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Growth and pigmentation of various species under blue light depletion

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The effect of blue light (400–500 nm) removal on the morphology and pigmentation of evergreen *Pinus sylvestris* seedlings of northern (67°N) and southern (62°N) origins, deciduous *Betula pubescens* spp. *czerepanovii* and *B. pubescens* f. *rubra* seedlings, and herbaceous *Epilobium angustifolium* and *Glechoma hederacea* plants was studied. The plants were grown at the latitude 69°N. The blue wavelengths were removed from sunlight by orange plexiglass chambers. The results suggest that the northern origin *Pinus sylvestris* seedlings are more sensitive to blue light as compared with the southern origin seedlings. The changes in the morphology of shade-intolerant *Pinus sylvestris*, both *Betula* seedlings and *Epilobium angustifolium* were similar and more pronounced under blue light depletion, as compared with the more shade-tolerant *Glechoma hederacea*. This indicates that the morphological responses of shade-intolerant species to blue light depletion are quite conservative across life forms.

Introduction

Shade below a vegetation canopy is composed of at least three components: reduced light quantity and changed light quality i.e. a reduced red (600–700 nm) to far-red (700–800 nm) light ratio (R/FR) and a reduced amount of blue light (400–500 nm). The changes in light conditions occur because the forest canopy foliage effectively absorbs red and blue wavelengths from sunlight (Smith 1982). Reduction both in light quantity and in the R/FR ratio increases stem elongation in many plant species (McLaren and Smith 1978, Warrington *et al*. 1988, Smith and Whitelam 1997). Artificial removal of blue wavelengths from sunlight also increased the stem elongation of *Pinus sylvestris* seedlings (Taulavuori *et al*. 2005, Sarala *et al*. 2007). The increase in elongation was observed especially at subarctic latitudes, while at mid-boreal latitudes the response was smaller or absent (Taulavuori *et al*. 2005, Sarala *et al*. 2007, Sarala *et al*. 2009). The obvious reason for the different responses between the latitudes are a result of a relatively high proportion of blue light during the night hours of polar summer (Taulavuori *et al*. 2010).

Northern populations of *Picea abies* require more far-red light to maintain epicotyl extension

growth than southern populations (Clapham *et al*. 1998, Mølmann *et al*. 2006). Far-red light is also needed to maintain secondary needle extension growth in northern populations of *Pinus sylvestris* (Clapham *et al*. 2002). The result was not observed in southern populations. In addition, red and far-red light de-etiolated more the hypocotyls of northern populations of *Arabidopsis thaliana* than southern populations (Stenøien *et al*. 2002). This suggests that plants from northern provenances may be more sensitive to quality of light.

Pinus sylvestris is an evergreen coniferous tree species with a strong shade-avoiding tendency (de la Rosa *et al*. 1998). In addition to increasing stem elongation in low-light conditions (Atkinson 1984, Taylor and Davies 1988, Warrington *et al*. 1988), shade-avoiding species reduce branching and accumulation of chlorophylls and carotenoids and change leaf size and allocation of growth resources (McLaren and Smith 1978, Atkinson 1984, Warrington *et al*. 1988, de Kroon and Knops 1990, Begna *et al*. 2002, Cookson and Granier 2006). In a low R/FR ratio, shade-intolerant plants increase petiole length and reduce leaf area, branching and chlorophyll synthesis (Smith and Whitelam 1997). In our earlier studies, the removal of blue wavelengths increased stem elongation, but it also increased the elongation of lateral branches, needle area and the biomass of the new stem and needles of *Pinus sylvestris* seedlings (Taulavuori *et al*. 2005, Sarala *et al*. 2007), which indicates that the response is not an etiolation process.

The aim of this research was to test if the elongation response to blue light depletion is similar (1) among *Pinus sylvestris* from northern and southern provenances, (2) among different life forms (i.e. evergreen *vs*. deciduous, woody *vs*. herbaceous), and (3) between sun- and shade-preferring species. Additionally, we tested (4) if the removal of blue light affect pigmentation of various species. This was done to find out whether the increased elongation under blue-light depletion is rather a photomorphogenic regulation response of metabolism instead of a simple etiolation response as was proposed by our previous study (Sarala *et al*. 2009). In addition, the effect of blue-light removal on anthocyanin concentration was studied, since anthocyanins absorb light in the blue/UV-region, thus protecting plants from a high level of short-wavelength sunlight (Mohr and Drumm-Herrel 1983).

Materials and methods

Experimental design

Four orange and transparent plexiglass chambers (bottom area 1 × 2 m and height 1 m) and ambient control plots (n = 4) were built at the study site (69°3´N, 20°47´E, 473 m a.s.l.). The chambers were built in an open area, in the same direction and at equal distances from each other. The orange chamber (–B; minus blue) removed the blue wavelengths (400–500 nm), while the transparent chamber allowed penetration of all wavelengths (CC; chamber control). There were also ambient control plots, which were wooden tables without chambers. However, these results are omitted since the growth of all species was retarded due to cold, growth-inhibiting weather. The removal of blue wavelengths decreased the amount of incoming visible light (400–800 nm) by approximately 30%. A more detailed description of the experimental design including chamber ventilation and an example of the spectra in the chambers is given in Taulavuori *et al*. (2005). The maximum temperatures inside the chambers were +40 °C (CC) and +37 °C (–B) (data not shown). The minimum temperature was –6 °C in both chambers. In the ambient control plots, the temperature maximum reached +26 °C and the minimum –6.5 °C. Relative humidity inside the chambers varied between 22% and 100%, and in the ambient control plots between 27% and 100% (data not shown).

The following four tests were carried out:

Test 1. The effect of blue light depletion on the morphology of *Pinus sylvestris* seedlings from two provenances: One-year-old *Pinus sylvestris* seedlings of northern (67°N) and southern (62°N) origins were studied for changes in morphology. The seedlings were placed in four (n = 4) replicate chambers and control plots with 20 seedlings from both origins in each on 18 May 2008 (Julian day 139). The seedlings were in the experiment

until 28 July (Julian day 210). By that time, stem elongation of the chamber seedlings had ceased.

