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Microscopic studies of cavity formation by soft rot fungi *Allescheria terrestris* Apinis, *Margarinomyces luteo-viridis* v. Beyma and *Phialophora richardsiae* (Nannf.) Conant

Mikroskopiska studier över kavitetsbildningen hos mögelrötesvamparna Allescheria terrestris Apinis, Margarinomyces luteo-viridis v. Beyma och Phialophora richardsiae (Nannf.) Conant

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Abstract

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The ability of three soft rot fungi to form cavities in birch sapwood (Betula sp.), was investigated, viz. Allescheria terrestris Apinis (strain Apinis and strain H63-1), Margarinomyces luteo-viridis v. Beyma (strain Beyma and strain M74-IV) as well as Phialophora richardsiae (Nannf.) Conant (strain BB40-V). The primary aim of the studies was to elucidate how rapidly the fungal hyphae form cavities after infection of the wood. The role of nitrogen in cavity formation was studied by comparing the time taken for cavity formation in untreated wood and in wood impregnated with various nitrogen compounds. Phialophora richardsiae reacted most strongly to the addition of nitrogen compounds to the wood, forming cavities after three days, as compared with six days in untreated wood. Allescheria terrestris and Margarinomyces luteo-viridis formed cavities two days after infection of the wood, regardless of whether nitrogen was employed or not. However, in nitrogen-impregnated wood, cavity formation began at a more uniform rate and to a greater extent than in untreated wood. The "T-shaped branching" form of attack frequently occurred, in various forms, in that the bore-holes were not always symmetrically related to the long edge of the cavity. The ability of Allescheria terrestris and Margarinomyces luteo-viridis to form cavities in 25 different plants with lignified cells was studied. Cavity formation differed widely between softwood where there was little and hardwood where cavities were very numerous, although with some small variations.

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1 Introduction

The ability of fungi hyphae to develop in the cell walls of wood and so destroy them, thus forming cavities, was described as early as the 1850s by Schacht (1850, 1863). Bailey and Vestal (1937) demonstrated that the hyphae develop in the secondary walls of the wood cell and that wood from both gymnosperms and angiosperms is attacked. Savory (1954), who gave this type of rot its English name "soft rot", isolated a large number of cavity-forming fungi in wood which had been attacked by soft rot. He found that the fungi isolated belonged to the Ascomycetes and Fungi Imperfecti. Later studies have shown that two Basidiomycetes display a rotting effect typical of soft rot, namely *Poria nigrescens* (Duncan, 1960) and *Stereum purpureum* (Nilsson, personal communication).

In addition to cavity formation, erosion of the cell wall from the lumen can also occur in soft rot. However, this type of attack has not been studied in detail in the present paper.

The penetration of a hypha into a wood cell and its method of forming a cavity by means of a T-shaped attack has been described by Corbett and Levy (1963). The variations in the form of cavities in different wood cells and types of wood were studied by Courtois (1963). Very little study has been devoted to the time taken for cavity formation after a hypha has come into contact with the wood. Greaves and Levy (1965) state that after a week *Chaetomium globosum* formed cavities in *Fagus sylvatica,* after two weeks in *Betula* sp. and after four weeks in *Pinus sylvestris.*

The time required for cavity formation by some soft rot fungi in wood cells has been especially studied in this investigation. The soft rot fungi employed are a thermophilic Ascomycete: *Allescheria terrestris* Apinis (strain Apinis and strain H63-1), two Fungi Imperfecti: *Margarinomyces luteo-viridis* v. Beyma (strain Beyma and strain M74-IV), as well as *Phialophora richardsiae* (Nannf.) Conant (strain BB40-V). Strain H63-1 was isolated from aspen chips in 1965 and strain M74-IV and strain BB40-V from birch chips in 1964. The collection was made by T. Nilsson. Strain Apinis has been supplied by A.E. Apinis and strain Beyma has been obtained from C B S in Baarn.

2 Materials and methods

The test fungi were cultivated in sloping agar tubes. The following temperatures and cultivation times before rotting were employed for the various fungi: *Allescheria terrestris* strain H63-1 and strain Apinis at 45° C for five days, *Margarinomyces luteoviridis* strain M74-IV and strain Beyma at 30° C for approximately 20 days and *Phialophora richardsiae* strain BB40-V at 25° C for approximately 20 days. The same temperatures were employed both for the cultivation of the mycelium and for the tests and were determined on the basis of the optimal temperatures for extension growth.

Blocks (2×0.5×0.5 cm) of *Betula verrucosa* Ehrh. or *Betula pubescens* Ehrh. were placed on the developed mycelium. The fresh wood samples were taken at a height of 0—2 m from the stems of trees with a maximum diameter of 25 cm. The stored air-dry wood samples were taken at an unknown stem height from trees with an unknown diameter. These samples were then either sterilised in an autoclave or not sterilised at all. When impregnating the wood with nitrogen, the following compounds were employed: ammonium chloride, ammonium nitrate, ammonium tartrate, asparagine and casein hydrolysate. The nitrogen impregnation was carried out in a desiccator under air suction for 50 minutes and vacuum for 50 minutes. The nitrogen compounds were mainly in a 0.6 per cent aqueous solution. The blocks were sliced with a razor blade for microscopic study. The cut was stained with a safranin solution (safranin dissolved in 70 per cent alcohol). A light microscope was primarily employed for the microscopic studies, but a Cambridge Stereoscan Electron Microscope was also used to a lesser extent. A method of studying cavity formation by the mycelium in the wood *in vitro* was also developed and utilised (Lundström, 1970). "Kjeldahl's macro-Se method" was employed for analyses of the total nitrogen content of the wood.

3 Cavity formation in birch wood

The studies of cavity formation primarily concern *Allescheria terrestris*. *Margarinomyces luteo-viridis* and *Phialophora richardsiae* have been utilised for some comparative tests. This was done in order to determine the range of the variation in time required for cavity formation by soft rot fungi with different temperature requirements for optimal extension growth. No large and clearly demonstrated differences in the time required for cavity formation were discovered among the types and strains of fungi studied here, with the exception of *Phialophora richardsiae* (see Table 1).

In some of the tests, cavities tended to develop more rapidly in sapwood than in the inner parts of the stem (including the pith). This may possibly depend on variations in the amounts of nitrogen. Merrill and Cowling (1966) investigated the radial distribution of nitrogen in a number of wood species and found wide variations. The highest amounts of nitrogen were found in the outer sapwood and the inner parts of the stem (pith region) and significantly smaller amounts in the wood between these two regions. They also found that early wood has a higher nitrogen content than late wood.

Two birches (*Betula verrucosa*) from which a major portion of the test blocks were obtained, were analysed for their nitrogen content (see Table 2). The nitrogen content proved to be lower in the sapwood than in the inner parts of the stem where cavity formation was somewhat poorer. The tendency towards rather slow cavity formation in the inner parts of the stem, observed in some cases, was probably caused by factors other than the nitrogen, and these are evidently of importance for cavity formation.

After impregnation of the sapwood with nitrogen, thus increasing the nitrogen content considerably (see Table 2), cavity formation began more rapidly and to a greater extent than in untreated wood (see Table 1). Only sapwood was employed in the studies of cavity formation referred to here. The first cavities in the blocks were formed on the sides which were in direct contact with the mycelium.

Since approximately 60—70 per cent of birch wood consists of fibres (Thunell and Perem, 1952), it was considered the simplest way to follow cavity formation in them. The studies have therefore almost exclusively come to concern cavity formation in fibres.

