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Microscopic studies on the degradation of cellophane and various cellulosic fibres by wood-attacking microfungi

Mikroskopiska undersökningar av vedangripande mikrosvampars nedbrytning av cellofan och olika cellulosahaltiga fibrer

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Abstract

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Microscopic studies have been carried out on the degradation of cellophane and various cellulosic fibres by a number of wood-attacking microfungi. The formation of cavities of soft rot type was especially studied. The following fibre materials were studied: birch wood (Betula verrucosa Ehrh.), a sulphate pulp (from Pinus silvestris L.) with a lignin content of 10.8 %, spruce holocellulose (from Picea abies (L.) H. Karst.), jute, sisal, kapok and cotton. All of the wood-degrading fungi were found to be able to degrade cellophane. Cavities of the soft rot type were formed in all the tested fibres. Most of the fungi failed, however, to form cavities when cultured in liquid media. Cavities were most easily formed in the intact lignified fibres. The number of cavities formed in the delignified wood fibres and in the cotton fibres was far less. The cavity-forming ability varied considerably among the different species. Only the species which were able to form cavities in birch wood were found to form cavities in the other fibres tested. Most of the species also produced an erosion-type attack in the delignified wood fibres and the cotton fibres. The degradation of cellophane and cotton by all the wood-degrading species demonstrated that they are able to degrade both cellulose in wood and pure cellulose.

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1. Introduction

This is the last of a series of papers concerning the degradation of woody and non-woody cellulosic fibres by microfungi. The cellulolytic activity and wood-degrading capability of 160 species of microfungi were studied and the results are reported in the first paper (Nilsson 1973). The ability of one species, *Humicola alopallonella* Meyers & Moore, to form soft rot cavities in various cellulosic fibres has also been studied (Nilsson 1974 a). One of the papers concerns the degradation of cellulose and the production of cell wall degrading enzymes by thirty-six species of microfungi (Nilsson 1974 b).

It was demonstrated in the first paper (Nilsson 1973) that the wood-degrading microfungi exhibited two different decay patterns. This had previously been shown by Corbett (1965). Her terminology was adopted for the two types of attack. Thus, Type 1 attack represented the formation of soft rot cavities in the secondary cell walls and Type 2 represented erosion of the wood cell walls.

In the same paper it was also observed that all species which caused attack of Type 2 in birch wood, with or without simultaneous attack of Type 1, exhibited cellulolytic activity when tested with the methods employed (Nilsson 1973 and 1974 b). Among the species which exclusively produced Type 1 attacks, some showed cellulolytic activity but several did not. The cavity-forming species which did not show cellulolytic activity were referred to as "non-cellulolytic" soft rot fungi (Nilsson 1974 b). The assays for cellulolytic activity employed the following procedures: 1) measurements of clearing zones in cellulose agar; 2) determination of weight losses of purified cellulose substrates in liquid media; and 3) assays of cellulase in culture filtrates from liquid cultures.

It was suggested that the inability of the "non-cellulolytic" soft rot fungi to demonstrate cellulolytic activity in the experiments performed (Nilsson 1974 b) was due to one or more of the following factors: 1) the culture conditions were unsuitable for cellulase production; 2) the induction of cellulase only occurs on cellulose associated with lignin and hemicellulose and not on pure cellulose; and 3) cellulase production is regulated by the physical structure of the wood fibres. The latter explanation was based on the observation that the cavity-forming hyphae (=the enzyme-producing hyphae) inside the secondary cell wall are always aligned parallel to the orientation of the cellulose microfibrils. It was then assumed that this relation between the hyphae and the microfibrils in some way induces the production of cellulase.

The fungus *Humicola alopallonella* was shown to be able to form cavities of soft rot type in a great number of cellulosic fibres, including a lignin-free fibre such as cotton. This indicated that soft rot cavities can be formed without the presence of lignin.

The purpose of the present study was to examine the influence of different culture conditions on the ability of a number of microfungi to degrade various cellulosic substrates and in particular to study the formation of soft rot cavities in various cellulosic fibres. The microfungi employed in the present study were selected to include representatives of different categories with respect to the degradation of birch wood and purified cellulose. The fibre materials studied were: wood fibres, de-

lignified wood fibres, non-wood lignified fibres and a pure cellulose fibre, viz. cotton. The degradation of cellophane was also studied.

Interest was primarily focussed on the "non-cellulolytic" soft rot fungi in order to obtain an explanation of their anomalous behaviour in the degradation of cellulose.

2. Material and methods

2.1 Organisms

Twenty-seven different species of microfungi were tested. All species are listed in Table 1. Eight species, including *Humicola alopallonella,* were only tested on cellophane. The results concerning the cavity formation by *H. alopallonella* shown in Table 4 are taken from a previous publication (Nilsson 1974 a).

Data on isolation, type of wood attack, weight losses of wood and cellulolytic activity can be obtained from two previous publications (Nilsson 1973 and 1974 b). The strain of *Chaetomium globosum* (F171—1) used in the present study had the same characteristics as the strain used previously (strain H56—A—2). Strain F171—1 was isolated from cotton fibres which had been placed in unsterile soil in a laboratory experiment. Typical soft rot cavities were observed in these cotton fibres.

The species used were selected so that different degradation patterns were represented. Previous data (from Nilsson 1973 and 1974 b) for the different species on the type of attack in birch wood and cellulolytic activity are given in Table 3. Ten species produced both erosion and cavities in birch wood and four species produced only erosion of the wood cell walls. All these species had shown cellulolytic activity in previous tests. Twelve species produced cavities only in birch wood. Only four of these species had shown cellulolytic activity while no activity had been found for the remaining eight species. One species was unable to degrade birch wood and had not shown cellulolytic activity.

2.2 Substrates

Cellophane Prepared in an experiment machine without the addition of plasticizers.

Lignin-cellophane 10—12 percent milled-wood lignin (from spruce *Picea abies*) was dispersed in this cellophane. The film was prepared by a glass plate method.

Both cellophane samples were obtained from Dr. E. Treiber at the Swedish Forest Products Research Laboratory.

Birch wood (*Betula verrucosa*) Wood blocks of the size 5×15×30 mm were used.

Sulphate pulp C (from *Pinus silvestris*). The lignin content was 10.8 %.

Spruce holocellulose (from *Picea abies*). Prepared by treating spruce wood with sodium chlorite at 70°C.

The sulphate pulp and the spruce holocellulose were prepared at the Swedish Forest Products Research Laboratory. These fibres were also used in a previous study on the formation of soft rot cavities in various cellulose fibres by *Humicola alopallonella* Meyers & Moore (Nilsson 1974 a).

Jute fibres (from *Corchorus olitorius*). These fibres were obtained from Dr. N. J. Poole at the School of Agriculture, Aberdeen.

Sisal (from *Agave sisalana*). Commercial sample.

Kapok (from *Ceiba pentandra*). Commercial sample.

Cotton. Chemically pure cotton (Kebo AB).

The chemical composition of the test fibres is given in Table 2.

2.3 Culture media

Malt extract agar: Malt extract 25 g, agar 15 g and deionized water 1000 ml.

F6A cellulose agar: Avicel 10 g, NH_4NO_3

Table 1. List of organisms

	Strains
Acremonium atro-griseum (Panasenko) W. Gams	SP35—7 (= CBS 981.70)
Bispora betulina (Corda) Hughes	P175—26
Ceratocystis albida (Mathiesen-Käärik) Hunt	B—23
Chaetomium globosum Kunze ex Fr.	F171—1
Chrysosporium pannorum (Link) Hughes	SP47—16
Cladorrhinum sp. A	600—2
Coniothyrium fuckelii Sacc. var sporulosum W. Gams & Domsch	CBS 218.68
Cordana pauciseptata Preuss	B63—A—25
Dictyosporium elegans Corda	SP5—16
Gonatobotrys sp. A	SP37—6
Graphium sp. A	B68—A—16
Humicola alopallonella Meyers & Moore	CBS 207.60
Humicola grisea Traaen	SP37—22
Mollisia sp. A	T694C
Petriellidium boydii (Shear) Malloch	SP31—4
Phialocephala sp. A	P152—55 (= CBS 390.71)
Phialophora fastigiata (Lagerb. & Melin)	731—1—3b
Phialophora hoffmannii (van Beyma) Schol-Schwarz	SP33—4
Phialophora verrucosa Medlar	P152—8 (= CBS 839.69)
Phialophora sp. A	SP35—1 (= CBS 882.73)
Pseudeurotium zonatum van Beyma	SP17—2
Rhinocladiella anceps (Sacc. & Ellis) Hughes	SP35—15
Rhinocladiella sp. A	P160—14
Scytalidium lignicola Pesante	H97—1
Wardomyces inflatus (March.) Hennebert	P180—62 (= CBS 412.68)
Xylogone sphaerospora v. Arx & Nilsson	E7—2 (= CBC 186.69)
Fungus A	A40—1

Strain numbers within parenthesis refer to species which have been included in the culture collection at "Centraalbureau voor Schimmelcultures" in Baarn.

Table 2. Chemical composition (percentage of dry weight) of the test fibres

Fibres	Cellulose	Hemicellulose	Lignin	References
Birch wood	44.9	32.7	19.3	Cowling & Brown (1969)
Sulphate pulp C	+ [a]	+ [a]	10.8	Boutelje & Hollmark (1972)
Spruce holocellulose	+ [b]	+ [b]	0.88	
Jute	65.2	22.2	10.8	Poole & Taylor (1973)
Sisal	71.5	18.1	5.9	Poole & Taylor (1973)
Cotton	96	1	0	Roelofsen (1959)
Kapok	37	40	12	Roelofsen (1959)

[a] Cellulose and hemicellulose were present, but the amounts were not known.
[b] The spruce holocellulose contained approx. 84 per cent glucose, 0.3 per cent arabinose, 5 per cent xylose, 10 per cent mannose and traces of galactose. The exact composition is given by Boutelje and Hollmark (1972).

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 deionized water.

B-VII cellulose agar (slightly modified after Bravery's (1968) medium VII): Avicel 10 g, $(NH_4)_2SO_4$ 0.543 g, KH_2PO_4 1.0 g, KCl 0.5 g, $MgSO_4 \cdot 7H_2O$ 0.2 g, $CaCl_2$ 0.1 g, thiamine hydrochloride 0.001 g, agar 15 g and 1000 ml deionized water.

Nutrient solution C: NH_4NO_3 1.5 g, K_2HPO_4 1.0 g, KH_2PO_4 1.2 g, $MgSO_4 \cdot 7H_2O$ 0.5 g and 1000 ml deionized water.

