SOME EXAMPLES OF THE INTERRELATION BETWEEN WOOD STRUCTURE AND THE MODE OF ATTACK OF MICROORGANISMS AND CELLULASE PREPARATIONS*

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SUMMARY

Microorganisms are used for gaining a better insight into the structure of the woody cell wall. For obtaining maximal effects from this branch of studies, microorganisms also need to be studied more intensively. Examples are given where microorganisms and cellulase preparations degraded the woody cell wall: differences in soft rot attack in Angiosperm and Gymnosperm fibre walls; soft rot attack in wood which was used in *Teredo* tests in coastal waters; the influence of ponding and immersion in cellulase preparations. It is proposed that the mucous layer around the hyphae of *Chaetomium globosum* and other soft rot fungi plays a role in producing the geometrical cavities. It was found that bacteria attacking ponded wood destroy the very thick membrane of the half bordered pit-pair to a considerable degree, leaving a thin membrane which is considered to be the S2 layer of the parenchyma cell wall. Bacteria also remove the membranes of the bordered pit pairs, most probably by digesting the margo, leaving the torus free to float away. The cellulase preparations are able to digest the margo-fibrils of the bordered pits.

1. INTRODUCTION

It is realized more and more that microorganisms and enzyme preparations can be useful to elucidate the structure of the woody cell wall. This goes back to the ideas of BAILEY & VESTAL (1937) concerning the attack of a certain type of wood destroying fungi in the cell walls of wood. They observed a spectacular destruction pattern in the secondary layer as revealed in polarized light between crossed nicols in longitudinal sections. The authors explained these regular cavities with conical ends by accepting two predetermined sets of planes of enzymatic activity, the first oriented parallel to the long axis of the fibrils, the second at an angle of 20–25 degrees to this axis. SAVORY (1954) introduced the name "soft rot fungi" for this special group, because they softened the surface so that it could easily be dug away with the point of a pen knife.

COWLING (1965) put the idea forward that microorganisms and their enzymatic systems could be used as tools in wood anatomy. By their selective

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* Dedicated to Professor Dr. Wim Karstens, to honour him with great respect and friendship on the occasion of his retirement.
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digestion of cell wall components, characteristic patterns develop which lead to a better understanding of the molecular structure of the cell wall. This attitude is quite a change from former interests in wood destroying micro-organisms, which were only focused on preventing their entrance into the wood.

The mode of attack of *Chaetomium globosum*, one of the soft rot fungi, is different for Gymnosperm and Angiosperm fibre walls. In both groups the S2 layer becomes degraded, but in Angiosperms the S3 layer is primarily attacked, whilst in Gymnosperms the decomposition is localized in the S2 layer. This behaviour is attributed to fundamental differences in the nature of the S3 layers in the different groups (CORBETT 1963, 1965; LEVY 1965; LIESE 1970b, MEIER 1955).

It has been found that soft rot fungi also play an important role in the attack of wood by shipworm. It is a fact that wood invaded by these molluscs is often found to be very soft. This is the result of the simultaneous attack of marine soft rot fungi, which cause the same type of destruction as the terrestial type (BARGHOORN & LINDER 1944; KAMPF et al. 1959; KOHLMEYER 1958).

ELLWOOD & ECKLUND (1959) found wood destruction by bacteria. This was a surprise, because it was more or less accepted that the woody cell wall is resistant to attack by bacteria. After the above discovery an increasing amount of information has been published on the destructive action of bacteria on wood. This phenomenon was especially studied because of the damage they cause in ponded logs. With this treatment the outer part of the logs shows a higher absorption for liquids than does its unponded equivalent (ADOLF et al. 1972; COURTOIS 1966; ELLWOOD & ECKLUND 1959; JUTTE 1971; KNUTH 1964; LIESE & KARNOP 1968). While studying the mode of attack of microorganisms on woody cell walls, it is a necessity to gain also full information on the fungi and bacteria in question. After all, they provide the surfaces from which the degrading processes originate. For soft rot fungi this aspect has been studied by LEVY (1967), LIESE (1964, 1967, 1970a, b), MARET (1972) and SCHMID & LIESE (1966).

