

Adaptive Growth of Gymnosperm Branches-Ultrastructural and Micromechanical Examinations

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ABSTRACT

The top, the lateral and the underside of basal branch segments of two gymnosperm species, spruce (*Picea abies* [L.] Karst.) and yew (*Taxus baccata* L.), were studied with respect to possible adaptation in structural and mechanical properties. Microtensile tests were performed on thin wet foils, which were removed from the periphery of the branches. Structural parameters such as density and the microfibril angle in the S2-layer were examined to investigate the structure-function relationships of the branch wood. The top, the lateral and the

underside of both branches showed significant differences in their structural and mechanical properties. However, no significant variations were observed as a function of age and size development. The findings were discussed in view of adaptive growth strategies of trees, including biomechanical constraints of a horizontally growing branch.

Key words: Gymnosperm branch; Compression wood; Opposite wood; Microtensile properties; Microfibril angle; Adaptive growth

INTRODUCTION

The morphology of trees and the anatomy of wood are results of an adaptive growth to maintain mechanical stability and water transport (Niklas 1992). To cope with the requirements of horizontal growth and to allow active spatial orientation, gymnosperm trees evolved compression wood on the underside of leaning stems and branches (Wardrop 1965; Fournier and others 1994). During their differentiation, compression wood cells generate compressive stresses, which allow the branch to grow in a horizontal direction. In comparison to

tracheids of the “normal” stem wood, compression wood tracheids show several characteristic features. The cells have a round shape and there is no S3-layer in the secondary cell walls (Cote and Day 1965; Yoshizawa and Idei 1987; Timell 1983). The lignin content is increased (Timell 1973a) and the cellulose microfibrils in the secondary cell wall 2 (S2) are wound in a less steep helix compared to “normal” tracheids (Cote and Day 1965; Reiterer and others 1999). Although the mechanism of compressive stress generation is not clearly understood yet, Yamamoto (1998) showed that a microfibril angle above 30° is needed to generate compressive forces during cell differentiation.

To understand the adaptive growth of branches, the structural aspects and mechanical properties of the opposite wood at the top side and of the wood at

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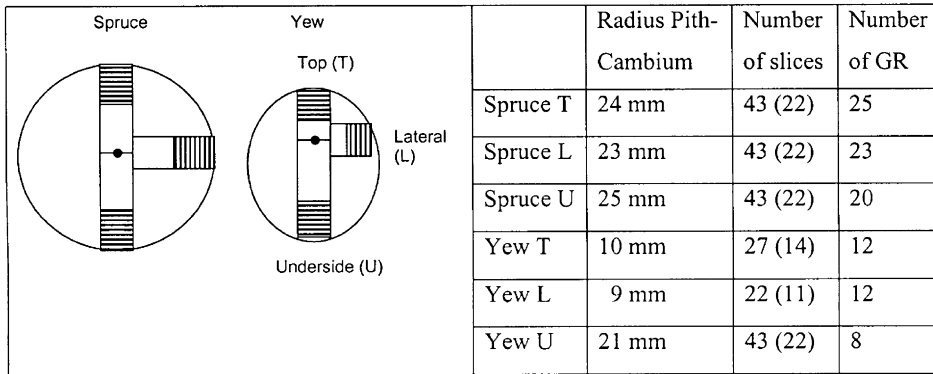


Figure 1. Schematic drawing of the cross-sections of the spruce and the yew branch. Distance (radius) between the pith and the cambium, the number of slices cut and (examined) the approximate number of growth rings (GR) in the investigated peripheral parts of the sections given for the top, the lateral and the underside of the branches.

the lateral sides also have to be studied. Only a few investigations have been performed on opposite wood at the top side of gymnosperm branches. In an investigation of the anatomy of opposite wood of three gymnosperm species Timell (1973b) reported that the growth rings were rather narrow but varied greatly in width. Park (1986) showed that in contrast to compression wood, the opposite wood tracheids have a S3 layer that is approximately 2-3 times thicker than in "normal" wood. Furthermore, opposite wood has a significantly lower microfibril angle than compression wood (Park 1984; Reiterer and others 1998; Färber and others 2001). The lignin content and the hemicelluloses composition of opposite wood and "normal" wood at the lateral side are almost the same (Timell 1973a). However, "normal" branch wood should not be equated with normal adult wood of the stem because for example, the tracheid length of the branch wood is significantly shorter and the microfibril angle is much higher (Park 1984).

