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Is the orientation of the fibrillar helix in the main layer of cell walls constant or variable within a tree?

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Abstract

The study material consisted of samples from 270 *Picea excelsa* and 54 *Abies alba* trees with variable type of wood grain. No correlation was found between the orientation of the fibrils and the type of grain. In all the samples in which the fibrillar helix formed an angle with the cell axis greater than 10° (80% of the total) the helix was of Z type. In the other samples undulations commonly occured in the orientation of fibrils within a cell. These were in the form of local deviations to the left or right of the general trend to develop the Z type of spiral.

INTRODUCTION

In plants several phenomena and structures occur in two orientational variants the „right" one and the „left" one (see Schmucker 1924). As examples one can mention wood spiral grain, phyllotactic helices, pseudotransverse anticlinal division in cambial initials, overlapping of intrusively growing cell tips in cambium, etc. The description "right" or "left" is applied to alternative configurations of structures or phenomena, where one corresponds to the mirror image of the other. A simple example are the middle strokes of the letters Z and S. We can use Z as the symbol for "right" and S as the symbol for "left". In order to describe a configuration of a body with this symbols one has to identify in it two directions, for example an axis and some direction forming an angle α with this axis, $0° < \alpha < 90°$. For example, in the case of the fibrillar helix in the cell wall we can consider the axis of the cell as one direction and the projection of the spiral on an axial plane as the other. In the case of pseudotransverse anticlinal divisions in cambium we can consider the axis of the parent cell and the axis of the newly formed cell wall. When describing what is "left" and what is "right" one has to decide from which side one is observing the orientation. For example the type of fibrillar helix in the cell wall is described when the cell is observed from the outside. When describing the pseudotransverse divisions of the cambium it is insufficient to say that the cell is observed from the outside, one has to decide from which side one is looking at the cambium. We can therefore say that in the first case the differentiation into "left" and "right" is determined on the level of the cell, whereas in the second it is on the tissue level, as is the case in cambium.

The type of alternative configuration can be a genetically determined character, and then only one of the types occurs in an individual. In other cases both types can occur, depending on the internal or external factors (see Dormer 1964). An example of the occurrence of both types can be found in the cambium, where two kinds of orientational domains (Z and S) exist (see Hejnowicz and Krawczyszyn 1969).

The question arises whether the variation in alternative configurations occurs also on the level of the cell in the fibrillar helix. The further question arises whether correlation can exist between the type of spiral grain and the type of fibrillar helix. Bases for the suggestion, that such a correlation can exist are to be found in the data of Jaccard and Frey (1928), who suggested that in *Picea excelsa* the fibrillar helix was of S type in a tree with an S spiral grained wood and Z type in Z spiral grained wood whereas in straight grained wood either Z or S type fibrillar helices were found.

In coniferous tracheids it is possible to distinguish three layers of secondary wall: the outer (S1), middle (S2) and inner (S3). The middle layer is the thickest and constitutes the bulk of the cell wall. The fibrillar orientation in the S2 layer varies from vertical to a deviation of 30° or more from the cell axis. The orientation of the fibrils in this layer is most significant and thus may control the behaviour of the entire tracheid cell wall. The layers S1 and S3, although much thinner than S2, are orientationally more complex, containing some number of lamellae of alternating S and Z orientation. In the S2 layer, which also consists of numerous concentric lamellae, the fibrillar orientation appears to be similar (see Wardrop 1964; Panschin et al. 1964). The orientation of the fibrils in the S2 layer determines the orientation of the flattened pit canals. Also radial cracks within the cell wall follow the microfibrils in the S2 layer. Sanio (1863) reported that a Z arrangement of the flattened pit canals, both in coniferous and in broadleaved woods, is the rule. Krieg (1907) reported however, that in the numerous representatives of coniferous trees that he had studied, including *Picea excelsa,* the strands in the tracheid walls, corresponding to fibrils, always had an S orientation. Schmucker (1924) confirmed the observations of Sanio with regard to *Pinus* and *Taxus* without mentioning other genera. Bucher (1957) found a Z spiral in S2 in 7 of the studied species, including *Picea excelsa,* Wardrop and Dadswell (1957) reported that the fibrillar helix in the S2 of *Pinus radiata* tracheids is of type S, however, in the studies of Meier (1957) also in this species a Z type spiral was observed. Panschin, De Zeeuw, and Brown (1964) reported that in the majority of coniferous species a Z type helix occurs in S2 and an S type in S3.

