REACTION WOOD FORMATION
DURING STEM GRAVITROPIC RESPONSE
OF YOUNG PICEA ABIES (L.) KARST. TREES

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with cooperation of

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Wydawnictwo SGGW
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Introduction

In ancient Chinese art (4th–13th centuries), landscapists often glorified the serenity of nature (Figs. 1 and 2). Compositions of rocks, wood ponds, and solitary trees provided a background for small human figures lost in contemplation.

The subtle strokes of the artist’s paintbrush portraying contorted trees stand in sharp contrast to the invisible enormous stresses at work within the trunk and branches. The truth is that trees growing on mountain slopes, with their crowns shaped by avalanches, light vectors inviting them towards abysses, and exposed to rapid currents of water, constantly fight for survival. While the development of every plant is a continuous response to its environment, the response of trees found on mountain ridges, in crevices, and at the timberline, is very pronounced and dramatic, fascinating not only to painters, but also to botanists. The language of this response is anatomy, expressing itself in the formation of special tissue, known as reaction wood.

**Figure 1.** Landscape with great pine Ma Lin 13th century, China (Courtesy of Metropolitan Museum of Art, New York)

**Figure 2.** Dragon pine Wu Boli 14th century, China (Courtesy of Metropolitan Museum of Art, New York)
1.1. The causes of reaction wood formation

Reaction wood is formed in the stems, branches (Wilson and Archer 1977; Timell 1986; Nicoll and Ray 1996; Coutand et al. 2007; Gardiner et al. 2014), and leaves (Sperry 1982; Tomlinson and Fisher 2005) of both coniferous and deciduous trees. Its functions include:

a) maintaining the existing spatial orientation of the organs, e.g. as the weight of the lateral branches increases;

b) reorienting the branches or the stem (righting of the stems of trees growing on slopes, inclination of the branches of trees in coastal areas, taking over of apical dominance by lateral branches).

There is still some controversy as to the formation of reaction wood in the roots; according to most authors it does not occur (Hsu et al. 2006) or occurs only if roots are exposed to light (Westing 1965; Timell 1986).

There is a fundamental difference in both the anatomy and function of reaction wood between coniferous and deciduous trees. Conifers form compression wood, which generates compressive stress along the stem axis, so by necessity it is located on the lower side of inclined stems or lateral branches. This stands in contrast to tension wood (reacting to tensile stress) in deciduous trees, which is formed on the upper side of the organ in the process of reorientation.

It should be emphasized that reaction wood gives rise to asymmetry in dimensions, geometry, and stresses in the anatomical structures of the plant organ, which add up to the “reaction” of the plant living in dynamically changing environment. Another question is whether such development should be termed reorientation, as in many cases this only leads to maintaining the system in a state of minimum energy. From this point of view, true reorientation would occur if the lateral branches yielded to increasing self-weight or if the plant did not grow towards the light source.

It is beyond any doubt that reaction wood is a major evolutionary innovation thanks to which woody plants gained greater capacity of adaptive growth, enabling tree crowns to actively change their geometry and main stems orientation reach a form parallel to the gravity vector. As reaction wood may have various anatomical forms, differences in their function between evolutionarily young and old woody plants are considered an important element of scientific inquiry in the evolutionary development of species.

For instance, orders such as Cycadales, which are primitive gymnosperms with xylem in the form of tracheids with scalariform pitting (Carlquist 2001) do not form compression wood in their stems (Timell 1986; Fisher and Marler 2006) but, e.g. plants of the genus Cycas develop gelatinous fibers in the root phloem (Tomlinson...
et al. 2014). In this respect, they are similar to Gnetales, which are considered an intermediary group between gymnosperms and angiosperms. Tomlinson, who studied *Gnetum gnemon*, found eccentric gelatinous fibers that may function as tension wood in the stem cortex and secondary phloem (Tomlinson 2001, 2003).

On the lower side of lateral branches, plants of the genus *Buxus* (an early-diverging lineage of angiosperms) develop tissue similar to compression wood with substantially greater lignification of the cell walls but without helical cavities or the S3 layer (Yoshizawa et al. 1992, 1993; Baillères et al. 1997). The tension wood of *Magnolia* and *Liriodendron* is characterized by the absence of the G layer, even though the eccentric pith and the increased division frequency in the upper portion of lateral branches, leading to wood with a very small microfibril angle, a high proportion of cellulose, and measurable tensile stress due to fiber contraction are thought to be characteristic of tension wood (Okuyama et al. 1994; Yoshizawa et al. 2000; Du and Yamamoto 2007). There are also some surprising forms of reaction wood; for instance, on the lower side of leaning stems, the angiosperm *Hebe salicifolia* develops reaction wood that is anatomically similar to normal wood, but the microfibril angle is highly increased in the cell walls, which generates a mechanical response similar to that found in compression wood (Kojima et al. 2012).

**1.2. Anatomy of reaction wood**

Reaction wood differs significantly from normal wood in a number of anatomical features, which again are very different for tension wood in deciduous trees (generating axial contraction) and compression wood in conifers (generating axial compression). Tension wood is characterized by an eccentric pith position, with its most important properties being the absence of the S3 layer in the cell wall, a small microfibril angle, and the presence of gelatinous fibers. It should be remembered that while almost half of angiosperms do not form gelatinous fibers, due to great plant diversity there are many exceptions to the rule, which are the subject of a considerable body of research. As this paper is devoted to compression wood, its anatomical structure will be discussed at length.  

**The defining characteristics of compression wood:**

1. Eccentric pith and darker growth rings (Fig. 3). The cambial cells of an inclined organ divide much more intensively on the side corresponding to compression wood development (the bottom side of a main stem), which leads to a clearly eccentric pith location. Compression wood layers are characterized by a much darker color, observable by the naked eye, which makes it easy to distinguish from normal wood (Timell 1986; Gryc and Horáček 2007).
2. Tracheid shape. Tracheids in compression wood are shorter than in normal wood, but in opposite wood they are usually slightly longer. Compression wood tracheids are clearly rounded, leading to intercellular spaces, which are absent from normal wood characterized by tracheids with quadrilateral cross-sections (Wardrop and Davies 1964; Timell 1973; Calbo et al. 1995; Calbo and Nery 2001).

3. Absence of the S3 layer. Compression wood tracheids lack the S3 layer; however, this is not the case in the mildest form of reaction wood, which shares some characteristics of both normal and reaction wood (Ruelle 2014).

4. Compression wood tracheids have a much thicker S2 layer, divided into two parts, with the external one being more lignified, and the internal one often containing helical cavities or ridges. Helical thickenings in the form of thin bands in the S3 layer, typical of *Taxus*, *Torreya*, and *Cephalotaxus*, are preserved in their compression wood, but in that of *Pseudotsuga*, *Larix*, and *Picea* these anatomical features take the form of helical ridges and cavities. In all cases, the chirality of the helix changes from right- to left-handed and vice versa. Interestingly, in the change in the microfibril angle in the cell walls of compression wood does not significantly affect the angle of the additional layer of helical ridges or cavities, which suggests that in *Pseudotsuga* they are formed more independently of the microfibril angle. No helical cavities have been found in the wood of *Ginkgo* or *Taxus* (Yoshizawa et al. 1985; Timell 1986; Fromm 2013).

5. Microfibril angle (MFA). It is known that the microfibril angle is regulated by the arrangement of cellular microtubules. However, the MFA differs between the various layers of the secondary wall of tracheids. As a rule, in the S1 and S3 layers microfibrils are arranged almost horizontally (approx. 75°), and in S2 almost vertically (approx. 10–30°). In contrast, in the S2 layer of compres-
sion wood, the angle is much larger (40–70°) as compared to and is strongly correlated with the content of hemicelluloses and higher lignification, affecting microfibril orientation. A lower MFA means reduced rigidity of the cells and their greater axial shrinkage. Many studies have reported that the MFA in lateral branches decreases from base to tip, both on the sides of compression and normal wood. This implies that branch wood is more flexible close to stem. However, it has also been indicated that the MFA varies considerably, especially in lateral branches, which may reflect the dynamic growth response of those organs to increasing weight or size (including aerodynamic resistance). Moreover, the MFA tends to decrease with age, and it is much lower in slow-growing trees as compared to fast-growing ones. It has been reported that the greater the inclination of the trunk, the greater the MFA (Lichtenegger et al. 1999; Plomion et al. 2000; Färber et al. 2001; Saren 2001; Thibaut et al. 2001; Barnett and Bonham 2004; Sedighi-Gilani et al. 2005; Rosner et al. 2007; Yamashita et al. 2007; Gierlinger et al. 2010; Sharma and Altaner 2014; Ruelle 2014).

6. Compression wood cell walls are significantly more lignified. Lignin is mostly composed of guaiacyl units (G-units). Compression wood lignin is enriched in p-hydroxyphenylpropane units (H-units), which are almost absent from normal wood (Yoshizawa et al. 1993; Baillères et al. 1997; Singh and Donaldson 1999; Selig et al. 2012; Villalobos et al. 2012; Aiso et al. 2013; Donaldson and Radotic 2013; Chavan et al. 2015).

7. Compression wood contains characteristic polysaccharides belonging to the group of galactans. β(1,4)-galactan is composed of crystallized galactose with β(1,4) bonds. In compression wood, the content of galactans is close to 10%, in contrast to normal wood, where they are present in much smaller quantities or entirely absent. These differences become apparent already during the formation of the S1 layer, and galactan content decreases with the development of S2. Interestingly, in Cryptomeria japonica galactan was not detected in the S2 layer in compression wood (as it is the case in normal wood walls). On the other hand, in Pinus radiata β(1,4)-galactan was found to occur uniformly in the cell walls of the strongly lignified tracheids of compression wood. This variation proves that the content of galactans is not related to lignification in compression wood. However, the literature also reports some data to the contrary, where increased lignification is correlated with higher galactan concentration in compression wood and a concurrent lower content of xylans and mannans. β(1,3)-glucan amounts to approx. 3% of the chemical composition of compression wood,
occurring mostly in the helical cavities of the S2 layer. The hypothesis that axial expansion of compression wood tracheids is attributable to the hydrophilicity of β(1,3)-glucan and high swelling properties was studied in the 1970s and 1980s. However, nowadays this theory is no longer thought to fully explain the compression wood mechanism of action. The invariably more pronounced lignification of the cell walls and the fact that laricinian is present only in the helical cavities of the S2 layer (but it may also be entirely absent from compression wood) are strong counterarguments to the hypothesis that β(1,3)-glucan is the cell wall component generating sufficient compressive stress to reorient plant organs. Also in this case, what should be taken into account, is not only the chemical composition of the cell wall, but also its dimensions and geometry. For instance, it is known that wood shrinkage is primarily proportionate to the thickness of cell walls, so this shrinkage is greater in compression wood mostly for that reason (Brodzki 1972; Hoffmann and Timell 1972; Boyd 1978; Włoch and Hejnowicz 1983; Zhang et al. 2000; Kelley et al. 2004; Mast et al. 2009; Altaner et al. 2010; Kim et al. 2010; Kojima et al. 2012).

8. Many authors have reported lower lignification of the middle lamella of compression wood tracheids (Côté et al. 1966; Yoshizawa et al. 1993; Singh and Donaldson 1999; Du and Yamamoto 2007; Aiso et al. 2013).

9. In compression wood tracheids, pits are less frequent and smaller; they are ellipsoidal and aligned with the microfibril angle (Mayr et al. 2006).

10. Tracheid ends in compression wood are often deformed, similarly to tracheids in traumatic xylem (Lee and Eom 1988; Zajączkowska 2014a, b).

11. Compression wood has a decreased content of cellulose, which is characterized by a lower degree of crystallization and shorter chains (Tanaka et al. 1981).

12. The width of macrofibrils (microfibril aggregates with a diameter of approx. 15 to 25 nm) is positively correlated with the degree of lignification in compression wood in Pinus radiata. The more pronounced the compression wood, the wider the S2 macrofibrils (Donaldson 1998).

13. Compression wood may contain numerous traumatic vertical resin ducts (Core et al. 1961).


15. In differentiating compression wood, cell walls contain a different make-up of proteins responsible for the formation of the cell wall, lignification, and polysaccharide formation (McDougall 2000; Plomion et al. 2000; Zhang et al. 2000).
1.3. Mechanism of reaction wood formation

1.3.1. Physical forces

In early research on the mechanism of reaction wood formation, many authors, mostly guided by their scientific intuition, hypothesized that physical forces directly affect cambium, leading to a special type of wood capable of counteracting the changing mechanical conditions. Even though it is now known that plant weight does influence wood induction (e.g. in *Arabidopsis*), there are also many stress-related genes that contribute to wood formation in *Arabidopsis* (Ko 2004; Sehr et al. 2010), and it is believed that reaction wood formation is not directly associated with the stresses acting on cambium. This was proven in an interesting experiment, conducted in 1938 by Jaccard, who bent tree stems into loops. In conifers, compression wood always formed at the lower side of the bent stem, which involved both the compressed side (the upper part of the loop) and the stretched side (the bottom part of the loop) (Jaccard 1919, 1938, 1939). Interestingly, compression wood was not formed in those parts of the loop that were positioned vertically. This was explained by the function of this special type of wood in organisms characterized by a polar structure (in the polar field of gravity, plants tend to reorient their positions to attain a minimum energy state). While this is largely true, the elaboration of this issue claiming that polarity and gravity also affect water and sugar transport, and so another function of reaction wood is to facilitate these processes, is for the most part false. The stem and branch loop experiments have been repeated many times in different configurations (Sinnott 1952; Schopfer 2006; Du and Yamamoto 2007).

