

# **ELECTRON MICROSCOPY OF WOOD: THE PIONEERING YEARS**

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## **SUMMARY**

The electron microscopy of wood started around 1951 in a few laboratories. Special preparation methods had to be developed for viewing the cell wall structures. The treatise describes the results obtained during the first five years with emphasis on the structure of bordered pits, the tertiary wall layer, the warty structure and fungal effects on the cell wall.

**Key words:** Electron microscopy, cell wall structures, bordered pits, tertiary wall, warty structure.

## **INTRODUCTION**

This workshop on Plant Cell Walls in Hamburg at an institution with considerable tradition and production in the electron microscopy of wood awakes personal memories on the pioneering years. Participating right from the beginning in exploring the secrets of cell wall structures, I feel a certain obligation to describe these early developments, particularly as they can hardly be retrieved and I am the last one from this period still around. Necessarily, such a retrospective is personal and incomplete, since I did not collect the old documents purposefully and a selection had to be made from the huge pile of notes and photos that exist.

### ***State of the art (1951-1956)***

With these limitations some results and memories for the first years from 1951 until 1956 will be presented, thus demonstrating the state of knowledge dating back 50 years.

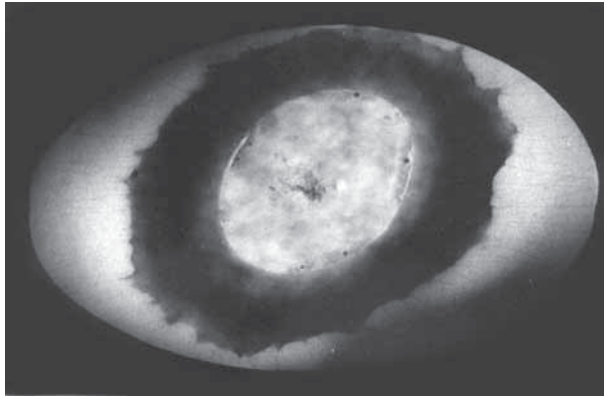
Until 1950 the nature of the wooden cell wall was investigated mainly by methods such as light-microscopy (phase-contrast-, fluorescence- and polarizing microscopy), staining and swelling reactions, maceration and X-ray diffraction. Remarkable amazing results were obtained, and Bailey & Kerr (1935), Frey-Wyssling (1951), Preston (1952) and Ziegenspeck (1951) should be mentioned as main contributors to the information from this period.

At this time it was well known that the woody cells are bound together by a non-cellulosic, amorphous middle lamella. Each cell is surrounded by the primary wall, followed by the secondary wall of three layers of a different thickness and fibril orientation, called outer, middle and inner layer (Bailey & Kerr 1935). The introduction of the electron-microscope with hundred times higher magnification than the light-microscope and its superior resolution opened up

a new dimension. However, considerable technical obstacles had to be overcome before viewing a specimen in an electron-microscope, such as need for thorough desiccation of the object, the required thinness of the specimen and the viewing restricted to a small area.

### Preparation methods

In early trials thin sections were obtained with a razor blade by Huber and Kolbe (1948) who observed sieve tubes of some soft- and hard-woods and by Liese (1951) who focussed on the structure of bordered pits. The micrograph in Fig. 1, which was taken in December 1950 at the institute of Ernst and Helmut Ruska in Berlin, shows the pit membrane between two pori in *Pinus sylvestris*. Ernst Ruska was later awarded the Nobel Prize for inventing this wonderful research tool.

