Wood and Tracheid Properties of Norway Spruce (*Picea abies* [L] Karst.) Clones Grown on Former Agricultural Land in Latvia

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Abstract

Norway spruce (*Picea abies* (L.) Karst) is an economically important tree species targeted for tree breeding programmes and also used in afforestation of former agricultural lands. To achieve the highest revenue while selecting clones for plantations, it is important to analyze not only growth traits but also wood and tracheid properties. Nine 31-year-old Norway spruce clones, represented by 3 ramets (clonal copies), were grown in a single stand field trial on former agricultural land. The tree height of all clones strongly correlated with diameter at breast height (DBH) (r = 0.77) and ring width (RW) (r = 0.80). Density negatively correlated with tree height (r = -0.65), DBH (r = -0.68), and RW (r = -0.73). Average tracheid diameters (earlywood and latewood) moderately correlated with DBH, tree height, and RW. No correlation between DBH and tracheid wall thickness was determined. Significant differences in lignin content between tracheids near pith and cambium zone among the clones were observed. In contrast, a variation gradient in lignin content from the pith toward the cambium within individual trees was not observed.

Key words: Norway spruce clones, Wood properties, Tracheid dimensions, Earlywood; Latewood, Lignin localization

Introduction

Norway spruce (*Picea abies* (L.) Karst) is one of the most important commercial coniferous species of the boreal zone, planted mostly on fertile forest soils and former agricultural lands, ensuring high growth rates. Intensive tree breeding programmes for this species have been established in Scandinavian and Baltic States.

The physical and chemical properties of wood are important for wood processing. Wood properties are influenced by environmental conditions (Raiskila et al. 2006), forest structure, silvicultural management (Ikonen et al. 2008), and genetic variation (Ivkovich et al. 2002, Kilpeläinen et al. 2010). A significant genetic and environmental variation influences wood stiffness, strength, and related wood traits such as latewood (LW) proportion, wood density, spiral grain, microfibril angle, and lignin content (Steffenrem et al. 2007). Silvicultural treatments, including fertilization, affect wood density by modifying the environmental conditions controlling tree growth. Fertilization increases radial growth more than threefold, especially earlywood (EW) width, and decreases wood density by over 20% (Mäkinen et al. 2002). Fertilization and irrigation treatments have been reported to decrease cell wall thickness and increase ring width, radial cell width, and lignin concentration (Lundgren 2004, Kaakinen et al. 2009).

Differences in tracheid length, diameter, and cell wall thickness reflect the processes occurring in the cambium. After completion of cell enlargement, the secondary wall begins developing. The differentiation of tracheids includes their maturation process from birth at the cambial zone to death after the secondary wall formation, by which both water-conducting functions and mechanical properties are provided by the tracheid (Fujita and Harada 2001, Schuetz et al. 2013).

Because of the differences in diameter, wall thickness, and coarseness, EW and LW cells differ consid-

erably in their papermaking properties. Separation into EW and LW classes is proposed to yield the lowest variances in raw material (Havimo et al. 2008, Havimo 2010). In papermaking, thick-walled LW fibers from dense wood are stiff and produce paper with high tear resistance. Alternatively, thin-walled EW fibers from low-density wood collapse, which leads to the formation of a dense, nonporous, opaque sheet with high tensile and burst strengths (Kellomäki 2009).

The chemical composition of cell walls also influences the properties of wood products. EW and LW cells of softwoods have different morphologies; the thick-walled LW cells contain more holocellulose and less lignin than EW cells (Bertaud and Holmbom 2004). In chemical pulping, a higher lignin content yields lower amounts of pulp (Kellomäki 2009).

Wood density is an important variable for the prediction of solid wood quality and the pulp yield from a volume unit of a digester. It correlates with the mechanical strength of sawn timber and other solid wood products. The density of spruce wood increases with decreasing width of growth rings (Kellomäki 2009). The increasing wood density from the pith outward is related to increasing LW density and LW percentage, whereas the EW density increases only slightly (Jyske et al. 2008). A close relationship has been found between the wood density and fiber properties, especially with the proportion of the cell wall in a cross section of each annual ring, as well as with fiber and lumen width (Mäkinen et al. 2002). However, it is difficult to achieve simultaneous desirable changes in growth, fiber, and wood traits (better growth, thinner but longer fibers, and higher density) due to negative genetic correlations, especially between growth traits and wood density (Hannrup et al. 2004, Fries 2012).

The trend in silviculture in recent decades has been toward enhanced growth and shorter rotations (Barnett and Jeronimidis 2009). The raw material, therefore, comes with an increasing proportion of juvenile wood. Data on the properties of juvenile spruce wood grown on former agricultural lands are scarce. The aim of this study was to assess the genetic differences in wood and tracheid properties of 31-year-old Norway spruce clones grown in a field trial on former agricultural land in the hemi-boreal zone. For this purpose, physical properties, tracheid parameters in EW and LW, lignin distribution in cell walls, and tracheid dimensions from the pith toward the cambium were characterized. The results of the study can contribute to an understanding of the future prospects for using spruce clones with desirable wood properties on a wider scale with similar soil and climatic conditions.

