Effects of altitude on tracheid differentiation and lignification of Norway spruce

W. Gindl, M. Grabner, and R. Wimmer

Abstract: The effect of altitude on lignification is important in developing an understanding of what drives natural variation in wood properties. Cambia of two Norway spruce trees, growing at altitudes of 580 and 1260 m a.s.l., were periodically sampled to measure tracheid dimensions and cellular lignin content. The low-elevation tree showed a higher rate of cell division with thicker cell walls and wider growth rings. The maturation phase of tracheids at high elevation was completed by the end of October while low elevation latewood tracheids were still lignifying. As revealed by ultraviolet microscopy, lignin content in single cells, as well as in complete tree-rings, was higher at high elevation. Rank correlation analysis indicated high negative correlation between lignin content of tracheid cell walls and corresponding wall thickness. It is hypothesized that trees growing at higher altitudes compensate for the thinner cell walls with an increased lignin content which helps to maintain mechanical integrity of the xylem.

Key words: altitude, lignin, tracheid, wood formation, ultraviolet microscopy.

Résumé : Pour mieux comprendre ce qui contrôle la variation naturelle des propriétés du bois, il est opportun d'examiner les effets de l'altitude sur la lignification. Afin de mesurer les dimensions des trachéïdes et leur teneur cellulaire en lignine, les auteurs ont périodiquement échantillonné les cambiums de deux épinettes de Norvège poussant à des altitudes de 580 et 1260 m, à la même latitude. L' arbre à basse élévation montre un taux plus élevé de divisions cellulaires, avec des parois cellulaires plus épaisses, et montre des anneaux de croissance plus larges. A haute altitude, la phase de maturation des trachéïdes est complétée vers la fin d'octobre, alors qu'à faible altitude les dernières trachéïdes sont encore en voie de lignification. La teneur en lignine des cellules individuelles ainsi que des anneaux de croissance complets, est plus importante en altitude. L'analyse des corrélations ordonnées indique une forte corrélation négative entre la teneur en lignine des parois cellulaires des trachéïdes et l'épaisseur des parois correspondantes. On formule l'hypothèse que les arbres poussant à haute altitude compensent pour des parois cellulaires plus minces, par une teneur accrue en lignine qui aide à maintenir l'intégrité mécanique du xylème formé.

Mots clés : altitude, lignine, trachéïdes, formation du bois, microscopie en ultraviolet.

[Traduit par la Rédaction]

Introduction

Wood formation is driven by a number of factors (Larson 1962; Zimmermann 1964) resulting in considerable variability of wood properties within a given species (Zobel and van Buijtenen 1989). The genetically determined tendency of an individual tree to form high-density wood (Rozenberg and Cahalan 1997) may be enhanced or weakened by mechanical stresses (Timell 1986); climatic variability (Fritts 1976); availability of light, nutrients, and water supply (Kozlowski and Pallardy 1997); or silvicultural practices including fertilization (Cown and McConchie 1981).

In softwoods, tracheid morphology is influenced by photoperiod, light intensity (Jenkins et al. 1977; Wilkes 1987), and temperature (e.g., Denne 1976). At higher elevations or latitudes, temperature and seasonal length become increasingly limiting for tree growth (Tranquillini 1979), and a general decrease in ring width, cell wall thickness, latewood percentage, and wood density is evident (Panshin and De Zeeuw 1964; Lassen and Okkonen 1969; Liese and Dujesiefken 1986). Hakkila (1969) showed that geographical patterns of wood structural properties of Scots pine (*Pinus sylvestris* L.) and Norway spruce (*Picea abies* (L.) Karst.) were predictable with latitude but not with longitude.

