

# VARIATION OF CROSS-SECTIONAL PROPERTIES WITHIN SINGLE NORWAY SPRUCE TRACHEIDS

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## ABSTRACT

The variation of cross-sectional tracheid properties along the tracheid length axis was studied in two Norway spruce (*Picea abies*) samples with varying cambium maturity. Twenty tracheids from both samples were measured for tracheid cross-sectional cell-wall area ( $A$ ), perimeter ( $P$ ), cell-wall thickness, and cross-sectional compactness ( $4\pi A/P^2$ ) at intervals of 5% of tracheid length using a confocal laser scanning microscope.

Tracheid dimensions were largest in the middle parts of the tracheid. The rate of change was greatest in the vicinity of the tracheid tips. The cross-sectional compactness was quite invariant along the tracheid length axis. Tracheids were symmetrical with respect to their midpoint, and this symmetry was not affected by cambium maturity. The most representative location along the tracheid length axis for the mean of the whole tracheid appeared to be around 20% of tracheid length from tracheid tip. The results were consistent between the samples. On the average, the deviation from the mean varied from -60% (near tracheid tip) to +30% (middle of the tracheid), depending on the property in question. Naturally, the former deviation further increases towards the tracheid tips.

Tracheid-to-tracheid correlations between the tracheid properties were not affected by the location of the measurement point within the tracheid length axis. The only exceptions were the locations near the tracheid tip, which often resulted in erroneous correlations.

*Keywords:* Tracheid properties, cross-sectional properties, *Picea abies*.

## INTRODUCTION

Tracheid properties differ, depending on whether they are located in the roots, branches, or stem of a tree. In the stem they vary within and between annual rings (Mork 1928; Atmer and Thörnqvist 1982; Sirviö and Kärenlampi 2000b), as well as within single tracheids. In addition, reaction wood, i.e., tension or compression wood, also shows different properties (Ollinmaa 1955, 1959). This paper considers the variation in cross-sectional properties along the length axis of single softwood tracheids.

Knowledge of the variation in properties in single tracheids is important, for example, in the determination of sample sizes or a representative location for measuring cross-sectional tracheid properties. Furthermore, variation in cross-sectional properties along the length axis may cause differences in mechanical be-

havior of the various tracheid segments. Thus, many wood- and paper-structure models may become more realistic by taking this kind of information into account.

Knowledge of the variation in properties along the length axis of tracheids is rather limited, at least in the case of quantitative information. The appearance of pits, i.e., their size and density, have been studied to some extent (Thomas and Scheld 1967; Takizawa and Ishida 1972; Takizawa 1974, 1979; Lin 1989; Sirviö and Kärenlampi 1998), but the variation in the cross-sectional dimensions and geometry of tracheids is not well known. It is well known, however, that tracheid width increases from the tips towards the middle parts of the tracheid. Some observations (Wardrop and Harada 1965; Okumura et al. 1974) suggest that cell-wall thickness also behaves in the same manner. However, the flexibility of fi-

bers, i.e., of tracheids removed from the wood-matrix, cannot be evaluated by these speculations alone.

The purpose of this paper is to clarify the variation of the tracheid cross-sectional cell-wall area, circumference (perimeter), cell-wall thickness, and cross-sectional compactness along the length axis of Norway spruce (*Picea abies*) tracheids. Cross-sectional compactness of a tracheid, indicating flexibility and collapsibility, was determined as  $4\pi A/P^2$ , where  $A$  is the cell-wall area and  $P$  is the perimeter in a tracheid cross-section.

#### MATERIALS AND METHODS

##### Materials

Two wood specimens of varying cambium maturity were taken from a Norway spruce (*Picea abies* L. Karst.) tree at breast height. Both specimens consisted of five entire growth rings; the first sample was taken from rings 13–17 and the second one from rings 53–57. Thus, the first sample is termed a low-cambium-maturity sample and the second one a high-cambium-maturity sample. The wood specimens were cut to match-sized sticks and macerated in a mixture of glacial acetic acid and hydrogen peroxide (1:1 (vol.), 24 h, +60°C). After maceration the tracheid specimens were washed and dyed in acridine orange, and 20 randomly selected unbroken tracheids from both samples were deposited onto microscope slides. Tracheid samples were allowed to dry overnight (+22°C, 16% RH) before mounting medium and cover slips were placed on the tracheids. The length of each tracheid was measured using an image analysis system attached to a PC, and the measuring points for cross-sectional properties were located. Twenty measuring points were located for each tracheid at intervals of 5% of tracheid length, starting at 2.5% from a randomly selected tracheid tip. A cross-sectional image from each measuring point was taken using a confocal laser scanning microscope CLSM (see Jang et al. 1992; Moss et al. 1993). The cross-sectional cell-wall area  $A$  and tracheid

perimeter  $P$  were measured from each CLSM-image.

