Effect of thinning on anatomical adaptations of Norway spruce needles

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Conifers and other trees are constantly adapting to changes in light conditions, water/nutrient supply and temperatures by physiological and morphological modifications of their foliage. However, the relationship between physiological processes and anatomical characteristics of foliage has been little explored in trees. In this study we evaluated needle structure and function in Norway spruce families exposed to different light conditions and transpiration regimes. We compared needle characteristics of sun-exposed and shaded current-year needles in a control plot and a thinned plot with 50% reduction in stand density. Whole-tree transpiration rates remained similar across plots, but increased transpiration of lower branches after thinning implies that sun-exposed needles in the thinned plot were subjected to higher water stress than sun-exposed needles in the control plot. In general, morphological and anatomical needle parameters increased with increasing tree height and light intensity. Needle width, needle cross-section area, needle stele area and needle flatness (the ratio of needle thickness to needle width) differed most between the upper and lower canopy. The parameters that were most sensitive to the altered needle water status of the upper canopy after thinning were needle thickness, needle flatness and percentage of stele area in needle area. These results show that studies comparing needle structure or function between tree species should consider not only tree height and light gradients, but also needle water status. Unaccounted for differences in needle water status may have contributed to the variable relationship between needle structure and irradiance that has been observed among conifers.

Keywords: light condition, needle cross-section, needle structure, tree height, water stress.

Introduction

Environmental conditions change continuously with stand development, and trees are constantly adapting to these changes by modifying the physiological and morphological traits of their foliage. Trees show substantial variation in leaf form and function at different canopy heights and along other light gradients in the canopy (Niinemets et al. 1999, Koike et al. 2001, Woodruff et al. 2004). The steep vertical gradients of photosynthetically active radiation within Norway spruce (Picea abies) crowns, for example, lead to differentiation of typical sun- and shade-adapted needles (Špunda et al. 1998). Modifications in needle morphology and acclimation of the photosynthetic apparatus allow needles to photosynthesize efficiently across the very variable distribution of light within the canopy (Terashima and Hikosaka 1995, Mitchell 1998).

Conifer needles often show a positive scaling of needle dry mass per unit needle area ($M_A$) with increasing radiation, i.e., shaded needles tend to have lower density than sun-exposed needles (Niinemets and Kull 1995, Sprugel et al. 1996, Stenberg et al. 1998). On the basis of $M_A$ values, the crown can thus be differentiated into sun- and shade-adapted zones...
thinning is a commonly used forestry practice and it is important to understand its effect on needle characteristics and photosynthetic efficiency. Other common morphological adaptations of conifer needles to shade are decreasing needle thickness (Ninemets 1997, Stenberg et al. 1998, Messier et al. 1999), needle width (Ninemets 1997) and mesophyll thickness (Youngblood and Ferguson 2003). Different light intensity not only leads to changes in needle morphology, but also affects physiological parameters, such as photosynthetic rates (Jordan and Smith 1993), stomata reaction time (Hinckley et al. 1978, Sellin 2001) and leaf conductance (Hinckley et al. 1978, Sellin 2001).

Recent research has begun to focus on the connections between leaf anatomical characteristics and physiological function. This includes correlations between leaf architecture and water transport efficiency (Aasamaa et al. 2005, Sack and Frole 2006), leaf architecture and gas exchange (Salleo et al. 2001, Sack et al. 2003, Brodribb et al. 2007), and needle anatomy and photosynthetic rates (Yoder et al. 1994, Apple et al. 2002). Still, the dependency of physiological processes on leaf or needle size has been little explored (Mencuccini 2002).

To address this shortcoming, the first aim of this study was to describe the structural and physiological changes that take place in conifer needles exposed to increasing illumination after thinning. The second aim was to compare sun-exposed and shaded needles within trees, i.e., to carefully determine needle structure at two extreme points of light availability. In addition to increasing the knowledge about light adaptation in conifer needles, this study also has practical implications, since thinning was done one year before needles were sampled. The other plot, situated ~15 m away, was an unthinned control plot. In each plot one tree of each of the same three families was selected (Table 1). The parents of the families come from the following locations and altitudes in south Norway: Family no. 15: ♀ 2598, Kråderad, Buskerud, 440 m, ♂ 5262, Gol, Buskerud, 395 m; Family 27: ♀ 2526, Flesberg, Buskerud, 455 m, ♂ 5262, Gol, Buskerud, 395 m; Family 29: ♀ 2627, Brandbu, Oppland, 430 m, ♂ 2767, Atna, Stor Elvdal, Hedmark, 400 m. The use of similar-sized trees of the same family reduces phenotypic variation between plots and facilitates the comparison of needle characteristics in the control and thinned plots.