While wood structural properties seem to have clear trends with elevation and latitude, patterns of chemical wood properties are unclear. Trendelenburg and Mayer-Wegelin (1955) and Kim et al. (1989) report a decrease in lignin content with greater latitudes. However, these findings seem to contradict Nylinder and Häggłund (1954) who report that cellulose yields of Norway spruce, as an inverse measure of lignin content, were lower as latitude and elevation became greater. A significant decrease in kraft pulp yield for *Eucalyptus globulus* Labill. and *Eucalyptus nitens* (Dean & Maid.) Maid. with increasing elevation was also reported by Beadle et al. (1996).

This study presents a comparison of tracheid formation and lignification throughout the 1998 growth season mea-

Received January 3, 2001. Published on the NRC Research Press Web site at http://cjb.nrc.ca on June 26, 2001.

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 DOI: 10.1139/cjb-79-7-815

sured in Norway spruce trees grown at altitudes of 580 and 1260 m a.s.l., respectively. Cell wall thickness and cellular lignin content of newly formed tracheids are determined and analysed and altitudinal trends are compared with gross lignin contents.

Materials and methods

Study sites and sample trees

Two Norway spruce sites located at the north-facing slope of Hochanger Mountain near the town, Bruck an der Mur (47°25′N, 15°16′E; Fig. 1) were selected: the valley site was at 580 m a.s.l., and the mountain site was at 1260 m a.s.l. The soil at the valley site was deep with moderate clay content, while the mountain site had a higher proportion of coarse-grained gneiss minerals. Water-holding capacity was high on both sites. The valley site is a spruce–larch (*Picea–Larix*) forest with mountain maple (*Acer pseudoplatanus* L.) and European ash (*Fraxinus excelsior* L.) in the understory. The upper site is basically a spruce forest with larch (*Larix decidua* Mill.) added and rowantree (*Sorbus aucuparia* L.) in the understory. Stocking density was 0.8 for both sites. The area belongs to the central Austrian Alps. Bruck an der Mur, 482 m a.s.l. receives an average annual rainfall of 774 mm. The annual mean air temperature measured at 482 m a.s.l. is 8.6°C. The climate diagram (Fig. 2) shows that the area has a moderate continental climate. Because of the similar site characteristics at both altitudes, including soil water-holding capacity, aspect, and irradiation, differences in growth between the two sites are mainly due to temperature and length of growing season. Mean temperature at the mountain site during the 1998 growing season was 5.8°C lower than that measured at the valley site (Fig. 2).

Measurement of ring width

Increment cores were taken from 18 trees from each site, with two cores per tree. After drying and polishing with sanding paper, samples were cross-dated (Cook and Kairiukstis 1990) and earlywood and latewood width measured to the nearest 10 μm on a LINTAB measuring stage connected to a computer using TSAP software (Rinn 1996). Ring width was calculated by adding earlywood and latewood width; mean radial growth curves were calculated for both sites.

Ultraviolet microscopy

Two representative trees, i.e., straight trees with no visible defects exhibiting average growth rate according to increment cores, were chosen for lignin analysis. The two trees (V, valley; M, mountain) were repeatedly visited and sampled during the 1998 growing season on the following dates: 4 April (only V), 30 April, 22 May, 28 June, 12 July, 1 August, 8 September, 20 October, and 28 December. Sample blocks cut from breast height included the outer xylem, the cambium, and part of the phloem. Sample sizes were 25 mm in the axial direction, 10 mm tangentially, and 10 mm in the radial direction, containing up to eight growth rings. Samples were immersed in FAA (formaldehyde – acetic acid – alcohol) (Gerlach 1984) immediately after removal and stored for 2–4 weeks. To minimize effects of wounding, the consecutive samples were taken from the stem in an upward spiral fashion, keeping a minimum distance of 10 cm. After rinsing the sample blocks with distilled water, small pieces were cut, dehydrated in a graded series of ethanol (40, 60, 80, and 100%) and acetone (100%), and embedded in epoxy resin (Spurr 1969). These samples contained the completed 1997 growth ring, all the cells formed during the 1998 growing season, the cambium cells, as well as some phloem cells. Transverse sections (1 μm) were cut on a Leica ultramicrotome equipped with a diamond knife. Sections were placed on quartz glass slides and covered with quartz cover slips. With a ZEISS MPM-800 photometer–microscope, ultraviolet (UV) absorbance was determined in the secondary cell walls (S2) of at least four parallel radial cell files spanning the entire increment in the xylem formed during 1998. Absorbance of the completed tracheids in the 1997 tree ring was also determined in an identical way. Lignin content was calculated from absorbance measurements according to Fergus et al. (1969).

