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## ACCLIMATION OF SILVER FIR (*ABIES ALBA* MILL.) SEEDLINGS TO IRRADIANCE CONDITIONS UNDER CANOPIES OF DIFFERENT TREE SPECIES IN SUDETY MTS. (SOUTHERN POLAND)

**ABSTRACT:** To determine the influence of tree canopy composition on growth and physiological performance of silver fir (*Abies alba* Mill.) in Karkonoski National Park (Sudety Mountains, Southern Poland), three-year-old fir seedlings were planted in five forest stands dominated by *Betula pendula*, *Fagus sylvatica*, *Larix decidua*, *Picea abies* or *Pinus sylvestris*. The stands differed in canopy openness such that young fir plants experienced drastically different light environments. After three years of acclimation to site conditions, length of shoots, seasonal changes in chlorophyll *a* fluorescence parameters, and the contents of chlorophyll, carotenoids and nutrients in the needles were studied to evaluate the seedling performance. Growth and photosynthetic characteristics of the young seedlings responded strongly to local light conditions. The lengths of leader shoot and twigs of the upper whorl reflected variation in canopy openness during the growing season and were highest under *Larix*. The potential quantum yield of photosystem II ( $F_v/F_m$ ) showed a strong depression in the spring, especially under the leafless canopies of *Betula* and *Fagus*, probably because of the occurrence of low air temperatures. Later in the season  $F_v/F_m$  showed substantial recovery in all stands. Effective quantum yield  $\Phi_{PSII}$  measured under actinic light also showed a growing trend throughout the season with the lowest levels noted in the spring, particularly under *Fagus* and *Betula*. Fluorescence quenching

revealed complex seasonal behaviour with little obvious relationship with stand illumination, except for consistently low value of photochemical quenching, and immediate or high nonphotochemical quenching under the dark *Picea* canopy, probably reflecting photoprotective acclimation to sunflecks. Although needle chlorophyll content showed no relationship to ambient light, the ratio of carotenoids to chlorophyll was positively linked to canopy openness suggesting the existence of leaf photoprotection that evaded detection by quenching analysis. Nutrient contents in needles depended on site conditions. In the light-transparent *Larix* stand, the fir needles were strongly depleted in Mg and Ca. Overall, however, all fir plants from experimental plots had much lower needle nutrient content than nursery-raised plants. Needle chlorosis found in *Betula* and *Pinus* stands was, however, not caused by macronutrient deficiencies. Considering all variables, conditions most conducive for fir growth and good vigor were found under larch and pine canopies where light penetration was intermediate to high. In contrast, the poorest conditions were found in the *Betula* stand, where high light penetration in the spring was followed by shading of firs during the growing season.

**KEY WORDS:** *Abies alba*, acclimation to light, reintroduction, canopy density, pigment content, chlorophyll fluorescence, nutrient content

## 1. INTRODUCTION

Forests in the Sudety Mts. (Southern Poland) have been drastically transformed through human-related activities, such as intense logging, grazing by domestic animals, industrial development, as well as certain unfortunate forest management techniques practiced in silviculture from the 13th until the 20th century (Fig. 1). In the seventies and eighties of the last century, forests declined over a large area of the mountains because of widespread industrial pollution. The massive scale of this phenomenon revealed not only a high level of pollutants but also an extreme vulnerability of monoculture conifer stands, here formed mostly by Norway spruce (*Picea abies* Karst.). Deciduous trees, such as oak *Quercus robur* L., hornbeam *Carpinus betulus* L., beech *Fagus sylvatica* L., and sycamore *Acer pseudoplatanus* L., which might

be more tolerant to pollution, had been largely cut down and replaced by the spruce (Wilczkiewicz 1982). Another important tree species that has become almost entirely replaced by spruce is the silver fir *Abies alba* Mill. (Wilczkiewicz 1976).

*Abies alba* has been declining throughout its natural European range since the 18th century (Bernadzki 1983). The reasons for its gradual disappearance are presumably natural and are attributable to an interaction of environmental stressors: drought, low temperatures, pathogenic fungi (predominantly *Armillaria mellea* (Vahl.) Quel.), viruses and phytophagous insect gradations (reviewed by Dobrowolska 1998). Another serious cause of fir decline is linked to acid rains, which have caused changes in soil chemistry with a drop of soil pH leading to solubilization of  $Al^{3+}$  and restriction of  $Ca^{2+}$  and  $Mg^{2+}$  availability. In the Sudety Mts., im-

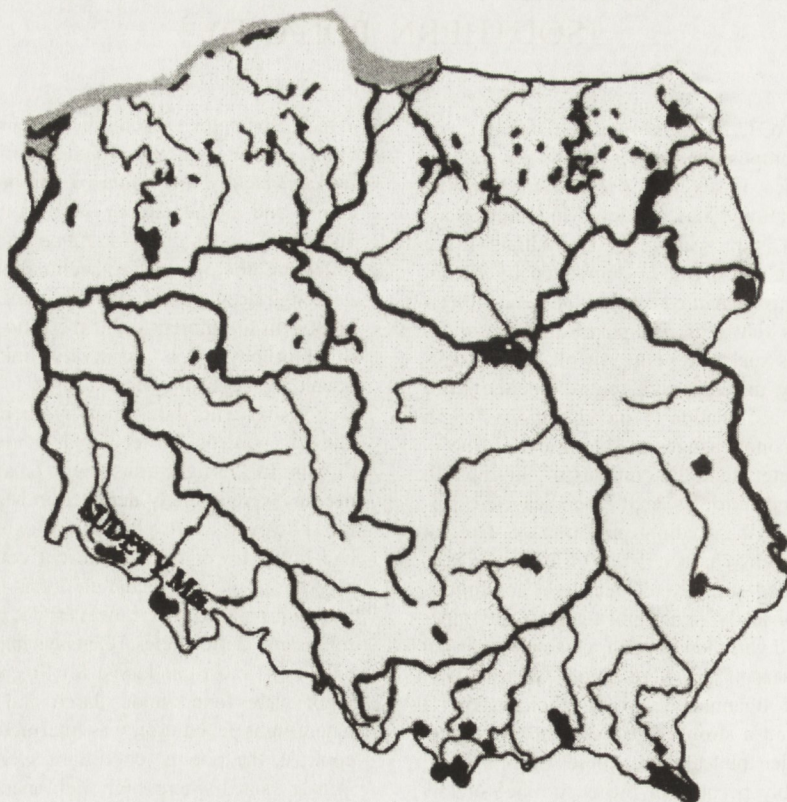


Fig. 1. General location of the study area – Sudety Mts. (Southern Poland)

proper silvicultural practices and a large deer population have also contributed to the decline of silver fir. Moreover, the genetic variability of *Abies alba* appears to be relatively low, which accounts for its low evolutionary resilience in the face of a changing environment (Bernadzki 1983).