Materials and Methods

Sample trees

Sample trees were selected from a single stand clonal trial, located in the eastern part of Latvia, consisting of 80 Norway spruce clones collected across Latvia. The experiment was started in 1975 with the establishment of a mother-plant collection and preparation of grafts. Rooting of grafts was finalized and the trial planted in 1983 with a spacing of 2 × 2 m (typical for spruce plantations on former agricultural lands and fertile drained forest lands). No thinning was carried out prior to the collection of material in 2010 (at the age of 31 years). Seven fast-growing Norway spruce (*Picea abies* (L.) Karst.) clones (26, A10, A15, B6, B10, B15, V7) and two slow-growing clones (KR13 and V9) were destructively sampled. Three trees per clone (27 trees altogether) were randomly selected to represent the diameter distribution in the trial. Only trees without visible defects like spike knots, double stems, notably asymmetric crown, or cracks were chosen.

Wood anatomy

Two-centimeter-thick discs were cut-off at a 1.3-m height of sample trees and treated with No. 150 sandpaper. The discs were dried at room temperature and scanned (Canon 4400) using calibrated software (Leica Image-Pro plus 6.0, Media Cybernetics, Inc.). Diameter at breast height (DBH), ring width (RW), and latewood proportion (LWP) were determined.

Wood density

Dry wood density was determined according to the standards DIN 50014-20/65-1: 1985 and DIN 68364: 1979. Wood specimens of size 20 × 20 × 10 mm^3 were cut and conditioned at 20 °C ± 2 °C and 65 ± 5% RH to constant weight. The size of samples was determined with a precision of 0.01 mm using an electronic caliper. Density was expressed as kg/m^3. A total of 14 samples were measured for each tree (ramet or clonal copy).

Tracheid dimensions

A two-centimeter-wide radial strip was cut-off from each sample disc in the direction from the pith to the bark. The strips contained an average of 23 annual rings. Each was divided into three equal blocks. The wood blocks were saturated with distilled water prior to sectioning. Thin cross sections (15 to 20 µm) were obtained from each block, and images were captured with a video camera (Leica DFC490) attached to a light microscope (Leica DMLB). Cross-sectional dimensions of individual tracheids were measured by calibrated image analysis software (Image-Pro plus 6.3, Media

Cybernetics, Inc.). Under measuring conditions at 400× magnification, the spatial calibration system corresponded to 0.1 µm/ pixel.

Lumen area, lumen diameter, mean tracheid diameter (radial and tangential/2), and cell wall thickness (radial and tangential/2) were measured in 150 randomly selected LW and 150 EW tracheids of each radial strip (Figure 1). Three strips of each clone were analyzed, comprising 450 tracheid measurements in both EW and LW. In total, the cross- sectional dimensions of 900 tracheids (EW and LW) for each clone were measured. Only characteristic EW and LW cells near the pronounced border between both growth rings were chosen.

water, and the length of fibers was determined using a Fiber tester (Lorentzen & Wettre).

UV microspectrophotometry (UMSP)

For UV-spectroscopic analyses, five replicates of sample blocks (1 × 1 × 5 mm^3) from each clone containing annual rings of year 3 and year 21 were prepared. The specimens were dehydrated and embedded in Spurr's epoxy resin (Spurr 1969) under vacuum conditions. Semi-thin transverse sections (1 µm) were prepared with a diamond knife, transferred to quartz microscope slides, immersed in a drop of non-UV-absorbing glycerin, and covered with a quartz cover slip.

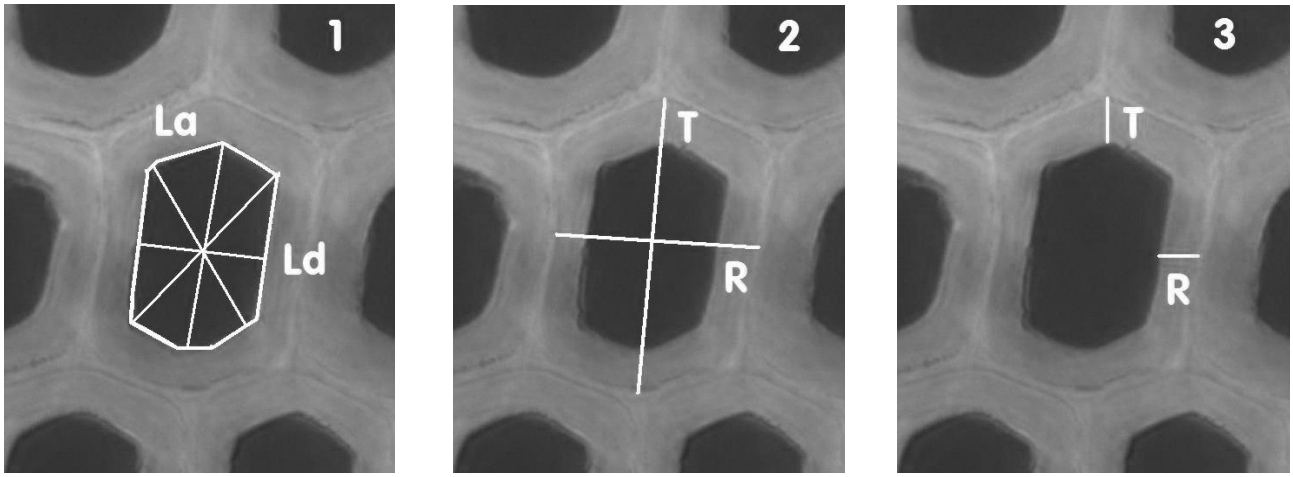


Figure 1. Measurement example of lumen area (La) and lumen diameter (Ld) (1), tracheid radial (R) and tangential (T) diameters (2), and cell wall thickness (radial R and tangential T) (3)

To obtain the mean cell parameters for each disc, the average arithmetical values of three blocks were re-calculated as average-weighted values by taking into account the specific weight of each block in a disc and its influence to the whole tree properties. The calculations have been described in a previous study (Irbe et al. 2013).

