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Formation of soft rot cavities in various cellulose fibres by *Humicola alopallonella* Meyers & Moore

Kaviteter i olika cellulosafibrer bildade av mögelrötesvampen Humicola alopallonella Meyers & Moore

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Abstract

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The ability of the soft rot fungus Humicola alopallonella Meyers & Moore to form cavities in various cellulose fibres has been studied. The following fibre materials were tested: wood of aspen, beech, birch, pine and spruce, three sulphate pulps with different lignin content, spruce holocellulose, Avicel, Sigmacell T38, flax, jute, ramie, sisal, cotton, kapok, seed hairs of Salix pentandra L. and two viscose rayon fibres. In addition the degradation of cellophane was studied.

Typical soft rot cavities were formed in all the natural fibres except for spruce holo cellulose tracheids. The cavity formation in fibres like Avicel, Sigmacell T38 and cotton which contain no or possibly minute amounts of lignin and hemicelluloses, shows that these substances are not needed for cavity formation per se. The shape of the cavities is rather similar in all the natural fibres, indicating that the explanation of the form of the cavities must be sought in the crystalline structure of the cellulose.

Various aspects of the process of cavity formation are discussed in the light of the new findings.

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Introduction

Soft rot fungi are generally characterised by the formation of cavities around hyphae growing in the secondary cell walls of wood. Studies on cavity formation by soft rot fungi have by most workers been performed with different wood species. Yet already Baker (1939) reported that cavities, similar to those found in wood by Bailey and Vestal (1937), also occurred in the secondary cell walls of vegetable materials from animal faeces, composts and dunghills. He also published some pictures showing typical cavities in a plant hair. He did not, however, mention the origin of the plant materials in which he had found the cavities.

Courtois (1963) reported in a study on the micromorphological decay patterns of soft rot fungi on wood that he had also made some experiments with cotton, but no cavities were observed in this fibre. Corbett (1967) examined the attack on ramie and cotton fibres by the well-known soft rot fungus *Chaetomium globosum* Kunze ex Fr. In the ramie fibre she found an attack which she claims was comparable with that produced in the wood of Scots pine by the same fungus. In a recent report Poole and Taylor (1973) demonstrated that *Humicola grisea* Traaen forms cavities in roselle fibres (from *Hibiscus sabdariffa*).

Although extensive studies have been made by various workers on the degradation of such fibres as cotton and jute, there appear to be no reports on soft rot cavity formation in these fibres. Basu and Ghose (1962) who made a microscopical study on the degradation of jute fibres by fungi and bacteria, reported that hyphae of *Chaetomium indicum* Corda penetrated into the cell wall and branched to form a "T-branch". The "T-branching" is known to be one of the first steps in the process of cavity formation in wood (Corbett 1965). No mention, however, of fully developed cavities was made in their report.

During studies at this laboratory on formation of soft rot cavities in various cellulose fibres it was found that a strain of *Humicola alopallonella* Meyers & Moore had an extraordinary ability to form cavities in the most diverse cellulose fibres. The degradation of twenty different cellulose fibres have been studied and the results are reported in this paper.

Meyers and Reynolds (1960) found that *Humicola alopallonella* was cellulolytic and that it reduced the tensile strength of manila twine. Eaton and Gareth Jones (1971) observed that the fungus produced soft rot cavities in beech and pine wood. Eaton and Irvine (1972) reported that *H. alopallonella* caused a weight loss of 29.8 percent of beech wood blocks after 15 weeks at 28°C. Nilsson (1973), however, obtained only 3.1 percent weight loss of birch wood after 12 weeks at 24—26°C.

Material and methods

The strain CBS 207.60, which is the type culture of *Humicola alopallonella,* was obtained from "Centraalbureau voor Schimmelcultures", in Baarn. This strain was isolated by Meyers and Moore (1960) from decaying wood of *Tilia americana* submerged in sea water.

The various cellulose fibres which have been tested are listed in Table 1. Since both lignified and nonlignified fibres were used the lignin content, if known, is also given in the table. The fibres can according to their origin be classified as wood fibres, bast fibres, leaf fibres, hair fibres and regenerated fibres. No information on the origin of Avicel could be obtained from the manufacturer (E. Merck AG). The orientation of the microfibrils in the cellulose particles do, however, indicate that Avicel is prepared from wood. In addition to the mentioned fibres degradation of cellophane was also studied.

The cavity formation in the microcrystalline celluloses, Avicel and Sigmacell T38 which are in powder form, was studied by inoculation of a small portion (approx 100 mg) of the cellulose moistened with a mineral salt solution. These experiments were carried out in 100 ml Erlenmeyer flasks. The salt solution had the following composition: NH_4NO_3 3.0 g, KH_2PO_4 1.5 g, $MgSO_4 \cdot 7H_2O$ 0.5 g and 1000 ml of water. The flasks were equipped with cotton plugs and autoclaved. The sterile flasks were then inoculated with a drop of a spore suspension from the fungus. After the incubation period a small amount of the cellulose powder was taken out from the flasks and spread on a glass slide and viewed under a microscope in polarized light.

For testing of the other fibres a different method was used. Agar slopes were prepared in 18 mm (diam.) test tubes. The agar medium consisted of Avicel 10 g, asparagine 1.0 g, NH_4NO_3 1.0 g, KH_2PO_4 1.0 g $MgSO_4 \cdot 7H_2O$ 0.5 g, $FeSO_4 \cdot 7H_2O$ 0.01 g, $ZnSO_4 \cdot 7H_2O$ 0.01 g, yeast extract (Difco) 0.5 g, glucose 2.5 g, agar 15 g and 1000 ml of water. The tubes were fitted with cotton plugs and autoclaved. The agar slopes were inoculated with small pieces of mycelium and agar taken from petri-plate cultures of the fungus growing on malt extract agar. When some growth had occurred the sterilised fibres were introduced and placed upon the mycelium. For the studies of cavity formation in natural wood fibres small wood blocks (approx 5×5×10 mm) were used. Small pellets were prepared of the other fibres. The test materials were sterilised by autoclaving in petri dishes immersed in a solution containing 3.0 g NH_4NO_3, 1.5 g KH_2PO_4 and 0.5 g $MgSO_4 \cdot 7H_2O$ per 1000 ml of water. The viscose fibres were used both in dried and "never-dried" condition.

After different periods of time the fibres were taken out from the test tubes for microscopical studies. Thin cross and longitudinal sections were prepared from the wood blocks. In the case of the other fibres small amounts were placed on glass slides and the fibres were separated with a pair of needles. The hyphae in the fibres were stained with Chlorazol Skye Blue (Imperial Chemical Industries Ltd), according to a method described by Simpson and Marsh (1969). The fibres were studied with a light microscope, usually under polarized light. To ascertain that the cavities were situated inside the cell wall, cross sections were also made of most fibres. For this purpose the fibres were embedded in polyethylene glycol 4000.

Table 1. The examined cellulose fibres and their lignin content.

Fibres	Lignin content %	Reference
Wood fibres		
Aspen wood (*Populus tremula*)	17.9—21.2	Henningsson 1967
Beech wood (*Fagus silvatica*)	21	Levi and Preston 1965
Birch wood (*Betula verrucosa*)	19.6	Henningsson 1962
Pine wood (*Pinus silvestris*)	28.1	Henningsson 1962
Spruce wood (*Picea abies*)	27.1	Henningsson 1962
Sulphate pulp A (from *Pinus silvestris*)	26.6	[1]
Sulphate pulp B (from *Pinus silvestris*)	13	[1]
Sulphate pulp C (from *Pinus silvestris*)	10.8	[1]
Spruce holocellulose (from *Picea abies*)	0.88 [2]	Boutelje and Hollmark 1972
Avicel (E. Merck AG)		
Sigmacell T38 (Sigma Chemical Co.)		
Bast fibres		
Flax (*Linum usitatissimum*)	2.23	Kleinert 1972
Jute (*Corchorus sp.*)	11	Roelofsen 1959
Ramie, bleached (*Boehmeria nivea*)	0.6	Stoves 1957
Leaf fibres		
Sisal (*Agave sisalana*)	7.1—7.8	Bagby et al. 1971
Hair fibres		
Cotton (*Gossypium hirsutum*)	0	Roelofsen 1959
Kapok (*Ceiba pentandra*)	12	Roelofsen 1959
Seed hairs of *Salix pentandra*		
Regenerated fibres		
Viscose rayon (10 % stretch)[3]		
Viscose rayon (90 % stretch)[3]		

[1] The pulps were prepared and analyzed at the Swedish Forest Products Research Laboratory, Stockholm.
[2] Klason lignin 0.28 % and acid-soluble lignin 0.6 %.
[3] Used in dried and "never-dried" condition.