Within Karkonoski National Park, a 55 km<sup>2</sup> area which protects the most valuable part of the Sudety Mts., *Abies alba* has fared extremely poorly. Almost entire natural population has disappeared with only a few reproductive trees left. Both the amount and the frequency of their seed output are insufficient to guarantee a substantial natural regeneration and are far too low to ensure the survival of this species within Karkonoski National Park. To remedy the situation, a large program of silver fir restitution has been undertaken in the Park, which involves seed collection from the remaining naturally growing trees, raising seedlings in local nurseries and planting them in sites chosen for stand reconstruction. As shown by long-term observations, firs are best planted under the canopy of other tree species (Jaworski 1995). Young firs experience a variety of microhabitat conditions, determined by local soil, exposure and other topographic features, but largely modified by the tree species composition of the local canopy. The tree species dominating the canopy significantly influences the conditions that prevail on the forest floor, mostly through its effect on transmission of radiation but also by affecting throughfall, evapotranspiration, soil nutrient relations, litter quality and the biotic environment (Puchalski and Prusinkiewicz 1990; Modrzyński unpublished).

The main purpose of this study is to compare the effect of stand characteristics, and especially the influence of the dominant canopy species, on ecophysiological performance of young silver fir plants in Karkonoski National Park. By measuring chlorophyll *a* fluorescence parameters in the field, we monitored the state of the photosynthetic apparatus in needles of silver fir growing in stands dominated by spruce, pine (*Pinus sylvestris* L.), larch (*Larix decidua* Mill.), birch (*Betula pendula* Roth.) or beech (*Fagus sylvatica* L.). Both potential and effective quantum yields, as well as the fluorescence quenching pathways determined by these techniques can be interpreted in terms of plant's stress responses and vitality (reviewed by Maxwell and

Johnson 2000). In addition, we studied the contents of photosynthetic pigments and nutrients in the needles, and the growth of fir plants *in situ*. An integrated view of all these measures of plant's physiological health allowed us to determine to what extent young silver fir plants are able to acclimate to the different site conditions. We use these data to select an optimal canopy species for best regeneration of this shade-adapted tree to aid in future fir restitution efforts. In a broader context, the knowledge of ecophysiological relationships between canopy composition and seedling performance will contribute to the understanding of causes of fir decline throughout its natural range.

## 2. MATERIALS AND METHODS

### 2.1. Plant material

Seeds of silver fir (*Abies alba* Mill.) were collected from trees growing in Karkonoski National Park and sown in a nursery in Jelenia Gora – Jagniątkow (600 m a.s.l. 50.5° 22' N, 15.5° 15' E). Substrate was composed of a local forest loam with the addition of peat and without any fertilizer. Plants were maintained under standard growing tunnels covered with a neutral shading cloth (25% light transmittance) for three years.

### 2.2. Experimental design

In March 1999, 400 root-balled seedlings were planted in each of five fenced wooded plots, situated in the proximity of the nursery. The canopy of each plot was dominated by one of five different tree species: *Betula pendula*, *Fagus sylvatica*, *Larix decidua*, *Picea abies*, or *Pinus sylvestris*, with the dominant species forming at least 80% of the stand. Overall, the plots were uniform with respect to other environmental characteristics, except for the birch stand with its unique, gley-type soil. The topographic situation, soil and vegetation cover of the plots are described in Table 1.

### 2.3. Light environment

To determine how tree species dominating the canopy modified light conditions on the forest floor, the canopy density was first measured using Lemon's densiometer, a de-

Table 1. Description of the experimental plots

Altitude a.s.l. (m)	Stand description				Site description				
	Stand composition	Age (year)	Proportion of stand mixture	Crown closure	Forest community	Site type	Inclination and exposition	Soil type	Floor cover
570–650	<i>Fagus</i> , <i>Picea</i> single <i>Larix</i>	122	Single and group mixture	Moderate crown closure	<i>Luzulo-Fagetum</i>	M(ixed) M(ountain) F(orest)	Steep slope N-E	Brown acid soil	Litter, occasional <i>Oxalis</i>
620–640	<i>Pinus</i> single <i>Larix</i> , <i>Betula</i> rarely <i>Picea</i> , <i>Fagus</i>	62	–	Patchy to moderate crown closure	<i>Luzulo-Fagetum</i>	MMF	Ridge E and W	Brown acid soil	Abundant weedy soil cover
630–640	<i>Picea</i> rarely <i>Pinus</i> , <i>Betula</i> , <i>Larix</i> , <i>Fagus</i> , <i>Alnus</i> , <i>Sorbus</i>	72	–	Patchy crown closure	<i>Luzulo-Fagetum</i>	MMF	Gentle slope, in places steep slope N	Brown acid soil	Turf cover
640–740	<i>Betula</i> , <i>Picea</i> , <i>Larix</i> , single <i>Acer</i> , <i>Fagus</i> , groups of <i>Sorbus</i>	12	Group, single and small cluster mixture	Moderate crown closure	<i>Abieti-Piceetum montanum</i>	Mixed Mountain Coniferous Forest	Flat	Gley soil	Abundant weedy soil cover
690–710	<i>Larix</i> , <i>Picea</i> single <i>Fagus</i>	142	Group and single mixture	Patchy crown closure	<i>Luzulo-Fagetum</i>	MMF	Leaning slope N-W	Brown acid soil	Turf cover

vice which allows estimation of the proportion of canopy openings in total projected canopy area (Lemon 1957). In addition, light penetration through the canopy was determined by comparing photosynthetic photon flux densities (PPFD) measured with quantum sensors (Spectrum Technologies, Inc. Plainfield, USA) in each stand and, simultaneously, in a nearby clearing.

#### 2.4. Growth of leader shoot and twigs

The length of leader shoot and twigs of the upper whorl were measured on 50 seedlings in each stand. The apical dominance ratio was calculated for each plant by dividing the length of leader shoot by mean length of the twigs of the upper whorl (Jaworski 1995, Niemtur *et al.* 1996).