3.1 *The preliminary stage of cavities*

The initial stage of cavity formation in the wood cells caused by fungal hyphae cannot always be easily distinguished in the light microscope. A very thin, oblong formation (see Figure 1) often appears and may be designated as a pre-cavity stage. Such preliminary stages can be encountered in wood fibres 24 hours after the mycelium has come into contact with the wood.

3.2 *Observation of the first cavity*

Two days (50 hours) after the mycelium came into contact with the wood, cavities were encountered in both the untreated and the nitrogen-impregnated wood (Figure 2). Greaves and Levy (1965) observed cavities in birch wood (*Betula* sp.) after a two-week attack by *Chaetomium globosum*. The shape of the cavities varies but the short ends are usually more or less pointed at an angle of approximately 45°. The term "cavity" was not employed in this study

Table 1. Cavity formation under optimal conditions in untreated and nitrogen-impregnated birch sapwood. The nitrogen impregnation has been carried out with ammonium chloride, ammonium nitrate, ammonium tartrate, asparagine or casein hydrolysate in 0.6 % solutions.

The treatment of the wood before rotting	Fungus	Cavity formation after			
		2 days	3 days	4—5 days	6—10 days
Untreated	Allescheria terrestris Apinis	(+)	(+)	+ +	+ +
Untreated	Allescheria terrestris H63-1	(+)	(+)	+ +	+ +
Nitrogen-impregnated	Allescheria terrestris Apinis	+	+ +	+ + +	+ + +
Nitrogen-impregnated	Allescheria terrestris H63-1	+	+ +	+ + +	+ + +
Untreated	Margarinomyces luteo-viridis Beyma	0	(+)	(+)	+
Untreated	Margarinomyces luteo-viridis M74-IV	(+)	(+)	+	+ +
Nitrogen-impregnated	Margarinomyces luteo-viridis Beyma	+	+	+ +	+ + +
Nitrogen-impregnated	Margarinomyces luteo-viridis M74-IV	+	+	+ +	+ + +
Untreated	Phialophora richardsiae BB40-V	0	0	0	+
Nitrogen-impregnated	Phialophora richardsiae BB40-V	0	(+)	+	+ +

0 No cavity formation.
(+) Extremely sporadic cavity formation. Individual cavities.
+ Sporadic cavity formation. Individual cavities.
+ + Somewhat more regular cavity formation. Cavity chains begin to form.
+ + + Regular cavity formation. Cavity chains.

Table 2. The total amount of nitrogen in the central wood and sapwood of *Betula verrucosa*. The sapwood is analysed before and after impregnation with nitrogen salts. Analysis method: "Kjeldahl's macro-Se method".

Date of sampling	Section of trunk	% N	Preparation of the wood
27 6 68	Central wood	0.12	Untreated
	Sapwood	0.07	Untreated
23 9 68	Central wood[a]	0.13	Untreated
	Sapwood[a]	0.09	Untreated
	Sapwood[a]	0.27	Imp. ammonium nitrate (0.6 % solution)
	Sapwood[a]	0.15	Imp. ammonium tartrate (0.6 % solution)
	Sapwood[a]	0.20	Imp. asparagin (0.6 % solution)
	Sapwood[a]	0.15	Imp. casein hydrolysate (0.6 % solution)

[a] Same cross-section of the stem.

before these formations could be perceived in the light microscope. In some individual cases cavities were formed within approximately 30 hours in nitrogen-impregnated wood. The cavities occur only very sparingly. It is rather common to find cavities in wood fibres in the vicinity of a ray. Cavity formation is always more rapid and more regular in wood which has been impregnated with nitrogen than in untreated wood. In the latter case three to six days may elapse before the first cavity is observed.

Margarinomyces luteo-viridis formed cavities after two days (50 hours) in both nitrogen-impregnated and untreated wood. On the other hand, no cavities caused by

Phialophora richardsiae were encountered until after three days in nitrogen-impregnated wood and after six days in untreated wood. The rapid cavity formation described above took place under optimal temperatures for extension growth.

Enlargement of the cavity takes place after its formation. This has been determined by an *in vitro* study of the cavity formation of *Margarinomyces luteo-viridis* (Lundström, 1970). It is uncertain how long the process continues. However, enlargement appears to continue after new cavities have been formed at the ends of the initial cavity. When an initial cavity is formed, it is normal that the fungal hyphae grow into secondary wall of the wood fibre at an angle of 90° (Figure 3). It was impossible to study the penetration of the outermost hypha tip into the initial cavity on the cut. Having penetrated the secondary wall, the hypha branches upwards and downwards and the cavity is enlarged rather similarly in both directions. This T-shaped penetration of the hypha into the wood cell was first described by Corbett and Levy (1963) in respect of an attack by *Chaetomium globosum* in *Pinus sylvestris*. The T-shaped penetration has been studied by Findlay (1970) with the electron microscope.

The position of the bore-hole in relation to the cavity is, however, not always near the centre and at right-angles to the long side of the cavity (see Figures 4, 9 and 11). Sometimes several bore-holes may be found in conjunction with one cavity (Figures 5, 6). In such cases it often appears as if cavity chains were not formed. Instead, the hypha grows further in the cell wall and into an adjacent wood cell after having formed only a single cavity. In some cases, it was observed that the bore-hole continued directly to the opposite long side of the cavity (Figure 7). The majority of the initial cavities occur without bore-holes. It is probable that they exist, but that their position in relation to the cavity is such that they cannot be perceived in the light microscope. The mycelium may possibly have grown inward from the lumen so that the bore-hole is very short, in contrast to the cases where the mycelium grows through the entire cell wall.

In addition to the cavities in wood fibres, cavities have also been observed in vessels after 100 hours (Figure 8). However, such cavities seldom occur. Levy and Stevens (1966) have made the same observation, i.e. that soft rot fungi attack fibres rather than vessels in hardwoods.

3.3 *The occurrence of cavity chains*

The cavity chains develop in two different ways: 1) a new cavity is formed at *both* ends of the initial cavity (see Figure 9), 2) new cavities are formed at *one* end of the initial cavity (see Figure 10). Cavity chains with three cavities were formed in nitrogen-impregnated wood (see Figure 11) 70 hours after the mycelium had come into contact with the wood and cavity chains with four cavities had been formed after 100 hours (see Figure 12). Cavity chains with three cavities were encountered in untreated wood after 100 hours of attack (see Figure 9). As in the case of the initial cavity, cavity formation here took place at a slower rate if the wood had not been supplied with nitrogen.

After 100 hours it began to be difficult to follow cavity formation in nitrogen-impregnated wood. New formation of initial cavities took place continuously and short and long cavity chains began to coalesce.

The formation of cavities in the cell wall sometimes took place very close to the lumen, so that the cavity shape could be observed directly from the lumen (Figure 13). It is probable that the thin wall between the cavity and the lumen was destroyed during the growth of the cavity, or that cavities were also formed in the wall of the lumen. Since the photograph was taken during studies with a Cambridge Stereoscan Electron Microscope, a thin wall may possibly have been destroyed during the preparation of the specimen or by the electron beam. Levy (1971) has pointed out this possibility.

4 The effect of temperature on cavity formation

Earlier observations have indicated that *Allescheria terrestris* does not form cavities in birch sapwood at temperatures between 35° C and 55° C. At these temperatures it has been reported that the fungus only erodes the cell walls (Bergman and Nilsson, 1967, 1968).

In the present investigation *Allescheria terrestris* formed cavities in birch sapwood between 25° C and 50° C—temperatures which closely approach the minimum and maximum temperatures, respectively, for the growth of the fungus. When *Allescheria terrestris* attacked birch wood at 50° C, the mycelium, in addition to forming cavities, also greatly eroded the cell wall.

