THE STRUCTURE AND DEGRADATION OF BORDERED PITS IN GYMNOSPERM TRACHEIDS AS REVEALED BY THE SCANNING REFLECTION ELECTRON MICROSCOPE

SUSANNE M. JUTTE[1] and JOHN F. LEVY

Imperial College of Science and Technology, Dept. of Botany, London SW 7

SUMMARY

A study has been made of the bordered pit structure in the tracheids of *Picea* species, *Pinus sylvestris* and *Pinus radiata* with the aid of the scanning reflection electron microscope. Cellulase treatment was applied to gain more detailed information on the pit morphology.

It was found that the pit membrane on both sides was covered with a thin membranous layer. The margo fibrils were digested in the cellulase preparation in nearly all samples.

The central part of the *Pinus* torus was often found weakened and very flexible: in many pits it had entirely disappeared, as was found by STEMSRUD (1956). After enzyme treatment the amount of this type of torus increased. In the branch wood of *Pinus sylvestris* many pits with aberrant membrane structures were found.

As a whole the overall structure of the pit dome as described by JUTTE & SPIT (1963) and MURMANIS & SACHS (1969) was confirmed.

The pit porus in *Picea* species often seemed to be strengthened by the presence of a thick fibrillar strand, possibly representing the S_3 layer.

In the cellulase preparation the lignified cell walls looked smoother, proving that some changes had occurred in their chemical components. After cellulase treatment the inside of the pit domes in both *Pinus* species were readily lifted or peeled off in a flat helix, but not in *Picea* sp. The tori in the aspirated pits were very tightly attached to the porus whose edge seemed to be pushed into the torus. The torus was not removed during cellulase treatment.

1. INTRODUCTION

The structure of the bordered pits in Gymnosperm tracheids is still in the focus of interest. The anatomy is not yet totally clarified, although it has often seemed likely that there were only a few minor details left to be elucidated (ALEXANDROV 1927; FREY WYSSLING et al. 1956). As early as the 19th century DIPPEL (1860) and SANIO (1873) discussed what is still a subject of contradiction, namely, the architecture of the pit dome. By means of the light microscope they observed the same differences which remain a matter of controversy even with the aid of a transmission electron microscope. By then also the structure of the closing membrane and the existence of a torus were discussed. The membrane was thought to be without perforations. It was BAILEY (1913) who suggested that quite substantial particles were able to pass the pit membranes, thus showing that the membranes were perforated layers. The bordered pit structure and its function have been studied intensively in recent years, mainly because it is thought to be of major interest for the permeability of wood (BANKS 1971, CÔTÉ 1968, CÔTÉ

[1] On leave of absence from the Organization for Industrial Research, T.N.O., The Hague Holland.

& DAY 1969, EICKE 1954, FREY WYSSLING *et al.* 1956, HARADA & CÔTÉ 1967, JUTTE & SPIT 1963, LIESE 1951, 1953, 1965, LIESE & BAUCH 1967, LIESE & FAHNENBROCK 1952, STEMSRUD 1956, THOMAS 1967, 1969).

These studies were often accompanied by research in penetration which is in most cases carried out with pressure treatment (BAILEY 1965, BAUCH *et al.* 1970, LIESE 1956, LIESE & BAUCH 1964, WARDROP & DAVIES 1961).

Recently MURMANIS & SACHS (1969a, b) published papers on the development and structure of the bordered pits in *Pinus strobus* L. BAUCH *et al.* (1968), FENGEL (1966), FREY WYSSLING *et al.* (1956), WARDROP & DADSWELL (1957) have also studied the ontogeny of the bordered pit structure in Conifers.

This paper gives some results of a study of the structure of bordered pits in the tracheids of softwoods with the aid of the scanning reflection electron microscope. As the bordered pit is such a relatively elaborate and spacious structure it was thought that this type of electron microscopy (S.E.M.) could help to elucidate more details of the structures present. The main advantages of this instrument are the great depth of focus which enables a three dimensional picture to be taken, the relatively large area which can be viewed at the same time, and a simple preparation technique. The chief disadvantage compared with the transmission electron microscopy (T.E.M.) is the resolution of the instrument, 100 to 300 Å, while for the T.E.M., depending on specimen preparation, it is less than 50 Å (min. 5 Å). The S.E.M. has already proved to be very useful in clarifying three dimensional wood structures (BANKS 1971, COLLETT 1970, FINDLAY & LEVY 1969, 1971, JUTTE & LEVY 1971, RESCH & BLASCHKE 1967). In this investigation a cellulase preparation was also used to partially degrade the wood since such a technique has already proved to give a better understanding of structural details in wood by its selective degradation which shows up more aspects of the structure (BAUCH *et al.* 1970, KING 1968, JUTTE 1969, JUTTE & WARDROP 1970, MCQUIRE 1970, NICHOLAS & THOMAS 1968).