Since the experimental trees were part of a long-term drought stress experiment, a plastic roof was set up under the tree crowns in the thinned plot in 2009 and a 30 cm deep trench was dug around the plot to intercept the precipitation. However, no clear differences in soil water potential, soil humidity or sap flow became apparent between the thinned plot and the control during the course of the experiment.

### Soil moisture

We monitored soil water potential (SWP) and soil humidity in triplicate measurements on both plots. While SWP was measured at 10 and 30 cm soil depths, volumetric soil moisture was measured as a mean value for the upper 30 cm of soil. Soil water potential was measured using gypsum blocks (Delmhorst Inc., Towaco, NJ, USA) and soil humidity was measured using CS-616 TDR probes (Campbell Scientific, Logan, UT, USA). Data were acquired every 10 min and stored in a data logger (Modulog 1037, EMS Brno, Czech Republic). Soil characteristics were measured from the beginning of the vegetation period until all samples were taken (i.e., from May to August 2009).

<table>
<thead>
<tr>
<th>Family</th>
<th>15</th>
<th>27</th>
<th>29</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Control plot</strong> (10,000 trees ha⁻¹)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tree diameter (cm)</td>
<td>9.1</td>
<td>9.4</td>
<td>9.2</td>
</tr>
<tr>
<td>Tree height (m)</td>
<td>10.8</td>
<td>9.7</td>
<td>9.8</td>
</tr>
<tr>
<td>Projected crown area (m²)</td>
<td>1.5</td>
<td>2.8</td>
<td>2.3</td>
</tr>
<tr>
<td><strong>Thinned plot</strong> (5000 trees ha⁻¹)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crown length (m)</td>
<td>6.3</td>
<td>5.7</td>
<td>5.0</td>
</tr>
<tr>
<td>Tree diameter (cm)</td>
<td>10.9</td>
<td>9.5</td>
<td>9.8</td>
</tr>
<tr>
<td>Tree height (m)</td>
<td>9.7</td>
<td>9.2</td>
<td>10.0</td>
</tr>
<tr>
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<td>2.3</td>
<td>1.3</td>
</tr>
<tr>
<td>Crown length (m)</td>
<td>6.8</td>
<td>5.6</td>
<td>5.6</td>
</tr>
</tbody>
</table>
Sap flow measurement

Sap flow in all six experimental trees was monitored using EMS 51 sap flow meters (EMS Brno) working under the trunk heat balance principle (Čermák et al. 1973, Kučera et al. 1977). Data were recorded at 10-min intervals and processed (by baseline subtraction) by Mini32 software (EMS Brno). Transpiration was normalized per unit of crown projected area according to Cienciala et al. (1998) and Čermák et al. (2004). Mean transpiration for each plot was calculated as the arithmetic mean of the three experimental trees per plot. The significance of the difference between plots was tested using the t-test, as described further in the Materials and methods section.

Light intensity

The photosynthetic quantum flux density \( Q_{pq} \) in the two stands was measured under cloudless conditions on 4 August 2010. \( Q_{pq} \) measurements were done twice (i.e., around noon and in the late afternoon) to assess radiation interception at different elevations of the sun. Measurements were done with two \( Q_{pq} \) sensors (Minikin QT, EMS Brno): one sensor was left in the open area outside the forest to measure ambient solar radiation, and the other sensor was held by a person walking slowly through the thinned plot and the control plot. The measurement interval was set to 3 s and mean \( Q_{pq} \) values were calculated for ambient conditions, the thinned plot and the control plot, respectively. Plots were compared using ratios (%) of plot \( Q_{pq} \) to ambient \( Q_{pq} \).

Needle material

One year after thinning (7 August 2009), we collected five current-year shoots at two different crown heights on each tree: within 1–3 m from the tree top (sun-exposed needles) and ~4 m above ground (shaded needles). All samples were taken from branches on the outer, southern part of the crown, in order to minimize the effect of any microclimatic differences within the stand or canopy (Lhotáková et al. 2007). The samples were fixed in 5% formaldehyde-acetic acid alcohol solution (90 ml 70% ethanol, 5 ml acetic acid and 5 ml 40% formaldehyde) according to Němec et al. (1962).