Microorganisms degrade wood by means of the secretion of digestive enzymes. Cellulase is one of these effective enzymes, and preparations of this enzyme also have been used in cell wall studies (BAUCH et al. 1970; JUTTE 1969; JUTTE & WARDROP 1970; NICHOLAS & THOMAS 1968; WARDROP & JUTTE 1968).

It is the aim of the present paper to give some results of the cell wall attack by soft rot fungi, bacteria, and enzyme preparations, which was studied by means of the Transmission electron microscope (TEM) and the Scanning reflection electron microscope (SEM).

2. MATERIAL

The materials selected were: *Pinus sylvestris* (Scots pine) sapwood and *Fagus sylvatica* (Beech) which were attacked heavily by a culture of *Chaetomium globosum*.

A *Gluta* species (Rengas) heartwood sample was used for studying its Teredo

resistance in coastal waters of the Netherlands. After 12 years the wood showed many shipworm boreholes and its consistency was soft.

Picea abies (Spruce) from the outer part of the stem (which can be expected to be sapwood) including sound wood and wood which had been ponded for some years or was treated with cellulase from *Trichoderma viride* (Onozutia, Japan) for 2 days.

The cellulase preparation was prepared and applied as described by JUTTE (1969) and JUTTE & WARDROP (1970).

3. METHODS

Both Scots pine and Beech were embedded in araldite and their structure studied with a Siemens Elmiskop 2A as described by JUTTE (1969) and JUTTE & WARDROP (1970).

Spruce was embedded in methacrylate, without pretreatment. Its structure was studied with a Philips E. M. 100C, as described in JUTTE & SPIT (1963). Spruce and Rengas were split radially and studied with a Cambridge Scientific Instruments Stereoscan, as described by JUTTE & LEVY (1971a).

4. RESULTS

4.1. Scots pine and Beech were seriously damaged by *Chaetomium globosum*. *Figs. 1* and *2* give an impression of the mode of attack in the fibres of Beech and Scots pine, respectively. *Fig. 1* shows clearly how the fungus changes the organized cell wall of Beech wood into a foamy disorganized mass. The S3 cannot any more be recognized from the S2 layer which also has been degraded on its inner part. In Scots pine, on the contrary (*fig. 2*), the S3 layer is still present, it is not even removed in the last stage of attack. The S2 layer, however, has large holes containing the remnants of degraded wood and perhaps fungi (JUTTE & WARDROP 1970).

4.2. Rengas heartwood, which normally has a mahogany-like colour, had lost this feature. The attacked sample was brownish grey. It was heavily infected by soft rot fungi, which must have been of marine origin. *Figs. 11, 12, 14* and *15* show the attack in the secondary cell wall. It is quite possible that here is dealt with a mixed infection of fungi and perhaps bacteria. The soft rot degrading, however, was the most pronounced. Not all phenomena which were observed could be fully explained. For instance, *fig. 14* pictures at high magnification a cell from which a certain substance, bordering the lumen has peeled off, showing a part of a degraded layer. Many of the fibres studied showed this apparently structureless swollen substance, which undoubtedly is the layer covering the fibre wall on the lumen side. The most obvious explanation is that the fungus has followed the same procedure as it did in Beech. The wood has a considerable amount of extraneous material, which partly may have leached out, but a certain amount of which is left. The fungus makes the S3 layer swell

