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Interactions between micro-organisms found in birch and aspen pulpwood

Samspelet mellan mikroorganismer från björk- och aspmassaved

by

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I. Introduction

Wood, although it is a comparatively poor substrate for micro-organisms, may under certain conditions be invaded by a number of microbes, e.g. when it is stored as unpeeled pulpwood (see Tab. 1), when it is in contact with the soil (MERRILL & FRENCH 1966) or when it is stored as pulpwood chips (BERGMAN & NILSSON 1966). While the invading microflora consists mainly of fungi, usually bacteria and sometimes even actinomycetes occur in the wood (KNUTH & MCCOY 1962, BERGMAN & NILSSON 1966, SHIGO 1965 and 1966). Many of these organisms live intermixed with one another or colonise adjacent parts of the wood. Thus they live in more or less intimate contact, and are in a position to influence one another's activities.

Studies on wood-destroying micro-organisms have generally been focussed on single cultures of various species. Certain species, as for instance many *Basidiomycetes*, have frequently been investigated, whereas others—*Ascomycetes, Fungi Imperfecti* and especially *Bacteria* and *Actinomycetes*—have only recently become of interest. The study of the very important inter-relationships of these microbes—the microecology of wood—has been greatly neglected in the past, although inspiring experiments with mixed cultures of wood-inhabiting fungi were made at the beginning of this century (HARDER 1911, ZELLER & SCHMITZ 1919). During the past few years, however, a more intense interest has arisen in wood microecology and in the phenomena of interaction in wood, as witness the increasing number of scientific papers in this field.

When the inter-relationships of two or more organisms have been studied, antagonistic activity has usually been recorded. Thus *Coryne sarcoides, Trichoderma viride, Scytalidium* spp., *Tympanis hypopodia, Gliocladium* sp, *Cryptosporiopsis* sp. and various wood bacteria are known to exhibit antagonistic activity against decay fungi. (ETHERIDGE, 1957; PERSSON-HÜPPEL, 1963; SHIELDS & ATWELL, 1963; KLINGSTRÖM, 1965; SHIGO, 1965, BASHAM, 1966; STILLWELL, 1966 and BERGMAN & NILSSON 1967). Reports of stimulation effects are much rarer in the literature. However, such effects in wood-inhabiting microbes have been demonstrated by, amongst others, FRIES (1938), BOURCHIER (1961) and PENTLAND (1965).

II. Materials and methods

The same fungal strains as were used in the physiological studies reported by HENNINGSSON (1967 b) were also used in the following interaction studies. The bacteria, which were all of the Bacillus-type, were isolated from pulpwood of birch and aspen *(Betula verrucosa, Betula pubescens* and *Populus tremula)*, by placing sterilely-taken increment cores on malt agar plates. This substrate was chosen for isolation of the wood-inhabiting fungi, but is certainly not the most suitable one for culturing bacteria in general. However, the wood bacteria concerned grew excellently on malt agar. It may be worth mentioning that their pH-optima varied between pH 5 and pH 7, which is surprisingly low for bacteria.

The organisms were allowed to grow together on agar substrate, in fluid media or in sapwood of birch and aspen. The composition of the media, the size of the test blocks and the incubation conditions were identical with those reported by HENNINGSSON (1967 b). The reactions were continuously observed and measured as increase or decrease in radial growth, mycelial yield or weight loss of the attacked wood samples.

III. Results

A. Observation of the microflora in unpeeled birch pulpwood

As was pointed out in the introduction, many different micro-organisms invade wood under suitable conditions. Tab. 1 presents the microflora which developed in unpeeled birch pulpwood during 30 months' outside storage. As regards material, storage conditions etc., see HENNINGSSON (1967 e). The example in Tab. 1 demonstrates that decay fungi could already be isolated from the wood after three months' storage. It is further shown that during the storage period the decay fungi invaded the pulpwood in a certain sequence. The first decay fungi to invade the wood were the *Corticium* species (*C. laeve, C. confluens* and perhaps others), *Libertella betulina* and *Stereum purpureum.* After the invasion of these primary decay fungi, *Stereum hirsutum, Polyporus zonatus* and *Polyporus hirsutus* became established in the wood. Among the decay fungi which were not recorded until a late stage, *Lenzites betulina, Panus torulosus, Polyporus adustus* and *Stereum rugosum* may be mentioned. It should also be noted that bacteria and "other fungi" were isolated during the whole period of storage. "Other fungi" is a group consisting of unidentified, mostly filamentous, non-*Basidiomycetes,* which were isolated from the wood as mycelia.

A further interesting observation made was that the total number of micro-organisms recorded per increment core taken increased up to 18 months' storage; thereafter it decreased, and the *Corticium* species were not isolated as frequently as before. Instead, species such as *Stereum hirsutum* and *Polyporus zonatus* were most frequently isolated. This indicates that the wood was invaded by an increasing number of micro-organisms until a certain stage was reached at which a few competitive organisms tended to predominate over the others.

B. Interactions between decay fungi

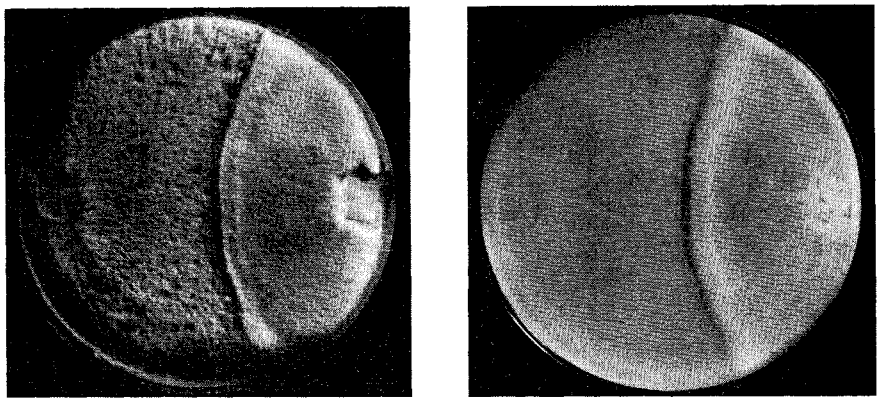
Of all micro-organisms which invade wood, the decay fungi are doubtless responsible for causing the most damage. Consequently, interactions between the various decay fungi or between decay fungi and other micro-organisms are of particular interest from the point of view of wood preservation.

Tab. 1. Micro-organisms occurring in birch pulpwood during 30 months' storage.