Variations of the mechanical properties of stem, branch and root systems have been investigated with regard to adaptive growth under biomechanical constraints (Stokes and Mattheck 1996; Niklas 1999; Hoffmann and others 2003). Our study focused on the structure-function relationships at the base of a horizontally growing branch. The top (opposite wood), the lateral and the underside (compression wood) of spruce (*Picea abies* [L.] Karst.) and yew (*Taxus baccata* L.) were examined. Thin wood foils of the branch wood were studied by performing microtensile tests, density measurements and examinations of the microfibril angle by using wide angle X-ray diffraction. Considering former studies on the distribution of microfibril angles in spruce branches (Reiterer and others 1998; Färber and others 2001), adaptive growth strategies with increasing age and size of the branch and thus, an increasing load were discussed.

MATERIAL AND METHODS

Straight segments 60 mm long were cut out from the base of branches of spruce and yew not directly but relatively close to the stem connection (less than 20 cm distance). Fresh segments were stored in foils in a freezer to avoid subsequent tissue dehydration until further sample preparation. The radial distance between pith and cambium was measured at the top, the lateral and the underside of the green branch segments. The segment of the yew branch had an eccentricity of the growth, whereas the spruce showed only a slightly greater distance between pith and cambium at the underside (compression wood). The underside of the branch segments contained almost exclusively compression wood. The wood on the top side was categorized as opposite wood, the wood of the lateral side as "normal" branch wood.

The branch segments were trimmed to produce rectangular wood blocks 60 mm long, 5 mm wide and thickness according to the radial distance between pith and cambium (Figure 1). The inner part (close to the pith) of the wet wood blocks was clamped in the specimen holder of a sledge microtome. Approximately 220 μm thick tangential foils were sliced continuously until approximately 9 mm from the periphery of each section of the branches were removed (for the thinner top and lateral side of yew as many slices as possible were prepared). The peripheral parts of the branches were selected for examination, because a horizontally growing branch is predominately stressed in bending. Therefore, the peripheral parts of the branches contribute a disproportionately higher second moment of area than the unexamined inner parts.

Every second sliced wood foil (dimensions: L: \sim 60 mm T: \sim 5 mm R: \sim 0.22 mm) was selected for micromechanical investigations. Microtensile tests were carried out on a micro-tensile device similar to conventional uniaxial testing machines to deter-

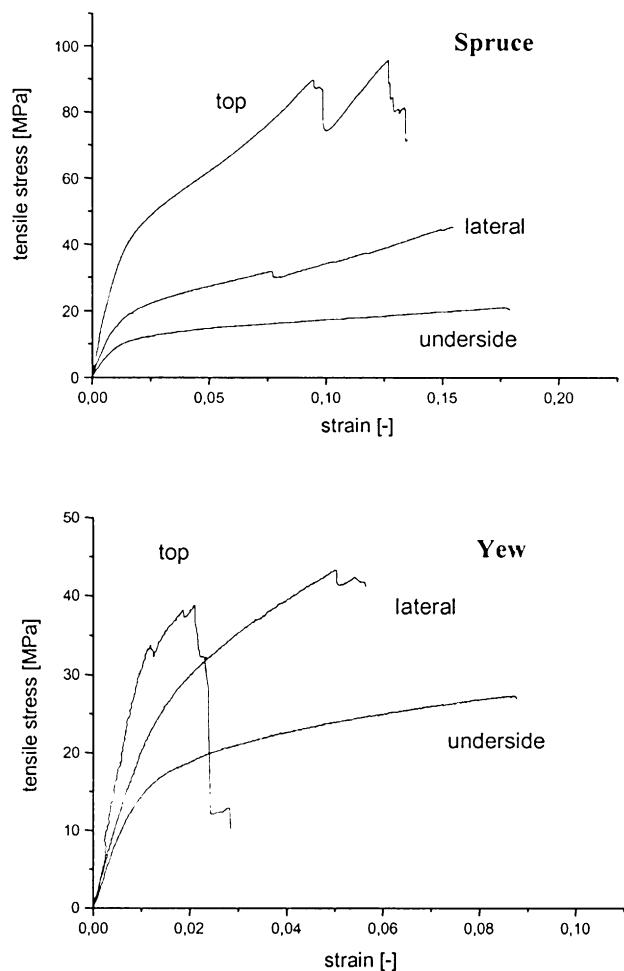


Figure 2. Representative stress-strain diagrams of wet thin tissue foils of spruce and yew; top side (opposite wood), lateral side and underside (compression wood).