1.3.2. Auxin

Many papers have addressed the question of whether auxin is the most important factor affecting cambium behavior in the process of compression wood formation. However, it is still not known whether the cambial cells giving rise to compression wood are molecularly determined at the very beginning of their arising, or perhaps there is a gradient between cambium and differentiating wood cells, which, similarly to the PCD (programmed cell death) process in wood, is responsible for cell death, and thus for the structural features of the tracheid (radial dimension or wall thickness). Therefore, many experiments have been conducted to elucidate the role of auxin in the process of compression wood differentiation, but no clear answers have been provided. On the one hand, there is some evidence that compression wood is
formed when the concentration of endogenous auxin in cambium is increased on the lower side of branches (Funada et al. 1990; Du et al. 2004), but there are also some reports to the contrary (Wilson 1986; Hellgren et al. 2004). On the other hand, compression wood has been reported to be stimulated by exogenous auxin (Wilson and Archer 1977; Timell 1986; Sundberg et al. 1994). Furthermore, an increase in the activity of the cambium giving rise to reaction wood is not always coincidental with an increase in the number of cambial cells (Sundberg et al. 1994; Little and Eklund 1999; Plomion et al. 2000; Du and Yamamoto 2007).

1.3.3. Ethylene

Many authors have reported that the emergence of reaction wood is accompanied by higher production of ethylene; however, exogenous ethylene does not lead to the formation of compression wood (Jaffe 1980; Telewski and Jaffe 1986; Timell 1986; Little and Eklund 1999; Du and Yamamoto 2003). It is noteworthy that an experiment involving inclined stems of *Pinus radiata* seedlings demonstrated the opposite: ethylene did stimulate the growth of compression wood (Ramos and Herrera 2013) or tension wood in *Populus tremula × P. tremuloides* (Andersson-Gunneras et al. 2003).

1.3.4. Transport

Compression wood constitutes a unique hydraulic system in trees and is an important part of studies upon reaction wood. Since this specific type of wood can occupy a larger cross-sectional area in the trunk region bent through gravitropic reaction in trees growing on slopes (Mattheck et al. 1994; Barij et al. 2007), the question arises as to the efficiency of water transport within this type of wood. It is known that the fundamental anatomical characteristic of xylem, responsible for specific hydraulic conductivity – $k_s$ (m²/s·Pa), is the lumen area of tracheids, which is significantly smaller in reaction wood (Timell 1986).

According to Mayr et al. (2006) $k_s = \frac{Q_l}{A_c \Delta P}$, where $Q_l$ is the volume flow rate (m³/s) in a segment of length – l (m), $A_c$ is the inner cannula cross-section (mm²), and $\Delta P$ is the pressure differential in the studied element (Pa). Experiments devoted to this issue have been conducted on seedlings, shoots, or directly on the trunks of growing trees. Reaction wood has been reported to exhibit decreased hydraulic conductivity – $k_s$; for instance, $k_s$ is by 52% lower in the compression wood of *Pseudotsuga menziesii* seedlings, as compared to opposite wood (Spicer and Gartner 2002). Interestingly, the same study also showed that the osmotic potential in the
leaves of inclined seedlings did not differ from the control plants, which suggests that from the point of view of the soil-tree-atmosphere system equilibrium is preserved and plants with reaction wood do not suffer from water stress. Mayr and Cochard (2003) found that the reaction wood in spruce shoots, whose conductivity was lower by 75% relative to opposite wood, was more susceptible to embolism (as determined by the hydraulic parameters obtained from a “Micro-Sperry apparatus”). The authors summarized their study with the conclusion that reaction wood as such had a primarily mechanical function. However, as their method allowed for analysis of only very small xylem areas (the probe diameter was 1.5 mm), they acknowledged that their results could not be the basis for making inferences about water transport in whole shoots, not to mention trees. A new study into the effect of compression wood structure on transport efficiency in spruce trees focused on an interesting anatomical property of annual rings containing reaction wood. It was found that at the beginning of each growth increment containing a pronounced layer of reaction wood (constituting the majority of the ring width) there was a light band (LB) composed of a row of tracheids that were almost identical to normal tracheids of earlywood (rectangular in cross-section, with thin cell walls, and with cellular lumen and pit apertures almost 3 and 1.7 times as large as those in the adjoining compression wood, respectively (Mayr et al. 2006). The hydraulic conductivity ($k_s$) of typical compression wood amounted to only 27% of that of opposite wood, in contrast to LB wood (8%). This shows that compression wood is not uniform either in anatomical or functional terms. By the same token, compression wood was also recognized as a tissue playing an important role in water conduction.

### 1.3.5. Molecular foundations of compression wood formation

Compression wood differs in terms of many anatomical and biochemical features from normal wood. As such, it provides a good model for studies into the activation of genes involved in cambium division, cell growth, lignification, polysaccharide synthesis, and the influence of phytohormones on the above processes. Thus, compression wood is also an excellent model for research on the molecular foundations of xylogenesis (Plomion et al. 2000, 2001). There are hundreds of genes and proteins whose activation is linked to the formation of reaction wood, and their abundance is positively correlated with the share of compression wood, the formation of non-cellulosic polysaccharides, and MFA (Mast et al. 2010; Sato et al. 2013).
In leaning trunks of the maritime pine (*Pinus pinaster*), genes responsible for intercellular communication and signal transduction were found to be activated 2.5 h after deviation from the vertical, both in the upper and lower regions of the trunk. After 10 h, while the molecular response in the upper region of branches did not change, gene activation in the lower trunk region was exclusively linked to proteins with a binding function. After 24 h and throughout the following month, proteins with a binding function were coded in both regions (Ramos et al. 2012). The transcripts occurring in small quantities in the compression wood of *Chamaecyparis obtusa*, and in large quantities in normal wood, typically had an inhibiting effect on the development of compression wood. For instance, the abundance of transcript Cod4u2258901, responsible for the process of cell wall lignification in upright trees was high, while it was low in inclined trees (Yamashita et al. 2009). The activation of α- and β-tubulin genes responsible for the orientation of microfibrils in the cell walls controlled by microtubule-associated proteins was observed in lateral branches of the radiata pine (*Pinus radiata*), while microarray gene transcription showed that as many as 29% of xylem transcriptomes were remodeled (Li et al. 2013).

### 1.4. The function of compression wood

Compression wood differs from normal wood not so much in compressive strength, but in its ability to create strong compressive stresses along the axis of the plant organ. There are many theories describing the formation and effects of compressive stresses in reaction wood, but the matter still does not seem to have been fully elucidated. One of the more prominent, but still disputable, theories proposes cellular elongation in the course of lignin accumulation between the oblique microfibrils. By filling the available spaces, amorphous lignin leads to axial compression with the neighboring tracheids blocking the process of tracheid elongation. This process is deemed to be correlated with the amount of lignin in the cell walls (Boyd 1972; Abasolo et al. 1999). This theory has been heavily criticized. First of all, it is thought that while cell wall swelling due to lignin deposition may somewhat contribute to compressive stress, this is only the case if the MFA is greater than 40° and, second, this phenomenon should also be reflected in cross-sectional cell geometry (xylem cells are more or less quadrilateral following cambial division, so theoretically they should preserve this shape also during transverse growth due to swelling; however, in reality tracheids are rounded in cross-section). In turn, according to Bamber’s helical spring theory, microfibrils in the cell walls of gymnosperms are in a compressed state, due to which they exert compressive stresses along the axis of plant organs (Bamber 2001). This microfibril mechanism is fully functional for tra-
cheids that are rounded, rather than quadrilateral, in cross-section. In addition, reaction wood arises under conditions of axial compression, tracheid ends are deformed, and the MFA does not reach low values in the course of the mechanically constrained process of tracheid maturation. According to Bamber (2001), lignin is not an active element, but rather a factor providing rigidity to the microfibril system, increasing its compressive strength. The author compares the function of lignin to that of cement in pre-stressed reinforced concrete structures. Yet another theory suggests that reaction wood is a special tissue whose function is to reduce local stress maxima, which would explain pronounced growth ring eccentricity in organs containing reaction wood (Archer and Wilson 1970, 1973).

1.5. Aim of work

It follows from the above brief review of the literature that the compression wood differs from the normal wood in number of characteristics of the tracheids. However, various changes in the tracheid parameters occur also in normal wood with increasing the age of a tree stem. Particularly marked changes take place during juvenile period of life comprised within the range of a few to several years (Fabisiak 2005). The result of this diversity is the formation of so-called juvenile wood, which in some species is significantly different from the wood formed later called mature wood (Zobel and Sprague 1998). The present paper attempts to describe the changes in the structure of reaction wood occurring with age during juvenile period of tree growth as compared to the structural changes of tracheids in normal wood. In order to accomplish this task the field experiment with young Norway spruce trees was performed. The trees were induced to form compression wood by planting them into the ground with the main stems oriented obliquely. Studies of the structure of wood concerned period of five years from the start of the experiment, during which due to the gravitropic reaction the upper part of the stem reoriented into the vertical position.

Analysis of wood structure was performed using a unique technique SilviScan which enables fast comprehensive measurements of various characteristics in the same radial files of tracheids. The measurements of were performed in Innventia Wood and Fibre Measurement Centre in Stockholm. The experimental work as well as additional comparative wood measurements with applying standard WinDENDRO and WinCELL techniques were made at the Warsaw University of Life Sciences – SGGW. This cooperation was supported by the project co-funded by the European Union Seventh Framework Programme FP7 under grant agreement No. 284181 Trees4Future, Transnational Access.
Materials and methods

The field study was conducted at the Arboretum of the Warsaw University of Life Sciences – SGGW in Rogów (central Poland), while laboratory work at the Inventia Wood and Fibre Measurement Centre in Stockholm (Sweden) and at the Department of Forest Botany of the Warsaw University of Life Sciences – SGGW (Poland). The studied material consisted of wood samples from young Norway spruce [Picea abies (L.) Karst.] trees.

2.1. Experimental

In 2005, at the Arboretum of the Warsaw University of Life Sciences – in Rogów, six five-year-old Picea abies saplings were planted experimentally at an angle of approx. 45° (Fig. 4) to induce the formation of reaction wood, which tends to reorient the stems back to a position parallel to the vector of gravity.

Figure 4. Norway spruce trees planted experimentally at stem axis angle 45° to induce formation of compression wood. Arboretum of the Warsaw University of Life Sciences – SGGW in Rogów (central Poland)
Over the years, most of the stems became indeed reoriented to the upright position (Fig. 5), with only the basal part of the stem tilted, and remained so up to the year 2012, when they were cut. Cross-sectional discs were obtained from three levels of the stem: (A) from the basal part, close to the ground, which remained inclined, (B) from the region intermediate between the inclined and upright parts, at a distance of about 50 cm from A, and (C) from the upper segment of the tree, which was in the upright position (about 50 cm distant from the B). The sample discs thus obtained were used for wood structural studies employing SilviScan, WinCELLL, and WinDENDRO techniques.

Figure 5. Norway spruce tree with main stem reoriented to the upright position after five years of the experiment. Arboretum of the Warsaw University of Life Sciences – SGGW in Rogów

Due to the large number of parameters concerning individual tracheids and the limited time of SilviScan usage, structural examinations were conducted for discs from three trees selected randomly from the six trees used in the experiment. The study involved radial measurements of annual rings in four orthogonal directions of stem discs, which were termed as follows: RW – reaction wood (lower side), OW – opposite wood (upper side), L – left wood (lateral left side), and R – right
wood (lateral right side). The samples were polished to obtain a smooth surface with well-visible annual growth rings. The final preparation process, involving sample polishing and scanning, was conducted at the Innventia laboratory in Stockholm, and the samples were examined using the SilviScan instrument. The same samples were re-scanned at the laboratory of the Department of Forest Botany of the Warsaw University of Life Sciences – SGGW and analyzed using the WinDENDRO software. Finally, microscopic mounts were made from the samples, which were used for analysis with the WinCELL software.

Field observations and preliminary analysis of the obtained results showed that stem reorientation to the vertical position (at level C) mostly occurred within five consecutive growth seasons from the onset of the experiment, so the studies focused on data concerning structural changes of the xylem five annual rings formed in the course of stem reorientation.

### 2.2. Methods of wood structure analysis

Anatomical observations of wood were done using optical and SEM microscopy. The main method of the wood structure analysis was the SilviScan technique which enables measurements of various characteristics in the same radial files of tracheids. The measurements were performed at the Innventia Wood and Fibre Measurement Centre in Stockholm. Additional comparative wood measurements with applying standard WinDENDRO and WinCELL techniques were made at the Warsaw University of Life Sciences – SGGW.

#### 2.2.1. Anatomical observations

Microscopic observations of stem anatomy were performed on transverse and tangential sectional samples of stems in an Olympus BX-61 optical microscope with UV light and using scanning electron microscopy (FEI QUANTA 200) at 25 kV.

#### 2.2.2. SilviScan

The SilviScan is considered to be one of the most efficient instruments for detailed characterization of wood and fiber properties. The SilviScan is designed for high capacity measurement of a large number of important properties to enable the study of sufficiently large sets of samples for good representability. In many ways, this instrument is 10–500 times more efficient than traditional laboratory methods.
The SilviScan technology was developed by Dr. Robert Evans. During the 1990s, the first instrument for softwood measurement was built at CSIRO in Melbourne, Australia, followed by a second instrument with enhanced functionalities, dedicated especially to hardwood. In these instruments, all measurements are performed in the same unit. The current (third) version with three separate units permits parallel measurements. It was designed, with further improvements, by Dr. Evans and colleagues within a joint project with Innventia (then STFI), where the first instrument of the kind was installed in 2004. Since the mid-1990s the SilviScan technology has been used in numerous research projects representing a wide spectrum of research fields related to wood growth and formation, tree improvement and genetics, fibers and vessels, and optimal use of wood for sawn products as well as pulp and paper.