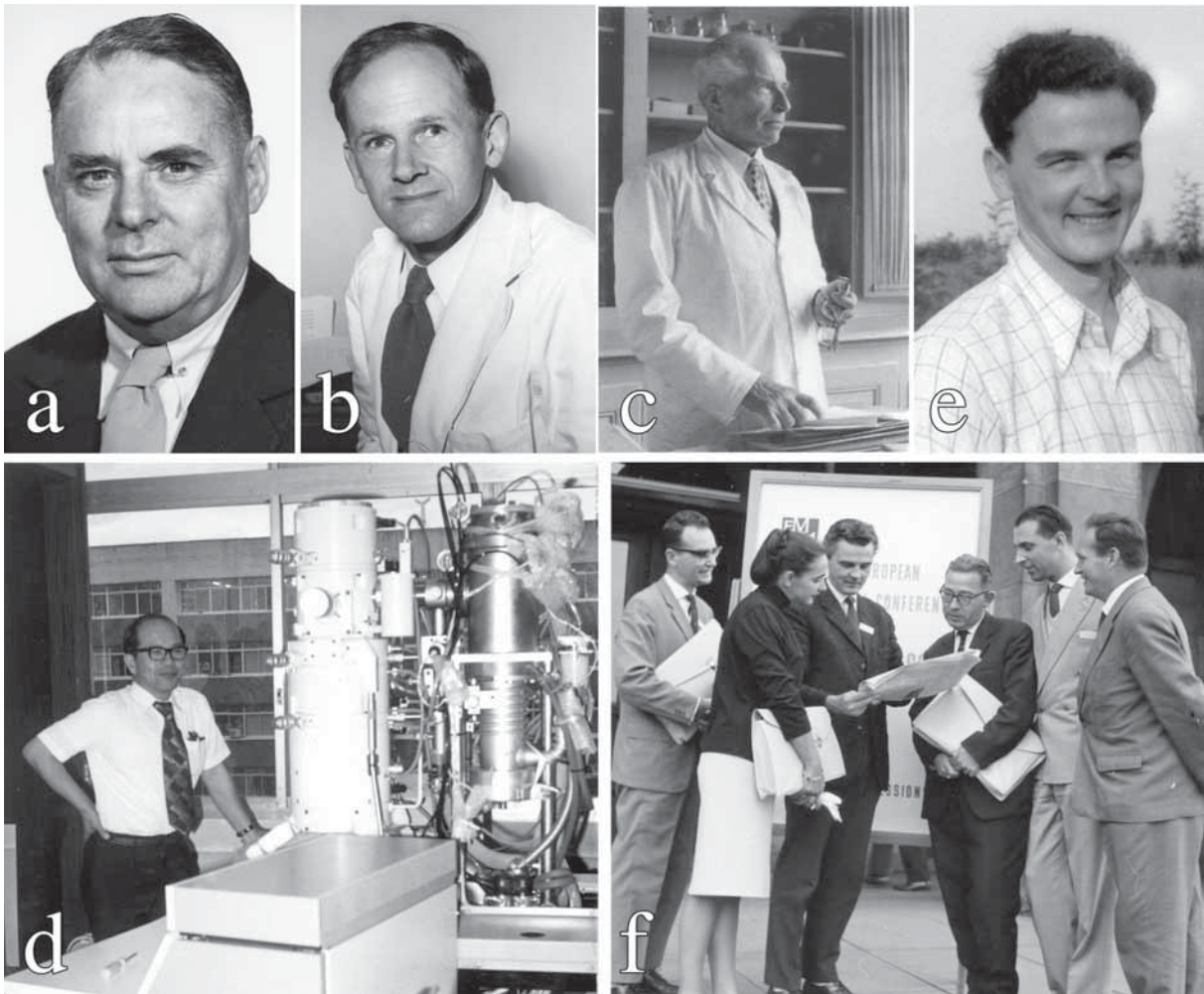


**Fig. 1:** Pit membrane with the torus between two pori, *Pinus sylvestris*, razor blade section.

During the years that followed, several new preparation techniques were developed. The use of a glass edge for thin sections was introduced by Latta and Hartman (1950) and of diamonds by Fernandez-Moran (1953). The fixation of plant tissue for electron-microscopy was investigated by Palade (1952). A sliding microtome with thermal expansion enabled thinner sections to be obtained (Sjöstrand 1954; Sitte 1955). Further advances were made by maceration, dispersion and suspension of fibres and developing cell walls, followed by metal shadowing with chromium or palladium. Most useful was the replica technique for wood first tried by Varossieau (1950). The surface of the object was covered with methylmetacrylat "Plexiglas" which after polymerization was lifted up. The inner surface of this cast reflected a perfect image of the structural details. It was then shadowed with silicium monoxide or chromium, the methylmetacrylat dissolved in toluene and the remaining thin film investigated in the electron-microscope (Fig. 2). The replica technique became quite common in these years, and also used it for all my electron-microscopic studies during the reference period. Bosshard (1952) applied the same method in his thesis under Frey-Wyssling on the cell wall structure of *Fraxinus*.

