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Reference:

Gebauer Roman, Čermák Jan, Plichta Roman, Špinlerová Zuzana, Urban Josef, Volařík Daniel, Ceulemans Reinhart.-
Within-canopy variation in needle morphology and anatomy of vascular tissues in a sparse Scots pine forest
Trees: structure and function - ISSN 0931-1890 - 29:5(2015), p. 1447-1457
Full text (Publishers DOI): <http://dx.doi.org/doi:10.1007/s00468-015-1224-1>
Handle: <http://hdl.handle.net/10067/1276470151162165141>

1 Within-canopy variation in needle morphology and anatomy of vascular tissues in a sparse Scots pine
2 forest

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15 Abstract

16 Key message

17 Azimuthal sides of the canopy affected neither needle morphology nor vascular anatomy. However, a
18 significant effect was found to be associated to canopy height. The needle morphological and anatomical
19 parameters of vascular tissues were scaled up to the forest stand level.

20 Abstract

21 In conifers the needle is the key organ for photosynthesis and transpiration, and these two processes
22 are influenced by the morphological and anatomical structure of the needle. Although many factors
23 are involved in needle development, long-term irradiance gradients through the canopy as well as
24 across the forest are among the main drivers. The present study had two principal objectives: (i) to
25 obtain a better knowledge of the morphological and anatomical parameters of sun, transient and
26 shade needles taken from different azimuthal orientations of a sparse Scots pine (*Pinus sylvestris* L.)
27 stand; and (ii) to scale up these needle data to the forest stand level. One year old needles were
28 collected from mature Scots pine trees from branches on the south- and the north-facing azimuthal
29 sides of the canopy at three different canopy heights. Needle structural parameters were measured
30 on cross sections at the needle base. Azimuthal sides of the canopy had no effect on the needle
31 morphology and anatomy since the irradiation was similar on both canopy sides due to the low leaf
32 area index (1.31) of the sparse Scots pine forest. However, sampling height (i.e. sun versus shade
33 needles) appeared to have a significant effect on the studied parameters, the largest differences
34 being needle stele area, xylem area, phloem area and number of tracheids. Tracheid frequency, leaf

1 specific hydraulic conductivity and needle density were the only parameters which were not
2 influenced by canopy height. Our measurements revealed that in a given ha of pine forest stand,
3 water was transported through approximately 40×10^9 tracheids situated in 380×10^6 needles,
4 whose xylem area was 4.34 m^2 . This type of data could be helpful for modelling and could provide a
5 better understanding of the forest stand environment, of tree hydraulic systems or of eddy
6 covariance flux measurements of this stand , an established ecosystem site within the European
7 ICOS network.

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10 Keywords: anatomy, forest stand, Hagen-Poiseuille law, *Pinus sylvestris*, sun and shade needles,
11 tracheid.

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1 Introduction

2 All tree canopies, including coniferous, have a complex heterogeneous structure, both in terms of
3 foliage architecture and physiology. The needle is the key organ for photosynthesis and transpiration,
4 and is also a key component for understanding rates of energy and material exchange between the
5 canopy and the atmosphere (Vose et al. 1994). Reliable estimates of conifer canopy gas exchange
6 therefore require an accurate characterization of the needle morphology, anatomy and cell
7 distribution in the canopy (Op de Beeck et al. 2010). Needle parameters are also very important for
8 studying forest productivity as well as for ecosystem modelling (Niinemets and Kull 1995; Niinemets
9 et al. 1999).

10 Morphological and anatomical characteristics of conifer needles are strongly related to long
11 term irradiance availability gradients through the canopy as well as across the forest. In particular,
12 there is a positive relationship of needle dry mass per unit needle area (LMA) with increasing
13 irradiance in conifers (Gebauer et al. 2011). An increase in the number of needles per branch length
14 with increasing irradiance has been reported for various coniferous species, i.e.: Scots pine
15 (Kellomäki and Oker-Blom 1983), Engelmann spruce, subalpine fir, lodgepole pine (Carter and Smith
16 1985) and Norway spruce (Niinemets and Kull 1995). Tracheid size and distribution are other
17 important functional parameters that are affected by irradiance intensity. Sun needles have larger
18 and more tracheids than shade needles (Protz et al. 2000). Larger and more numerous tracheids are
19 also related to the higher hydraulic conductivity of sun needles, as the hydraulic conductivity
20 increases with the conduit diameter (Tyree and Zimmermann 2002; Sperry et al. 2006). Thus, the
21 study of tracheid size and distribution is needed for a better knowledge of the water transport
22 efficiency through a tree and is a prerequisite for the correct interpretation of sap flow data obtained
23 in forest stands. Hydraulic conductivity calculated from measurements of conduit radii often
24 overestimate measured conductivity as tracheid anatomy and their interconnections are more
25 complex than a simple series of parallel straight-walled tubes (Tyree and Zimmermann 2002).
26 Hydraulic conductivity is usually measured in shoots and is 30-50 % of the theoretical conductivity (Sperry et al. 2006) However, a new technique was recently developed enabling the measurement of
27 needle hydraulic conductivity (Charra-Vaskou and Mayr 2011; Charra-Vaskou et al. 2012).