Nutrient solution EP and B-VII-L: The composition of these media has been given in a previous paper (Nilsson 1974 b).

2.4 **Test methods**

Degradation of cellophane

Agar plates were prepared in petri dishes with three different agar media. Malt extract agar was used only for those species which had failed to show cellulolytic activity in the previous tests (Nilsson 1973 and 1974 b).

The cellophane was cut into small pieces (approx. 10×10 mm). These pieces were placed in glass petri dishes and some drops of water were added. The petri dishes were then autoclaved. The sterilised cellophane pieces were placed on the surface of the agar plates in the centre of the dish. The agar plates were inoculated with small pieces (approx. 1×1 mm) of mycelium and agar taken from cultures growing on malt agar. The inocula were placed close to the cellophane pieces. The agar plates were then incubated at the ambient room temperature (23°—25°C).

After incubation for different periods of time, the cellophane membranes were removed from the plates and the mycelium was scraped off. The membranes were mounted in lactophenol containing Chlorazol Skye Blue (Imperial Chemical Industries Ltd) and viewed under a light microscope using polarised light. The incubation periods were not fixed but varied between the different species. The less active species were incubated for up to 84 days.

Degradation of cellulosic fibres

Three different test methods were used.

Method A. The tests were carried out on cultures growing on agar slopes in test tubes. The method was described earlier (Nilsson 1974 a).

Method B. The tests were carried out in 100 ml Erlenmeyer flasks with 20 ml of either medium EP or medium B-VII-L. The composition of these media are given by Nilsson (1974 b). A small amount (approx. 100 mg) of each fibre to be tested was added to each flask. The flasks were fitted with cotton plugs and autoclaved. The sterilised flasks were then inoculated and incubated for up to 64 days at the ambient room temperature. Only stationary cultures were employed.

Method C. The tests were carried out in wide-necked 100 ml Erlenmeyer flasks with a layer of vermiculite (10 g) in the bottom. The fibres to be tested were placed on the flat surface of the birch wood blocks. Approximately 25 mg of each fibre was used. Occasionally two or more different fibres were placed on the same wood block. The fibres were attached to the wood blocks by cotton yarn. The arrangement of the fibres on the wood blocks is shown in Fig. 1.

One wood block was placed in each flask. The wood blocks were arranged so that the upper flat surface on which the fibres were attached was at the same level as the vermiculite surface. 25 ml of the nutrient solution C was added to each flask. The flasks were fitted with cotton plugs and autoclaved. They were then inoculated with the test species and incubated at the ambient room temperature for different periods of time. The less active species were incubated for up to 56 days. The average moisture content of the wood blocks plus fibres was 50 percent after autoclaving. The variations were, however, considerable—from 42 to 57 percent. The moisture contents after incubation were not determined.

The fibres were examined by means of a light microscope for evidence of degradation as described previously (Nilsson 1974 a). Cavity formation was studied in all the fibres except for the earlywood tracheids of sulphate pulp C and spruce holocellulose. The occurence of the erosion type of degradation (Type 2 attack) was studied only in birch wood, sulphate pulp, spruce holocellulose and cotton fibres.

3. Results

3.1 **Degradation of cellophane**

Table 3 shows the results of the experiments with cellophane on three different types of agar media. Previous results (Nilsson 1973 and 1974 b) concerning the cellulolytic activity and degradation patterns in birch wood are also given in the table. Of all species listed in the table, only one, *Phialophora verrucosa,* was unable to degrade birch wood. This species had also failed to show cellulolytic activity in all the previous tests. Eighteen of the species had shown cellulolytic activity in previous tests. Of these, Fungus A had failed to produce clearing zones in cellulose agar. The fungus had, however, been able to cause weight losses of pure cellulose substrate in liquid media. The remaining eight species belonged to the category: "non-cellulolytic" soft rot fungi. These fungi were inactive in previous assays of cellulolytic activity.

All species were able to penetrate the cellophane and form small bore holes not exceeding 1—2 μ diam (see Fig. 2). It was assumed that this penetration did not represent an active degradation of the cellulose since it could be purely mechanical. The types of degradation illustrated in Figures 3—9, viz. large bore holes, broad lysis zones along hyphae and general erosion of the cellophane surface, were all considered to be active degradation of cellulose.

The amount of degradation varied considerably between the different species but little interest has been paid to these variations. Instead, interest was focussed on whether degradation had occurred or not. Thus Table 3 shows if any active degradation had occurred during the incubation period which was extended to 84 days for the less active species.

The fungi were first tested on cellophane placed on F6A cellulose agar. As can be seen in the table, all species which had shown cellulolytic activity in the previous tests, except for Fungus A, were able to actively degrade cellophane. Most of these species degraded the cellophane fairly rapidly. Active species such as *Cladorrhinum* sp. A, *Coniothyrium fuckelii* var. *sporulosum, Dictyosporium elegans, Humicola grisea, Phialophora hoffmannii* and *Xylogone sphaerospora* had degraded most of the cellophane after about 30 days. *Rhinocladiella anceps* was the least active species. No degradation was visible after 55 days and very little degradation had occurred even after 84 days.

All of the species penetrated the cellophane film and formed bore holes when cultured on F6A cellulose agar. Some hyphae penetrated through the cellulose film while others grew parallel to the plane of the film. The bore holes of the "non-cellulolytic" soft rot fungi remained small. Lysis zones were formed around the hyphae of the cellulolytic species, leading to enlargement of the bore holes (Fig. 4). Lysis zones also developed along hyphae growing on the surface of the cellophane. "Rooting branches" of the type described by Tribe (1960) also occurred but were less common. Most of the species also produced a general erosion of the surface of the cellophane (see Figure 4) which was not restricted to the vicinity of the hyphae. This erosion was probably caused by cellulases which had been secreted by the hyphae and diffused out over the whole cellophane surface. Sometimes, but not always, the erosion could be seen to be influenced by the molecular orientation of the cellulose film.

The growth of *Phialophora verrucosa,*

Table 3. The degradation of cellophane

Species	Type of attack in birch wood[a]		Cellulolytic activity[a]	Cellophane degradation		
	Cavities	Erosion		Malt extract agar	F6A cellulose agar	B-VII cellulose agar
Acremonium atro-griseum	+	—	+		+	+
Bispora betulina	+	—	—	—	—	+
Ceratocystis albida	+	—	—	NT	NT	NT
Chaetomium globosum	+	+	+		+	+
Chrysosporium pannorum	+	+	+		+	+
Cladorrhinum sp. A	+	+	+		+	+
Coniothyrium fuckelii var. sporulosum	+	+	+		+	+
Cordana pauciseptata	+	+	+		+	+
Dictyosporium elegans	+	+	+		+	+
Gonatobotrys sp. A	+	—	—	—	—	+
Graphium sp. A	+	—	—	—	—	+
Humicola alopallonella	+	—	+		+	+
Humicola grisea	+	+	+		+	+
Mollisia sp. A	+	—	—	—	—	+
Petriellidium boydii	+	—	+		+	+
Phialocephala sp. A	+	—	—	—	—	+
Phialophora fastigiata	+	+	+		+	+
Phialophora hoffmannii	+	+	+		+	+
Phialophora verrucosa	—	—	—	—	—	—
Phialophora sp. A	+	—	—	—	—	+
Pseudeurotium zonatum	—	+	+		+	+
Rhinocladiella anceps	—	+	+		+	+
Rhinocladiella sp. A	+	—	—	—	—	+
Scytalidium lignicola	—	+	+		+	+
Wardomyces inflatus	+	+	+		+	+
Xylogone sphaerospora	—	+	+		+	+
Fungus A	+	—	+	—	—	+

[a] Data from Nilsson (1973 and 1974 b). The cavity-forming species which lacked cellulolytic activity are referred to as "non-cellulolytic" soft rot fungi.
+ activity present
— activity absent
NT not tested

Fungus A and all the "non-cellulolytic" soft rot fungi was rather sparse on F6A cellulose agar. This was also reported earlier (Nilsson 1974 b). None of the species were able to degrade the cellophane on F6A cellulose agar. All species formed, however, small bore holes (see Fig. 2), but these were never seen to enlarge, not even after extended periods of incubation.

All of these species were then tested on pieces of cellophane which had been placed on malt agar and B-VII cellulose agar. The species which had actively degraded the cellophane on the F6A cellulose agar were also tested on the B-VII medium for comparison. The B-VII cellulose agar is a rather poor medium which, in contrast to F6A, contains no other carbon source than cellulose.

The species which were tested on malt agar showed good growth and numerous small bore holes were formed but active degradation could not be observed in any case. When the fungi were cultured on B-VII cellulose agar they produced much less growth than on malt agar and F6A cellulose agar. The poor growth on B-VII medium as compared with the other two media has also been reported earlier (Nilsson 1974 b). As can be seen in Table 3,

all species except *Phialophora verrucosa* were able to actively degrade the cellophane when growing on B-VII cellulose agar. However, several species produced a very small amount of degradation. Most of the species which were active on the F6A medium showed considerably less activity on the B-VII medium. Only *Cordana pauciseptata* showed slightly higher activity on the B-VII medium than on the F6A medium. *Rhinocladiella anceps* which had shown a positive response to the medium B-VII when cultured on cellulose in liquid media (Nilsson 1974 b) produced approximately the same weak attack on both types of cellulose agar.

Fungus A and the "non-cellulolytic" soft rot fungi produced similar degradation patterns in the cellophane, as did the species which had been active on the F6A medium. Some species such as *Bispora betulina, Mollisia* sp. A, *Phialocephala* sp. A, *Phialophora* sp. A and Fungus A formed a great number of large bore holes (Fig. 3). Lysis along hyphae or strands of hyphae growing on the cellophane surface (Figures 5 and 6) and general erosion of the cellophane surface (Fig. 7) was also observed.

A feature which was more common for Fungus A and the "non-cellulolytic" soft rot fungi was the formation of "rooting branches" (Fig. 8) of the type described by Tribe (1960) around which lysis of the cellophane occurred. Several species formed a more compact aggregate of hyphae like a ball within the cellophane. Lysis also occurred around these aggregates (Fig. 9). These complex aggregates of hyphae were especially common for the following species: *Graphium* sp. A, *Gonatobotrys* sp. A and *Rhinocladiella* sp. A. The two latter species formed this type of degradation almost exclusively.

Some of the species penetrated the cellophane with a single hyphae which grew in the plane of the film. Small lysis zones developed along these hyphae and the degradation pattern was sometimes reminescent of soft rot cavities in wood fibres (Fig. 10).

The conclusion which can be drawn from the experiments described is that all species which were able to degrade wood could also degrade cellophane. The degradation of cellophane by Fungus A and the "non-cellulolytic" soft rot fungi was, however, profoundly influenced by the type of medium on which the fungi were cultured.