Test 2. The effect of blue light depletion on deciduous trees: *Betula pubescens* ssp. *czerepanovii* and *Betula pubescens* f. *rubra* were studied for changes in morphology. *Betula pubescens* ssp. *czerepanovii* is a subspecies of a deciduous tree species *Betula pubescens*, which is shade intolerant especially in its early stage (Ellenberg 1988). *Betula pubescens* f. *rubra* is a mutant form of *Betula pubescens*, which exhibits a special springtime phenology: the young leaves are green but turn to red soon after leaf burst because of anthocyanin accumulation. During the summer, as the amount of chlorophyll increases, the content of anthocyanins also increases further, deepening the red colour of the leaves (Kauppi and Ulvinen 1989). In the beginning of March, *Betula pubescens* ssp. *czerepanovii* originating from 69°N and *Betula pubescens* f. *rubra* originating from 64°N were micro-propagated and placed in growing rooms until included in the experiment on 17 June 2008 (Julian day 169), with 10 seedlings in four replicates of each treatment. The seedlings were in the experiment until July 18 (Julian day 200).

Test 3. The effect of blue light depletion in response to shade tolerance: Two herbaceous species, *Epilobium angustifolium* and *Glechoma hederacea*, were studied for changes in morphology. A light (L) value for shade tolerance of plant species has been determined by Ellenberg (1988). The value ranges from 1 to 9, number 1 being a very shade-tolerant plant and number 9 very intolerant. According to Ellenberg's (1988) classification, *Epilobium angustifolium* belongs to shade-intolerant (L = 8) pioneer species having strong demand for light, and therefore inhabiting open areas very fast. *Glechoma hederacea* is a more shade-tolerating species with L = 6, although Sparks *et al*. (1996) suggested changing the value to 3. It grows in undervegetation, where it rapidly forms wide patches with stolons. The leaves of *Glechoma hederacea* are green when growing in shade, but under greater light intensity conditions, the leaves turn to purple because of anthocyanin accumulation. *Epilobium angustifolium* plants were grown from seeds and *Glechoma hederacea* from stolons (both originating from 65°N) in growing rooms before placing 20 *Epilobium angustifolium* and 14 *Glechoma hederacea* plants in four replicates of each treatment on 25 June 2008. The plants were included in the experiment until July 25.

Test 4. The effect of blue light depletion on pigmentation (chlorophyll *a* and *b*, carotenoid and anthocyanins): The pigment concentrations were studied in *Pinus sylvestris* seedlings of northern and southern origins (Test 1), *Betula* seedlings (Test 2), *Epilobium angustifolium* and *Glechoma hederacea* (Test 3). All the seedlings were well watered and were positioned in the treatments so that no mutual shading occurred. All seedlings were grown in plastic pots. The diameter of the pots was 10 cm for southern *Pinus sylvestris* seedlings and 8 cm for northern *Pinus sylvestris* seedlings and other plant species. *Pinus* seedlings were planted in a peat–sand mixture [2/3 standard fertilized peat (N-P-K: 12%-9%-18%), 1/3 sand] and all the other species in fertilized peat. No other fertilization was given to the plants during the experiment.

Morphological measurements

Shoot elongation of *Pinus sylvestris* seedlings was measured principally once a week. The elongation of the seedlings is expressed in percentages to clarify the effect of blue light removal, since the seedlings of southern origin were much larger and they also grew larger during the experiment. The percentages were calculated against the elongation of the seedlings grown in the CC chambers. Elongation of *Betula* seedlings was measured three times during the experiment. Shoot elongation of *Epilobium angustifolium* and elongation of two selected stolons of *Glechoma hederacea* were measured at the beginning and at the end of the experiment. The initial growth was subtracted from the growth at the end to obtain the amount of elongation that occurred during the experiment.

Lateral branch elongation of *Pinus sylvestris* seedlings of both origins and *Epilobium angustifolium* and length of stolons of *Glechoma hederacea* that had grown only during the experiment were measured from three of the longest branches or stolons of four plants per chamber or control plot.

Old stem or stolon diameter of all the plants was measured 30 mm the above soil level and new stem diameter at the mid-point of the new growth. Due to slow growth of the *B. pubescens* ssp. *czerepanovii* seedlings, the diameter increment measurements were not performed.

An internode number was calculated from four *Betula pubescens* f. *rubra* seedlings per chamber or control plot from the part of stems that had grown during the experiment. As the *Betula pubescens* ssp. *czerepanovii* seedlings elongated very little or not at all, an internode number was not calculated from this sub-species.

Needle length was measured from 3–4 needles per plant and four seedlings per chamber or control plot. Needle width and thickness were measured from the middle of the same needles. All the measurements described so far from every plant species were performed with Vernier's calliper (accuracy ± 0.1 mm).

Leaf length, width and area of *Betula* sp. seedlings and *Epilobium angustifolium* were determined from photographs (taken with a Canon IXUS 500 digital camera) of the three uppermost leaves of four plants per chamber or control plot with ImageJ (NIH). Leaf length, width and area of *Glechoma hederacea* were measured from photographs of three randomly selected leaves from the leaves grown during the experiment (new) and the leaves that already existed at the beginning of the experiment (old) of four plants per chamber or control plot with ImageJ. Leaf width of all species was determined from the widest part of the leaf. The leaves used for size measurements were also used for petiole elongation measurements. Total leaf number was calculated from four *Epilobium angustifolium* plants and the number of new leaves from one *Glechoma hederacea* plant per chamber or control plot.

