GROWTH RATE EFFECTS ON INTRA-RING AND INTER-RING TRAJECTORIES OF MICROFIBRIL ANGLE IN NORWAY SPRUCE (PICEA ABIES)

by

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SUMMARY

Fourteen Norway spruces [Picea abies (L.) Karst.], randomly sampled as 7 fast-grown and 7 slow-grown trees, were used to test whether an increased tree growth rate in circumference affects the intra-ring and inter-ring trajectories of the microfibril angle in the S2 layer of the tracheid wall. Those trajectories describe the fluctuations of the S2 microfibril angle, respectively, from earlywood to latewood within rings and from pith to bark among rings. Using the cross-field pit apertures, intra-ring measures of microfibril angle were made at 11 equally-spaced sampling sites over each of 8 growth rings, following an 11 x 8 doubly repeated measures design with the tree as the ‘subject’ on which repeated measures were made. All the intra-ring trajectories of microfibril angle decreased linearly from earlywood to latewood, whereas the inter-ring trajectories showed significant year effects. Both types of trajectories are significantly affected by the growth rate after first thinning, as the fast-grown spruces showed a systematically larger microfibril angle and a mean microfibril angle of 29° compared to 21° for the slow-grown spruces. Thus, lower tensile and tear strengths of tracheids as well as some modifications of the mechanical properties of solid wood and paper can be expected from Norway spruces growing faster than 2.2 cm/year in circumference.

Key words: Growth rate effects, intra-ring and inter-ring trajectories of microfibril angle, Norway spruce, Picea abies (L.) Karst., cross-field pit aperture.

INTRODUCTION

Silvicultural practices such as heavy thinnings, planting with wide spacing and fertilisation of forest stands result in increased tree growth rate because of reduced competition for light, soil moisture and nutrients (Kozlowski 1971; Bodner 1984; André et al. 1994). As the physiology of the whole tree, from roots to crown, is affected by changes in the environment (Zobel & Van Buijtenen 1989), the resulting increased

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growth rate influences the pattern of wood formation and, hence, its anatomical and technological properties (Senft et al. 1985; Bendtsen & Senft 1986; Keith & Chauret 1988; De Kort et al. 1991; Lindström 1996). In particular, it is acknowledged that for most fast-grown softwoods, the cambium activated by a higher level of growth hormones will produce tracheids with larger lumen diameter and thinner cell walls (Larson 1969; Brown 1970; Kramer & Kozlowski 1979; Megraw 1985; Wilkes 1987).

Changes in cell wall formation and thickening can be expected from increased growth rate since the cellulose deposition is known to depend on growing conditions of the tree, such as light, temperature, soil moisture, and obviously, the physiological conditions of the tree itself (Hiller 1964; Kozlowski 1971; Panshin & De Zeeuw 1980). Therefore, the orientation of cellulose microfibrils, particularly in the secondary wall, may be affected by the increased growth rate of the tree. However, cellulose microfibrils of the S2 layer, which is the most important in terms of volume of the whole cell wall (Panshin & De Zeeuw 1980), are strongly linked to the physical and mechanical strength properties of solid wood as well as to the modulus of elasticity (MOE) of isolated fibres (Barber & Meylan 1964; Armstrong et al. 1977; Boyd 1980). In fact, stiffness of solid wood and fibres in pulp and paper arises from the cellulose content and the way this is distributed within the cell wall. Cellulose occurs as very long crystalline microfibrils that have very high stiffness in the direction of the microfibril axis (Cave & Walker 1994). The microfibril angle related to the cell axis varies from 10 to 30° among species (Walker 1993). The larger it is, the lower the rigidity, the lower the tensile and tear strengths of the tracheid, and the higher its longitudinal shrinkage (Hiller 1964; Harris & Meylan 1965; Horn 1974; Hitchings 1984; Boyd 1985; Megraw 1985; Bendtsen & Senft 1986; Stuart & Evans 1995).

The microfibril angle of the S2 layer is considered by many authors as one of the most relevant characteristics for selecting wood for industry and paper mills (Horn 1974; Armstrong et al. 1977; Shupe et al. 1996; Walker & Butterfield 1996; Butterfield 1998). Accordingly, this variable is widely taken into account in studies of the morphology of tracheids. In the literature, the following is reported:

1) From pith to bark: the microfibril angle is large in the first rings near the pith and gradually decreases over years, as classically does the ring width, while the cambium progresses to maturity (Hiller 1964; McMillin 1973; Megraw 1985; Donaldson 1992; Walker 1993; Cave & Walker 1994). A large microfibril angle associated with a short tracheid length and a low latewood percentage could explain the lesser strength and lower stiffness of juvenile wood in conifers (Bendtsen & Senft 1986; Kennedy 1995).

2) From earlywood to latewood: earlywood tracheids show a very large microfibril angle, whereas those of latewood have microfibrils quite parallel to the cell axis. Between these extremes, microfibril angle is supposed to decrease, providing a linear trend (Hiller 1964; McMillin 1973; Panshin & De Zeeuw 1980; Bucur 1982; Cave & Walker 1994).

3) Within a single tracheid: microfibril angle is larger in radial than in tangential walls (Bucur 1982; Cave & Walker 1994), and may vary along the longitudinal axis of the tracheid (Panshin & De Zeeuw 1980).
In addition, it is generally accepted that large microfibril angles in the S2 layer of the cell wall are observed in short tracheids or in earlywood tracheids with a large diameter associated with thin walls (Hiller 1964; Erickson & Arima 1974; Panshin & De Zeeuw 1980; Megraw 1985).