The cellophane which contained 10 to 12 percent milled-wood lignin was tested as described above in order to study the influence of lignin on the cellulose degradation. Microscopic examinations did not, however, reveal any differences in the way in which the two types of cellophane were degraded.

3.2 **Degradation of cellulosic fibres**

The degradation of the cellulosic fibres was followed by microscopical observations. No attempts were made to determine the amount of degradation by measuring the losses.

All the degradation patterns observed were classified either as Type 1 attack (formation of soft rot cavities) or as Type 2 attack (erosion). The formation of small bore holes was not regarded as an active form of degradation since it could not be determined whether the penetration was mechanical or enzymatic.

The term "cavity" requires a definition since the form of the cavities varied considerably between different fibres and different species of fungi. A "cavity" is defined here as a zone of lysis of the cell wall substance produced by hyphae which are growing within the fibre cell walls parallel to the presumed direction of the cellulose microfibrils. The most common forms of cavities observed are shown in Figure 11. Cavities of type A are referred to as "normal cavities" since this form is that most commonly observed in wood fibres. Cavities of type B and C have also been observed in wood, whereas cavities of type D have never been observed in the fibres of intact wood, jute, sisal or kapok. The cavities of type D are characterised by their irregular ends with several pointed tips. The cavities of the other types have

smooth ends and a single tip at each end. Except for the patterns described above as cavity formation, all other types of active degradation were regarded as Type 2 attack. Thus Type 2 attack, in the sense used here, includes thinning of cell walls, transverse and helical cracks in the fibre walls and irregular erosion both from the outside of the fibres and from the lumen side. The different forms of Type 2 attack were not studied in any detail. Figure 12 illustrates Type 2 attack in a tracheid from spruce holocellulose.

All species which have been previously reported to show cellulolytic activity on pure cellulose (see Table 3) will in the remainder of this paper be referred to as cellulolytic species. The cavity-forming species which failed to exhibit cellulolytic activity in previous tests (see Table 3) will be referred to as "non-cellulolytic" soft rot fungi.

The experiments described here were preceded by a large number of tests using different methods. Only the results from the most successful method with respect to cavity formation will be reported in any detail in this paper. However, the numerous pre-tests clearly demonstrated that the culture conditions exercise a profound influence on the ability of the microfungi to degrade cellulosic fibres.

The first experiments were carried out according to method B where the fungi are cultured in liquid media together with the fibres to be tested. The media EP and B-VII-L were primarily employed but media of other compositions were also tried. The results showed that all the cellulolytic species were able to produce an erosion-type of attack on the fibres, especially on the lignin-free cotton fibres and on the delignified wood fibres. The lignified jute, sisal and kapok fibres were degraded to a much lesser degree. Kapok fibres appeared to be especially resistant.

It might already be mentioned here that *Scytalidium lignicola* and *Xylogone sphaerospora,* which have proved incapable of forming soft rot cavities in birch wood, were only able to degrade the other fibres by Type 2 attack. These species were unable to form cavities of the soft rot type in any of the fibres tested and under all test conditions. Not even "T-branches" or growth of the hyphae within the cell walls in the longitudinal direction of the fibres was observed.

Most of the species formed no cavities when cultured in liquid media. However, *Ceratocystis albida, Dictyosporium elegans, Petriellidium boydii* and *Wardomyces inflatus* formed cavities in jute and sisal fibres. *Ceratocystis albida* and *Dictyosporium elegans* also formed cavities in the kapok fibres. No, or very few, cavities were formed in the two delignified wood fibres and in the cotton fibres. Some tests were also carried out on the test fibres in liquid media with *Humicola alopallonella.* This fungus formed, in contrast to the other species, numerous cavities in all of the different fibres.

Test method A was then tried. This method was previously used successfully for studies of the cavity formation by *Humicola alopallonella* (Nilsson 1974 a). The tests were carried out in test tubes on agar slopes with two agar media of different composition. One was F6A cellulose agar and the other was malt extract agar. It was found that all the cellulolytic species, with the exception of Fungus A, formed cavities in jute and sisal fibres regardless of the medium used. The species listed in Table 4 as being able to form cavities in kapok fibres also formed cavities in this fibre when method B was employed. Most of the cellulolytic species formed, however, very few cavities in the other fibres. These fibres were instead degraded by Type 2 attack when the fungi were grown on F6A cellulose agar. When cultured on malt agar most of the species produced very little Type 2 attack.

Fungus A and the "non-cellulolytic" soft rot fungi formed cavities in the jute and sisal fibres, some species also in the kapok fibres, when grown on malt agar. No, or very few, cavities were formed in the other fibres. In some cases hyphae of *Bispora betulina, Phialophora* sp. A and Fungus A

could be observed to penetrate into the cell walls of the fibres from sulphate pulp C and spruce holocellulose and to change direction to grow in the longitudinal direction of the fibres. "T-branches" of the type described by Corbett and Levy (1963) were often seen, but the hyphae could also make a direct change in the direction of growth. Some of the hyphae continued to grow in the longitudinal direction of the fibres but no lysis zones could be seen around these hyphae. Fig. 13 shows a T-branch formed by *Bispora betulina* in a fibre from sulphate pulp C. No lysis is apparent around the hyphae within the cell wall.

When Fungus A and the "non-cellulolytic" soft rot fungi were cultured on F6A cellulose agar no, or very little, attack in the form of cavities was observed. Very few cavities were formed even in the jute and sisal fibres. Attack of Type 2 was not observed at all. The weak attack was probably due to the sparse growth produced by the aforementioned fungi on F6A cellulose agar.

Since the methods described above were relatively unsuccessful because only a very limited degradation was obtained, other methods were tried. An attempt was first made to place the fibres in or on a vermiculite layer in Erlenmeyer flasks and add a nutrient solution. Although this method met with some success with the cellulolytic species, it proved to be an unsuitable method for obtaining cavity formation in the delignified wood fibres and in the cotton fibres by Fungus A and the "non-cellulolytic" soft rot fungi. Some of the latter species, viz. *Bispora betulina, Phialocephala* sp. A, *Phialophora* sp. A and Fungus A, produced a weak attack of Type 2 in the latter fibre.

The next method to be tried was method C where the fibres were attached to birch wood blocks placed in a vermiculite layer in Erlenmeyer flasks. This method was the most successful with respect to both Type 1 and Type 2 attack by the "non-cellulolytic" soft rot fungi. The results of the experiments carried out according to method C are presented in Table 4. The birch wood blocks to which the test fibres were attached were also examined microscopically for evidence of Type 1 and Type 2 attack. Some comparisons were made between the amount of attack on the birch wood fibres and the fibres attached to the surface of the wood blocks.

Table 4 shows the occurrence of the different types of attack in the fibres tested. The occurrence of Type 1 attack is given for all fibres except for earlywood tracheids of sulphate pulp C and spruce holocellulose. The occurrence of Type 2 attack is given only for fibres of birch wood, sulphate pulp C, spruce holocellulose and cotton. Type 2 attack was also observed in jute, sisal and kapok fibres, but since no detailed studies were carried out no results are presented in the table for these fibres, except for the fungi which caused Type 2 attack exclusively.

The results of earlier experiments with birch wood which were carried out according to method B (Nilsson 1973 and 1974 b) are shown in Table 3. When these results are compared with the results shown in Table 4 it is evident that some species exhibited a different degradation pattern when tested according to method C. *Bispora betulina, Ceratocystis albida, Phialocephala* sp. A, *Phialophora* sp. A and Fungus A caused, when tested according to method C, a weak erosion of the wood cell walls which had not been previously observed. Earlier, these species had been found to produce exclusively Type 1 attack in birch wood. *Acremonium atro-griseum, Gonatobotrys* sp. A, *Graphium* sp. A, *Petrielli-dium boydii* and *Rhinocladiella* sp. A, which had also been found to produce exclusively Type 1 attack in birch wood, were not able to produce any erosion of the birch wood cell walls when tested according to method C.

Degradation of jute, sisal and kapok

All the tested species, except *Scytalidium lignicola* and *Xylogone sphaerospora,* were able to form cavities in jute and sisal fibres (Table 4). Most cavities were very

Table 4. Types of degradation observed in the various cellulose fibres

Species	Birch wood		Sulphate pulp C[a]		Spruce holocellulose[a]		Jute		Sisal		Kapok		Cotton	
	Cavities	Erosion	Cavities	Erosion	Cavities	Erosion	Cavities	Erosion	Cavities	Erosion	Cavities	Erosion	Cavities	Erosion
Acremonium atro-griseum	+	—	+	+	+	+	+	0	+	0	+	0	+	+
Bispora betulina	+	+	+	+	+	+	+	0	+	0	+	0	+	+
Ceratocystis albida	+	+	+	—	+	—	+	0	+	0	+	0	+	+
Chaetomium globosum	+	+	+	+	—	+	+	0	+	0	—	+	+	+
Cordana pauciseptata	+	+	—	+	—	+	+	0	+	0	—	+	—	+
Dictyosporium elegans	+	+	+	+	+	+	+	0	+	0	—	+	+	+
Gonatobotrys sp. A	+	—	+	—	+	—	+	0	+	0	+	0	+	—
Graphium sp. A	+	—	+	+	+	+	+	0	+	0	- -	—	—	+
Humicola alopallonella[b]			+	+	+	+	+	0	+	0	+	0	+	+
Humicola grisea	+	+	+	+	+	+	+	0	+	0	+	0	+	+
Petriellidium boydii	+	—	+	+	+	+	+	0	+	0	+	0	+	+
Phialocephala sp. A	+	+	+	+	+	+	+	0	+	0	+	0	+	+
Phialophora fastigiata	+	+	+	+	+	+	+	0	+	0	—	+	+	+
Phialophora hoffmannii	+	+	—	+	—	+	+	0	+	0	—	+	—	+
Phialophora sp. A	+	+	+	+	+	+	+	0	+	0	+	0	+	+
Rhinocladiella sp. A	+	—	+	—	+	—	+	0	+	0	+	0	+	—
Scytalidium lignicola	—	+	—	+	—	+	—	+	—	+	—	+	—	+
Wardomyces inflatus	+	+	—	+	—	+	+	0	+	0	+	0	+	+
Xylogone sphaerospora	—	+	—	+	—	+	—	+	—	+	—	+	—	+
Fungus A	+	+	+	+	+	+	+	0	+	0	+	0	+	+

[a] Cavity formation was studied only in the latewood tracheids
[b] Data from Nilsson (1974 a)
\+ activity present
— activity absent
0 not studied

similar to those formed by the fungi in birch wood, although some of the cavities in the sisal fibres had a more irregular form. Ten species formed cavities in the kapok fibres. These cavities tended to be more narrow than those formed in birch wood. Eight species, viz. *Chaetomium globosum, Cordana pauciseptata, Dictyosporium elegans, Graphium* sp. A, *Phialophora fastigiata, Phialophora hoffmannii, Scytalidium lignicola* and *Xylogone sphaerospora* failed to produce cavities in the kapok fibres even if the incubation was extended to 56 days. With the exception of *Graphium* sp. A, all these species eventually caused a weak erosion of the cell walls. Bore holes were also formed in the kapok fibres but no T-branching was observed. When studying cross-sections of the kapok fibres, it was observed that the cell walls were very thin in comparison with the cell walls of the other fibres. Cross sections of the kapok fibres were therefore prepared and the thickness of the cell walls was measured. The cell walls were found to have an average thickness of 1 μ. The thickness varied from 0.6 to 1.7 μ. The variation in thickness is due to the variation between the basal and the upper sections of the kapok fibre. Thus, the cell wall is very thin and for comparison it might be mentioned that the thickness of cell walls of earlywood tracheids of pine wood (*Pinus silvestris*) have been reported to be 3.0 μ (Courtois 1963 a).