and this mass together with the extraneous material gives it a crumbly amorphous appearance. Where this layer has peeled off, a heavily degraded part of the cell wall appears, being the S2 layer that shows two initial bore holes. *Fig. 11* gives an overall picture of the soft rot cavities. It is clear from their steep direction that the S3 layer has been removed, most possibly in the same way as shown in *fig. 14*. The hypha penetrating a whole series of tangential walls is most probably a soft rot fungus. One can only guess what causes the hyphal mechanism to pass nearly horizontally through so many layers. This type of penetration is often found (KOHLMEYER 1958, his fig. 6). Hyphae very often seem to branch before entering the wall. *Fig. 13* depicts a stage of the cell wall where the S3 layer is still intact. The lower branch is ready to attack the S3 layer and the top one may be the original hypha growing further over the cell wall surface. It might also be that soft rot hyphae in Angiosperms branch before their attack on the fibre wall into hyphae with different teleological affinities, one for attacking the S3 layer, the other to go through directly to the S2 layer. In *fig. 10* the fungus has already penetrated the cell wall and has branched in it to build up its usual pattern. It can be accepted, that because of the heavy deterioration of the wood the S3 layer and part of the S2 layer have been removed.

Figs. 12 and *15* show the destruction of the cell walls. In the left fibre of *fig. 12* cavities can be seen. A part of the swollen layer is present on the left plus some remains of the destroyed transition lamellae between the S2 and S3 layers, as also can be seen from *fig. 14*. How heavily the S2 layer is degraded is shown in *fig. 15*, where in the fibre the holes along the S2 microfibrils are obvious. They are covered with debris remaining from the destructive activities of the fungus. From the left fibre the S2 layer is broken off, just leaving us a part for orientation. The fairly smooth surface here is from the S1 layer.

4.3. In Spruce both the half-bordered pit-pairs and the bordered pits in the tracheids were investigated.

4.3.1. Half-bordered pit-pairs. In untreated Spruce the half-bordered pit-pairs all showed very thick membranes, especially on the side of the parenchyma cell (*fig. 3*). In ponded wood this membrane was for the greater part digested, still leaving a very thin membrane (*fig. 4*), which does not originate from the middle lamella but from one of the central layers of the parenchyma wall. Ponded Spruce is also shown in *fig. 9*, and there it can be observed that the pit membranes are still present in the half bordered pit-pairs. If the inner part of the cell wall is split off, it removes with it the membranes. *Fig. 8* depicts wood treated with cellulase. From the tracheid side the pit membranes of the half-bordered pit-pairs cannot be observed, which does not mean that they are absent. Their presence is obvious on the inside of the parenchyma wall. On the right, parts of the cell walls have been broken off, taking the membranes from three pit-pairs away.

4.3.2. Bordered pit-pairs. In untreated Spruce (*fig. 5*) the bordered pit membrane shows up clearly with its torus and radial margofibrils. In ponded wood on the contrary, the whole membrane was removed (*fig. 7*). Not a single membrane could be found in the investigated material, neither in the TEM nor in the SEM. *Fig. 6* shows Spruce treated with the cellulase preparation. The aspirated torus is still present, but the margo fibrils have been digested.

5. DISCUSSION

The examples of the cell wall degradation described above give some insight in the problems of the interrelationship between wood and microorganisms or their isolated products although it is clear from the material studied that we are left with many problems, not only pertaining to the structure of the woody cell wall, but also with the actual behaviour of the microorganisms. It is for instance known since BAILEY & VESTAL's (1937) discovery that a certain type of fungi is able to produce geometrically shaped cavities. Since 1954 soft rot fungi were found to be a group of microorganisms which are able to achieve this type of architecture (SAVORY 1954). Now it is known that Ascomycetes and Fungi imperfecti are involved in the creation of holes with 'pointed ends' in the S2 layer. As was shown in *figs. 10–15*, this type of degrading also exists in Angiosperms, however, in combination with the destruction type in *fig. 1*. The mode of attack of *Chaetomium globosum*, an Ascomycete, has been investigated most thoroughly (CORBETT 1963; CORBETT & LEVY 1963; LEVI & PRESTON 1965; LEVY 1965).