Only a few papers are dedicated to the influence of tree growth rate on the orientation of microfibril angle in the S2 layer. Working with Douglas firs which were 27 to 31 years of age and whose growth had been stimulated by fertiliser treatment and thinning, Erickson and Arima (1974) report that microfibril angle tends to increase slightly from pith to bark over a few years after accelerated growth. No growth rate effect on the S2 microfibril angle is reported for *Pinus glabra* (Manwiller 1972) and *Cryptomeria japonica* (Hirakawa & Fujisawa 1995). For *Pinus ponderosa*, large differences in radial growth induced by thinning treatments were not accompanied by significant differences in microfibril angle (Markstrom et al. 1983). However, Oda (1983) reports that thinning increases microfibril angle in *Pinus luchuensis*.

Using 14 Norway spruces (*Picea abies*) from an even-aged, plantation-grown stand located in the Belgian Ardennes, this study has been designed to test whether an increased tree growth rate in circumference affects the *intra-* and *inter-* ring trajectories of the microfibril angle in the S2 layer of the tracheid wall. Those trajectories describe the fluctuations of the S2 microfibril angle, respectively from earlywood to latewood within rings and from pith to bark among rings. Seven fast-grown spruces and seven slow-grown spruces were randomly sampled. The definition of the two growth categories is based on an average annual increment in circumference (Herman et al. 1998a, b). If fast growth in softwood results in an increased proportion of earlywood-type tracheids (Larson 1969) and this type of tracheids is associated with a larger S2 microfibril angle, the *intra-* and *inter-* ring trajectories for fast-grown spruces should be above those of slow-grown spruces. Using the cross-field pit apertures, *intra-* ring measures of microfibril angle were made at 11 equally-spaced sampling sites over each of 8 growth rings, following an $11 \times 8$ doubly repeated measures design with the tree as the ‘subject’ on which repeated measures were made (Dutilleul 1998a). The autocorrelation (i.e., lack of independence) and heteroscedasticity (i.e., heterogeneity of the variance) that characterise such data (Dutilleul 1998b) were taken into account in the statistical analyses, among sampling sites within a growth ring and among growth rings. The growth rate effects observed on the *intra-* and *inter-* ring trajectories of the S2 microfibril angle are discussed from the perspectives of forest management and paper production.

**MATERIALS AND METHODS**

*Growth rate, growth category and tree sampling*

The ‘growth rate’ and ‘growth category’ concepts used in this study have been detailed in previous papers (Herman et al. 1998a, b). Basically, growth rate does not refer to the average ring width of a tree, but rather to an average annual increment in circumference of the trunk at a given height. It is defined over a given number of years as the ratio of the increment in circumference to the corresponding number of
years. The growth rate in circumference provides an expression of the average growth speed of the tree.

In Belgium, Norway spruce stands managed as even-aged high forest are characterised by an average growth rate in circumference of 2.2 cm/year. Considering this figure as a reference, a Norway spruce is said to be fast-grown or slow-grown, depending on whether its average annual increment in circumference is above or below 2.2 cm/year (Herman et al. 1998a, b).

Thirty-eight-year-old trees have been harvested in an experimental stand planted in 1949 and in which different thinning intensities have been conducted since 1969 (André 1976; André et al. 1996). An average annual increment of circumference at breast height (1.5 m) was computed for each individual tree of the stand over the growing period between 1970 (year after first thinning) and 1987. For this study, seven spruces were randomly sampled within each growth category. The slow-grown spruces had a mean circumference of 76.4 cm and a growth rate ranging from 1.3 to 2.2 cm/year for a mean growth rate of 1.6 cm/year, whereas the fast-grown spruces had a mean circumference of 99.4 cm and a growth rate ranging from 2.5 to 3.1 cm/year for a mean growth rate of 2.9 cm/year. We refer to Herman et al. (1998b: Table 1) for further information (e.g. total height, height of first living branch) about the 14 trees whose numbers are 325, 328, 330, 331, 334, 335, 340 for the slow-grown spruces and 321, 322, 329, 332, 333, 337, 338 for the fast-grown spruces.

Measurement of microfibril angles

For each sampled tree, a 15 cm-thick slice was cut from the trunk at 2.3 m height because the lower base of the log was used for other studies. On each cross-sectional disc, a 2 cm-thick strip of wood was sawn from pith to bark along the north radius, so that rings were at right angle to this radius. The north direction was chosen in order to minimise the occurrence of compression wood due to westerly prevailing winds.

Among the 33 annual rings available for measurement (1955–1987; see Herman et al. 1998a, b), 8 growth rings per sampled tree were retained for analysis. These rings were equally spaced every four years from 1959 to 1987. The first 3 rings (1959, 1963, 1967) belong to the period before first thinning (i.e. from 1955 to 1969) and the latter 5 rings (1971, 1975, 1979, 1983, 1987), to the period after first thinning (i.e. from 1970 to 1987). Following Zobel and Van Buijtenen (1989) and according to the year of plantation (1949) and our fibre length measurements (Herman et al. 1998a, b), those periods correspond to the juvenile and mature wood, respectively.

