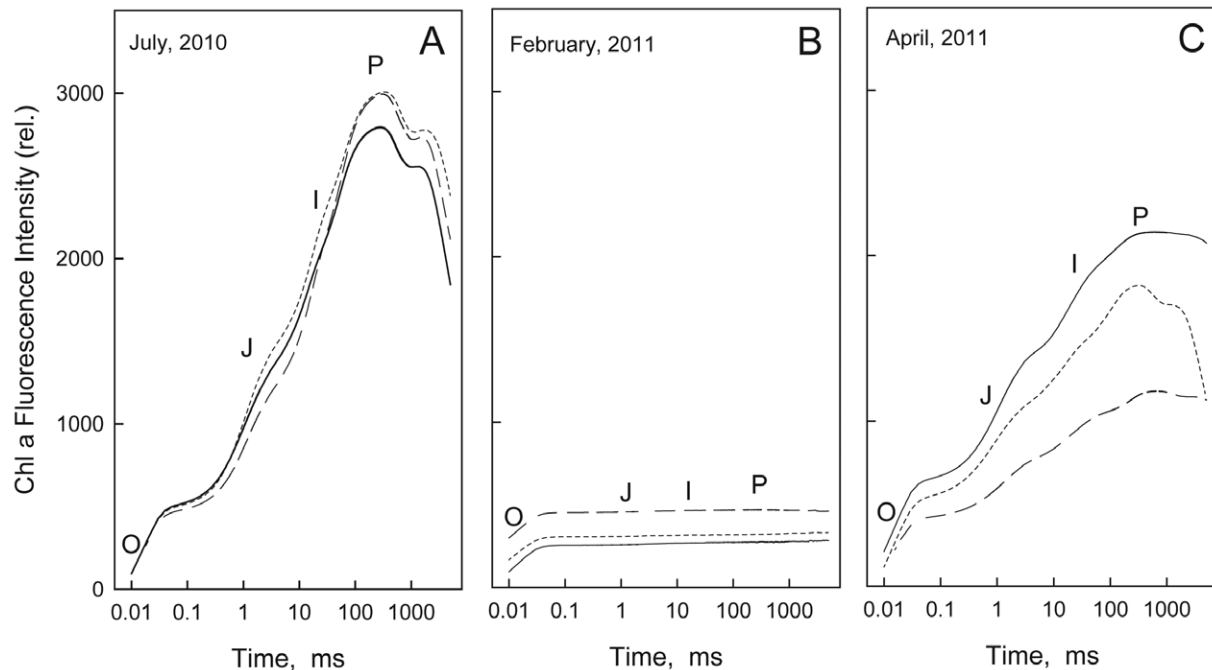


FIGURE 2. Characterization of the Chl *a* fluorescence transient O-J-I-P from needles of Korean fir trees growing at the altitudes of 1500 m (---), 1671 m (.....), and 1800 m (—) in (A) July, (B) February, and (C) April. Each transient represents the mean of five separate measurements.