Margarinomyces luteo-viridis formed cavities at temperatures between 5° C and 35° C and *Phialophora richardsiae* between 5° C and 30° C—temperatures which approach the minimum and maximum temperatures for the growth limits of these fungi.

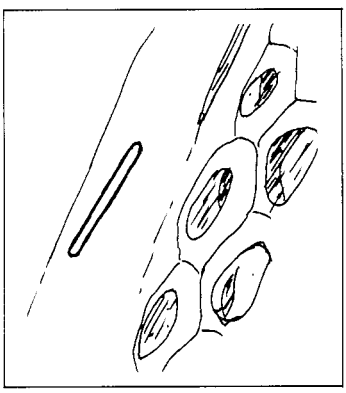


Figure 1 Pre-stage of a cavity formed by *Allescheria terrestris* in birch sapwood. Impregnated with a 0.6 per cent solution of ammonium tartrate. Approx. 875×.

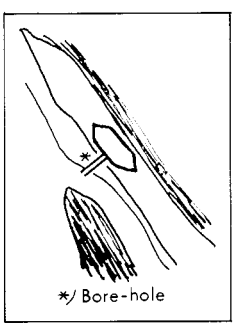
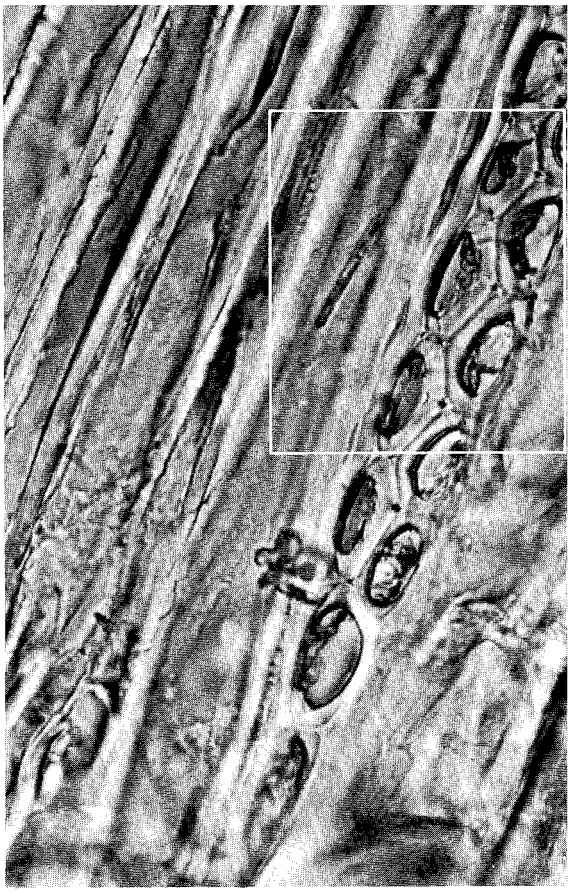
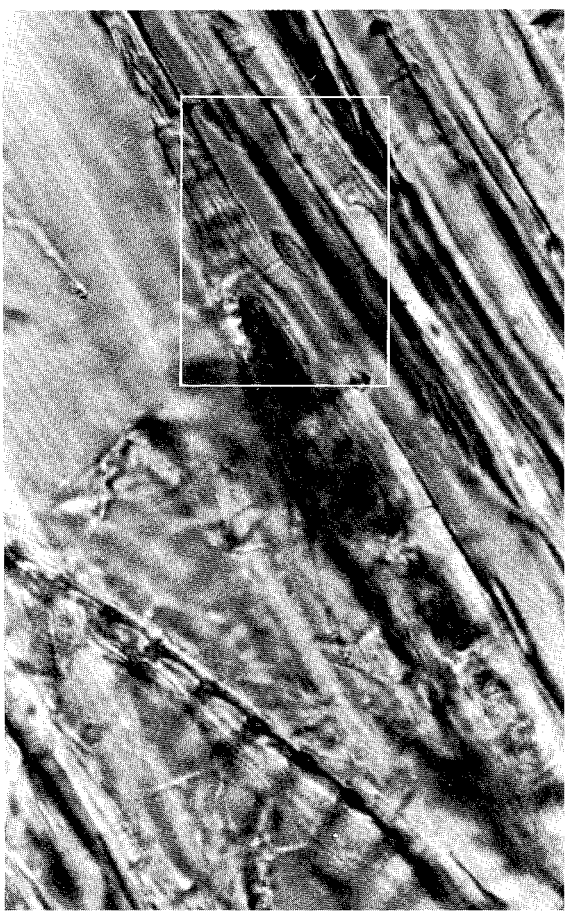
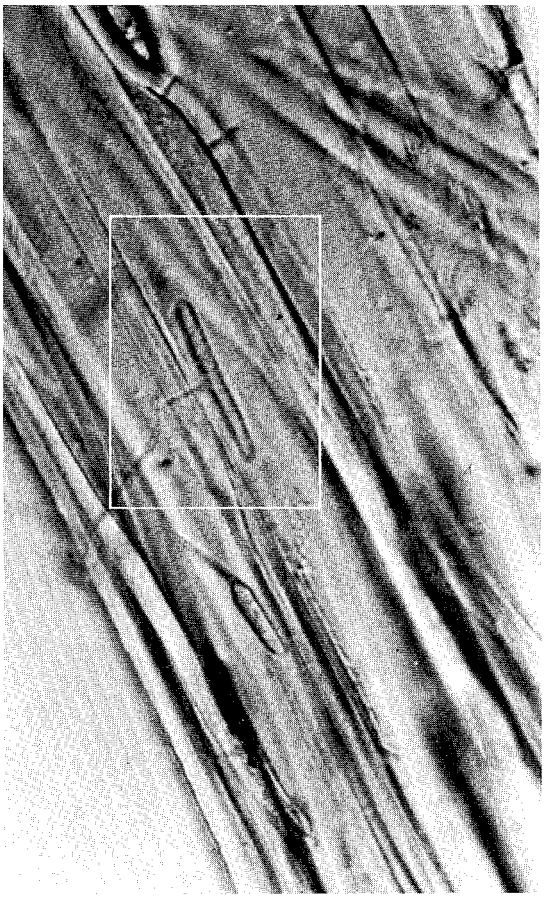


Figure 2 Cavity formed by *Allescheria terrestris* in birch sapwood after 50 hours of attack. Impregnated with a 0.6 per cent solution of asparagine. Approx. 875×.







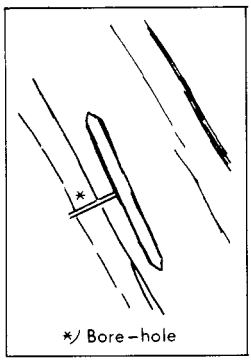
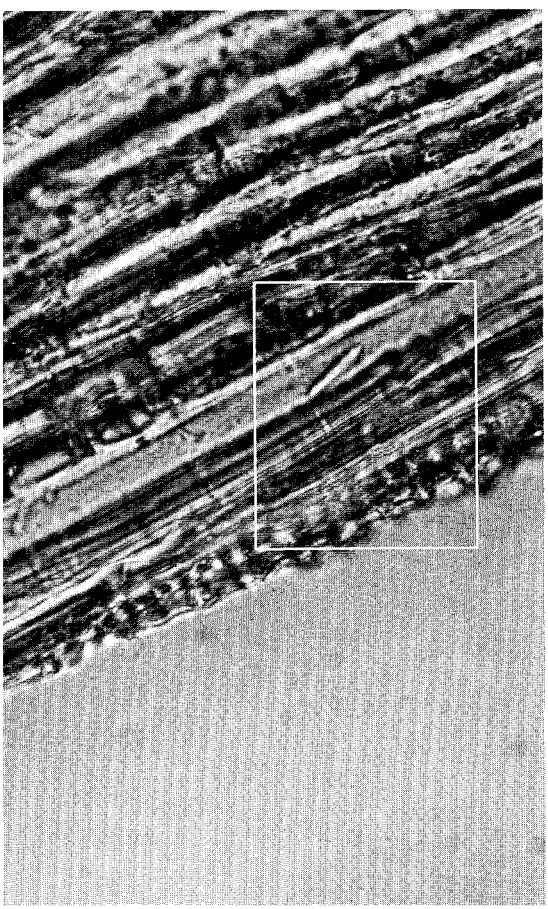


Figure 3 T-formed penetration (Corbett's Type I attack) by *Allescheria terrestris* in a birch wood fibre. Approx. 875×.