Needle anatomy

Subsamples of five randomly selected needles per shoot (i.e., altogether 25 needles per tree height) were taken for microscopic analysis. For histological analysis, cross-sections were made by hand at the needle base, examined under a bright-field microscope (Olympus BX51, Olympus Czech Group, Czech Republic) at magnifications up to ×400, and photographed using a digital camera (Olympus E-330, Olympus Czech Goup, Czech Republic) connected to a computer by QuickPhotomicro 2.3 software (Promicra, Czech Republic). Cross-sectional needle area \( A_n \) and stele area \( A_s \), as well as needle thickness \( T_n \) and needle width \( W_n \) (Figure 1) were determined using ImageTool 3.00 image analysing software (The University of Texas Health Science Center, San Antonio, TX, USA).

Needle cross-sectional shape (i.e., needle flatness \( F_l \)) was calculated according to Apple et al. (2002) and Sellin (2001) as the ratio of needle thickness to needle width \( F_l = T_n / W_n \). A needle with a round or square cross-section will have \( F_l \) close to 1, and larger ratios characterize more or less flattened needles. Needle density \( I_D \) was characterized according to Niinemets and Kull (1995) and Sellin (2001):

\[
I_D = \frac{M_s}{A_n} \left( \frac{T_n^2 + W_n^2}{2} \right) \left( \frac{A_n}{A_n} \right)^{1/2} (g \text{ cm}^{-3})
\] (1)

Figure 1. Measured and calculated needle characteristics: needle cross-sectional area \( A_n \) (solid line), stele area \( A_s \) (dashed line), needle thickness \( T_n \), needle width \( W_n \), needle flatness \( F_l \) and percentage of stele area in needle cross-section \( A_s^p \). \( W_{PLA} \) shows the projected needle width which is used to calculate projected leaf area \( A_p \).
Leaf mass per area and nitrogen content per unit leaf area

To assess leaf mass per area ($M_a$), 20 randomly selected sun-exposed needles and 20 shaded needles were taken from each tree, scanned to determine the projected leaf area ($A_p$) and needle length ($L_n$), and dried at 85 °C for 48 h to determine needle dry mass ($M_n$). $A_p$ and $L_n$ were determined using ImageTool 3.00 software. $M_n$ was calculated from $N_A$ and $M_A$.

Another sample of randomly selected sun-exposed needles was dried (85 °C, 48 h) for nitrogen analysis. Total nitrogen content per needle dry mass ($N_A$) was determined in an authorized laboratory (Ekola Bruzovice Ltd, Bruzovice, Czech Republic). The nitrogen content per unit leaf area ($N_A$) was calculated from $N_A$ and $M_A$.

Statistical analysis

Differences between plots (control and thinned) and between sun-exposed and shaded needles were evaluated using the t-test or Mann–Whitney test as appropriate. Statistical analysis was carried out using the R statistical program (R Development Core Team 2010). All parameters used throughout the study are explained in Table 2.

Results

Soil water potential and soil humidity

Mean soil moisture for the whole study period was 15.7 ± 2.2 and 18.1 ± 2.2% in the control and thinned plot, respectively ($P = 0.34$). Soil moisture gradually decreased from the beginning of May until the end of July, from 22% in both plots to 15 and 16% in the control and thinned plot, respectively. Similarly, SWP decreased progressively from May towards the beginning of August, from an initial value of −0.2 to −1.1 MPa, reaching the wilting point and the lower limit of our sensors. There was a temporary increase in SWP at 10 cm soil depth in the control plot after a rain event on 10 July, but SWP returned to −1.1 MPa after 8 days. There was no change in SWP at 30 cm soil depth in response to the rain.

Transpiration rate

Mean accumulated tree transpiration per unit of crown projected area was 83 ± 17 mm (mean value ± SE) in the thinned plot and 67 ± 28 mm in the control plot over the 3-month study period (from early May until early August). The difference between plots was not statistically significant ($P = 0.63$).

Light intensity

In the middle of the day, the light intensity under the canopy was 5-fold higher in the thinned plot than in the control plot (Table 3). Around midday, 16% of the ambient radiation reached the lowest branch level in the thinned plot, compared with only 3% in the control plot. In the evening, the light intensity was more similar (but very low) in both plots.

Differences in needle characteristics between thinned and control plots

Needle morphology and histology differed less between experimental plots than between needle types (Figure 2). For most parameters the largest between-plot differences were observed in Family 29 for shaded needles and in Family 27 for sun-exposed needles.

Leaf mass per area and nitrogen content per unit leaf area

$M_a$ in sun-exposed needles was higher in the thinned plot than in the control plot, whereas there were no clear differences between plots for shaded needles (Table 4). Sun-exposed needles in the thinned plot also had somewhat higher $N_A$ than in the control plot, except for Family 27 (Table 4).