The angles of the cavity chains in relation to the fibre axis were measured on the microphotographs taken.

The attack on thin transverse sections of birch wood (approx 10μ thick) was also studied. The thin wood sections were sterilised by autoclaving in petri dishes. A drop of water was added to each section before autoclaving to prevent drying out. The sterile sections were then placed upon the mycelium of the fungus growing on malt extract agar in a petri dish. The section was placed a few millimeters from the margin of the colony where the mycelial mat was rather thin. When studies were to be made under the microscope, the lid of the petri dish was removed and the wood section was covered with a heat-sterilised cover slip. The petri dish was then placed under the microscope. Under these conditions the attack on the section could be carefully studied and microphotographs could be taken. After the observations ended the cover glass was removed while the section was left in its position on the mycelium. This process could be repeated

several times after different periods of time, giving an opportunity to study the continuing attack on the same section. If too much aerial mycelium tended to overgrow the section and obscure the observations some of the mycelium was scraped off with a sterile needle.

The section could also be moved towards the new-formed margin of the colony. We found the risk of contamination was small if the petri dishes were not left open too long.

The degradation of cellophane was studied by growing the fungus on a cellophane membrane. The same cellulose agar which was used for the test tubes described above was used to prepare agar plates in petri dishes. The cellophane was cut into pieces (approx 30×30 mm) and sterilised by autoclaving. The cellophane membranes were then placed on the top of the agar plates. The agar plates were inoculated with small pieces of mycelium and agar taken from cultures of the fungus growing on malt extract agar.

After incubation for different periods of time, the cellophane membranes were removed from the agar plates and placed on glass slides for microscopic studies.

Results

Soft rot in wood is characterised by the formation of cavities within the secondary cell walls. The cavities are produced around hyphae which grow longitudinally in the wall. The cavities normally occur in chains which apparently follow the direction of the cellulose microfibrils. When longitudinal sections of soft-rotted wood are observed, the attack is seen as cylindrical cavities with conical ends or as biconical cavities (Fig. 1). In transverse sections the cavities are seen as round or oval holes in the cell wall (Fig. 2).

The process of cavity formation in wood has been closely studied by Corbett and Levy (1963), Corbett (1965), Levi (1965), Levy and Stevens (1966), Findlay (1970), Casagrande and Ouellette (1971) and Lundström (1972). The first step in the process of cavity formation is initiated by hyphae which grow in the cell lumen. The hyphae branch to give rise to lateral hyphae which penetrate the cell wall, usually at right angles to the long axis of the fibre. When a hypha penetrates the cell wall a considerable constriction of its diameter usually occurs. The penetrating hypha give rise to bore holes which are very little wider than the hyphae. These bore holes never enlarge. Quite often the penetrating hyphae pass completely through the whole cell walls without formation of cavities. It seems to be necessary for the penetrating hypha to align itself in the direction of the cellulose microfibrils when a cavity is to be formed. This can be brought about in different ways. The hypha might branch vertically inside the wall to form a T-shaped branch (Corbett and Levy 1963) or the hypha might pass completely through the cell walls forming two lateral branches within one of the walls. A direct turn of the hypha without branching has also been observed (Levi 1965). Casagrande and Ouellette (1971) have illustrated some of the different ways for initiation of cavities.

The cavities are then formed by dissolution of the cell wall material around the penetrating hyphae which grow more or less longitudinally in the fibre wall. New cavities are initiated when the hypha in the first cavity continues to grow longitudinally within the cell wall.

It has been observed by Corbett (1965) and Nilsson (1973) that microfungi produce two morphologically different types of attack in wood. The first type of attack, designated as "Type 1" by Corbett (1965) is represented by the cavity formation described above and the second, which Corbett called "Type 2" attack, is an erosion of the cell walls brought about by lumenal hyphae. Studies on the attack of *Humicola alopallonella* on five different wood species were reported in a previous paper (Nilsson 1973). Only attack of "Type 1" was observed.

In this study it was found that *H. alopallonella* could produce typical soft rot cavities in all the cellulose fibres tested, except for the two viscose fibres. Cavities were formed even in a completely lignin-free fibre such as cotton. The initiation of the cavities was in most cases brought about by a direct turn of the penetration hyphae. "T-branching" also occurred but was less common. When single-cell fibres were used it was found that the hyphae which penetrated the wall and initiated the cavities, could develop either from hyphae inside the cell lumen or from hyphae outside the fibre. This was also found for the sulphate pulp and spruce holocellulose fibres which had been defibrated chemically. The fungus also formed numerous penetrating hyphae which only produced bore holes through

the cell walls and no cavities. These bore holes could not be seen to enlarge in any of the fibres studied except for the viscose fibres. In these fibres a type of attack occurred which possibly could be interpreted as enlargement of bore holes.

Cavity formation in the various fibres

Wood fibres

Aspen, beech and birch wood

Cavity chains were formed which were arranged in a very steep Z-helix (right-handed) in the fibre walls (Fig. 1). In some fibres the cavity chains appeared to be nearly parallel to the long axis of the fibres. Numerous cavity chains were also formed in the vessel cell walls. These cavities were considerably narrower than the cavities in the fibres.

The arrangement of the cavity chains in the vessel walls was complicated due to the presence of numerous pits. But generally the cavity chains were oriented in a flatter Z-helical spiral than those in the fibres. In the vessel walls of the aspen and beech woods cavities were observed encircling the pits in the border region indicating a circular arrangement of the cellulose microfibrils. In an electron micrograph of the vessel wall of beech published by Harada (1965 a), a corresponding circular arrangement of the microfibrils can be observed around the bordered pits. No lumenal erosion of the cell walls was observed in any of the hardwoods.

Birch wood cross sections

The thin 10μ birch wood cross sections were used to see whether the fungus would attack the whole transverse surface of the secondary wall or if it would produce cavities under these unusual conditions. The reason for using thin transverse sections is that the fungus does not have to overcome any hindering effects of the S_3 layer but has free access to the secondary wall.

The fungus grew over the sections and some of the hyphae were seen to penetrate into the exposed secondary wall longitudinally. Then typical lysis zones developed around the penetrating hyphae. When viewed under the microscope the attack looked quite similar to that observed in transverse sections cut from wood blocks previously decayed by the fungus (Fig. 3). After longer exposure of the sections to the fungus, the lysis zones enlarged and numerous new cavities developed (Fig. 4). Even after three weeks exposure no erosion type of attack was observed. Some of the transverse sections were macerated using a method described by Burkart (1966) and the loosened short fibres were spread out on a slide and observed in polarised light. A certain amount of the fibres were oriented longitudinally on the slide whereas others were oriented cross-wise. In those laying longitudinally, numerous cavities were seen of which some were open in one end, and the typical tapering of the cavity was seen only in the other end. In some cases two cavities had developed of which one or both were open at the ends. Some cavities were as long as the fibres themselves and in these cases both of the cavity ends were open. Thus these cavities had no tapered ends but could be regarded as tubes going longitudinally through the fibres. Fig. 5 illustrates the different types of cavities observed.

This experiment shows, that although the hyphae which grew over the section were in close contact with the exposed secondary wall, they did not produce any visible erosion (Type 2) attack. Only when the hyphae had oriented themselves parallel with the cellulose microfibrils by growing into the section were they able to degrade the wood. This seems to indicate that the enzyme production is greatly stimulated when the hyphae grow inside the wall parallel to the microfibrils.

Pine and spruce wood

Cavities were easily formed in both early- and latewood tracheids. No erosion of the cell walls was seen. It was observed that most of the first cavities in the earlywood

tracheids were formed in the part of the secondary wall which is adjacent to the cell corners.