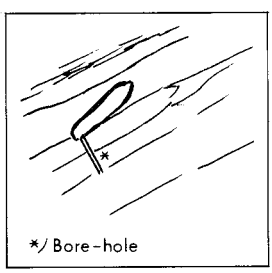


Figure 4 Cavity formed by *Allescheria terrestris* in sallow wood (*Salix caprea*) where the bore-hole adjoins one end of the cavity. Approx. 875×.

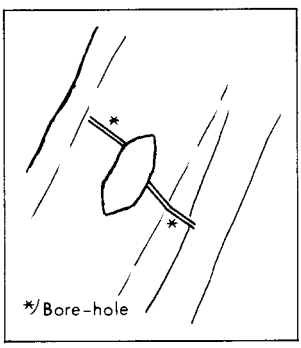


Figure 5 Cavity with two bore-holes formed in birch wood fibre by *Phialophora richardsiae*. Approx. 875×.

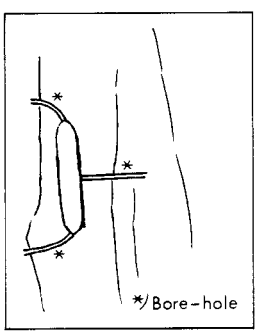
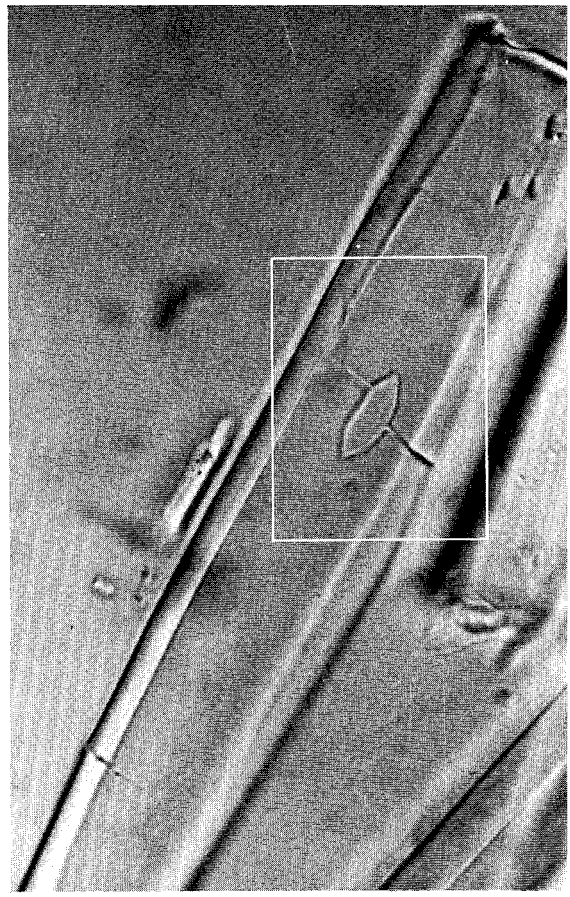
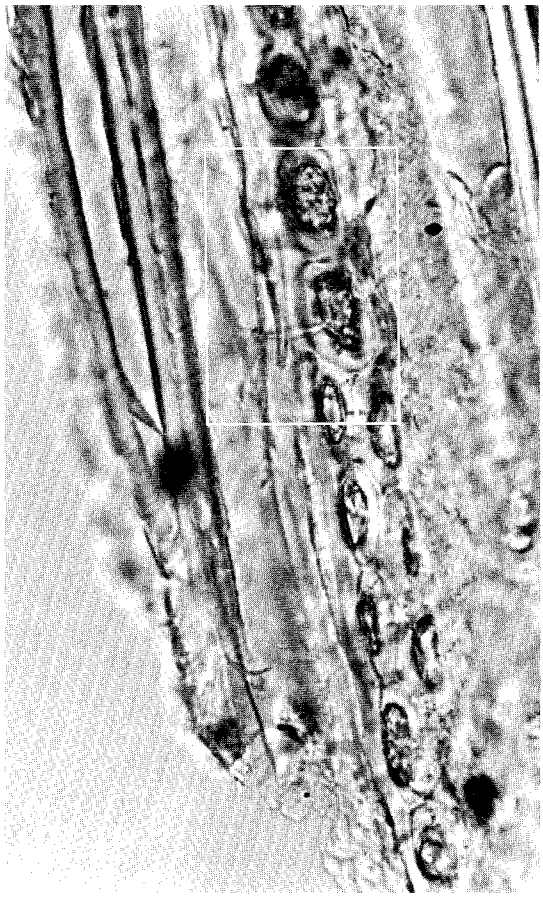


Figure 6 Cavity formed in birch wood fibre by *Allescheria terrestris* with three bore-holes, one in each end of the cavity and one in the centre of the long edge. Approx. 875×.





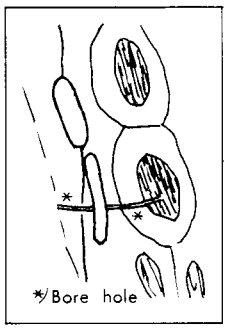
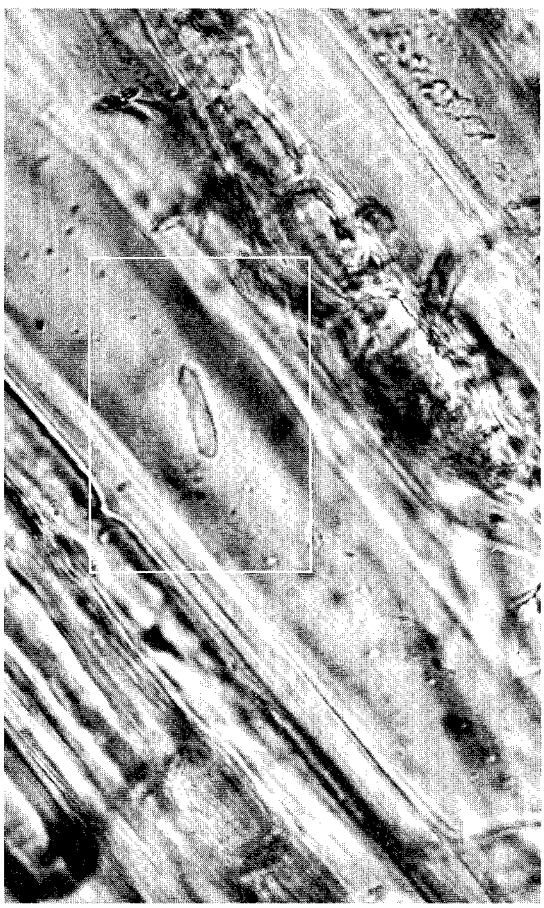


Figure 7 Cavity formed by *Allescheria terrestris* with bore-holes opposite each other on the long edges of the cavity. Approx. 875×.