The degradation patterns observed in the other fibres when using method C are reported in more detail below.

Fibres from sulphate pulp C. Strong erosion type of attack was observed with the following species: *Acremonium atro-griseum, Bispora betulina, Chaetomium globosum, Cordana pauciseptata, Dictyosporium elegans, Humicola grisea, Petriellidium boydii, Phialocephala* sp. A, *Phialophora fastigiata, Phialophora hoffmannii, Phialophora* sp. A, *Scytalidium lignicola, Wardomyces inflatus, Xylogone sphaerospora,* and Fungus A (Table 4). *Graphium* sp. A caused a rather weak erosion-type attack. *Ceratocystis albida, Gonatobotrys* sp. A and *Rhinocladiella* sp. A caused no erosion at all.

Cordana pauciseptata, Phialophora hoffmannii, Scytalidium lignicola, Wardomyces inflatus and *Xylogone sphaerospora* failed to produce cavities in the fibres of sulphate pulp C. Only one species, viz. *Acremonium atro-griseum,* was able to form numerous cavities in these fibres. Most of the cavities assumed the same shape as cavities formed by the same species in pine wood (Fig. 14). In most cases they were, however, formed singly. Only occasionally were cavity chains seen. The remaining species were all able, as can be seen in Table 4, to form cavities in the fibres of sulphate pulp C. But usually no species formed more than a few cavities. Cavity chains occurred as shown in Fig. 15 but they were very rare. Some cavities of type A were observed but most of the cavities were of type C or D. Cavities of type B were also occasionally observed.

Some hyphae of *Chaetomium globosum, Dictyosporium elegans, Humicola grisea* and Fungus A penetrated the cell walls of the fibres and changed direction to grow longitudinally in the fibre cell walls. These hyphae could continue to grow within the fibre walls without the formation of lysis zones. A similar phenomenon was described earlier. Later, lysis zones developed around some of these hyphae, giving rise to cavities, usually of type C while other hyphae appeared unable to produce lysis zones.

Fibres from spruce holocellulose

Essentially, the same results were obtained with these fibres as with the fibres of sulphate pulp C. The species which had caused an erosion type of attack in the former fibres also did so in the fibres from spruce holocellulose (Table 4). The erosion type of attack tended to be more extensive in the latter fibres but *Graphium* sp. A also produced a rather weak attack in these fibres. The species which failed to produce an erosion-type attack on the fibres of sulphate pulp C also failed on fibres from spruce holocellulose.

The same species which had failed to

form cavities in the fibres of sulphate pulp C also failed to form cavities in fibres of spruce holocellulose. *Chaetomium globosum* also failed to produce cavities in these fibres. The remaining species formed far fewer cavities in fibres of the latter type. Most cavities were of type C and cavity chains were very rare. Only a very few cavities of type B and D were found and none of type A. Hyphae growing in the longitudinal direction of the fibres without the formation of lysis zones were more common in the fibres of spruce holocellulose than in the fibres of sulphate pulp C.

Cotton

With respect to the erosion-type attack, similar results were obtained as in the fibres treated above but the degradation of the cotton fibres appeared to be less extensive.

Most of the species were able to produce cavities in cotton (Table 4). Only five species failed, viz. *Cordana pauciseptata, Graphium* sp. A, *Phialophora hoffmannii, Scytalidium lignicola* and *Xylogone sphaerospora.* The first cavities to be formed were almost exclusively of type B and they were often rather small (Fig. 16). Single cavities were observed in the early stages, later chains of type B cavities were formed. *Bispora betulina, Phialocephala* sp. A and *Phialophora* sp. A were found to produce only the small cavities of type B, whereas the other species formed larger cavities in the later stages. *Acremonium atro-griseum, Ceratocystis albida, Chaetomium globosum, Dictyosporium elegans, Humicola grisea, Petriellidium boydii, Phialophora fastigiata* and *Wardomyces inflatus* also formed cavities of type A but they were extremely rare. Still rarer was the formation of chains of type A cavities, although the species *Ceratocystis albida, Chaetomium globosum* and *Phialophora fastigiata* tended to form more cavity chains than the other species. Fig. 17 shows large cavities formed by *Ceratocystis albida* in a cotton fibre.

Even if all the tested species were able to degrade the cotton fibres when method C was employed, the attack was extremely weak for some of the species. Especially *Gonatobotrys* sp. A, *Graphium* sp. A and *Rhinocladiella* sp. A caused very little degradation of the cotton fibres. When the underlaying surface fibres of the birch wood blocks were compared with the cotton fibres, it was found that the attack on the wood fibres was much more intensive than the attack on the cotton fibres. Other "non-cellulolytic" soft rot species such as *Bispora betulina, Phialocephala* sp. A, *Phialophora* sp. A and Fungus A caused a rather strong erosion of the cotton fibres. Judging from the microscopic examinations, the amount of attack on the wood fibres and the cotton fibres was about the same, although the former fibres were degraded mainly by cavity formation and the latter mainly by an erosion-type attack.

It is difficult to quantify the observations carried out with a microscope. But if the cavity formation in the different fibres is compared, it is obvious that cavities formed more readily in some of the fibres. Cavities appeared to form with about the same ease in fibres from birch wood, jute and sisal. The species which were able to produce cavities in the kapok fibres appeared to form them almost as easily as in the aforementioned fibres.

If the fibres of the sulphate pulp C, spruce holocellulose and cotton are compared, the following order could be established with respect to decreasing cavity formation: sulphate pulp C→cotton→spruce holocellulose. The difference between the first two fibres was minimal. The difference between these fibres and spruce holocellulose was definite.

There was also a clear difference between the tested species with respect to cavity-forming ability. None of the species tested here had the same extraordinary ability to form cavities as *Humicola alopallonella* (cf. Nilsson 1974 a). Some species like *Ceratocystis albida, Dictyosporium elegans* and *Phialophora fastigiata* were judged to have a slightly better cavity-forming ability than the other species.

4. Discussion

The results of the present study confirm the results of previous experiments (Nilsson 1974 a), i.e. that cavities of the soft rot type can be formed not only in wood fibres but also in non-wood cellulosic fibres. Further, the results confirm the earlier observation that cavities can also be formed in delignified wood fibres and in lignin-free fibres such as cotton if the culture conditions are suitable. The present study also clearly demonstrates that the culture conditions exercise a decisive influence on the cavity-forming ability of most of the soft rot fungi. Earlier attempts to obtain cavity formation in cotton (Courtois 1963 a and Corbett 1967) and in delignified pine wood (Findlay 1970) failed, probably because culture conditions were unsuitable for cavity formation. The various aspects which are connected with the formation of cavities, e.g. why the hyphae penetrate the cell walls and change their growth direction, why and how the cavities are formed etc., will not be discussed in this paper since these questions have been treated in detail in a previous publication (Nilsson 1974 a).

Experiments with the two species which were not able to form cavities in birch wood, viz. *Scytalidium lignicola* and *Xylogone sphaerospora,* showed that these species also lacked the ability to form cavities in the other types of fibres tested. The ability to form soft rot cavities in cellulosic fibres thus appears to be an inherited characteristic which is specific to certain species. The ability to form T-branches and to grow within the cell walls parallel to the direction of the cellulose microfibrils is connected with the ability to form cavities. Each soft rot species can be said to have a certain cavity-forming ability. This ability is evidently a species characteristic. *Humicola alopallonella* appears to possess an outstanding cavity-forming ability, as compared with other soft rot species (cf. Nilsson 1974 a).

The main part of the discussion concerns the cellulolytic activity of the tested fungi. The evidences of cellulolytic activity were obtained only by means of microscopic studies of the attacked fibres and no attempts were made to measure enzyme activities. The formation of soft rot cavities will thus be discussed in this section since these cavities arise as a result of the cellulolytic activity.

The degradation of cellophane and cotton clearly demonstrates that all of the wood-degrading fungi tested are able to degrade not only wood cellulose but also a pure cellulose substrate. This also applies to the species referred to as "non-cellulolytic" soft rot fungi which had failed to show cellulolytic activity in previous experiments (Nilsson 1973 and 1974 b). The present study shows that the cellulase production of these species is profoundly influenced by factors such as: 1) the presence of alternative carbon sources in the culture medium; 2) the moisture content of the fibres; 3) the physical structure of the cellulosic substrate; and 4) the presence of hemicellulose and lignin in the substrate. The cellulase production of the cellulolytic species is, of course, also influenced by these factors but these species appear to be able to produce cellulase within wide ranges of varying conditions.

The influence of alternative carbon sources

Bravery (1968) showed that small amounts of freely available carbon sources such as asparagine and yeast extract hindered certain soft rot fungi from producing clearing

zones in cellulose agar. Nilsson (1973 and 1974 b), who assayed a large number of microfungi for cellulolytic activity, obtained similar results with some of the species. But the "non-cellulolytic" soft rot fungi were inactive, regardless of whether freely available carbon sources such as glucose, asparagine and yeast extract were present or not. In the present study it was possible, however, to demonstrate the inhibitory effect of small amounts of glucose and yeast extract on the cellulolytic activity of the "non-cellulolytic" soft rot fungi. The studies of the degradation of cellophane showed that all the "non-cellulolytic" soft rot fungi failed to degrade cellophane when cultured on cellulose agar containing small amounts of glucose and yeast extract. But when these species were cultured on a medium which contained no carbon sources other than cellulose (medium B-VII), all were able to degrade the cellophane.