Radial sections 20 μm thick were cut for each of the 112 growth rings, briefly immersed in a 75% ethanol solution, and mounted in glycerine-alcohol. They were viewed through a light microscope (lens × 25) equipped with a rotating stage graduated in degrees and an eyepiece micrometer.

Intra-ring measurements of microfibril angle were made at regular intervals across the width of radial microtomed sections. For each ring analysed, 11 equally-spaced positions, called ‘sampling sites’ hereafter, were defined along a radial transect from earlywood to latewood; the space between successive sampling sites was about 10% of the radial ring width. This constant number of sampling sites per annual ring and their equal spacing allowed appropriate statistical analysis (see the next two sections).
At each *intra*-ring sampling site, microfibril angle is obtained by the measurement of the orientation, related to the longitudinal axis of the tracheid, of the pit apertures viewed in the cross-field. In Norway spruce, these cross-field pits are piceoid (Core et al. 1979) and give a clear indication of the angle because of the elliptic shape of their aperture in the S$_2$ layer of the tracheid wall (Panshin & De Zeeuw 1980; Senft & Bendtsen 1985; Donaldson 1991). The average of two microfibril angle values in the same cross-field was used as datum at each sampling site. Although rings were radially sectioned, continuous measurement on the same ray across the whole growth ring was not always possible. At a few sampling sites, a displacement along the longitudinal axis of the tracheids was required to meet another ray for further radial measurements.

Compared to the X-ray diffraction method adapted for microfibril angle measurement by Cave (1966) and Boyd (1977), the method that we used is tedious but remains one of the most accurate for obtaining point *intra*-ring observations for microfibril angle. At least, the use of cross-field pit apertures requires less preparation than the technique of polarised light (Preston 1934; Manwiller 1966; Page 1969; El-Hosseiny & Page 1973; Leney 1981) or that of Senft & Bendtsen (1985), which involves inducing checks in the cell wall and precipitation of iodine crystals within the checks that follow the microfibril angle. However, the use of cross-field pit apertures for indirect measurement of S$_2$ layer microfibril angle is limited to species with pinoid or piceoid cross-field pit apertures (Hiller 1964; Markstrom et al. 1983).

**Data sets**

The S$_2$ microfibril angle data were collected following an $11 \times 8$ doubly repeated measures design. In fact, measurements were made repeatedly on the same tree, at 11 equally spaced sampling sites within a growth ring for each of 8 sampling years. Whereas the annual growth ring defines a temporal repeated measures factor with 8 levels, the sampling site within a growth ring tends to represent a spatial rather than temporal repeated measures factor. Thus, the combination of the two factors provides doubly repeated measures (Dutilleul 1998a). The number of sampling sites within a growth ring was constantly 11 and their equal spacing was fixed to 10% of the ring width in order to adjust for differences in ring width among trees for a given sampling year.

The concept of trajectory, which originates from time series analysis (Brillinger 1981: 18) and refers to the realisation of a time series or an ordered sequence of observations with a beginning and an end, was used in a purely temporal framework in Herman et al. (1998a, b). It is extended here to describe the fluctuations of the S$_2$ microfibril angle from earlywood to latewood within rings (*intra*-ring trajectories) and from pith to bark among rings (*inter*-ring trajectories). A total of $11 \times 8 = 88$ repeated measures data of S$_2$ microfibril angle were collected on each of the 14 trees. For technical reasons related to the number of subjects relative to the number of repeated measures (Crowder & Hand 1990), it was not possible to reliably analyse the 88 repeated measures data at once. Thus, the data analysis had to be split into two main types of analysis: an *intra*-ring and an *inter*-ring analysis.
For the *intra*-ring analysis, the process of measurement of S₂ microfibril angles provided eight $14 \times 11$ data tables. Each row in a $14 \times 11$ table represents a tree (7 per growth category), whereas a column refers to a sampling site in the ring, along a radial transect from earlywood (site 1) to latewood (site 11). In the jargon of repeated measures analysis (Crowder & Hand 1990; Dutilleul 1998a, b), a ring within a tree, rather than the tree itself, is a ‘subject’ in this particular context and each row in a $14 \times 11$ table of S₂ microfibril angles here defines an *intra*-ring ‘profile’. For each growth category, a mean profile (i.e. mean *intra*-ring trajectory) has been computed over the 7 individual *intra*-ring profiles for each sampling year.

For the *inter*-ring analysis, one $14 \times 8$ data table was prepared. A cell of this table is the average of *intra*-ring observations for one of the 14 sampled trees in one of the 8 sampling years. A sampled tree is a ‘subject’ in this context and each row of the $14 \times 8$ table is an *inter*-ring profile. For each growth category, a mean profile (i.e. mean *inter*-ring trajectory) has been computed over the 7 individual *inter*-ring profiles.

**Statistical analyses**

Classical $t$ tests for equality of two means and classical F tests for equality of two variances (Sokal & Rohlf 1995) were carried out between growth categories, site by site within a ring and year by year. Both tests were performed with the TTEST procedure of SAS Version 6 (SAS Institute Inc. 1989).