Kraft pulping

Delignified fibers were obtained by the Kraft pulping procedure in a 2-L laboratory digester at 170 °C; white liquor contained 57.4 g/L active alkali as NaOH, the sulfidity was 29.8%, and the liquor to wood ratio was 4.5:1. After the cooking procedure, delignified fibers were washed with water and treated in a standard PTA disintegrator for 30,000 revolutions, then filtered and collected on a Büchner funnel. Pulp fiber samples were dried overnight at 50 °C, and moisture content was measured to determine fiber weight for the measurement of dimensions. Accurately weighed samples were then re-suspended in 20 mL of de-ionized distilled

The analysis was carried out using a microspectrophotometer (Zeiss-UMSP 80) equipped with a scanning stage (Koch and Kleist 2001), which enabled the determination of UV image profiles at constant wavelengths, e.g., for conifer wood lignin, at the absorbance maximum of 280 nm. The semi-thin sections were studied by means of point measurements with a spot size of 1 $µm^2$ between 240 nm and 400 nm wavelengths using the program LAMWIN (Zeiss) to characterize the UV absorbance of lignin.

In addition, specimens were also scanned with a defined wavelength of 280 nm using the scan program APAMOS (Zeiss). This program digitizes rectangular fields of the tissue with a local geometrical resolution of 0.25 × 0.25 µm and yields a photometric resolution of 40,966 grey scale values, which are converted into 14 basic colours to visualize the absorbance intensities of individual cell types and cell wall layers (Koch and Grünwald 2004). The scans were depicted as two- and three-dimensional image profiles with statistical evaluation of the UV absorbance values.

Statistical analysis

Statistical software (SPSS 17.0) was used to estimate descriptive statistics. Analysis of variance (ANOVA) was used to detect the influence of various factors, such as the individual spruce clone and the distance from the pith, on wood and tracheid properties, as well as the lignin distribution in the cell wall. A level of significance of $P \leq 0.05$ was applied in all cases.

Results

Physical properties

The physical parameters of the spruce clones are shown in Table 1. The average values of all clones were as follows: DBH was 15.8 cm, tree height was 15.9 m, density was 420 kg/ m^3, and RW and LWP were 3.5 mm and 42%, respectively.

Table 1. Growth and physical properties of Norway spruce clones. DBH, diameter at breast height; RW, ring width; LWP, latewood proportion

Clone	DBH (cm)	Tree height (m)	Density (kg/m^3)	RW (mm)	LWP (%)
26	18.3 ± 1.6	16.7 ± 1.9	395 ± 33	4.0 ± 0.3	46.7 ± 3.5
A10	16.9 ± 1.5	15.0 ± 0.7	433 ± 11	3.6 ± 0.2	43.5 ± 1.0
A15	13.8 ± 2.4	15.7 ± 1.8	408 ± 38	3.2 ± 0.3	46.8 ± 1.6
B6	17.1 ± 1.0	15.8 ± 0.4	399 ± 25	3.7 ± 0.1	39.8 ± 1.0
B10	17.9 ± 1.2	18.1 ± 0.4	406 ± 13	4.0 ± 0.1	39.7 ± 0.6
B15	16.9 ± 2.6	17.4 ± 0.8	409 ± 21	3.7 ± 0.7	40.8 ± 0.6
KR13	12.0 ± 2.3	13.9 ± 1.1	502 ± 17	2.7 ± 0.5	42.3 ± 0.5
V7	17.5 ± 3.4	16.9 ± 1.7	388 ± 30	4.0 ± 0.7	39.5 ± 0.7
V9	11.9 ± 1.8	13.8 ± 1.0	443 ± 30	2.7 ± 0.3	38.8 ± 1.5
Average of all clones	15.8 ± 20	15.9 ± 1.1	420 ± 24	3.5 ± 0.4	42.0 ± 1.2

The tree height of all clones strongly correlated with the DBH ($r = 0.77$; $P < 0.05$) and RW ($r = 0.80$; $P < 0.05$), in agreement with an earlier study (Gerendiain et al. 2008) of juvenile Norway spruce clones.

The average density of clones was lower (420 kg/m^3) than that reported in the literature, i.e., 440 kg/m^3 (Green et al. 1999). This could be explained by the juvenile age of trees. However, both slow-growing clones, KR13 and V9, demonstrated a higher density than the fast-growing clones. These clones also possessed the smallest DBH, tree height, and RW.

Density was negatively correlated with tree height ($r = -0.65$; $P < 0.05$), DBH ($r = -0.68$; $P < 0.05$), and RW ($r = -0.73$; $P < 0.05$). In earlier studies (Ivkovich et al. 2002, Gerendiain et al. 2007), strong, negative, and significant genetic correlations have been determined between the stem volume and wood density traits. Hannrup et al. (2004) concluded that the clearest adverse effect following the selection of volume would be a reduction in wood density.

RW varied from 2.7 to 4.0 mm, depending on the clone. This parameter strongly correlated ($r = 0.98$; $P < 0.05$) with DBH. In contrast, no correlation was found between RW and LWP, which could be explained by a larger proportion of EW in growth rings. Steffenrem et al. (2007) found an intermediate correlation ($r = -0.42$) between the RW and LWP of Norway spruce families.