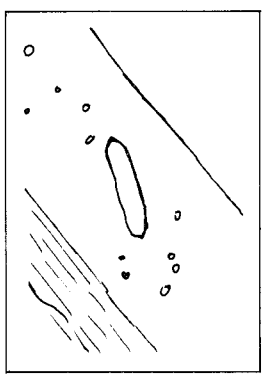


Figure 8 A cavity formed in a birch wood vessel after 100 hours of attack by *Allescheria terrestris*. Approx. 875×.

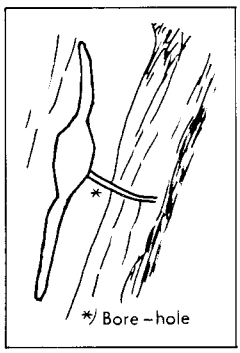
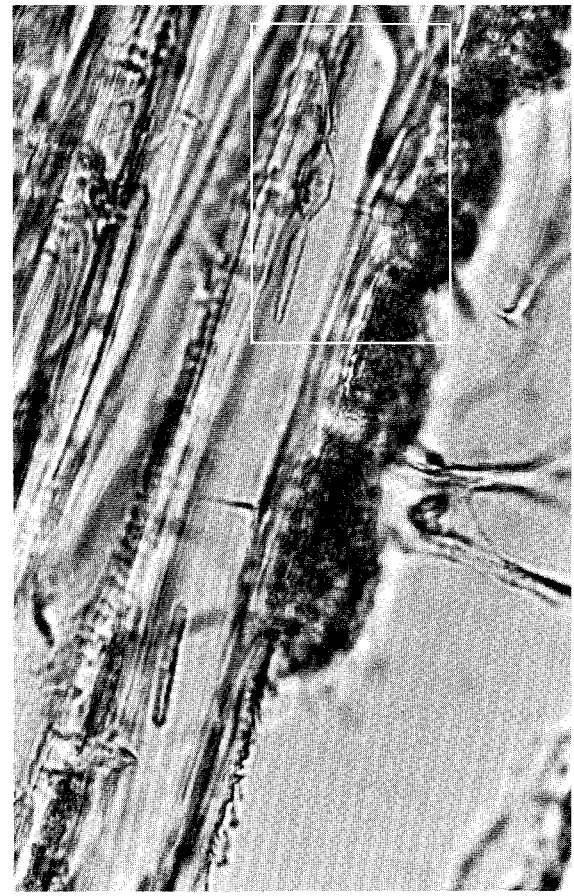
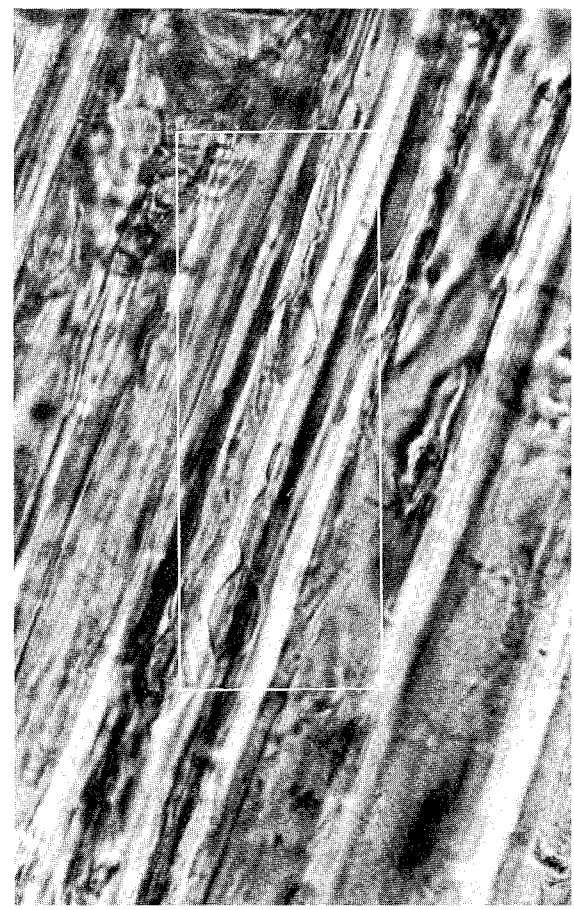
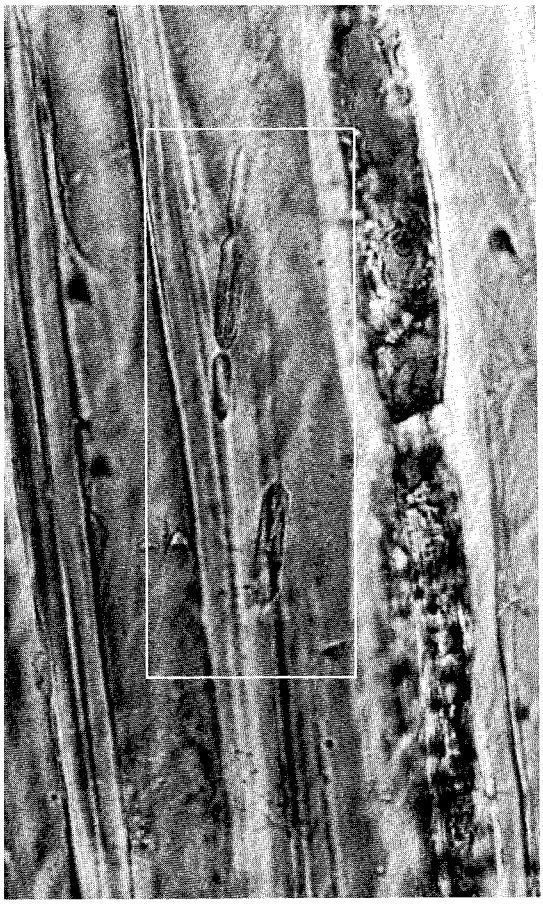


Figure 9 Growth of a cavity chain by means of new formation of cavities on both short ends of the initial cavity. Birch sapwood (not treated with nitrogen) attacked 100 hours by *Allescheria terrestris*. NOTE: The bore-hole is not at right-angles to the cavity. Approx. 875×.

Figure 10 Growth of cavity chains by means of cavities formed at one end of the initial cavity. *Allescheria terrestris* has been allowed to attack birch sapwood treated with a 0.6 per cent solution of asparagine for 100 hours. Approx. 875×.