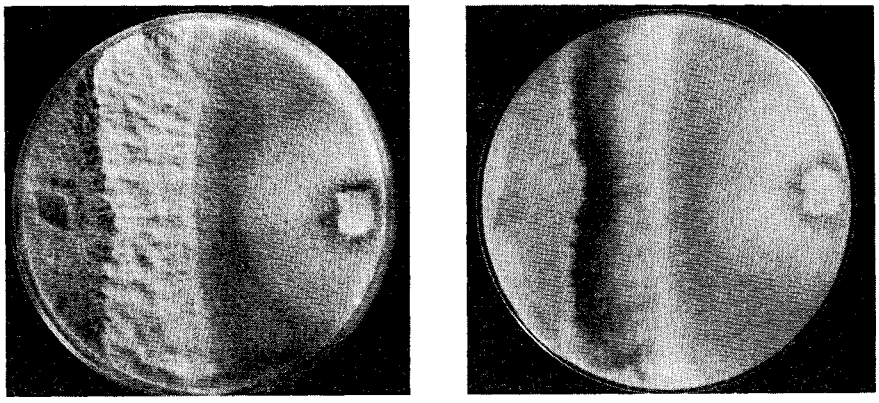
The experimental material was cut and piled in April 1963 at Ryd in south-eastern Sweden. Owing to the sampling technique, moulds such as *Penicillia*, *Aspergilli* and others growing superficially only have not been reported. Earliest observation of a species is indicated by underlining.

Storage time months	Micro-organisms isolated from the wood or observed as sporophores on the wood
3	Corticium spp., Libertella betulina, Stereum purpureum, Stereum hirsutum, other fungi (2), bacteria (1).
6	Corticium spp., Libertella betulina, Stereum purpureum, Peniophora incarnata, unknown Basidiomycete (1), Trichoderma sp, other fungi (4), bacteria (2).
12	Corticium spp., Libertella betulina, Stereum purpureum, Stereum hirsutum, Peniophora incarnata, Polyporus zonatus, Trichoderma sp, other fungi (3), bacteria (1).
15	Corticium spp., Libertella betulina, Stereum purpureum, Stereum hirsutum, Peniophora incarnata, Polyporus zonatus, Polyporus hirsutus, Tremella foliacea, Trichoderma sp., other fungi (2), bacteria (2).
18	Corticium spp., Libertella betulina, Stereum purpureum, Stereum hirsutum, Stereum rugosum, Stereum sanguinolentum, Peniophora incarnata, Polyporus zonatus, Polyporus hirsutus, Polyporus adustus, Polyporus sp., Tremella foliacea, Schizophyllum commune, Phlebia radiata, Coryne sarcoides, Phialophora sp., Trichoderma sp., other fungi (5), bacteria (2).
24	Corticium spp., Libertella betulina, Stereum purpureum, Stereum hirsutum, Stereum rugosum, Peniophora incarnata, Polyporus zonatus, Polyporus hirsutus, Polyporus adustus, Lenzites betulina, Tremella foliacea, Panus torulosus, Ceratocystis sp., Trichoderma sp., other fungi (4), bacteria (3).
30	Corticium spp., Libertella betulina, Stereum hirsutum, Stereum rugosum, Stereum sp., Peniophora incarnata, Polyporus zonatus, Polyporus hirsutus, Polyporus adustus, Lenzites betulina, Schizophyllum commune, Hypoxylon multiforme, Tremella foliacea, Panus torulosus, Trichoderma sp., other fungi (4), bacteria (2).

Interactions between decay fungi growing on malt agar were studied, the fungi being inoculated opposite each other in petri dishes. Four dishes of each combination were incubated at 10°, 23° and 32°C for two months, during which period the dishes were continuously observed. Tab. 2 summarises the results, from which the following conclusions may be drawn:



23°C



30°C

Fig. 1. Interaction phenomena between *Stereum purpureum*, inoculated to the left, and *Polyporus versicolor*, inoculated to the right, growing together on malt agar plates at two temperatures. The two plates to the left are seen from the upper side and the two plates to the right from the under side. Note that *Polyporus versicolor* is overgrowing *Stereum purpureum* and that the bluish zones are formed under the overgrown part of the latter colony.

1. Mycelia from identical strains easily grew into each other and intermingled.

2. Mycelia from *Polyporus zonatus, Polyporus versicolor* and *Lenzites betulina* easily grew into each other and intermingled.

3. In most combinations, one of the two species in a dish finally suppressed or destroyed the other.

4. The temperature had a decisive effect on the development of interaction phenomena.

5. *Corticium laeve* was almost always suppressed by the other decay fungi.

Tab. 2. Interaction phenomena between decay fungi growing together on malt agar plates.

Fungus	Temp. C°	Corticium leave	Stereum purpureum	Stereum hirsutum	Lenzites betulina
Polyporus betulinus	10	2, 6 weak brown	3 P.b. 7 S.p., yellow-brown	4, 6 yellow-brown	3 L.b., (4), 6 brown
	23	3 P.b., 7 C.l.	3 P.b., 7 S.p. 6 yellow	3 S.h., 6 brown	4, (3 L.b.), 6 yellow-brown
	32	No growth C.l.	Too slow growth	Too slow growth	5, 6 yellow
Polyporus marginatus	10	3. P.m.	3 P.m., 7 S.p.	4, 6 yellow	3 L.b.
	23	3. P.m.	3 P.m., 7 S.p.	3 P.m. (4), 6 yellow	3 P.m., (4)
	32	No growth C.l.	3 P.m.	5, 6 yellow-brown	3 P.m., (2), 6 grey-brown
Polyporus hirsutus	10	3 C.l.	3 S.p., 6 yellow	3 S.h., 6 yellow	3 L.b., 6 weak brown
	23	3 P.h.	3 S.p., 6 brown	3 S.h., 6 yellow-brown	3 L.b.
	32	No growth C.l.	3 P.h., 6 blue-brown	5	5, 6 yellow
Polyporus versicolor	10	3 P.v.	3 P.v., 6 blue-green	3 P.v., 6 yellow	2
	23	3 P.v.	3 P.v., 6 black-blue	4, (3 P.v.), 6 yellow	2
	32	No growth C.l.	3 P.v., 6 black-brown	4, (3 P.v.), 6 brown	2
Polyporus zonatus	10	3 P.z., 6 yellow	3 P.z., 6 blue-green	3 P.z., (4), 6 yellow	2
	23	3 P.z.	3 P.z., 6 blue-black	3 P.z., 6 yellow	1, (2)
	32	No growth C.l.	4, 6 blue-brown	4, 6 yellow	2
Polyporus adustus	10	2, (3 P.a.)	3 S.p.	3 S.h., 6 yellow	3 L.b.
	23	3 P.a.	3 P.a., (2), 6 yellow	3 P.a., 6 yellow	3 P.a., (4)
	32	No growth C.l.	3 P.a.	3 P.a., (5), 6 brown	3 P.a., 6 yellow
Lenzites betulina	10	3 L.b., 6 yellow	4 (2)	3 L.b., 6 yellow	1
	23	3 L.b., 6 yellow	3 S.p., 6 yellow-brown	4, 6 yellow-brown	1
	32	No growth C.l.	4, 6 brown	5, 6 yellow-brown	1
Stereum hirsutum	10	2, (3 S.h.)	4, 6 yellow-brown	1	
	23	3 S.h.	4, (3 S.h.), 6 blue-brown	1	
	32	No growth C.l.	Too slow growth	Too slow growth	
Stereum purpureum	10	2, (3 S.p.) 6 grey-yellow	1		
	23	3 S.p., 7 C.l., 6 yellow-brown	1		
	32	No growth C.l.	Too slow growth		
Corticium leave	10	1			
	23	1			
	32	No growth			