28 Scots pine (*Pinus sylvestris* L.) is the most widely distributed pine species and one of the most
30 important timber species in Eurasia (Poyatos et al. 2007). Previous studies have provided useful

1 descriptions of canopy architecture as well as of allometric relationships for the overall needle
2 distribution in the canopy (Čermák et al. 1998; Xiao et al. 2003; Urban et al. 2015). However, the
3 variability in needle morphology and anatomy in the different vertical and azimuthal positions within
4 the canopy has seldom been reported for mature pine trees. Thus, the establishment of appropriate
5 relationships based on data from individual canopy heights are necessary to scale up needle
6 morphological and anatomical parameters to the tree or stand level.

7 Given these existing gaps in our knowledge, the present study aimed to: (i) to obtain better
8 knowledge of the morphological and anatomical parameters (associated with the vascular system) of
9 sun, transient and shade needles from different azimuth sides of mature Scots pine trees; and (ii) to
10 scale up the studied needle parameters to the forest stand level. To attain these objectives, the
11 number, size and distribution of tracheids were studied in Scots pine needles from three canopy
12 heights on the south- and north-facing azimuth angles of the canopies.

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14 Materials and Methods

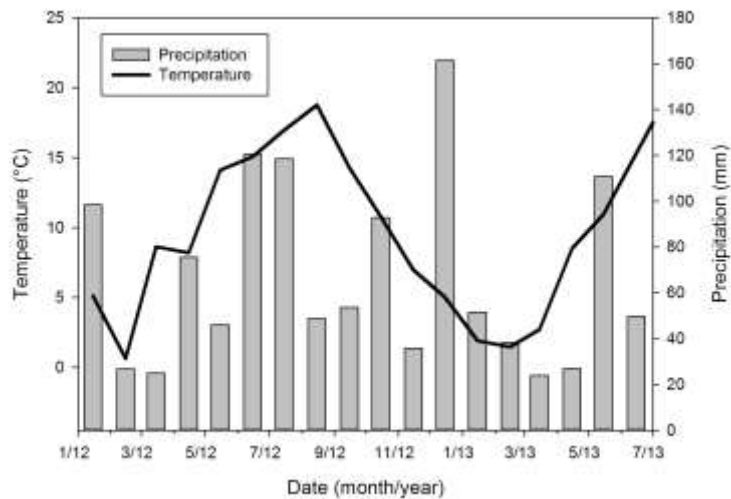
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16 Study site

17 The study site is an even-aged, 2 ha Scots pine (*Pinus sylvestris* L.) stand, representing a portion of
18 the 150 ha mixed coniferous/deciduous regional forest 'De Inslag' located in Brasschaat, in the
19 Campine region of the province of Antwerp, Belgium (51°18'3'' N and 4°31'14'' E, altitude 16 m). In
20 this forest, the ecosystem fluxes of carbon, energy, and water are monitored from a meteorological
21 tower using the eddy covariance technique within the framework of the European CARBO-EUROFLUX
22 program (between 1996 and 2013) and the European ICOS network (from 2013 onward;
23 <http://www.icos-infrastructure.eu>). The Campine region has a temperate maritime climate. The
24 mean annual temperature at the site is 9.8°C with mean temperatures for the coldest and the
25 warmest months of 3 and 18°C, respectively. Mean annual precipitation was 767 mm. Mean annual
26 temperature was 10.4°C and the sum of precipitation was 908 mm in 2012 (Figure 1).

27 The stand was planted in 1929, and in 2011 the stock density was 360 trees ha⁻¹ (Gielen et al.
28 2013). In 2011, mean diameter at breast height (DBH) was 32.9 cm, and mean tree height was 21.4 m
29 (Gielen et al. 2013). The stand canopy was sparse, with a projected leaf area index (LAI) of 1.31 in
30 2007, and the pines bear only two needle age classes (Op de Beeck et al. 2010). Needle analysis of
31 the stand showed low levels of magnesium and phosphorus. The concentration of magnesium was
32 about 700 mg kg⁻¹ of dry mass and 310 mg kg⁻¹ in needles and litter, respectively. The concentration
33 of phosphorus was about 1100 mg kg⁻¹ and 446 mg kg⁻¹ in needles and litter, respectively (Janssens et
34 al. 1999). The site is located in an area with high NO_x and ammonia deposition, with an average total

1 atmospheric N deposition of 48 kg ha⁻¹ year⁻¹ (Neiryck et al. 2008). The soil was classified as an Albic
 2 Hypoluvic Arenosol. The pH (CaCl₂) values varied from 2.7 in the forest floor, to 3.1 in the top soil and
 3 3.6 deeper in the soil profile. Base saturation was lower than 10% throughout the soil column. The
 4 C:N ratio of the litter was 31, whereas values in the mineral soil fell below 20 (Neiryck et al. 2008).
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 7 *Figure 1 Monthly mean air temperatures and monthly sums of precipitation from 1.1.2012 to*
 8 *30.6.2013.*
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10 Sampling of needle material and determination of needle parameters