Projected leaf area, needle length, needle dry weight and needle density

$A_p$ was similar between plots for shaded needles. For sun-exposed needles $A_p$ was larger in the control plot than in the

| Table 2. Needle parameters, their abbreviations and units used throughout the study. |
|--------------------------|--------------------------|--------------------------|
| Variable                | Explanation              | Unit                     |
| $M_n$                   | Mean needle dry mass      | g                        |
| $A_p$                   | Mean needle projected area| m²                       |
| $M_A$                   | Leaf mass per area        | g m⁻²                    |
| $N_A$                   | Nitrogen content per unit leaf area | g m⁻² |
| $l_0$                   | Needle density (Eq. (1))  | g cm⁻³                   |
| $L_n$                   | Needle length             | mm                       |
| $A_n$                   | Needle cross-sectional area (Figure 1) | mm² |
| $A_s$                   | Needle stele area (Figure 1) | mm² |
| $T_n$                   | Needle thickness (Figure 1) | mm |
| $W_n$                   | Needle width (Figure 1)   | mm                       |
| $A_s$%                  | Percentage of stele area in needle area ($A_s/A_n$ x 100) | % |
| $F_l$                   | Needle flatness ($T_n/W_n$) | - |

| Table 3. Photosynthetic quantum flux density ($Q_{eq}$: μmol m⁻² s⁻¹) in the thinned Norway spruce plot and the control plot on 4 August 2010. Radiation in the experimental plots is also expressed as a proportion (%) of that in an adjacent open field. |
|-----------------|-----------------|-----------------|-----------------|
|                | **Middle of day (11:45)** | **Evening (17:00)** |
| $Q_{eq}$       | %               | $Q_{eq}$       | %               |
| Control plot   | 57              | 3              | 11             |
| Thinned plot   | 286             | 16             | 15             |
| Open field     | 1809            | 100            | 791            |

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thinned plot, except for Family 29 which had the opposite pattern (Table 4).

$L_n$ of both shaded and sun-exposed needles was 1.2–1.3 times longer in the control than in the thinned plot for Families 15 and 27. The opposite pattern was found for Family 29, where both needle types were 1.15 times longer in the thinned plot.

$M_n$ showed no clear differences between plots, either for shaded needles or for sun-exposed needles (Table 4).

$I_D$ of shaded needles did not differ between plots, but for sun-exposed needles $I_D$ was 1.2–1.4 times higher in the thinned plot than in the control for the different families (Table 4).

Needle width, needle thickness and needle flatness
Shaded needles were wider ($W_n$) in the thinned plot than in the control plot, except for Family 15 where there was no significant difference between plots (Table 5, Figure 2c). Sun-exposed needles were also somewhat wider in the thinned plot, but the difference was statistically significant only for Family 27 (Table 5, Figure 2d).

Differences in $T_n$ between plots showed opposite patterns for shaded and sun-exposed needles. Shaded needles were significantly thicker in the thinned plot than in the control plot (except for Family 27), whereas for sun-exposed needles it was the other way around (except for Family 15) (Figure 2c and d).

Shaded needles were significantly flatter ($F_l$) in the thinned plot than in the control for Family 15, but for the other families there were no significant differences between plots (Figure 2c). Sun-exposed needles were significantly less flat in the thinned plot compared with the control plot, except for Family 15 (Figure 2d).

Needle cross-sectional area, stele area and proportion of stele area
Shaded needles had a somewhat larger $A_n$ and $A_s$ in the thinned plot than in the control, but the difference was statistically significant only for Family 29 (Figure 2c). Sun-exposed needles had more similar $A_n$ in both plots, but with significantly larger values for Family 27 in the thinned plot (Figure 2d). Stele area was also significantly larger in the thinned plot than in the control for sun-exposed needles in Family 27 (Figure 2d).

The proportion of needle cross-sectional area that was made up by stele area ($A_s^\%$) was quite similar between plots for shaded needles, but with significantly lower values in the thinned plot for Family 15 (Figure 2c). For sun-exposed needles $A_s^\%$ was significantly higher in the thinned plot than in the control plot for all families except Family 15 (Figure 2d).

Differences in needle characteristics between sun-exposed and shaded needles
In general, needle morphological and histological parameters had higher values in sun-exposed needles than in shaded needles. When there were family differences, Family 15 generally displayed the largest differences between sun-exposed and shaded needles and Family 29 the smallest differences.

Leaf mass per area
Sun-exposed needles had higher $M_A$ than shaded needles in all families, both in the control plot (1.4–1.6 times higher for the different families) and in the thinned plot (1.3–1.9 times higher) (Table 4).