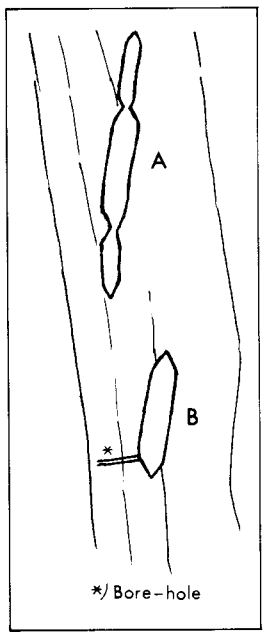
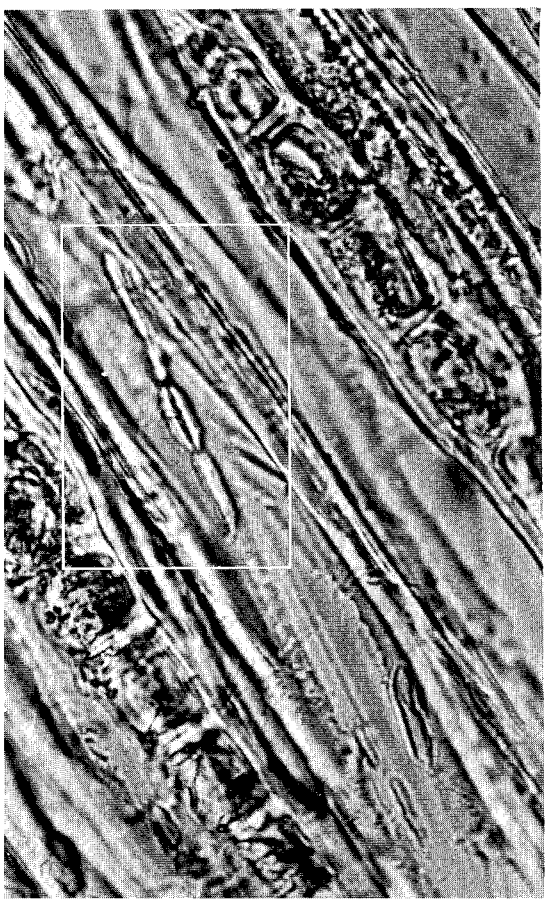


Figure 11 A. Cavity chain with three cavities formed by *Allescheria terrestris* after 70 hours' attack of birch sapwood impregnated with a 0.6 per cent solution of ammonium tartrate.
B. Cavity with bore-hole at one of the cavity's short ends. Approx. 875×.



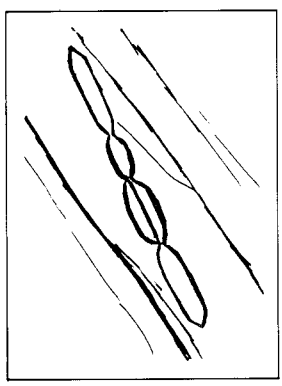


Figure 12 Cavity chain with four cavities formed by *Allescheria terrestris* after 100 hours' attack on birch sapwood impregnated with a 0.6 per cent solution of ammonium tartrate. Approx. 875×.

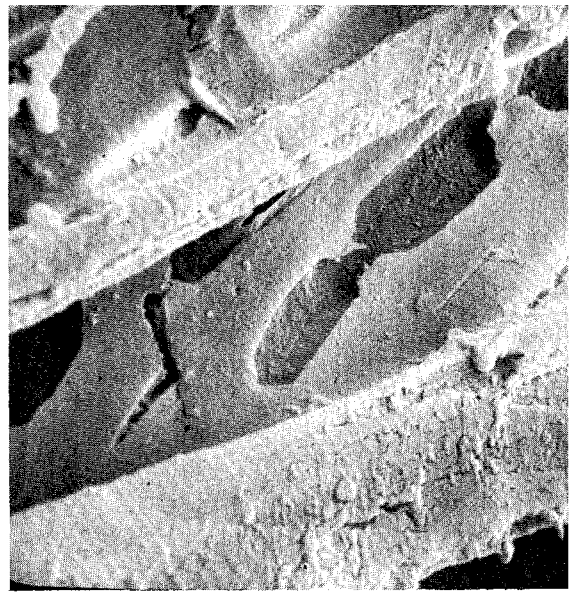


Figure 13 Cavity formed by *Allescheria terrestris* in the wall of the lumen. Approx. 10 600×. (Cambridge Stereoscan Electron Microscope.)

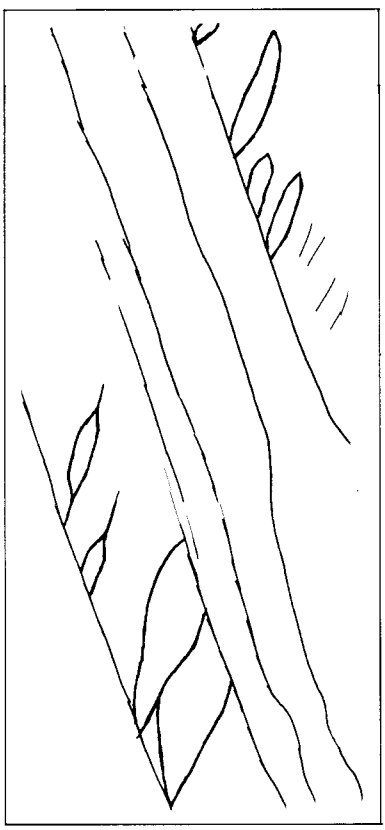
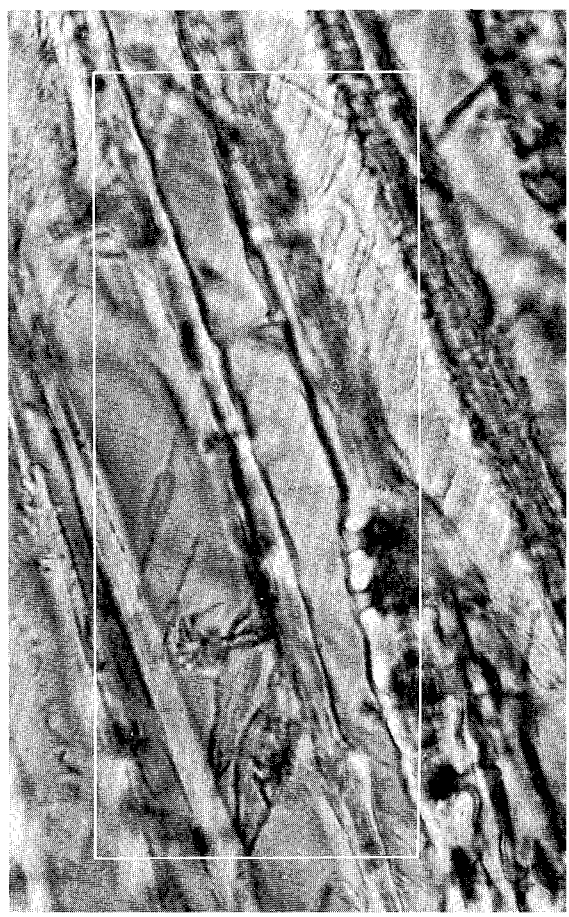
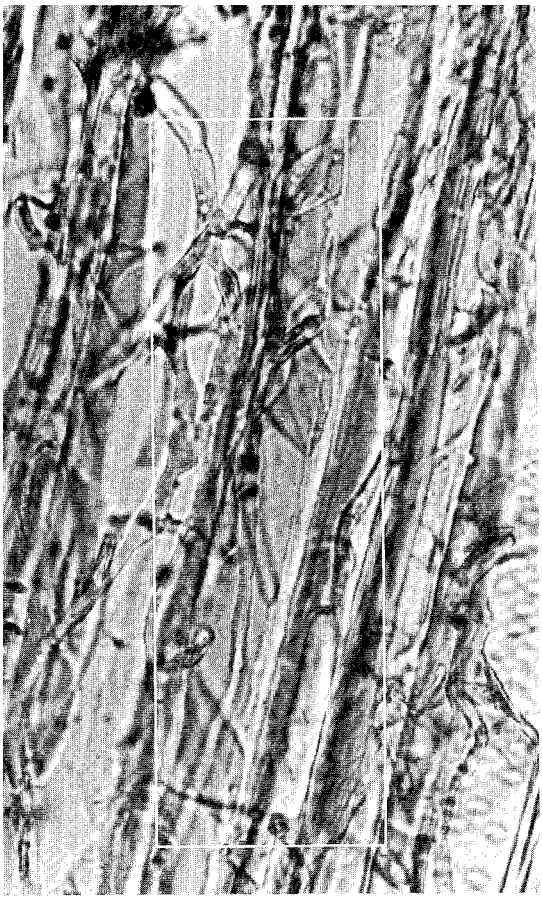


Figure 14 Cavities formed by *Margarinomyces luteo-viridis* in *Juniperis communis*. Approx. 875×.