The univariate approach to the analysis of variance for repeated measures (ANOVAR) (Crowder & Hand 1990; Dutilleul 1998b) was followed to assess (i) the between-subjects effects of growth category and (ii) the within-subject effects of sampling site within a growth ring and its interaction with growth category in the *intra*-ring analysis, and of sampling year and its interaction with growth category in the *inter*-ring analysis. Two types of *inter*-ring analysis were carried out: the main one, using averages over the 11 sampling sites, and a second, carried out separately for the first and the last sampling sites followed over the 8 years. The SAS procedure used was PROC GLM with the REPEATED statement (SAS Institute Inc. 1989).

In the ANOVAR, the probabilities of significance for the F tests of site- or year-related effects were adjusted by using Greenhouse & Geisser’s (1959) estimate of Box’s (1954a, b) epsilon correction factor. This correction applied to the number of degrees of freedom of the F-test statistic takes into account the autocorrelation and heteroscedasticity among sampling sites and among sampling years, respectively. We used the Greenhouse & Geisser’s (1959) estimate instead of Huynh & Feldt’s (1976) which is recommended only when the number of subjects is smaller than two times the number of repeated measures and the epsilon value is greater than 0.75 (Crowder & Hand 1990: 55).

Polynomial and profile contrast analyses (Von Ende 1990) were carried out for sampling site within a ring in the *intra*-ring analysis, using the GLM procedure with the POLYNOMIAL and PROFILE statements (SAS Institute Inc. 1989). The profile contrast analysis refers to the flatness hypothesis of whether there is a site effect on the mean microfibril angle between successive sampling sites, whereas the polynomial contrast analysis provides a more global information as to the existence of lin-
ear, quadratic, etc. trends in the trajectory of the variable and possible growth rate effects on existing trends.

RESULTS AND DISCUSSION

_Intra-ring trajectories of microfibril angle_

Microfibril angle (MFA) values in earlywood are large (43° on average for a range of 35 to 54°), compared to those in latewood (7° on average for a range of 3.5 to 11°) (Fig. 1a, b). The difference observed between earlywood and latewood is in accordance with the literature (e.g., McMillin 1973; Panshin & De Zeeuw 1980). However, the ranges that we report for Norway spruce are wider than those for other forest species of the genus _Pseudotsuga_, _Picea_ and _Pinus_, which are about 25 to 30° in earlywood and 10 to 12° in latewood tracheids (Erickson & Arima 1974; Panshin & De Zeeuw 1980; Bucur 1982; Bendtsen & Senft 1986; Walker 1993; Donaldson 1996). Between the extreme values, a linear and gradual decrease appears to be the rule, and the _intra_-ring fluctuations of the MFA follow the same pattern, whatever the annual ring (Fig. 1a, b). Indeed, the Site main effects are always highly significant (Table 1). The polynomial contrast analysis provides that the linearity of _intra_-ring trajectories was always highly significant, whereas no growth category effect was observed on this linear decreasing pattern (numerical results not reported here). This is consistent with other studies on softwood species (Hiller 1964; Cave & Walker 1994).

The analysis of successive differences between sampling sites sheds light on other characteristics of the _intra_-ring trajectory of MFA. Some of the years analysed (i.e. 1971, 1975) tend to show more significant differences between successive sampling sites in the second half of the growth ring, whereas some other years show significant differences between successive sampling sites already at the beginning of the growth ring (Table 2). Thus, the decreasing pattern of MFA is gradual, from high values in earlywood to lower values in latewood, but the _intra_-ring effects of sampling site may vary from one year to another and are not clearly linked to the location of the increment analysed, in the juvenile wood (Fig. 1a) or in the mature wood zone (Fig. 1b).

These observations also suggest the presence of a transition xylem whose tracheids have developed intermediate ultrastructural characteristics, between those of true earlywood and true latewood, in Norway spruce. This concept has already been developed for morphological characteristics of softwood tracheids, such as lumen area, cell wall thickness and cell diameter (Larson 1969; Core et al. 1979), and emphasises the natural _intra_-ring heterogeneity of the morphology of wood tracheids. In addition, no significant interaction between growth category and sampling site within a ring was observed in the contrast analyses (numerical results not reported here). Thus, there was no evidence against a parallel decrease of MFA within rings in both growth categories.

Before first thinning, while homoscedasticity is the rule except for three sites in 1967, significant differences in mean MFA between growth categories are observed for a few sites along the _intra_-ring trajectories of 1959 and 1967 (Fig. 1a). In view of Table 1, G C main effects were significant in 1959, whereas the interaction between G C and Site was never significant before first thinning.
Fig. 1. Year-by-year mean *intra*-ring trajectories from earlywood (sampling site 1) to latewood (sampling site 11) for each growth category (n = 7 spruces): a) before first thinning and b) after first thinning. Bounds of vertical intervals = sample mean ± standard error. The stars and crosses indicate the sampling sites at which the growth categories differ, either in the mean (stars) or in the variance (crosses): *, + = the difference is significant at the 0.05 level; **, ++ = the difference is significant at the 0.01 level.
Table 1. Year-by-year *intra*-ring repeated measures analysis of variance of microfibril angle in Norway spruce: Results in terms of significance probabilities (p) of the F tests. * = significant effect at 0.05 level; ** = significant effect at 0.01 level.