General description of the technique

The instrument provides data on radial variations from pith to bark or parts thereof. It integrates three measurement principles for efficient characterization of many wood and fiber properties from the same wood sample, applied in three separate units: (A) image analysis of cross-sections of fibers and vessels, (B) X-ray absorption for microdensitometry and (C) X-ray diffraction for orientation in wood. Measurements are performed on 2 mm thick sample strips extending in the radial direction of wood (in this study the strips were produced from wood discs collected from the main stem of the trees). For each project, the measurements are optimized by using the units and resolutions allowing for the characterization of more samples and reducing the cost.

One important feature of the SilviScan technology is that the measurement of all parameters is performed from the same sample. This ensures compatibility of the different data sets and enables precise combination of the data for the calculation of further properties. This enhances the investigation of relationships between stem radial growth and wood properties, the influence of cambial age, and other evaluations. This is also helpful in obtaining better measurements: image analysis performed in unit A also provides information about orientation in the wood sample. Based on this, the sample strip can be subsequently rotated during scanning from pith to bark so that the X-ray beam is always oriented in parallel with each annual ring. This way, the highest resolution data can be obtained for rings and for thin features such as narrow bands of latewood.

Using the SilviScan technique, radial variations in a large number of properties may be analyzed (Evans 1994, 2006) including:

- wood density, fiber width and wall thickness, with a radial resolution of 25 μm for softwood,
Reaction wood formation during stem gravitropic response...

- microfibril angle and wood stiffness,
- number and dimensions of vessels in hardwood,
- width of annual rings, as well as their earlywood and latewood components,
- mean values for each ring and its component,
- number of cells and other features formed per year.

**Preparation of samples**

Samples were delivered in the form of sawn discs. The samples were sawn into four specimens for each disc. Two samples went through the pith and two were perpendicular, without pith. Standard sample strips were produced: 2 mm wide and 7 mm high, starting as close as possible to the pith and extending towards the bark. Resin was removed with acetone to avoid random disturbances in the measurement of the density of wood itself and of tracheid wall thickness. The top surface of the sample strips was polished to allow measurement of cross-sectional tracheids dimensions.

**Measurements**

The measurements have been performed on samples in equilibrium under the conditioned atmosphere of the laboratory: 23°C and 43% relative humidity. The average density of each sample strip was determined by a standard gravimetric procedure. The sample strips were analyzed on the: (i) cell scanner – 25 μm resolution, (ii) density scanner – 25 μm resolution, (iii) diffractometer scanner with the highest possible resolution – 0.2 mm.

**Ring analysis.** The position of annual rings was identified from the steep change in wood density at the transition from latewood to earlywood. The distinction between true and false annual rings (latewood bands) was supported by visual examination of images from the CellScanner. The earlywood, transition wood and latewood parts of each ring were identified from wood density variation within each ring. Earlywood was defined as the part of the ring with wood density amounting to 0–20% of the minimum-to-maximum range within the ring, latewood as the part with density corresponding to 80–100% of that range, and the rest was considered transition wood. Averages were calculated for all the annual rings analyzed as well as for parts of the rings.

**Definitions and units.** Data based on measurements with a radial resolution of 25 μm: position – mm, wood density – kg/m³, radial tracheid width – μm, tracheid wall thickness – μm.
Materials and methods

Data based on measurements with a radial resolution of 0.2 mm: microfibril angle (MFA) – degrees; wood stiffness – modulus of elasticity (MOE) – GPa (estimated acoustic MOE).

2.2.3. WinDENDRO

WinDENDRO is software enabling macroscopic analysis of annual rings. It graphically analyzes scanned cross-sectional wood samples. Of critical importance in using this software is good polishing of the sample and high resolution scanning. WinDENDRO offers both automatic analysis of the sample as well as manual marking of annual rings. The software provides a wealth of data obtained from sample analysis. The user can define the area to be analyzed, select the parameters to be measured, and enter additional data, such as age, etc. The acquired data can be exported in the form of a spreadsheet together with the elements selected on the sample scan, which makes it possible to save the measurements at any time and return to them and continue at a later time.

In the present work, the samples analyzed with WinDENDRO were previously examined with the SilviScan. The samples were re-scanned at the laboratory of the Department of Forest Botany of the Warsaw University of Life Sciences – SGGW, using an Epson scanner (Perfection v700 PHOTO) at a resolution of 800 DPI. WinDENDRO analysis involved uploading of the scans, creating a text file for storing data from measurements, selecting the area to be analyzed, and introducing some basic information concerning the sample. In the next step, the user selected the parameters to be measured and statistics to be calculated, as well as marked annual rings in the studied area. All wood samples were analyzed in this way, with the results exported to MS Excel spreadsheets.

In this work WinDENDRO was used for measurement of ring width and share of early- and latewood. The distinction between the two types of wood was based upon darker color of the latewood layer.

2.2.4. WinCELL

WinCELL software performs comprehensive analysis of microscopic images. First, a photomicrograph of the studied object must be acquired. Following horizontal and vertical calibration, the user can select the area to be examined. The software can also be comprehensively configured, from the size of elements to be measured to pixel parameters. Similarly to WinDENDRO, the results can be exported in the form of a spreadsheet, which substantially facilitates their processing. While WinDENDRO
is limited to processing whole samples of wood, WinCELL can be used to acquire data concerning the constituent cells. In the case of both software applications, reliable results can only be obtained if the samples are well-calibrated and the software is correctly configured and monitored.

In the present work WinCELL analysis was performed on microscope slides of cross-sectional specimens prepared from the samples previously analyzed by the SilviScan and WinDENDRO. Slide preparation was preceded by the samples being boiled in water for several hours in order to soften them and remove air. Subsequently, the samples were divided into smaller specimens to fit the microscope slides. Cross-sections were made using a sliding microtome. The sections were mounted in glycerin and photographed under an Olympus BX61 optical microscope using Cell^P software, following microscope calibration. The photomicrographs were taken in UV light to improve image sharpness and better visualize all elements of the examined xylem. The images were processed using Adobe Photoshop software: converted to grayscale, cropped, rotated to the correct position, and adjusted with such parameters as brightness, contrast, clarity, exposure, and shadow and highlight correction. The images were saved in TIFF format with 8 bit color depth, and then analyzed with WinCELL.

Microphotograph analysis with WinCELL included software calibration depending on the image scale, both in horizontal and vertical positions, as well as the setting of program parameters. Subsequently, as in the case of WinDENDRO, a text file was created for measurement recording and the parameters to be measured and statistics to be calculated were defined. Prior to measurement, a radial row of cells was selected. Despite the fact that WinCELL measurements were conducted in automatic mode, they required continuous monitoring of such parameters as the width of the cells and the brightness of the pixels analyzed (due to differences in cell sizes and in brightness across the image). WinCELL measurements provided data concerning the radial diameter and cell wall thickness of tracheids found in the samples. In addition, width of the annual rings and share of early- and latewood were also estimated. The criterion for the latewood tracheid was Mork’s (1928) definition in the form as follows: latewood consists of tracheids with a lumen equal to or smaller than double cell wall thickness multiplied by two. Similarly to WinDENDRO, the data were exported to MS Excel spreadsheets. Subsequently, the spreadsheets were collated and the relevant parameters were selected for further study, these included: annual ring width and early- and latewood proportions acquired using WinDENDRO, WinCELL, as well as SilviScan technology.
2.3. Statistics

The average values, standard deviation or standard error characteristics were applied in order to compare the results of the measurements of wood parameters in the consecutive annual rings, in different directions on stem cross-sections, at various stem levels of individual trees. Correlation coefficients were calculated in order to find relations between the anatomical and physical characteristics of the tracheids formed during the experiment.
The observations on the morphology of trees after the 5-year experiment has shown that during this period all the trees showed a similar geotropic reaction. It was manifested by the characteristic growth of the main stem, leading to adjust the vertical position of the upper part of the stem (level C). At the lowest level, near the surface of the soil (level A), the stem axis still remained inclined, and at the intermediate level (B) was at the transition region between the inclined and vertically oriented zones.

Numerous authors in their studies on the spatial distribution of compression wood on cross-sections of the inclined trunks and branches have used the method of manually selecting the layer of reaction wood, guided by the criterion of the darker color of this type of wood (see Timell 1986). During preliminary analysis of the material collected in this experiment, an attempt was made to apply this method to determine the spatial distribution of the zones of compression wood on cross-sections of the inclined trunks of Norway spruce. It turned out, however, that in many cases in the cross sections the juvenile stems of Norway spruce the compression wood like zones distinguished poorly in terms of color from the normal wood. Sometimes the zones looked very narrow and distributed rather randomly around ring circumference. Guided by these criteria, it was rather difficult to unambiguously determine the actual distribution of compression wood in whole material. The selected example of such an analysis based on the stem cross-sections where the compression wood like zones were relatively distinct are illustrated in 3D model shown in Figure 6.

Taking into account the above difficulties we decided to made the detailed analyses of wood on basis the data obtained by the SilviScan technique. The studies performed by this technique have shown that despite the similar geotropic reaction, among trees there were differences between such wood parameters as the width of the annual ring, the radial diameter of tracheids, cell wall thickness, wood density, microfibril angle and the modulus of elasticity. Detailed measurements revealed that the geotropic response in trees was accompanied by differences between wood parameters with respect to the position along the stem and direction of around the cross-section of the stem with respect to the initial inclination of the stem as well as the changes
Results

Figure 6. Three-dimensional model of spatial distribution of compression wood zones (colored layers) in the successive annual rings formed in the inclined stem of young Norway spruce tree. The model was prepared using AutoCAD 2010 software and based on data obtained from scanning cross-sectional stem discs cut at various distances from the base of the stem segment of a length of 50 cm, collected near the ground (level A). The segment of the trunk to make the sample model was selected for good visibility of compression wood zones in six annual increments (for full analysis of wood characteristics five annual rings were used).
that occurred with age in successive annual rings during the 5-year experiment. This diversification has occurred both between the trees as well as within individual trees. Therefore, the description of the results concerning the changes and differences between the measured wood characteristics is presented subsequently with regard to data from the three stem levels (A, B, C) of each of the three tested trees, the radial direction on stem cross-section (RW, L, R, OW) throughout the whole 5-year period of experiment, and separately in the successive five annual rings.

### 3.1. Width of the annual ring

The results of measurements have shown that the average width of the annual rings of wood estimated for the 5-year period of experiment differed between the trees (Fig. 7). The widest rings were noted in trees No. 1 and 3 and the narrowest in tree No. 2. The average ring width usually increased in consecutive five years of the experiment (Fig. 8). In the first two years of the width of the ring ranged from 2.7 to 4.5 mm in tree No. 1, from 2.7 to 4 mm for tree No. 2, and from 4.1 to 4.6 mm at the levels A and B of tree No. 3. An exception was the first annual ring of tree No. 3 at the C level, which reached the width of 5.9 mm. The annual wood increments recorded in the subsequent three years showed that the broadest annual rings were in tree No. 1, wherein the size of those are in the range of 7–9 mm. The lowest values were found for the tree No. 2, where the width of the ring was in the range of 4.0–4.7 mm. In the case of a tree No. 3, the widths of growth increments were in the range of 5.9–8.1 mm. However, there was no clear trend between the widths of the annual rings of wood at different levels along the tree stem.

**Figure 7.** Average width of annual ring of wood formed at three stem levels (A – basal, B – middle, C – upper) of the inclined stems of three Norway spruce trees (1–3). Average values of five annual increments measured in four radial directions on the stem cross-sections. Error bars represent standard error.
Figure 8. Changes of width of annual rings of wood in the inclined stems of three Norway spruce trees (1–3) during successive five years of experiment. Averages data for three stem levels (A – basal, B – middle, C – upper) obtained from four radial directions on stem cross-sections. Error bars represent standard error.
A comparison of the average width of the rings for the 5-year period at four radial directions of the stem cross-section provides that, in a tree No. 1, at the levels A and B of the widest rings were on the lower side (RW) of the inclined stem (Fig. 9). In the case of samples collected from the highest stem level (C) the differences between the mean ring widths in four directions on the stem cross-sections were slight (ranged from 6.2 to 6.7 mm). Similar relationships were found in the stem of tree No. 3. In the case of tree No. 2 differences in the ring width between the RW and the other directions were accentuated most clearly at the lowest stem level (A). The narrowest rings usually were noted on the upper side of the inclined stem referred to as OW (opposite wood). It was observed markedly at levels A and B of trees Nos. 2 and 3. In particular, it was especially strongly manifested in tree No. 2.

Detailed results of the width measurements of the successive annual increments in four directions on the stem cross-sections (RW, R, L, OW) on three stem levels (A, B, C) in three individual trees are shown in Figures 10–12.

In the tree No. 1 at the lowest trunk level (A), in the first year of the experiment, the widest growth was ring recorded in the RW while the narrowest increment on the OW (Fig. 10). The smallest increment on the OW continued also in the second year. In other years the differences between the directions were minor and did not show any clear tendency. At a higher level (B) of the same tree (No. 1) the widest ring in the RW direction occurred in the first, second and fourth year of the experiment. In the first year of the narrowest increments were found in the directions L and R while during the period from the second to the fourth year, the smallest width of the ring was revealed in the OW direction. It is interesting to note that in the last (fifth) year the highest value of the width of annual ring was recorded just in this OW direction, albeit only slightly exceeding the value recorded for the RW and the other two directions. In the case of the highest level (C), in the first year, the largest width of annual ring occurred in the direction of RW. The relatively high value was noted in the OW, while the widths of the annual ring the directions R and L were significantly smaller. In the subsequent years, the differences between the widths of the annual increments measured in different directions around the stem circumference were small.