11 Five 85-year old co-dominant pine trees with a mean tree height of 21.5 m and diameters at breast
 12 height (DBH) of 29, 33, 34, 38 and 41 cm were sampled on June 4, 2013. The canopy length of all
 13 trees was around 4.5 m with a slightly higher canopy for larger trees. The rope technique was used
 14 for getting to the top of trees to avoid stem damage. Five one-year old shoots with fully developed
 15 needles from branches on the south-facing azimuthal side and five from the north-facing azimuthal
 16 side of the canopy, from three different canopy heights at the outer part of the canopy, were
 17 collected from each of the five trees (i.e. 30 shoots/tree x 5 trees). The three canopy heights were
 18 taken at (i) 0–1.5 m from the tree top (upper canopy; sun needles); (ii) 1.5–3.0 m from the tree top
 19 (middle canopy; transient needles); and (iii) 3.0–4.5 m from the tree top (lower canopy; shade
 20 needles). Shoots were collected from the middle part of each layer. In the field, the samples were
 21 fixed in 5 % FAA solution (90 ml 70% ethanol, 5 ml acetic acid and 5 ml 40% formaldehyde).
 22 Subsequently, from each of the collected shoots, two randomly selected fascicles (with two needles
 23 per fascicle, thus yielding 20 needles per position for each tree) were taken for further analysis. One
 24 needle from each fascicle was used for the anatomical analysis (10 needles), and one needle for
 25 measurements of needle morphology.

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Needle morphology

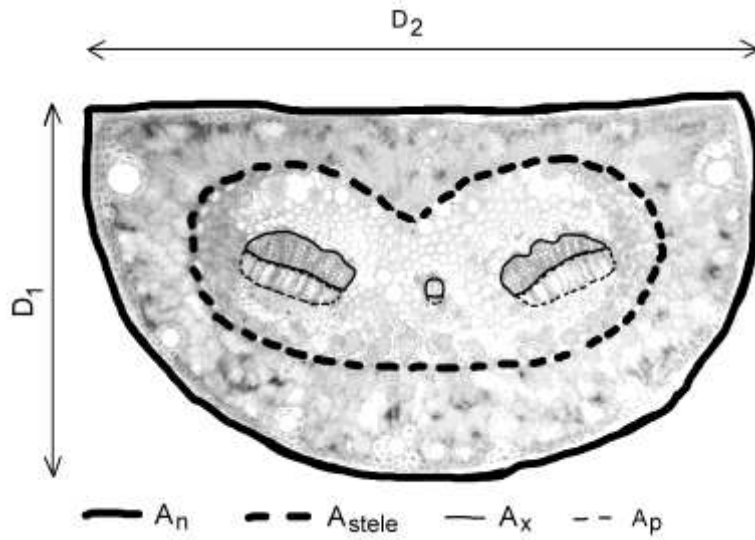
Fresh needles were scanned to determine the projected leaf area (PLA) and individual needle length (L_n). They were then oven dried at 85 °C for 48 hours to determine needle dry mass (DM). PLA and L_n were determined using the ImageTool 3.00 software (The University of Texas Health Science Centre, San Antonio, TX, USA). LMA [g m^{-2}] was calculated as DM divided by PLA. Needle density (N_d) was calculated according to Equation 1:

$$N_d = \frac{DM}{A_n L_n} \quad [\text{g cm}^{-3}] \quad (\text{eq. 1})$$

where DM is needle dry mass (g); A_n is cross-sectional needle area (cm^2); and L_n is needle length (cm).

Needle anatomy

Cross sections for anatomical analyses were taken at the needle base and stained for lignin using phloroglucinol and HCl. Stained sections were examined under a light microscope (Olympus BX51, Olympus Czech Group Corp.) at magnifications of up to $\times 400$, and photographed using a digital camera (Olympus E-330, Olympus Czech Group Corp.) connected to a computer by QuickPhotomicro 2.3 software (Promicra, Czech Republic). The following parameters were measured: cross-sectional needle area (A_n), stele area (A_{stete}), phloem area (A_p), xylem area (A_x), needle thickness (D_1) and needle width (D_2) using ImageTool 3.00 image analysis software (Figure 2). The xylem/phloem area ratio (R_a) was also calculated. The tracheid lumens were manually coloured using Adobe Photoshop 9.0 (Adobe Systems, USA). The tracheid lumen area (A_{lum}), and maximum and minimum lumen diameter (d_{max} and d_{min} , respectively) were measured using the ImageTool3.00 software. We also quantified the number of tracheids per needle cross section (T_{num}) as well as the tracheid frequency (T_f), which was T_{num} divided by A_n .



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Figure 2. Measured needle parameters: needle thickness (D_1), needle width (D_2), cross-sectional needle area (A_n ; solid bold line), stele area (A_{stele} ; dashed bold line), xylem area (A_x ; solid fine line) and phloem area (A_p ; dashed fine line). Studied needles were for 95% constructed by two vascular cylinders and the rest of the needles had three vascular cylinders as shown.