The LWP ranged from 38.8 to 46.8%. The LWP did not correlate with wood density. This could be attributed to the juvenile age of trees because positive linear correlations between density and LWP in mature hardwood have been observed (Adamopoulos et al. 2010). However, Koga and Zhang (2002) found significant correlations between wood density and LWP in both juvenile and mature balsam fir wood.

Clones 26, B10, and V7 demonstrated higher yield traits (DBH, tree height, and RW) than other clones. These clones could be considered more appropriate for biomass production. In turn, slow-growing KR13 and V9 with a higher density would be suitable for production of high-grade structural timber because density is closely related to wood strength properties. Fast-growing clones also fulfilled the mean density criteria for timber strength classes C14-C24 (EN 338: 2009); therefore, additional wood density, compromising the productivity, could be desirable only if there is a market demand or it is linked to other properties such as being important for paper production.

Tracheid micromorphology

EW and LW tracheids

The micromorphological characteristics of spruce clones are shown in Table 2. Significant differences

Clone	Lumen area (μm^2)		Lumen diameter (μm)		Mean tracheid diameter (μm)		Cell wall thickness (μm)	
	EW	LW	EW	LW	EW	LW**	EW	LW
26	947 ± 72	183 ± 62	34.2 ± 1.3	14.3 ± 2.6	37.0 ± 1.8	26.3 ± 3.2	2.3 ± 0.2	5.9 ± 0.7
A10	821 ± 56	131 ± 32	31.8 ± 1.1	12.2 ± 1.7	35.5 ± 0.9	24.9 ± 0.7	2.5 ± 0.2	6.4 ± 0.7
A15	898 ± 45	150 ± 8	33.2 ± 0.8	13.0 ± 0.4	36.0 ± 1.5	25.4 ± 0.5	2.3 ± 0.3	6.1 ± 0.3
B6	1028 ± 27	164 ± 12	35.6 ± 0.5	13.6 ± 0.7	37.7 ± 0.6	24.5 ± 1.3	2.2 ± 0.0	5.3 ± 0.6
B10	1010 ± 84	161 ± 6	35.3 ± 1.5	13.3 ± 0.3	37.6 ± 1.4	24.9 ± 0.1	2.0 ± 0.0	5.4 ± 0.1
B15	1018 ± 88	172 ± 34	35.3 ± 1.5	13.7 ± 1.6	37.5 ± 1.6	25.7 ± 0.7	2.1 ± 0.1	5.6 ± 0.9
KR13	838 ± 13	112 ± 10	32.2 ± 0.2	11.1 ± 0.6	34.5 ± 0.4	23.6 ± 0.5	2.2 ± 0.2	6.0 ± 0.4
V7	978 ± 56	172 ± 8	34.8 ± 1.0	13.9 ± 0.4	37.4 ± 1.3	25.6 ± 1.4	2.3 ± 0.1	5.8 ± 0.6
V9	830 ± 85	159 ± 19	32.0 ± 1.6	13.3 ± 0.9	34.1 ± 1.4	24.3 ± 0.4	2.0 ± 0.0	5.2 ± 0.2

Table 2. Tracheid dimensions of Norway spruce clones. Number of trees per clone n = 3; $P < 0.05$, except mean tracheid diameters in LW** with $P > 0.05$. Each clone was represented by 450 earlywood (EW) and 450 latewood (LW) cell measurements

in EW and LW tracheid micromorphology were observed among all clones, except the mean tracheid diameter in LW. The lumen diameter in EW and LW tracheids ranged from 31.8 to 35.6 µm and from 11.1 to 14.3 µm, respectively. The EW tracheid diameters varied between 34.1 and 37.7 µm. LW tracheids had diameters between 23.6 and 26.3 µm. The cell wall thicknesses of EW and LW tracheids were 2.0 to 2.5 µm and 5.2 to 6.4 µm, respectively.

EW tracheid diameter was strongly correlated with the lumen area ($r = 0.94$; $P < 0.05$) and lumen diameter ($r = 0.94$; $P < 0.05$), but it was not correlated with the cell wall thickness. In LW, a moderate correlation was found between tracheid diameter and lumen parameters such as area ($r = 0.60$; $P < 0.05$) and diameter ($r = 0.55$; $P < 0.05$). LW tracheid diameter was moderately correlated ($r = 0.53$; $P < 0.05$) with the cell wall thickness.

Clones B6, B10, and B15 possessed EW tracheids with the largest lumen parameters and tracheid diameters, while the cell wall thickness displayed the lowest values. In turn, clones 26, B15, and V7 had LW tracheids with the largest lumen dimensions and tracheid diameter, while the cell walls were of average thickness. The thickest EW and LW cell walls were observed in clone A10. The clones with the largest EW tracheid dimensions were different from those with the largest LW tracheid dimensions. Only clone B15 displayed large lumen and tracheid diameters in both EW and LW (Table 2).

Average tracheid parameters for EW and LW

The average tracheid parameters for the EW and LW of clones are summarized in Table 3. Statistical analysis confirmed significant differences in average tracheid dimensions among all clones.

Average tracheid diameter was strongly and significantly correlated with lumen area ($r = 0.87$; $P < 0.05$) and lumen diameter ($r = 0.84$; $P < 0.05$), while no correlation with the wall thickness was found ($r = 0.11$; $P > 0.05$).