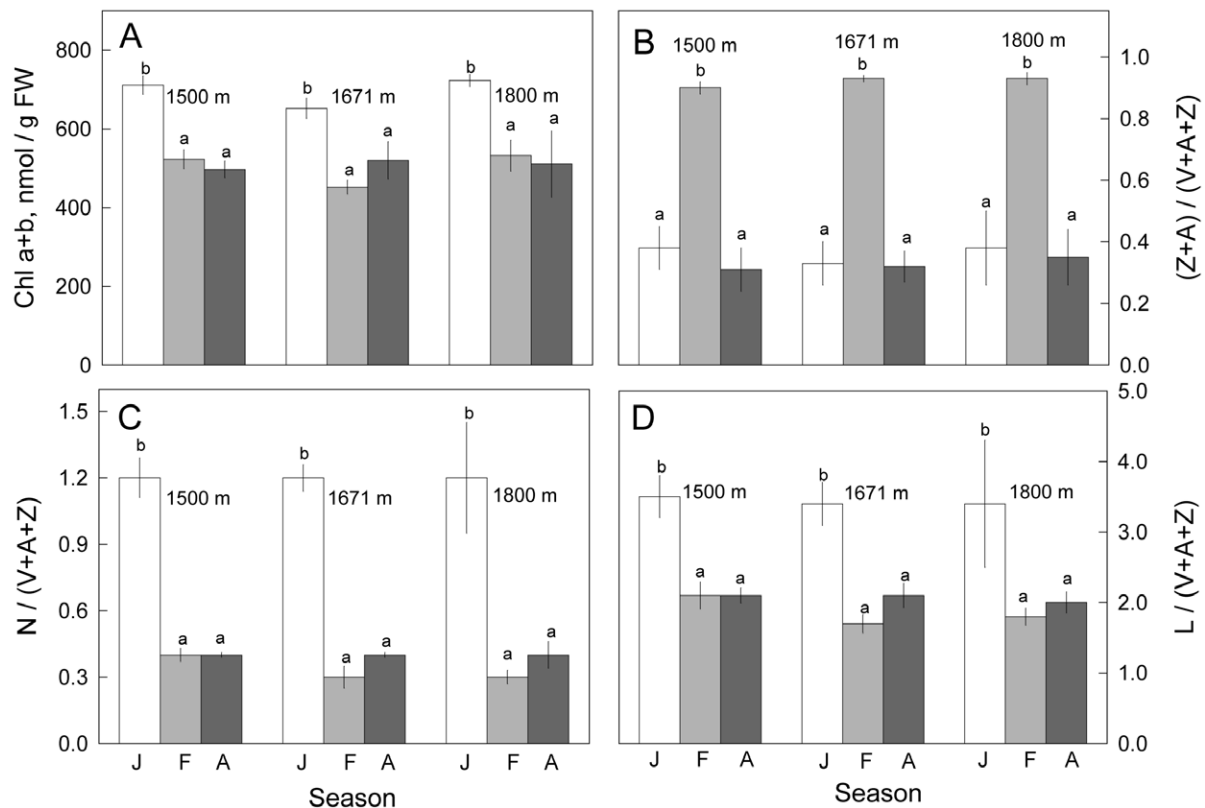


FIGURE 3. (A) Total Chl *a+b* relative to needle fresh weight, (B) the conversion state of the xanthophyll cycle, (C) neoxanthin relative to the total xanthophyll cycle pool, and (D) lutein relative to the total xanthophyll cycle pool in needles of Korean fir growing at three altitudes and collected during three seasons (July = J, February = F, or April = A). V = violaxanthin, A = antheraxanthin, Z = zeaxanthin, N = neoxanthin, and L = lutein. Each column and error bar represent the mean \pm standard deviation of three different trees. Significant differences (determined by two-way ANOVA and Duncan's multiple range test, $p < 0.05$) among the means are indicated by the different letters.

TABLE 1

Chlorophyll contents on a fresh weight (FW) basis and chlorophyll *a*/chlorophyll *b* ratio from needles of Korean fir trees growing at three altitudes on Mount Halla and collected during three seasons. Each value represents the mean \pm standard deviation of three different trees. Within each column, significantly different values (evaluated by two-way ANOVA and Duncan's multiple range test, $p < 0.05$) are indicated by the different letters.