Theoretical hydraulic conductivity

The tracheid theoretical hydraulic conductivity (K_{th_t}) was calculated according to the Hagen-Poiseuille law (Equation 2) (Cruiziat et al. 2002). Since the tracheid lumen is usually not circular, a minor modification to the formula was applied, as recommended by Martre et al. (2000) (Equation 3).

$$K_{th_t} = \frac{\pi \rho}{8 \eta} r_{lum}^4 \quad [\text{kg m s}^{-1} \text{MPa}^{-1}] \quad (\text{Eq. 2})$$

$$r_{lum}^4 = \frac{d_{max}^3 d_{min}^3}{8 d_{max}^2 + 8 d_{min}^2} \quad (\text{Eq. 3})$$

where ρ is the density of water at 20°C (998.2 kg m⁻³), r_{lum} tracheid lumen radius and η is the viscosity of water at 20 °C (1.002 e⁻⁹ MPa s).

The needle theoretical hydraulic conductivity (K_{th_n}) was calculated as the sum of all K_{th_t} per needle. Then the xylem-specific hydraulic conductivity (K_{s_xylem}) was estimated as K_{th_n} divided by the xylem area of the needle (A_x) and needle-specific hydraulic conductivity (K_{s_leaf}) was estimated as K_{th_n} divided by the PLA of the needle.

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Scaling-up from needle level to forest stand level

To scale up the needle data to the forest stand level we calculated the vertical needle dry mass distribution for a mean tree (DBH = 32.9 cm; tree height = 21.4 m and canopy height = 4.5 m) according to the allometric relationship previously established (and already published) for mature Scots pines at the study site (Čermák et al. 1998) (Equation 4).

$$y = \frac{P_1(h_{top} - h_i)}{P_2} \exp[-(h_{top} - h_i)^{P_3}] \quad [\text{kg}] \quad (\text{Eq. 4})$$

where P_1 , P_2 , P_3 and P_4 are coefficients derived on the basis of DBH; h_{top} is tree height; and h_i are different canopy heights above ground.

Needle dry mass distribution per canopy height (1.5 m thick) was assessed for three canopy heights representing sun, transient and shade needle classes of the sampled trees. The needle dry mass per canopy height was divided by the DM of one needle to obtain the number of needles per canopy height (N_m). The needle anatomy data were then multiplied by N_m and by the forest stock density (360 trees ha^{-1}) to scale up from the needle level to the forest stand level.

All acronyms, abbreviations and symbols are defined in Table 1.

Data analysis

Because samples were collected from five different trees, linear mixed effect models (LME) were used (Zuur et al. 2009). These models enabled inclusion of variation among trees by specifying the individual tree as a random effect. Some parameters (L_n , PLA , A_{steler} , A_p and A_x) did not conform to the assumption of homogeneity for LME. We observed that variance increased with mean values for those parameters. For these five parameters, we used generalized linear mixed effect models (GLMM) with a gamma distribution and a log-link function or simpler (general) LME. To test for the significance of the random effect of individual trees and for the significance of the fixed effects of canopy height and azimuthal sides of the canopy, as well as to obtain appropriate p-values, we applied the approach described by Zuur et al. (2009). Briefly, we used likelihood ratio tests to compare each simpler model with a relevant more complex model. Some studied parameters (DM, K_{s_leaf} and N_D) were calculated at the whole-tree level. For these parameters, we used simple linear models (LM). Statistical analyses were carried out in the R software environment (R Core Team 2014), using the package 'lme4' (Bates et al. 2014) for the LME and GLMM calculations, and the 'MuMIn' package (Bartoń 2014) for the R^2_{GLMM} calculations.

1 Results

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4 Needle morphology and anatomy

5 We found a strong impact of canopy height – and thus of irradiation – on almost all studied
6 parameters (Tables 2 and 3). The largest influences of canopy height were found for A_{steler} , T_{num} , A_x , A_p ,
7 DM and K_{th} (Figure 3). A larger increase of A_p than A_x with increasing tree height resulted in a
8 significant decline of R_a with increasing tree height. We also found a significant interaction effect
9 between canopy height and L_n , K_{s_xylem} , LMA, PLA and A_n (Figure 3). Parameters T_f , K_{s_leaf} and N_D were
10 the only ones that were not significantly influenced by canopy height (Tables 2 and 3). However,
11 although there were significant effects of canopy height on the studied parameters, this trend was
12 not continuous from canopy base to tree top, as shade and transient needles had almost the same
13 values (Figure 3). Only A_{steler} , D_2 , PLA and R_a significantly differed between shade and transient
14 needles. In the follow-up analysis, transient and shade needles were therefore combined, and this
15 group was referred to as shade needles. In contrast to the significant impacts of the individual tree or
16 of canopy height on the studied parameters (A_x , A_{steler} , T_{num} , A_x , A_p , d_{max} , d_{min} , DM or K_{th}), there was no
17 significant influence of azimuthal side, except for on R_a (Tables 2 and 3). For this reason, our follow-
18 up analysis ignored the influence of individual azimuthal sides and instead combined the data from
19 the north- and south-facing azimuthal sides of the canopy.