Projected leaf area, needle length, needle dry mass and needle density index
Sun-exposed needles generally had larger $A_p$ than shaded needles, but not in all families. In the control plot sun-exposed needles had 1.4–1.5 times larger $A_p$ than shaded needles in...
Table 5. Anatomical characteristics of sun-exposed and shaded Norway spruce needles (n = 25; mean ± SE) in the thinned plot and the control plot. For an explanation of needle parameters, see Figure 1 and Table 2.

<table>
<thead>
<tr>
<th>Characteristic (units)</th>
<th>Family</th>
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<th>Shaded needles</th>
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<tr>
<td></td>
<td></td>
<td>Thinned</td>
<td>Control</td>
<td>Thinned</td>
<td>Control</td>
</tr>
<tr>
<td>( M_a ) (g m(^{-2}))</td>
<td>15</td>
<td>279</td>
<td>214</td>
<td>149</td>
<td>157</td>
</tr>
<tr>
<td></td>
<td>27</td>
<td>230</td>
<td>221</td>
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<td>29</td>
<td>261</td>
<td>246</td>
<td>194</td>
<td>163</td>
</tr>
<tr>
<td>( N_a ) (g m(^{-2}))</td>
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<td>2.65</td>
<td>2.4</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
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<td>2.48</td>
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<td>—</td>
</tr>
<tr>
<td>( A_p ) (mm(^2))</td>
<td>15</td>
<td>10 ± 1**</td>
<td>13 ± 2</td>
<td>7 ± 1**</td>
<td>9 ± 2</td>
</tr>
<tr>
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<td>12 ± 2</td>
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<tr>
<td></td>
<td>29</td>
<td>14 ± 2**</td>
<td>11 ± 2</td>
<td>12 ± 3</td>
<td>11 ± 4</td>
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<tr>
<td>( L_n ) (mm)</td>
<td>15</td>
<td>10.6 ± 1.2**</td>
<td>12.4 ± 2.9</td>
<td>8.5 ± 0.7**</td>
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<td>11.7 ± 1.2</td>
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<td>10.8 ± 1.4</td>
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<td>( M_n ) (mg)</td>
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<td>0.28</td>
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</tbody>
</table>

Differences between thinned and control plot within each needle class are statistically significant at **\( P < 0.001 \) and *\( P < 0.05 \).
Families 15 and 27, but in Family 29 $A_p$ was similar across needle types. In the thinned plot, $A_p$ was 1.2–1.4 times larger in sun-exposed needles than in shaded needles in Families 15 and 29, but in Family 27 there was no difference between needle types (Table 4).

Sun-exposed needles were ~1.2 times longer ($L_n$) than shaded needles in both plots in Families 15 and 27, whereas there was no difference between needle types in Family 29. $M_n$ was higher in sun-exposed needles than in shaded needles in all families, both in the control plot (1.6–2.3 times higher) and in the thinned plot (1.5–2.5 times higher) (Table 4). Family 29 had the smallest difference in $M_n$ between sun-exposed and shaded needles in both plots. $L_o$ was also higher in sun-exposed needles than in shaded needles in all families, and the difference between needle types was somewhat larger in the thinned plot (1.5–2.2 times higher) than in the control plot (1.4–1.6 times higher) (Table 4). Again, Family 29 had the smallest difference between needle types in both plots.

**Needle width, needle thickness and needle flatness**

Sun-exposed needles were wider ($W_n$) than shaded needles in all families, both in the control plot (1.6–2.1 times wider) and in the thinned plot (1.4–2.4 times wider) (Table 5, Figure 2a and b). Family 29 had the smallest difference between needle types in both plots, and Family 15 had the largest difference.

In the control plot sun-exposed needles were somewhat thicker ($T_n$) than shaded needles in all families (1.1–1.2 times thicker), whereas in the thinned plot needle thickness was very similar between needle types (Table 5, Figure 2a and b).

Shaded needles were much flatter in cross-section than sun-exposed needles. $F_n$ was higher in shaded needles in all families, both in the control plot (1.2–1.4 times higher) and in the thinned plot (1.3–1.5 times higher) (Table 5, Figure 2a and b). Family 29 had the smallest difference between needle types in both plots, and Family 15 had the largest difference.

**Needle cross-sectional area, stele area and percentage of stele area in needle area**

Sun-exposed needles had a larger $A_n$ than shaded needles in all families, both in the control plot (1.5–2.3 timer larger) and in the thinned plot (1.4–2.2 times larger) (Table 5, Figure 2a and b). The largest difference between needle types was found in Family 15 in both plots (Figure 2a and b).

Sun-exposed needles also had a substantially larger $A_n$ than shaded needles in all families and in both plots (2.1–3.0 times higher in the control and 1.7–3.1 times higher in the thinned plot) (Table 5, Figure 2a and b). In both plots Family 29 showed the smallest and Family 15 the largest difference between needle types.