The cellulolytic species were able to degrade the cellophane on both types of media. Degradation by these species was even enhanced on the cellulose agar which contained glucose and yeast extract.

Inhibition of a part of the cellulolytic activity by freely available carbon sources, viz. glucose, yeast extract and malt extract, might also explain the differences in the degradation patterns observed in birch wood in the present study as compared with previous studies (Nilsson 1973 and 1974 b). The previous decay tests on birch wood were all carried out on agar slopes in test tubes. Two types of agar media were used, i.e. malt extract agar and a cellulose agar (F6A) of similar composition as that employed in the present study. All of the "non-cellulolytic" soft rot fungi showed sparse growth and caused virtually no attack when cultured on cellulose agar. Cavity formation was, however, prominent on malt agar but erosion-type attack was not found on any of the two media. The experiments with birch wood in the present study were carried out in vermiculite. Only a mineral salt solution and no carbon-containing compounds were added to the wood blocks. Five species which had previously produced only cavities in birch wood were found to cause an erosion-type of attack as well when cultured on birch in vermiculite. It is probable that the cellulolytic activity which gives rise to erosion of the cell walls was inhibited by the carbon sources present in the agar media used in the previous experiments. The erosion type of attack is produced by hyphae growing in the cell lumina. These hyphae were thus subjected to the inhibition of cellulolytic activity whereas the cavity-forming hyphae within the cell walls were not affected.

It is difficult, however, to explain why the enzymic activity giving rise to cavities should not be influenced by the alternative sources of carbon if lignified fibres are used as a substrate. The present study shows that the jute, sisal and kapok fibres are easily degraded by cavity formation even if the fungi are cultured on malt agar. This was also the case with birch wood, as mentioned above. When delignified wood fibres or cotton fibres were used, however, no or very few cavities were formed.

Although all of the "non-cellulolytic" soft rot species could degrade cotton fibres, the attack by some of the species was very weak. Some species failed to attack the cotton fibres when they were placed directly in the vermiculite without the support of the wood blocks. It appeared that the degradation of the fibres was so weak that it could not support sufficient growth of the fungi. But when the cotton fibres were placed on the birch wood blocks (method C), the wood served as a substrate which supported the growth of the fungi and enabled them to degrade the fibres. Wood is a complex organic material which is only slowly hydrolysed by the soft rot species and the carbon-containing products of hydrolysis thus evidently exercise no inhibiting effects. Similar results to those obtained with cotton fibres were also obtained with delignified wood fibres.

The influence of the moisture content of the fibres

In a previous paper (Nilsson 1974 b) it was shown that the "non-cellulolytic" soft rot fungi were unable to degrade cotton in liquid media. In the same paper it was also reported that two such species, viz. *Bispora betulina* and *Phialophora* sp. A were unable to degrade jute fibres or birch wood meal in liquid media. One "non-cellulolytic" species, *Ceratocystis albida,* was, however, able to cause significant weight losses of jute fibres in liquid media. Under these conditions this species formed numerous soft rot cavities in the fibres. Cellulase could not be found in culture filtrates from any of the experiments mentioned.

The present study shows that *Bispora betulina, Phialophora* sp. A and the other cavity-forming species easily formed cavities in jute fibres when the fibres were placed on a vermiculite layer or on wood blocks in vermiculite, as well as on fungal colonies growing on malt agar slopes in test tubes. This demonstrates that although these fungi had the ability to form cavities in jute fibres, they were prevented from doing so when cultured in liquid media. The results with *Ceratocystis albida* and especially *Humicola allopallonella* show that not all cavity-forming species react in the same way. *H. alopallonella* might be considered as being adapted to a liquid environment since it is a marine species.

The importance of the moisture content is also illustrated in the results obtained with cotton fibres. The "non-cellulolytic" soft rot fungi all failed to degrade cotton when cultured in liquid media. However, degradation occurred when the cotton fibres were placed on vermiculite or on wood blocks in vermiculite. It was often observed that the heaviest degradation of cotton was found in the drier parts of the fibre pads. Cotton degradation was not observed in the fungal cultures on malt agar slopes but this was in all probability due to inhibition by the malt extract.

The above results indicate that the moisture content of the fibres is of considerable importance for the degradation ability of the "non-cellulolytic" soft rot fungi. Thus some species which actually can degrade a fibre will not do so if the moisture content is too high. Unfortunately, no exact measurements of the moisture contents of the fibres was made. However, the moisture content of the wood blocks plus fibres in method C was around 50 percent. In the future it will be necessary to study the influence of various moisture contents on the degradation under carefully controlled conditions and using weight loss or loss in the strength of fibres as indications of activity.

It appears that the cellulolytic activity of the category of fungi referred to as "non-cellulolytic" is repressed during cultivation in liquid media. This effect is not easily explained. It was previously shown (Nilsson 1974 b) that the actual fungi could grow in liquid cultures if provided with glucose as a source of carbon. Although most of the fibres and cellulose were immersed in the nutrient solutions, parts of them floated. The liquid layer was also rather shallow and shake cultures were also employed. It therefore seems unlikely that the inhibition of cellulolytic activity in liquid media was due to oxygen deficiency. This could possibly be the case as regards the formation of cavities where the hyphae act within the fibre cell walls. But since the erosion-type attack is caused by hyphae acting on the outside of the cell walls, it is unlikely that these hyphae lacked sufficient amounts of oxygen.

The influence of the physical structure of the substrate

A characteristic feature of the soft rot fungi is that the cavity-forming hyphae follow the direction of the cellulose microfibrils in the fibre walls (cf. Bailey and Vestal 1937 and Nilsson 1974 a). It was previously shown (Nilsson 1974 a) that *Humicola alopallonella* was stimulated to produce cellulase when the hyphae grew parallel to the cellulose microfibrils in birch wood and cotton fibres. Similar phe-

nomena were observed in the present study. Several of the species tested here produced considerably more degradation in the form of cavities than in the form of erosion. *Gonatobotrys* sp. A and *Rhinocladiella* sp. A degraded the delignified wood fibres and the cotton fibres exclusively through cavity formation. No erosion-type attack was observed. These results also indicate a stimulation of the cellulolytic activity of the hyphae when they grow parallel to the cellulose microfibrils within the wood cell walls. The soft rot fungi thus appear to have a certain requirement, which may be more or less pronounced, for an ordered fibrillar structure of the substrate. The rather sparse cavity formation obtained in the two delignified wood fibres could be due to a disorganisation of the fibrillar structure produced in the delignification treatments. Such disorganisation of the cell wall structure following delignification by sodium chlorite has been reported by Findlay (1970) for pine wood.

The influence of hemicellulose and lignin

The influence of the presence of lignin and hemicellulose in jute fibres on the degradation by microfungi was extensively studied by Basu and Ghose (1952 and 1960). They found that delignified jute fibres were more susceptible than lignified jute fibres. Jute holocellulose was more susceptible than cotton and jute α-cellulose. This indicated a stimulatory effect of the hemicellulose on the degradation of cellulose. Hemicellulose was also found to stimulate the production of cellulase in liquid cultures. Similar effects might possibly have been found for the cellulolytic species tested here but this was not studied. No such effects could, however, be found on the degradation of cellulose by the "non-cellulolytic" soft rot fungi. Delignification led to an increased amount of the erosion-type attack but the cavity formation decreased considerably. The hemicellulose present in the spruce holocellulose had, as far as could be judged from the microscopic observations, no stimulatory effects on the degradation.

Lignin is generally considered to increase the resistance of cellulosic fibres to degradation by cellulolytic microorganisms (cf. Siu 1951 and Basu & Ghose 1952). However, it was demonstrated both earlier (Nilsson 1974 b) and in the present study that certain soft rot fungi attack lignified fibres more readily than non-lignified. A similar phenomenon has been observed in connection with the brown-rotting basidiomycetes. These fungi are well-known for their rapid degradation of the cellulose in wood but most of the species tested have failed to degrade pure cellulose substrates such as cotton fibres (Reese & Levinson 1952) and filter paper (Yokota 1955). Even if the presence of lignin decreases the resistance of the cellulosic substrate to degradation by some of the soft rot fungi, the amount of lignin present appears to be less important since soft rot cavities were easily formed in fibres with varying lignin content (cf. Tables 2 and 4).

The ability of the soft rot fungi to form cavities is evidently connected with the decreased resistance of the lignified fibres since it appears that the presence of lignin facilitates the formation of cavities. However, if the other type of attack produced by soft rot fungi is considered, viz. the erosion-type attack, lignification appears to increase the resistance to degradation. This observation is supported by a previous study (Nilsson 1974 b) where the wood-degrading ascomycete *Xylogone sphaerospora,* which is unable to form cavities and thus only produces an erosion-type attack, caused considerably higher weight loss in cotton fibres than in lignified jute fibres. Courtois (1963 b), Bailey et al. (1968), Nouvertné (1968) and Findlay (1970) have demonstrated that even a mild delignification of softwood will considerably increase the amount of attack by soft rot fungi. It is likely that the fungi tested were able to produce erosion-type attack in the wood and that this type of attack increased after the delignification treatments. This view is supported by Findlay's observations (1970).

He found that *Phialophora fastigiata* degraded delignified pine wood by a type of attack which can be considered to be of an erosion type. Soft rot cavities were not observed. It has been shown by the present author (Nilsson 1973) that *Phialophora fastigiata* is able to produce an erosion-type attack in wood.

The role of lignin in the process of cavity formation is not fully understood. The attempts to use cellophane containing lignin for studies of the influence of lignin on the activities of the soft rot fungi failed, probably because the incorporated lignin was not evenly distributed in the cellulose. It was shown both earlier (Nilsson 1974 a) and in the present study that soft rot cavities can also be formed in a lignin-free fibre such as cotton. The cavities were, however, formed much more easily in the lignified fibres. Cavity formation was sparse in the fibres of sulphate pulp which contained an appreciable amount of lignin (10.8 %) and in fibres of spruce holocellulose which can be considered to contain most of the hemicellulose in the intact wood. This suggests that the stimulatory effect exerted by lignin and hemicellulose on the formation of cavities is not related to their chemical composition but rather to their role as structural components giving the fibre walls a certain structure which is suitable for cavity formation. However, it is also possible, as mentioned above, that the sparse formation of cavities in the delignified wood fibres was due to disorganisation of the fibrillar structure. Delignification also increases the porosity of the cell walls and this might also influence the cavity formation. The increased porosity of the cell walls appears to be reflected in the shape of the type C cavities which were observed in the sulphate pulp fibres. Normal cavities have only one pointed tip at each end whereas cavities of type C have several pointed tips (see Fig. 11). This indicates that the cell wall degrading enzymes have been able, due to the increased porosity of the cell wall, to diffuse longitudinally along the cellulose microfibrils.

