Acta Bot. Neerl. 22(2), April 1973, p. 100–105.

HELICAL THICKENINGS IN THE TRACHEIDS OF TAXUS AND PSEUDOTSUGA AS REVEALED BY THE SCANNING REFLECTION ELECTRON MICROSCOPE

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SUMMARY

Helical thickenings in the tracheids of *Pseudotsuga* and *Taxus* were studied by the scanning reflection electron microscope. From this it was found that both were part of the S_3 layer. In *Pseudotsuga* they were branched in the radial wall, there having a much smaller angle of inclination than the spirals in the tangential wall, which were not branched. Their direction is the same as from the "streaming figure" in the bordered pit. The thickenings in *Taxus* showed up as sharply demarcated ridges of about the same width at regular intervals in the radial plane. The tangential plane was not studied. The helices here ran in a direction opposite to the "streaming figure" around the pit apertures. They seemed to be loosely attached to the S_3 layer but no proof of that could be found.

1. INTRODUCTION

The structure of the cell wall of Gymnosperm tracheids has been studied intensively during the last decades. The pioneering work of BAILEY (1913), BAILEY & KERR (1935), BAILEY & VESTAL (1937), FREY-WYSSLING (1959), LIESE (1953), PRESTON (1934, 1947, 1948), PRESTON & WARDROP (1949), ROELOFSEN (1959), WARDROP (1958, 1964), WARDROP & DADSWELL (1951), WARDROP & PRESTON (1947) has for the greater part elucidated its basic structure.

According to WARDROP (1964) the cell walls of tracheids consist of a middle lamella, primary wall and secondary wall. The latter (S) can in most cases be divided into an S_1, S_2, and S_3 layer, respectively, each of which consists of very thin concentric lamellae.

The S_3 layer in some species is lacking or very thin. This layer in many genera is not smooth but covered with warts, on which WARDROP (1964) and LIESE (1965) have made a thorough study.

Another type of sculpturing of the tracheid walls is due to spiral thickenings, which according to I.A.W.A. (1964) are: "helical ridges on the inner face of, and part of, the secondary wall." These helices occur in *Taxus*, *Torreya* and *Cephalotaxus*, all members of the Taxaceae, and in *Pseudotsuga* of the Pinaceae. In all the above genera they characterize the tracheids, though in the latter they may be absent in the outer portion of the late wood of the growth ring (ANONYMOUS 1925). A special study of the spirals has been made for *Taxus* by WERGIN & CASPERSON (1961) and WARDROP & DAVIES (unpublished) and for *Pseudo-*

tsuga by LIESE & HARTMANN-FAHNENBROCK (1953), CÔTÉ (1967), HODGE & WARDROP (1950). WARDROP (1964) gave information about both genera.

GREGUSS (1955) observed spiral thickenings in other genera and also in ray-tracheids, but not as a characteristic.

Whilst studying the bordered pits of *Pseudotsuga taxifolia* in the scanning reflection electron microscope (S.E.M.), its spirals were seen to be branched. It was therefore thought to be of interest to study also the helices in *Taxus* by the same method, so as to be able to make a comparison between both.

2. MATERIAL AND METHODS

Small air-dried samples were used from the fully developed sapwood of *Pseudotsuga taxifolia* and *Taxus wallichii* and from the heartwood of *Taxus baccata*. The wood samples were prepared by the method described by JUTTE & LEVY (1971) for examination in the S.E.M.

3. RESULTS

In *Pseudotsuga taxifolia* it could be seen that the thickenings on the radial wall were not pronounced helices as might be concluded from light microscopy. As can be seen from *fig. 1* it is clear that there is a main tendency towards helical orientation, though many more or less developed branching thickenings were formed. These always run over the pit border, in most cases coming from two directions and meeting on its top, just leaving space for the pit aperture (*fig. 2*). The thickenings have a very broad basis and it is clear that they are part of the S_3-layer. They always run in the direction of the longest axis ("streaming figure") of the aperture. The tangential walls also showed the spirals (*fig.* 3), but here they were not branched and their direction was almost horizontal. They seemed to be very shallow ridges in a thin S_3, the direction of the S_2-layer microfibrils showing through. That the S_3-layer can be very thin indeed or lacking in places was also very well demonstrated in CÔTÉ's plate 2 (1967).