Fig. 6 shows cavities formed in latewood tracheids of spruce. The cavities were often broad and large. Several of them had rather sharply pointed ends. The cavity chains in the latewood tracheids run in a very steep Z-helix, in some cases almost parallel to the long axis of the fibre. Fig. 7 shows cavities formed in earlywood tracheids of spruce. These cavities were considerably narrower than those formed in the latewood. The orientation of the cavity chains was more varying in the earlywood tracheids than in the latewood tracheids. In the tangential cell walls the cavity chains were rather regularly arranged in a Z-helix making an angle of approx 20—30° to the long axis of the fibre. In the radial cell walls the cavity chains were arranged more irregularly due to the arrangement of the microfibrils around the bordered pits (see Harada 1965 b, Harada & Côté, 1967 and Okumura et al. 1973). Fig. 8 illustrates some of the different orientations of the cavities observed in radial sections. Cavities observed at the sides of the tracheids, probably representing the cell corner region, were oriented almost parallel to the long axis of the tracheids (Fig. 8 Cavity A). Cavities which had developed in the part of the tracheid between two bordered pits lay in a Z-helical spiral at an angle of approx 30° to the long axis of the tracheids (Fig. 8. Cavity B). In the pit border region some cavities crossed over the pit border in a streamline pattern (Fig. 8. Cavity C) while others encircled the pits more or less completely (Fig. 8. Cavities D, E and F), in the same way as was observed in the pit borders of the vessels in aspen and beech wood. The orientation of the cavity chains indicates that at least two wall layers with different orientation of the microfibrils exist in the bordered pit region of pine and spruce earlywood tracheids. Both of the layers are apparently sufficiently thick to permit cavity formation. When the pits were observed from the lumen side it was found that the cavities which encircled the pits were formed underneath the cavities with the other orientation. Thus the circular arrangement of the microfibrils occurs in the outer part of the cell wall.

Similar microfibrillar orientation in the bordered pit region of softwoods, has also been observed on electron micrographs by Harada (1965 b), Harada and Côté (1967) and others. According to Harada and Côté (1967) the S_1 layer of the bordered pits is relatively thick whereas the initial pit border thickening is considerably thinner. Thus the cavities seem to have developed in the S_1 layer, a layer which in the other parts of the tracheid walls, is too thin to permit cavity formation.

Sulphate pulps A, B and C (from *Pinus silvestris*)

Cavities were easily formed in all of the three sulphate pulps despite the varying lignin content. The morphological pattern was very similar to that which was found in the pine wood. Even in the bordered pit region the same patterns were observed. Fig. 9 shows cavities in an earlywood tracheid from sulphate pulp B (lignin content approx 13 %).

In the fibres from pulp C, which had the lowest lignin content (10.8 %), erosion of the cell walls was also observed. The erosion was produced by hyphae in the cell lumen. Some erosion also occurred in fibres from pulp B, while very little erosion was observed in fibres from pulp A (lignin content 26.6 %).

Spruce holocellulose (from *Picea abies*)

Only a few cavity initials, which never enlarged, were formed in the earlywood tracheids. After extended incubation these tracheids were degraded by the erosion type of attack and not by cavity formation.

Cavities were more easily formed in the latewood tracheids. Most of the cavities were, however, formed singly, although chains consisting of two to six cavities also were observed. The cavities in the holocellulose had very sharply pointed ends.

Fig. 10 shows cavities in a latewood tracheid.

Avicel and Sigmacell T38

These fibre particles consist of nearly pure cellulose. Lignin may occur only in minute amounts. Avicel and Sigmacell are commercial microcrystalline cellulose for chromatography purposes. According to the manufacturers they have an average particle size of 38μ.

Fig. 11 shows cavities in an Avicel particle and Fig. 12 shows cavities in a particle of Sigmacell T38. Typical soft rot cavities were formed in chains in the two fibres. The chains were almost parallel to the fibre axis both in Avicel and Sigmacell.

Erosion of the cellulose was also evident. Most of the degradation of the Avicel and Sigmacell particles seemed to occur at the open fibre ends.

Bast fibres

Flax

The cavities were often formed singly or in chains of two to four cavities. Fig. 13 shows a cavity in a flax fibre. Note the extended end of the cavity which is sharply pointed. According to Roelofsen (1959) the greater part of the microfibrils in flax are oriented in a steep S-helix. Most of the cavity chains were also oriented parallel to the fibre axis, or oriented at a very low angle to the fibre axis in a very steep S-helix (left-handed). Slight lumenal erosion was observed in the flax fibres.

Jute

Typical soft rot cavities were formed in chains which were aligned parallel to the long axis of the jute fibres. This agrees with the report by Heyn (1966) where it was shown that the cellulose microfibrils in jute were oriented parallel to the long axis of the fibre. Only very slight lumenal erosion was observed. Fig. 14 shows a cross section of a bundle of jute fibres attacked by *H. alopallonella*. Some of the fibres are heavily degraded but in other fibres the individual cavities can be seen. The wall layer closest to the lumen was more resistant than the rest of the wall and remained intact when the secondary wall had been degraded. The resistant wall layer represents the tertiary wall and it is known from studies on soft rot attack in wood that this layer is very resistant to degradation by microfungi.

Ramie (bleached)

Chains with typical cavities occurred as well as single cavities with very pointed ends. The chains were oriented parallel to the fibre axis. According to Heyn (1966) the microfibrils in ramie are also aligned parallel to the long axis of the fibre. Fig. 15 and 16 show cavities in ramie fibres.

Leaf fibres

Sisal

Cavities were formed in chains running in a Z-helix at approx 20° to the long axis of the fibre. According to Preston and Middlebrook (1949) the spiral angle of the central layer in sisal fibres amounted to approx 20°.

Hair fibres

Cotton

Cavity chains were easily formed in the cotton fibres. Lumenal erosion also occurred but was rather weak. The cavity chains apparently followed the microfibrils very closely in alternating S- and Z-helices. Fig. 17 shows cavities in a cotton fibre and in Fig. 18 the chains can be seen to reverse in direction, evidently following a reversal in direction of the cellulose microfibrils in the cell wall. The shape of the cavities was remarkably similar to that of the cavities formed in the hardwoods.

Kapok

Figs. 19 and 20 show cavities in kapok fibres. It is clearly illustrated that two different orientations of the cavity chains occur. The cavity chains in the thickened basal part of the hair are oriented at an angle of 60 to 90° (Fig. 19) to the long axis of the fibre, whereas the cavity chains in the other parts of the hair are oriented nearly parallel or at an angle of up to 30° to the long axis of the fibre (Fig. 20). The two different orientations of the cavity chains could be seen to overlap each other towards the lower part of the hair. This indicates the existence of two wall layers with different orientation of the cellulose microfibrils. Both of the layers are sufficiently thick to permit cavity formation. The layer with the flat orientation of the microfibrils surrounds the other layer. The cavities in the outer layer were distinctly broader than the cavities in the inner layer. The cavity chains were oriented in S- or Z-helices. No lumenal erosion was observed in the fibres.

Seed hairs of *Salix pentandra*

The same pattern was found in these fibres as in the kapok fibres.

Regnerated fibres

Viscose fibres

These fibres were extensively degraded by *H. alopallonella*. Numerous bore holes were formed, most of them penetrating more or less at right angle to the long axis of the fibre, but also irregular penetration occurred (Fig. 21). Erosion of the fibre also occurred around some of the transverse bore holes. Some T-branches were observed and cavity-like figures occurred as illustrated in Fig. 22. These "cavities" were formed singly, or occasionally two or three in each chain and had very sharply pointed ends. No difference in the degradation pattern could be detected between the two types of fibres used, nor between dried and "never-dried" fibres.

Degradation of cellophane

Examples of the degradation of cellophane are shown in Figs. 23 and 24. Numerous hyphae penetrated the cellophane and formed small bore holes. Through the lysis of the cellophane around the penetrating hyphae the bore holes were enlarged to lysis zones. In Fig. 23 it can be seen that the lysis was restricted to zones just around the hyphae indicating limited diffusion of the cellulases. The lysis zones were also restricted in length. This and the fact that the lysis zones are produced by a single central hypha indicates a resemblance to soft rot cavities. In Fig. 23 even T-branching of some hyphae can be observed, but no tapering of the lysis zones can be seen. But as the attack proceeds and the lysis zones enlarge, tapering of the ends is evident as seen in Fig. 24. The edges of the lysis zones are, however, rather irregular. Fig. 23 shows that the lysis zones evidently are randomly oriented.

Discussion

The formation of soft rot cavities is a rather complicated process which involves several phenomena which are very little understood. These phenomena have been discussed during the years in a number of papers by several authors. In the following discussion the conclusions drawn from the results obtained with *Humicola alopallonella,* will be compared with some of the hypothesis which have been put forward.