##### Calculations

The measurements of the cross-sectional cell-wall area  $A$  and tracheid perimeter  $P$  were used in determining the mean cell-wall thickness

$$T = \frac{P - \sqrt{P^2 - 16A}}{8} \quad (1)$$

and the cross-sectional compactness

$$C = \frac{4\pi A}{P^2}. \quad (2)$$

In Eq. 1 it is assumed that the shape of a tracheid cross-section is rectangular and that the cell-wall thickness is constant between the radial and tangential walls. These assumptions do not fully correspond to the reality (see Saiki 1970; Okumura et al. 1974; Saranpää et al. 1997), but in this way a mean value for the cell-wall thickness in a tracheid cross-section may be produced without extra measurements on CLSM-images. Cross-sectional compactness (Eq. 2) is a dimensionless measure for the cross-sectional geometry of a tracheid, and it measures the relative proportion of the cell-wall area to the total area within a tracheid cross-section.

The relative changes in cross-sectional properties were studied as functions of the relative tracheid length axis. In order to observe the possible asymmetry of the tracheid cross-sectional properties with respect to tracheid midpoint more easily, the tracheids were turned lengthwise to have the maximum value of the considered cross-sectional property after the midpoint of the tracheid. Furthermore, the cross-sectional measures were normalized by the maximum value of the considered property within that particular tracheid.

The amount of variation in the cross-sectional properties in any given tracheid was described with a coefficient of variation, CV, and its dependence on the corresponding mean tracheid value was examined. The dimensionless

CV was preferred instead of using the standard deviation in order to facilitate the comparisons between the studied properties.

### Statistical analyses

Statistical significance of the deviations in mean values and coefficients of variation of the tracheid properties between the samples was evaluated using the *U*-test developed by Mann and Whitney, also known as the Wilcoxon test (see Wonnacott and Wonnacott 1985; Ranta et al. 1991).

An appropriate sample size (*N*) for accurate determination of the cross-sectional properties of a tracheid may be clarified as

$$N = (a\sigma/d)^2, \quad (3)$$

where *a* is a parameter depending on the required confidence level,  $\sigma$  is the standard deviation of the property, and *d* is the allowable error in measurement units (see Wonnacott and Wonnacott 1985; Ranta et al. 1991). Equation 3 can be rewritten as

$$N = (aCV/d_r)^2 \quad (4)$$

in order to determine the sample size using the coefficient of variation and relative allowable error ( $d_r$ ) instead of the standard deviation and actual allowable error, respectively.

For the mean value of the whole tracheid, the most representative measuring location along the tracheid length axis was examined as follows: The squares of errors between individual observations and the mean value of the tracheid were grouped by their relative distance from the tracheid tip. The location having the minimum sum of squares was considered to be the most representative measurement location.

The dependence of the tracheid-to-tracheid correlations on the measurement location within a tracheid was investigated as follows: Combinations of the cross-sectional properties were grouped by their relative distance from the tracheid tip. This yielded in 10 groups having 40 observations per sample. The differences between the coefficients of correlation

TABLE 1. The mean tracheid length, cross-sectional cell-wall area, tracheid perimeter, cell-wall thickness, and cross-sectional compactness of the samples. The mean values of the properties, except that of the tracheid perimeter, were statistically different ( $P < 0.05$ ) between the samples.

Property	Low-cambium-maturity	High-cambium-maturity
Length, mm	2.25	3.01
Area, $\mu\text{m}^2$	195	295
Perimeter, $\mu\text{m}$	68.1	75.1
Thickness, $\mu\text{m}$	3.41	5.28
Compactness	0.51	0.67

(Pearson's product moment correlation coefficient) of the 10 groups in both samples were statistically evaluated using the Fisher transformation (see Steel and Torrie 1980; Ranta et al. 1991).