Altitude (m)	Month	Chl <i>a</i>	Chl <i>b</i>	Chl <i>a/b</i> ratio
		nmol/g FW		
1500	Jul	410 ± 1 ^b	301 ± 25 ^{bc}	1.38 ± 0.12 ^a
	Feb	381 ± 14 ^{ab}	142 ± 11 ^a	2.70 ± 0.13 ^c
	Apr	343 ± 13 ^{ab}	154 ± 8 ^a	2.24 ± 0.03 ^{bcd}
1671	Jul	395 ± 14 ^{ab}	257 ± 13 ^b	1.54 ± 0.05 ^a
	Feb	325 ± 20 ^a	127 ± 4 ^a	2.57 ± 0.22 ^{de}
	Apr	357 ± 36 ^{ab}	163 ± 12 ^a	2.18 ± 0.11 ^{bc}
1800	Jul	409 ± 4 ^b	313 ± 19 ^c	1.32 ± 0.09 ^a
	Feb	380 ± 27 ^{ab}	152 ± 13 ^a	2.51 ± 0.07 ^{cde}
	Apr	336 ± 50 ^{ab}	175 ± 34 ^a	1.95 ± 0.08 ^b

phyll *b* levels declined, lutein remained constant regardless of chlorophyll *b* content (Fig. 5, part C). However, V + A + Z shows an inverse relationship with chlorophyll *b* content, becoming highly enriched relative to chlorophyll *b* as chlorophyll *b* content declined (Fig. 5, part D).

Discussion

The sun-exposed needles of Korean fir growing on Mount Halla exhibited adjustments in chloroplast pigment composition and chlorophyll fluorescence characteristics indicative of acclimatory restructuring of the photosynthetic apparatus over the course of the year—with greatest light harvesting potential in the summer

(higher chlorophyll content, decreased Chl *a/b* ratio, and high intrinsic PSII efficiency) and pronounced employment of photoprotective energy dissipation in the winter (increased total xanthophyll cycle carotenoids, 90% or greater dark-sustained conversion of the xanthophyll cycle to zeaxanthin and antheraxanthin, sustained decrease in PSII efficiency involving depressions in both F_o and F_m ; Kitajima and Butler, 1975).

The chlorophyll fluorescence OJIP transients provide additional information that is consistent with both the F_o and F_m fluorescence data, as well as with the pigment data. The OJIP transients serve to further support the differences in F_o (minimal fluorescence emission at open PSII reaction centers with oxidized electron acceptors, corresponding to the O phase) and F_m levels (maximal fluorescence emission at closed PSII reaction centers with reduced electron acceptors). The P plateaus of the OJIP transients approach but do not necessarily reach the full maximal F_m level. Additional information is offered by the transients in that the intermediate levels during the OJIP transitions are all severely compressed in winter (February) and are all proportionally intermediate during spring recovery in April. According to e.g. Yusuf et al. (2010), the O–J phase is thought to reflect reduction of PSII reaction center electron acceptor Q_A , and to be influenced by donor side and acceptor sides of PSII, and the J–I phase is thought to reflect reduction of the next electron acceptor in line, Q_B . The present findings of proportional depressions in O, J, I, and P levels during severe winter depression in fluorescence emission and subsequent spring recovery indicate that all components within, and those associated with, the PSII reaction center are inactivated or degraded, and subsequently re-activated or re-assembled, concomitantly. This conclusion of coordination in the disassembly and reassembly of multiple components of the PSII complex is likely a sound interpretation of the changes in the OJIP chlorophyll fluorescence transients, even though there is considerable disagreement regarding the interpretation of what influences the different stages of the transients and what changes in those transients actually reflect among different

TABLE 2

Carotenoid contents on a fresh weight (FW) basis and the ratio of zeaxanthin (Z) to total xanthophyll cycle pool (violaxanthin + antheraxanthin + zeaxanthin = V + A + Z) in needles of Korean fir trees growing at three altitudes on Mount Halla and collected during three seasons. N = neoxanthin, L = lutein, and β -C = β -carotene. Each value represents the mean \pm standard deviation of three different trees. Within each column, significantly different values (evaluated by two-way ANOVA and Duncan's multiple range test, $p < 0.05$) are indicated by the different letters.