Taxus shows a great difference from *Pseudotsuga* in its radial plane. The helices are never branched and have a small base, as if they have been attached to the cell wall as a separate coil at quite even thickness (*fig. 4*). In splitting the wood in a perfect radial direction the S_3-layers very often split off, taking the spiral thickening away. It was therefore necessary to split the wood slightly out of the true axial plane, so the inner tracheid surface was kept intact. At first it seemed as if the spirals could very easily be torn away from the cell wall. This is not the case. Moreover, with higher magnification it was occasionally found that the S_3-layer was thickened at one side of the spiral, giving quite an obvious sculpturing, with a fairly heavy shadow, due to the coating mechanism (*fig. 5*). The spirals did not usually run over the pit borders, and when they did, it was not close to the aperture. They also were not laid down in the direction of the longitudinal axis of the pit, but contrary to it (*fig. 4*).

In *Taxus baccata*, where heartwood was used, the coils seemed to be covered by extraneous material, which in some cases was broken away, leaving a "core" of the original coil (*fig. 6*). The spirals in both *Pseudotsuga* and *Taxus* were laid down in S helices.

4. DISCUSSION

In making a comparison between the helical thickenings of the genera *Pseudotsuga* and *Taxus*, they were found with the S.E.M. to differ in shape and appearance. Neither of them were found to be easily detached from the cell wall as WARDROP (1964) found, although the spirals in *Taxus* can very readily be removed, together with the S_3-layer, leaving the S_2. PRESTON (1934, 1948) noted for the S_2-layer that the angle of inclination of micelles in the radial walls of the tracheids was greater than in the tangential walls; this was confirmed by WARDROP & DADSWELL (1951). The same phenomenon was also found to be true for the helices in *Pseudotsuga* (WARDROP & DADSWELL 1951). This was not confirmed for the thickenings in *Pseudotsuga* under investigation, where angles of inclination to the cell axis in the tangential plane were almost 90 degrees, and in the radial plane much less.

In the literature there is no mention of the fact that the helices in *Pseudotsuga* tracheids may be branched, although it is clear from plates 1 and 2 in CÔTÉ's atlas (1967) that this is the case. The branching does not make it easy to measure the angle of inclination from the S.E.M. pictures. This can best be done from the light microscopic image, where only the main helical directions seem to show up. It is clear from the above pictures that the thickenings are part of the S_3-layer, as stated by CÔTÉ (1967), his plates 3 and 4 giving evidence. WARDROP (1964) has stated that the S_3 layer is constructed of 0 to 6 lamellae, every lamella having its microfibril direction in the opposite pitch, that is to say in alternating S and Z spirals. From the scanning electron micrographs it could be concluded that the S_3 layer is very thin, possibly consisting of only two lamellae. The lamella closest to the S_2 layer has its microfibrils running in the Z direction while the microfibrils of the second lamella (closest to the lumen) appear to flow more or less together to form branched helices running in the S direction. As can be concluded from the streaming figure of the pit apertures in the S_2 layer, microfibrils there are also oriented in S direction. It is interesting that the helices mostly run over the top of the pit borders very close to the