General conclusions

The following conclusions concerning the degradation of cellulosic substrates by microfungi can be reached on the basis of previous results and the results of the present study:

1) Soft rot fungi produce two different types of attack in cellulosic fibres. One is the formation of soft rot cavities, the other is an erosion-type attack.

2) The ability to form soft rot cavities is an inherited characteristic which is restricted to certain species. The cavity-forming ability varies among different species.

3) Soft rot cavities are much more readily formed in lignified than in lignin-free fibres. Delignification results in decreased cavity formation. The erosion-type of attack appears to be increased by delignification.

4) Wood-degrading microfungi are not only able to degrade the cellulose in wood but can also degrade pure cellulose substrates. However, some species referred to as "non-cellulolytic" soft rot fungi have very special requirements. The cellulolytic activity of these species appear to be inhibited by small amounts of freely available alternative carbon sources and by an excessively high moisture content of the substrate. The cellulolytic activity seems to be stimulated by a substrate which is lignified and which has an oriented fibrillar structure.

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Sammanfattning

Nedbrytningen av cellofan och olika cellulosahaltiga fibrer förorsakad av ett antal mikrosvampar har studerats med mikroskop. Följande fibermaterial undersöktes: björkved (*Betula verrucosa* Ehrh.), en sulfatmassa (från *Pinus silvestris* L.) som innehöll 10.8 % lignin, holocellulosa från gran (*Picea abies* (L) H. Karst.), jute, sisal, kapok och bomull.

Tjugosju olika arter av mikrosvampar ingick i försöket. De hade utvalts med ledning av tidigare resultat (se Nilsson 1973 och 1974 b) så att svampar med olikartad förmåga att bryta ned björkved och cellulosa var representerade. En av svamparna, *Phialophora verrucosa,* hade visat sig sakna förmåga att bryta ned både björkved och cellulosa. De övriga arterna hade åstadkommit angrepp på björkveden antingen i form av soft rot kaviteter eller erosion av cellväggarna. Några arter hade förorsakat båda typer av angrepp simultant. Ett antal av de vednedbrytande arterna, vilka endast hade förorsakat soft rot kaviteter, hade i de tidigare försöken inte uppvisat någon cellulolytisk aktivitet på rena cellulosasubstrat. De kallades därför "icke-cellulolytiska" soft rot svampar medan de som visat aktivitet benämndes cellulolytiska.

Cellofannedbrytningen studerades genom att odla svamparna på en bit cellofan vilken hade placerats på ett agarsubstrat. Tre olika agarmedia användes, nämligen vanlig maltagar samt två olika typer av cellulosaagar. Av de senare innehöll ett endast cellulosa medan det andra dessutom innehöll små mängder glukos och jästextrakt. De cellulolytiska svamparna odlades endast på de två cellulosaagarsubstraten. Samtliga arter bröt ned cellofan oberoende av vilket substrat de odlades på. *Phialophora verrucosa* och de "icke-cellulolytiska" soft rot svamparna odlades på maltagar och på de två cellulosaagarsubstraten. De senare förorsakade nedbrytning av cellofanet endast på det cellulosaagarmedium som saknade tillsats av glukos och jästextrakt. *Phialophora verrucosa* saknade helt förmåga att bryta ned cellofan.

Nedbrytningen av ett speciellt tillverkat cellofan som innehöll 10—12 % lignin studerades i ett försök att undersöka ligninets inverkan på nedbrytningen av cellulosa. Några skillnader i nedbrytningen av detta cellofan och "vanligt" cellofan kunde emellertid inte upptäckas. Nitton av de tjugosju mikrosvamparna användes vid undersökningarna av nedbrytningen av de olika fibermaterialen. Sjutton av svamparna hade i tidigare försök visat förmåga att bilda soft rot kaviteter i björkved medan två av arterna saknade denna förmåga. Dessa två hade endast förorsakat angrepp av erosionstyp.

Svamparnas förmåga att bilda kaviteter av soft rot typ i de olika fibermaterialen undersöktes. Förekomsten av andra typer av angrepp studerades huvudsakligen hos björkveden, de delignifierade vedfibrerna och bomullsfibrerna. Dessa angrepp bestod bland annat av erosion av cellväggarna inifrån cellumen eller från fibrernas utsida och av tvärgående eller spiralformigt löpande sprickor i fibrernas cellväggar. De sistnämnda angreppstyperna karaktäriserades samtliga som angrepp av erosionstyp.

Följande tre metoder användes för studier av svamparnas fibernedbrytning:

A) Svamparna odlades på snedagarrör. När svamparna vuxit ut placerades fibermaterialet ovanpå mycelen. Två olika agarsubstrat användes. Det ena var vanlig maltagar och det andra ett cellulosaagarsubstrat.

B) Svamparna odlades tillsammans med fibrerna i Erlenmeyerkolvar med olika när-

lösningar. Endast stationära kulturer användes.

C) Fibrerna fästes ovanpå björkklotsar vilka placerades i ett vermiculitlager i Erlenmeyerkolvar till vilka en viss mängd närlösning tillsattes. Fukthalten hos klotsar plus fibrer var efter autoklavering ca 50 %.

När metod A användes förorsakade de svampar, som tidigare hade visat sig ha förmåga att bilda kaviteter i björkved, kraftiga angrepp i form av kaviteter i de lignifierade fibrerna. Kavitetsbildningen i de delignifierade vedfibrerna och i bomullsfibrerna var däremot mycket sparsam. De cellulolytiska arterna förorsakade sådana angrepp oberoende av typ av odlingssubstrat. Dessa svampar förorsakade vid odling på cellulosaagar även kraftiga angrepp av erosionstyp i de delignifierade vedfibrerna och i bomullsfibrerna. De "icke-cellulolytiska" soft rot svamparna bildade rikligt med kaviteter endast vid odling på maltagar. Vid odling på cellulosaagar växte svamparna dåligt och förorsakade endast obetydliga angrepp.

Vid odling i närlösning (metod B) förorsakade de flesta cellulolytiska arterna angrepp av erosionstyp i de delignifierade vedfibrerna och i bomullsfibrerna. De "icke-cellulolytiska" soft rot svamparna förorsakade däremot inga angrepp av erosionstyp. Flertalet svampar bildade mycket sparsamt med kaviteter vid odling i närlösningar. Enstaka arter bildade dock rikligt med kaviteter under dessa förhållanden men endast i de lignifierade fibrerna.

Den kraftigaste nedbrytningen erhölls med metod C. De cellulolytiska arterna och flertalet av de "icke-cellulolytiska" förorsakade angrepp av erosionstyp i björkved, de två delignifierade vedfibrerna och i bomullsfibrerna.

Kaviteter av soft rot typ bildades i samtliga typer av fibrer även om kavitetsbildningen var betydligt rikligare i de lignifierade fibrerna än i de delignifierade vedfibrerna och bomullsfibrerna.

Förmågan att bilda kaviteter varierade avsevärt mellan de olika svamparna. De två svamparna, som i tidigare försök ej bildat kaviteter i björkved, saknade även förmåga att bilda kaviteter i övriga undersökta fibrer. Några arter vilka utan svårighet bildade kaviteter i björkved, jute och sisal, kunde ej bilda kaviteter i kapok. Detta kan bero på att cellväggen i kapokfibrerna är alltför tunn för kavitetsbildning av vissa arter. Ingen av de här undersökta arterna hade samma framstående förmåga att bilda kaviteter som *Humicola alopallonella* (jfr Nilsson 1974 a).

Följande slutsatser har dragits av resultat från tidigare undersökningar (Nilsson 1973, 1974 a och 1974 b) och resultat från föreliggande undersökning:

1) Soft rot svampar kan förorsaka två olika typer av angrepp i cellulosahaltiga fibrer. Det ena är bildningen av soft rot kaviteter och det andra ett angrepp av erosionstyp.

2) Förmågan att bilda kaviteter är en nedärvd egenskap som är begränsad till vissa arter. Denna förmåga varierar mellan olika arter av mikrosvampar.

3) Soft rot kaviteter bildas i lignifierade fibrer och i lignin-fria fibrer. Kaviteterna bildas dock betydligt lättare i lignifierade fibrer. Delignifiering resulterar i minskad kavitetsbildning. Angrepp av erosionstyp tycks däremot öka efter delignifiering.

4) Vednedbrytande mikrosvampar kan inte endast bryta ned cellulosa i ved utan också rena cellulosasubstrat. De svampar som benämnts "icke-cellulolytiska"soft rot svampar har dock mycket speciella krav på substrat och odlingsbetingelser. Den cellulolytiska aktiviteten hos dessa arter tycks hämmas av små mängder lättillgängliga alternativa kolkällor och av en alltför stor fukthalt hos substratet. Aktiviteten tycks stimuleras av ett substrat som är i lignifierat och där cellulosan är ordnad i en fiberstruktur.

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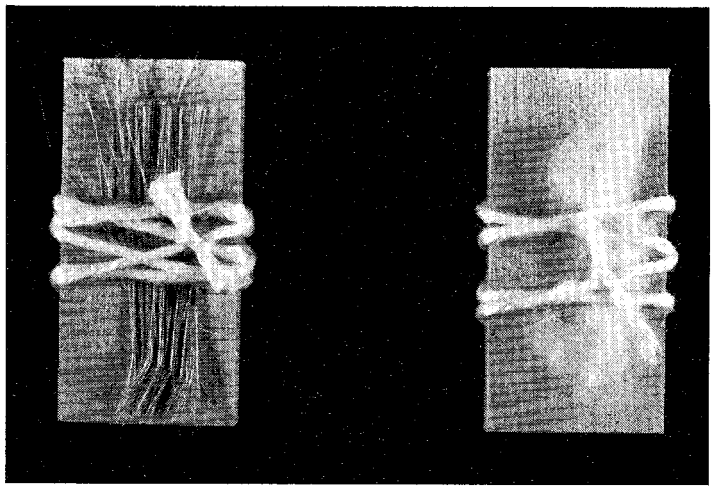

Figure 1
Arrangement of test fibres on the birch wood blocks (test method C).