2. MATERIALS AND METHODS

Wood: small air-dried sapwood blocks (1 × 1 × 1 cm) of *Pinus sylvestris* L., *Pinus radiata* D. Don. and *Picea* species were used and in addition wood from the last formed growth ring of a 4 year old air-dried branch of *P. sylvestris* L. The wood samples were prepared by the method described by JUTTE & LEVY (1971).

Enzyme: a *Trichoderma viride* cellulase ("Onozutia" Japan) preparation was used as described by JUTTE (1969) and JUTTE & WARDROP (1970). The incubation time varied from two days to two months. After treatment the samples were air-dried and mounted for examination in the S.E.M.

3. RESULTS

The wood was usually split along the middle lamella or its adjacent layers, showing the inside of the pit chamber, though quite often the wood surface dis-

played the lumen side of the split tracheids. The pits were very often aspirated, so that the closing apparatus could be studied. Occasionally the pit membranes were split off and the inner structure of the dome was exposed. Parts of the S_2 layer were often split off from the lumen side, giving information of the dome structure. Of the hundreds of pits examined only very few were unaspirated or only partly aspirated.

3.1. Wood without enzyme treatment

The aspirated pits were studied from the inside of the pit dome, and in all three species the same phenomenon was observed. The torus was mostly prominent, but the fibrillar structure of the margo was not as clearly defined as could be expected from replica studies with the T.E.M. (CÔTÉ & DAY 1969, JUTTE & SPIT 1963, LIESE 1965). The fibrils appeared to be more or less embedded and rather indistinct in the inner dome surface (*figs. 1* and *9*). Since they must be stretched or extended in the aspirated condition *(fig. 1)* one would not expect them to be so closely appressed to the inner dome surface. This suggests that other factors are acting. The microfibrils appear to be covered with a thin, more or less diaphanous layer, and this is also true of the unaspirated pit condition. This layer cannot be seen covering the stressed fibrils in *fig. 1*.

3.1.1. *Pinus sylvestris* - stem sapwood

The central part of the torus overlying the porus seems to lack support from microfibrils. It does not appear to be stretched as would be expected from pictures in the T.E.M. The central part seems to be pushed through the porus into the lumen or projecting as if it had been pulled into the pit-chamber.

Occasionally the central part of the torus is a hole of the same diameter as the aperture. No detailed structure can be detected in the torus, although T.E.M. pictures would suggest that the fibrillar pattern of the pit membrane continues within the torus (CÔTÉ & DAY 1969, LIESE 1965).

An interesting observation was made on an aspirated pit of which the margo and the torus were split along the middle lamella. The closing membrane thus showed up in two separate discs, each representing individual parts of the pit pair *(fig. 5)*. The one half was turned over from its original position, so that the inside of both halves could be observed. Unfortunately in the electron beam this half collapsed very quickly at its edge. There was, therefore, very little time for bringing the whole constellation into focus before the collapse occurred. It was only in this picture *(fig. 5)* that some inter-connecting fibrils were seen which suggested that both halves were part of one pit membrane. This phenomenon was only found once more in untreated *Pinus sylvestris*. After disintegration more pictures were made, with both halves in focus. From these it can be seen that both halves appeared to be solid discs. The fibrils visible from the inside of both discs were more prominent than when the outer part of the membrane was seen, but some shrinking effects were also apparent, originating in wrinkling which looked very similar to fibrils. From the inside of both discs the central part of the torus was without oriented structure.

In some cases as seen from the lumen side, the cell wall layers were torn off, leaving the central dome, looking more or less like a usual pit border, but with its aperture of about the size of the torus. It could be concluded from the fibril direction that this dome is part of the S_1 layer. In case of aspirated pits the upper part of the S_2 layer can be lifted, leaving the S_1, the inner S_2 covering the pit dome with circular orientated fibrils, and the torus. The inside of the pit dome as well as its lumen side were covered with warts.