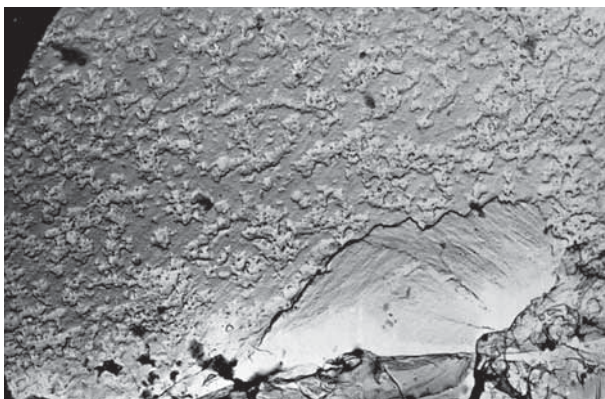
Electron-microscopy of cell wall structures was developed within these five years mainly in Australia by Dadswell and Wardrop (Fig. 3a, b), in Switzerland by Frey-Wyssling (Fig. 3c) and Mühlethaler, in Japan by Harada (Fig. 3d) and in Germany by Liese (Fig. 3e); among the pioneers Nécsey in Slovakia should also be mentioned (Fig. 3f).

Before outlining some of the observations made, I want to mention briefly my own whereabouts during this dynamic time. After my thesis on the treatability of spruce in the spring of 1951, I worked in Düsseldorf on root physiology. By a lucky incidence the brother-in-law of the Ruskas, Bodo von Borries, had founded an "Institut für Übermikroskopie" in Düsseldorf, mainly for the purpose of the metal industry. To prove usefulness of this costly instrument, von Borries was interested in further extending its application on other fields. So my intention to continue the trials in Berlin on electron-microscopy of



**Fig. 3:** a) Erik Dadswell (1903-1964); b) Alan Wardrop (1921-2003); c) Albert Frey-Wysling (1900-1988); d) Hiroshi Harada (1923-1991); e) Walter Liese (1951); f) D. Fengel, R. Schmid, W. Liese, B. J. Spit, G. Caspersson and V. Nêcesaný at the European Electronmicroscopy Conference, Prague (1964)..

wood was much welcomed. I became his local customer for one year, before moving to the wood preservation industry in Mannheim to work for a year and then in 1953 to the Institute for Forest Botany in Freiburg. At all these places and in spite of considerable logistic difficulties, the generous contact with von Borries could be maintained as my only access to electron-microscopy. The objects investigated embraced the wooden cell wall in its widest sense, but fortunately also samples of bamboo. In Düsseldorf I was briefly involved in testing bamboo imported as replacement for mining timber, but the bamboo culms cracked under pressure, proving useless for the operational safety in the underground. Out of curious, I took some electron



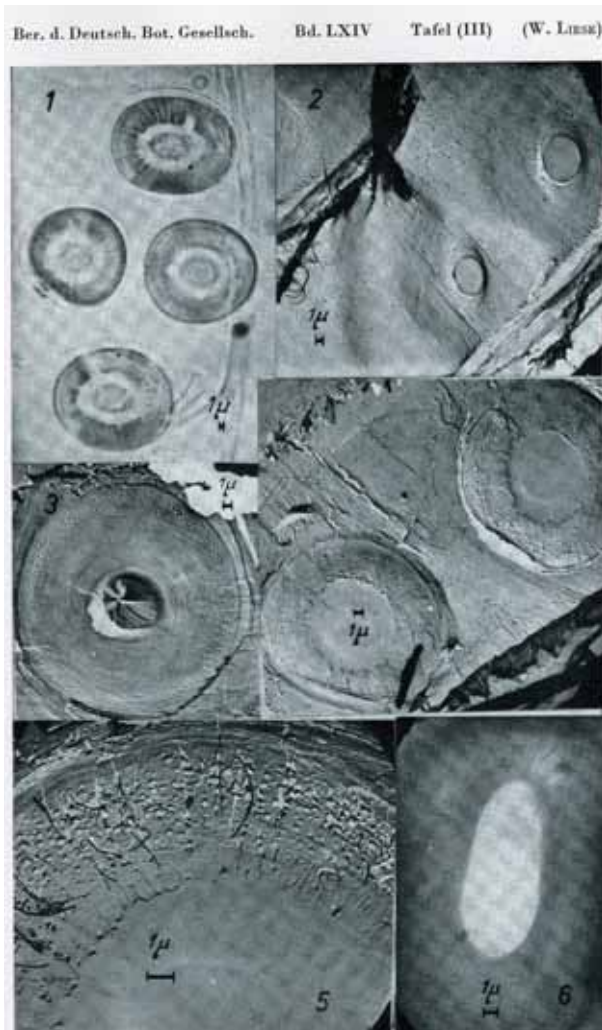
**Fig. 4:** Parenchyma cell, *Bambusa vulgaris* (1952).