<table>
<thead>
<tr>
<th>Year</th>
<th>Effect</th>
<th>ANOVA(^1)</th>
<th>Modified ANOVA(^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1959</td>
<td>GC(^3)</td>
<td>0.036*</td>
<td>Epsilon = 0.435</td>
</tr>
<tr>
<td></td>
<td>Site(^4)</td>
<td>&lt; 0.001**</td>
<td>G C * Site = 0.144</td>
</tr>
<tr>
<td></td>
<td>G C * Site(^5)</td>
<td>0.144</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Epsilon =</td>
<td>0.435</td>
<td></td>
</tr>
<tr>
<td>1963</td>
<td>G C</td>
<td>0.873</td>
<td>Epsilon = 0.463</td>
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<tr>
<td></td>
<td>Site</td>
<td>&lt; 0.001**</td>
<td>G C * Site = 0.607</td>
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<tr>
<td></td>
<td>G C * Site</td>
<td>0.607</td>
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</tr>
<tr>
<td></td>
<td>Epsilon =</td>
<td>0.463</td>
<td></td>
</tr>
<tr>
<td>1967</td>
<td>G C</td>
<td>0.133</td>
<td>Epsilon = 0.441</td>
</tr>
<tr>
<td></td>
<td>Site</td>
<td>&lt; 0.001**</td>
<td>G C * Site = 0.347</td>
</tr>
<tr>
<td></td>
<td>G C * Site</td>
<td>0.347</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Epsilon =</td>
<td>0.441</td>
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</tr>
<tr>
<td>1971</td>
<td>G C</td>
<td>0.002**</td>
<td>Epsilon = 0.423</td>
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<td>G C * Site = 0.504</td>
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<td></td>
<td>G C * Site</td>
<td></td>
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<tr>
<td>1975</td>
<td>G C</td>
<td>0.002**</td>
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<td>G C * Site = 0.122</td>
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<tr>
<td></td>
<td>Epsilon =</td>
<td>0.387</td>
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</tr>
<tr>
<td>1979</td>
<td>G C</td>
<td>0.001**</td>
<td>Epsilon = 0.382</td>
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<td>G C * Site = 0.386</td>
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<td>G C * Site</td>
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<td></td>
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<td>G C * Site</td>
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<tr>
<td></td>
<td>G C * Site</td>
<td></td>
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</table>

1) ANOVA = univariate analysis of variance (between-subjects effects; one subject is a ring within a tree in a given year).

2) In the modified ANOVA procedure (within-subject effects), the probabilities of significance are adjusted by using Greenhouse & Geisser’s (1959) estimate of Box’s (1954a, b) epsilon correction factor. The correction applied to the number of degrees of freedom of the F test statistic aims to take the among-site autocorrelation and heteroscedasticity into account.

3) G C = growth category.

4) Site = sampling site within a ring.

5) G C * Site = growth category-by-site interaction.

Differences in MFA between growth categories were not expected to be large, on average, over the 1955–1969 period during which all trees were supposed to grow under similar stand conditions. However, fast-grown Norway spruces, which were selected for their superior average growth rate in circumference over the 1970–1987 growing period, show lower microfibril angles in 1959 than slow-grown trees, whereas the tendency is reversed in 1967. The mean *intra*-ring trajectories of the two growth categories are confluent in 1963. The significant growth category effects observed in
Table 2. Intra-ring profile analysis based on successive differences between sampling sites 1–10\(^1\); Results in terms of significance probabilities (p) of the F tests. * = significant effect at 0.05 level; ** = significant effect at 0.01 level.

<table>
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<tr>
<th>Year</th>
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<td>0.073</td>
<td>0.214</td>
<td>0.078</td>
<td>0.627</td>
<td>0.146</td>
<td>0.952</td>
<td>0.277</td>
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<td>0.074</td>
<td>0.037*</td>
<td>0.090</td>
<td>0.058</td>
<td>0.039*</td>
<td>0.475</td>
<td>0.010*</td>
<td>0.255</td>
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<td>1967</td>
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<td>0.002**</td>
<td>0.404</td>
<td>0.008**</td>
<td>0.007**</td>
<td>0.084</td>
<td>0.002*</td>
<td>0.013*</td>
<td>0.072</td>
<td>0.225</td>
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<td>1971</td>
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<td>0.022*</td>
<td>0.457</td>
<td>0.287</td>
<td>0.100</td>
<td>0.116</td>
<td>0.006**</td>
<td>0.835</td>
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<tr>
<td>1975</td>
<td>0.974</td>
<td>0.064</td>
<td>0.003**</td>
<td>0.476</td>
<td>0.016*</td>
<td>0.105</td>
<td>0.044*</td>
<td>0.255</td>
<td>0.027*</td>
<td>0.028*</td>
</tr>
<tr>
<td>1979</td>
<td>0.632</td>
<td>0.028*</td>
<td>0.059</td>
<td>0.038*</td>
<td>0.504</td>
<td>0.035*</td>
<td>0.859</td>
<td>0.232</td>
<td>0.014*</td>
<td>0.081</td>
</tr>
<tr>
<td>1983</td>
<td>0.525</td>
<td>0.114</td>
<td>0.007**</td>
<td>0.562</td>
<td>0.011*</td>
<td>0.108</td>
<td>0.008**</td>
<td>0.578</td>
<td>0.006**</td>
<td>1.000</td>
</tr>
<tr>
<td>1987</td>
<td>0.123</td>
<td>0.578</td>
<td>0.111</td>
<td>0.123</td>
<td>0.004**</td>
<td>0.694</td>
<td>0.001**</td>
<td>0.078</td>
<td>0.070</td>
<td>0.033*</td>
</tr>
</tbody>
</table>

\(^1\) The successive differences between sampling sites are contrasts calculated and tested across growth categories. Values reported refer to a test of the flatness hypothesis of whether there was a significant ‘Site’ effect on the mean microfibril angle between successive sampling sites.