3.1.2. *Pinus sylvestris* – branch wood

Special attention was given to the pit structure in the last-formed growth season, from which the following information was obtained. In viewing many pits, hardly any could be found to contain the structure seen in mature stems. In the early wood, rows of pits of about the same aberrant structure could be found. In these the torus was not disc-like, but on the contrary appeared to be a radially orientated formation (*fig. 3*). No structure apart from what seemed to be shrinkage effects could be detected in this torus-type. Only a few very fine fibrils could be seen radiating from the edge of the torus to the rim of the pit cavity, and it looked as if these were embedded in a very thin layer of material. The torus of a number of other pits was very irregular in outline, from which parts were connected with the rim of the pit by means of fibrils. The coarser fibrils were clearly visible in part, although it appeared as if they were covered with an extremely thin layer which was rippling in some places where the margo fibrils were not. The tori here appeared to be retracting thick layers. Another type of pit was found in which the torus in the normal sense was present, but only in the very centre of the pit membrane. The pits were aspirated and it looked as if the usual torus was contracted to its minimum size, forming an irregularly shaped bubble. The radially oriented margo fibrils were fairly prominent.

Fig. 1. *Pinus sylvestris* (untreated). Aspirated pit with most of the margo fibrils lying against the pit dome and covered with a thin membrane. Part of the margo fibrils still have a "stretched" appearance. × 5,200.

Fig. 2. *Pinus sylvestris* (cellulase treated). Aspirated pits. From the pit on the right top of the figure the S_2 layer on the lumen side is removed leaving the S_1 dome with inside S_2 layer and attached torus. From the other pits the margo fibrils are digested. Of the five tori, four are split and in one a central hole is present. × 950.

Fig. 3. *Pinus sylvestris* (branch; early wood). The torus of the aspirated pit is a somewhat radially oriented structure rather than a disc. The very fine margo fibrils can scarcely be seen. × 5,500.

Fig. 4. *Pinus sylvestris* (branch; late wood). Unaspirated pit membrane containing large holes towards the edge, leaving in the centre a large torus like structure. On its surface are warts of even dimension in regular arrangement; they may be associated with plasmodermata with organelle remnants (arrows). × 11,000.

Fig. 5. *Pinus sylvestris* (untreated). Two halves of a pit membrane showing some connecting fibrils (arrow). The central part of the torus does not show a fibrillar structure. ×4,400.