It has been discussed (Levy 1965, Levi 1965, Fuller 1970) whether the initial penetration of the cell wall which precedes cavity formation occurs randomly or as a response from a stimulus in the cell wall. Levy (1965) and later Lundström (1972) have suggested that the possible presence of plasmodesmata within the wood cell walls would provide a stimulus and a pathway for hyphae penetrating the wall. The numerous bore holes formed by *Humicola alopallonella* in viscose fibres and cellophane, show that the fungus certainly also can penetrate wood cells without being stimulated by plasmodesmata. So the penetration can occur randomly. However, it can not be excluded that the hyphae penetrate the wood cells at certain points due to a stimulus of some kind originating from the cell wall. The present investigation gives no answer to this problem. It just shows that the hyphae might penetrate equally well without stimuli. This investigation also shows that penetration evidently occurs equally well from lumenal hyphae and from hyphae on the outside of the fibres.

A more intriguing question is why the penetrating hyphae change direction within the wood cell wall. Some hypotheses have been put forward by Levy (1965), Levi (1965), Fuller (1970) and Lundström (1972). As possible responsible factors plasmodesmata or other wall capillaries, barrier action of the S_3 layer to continued growth and local regions of low resistance have been mentioned. The present investigation shows that a change in direction of penetrating hyphae occurred in all the tested fibres. T-branching was seen even in the viscose fibres and in cellophane (Fig. 23) but was not related to any fibrillar structure as in the natural fibres. The thin transverse sections of birch wood were penetrated and cavities formed without a change in direction within the cell wall of the penetrating hyphae (Fig. 5). The change in direction occurring in natural fibres causing the hyphae to grow along the microfibrils and form cavities may be a way of more efficiently utilizing the carbon source cellulose. It is evident from the present investigation that *H. alopallonella* has a very weak activity on the lumen cell walls of the tested fibres and even when the secondary cell wall was exposed as in the case of the thin transverse sections of birch wood, no visible degradation occurred except for the cavities. Thus, the formation of cavities seems to be the most efficient way for *H. alopallonella* to degrade the cellulose in the fibres. The cellulolytic activity of the hyphae in the cavities is evidently greater than the activity of the lumenal hyphae. Levy and Stevens (1966) suggested that the cavity hyphae of soft rot fungi acted as haustoria in the wood cell walls. Haustoria, possessed by plant parasitic fungi, are hyphae that are specially modified to absorb nutrients from the hosts. The cavity hyphae of *H. alopallonella* can be regarded to have the same functions as the haustoria of parasitic fungi. The change in direction of the cavity-forming hyphae is then an adaption to their function as specialized nutrient-absorbing hyphae. It seems that the hyphae, when growing along the micro-

fibrils within the wall, are stimulated to produce cellulase.

In this study it was found that *Humicola alopallonella* is able to produce cavities in all the tested fibres of natural origin. The cavity formation in fibres which contain no or only minute amounts of lignin, such as spruce holocellulose, Avicel, Sigmacell T38 and cotton, clearly shows that lignin is not necessary for formation of soft rot cavities *per se*. The same applies to the hemicelluloses. It might be noted that these results refer to studies made with *H. alopallonella* and can not be applied to all species of soft rot fungi. In a later paper it will be demonstrated that although some soft rot fungi are able to form soft rot cavities in cotton, these cavities usually differ from the cavities produced in wood. The cavities formed in cotton are usually single and not in chains. They are often small and have a biconical shape.

Roelofsen (1956) tried to explain the shape of the cavities with a hypothesis which was based on several assumptions. He postulated that the rate of decomposition is governed by cellulose decomposition and not by lignin decomposition. He also postulated that the cellulases can not diffuse freely because they are strongly adsorbed by the cellulose and can only migrate with simultaneous dissolution of the cellulose. Longitudinal dissolution of the cellulose microfibrils and transverse dissolution of microfibrils, which were supposed to touch each other occasionally, would, if the longitudinal dissolution was more rapid than the transverse, lead to cavities with conical ends. According to his hypothesis no cavities could be formed in fibres with too long or too short distance between the microfibrils. This would according to him explain why typical cavities never had been found in pure cellulose fibres. Roelofsen's explanation is not entirely correct, since cavities in this investigation were found in cotton.

Levi and Preston (1965) used Roelofsen's hypothesis to explain the shape of the cavities, but they assumed that the rate of decomposition was governed by lignin modification rather than cellulose decomposition. The transverse dissolution would be slower than the longitudinal because it had to proceed through the lignin deposited between the microfibrils. But this can not apply to cotton which contains no lignin. It is evident that the rate of decomposition of cotton is governed by the decomposition of cellulose. It is remarkable how similar the cavities are for instance in cotton, in the microcrystalline celluloses and in the hardwoods. Thus it can be assumed that the shape of the cavities depends on the structure of cellulose itself and not of lignin and hemicelluloses. It has also been suggested by Frey-Wyssling (1938 and 1956), Wardrop and Jutte (1968), and Jutte and Wardrop (1970) that the explanation of the shape of the cavities is to be sought in the structure of cellulose.

It has also been discussed why chains of cavities are formed instead of a continuous cylinder of dissolution. It has been suggested (Courtois 1963 and others) that this could be due to variations in the enzyme secreting activity along the hyphae within the wall. The thin hyphal parts which connect the individual cavities are supposed to lack enzymic activity. Various explanations to this have been proposed such as absence of enzyme secretion at the septa (Levi 1965), rythmic behaviour of the whole organism (Bailey et al. 1968), ageing of the cavity hypha (Liese 1970), and inhibition of enzyme secretion by toxic breakdown products like free phenolic groups (Liese 1970) and polyphenols (Fuller 1970).

It is unlikely that there is a much decreased secretion of enzymes in the vicinity of the septa leading to the constrictions between the cavities, since it has been observed that the hyphae within the cavities often have several septa.

The phenolic substances which are supposed to be formed during the degradation of wood do not occur in the lignin-free fibres examined here. Since cavity chains also occurred in for instance cotton other explanations must be sought.

Two new explanations to the phenomenon are suggested here: 1) The inhibition

of enzyme synthesis could be due to catabolic repression by the cellobiose or glucose released during the lysis of the cell wall around the hyphae. 2) It might be possible that the enzymes are produced only when a hypha in the cell wall is growing exactly parallel to the microfibrils. Even a slight deviation of the hypha would make the enzyme production cease. As the hyphal tip grows further in the wall it later resumes its orientation parallel with the microfibrils and a new cavity is formed. It has already been shown that the fungus depends on the orientation of the cellulose microfibrils in order to degrade the fibres efficiently, i.e. to produce cavities. It is also possible that the phenomenon is due to several related or independent factors which combined give rise to the chains of individual cavities.

It is an open question why soft rot cavities in much studied fibres such as cotton and jute have not been reported before. It is possible that cavities have been seen, but that the observations have been misinterpreted. This study shows that soft rot cavities can be formed in a large number of different types of fibres. It can be postulated that soft rot cavities might be formed in all cellulose fibres which have an ordered orientation of the cellulose microfibrils and a cell wall of a certain thickness. The degradation pattern known as soft rot is thus likely to be very common in nature and not restricted to wood. This assumption is supported by the observations on plant materials made by Baker (1939). In an experiment at this laboratory a pellet of cotton was placed on a sample of unsterile soil. After some time typical soft rot cavities had been formed in the cotton fibres. *Chaetomium globosum* Kunze ex Fr. was isolated from the fibres and could be shown to be able, in pure culture, to produce soft rot cavities in cotton fibres. Typical soft rot cavities have also been found in materials such as leaves, stems of annual plants and pine cones. These materials were sampled when already degraded under natural conditions.

Cowling (1965) has suggested that microorganisms and microbial enzyme systems could be used as selective tools in wood anatomy. The extraordinary ability of *Humicola alopallonella* to form cavities in different types of fibres may possibly be utilized in anatomical research on fibre structures.

Sammanfattning

Vid studier av ett antal mögelrötesvampars förmåga att bilda kaviteter i olika typer av cellulosafibrer upptäcktes det att en svamp, *Humicola alopallonella* Meyers & Moore, hade en ovanlig förmåga att bilda kaviteter i de mest skilda typer av cellulosafibrer.