Polyporus adustus	Polyporus zonatus	Polyporus versicolor	Polyporus hirsutus	Polyporus marginatus	Polyporus betulinus
3 P.b., 7 P.a., 6 yellow-brown	3 P.z., 6 yellow-brown	3 P.v., 6 yellow-brown	4	4, 6 brown	1
3 P.b., (2), 6 yellow-brown	3 P.b., (4), 6 yellow	3 P.v., (4), 6 yellow	4, 6 yellow-brown	4, 6 yellow-brown	1
3 P.a. 6 grey-brown	4, 6 yellow-grey	4, 5, 6 yellow-brown	3 P.h.	3 P.m., 6 yellow	Too slow growth
3 P.m.	4, 6 weak yellow	2, 6 weak brown	3 P.m. 6 weak yellow	1	
3 P.m.	3 P.m., 6 yellow	2, (3 P.m.), 6 yellow	3 P.m., 6 yellow	1	
3 P.m.	3 P.m.	3 P.m., 6 yellow	5, 6 brown	2	
3 P.a.	5, (3 P.z.)	4, 5, 6 yellow	1		
3 P.a., 6 weak yellow	4, (3 P.h.)	4, 6 brown	1		
3 P.h., 6 yellow-grey	5, 6 yellow-brown grey-green	4, 6 yellow	2		
3 P.v.	1	2			
3 P.v.	2	1			
3 P.a., 6 yellow	2	1			
3 P.z.	1				
3 P.a., (2), 6 weak-brown	2				
3 P.a., 6 yellow-brown	1				
1					
1					
1					

Explanation:

1. Mycelia advancing into each other without visible difficulty.
2. Growth of the mycelia temporarily stopped at or before reaching contact. After a certain period the mycelia grow into each other without any sign of dominance.
3. One mycelium dominates and overgrows the other mycelium either directly or after a temporary delay after reaching contact. In the table, e.g. 3 P.m. means that *Polyporus marginatus* predominated over the other fungus.
4. Growth of both mycelia permanently (at least two months) stopped after reaching contact.
5. Growth of both mycelia permanently stopped before reaching contact, resulting in an antibiotic zone between the mycelia.
6. Formation of coloured zones in the substrate when the mycelia approach or reach one another.
7. One of the mycelia undergoes intensive lysis after reaching contact, resulting in the formation of a lysis zone. In the table, e.g. 7 S.p. means that mycelium of *Stereum purpureum* was lysed by the action of the opposite mycelium.

6. *Polyporus marginatus, Polyporus zonatus, Polyporus versicolor* and *Lenzites betulina* were the most competitive species.

7. *Polyporus hirsutus* and *Polyporus adustus* were most competitive at the highest temperature studied.

8. The formation of coloured contact-zones in the substrate was a very common phenomenon.

9. Yellow-brown zones were formed in all combinations in which *Stereum hirsutum* was a partner.

10. Bluish zones were formed when *Polyporus hirsutus, Polyporus versicolor* or *Polyporus zonatus* was cultured against *Stereum purpureum* (Fig. 1).

Many of these results seem to correspond with phenomena observed in stored pulpwood in the field. The formation of coloured zones between two fungi is, for instance, very common in stored pulpwood of birch and aspen. Bluish zones occurring between *Stereum purpureum* and some other fungi appear greenish in the wood, and are very common.

Corticium laeve was one of the most common species isolated from the pulpwood during the first months of storage. In later stages *Stereum hirsutum, Polyporus zonatus* and other more competitive species were isolated most frequently, indicating that these species had suppressed *Corticium*. This is in good agreement with the results of the plate tests.

Polyporus adustus and *Polyporus hirsutus,* which in the plate tests appeared to be most competitive at high temperatures, were found almost exclusively in the uppermost parts of the pulpwood piles, where the temperature must often have been rather high.

The culturing of decay fungi together on a fluid medium gave results similar to those of the combined cultures on malt agar. When antagonistic fungi were cultured together as stationary floating cultures, their combined mycelial production was usually lower than that of the most rapidly growing of the two fungi. This is shown clearly in Fig. 2 for *Polyporus versicolor* and *Stereum purpureum*. Erlenmeyer flasks (100 ml) containing 20 ml of medium B (HENNINGSSON 1967 b) were used. Every flask was inoculated with two small pieces from malt agar cultures. The two inocula were taken either from the same fungus or from each of the tested fungi. The flasks were incubated at +25°C.