MATERIALS AND METHODS

The samples originated from 324 trees *Picea excelsa* and *Abies alba* growing in the Tatras (270 trees of *Picea* and 54 of *Abies*), aged 30—80 years and differing in the type of wood grain: straight, spiral (helical) — Z and S with different slope of grain. The type of grain was determined on the basis of fissures on the surface of the debarked trunks.

From 321 trees single samples were collected from a height of 1.5 m above the ground. From two *Picea* stems about 80 years samples were collected every 1.5 m (12 and 13 samples from a tree). One of these stems had a Z spiral grain for 6 meters upward from the base, then straight grain for 2 m, and S-spiral grain in the following 10.5 m. Four samples from this trunk originated from the region with a Z-spiral grain, one from the straight grain segment, and seven from the S-spiral grain.

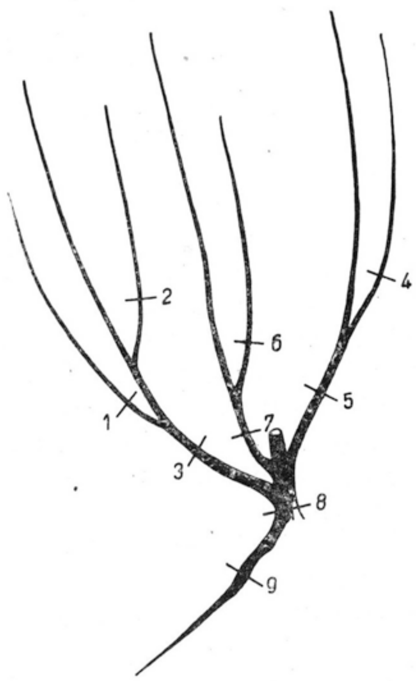


Fig. 1 Sampling of xylem from a 16-year-old *Picea.*

The second stem had for its full length a distinct Z-spiral grain. From one 16-year-old *Picea* tree, which was decapitated in the 7th year, 8 samples were collected from various parts of the crown and one from the root (Fig. 1).

The orientation of the fibrillar helix was determined on macerated tracheids and on transverse-medial oblique sections.

The maceration was performed on thin strips in a mixture of hydrogen peroxide solution (commercial, circa 30%) and glacial acetic acid 1 : 1 at a temperature of 100° C. Adequate maceration required about 1.5 hours.

The oblique microtome sections were prepared from specially treated wood. The treatment, the purpose of which was to soften the wood as well as to prevent the separation of various layers of the cell wall during cutting, consisted of placing the wood blocks (5 × 5 × 50 mm) into a mixture of hydrogen peroxide and acetic acid 1 : 1 at a temperature of 100° C for a period of one hour, then washing them thoroughly under running water for about 20 hours and transferring to a 50% solution of gelatine, in which the blocks were left for two days at a temperature of 60° C until completely saturated with gelatine. Later, when the blocks had attained a solid consistency at room temperature, microtome sections were cut from them, 10—15 microns thick. These were later embedded in glicero-gelatine.

RESULTS

In the studies conducted on macerated material the direction of the flattened pit canals and the course of cell wall fissuring were considered. The direction of flattened pit canal inclination was determined only in such fragments of the cells, in which the pits occurred on both opposite cell walls (Plate I, fig. *G—K*). When the pits perforate only one of the walls it is very easy to determine erroneously the side that one is observing. The studies of of pit orientation were conducted primarily in the cross fields in early tracheids, in view of the greater inclination of the flattened pit canals relative to the cell axis than is the case in late tracheids. When it was not possible to determine the orientation of the pit canals in early tracheids, for example when the canals were not markedly flattened (particularity in *Abies*), then the tracheids from late wood were taken. Results of the observations on macerated tissues are presented in table 1.