The proportion of needle cross-sectional area that was made up by stele area ($A_s^*$) was also higher in sun-exposed needles than in shaded needles (Table 5, Figure 2a and b). This was true for the thinned plot (1.3–1.6 times higher) and for Families 15 and 27 in the control plot (1.2–1.4 times higher) (Table 5, Figure 2a and b). Again, Family 29 had the smallest difference between needle types in both plots.

**Discussion**

**Structural and physiological differences in sun-exposed needles between plots**

**Sap flow**

The transpiration rate (the physical process of water vaporization) depends mostly on the amount of available solar radiation that is intercepted in the canopy. In a fully closed forest most of the radiation is intercepted by the uppermost needle and branch layers, and there is a striking difference in interception of solar energy at different canopy heights. For example, Kucera et al. (2002) found that in a fully closed Norway spruce canopy as much as 50% of the incoming solar radiation is intercepted by the uppermost 10% of needle biomass. Higher radiation interception in the upper canopy in turn leads to higher transpiration of the tree tops (Morén et al. 2000). However, after thinning, solar radiation penetrates deeper into the canopy and increases the transpiration of the lower branches (Morén et al. 2000). Thinning thus tends to increase whole-tree transpiration (Lagergren and Lindroth 2004)—although insignificantly over the time frame of our study. Most of this increased water flow is due to hydraulical constraints of the xylem, maintained by the lower branches. Consequently, upper branches experience stronger water stress than branches at the tree base. This mechanism is commonly observed in trees on a clear-cut that are left as seed trees: water is predominantly transpired by the lower branches and the tree top is subjected to severe water stress and dries. Similarly, the upper branches of extremely tall trees are more water stressed than the lower branches (Ryan et al. 2006, Ambrose et al. 2010) due to increased transporting height and path length (Kozlowski and Pallardy 1997, Rijkers et al. 2000). Because whole-tree transpiration rates remained similar across plots in our study, this implies that sun-exposed needles in the thinned plot were subjected to higher water stress than sun-exposed needles in the control plot, although they were growing in the same light conditions. This means that we could separate the effects of altered water status and increased light on needle morphology and anatomy.

**Leaf mass per area and nitrogen content per leaf area**

$M_n$ is an easy-to-measure and useful proxy for plastic structural characteristics and underlying physiological properties that are more difficult to quantify (Temesgen and Weisittel 2006). The range of $M_n$ values found in this study (142–279 g m$^{-2}$) is at the
low end of the range reported for Norway spruce at other sites (Hager and Sterba 1985, Stenberg et al. 1999). One reason for this could be that we sampled current-year shoots from relatively young trees and that $M_A$ generally increases with leaf age (Gulmon and Chu 1981). Our finding that $M_A$ of sun-exposed needles was higher in the thinned plot (Table 4), even though light conditions in the upper crown did not change after thinning, shows that not only light conditions but also water stress may increase $M_A$ of Norway spruce needles.

Although foliar nitrogen content is relatively constant throughout tree canopies (Ellsworth and Reich 1993, Niinemets et al. 1998), $N_A$ is positively scaled with irradiance due to the strong effects of light on $M_A$ (Ninemets et al. 2002). In both deciduous trees (Ellsworth and Reich 1993, Takahashi et al. 2005) and conifers (Ninemets et al. 2002), $N_A$ has been found to be a good indicator of the foliage’s assimilation capacity, because photosynthetic enzymes such as RuBP carboxylase/oxygenase contain large amounts of nitrogen. Net production per unit leaf area does for example increase with increasing $N_A$ in open crowns (Takahashi et al. 2005). Because $N_A$ was higher in sun-exposed needles in the thinned plot than in the control plot (Table 4), it seems that increasing irradiance is not the only factor affecting $N_A$. Other factors, such as needle water status and anatomical structure, also seem to influence $N_A$ and hence photosynthetic activity in Norway spruce.

Projected leaf area, needle length, needle dry weight and needle density

$I_D$ of sun-exposed needles was higher in the thinned plot than in the control plot due to higher $W_n$ and lower $A_p$ (Tables 4 and 5). An extensive review of the literature has demonstrated that the relationship between needle density and irradiance is variable among conifers (Ninemets 1997). The review also concluded that increasing needle density in the crowns of large trees may be a consequence not only of increased irradiance, but also of increasing water limitation. This fits with our observation of higher density in sun-exposed needles in the thinned plot.

Needle thickness, needle width and needle flatness

Sun-exposed needles in the thinned plot showed an increase in needle width, and a decrease in needle thickness and needle flatness compared with the control plot. These changes are probably other examples of structural responses to altered needle water status after thinning, since, as already mentioned, light conditions in the upper crown were little affected by thinning.