Svampens förmåga att bilda kaviteter i tjugo olika cellulosafibrer har undersökts med hjälp av ljusmikroskop. De cellulosafibrer som studerats är: normala vedfibrer, vedfibrer i olika delignifieringsstadier, fiberfragment från mikrokristallina cellulosapreparationer (Avicel och Sigmacell), lin, jute, ramie, sisal, bomull, kapok, fröhår från *Salix pentandra* och två olika viskosfibrer. Dessutom har nedbrytningen av cellofan studerats.

En metod för kontinuerliga mikroskopiska studier av kavitetsbildningen i tunna tvärsnitt av björkved (ca 10μ tjocka) har använts. Svampen växte över snitten och hyfer penetrerade de sekundära cellväggarna i vedfibrernas longitudinella riktning. Genom upplösning av cellväggssubstansen runt hyferna bildades typiska kavitetshål.

Typiska kaviteter bildades i samtliga naturliga cellulosafibrer, alltså även i ligninfria eller nästan ligninfria cellulosafibrer såsom bomull, granholocellulosa, Avicel och Sigmacell. Detta visar klart att kaviteter kan bildas i rena cellulosafibrer. Kavitetsbildningen är således ej beroende av närvaron av lignin.

Kaviteternas form i så olika material som bomull och lövvedsfibrer är tämligen likartad. Från- eller närvaron av lignin tycks således ej påverka kaviteternas utseende. Detta antyder att en förklaring till kaviteternas form måste sökas i cellulosans mikrostruktur.

Orienteringen hos de bildade kavitetskedjorna har jämförts med orienteringen av cellulosamikrofibrillerna i de fibrer där fibrillriktningen är känd. I samtliga av dessa fibrer tycks orienteringen av kavitetskedjorna mycket nära följa orienteringen av cellulosamikrofibrillerna.

Olika allmänna aspekter på kavitetsbildning i cellulosafibrer diskuteras i anslutning till en redogörelse för tidigare resultat och hypoteser.

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Figures

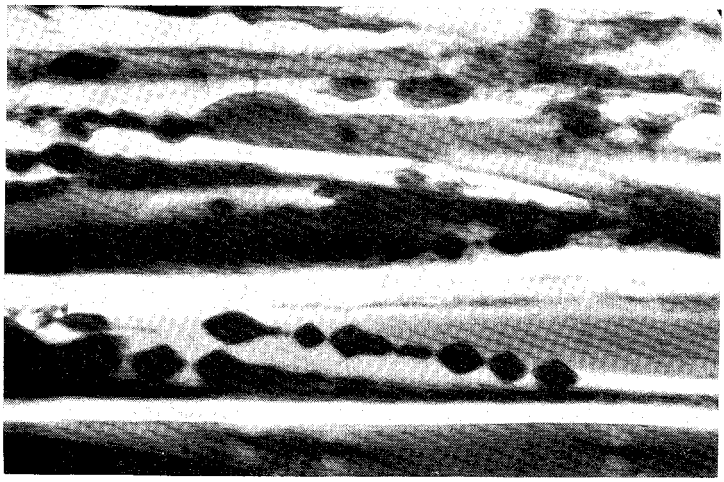

Fig. 1. Longitudinal section of birch wood showing cavity chains. Polarized light; Magn.: 810: 1.

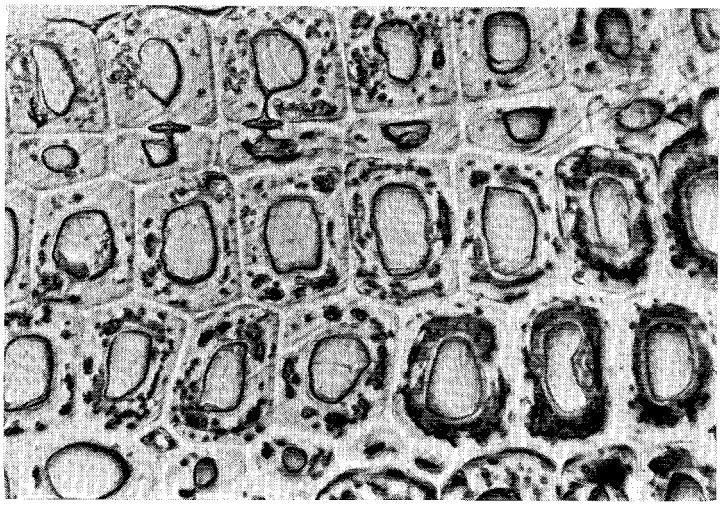

Fig. 2. Transverse section of pine latewood showing numerous cavity holes in the secondary cell walls. Magn.: 460: 1.

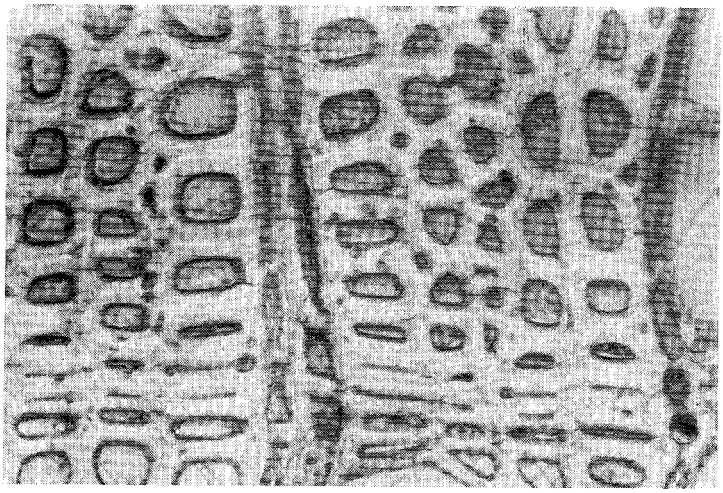

Fig. 3. Cavities formed in a thin transverse section of birch wood. Magn.: 460: 1.

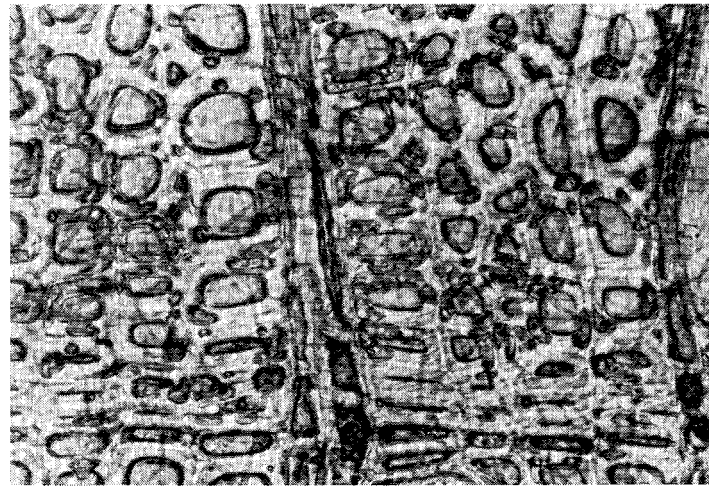

Fig. 4. The same section as in Fig. 3, but 12 days later. Note the new-formed cavities and the enlargement of the first-formed cavities. Magn.: 460: 1.

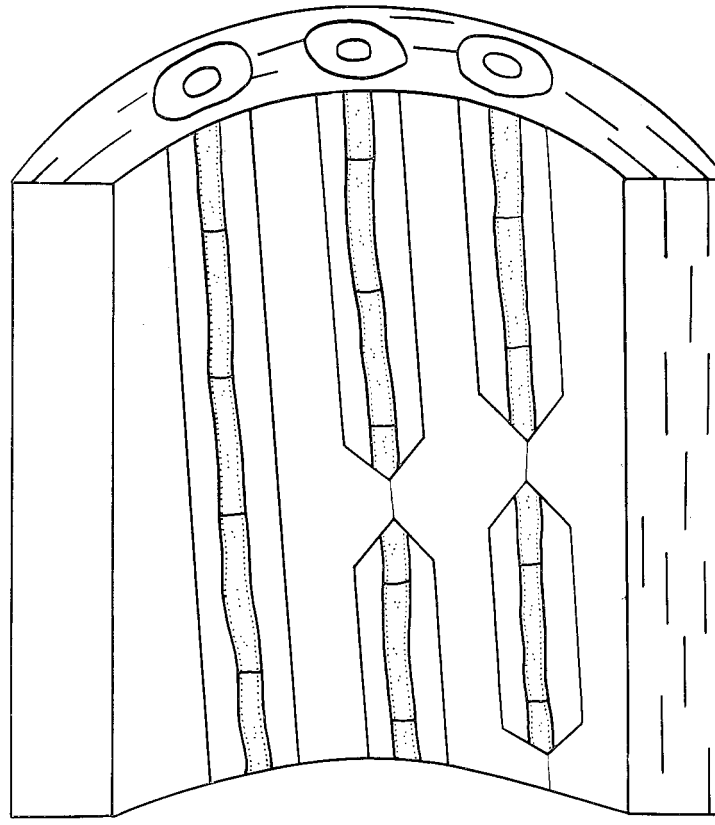

Fig. 5. Drawing illustrating different types of cavities formed in a thin transverse section of birch wood.