1959 may be partly explained by the presence of a ‘false ring’ which appears more clearly in fast-grown spruces (1959, sites 6 & 7) than in slow-grown spruces (1959, site 8). Indeed, latewood-type tracheids in general, and those of false rings in particular, are known to show a lower microfibril angle (Hiller 1964; Erickson & Arima 1974; Panshin & De Zeeuw 1980; Megraw 1985).

After first thinning, the MFA mean intra-ring trajectories of the fast-grown spruces are systematically above those of the slow-grown spruces (Fig. 1b). In 1971, 1975, and 1979, most of the sampling sites within a growth ring show significant differences between growth categories in mean MFA. Thus, the MFA intra-ring trajectory is significantly affected by the increased growth rate of the tree in these years, but essentially in its overall mean level because the G C main effects are significant, while the interaction between G C and Site is not (Table 1). The fast-grown spruces have tracheids whose S\(_2\) layer is composed of microfibrils lying more horizontally in relation to the tracheid axis. In this study, the magnitude of a significant difference at the 0.05 level is about 9 to 10°, or more. In addition, the condition of homoscedasticity is satisfied at all sampling sites, with only two exceptions at the end of the 1983 growth ring.

Some researchers support that an increased growth rate of softwood, and in particular of Norway spruce, will anatomically be reflected by an increased proportion of earlywood-type tracheids which are characterised by a greater lumen area, a thinner wall, and a larger radial lumen diameter on the one hand (e.g. Zobel & Van Buijtenen 1989), and a larger MFA on the other hand (Hiller 1964; Erickson & Arima 1974; Panshin & De Zeeuw 1980; Megraw 1985). However, a study similar to this did not show any significant growth category effects in the intra-ring variations of lumen area, wall thickness, and radial lumen diameter (Herman 1997). On that basis, it might be thought that an increased growth rate of Norway spruce affects, independently of
cell morphology, the ultrastructural organisation of cell wall, by modifying some physiological mechanisms of the living cell that govern the microfibril orientation in the S_2 layer; these mechanisms are still under investigation (Chafe 1978; Meylan & Butterfield 1978; Boyd 1985; Abe et al. 1995). According to Boyd (1985), because genetic factors control the basic character of wall architecture during the extension growth of the cell, the major biophysical factor which determines the orientation of new microfibrils added to the wall is the magnitude and direction of principal strain on the forming cell wall and the plasmalemma at that particular time. Therefore, if the growth pattern of a cell is changed by any treatment affecting the growth rate of the tree, this would affect the elementary structure of the cell wall. Based on our *intra-*ring analysis of MFA, this seems to be the case for Norway spruce whose growth rate in circumference is above 2.2 cm/year under Belgian climatic and silvicultural conditions.

*Inter-ring trajectories of microfibril angle*

The fluctuations of microfibril angle from pith to bark classically reported in the literature (Hiller 1964; McMillin 1973; Erickson & Arima 1974; Megraw 1985; Donaldson 1992; Walker 1993; Cave & Walker 1994; Walker & Butterfield 1996; Butterfield 1998) seem to be applicable only to the slow-grown spruces in our study (Fig. 2). In fact, higher values of yearly mean MFA are observed for the slow-grown spruces in the first formed rings near the pith; they are followed by a gradual decrease before stabilising after first thinning, reaching a trough in 1979 and tending to increase at the end of the 1970–1987 growing period. In contrast, the mean *inter-*ring

![Graph showing mean inter-ring trajectory of microfibril angle in S2 layer wall from pith to bark for each growth category. For legends, see Fig. 1.](image)
trajectory of the fast-growth category is stationary from 1959 to 1967, before an increase in 1971. Thereafter, fast-grown spruces follow a pattern similar to slow-grown spruces, with the exception of a trough in 1983 instead of 1979.

More specifically, the difference between growth categories is significant only in 1959 before first thinning (Fig. 2), as already suggested by the intra-ring analysis for that year (Table 1). As discussed above, this difference is likely to be related to the presence of a false ring instead of actual growth rate effects, since the trees were supposed to grow in similar stand conditions over the 1955–1969 period. Accordingly, non-significant G C main effects are reported for this period (Table 3). On the other hand, Year main effects are significant (Table 3), but must be due for a good part to the decreasing pattern of slow-grown spruces before first thinning.

A significant interaction between G C and Year is reported before first thinning (Table 3). In fact, the MFA mean inter-ring trajectories of the two growth categories are crossing in 1963 (Fig. 2). Thus, tracheids of trees classified as fast-grown on the basis of their circumference increment between 1970 and 1987 seem to have, on average, smaller microfibril angles related to the cell axis in the very first juvenile rings (i.e. until 1963), before the tendency is reversed in the rings formed later.