In the case of a tree No. 2 at stem level close to the soil surface (A) the widest width of the annual ring was found at the stem lateral sides (directions R and L) (Fig. 11). At the direction of the RW, at the lower stem side, the width of the rings formed during the seasons from second to fifth was only slightly lower than on the lateral sides. During all five years the narrowest rings were recorded in the OW, on the upper side of stem. At a higher trunk level (B) in the first three seasons the greatest width occurred in the direction of RW and in the next two seasons slightly larger
Figure 9. Width of annual ring of wood formed in four radial directions (RW, L, R, OW) on the cross-section of three levels (A – basal, B – middle, C – upper) of the inclined stems of three Norway spruce trees (1–3). Average values for five annual increments formed during the whole period of experiment. Error bars represent standard error.
Figure 10. Width of annual rings of wood measured in four radial directions (OW, R, L, RW) on the cross-section of the inclined stems of Norway spruce. Data for three stem levels of tree No. 1 (A – basal, B – middle, C – upper). Values estimated for the successive annual rings formed during five years of experiment.
Figure 11. Width of annual rings of wood measured in four radial directions (OW, R, L, RW) on the cross-section of the inclined stems of Norway spruce. Data for three stem levels of tree No. 2 (A – basal, B – middle, C – upper). Values estimated for the successive annual rings formed during five years of experiment.
Figure 12. Width of annual rings of wood measured in four radial directions (OW, R, L, RW) on the cross-section of the inclined stems of Norway spruce. Data for three stem levels of tree No. 3 (A – basal, B – middle, and C – upper). Values estimated for the successive annual rings formed during five years of experiment.
in width was noted in the lateral sides (R and L). The narrowest rings in all years, except in the third, were observed in OW direction. In the third year of slightly less ring width value than in OW was recorded in the direction of L. In the zone located closer the tree top (C), in all the examined annual growth rings, with the exception of the fourth, the highest width of the ring was recorded on the RW. The smallest ring width in the first two years have occurred in the stem lateral sides of R and L. In the second year a narrow ring appeared also in the OW. In the subsequent years, this direction showed the lowest recorded value of the width of the annual ring.

Measurements of the annual rings of wood in tree No. 3 at the lowest stem level (A) have shown that during the five seasons, the annual increments achieved the greatest width in the direction of RW (Fig. 12). Over the last three years, the smallest width of the ring was recorded at the OW. Also in the second season the ring width measured in this direction was only slightly higher than in the two lateral directions (R and L). At a higher level of the trunk (B) the highest values of the ring width, with the exception of the third year, was recorded at the RW, and the smallest, with the exception of the first season, at the OW. In the first year the smallest width of the ring was noted at the direction L, but in the third year the annual ring measured the same direction was the widest. At the highest tested zone of the trunk (level C) there were no clear ring width variation on various directions around the stem circumference. In contrast to the lower stem levels (A and B) the largest value of ring width was not observed on the RW and the smallest in the OW directions.

3.2. Share of earlywood and latewood in annual rings

As described in the section “Material and methods”, the earlywood, transition wood and latewood parts of each ring were identified from wood density variation within each ring. Earlywood was defined as the part of the ring with wood density amounting to 0–20% of the minimum-to-maximum range within the ring, latewood as the part with density corresponding to 80–100% of that range, and the rest was considered transition wood. The data presented in Figure 13 concern average values for the 5-year period, calculated for four radial direction on the stem cross-section (OW, L, R, RW) at three stem levels (A, B, C) of three trees (1–3). It is seen that share of so defined earlywood was on average about 50%, and ranged from about 30% (2B) to about 70% (1C). Usually the share of latewood did not exceed 10%, and in many cases as close to a value of about 5%. The exception was the second stem level (B)
Figure 13. Share (percent) of earlywood (EW), transition wood (TW) and latewood (LW) in annual rings in four radial directions (OW, R, L, RW) on the cross-sections of the inclined stems of three Norway spruce trees (1–3). Data for three stem levels (A – basal, B – middle, C – upper). Average values for five annual rings formed during the whole period of experiment.
Figure 14. Share (percent) of earlywood (EW), transition wood (TW) and latewood (LW) in annual rings in four radial directions (OW, R, L, RW) on the cross-sections of the inclined stems of Norway spruce. Data for three stem levels of tree No. 1 (A – basal, B – middle, C – upper). Values for the successive annual rings formed during five years of experiment.
Figure 15. Share (percent) of earlywood (EW), transition wood (TW) and latewood (LW) in annual rings in four radial directions (OW, R, L, RW) on the cross-sections of the inclined stems of Norway spruce. Data for three stem levels of tree No. 2 (A – basal, B – middle, C – upper). Values for the successive annual rings formed during five years of experiment.
Figure 16. Share (percent) of earlywood (EW), transition wood (TW) and latewood (LW) in annual rings in four radial directions (OW, R, L, RW) on the cross-sections of the inclined stems of Norway spruce. Data for three stem levels of tree No. 3 (A – basal, B – middle, C – upper). Values for the successive annual rings formed during five years of experiment.
of tree No. 2, where in one of the lateral directions (R) the contribution of late wood was about 30%. No clear trends can be observed between share of different types of wood and stem level position or the radial direction on the stem cross-section.

Detailed data for individual trees concerning successive five annual rings formed during the experiment, at three stem levels (A, B, C) separately for each radial direction on the stem cross-section (OW, L, R, RW) are shown in Figures 14–16. The share of earlywood (defined as above) in the particular years showed the broad range from about 0–5% in the first year up to approximately 75% in the fifth year (Tree No. 1A R). However, the increasing share of earlywood with age was not clear and in a number of cases, also in the fourth and fifth year, the earlywood contribution was low and amounted to about 20%. The share of latewood generally decreased with time from the beginning of the experiment. In the first and second years, this share reached 40–50%, and in the successive years often fell to around 5%, or even to 0%. It is interesting to note that at the highest stem level (C) of tree No. 1, in the OW direction on the disc cross-section in the first year the zone “latewood” (estimated according to the applied definition) covered the whole ring width, but in subsequent annual increments this share was already low and amounted about 5%.

3.3. Comparison of annual ring width measurements done using the SilviScan and WinDENDRO and WinCELL techniques

A comparison of annual ring width and share of earlywood width results estimated using the SilviScan techniques with those obtained using the widely available WinDENDRO and WinCELL systems are presented in Table 1 which gives differences between average values, as well as standard deviations for those differences. The data refer to individual trees and include measurements for all the three studied annual rings at three stem levels and for the four cross-sectional directions (L, R, OW and RW).

Differences between the average annual ring widths obtained for the three studied trees using the aforementioned systems were rather small in the case of data aggregated for all the four cross-sectional directions. The difference of WinDENDRO results ranged from −0.21 to +0.08 mm, while that of WinCELL results was from −0.18 to 0.12 mm. The standard deviations for those values were 0.68–1.09 mm for WinDENDRO and 0.48–0.99 mm for WinCELL. Differences between the different measurement systems were larger for individual cross-sectional directions:
for WinDENDRO they were from −0.43 to +0.40 mm with standard deviations of 0.08–1.86 mm, and for WinCELL they ranged from −0.98 to +0.68 mm with standard deviations of 0.13–1.77 mm.

Table 1. Comparison of the results of annual ring width measurements made by the SilviScan, WinDENDRO and WinCELL techniques. Data for four radial directions on stem cross-sections (L, R, OW, RW). Figures (mm) denote differences between mean values of annual ring width measurement made using WinDENDRO or WinCELL and the SilviScan. Average data from five annual rings of three stem levels. SD – standard deviation

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<th>WinCELL</th>
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In the case of earlywood width results, differences were greater than for measurements of entire annual rings: for WinDENDRO differences between averages calculated for all four cross-sectional stem directions ranged from 1.81 to 2.06 mm with standard deviations of 1.38–1.43 mm, and for WinCELL these differences were from 2.25 to 2.50 mm with standard deviations of 1.42–1.52 mm (Table 2). Differences in the width of earlywood layers for individual cross-sectional directions were from 0.02 to 3.28 mm for WinDENDRO and from 1.49 to 2.96 mm for WinCELL, with standard deviations being 0.67–1.50 mm and 0.72–1.78 mm, respectively.
Table 2. Comparison of the results of earlywood width measurements made by the SilviScan technique and the WinDENDRO and WinCELL techniques. Data for four radial directions on stem cross-sections (L, R, OW, RW). The figures (mm) denote differences between mean values of earlywood width measurements made using WinDENDRO or WinCELL and the SilviScan. Average data from five annual rings of three stem levels (A, B, C). SD – standard deviation.

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<td>1.45</td>
</tr>
</tbody>
</table>

3.4. Anatomical observations

Microscopic observation revealed that wood formed in stems of the experimental trees has a typical structure of juvenile wood of Norway spruce. The tracheids seen on the stem cross-sections were arranged in radial rows (Fig. 17). Near the annual ring boundary the latewood tracheids have smaller radial diameter radial and thicker cell wall as compared to the earlywood tracheids formed in at the beginning of the next annual ring. However, within the annual increment the transition from early- to latewood was gradual and no a clear boundary between the two types of wood are seen. The annual rings formed on the lower side of the tilted stems often have a structure similar to the compression wood, and are characterized by thick-walled tracheids of rounded shape with visible intercellular spaces (Figs. 18–20). Frequently, however, in the portion of annual ring with the compression wood the tracheids
Figure 17. Compression (A) and opposite (B) wood formed on lower and upper sides of the inclined Norway spruce stem, respectively. No intercellular spaces are seen in the zone of slightly rounded (white arrows) and polygonal (black arrows) shape of tracheids in the zone of compression wood (B) nor polygonal shape tracheids of opposite wood (B). Transverse sections of wood samples collected from the upper portion of the main stem of tree No. 3; annual rings formed during the second year of experiment. Scale bars 200 μm

Figure 18. Examples of compression wood formed on the lower side of the main stem during gravitropic response of the inclined stems of young Norway spruce trees. Radial arrangement of round shaped thick-walled tracheids with intercellular spaces (A, B, D) and the tracheids of the polygonal shape without intercellular spaces (C). Some of the round shaped tracheids show extremely high radial diameter (D). Transverse sections wood from lower part of stem Tree No. 1 seen under optical microscope. Scale bars 50 μm
Figure 19. Structure of compression wood formed on the lower side of the main stem during gravitropic response of the inclined stems of young Norway spruce trees. Round shaped thick-walled tracheids with intercellular spaces seen on transverse sections (A, B). Tangential (C) view of tracheids and xylem rays with (white arrow) and without (black arrow) radial resin canals. Radial view of the successively formed tracheids with visible direction of microfibril orientation in the cell wall. Samples of wood collected from lower sides of the main stem tree No. 2 near the ground level seen under SEM. Scale bars 50 μm for A, 20 μm for B and 100 μm for C and D.

Figure 20. Wood formed on the upper (A) and lower (B) sides of inclined Norway spruce stem. Slightly rounded (white arrows) and polygonal (black arrows) shape of tracheids in the zone of compression wood (B). Polygonal shape of the opposite wood tracheids of earlywood thin-walled (black arrow) and latewood thick-walled tracheids. Transverse sections of wood samples collected from the middle fragment of the main stem of tree No. 2; annual rings formed during the second year of experiment. Scale bars 200 μm.
with thick cell walls have a polygonal shape and no clear intercellular spaces were seen. In the zone of compression wood occasionally a single tracheids of rounded shape with a significantly greater diameter occurred (Fig. 18D). In some cases on lower side of the tilted stem at the beginning of the annual ring an abnormal wood with irregularly arranged cells of various diameters were also noted (Fig. 21C).

Figure 21. Relatively narrow annual ring with compression wood (A) and wider ring of opposite wood (B) of the inclined Norway spruce stem. C – zone of abnormal wood consisting of irregularly ordered cells. Transverse sections of wood samples collected from the basal portion of the main stem of tree No. 2; annual rings formed during the first (C) and the third (B, C) year of experiment. Scale bars 250 μm for A and B, 500 μm for C
Oblique orientation of the microfibrils in the secondary walls of tracheids was clearly seen under SEM on the tangential and radial sections made in the zone of compression wood (Fig. 20C, D).

The asymmetric rings in the tilted stems were usually wider on lower side inclined stem. In few cases, however the ring width in the RW direction was narrower as compared to width in the OW direction (Fig. 21A, B). In the broad rings on the lower side of the inclined stems the compression wood appeared often in the form relatively narrow zones of thick-walled cells with slightly rounded or polygonal shape without clear intercellular spaces (Figs. 17, 20).

3.5. Characteristics of tracheids formed successively in annual rings

The tracheids formed successively throughout the growing season differed in terms of the studied parameters, exhibiting specific changes within annual rings. Examples of the seasonal patterns of such changes at two radial directions on upper (OW) and lower sides (RW) of the inclined stems of three trees (Nos. 1–3) at three stem levels (A, B, C) are given in Figures 22–30.

As can be seen in the plots concerning opposite wood (OW) the tracheids parameters follow the normal pattern of conifer wood observed by numerous authors. The tracheids formed at the beginning of annual growth had a large radial diameter, which was significantly smaller in those formed later in the season. The wall thickness of tracheids in the initial layers of annual rings was much smaller than that in layers developing towards the end of the season. This typical seasonal pattern was usually modified in the annual rings formed on the lower side of the inclined stem (RW), where tracheids with thicker cell walls occupied wider layers of annual rings. The seasonal patterns of wood density and modulus of elasticity were similar to that for cell wall thickness. Of particular interest are differences in microfibril angle in the tracheids formed successively throughout the growing season, which revealed a decreasing tendency. However, in tracheids on the lower side of the inclined stem (RW), this angle was higher than in the opposite side (OW).