#### 2.5. Chlorophyll fluorescence

Chlorophyll *a* fluorescence was determined in needles of silver fir on single occasions in April, June, July and October of the 2000 growing season using Fluorescence Monitoring System (FMS 2, Hansatech, Norfolk, UK). Fluorescence measurements were taken *in situ* on needles of 50 seedlings in each of the five stands using mature needles of the upper whorl. Each time, measurements were conducted over two consecutive days during morning hours. The protocol used for fluorescence induction followed the standard procedure of Hansatech (2000) according to Genty *et al.* (1989). First, needles were arranged tightly within a factory-provided clip to fill the entire aperture and dark adapted for 30 minutes. Following the adaptation period, a fiberoptics surrounded by a light-tight chamber was inserted onto the leaf clip and the needles were exposed to modulated measuring light of  $0.05 \mu\text{mol m}^{-2} \text{s}^{-1}$ . After reading the background fluorescence level  $F_0$ , a saturating 0.7s pulse of actinic light (Photosynthetic Photon Flux Densities – PPFD =  $15.300 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) was delivered to provide a maximal dark-adapted fluorescence reading ( $F_m$ ). Next, a light-adapted measurement was taken using another leaf clip not equipped with a dark chamber which allowed exposing the needles to ambient light. Leaf-level ambient light intensities during the measurements were very low because of the overcast skies ( $8\text{--}58 \mu\text{mol m}^{-2} \text{s}^{-1}$  in April,  $5\text{--}75 \mu\text{mol m}^{-2} \text{s}^{-1}$  in July and  $8\text{--}41 \mu\text{mol m}^{-2} \text{s}^{-1}$  in October) and were therefore augmented

by turning on the actinic light (PPFD =  $300 \mu\text{mol m}^{-2} \text{s}^{-1}$ ). After 120 sec, when fluorescence signal reached a stable value ( $F_s$ ), another 0.7s pulse of saturating pulse was delivered and maximal light-adapted fluorescence ( $F_m'$ ) was determined. In the field conditions, it was difficult to measure  $F_0'$  (quenched background fluorescence), which requires the turning off of actinic light, darkening of the leaf and measuring fluorescence level under far-red illumination. We assumed that dark-adapted  $F_0$  values are equivalents of  $F_0'$  in calculating fluorescence quenching parameters, with the understanding that a slight overestimation of  $F_0'$ , may result. The following values were calculated using built-in software:

- Potential (dark adapted) quantum yield =  $F_v / F_m$ , where  $F_v = F_m - F_0$  is variable fluorescence
- Effective (light adapted) quantum yield  $\Phi_{\text{PSII}} = (F_m' - F_s) / F_m'$
- Photochemical quenching coefficient  $qP = (F_m' - F_s) / (F_m' - F_0)$
- Non-photochemical quenching coefficient  $q_{\text{NP}} = (F_m - F_m') / (F_m - F_0)$ .

For logistical reasons the  $F_v/F_m$  measurements in June were conducted with Plant Efficiency Analyser (PEA, Hansatech) according to the protocol of Strasser *et al.* (2000). No light-adapted measurements were taken at that time. As with FMS 2, needles were dark adapted in leaf-clips for 30 minutes, but maximal induced fluorescence yield was measured under a 1s pulse of  $4000 \mu\text{mol m}^{-2} \text{s}^{-1}$  red light. Prior to measurements, we determined that results obtained by both fluorescence meters were in close agreement.

A “photosynthetic plasticity” index was calculated for each stand and each sampling period by dividing the difference between average maximum and minimum yield values by the average maximum yield (Valladares *et al.* 2002).

#### 2.6. Photosynthetic pigments

Analyses of chlorophyll and carotenoid contents were conducted in the laboratory of Institute of Dendrology Polish Academy of Sciences in Kornik. In July, ten needles (40–50 mg FW) were collected from the upper whorl of twenty seedlings per plot, and additionally from several seedlings showing signs of chlorosis. They were quickly frozen and taken to the laboratory, where they were cut into 2 mm pieces and incubated for 5 hours in 5 ml 100% dimethylsulfamide

(DMSO) at 60° C in water bath until the solution became translucent. The absorbance of the extract was measured spectrophotometrically at 665, 648 and 470 nm. Chlorophyll *a*, *b* and total carotenoid contents were calculated using the formula given by Barnes *et al.* (1992).

## 2.7. Nutrients

On 25 July 2000, about 1 g FW of needles were collected from ten seedlings per plot (and additionally from several seedlings showing signs of chlorosis) and frozen at -20° C. In laboratory they were dried for 72 hours at 70° C and ground to fine powder in a mortar. Nutrient contents were determined in the laboratory of Department of Agricultural Chemistry, Agricultural University in Poznań. For total nitrogen content, samples were analyzed spectrophotometrically after Kjeldahl digestion. The remaining elements were determined in dry-ashed samples dissolved in HCl. The phosphorus content was determined spectrophotometrically, potassium and calcium were analysed using flame spectroscopy and magnesium content was determined using atomic absorption spectrometry. Nutrient contents were expressed on the dry weight basis.

## 2.8. Statistics

Data were analysed with one-way ANOVA using General Linear Models procedure. Significant ANOVA effects ( $P < 0.05$ ) were followed by *a posteriori* Tukey's test at global  $\alpha = 0.05$ . Relationships between fluorescence parameters were evaluated with Pearson's coefficients of linear correlation ( $r$ ). All analyses were carried out using "Statistica 5.5" software.

# 3. RESULTS

## 3.1. Light environment

Both methods of measuring light conditions on the forest floor showed wide ranging differences between the different canopies, which, for deciduous species varied with time of year (Table 2). While under *Picea* and *Pinus* light conditions remained relatively steady, light level under deciduous canopies was reduced severalfold upon canopy closure (e.g. over six-fold decrease in canopy open-

ness in the *Fagus* stand). During the growing season, when most of our fluorescence measurements were carried out, *Larix* canopy was the most light-transparent and *Fagus* was the darkest. Remarkably, both measuring methods gave identical ranking of sites on both dates, indicating their good reliability.

## 3.2. Growth

The length of the apex and the average twig length were lowest in the *Fagus* stand, highest in the *Larix*, and the ranking of canopy types was the same with respect to both measures of fir growth (Table 3). The growth rate closely reflected light transmission through the canopy during growing season. At the same time, the values of the apical dominance ratio indicated greater dominance of apical extension over twig extension with increasing light (Tables 2 and 3).

## 3.3. Chlorophyll fluorescence

Both potential and effective quantum yield of PSII in needles of young silver firs displayed strong seasonal trends and were significantly affected by tree species dominating the canopy (Tables 4 and 5). The levels of  $F_v/F_m$  were consistently lowest in April and showed an increasing tendency in the summer, or, in the case of *Pinus* and *Larix* canopies, even until October (Table 4). Remarkably, at almost all times the  $F_v/F_m$  values were much lower than the theoretical optimum of 0.83 (Björkman and Demmig 1987). The only fir plants in which this value was exceeded (and only in July) were those growing in the *Picea* stand, i.e. where canopy was closed and incident light level was very low and independent of season (Table 2). The variability of  $F_v/F_m$  values was particularly pronounced in April, where, in the *Betula* stand, a very low level of 0.458 was measured. This was also the time before leafing out of the two deciduous species *Betula* and *Fagus* whose canopy was then very open (over 70% openness and over 40% incident light penetration, Table 2). The April depression of  $F_v/F_m$  under the evergreen *Picea* and *Pinus* was much less pronounced, with deciduous *Larix* having an intermediate effect. In later months the influence of canopy species became less clear until quite homogeneous values of  $F_v/F_m$  were measured in October, when significant depression was found only under the leafless *Betula*.