In the majority of trees (81%), regardless whether the trees had S or Z spiral grain or were straight grained, all the pits were inclined in the Z direction (looking on the external surface of the wall, Plate I, *G*). It is emphasized that such a situation occurred everywhere where the

Table 1

Orientation of microfibrillar helix in tracheids determined in basis of flattened pit canals

Species		Type of grain	Number of trees	Number of samples	Number of samples. Pit canals form angle with tracheid axis when observed on on external surface					
					to the right Z**		to the left S		either right or left Z—S	
					>10°	<10°	>10°	<10°	>10°	<10°
Picea excelsa	single sampling	Z	112	112	97	9	—	3	—	3
		straight	88	88	76	10	—	—	—	2
		S	67	67	53	12	—	—	—	2
	single sampling	Z	1	11	11	—	—	—	—	—
		mixed*	1	12	12	—	—	—	—	—
	young tree		1	9	9	—	—	—	—	—
Abies alba	single sampling	Z	9	9	7	2	—	—	—	—
		straight	17	17	11	5	—	—	—	1
		S	28	28	17	11	—	—	—	—

* Z-spiral along the first 4 m the bottom, straight grain along the next 2 m and S spiral in the remaining 10 m

** the angle greater and smaller than 10° respectively.

angle between the flattened canals and the axis of the tracheid was on the average greater than 10°.

This situation was also evident in the trees from which serial samples were taken (two old *Picea* trees and one young one described in fig. 1). Thus we can conclude that the S2 layer in the tracheids was characterized by a fibrillar helix of the Z type. In 60 trees from which wood samples were obtained from the peripheral part of the trunk the inclination of the flattened pit canals to the axis of the tracheid was on the average bellow 10°. In 49 samples from this group the pit canals were inclined in the Z direction or else were positioned parallel to the tracheid axis (Plate I, *K*). In three samples, the majority of pit groups had canals inclined in the S direction (Plate 1, *H, J*). In the 8 remaining samples besides the parallel pit canals there occurred those inclined in the Z or S direction with approximately equal frequency. (Plate I, *I*). Studies on the arrangement of pit canals in the last group indicate that the arrangement of the microfibrils in the S2 layer is either more or less parallel to the axis with local S or Z deviations, or spiral of the Z type, with a small angle of inclination, with local variations so that in places the microfibrils are inclined more in the S direction than on the average in the Z direction (Fig. 2, Plate I, *F, H, I*). It is not untikely that even in the three samples where the majority of pit canals were inclined in