Microfibril angle is known to be inversely related to tracheid length, with longer tracheids having steeper angles (Erickson & Arima 1974; Donaldson 1992), and the pattern of tracheid length from pith to bark reflects that of microfibril angle in the S2 layer (Hiller 1964; Marton et al. 1972; Megraw 1985; Cave & Walker 1994; Walker & Butterfield 1995). In our study, these rules seem to be readily applicable to the slow-grown spruces, but not to the fast-grown spruces. However, as mentioned above, the MFA mean inter-ring trajectories of the two growth categories are crossing in 1963. This crossing is mirrored by that of the inter-ring trajectories of mean tracheid length in the same year in related studies (Herman et al. 1998a, b). The combination of the two crossings does suggest an inverse relationship between MFA and tracheid length.

After first thinning, the MFA yearly mean increases in fast-grown spruces and the difference in mean between growth categories is significant in four of the five sampling years (Fig. 2). Since the application of first thinning and the transition from juvenile wood to mature wood overlap in this study (see the dotted line in Fig. 2), it

Table 3. Inter-ring repeated measures analysis of variance across sampling sites: Results in terms of significance probabilities (p) of the F tests.1

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<tr>
<td></td>
<td>ANOVA</td>
<td>Modified ANOVA</td>
</tr>
<tr>
<td>G C Year</td>
<td>0.427</td>
<td>0.015*</td>
</tr>
<tr>
<td>G C * Year</td>
<td>0.011*</td>
<td>Epsilon = 0.763</td>
</tr>
</tbody>
</table>

1) See Table 1 for notations.
may be held that the average MFA for fast-grown spruces increases from 26° in juvenile wood (i.e. before first thinning) to 29° in mature wood (i.e. after first thinning). Reversely, the average MFA for slow-grown spruces decreases from 29 to 21°; this pattern is more in accordance with the one usually observed (Butterfield 1998). Growth Category main effects are highly significant after first thinning (Table 3). Thus, the increased growth rate of Norway spruce negatively affects the inter-ring trajectory of microfibril angle in that spruces growing faster than 2.2 cm/year in circumference have, on average, significantly larger microfibril angles (+8°) than slow-grown spruces, which represents an increase of 38%. These results are consistent with those reporting significant effects of increased growth rate by fertilisation and thinning treatments on microfibril angle, in Douglas fir (Erickson & Arima 1974) and Pinus luchuensis (Oda 1983).

Significant Year main effects are reported for the 1970–1987 growing period (Table 3). This underlines the non-stationarity (i.e. non-constancy) of the MFA yearly mean over time and hence, the importance of taking this feature into account in other data analyses (e.g. in correlation analysis, see Dutilleul et al. 1998). However, the interaction between G C and Year was not significant after first thinning, whereas the G C main effects were significant (Table 3). Thus, the MFA mean inter-ring trajectories of the two growth categories are parallel, with one category (i.e. fast-growth) above the other (i.e. slow-growth) since the growth categories differ in the overall mean of MFA. The fluctuations of microfibril angle from sampling year to sampling year (see the significant Year main effects in Table 3, both before and after first thinning) may reflect a predominant influence of climate on cell physiology instead of genetic factors which control the basic character of wall architecture (Boyd 1985).

Microfibril angles at first and last sampling sites within a growth ring

Growth Category main effects on the MFA of first formed tracheids are significant in the rings observed after first thinning (Table 4). On average, the MFA of these earlywood tracheids is significantly larger (+10°) in Norway spruces growing faster than 2.2 cm/year in circumference than in those with lower growth rate. Given the acknowledged importance of genetic factors in the control of microfibril orientation in the cell wall (Boyd 1985), growth rate effects could be less influential at the beginning of a new vegetation period. Our results show that an increased growth rate affects not only the intra-ring trajectory of microfibril angle, but also the cell wall formation of the first formed tracheids in earlywood. The absence of significant interaction between G C and Year at the first sampling site within a growth ring (Table 4), combined with the absence of significant interaction between G C and Site in the intraring analysis (Table 1), suggests that the MFA of first formed tracheids determines the level of the whole intra-ring trajectory for each growth category.

For the 1970–1987 growing period, growth category effects on the MFA at last sampling site within a growth ring are not significant, although the significance probability (p = 0.060) is close to the 0.05 significance level (Table 4). In fact, fast-grown spruces show a larger MFA in latewood tracheids (+5° on average) than slow-grown spruces, which represents a difference of less magnitude than for the first formed tra-
Table 4. *Inter*-ring repeated measures analysis of variance for first and last sampling sites within a growth ring: Results in terms of significance probabilities (p) of the F tests.¹

<table>
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<tbody>
<tr>
<td></td>
<td>ANOVA</td>
<td>Modified ANOVA</td>
</tr>
<tr>
<td>First site (earlywood)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G C</td>
<td>0.349</td>
<td></td>
</tr>
<tr>
<td>Year</td>
<td>0.004**</td>
<td></td>
</tr>
<tr>
<td>G C * Year</td>
<td>0.065</td>
<td></td>
</tr>
<tr>
<td>Epsilon =</td>
<td>0.961</td>
<td>Epsilon =</td>
</tr>
<tr>
<td>Last site (latewood)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G C</td>
<td>0.833</td>
<td></td>
</tr>
<tr>
<td>Year</td>
<td>0.003**</td>
<td></td>
</tr>
<tr>
<td>G C * Year</td>
<td>0.359</td>
<td></td>
</tr>
<tr>
<td>Epsilon =</td>
<td>0.840</td>
<td>Epsilon =</td>
</tr>
</tbody>
</table>

¹ See Table 1 for notations.