The same plants of all species that had been used for the other measurements were also used for biomass determination. The plants were cut into pieces (old stem from the soil level), the roots carefully washed and all parts oven dried before weighing. Root elongation of *Pinus sylvestris* seedlings of both origins was measured from three of the longest roots.

Pigment analyses (Test 4)

Pigment concentrations were analyzed from both previous and current year needles of *Pinus sylvestris* seedlings. From *Betula pubescens* ssp. *czerepanovii*, *Betula pubescens* f. *rubra* and *Epilobium angustifolium*, the leaves that had grown before placing them into the experiment and leaves grown during the experiment were mixed for pigment analyses to obtain an adequate amount of sample. From *Glechoma hederacea*, the leaves that had grown before and during the experiment were collected and analyzed separately. The needles and leaves were frozen in liquid nitrogen after collection and stored in a deep freezer (70 °C). Chlorophyll *a* and *b* and carotenoid pigments were analysed spectrophotometrically according to Soukupova *et al*. (2000), with the exception that the 0.1 g of sampled frozen needles and leaves were cut into < 2 mm pieces before placing them into 2 ml of dimethylformamide for 2–3 days. The concentrations of chlorophylls and carotenoids were calculated according to Wellburn (1994). Anthocyanin content was analysed spectrophotometrically using a slightly modified Hodges *et al*.'s (1999) method. For this work, 0.1 g of sampled frozen needles and leaves were homogenized in 2 ml of methanol–1% HCl (v/v). Total anthocyanins were determined as the difference between the absorbance at 536 and 600 nm (corrected for phaeophytin) and the results are expressed as cyaniding-3-glucoside equivalents through its molar absorption coefficient.

Statistical analyses

Differences in seedling growth and pigment concentrations between CC and –B chambers were analysed with an independent samples *t*-test at the end of the experiment ($n = 4$ chambers/plots). The equality of variances was tested with Shapiro-

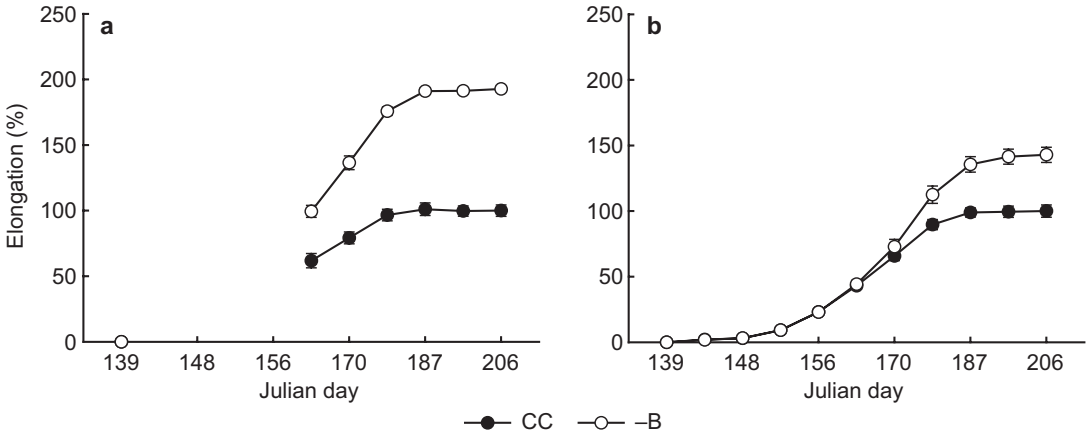

Fig. 1. Elongation growth of one-year-old *Pinus sylvestris* seedlings of (**a**) northern and (**b**) southern origins grown in transparent (CC) and orange (–B) chambers (n = 4). The percentages are calculated against the elongation growth of the seedlings grown in CC chambers. Error bars represent SE. Julian day 153 denotes 1 June 2008.

Wilk's test. The statistical analyses were performed using the SPSS 16.0 software package.

Results

Changes in morphology under blue light depletion

Test 1

The elongation of both origins of *Pinus sylvestris* was significantly (northern origin: t-test t_6 = 19.132, $p < 0.001$ and southern origin: $t_6 = 5.74$, $p < 0.001$) increased by the removal of blue light (Fig. 1). In the northern origin *Pinus sylvestris*, the elongation under blue light depletion was 92% higher as compared to the seedlings in CC (Fig. 1a) being 90.4 ± 0.8 mm in –B and 46.9 ± 2.1 mm in CC. The elongation of the southern origin *Pinus sylvestris* was 143.8 ± 5.8 mm in –B and 100.7 ± 4.9 mm in CC, indicating that the seedlings grew 43% higher under blue light depletion (Fig. 1b).

The removal of blue light significantly increased branch ($t_6 = 2.693$, $p < 0.05$) and needle (t_2= 4.441, $p < 0.05$) lengths of northern origin *Pinus sylvestris* (Table 1). Also the elongation of the three longest roots was slightly increased under blue light depletion. No differences between the chambers were found in the following: diameter of new and old stem, needle width and thickness, biomass of the new and old stem and needles, new branches and roots. In the southern origin *Pinus sylvestris*, there was a significant increase in the diameter of the new stem (t_6 = 3.996, $p < 0.01$) and needle length (t_4 = 5.536, $p < 0.01$) and a reduction in the biomass of the roots (t_3 = –4.943, $p < 0.05$) in seedlings grown under blue light depletion (Table 1). Elongation of branches was slightly increased by light manipulation. No differences between the chambers were found in old-stem diameter, needle width and thickness, root elongation, biomass of the new and old stem and needles or new branches.