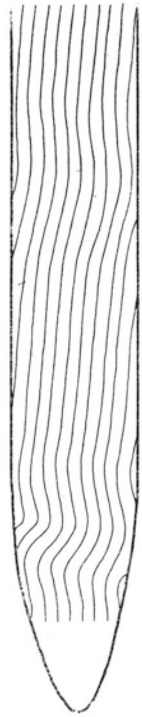


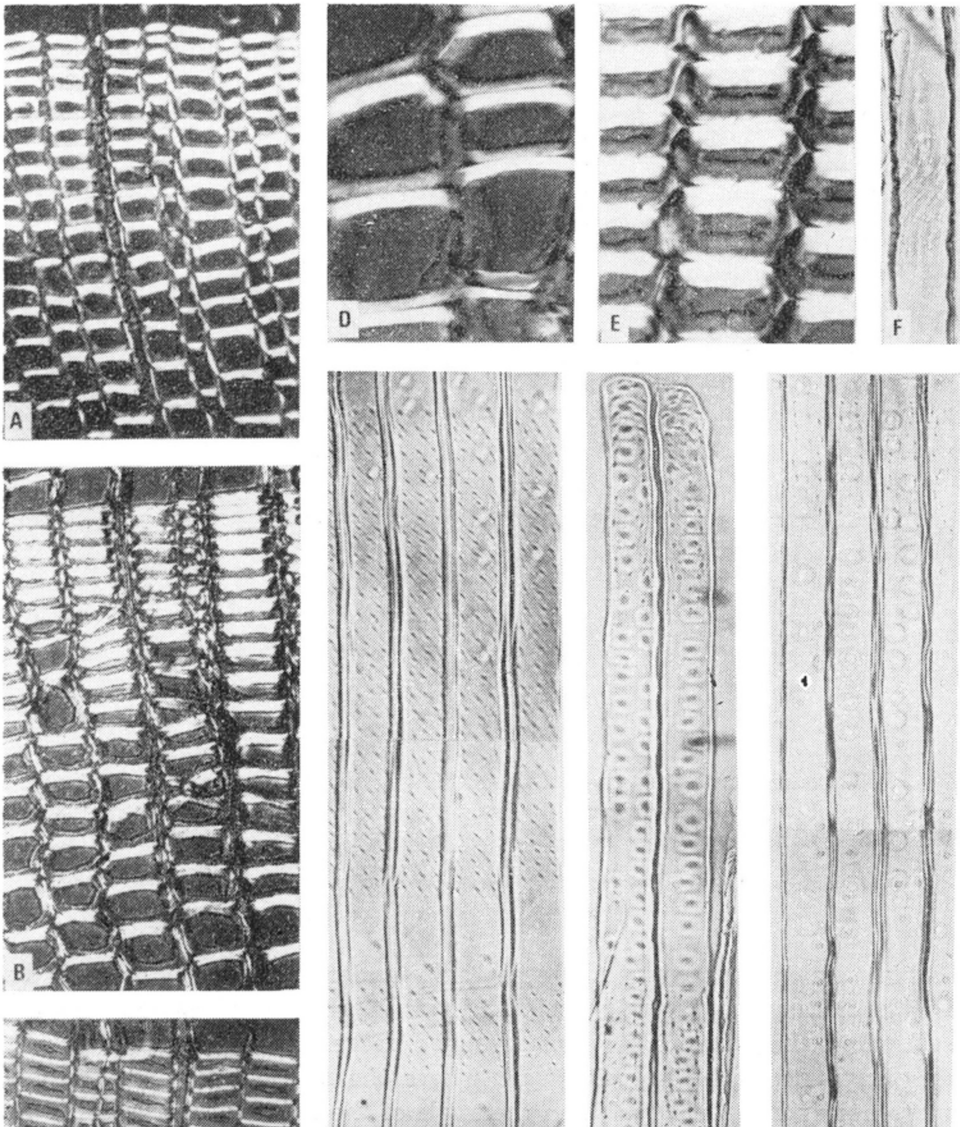
Fig. 2 Interpretation of the variable inclination of microfibrils in a tracheid with a very steep Z-spiral

the S direction we were in fact dealing with local deviations of microfibrils without there being a consistent helix of the S type.

The method of studying pit canals generally is destructive of the natural arrangement of cells (the groups of cells represented in Plate I have been selected from a large number of single cells). In particular it is not known what type of fibrillar helix occurs in the neighbors of a cell in which the type has already been established.

Plate I

A-Abies alba, *B-K* — *Picea excelsa*. *A-E* — oblique radial-transverse sections between crossed Nicols (the difference in brightness of adaxial and abaxial walls is seen). *A*, *B*, *D*, *E*-reveal the Z-spiral of microfibrils in S_2, *C* — locally bright adaxial walls indicating S inclination of the microfibrils. *F-K* — tracheids from macerated xylem illustrating the arrangement of flattened pit canals. *C*, *H* and *J* from the same sample, similarly *B* and *G*. In *G* the flattened pit canals are seen on the lower wall, in others on the upper wall. Enlargement: *A-C* and *F-I*, *D*, and *E*, *J* and *K*


A
B
C
D
E
F
G
H
I
J
K

In order to study these neighbourhood relationships oblique sections were studied under a polarizing microscope (see: Preston 1952). The principle of this is as follows: in S2 the fibrills are arranged more or less obliquely to the vertical axis, because of their spiral arrangement. The tangent to the microfibril in the pithward wall crosses with a similar tangent in the cambialward wall (Fig. 3). When an oblique transverse-