Correlation coefficients between the measured characteristics of the successive tracheids formed during five years in radial files on lower (RW) and upper (OW) sides of the inclined stems at three stem levels (A, B, C) of three (1, 2, 3) trees are presented in Table 3. It is seen that the highest positive values of the coefficients were found between cell wall thickness and wood density (from 0.925 to 0.980), between wall thickness and MOE (from 0.615 to 0.952), and between wood density and MOE.
Figure 22. Anatomical and physical characteristics of successive tracheids formed in radial files on the lower (RW) and upper (OW) sides of the inclined stems of Norway spruce during the 5-year experiment. The plots show patterns of the following characteristics: tracheid radial diameter, cell wall thickness, wood density, microfibril angle (MFA) and modulus of elasticity (MOE). Results of SilviScan measurements of five annual rings of tree No. 1 at the stem level near the base (A)
Figure 23. Anatomical and physical characteristics of successive tracheids formed in radial files on the lower (RW) and upper (OW) sides of the inclined stems of Norway spruce during the 5-year experiment. The plots show patterns of the following characteristics: tracheid radial diameter, cell wall thickness, wood density, microfibril angle (MFA) and modulus of elasticity (MOE). Results of SilviScan measurements of five annual rings of tree No. 1 in the middle portion of the stem (B)
Figure 24. Anatomical and physical characteristics of successive tracheids formed in radial files on the lower (RW) and upper (OW) sides of the inclined stems of Norway spruce during the 5-year experiment. The plots show patterns of the following characteristics: tracheid radial diameter, cell wall thickness, wood density, microfibril angle (MFA) and modulus of elasticity (MOE). Results of SilviScan measurements of five annual rings of tree No. 1 in the upper part of the stem (C)
Figure 25. Anatomical and physical characteristics of successive tracheids formed in radial files on the lower (RW) and upper (OW) sides of the inclined stems of Norway spruce during the 5-year experiment. The plots show patterns of the following characteristics: tracheid radial diameter, cell wall thickness, wood density, microfibril angle (MFA) and modulus of elasticity (MOE). Results of SilviScan measurements of five annual rings of tree No. 2 at the stem level near the base (A)
Figure 26. Anatomical and physical characteristics of successive tracheids formed in radial files on the lower (RW) and upper (OW) sides of the inclined stems of Norway spruce during the 5-year experiment. The plots show patterns of the following characteristics: tracheid radial diameter, cell wall thickness, wood density, microfibril angle (MFA) and modulus of elasticity (MOE). Results of SilviScan measurements of five annual rings of tree No. 2 in the middle portion of the stem (B)
Figure 27. Anatomical and physical characteristics of successive tracheids formed in radial files on the lower (RW) and upper (OW) sides of the inclined stems of Norway spruce during the 5-year experiment. The plots show patterns of the following characteristics: tracheid radial diameter, cell wall thickness, wood density, microfibril angle (MFA) and modulus of elasticity (MOE). Results of SilviScan measurements of five annual rings of tree No. 2 in the upper part of the stem (C)
Figure 28. Anatomical and physical characteristics of successive tracheids formed in radial files on the lower (RW) and upper (OW) sides of the inclined stems of Norway spruce during the 5-year experiment. The plots show patterns of the following characteristics: tracheid radial diameter, cell wall thickness, wood density, microfibril angle (MFA) and modulus of elasticity (MOE). Results of SilviScan measurements of five annual rings of tree No. 3 at the stem level near the base (A)
Figure 29. Anatomical and physical characteristics of successive tracheids formed in radial files on the lower (RW) and upper (OW) sides of the inclined stems of Norway spruce during the 5-year experiment. The plots show patterns of the following characteristics: tracheid radial diameter, cell wall thickness, wood density, microfibril angle (MFA) and modulus of elasticity (MOE). Results of SilviScan measurements of five annual rings of tree No. 3 in the middle portion of the stem (B)
Figure 30. Anatomical and physical characteristics of successive tracheids formed in radial files on the lower (RW) and upper (OW) sides of the inclined stems of Norway spruce during the 5-year experiment. The plots show patterns of the following characteristics: tracheid radial diameter, cell wall thickness, wood density, microfibril angle (MFA) and modulus of elasticity (MOE). Results of SilviScan measurements of five annual rings of tree No. 3 in the upper part of the stem (C).
Table 3. Correlations coefficients between anatomical and physical characteristics of tracheids formed successively in radial files at three stem levels (A – basal, B – middle, C – upper) of three trees (1, 2, 3) on the lower (RW) on upper (OW) sides of the inclined stems of Norway spruce during the 5-year experiment. The correlation coefficient values were calculated from the original data presented in Figs. 22–30.

<table>
<thead>
<tr>
<th>Tree No. and stem level</th>
<th>Correlation coefficients between wood characteristics</th>
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<tr>
<td></td>
<td>WThick - tracheid cell wall thickness, RDiam - tracheid radial diameter, Density - wood density, MFA - microfibril angle, MOE - modulus of elasticity.</td>
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<tr>
<td></td>
<td>WThick</td>
</tr>
<tr>
<td></td>
<td>RDiam</td>
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<td>1A OW</td>
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</tr>
<tr>
<td>1B OW</td>
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</tr>
<tr>
<td>RW</td>
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</tr>
<tr>
<td>1C OW</td>
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</tr>
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<td>RW</td>
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<tr>
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**Figure 31.** Average values wood five characteristics of wood (I–V) formed at three stem levels (A – basal, B – middle, C – upper) of the inclined stems of three Norway spruce trees (1–3). Average values of five annual increments measured in four radial directions on the stem cross-sections. Error bars represent standard error.
Reaction wood formation during stem gravitropic response...

(from 0.470 to 0.932). The correlation coefficients between cell wall thickness and tracheid radial diameter were negative and ranged from −0.436 to −0.806. Negative values of the coefficients were also obtained when correlations between tracheid radial diameter and the following characteristics were calculated: density (from −0.719 to −0.908) and MOE (from −0.105 to −0.775). Relatively strong negative correlation was obtained between MFA and MOE (from −0.345 to −0.777). In most cases practically no correlation was observed between MFA and wall thickness, between MFA and radial diameter and between MFA and density. No clear trends of the correlation coefficients values were manifested with respect to the stem levels and upper or lower sides of the inclined stems.

Average values of these five characteristics of wood formed at three stem levels (A, B, C) of the inclined stems of three Norway spruce trees (Nos. 1–3) are shown in Figure 31. The values were calculated as means of five annual increments measured in four radial directions on the stem cross-sections. It is seen that with increasing distance from the stem base the decrease of tracheid cell wall microfibril angle value occurred. When tracheid cell wall thickness and wood density were analyzed the similar tendency occurred only in tree No. 2. Opposite relation in stems of all three trees was observed in case of modulus of elasticity. In case of tracheid diameter there was not clear trends.

Detailed results concerning each of the characteristics with respect to the cambial age, radial direction on the stem cross-section are presented in the following sections.

3.6. Tracheid radial diameter

In all of the trees at each of the stem levels the average radial diameters of the tracheids formed in successive years clearly increased (Fig. 32). This parameter was in the range of 24–36 μm and the differences between these values for the first and fifth ring at a given stem level of the individual tree reached the limit of 4–8 μm. The highest tracheid diameter values were recorded for the tree No. 1 and reached values close to 36 μm, and the smallest values which did not exceed 30 μm were recorded in the tree No. 2. There were, however, statistically significant differences between the tracheid diameters formed in successive annual rings at different stem levels (A–C) of the same tree.

A comparison of the average values of tracheid diameter for the 5-year periods, did not reveal a clear trend with respect to different directions around the stem circumference. The maxima and minima values of this parameter at different stem levels of individual trees were noted on different sides (RW, R, L or OW) of the tilted stem (Fig. 33).
Figure 32. Changes of the tracheid radial diameter in the inclined stems of three Norway spruce trees (1–3) during successive five years of experiment. Averages data for three stem levels (A – basal, B – middle, C – upper) obtained from four radial directions on stem cross-sections. Error bars represent standard error.
Figure 33. Radial diameter of tracheids formed in four radial directions (RW, L, R, OW) on the cross-section of three levels (A – basal, B – middle, C – upper) of the inclined stems of three Norway spruce trees (1–3). Average values for five annual rings formed during the whole period of experiment. Error bars represent standard error.
In shown in Figures 34–36 detailed data on the average of the diameters of the tracheids in successive five rings in four directions at different heights it provides that, in each of the three test trees, there is no clear trend in differences in the value of the parameter depending on the direction of the stem cross-section. None of the four directions does not really stand out clearly in terms of the size of the tracheid diameter at any level of a trunk. Recorded for some years, slightly higher values of this parameter refer to the lower and upper stem sides (RW, OW) and the lateral sides (R, L) did not show clear trends.

3.7. Tracheid cell wall thickness

Tracheid cell wall thickness values calculated for the successive five annual growth rings of inclined stems of the trees ranged from 1.7 to 2.5 μm (Fig. 37). In case of tree No. 1 on three stem levels studied (A–C) the wall thickness decreased in the successively formed annual rings. A similar trend, though less pronounced was revealed also in trees Nos. 2 and 3.

Average for the 5-year period, the wall thickness of the tracheids in different directions on stem cross-section showed little variation both within the stem level and between the levels (Fig. 38). Only in tree No.1 at B level and in tree No. 2 at A level, the cell walls of tracheids from the lower side of the stem were significantly greater as compared to both annual rings at different stem levels of the same tree as well as stems of other trees.

The data of the average wall thickness of the tracheids in the successive annual rings revealed no clear trends in the changes of the parameter depending on the direction of a tree stem cross-section (Figs. 39–41). In some cases, the differences between the wall thickness of the tracheids formed in various directions on the stem cross-sections, however, are quite large. In the case of tree No. 1 in the lowest two stem levels (A and B) in the first year, the largest wall thickness of the tracheids was estimated in the lateral stem side (R and L) and in the (RW) (Fig. 39). In the next years however, the direction RW is also distinguished by high values of wall thickness of at the B stem level. At the lowest stem level (A) of tree No. 2 in the first four rings the maxima of cell wall thickness occurred in RW direction (Fig. 40). At the upper stem level (B) of the tree No. 2 in successive rings the maximum wall thickness of the tracheids occurred in different directions on the stem circumference: in the first and fourth rings the largest cell wall thickness was noted on the upper side (OW) and in the fifth year in this respect dominated tracheids formed in the RW side. At the highest stem levels (C), the tracheids formed in the RW direction were
Figure 34. Radial diameter tracheids formed in four radial directions (OW, R, L, RW) on the cross-section of the inclined stems of Norway spruce. Data for three stem levels of tree No. 1 (A – basal, B – middle, C – upper). Average values for the successive annual rings formed during five years of experiment.
Figure 35. Radial diameter tracheids formed in four radial directions (OW, R, L, RW) on the cross-section of the inclined stems of Norway spruce. Data for three stem levels of tree No. 2 (A – basal, B – middle, C – upper). Average values for the successive annual rings formed during five years of experiment.
Figure 36. Radial diameter tracheids formed in four radial directions (OW, R, L, RW) on the cross-section of the inclined stems of Norway spruce. Data for three stem levels of tree No. 3 (A – basal, B – middle, C – upper). Average values for the successive annual rings formed during five years of experiment.
Figure 37. Changes of the tracheid cell wall thickness in the inclined stems of three Norway spruce trees (1–3) during successive five years of experiment. Averages data for three stem levels (A – basal, B – middle, C – upper) obtained from four radial directions on stem cross-sections. Error bars represent standard error.
Figure 38. Cell wall thickness of tracheids formed in four radial directions (RW, L, R, OW) on the cross-section of three levels (A – basal, B – middle, C – upper) of the inclined stems of three Norway spruce trees (1–3). Average values for five annual rings formed during the whole period of experiment. Error bars represent standard error.
Figure 39. Cell wall thickness of tracheids formed in four radial directions (OW, R, L, RW) on the cross-section of the inclined stems of Norway spruce. Data for three stem levels of tree No. 1 (A – basal, B – middle, C – upper). Average values for the successive annual rings formed during five years of experiment.
Figure 40. Cell wall thickness of tracheids formed in four radial directions (OW, R, L, RW) on the cross-section of the inclined stems of Norway spruce. Data for three stem levels of tree No. 2 (A – basal, B – middle, C – upper). Average values for the successive annual rings formed during five years of experiment.
Figure 41. Cell wall thickness of tracheids formed in four radial directions (OW, R, L, RW) on the cross-section of the inclined stems of Norway spruce. Data for three stem levels of tree No 3. (A – basal, B – middle, C – upper). Average values for the successive annual rings formed during five years of experiment.
distinguished by the greatest value of wall thickness only in the first year of the experiment; the opposite direction (OW) dominated in this respect in the fifth year at the stem B level. In the stem of tree No. 3, there were no major differences between the directions, although at the B level a slightly higher wall thickness can be RW observed in the first, second and fifth annual rings (Fig. 41).

### 3.8. Wood density

Wood density in the experimental trees stems showed trends similar to those observed in the case of the cell wall thickness. During five years of the experiment wood density showed a decreasing tendency in the successively formed annual rings (Fig. 42). The maximum value of this parameter in the first annual increment varied in the range of 420–540 kg/m³, while in the fifth increment these values ranged from 340 to 390 kg/m³. The data calculated for the 5-year period showed no significant relations between the values of this parameter and the position around stem circumference (Fig. 43). Similarly as in the case of the cell wall thickness, greater variation of this parameter occurred in the tree No.1 at the B and in tree No. 2 at A and B levels (Figs. 44–46). In tree No. 1 (B level) and at A level of tree No. 2 the highest density was recorded at the bottom side the stem (RW) while in the tree No. 2 at B level the maximum density was recorded in wood on the upper side (OW).