Fig. 6. *Pinus radiata* (enzyme treated). The inner pit dome peels off in a flat helix around the firmly attached torus. × 2,600.
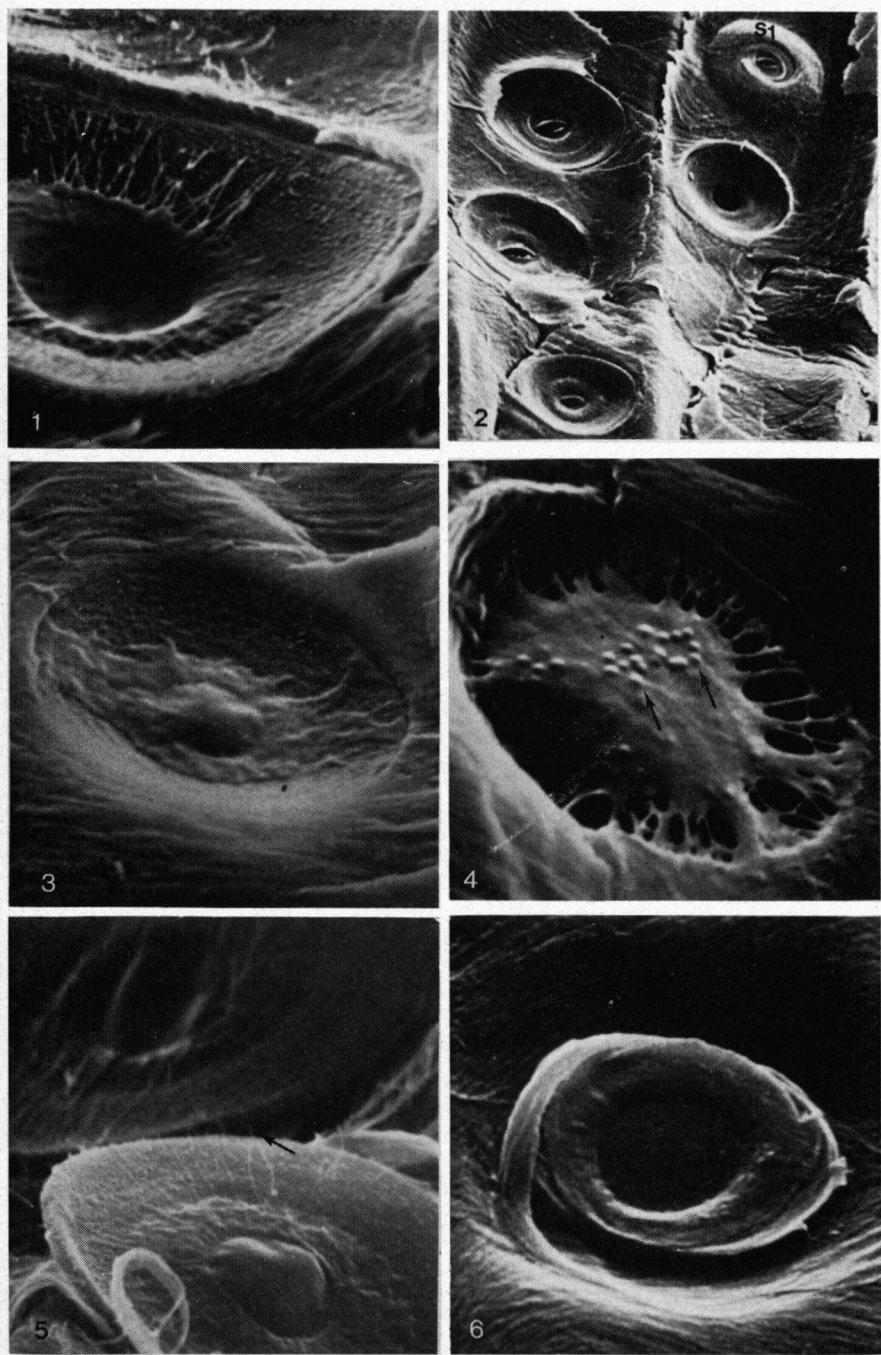
In the late wood aspirated as well as unaspirated pits were found of a smaller size than those occurring in the early wood. The unaspirated closing membranes appeared as large layers with a remarkable set of "warts" in a more or less circular orientation (*fig. 4*). Towards the edges large holes could be seen giving rise to a very coarse margo-like structure. In aspirated pits the torus occupied the greater part of the pit dome, looking like the unaspirated torus and showing besides the thick substantial radial bands, coarse margo-like fibrils and finer fibrils of the normal type.

3.1.3 *Pinus radiata* – stem sapwood

As a whole this species gave the same overall picture as *Pinus sylvestris* stem wood. There were two main differences in the central part of the torus. In the first place holes of the size of the porus were often present (*fig. 7*). This could be observed in both unaspirated and aspirated pits. Secondly the centre of the torus could appear more swollen and voluminous than the rest of the torus. It looked somewhat bubbly, so that the whole torus did not have the usual disc-like appearance. From the lumen side the layers had frequently been lifted from the wall, leaving the S_1 dome, as already mentioned for *Pinus sylvestris* (*fig. 7*).

3.1.4. *Picea species* – stem sapwood

Aspirated pits were usually seen. Only very few pits were unaspirated. It was striking that in the unaspirated pits the margo showed up as a much more massive and densely "woven" structure than is usually thought, with its main orientation in the radial direction. It was as if in these membranes some thin ragged substance was interconnected with the fibrils. If the pits were aspirated the margo gave a more densely packed impression than is the case in both *Pinus* species (*fig. 9*). In contrast to the pines, the torus of spruce showed a fairly rough, more or less granular surface. Wart-like structures covered the torus, as is the case in pine. The torus part laying over the porus seems to be firm, and neither holes nor cracks could be observed. When seen from the lumen side a lifted pit dome was occasionally present.

3.2. Wood reacted with enzymes

It is known that lignin is present between the cellulose microfibrils and probably penetrates within the microfibrils (WARDROP 1965). This presents the large cellulase molecules acting on the cellulose microfibrils in the cell wall (COWLING 1965, FULLER 1970, JUTTE 1969). Therefore, it is striking that the surfaces of the enzyme-treated samples look much "cleaner" or "smoother", indicating that at least some superficial change must have taken place. This phenomenon was also noticed by McQUIRE (1970) who used cellulase in studying the radial penetration of fluids into wood.