### RESULTS

The mean tracheid properties of the samples are presented in Table 1. All the studied properties show greater values within the high-cambium-maturity sample. Except for the tracheid perimeter, the mean values of the tracheid properties between the samples differed statistically significantly ( $P < 0.05$ ).

Cross-sectional tracheid dimensions decrease towards the tracheid tips, but cross-sectional compactness seems to be invariant along the tracheid length axis (Fig. 1). The tracheids seem to be symmetrical with respect to their midpoint, and this symmetry is independent of cambium maturity.

Coefficients of variation (CV) of tracheid cross-sectional properties as functions of those properties are presented in Fig. 2. The mean CVs of the different samples are statistically the same, except in the case of the cross-sectional compactness, where the CV in the high-cambium-maturity sample is lower ( $P < 0.05$ ). This phenomenon is due to the fact that the CV of cross-sectional compactness decreases with an increasing mean value of tracheid compactness and that the mean compactness of all tracheids is higher in the high-cambium-maturity sample. It can be further observed

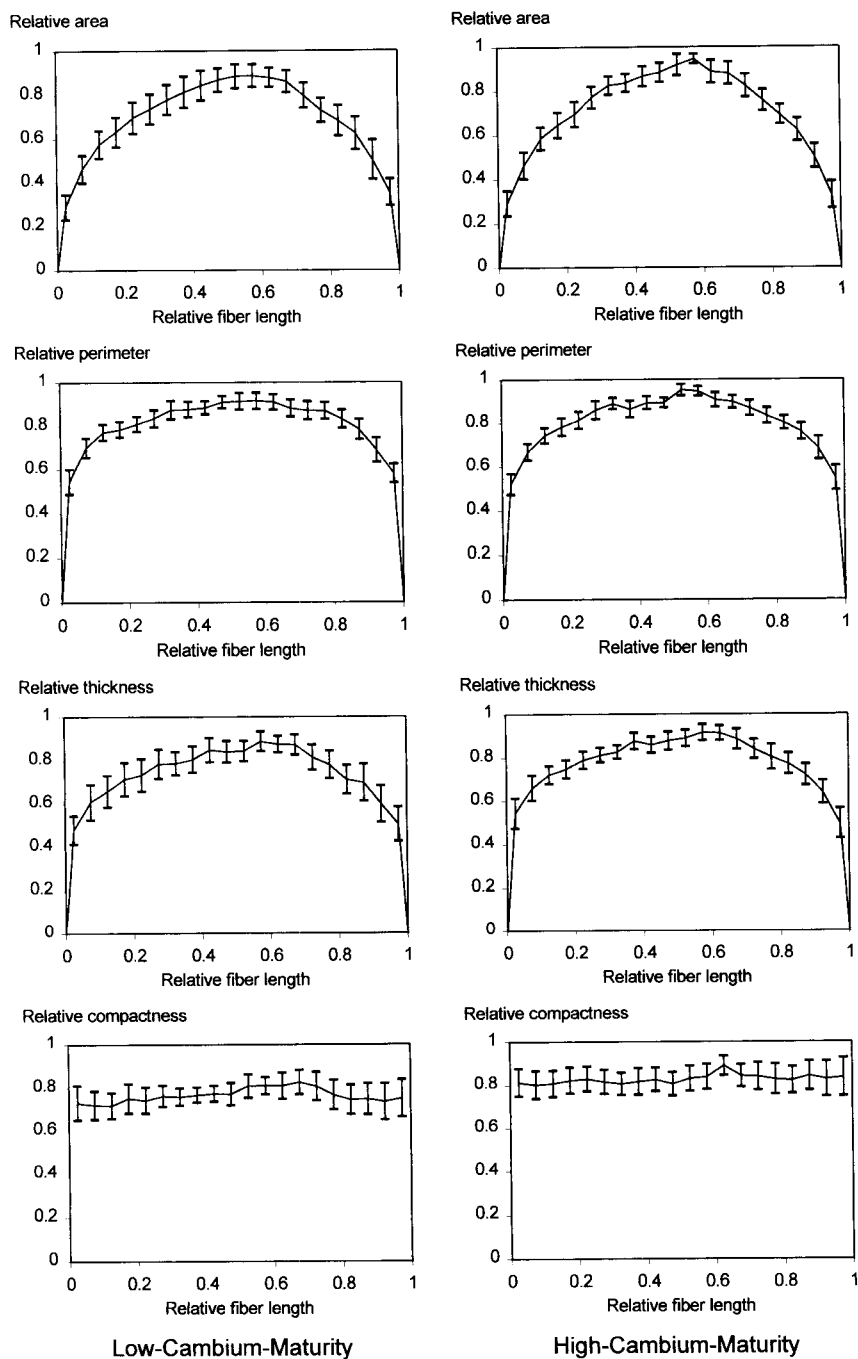


FIG. 1. Relative change of the cross-sectional cell-wall area, tracheid perimeter, cell-wall thickness, and cross-sectional compactness as a function of relative tracheid length. Low-cambium-maturity sample on the left, high-cambium-maturity sample on the right. The vertical lines indicate the 95% confidence levels of the mean.

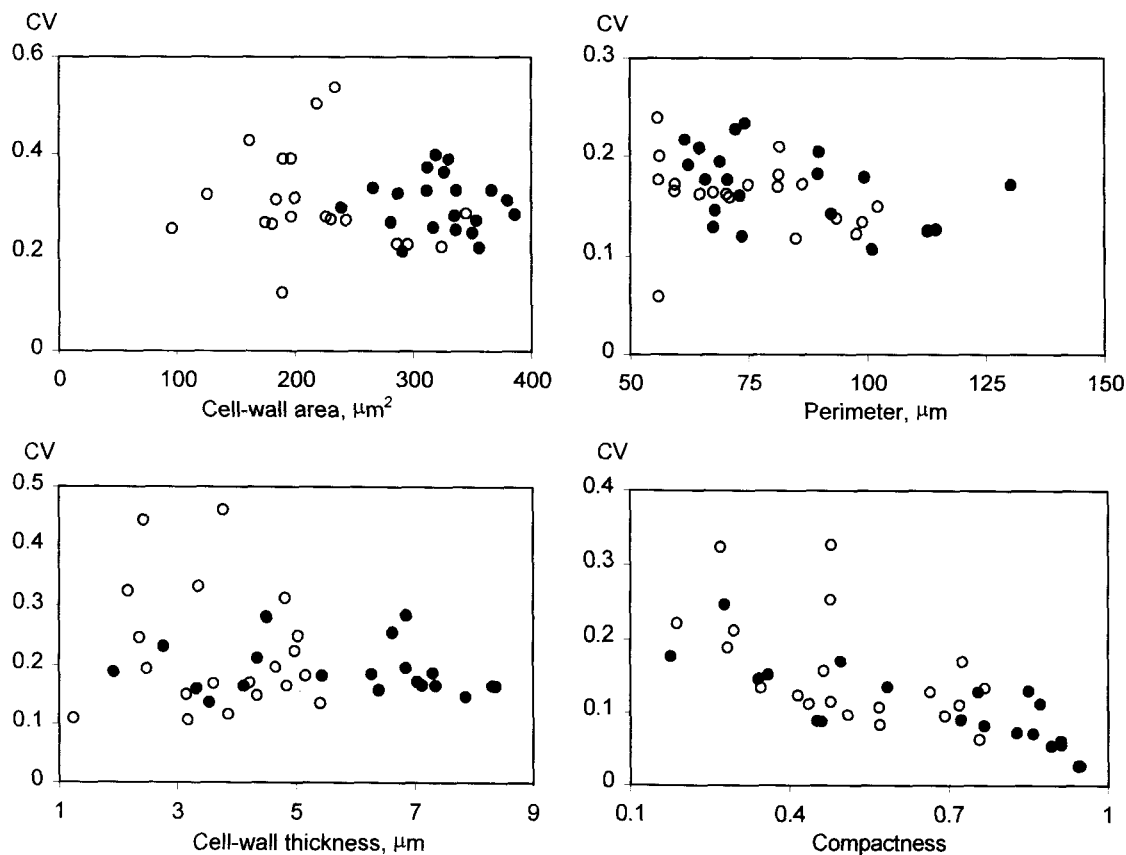


FIG. 2. Coefficient of variation (CV) of the cross-sectional cell-wall area, tracheid perimeter, cell-wall thickness, and cross-sectional compactness as a function of its mean value in a tracheid. Open circles = low-cambium-maturity sample, filled circles = high-cambium-maturity sample.