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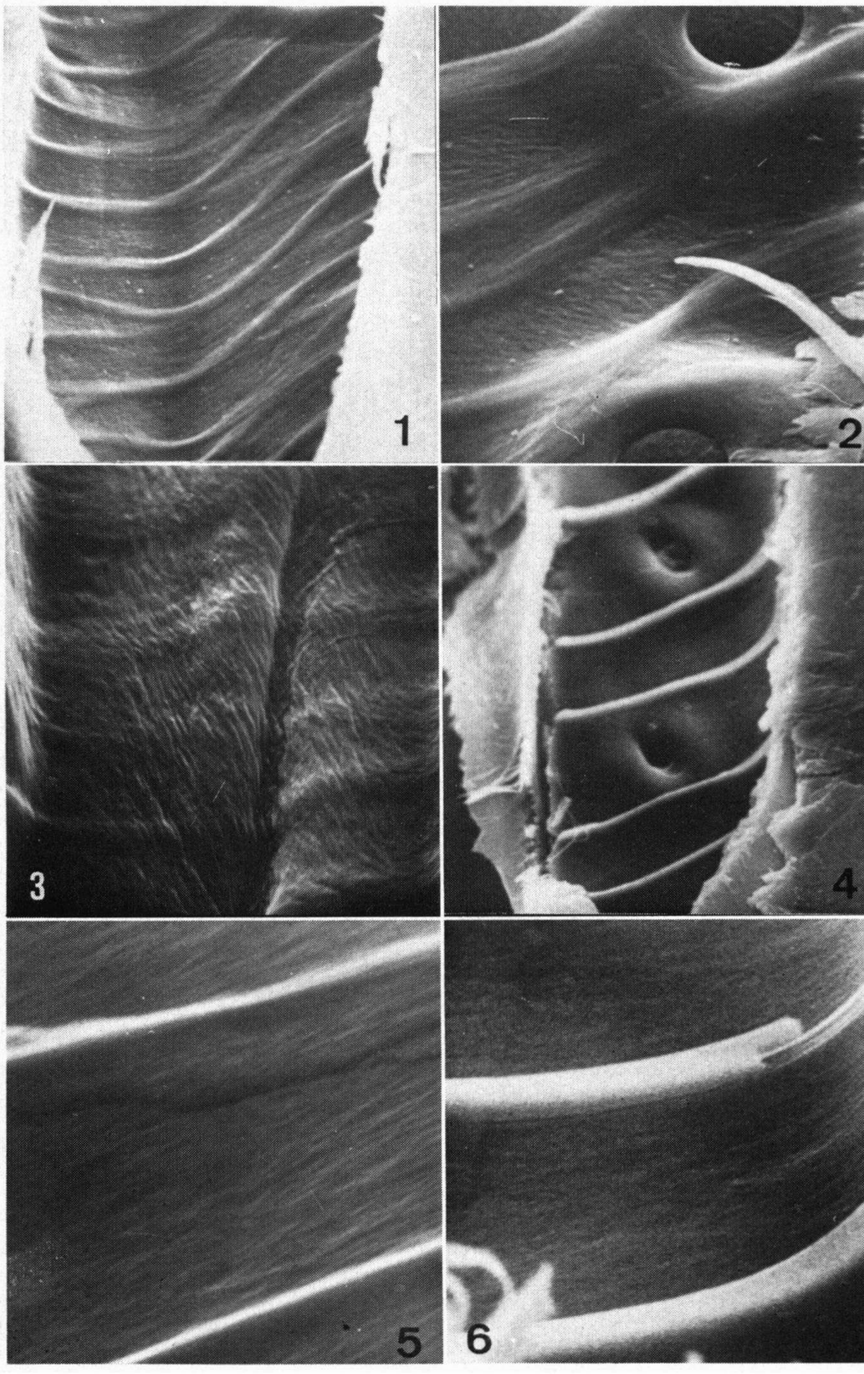
Fig. 1. *Pseudotsuga taxifolia.* Branched helical thickenings on the radial wall (× 1,300).
Fig. 2. *Pseudotsuga taxifolia.* Helical thickenings running over the pit border in the direction of the longest axis of the pit aperture (× 2,500).
Fig. 3. *Pseudotsuga taxifolia.* Flat helical thickenings on the tangential wall (× 2,700).
Fig. 4. *Taxus baccata.* Helical thickenings on the radial wall, running in opposite direction of the longest axis of the pit aperture (× 1,500).
Fig. 5. *Taxus wallichii.* Helical thickening with wall sculpturing underneath (× 5,000).
Fig. 6. *Taxus baccata.* Helical thickening from which the outside part is broken off, leaving a "core" of the original coil (× 5,000).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aperture and in these spots there mostly is a junction of two thickenings. It would be worthwhile to study the inside of the dome of these pits more carefully, to see if the S_3-layer on these is also inclined to collect its fibres in, for instance, ridges or sheaves in a more or less circular way.

In *Taxus* the helices could not really be lifted from the S_3-layer although their bases are much narrower than those in *Pseudotsuga*. No microfibrils were detected in these thickenings but these were not very clear either in the S_3-layer itself. While following the concept of the cell wall organization proposed by WARDROP (1964) the S_3 layer in the tracheids of *Taxus* are possibly composed of more lamellae than in *Pseudotsuga*. The last-formed lamella of the S_3 layer, from which the helical thickenings originate, has its microfibrils running in the S direction. The microfibrils in the layer S_2 run in the opposite (Z) direction, as can be concluded from the streaming figure around the pit aperture.

In this connection it is interesting that in compression wood where the S_3 layer is absent or only feebly developed (WARDROP & DADSWELL 1950), WERGIN & CASPERSON (1961) found thickenings which they concluded to be part of the S_2-layer. These helices, however, run in the same S direction as do the S_3 spirals in normal tracheids. The main microfibrillar direction in the S_2 layer of compression wood has thus changed from a Z to an S helix. This requires special attention in relation to the changes in the protoplast occurring in the developing compression wood tracheids (WARDROP & DAVIES 1964). How does the compression wood of *Pseudotsuga* show up? Does it also change its S_2 microfibril direction?

It seems indeed that the helical thickenings in Gymnosperm tracheids need more investigation, taking into account that the members of the Taxaceae which is an "older" plant unit than the Pinaceae still have this characteristic feature. Could this type of helices be the classic one, while that of *Pseudotsuga* might just be at the stage of evolutionary development where this feature is disappearing, probably leaving in the next period of time only a collection of warts at the spot where it first developed spirals?