The coloured zones typical of certain combinations in malt agar cultures and in the wood were also obtainable in fluid media. This was, for instance, the case with the bluish-coloured zone between *Stereum purpureum* and some other fungi. However, the blue com-

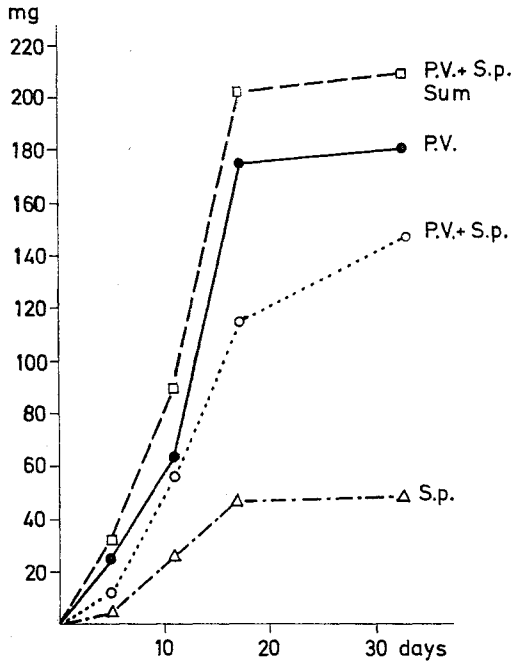
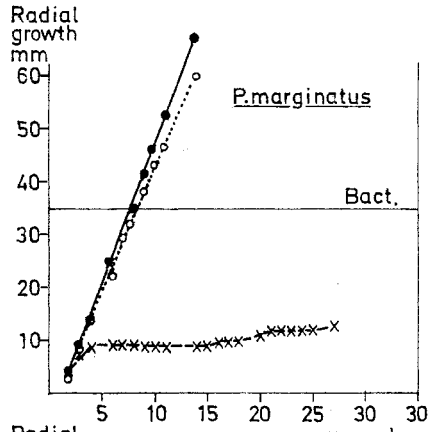


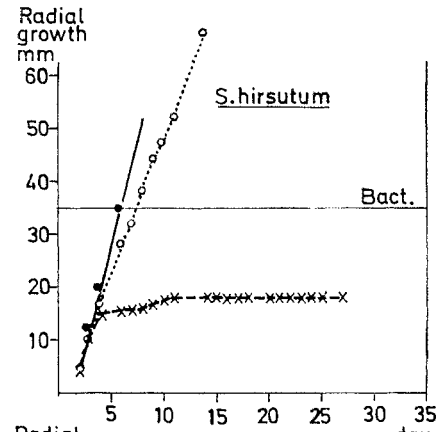
Fig. 2. Mycelial production of *Stereum purpureum* and *Polyporus versicolor* growing both separately and together in medium B.

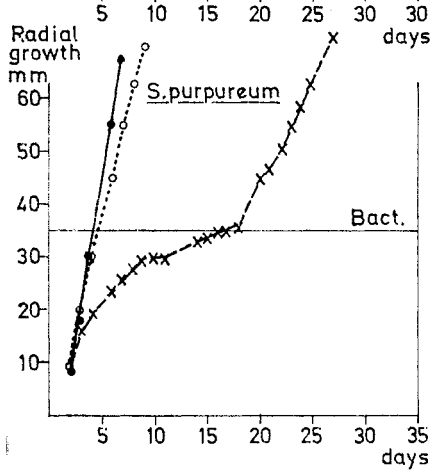
pound was not produced in shake cultures. The blue compound dissolved in ethanol or acetone and had an absorption maximum at a wave length of about 630 mμ. The blue colour was not stable in these solvents at room temperature. Complete decoloration occurred within 20 hours and after a further 24 hours, the samples had become weakly red-brown in colour. No attempt was made to identify the bluish substance.

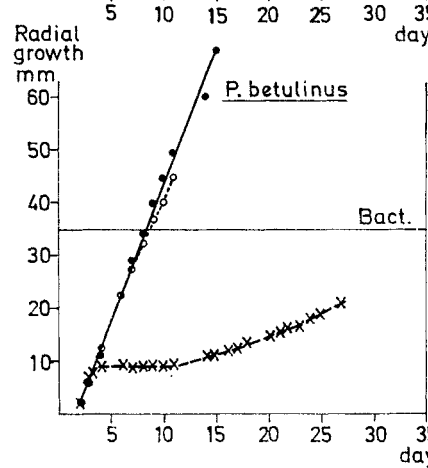
C. Interactions between bacteria and decay fungi

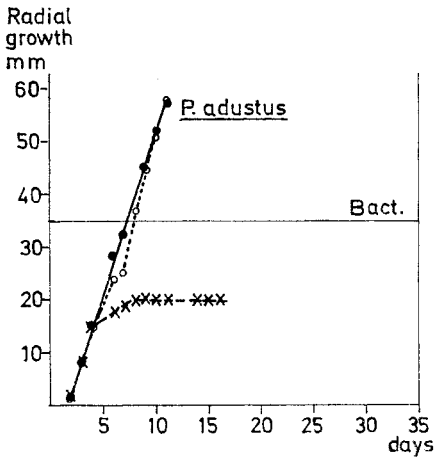
Interactions between bacteria and decay fungi were studied on agar plates, in fluid media and in wood. The antibiotic activity of two bacteria isolated from birch and aspen pulpwood against ten decay fungi was studied, using malt agar plates. A straight bacterial streak was made across the plate, 35 mm from the fungal inoculum. The plates were incubated at 23°C, and the daily radial growth of fungi was measured. The results presented in Fig. 3 show that a very strong antibiotic activity occurred in certain combinations. Under the cultural conditions used, bacterium B 64 D exhibited very little antibiotic activity as compared to bacterium A 64 Q. A complete, or extremely