The problem is now: how does the fungal hypha exactly bring about this geometrical pattern? CORBETT (1963, 1965) found that when the hypha has entered the S2 layer, it follows the direction of the steep microfibrils. The hypha, while growing, dissolves a tunnel by secreting enzymes, apparently from the apex (LIESE 1970a). After a while its growth practically comes to a standstill, and the cavity with its conical ends develops. Theories to explain the origin of the conical ends have been introduced and controversies arisen (FREY-WYSSLING 1938; LEVI & PRESTON 1965; ROELOFSEN 1956; WARDROP & JUTTE 1968). Even when one of the above theories is accepted, it still remains unsolved why the hypha produces a cone at its growing apex as well as at the "tail" of the cavity. And why is the degraded central part so smooth-walled? The cellulose lattice could by the same token have been degraded in a zig-zag pattern. Why should the enzyme not degrade at both ends cones with different angles or different bases? These problems have been and still are wonderful challenges for our imagination.

Fungal hyphae have often been found to be surrounded by a mucous layer (SCHMID & BALDERMANN 1967; SCHMID & LIESE 1965, 1966; LIESE 1970b; MARET 1972). SCHMID & LIESE (1965, 1966) suggest that this layer may carry extracellular enzymes away from the hypha to the substrate and may take up substances from the substrate. It can be visualized that the enzyme release to the substrate is dependent on the nature of the mucous layer, which develops when

the apical growth of the hypha practically stops. The layer may be closely linked to the existence of the geometrical pattern. During its growth it could distribute evenly the enzyme released by the fungus, its smooth surface causing a smooth degradation pattern of the S2 layer, with which it may be in contact. It is also possible that the mycofibrils play a role in this process (SCHMID & LIESE 1966; MARET 1972). While the layer acts over the whole length of the hypha, the tips degrade the wood in a conical shape, along the cellulose lattice (FREY-WYSSLING 1938; WARDROP & JUTTE 1968) breaking off parallel fibrils from the centre. From this it could be understood why *Chaetomium* has its best development under very wet conditions, which keep the layer swollen to its maximal extent. It would be interesting to study *Chaetomium* in vivo, as proposed by LUND-STRÖM (1970), because in all pictures published up to now the mucous layer has shrunk considerably during its treatment, so that its real extension remains unknown. Another problem is the mechanism of the enzyme secretion itself (LAMPEN 1972) and the entering of the enzyme molecules into the wood micro-fibrils (COWLING & BROWN 1970). It would lead us too far to discuss these problems here.

The understanding of bacterial attack on wood is still in its infancy, although much research has been done since its discovery by ELLWOOD & ECKLUND (1959). This type of degradation was studied for ponded wood from different points of view in Europe and in the New World. JUTTE (1971) in a literature survey gave information on this problem. Coniferous wood, especially Spruce and Pine, has been investigated more intensively than Angiosperm wood. Pioneering work was done, amongst others, by ADOLF et al. (1972); BAUCH et al. (1970); COURTOIS (1966); GREAVES (1965, 1969); KARNOP (1967); KNUTH (1964) and LIESE & KARNOP (1968).

As shown by *fig. 3*, it is clear that the pit membranes, which according to the author are too thick to be called by this name, contain some deposits. *Fig. 4* shows that at least this part has been degraded, starting from the side of the tracheid. The destruction from the parenchyma side seems to be less intensive. The thick membrane becomes much thinner. It is clear that the middle lamella has gone. Interesting is the remaining thin layer originating from the centre of the parenchyma wall. *Fig. 9* shows the inside of parenchyma cells from which parts have been broken off, taking with them the membrane, which looks quite dense in surface view. HARADA & WARDROP (1960) and HARADA (1965) reported that the secondary parenchyma walls of *Cryptomeria* rays consist of S1, S2 and S3 layers, of which the S2 layer is thin, its microfibrils running parallel to the cell axis. If the parenchyma cell walls of *Picea* are built up after the same pattern, then the thin S2 layer might be more resistant. This again is a tempting problem offered by bacterial attack. The cellulase preparation did not degrade the membranes of the half bordered pit-pairs, either (*fig. 8*). It would be interesting to study the results by TEM in the same way as the ponded wood.

The membranes of the bordered pit-pairs (*fig. 5*) were wholly or partly re-moved by bacterial action (*fig. 7*). Most probably the bacteria digest the margo first, as the cellulase preparation does (*fig. 6*), leaving the torus without contact

with the cell walls. That in *fig. 6* the tori are still present is because the wood was dried before the cellulase treatment, leaving the membrane in aspirated position.