Test 2

No statistically significant difference in stem elongation of *Betula pubescens* ssp. *czerepanovii* and *Betula pubescens* f. *rubra* was found, even though the height growth of the seedlings grown under blue light depletion was slightly increased as *Betula pubescens* ssp. *czerepanovii* elongated 3.4 ± 0.2 mm in –B and 2.8 ± 0.6 mm in CC and *Betula pubescens* f. *rubra* 20.5 ± 3.8 mm and 14.2 ± 3.0 mm in –B and CC (Fig. 2). There were no statistically significant differences in any variable between the chambers in *Betula pubescens* ssp. *czerepanovii* (Table 2). However, leaf area, leaf length and width and petiole

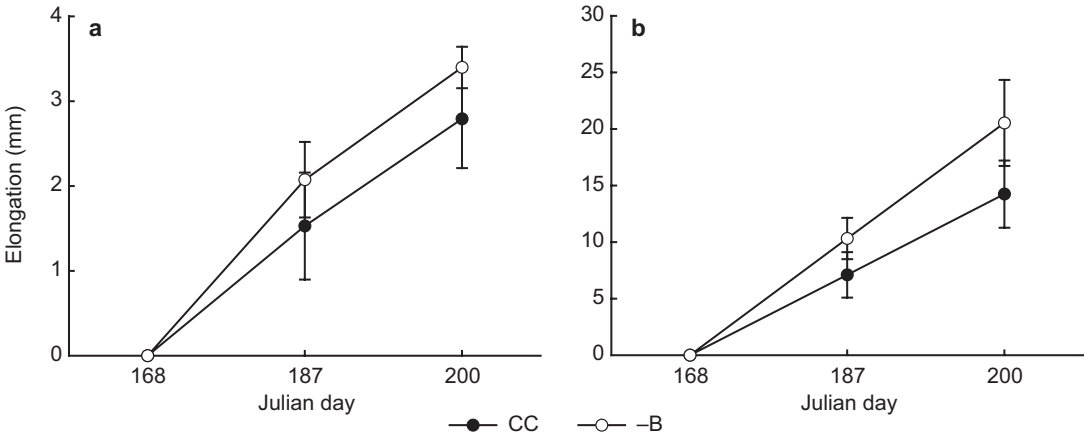

Fig. 2. Elongation of (**a**) *Betula pubescens* ssp. *czerepanovii* and (**b**) *Betula pubescens* f. *rubra* in transparent (CC) and orange (–B) chambers (n = 4). Error bars represent SE. Julian day 169 denotes 17 June 2008.

length were slightly increased by the removal of blue wavelengths. In *Betula pubescens* f. *rubra*, blue light depletion significantly increased leaf area (t_6 = 2.847, $p < 0.05$), leaf length (t_6 = 3.071, $p < 0.05$) and petiole length (t_6 = 3.729, $p < 0.01$) (Table 2). No difference between the

Table 1. Growth characteristics of northern (67°N) and southern (62°N) origins of one-year-old *Pinus sylvestris* seedlings grown in the transparent (CC) and orange (–B) chambers (n = 4). One (*) and two (**) asterisks indicate significant difference (independent samples t-test) between CC and –B at $p < 0.05$ and $p < 0.01$, respectively.

	CC ± SE	–B ± SE
***Pinus sylvestris* northern**		
Branch elongation (mm)	10.43 ± 1.16	22.42 ± 4.3*
New stem diameter (mm)	2.11 ± 0.07	2.13 ± 0.04
Old stem diameter (mm)	2.88 ± 0.09	2.88 ± 0.1
Needle length (mm)	32.02 ± 1.6	45.44 ± 4.59*
Needle width (mm)	1.08 ± 0.045	0.97 ± 0.034
Needle thickness (mm)	0.45 ± 0.016	0.43 ± 0.043
Root elongation (mm)	139.54 ± 25.02	201.83 ± 17.33
New stem biomass (g)	0.13 ± 0.011	0.16 ± 0.005
Old stem biomass (g)	0.27 ± 0.012	0.27 ± 0.004
New needle biomass (g)	0.48 ± 0.047	0.42 ± 0.032
Old needle biomass (g)	0.48 ± 0.024	0.45 ± 0.028
New branch biomass (g)	0.02 ± 0.001	0.021 ± 0.004
Root biomass (g)	0.74 ± 0.038	0.79 ± 0.088
***Pinus sylvestris* southern**		
Branch elongation (mm)	19.97 ± 3.19	28.7 ± 4.67
New stem diameter (mm)	2.19 ± 0.02	2.5 ± 0.07*
Old stem diameter (mm)	3.32 ± 0.12	3.43 ± 0.05
Needle length (mm)	32.1 ± 1.3	41.71 ± 1.16**
Needle width (mm)	1.15 ± 0.05	1.05 ± 0.03
Needle thickness (mm)	0.5 ± 0.03	0.48 ± 0.02
Root elongation (mm)	141.7 ± 19.08	145.7 ± 32.0
New stem biomass (g)	0.26 ± 0.023	0.32 ± 0.027
Old stem biomass (g)	0.53 ± 0.014	0.49 ± 0.013
New needle biomass (g)	0.93 ± 0.06	0.97 ± 0.05
Old needle biomass (g)	1.06 ± 0.09	0.67 ± 0.17
New branch biomass (g)	0.047 ± 0.015	0.055 ± 0.011
Root biomass (g)	1.56 ± 0.1	1.07 ± 0.02*

chambers was found in new stem diameter or in the number of internodes. Also, none of the treatments had an effect on old stem diameter, leaf width and total biomass of stem, leaves or roots.

Test 3

The removal of blue light significantly (t_6 = 6.535, $p < 0.001$) increased stem elongation of *Epilobium angustifolium* (Table 3). Also, the leaf area (t_6 = 3.211, $p < 0.05$) and the diameter of old stem (t_6 = 3.222, $p < 0.05$) were significantly increased by the applied light manipulation. In addition, the leaf length was slightly, although not significantly, higher under blue light depletion. The removal of blue wavelengths significantly lowered the number of branches ($t_6 = -2.523$, $p < 0.05$) and the biomass ($t_6 = -3.721$, $p < 0.01$) and number of leaves ($t_6 = -5.211$, $p < 0.01$). No differences between the treatments were found in the length of three of the longest branches, diameter of new stem, total stem biomass and leaf width.