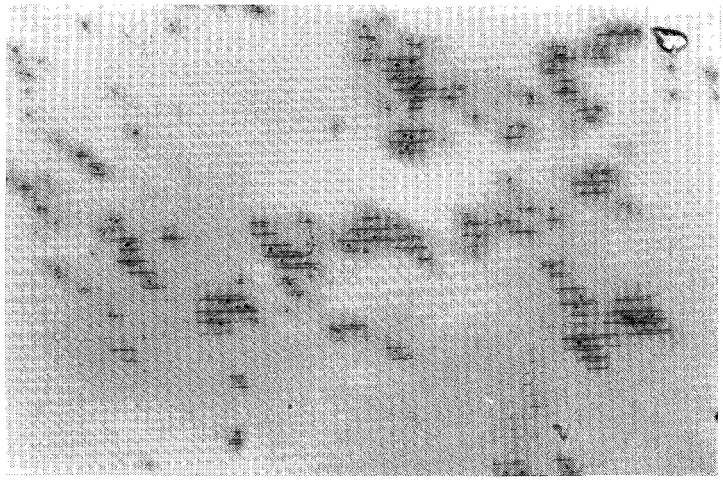

Figure 2
Small bore holes produced in the cellophane by *Bispora betulina* on F6A cellulose agar. These small bore holes were not regarded as an active form of degradation since the formation of these bore holes could be purely mechanical. Polarised light. Magn.: 490: 1.

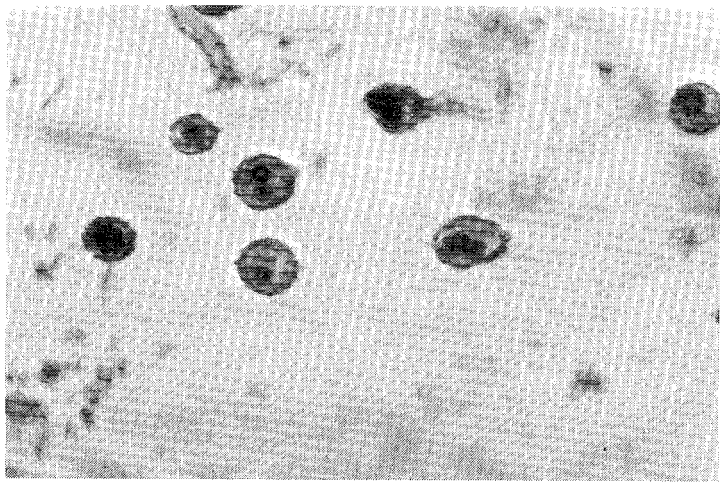

Figure 3
Large bore holes produced by *Bispora betulina* on B-VII cellulose agar. Polarised light. Magn.: 490: 1.

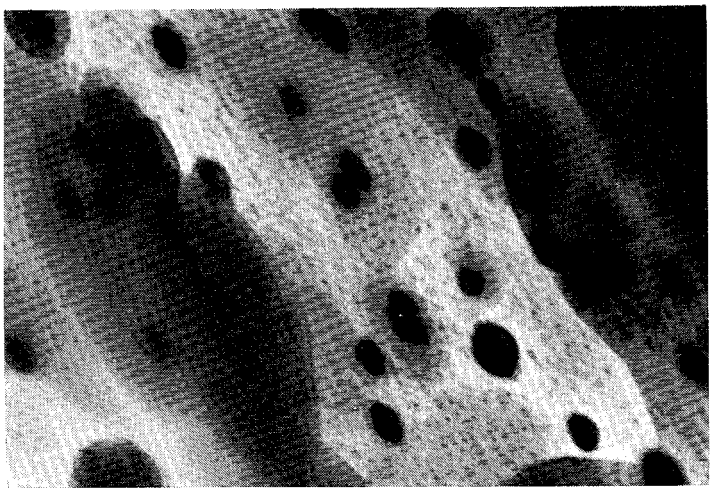

Figure 4
General erosion of the cellophane surface and large bore holes. Attack by *Dictyosporium elegans* on F6A cellulose agar. Polarised light. Magn.: 490: 1.

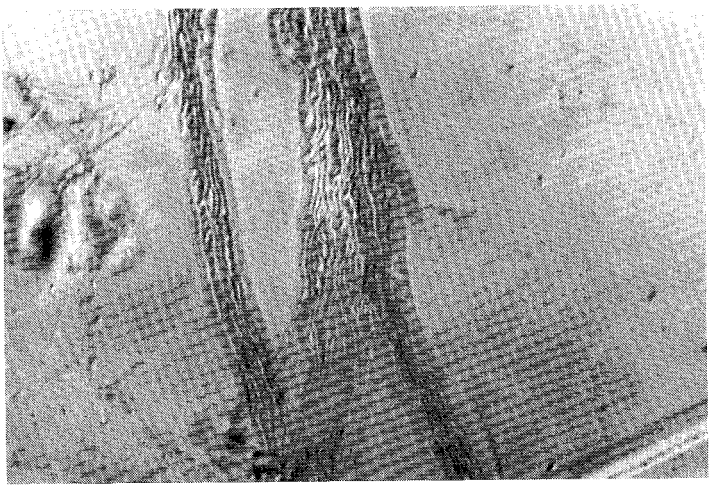

Figure 5
Lysis zones produced in cellophane along hyphal strands of *Graphium* sp. A. B-VII cellulose agar. Polarised light. Magn.: 490: 1.

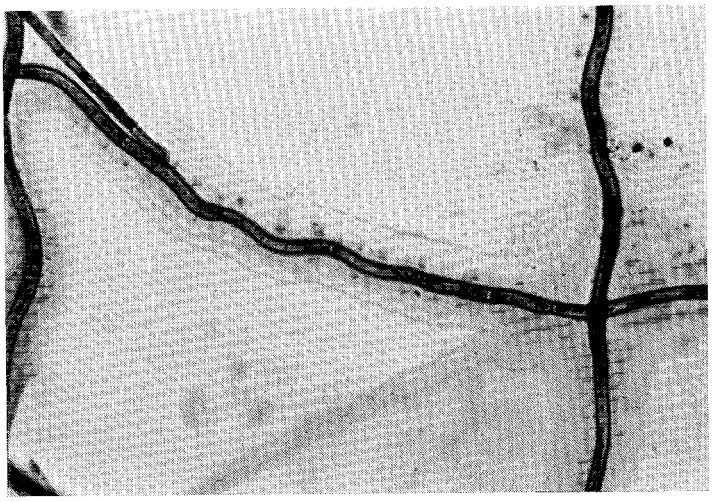

Figure 6
Lysis zones produced in cellophane along hyphae of *Phialophora* sp. A. B-VII cellulose agar. Polarised light. Magn.: 490: 1.

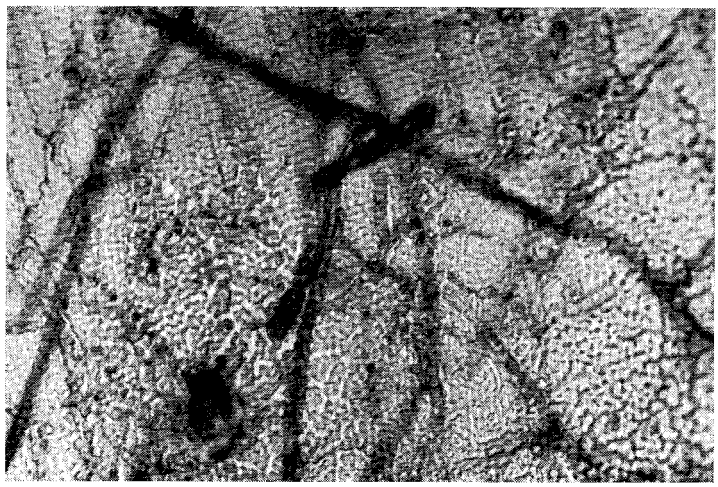

Figure 7
General erosion of the cellophane surface caused by *Bispora betulina* on B-VII cellulose agar. Polarised light. Magn.: 490: 1.

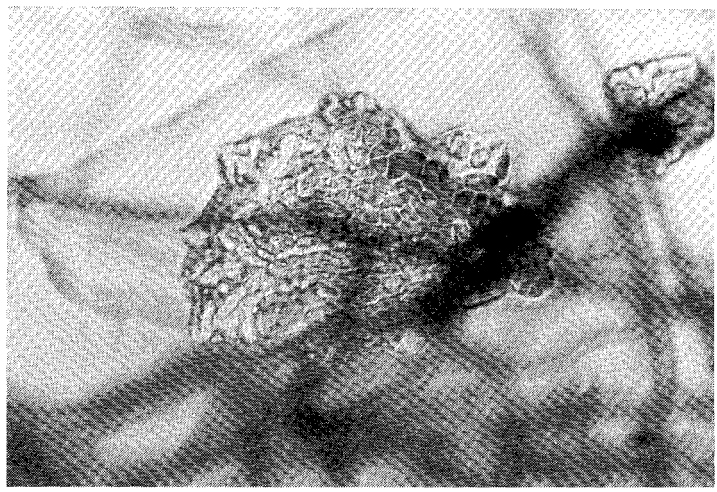

Figure 8
Lysis zones in cellophane produced by "rooting branches" of *Phialocephala* sp. A. B-VII cellulose agar. Polarised light. Magn.: 490: 1.

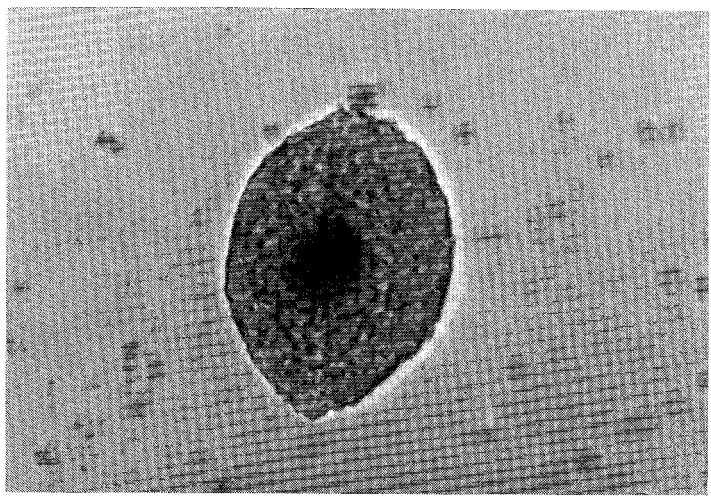

Figure 9
Lysis in the cellophane around a wind ball-like aggregate of hyphae. *Gonatobotrys* sp. A on B-VII cellulose agar. Polarised light. Magn.: 600: 1.