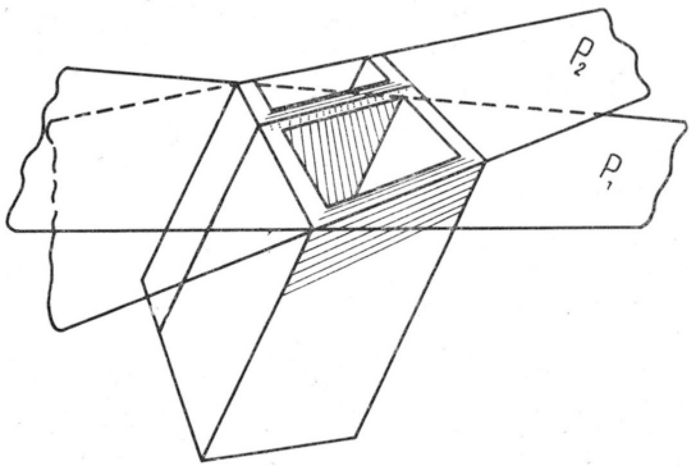


Fig. 3 Diagram illustrating the effect of oblique radial-transverse sectioning on orientation of microfibrils relative to the cut face in two opposite tangential walls; P1 — the plane perpendicular to the cell axis, P2 — the plane parallel to the microfibrils in the frontal wall.

-radial section is observed microscopically under polarized light the walls will be not equally birefringent. That wall in which as a result of oblique sectioning the microfibrils are arranged more nearly vertical will be less bright than the opposite wall, where the microfibrils are arranged more nearly horizontally. When the angle of the cut is Z (that is when we look at the wood sample from the side of the cambium), and the microfibrillar helix is also Z the external wall will be more bright. When the slope of the cut is changed or the orientation of the helix is opposite (but not both these factors simultaneously) then the internal wall will be brighter. The angle of the oblique cut, giving the maximal contrast between the walls, that is resulting in one of the tangential walls remaining dark, also provides information about the angle of microfibrils to the cell axis, or the slope of the helix. In the studied material the helices were rather steep. The method worked when the helical angle was greater than 10°. In instances in which the angle of microfibril inclination was less than 10° differences in brightness of walls were not distinguishable.

In this study we used an oblique cut of the Z type with an inclination

Table 2

Orientation of microfibrillar helix in the tracheids determined on oblique transverse-radial section in polarizing microscope

Species		Type of grain	Number of trees	Number of samples	Number of samples. The difference in brightness between pith and cambialward walls: widespread and distinct, the brighter wall: cambialward (Z)	widespread and distinct, the brighter wall: pithward (S)	no difference or nonconsistent one
Picea excelsa	single sampling	Z	32	32	19	—	13
		straight	41	41	32	—	9
		S	16	16	12	—	4
	serial sampling	Z	1	12	12	—	—
		mixed*	1	11	11	—	—
	young tree		1	9	9	—	—
Abies alba		Z	2	2	2	—	—
		straight	7	7	2	—	5
		S	5	5	2	—	3

* Z-spiral along the first 4 m from the bottom, straight grain along the next 2 m and S-spiral in the remaining 10 m.

usually of 70°, but in some samples it was more convenient to use smaller or greater angles. Results of these studies are presented in table 2.

In all the samples, in which on macerated tracheids the angle between the microfibril and cell axis was found to be greater than 10° the brighter layer of S2 was found on the cambialward wall (Plate I, *A, B, D, E*), from which it can be concluded that the fibrillar helix in S2 was of type Z. The difference in wall brightness was universal troughout the section, however, it was not obvious to the same extent in early and late tracheids after cutting at various angles. Thus the conclusion from the first part of the study is confirmed, that a microfibrillar helix occurs regardless of type of wood grain. In the sample in which the angle of pit canal inclination was less than 10° the differences in the brightness of pithward and cambialward walls occurred only in some parts of the sections, and locally this difference could have been in favour of the pithward wall. These samples are referred to in column 8 of table 2. In three samples from this group, that is in those in which the S type of pit canal inclination dominated, the local differences in the brightness of adjacent walls indicated an S helix also (Plate I, C). In the remaining samples with a small angle of pit canal inclination, and therefore also of microfibrils inclination, the local differences in brightness indicated that the helix was of the Z type. Generally the samples in column 8 are those having a more or less longitudinal arangement of microfibrils with local deviations to the one side and to the other. It is emphasized that the material for this study consisted primarily of samples from the external growth rings of old stems, in which the angle of microfibrillar inclination is small, and therefore local undulations of the microfibrils produce local nonconformities of the spiral type. For such material, therefore, the method of oblique sectioning for determination of the direction of the microfibrillar helix is not the best one. It is, however, very good for material in which the angle of microfibrillar helix is shallow.