ACKNOWLEDGEMENTS

We wish to thank Miss Jo Fillery for valuable advice on S.E.M. techniques. We also thank Miss D. Catling, Metropolitan Police Lab., London and Drs. P. Baas, Rijksherbarium, Leiden for supplies of *Taxus baccata* and *Taxus wallichii*.

REFERENCES

ANONYMOUS (1925): Spiral tracheids and fiber tracheids. *Tropical Woods* 3: 12–16.

BAILEY, I. W. (1913): The preservative treatment of wood. II. The structure of the pit membranes in the tracheids of Conifers and their relation to the penetration of gases, liquids and finely divided solids into green and seasoned wood. *Forestry Quarterly* **11**: 12–20.

— & T. KERR (1935): The visible structure of the secondary wall and its significance in physical and chemical investigations of tracheary cells and fibers. *J. Arnold Arboretum* **16**: 273–300.

BAILEY, I.W. & M. VESTAL (1937): The orientation of cellulose in the secondary wall of tracheary cells. *J. Arnold Arboretum* **18**: 187–195.

CÔTÉ, W. A. (1967): *Wood ultrastructure. An atlas of electron micrographs.* Univ. of Washington Press, Seattle and London.

FREY-WYSSLING, A. (1959): *Die pflanzliche Zellwand.* Springer-Verlag. Berlin.

GREGUSS, P. (1955): *Identification of living Gymnosperms on the basis of xylotomy.* Akadémiai Kiadó, Budapest.

HODGE, A. J. & A. B. WARDROP (1950): An electron-microscopic investigation of the cell-wall organization of Conifer tracheids. *Nature* **165**: 272–273.

I. A. W. A. (1964): *Multilingual glossary of terms used in wood anatomy.* Comm. of Nomenclature. Verlagsanstalt Konkordia, Winterthur, Switzerland.

JUTTE, S. M. & J. F. LEVY (1971); Scanning reflection electron microscopy on studies of wood structure and its degradation. *I.A.W.A. Bull.* No. **1**: 3–13.

LIESE, W. (1953): Über die Hoftüpfel der Koniferen. *Ber. Deut. Botan. Ges.* **66**: 203–211.

— (1965): The fine structure of bordered pits in softwoods. In: W. A. CÔTÉ, *Cellular ultrastructure of woody plants.* Syracuse University Press, Syracuse and New York.

— & HARTMANN-FAHNENBROCK (1953): Elektronenmikroskopische Untersuchungen über die Hoftüpfel der Nadelhölzer. *Biochimica et Biophysica Acta* **11**: 190–198.

PRESTON, R. D. (1934): The organization of the cell wall of the Conifer tracheid. *Phil. Trans. Roy. Soc.* B. **224**: 131–173.

— (1947): The fine structure of the wall of the Conifer tracheid. II. Optical properties of dissected walls in Pinus insignis. *Proc. Roy. Soc.* **134**: 202–218.

— (1948): The fine structure of the wall of the Conifer tracheid. III. Dimensional relationships in the central layer of the secondary wall. *Biochimica et Biophysica Acta* **2**: 370–383.

— & A. B. WARDROP (1949): The submicroscopic organization of the walls of Conifer cambium. *Biochimica et Biophysica Acta* **3**: 549–559.

ROELOFSEN P. A. (1959): *The plant cell wall.* Gebr. Borntraeger, Berlin.

WARDROP, A. B. (1958): Organization of the primary wall in differentiating Conifer tracheids. *Australian J. Botany* **6**: 299–305.

— (1964): The structure and formation of the cell wall in xylem. In: M. H. ZIMMERMANN, *The formation of wood in forest trees.* Academic Press, New York-London.

— & H. E. DADSWELL (1950): The nature of reaction wood. II. The cell wall organization of compression wood tracheids. *Aust. J. Sci. Res.* Ser. B. **3**: 1–13.

— & H. E. DADSWELL (1951): Helical thickenings and micellar orientation in the secondary wall of Conifer tracheids. *Nature* **164**: 610–612.

— & G. W. DAVIES (1964): The nature of reaction wood. VIII. The structure and differentiation of compression wood. *Australian J. Botany* **12**: 24–38.

— & R. D. PRESTON (1947): Organization of the cell walls of tracheids and wood fibres. *Nature* **160**: 911–913.

WERGIN, W. & G. CASPERSON (1961): Über Entstehung und Aufbau von Reaktionsholzzellen. 2. Mitt. Morphologie der Druckholzzellen von Taxus baccata L. *Holzforschung* **15** (2): 44–49.