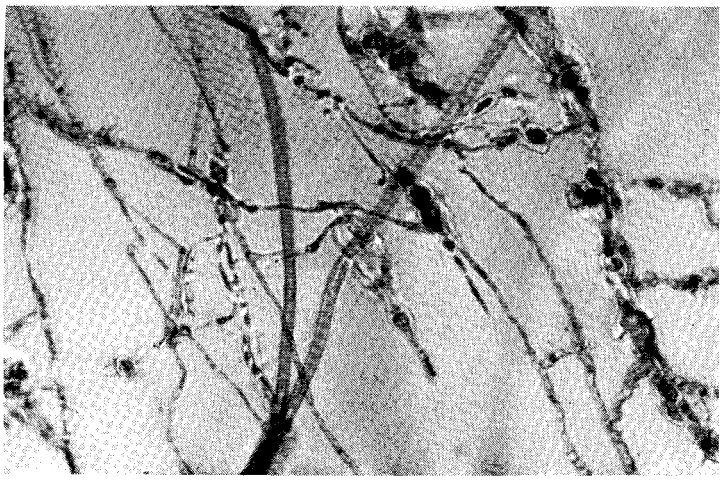

Figure 10
Cavity-like degradation pattern produced in cellophane by *Bispora betulina* on B-VII cellulose agar. Polarised light. Magn.: 490: 1.

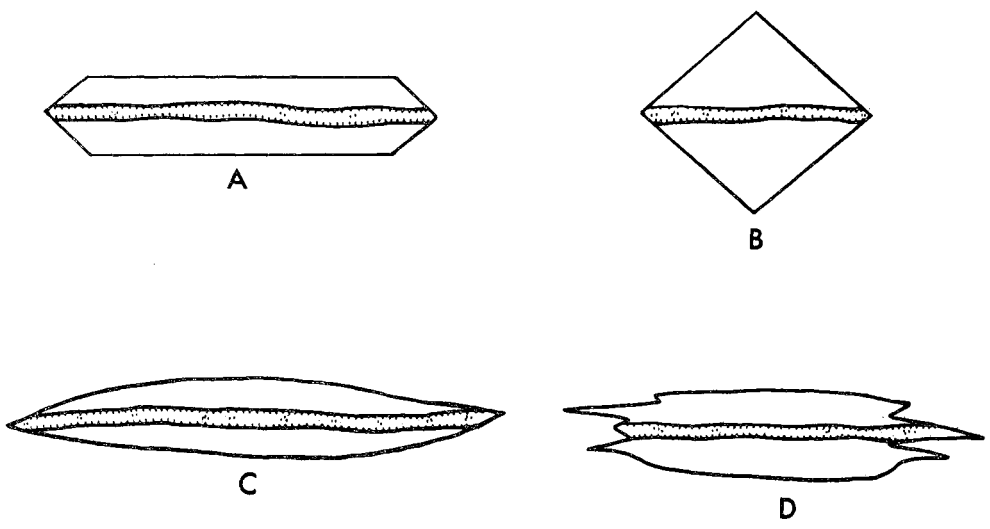

Figure 11
Drawing illustrating different types of cavities observed in the various fibres.

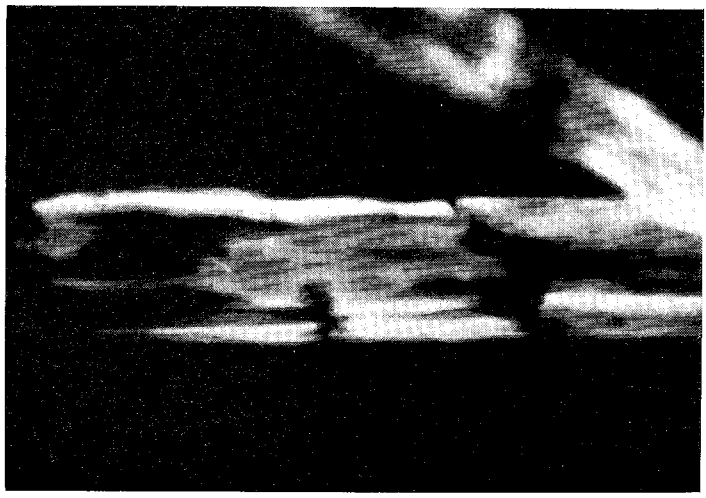

Figure 12
Erosion-type attack on a tracheid from spruce holocellulose. The attack was caused by *Phialophora* sp. A. Polarised light. Magn.: 810: 1.

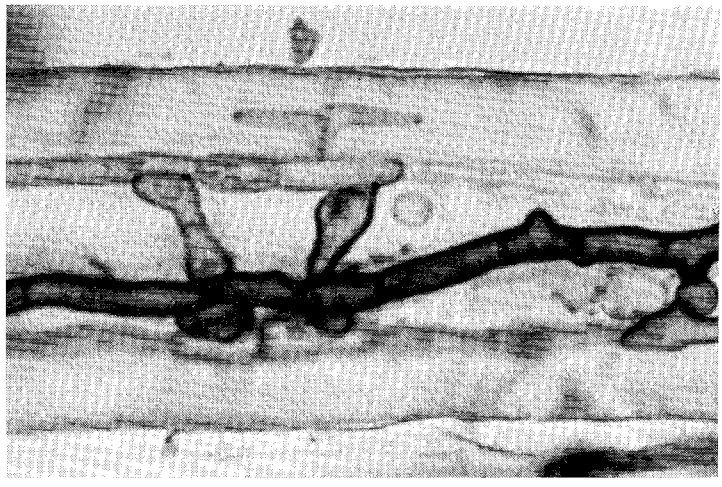

Figure 13
T-branch produced by *Bispora betulina* in a latewood tracheid from sulphate pulp C. No lysis is evident around the hypha which grows longitudinally within the cell wall. Magn.: 1150: 1.

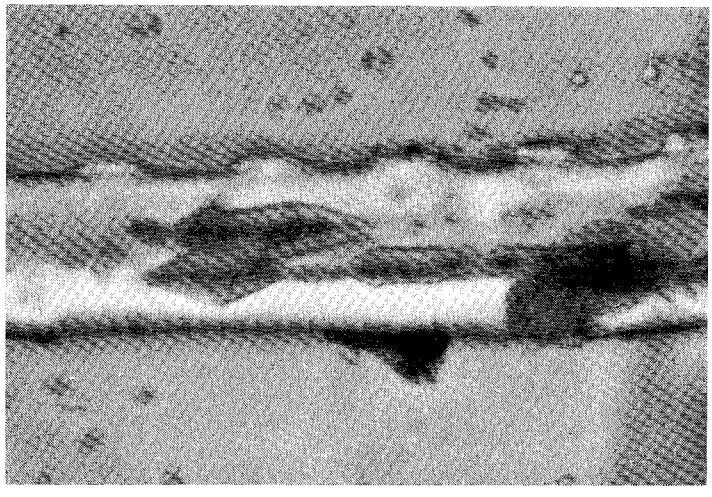

Figure 14
Cavities formed in a latewood tracheid from sulphate pulp C by *Acremonium atrogriseum*. Magn.: 810: 1.

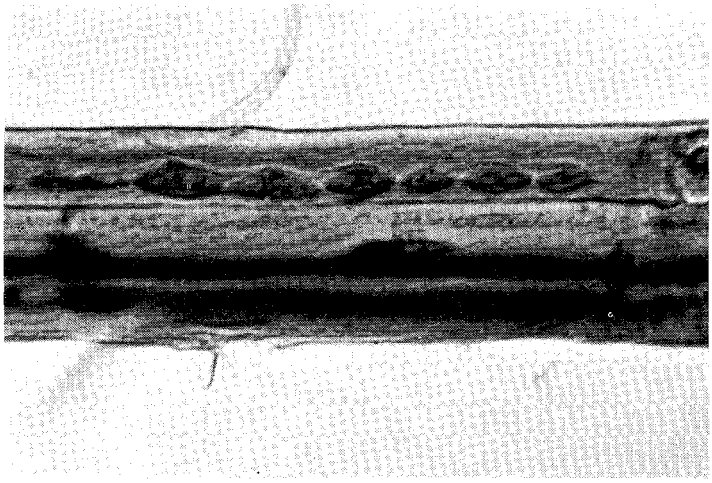

Figure 15
Cavity chain formed in a latewood tracheid from sulphate pulp C by *Bispora betulina*. Magn.: 950: 1.

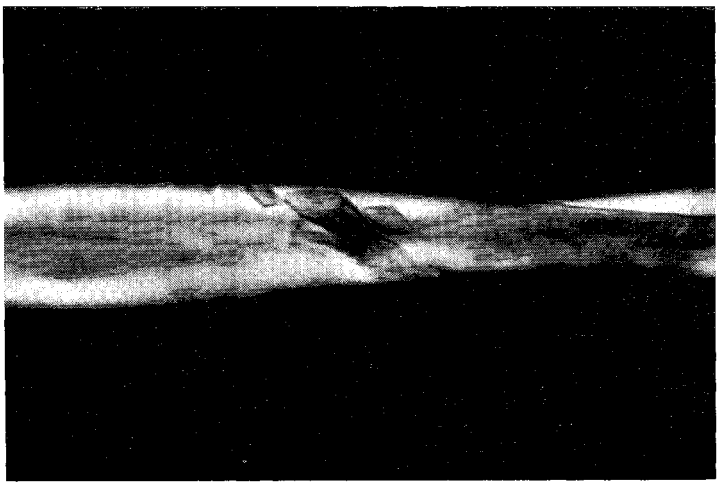

Figure 16
Cavities formed in a cotton fibre by *Ceratocystis albida*. Polarised light. Magn.: 810: 1.

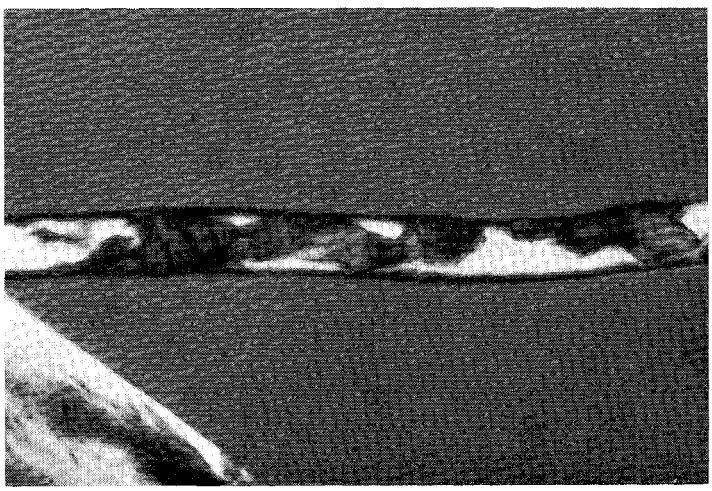

Figure 17
Cavities formed in a cotton fibre by *Ceratocystis albida*. Polarised light. Magn.: 710: 1.