In *Glechoma hederacea*, there were no significant differences between the chambers in the elongation of selected old stolons and new stolons grown after the start of the experiment (Table 3). The length of new petiole was, however, significantly ($t_6 = 5.142$, $p < 0.01$) increased under blue light depletion. Also, the leaf area and width of new leaves were slightly, but not significantly, increased by the removal of blue light. However, the length of new leaves was the same in both chambers. There were also no significant differences between the chambers in the number of over 1-cm- and over 20-cm-long stolons, length of old petioles, old stem diameter, biomass of old stem and old leaves or number of new leaves.

A summary of the effect of blue light removal on the morphology of all species is presented in Table 4.

Changes in pigmentation under blue light depletion (Test 4)

Accumulation of Chl *a* and *b* and Car of the previous year's needles of both origins of *Pinus sylvestris* seedlings was unaffected by the applied light manipulation (Table 5). However, the anthocyanin concentration of the previous year's needles of northern origin *Pinus sylvestris* grown

Table 2. Growth characteristics of *Betula pubescens* ssp. *czerepanovii* and *Betula pubescens* f. *rubra* seedlings. Abbreviations are as in Table 1 and $n = 4$. * = significant difference (independent samples *t*-test) between CC and –B at $p < 0.05$.

	CC ± SE	–B ± SE
Betula pubescens* ssp. *czerepanovii		
Stem biomass (g)	0.03 ± 0.008	0.034 ± 0.008
Leaf biomass (g)	0.08 ± 0.021	0.09 ± 0.029
Root biomass (g)	0.17 ± 0.044	0.16 ± 0.04
Leaf area (mm^2)	99.06 ± 22.23	154.69 ± 49.2
Leaf length (mm)	15.08 ± 1.73	18.48 ± 3.41
Leaf width (mm)	15.77 ± 1.95	19.07 ± 3.91
Petiole length (mm)	2.78 ± 0.5	3.63 ± 0.63
Betula pubescens* f. *rubra		
Internode no.	4.75 ± 0.23	4.5 ± 0.1
New stem diameter (mm)	0.98 ± 0.05	1.05 ± 0.07
Old stem diameter (mm)	1.18 ± 0.03	1.18 ± 0.04
Stem biomass (g)	0.04 ± 0.008	0.04 ± 0.003
Leaf biomass (g)	0.13 ± 0.018	0.11 ± 0.002
Root biomass (g)	0.12 ± 0.013	0.1 ± 0.004
Leaf area (mm^2)	146.1 ± 19.4	206.97 ± 8.92*
Leaf length (mm)	18.76 ± 1.17	22.67 ± 0.51*
Leaf width (mm)	18.7 ± 1.19	20.9 ± 0.32
Petiole length (mm)	3.9 ± 0.27	5.0 ± 0.15*

under blue light depletion was significantly (t_6 = –3.772, $p < 0.05$) reduced, as was also the anthocyanin content of the seedlings from southern provenance, although statistically significant difference in the southern seedlings were not found (Table 5). The Chl *a* concentration in old leaves of *Glechoma hederacea* was not affected by the applied light manipulation (Table 5). No differences between the treatments were found in Chl *b* and Car concentrations. The anthocyanin concentration was 0.0 μmol g^{-1} FW under blue light depletion. The content was markedly increased in CC, although significant differences between the chamber treatments were not found.

There were no differences between the treatments in accumulation of Chl *a* and *b* and Car of the current year's needles of both origins of *Pinus sylvestris* seedlings (Table 6). Differences between the treatments were also not found in the anthocyanin concentration of both origins of *Pinus sylvestris*. Significantly reduced by the removal of blue wavelengths were the Chl *a* (t_6 = –4.101, $p < 0.01$) and *b* (t_6 = –4.301, $p < 0.01$) and Car (t_6 = –2.903, $p < 0.05$) concentrations of *Betula pubescens* f. *rubra*, as well as the anthocyanin content of *Betula pubescens* ssp. *czerepanovii* (t_6 = –5.25, $p < 0.01$) (Table 6). The anthocyanin concentration of *Betula pubescens* f. *rubra* seedlings was 104.7 ± 8.2 μmol g^{-1} FW at the beginning of the experiment, but decreased to 89.0 ± 10.4 μmol g^{-1} FW in –B and increased significantly (t_6 = –13.465, $p < 0.001$) to 294.8 ± 11.2 μmol g^{-1} FW in CC by the end of the experiment (Fig. 3). In *Epilobium angustifolium*, leaves having no differences between the treatments in Chl *a* and *b* and Car concentrations were found, and the removal of blue light had no effect on anthocyanin concentration (Table 6). There were

Table 3. Growth characteristics of *Epilobium angustifolium* and *Glechoma hederacea* plants. Abbreviations are as in Table 1. One (*) and two (**) asterisks indicate significant difference (independent samples *t*-test) between CC and –B at $p < 0.05$ and $p < 0.01$, respectively.