Table 2. Light environment of fir plantings under canopies of five different tree species measured in April and June 2001. Two measures of light environment are presented: (1) canopy opening (100 – canopy density [%]) determined with Lemon's densiometer and (2) percentage of PPF (Photosynthetic Photon Flux Densities) reaching the forest floor, relative to an adjacent clearing. The different letters show statistical differences between the mean values of canopy openness and % PPF for all the plots determined by Tukey's test at global  $\alpha = 0.05$ .

Date	Principal canopy species	Canopy openness (mean $\pm$ SD)	% PPF (mean $\pm$ SD)
April	<i>Picea abies</i>	18 $\pm$ 3a	5 $\pm$ 2a
	<i>Betula pendula</i>	74 $\pm$ 13b	46 $\pm$ 16bd
	<i>Pinus sylvestris</i>	24 $\pm$ 3a	25 $\pm$ 8cd
	<i>Larix decidua</i>	52 $\pm$ 14c	36 $\pm$ 14bcd
	<i>Fagus sylvatica</i>	72 $\pm$ 4b	40 $\pm$ 8bd
June	<i>Picea abies</i>	17 $\pm$ 3a	11 $\pm$ 2a
	<i>Betula pendula</i>	18 $\pm$ 4a	17 $\pm$ 4b
	<i>Pinus sylvestris</i>	24 $\pm$ 3b	22 $\pm$ 4b
	<i>Larix decidua</i>	29 $\pm$ 3c	41 $\pm$ 6c
	<i>Fagus sylvatica</i>	11 $\pm$ 2d	9 $\pm$ 4a

Table 3. Length of the leader shoot, averaged length of twigs in the upper whorl, and apical dominance ratio for young silver firs growing under canopies of five different tree species (means  $\pm$  SD). Statistical significance of differences among the mean values of each parameter determined for all the plots was tested using one-way analysis of variance ( $P < 0.05$ ) followed by pairwise comparisons using Tukey's test (global  $\alpha = 0.05$ ; different letters indicate significant differences)

Principal canopy species	Apex (mm)	Twigs (mm)	Apical dominance ratio
<i>Picea abies</i>	52 $\pm$ 13a	83 $\pm$ 13a	0.63 $\pm$ 0.14b
<i>Betula pendula</i>	64 $\pm$ 33ab	98 $\pm$ 24b	0.63 $\pm$ 0.23b
<i>Pinus sylvestris</i>	74 $\pm$ 26bc	103 $\pm$ 25bc	0.70 $\pm$ 0.15b
<i>Larix decidua</i>	81 $\pm$ 30c	110 $\pm$ 20c	0.72 $\pm$ 0.18b
<i>Fagus sylvatica</i>	39 $\pm$ 10a	74 $\pm$ 10a	0.52 $\pm$ 0.10a
<i>P</i> *	0.000	0.000	0.000

\* *P* – probability obtained as a result of analysis of one-way ANOVA. When  $P < 0.05$ , the canopy effect was statistically significant.

Table 4. Potential quantum yield PSII ( $F_v/F_m$ ) (mean  $\pm$  SD) in needles of young silver firs growing under canopies of five different tree species measured in April, June, July and October 2001. Statistical significance of differences among means was tested separately for each sampling interval using one-way analysis of variance ( $P < 0.05$ ) followed by pairwise comparisons using Tukey's test (global  $\alpha = 0.05$ ; different letters indicate significant differences)

Principal canopy species	April	June	July	October
<i>Picea abies</i>	0.735 $\pm$ 0.1a	0.787 $\pm$ 0.04a	0.836 $\pm$ 0.01a	0.819 $\pm$ 0.01a
<i>Betula pendula</i>	0.458 $\pm$ 0.1b	0.784 $\pm$ 0.03a	0.802 $\pm$ 0.04a	0.796 $\pm$ 0.03b
<i>Pinus sylvestris</i>	0.654 $\pm$ 0.1c	0.752 $\pm$ 0.03b	0.713 $\pm$ 0.06b	0.820 $\pm$ 0.01a
<i>Larix decidua</i>	0.648 $\pm$ 0.1c	0.772 $\pm$ 0.02c	0.755 $\pm$ 0.04b	0.812 $\pm$ 0.02ab
<i>Fagus sylvatica</i>	0.509 $\pm$ 0.1b	0.777 $\pm$ 0.03ac	0.819 $\pm$ 0.02b	0.821 $\pm$ 0.004ab
<i>P</i>	0.000	0.000	0.000	0.000

Table 5. Effective quantum yield ( $\Phi_{\text{PSII}}$ ) of PSII (means  $\pm$  SD) in needles of young silver firs growing under canopies of five different tree species measured in April, July and October. Measurements were taken under  $300 \mu\text{mol m}^{-2} \text{s}^{-1}$  actinic light superimposed on very low ambient light levels (see Materials and Methods). Statistical analysis as in Table 4

Principal canopy species	April	July	October
<i>Picea abies</i>	0.382 $\pm$ 0.1a	0.326 $\pm$ 0.06a	0.437 $\pm$ 0.07c
<i>Betula pendula</i>	0.331 $\pm$ 0.1ab	0.397 $\pm$ 0.07a	0.562 $\pm$ 0.07b
<i>Pinus sylvestris</i>	0.449 $\pm$ 0.1a	0.395 $\pm$ 0.09a	0.655 $\pm$ 0.05a
<i>Larix decidua</i>	0.435 $\pm$ 0.1a	0.506 $\pm$ 0.09b	0.684 $\pm$ 0.06a
<i>Fagus sylvatica</i>	0.279 $\pm$ 0.1b	0.385 $\pm$ 0.07a	0.648 $\pm$ 0.02ab
<i>P</i>	0.000	0.000	0.000

The performance of photochemical yield of PSII under illumination ( $\Phi_{\text{PSII}}$ ) is summarized in Table 5. A tendency is again present for the yield to be lowest in April and highest in October in all tree stands. Similar to  $F_v/F_m$ , the strongest depression of  $\Phi_{\text{PSII}}$  in firs in April was noted under the leafless deciduous trees *Betula* and *Fagus*. In July and October, the variability in  $\Phi_{\text{PSII}}$  was not clearly related to the predominant light conditions, although the highest level of effective quantum yield was detected under *Larix*, a deciduous species with the most light-permeable canopy when in leaf.

Also the photochemical quenching coefficient  $qP$ , a measure of photosynthetic light utilization, exhibited a pronounced seasonal variation and a dependence on canopy composition (Fig. 2). While in April a high level of  $qP$  was found in firs growing in all stands except *Picea* (Fig. 2a), on later dates there was a considerable variability among stands, largely explained by the relationship between the effective quantum yield and  $qP$  (Fig. 2c, e).

Thus, plants that were able to maintain a high  $\Phi_{\text{PSII}}$ , were also able to utilize the absorbed light to drive photochemistry.