Needle cross-sectional area, stele area and percentage of stele area in needle cross-sectional area

$A_n$ and $A_s$ seemed to be independent of needle water status, as there was no difference in these parameters between sun-exposed needles in the two plots. On the other hand, $A_s^{50}$ increased in sun-exposed needles after thinning. A proportionally larger vascular cylinder in upper needles probably contributes to counteract water stress associated with increasing tree height (Ryan and Yoder 1997, Bauerele et al. 1999) and increasing transpiration from the lower crown following thinning. The percentage stele area is also a measure of the proportion of stele to mesophyll in the needle, and a higher proportion of stele means that the needle is better able to supply the mesophyll with water and remove photosynthate (Richardson et al. 2000, Ninemets et al. 2007). A larger proportion of mesophyll may, on the other hand, increase needle resistance to water transport, as the resistance of the mesophyll is similar to that of the leaf xylem pathways (Gascó et al. 2004, Nardini et al. 2005).

Structural differences between sun-exposed and shaded needles

Leaf mass per area

Variation in $M_A$ within tree canopies is commonly attributed to light gradients (e.g., Hollinger 1989, Ellsworth and Reich 1993), and $M_A$ generally decreases with decreasing solar radiation (Stenberg et al. 1999, Sellin 2001). Our finding that $M_A$ of sun-exposed Norway spruce needles was ~1.5 times greater than for shaded needles thus agrees with previous results (Ellsworth and Reich 1993, Temesgen and Weiskittel 2006), but is lower than the plasticity in $M_A$ found by Niinemets (1997). Ninemets observed a fourfold variation in $M_A$ across the light gradient in Norway spruce crowns, but he studied trees at a different ontogenetic stage than our trees. $M_A$ has been found to differ by only ~1.5-fold between sun-exposed and shaded needles in other conifers, such as Douglas fir (Pseudotsuga menziesii) and Western hemlock (Tsuga heterophylla) (Bond et al. 1999). Sun-exposed needles in our study had larger $A_n$ than shaded needles and this contributed most to their higher $M_A$ (Table 5, Figure 2a and b). $M_A$ was also related to $I_D$ and $T_n$, as has been found by others (Sellin 2001, Sack and Holbrook 2006). The highly determined spatial variation pattern in $M_A$ across the crown is a manifestation of an important canopy acclimatization response that enhances the foliage’s photosynthetic capacity in high-light conditions and its light-harvesting efficiency in low-light conditions (Gutschick and Wiegel 1988, Niinemets et al. 1998, Bond et al. 1999).

Projected leaf area, needle length, needle dry weight and needle density

$I_D$, $W_n$, and $A_p$ contributed most to the higher $I_D$ of sun-exposed needles compared with shaded needles. $I_D$ increases with irradiance also in other conifer species (Richardson et al. 2001, Niinemets et al. 2007). $I_D$ was influenced by $T_n$ and $A_p$ (according to Eq. (1)), but these parameters differed less between sun-exposed and shaded needles than $M_A$ and $W_n$ (Tables 4 and 5, Figure 2a and b). The hydraulic limitation hypothesis of
Ryan and Yoder (1997) suggests that tree height is constrained by water supply to leaves, which becomes increasingly difficult with increasing tree height. If tree tops are water stressed, $L_n$ would also be expected to decrease with tree height, because cell expansion and division are negatively affected by low water potentials (Dale 1988). On the other hand, we found that sun-exposed needles in the upper canopy were longer than shaded needles at the bottom of the crown. This corresponds with the results of others, as long as the trees are below ~20 m (Meinzer et al. 2008, Woodruff et al. 2008).

**Needle thickness, needle width and needle flatness**

$T_n$ in Norway spruce is usually larger (1.15–1.5 mm) and less variable than $W_n$ (0.4–1.5 mm) with increased irradiance (Frey 1978, Sellin 2001), and our data replicated this pattern. On the other hand, $W_n$ and also $T_n$ have been found to increase with increased irradiance in other conifer species (Richardson et al. 2000, 2001, Niinemets et al. 2007). Our data agree with those of Stenberg et al. (1999) and Sellin (2001), who found that the width and thickness of Norway spruce needles increase with canopy openness, but that needle thickness is less influenced by increasing light intensity than needle width. Niinemets et al. (2002) observed a different pattern in pines, where needle thickness was positively related to light intensity in three species, but needle width only in two species. These contrasting results could be due to differences in needle anatomy between pine and spruce, since pine needles are wider than they are thick (i.e., $F_l < 1$) (Niinemets et al. 2002).