	CC ± SE	–B ± SE
Epilobium angustifolium		
Elongation (mm)	52.8 ± 3.06	93.8 ± 5.48*
Branch length (mm)	74.2 ± 3.3	78.4 ± 6.5
Branch no.	20.0 ± 1.2	15.4 ± 1.4*
New stem diameter (mm)	2.43 ± 0.14	2.38 ± 0.1
Old stem diameter (mm)	1.7 ± 0.08	2.1 ± 0.09*
Stem biomass (g)	0.17 ± 0.007	0.16 ± 0.02
Leaf biomass (g)	1.02 ± 0.05	0.72 ± 0.06**
Leaf area (mm^2)	226.7 ± 25.34	355.0 ± 31.0*
Leaf length (mm)	13.5 ± 1.94	18.7 ± 3.61
Leaf width (mm)	3.9 ± 0.92	4.2 ± 0.71
Leaf no.	179.5 ± 12.8	95.3 ± 10.0*
Glechoma hederacea		
Old stolon elongation (mm)	31.1 ± 2.83	36.7 ± 1.00
New stolon length (mm)	26.8 ± 2.04	28.5 ± 1.52
Stolon no. over 1 cm long	44.0 ± 3.5	39.2 ± 1.3
Stolon no. over 20 cm long	8.5 ± 2.25	14.9 ± 3.07
New petiole length (mm)	1.6 ± 0.17	5.6 ± 0.75**
Old petiole length (mm)	5.4 ± 0.86	6.2 ± 0.84
New stem diameter (mm)	1.4 ± 0.1	1.2 ± 0.55
Old stem diameter (mm)	2.0 ± 0.6	1.5 ± 0.76
New stem biomass (g)	1.8 ± 0.28	1.5 ± 0.11
Old stem biomass (g)	0.6 ± 0.03	0.6 ± 0.08
New leaf biomass (g)	1.9 ± 0.36	1.8 ± 0.27
Old leaf biomass (g)	1.4 ± 0.11	1.3 ± 0.31
New leaf area (mm^2)	223.5 ± 20.7	344.5 ± 48.03
New leaf length (mm)	4.3 ± 0.54	4.7 ± 0.29
New leaf width (mm)	5.7 ± 0.3	6.9 ± 0.48
New leaf no.	287.8 ± 22.1	251.5 ± 23.0

no differences between the treatments in the Chl *a*, Chl *b* and Car concentration of the new leaves of *Glechoma hederacea* (Table 6). The anthocyanin concentration was 0 μmol g^{-1} FW in –B. The content was slightly increased in CC, although not statistically significantly.

In summary, blue light removal did not affect the concentrations of chlorophylls and carotenoids, except in *Betula pubescens* f. *rubra*. The anthocyanin content of all species decreased under blue light depletion.

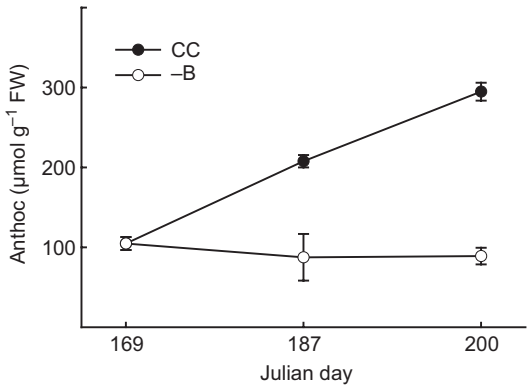

Fig. 3. Anthocyanin concentration in the leaves of *Betula pubescens* f. *rubra*. Error bars represent SE. Abbreviations are as in Fig. 2 and $n = 4$. Julian day 169 denotes 17 June 2008.

Discussion

Elongation

The elongation of both northern and southern origin *Pinus sylvestris* seedlings was increased

Table 4. Effect of blue light removal on the morphology of one-year-old *Pinus sylvestris* seedlings from northern (67°N) and southern (62°N) origins, *Betula pubescens* ssp. *czerepanovii*, *Betula pubescens* f. *rubra*, *Epilobium angustifolium* and *Glechoma hederacea*. ↑ = increase, ↓ = decrease, (↑) = slight but not statistically significant increase (↑), 0 = no effect in the morphological variable under blue light depletion (–B chamber) as compared with the control chamber (CC). – = no measured data.

Variable	*Pinus sylvestris*		*Betula pubescens*		*Epilobium angustifolium*	*Glechoma hederacea*
	northern	southern	ssp. *czerepanovii*	f. *rubra*		
Stem/stolon elongation	↑	↑	(↑)	(↑)	↑	0
Branch/new stolon elongation	↑	(↑)	–	–	0	0
Branch/stolon no.	–	–	–	–	↓	0
Old shoot/stolon diameter	0	0	–	0	↑	0
New stem/stolon diameter	0	↑	–	0	0	0
Internode no.	–	–	–	0	–	–
Needle length	↑	↑	–	–	–	–
Needle width	0	0	–	–	–	–
Needle thickness	0	0	–	–	–	–
New leaf length	–	–	0	↑	(↑)	0
New leaf width	–	–	(↑)	0	0	(↑)
New leaf area	–	–	(↑)	↑	↑	(↑)
Old petiole length	–	–	–	–	–	0
New petiole length	–	–	(↑)	↑	–	↑
Total leaf no.	–	–	–	–	↓	–
New leaf no.	–	–	–	–	–	0
Old shoot/stolon biomass	0	0	–	–	–	0
New stem/stolon biomass	0	0	–	–	–	0
Total stem biomass	–	–	0	0	0	–
Old needle/leaf biomass	0	0	–	–	–	0
New needle/leaf biomass	0	0	–	–	–	0
Total leaf biomass	–	–	0	0	↓	–
New branch biomass	0	0	–	–	–	–
Root biomass	0	↓	0	0	–	–
Root elongation	(↑)	0	–	–	–	–

by the removal of blue light (Fig. 1). This result is supported by our earlier work (Taulavuori *et al*. 2005, Sarala *et al*. 2007, Sarala *et al*. 2009) and is in line with the results of Fernbach and Mohr (1990). However, the increase in stem, branch and root elongation was more pronounced in the northern provenance (Fig. 1 and Table 1). This suggests that they may be more sensitive to blue light than the seedlings of southern provenance. This is in accordance with the findings that northern populations are more sensitive to changes in light quality (Clapham

Table 5. Concentrations (± SE) of chlorophyll *a* and *b*, carotenoids and anthocyanins in the previous year's needles of one-year-old northern (67°N) and southern (62°N) origin *Pinus sylvestris* seedlings and in the *Glechoma hederacea* leaves that already existed at the beginning of the experiment. CC denotes transparent and –B orange chamber ($n = 4$). * = significant difference (independent samples *t*-test) between CC and –B at $p < 0.05$.