Table 3. Average tracheid dimensions (earlywood and latewood) of spruce clones. Number of trees per clone n = 3; $P < 0.05$. The data are based on measurements of the cross-sectional dimensions of 900 tracheids per clone

Clone	Lumen area (µm²)	Lumen diameter (µm)	Mean tracheid diameter (µm)	Cell wall thickness (µm)	Fiber length (mm)
26	594 ± 82	24.9 ± 2.2	32.0 ± 2.6	4.0 ± 0.3	2.5 ± 0.1
A10	522 ± 25	23.3 ± 0.3	30.9 ± 0.5	4.2 ± 0.3	2.3 ± 0.1
A15	548 ± 31	23.8 ± 0.6	31.1 ± 1.1	4.1 ± 0.2	2.4 ± 0.3
B6	685 ± 21	26.8 ± 0.4	32.5 ± 0.8	3.4 ± 0.3	2.4 ± 0.0
B10	674 ± 54	26.6 ± 1.0	32.5 ± 0.9	3.4 ± 0.1	2.6 ± 0.1
B15	662 ± 40	26.2 ± 0.5	32.5 ± 0.9	3.6 ± 0.4	2.2 ± 0.1
KR13	531 ± 4	23.3 ± 0.2	29.9 ± 0.3	3.8 ± 0.2	2.5 ± 0.1
V7	659 ± 37	26.5 ± 0.7	32.7 ± 0.9	3.6 ± 0.3	2.5 ± 0.2
V9	568 ± 46	24.7 ± 1.0	30.0 ± 0.8	3.3 ± 0.0	2.3 ± 0.2
Average of all clones	606 ± 75	25.1 ± 1.7	31.7 ± 1.5	3.7 ± 0.4	2.4 ± 0.2

The largest average lumen parameters and tracheid diameters were determined for clones B6, B10, B15, and V7. However, the cell wall thicknesses of those clones showed the lowest values. This is in accordance with an earlier study (Ivkovich et al. 2002), in which growth rings with larger tracheids did not possess significantly thicker walls.

The thickest average cell walls (4.2 µm) were produced by clone A10, and this parameter positively affected the wood density (Tables 1 and 3). However, the average wall thickness of all clones did not correlate with density ($r = 0.13$; $P > 0.05$). Our study showed that the wood density was affected by RW rather than wall thickness. Mäkinen et al. (2002) reported that in both earlywood and latewood, no clear relationship was found between cell wall thickness and wood density. However, rather large variations were found (Mäkinen et al. 2002) between individual trees concerning the relationship between wood density and fiber properties.

Significant differences among the clones in fiber length were determined (Table 3). The fibers were shorter (2.2 to 2.6 mm) than reported (Kellomäki 2009) for mature spruce wood, with an average of 3.4 mm. In turn, Mäkinen et al. (2008) found no significant differences in tracheid length between EW and LW, while the length increased rapidly from the pith outward.

Clone B10 demonstrated a larger average lumen area (674 µm²), lumen diameter (26.6 µm), and tracheid diameter (32.5 µm), thinner cell walls (3.4 µm), and longer fibers (2.6 mm) than other clones (Table 3). These micromorphological properties could favor the production of strong paper with excellent fiber-to-fiber bonding properties. Clone B15 displayed high average lumen and tracheid diameter values, although it had the shortest fibers (2.2 mm). This clone could be considered appropriate for paper production with good paper surface and strength properties.

Correlations between tracheid dimensions and wood growth, and physical parameters

The following correlations were determined between tracheids and growth parameters of clones: average lumen diameter was negatively correlated with LWP ($r = -0.62$; $P < 0.05$); average tracheid diameter was moderately correlated with DBH ($r = 0.56$; $P < 0.05$), tree height ($r = 0.45$; $P < 0.05$) and RW ($r = 0.56$; $P < 0.05$). A significant correlation between fiber width, DBH, and RW was reported also for narrow crowned spruce clones (Gerendiain et al. 2009), although the correlation was not found in normal crowned spruce clones. Molteberg and Høibø (2006)

reported that DBH was negatively correlated with basic density and positively correlated with fiber width. Additionally, a negative correlation was found between DBH and fiber wall thickness. In contrast, our study showed no correlation between DBH and wall thickness.

Clone V7 with pronounced tree height, DBH, RW, tracheid lumens, tracheid diameters, long fibers, and low lignin content (see section Topochemical detection of lignin) could contribute to the production of high-quality paper products.

Clones B6, B10, and V9, with the lowest LWP (38.8 to 39.8%) and thinnest average cell walls (3.3 to 3.4 ģm), could be considered for the manufacture of high-quality paper products. Clone B10 also possessed the longest fibers (2.6 mm), which would positively influence the paper properties.

Clone A10, with the thickest average cell walls and high LWP, density, and lignin content, displays good mechanical properties for timber production.

The wood material in the present study was obtained from a clonal trial without prior stand thinning. Consequently, the results do not provide information on how the differences in wood properties of clones could change in trials with a different stand density or thinning regime. The effect of thinning in slow- and fast-grown Norway spruce has been studied (Dutilleul et al. 1998). It was found that the correlation between the average RW and the average wood density depends on the growth rate of the tree rather than thinning. Jaakkola et al. (2005) reported that thinning intensity enhances growth rate but has a rather small effect on tracheid dimensions. Moreover, growth suppression has been shown to produce more uniform wood density and tracheid wall thickness, which may be suitable for pulp and paper production (Zhu et al. 2008).