The above study shows clearly that microorganisms and their enzyme preparations are useful for elucidating the structure of the woody cell wall. It also stresses, however, the need for a more intensive research in this field on a much broader basis.

This study was carried out in three different periods: One, while in receipt of a General Motors Holden's Scholarship at La Trobe University, Melbourne, Australia in cooperation with Prof. A. B. Wardrop (*figs. 1 & 2*), another at the Forest Products Research Institute TNO, Delft, Holland in cooperation with Ir. B. J. Spit, Technical Physics Dept. TNO & TH, Delft, Holland (*figs. 3, 4 & 7*), the third at the Imperial College, Botany Dept., London, England, in cooperation with Dr. J. F. Levy (all 10 scanning electron micrographs).

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Fig. 1. *Fagus sylvatica*. Transverse section of fibre, the S3 layer and part of S2 layer of which have been degraded by *Chaetomium globosum*. Transm. electron micrograph (reversed negative). Araldite embedding. × 10,000

Fig. 2. *Pinus sylvestris*. Transverse section of tracheid from which S2 layer has been degraded by *Chaetomium globosum*. Transm. electron micrograph (reversed negative). Araldite embedding. × 10,000

Fig. 3. *Picea alba*, sound wood. Transverse section. Part of ray parenchyma cell walls with neighbouring tracheid walls. Half-bordered pit-pairs, their thick "membranes" show up darker than the rest of the cell walls. Transm. electron micrograph. Methacrylate embedding. × 4,200

Fig. 4. *Picea alba*, ponded wood. Transverse section. Part of ray parenchyma and tracheid wall. Half-bordered pit-pairs: thick "membranes" are degraded considerably, leaving a thin membrane which originates from central layer of parenchyma cell wall. Transm. electron micrograph. Methacrylate embedding. × 3,000

Fig. 5. *Picea alba*, sound wood. Radially split surface. Inside of aspirated bordered pit with intact membrane (torus + margo). Scanning electron micrograph. × 5,000

Fig. 6. *Picea alba*, sound wood treated with cellulase preparation. Radially split surface. Inside of aspirated bordered pits margofibrils have been digested, tori still present. Scanning electron micrograph. × 1,900

Fig. 7. *Picea alba*, ponded wood. Transverse section. Tracheids, bordered pit-pair from which membrane has been removed. Transm. electron micrograph. Methacrylate embedding. × 1,200

Fig. 8. *Picea alba*, sound wood treated with cellulase preparation. Radially split surface. Membranes of half-bordered pit-pairs are still present: on the right three have been split off. Scanning electron micrograph. × 460

Fig. 9. *Picea alba*, ponded wood. Radially split surface. Ray with central layer of parenchyma cells, still showing pit membranes. Parts of cell wall have been broken off, taking membranes away. Scanning electron micrograph. × 600

Figs. 10–15. *Gluta* species, heavily attacked by soft rot fungi and shipworm. Radially split surfaces. Scanning electron micrographs.

Fig. 10. Hypha originally branching in cell wall, which has partly been broken off. Arrow in axial direction of fibre. × 5,000

Fig. 11. Fibres with soft rot cavities in S2 layer, covering S3 layer has been removed. A soft rot hypha passes nearly horizontally through fibre walls. × 1,200

Fig. 12. Fibres with soft rot cavities in S2 layer in direction of microfibrils, S3 layer is absent. × 2,200

Fig. 13. Fungus with branched hypha. Lower branch seems ready for destructive activity; upper branch attached to cell wall surface. × 4,800

Fig. 14. Lumen side of fibre with on upper side swollen parts of S3 layer, combined with extraneous material. Lower side with initiated holes of soft rot fungus in S2 layer and remnants of the transmission layer between S3 and S2. × 10,000

Fig. 15. Two fibres, the right one with S2 layer heavily degraded by soft rot. From left one the S3 and most of the S2 have been split off, leaving the S1 layer. × 5,000