Altitude (m)	Month	N	L	V	A	Z	β -C	V + A + Z	Z/(V + A + Z) ratio
		nmol/g FW							
1500	Jul	59 \pm 1 ^{cd}	167 \pm 7 ^{ab}	29 \pm 2 ^{ab}	19 \pm 5 ^a	0 ^a	50 \pm 1 ^b	48 \pm 3 ^{ab}	0 ^a
	Feb	31 \pm 2 ^a	160 \pm 17 ^{ab}	8 \pm 1 ^a	13 \pm 4 ^a	59 \pm 11 ^b	19 \pm 1 ^a	80 \pm 13 ^{bcd}	0.74 \pm 0.04 ^c
	Apr	31 \pm 2 ^a	158 \pm 22 ^{ab}	52 \pm 11 ^{bc}	15 \pm 3 ^a	7 \pm 3 ^a	19 \pm 2 ^a	74 \pm 8 ^{abc}	0.10 \pm 0.03 ^{ab}
1671	Jul	50 \pm 0 ^c	142 \pm 5 ^a	28 \pm 1 ^{ab}	14 \pm 4 ^a	0 ^a	55 \pm 10 ^b	42 \pm 2 ^a	0 ^a
	Feb	30 \pm 3 ^a	179 \pm 35 ^{abc}	6 \pm 1 ^a	13 \pm 5 ^a	86 \pm 20 ^c	20 \pm 3 ^a	106 \pm 23 ^{cd}	0.82 \pm 0.03 ^c
	Apr	33 \pm 1 ^{ab}	162 \pm 7 ^{ab}	53 \pm 1 ^{bc}	16 \pm 5 ^a	10 \pm 1 ^a	26 \pm 3 ^a	79 \pm 4 ^{bcd}	0.12 \pm 0.01 ^b
1800	Jul	63 \pm 3 ^d	178 \pm 6 ^{abc}	39 \pm 15 ^b	21 \pm 7 ^a	0 ^a	87 \pm 11 ^c	60 \pm 14 ^{ab}	0 ^a
	Feb	36 \pm 1 ^{ab}	198 \pm 9 ^{bc}	8 \pm 1 ^a	16 \pm 6 ^a	87 \pm 12 ^c	29 \pm 1 ^a	112 \pm 9 ^d	0.78 \pm 0.07 ^c
	Apr	41 \pm 6 ^b	220 \pm 11 ^c	72 \pm 13 ^c	22 \pm 9 ^a	17 \pm 3 ^a	30 \pm 2 ^a	112 \pm 10 ^d	0.15 \pm 0.02 ^b

TABLE 3

The ratios of neoxanthin (N), lutein (L), zeaxanthin (Z), the total xanthophyll cycle pool violaxanthin + antheraxanthin + zeaxanthin (V + A + Z), and β -carotene (β -C) to Chl *a* + *b* in needles of Korean fir trees growing at three altitudes on Mount Halla and collected during three seasons. Each value represents the mean \pm standard deviation of three different trees. Within each column, significantly different values (evaluated by two-way ANOVA and Duncan's multiple range test, $p < 0.05$) are indicated by the different letters.

Altitude (m)	Month	N/(Chl <i>a</i> + <i>b</i>)	L/(Chl <i>a</i> + <i>b</i>)	Z/(Chl <i>a</i> + <i>b</i>)	(V + A + Z)/(Chl <i>a</i> + <i>b</i>)	β -C/(Chl <i>a</i> + <i>b</i>)
		mmol/mol Chl <i>a</i> + <i>b</i>				
1500	Jul	83 \pm 3 ^d	233 \pm 7 ^a	0 ^a	67 \pm 7 ^a	70 \pm 0 ^{cd}
	Feb	57 \pm 3 ^a	303 \pm 35 ^{abc}	113 \pm 19 ^b	150 \pm 20 ^{ab}	37 \pm 3 ^a
	Apr	63 \pm 3 ^{ab}	317 \pm 34 ^{abc}	15 \pm 5 ^a	150 \pm 12 ^{ab}	40 \pm 6 ^{ab}
1671	Jul	77 \pm 3 ^{bcd}	220 \pm 17 ^a	0 ^a	63 \pm 3 ^a	83 \pm 12 ^d
	Feb	67 \pm 9 ^{abc}	400 \pm 85 ^{bc}	195 \pm 49 ^c	237 \pm 60 ^b	43 \pm 9 ^{ab}
	Apr	63 \pm 3 ^{ab}	317 \pm 47 ^{abc}	19 \pm 3 ^a	153 \pm 12 ^{ab}	53 \pm 7 ^{abc}
1800	Jul	87 \pm 3 ^d	247 \pm 13 ^{ab}	0 ^a	83 \pm 20 ^a	120 \pm 12 ^c
	Feb	67 \pm 3 ^{abc}	380 \pm 46 ^{abc}	169 \pm 37 ^{bc}	213 \pm 30 ^b	57 \pm 3 ^{abc}
	Apr	80 \pm 6 ^{cd}	457 \pm 83 ^c	35 \pm 8 ^a	233 \pm 41 ^b	63 \pm 9 ^{bcd}