micrographs of a few samples which were then filed away and nearly forgotten (Fig. 4). Since the electron-microscope was at this time considered a miracle tool for all sorts of problems, I took the bamboo micrographs six years later to India as a so-called "FAO expert for bamboo preservation". I had never seen a bamboo plant before arriving in India. However, I could solve some problems and my activities on bamboo have continued till to date. Without this bamboo sample my life would have taken a different course, especially after retirement 15 years ago.

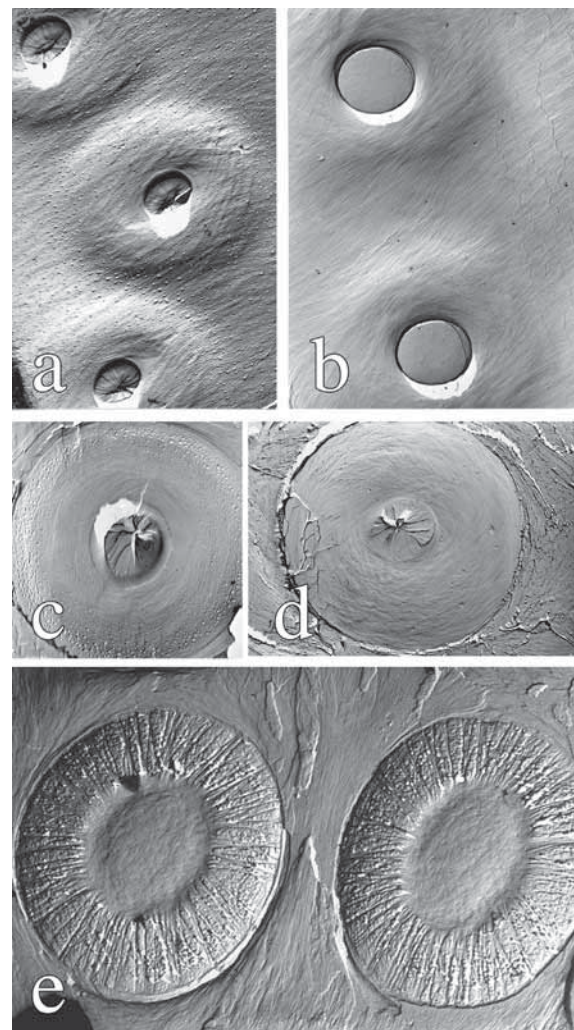
## OBSERVATIONS

***Bordered pits***

Few examples presented here demonstrate our knowledge on the woody cell wall approx. 50 years ago: the structure of bordered pits, the tertiary wall, the warty layer, and the effects of fungi on the cell wall. One of the first reports on cell wall structures had been a lecture on the fine structure of bordered pits in softwoods at the German Botanical Association in Berlin (Liese 1951). Fig. 5 shows split bordered pits with a margo consisting of a torus with radial strands attached to the pit chamber. The visibility of its microfibrils with a warty structure gave evidence for openings between the margo strands for free water movement in the functional state. The structure of bordered pits was further elaborated for a number of conifers (Fig. 6a - e) (Liese 1953, Liese & Hartmann-Fahrenbrock 1952, 1953a). The conclusion of free spaces between