20 On average, sun needles were observed to be 9, 8, 7, 28 and 7% longer, thicker, wider,
21 heavier and denser than shade needles (Table 4). An average sun needle also had a 12% higher
22 K_{s_xylem} and a 17% larger LMA (Table 4). The A_{steler} , A_x and A_p of a mean sun needle represented 32%,
23 1.9% and 2.2%, respectively, of the A_n (Table). Almost the same values were found for shade
24 needles. Only the representation of A_p in the A_n was 18% higher in sun needles than in shade
25 needles. The sum of A_{lum} in the sun and shade needles occupied approximately 35% of the A_x .

26

27 Scaling-up of needle parameters to the mean tree and the forest stand levels

28 A mean pine tree had 0.21×10^6 sun and 0.84×10^6 shade needles (Table 5). Thus, on average, 1 ha of
29 forest stand has 76×10^6 sun and 302×10^6 shade needles. Sun needles of a mean tree had an A_n of
30 0.16 m^2 and their A_x was 0.0029 m^2 , comprising 25.5×10^6 of tracheids (Table 5). Thus, the A_x of sun
31 needles per 1 ha of forest stand was 1.06 m^2 , and theoretically water could be transported in the sun
32 needles through approximately 9.2×10^9 tracheids (Table 5). The total A_{lum} (theoretical functional
33 area for water transport) of sun needles of a mean tree and of a 1 ha forest stand were only 0.0011
34 m^2 and 0.38 m^2 , respectively (Table 5). According to our results, trees of the 1 ha forest stand

1 transported water through the approximately 38×10^9 tracheids situated in the 378×10^6 needles,
 2 whose xylem area was only 4.34 m^2 . About a quarter of the needles accounting for these values are
 3 sun needles, with shade needles accounting for the rest (Table 5).

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Table 1. Overview of the needle parameters, their abbreviations, definitions and units used throughout this study.

Parameter	Explanation	Unit
A_{lum}	tracheid lumen area	μm^2
A_n	needle cross-sectional area	mm^2
A_p	needle phloem area	μm^2
A_{stele}	needle stele area	mm^2
A_x	needle xylem area	μm^2
D_1	needle thickness	mm
D_2	needle width	mm
d_{max}	maximum lumen diameter	μm
d_{min}	minimum lumen diameter	μm
DM	needle dry mass	mg
K_{th_n}	theoretical needle hydraulic conductivity	$\text{kg m s}^{-1} \text{MPa}^{-1}$
K_{s_leaf}	leaf-specific hydraulic conductivity	$\text{kg m}^{-1} \text{s}^{-1} \text{MPa}^{-1}$
K_{s_xylem}	xylem-specific hydraulic conductivity	$\text{kg m}^{-1} \text{s}^{-1} \text{MPa}^{-1}$
L_n	needle length	cm
LMA	leaf mass per area (DM/PLA)	g m^{-2}
N_D	needle density	g cm^{-3}
PLA	needle projected area	cm^2
R_a	ratio A_x/A_p	-
T_f	tracheid frequency	unit mm^{-2}
T_{num}	tracheid number per needle	-
Q_{calcu}	theoretical maximum sap flow rate of tree	kg h^{-1}
Q_{measu}	measured maximum sap flow rate of tree	kg h^{-1}

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1 Table 2. P-values from likelihood ratio tests showing the significance of the random effect of the individual tree
 2 and of the fixed effects of canopy height (sun, transient and shade needles) and of south-facing and north-
 3 facing azimuthal sides of the canopy. Only parameters that were tested by linear models (LM) are shown. For
 4 an explanation and definition of parameters, see Table 1.
 5

Parameters	Tree	Canopy height	Azimuthal side	Canopy height + Azimuthal side
DM	-	0.005	0.95	0.94
LMA	-	0.001	0.32	0.72
N _D	-	0.302	0.53	0.45
K _{S_leaf}	-	0.187	0.77	0.34

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9 Table 3. P-values from likelihood ratio tests showing the level of significance of the random effect of the
 10 individual tree and of the fixed effects of canopy height (sun, transient and shade needles) and of south-facing
 11 and north-facing azimuthal sides of the canopy. Parameters that were tested by linear mixed effect models
 12 (LME) or by generalized linear mixed effect models (GLMM) are shown. For an explanation and definition of the
 13 parameters, see Table 1.