After measurements of UV absorbance, the sections were immersed in a solution of 1% aqueous gentian violet for 1 min. This resulted in an intense violet staining of mature and differentiating cell walls. Excess staining solution was removed by rinsing twice with distilled water. Thereafter, the sections were placed onto glass slides using a drop of distilled water as embedding medium. Thickness of the tangential tracheid walls and lumen width were determined to the nearest 0.2 μm on successive cells in a radial direction using a microscope equipped with a video camera connected to a computer using the public domain NIH IMAGE program (devel-

Fig. 1. Location of the study area in Austria. V, valley site; M, mountain site

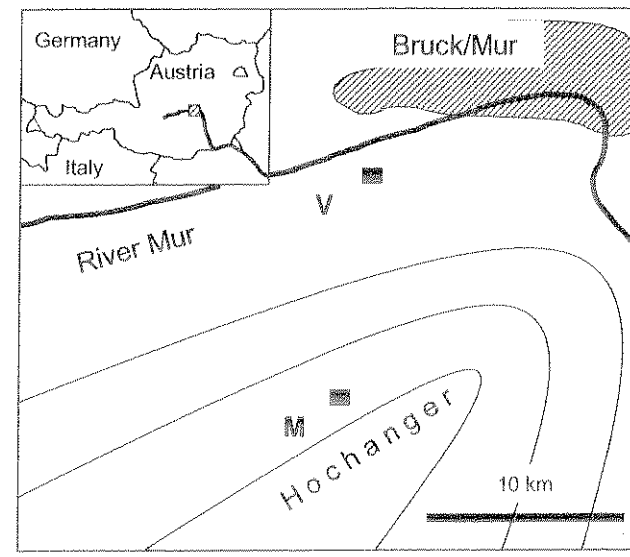
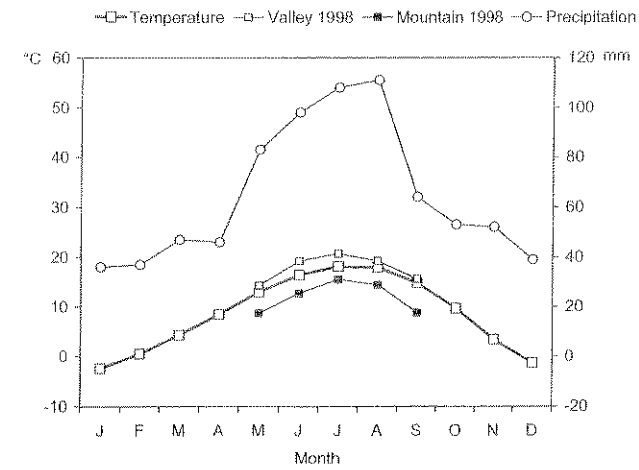


Fig. 2. Temperature and precipitation (1961–1990 mean) of the study area measured at 482 m a.s.l., and the 1998 temperature curves measured directly at the sample sites: 580 m (valley) and 1260 m (mountain) a.s.l., respectively.



oped at the U.S. National Institute of Health and available on the Internet at http://rsb.info.nih.gov/nih-image/). According to Denne (1988), tracheids with a lumen smaller than half of the tracheid diameter measured in radial direction were classified as latewood.