laboratories investigating this phenomenon (for a recent review, see Stirbet and Govindjee, 2012). This conclusion is furthermore consistent with the report of winter degradation and spring re-assembly of whole PSII center units, including the oxygen-evolving complex (OEC, as the PSII donor side) and PSII core proteins (like the D1 protein) in overwintering conifers in the Colorado Rockies at high altitudes that also show severe depression of (light- and CO₂-saturated) maximal rates of photosynthetic oxygen evolution in the winter (Zarter et al., 2006a, 2006b). The differences in chlorophyll and carotenoid needle levels in summer (July), winter (February), and the spring transition month of April (see below) are also consistent with the coordinated

PSII disassembly and reassembly scenario suggested by the OJIP transients.

PATTERNS OF SEASONAL CHANGES IN LIGHT HARVESTING EFFICIENCY AND PIGMENT COMPOSITION IN KOREAN FIR IN COMPARISON WITH OTHER HIGH-ALTITUDE CONIFEROUS FORESTS

The general pattern observed in the present study, with enhanced light harvesting during the growing season with mild (warm) temperatures versus pronounced employment of sustained photoprotective energy dissipation in the cold winter, is consistent with patterns in a high-altitude (subalpine) coniferous forest in

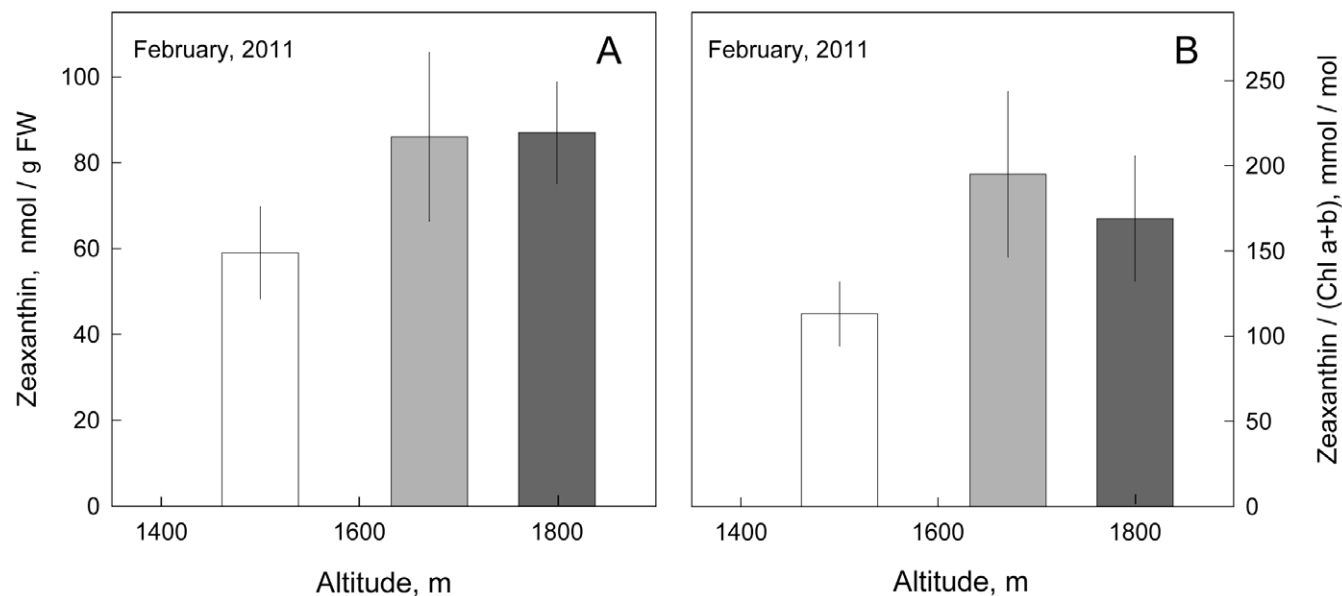


FIGURE 4. Zeaxanthin content of needles from Korean fir trees growing at three altitudes in February 2011 (A) on a fresh weight basis, and (B) relative to the total chlorophyll *a* + *b* content. Each column and error bar represents the mean \pm standard deviation of three different trees. For significant differences, see Tables 2 and 3.