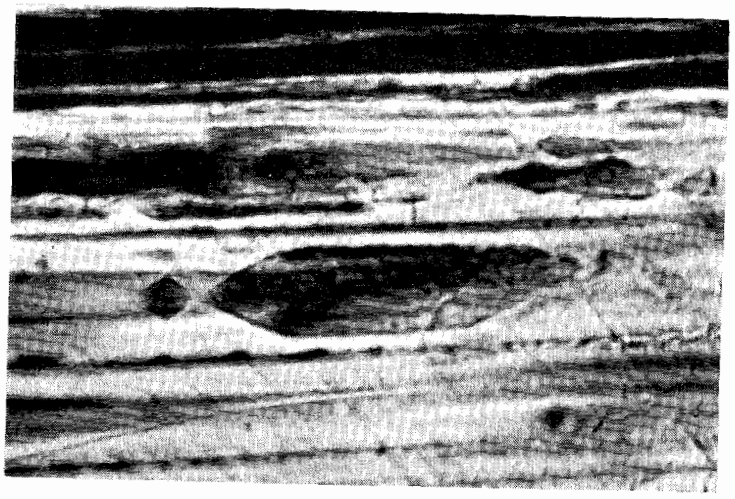

Fig. 6. Longitudinal section of spruce latewood showing cavities. Polarized light; Magn.: 850: 1.

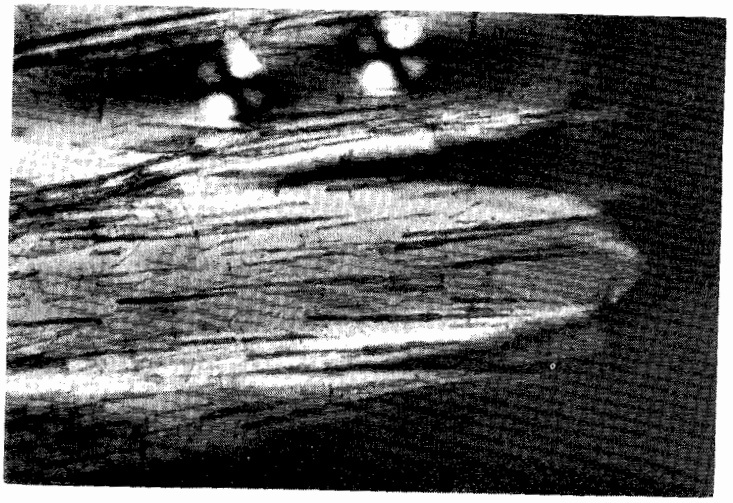

Fig. 7. Longitudinal section of spruce earlywood showing cavities. Polarized light; Magn.: 460: 1.

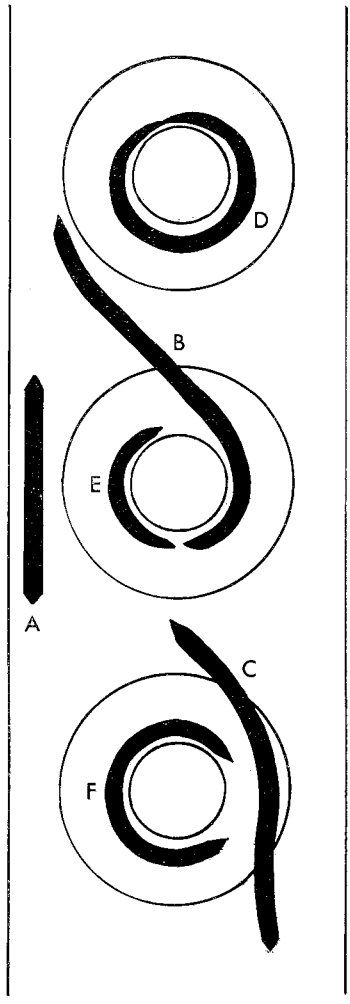

Fig. 8. Drawing illustrating the orientation of the cavities around the bordered pits in pine and spruce wood, when observed in radial sections.

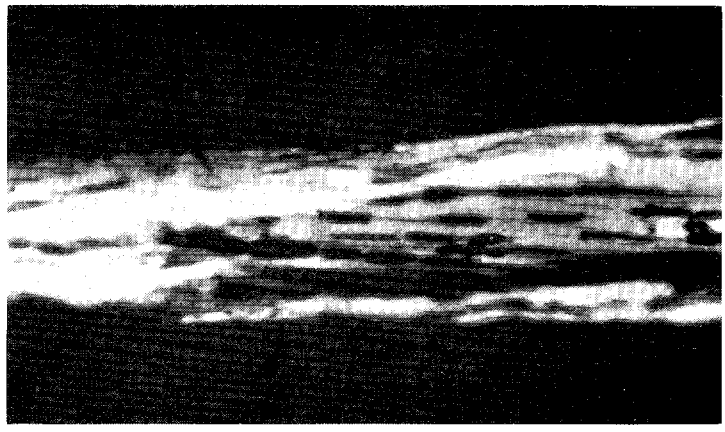

Fig. 9. Cavities formed in an earlywood tracheid from sulphate pulp B (from *Pinus silvestris*). Lignin content approx. 13 %. Polarized light; Magn.: 810: 1.

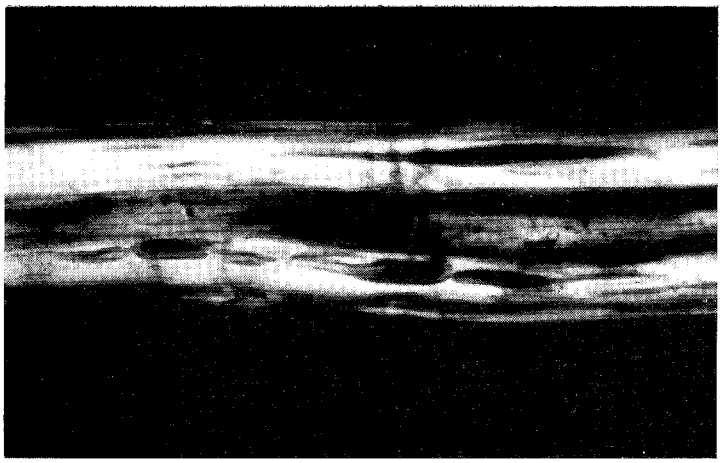

Fig. 10. Cavities formed in a latewood tracheid of spruce holocellulose (from *Picea abies*). Polarized light; Magn.: 850: 1.

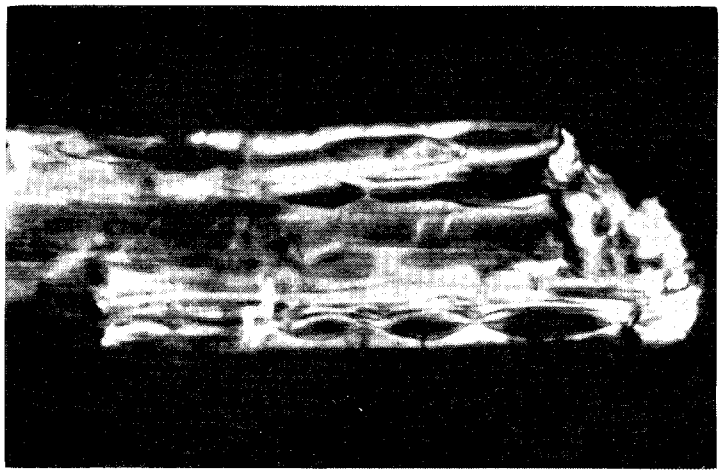

Fig. 11. Cavity chains formed in an Avicel particle. Polarized light; Magn.: 850: 1.