Parameters	Tree	Canopy height	Azimuthal side	Canopy height + Azimuthal side
A _{lum}	< 0.001	< 0.001	0.76	0.74
A _n	< 0.001	< 0.001	0.79	0.75
A _p *	< 0.001	< 0.001	0.27	0.32
A _{stete} *	< 0.001	< 0.001	0.59	0.68
A _x *	< 0.001	< 0.001	0.80	0.60
D ₁	0.002	< 0.001	0.85	0.82
D ₂	< 0.001	< 0.001	1.00	1.00
d _{max}	< 0.001	0.002	0.86	0.86
d _{min}	< 0.001	< 0.001	0.92	0.92
K _{th}	< 0.001	< 0.001	0.88	0.88
K _{s_xylem}	< 0.001	0.002	0.92	0.93
L _n *	< 0.001	< 0.001	0.87	0.78
PLA*	< 0.001	< 0.001	0.50	0.60
R _a	< 0.001	< 0.001	0.03	0.03
T _f	< 0.001	0.090	0.26	0.25
T _{num}	< 0.001	< 0.001	0.94	0.95

14 * GLMM

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1 Table 4. Mean values (95% confidence interval) for sun, transient and shade Scots pine needles. For an
 2 explanation and definition of the parameters, see Table 1.
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Parameters	Mean (95% confidence interval) ⁴		
	Sun needles	Transient needles	Shade needles
A_n	0.74 (0.7-0.78)	0.60 (0.56-0.64)	0.62 (0.58-0.66)
A_p ($\times 10^3$)	16.0 (12.7-20.1)	11.4 (9.0-14.3)	11.0 (8.8-13.9)
A_{stele}	0.24 (0.21-0.28)	0.19 (0.16-0.21)	0.20 (0.17-0.23)
A_x ($\times 10^3$)	14.1 (11.5-17.2)	10.5 (8.6-12.8)	11.1 (9.1-13.6)
D_1	0.72 (0.71-0.74)	0.65 (0.64-0.67)	0.66 (0.64-0.67)
D_2	1.24 (1.21-1.28)	1.12 (1.08-1.15)	1.15 (1.11-1.18)
d_{max}	8.48 (8.04-8.92)	8.20 (7.76-8.64)	8.21 (7.77-8.66)
DM	22.0 (19.1-24.8)	15.2 (12.3-18.0)	15.6 (12.7-18.5)
K_{th} ($\times 10^{-9}$)	9.7 (8.4-10.9)	6.5 (5.3-7.7)	6.8 (5.6-8.0)
K_{s_leaf} ($\times 10^{-4}$)	1.26 (1.05-1.47)	1.05 (0.84-1.25)	1.01 (0.80-1.21)
L_n	6.5 (5.7-7.4)	5.8 (5.1-6.6)	5.9 (5.2-6.7)
LMA	275 (259-291)	239 (223-255)	227 (211-243)
N_D	0.45 (0.42-0.47)	0.43 (0.40-0.46)	0.42 (0.39-0.44)

5

6 Table 5. Scaled-up sun and shade needle parameters of Scots pine to the tree and the forest stand levels (360
 7 trees ha^{-1}). For an explanation and definition of the parameters, see Table 1.
 8

Parameters	Mean tree		Forest stand (= 1 ha)	
	sun needle	shade needle	sun needle	shade needle
Number of needles (10^6)	0.21	0.84	76	302
T_{num} (10^6)	25.52	80.57	9187	29004
A_{lum} (m^2)	0.0011	0.0031	0.38	1.12
A_n (m^2)	0.16	0.51	56	185
A_p (m^2)	0.0033	0.0094	1.21	3.40
A_x (m^2)	0.0029	0.0091	1.06	3.28
A_{stele} (m^2)	0.051	0.162	18	58
PLA (m^2)	16.40	54.56	5905	19640

1 Discussion

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3 Canopy height and azimuth angle of the canopy sides

4 For both ecologists and physiologists, foliar morphology and anatomy are important for a better
5 understanding of the whole tree physiology (Yan et al. 2012). However, only limited information is
6 available with regard to potential effects of canopy azimuth angle on the heterogeneity of foliar
7 properties. A significant azimuthal angle effect on needle structure and physiology has been reported
8 in only a few studies on coniferous trees (Peters et al. 2008; Yan et al. 2012), while other studies
9 found no anatomical or functional differences between the north- and the south-facing sides of the
10 canopy in a sparse forest stand (Oliveira et al. 1996) and in the upper, sunlit part of the canopy
11 (Lhotáková et al. 2007). Our observations correspond with these findings as we did not observe an
12 azimuthal side effect on the studied needle parameters (except for R_a). Part of the reason for the
13 absence of a difference is that in our sparse forest stand with a low LAI of only 1.31 (Xiao et al. 2003),
14 sufficient irradiance reached the needles situated on the north azimuth side of the canopy.

15 Our results on the effects of canopy height (i.e., increase/decrease of values of studied
16 needle parameters between transient and sun needles) were consistent with those from other
17 studies on Scots pine (Čermák et al. 1998; Lin et al. 2002; Op de Beeck et al. 2010) and other conifers
18 (Niinemets and Kull 1995; Peters et al. 2008; Yan et al. 2012). However, shade and transient needles
19 had almost the same values for the given morphological and anatomical parameters. The reason is
20 that the sparse canopy and rather limited canopy depth enabled the penetration of sufficient
21 irradiance into the lower canopy (Čermák et al. 1998).