Tracheid dimensions from pith to cambium

A variation in spruce EW and LW tracheid dimensions toward the cambium is shown in Figure 2. EW tracheids demonstrated a significant increase in tracheid diameter, lumen area, and diameter up to growth ring 23. LW tracheid diameter also increased significantly until ring 23, while lumens did not show any pronounced increase ($P > 0.05$). EW wall thickness did not increase significantly with increasing tree age. In contrast, the increment of LW cell walls from the pith toward the cambium was significant. The enlargement of the EW tracheid diameter toward the cambium did not cause any significant increase in the size of the cell wall, leading to larger tracheid lumens. In turn, the enlargement of the LW tracheid diameter was accompanied by thicker cell walls and the formation of smaller lumens.

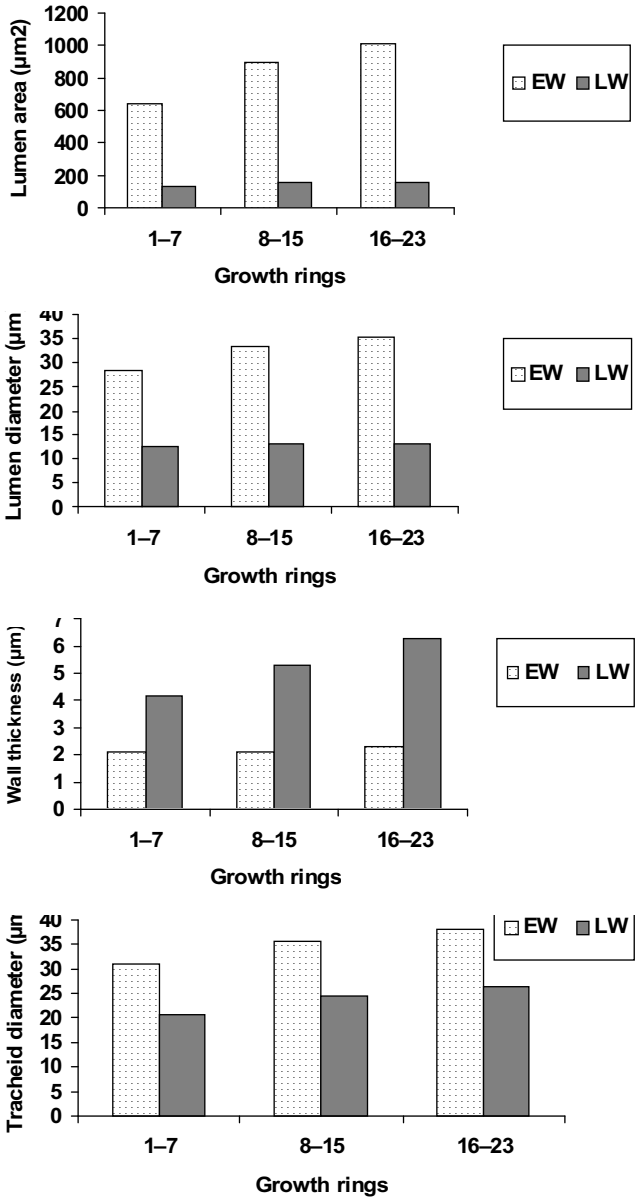


Figure 2. Lumen area (A), lumen diameter (B), cell wall thickness (C), and tracheid diameter (D) of spruce wood *vs.* growth rings from pith to cambium. EW is earlywood; LW is latewood. Each graph represents average results of 4050 EW and 4050 LW tracheids

Reme and Helle (2002) found that the cell perimeter increased toward the cambium in 80-year-old Norway spruce, while the cell wall thickness increased for the first 20 growth rings and then stabilized or decreased. In contrast, Molteberg and Høibø (2006) reported a decrease in average fiber width from rings 10 to 12 outward. This trend was attributed to different RW in juvenile Norway spruce wood.

The trees with 23 growth rings in this study represented the juvenile phase of stem development. The

period of juvenile wood formation is variable for different species. The characteristics of juvenile wood, as compared to mature wood, depend to some extent on growth conditions and genetic factors (Maeglin 1987). Juvenile wood of Scots pine and Norway spruce consists of 15 to 25 growth rings (Kellomäki 2009). It differs in structure from mature wood by having shorter tracheids, thinner cell walls, a lower proportion of latewood, a higher proportion of lignin, and a lower basic density (Kellomäki 2009).

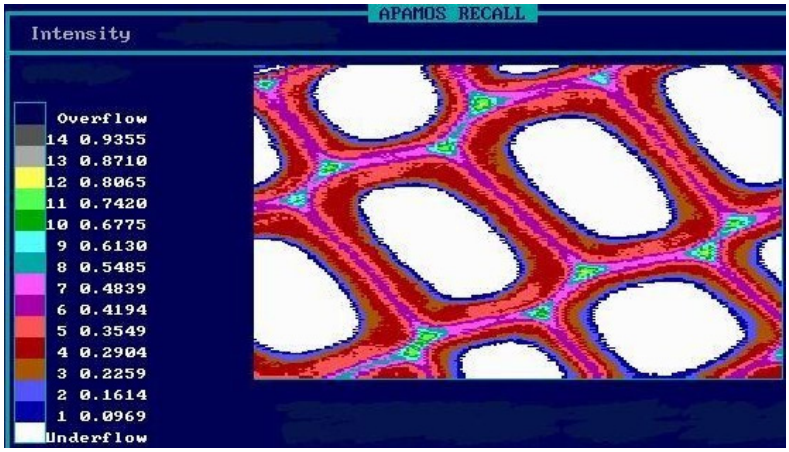
Topochemical detection of lignin

The lignin distribution within different wall layers of different spruce clones was analyzed by scanning UV microspectrophotometry at a constant wavelength of 280 nm.