3.2.1. *Pinus sylvestris* – stem sapwood

It was obvious that the central part of the torus was affected by the cellulase treatment. This shows up as a hole as large as the porus, or this part is split in

the main direction of the orientation of the microfibrils of the S_2 layer on the lumen side (*fig. 2*). Sometimes the torus as a whole was so thin that it could hardly be seen. The margo fibrils were always digested or so thin that they hardly could be recognized. Near the torus the margo fibrils are thickened: this part remained. When the porus is in an aspirated position, it remained attached to the porus. The inner side of the dome could either be lifted off or peeled off in a very flat helix. If the pit was aspirated, this inner layer was lifted off or peeled off around the torus (*fig. 6*). Only very occasionally the torus partly was lifted. It seemed as if the torus fitted over an extra ridge, being thus somewhat strengthened, which, after aspiration, gave a kind of push-button effect (*fig. 8*). From the lumen side the layers of the walls were stripped off in a similar manner as in untreated wood. The peeling starts with the S_3 with attached warty layer, and then the upper S_2 layer having its main orientation (*fig. 2*) in the direction of the corresponding cell wall layer comes off, leaving the S_2 layer in the dome often unattached, thus showing the border between the S_1 and inner S_2 domes. It can also be seen that the pit border is lifted as a whole or in part.

3.2.2. *Pinus radiata* – stem sapwood

The margo cannot usually be detected and the thickness of the torus is reduced. On the other hand in the wood from one sapwood block the margo fibrils showed up much more clearly. They appeared much thicker and the torus showed up as a prominent disc with the edges sharply outlined. In the centre of the torus a hole is very often present as in untreated wood; this part also can be split in the same way as in *Pinus sylvestris*. On the torus coarse warts can be seen, as in *Pinus sylvestris*.

Whether the inside layer of an aspirated pit peels off or whether it is lifted as a whole, this always occurs around the torus which is very firmly attached to the porus (*fig. 8*). In the case of unaspirated pits the inner S_2 layer of the dome is also lifted or peeled off, taking the covering layers with it, as in *Pinus sylvestris*, and leaving the S_1 and S_2 layers towards the lumen side of the pit dome.

3.2.3. *Picea species* – stem sapwood

The margo fibrils are always entirely or at least for the greater part digested, leaving only the coarse interconnection points with the torus (*fig. 10*). Only in one case was the margo still present, namely in two pits which were situated side by side. In these twin-pits it is striking that the margo fibrils are more clearly visible individually than in untreated wood. The part of the torus which is over the porus largely keeps its somewhat granular structure but in some cases it seems to be somewhat drawn out. A hole in the torus is never present, as may be the case in the *Pinus* species. The central part can occasionally be split, but this gives rise to triangular ruptures.

The removal or degrading of the pit dome occurs in a way different from that in *Pinus*. It is more prominent from the lumen side than from the inner dome side. In none of the cases was a peeling off from the inner dome or a lifting of this layer noticed. From the pictures showing the degrading from the lumen side

(*figs. 11* and *12*) it could be concluded that the top layer which seems to be very thin can be peeled off. This gives rise to a sort of a crater-like structure with a more or less concentric orientation pattern from the remaining inner S_2. In its centre a more solid ring borders the porus, against which the granular torus is attached (*fig. 11*).

In a few cases the fine S_2 layers on the lumen side were still partly connected with the porus. These pits were aspirated and from the lumen side the torus could be seen peeping through the porus with its thickened seam (*fig. 12*).

4. DISCUSSION

Studies of the bordered pits of the two *Pinus* species and of *Picea* with the S.E.M. have led to a better understanding of their structure, although it cannot be elucidated as a whole. There are still problems remaining to be solved and some of these new observations pose further problems.

4.1. Structure of the pit border

This study confirmed the overall structure of the pit dome as observed by JUTTE & SPIT (1963) in *Picea alba* Karst. and *Araucaria angustifolia* O. Ktze and by MURMANIS & SACHS (1969) in *Pinus strobus* L.