mine the modulus of elasticity. Because the specimens were not tapered, the tensile strength was not calculated. The specimens were clamped into jaws (span length of the specimens ~ 23 mm), which were driven by two spindle crossheads. The machine was driven by a DC motor and a gear which enabled displacement rates of $10 \mu\text{m/s}$. For further details see Fröhmann and others (2003). To obtain accurate information about the elongation of the specimen and to avoid errors by loose fits of the threads or the jaws, the microtensile device was combined with video extensometry.

With respect to the structural parameters, the density (ρ) and the cellulose microfibril angle in the secondary cell wall 2 (S2) were measured. Density was determined by dividing the oven dry weight of a part of the specimen by its wet volume. For determining the microfibril angle in the S2 unstrained parts of the thin wood, tissue foils were

examined by wide angle X-ray diffraction using a position-sensitive detector in transmission geometry. The microfibril angle (MFA) was evaluated on the basis of cellulose (110) reflections (Lichtenegger and others 1998). For yew, the microfibril angle was determined for all mechanically tested foils, for spruce every second specimen was examined.

RESULTS AND DISCUSSION

Representative stress-strain curves of the top, lateral and underside of the spruce and the yew branch are shown in Figure 2. Except for the top side of the yew, all curves show two distinctly different slopes.

After a relatively short initial linear stage, a typical yield point was observed. Beyond the yield point the wood specimens went through large deformations. This appears to be a typical tensile behavior of wet plant tissues with a high microfibril angle (Navi and others 1995; Spatz and others 1999; Köhler and Spatz 2002; Keckes and others 2003; Burgert and others 2004). For spruce, the top side of the branch had the stiffest wood, whereas the wood of the underside was the most flexible. In accord with its position in the branch, the lateral side showed intermediate properties. The stress-strain curves of yew indicated a similar behavior but less pronounced than for spruce, because the stiffness in the initial stage differed less among the three tissue types. The main difference between the lateral side and the underside was that the specimen of the lateral side reached the yield point at a higher stress level and the stiffness beyond the yield point was significantly higher. For spruce, the tissue properties of the lateral side seemed to be more closely related to compression wood, whereas for yew, the stress-strain levels of the curves of the opposite wood and the lateral wood were more corresponding. This might be related to the fact that the lateral side of the yew branch was shifted to the top because of the eccentricity of growth. Comparing the stress-strain diagrams of spruce and yew with respect to the maximum strain levels, the spruce samples of all three segments (top, lateral, underside) went through larger deformations than the yew samples. However, deviating from a former investigation on different compression wood types (Burgert et al. 2004), the underside of yew showed a slightly higher stiffness before and only half the deformation beyond the yield point, which may be due to a small difference in microfibril angle.

In Figure 3 the modulus of elasticity before the yield point, the microfibril angle and the density of the branch sections of spruce and yew are shown by