Frey (1978) proposed $F_l$ as a morphological index of light adaptation of spruce foliage, as it is both easy to measure and determined by light availability. Needles with a flatness value around 1.0 have the greatest area for gas exchange of water vapour and CO$_2$ relative to needle volume. For typical sun-exposed shoots in Norway spruce $F_l$ ranges from 1.0 to 1.3, and for typical shaded shoots from 1.8 to 2.8 (Frey and Ivask 1983 in Sellin 2001). According to this needle flatness criterion, 15 of the 30 shoots we sampled from the bottom crown were typical shaded shoots, and 25 of the 30 sampled shoots from the upper crown were typical sun-exposed shoots (the remaining shoots were transitional shoots sensu Sellin 2001). Sellin (2001) found that $F_l$ decreased from 1.9 to 1.1 with increasing light availability in Norway spruce, and this is very similar to our observation of a decrease from 1.8 to 1.1 (Table 5).

**Needle cross-sectional area, stele area and percentage of stele area in needle area**

Structural characteristics of conifer needles are often strongly related to gradients in long-term light availability within canopies and across stands (Richardson et al. 2000, 2001, Niinemets et al. 2002, 2007). Lhotáková et al. (2007) found that the $A_s$ of Norway spruce needles is 0.95 mm$^2$ at the needle base, which is close to our figure for sun-exposed needles, but about twice as high as for our shaded needles (Table 5). $A_s$ seems to be determined not only by light gradients, since improved light conditions after thinning did not significantly influence the cross-sectional area of either needle type. The higher $A_s$ of sun-exposed needles compared with shaded needles may be a result of the higher photosynthetic activity of sun-exposed needles (Bertamini et al. 2006) and the need to transport photosynthates from source to sink or to increase water transport efficiency (Niinemets et al. 2007).

**Scaling up from needle parameters to the crown, tree and forest level**

Plant structure is generally tightly related to function (Tyree and Zimmermann 2002), and any structural changes in tree organs such as roots, stems, branches or needles will influence tree function. Many of the structural adaptations observed in needles in response to altered illumination and water supply may be related to functional traits such as photosynthesis and water relations.

The increase in $M_s$ and $I_s$ with increasing tree height (Table 4) suggests that the construction costs are higher in the upper crown, but this is to some extent balanced by the higher photosynthetic activity of sun-exposed needles (Niinemets et al. 1998, Bond et al. 1999). Higher assimilation capacity of sun-exposed needles was also indicated by an increase in $A_s$ (Table 5), as an increased phloem area (a part of $A_s$) suggests a greater need to translocate photosynthates (Niinemets et al. 2007). Nevertheless, we hypothesize that higher construction costs in the upper crown could reduce tree growth under stress conditions.

Increased transpiration of the lower crown after thinning may result in increased water stress in the upper crown. In our study this was indicated by increased xeromorphy ($M_s$ and $I_s$; Table 4) of sun-exposed needles on the thinned plot. Increased transpiration of the lower crown after thinning was also indicated by an increase in $A_s$ as an increased xylem area (a part of $A_s$) suggests greater evaporative demand in the lower crown (Niinemets et al. 2007). Hence, if thinning is done during dry periods this could result in reduced growth of the upper crown, or even top dying. Tree top dying in response to drought stress has been reported in several studies (Rundel 1973, Diamandis 1979, Kołoski and Pallardy 1997, Ueda et al. 2006). Our findings thus have implications for forest managers, as they identify possible negative effects of thinning that may have to be balanced against the positive effects of stem growth release.

**Conclusions**

In this study we have demonstrated substantial variation in needle form within individual Norway spruce trees along height and light gradients in the canopy. In particular, increasing tree height and light intensity increased most morphological and histological needle parameters (including leaf mass per area,
needle thickness, needle width, needle length, needle weight, needle density, needle cross-sectional area, needle stele area and percentage of stele area in needle area) and decreased needle flatness. Of all these parameters, needle width, needle cross-sectional area, needle stele area and needle flatness differed most between the upper and lower canopy.

Lower needle water status in the thinned plot resulted in an increase in leaf mass per unit leaf area, nitrogen content per leaf area, needle density and percentage of stele area in needle area, and a decrease in needle thickness and needle flatness. The parameters that were most sensitive to altered needle water status were needle thickness, needle flatness and percentage of stele area in needle area. These results show that when comparing needle structure or function between tree species, it is important to consider not only tree height and light gradients, but also needle water status. Unaccounted for differences in needle water status may have contributed to the variation found among conifers in the relationship between needle structure and irradiance.

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References


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