### 3.9. Microfibril angle (MFA)

Average values of the microfibril angle (MFA) in the cell walls in the consecutive annual rings formed during the 5-year period of the experiment were contained in a wide range from 6 to 29° (Fig. 47). Tree No. 3 in every season, on each of the three investigated stem levels was distinguished by significantly lower microfibril angle values as compared to the other two trees. The highest average value of the MFA estimated for this tree (No. 3) did not exceed 20°. Comparing the MFA values for individual trees estimated different stem levels in the particular years can be seen that the tracheids from a higher parts of the stem distinguished by a smaller MFA values as compared to the tracheids from the stem zones situated closer to the base of the trunk. Mean values for the MFA of tracheids at given stem level formed in the successive years were gradually smaller. This tendency was demonstrated at all three stem levels of the trees Nos. 1 and 3 and at the top level (C) of tree No. 2. In the case of the two lower stem levels (A and B) of the tree No. 2 the MFA value in particular years does not show clear trends.
Figure 42. Changes of the density of wood tracheids in the inclined stems of three Norway spruce trees (1–3) during successive five years of experiment. Averages data for three stem levels (A – basal, B – middle, C – upper) obtained from four radial directions on stem cross-sections. Error bars represent standard error.
Figure 43. Density of wood tracheids formed in four radial directions (OW, R, L, RW) on the cross-section of tree levels (A – basal, B – middle, C – upper) of the inclined stems of three Norway spruce trees (1–3). Average values for five annual rings formed during the whole period of experiment. Error bars represent standard error.
Figure 44. Density of wood tracheids formed in four radial directions (OW, R, L, RW) on the cross-section of the inclined stems of Norway spruce. Data for three stem levels of tree No. 1 (A – basal, B – middle, C – upper). Average values for the successive annual rings formed during five years of experiment.
Figure 45. Density of wood tracheids formed in four radial directions (OW, R, L, RW) on the cross-section of the inclined stems of Norway spruce. Data for three stem levels of tree No. 2 (A – basal, B – middle, C – upper). Average values for the successive annual rings formed during five years of experiment.
Figure 46. Density of wood tracheids formed in four radial directions (OW, R, L, RW) on the cross-section of the inclined stems of Norway spruce. Data for three stem levels of tree No. 3 (A – basal, B – middle, C – upper). Average values for the successive annual rings formed during five years of experiment.
Figure 47. Changes of microfibril angle (MFA) in the cell wall of tracheids in the inclined stems of three Norway spruce trees (1–3) during successive five years of experiments. Averages data for three stem levels (A – basal, B – middle, C – upper) obtained from four radial directions on stem cross-sections. Error bars represent standard error.
Results

Analysis of the MFA average values for five years for different directions on the stem cross-section indicates that the highest values of MFA generally occurred in the tracheids formed on the lower side (RW) of the inclined trunk (Fig. 48). The only exception were the tracheids on the lowest level (A) of the tree No. 1 and the highest level (C) of tree No. 3, where there was a no clear trends in diversification of the MFA values around the stem circumference. The lowest MFA values were found mainly on the upper side of the inclined trunk corresponding to the zone OW. It is also seen that the average MFA values for the 5-year periods in certain directions around the circumference of the trunk were generally smaller at a higher levels of the trunk (from A to C). It should also be noted that in the tree No. 3 on all three levels, the MFA of the tracheids in the four radial directions on the stem cross-sections (RW, R, L, OW) were significantly lower than the corresponding values for the trees Nos. 1 and 2.

The results of MFA estimation in the walls of the tracheids formed in the consecutive years in four directions on the stem cross-sections at three stem levels of the inclined trees are shown in Figures 49–51.

The highest MFA values (about 40°) were found in trees Nos. 1 and 2, whereas in the tree No. 3 the maximum MFA value was about 10 degrees lower.

In the tree No. 1, at the lowest level (A) in the first two years of the highest values were recorded on the lower side (RW) and in the next three years, the highest MFA values found in the lateral stem side (direction L). The smallest microfibril angles in all annual rings (except the first) occurred from the upper side of the inclined trunk (OW). In the first annual ring a slightly lower MFA value than on the OW direction was detected on the lateral side (L). At the higher level (B) in the first year there was virtually no difference between the MFA values of tracheids formed in different directions on the trunk, but in the subsequent four years the maximum angle values were found for the RW, and the minimum value on the upper side (OW). In the upper stem level (C), in the first two rings, the highest MFA values were recorded in the tracheids formed on the lower side of the stem (RW), and the next three annual rings the maximum value of this parameter was observed in the tracheids formed at the lateral side (L) of the tilted stem. Throughout the 5-year period of the experiment the lowest MFA values were observed in the tracheids from the upper side of the trunk (OW).

At the lowest stem level (A) of tree No. 2 the highest MFA values in the successive years, were observed in the tracheids from the bottom side of the trunk (RW) although in the fifth ring the MFA value from this side was only slightly higher than the values estimated for the lateral stem sides (R and L). The minimum MFA values for the first three years were observed in the tracheids from the lateral stem side (R) and in the consecutive two rings in the tracheids from the upper side of the trunk.
**Figure 48.** Micribril angle (MFA) in cell wall of tracheids formed in four radial directions (RW, L, R, OW) on the cross-section of three levels (A – basal, B – middle, C – upper) of the inclined stems of three Norway spruce trees (1–3). Average values for five annual rings formed during the whole period of experiment. Error bars represent standard error.
Figure 49. Microfibril angle (MFA) in cell wall of wood tracheids formed in four radial directions (OW, R, L, RW) on the cross-section of the inclined stems of Norway spruce. Data for three stem levels of tree No. 1 (A – basal, B – middle, C – upper). Average values for the successive annual rings formed during five years of experiment.
**Figure 50.** Microfibril angle (MFA) in cell wall of wood tracheids formed in four radial directions (OW, R, L, RW) on the cross-section of the inclined stems of Norway spruce. Data for three stem levels of tree No. 2 (A – basal, B – middle, C – upper). Average values for the successive annual rings formed during five years of experiment.
Figure 51. Microfibril angle (MFA) in cell wall of wood tracheids formed in four radial directions (OW, R, L, RW) on the cross-section of the inclined stems of Norway spruce. Data for three stem levels of tree No. 3 (A – basal, B – middle, C – upper). Average values for the successive annual rings formed during five years of experiment.
(OW). At the higher stem level (B) in the first four rings, the highest MFA values were observed in the tracheids from lower side of the trunk (RW), while in the fifth ring the maximum value of this parameter was revealed on the upper side (OW). The minimum MFA values were estimated on the lateral sides of the inclined stem (L or R).

At the lower stem levels (A and B) of tree No. 3, the highest MFA values in the consecutive five rings were found in the tracheids from the lower side of the trunk (RW). Throughout the 5-year experimental period the lowest MFA values were usually recorded in tracheids from the upper side of the trunk (OW), although in some annual rings the differences between the MFA values in tracheids from the upper and lateral (L and R) sides were very small. In case of the uppermost part of the stem (C) no clear relation between MFA and the position on stem circumference is seen.

3.10. Modulus of elasticity (MOE)

The acoustic modulus of elasticity (MOE) estimated using the SilviScan technology varied both with age (between consecutive annual rings) as well as between the different stem heights (Fig. 52). The average MOE values calculated for the 5-year period of the experiment ranged from 7.9 to 11.8 GPa. The highest MOE was found for tree No. 3. Within each tree the highest value of the modulus of elasticity occurred at the uppermost stem level (C), while the lowest MOE was observed at the stem base (A). Changes in MOE between the consecutive years of study exhibited a clear decreasing tendency only in the bottommost level of the stem (A). At the higher level (B), this trend was less marked, and at the uppermost stem level no such tendency was found. Average MOE values calculated for the 5-year period for different cross-sectional stem directions show the smallest and greatest modulus of elasticity for the xylem formed on the lower (RW) and upper (OW) sides of the leaning stem, respectively (Fig. 53).

Detailed results of MOE measurements for the xylem formed in consecutive annual rings in the four cross-sectional stem directions and at the three stem height levels of the three studied trees are given in Figures 54–56. In tree No. 1, at the base of the leaning stem (level A), in the first two years of experiment the lowest MOE was observed in the wood formed on the lower side (RW) (Fig. 54). Over the subsequent three seasons, the minimum MOE values were found in the R region. Throughout the period of study except for the first season, the greatest MOE occurred on the upper side of the stem (OW). At level B, differences in MOE between the four cross-sectional directions were relatively small in the year, while in the following four years the lowest values were found for the lower side of the inclined trunk
Figure 52. Changes of modulus of elasticity (MOE) of wood tracheids in the inclined stems of three Norway spruce trees (1–3) during successive five years of experiments. Averages data for three stem levels (A – basal, B – middle, C – upper) obtained from four radial directions on stem cross-sections. Error bars represent standard error.
Figure 53. Modulus of elasticity (MOE) of the wood tracheids formed in four radial directions (RW, L, R, OW) on the cross-section of three levels (A – basal, B – middle, C – upper) of the inclined stems of three Norway spruce trees (1–3). Average values for five annual rings formed during the whole period of experiment.
Figure 54. Modulus of elasticity (MOE) of the wood tracheids formed in four radial directions (OW, R, L, RW) on the cross-section of the inclined stems of Norway spruce. Data for three stem levels of tree No. 1 (A – basal, B – middle, C – upper). Average values for the successive annual rings formed during five years of experiment.
Figure 55. Modulus of elasticity (MOE) of the wood tracheids formed in four radial directions (OW, R, L, RW) on the cross-section of the inclined stems of Norway spruce. Data for three stem levels of tree No. 2 (A – basal, B – middle, C – upper). Average values for the successive annual rings formed during five years of experiment.
Figure 56. Modulus of elasticity (MOE) of the wood tracheids formed in four radial directions (OW, R, L, RW) on the cross-section of the inclined stems of Norway spruce. Data for three stem levels of tree No. 3 (A – basal, B – middle, C – upper). Average values for the successive annual rings formed during five years of experiment.
Reaction wood formation during stem gravitropic response...

(RW), and the highest values on the upper side (OW). At the highest stem level (C), the MOE was the smallest on the RW side only in the first season, while over the subsequent years the lowest modulus of elasticity in occurred the L region. In all the studied growing seasons except for the first one the maximum MOE was identified on the OW side.

Examination of samples from the stem base (level A) of tree No. 2 showed that throughout the period of study (except for fifth year), the lowest MOE occurred in the wood generated on the RW side, and in the last year – on the R side (Fig. 55). The wood formed on the OW side exhibited the highest MOE in the first, fourth, and fifth seasons. In the annual rings formed in the second and third years, the greatest modulus of elasticity was found on the lateral, R and L sides, respectively. At the higher level (B), the first four annual rings of wood on the lower side (RW) was also characterized by the lowest MOE, in contrast to the fifth year, when the values of this parameter on the RW was similar to those on R and OW sides. In samples from this level (B), the maximum MOE occurred in the lateral directions: on the R side of the stem in the first two years and on the L side in the remaining three years. At the highest level (C), throughout the whole period of study the smallest MOE was found for the wood formed on the RW side, and the largest on the OW side, except for the third annual ring in which modulus of elasticity was slightly higher on the L side.

In tree No. 3 the smallest MOE values during the whole 5-year period were estimated in RW direction at lowest (A) stem level (Fig. 56). Low values of this characteristic at the lower stem side (RW) were detected also at the upper (B) level. At the two levels the MOE values in the OW direction were high. At uppermost stem region (level C) the differences between four directions on stem cross-sections were less pronounced.

3.11. Comparison of the results of measurements of the tracheid radial diameter and the cell wall thickness obtained by the SilviScan and WinCELL techniques

Comparison of the results was made separately for the three studied stem levels (A, B and C) of three trees using average data from five annual growth rings in the four directions on the stem cross-section (RW, L, R and OW). The data presented in Table 4 refer to the differences between the average (for the entire annual ring)
Table 4. Comparison of the results of tracheid radial diameter measurements made by the SilviScan (S) and WinCELL (W) techniques. Data for four radial directions on stem cross-sections (RW, L, R, OW) of three stem levels (A, B, C) of three Norway spruce trees (1–3). Figures denote mean values (S, W) and the differences (W-S) between the results of radial tracheid annual measurement made by the WinCELL and SilviScan techniques. Average values for five annual rings formed during the whole period of experiment.

<table>
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<tr>
<th>Tree No.</th>
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<th>R</th>
<th>OW</th>
<th>Average</th>
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</tr>
<tr>
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values of the tracheid radial diameter obtained by the two techniques. The results indicate that the differences are relatively small and do not exceed the value of 2.05 μm. When considering data relating to a total of four directions of the trunk of the differences between the values of the radial diameter are in the range from 0.13 to 0.63 μm, from 0.59 to –1.28 μm, and from –0.12 to –0.95 μm, for trees Nos. 1, 2 and 3, respectively. When the data for each of the four directions on the stem cross-section where compared the differences are greater. The differences between these type values are in the range from –0.68 to 1.89 μm, from –2.04 to –0.03 μm, and from –1.36 to 0.07 μm for trees Nos. 1, 2 and 3, respectively.

Concomitantly with estimation the tracheid radial diameter the WinCELL supplied also numerical data related to the cell wall thickness. Results of the comparison this method with the SilviScan is presented in Table 5. The differences in estimation average tracheid cell wall thickness ranged from –0.485 μm to 0.53 μm. However,

Table 5. Comparison of the results of tracheid cell wall thickness measurements made by the SilviScan (S) and WinCELL (W) techniques. Data for four radial directions on stem cross sections (RW, L, R, OW) of three stem levels (A, B, C) of three Norway spruce trees (1–3). Figures denote mean values (S, W) and the differences (W-S) between the results of tracheid cell wall thickness measurement made by WinCELL and the SilviScan techniques. Average values for five annual rings formed during the whole period of experiment.