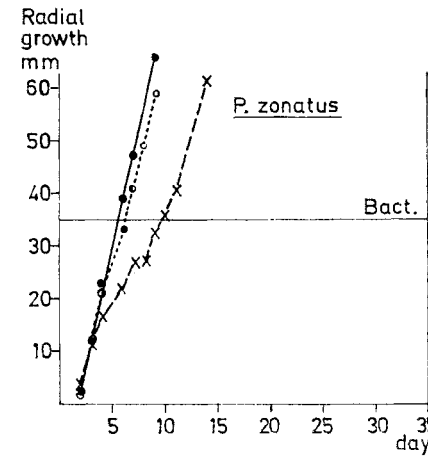

Radial growth mm
P.marginatus
Bact.
days


Radial growth mm
S.hirsutum
Bact.
days


Radial growth mm
S.purpureum
Bact.
days


Radial growth mm
P. betulinus
Bact.
days


Radial growth mm
P. adustus
Bact.
days


Radial growth mm
P. zonatus
Bact.
days

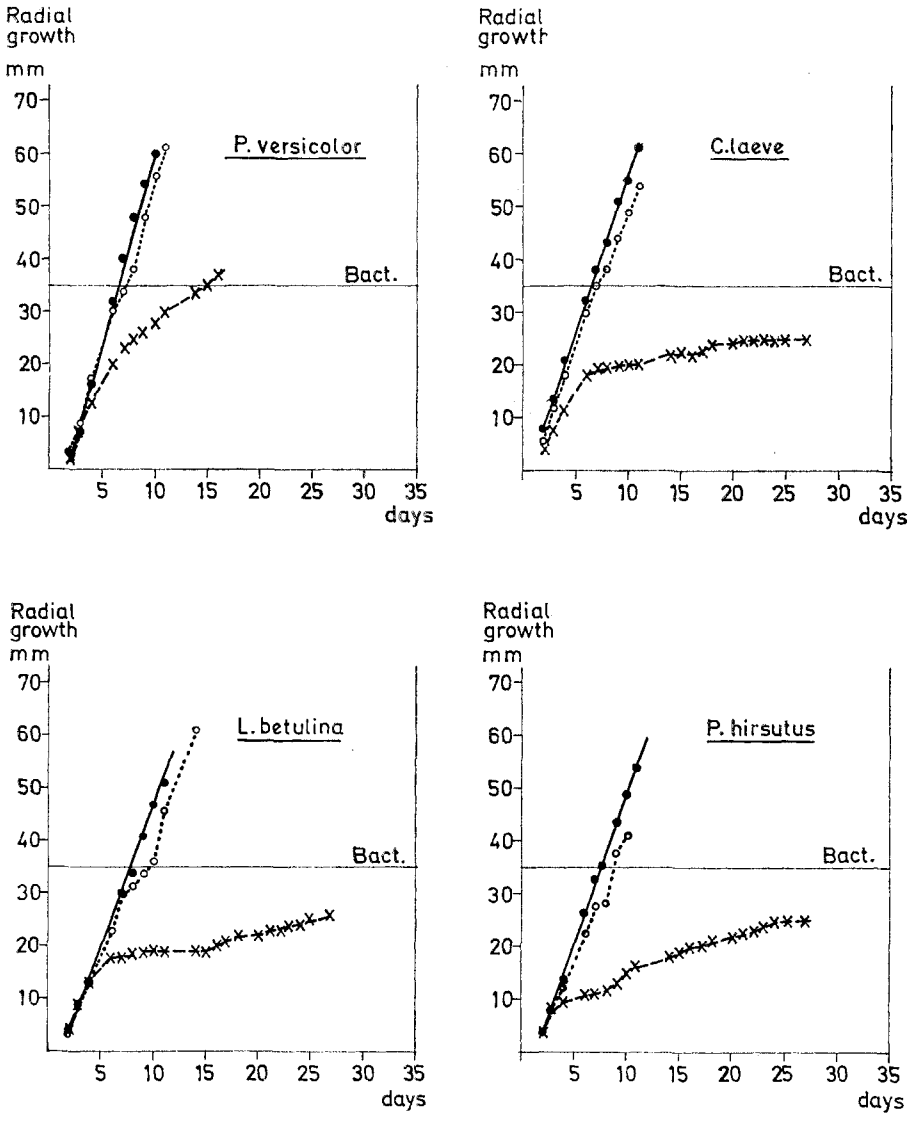


Fig. 3. Radial growth of various decay fungi on malt agar plates on which a bacterial streak was made 35 mm from the fungal inoculum. Growth of the fungi is indicated as follows: unbroken lines = plates without bacteria, broken lines = plates with bacterium A 64 Q, dotted lines = plates with bacterium B 64 D.

strong inhibition of mycelial growth was caused by A 64 Q on *Stereum hirsutum, Polyporus marginatus, Polyporus adustus, Corticium laeve, Lenzites betulina, Polyporus betulinus* and *Polyporus hirsutus,* while *Polyporus zonatus* and *Polyporus versicolor* were only slightly affected by the bacterium.

The way in which the fungi passed the bacterial cultures varied. Since the bacteria grew only superficially on the agar substrate, some fungi grew down into the agar, beneath the bacterial colony and up to the surface again on the other side. Some fungi grew straight through and others built a bridge of hyphae over the bacterial colony.

Summarising the results of these experiments, it is evident that wood bacteria can produce antibiotic substances, which, if favourable conditions exist, can diffuse and inhibit the growth of decay fungi at considerable distances from the bacterial colony.

The influence of temperature on the antagonistic activity of the two bacteria B 64 D and A 64 Q was studied in a series of experiments. A bacterial suspension was poured into the malt agar when the plates were prepared. The bacteria were allowed to develop for three days at 23°C, after which some of the plates were autoclaved. All plates were then inoculated with various decay fungi and incubated for ten days at 10°, 23° and 32.5°C.

The results showed that in plates on which the bacteria had been killed by autoclaving, the growth of the decay fungi was only slightly inhibited, or—occasionally—not inhibited at all. In plates in which the bacteria were still alive, however, there was usually a substantial inhibition of the growth of the decay fungi. The inhibition was always more pronounced at 10° and 23°C. This is clearly demonstrated in Fig. 4. These results thus indicate that the antagonistic influence was more active at low than at high temperatures.

Temperature effects similar to those in the plate tests were obtained when bacterial influence on the fungal wood decomposition was measured. Tab. 3 gives the results from a decay experiment. Four fungi and bacterium B 64 D, all occurring in birch pulpwood, were allowed to attack sapwood of birch, and three fungi and bacterium A 64 Q, all isolated from aspen pulpwood, were allowed to attack sapwood of aspen. The experiment was carried out according to HENNINGSSON (1967 b), the test samples being placed in a mixture of vermiculite and malt extract solution. The bacteria were inoculated 14 days before the fungi were inoculated. The results reported in Tab. 3 show that reduction in decay activity, caused by the presence of bacteria in the wood, was more pronounced at 10° than at 20° and 30°C. This was especially apparent when *Polyporus zonatus* attacked birch sapwood which had already been invaded by the birch bacterium B 64 D.