TABLE 2. Required sample size ( $N$ ) per tracheid for the mean value of the cross-sectional cell-wall area, tracheid perimeter, cell-wall thickness, and cross-sectional compactness of a tracheid, using randomly located measurements along the tracheid length axis.  $N$  is determined by Eq. 4. Mean coefficients of variation (CV) are calculated from the study material, the confidence level is 95% and the relative allowable error is 5%.

Property	CV	N, pcs.
Area	0.30	138
Perimeter	0.17	44
Thickness	0.21	68
Compactness	0.13	26

from Fig. 2 that the CVs of cross-sectional dimensions are rather independent of the size of the tracheids.

For sample-size determination, we need to know the coefficient of variation (CV) of the property in question. We also need to decide the confidence level we want to achieve and the relative error we can allow (Eq. 4). The independence of CV in connection with the mean cross-sectional tracheid dimensions can be seen in Fig. 2. A reasonably normal confidence level is considered to be 95%. If we can then accept a 5% relative error in mean value, we can calculate the required sample size for any property. The calculated sample sizes for the tracheid properties studied are presented in Table 2.

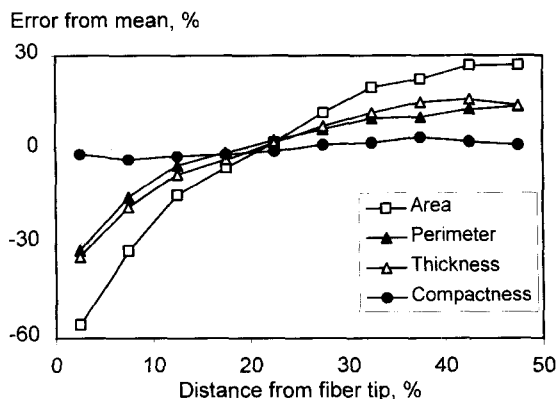


FIG. 3. The dependence of the mean relative deviation from the mean value of the whole tracheid as a function of the relative distance from the tracheid tip for cross-sectional cell-wall area, tracheid perimeter, cell-wall thickness and cross-sectional compactness.

Table 2 shows that many randomly located measurements must be done for even a single tracheid in order to get an accurate mean value of the property being investigated. This is, however, very time-consuming without reliable, automated measuring devices. Another way to deal with this problem is to select a representative measurement location using knowledge of the systematic variation of tracheid properties along the tracheid length axis.

A representative measurement location, determined by the method of the least sum of squares, appeared to be 22.5% of tracheid

length from tracheid tip in the case of a cross-sectional cell-wall area, 17.5% for tracheid perimeter and cell-wall thickness, and 42.5% for cross-sectional compactness. These results were identical for both samples.

Because of the symmetry of the tracheid with respect to its midpoint, the error due to improper measurement location varies as a function of that location. The relative error caused by the measurement location is greatest in the case of the cross-sectional cell-wall area, while in the case of cross-sectional geometry, the error varies only slightly (Fig. 3). On the average, measurement locations near tracheid tips give underestimates up to 30%, and locations near the midpoint of the tracheid overestimates of 10–15% for tracheid perimeter and cell-wall thickness.

Tracheid length does not correlate with cross-sectional tracheid properties at any measurement location (Tables 3 and 4). The only observed exception to this is the positive correlation between tracheid length and cross-sectional cell-wall area in the low-cambium-maturity sample (Table 3).

A positive correlation exists between the cross-sectional cell-wall area and other cross-sectional dimensions in the low-cambium-maturity sample regardless of the measurement location (Table 3). In the high-cambium-maturity sample, a correlation between the cross-

TABLE 3. The influence of measurement location, as a relative distance from the tracheid tip, on the coefficients of correlation between tracheid properties in the low-cambium-maturity sample. A = cross-sectional cell-wall area, P = tracheid perimeter, T = cell-wall thickness, C = cross-sectional compactness, and L = tracheid length. The coefficients of correlation greater than 0.31 are statistically different from 0.00 ( $P < 0.05$ ) (Ranta et al. 1991).