The initial pit border which according to the latter authors lies in the centre of S_1 could not be observed in our S.E.M. investigations. According to FENGEL (1966), however, the initial pit border cannot be recognized separately after the S_1 layer has been laid down. From both the untreated and the enzyme-treated wood it was clear that whenever the dome was split this occurred between the S_1 and S_2 layers as in the other parts of the cell wall. WARDROP (1962) has already shown that this transitional region is the weakest, which gives rise to failures during mechanical treatment.

Fig. 7. *Pinus radiata* (untreated). From the two pits on the right only the S_1 dome is left. From the pit on the lower left the S_1 dome is left and also the unaspirated membrane showing a hole in its centre (arrow). × 1,300.

Fig. 8. *Pinus radiata* (enzyme treated). Two pits in which the inner part of the pit border has disappeared together with the margo leaving the tori firmly attached to the pori. × 2,000.

Fig. 9. *Picea* sp. (untreated). Aspirated pit showing a densely interwoven granular torus and a dense margo covered by a thin membrane. × 5,000.

Fig. 10. *Picea* sp. (enzyme treated). An aspirated pit with the margo digested, the inside of the border remained intact. × 5,000.

Fig. 11. *Picea* sp. (enzyme treated). Degradation of the aspirated pit from the lumen side leaving around the porus a thick seam (arrows). Parts of the S_2 layers with different fibril directions show up. × 5,000.

Fig. 12. *Picea* sp. (enzyme treated). The border of the aspirated pit is partly split off from the lumen side showing different layers of which the partly peeled off S_2 forming the outside dome is the most prominent. The torus bulges through the porus with its strengthened border. × 2,000.
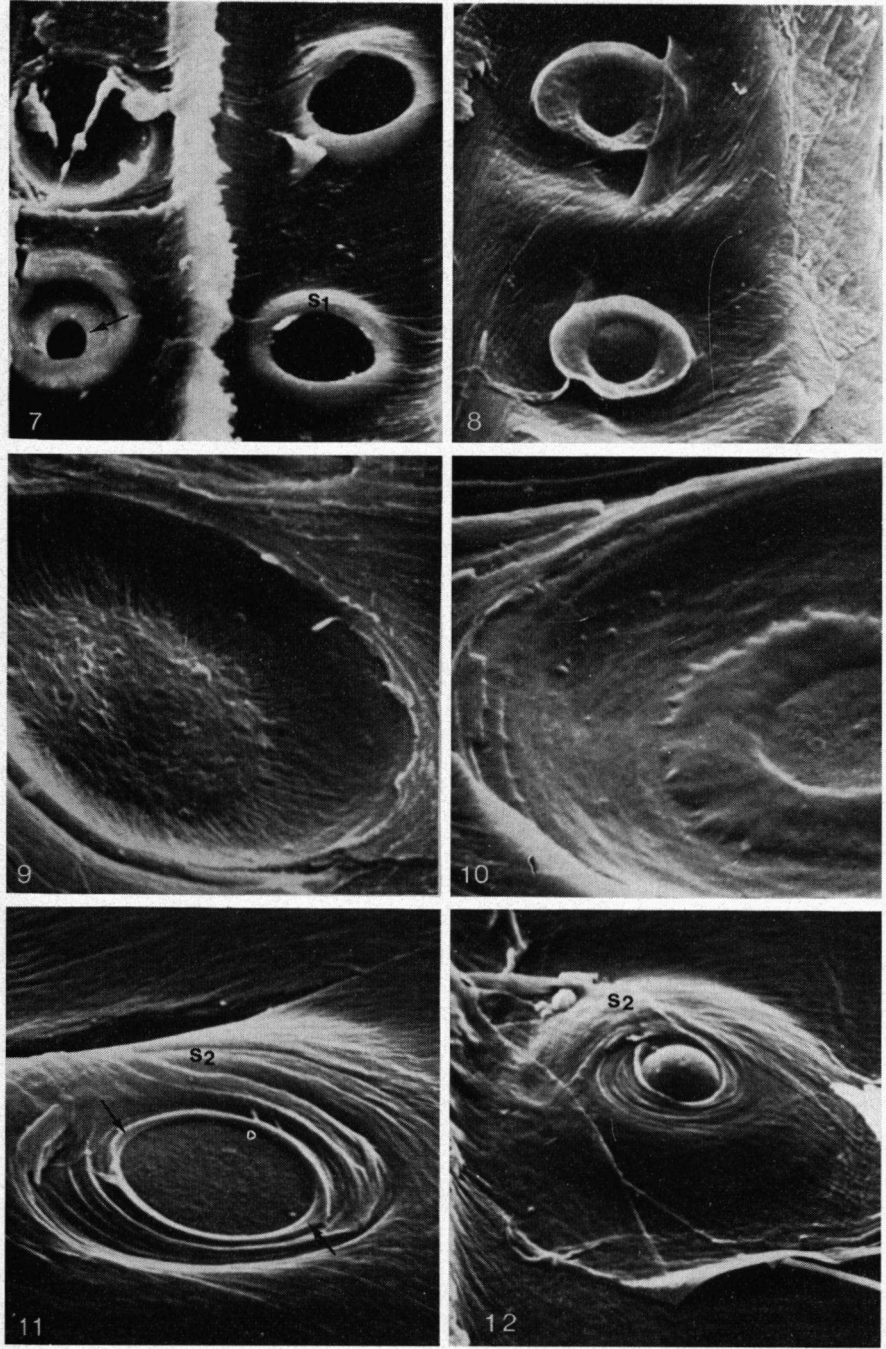
4.1.1. Lumen side