Several experiments on fluid media and on wood were carried out to determine the effect of the time at which the bacteria were inoculated relative to the inoculation of the fungi. It appeared that if wood were

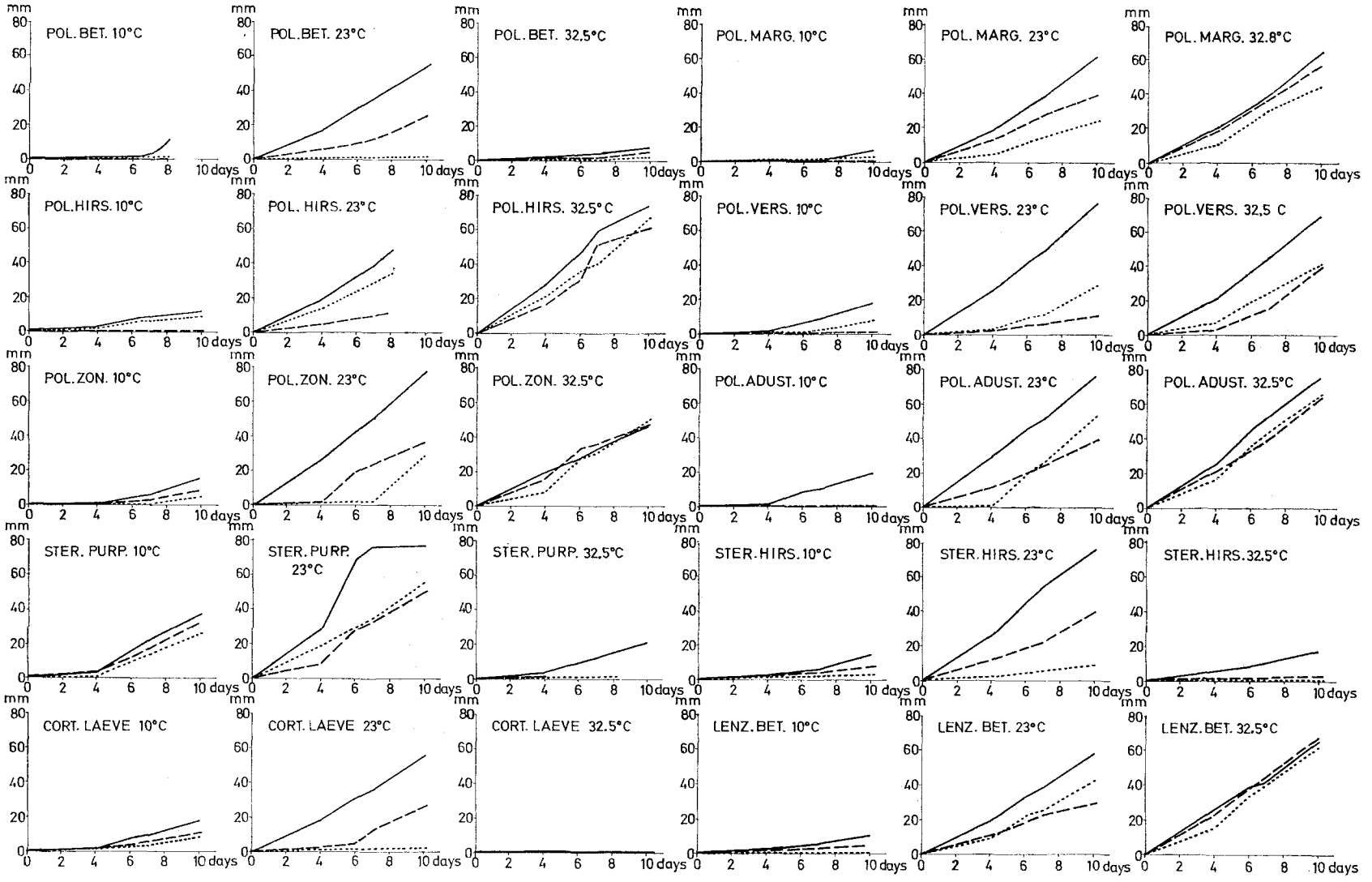


Fig. 4. Influence of temperature on radial growth of various decay fungi on malt agar plates. The bacteria were suspended in the agar and allowed to grow for three days at + 23° C before fungal inoculation. Growth of the fungi is indicated as follows: unbroken lines = plates without bacteria, broken lines = plates with bacterium A 64 Q, dotted lines = plates with bacterium B 64 D.

Tab. 3. The influence of temperature on the effect of wood bacteria on the wood decomposition produced in 60 days by various decay fungi in medium E.

The bacteria were incubated for 14 days at + 23° C before the fungal inoculation was performed. Bacterium B 64 D was used for birch sapwood and bacterium A 64 Q for aspen sapwood. S.E. = standard error.

Type of wood	Fungus	Weight loss at various temperatures					
		10°C		20°C		30°C	
		bact.	no bact.	bact.	no bact.	bact.	no bact.
Birch sapwood	Lenzites betulina	*0.0*	*2.9*	*18.4*	*18.6*	*28.2*	*35.1*
	S.E.		0.6	1.9	1.9	1.7	2.3
	Polyporus hirsutus	*0.0*	*6.5*	*19.8*	*24.2*	*29.5*	*32.5*
	S.E.		0.8	1.1	1.9	1.2	1.4
	Polyporus versicolor	*1.1*	*13.1*	*35.6*	*41.1*	*36.7*	*42.5*
	S.E.	0.5	1.0	1.3	1.2	3.9	1.3
	Polyporus zonatus	*0.1*	*5.2*	*37.3*	*41.2*	*41.1*	*43.9*
	S.E.		0.7	1.0	1.5	1.1	1.4
	Bacterium B 64 D	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*
Aspen sapwood	Polyporus zonatus	*0.0*	*2.4*	*23.3*	*33.8*	*39.2*	*35.9*
	S.E.		0.5	1.4	1.3	2.5	2.1
	Schizoph. commune	*0.0*	*0.0*	*1.7*	*1.5*	*7.3*	*8.1*
	S.E.			0.3	0.3	0.6	0.7
	Stereum purpureum	*0.0*	*0.0*	*1.1*	*1.5*	*0.0*	*1.7*
	S.E.			0.3	0.3		0.1
	Bacterium A 64 Q	*0.0*		*0.0*		*0.0*	

inoculated with bacteria from two to 20 days before inoculation with the fungi, a reduction in the rate of wood decomposition always resulted. But if the bacterial inoculation was done at the same time as the fungal inoculation or after it, the results varied considerably, from a slight reduction to a significant increase in the rate of decomposition. In fluid media, a bacterial inoculation before the fungal inoculation, or at the same time as it, resulted in a substantial decrease in growth rate, or even in a complete inhibition of growth. If the bacteria were inoculated some days after the fungi, there was usually only a slight influence, or no influence at all, on fungal growth. These results are illustrated by the data in Tab. 4 and Fig. 5.

The results of a combined attack by three micro-organisms on birch

Tab. 4. The influence of the time of bacterial inoculation on the wood decomposition produced by three decay fungi in medium E.

Stereum purpureum and *Polyporus zonatus* were grown on aspen sapwood and *Stereum hirsutum* on birch sapwood. S. E. = standard error.