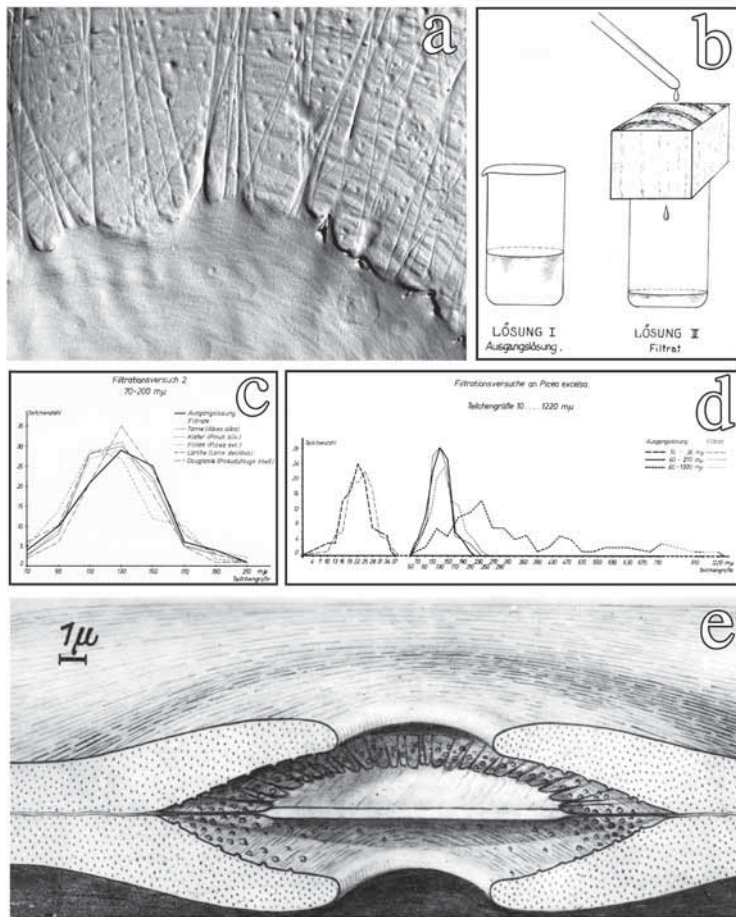


**Fig. 5:** Structures of bordered pit membranes, *Pinus sylvestris* (Liese 1951).

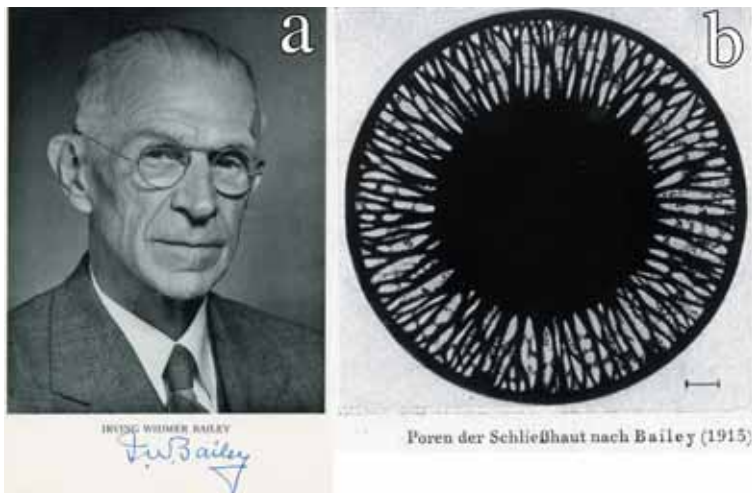


**Fig. 6a - e:** a, b) Bordered pits from the tracheid lumen, *Pinus sylvestris*, *Picea excelsa*; c, d) Pit chamber, *Pinus sylvestris*, *Picea excelsa*; e) Margo of the pit membrane attached to the pit chamber, *Pinus sylvestris*.

the fibrils involved a rather heated dispute with Frey-Wyssling, the then unquestioned authority on cell wall structures. He argued that the replica method could not reveal the fine network of the real pit membrane between the radial strands with no spaces in-between (Frey-Wyssling 1953, Frey-Wyssling & Bosshard 1953). Our following work with the replica technique and especially with filtering experiments using particles of titanium dioxide (70-200  $\mu\text{m}$ ) confirmed the open passage between the margo fibrils for liquids with small particles (Fig. 7) (Liese 1954a, Liese & Johann 1954a). This open texture of the margo was supported by Eicke (1954) from a



**Fig. 7a - e:** a) Margo fibrils attached to the pit chamber with warts; b - e) Filtration of particles to prove the porous margo structure of the pit membrane as shown in drawing e.