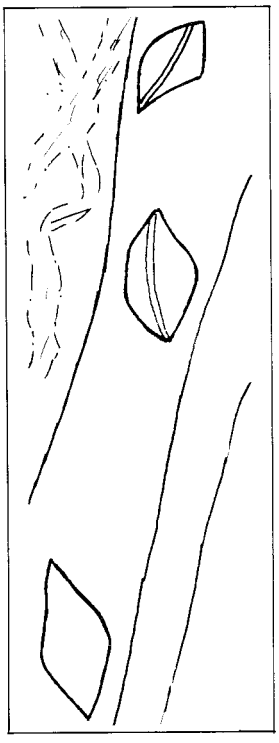


Figure 15 Cavity formed by *Margarinomyces luteo-viridis* in *Ledum palustre*. Approx. 875×.

5 Cavity formation in lignified stems

A large number of plants with lignified stems were infected with *Allescheria terrestris* and *Margarinomyces luteo-viridis* (see Table 3). A total of approximately 300 samples was studied. The summary in Table 3 shows that the ability to form cavities in the various woods differs between the two fungi tested. This is especially true of softwood, where the capacity of *Allescheria terrestris* to form cavities is poorer than that of *Margarinomyces luteo-viridis*.

A number of investigations, such as those by Savory (1954), Greaves and Levy (1965) and Findlay (1970), have shown that cavity formation in softwood is slower than that in hardwood and may be completely absent. Variations in the intensity of cavity forma-

Table 3. Cavity formation in plants with lignified cells, exposed to attack by *Allescheria terrestris* at 45° C and *Margarinomyces luteo-viridis* at 30° C for approx. 1 month.

Species	Allescheria terrestris	Margarinomyces luteo-viridis
Acer platanoides L.	+ + +	+ + +
Andromeda polifolia L.	0	+
Alnus glutinosa (L.) Gaertn.	+ +	+ + +
Betula nana L.	+ + +	+ + +
Betula tortuosa Ledeb.	+ + +	+ + +
Corylus avellana L.	+ + +	+ + +
Fagus silvatica L.	+ + +	+ + +
Fraxinus excelsior L.	+ +	+ +
Hedera sp.	+ + +	+ + +
Ledum palustre L.	+[2]	+ +
Parthenocissus tricuspidata (Siebold & Zucc.) Planchon.	+ + +	+ + +
Populus tremula L.	+ +	+ +
Prunus spinosa L.	+ + +	+ + +
Quercus sp.	+ + +	+ + +
Salix caprea L.	+ +	–
Salix fragilis L.	+ +	+ +
Salix pentandra L.	+ +	–
Sambucus racemosa L.	+ + +	+ + +
Sorbus aucuparia L.	+ + +	+ + +
Tilia sp.	–	+ + +
Ulmus sp.	–	+ +
Vaccinium myrtillus L.	0	+
Juniperus communis L.	[1]	+
Picea abies (L.) H. Karst.	0	+[2]
Pinus silvestris L.	+[2, 3]	+

\+ sparse
\+ + occasional
\+ + + plentiful
0 none occurring
– not studied

[1] Cavities not found with complete certainty.
[2] Only upon impregnation with 0.6 % NH_4NO_3.
[3] After 2 months of rotting.

tion in hardwood are also discussed. The fact that softwood is more resistant to the attack of soft rot fungi than hardwood is assumed to be due to the higher lignin content, to a different chemical composition of the lignin, etc.

The present study, however, showed that even if there were great differences in the intensity of cavity formation, the general appearance of the cavities formed in the various plant species did not differ from those formed in birch wood.

The orientation of the cavities and cavity chains varied widely among the various types of plant but also within the same species of plant. On the other hand, in *Vaccinium myrtillus, Juniperus communis, Andromeda polifolia, Parthenocissus tricuspidata* and *Ledum palustre* the cavities encountered were always orientated at an angle of approximately 30° to 60° in the wood cell (Figures 14 and 15). The cavities were usually orientated in the same manner in *Betula nana*. Orientation of the cavities and cavity chains is probably highly dependent on the direction of the microfibrils in the cell wall (Courtois 1963, Levy 1965). It should therefore be possible to determine approximately the direction of the fibrils in the cell walls of the attacked wood fibres, on the basis of the orientation of the cavities. Thus, the microfibrils in the four above-mentioned plants should primarily be found within the approximate angle of 30° to 60° in the section of the secondary cell wall where the cavities were formed.

6 Discussion

If rot conditions are very favourable, with a high moisture content in the wood and an optimal growth temperature, the soft rot fungi employed in this study form cavities in birch wood fibres very rapidly after the mycelium has grown into the wood. *Allescheria terrestris,* with which the study primarily deals, forms individual cavities two days after the wood comes into contact with the mycelium. After an additional day, some individual cavity chains are formed. A week after the penetration of the mycelium into the wood, cavities and cavity chains appear in great numbers in the wood which was first attacked. *Margarinomyces luteo-viridis* forms cavities just as rapidly as *Allescheria terrestris,* while *Phialophora richardsiae* is a soft rot fungus which forms cavities at a somewhat slower rate. The holes in the form of cavities and bore-holes, which the mycelium creates in the walls of the wood cells, reduce the strength of the wood even at an early stage of attack. This occurs long before a weight loss can be registered in the wood (Liese and Pechmann 1959, Liese and Ammer 1964, Henningsson 1967). This is also true of several of the soft rot fungi studied.

Two types of attack, 1 (cavity formation) and 2 (erosion) were described by Corbett (1965). Of these, type 1 occurred most frequently in the wood fibres during this study. Type 1, with the typical "T-shaped branching" appearance, often occurs in somewhat different forms in that the bore-hole is not always symmetrically situated on the long edge of the cavity. The hypha which forms the lateral cavity sometimes grows farther along the opposite long side of the cavity after, or in connection with, cavity formation.

The fungal hypha's actual penetration of the cell wall, especially by means of pits, and whether this is passive or active, has been studied and discussed by Corbett (1965), Levy (1965), Greaves and Levy (1965), Levy and Stevens (1966), Findlay (1970) and others. The problem of studying the hypha itself before and during penetration of the cell wall and the formation of an initial cavity with the aid of a light microscope, is technically rather difficult. However, it is apparent that cellulolytic activity exists in the hypha when the initial cavity is enlarged.

The role of the plasmodesmata during a hypha attack (type 1) on a wood cell has been discussed by Levy (1965) and Levi (1966) and others. Since at that time no known studies of the plasmodesmata indicated that they passed through the fully formed walls of the wood cells, the significance of the plasmodesmata for the hypha's penetration of the cell wall was uncertain. Corbett (1965) considered that the hypha only attacks enzymatically and is not dependent on plasmodesmata or any other "physical discontinuity".

According to recent literature plasmodesmata form during the embryonic development of the cells and constitute the connections between the cells. During the growth of the cells, the plasmodesmata collect within certain limited areas and form the pits of the cells, Cronshaw (1964), Clowes and Juniper (1968).

The question is whether all plasmodesmata in the cell are utilised for pit formation or if some remain and are incorporated into the cell wall. Robards's (1968) electron-microscopic photographs of the plasmodesmata in the ray cells of *Salix fragilis* show that the plasmodesmata are present in very large numbers, which is a prerequisite for the existance of surplus plasmodesmata after pit formation. Burgess (1971) has

established that the plasmodesmata are reduced during the growth of the cells, which should indicate that they are incorporated into the cell walls.