<table>
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<th>Tree No.</th>
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<th>R</th>
<th>OW</th>
<th>Average</th>
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</table>

taking into account that average values of the cell wall thickness were relatively small (about 2 μm), the observed differences are high (about 25%). Seemingly, this was related to the difficulty of precise determining the cells lumen in the microscopic images containing successively formed tracheids in the very long radial files which were used for the WinCELL analysis. On the other hand, also with the SilviScan technique the difficulty to define the inner edge of the cell wall is recognized and the local wood density (mass) is used together to compensate this deficiency.
Discussion

The SilviScan studies of the structure of wood formed during the five years in which the inclined stems of Norway spruce (*Picea abies*) young trees reoriented to the vertical position revealed differences in several wood characteristics resulting both from natural changes in xylogenetic processes occurring during the course of ontogenesis and the direct gravitropic response of the stems. In order to keep the effects of these two processes separate, this work presents a comparison of wood structure between different cross-sectional directions of inclined stems, with a particular focus on their lower and upper sides, as it is generally accepted that compression wood forms on the lower side, with so-called opposite wood develops on the upper side. Xylem structure on the lateral sides of the stem, where neutral stresses prevail, should correspond to normal wood. The study involved samples from three stem heights collected at the end of the 5-year experimental period, which were: (A) the stem segment near the tree base, which was still inclined, (B) the intermediate segment, and (C) the apical segment, which had been reoriented in the upright position.

Publications examining the structure of annual rings in conifers usually make a distinction between early- and latewood. The former is characterized by tracheids with large radial diameters and thin cell walls while the latter is composed of tracheids with small radial diameters and thick cell walls. Macroscopically, latewood is usually characterized by a darker color as compared to earlywood. However, despite this seemingly simple and useful distinction, it is difficult to adopt clear-cut criteria for the two types of wood; a review of such criteria is given in the work of Creber and Chaloner (1984). In the case of coniferous species, researchers most often use the definition proposed by Mork (1928), which can be interpreted in two ways: latewood consists of tracheids with a lumen equal to or smaller than either (1) double cell wall thickness multiplied by two (Larson 1969; Kozlowski 1971; Koch 1972) or (2) double cell wall thickness (Fry and Chalk 1957; Fritts 1976; Timell 1986). According to Denne (1988), the divergent interpretations of Mork’s original definition by English-speaking authors may be due to the fact that the 1928 Mork’s paper published in German, contained a typographical error, and provided a rather vague description of the tracheid parameters in question. Denne’s short analysis (1988) shows that the percentage shares of latewood in annual rings calculated by the two
Formulas may be very similar in some species, while widely discrepant in others; for instance, in *Pinus contorta* latewood accounted for 29% according to version (1), but was entirely absent (0%) according to version (2), while in *Pinus strobus* latewood was missing (0%) for both versions. These examples show that fixed criteria for early- and latewood identification in annual rings may be inappropriate, and so the definition adopted by an author should correspond to the research objectives, especially for juvenile wood in which differences in tracheid wall thickness between xylem formed early and late in the growing season are much smaller than in mature wood (see Zobel and Sprague 1998).

As the spruce trees studied in the present work were young and their wood was juvenile, data concerning the relative proportions of early- and latewood in the annual rings are only of informational nature for general description of the wood structure. Furthermore, spruce wood is normally characterized by a gradual transition from early- to latewood. As a result, SilviScan measurements were conducted separately for three annual ring layers: earlywood, transition wood, and latewood. These layers were distinguished pursuant to the criteria developed for the SilviScan technology in which the parameter of wood density was used. It should also be noted that generally in the annual rings of conifers containing compression wood, distinguishing between the early- and latewood seems to be highly questionable. Thus, in this work the detailed analysis of the relationship between wood structure and gravitropic response was based on data for entire annual rings without any arbitrary earlywood–latewood distinctions.

An important feature of wood structure linked to the gravitropic response of tree stems and the formation of compression wood is annual ring eccentricity resulting from the development of wider increments on the lower side of the stem (containing compression wood) in contrast to increments on the upper side containing opposite wood (see Timell 1986). Differences in the width of annual rings were also observed in the present study over the 5-year period of reorientation to the vertical position of Norway spruce stems, and varied depending on the stem height. The fact that annual rings were commonly the widest on the bottom side of the lowest part of stem, which did not return to vertical by the end of the experiment, suggests that this phenomenon may be associated with an unfinished geotropic response in this portion of the trunk. In the uppermost stem segment, where the process of stem reorientation was completed, the annual rings on the lower side were wider only in the first years of the experiment. It should also be noted that in some instances the absence of marked differences between the neighboring directions of the stem may have been caused by the fact that the area occupied by reaction wood could have been larger around stem
circumference. The occurrence of such wide regions of reaction wood formation has been reported by a number of authors (see Timell 1986).

The results obtained in the present work show changes in some wood parameters (such as radial tracheid diameter or microfibril angle) between successive annual rings and these are essentially consistent with the findings of other researchers. It should be noted that the described processes concern the broader issue of wood morphogenesis linked to the mechanisms of cambial activity and the differentiation of cambial xylem derivatives. Age-related changes in the length of fusiform cells have been noted by many authors (see Larson 1994). Generally, in young small diameter stems the longer distance from the cambium to the stem pith, the greater length of the fusiform cells. Subsequently, with the increasing stem diameter the length of meristematic cells stabilizes, remaining more or less the same for many years and undergoing only minor seasonal or environment-induced alterations. Changes in the length of these cells are determined by the age-dependent relationship between the intensity of fusiform cambial cells and the rate of their intrusive growth (Romberger et al. 1993; Hejnowicz 2002). In coniferous wood, in which intrusive growth of cambial derivatives differentiating into tracheids is strongly limited after leaving the cambial zone, the age-dependent changes in the cambial fusiform cells are reflected in the tracheid length. Given that the present study involved young Norway spruce trees with a diameter at the beginning of experiment about 5 cm, one can assume that tracheid length increased in consecutive annual rings throughout the investigated 5-year period. Moreover, in a given season, the higher parts of the stem, characterized by a younger relative age of cambium, seemingly formed shorter tracheids than lower stem parts with relatively older cambium.

The SilviScan technique does not enable direct measurement of tracheid length, and so it was not investigated in the present work. However, it has been stated that a parameter that is closely associated with tracheid length is the angle of microfibrils in the cell wall. The relationship between these two parameters was suggested by Preston as early as in 1934; he proposed that the relationship between tracheid length (L) and microfibril angle (α) can be expressed by the formula: \[ L = \alpha + b \cot \alpha \] (Preston 1934). This hypothesis is in accordance with the results of a number of authors studying the relationship between cambial ageing, tracheid length, and microfibril angle (see Timell 1986). The decreasing trend of microfibril angle in the tracheids formed in consecutive annual rings observed in the present work, were thus probably caused by the aging process naturally occurring in xylogenesis. There is a number of studies showing relationship between the angle of cellulose microfibrils in the cell wall and the parameters affecting the physical properties of wood, such as Young’s modulus of elasticity (see Timell 1986) which was investigated in this paper. Taking into con-
sideration that this study was conducted on young spruce trees it can be assumed that the age-related changes in tracheid length could be involved in the observed changes of these wood characteristics.

The results of our experiment with tilted trees showed that the process of the main stem reorientation is associated with the formation of reaction wood. However, in case of juvenile wood of young Norway spruce trees, the reaction wood is not always manifested as typical compression wood with distinct asymmetry of annual rings and darker color of reaction wood bands on lower sides of the inclined stems. In a number of cases no characteristic wide layers of compression wood cells consisting of thick walled tracheids of rounded shapes on the stem cross-sections were observed. In the successive annual rings formed during the experiment the dynamic changes of various anatomical and physical characteristics of wood occur. These changes relate to such characteristics as the width of the ring, tracheid diameter and wall thickness, wood density, MFA and MOE. These changes were observed both at the bottom and the upper sides as well as on the lateral sides of the tilted stems. The results indicate that greater the value of MFA in the cell walls of tracheids wood was the basic difference between compression wood and opposite wood in the same annual ring. The absolute MFA values in juvenile wood cannot be considered as indicators reflecting gravitropic reaction of the main stem since reorientation takes place only if in a given annual ring there are differences between the MFA values in cell wall tracheids from the opposite sides of tilted stem.

Microfibril angle decreases with increasing age of cambium which is manifested in gradually lower MFA values in the tracheids formed in the successive annual rings of wood. The inverse trend is manifested when the MOE values are taken into account; the highest values of this characteristics occur in the tracheids formed in the outermost annual rings of wood. In the young Norway spruce trees the highest values of MFA were noted at the base and the lowest values at the uppermost part of the stem. The decrease of MFA is associated with the increase of MOE which may suggest lower flexibility of wood formed at the upper part of the stem of the experimental trees. In juvenile wood of stems of *Picea abies* trees the spatial and temporal trends of wood density changes were similar to that observed for MFA: the highest density values were found at the stem base and the lowest at the uppermost part of the stem; wood density decreased slightly with increasing distance from the pith in the successively five annual rings. Decrease of wood density, which can be assumed as indicator of stem stiffness, was associated with simultaneous increase of tracheid diameter and slight decrease of their cell wall thickness.

The present results indicate that SilviScan technology which allows simultaneous measurements of physical parameters and anatomical the individual tracheids
formed sequentially in the successive annual rings of wood offers new possibilities for studying physiological mechanisms of growth and response of forest trees to changing environment during the life span of a tree. The WinDENDRO and WinCELL techniques commonly used in studies on wood structure in the tree trunks enable to obtain data relating to the width of the annual ring and the anatomical characteristics such as wall thickness and tracheid diameter with comparable to SilviScan accuracy. These techniques, however do not allow the study of successively formed tracheids with respect to the very important for the tree stem biomechanics characteristics such as MFA, MOE and wood density.

Of particular note is the fact that in the present study compression wood was not always formed on the same side over consecutive years of reorientation of the leaning stem. While initially compressive wood formation occurred on the lower side, over time it was sometimes found on the upper or lateral sides, especially in the higher regions of stem, which returned to vertical orientation after the 5-year period of experiment. This suggests that the amount of compression wood generated by the geotropic response of the tree in a given year could lead to excessive reorientation resulting in the stem deviating in the opposite direction. That deviation would be in turn corrected by a compression wood layer on the opposite side of the stem. This may indicate that the formation of compression wood, causing small changes in stem inclination, constitutes a mechanism for the fine tuning of spatial orientation with respect to the gravity vector. This is in line with Darwin and Darwin’ early theory (Brown 1993) that the tropic movements of herbaceous plants enable continuous adjustment of the organ’s orientation with respect to the vector of the tropic stimulus. There is also some analogy here to the nutation of herbaceous plants or to the twining growth of lianas which permits ongoing modification of the orientation of the organs with respect to the objects they surround. There is also some similarity to the continuous response of plants to changes in the lunar–solar gravitational acceleration (Barlow 2015; Zajączkowska and Barlow 2016).

In turn, woody angiosperms develop tension wood, which provides a mechanism corresponding to compression wood formation. It should also be noted that in some woody species, the role of reaction tissue in geotropic processes can be fulfilled by tissues external to secondary xylem and cambium, located in the secondary phloem layer (Böhlmann 1971; Tomlinson 2003; Zajączkowska and Kozakiewicz 2016).

Despite the fact that the movement mechanisms of herbaceous plants are usually based on osmotic processes in the epidermis or collenchyma (Hejnowicz 2011) or also in trichomes, as it has recently been reported (Zajączkowska et al. 2015), the principles governing these mechanisms in herbaceous and woody plants are similar. They are linked to the tendency to achieve the lowest energy state sufficient to main-
tain a given plant structure subjected to the action of the various physical fields, such as gravity, light, humidity, wind, etc.

In the case of woody plants, the formation of reaction wood is one of the mechanisms responsible for the reorientation of organs within a plant body. During tree ontogenesis (e.g. at seedling stage), or at different stem regions of the same plant (e.g. the apical stem meristems), different morphogenetic mechanisms responsible for organ reorientation occur, and an important role can play e.g. changes in cell turgor, or uneven cell elongation growth. From this point of view the formation of reaction wood should be considered as part of an integrated morphogenetic mechanism responsible for the reorientation processes that allow to maintain the whole body of a woody plant in the state of minimum energy under changing environmental conditions.

Authors investigating these topics often use the conceptual framework of a morphogenetic field, which, over the decades, has been adopted for the description of different groups of organisms (Thompson 1917; Barlow and Carr 1984; Klebe et al. 1991; Wolfson et al. 2008) The morphogenetic field affects cell growth processes, typically resulting in symplastic growth in plants. A study on the mechanism of Douglas fir stump overgrowth (Zajęczkowska 2014b) indicated that the morphogenetic field (F) controlling this type of growth is an irrotational vector field (curl F = 0), while the occurrence of intrusive growth in the cambium (associated with anticlinal divisions controlling the length of cells in the axial system of the xylem) may be linked to local rotations of the morphogenetic field (curl F ≠ 0). It should be noted that fusiform cambial initials, which give rise to secondary xylem, are characterized by intrusive growth. Together with anticlinal divisions, they enable increase of stem circumference due to the radial growth of the stem. Given that compression wood formation is usually associated with locally greater stem radial growth, a higher frequency of anticlinal divisions, and more intensive intrusive growth in the region where xylem is formed (Hejnowicz 2002), one may hypothesize that compression wood arises in regions where the morphogenetic field is disturbed and local field rotations occur (curl F ≠ 0). On the other hand, the process of reaction wood formation in the main stem and in the lateral shoots plays a major role in the regulation of tree architecture (Zajączkowska 2013), and it can be affected by the presence of a morphogenetic field influencing the polar transport of auxin leading to the development of structures requiring minimum energy expenditure for their maintenance (Zajęczkowski and Wodzicki 1978; Zajączkowski et al. 1984). Such mechanisms optimize the shape of woody plant organs minimizing mechanical stresses within the entire organism (Mattheck and Kubler 1995; Mattheck 1997; Zajączkowska 2006).
The reorientation of the organs of woody plants through the formation of reaction wood is associated with cell division in the cambial region and differentiation of cambial xylem derivatives to achieve specific ordering of the newly formed cell arrangements in the secondary xylem. Even though tissue ordering processes have been discussed in several handbooks and monographs devoted to plant anatomy (e.g. Fahn 1990; Romberger et al. 1993; Hejnowicz 2002), until recently no research methods enabled the quantification of this process. This situation changed with the development of digital image processing based on the structure tensor (Jahne 1993; Bigun et al. 2004). Initially, such methods were used to measure the orientation of elastin fibers in human cerebral arteries (Fonck et al. 2009), as well as collagen orientation in arterial adventitia (Rezakhaani et al. 2012). Recently, digital image processing has also been successfully adopted for measurement of cellular ordering in regenerating plant tissues (Zajączkowska 2014a, b) and for investigation of xylemogenetic processes in callus cultures of woody plants (Zajączkowska 2015). It seems that the adaptation of these methods studying reaction wood structure with respect to cell ordering quantification may permit the characterization of the geotropic reaction mechanism in more general terms, taking into account the thermodynamic aspects of growth and differentiation processes in trees.