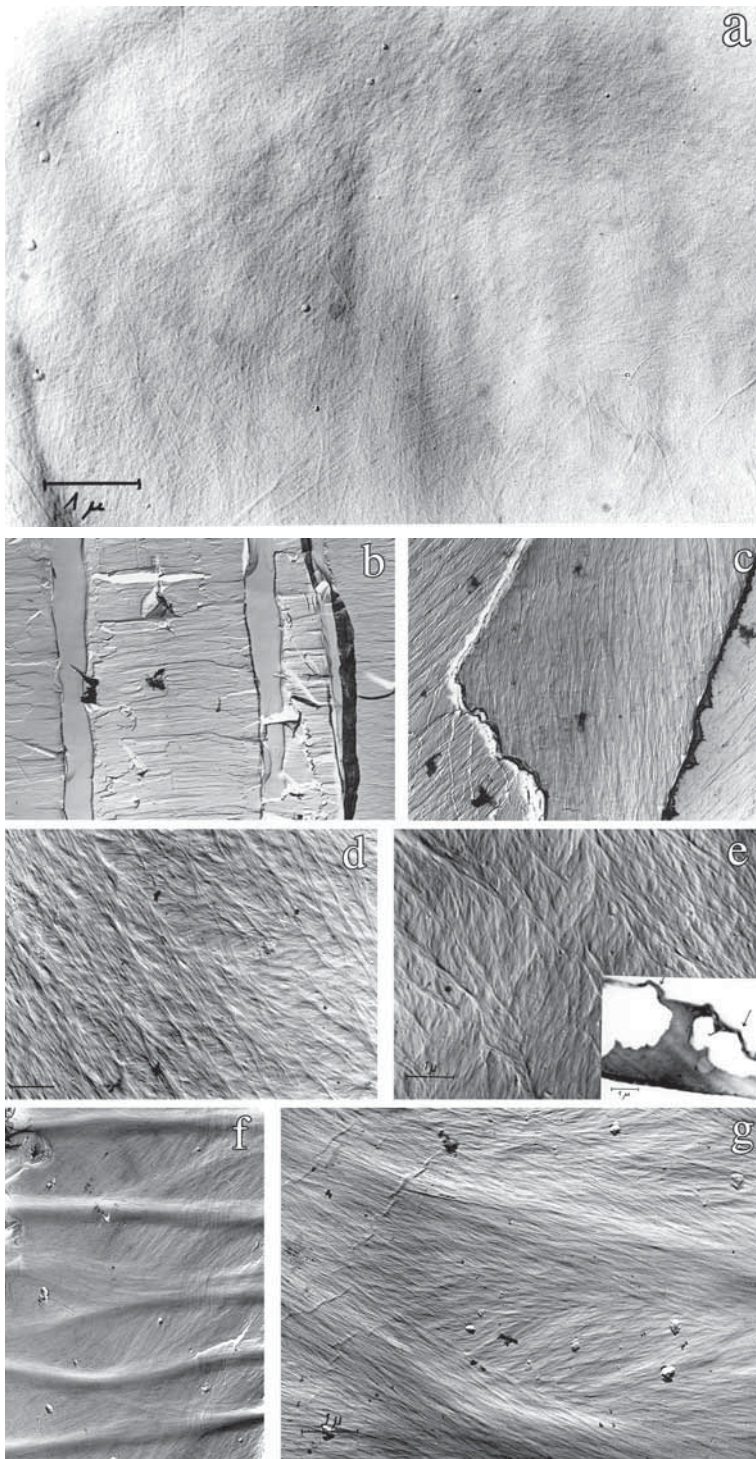
**Fig. 8:** a) I.W. Bailey (1884-1967); b) Sketch of the pit membrane structure (Bailey 1913).

between vessels contain a solid membrane without openings (Harada 1954, Liese 1956b). Their development as tyloses in *Fagus silvatica* has been studied by Nêcesaný (1955).

Another topic of early interest was the submicroscopic structure of the cell wall, the S1, with its horizontal fibril orientation (Fig. 9b), the varying layers of the S2 (Fig. 9c), and especially the inner lining, the S3 or tertiary wall (Hodge & Wardrop 1950).

stereoscopic view of replicas and confirmed by Harada (1953b, 1955) and Stemsrud (1956). A strong support in this dispute was given by a personal letter from I.W. Bailey in June 1956 where he fully agreed to my conclusions and elaborated his point of view in a paper printed in a German journal (Bailey 1956). Already in 1913 Bailey had presented a most remarkable sketch about the pit structure (Fig. 8). We met in 1962 at Harvard, when I investigated with Myron Ledbetter details of the warty structure of the innermost cell wall layer. Only later Frey-Wyssling admitted open spaces in the margo and explained his earlier statements on the basis that he had investigated less developed pit structures (Frey-Wyssling et al. 1956). In a lecture at the "Dreiländer Holztagung" in Baden-Baden in 1956 on "Electron-microscopy of Wood", he consequently outlined the free spaces within the margo, showing my micrographs (Frey-Wyssling 1956). Since the dispute between an authority like Frey-Wyssling and a youngster like me was well known within the scientific community, I had the nickname "Tüpfel-Liese".