Determination of gross lignin content

In spring of 1999, twelve millimetre thick cores were taken from eight trees at the valley site and eight trees at the mountain site, including the trees V and T sampled during 1998 for UV microscopy. The 1998 growth ring of all 16 trees, and the previously formed rings 1995, 1996, and 1997 of trees V and T, were separated into individual ring samples and extracted with water and acetone. Extractive-free samples were milled in a Retsch rotary mill and dried at 103°C. According to Johnson et al. (1961), 12.0–14.0 mg of the fraction passing a 400-µm sieve were completely dissolved in acetylbromide reagent. To overcome problems of weak repeatability (Dence 1992; Rodrigues et al. 1999), all samples were digested simultaneously. Ultraviolet absorbance at 280 nm was determined using a Pharmacia LKB Biochrom 4060 UV–visible spectrophotometer in reference to a blank solution prepared in parallel with the samples. Lignin concentration was calculated by relating the absorbance of unknown samples to the absorbance of spruce samples with known Klason lignin contents determined according to TAPPI standard T222 om-88.

Results

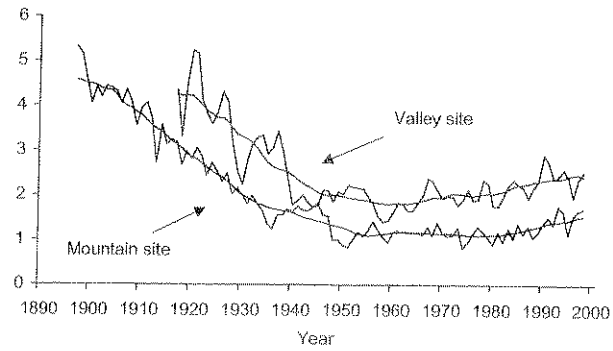
Average radial growth

Radial growth curves of trees sampled from the two sites are shown in Fig. 3. Both sites were fairly even aged. With a mean age of 98 ± 5 years the mountain trees were 16 years older than the valley trees (82 ± 5 years). The mean annual growth rate (cambium age at least 20 years) was 2.05 mm for the valley site and 1.27 mm for the mountain site. Mean latewood percentage of the valley site trees was 30 ± 4%, which was considerably higher than the 16 ± 3% measured at the mountain site.

Time course of cell wall thickening and lignification

On 9 April, the cambium at the valley site was still dormant, and the mountain site was not yet accessible because of persisting snow cover. On 30 April, the cambium of both trees V and M had resumed cell division, but newly produced xylem cells had not entered the phase of radial enlargement. On 22 May, the first cells entered the thickening phase in tree V, while in tree M tracheids were still enlarging without wall thickening. A month later (28 June) about 50% of the annual radial increment was completed in tree V. Between five and six earlywood tracheids had reached full lignification at this stage (Fig. 4*b*). Tree M only completed one third of the annual increment to this date (Fig. 4*a*) with no fully matured tracheids (Fig. 4*b*). In mid July, 80–90% of the earlywood tracheids were fully thickened in both sample trees. In tree V lignification started (i.e., lignin became first detectable) 11 cells behind wall thickening and in tree M lignification was lagged by 5 cells. By mid-July tree V had completed 47 xylem cells per radial file, while tree M had only completed 26 (Figs. 4*a*, 4*b*, and 5). Transition from earlywood to latewood commenced shortly before 12 July in tree V and a few days after this date in tree M. On 1 August the cambia were still active in both trees, and by 8 September, both cambia no longer showed evidence of new xylem

Fig. 3. Mean radial growth of the valley (580 m a.s.l) and the mountain (1260 m a.s.l.) site with each curve representing 18 spruce trees. A 15-year moving average is drawn through each curve.



being produced. During the first part of September, most latewood cells in tree M were fully thickened, while the same process had just started in tree V. On 20 October, wall thickening was completed in both trees (Fig. 4*a*), but lignin was still accumulating in the latewood cells of tree V (Fig. 4*b*). At the end of 1998, tree V had completed 58 tracheids per radial file, while tree M had 34 tracheids. In the most active period between 22 May and mid-July, the cambium of tree V produced 0.6 cells/day, while tree M showed a rate of 0.4 cells/day (rates calculated by dividing the total number of cells produced by the number of days). Mean cell wall thickness was 3.3 ± 0.2 µm in the earlywood and 6.4 ± 0.4 µm in the latewood of tree V. In tree M the mean cell wall thickness was 3.0 ± 0.2 µm in the earlywood and 5.1 ± 0.3 µm in the latewood.