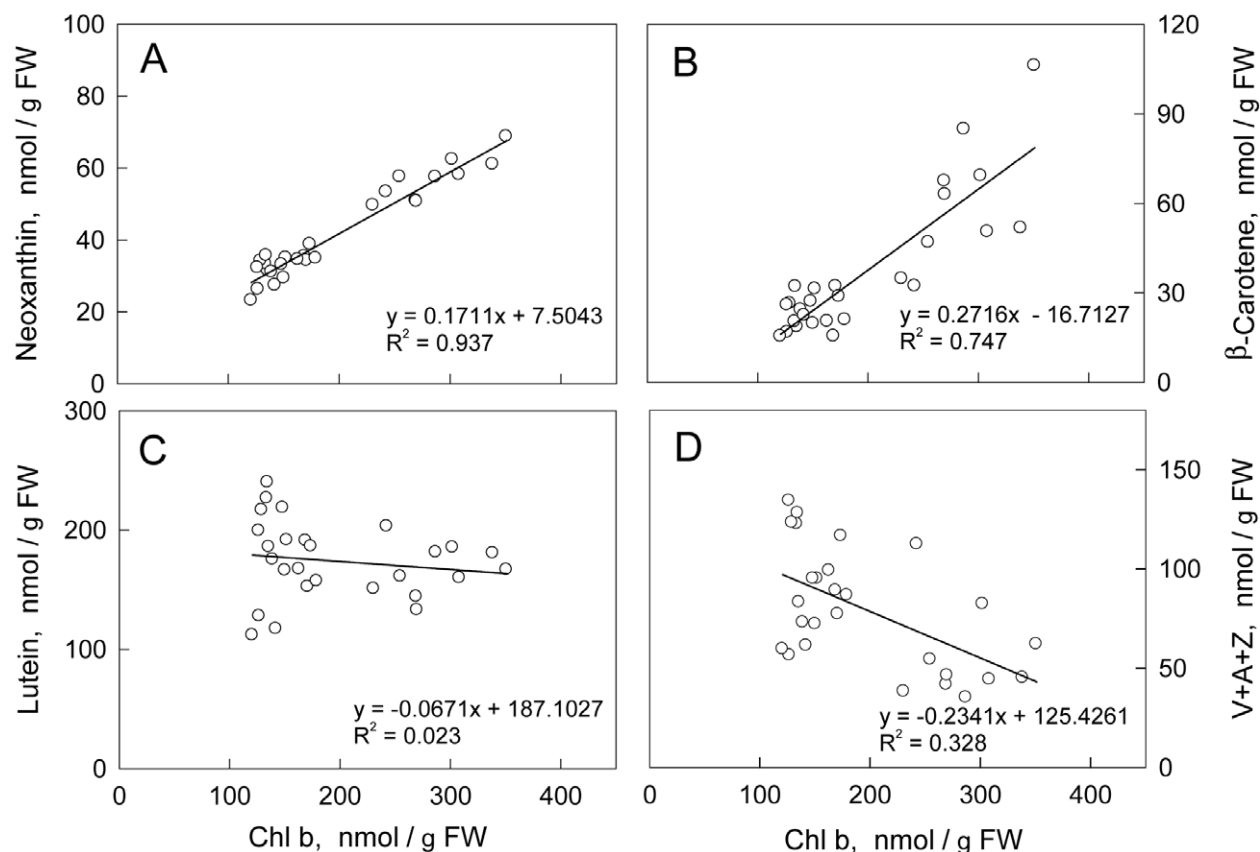


FIGURE 5. Relationships between chlorophyll *b* level and the levels of (A) neoxanthin, (B) β -carotene, (C) lutein, and (D) the total pool of xanthophyll cycle carotenoids in needles of Korean fir trees growing at three altitudes and collected in three seasons (July, February, and April). See Materials and Methods for additional information.

Colorado (Zarter et al., 2006a, 2006b). However, while high levels of sustained photoprotection were seen in the winter season in both forests, the time of year with the highest level of light harvesting differed between Colorado and Korea. In the Colorado climate, the highest levels of light harvesting (after rapid relaxation of sustained photoprotection) were seen in the mild spring season (Zarter et al., 2006b), where apparent water availability was higher than in the summer, while Korean fir exhibited evidence for higher light harvesting in the summer than in the spring. These differences suggest that precipitation patterns and water availability are key to when light harvesting and forest carbon gain are maximal, which is consistent with total forest carbon gain for Colorado (with moist springs and dry summers; Monson et al., 2002) versus conifers in regions with moist summers (Zha et al., 2004; Lagergren et al., 2005; Ueyama et al., 2006; Etzold et al., 2011). In Korean fir, the disengagement of photoprotection (return to a xanthophyll cycle conversion state of 30–35% accompanied by increases in F_o , F_m , and F_v/F_m) during the transition from winter to spring was not accompanied with an immediate return to overall summer pigment composition.

The lower levels of chlorophyll (and especially chlorophyll *b*) in both winter and spring needles (compared to summer needles) are suggestive of lower levels of the major, chlorophyll-*b*-rich light-harvesting chlorophyll protein complexes (Anderson and Osmond, 1987; Yamamoto and Bassi, 1996). The lower levels of β -carotene (with β -carotene being associated with the core proteins of the

photosystems; Yamamoto and Bassi, 1996) in the winter and spring, on the other hand, are consistent with decreased levels of the PSII core protein D1 and downregulation of photosynthetic electron transport capacity in evergreens exposed to winter conditions, including other species of fir (Ensminger et al., 2004; Zarter et al., 2006a, 2006b, 2006c; Koh et al., 2009). In addition, the fact that β -carotene levels in the summer were, in fact, highest in Korean fir at the highest altitude considered in the present study is (i) suggestive of higher levels of core antennae at the higher altitudes in the summer, and is (ii) consistent with the observation of higher maximal photosynthetic capacities at higher altitudes in Colorado conifers during the actual growing season (Koh et al., 2009). Enhanced carbon gain over a short period of time (as a result of higher maximal photosynthesis rates) should be beneficial at higher altitudes with shorter growing seasons.