Inoculation schedule	Incubation time			
	2 months		4 months	
	weight loss	S.E.	weight loss	S.E.
S. purpureum alone	1.3	0.13	3.4	0.23
» A 64 Q at the same time	2.0	0.18	3.0	0.22
» » 10 days later	1.8	0.12		
» » 18 » »	2.7	0.20		
» » 24 » »	2.4	0.19		
» » 2 months later			4.3	0.31
P. zonatus alone	31.7	1.1		
» A 64 Q at the same time	28.3	1.4		
» » 10 days earlier	23.9	1.4		
» » 20 » »	21.1	2.0		
S. hirsutum alone	18.8	0.81		
» A 64 Q at the same time	17.0	1.2		
» » 10 days later	25.0	1.0		
» » 18 » »	22.2	1.1		
» » 24 » »	23.6	0.85		

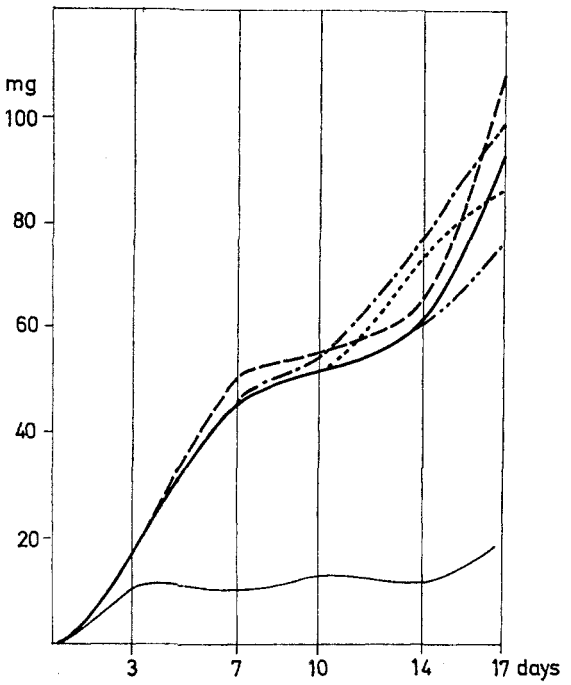


Fig. 5. The influence of the time of bacterial inoculation on the growth of *Stereum hirsutum* in medium B. Bacterium B 64 D was inoculated at the same time as the fungal inoculation or 3, 7 or 10 days after the fungal inoculation.

Tab. 5. The result of a combined attack by three micro-organisms on birch and aspen sapwood.

The bacteria were inoculated 14 days after inoculation of the fungi. S. E. = standard error. Incubation time: 90 days.

Organism	Weight loss	S.E.
Birch sapwood		
Corticium laeve	2.8	
Corticium laeve + B 64 D	2.5	
Corticium laeve + Stereum hirs. + B 64 D	39.9	1.3
Stereum hirsutum + B 64 D	38.5	1.2
Stereum hirsutum	48.0	1.1
B 64 D	0.5	
Aspen sapwood		
Stereum purpureum	1.8	
Stereum purpureum + A 64 Q	1.6	
Stereum purp. + Polyporus zon. + A 64 Q	53.0	0.8
Polyporus zonatus + A 64 Q	65.6	1.9
Polyporus zonatus	47.9	1.0
A 64 Q	0.2	

and aspen sapwood are shown in Tab. 5. The bacteria were inoculated 14 days after inoculation of the fungi. The incubation time was three months. It is clearly shown that *Stereum hirsutum*'s activity on birch wood decreased in the presence of bacterium B 64 D. *Corticium laeve*, which is a weak wood decomposer, did not significantly influence the

Tab. 6. The ability of culture filtrates from bacteria to replace thiamine in the nutrient solution for three decay fungi.

10 ml culture filtrate was added to 10 ml of a doubly concentrated medium B with and without thiamine.

Culture filtrate from bacterium no.	Thiamine added	Dry weight of mycelium mg/flask		
		Stereum hirsutum	Polyporus zonatus	Libertella betulina
A 64 A	+	74.1	51.9	34.4
»	—	26.4	36.7	67.3
A 64 B	+	106.9	54.9	94.5
»	—	4.5	14.4	70.6
B 64 C	+	97.0	66.7	69.6
»	—	38.6	73.5	74.3
B 64 D	+	94.8	51.7	78.3
»	—	36.0	50.3	71.1
A 64 E	+	64.0	56.8	76.0
»	—	13.3	17.9	69.2
no filtrate	+	70.9	41.5	64.2
»	—	6.8	12.9	47.2

total wood decomposition (weight loss). The activity of *Polyporus zonatus,* however, was highly stimulated by the presence of bacterium A 64 Q. The slow wood decomposer *Stereum purpureum* evidently reduced much of the stimulating influence of A 64 Q.

In view of the results obtained by FRIES (1938), showing that some bacteria can produce sufficient thiamine for the growth of certain decay fungi, a series of experiments was performed to find out whether the bacteria isolated from birch and aspen pulpwood could provide the decay fungi with thiamine. The bacteria were cultured in thiamine-free medium B (HENNINGSSON 1967 b) in a shaker. Five days after inoculation the bacteria were filtered off and the culture filtrates filter-sterilised. The filtrates were then added to a doubly concentrated medium B with or without thiamine. The results presented in Tab. 6 show that the bacterial culture filtrate could, to some degree at least, replace the thiamine of the nutrient solution. *Libertella betulina,* however, which is thiamine-autotrophic, was only slightly affected by the addition of culture filtrates from bacteria. The growth response obtained by adding culture filtrates from bacteria to fungal cultures consists of a complex of growth-promoting and antagonistic effects exerted by the filtrates. This is clearly demonstrated in Fig. 6. In both *Stereum hirsutum* and in *Polyporus zonatus,* the autoclaved culture filtrate from bacterium B 64 D could replace thiamine. An increase in the amount of filter-sterilised bacterial culture filtrate added resulted in a decrease in the mycelial yield of both *Stereum hirsutum* and the thiamine-autotrophic *Libertella betulina,* whereas growth increased in *Polyporus zonatus.* This indicates that a heat-instable antibiotic factor was very active in the case of *Stereum hirsutum* and *Libertella betulina,* but not in the case of *Polyporus zonatus.*

Bacteria generally have their pH-optimum near or somewhat above the neutral point. Since the pH of unpeeled birch and aspen pulpwood appeared to be betweeen pH 4 and 5 during storage, it was considered desirable to determine the pH requirements of the wood bacteria. Experiments with 15 bacterial isolates from birch and aspen pulpwood, grown on buffered medium D (HENNINGSSON 1967 b), showed that these bacteria grew excellently on acid media. Most of them had their optimal growth at pH 5—7. The bacteria isolated from birch grew only poorly or not at all at pH 3, whereas several aspen bacteria grew well even at pH 3.