22

23 Morphology and anatomy of sun and shade needles

24 Needle morphology and anatomy is often strongly related to gradients in the long-term irradiance
25 availability within canopies and across stands (Gebauer et al. 2011). LMA has been used as an easy-
26 to-assess index of the functional status of a leaf or a plant, and the variation in LMA within tree
27 canopies is commonly attributed to irradiance gradients (Niinemets and Kull 1995) and needle age
28 (e.g., Ishii et al. 2002; Yan et al. 2012). LMA generally decreases with decreasing irradiance intensity
29 (Xiao et al. 2006; Lin et al. 2001). Our observation that LMA of sun needles was approximately 1.2
30 times higher than that of shade needles agrees with previous results. Such plasticity of LMA within a
31 Scots pine canopy confirms earlier observations from the same stand (Xiao et al. 2006), although the
32 last mentioned study found by 10% lower LMA both for both sun and shade needles. A lower LMA of
33 shade needles relative to sun needles also means that they had a lower biomass cost for the
34 formation of the exposed surface area. The mean LMA of 252 g m^{-2} was well within the range of 182–

1 345 g m⁻² reported for Scots pine from various stands in Europe (Mencucini and Bonosi 2001; Xiao et
2 al. 2006). The spatial variation in LMA across canopy heights demonstrated an acclimatization
3 response of the canopy that enhances the foliage's photosynthetic capacity in high-irradiance
4 conditions and its light-harvesting efficiency in low-irradiance conditions (Bond et al. 1999). This also
5 improves the whole-canopy carbon gain for a given biomass investment in needles (Bond et al.
6 1999).

7 In the present study, the mean D₁ (0.68 mm) and D₂ (1.17 mm) values were well within the
8 range of 0.4-0.72 mm and 1.13-1.32 mm, respectively, reported for adult Scots pine trees (Lin et al.
9 2002; Luomala et al. 2005; Lukjanova and Mandre 2008). Similar to our study, D₁ and D₂ of other pine
10 species in the other studies were positively related to irradiance intensity (Niinemets et al. 2002;
11 Niinemets et al. 2007). A negative correlation with increasing tree height for D₂ was, however, was
12 observed in the same forest stand 12 years ago (Lin et al. 2002) while N_D was independent of
13 irradiance, which has been reported for *Pinus sylvestris* (Niinemets et al. 2002) as well as other
14 conifers (Whitehead et al. 1994; Niinemets 1997). However, some studies have found increases in N_D
15 with increasing irradiance intensity (Niinemets et al. 2007; Gebauer et al. 2011). The different trends
16 may be attributable to differences in within-canopy water stress gradients observed in individual
17 studies (Niinemets et al. 2002), as N_D increases in response to water limitations (Roderick et al. 2000)
18 and foliar water stress tends to increase from the bottom to the top of the canopy (Niinemets et al.
19 1999). If the tree top is water stressed, L_n should also decrease, because cell expansion and division
20 are negatively affected by low water potentials (Dale 1988). Our study showed, however, sun
21 needles to be longer than shade needles. This corresponded with results reported for other conifers,
22 if the canopy of the tree is located in shade (Lin et al. 2002; Gebauer et al. 2011). In our study, the
23 mean L_n was within the range of 1.5 - 8.4 cm found for other Scots pine needles (Junttila and Heide
24 1981; Čermák et al. 1998; Luomala et al. 2005; Primicia et al. 2014). The shortest needle grew at
25 extreme, poor or high altitude sites. Here we found a smaller difference between sun and shade
26 needles for L_n than was observed for the same forest stand by Lin et al. (2002), who also recorded
27 around 30% longer needles than those we observed. Thus, plasticity within the canopy seemed to be

1 influenced not only by the irradiance gradient, but also by other environmental parameters that may
2 play an important role in needle development. For example, L_n of Scots pine is strongly dependent on
3 the temperature of the current growing season (Junttila and Heide 1981). The needles of trees grown
4 at elevated temperature were thinner, shorter and had a smaller projected area than at ambient
5 temperature (Luomala et al. 2005). The temperature in the year preceding the sampling was by 0.6
6 °C higher than the long-term mean, which may have been the reason for the needles being shorter in
7 our study than in the previous work of Lin et al. (2002). Although the connection between
8 temperature and tree growth has been well documented by many authors (Way and Oren 2010),
9 studies about the relationship between needle growth and temperature are rare. As photosynthesis
10 and transpiration take place in needles, detailed research in this area is needed.