The existance of plasmodesmata which constitute a break in the heavily lignified sections of the cell walls, is an important condition which is of advantage to the hyphae when attacking the cell walls.

The following very hypothetical explanation can be given for the right-angled bore-holes made in most cases by the mycelium in the cell wall. In cases where diagonal penetration occurs, this may also be the result of plasmodesmata since there are anastomic forms (Robards, 1971). As the wood cell ages, new layers are laid down on the cell wall, and the plasmodesmata which are not incorporated into the pits become covered. Within these plasmodesmata the endoplasmic reticulum passes through the plasmodesmata (Robards, 1971, and others) and possibly at their openings, substances may be stored which may conceivably be diffusible and attractive to the mycelium. At moisture contents in excess of the fibre saturation point, the diffusible elements expand and penetrate the reinforced cell walls, entirely or in part. The mycelium is stimulated and grows up to the plasmodesmata and through them, penetrating the middle lamella and thus forming the central right-angled bore-hole. Once in the new wood cell, the mycelium can either branch out or grow further towards the lumen. Branching out may be presumed to occur when the mycelium must again find its way along the new cell wall.

Type 2, erosion of the cell wall from the lumen, was not observed during the earliest attack of the mycelium on the cell wall, where only cavities were noted. On the other hand, erosion of the cell walls appears after a longer period of attack. In certain cases it is difficult to determine whether pure erosion takes place. A larger number of hyphae can sometimes develop in the S_2 layer and destroy the cell wall towards the lumen, so that an erosion-like pattern is formed in the walls of the wood cell.

The rate at which the mycelium forms cavities in the test blocks has not yet been attained in the thin wood shavings which were used for studies of cavity formation *in vitro*. This may result from several causes. The intense heat radiation during microscopy and photographing may disturb the growth of the mycelium by drying out the wood. The mycelium itself may be damaged by the light. Since there are fewer intact wood cell rows in a shaving some 25 μ thick, there may have been too few wood cells. The hyphae simply grow on the outside of the wood.

Findlay (1970) has also described a technique for studying cavity formation *in vitro*. The procedure is simple in itself. Thin wood shavings are placed on a sheet of glass. Another sheet of glass is placed over the wood shaving. The wood shaving is moistened by means of a wick from a water container. The basic idea is the same as Lundström's (1970), i.e. to study cavity formation by means of thin wood shavings. Unfortunately, Findlay's cultivation technique has not proved very successful. The reason for this may again be that the mycelium is damaged by the light.

Existing studies show that soft rot fungi possess great cavity-forming capacity in lignified cells of various plants. Schacht noted cavities in wood cells of plants as early as 1863. Cavity formation in wood cells from a large number of trees has been reported by Duncan (1960), Courtois (1963), Levy (1969), Findlay (1970) and others. All of the cases referred to here concern dead wood cells. Cavity formation in wood cells from living plants is almost unknown. Oliver (1959) has, however, reported formations resembling cavities in living *Guarea* sp. and *Lovoa klaineana* Pierre.

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Sammanfattning

Mögelrötesvamparna angriper vedceller på två olika sätt, dels genom att erodera cellväggen direkt inifrån cellhåligheten varvid cellväggen förtunnas, dels genom att växa fram i cellväggens S_2-skikt och där bilda håligheter s.k. kaviteter. Variationer av dessa angreppsbilder förekommer hos olika mögelrötesvampar. De håligheter i form av kaviteter och borrhål, som mycelet skapar i vedcellernas väggar redan i tidigt skede vid rötangrepp, sänker vedens hållfasthet avsevärt och detta långt innan större viktsförluster av veden registrerats.

I denna studie har tidsförloppet speciellt studerats ifrån det att en mögelrötesvamp infekterat björkved till dess att mycelet bildat de första kaviteterna. Studierna har utförts på laboratoriet under olika optimala rötbetingelser för testsvamparna.

De studerade mögelrötesvamparna är en termofil Ascomycet *Allescheria terrestris* Apinis stam Apinis och stam H63-1, två Fungi Imperfecti *Margarinomyces luteoviridis* v. Beyma stam Beyma och stam M74-IV samt *Phialophora richardsiae* (Nannf.) Conant stam BB40-V. Studierna har främst gällt *Allescheria terrestris* varvid de två övriga mögelrötesvamparna har använts i jämförande studiesyfte.

Svamparna framodlades på snedagarrör i den optimala längdtillväxttemperaturen för varje svamp. Klotsar ($2 \times 0{,}5 \times 0{,}5$ cm) från splintveden av *Betula verrucosa* Ehrh. eller *Betula pubescens* Ehrh. lades därefter ut på det utvuxna mycelet. Vid mikroskopstudierna har klotsarna snittats med rakblad och färgats in med safranin löst i 70 % alkohol. Ett Cambridge Stereoscan Electron Microscope har också använts i mindre omfattning.

Den sparsamma tillgången på kväve i veden har visat sig vara en begränsande faktor för rötsvamparnas förmåga att angripa veden. Därför har kvävets roll vid kavitetsbildningen undersökts. Därvid har kavitetsbildningen tidsmässigt jämförts i obehandlad resp. kväveimpregnerad (ammoniumklorid, ammoniumnitrat, ammoniumtartrat, asparagin och kaseinhydrolysat) ved.

Phialophora richardsiae påverkades mest om kväve tillsattes i veden, då svampen bildade kaviteter efter 3 dygn mot 6 dygn i obehandlad ved. *Allescheria terrestris* och *Margarinomyces luteo-viridis* bildade kaviteter 2 dygn efter det att de infekterat veden oberoende om kväve tillsattes eller ej (figur 2). I kväveimpregnerad ved kom dock kavitetsbildningen i gång jämnare och i större omfattning än i obehandlad ved (tabell 1).

En vanlig angreppstyp vid mögelrötesvampars angrepp på cellväggen, är att hyfen växer in i en mer eller mindre 90° vinkel in i cellväggen och därefter förgrenar sig i fiberns bägge längdriktningar. Därmed bildas ett "T-format angrepp" i vedcellens sekundära cellvägg (figur 3). Hyfen och det efterlämnade borrhålet kommer därvid att ansluta mitt på initialkaviteten. Denna angreppsbild är mycket vanlig och har beskrivits tidigare. I denna undersökning har det emellertid visat sig att borrhålet kan ansluta var som helst utefter kavitetens sida (figurerna 4, 5, 9, 11).

En metod att följa kavitetsbildningen *in vitro* har utarbetats och publicerats separat (Lundström, 1970). Den går ut på att låta mycelet angripa tunna träspån utan att störa kavitetsbildningen. Träspånet sitter monterat över ett vertikalt fastsatt glasrör, som är fäst i botten på en glaspetriskål. Svampen ympas på maltagar som har ingjutits i petriskålen. Glasröret håller undan maltagarn så att träspånet kan belysas underifrån. Odlingen sker helt sterilt och fukten i träspånet (som är en absolut nöd-

vändighet för att mycelet skall angripa veden) uppsugs från agarn. Vissa problem kvarstår dock att lösa i samband med dessa studier, då mycelet störs av vissa yttre faktorer såsom ljus, värme m.m.

Allescheria terrestris och *Margarinomyces luteo-viridis* kavitetsbildande förmåga har studerats i 25 olika växter med förvedade celler (figurerna 14, 15). Kavitetsbildningen visade stora skillnader mellan barrved, där den var sparsam, och lövved, där den oftast skedde rikligt (tabell 3).