Figures 3 and 4 represent typical two- and three-dimensional UV image profiles of the lignin distribution within the individual cell wall layers of LW tracheids from the spruce clone B6. The UV image profiles of the tracheids displayed a typical lignin distribution, with the highest absorbance values in the compound middle lamellae (CML) (abs_{280nm} 0.45) for the annual ring of year 3 (pith zone) and (abs_{280nm} 0.55) for annual ring 21 (cambium zone). The adjacent secondary wall (S2) layers showed a lower lignin distribution (abs_{280nm}0.31) for the annual ring of year 3 and (abs_{280nm}0.34) for annual ring 21.

Point measurements were conducted to characterize the UV absorbance behavior of the scanned tissue. Figures 5 and 6 show typical UV absorbance spectra of the secondary cell wall (S2) of LW of two different annual rings for seven different clonal spruces. Our results revealed significant differences ($P <$ 0.05) among the clones in the lignin content of tracheids near pith and cambium. The UV spectra in all graphs showed the typical absorbance behavior of softwood lignin, with a distinct maximum at 280 nm and a local minimum at about 250 nm. The maximum absorbance at 280 nm usually indicates the presence of strongly absorbing guaiacyl lignins (Fergus and Goring 1970, Musha and Goring 1975, Fujii et al. 1987).

The lowest value (abs_{280nm} 0.31) in the annual ring of year 3 was semi-quantitatively recorded in clone V7,



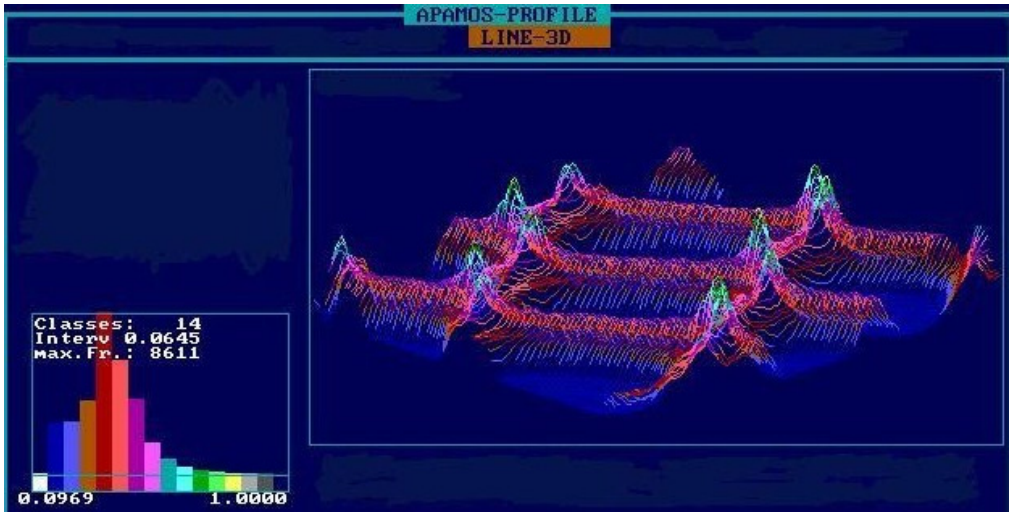


Figure 3. Representative UV microscopic scanning profile of individual cell wall layers of Norway spruce clone B6 from the annual ring of year 3 (size of the scanning area: 59.75 × 52.75 µm = 50,880 pixels or measuring points)

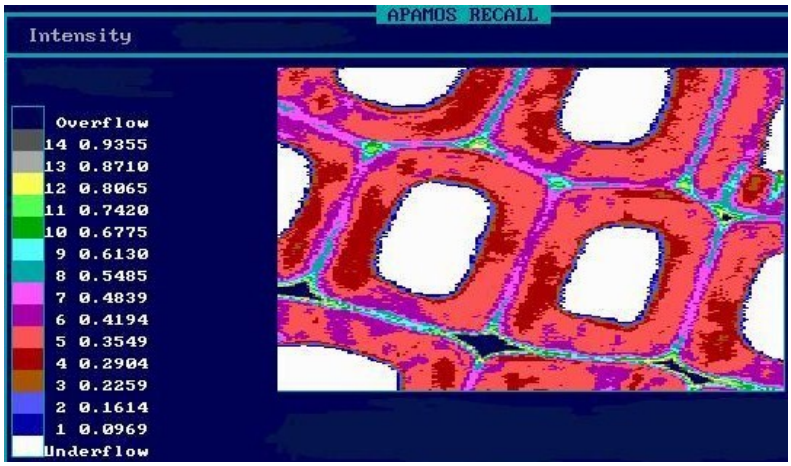
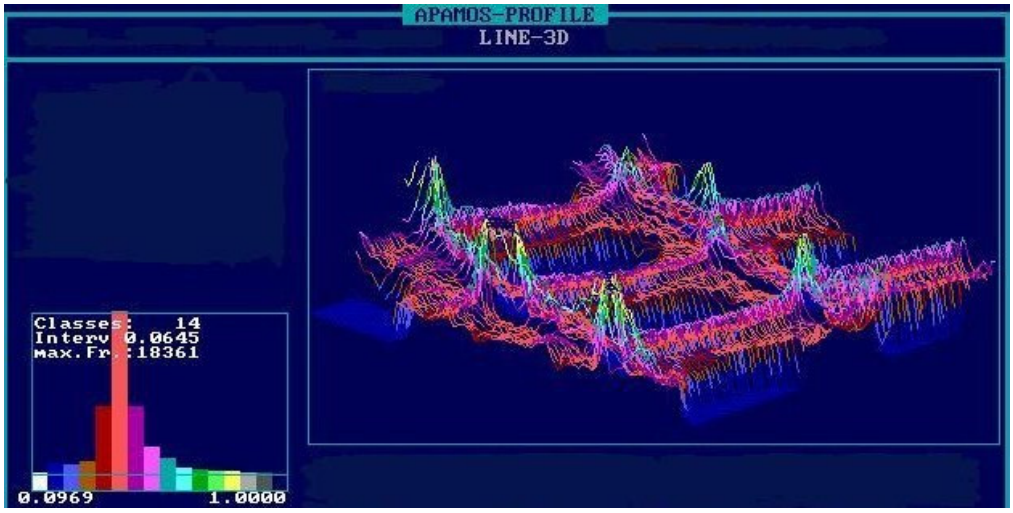