11 Site, climate conditions, tree age and nutrition also play important roles in the needle and
12 vascular cylinder development. Comparison with Scots pine needles growing on dunes in SW Estonia
13 shows that the A_n value of $320 \mu\text{m}^2$ (Lukjanova and Mandre 2008) was half that of our needles. On
14 the other hand, about 50% larger A_n relative to our values were found for Scots pine seedlings grown
15 in favourable greenhouse conditions (Lin et al. 2001). These differences could be related to variations
16 in tree age, but also to differences in the nutrition. Scots pine needles from the sites with limited
17 phosphorus availability (such as our site) were narrower, thinner, and had lower LMA than needles
18 from the nutrient-rich site (Niinemets et al. 2001). The 1.3 and 1.4 times higher A_p and A_x ,
19 respectively, of sun needles than shade needles may be the result of the higher photosynthetic
20 activity of sun needles and the need both to transport photosynthates from source to sink (Lin et al.
21 2001) and to increase water transport efficiency (Niinemets et al. 2007). However, the relative areas
22 of A_p and A_x per needle cross-section were only 1.1 times higher in sun needles than in shade
23 needles, which suggests that capacities for water transport and for translocation of photosynthates
24 were less affected.

25 Tracheid lumen diameter is a key parameter for determining water hydraulic conductivity, as
26 theoretical hydraulic conductivity increases with the fourth power of the conduit radius (Hagen–

1 Poiseuille law). The mean d_{\max} in our trees was 8.3 μm , which was less (by 3.3 μm) than in Scots pine
2 trees growing in France (Cochard et al. 2004). The number of tracheids in our needles (around 110)
3 was lower than reported for *P. ponderosa* (337), *P. palustris* (290) and *P. rigida* (211) (Zwieniecki et
4 al. 2006). We found no comparative studies of Scots pine needles. The higher K_{th} of sun needles in
5 our study corresponds with the observations of other authors (Sack et al. 2003; Gebauer et al. 2011).
6 In general, $K_{\text{s_leaf}}$ is a measure representing sufficiency of a specific tree part to supply water to a
7 distal part (hydraulic sufficiency; Tyree and Ewers 1991), and $K_{\text{s_xylem}}$ is a useful measure of the
8 porosity of a shoot segment (Cruziat et al. 2002). In our study, needle porosity was more plastic than
9 the hydraulic sufficiency, which was independent of canopy height. Our comparison of sun and shade
10 needles seems to support Sellin and Kupper's (2004) conclusion that differences in leaf conductance
11 between the lower and upper canopy are a result of differences in xylem anatomy. Finally, our
12 results agree with the hypothesis of Protz et al. (2000) that reduced photosynthesis in shade needles
13 leads to the development of smaller tracheids.

14

15 Scaling up from needle level to the tree and stand levels

16 Plant (and tree) structure is generally closely related to function (Tyree and Zimmermann 2002), and
17 a number of the morphological and anatomical adaptations observed in needles in response to
18 altered irradiance and water supply may be related to their functional traits, such as photosynthesis
19 and water transport (Gebauer et al. 2011). Although the general characteristics of conductive stem
20 systems at the whole-tree level have been well studied (Tyree and Zimmermann 2002), there is not a
21 great deal of detailed information available at the needle or stand level (Čermák et al. 2002). That
22 said, a new and promising technique was recently developed which enables direct measurements of
23 needle hydraulic conductivity by the flow-meter technique. This novel technique has thus allowed for
24 the quantification of specific hydraulic conductivity of the needle xylem and of the partitioning of
25 resistances within needles (Charra-Vaskou and Mayr 2011; Charra-Vaskou et al. 2012). With this
26 innovation, a relationship between calculated and measured needle hydraulic conductivity can and
27 should be studied in greater detail in future research. Moreover, the type of data such as the number
28 of needles, number of tracheids or xylem and phloem area of needles per 1 ha forest stand, which
29 has been rarely applied in traditional physiological studies, could be helpful, for example, for

1 biophysical calculations (Tributsch 1985) in studies on tree hydraulic systems (Čermák et al. 2002),
2 for modelling gas exchange (Op de Beeck et al. 2010) or in studies on isoprene emissions (Sharkey
3 and Yeh 2001). Our data provide a useful contribution to modeling the tree water regime and of
4 water relations within a forest ecosystem. Predictive simulation models are currently highly important
5 for forest ecosystem services since climate change is expected to accelerate drought-induced tree
6 susceptibility to pests and pathogens (Adams et al. 2009).

7

8 Author contribution statement

9 RG, JČ and RC conceived, designed and performed research; RG, RP, ZŠ and JU conducted the
10 experiment; DV, RG and RP analysed the data. All authors contributed to the interpretation of results
11 and editing of the manuscript.

12

13 Acknowledgments

14 This work was funded by Czech project MSMT COST LD 13017 under the framework of the COST
15 FP1106 network STReESS, Mendel University (Grant IGA 73/2013) and by the project “Indicators of
16 Tree Vitality” (Reg. No. CZ.1.07/2.3.00/20.0265) co-financed by the European Social Fund and the
17 Czech Republic. We would like to acknowledge the contribution of the COST Action FP1106, STReESS.
18 The forest field site has received support from the European Commission’s Fifth and Sixth Framework
19 Programs as the EUROFLUX and Carbo-Euroflux projects.

20

21 Conflict of interest

22 The authors declare that they have no conflict of interest.

23

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