Simultaneously with Harada (1953a) the pits of ray parenchyma in softwoods were also investigated as consisting of a simple membrane (Fig. 9a), whereas the membrane between ray tracheids and longitudinal tracheids has a torus with a margo. For hardwoods, the pits



**Fig. 9:** a) Membrane of a ray parenchyma, *Pinus sylvestris*; b) S1 with horizontally arranged microfibrils; c) S2 with lamella in different orientation; d) Tertiary wall. Microfibrils with interwoven texture; e) Microfibrils with interwoven texture, below right: resistance against soft-rot fungi; f, g) Spiral thickenings on tertiary wall, *Pseudotsuga menziesii*.

### Warty structure

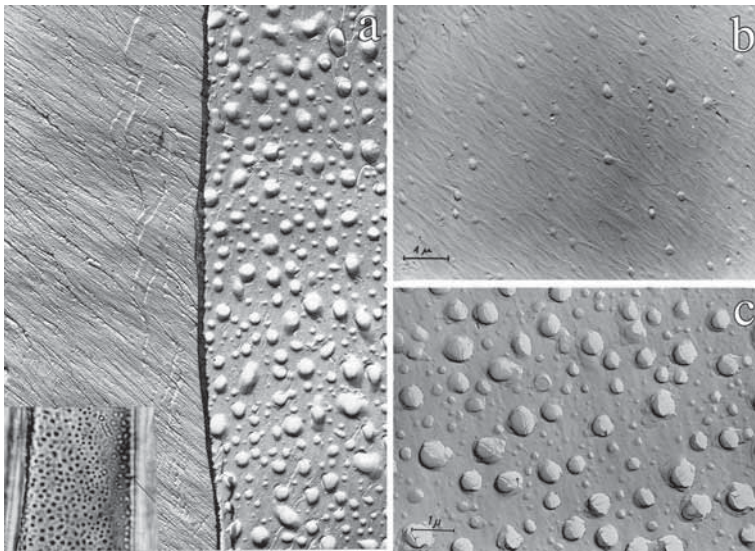
The tertiary wall is often covered by a wart-like structure (Fig. 10a). Already the first report on bordered pits showed little elevations in the inner pit chamber (Liese 1951) (Fig. 5). Around the same time Harada reported the existence of "peculiar structures" (Harada & Myazaki 1952), which led to our first personal contacts and we agreed on the term "warty structure or-layer" (Harada 1953b, Harada 1955). The occurrence, forms and systematic sig-

### Tertiary wall

The replica technique revealed a different texture of the tertiary wall in comparison with the other secondary wall layers. The fibrils are not parallel aligned, but oriented in an interwoven texture so the individuality of this layer should be emphasised by the term "tertiary wall" (Fig. 9d, e) (Liese 1954b, Liese & Johann 1954b). The common designation of S1, S2 and S3 was also questioned by Bucher (1953) in his thesis on "The tertiary wall of wood fibres and its appearance in coniferous woods". Delignified, swollen and stained fibres gave clear evidence for the special nature of the inner wall, called "Tertiärlamelle" in German. A different behaviour in pulping was stated by Meier and Yllner (1956), since it is well preserved in sulfate pulp, but largely destroyed in sulfite pulp with consequences for the diffusion of the cooking liquor.

Its specific nature became also obvious in wood decayed by soft-rot fungi. They easily destroy the secondary wall of tracheids, but not the tertiary wall (Fig. 9 e) (Savory 1954, Liese 1955, Meier 1955).

The replica technique showed also nicely the fibrillar structure of spiral thickenings on top of the tertiary wall (Fig. 9f, g) (Wardrop & Dadswell 1951, Liese & Hartmann-Fahnenbrock 1953a).