Distance, %	A - P	A - T	A - C	P - T	P - C	T - C	L - A	L - P	L - T	L - C
2.5	0.63	0.59	0.09	-0.18	-0.65	0.83	0.06	0.09	0.00	-0.01
7.5	0.60	0.57	0.17	-0.24	-0.63	0.88	0.16	0.02	0.16	0.14
12.5	0.54	0.58	0.19	-0.30	-0.68	0.87	0.29	0.05	0.24	0.14
17.5	0.43	0.57	0.23	-0.45	-0.75	0.91	0.33	0.07	0.17	0.09
22.5	0.42	0.50	0.19	-0.52	-0.78	0.92	0.31	0.03	0.17	0.10
27.5	0.53	0.47	0.10	-0.45	-0.76	0.90	0.29	-0.01	0.21	0.16
32.5	0.50	0.53	0.12	-0.43	-0.77	0.88	0.28	-0.02	0.27	0.16
37.5	0.57	0.49	0.09	-0.38	-0.73	0.88	0.33	0.00	0.24	0.20
42.5	0.58	0.33	0.05	-0.52	-0.76	0.94	0.47	0.16	0.24	0.12
47.5	0.60	0.44	0.05	-0.41	-0.74	0.89	0.46	0.14	0.34	0.12

TABLE 4. The influence of measurement location, as a relative distance from the tracheid tip, on the coefficients of correlation between tracheid properties in the high-cambium-maturity sample. A = cross-sectional cell-wall area, P = tracheid perimeter, T = cell-wall thickness, C = cross-sectional compactness, and L = tracheid length. The coefficients of correlation greater than 0.31 are statistically different from 0.00 ( $P < 0.05$ ) (Ranta et al. 1991).

Distance, %	A - P	A - T	A - C	P - T	P - C	T - C	L - A	L - P	L - T	L - C
2.5	0.64	0.37	-0.21	-0.41	-0.84	0.79	0.13	0.13	0.04	-0.08
7.5	0.41	0.32	-0.03	-0.68	-0.89	0.91	0.00	0.03	0.07	0.01
12.5	0.04	0.43	0.24	-0.83	-0.93	0.96	-0.08	0.06	-0.04	-0.03
17.5	-0.05	0.48	0.30	-0.81	-0.92	0.95	0.02	0.15	-0.03	-0.08
22.5	-0.14	0.50	0.34	-0.84	-0.93	0.97	0.02	0.22	-0.14	-0.18
27.5	-0.03	0.47	0.24	-0.82	-0.93	0.95	-0.11	0.08	-0.06	-0.06
32.5	-0.01	0.35	0.20	-0.88	-0.95	0.96	-0.03	0.13	-0.13	-0.11
37.5	-0.05	0.38	0.23	-0.86	-0.93	0.96	-0.12	0.10	-0.12	-0.07
42.5	-0.05	0.43	0.24	-0.85	-0.94	0.95	-0.01	0.12	-0.06	-0.07
47.5	-0.15	0.42	0.29	-0.90	-0.96	0.97	-0.14	0.11	-0.16	-0.11

sectional cell-wall area and tracheid perimeter does not exist, unless the measurement is located near the tracheid tip (Table 4).

A negative correlation exists between the tracheid perimeter and cell-wall thickness as well as between the tracheid perimeter and cross-sectional compactness, while there is a strong positive correlation between the thickness and compactness in both samples regardless of the measurement location (Tables 3 and 4). All these correlations were statistically significant ( $P < 0.05$ ).

Typically, the correlations between the tracheid properties among tracheids were statistically the same ( $P < 0.05$ ) irrespective of the measurement location. Erroneous results for the correlations between tracheid dimensions were observed only in the high-cambium-maturity sample, and only in the case of measurement locations nearest the tracheid tip.

#### DISCUSSION

Tracheid dimensions were largest in the middle parts of the tracheid (Fig. 1), while the rate of change was greatest in the vicinity of the tracheid tips. These kinds of changes have been reported also by Okumura et al. (1974). The cross-sectional compactness was quite invariant along the tracheid length axis (Fig. 1). Comparable data were not found in the literature.