Lignin content

Lignin concentration in the secondary walls across the annual growth ring was highest in earlywood with a linear decrease towards latewood (Fig. 4*b*). The mean lignin content in secondary walls of the growth rings 1997 and 1998 in tree V was 0.211 $g \cdot g^{-1}$, which was considerably lower than that of tree M with 0.234 $g \cdot g^{-1}$. This difference of lignin content was found also when determined after dissolution of wood in acetylbromide: the eight trees from the mountain site show higher gross lignin content than the eight trees from the valley site (Fig. 6).

In Fig. 7 the cell wall thickness of tracheids formed in 1997 and 1998 is plotted along with their corresponding lignin content. A rank correlation analysis revealed a negative relationship between cell wall thickness and lignin content of $r = -0.71$ ($p < 0.001$). For latewood (according to Mork's definition) the correlation coefficient was still –0.54, and for earlywood the correlation was –0.65.

Discussion

Wood formation studies in conifers focused primarily on the processes of cell division, cell enlargement, and cell wall thickening and less on lignification (e.g., Whitmore and Zahner 1966; Skene 1969; Wodzicki 1971; Horacek et al. 1999). Sequences of lignification have been considered only for short portions of the growing season (Kutscha and

Fig. 4. Sequence of (*a*) cell wall thickening and (*b*) lignification in the valley and mountain tree during the 1998 growing season. The distance from the end of the previous increment (growth ring boundary 1997–1998) provides the *x* axis. The dates given indicate the day of sampling. Arrowheads mark the transition from earlywood to latewood according to Denne (1988).