**Fig. 10:** a) Warty layer on the tertiary wall, *Cedrus deodara*; b) Tertiary wall, *Pinus sylvestris*, *Widdringtonia dracomontana*.

nificance of the warty structure were investigated for about 120 softwood and hardwood species (Fig. 10b, c) (Liese 1956 a, c, Liese & Johann 1954b). Again a dispute with Frey-Wyssling arose about their taxonomic evidence, especially their differentiation between haploxylon and diploxylon pines (Frey-Wyssling et al. 1955, 1956). Our detailed analysis of 81 well defined pine species showed the haploxylons mostly without a warty structure, but its common and distinct presence in the diploxylons (Liese 1956a). I may mention a later cooperation with

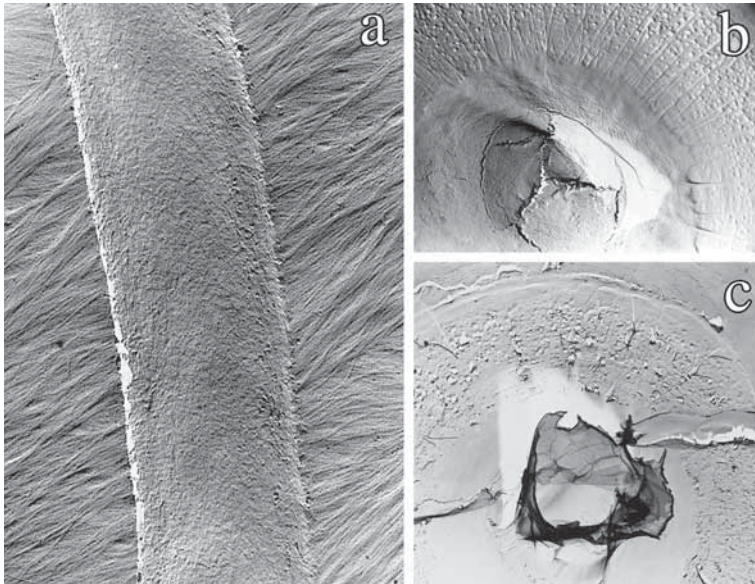
Alan Wardrop, Melbourne, in 1958 on the development and morphology of the warty structure in conifer tracheids (Fig. 11). The warts appear directly on the tertiary wall or are covered by a thin membrane (Wardrop et al. 1959).



**Fig. 11:** Alan Wardrop in his lab; Bob Davies and Jim Cronshaw, 1958.

### *Fungal effects on the cell wall*

Finally, few observations on the effects of fungi on the cell wall are worth mentioning. Already Varossieau (1950) used decayed wood samples for describing the replica method. Of special interest was the behaviour of blue-stain fungi, known for their discoloration of sapwood without enzymatically changing the wall structures. So the question was how the hyphae could invade into the cell lumen. By electron-microscopy it was revealed how hypha growing on the cell wall without any detectable effects (Fig. 12a) invaded the tracheids by penetrating the pit chamber and pushing through the torus (Fig. 12b, c) (Liese & Hartmann-Fahrenbrock 1953b). Later it became evident that these fungi invaded wood also by forming so-called microhyphae which grew through the secondary wall. Also the special cell wall degradation in cooling towers showed the participation of so-called soft-rot fungi and also the participation of bacteria (Liese 1955). A detailed survey about microscopical cell wall changes due to brown-rot, white-rot and soft-rot fungi provided the thesis by Meier (1955), with details about the cell wall structure and underlining the individuality of the tertiary wall.



**Fig. 12:** a) Hypha of a blue-stain fungus on tertiary wall; b, c) Penetration of the pit torus by blue-stain hypha.

The observations made over the first five years from thousands of electron micrographs were evaluated by Liese (1957) in his habilitation thesis. Since it appeared 49 years ago, it is not to be included in the 50 years period considered. It remained unpublished, as I went for bamboo work to India later in the year and than to Alan Wardrop in Melbourne, to investigate further the warty structure.

### *After 1956*

In the years after 1956 electron microscopy of wood was applied in many laboratories on a great number of topics. On the work done until 1960, a survey was given at the Fifth World Forestry Congress in Seattle together with Wilfried Côté (Liese & Côté 1960), with whom I had cooperated in 1958 in München (Fig. 13a). The state of art after 15 years was presented at an Advanced Science Seminar at Pinebrook, USA (Côté 1965) on "Cellular Ultrastructure of Woody Plants"; with 30 contributors it was the largest gathering of all colleagues in this field (Fig. 13b).



**Fig. 13:** a) Wilfried A. Côté; b) Participants of the Seminar on "Cellular Ultrastructure of Woody Plants", Pinebrook, USA, 1965.

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