If the tracheids were not symmetrical with

respect to their midpoint, either 1) the variation of the property in question would increase towards the tracheid tips (random tracheid orientation), or 2) the mean value of the property in question would increase towards one of the tracheid tips (systematic tracheid orientation). Neither of these phenomena is present in Fig. 1, so the tracheids are symmetrical with respect to their midpoint. This symmetry is not affected by the cambium maturity. Thus, the measurement of tracheid cross-sectional properties at the midpoint of the tracheid yields a systematic overestimation of the mean tracheid cross-sectional dimensions.

On average, the coefficients of variation (CV) of tracheid cross-sectional dimensions in the tracheids were independent of cambium maturity and the mean value of the property in question (Fig. 2). On average, the CV of tracheid cross-sectional geometry, described here with cross-sectional compactness, was slightly lower in the high-cambium-maturity sample (growth rings 53–57) than in the low-cambium-maturity sample (growth rings 13–17). However, the variability in CV of the cross-sectional dimensions was greater in the sample near the pith, but this was not the case for tracheid cross-sectional compactness.

The sample size for the determination of the mean value of the whole tracheid using randomly located measurements depends on the property in question, and naturally, on the re-

quired accuracy. With a confidence level of 95% and allowable error of 5%, the required sample sizes varied between 25 and 140 per tracheid (Table 2). Thus, the location of the measurement point along the tracheid length axis is not insignificant. The most representative location appeared to be 22.5% of tracheid length from tracheid tip for the cross-sectional cell-wall area and 17.5% for the other dimensions. For the cross-sectional compactness, the most representative location was 42.5% of tracheid length. These results were consistent between the samples. In spite of these results, the cross-sectional compactness may be measured at any location along the tracheid length axis, because of its relatively low variation from one tracheid tip to another (Figs. 1 and 3).

On average, the measurement error from the mean varied from -60% (near tracheid tip) to +30% (middle of the tracheid), depending on the property in question (Fig. 3). The former error, naturally, further increases towards the tracheid tips beyond our nearest measurement location to the tracheid tip, i.e., 2.5% of tracheid length. Because the cross-sectional compactness is rather invariant along the length axis of a tracheid, the relative error does not become remarkable even if the measurement is accomplished near the tips (Fig. 3).

Tracheid-to-tracheid correlations between the tracheid properties were not affected by the location of the measurement point in the tracheid length axis (Tables 3 and 4). The only exceptions were the locations very near the tracheid tip, which sometimes resulted in erroneous correlations. The tracheid-to-tracheid correlations between the tracheid properties in both samples were similar to those found for larger experimental material (Sirviö and Kärenlampi 2000).

The results presented in this paper may be useful in designing sampling methods for wood and fiber related studies. The results may be applicable not only to macerated tracheids, but also to solid wood sections, like microtome cuts. However, the meaning of these results depends on the purpose of each

individual study. Thus, they are not further analyzed in this paper.

Methods for measuring single tracheid strength typically consist of gluing or gripping of tracheids from their tips (Jayne 1959, 1960; Hartler et al. 1963). Because the cross-sectional dimensions were shown to decrease towards the tracheid tips, and because the density and size of the pits increase at the same time (Thomas and Scheld 1967; Takizawa and Ishida 1972; Takizawa 1974, 1979; Lin 1989; Sirviö and Kärenlampi 1998), the methods used for measuring mechanical properties of single tracheid obviously overestimate the mean strength of individual tracheids and underestimate their mean stretch.

The results may be useful to some pulp- and paper-related applications, too. Although the original wood tracheid dimensions and geometry are modified during various pulp- and papermaking processes, the tracheids are not totally destroyed. Thus, taking the original size and shape of tracheids into account, and evaluating the effects of processing on those, may yield to more realistic modelling or description of paper structure.

#### CONCLUSIONS

The variation of cross-sectional tracheid properties along the tracheid length axis was studied in two Norway spruce (*Picea abies*) samples with varying cambium maturity. Twenty tracheids from both samples were measured for tracheid length as well as for tracheid cross-sectional dimensions and geometry at intervals of 5% of tracheid length using a confocal laser scanning microscope.

Tracheid dimensions were largest in the middle parts of the tracheid. The rate of change was greatest in the vicinity of the tracheid tips. The cross-sectional tracheid geometry, described with compactness ( $4\pi A/P^2$ , where  $A$  is the cell-wall area and  $P$  is the tracheid perimeter in a tracheid cross-section), was quite invariant along the tracheid length axis. Tracheids were symmetrical with respect