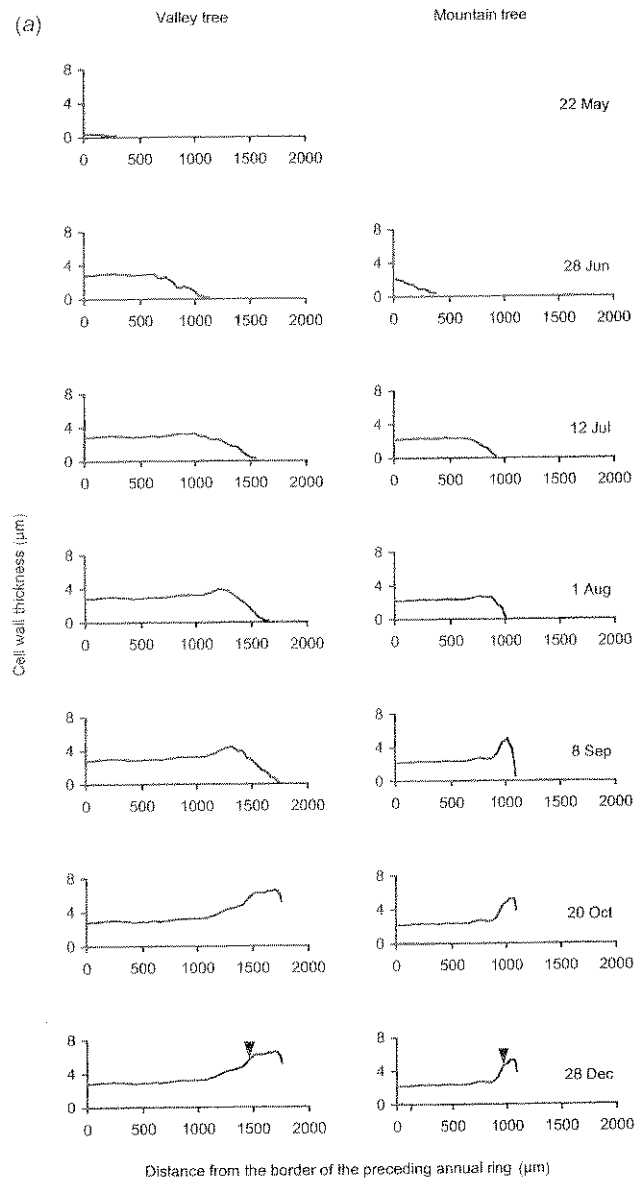
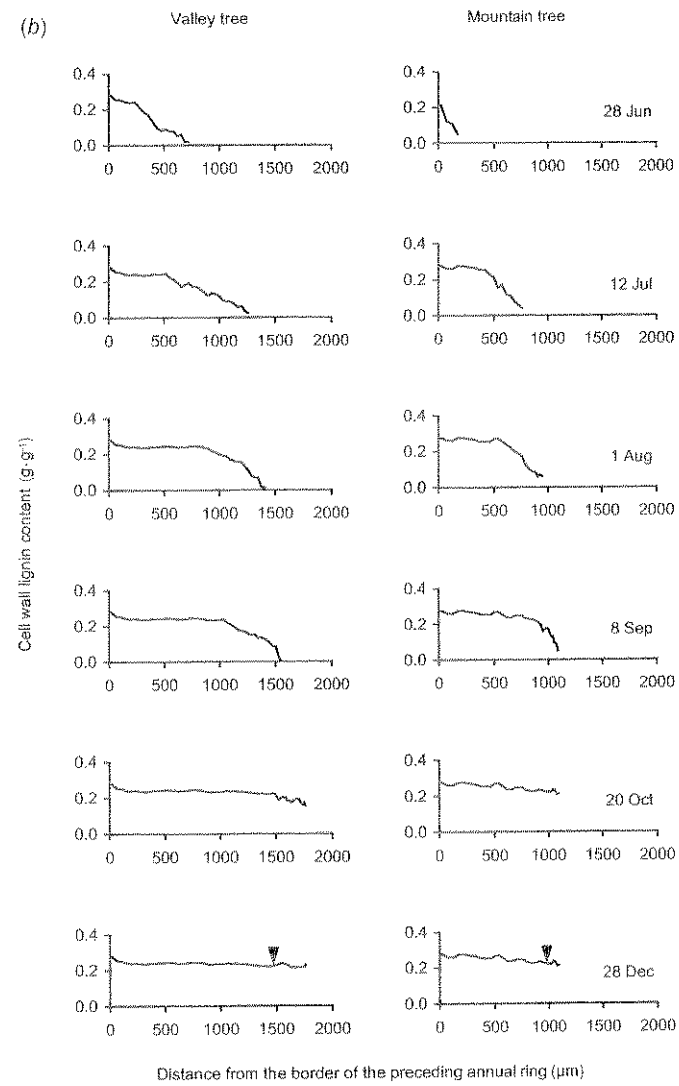


Fig. 4 (*concluded*).



Schwarzmann 1975; Imagawa et al. 1976). The results presented in Figs. 4*a*, 4*b*, and 5 describe the thickening as well as lignification of tracheid cell walls for a complete growing season.

Because of the longer interval between the first two samplings, a possible delayed resumption of cambial activity in tree M could not be detected. However, once cell production had initiated, tree V formed tracheids at a much higher rate (Fig. 4*a*). The higher cambium activity at the valley site is also reflected in its growth rates (Fig. 3). Latewood percentage, as a measure related to wood density (Bernhart 1964; Wimmer 1991, 1995) was higher in the valley trees. Since latewood percentage is highly susceptible to the influence of climate (Schweingruber 1989), this difference can be explained by the more favourable conditions in the valley, where the average annual temperature is 5.8°C higher.