In October, but not in April or July,  $\Phi_{\text{PSII}}$  was strongly linked to nonphotochemical quenching coefficient ( $qNP$ ), which measures the ability to dissipate energy through pathways alternative to photochemistry, mainly via the photoprotective xanthophyll cycle (Fig. 2b, d, f). A decline in  $\Phi_{\text{PSII}}$  was then associated with a linear increase of  $qNP$  suggesting an induction of light-protective mechanisms at least in the dark-acclimated firs growing under *Picea*. Notably however,  $qNP$  values measured in October were overall several times lower than in the two earlier sampling intervals.

Plasticity indices demonstrated considerable changes in all parameters throughout the growing season, but not in all tree stands (Table 6). Especially stable was  $qP$  in firs growing under *Pinus* or *Larix*, i.e. in relatively evenly bright conditions, and  $F_v/F_m$  under *Picea*, i.e. in uniformly dark conditions.

Table 6. Photosynthetic plasticity indices for needles of young silver firs growing under canopies of five different tree species. The index was calculated as the difference between the mean maximum and minimum value of  $F_v/F_m$ ,  $\Phi_{\text{PSII}}$ ,  $qP$  and  $qNP$  (see Methods) in each stand divided by the all-season maximum

Principal canopy species	Index of plasticity				
	$F_v/F_m$	$\Phi_{\text{PSII}}$	$qP$	$qNP$	Mean plasticity
<i>Picea abies</i>	0.123	0.250	0.230	0.771	0.344
<i>Betula pendula</i>	0.429	0.411	0.323	0.838	0.500
<i>Pinus sylvestris</i>	0.202	0.397	0.065	0.877	0.385
<i>Larix decidua</i>	0.202	0.364	0.038	0.870	0.368
<i>Fagus sylvatica</i>	0.380	0.569	0.358	-	0.436



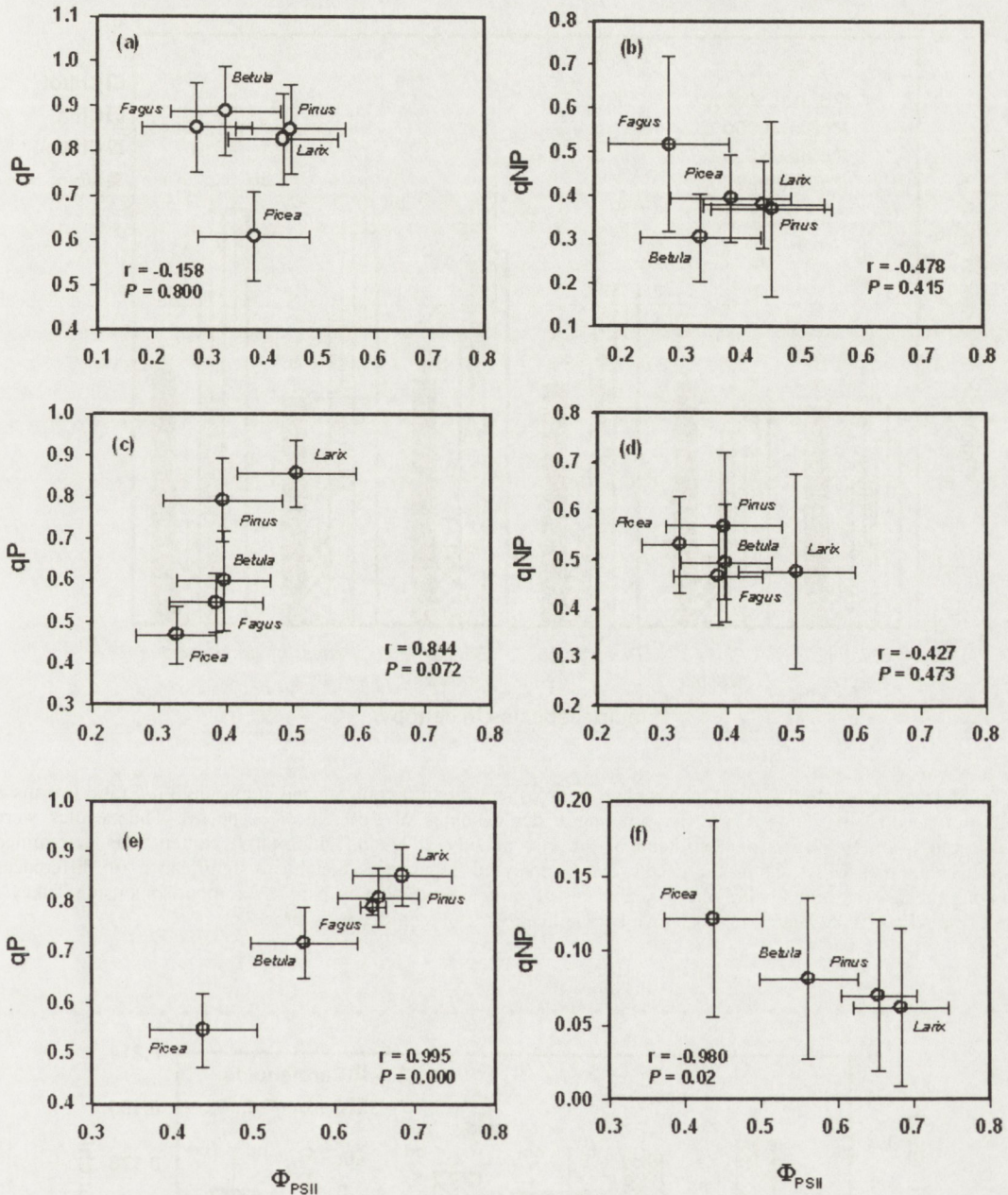


Fig. 2. Relationships between effective quantum yield ( $\Phi_{PSII}$ ), photochemical quenching (qP) and non-photochemical quenching of fluorescence (qNP) in April (2a, b), July (2c, d) and October (2e, f). The values of Pearson's coefficients of correlation (r) with associated significance level (P) are given.

### 3.4. Pigments

Chemical analysis of total chlorophyll content in needles collected in July revealed large differences among the stands, although there was no clear relationship between local light conditions and needle chlorophyll content (Fig. 3). The chlorophyll *a/b* ratio was similar in all stands, except for the enrich-

ment in chlorophyll *a* under *Larix* seen on comparison with *Fagus*. As expected, chlorotic needles were greatly depleted in chlorophyll. The ranking of tree stands with regard to fir needle carotenoid content (Fig. 4) was the same as for total chlorophyll. The ratio of carotene to chlorophyll varied significantly among the stands with the lowest value under *Fagus* and highest under *Pinus*.