CORRELATIONS BETWEEN SUSTAINED PHOTOPROTECTION AND INDIVIDUAL XANTHOPHYLLS

Employment of strong photoprotective thermal energy dissipation is thought to be necessary to allow leaves and needles to remain evergreen when utilization of absorbed light is greatly diminished under harsh winter conditions (Öquist and Huner, 2003; Adams et al., 2002, 2004, 2006). Multiple studies have reported high levels of thermal dissipation that are locked-in in evergreen leaves and needles overwintering at high altitudes, such that fluo-

rescence yield and intrinsic PSII efficiency do not relax (increase) as they do under summer conditions when leaves/needles are darkened and/or moved to warm temperatures (for a review, see Demmig-Adams and Adams, 2006). These latter studies also reported strong correlations between locked-in thermal dissipation and long-term retention of zeaxanthin (and antheraxanthin) in overwintering evergreen leaves/needles (see Demmig-Adams and Adams, 2006). The data reported here, with sustained low intrinsic PSII efficiency, sustained low F_o and F_m fluorescence yields, and, as an additional feature, altered fluorescence OJIP transients, are consistent with these previous results. Korean fir exhibited high levels of de-epoxidation of the xanthophyll cycle and strong down-regulation of PSII efficiency in winter, even following 15 min or more of dark adaptation. Although comparing absolute levels of fluorescence is somewhat problematic among needles or leaves with different chlorophyll contents, the strong decreases in F_m and F_o fluorescence observed in mid-winter compared to summer and the following spring support the interpretation that the low PSII efficiency in mid-winter was due to an increase in photoprotective energy dissipation (Kitajima and Butler, 1975) and is consistent with the seasonal analyses of these fluorescence parameters in other evergreen species (Adams and Demmig-Adams, 1994; Verhoeven et al., 1996).

Our comparison here, of the relative levels of different xanthophylls with the functional state of PSII in Korean fir, revealed that neoxanthin is closely and positively associated with overall chlorophyll *b* levels (and thus presumably overall LHCII levels) and high light harvesting efficiency, but is inversely correlated with sustained photoprotection in the winter, none of which provides any support for a function of neoxanthin in sustained photoprotection (see Introduction). Furthermore, since lutein levels in Korean fir did not decrease in the winter (with lutein thus becoming somewhat enriched relative to chlorophyll *b* levels in the winter), a role of lutein in sustained photoprotection in the winter is possible. However, the decreasing ratio of lutein to xanthophyll cycle pigments ($V + A + Z$) in the winter as well as the strong retention of zeaxanthin in winter both suggest that zeaxanthin plays the prominent role in winter photoprotection of the photosynthetic apparatus of Korean fir needles. These field correlations, however, do not allow an assessment of the relative roles of zeaxanthin in thermal dissipation versus direct antioxidation (Havaux and Niyogi, 1999; Havaux et al., 2007; Johnson et al., 2007).

EFFECTS OF ALTITUDE ON PSII FUNCTION AND PIGMENT COMPOSITION

The general emphasis in Korean fir on efficient light harvesting in the summer and on sustained photoprotective thermal dissipation in the winter was present at all three altitudes studied here. However, several features (some in mid-winter, some during the spring transition, and some in the summer) showed significant differences as a function of altitude, which is remarkable in light of the fact that the three sites considered here spanned an altitudinal range of only 300 m. In mid-winter, the presence at the highest altitudes of greater amounts of retained, locked-in zeaxanthin (as a putative catalyst of sustained, locked-in thermal dissipation in needles with sustained low PSII efficiency) provides evidence for somewhat harsher conditions at the higher versus lower altitudes.

The latter evidence provides support for the suggestion made by Kang et al. (1990), based on needle morphology, that needles of Korean fir might be exposed to harsher conditions with increasing altitude on Jeju Island.

In addition, the higher levels of β -carotene (as a marker of leaves with high maximal electron transport rates and high light-harvesting efficiency) at the higher altitudes in the summer suggests a discernibly shorter actual growing season (and greater need to compress net carbon gain into a very narrow window) at the higher versus lower altitudes characterized here. The latter conclusion is further supported during the spring transition, where both OJIP fluorescence transients and F_o and F_m levels provided evidence for a somewhat slower reactivation of photosynthesis in spring at the higher versus lower altitudes.

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