	Chl *a* (μg g^{-1} FW)	Chl *b* (μg g^{-1} FW)	Car (μg g^{-1} FW)	Anthoc (μmol g^{-1} FW)
Pinus sylvestris				
northern				
CC	528.6 ± 30.6	149.9 ± 7.7	163.1 ± 10.7	8.4 ± 1.6
–B	479.4 ± 19.6	126.3 ± 6.4	148.3 ± 6.0	2.0 ± 0.6*
southern				
CC	478.4 ± 27.1	131.8 ± 7.4	154.8 ± 4.4	7.4 ± 1.7
–B	494.7 ± 54.4	135.0 ± 19.2	137.7 ± 9.3	0.9 ± 0.22
Glechoma hederacea				
CC	1205.9 ± 119.8	441.6 ± 64.6	287.4 ± 35.5	66.3 ± 43.7
–B	1257.6 ± 87.1	460.5 ± 27.2	296.8 ± 14.6	0

Table 6. Concentrations (± SE) of chlorophyll *a* and *b*, carotenoids and anthocyanins in the current year's needles of one-year-old northern (67°N) and southern (62°N) origin *Pinus sylvestris* seedlings, in the leaves of *Betula pubescens* ssp. *czerepanovii* and *Betula pubescens* f. *rubra* seedlings, in the leaves of *Epilobium angustifolium* and in the *Glechoma hederacea* leaves grown during the experiment. CC denotes transparent and –B orange chamber ($n = 4$). – = missing data. One (*) and two (**) asterisks indicate significant difference (independent samples *t*-test) between CC and –B at $p < 0.05$ and $p < 0.01$, respectively.

	Chl *a* (μg g^{-1} FW)	Chl *b* (μg g^{-1} FW)	Car (μg g^{-1} FW)	Anthoc (μmol g^{-1} FW)
Pinus sylvestris				
northern				
CC	393.3 ± 49.1	118.5 ± 17.3	119.5 ± 7.8	0.9 ± 0.5
–B	329.3 ± 28.3	90.1 ± 6.8	115.8 ± 7.5	0
southern				
CC	307.6 ± 16.5	92.3 ± 4.5	105.2 ± 5.2	0
–B	291.7 ± 32.2	83.0 ± 9.5	88.1 ± 11.1	0
Betula pubescens				
ssp. *czerepanovii*				
CC	–	–	–	123.7 ± 15.7
–B	–	–	–	32.0 ± 7.6**
f. *rubra*				
CC	960.1 ± 86.0	294.8 ± 26.0	269.7 ± 19.5	–
–B	600.2 ± 17.7*	187.3 ± 6.1*	210.8 ± 5.6*	–
Epilobium angustifolium				
CC	697.1 ± 35.2	231.2 ± 11.3	227.9 ± 11.5	134.6 ± 14.2
–B	617.8 ± 64.7	222.1 ± 20.3	193.5 ± 17.5	109.7 ± 20.8
Glechoma hederacea				
CC	1502.7 ± 54.8	494.8 ± 21.2	364.4 ± 9.2	5.6 ± 2.4
–B	1548.6 ± 95.5	542.5 ± 33.8	360.1 ± 21.3	0

et al. 1998, Clapham *et al*. 2002, Stenøien *et al*. 2002, Mølmann *et al*. 2006).

The removal of blue wavelengths also slightly increased elongation of both *Betula* seedlings by the harvest day on 18 July (Fig. 2). However, elongation of both *Betula* seedlings was not completed by that time, which may explain the weak response. The critical night length for growth cessation of tree species originating from the 69°N latitude is two hours (Junttila 2007), while for *Betula pendula* at the 65°N latitude it is approximately 4 hours (Viherä-Aarnio *et al*. 2006). At 69°N latitude, a two-hour night length occurs at the end of July and only a week later the night length is 4 hours. Thus, if the seedlings had been in the chambers for a couple of weeks longer, the difference in the elongation might have become significant, at least with the quicker growing *Betula pubescens* f. *rubra*.

Shade-intolerant herbaceous species increase shoot elongation in low light quantity (de Kroon and Knops 1990, Begna *et al*. 2002). Similarly, the stem elongation of *Epilobium angustifolium* increased under blue light depletion (Table 3) being 78% higher as compared with that of the plants grown in CC. On the contrary, in experimental light conditions in which quantity of light and R/FR ratio were low, the total length of primary stolons of *Glechoma hederacea* was not affected (Price and Hutchings 1996). Consistently, also in our work, the elongation of old stolons and the longest stolons developed during the experiment were unaffected by the removal of blue light in *Glechoma hederacea* (Table 3).

Taken together, the removal of blue wavelengths increased stem elongation of evergreen *Pinus sylvestris* and deciduous *Betula* tree seedlings, which are all shade intolerant. In herbaceous species, the stem height increment of shade-avoiding *Epilobium angustifolium* also increased, while the elongation of stolons in the more shade-tolerant *Glechoma hederacea* was not affected by light manipulation.

Other morphology

Low light quantity mainly decreased many growth-related variables of *Pinus radiata* (Warrington *et al*. 1988). In our work, the removal of blue light had no effect on morphology (other than elongation), except for needle and branch length, which were increased in *Pinus sylvestris* of both origins (Table 1). In *Betula pubescens* f. *rubra*, the increase in leaf area (Table 2), however, resembled the response of *Betula pubescens* seedlings grown in low light (Atkinson 1984). Generally, elongation of all above-ground plant parts in both origins of *Pinus sylvestris* and both *Betula* seedlings was increased under blue light depletion.