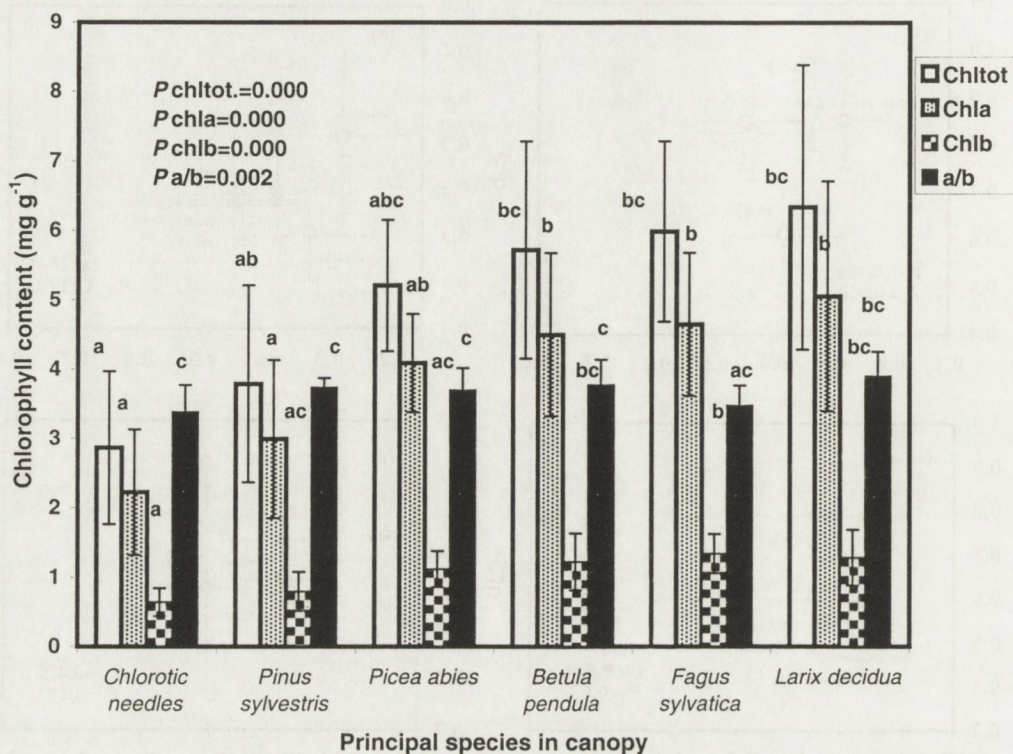


Fig. 3. Total chlorophyll (chl<sub>tot</sub>), chlorophyll *a* (chl<sub>a</sub>) and *b* (chl<sub>b</sub>) contents and chlorophyll *a/b* ratio (means ± SD) in needles of young silver firs growing under canopies of different tree species. The needles were collected from 20 plants in each stand at the end of July 2001 and chlorophyll content was determined spectrophotometrically. Chlorotic needles were analysed separately. Statistical significance of differences among means was tested using one-way analysis of variance followed by pairwise comparisons using Tukey's test (global  $\alpha = 0.05$ ; shared letters above bars indicate lack of significant differences).

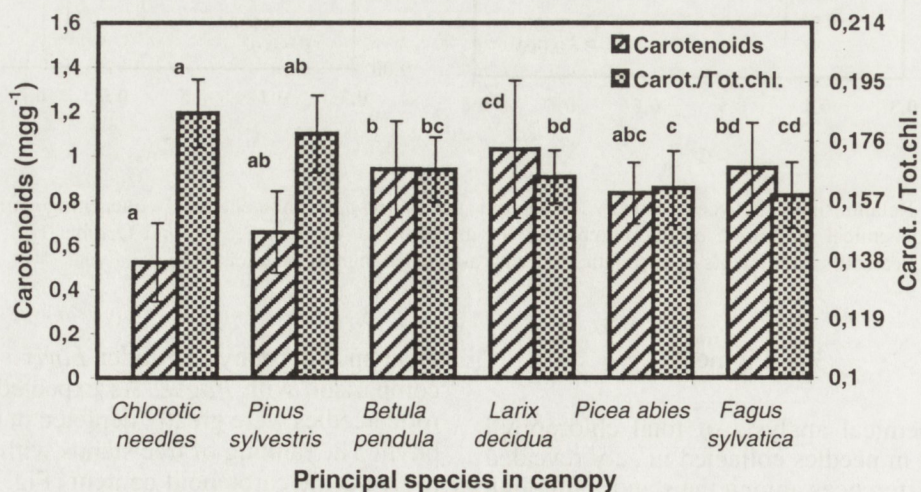


Fig. 4. Carotenoid content and carotenoids/total chlorophyll ratio (mean ± SD) in needles of silver fir seedlings growing under canopies of different tree species. Statistical analysis as in Fig. 2.

### 3.5. Nutrients

Needles of young firs growing under *Fagus* canopy had the highest contents of all nutrients except N, which did not vary among stands (Table 7). The lowest contents were consistently found under *Larix*. Chlorotic needles were depleted in nitrogen, but the contents of other nutrients were within the range typical for green needles.

As a reference, we provide an unpublished data set of nutrient contents in needles of silver fir seedlings growing in a nursery under variable solar irradiance and fertilized with a slow release fertilizer (Table 8). It is apparent that the nursery-grown plants show much higher levels of all nutrients than plants growing in Karkonoski National Park. An-

other observation that can be made is that differences in nutrient concentrations among drastically different light environments in the nursery are small and nonsignificant for N, P and K. Enrichment in Mg and Ca with lower incident light was noted in the nursery, but no such relationship existed in the field (Tables 2 and 7).

## 4. DISCUSSION

Young silver fir seedlings exposed to different light environments under canopies dominated by *Fagus sylvatica*, *Picea abies*, *Betula pendula*, *Pinus sylvestris*, and *Larix decidua* showed a remarkable degree of vari-

Table 7. Nutrient content (mean  $\pm$ SD) in needles of young silver firs growing under canopies of five different tree species. In each stand the needles were collected from 10 seedlings. Additionally, nutrient contents in chlorotic needles (three plants under *Betula* and five plants under *Pinus*) are presented in separate columns and means for all chlorotic seedlings are given in the last column. Different letters show statistically significant differences between means determined using Tukey's test (global  $\alpha = 0.05$ ). Statistically significant differences in mean nutrients content between chlorotic and green needles are marked with asterisks.