DISCUSSION

Determination of the microfibrillar orientation on a tracheid segment permits us to answer the question posed in the tittle to this paper only in those situations in which the microfibrillar helix forms a sufficiently large angle with the axis of the cell. Only then, does the general orientation of the microfibrils relative to the cell axis remain S or Z in spite of minor random variation.

Among the studied samples 19% had tracheids with very steep helices. In these, it is not known whether the variable orientation of the flattened pit canals should be interpreted as variable orientation of fibrillar helices or as undulations of nearly longitudinally arranged fibrils.

On the basis of the data from the samples in which the angle of the spiral was large, the answer to the question posed in the tittle should be that the type of fibrillar helix in the S2 layer of the tracheid wall within a tree is constant!

Within the population of species which we hawe studied, when a distinct helix occurred in the S2 layer it was of type Z.

Stability of the type of configuration in the S2 layer is interesting. This stability persists in spite of the well documented ability of a cell to form microfibrillar helices of both types. It is known that successive lamellae of layers S1 and S3 have alternate configurations of the spiral.

It is interesting that various authors repeatedly report only one type of microfibrillar helix in the S2 layer for a given species, yet according to some it is of type Z and according to others of type S. The results of the present study as well as those of Bucher (1957) are contradicted by the results of Krieg (1907), who found an S type spiral in *Picea excelsa*. A different pair of contradictory results can be found in the papers of Wardrop and Dadswell (1957) and Meier (1957) concerning *Pinus radiata* (S and Z type respectively). It is possible that the type of spiral is a genetic character of an ecotype.

In our studies we found no correlation between the type of spiral grain and the type of microfibrillar helix in the cell walls of the studied species. A correlation of this type reported earlier by Jaccard and Frey (1928) was probably the result of a coincidence, in which the deviations in the orientation of the microfibrils gave a local orientation in agreement with the type of spiral grain.

Wood of *Picea* from the same provenance as used for this study was studied earlier with regard to orientation of the anticlinal partitions in the xylem (Hejnowicz unpublished). These studies indicated, that regardless of the type of spiral grain S and Z domains existed in the cambium of the studied trees at the time of wood formation. If there were a correlation between the configuration on the cambial level with the configuration on the cellular level, then among the studied samples some would have been found having a microfibrillar helix of type Z or S, as well as those having both. Absence of such samples indicates that there is no correlation between these configurations on different levels. Absence of such correlation has already been indicated in a preliminary way by earlier study of Hejnowicz (1964).

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Kierunek spirali fibrylarnej w środkowej warstwie wtórnej ściany komórkowej

Streszczenie

W niniejszej pracy badano kierunek nachylenia (skrętność) fibryl w środkowej warstwie (S2) ściany komórkowej cewek *Abies alba* i *Picea excelsa*. Badania prowadzono na przekrojach skośnych w mikroskopie polaryzacyjnym oraz na materiale zmacerowanym w mikroskopie zwyczajnym. We wszystkich przypadkach, w których spirala fibrylarna tworzy z osią komórki kąt nie mniejszy od 10° (ok. 80% próbek), spirala ta była wyraźnie Z-owa, niezależnie od tego, czy próbki pochodziły z drzew o włóknie drewna typu Z, typu S, czy z drewna prostowłóknistego.

Gdy fibryle były nachylone pod kątem mniejszym niż 10° (20% przypadków) stwierdzono powszechne występowanie zawichrowań w przebiegu fibryl w obrębie jednej komórki w formie lokalnych odchyleń na lewo lub na prawo od ogólnego kierunku. Również w tych przypadkach dominujący był kierunek Z-owy, na 49 takich próbek kierunek S przeważał jedynie w trzech.