Figure 4. Representative UV microscopic scanning profile of individual cell wall layers of Norway spruce clone B6 from the annual ring of year 21 (size of the scanning area: 60.75 × 57.75 µm = 56,608 pixels or measuring points)



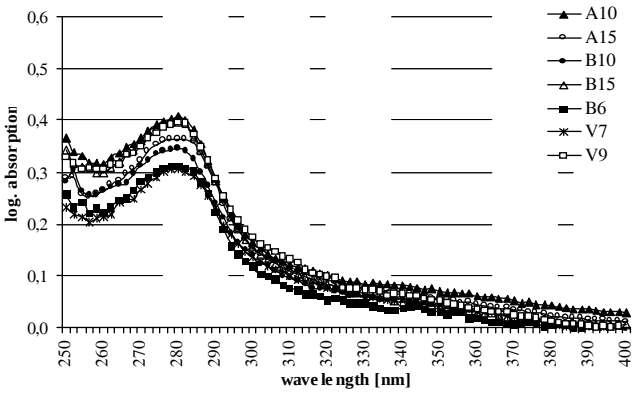


Figure 5. Representative lignin UV absorbance spectra of the S2 layer of clonal spruce tracheids from the annual ring of year 3

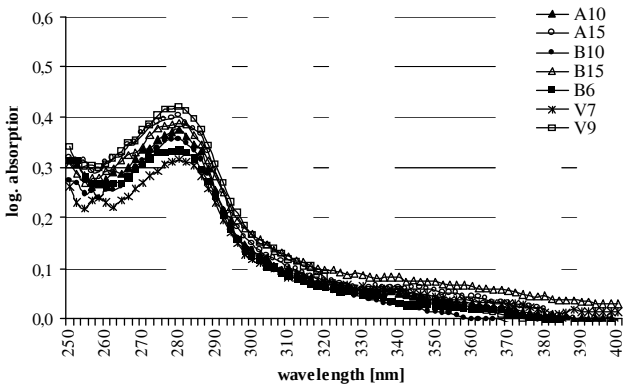


Figure 6. Representative lignin UV absorbance spectra of the S2 layer of clonal spruce tracheids from the annual ring of year 21

and the highest value (abs_{280nm} 0.41) was found in clone A10. Similarly, the analysis of annual ring 21 showed almost identical values: 0.32 (lowest value) in clone V7 and 0.42 (highest value) in clone V9. The values between the annual rings of the pith and cambium zones within trees did not differ significantly. Moreover, a strong correlation in lignin content between annual rings was found ($r = 0.85$; $P < 0.05$).

According to earlier studies carried out by Panshin and de Zeeuw (1980), the lignin content in xylem

decreases from pith to bark. In heartwood, the lignin content is generally higher than it is in sapwood (Bertraud and Holmbom 2004, Raiskila 2008), but no variations have been observed between annual rings. Our semi-quantitative determination of lignin contents in individual cell wall layers in spruce clones showed no variation gradient from pith to cambium. This is in agreement with a previous study by Irbe et al. (2012), in which the investigated 31-year-old spruce clones were of juvenile age and did not show a pronounced sapwood-heartwood boundary.

Conclusions

Wood and tracheid properties of young spruce clones grown on former agricultural land were significantly affected by their genetic differences. Differences among the clones were determined regarding wood properties (DBH, tree height, density, RW, and LWP), and tracheid properties of EW and LW (lumens, tracheid diameters and wall thickness).

Taller trees produced larger diameter stems with wider growth rings and larger tracheids, with reduced stem density.

Enlargement of the EW tracheid diameter toward the cambium did not show any significant increase in the thickness of the cell wall, while the increase of the LW tracheid diameter from the pith outward was accompanied by an enlargement of cell walls.

Semi-quantitative lignin content significantly differed in the pith and cambium zones among the clones. In contrast, no difference in lignin content from the pith toward the cambium within each tree stem was observed.

The increase of LW cell wall thickness from the pith toward the cambium did not cause any increase in lignin content in these cell walls.

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