Nutrients (mg g <sup>-1</sup> )	<i>Picea</i>	<i>Betula</i>	<i>Pinus</i>	<i>Larix</i>	<i>Fagus</i>	Chlorotic needles
N	10.63 $\pm$ 1.0ab	12.27 $\pm$ 4.3ab*	9.79 $\pm$ 1.2a	12.64 $\pm$ 1.5ab*	13.51 $\pm$ 1.7b*	8.09 $\pm$ 1.4*
P	1.55 $\pm$ 0.1a*	1.53 $\pm$ 0.2a	1.42 $\pm$ 0.1ab	1.250 $\pm$ 0.1b	2.000 $\pm$ 0.2c*	1.337 $\pm$ 0.1*
K	4.11 $\pm$ 0.6a	4.10 $\pm$ 0.8a	3.50 $\pm$ 0.5ab	2.870 $\pm$ 0.5b	6.260 $\pm$ 0.9c*	3.530 $\pm$ 0.4*
Mg	1.14 $\pm$ 0.1a*	0.89 $\pm$ 0.1b	1.07 $\pm$ 0.1a	0.770 $\pm$ 0.1b	1.370 $\pm$ 0.1c*	0.867 $\pm$ 0.2*
Ca	2.86 $\pm$ 0.4a	2.86 $\pm$ 0.5ab	2.91 $\pm$ 0.6ab	2.350 $\pm$ 0.5a	3.760 $\pm$ 1.1b	2.654 $\pm$ 0.7

Table 8. Nutrient contents (mean  $\pm$  SD) in needles of nursery-grown silver fir seedlings maintained for two years under four different levels of solar irradiance (100%, 48%, 18% and 8% of full irradiance) in a nursery. The seedlings were grown in 10 L pots filled with a mixture of sand and blond peat (5:2; v/v) and fertilized with 5 g L<sup>-1</sup> slow-release fertilizer "Nutricote" (N/P/K 13:13:13 supplemented with micronutrients). Statistical significance of differences among means was tested using one-way analysis of variance ( $P < 0.05$ , ns - non significant) followed by pairwise comparisons using Tukey's test (global  $\alpha = 0.05$ ; different letters indicate significant differences).

Nutrients (mg g <sup>-1</sup> )	100 % of full irradiance	48%	18%	8%	<i>P</i>
N	19.7 $\pm$ 14.46	20.47 $\pm$ 4.42	20.0 $\pm$ 63.23	19.75 $\pm$ 2.71	ns
P	2.07 $\pm$ 0.36	2.15 $\pm$ 0.32	2.24 $\pm$ 0.35	2.44 $\pm$ 0.23	ns
K	9.16 $\pm$ 1.57	9.10 $\pm$ 1.21	9.92 $\pm$ 0.52	10.68 $\pm$ 0.57	ns
Mg	1.44 $\pm$ 0.27a	1.70 $\pm$ 0.16 ab	1.86 $\pm$ 0.23b	1.82 $\pm$ 0.14b	0.018
Ca	6.78 $\pm$ 1.39a	8.86 $\pm$ 1.55ab	9.37 $\pm$ 1.53b	6.94 $\pm$ 0.42a	0.009

ability with regard to growth and physiological parameters. Growth rate is usually considered the best indicator of environment quality because it integrates numerous trophic and nontrophic factors experienced by the plant. In the case of silver firs, growth increments correlated positively with canopy openness showing that even for this shade tolerant species (Jaworski and Zarzycki 1983) per-plant carbon gains are light limited. This confirms earlier findings that *Abies alba* shows better growth under higher irradiance (Magnuski *et al.* 2001). Also an *in situ* study of responsiveness of *A. alba* seedlings to CO<sub>2</sub> indicated severe light limitation, although at lower light levels than encountered in our study (Hättenschwiler and Körner 2000). On the other hand, firs that in young age grew under high light levels suffer a decreased vitality as adult trees (Jaworski 1995). The effect of light environment on young fir trees needs therefore to be analyzed at multiple temporal scales. Ways of quantifying plant vigour other than simple growth measures should also be incorporated in the studies.

An integrated measure of plant's physiological health is provided by the potential quantum efficiency of photosystem II (Shangguan *et al.* 2000). A decrease in the  $F_v/F_m$  level below 0.83 is considered to indicate stress conditions affecting the photosynthetic apparatus. In silver firs, the  $F_v/F_m$  index was almost always below this critical value, and the pattern of its depression suggested an inverse link to the light level prevailing under the particular canopy. It appears that the light stress could have also interacted with cold temperature inhibition of photosynthesis (Taulavuori *et al.* 2000) related to radiative cooling of needles under the open canopies, such as those formed by *Betula* or *Fagus*. This is because the especially low value of  $F_v/F_m$  in April under *Betula* indicated an additional local stress factor, most likely a temperature drop, such as might have been caused by a mass of cold air stagnating in this flat depression. Thus, good illumination in the absence of canopy foliage in the spring, combined with low ambient temperatures and led to severe photoinhibition under deciduous canopies. In contrast, the evergreen *Picea* and *Pinus* canopies provided some shade in the spring when photosynthetic carbon fixation was temperature inhibited, thus attenuating light stress and possibly also reducing the extent of radiative leaf cooling in

young fir plants. With the progression of the growth season, stress indicated by depression of  $F_v/F_m$  diminished greatly, again suggesting a link to temperature. In a growth chamber study, significantly lower values of  $F_v/F_m$  were measured in needles of silver firs exposed for 24 h to 10° C, compared with plants acclimated to a higher temperature (Robakowski *et al.* 2002). The overall seasonal pattern of  $\Phi_{PSII}$  depression and recovery (Table 5) also suggests a role for ambient temperature in determining the extent to which young fir plants are able to utilize photosynthetically active radiation.

The reduction of quantum efficiency early in the season has been documented for *Pinus sylvestris* (Pukacki 2000) and *Pinus contorta* (Savitch *et al.* 2002). Although such reduction is normally thought to represent a stress symptom, it may also be considered as a beneficial adjustment linked to the lower photosynthetic ability in the cold season. Plants that remain actively growing in winter, such as wheat, maintain a high  $F_v/F_m$  (Savitch *et al.* 2002). In this study, a positive example of acclimation to high light environment is seen in the maintenance of highest  $\Phi_{PSII}$  in firs growing under the highly light-transparent *Larix* stand (Table 5).

Further insights into the question of acclimation to light is provided by fluorescence quenching analysis, especially by the seasonal course of qNP, the level of which is largely determined by the operation of a system of photoprotective xanthophyll pigments (Havaux and Tardy 1996; Müller *et al.* 2001). In April and July, qNP was both high and, surprisingly, independent of light level (Fig. 2). In October, however, an overall strong reduction in qNP was noted, again, contrary to the expectation that needles would adapt to the approaching dormant, stressful season (Ottander *et al.* 1995, Savitch *et al.* 2002). Admittedly, in October qNP showed a strong negative correlation with  $\Phi_{PSII}$  suggesting that photoprotective mechanisms efficiently operated in low-light sites (e.g. *Picea*, Fig. 2f). The role of qNP in providing protection against excessive light level during the winter in silver fir requires further study. Our data on total carotenoid content were collected only for summertime and show little indication of a link with the light level (Fig. 4). However, the carotenoid/chlorophyll ratio was clearly elevated in the better illuminated sites, especially under *Pinus*, as compared to e.g. the dark *Fagus*

(Fig. 4) suggesting a protective role against excessive light played by carotenoid compounds towards the chlorophyll.