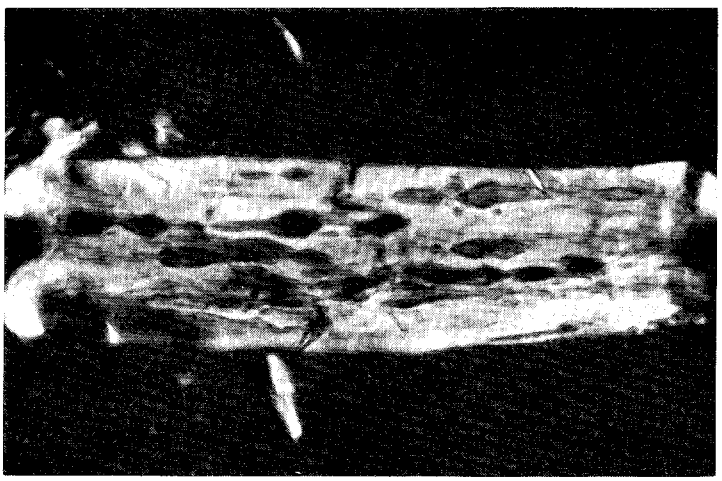

Fig. 12. Cavities formed in a Sigmacell T38 particle. Polarized light; Magn.: 690: 1.

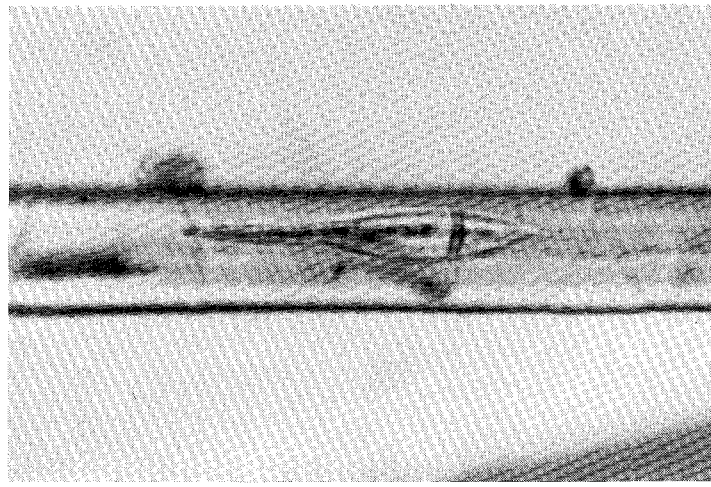

Fig. 13. Cavity formed in a flax fibre. Magn.: 940: 1.

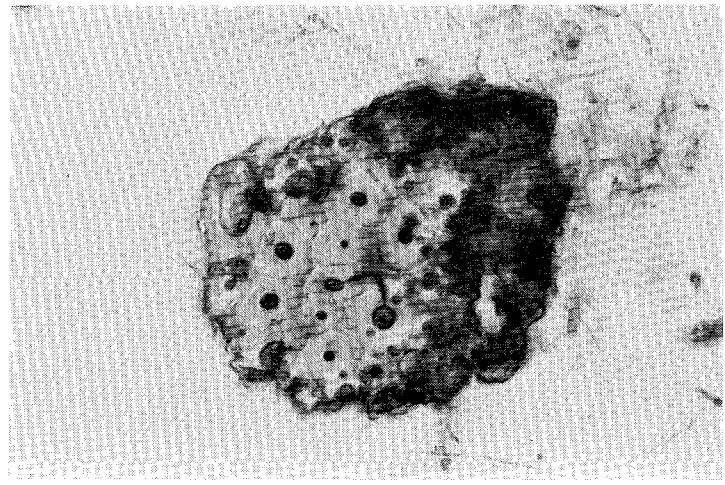

Fig. 14. Transverse section of jute fibres showing cavity holes in the secondary cell walls. Magn.: 460: 1.

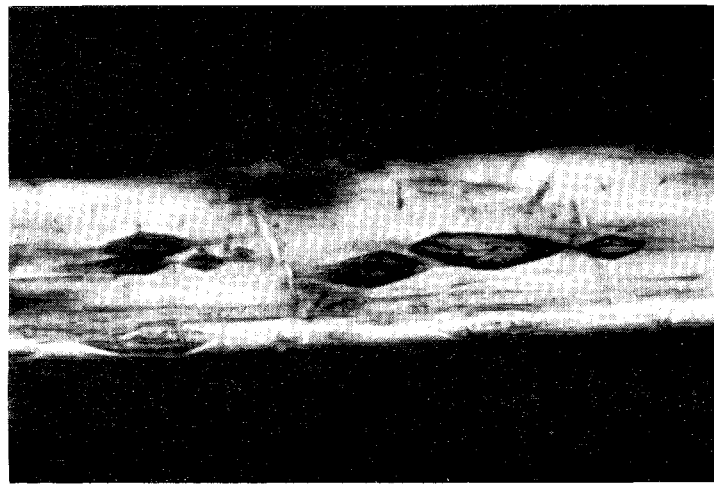

Fig. 15. Cavities formed in a ramie fibre. Polarized light; Magn.: 820: 1.

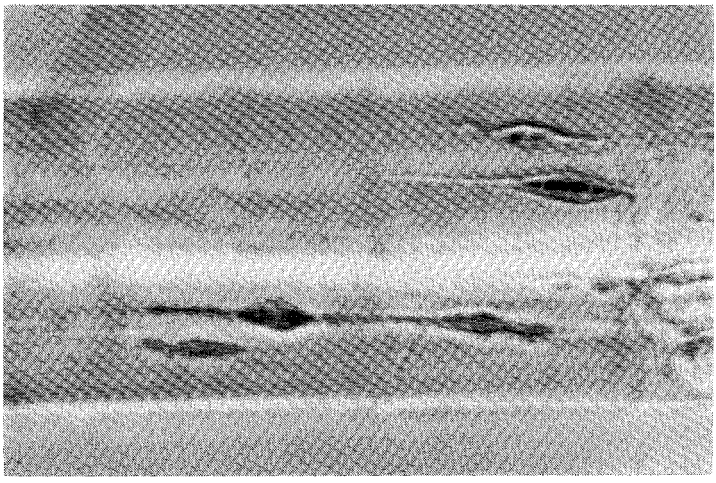

Fig. 16. Cavities formed in a ramie fibre. Magn.: 830: 1.

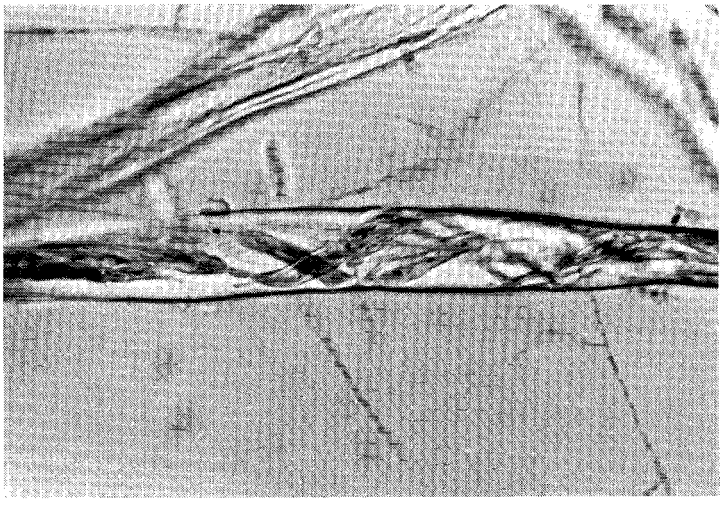

Fig. 17. Cavity chains formed in a cotton fibre. Magn.: 620: 1.

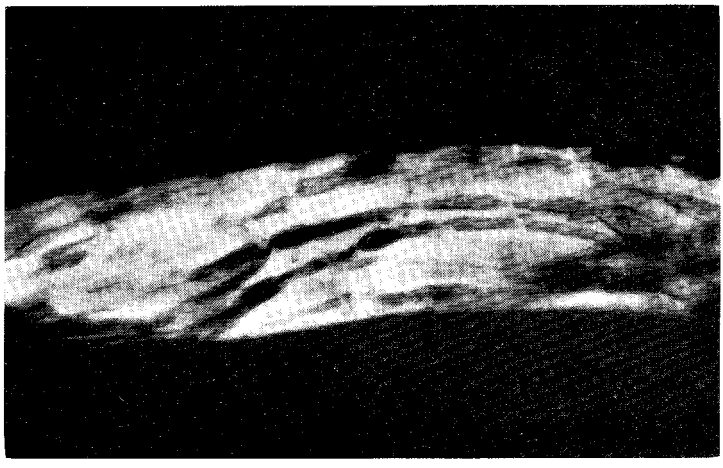

Fig. 18. Cavity chains formed in a cotton fibre. Note the reversal of direction of the cavity chains, evidently in parallel with reversal of direction of the cellulose microfibrils in the fibre. Polarized light; Magn.: 790: 1.