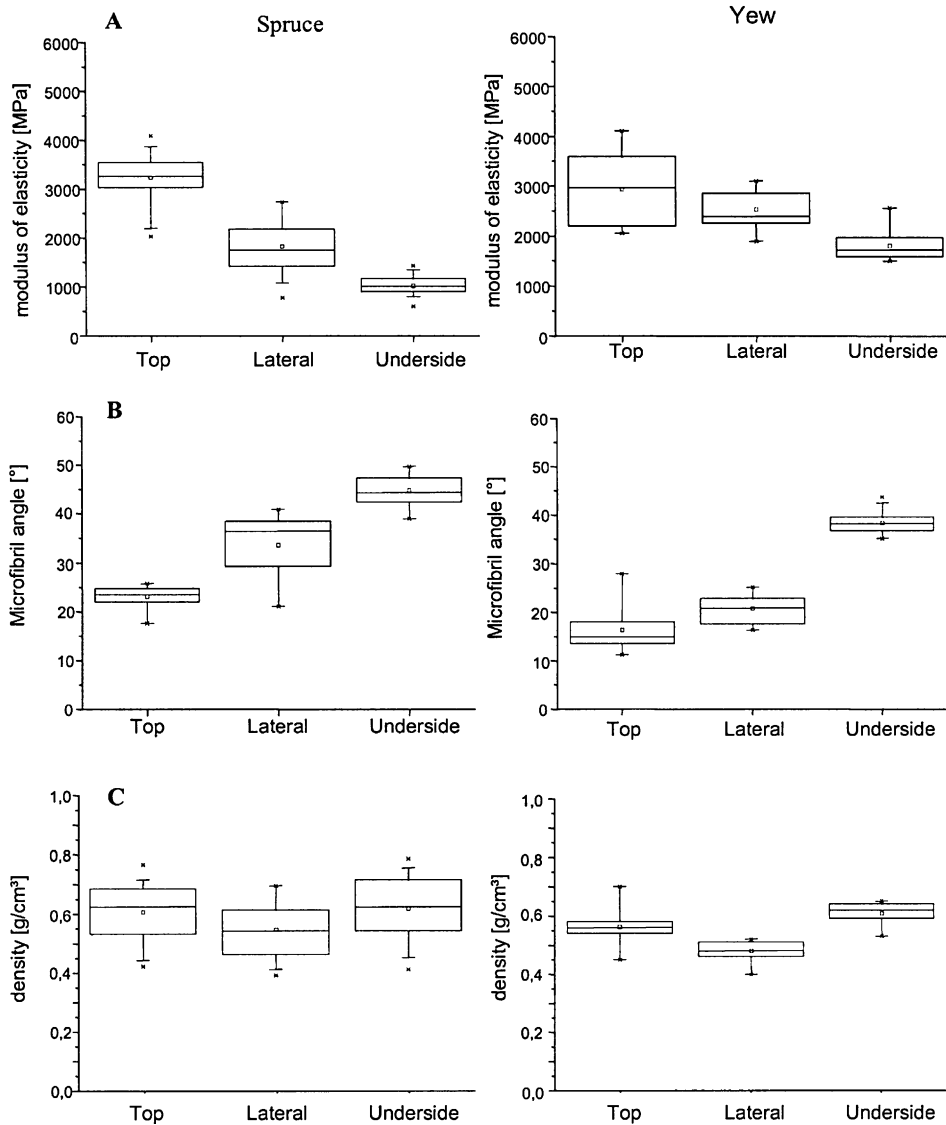


Figure 3. (A) Modulus of elasticity in the initial stage before the yield point of wet thin tissue foils, (B) microfibril angle in the secondary cell wall 2 (S2), (C) tissue density of spruce and yew of the top side (opposite wood), the lateral side, and the underside (compression wood) depicted as box-and-whisker plots; 50% of the values inside the box; median: line in the box; arithmetic mean: rectangle in the box.

box-and-whisker-plots on the basis of the raw material.

Spruce and yew corresponded greatly and showed the same branch section-related order. For both species the stiffness of the top side was the highest and the stiffness of the underside was the lowest (Figure 3A). However, the differences between the branch sections were more pronounced for spruce. The underside showed the lowest (standard) deviation. Obviously, particular for yew, the scatter of the data increased towards the top side. The microfibril angle showed a definite trend among the branch sections (Figure 3B). For both species the compression wood at the underside had the highest and the opposite wood at the top-side the lowest microfibril angle. Additionally, spruce and yew showed similar data trends with respect to the density (Figure 3C). The compression wood and

opposite wood of spruce had almost the same density, whereas the lateral side contained the lightest tissue. For yew the lateral side had the lowest density as well. In contrast to spruce the compression wood of yew showed a higher density than the opposite wood.

The highly corresponding data of spruce and yew indicate that the microfibril angle is the most important indicative feature of the mechanical behavior of each branch section and in consequence of the whole branch. Normally, wood tissue density serves as a good predictor of the modulus of elasticity according to the theory of cellular solids (Niklas 1997). However, the box-and-whisker-plots of the modulus of elasticity, density and microfibril angle show that for the branch wood of spruce and yew, the microfibril angle is the most effective structural parameter.