The qP coefficient, which is linearly related to the proportion of open PSII reaction centers (Maxwell and Johnson 2000) was positively related to the effective quantum yield on both July and October measuring dates (Figs. 2c, e). The link to site light conditions was, again, weak, but in these two months consistently lowest qP was found under the dark *Picea* canopy and the highest under the rather light-permeable *Larix*. It has been suggested that with low qP values (under 0.6, Öquist *et al.* 1992) a potential exists for photoinhibitory damage. Even with the light intensity slightly in excess of  $300 \mu\text{mol m}^{-2} \text{s}^{-1}$  that we used for light-adapted measurements, such values of qP were reached under *Picea*, clearly suggesting low photosynthetic capacity and therefore acclimation of firs growing at that site to shade, and at the same time their vulnerability to high light stress. Perhaps even more remarkably, photosynthetic characteristics of young firs growing under the bright canopy formed by *Larix*, showed both high qP and quantum yield, and should be considered as light adapted.

Analyses of foliar nutrient content in field-grown firs from Sudety Mts. (Karkonoski National Park) indicate an overall nutrient deficiency on comparison with well-nourished nursery grown plants (Tables 7 and 8). Admittedly, supra-optimal levels of nitrogen are frequently found in fertilized plants (e.g. 5–10 year old spruce trees, Landmann *et al.* 2000) reflecting luxury consumption, a phenomenon frequently found in slow growing late-successional plants (Chapin 1985). Such response to fertilization is not, however, likely for all nutrients, therefore we interpret nutrient levels in nursery plants as indicative of nutrient sufficiency. Unexpectedly low values of K and Mg were found under the *Larix* canopy, where growth increments were the highest. It is likely that both these nutrients became diluted by the accumulating biomass. In the spruce stands of Karkonosze Mts., a deficiency in magnesium was suggested to be a main cause of spruce crowns defoliation. However, the relations among nutrients are more complex. A deficiency in nitrogen, potassium, magnesium, calcium and iron was accompanied by an unbalance between the concentrations of nitrogen –

phosphorous, potassium – phosphorous, iron – phosphorous and by toxic effects of aluminium, lead and sulphur on photosynthetic apparatus (Stachurski *et al.* 1992, Zimka *et al.* 1995). Supplementary fertilization studies and soil nutrient data will be required to further elucidate the issue of nutrient deficiency in young firs. Interestingly, chlorotic needles collected from *Betula* and *Pinus* stands had nutrient contents within the green-needle range, except for a slight depletion of N (Table 7), suggesting a non-nutrient cause for chlorosis.

Indications of other stress factors not directly related to ambient light level, such as deer damage (*Fagus* stand) and soil flooding (*Betula* stand) were observed during this study, adding complexity to our field-based analysis of stress and vitality in young firs.

## 5. CONCLUSIONS

Our study revealed a significant influence of conditions created by composition of canopy on growth and physiological performance of young fir trees. Canopy type strongly modifies the light environment experienced by fir seedlings in a season-dependent manner. While growth shows a straightforward positive relationship with ambient irradiance level, the effect of canopy type on photosynthetic parameters is complex. In the spring, *Abies alba* experienced photoinhibition (probably as a result of photodamage aggravated by cold stress), particularly under deciduous canopies (esp. *Betula*). Late frost which in the Polish mountains may occur as late as June, along with radiative cooling occurring before the leafing out of deciduous canopies poses a danger of causing photoinhibitory damage. Some indications of acclimation to local light conditions are provided by both fluorescence quenching analysis and leaf carotenoid content. Considering growth responses and light acclimation ability of *Abies alba* seedlings under the different canopies, a tentative recommendation can be made that their performance is optimal under the more open canopies, such as those formed by *Larix* and *Pinus*. Nutrient budgets of *Abies alba* planted in Karkonosze Mts. appear to be uniformly strained, without clear connection to the ambient light. Mineral nutrition of these seedlings offers a fruitful field of future silvicultural studies.

Complex analyses of physiological performance in the field remain highly challenging but are needed for realistic evaluation of stress effects. Fluorescence analysis has become a valuable tool in such research, but a finer time resolution in seasonal sampling and integration with gas exchange studies, as well as intensively recorded environmental parameters will be needed to allow deconvoluting specific stress factors.

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## 6. SUMMARY

In Karkonoski National Park (Sudety Mountains, Southern Poland), three-year-old seedlings of *Abies alba* Mill. were planted in five forest stands dominated by *Betula pendula*, *Fagus sylvatica*, *Larix decidua*, *Picea abies* or *Pinus sylvestris* (Fig. 1). The stands differed in canopy openness such that young fir plants experienced drastically different light environments. After three years of acclimation to site conditions, length of shoots, seasonal changes in chlorophyll *a* fluorescence parameters, and the contents of chlorophyll, carotenoids and nutrients in the needles were studied to evaluate the seedling performance. Experimental sites and their light conditions are described in Tables 1 and 2. Growth and photosynthetic characteristics of the young seedlings responded strongly to local light conditions. Length of leader shoot and twigs of the upper whorl increased with growing irradiance (Table 3). The potential quantum yield of photosystem II ( $F_v/F_m$ ) showed a strong depression in the spring, especially under the leafless canopies of *Betula* and *Fagus*, probably because of the occurrence of low air temperatures (Table 4). Later in the season  $F_v/F_m$  showed substantial recovery in all stands. Effective quantum yield  $\Phi_{PSII}$  measured under actinic light also showed a growing trend throughout the season with the lowest levels noted in the spring, particularly under *Fagus* and *Betula* (Table 5). Photosynthetic plasticity of the seedlings based on chlorophyll fluorescence parameters significantly differed among the sites (Table 6) without showing a clear relationship with irradiance level. Fluorescence quenching revealed complex seasonal behaviour with little obvious relationship with stand illumination, except for consistently low value of photochemical quenching, and immediate or high nonphotochemical quenching under the dark *Picea* canopy, probably reflecting photoprotective acclimation to sunflecks (Fig. 2). Although needle chlorophyll content showed no relationship to ambient light, the ratio of carotenoids

to chlorophyll was positively linked to canopy openness suggesting the existence of leaf photoprotection that evaded detection by quenching analysis (Figs. 3 and 4). Nutrient contents in needles depended on site conditions but, again, without a direct link to light, except perhaps for the light-transparent *Larix* stand, where fir needles were strongly depleted in Mg and Ca (Table 7). Overall, however, all fir plants from experimental plots had much lower needle nutrient content than nursery-raised plants (Table 8). Needle chlorosis found in *Betula* and *Pinus* stands was, however, not caused by macronutrient deficiencies. Considering all variables, conditions most conducive for fir growth and good vigor were found under *Larix* and *Pinus* canopies where light penetration was intermediate to high. In contrast, the poorest conditions were found in the *Betula* stand, where high light penetration in the spring was followed by shading of firs during the growing season.

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