When the S_2 layer is split off from the unaspirated pits the S_1 is left and shows up as the inner dome with a large aperture. When viewed from the lumen side, the inner S_2 layer towards the pit chamber may also be visible, but usually the outer S_2 layer has removed the inner S_2 layer from the dome. Thus both S_2 layers are fairly closely connected, notwithstanding their differences in fibril orientation (JUTTE & SPIT 1963, MURMANIS & SACHS 1969). In aspirated pits from which the S_2 on the lumen side was removed, the inner S_2 showed up, because it was tightly held by the torus, being of about the same size as the porus of the S_1 dome. It is interesting that in the enzyme-treated spruce the porus seems to be lined with thick fibril bundles. This seems to be related to the closing mechanism, although no special evidence could be found. It may well be that the thick fibrils in *Picea* (*fig. 11*) have some relation to the S_3 layer which is usually absent in this wood.

4.1.2. Inner side

The insides of the pit showed up as sharply demarcated discs as is well known from literature. In untreated wood no splitting off of parts occurred. After enzyme treatment the connection between S_1 and S_2 was obviously weakened, resulting in the lifting or peeling off from the inner side of the pit dome in the Pine species. This could possibly be explained by the enzyme easily penetrating into the S_1 layer which has been shown to be the most porous part of the cell wall (WARDROP & DAVIES 1961), thus loosening the contact between S_1 and S_2. Since wood splits along its natural weak lines, this shows that the fibrils covering the dome and peeling off in a flat helix are not really oriented in perfect circles. This can be appreciated bearing in mind that the protoplast has a tendency to depose its microfibrils in a helix for reasons not understood. This pattern is repeated even in a smaller cavity such as the pit chamber, resulting in its characteristic structure. From the activity of the enzyme preparation in *Pinus* it can be concluded that the molecules react inside the layers of the pit-border, the cellulose microfibrils being more accessible than those in *Picea* in which pit-borders prove to be more stable in cellulase preparations. It also looked as if the layers in the pit borders of Spruce are more densely packed than in Pine. When only the aspirated torus remained in *Pinus*, the inner dome layer could be seen either lifted or peeled off around the torus which kept tight contact with the porus.

In aspirated pits the porus is very tightly connected with the torus which is only sporadically somewhat lifted. It seems as if the closing system works like a push-button, the border of the pit aperture being pressed into the torus. It is unusual to find that in the aspirated condition the margo fibrils are not normally stressed (*fig. 1*), but apparently embedded in the inner dome surface.

4.2. Pit membrane

Another phenomenon is that the pit membrane is covered by a thin diaphanous layer, as was found by BAILEY (1965) and BANKS (1971). JUTTE & SPIT (1963) also observed this layer as a thin veil issuing from the porus and interpreted it as