At the site level, at the tree ring level, and at the tracheid cell wall level, lignin concentration was consistently higher at the mountain site compared with the valley site (Fig. 6). When lignin is related to cell wall thickness, highly significant negative correlations were calculated (Fig. 7). The higher lignin concentrations found in wood of trees grown at the cooler mountain site seem to disagree with the finding that low temperature during later summer and autumn leads to lower lignin content of terminal latewood tracheids (Gindl and Grabner 2000; Gindl et al. 2000). However, since this is only true for the very final tracheids formed, the picture for the entire tree ring or even several tree rings is reversed. Our results are, therefore, consistent with research that reported lower pulp yields at higher elevation (Nylinder and Hägglund 1954; Beadle et al. 1996).

The mean lignin content (determined in the growth rings 1997 and 1998) in secondary walls of the valley tree was 0.211 $g \cdot g^{-1}$ and of the mountain tree 0.234 $g \cdot g^{-1}$, which

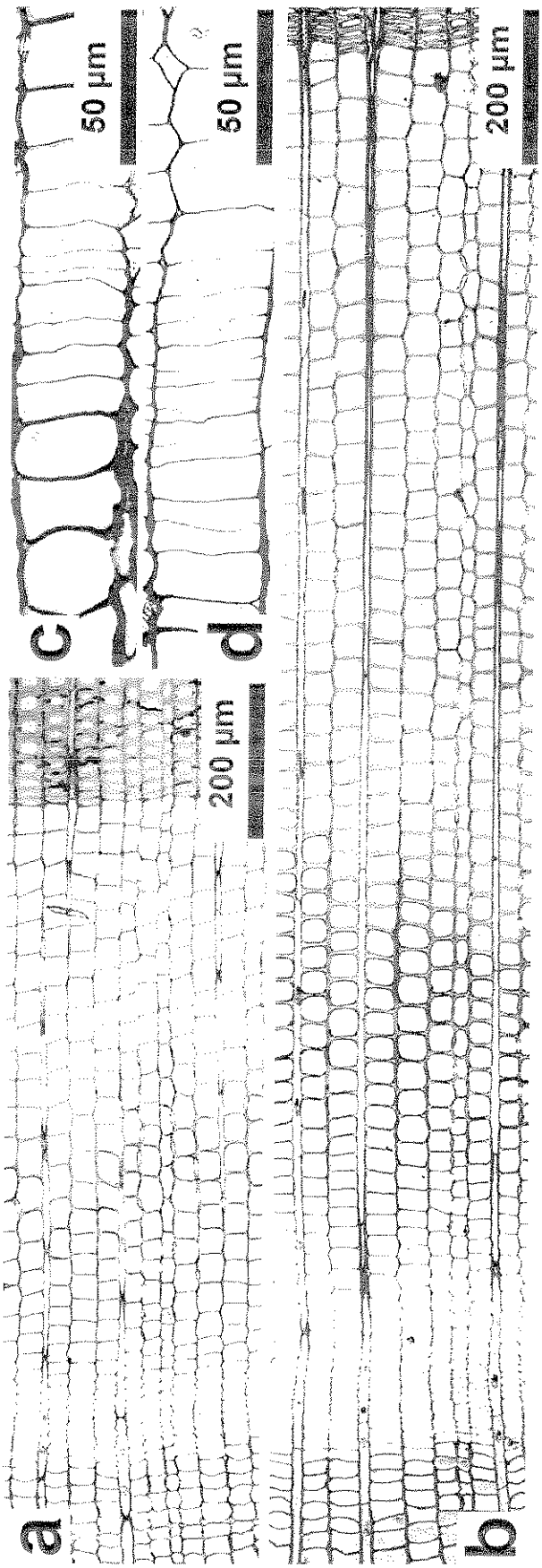


Fig. 5. Cross sections of samples taken on 12 June 1998: (*a*) cambium (left) and developing increment of the mountain tree; (*b*) cambium (left) and developing increment of the valley tree; (*c*) cambium of the mountain tree; and (*d*) cambium of the valley tree.