In herbaceous shade-avoiding plant species, branching and number of leaves has been reported to be reduced in low light quantity (Dong and de Kroon 1994, Cookson and Granier 2006). Similar phenomena occurred in shade-intolerant *Epilobium angustifolium* by the removal of blue wavelengths (Table 3). However, unlike would be expected for plants growing in low light (McLaren and Smith 1978, Begna *et al*. 2002, Cookson and Granier 2006) no reduction in leaf area and stem biomass was observed under blue light depletion. Thus, in *Epilobium angustifolium*, the removal of blue light decreased the amount of leaves, but increased the leaf size. In the more shade-tolerant *Glechoma hederacea*, experimental light competition increased petiole elongation (Price and Hutchings 1996). In the present work, the petiole elongation of new *Glechoma hederacea* leaves was also increased by the removal of blue light (Table 3). As the area of new leaves also slightly increased, the leaves of *Glechoma hederacea* grew higher and wider under blue light depletion. In low light and low R/FR ratio, morphological variables of more shade-tolerant plants mainly decrease (Price and Hutchings 1996, Vermeulen *et al*. 2008). The blue light removal, however, did not affect the morphology of *Glechoma hederacea*, except for the increased leaf area and petiole length.

In summary, in shade-intolerant *Epilobium angustifolium* the removal of blue light increased the stem and leaf elongation, as also occurred in *Pinus sylvetris* and *Betula* tree seedlings. In more shade-tolerant *Glechoma hederacea*, petiole elongation was increased instead of stolon elongation. The removal of blue wavelengths increased the leaf area growth of *Epilobium angustifolium*, *Glechoma hederacea* and *Betula*

seedlings. This is in accordance with Eskins (1992), who observed an increasing reduction in leaf area of *Arabidopsis thaliana* under an increasing amount of blue light. The results suggest that leaf area growth is controlled by blue light.

Pigmentation

Low light quantity affects chlorophyll *a* and *b* and carotenoid concentrations, as the contents of these pigments has been reported to decrease under low radiation (McLaren and Smith 1978). The removal of blue light did not affect chlorophyll and carotenoid contents of most of the studied species (Table 5 and 6). The results support the finding (Sarala *et al*. 2007) according to which etiolation alone is not the reason behind the increased elongation observed under blue light depletion. However, in *Betula pubescens* f. *rubra,* the chlorophyll and carotenoid levels were significantly reduced (Table 6). Thus, the birch in this study also responded stronger to quantity of light, which is in line with the observation that birch leaves are very sensitive to changes in PAR (photosynthetically active radiation) (Taylor and Davies 1988).

The lower total amount of sunlight passing through the –B chamber, nevertheless, may have induced minor etiolation especially in the new needles and leaves of the plants, as all individuals of all plant species were pale green and there were even small white patches on the needles of the northern origin *Pinus sylvestris*. Furthermore, as also occurs in low light quantity (McLaren and Smith 1978), the chlorophyll *a* contents in one-year-old northern origin *Pinus sylvestris* and *Epilobium angustifolium* was slightly decreased (Tables 5 and 6). On the other hand, in the southern origin one-year-old *Pinus sylvestris* seedlings, the contents of both chlorophylls were unaffected by light manipulation, and in *Glechoma hederacea*, the concentrations of the chlorophylls slightly increased (Table 6). However, although the quantity of light in –B chamber decreased, it still remained above the Scots pine's saturation level of photosynthesis (> 800 μmol m^{-2} s^{-1}; Wang *et al*. 1995) during the daytime. Thus, etiolation can be only a marginal factor in the increased elongation of seedlings under blue light depletion.

The removal of blue wavelengths reduced the anthocyanin content of most of the studied plants species (Table 6). This result supports the idea that anthocyanins are produced for protecting plants against short wavelength sunlight (Mohr and Drumm-Herrel 1983), and that sensitivity of anthocyanin synthesis increases towards northern ecotypes (Taulavuori *et al*. 2011). This is especially evident in *Betula pubescens* f. *rubra*, which synthesizes so much anthocyanins that the initially green leaves turn red soon after leaf burst. In this work, nevertheless, during the experiment the new leaves formed by seedlings grown under blue light depletion remained green (Fig. 3). Additionally, the old leaves of *Glechoma hederacea* plants grown in CC produced anthocyanins, while the old and also the new leaves of the plants grown under blue light depletion lacked them (Tables 5 and 6). Also, no anthocyanins were detected in the leaves of *Glechoma hederacea* at the beginning of the experiment (data not shown). In a work by Zhou *et al*. (2007), only UV-A induced anthocyanin accumulation in the swollen hypocotyls of *Brassica rapa*, while UV-B, blue, red and far-red light had no effect. They (Zhou *et al*. 2007) suggested that a distinct UV-A photoreceptor was involved, but not phytochrome, UV-A/blue photoreceptors or UV-B photoreceptors. In our work, the blue light depletion reduced the level of anthocyanins in almost all the species. Thus, different photoreceptors may be mediating the effect of light quality on anthocyanin biosynthesis in different species.

Conclusions

Both origins of *Pinus sylvestris* seedlings responded in a similar way to the removal of blue light, but the response was more pronounced in northern origin *Pinus sylvestris* seedlings. This suggests that they are more sensitive to blue light than the southern origin seedlings. As in evergreen *Pinus sylvestris*, the removal of blue wavelengths induced similar responses in the other shade-avoiding tree species as well, such as deciduous *Betula* seedlings. Also, shade-intolerant herbaceous *Epilobium angustifolium*

responded similarly under blue light depletion as the woody species, while in the herbaceous, more shade-tolerant *Glechoma hederacea*, a minor part of the measured variables were affected. The results indicate that the morphological responses of shade-intolerant species to blue light depletion are quite conservative across life forms. The results from morphological measurements and analyses of photosynthetic pigments suggest that the increased elongation of plant species under blue light depletion is only marginally a concern of etiolation. This result is in accordance with our previous study (Sarala *et al*. 2007). However, some species such as *Betula pubescens* f. *rubra* seemed to also be more sensitive to changes in PAR.

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