a remnant of the plasma membrane. Before their replica technique was applied, the wood was pretreated chemically to remove non-cellulosic material. This prevented observations of the details of the microfibril structure and arrangement. It is quite clear now that a plasma membrane covered the pit membrane and the inner side of the pit border. This thin layer was not really removed from the wood by its pretreatment but only loosened and while being held by the porus, for some unknown reason, it came out like a veil. In studying the pit ontogeny it could be seen from the figures in MURMANIS & SACHS' (1969) paper that the original young pit membrane was of considerable thickness and showed up as an even layer. In their transverse sections a very thin layer covering the pit membrane and the inner pit dome can be recognized. This also proves that the thin covering layers described in the present paper, are real. The thin plasma membrane can probably be damaged easily when it is not sufficiently supported, but where it is supported the damage may be much less. The cellulase was able to digest the margo in sapwood, as was also found by NICHOLAS & THOMAS (1967) and BAUCH et al. (1970), thus showing that the microfibrils were of cellulose origin. In addition the margo where it was not digested and the torus showed up more clearly after treatment which may be caused by the sample having been taken close to the heartwood where the margo fibrils become coarser (FREY WYSSLING et al. 1959). Both phenomena show that the cellulase was able to destroy the diaphanous layer. This seems strange, the plasma membrane being mainly of lipid protein origin. JUTTE (1969) found that cellulase originating from *Helix pomatia* was able to destroy this layer in Beech and Ash tension wood, but that the cellulase from *Trichoderma viride* was not. This could well be caused by differences originating from the composition of the protoplast. In relation to the above confusing results it would be interesting to treat the wood also with a protease as a control. If this thin membrane covering the torus and margo really exists, it seems strange that particles can be filtered through the whole structure in green sapwood (LIESE & BAUCH 1964). In working with small particles like TiO_2 which are not globules but have a very irregular shape with sharp edges, they may cut through the membranes and possibly through the margo fibrils as well. This can also be the case with small particles of other origin. It would be very interesting to study cross sections of the pit membranes after its filter process in the T.E.M.

4.3. Holes in the torus

The phenomena found by DAHLITZ (1953) and STEMSRUD (1956) in *Pinus silvestris* were confirmed by us. Moreover a torus pushed through the porus was found by DAHLITZ (pers. comm. by Spit 1966) in T.E.M. It is striking that in both Dahlitz's and Stemsrud's figures the central part of the torus is often seen as a layer of very loosely arranged fibrils in which perforations occurred. This was also found by THOMAS (1969) but only in steamed and pectinase treated Southern Pines. Stemsrud, who also found holes as large as the porus, worked with pretreated (macerated) wood. In the S.E.M. no central torus with smaller pores was found. It could well be that the lower resolution of the S.E.M. prevents ob-

servation of the thin microfibrils or that because of beam damage the fragile
fibrils disappeared, though this does not seem likely from the pictures, especially
of the unaspirated pits.

4.4. Split torus and margo

Most probably in the torus of *Pinus* a natural degrading process takes place
resulting in the partial degradation of the primary walls and middle lamella. It
can be thought that this action originates from the presence of plasmodesmata
piercing through the torus. Maybe there is an analogous process going on as
described by MURMANIS & EVERT (1967) for parenchyma walls in the phloem of
Pinus strobus L. Here plasmodesmata located in primary pit fields were con-
fluent in median nodules. The inner surfaces of the split membrane in *Pinus
sylvestris* show up as very smooth surfaces while according to CÔTÉ (1958) the
split middle lamella must show up at least in the torus. This is unusual because
of the granular character of the middle lamella (SACHS 1963). It proves that the
nature of the middle lamella has changed anyway and most probably by in-
trinsic enzyme reactions. When the pit membrane is split, the fibrils show up
more clearly. This is another reason for believing that the indistinctness of the
fibrils in the margo of the usual membrane has nothing to do with the middle
lamella but with layers sandwiching the margo and torus on both sides. It has
already been mentioned that it would be reasonable to accept that the plasma
membrane and other organelles may stay over the pit membrane, as in the case
of the tracheid lumen and pit chamber.

4.5. Pits in branch

From the appearance of the pits found in the *Pinus* branch it could be concluded
that the pit membrane was becoming mature. Its appearance did not fit, how-
ever, the ontogenetic picture in which FREY WYSSLING et al. (1956) postulated that
a central torus is laid down on the even membrane. It seems as if the branch pits
behave more in accordance with the hypothesis of JAYME et al. (1960) on the
origin of the torus, though no evidence was obtained from our study.

The warts on the torus cannot be fully understood without a more detailed
study. They often occur in a more or less pronounced pattern, especially in the
branch pits. They appear to be of the same diameter as the holes which THOMAS
(1969) found in the torus of Southern Pine and which he thought to be remains
of plasmalemma like structures. The most logical explanation could therefore
be that the warts have something to do with residual organelles from the existing
plasmodesmata.

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