Cheids (+10° on average). Further studies using a larger number of trees in each growth category would help clarify the almost significant effects that we observed.

Year main effects are highly significant at first and last sampling sites within a growth ring before first thinning, but totally disappear over the next growing period under thinning treatment (Table 4). For both growth categories, the MFA of first and last formed tracheids seems to be subjected to fluctuations in juvenile wood, but remains more or less constant over growth rings in mature wood (see the first and last points of the mean *intra*-ring trajectories in Fig. 1a, b). In juvenile wood, MFA values at the end of the vegetation period vary over years, and the observed pattern is a decrease from 1959 to 1967. As many studies report about microfibril angle in latewood tracheids, this part of our results is in accordance with the widely acknowledged gradual decrease outwards of this ultrastructural characteristic of cells (e.g., Hiller 1964; Shupe et al. 1983; Bendtsen & Senft 1986; Cave & Walker 1994; Butterfield 1998). The non-significant Year main effects at first and last sampling sites within a growth ring after first thinning (Table 4) emphasise growth rate effects in comparison with genetic and climatic factors.

**Practical consequences**

This study supports the hypothesis that an increased growth rate, from 1.6 to 2.9 cm/year in circumference on average, results in larger microfibril angles in the S2 layer of cell wall in Norway spruce. The difference in microfibril angle between growth categories appears significantly in the *intra*-ring trajectories after first thinning. Over the corresponding five sampling years, the fast-grown spruces show an average microfibril angle of 29°, which is 8° more than for the slow-grown spruces.
It has already been reported that wider microfibril orientation related to fibre axis goes together with lower tensile strength, lower tear strength and higher longitudinal shrinkage of fibres, and when solid wood is considered, lower stiffness (Hiller 1964; Horn 1974; Hitchings 1984; Boyd 1985; Megraw 1985; Bendtsen & Senft 1986; Stuart & Evans 1995). Compared to juvenile wood whose poor technological properties are well known (Armstrong et al. 1977; Bendtsen & Senft 1986), mature wood of fast-grown Norway spruces presents similar microfibril angles (26° versus 29°), whereas values reported for mature wood in slow-grown spruces are much lower (29° versus 21°) (Fig. 2). Assuming 30° is the cut-off point between acceptable and unacceptable wood quality (MOE < 1000 kg/mm²; Cave 1968) (Donaldson 1992), wood and fibre from fast-grown Norway spruces (growth rate > 2.2 cm/year) may be expected to have worse technological properties than slow-grown Norway spruces (growth rate < 2.2 cm/year) because the average microfibril angle of fast-grown spruces is closer to the cut-off point.

In the context of a broader study of the influence of increased growth rate on morphological characteristics of tracheids that can influence pulp and paper properties (e.g., lumen diameter, wall thickness, fibre length), some mechanical tests have been performed on Kraft pulp samplings made from wood of each growth category (Herman 1997). Whereas tear index as well as breaking length of pulp were not influenced by the growth rate of the trees, fast-grown spruces showed significantly lower burst index (−12.5%) and fibre strength (−18%). Since no morphological characteristic was found to be significantly different between growth categories, the only explanation for lower burst index and fibre strength would be in the larger microfibril angle in the S₂ layer of cell wall, as shown in this study. This sheds light on the importance of taking the microfibril angle into account when assessing the influence of increased growth rate on wood and fibre quality in softwoods, and on the need to extend conventional thinking about wood quality beyond primarily wood density (Walker & Butterfield 1996).

CONCLUSIONS

Growth rate effects have been assessed on intra- and inter-ring trajectories of microfibril angle in the S₂ layer of tracheid wall, in 14 Norway spruces classified as 7 fast- and 7 slow-grown trees according to Belgian growing conditions. While an increased growth rate strongly affects the annual ring width (Herman et al. 1998a), the gradual decreasing pattern of microfibril angle from earlywood to latewood is maintained. However, significant growth category effects are reported in the intra-ring analysis, as Norway spruces growing faster than 2.2 cm/year in circumference after first thinning systematically present larger microfibril angles. Significant differences between growth categories are also observed during the thinning period in the first formed earlywood tracheids of the growth rings analysed, while differences are almost significant in the last formed latewood tracheids.

Slow-grown Norway spruces show the classical decreasing pattern of microfibril angle from pith to bark, whereas microfibril angle increases after first thinning in fast-grown spruces. Thus, growth rate also affects significantly the inter-ring trajectory of
microfibril angle. When averaged over the five sampling years of the thinning period, microfibril angle is about 29° in fast-grown Norway spruces, which is 8° more than in slow-grown spruces. The inter-ring trajectories of microfibril angle are characterised by a crossing of the two growth categories in 1963.

Sampling site and sampling year effects are significant in the intra-ring analysis and in the inter-ring analysis, respectively. This underlines the importance of taking the structure of doubly repeated measures data adequately into account in performing their statistical analysis. However, variations of microfibril orientation in the first and last sampling sites within a growth ring are not significant over time after first thinning, which emphasises growth rate effects in comparison with genetic and climatic factors.

The comparison of slow-grown versus fast-grown Norway spruces characterised by an average growth rate in circumference of 1.6 and 2.9 cm/year, respectively, has demonstrated a significant increase of the S₂ microfibril angle that may negatively change the quality of wood as well as the strength properties of pulp and paper.

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