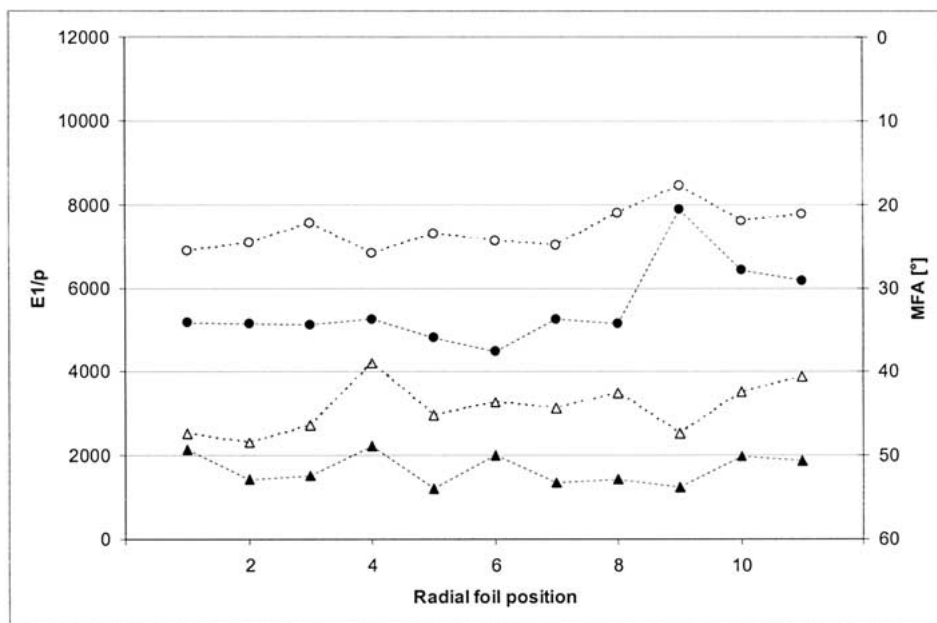


Figure 4. Modulus of elasticity in the initial stage before the yield point divided by density ($E1/p$) of wet thin tissue foils and microfibril angle (MFA) of spruce of the top side (opposite wood) and of the underside (compression wood). Symbols: Opposite wood: $E1/p$ (●), MFA (○); compression wood: $E1/p$ (▲), MFA (△). On the abscissa the radial positions of the thin tissue foils are given. Number 1 is defined as the radial position closest to the cambium, whereas higher numbers stand for positions closer to the pith. A second ordinate for the microfibril angle (MFA) with numbers in reversed order is given. To assign the matching data points, symbols are connected by dotted lines.

In Figure 4 the radial trend of the mechanical and structural properties of the top-side (opposite wood) and of the underside (compression wood) within the peripheral parts of the basal segment is shown exemplarily for the spruce branch. Because the microfibril angle was determined for every second foil, only every second stiffness data point is shown. For this detailed view on the mechanical and ultrastructural properties with respect to the radial position, the modulus of elasticity was divided by the density of the wood foils. The possible variation of mechanical properties caused by different densities had to be excluded because the approximately $220\ \mu\text{m}$ -thick foils were sliced continuously without distinguishing between the earlywood and latewood layers of the rather narrow growth rings. Therefore, the slices contained different relative volume fractions of earlywood and latewood influencing the variation of the modulus of elasticity.

Considering the variation of the parameters, the magnitude of the stiffness divided by the density ($E1/p$) varied partly in accordance with the microfibril angle. The stiffness and the microfibril angle did not show any specific radial trend, neither for the top nor for the underside. Thus, our data indicate that the microfibril angle and the mechanical properties in the peripheral parts at the base of the

branch did not change as a function of age and size development.

There is a wide variation in mechanical properties among the top, the lateral, and the underside of the branches. However, no radial trend of the cellulose microfibril orientation and the mechanical properties within a single branch section was observed. In a highly systematic study on the distribution of the cellulose microfibril angle in a spruce branch, Färber and others (2001) observed that the microfibril angle of the opposite wood showed a large variation with respect to the distance to the trunk. However, at the base of the branch they found no definite trend and only slight variations of the magnitude of the microfibril angle across the growth rings. For the underside (compression wood) of a spruce branch, Reiterer and others (1998) measured a microfibril angle of approximately 40° in the 3rd, 7th and 18th growth ring. Only the first two growth rings had a lower microfibril angle of about 32° . For the top side (opposite wood) the microfibril angle was constant across all growth rings.