Fig. 6. Lignin contents at the two elevations: A, lignin of the complete 1998 tree rings, eight trees per site (acetylbromide bromide method); B, lignin content of rings 1995 – 1898 of the valley and the mountain tree (acetylbromide bromide method); C, mean lignin content in S2 layer of single tracheids formed in 1998 (UV microscopy), one tree per site, 74 mountain and 126 valley tracheids. Error bars are SD.

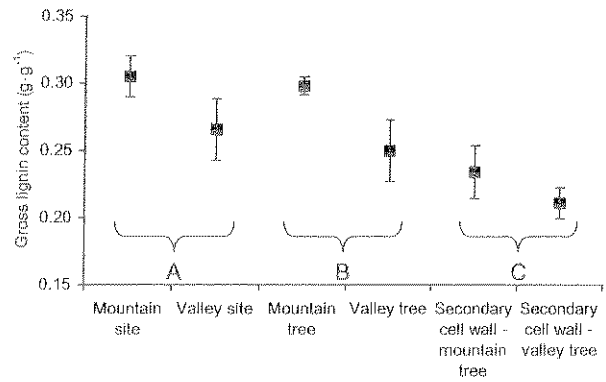
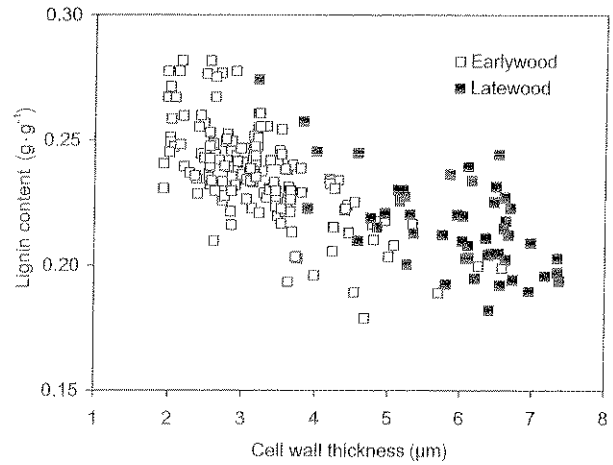


Fig. 7. Relationship between lignin content of the secondary cell wall and cell wall thickness in Norway spruce tracheids (n = 200).



gives a difference of 0.023 $g \cdot g^{-1}$. This difference is smaller than the one obtained through gross lignin determination (0.039 $g \cdot g^{-1}$). Coté (1968) as well as Sarkanen and Hergert (1971) propose a relationship between cell wall geometry and gross lignin content, which may explain the remaining difference (i.e., 0.016 $g \cdot g^{-1}$). An estimate leads to the result that the relatively larger contribution of the less lignified secondary wall layer in thick cell walls explains this difference. The estimate assumes no rays and resin ducts, constant tangential tracheid diameter of 30 µm, lignin concentration in the middle lamella as determined by Fergus et al. (1969), a thickness of the middle lamella as given by Fengel and Stoll (1973), and dimensions of the cell corner middle lamella area proposed by Wimmer and Lucas (1997).

With regard to mechanical strength, the findings of this study suggest a compensation of thin cell walls in wood grown at high altitudes through increased lignification. In future research this hypothesis needs to be verified through parallel measurements of cell wall mechanical properties

and lignin content as well as corresponding microfibril angles. It also seems reasonable that a high portion of the variability found in various lignin content studies are probably explained by different cell sizes, as already indicated by Wilson and Wellwood (1965), who consistently found higher lignin content in earlywood than in latewood. Therefore, future studies on lignin or pulp yield of trees as related to environmental effects should include cell morphological measurements to control for this effect.

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