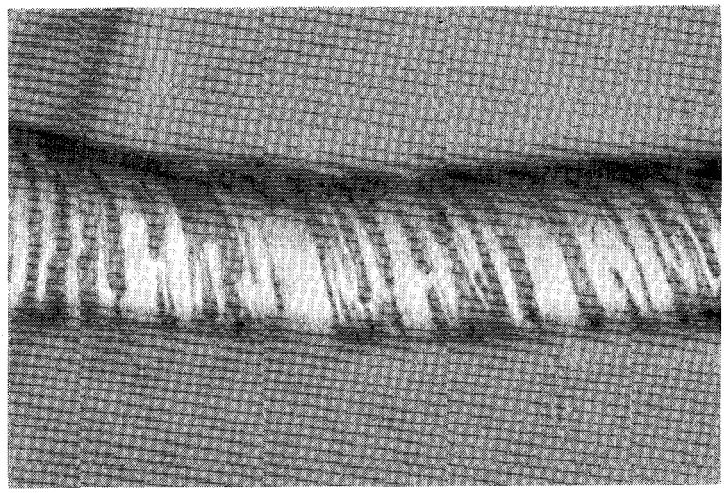

Fig. 19. Cavities formed in the outer wall layer of a kapok fibre. Polarized light; Magn.: 830: 1.

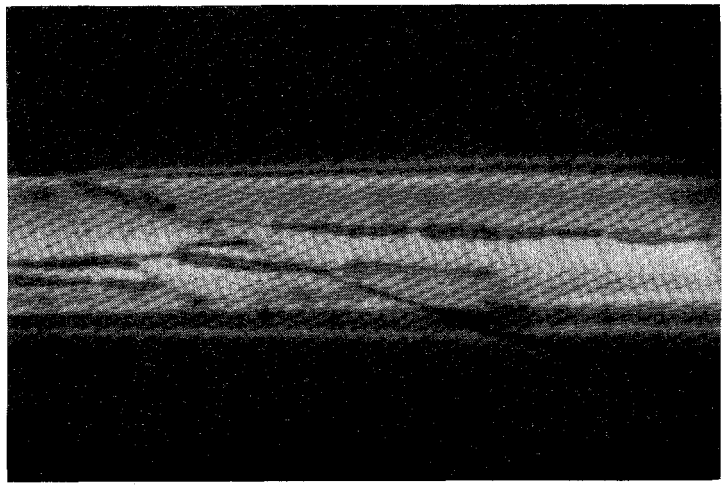

Fig. 20. Cavities formed in the inner wall layer of a kapok fibre. Polarized light; Magn.: 960: 1.

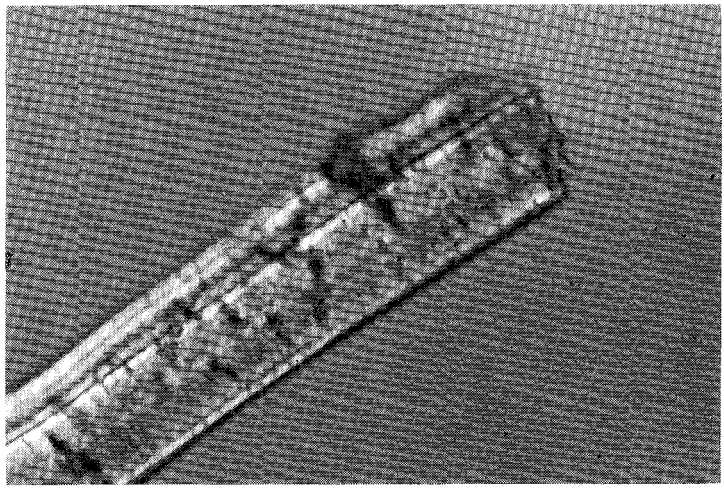

Fig. 21. Viscose fibre (10 % stretch) attacked by *Humicola alopallonella*. Note the numerous bore holes. Polarized light; Magn.: 460: 1.

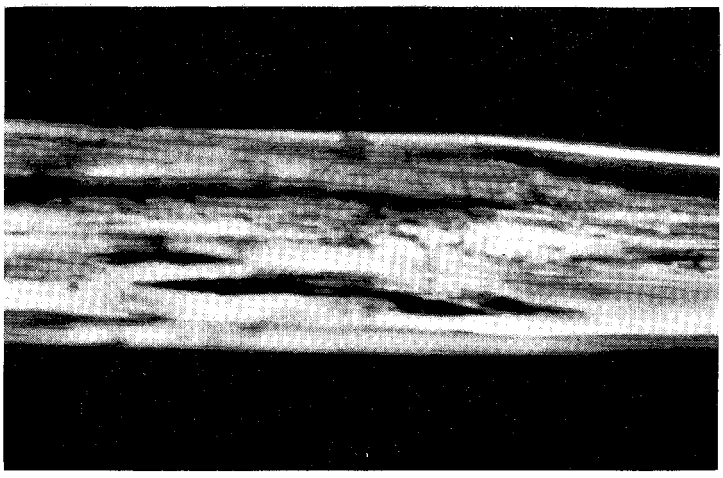

Fig. 22. Cavity-like degradation pattern in a viscose fibre (90 % stretch). Polarized light; Magn.: 830: 1.

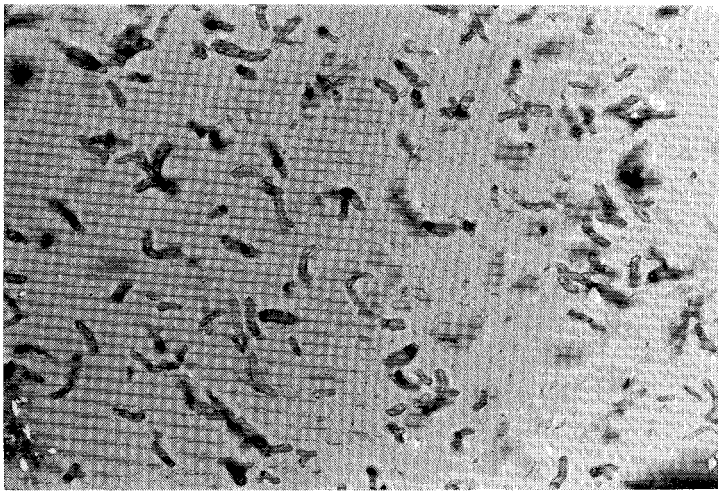

Fig. 23. Degradation pattern in cellophane. Polarized light; Magn.: 260: 1.

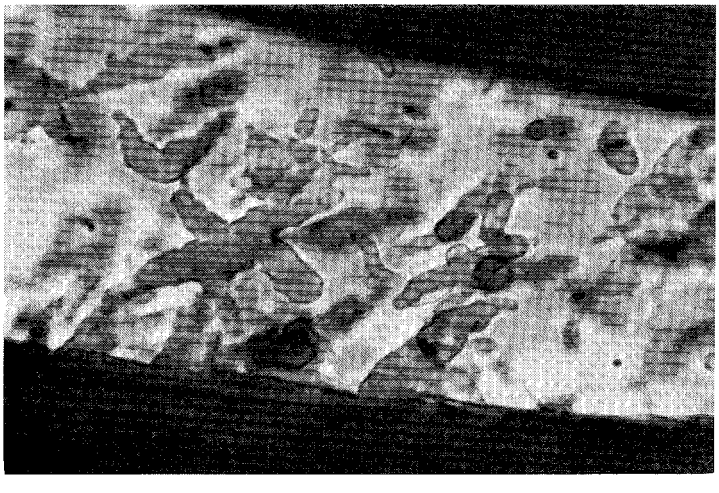

Fig. 24. Degradation pattern in cellophane. Note tapering of the ends of the lysis zones. Polarized light; Magn.: 460: 1.