Our measurements focused on the peripheral parts at the base of gymnosperm branches. However, for spruce more than $1/3$ of the radius from the pith to the cambium, and for yew because of the eccentricity and the smaller diameter of the branch -

for the top and the lateral side an even higher radial proportion was investigated. After comparing our data with the measurements performed by Färber and others (2001) and Reiterer and others (1998) it seems reasonable to conclude that the microfibril angle and the tensile stiffness at the branch base remain unchanged. In consequence, during the growth of the branch the tissue properties at its base require no size and age-dependent adaptation. Even though the branch is growing at the tip and the shape of the branch is changed, the newly formed wood still has the same microfibril angle and the same modulus of elasticity as the older one.

These findings lead to several implications with respect to adaptive growth and growth regulation. Comparing the growth pattern of stem and branches it seems reasonable to assume that trees have developed different growth strategies. Trunk and branches can be categorized as cantilever beams, growing in a vertical and horizontal direction, respectively. In the trunk the young tree forms juvenile wood with a large microfibril angle making the stem flexible enough to bend under external loads (Fratzl 1999). During further radial growth, the strategy has to be changed to withstand increasing bending forces and a much stiffer adult wood with lower microfibril angles is produced. The change of the structural and mechanical properties from juvenile wood to adult wood has been shown by means of the microfibril angle in the S2-layer (Lindström and others 1998) as well as the tensile properties of single wood fibers (Groom and others 2002).

In contrast to the trunk, at the base of a branch the microfibril angle—and therefore, also the mechanical (tensile) properties—remains almost constant across the radial distance between pith and cambium. The difference between the growth patterns of trunk and branches might be related to the influence of gravity. The horizontal growth of the branch requires a continuous generation of compressive stresses by the compression wood at the underside. The compressive stress generation in every single cell during differentiation relies on a microfibril angle in the S2 layer higher than 30° (Yamamoto 1998). In consequence, the compression wood is a tissue with a rather low stiffness compared to normal adult wood. With respect to external compressive forces the high microfibril angle is partly compensated by the increased lignin content of the compression wood. However, given the fact that the elastic modulus of the opposite wood would be much higher than the modulus of elasticity of the compression wood, the compressive stresses generated during cell differentiation would

not be sufficient to keep the branch in a horizontal position. Thus, the demand for compressive stress generation at the underside determines the growth pattern of the compression wood and the opposite wood simultaneously and continuously. For this reason stiffening of the top-side by formation of opposite wood with lower microfibril angles would be counter-productive at the base of the branch. However, gymnosperm trees still have to cope with an increasing load due to the axial and radial growth of the branch, although they seem to be limited with respect to mechanical adaptation at its base. Färber and others (2001) provided a map of microfibril angles all over the branch and could show that the microfibril angle decreased in the outer growth rings of the opposite wood at a certain distance (1-2 m) from the stem. In consequence, the stiffening of the opposite wood at the top side expected as a function of age and size development occurs not directly at the base of the branch but is shifted slightly towards its tip. Thus, gymnosperm branches have evolved an optimized distribution of structural and mechanical properties to maintain horizontal growth in spite of an increasing load.

CONCLUSIONS

There is a wide variation in mechanical and structural properties among the top, the lateral, and the underside within the branches of spruce and yew. However, it seems that these features do not change as a function of age and size development at the base of the branch. From a biomechanical point of view this can be explained by the fact that for the horizontal growth of a gymnosperm branch, compressive stresses have to be generated continuously at the underside. Because the generation of compressive stresses requires a high microfibril angle in the S2-layer, in consequence the stiffness of the compression wood is rather low. In consequence, the stiffness of the opposite wood cannot exceed the stiffness of the compression wood too much, because generation of compressive stresses would otherwise not be sufficient to hold the branch upright for horizontal growth. Therefore, from a biomechanical point of view the most promising growth strategy is to form tissues with almost constant properties at the base of a branch.

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