In their paper on graviresponse in land plants, Hoson and Wakabayashi (2015) noted that the development of the gravity resistance mechanism has played an important role in the acquisition of responses to various mechanical stresses and evolution of land plants. The cell wall is responsible for the final step of gravity resistance, with the gravity signal increasing its rigidity, which is also affected by changes in cell wall metabolism and the environment.

To sum up the discussion, the reorientation of tree stems and lateral branches in a gravitational field brought about by the formation of reaction wood, whose anatomical structure and physical properties allow the movement of structures with dimensions of up to more than 100 m height and a weight of several dozens of tons, is analogous to that found in small herbaceous plants, in which reorientation may occur, e.g. by differential cell growth on two sides of a responding organ. In all cases, plants tend to minimize the energy expenditure necessary to maintain the system. Researchers dealing with tree morphogenesis can now relate graviresponse to the widely accepted morphogenetic mechanisms described mostly for herbaceous plants (with a particular focus on the model organism Arabidopsis). New opportunities for research in this area have emerged thanks to the development of digital image processing enabling quantification of cell ordering, including the SilviScan technology, which can be used to quickly acquire a large body of data concerning a variety of anatomical and mechanical parameters for individual cells. Another promising
approach in the study of reaction wood is the recently developed techniques of transforming digital images of anatomical wood structures into digital sound recordings, which can subsequently be subjected to acoustic spectral analysis (Zajączkowska 2016). The available software makes it possible to quickly identify, in data series encompassing dozens of years, successive annual rings and wood regions with cells characterized by similar parameters.
Conclusions

The results of the present studies on gravitropic response of the inclined stems of young Norway spruce trees allow to present the following conclusions:

1. The process of main stem axis reorientation is associated with the formation of reaction wood. In case of juvenile wood of young Norway spruce trees, the reaction wood is not always manifested as typical compression wood with distinct asymmetry of annual rings and darker color of reaction wood bands on lower sides of the tilted stems. In a number of cases no characteristic wide layers of compression wood cells consisting of thick-walled tracheids of rounded shapes on the stem cross-sections were observed. The results of this study indicate that greater value the cellulose microfibril angle in the cell walls of tracheids wood is the basic difference between compression wood and opposite wood in the same annual ring.

2. In the successive annual rings formed in the region of juvenile wood the dynamic changes of various anatomical and physical characteristics of wood occur. These changes relate to such characteristics as the width of the ring, tracheid diameter and cell wall thickness, MFA, wood density and MOE. These changes occur both at the bottom and the upper sides of the tilted stem, as well as on the lateral sides. Therefore, any criteria for distinguishing reaction wood cannot be based on an assessment of the absolute values of these parameters, but on the basis of their diversification around the circumference of the same annual ring.

3. The absolute MFA values cannot be considered as indicators reflecting gravitropic reaction of the inclined main stem. The gravitropic reaction occurs only if in a given annual ring there are differences between the MFA values in cell wall tracheids from the opposite sides of inclined stem. In the studied young Norway spruce trees the highest values of MFA occur at the base of the tree trunk and the lowest values at the uppermost part of the stem. The decrease of MFA is associated with the increase of MOE which indicates decreased flexibility of wood formed at the upper part of the main stem. Microfibril angle decreases with increasing age of cambium which is manifested in lower MFA values in the tracheids formed in the successive annual rings of wood.

4. In juvenile wood of stems of young Norway spruce trees the spatial and temporal trends of wood density changes were similar to that observed for MFA: the highest density values usually were found at the stem base and the lowest at the up-
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Permost part of the stem; wood density decreased slightly with increasing distance from the pith in the successively formed five annual rings. Decrease of wood density was associated with simultaneous increase of tracheid diameter and slight decrease of their cell wall thickness.

5. With the decreasing of the MFA values the flexibility of stem increases, as indicated by the increased values of MOE. These changes are accompanied by increasing the stiffness of stem, which are an indicator of higher density. The higher density at the base of the stem is associated with a greater thickness of the cell wall and the smaller tracheid diameter.

6. The present results indicate that the SilviScan technology which allows simultaneous measurements of physical parameters and anatomical the individual tracheids formed sequentially in the successive annual rings of wood offers new possibilities for studying physiological mechanisms of growth and response of forest trees to changing environment during the life span of the tree.

7. WinDENDRO and WinCELL techniques commonly used in studies on wood structure in the tree trunks enable to obtain data relating to the width of the annual ring and the anatomical characteristics such as wall thickness and tracheid diameter with comparable to the SilviScan accuracy. These techniques however, do not allow the study of successively formed tracheids with respect to the very important for the tree stem biomechanics characteristics such as MFA, MOE and wood density.

8. In the case of woody plants, the formation of reaction wood is one of the mechanisms responsible for the reorientation of organs within a plant body. During tree ontogenesis (e.g. at seedling stage), or at different stem regions of the same plant (e.g. the apical stem meristems), different morphogenetic mechanisms responsible for organ reorientation occur, and an important role can play, e.g. changes in cell turgor, or uneven cell elongation growth. From this point of view the formation of reaction wood should be considered as part of an integrated morphogenetic mechanism responsible for the reorientation processes that allow to maintain the whole body of a woody plant in the state of minimum energy under changing endogenous and environmental conditions.
Authors contribution statement

Urszula Zajączkowska conceived and designed the research, performed the field experiment, collected and prepared wood samples, performed anatomical observations, analyzed all data, and wrote the manuscript. Sven-Olof Lundqvist in cooperation with Urszula Zajączkowska conducted measurements of wood characteristics with the SilviScan and prepared the section of manuscript describing the SilviScan technique. Urszula Zajączkowska and Mateusz Bujalski performed measurements with WinDENDRO and WinCELL techniques.
Literature cited


LARSON P.R. 1969. Wood formation and the concept of wood quality. Yale University, School of Forestry Bulletin 74.


Streszczenie

Tworzenie drewna reakcyjnego w czasie odpowiedzi grawitropicznej pni młodych drzew świerka pospolitego [Picea abies (L.) Karst]. Drzewa mają zdolność do zmiany orientacji przestrzennej pnia głównego i gałęzi bocznych dzięki możliwości tworzenia tzw. drewna reakcyjnego. Występuje ono zarówno u drzew iglastych, jak i liściastych, a jego działanie polega na wytworzeniu specyficznej asymetrii wymiarów, geometrii oraz naprężeń w strukturach anatomicznych organu. Między drzewami iglastymi i liściastymi istnieje zasadnicza różnica zarówno w strukturze drewna reakcyjnego, jak i w sposób jego działania. Drzewa iglaste tworzą tzw. drewno kompresyjne, które generuje naprężenia ściskające wzdłuż osi organu i występuje po stronie spodniej pochylonego pnia lub gałęzi bocznych. Odwrotnie funkcjonuje występujące u drzew liściastych tzw. drewno tensyjne, które wywołuje naprężenia rozciągające i jest tworzone zwykle po stronie górnej reorientującego się organu.

Celem niniejszej pracy było zbadanie zmian w strukturze anatomicznej drewna podczas reorientacji pnia głównego w eksperymencie z pochylonymi młodymi drzewami świerka pospolitego, z wykorzystaniem unikatowej aparatury SilviScan, znajdującej się w Innventia, Wood and Fibre Measurement Centre w Sztokholmie. Aparatura ta pozwala na równoczesny pomiar następujących parametrów: średnica i grubość ścian komórkowych kolejno tworzonych w kierunku promieniowym cewek, gęstość drewna, kąt mikrofibryli celulozy (MFA) oraz moduł sprężystości (MOE).

Badania terenowe wykonano w Arboretum SGGW w Rogowie, gdzie założono doświadczenie z pięcioletnimi drzewami świerka pospolitego [Picea abies (L.) Karst.], posadzonymi ukośnie (pod kątem ok. 45°) w celu zain dukowania tworzenia drewna kompresyjnego, w procesie reakcji grawitropicznej prowadzącej do reorientacji pnia głównego do pozycji pionowej. Po upływie pięcioletniego okresu doświadczenia, górne partie pnia przyjęły orientację pionową, a pochylona pozostała jedynie dolna część pnia znajdująca się u podstawy w pobliżu gleby. Do analiz struktury drewna pobrano próbki w postaci dysków wyciętych z trzech regionów pnia: (A) z pochylonej części dolnej, (B) ze strefy przejściowej oraz (C) z części górnej, która po pięciu latach przyjęła pozycję pionową.

Badania struktury drewna przeprowadzono pomiarami aparatury SilviScan (Innventia) oraz pomiarami wykonanymi pod mikroskopem optycznym (laboratorium Samodzielnego Zakładu Botaniki Leśnej SGGW), które przeanalizowano standardowymi technikami przy zastosowaniu programów WinDENDRO i WinCELL.
Obserwacje mikroskopowe wykazały, że reakcja grawitropiczną pnia w procesie reorientacji młodych drzew świerka pospolitego wiąże się z tworzeniem drewna o charakterze drewna reakcyjnego, które w drewnie juwenilnym świerka nie występuje, przypomina typowym dla drewna kompresyjnego, wyraźną asymetrią słojów i ciemniejszą barwą drewna po spodniej stronie pnia, a także charakterystycznymi dla drewna reakcyjnego, szerokimi warstwami grubościennych cewek o okrągłych kształtach na przekroju poprzecznym. W wielu przypadkach drewno to występuje w postaci stycznych warstw na granice poprzecznej, której można zwykle zauważyć, a także w postaci traumatycznych przewodów żywicznych, a czasem również różnicowanie się pojedynczych cewek o dużym świetle.

W ciągu pięcioletniego okresu doświadczenia w kolejnych rocznych słojach, wraz z postępującym wiekiem kambium, nastawały dynamiczne zmiany różnych parametrów takich jak: szerokość słoja, średnica cewek, grubość ścian komórkowych, MFA, a także takich parametrów fizycznych jak MOE czy gęstość drewna. W badanym okresie nastawały zrewizjowanie szerokości rocznych słojów drewna. Zmianom tym towarzyszyło zwiększenie się szerokości promieniowej cewek, grubość ściany komórkowej natomiast nie wykazywano wyraźnych zmian. W wyniku tych dwóch procesów nastawały zmniejszanie się gęstości drewna. Wraz z postępującym wiekiem kambium obserwowano także różnice w wielkości kąta mikrofibryli oraz wartości modułu sprężystości, które stwierdzano głównie w górnych partiach pnia. Porównanie parametrów struktury drewna w tych samych słojach rocznych z dolnej i górnej strony pochylonego pnia wskazuje, że reakcji grawitropicznej towarzyszyło zwykle zwiększanie MFA z dolnej strony pochylonego pnia, gdzie tworzyło się drewno kompresyjne. Najmniejsze wartości MFA notowano natomiast w części górnej, odpowiadającej strefie tzw. drewna przeciwwległego (OW – opposite wood). Zwiększonym wartościom MFA towarzyszyło zmniejszenie wielkości MOE.

Analiza parametrów drewna na różnych wysokościach pnia wskazuje, że w drewnie młodszych świerków największe wartości MFA występują u podstawy pnia, a najmniejsze w części wierzchołkowej. Wraz ze zmniejszeniem się wielkości MFA zwiększają się wartości modułu sprężystości (MOE). Zmianom tym towarzyszy zwiększenie się wartości gęstości drewna, które związane są ze zmniejszającą się grubością ściany komórkowej i większą średnią cewek. Wyniki te wskazują, że dzięki zróżnicowaniu procesów ksylogenezy w obrębie pnia głównego, wraz ze wzrostem młodych drzew świerka utrzymywanej jest duża elastyczność pnia ułatwiająca reakcję drzewa na zmienne warunki środowiska (np. wiatr) przy równocześnienym zachowaniu stosunkowo dużej sztywności pnia, zwiększającej wytrzymałość na rosnący ciężar całego drzewa.
Otrzymane w niniejszej pracy wyniki wskazują, że technologia SilviScan, umożliwiająca pomiary różnych parametrów anatomicznych i fizycznych na tych samych cewkach tworzonych kolejno w słoju rocznym, stwarza zupełnie nowe możliwości w badaniach fizjologicznych mechanizmów wzrostu oraz reakcji drzew leśnych na zmienne warunki środowiska w ciągu całego życia drzewa.

Obserwowane zmiany parametrów drewna wskazują, że jakiekolwiek kryteria wyróżniania drewna reakcyjnego nie mogą polegać na ocenie bezwzględnych wartości tych parametrów, lecz powinny być określone na podstawie różnicowania tych wielkości na obwodzie pnia w danym słoju rocznym.

Stosowane dotychczas zazwyczaj w badaniach struktury drewna pni drzew techniki z zastosowaniem programów WinDENDRO i WinCELL pozwalają na uzyskanie, że zbliżoną do SilviScan dokładnością, danych odnoszących się do szerokości słojów oraz takich parametrów anatomicznych jak grubość ściany czy średnica cewek. Zastosowanie tych technik jest jednak ograniczone, bowiem nie pozwalają one na badanie w kolejno tworzonych cewkach tak istotnych, dla funkcjonowania pnia biomechaniki drzewa, parametrów jak MFA, MOE czy gęstość drewna.