The influence of bacteria on the wood's pH was studied by incubating moist wood meal for from one to three weeks. It appeared from this that about half the number of bacteria tested caused an increase in

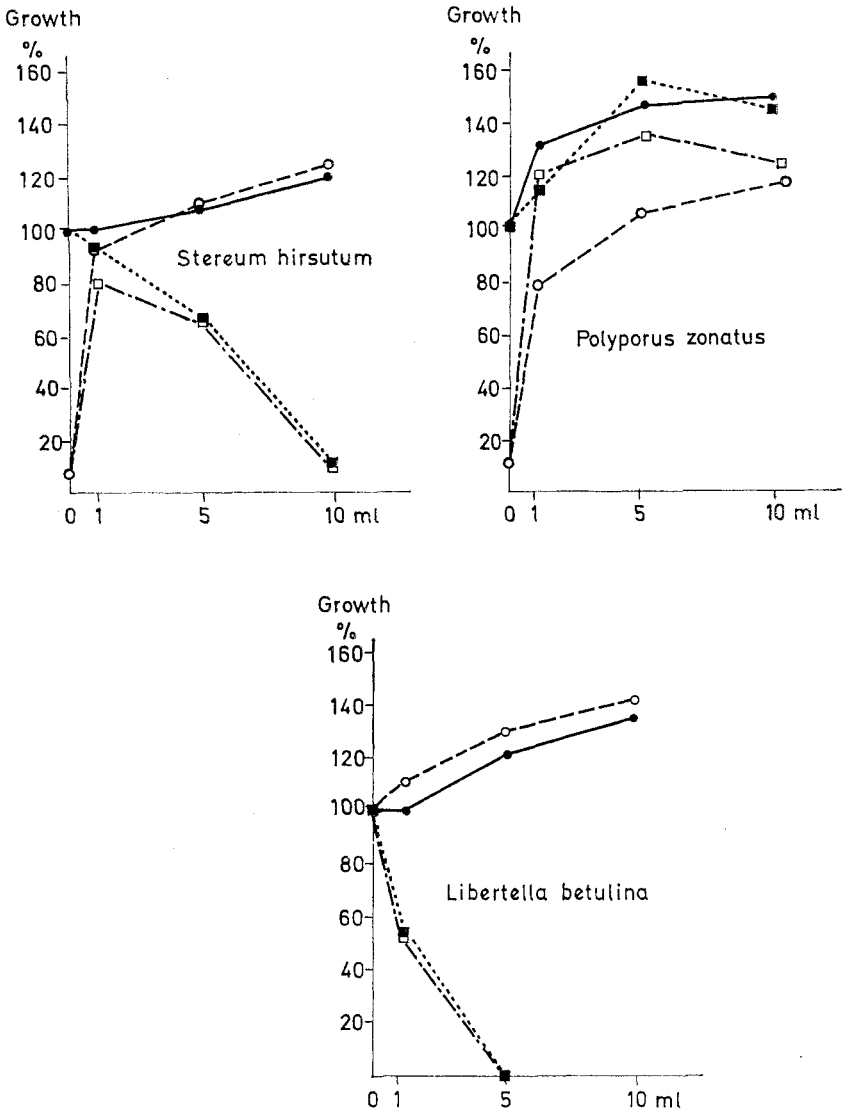


Fig. 6. The influence of various amounts of bacterial culture filtrate from B 64 D on the growth of three fungi in medium B with and without thiamine. Growth (mycelial weight) in complete medium B is assumed to be 100 per cent. Growth of the fungi is indicated as follows: unbroken lines = flasks with autoclaved culture filtrate and thiamine, broken lines = flasks with autoclaved culture filtrate but without thiamine, dotted lines = flasks with filter-sterilised culture filtrate and thiamine, dot-and-dash lines = flasks with filter-sterilised culture filtrate but without thiamine.

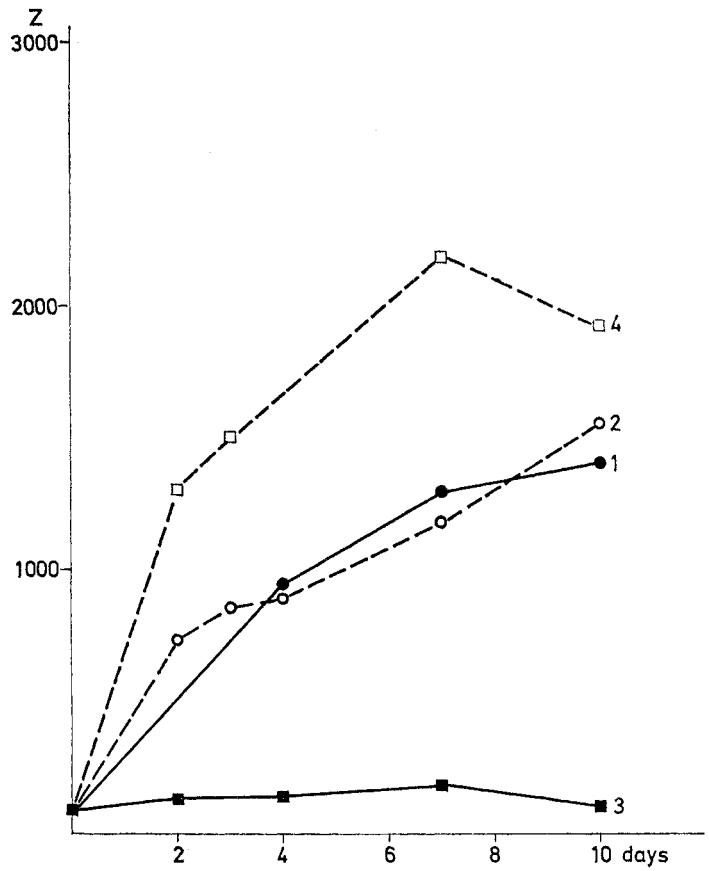


Fig. 7. Growth of two bacteria in medium B with cellobiose or glucose as the sole carbon source. Explanation of symbols: 1 = A 64 Q on D-glucose, 2 = B 64 D on D-glucose, 3 = A 64 Q on D-cellobiose, 4 = B 64 D on D-cellobiose.

the pH of the moist wood meal. This increase amounted to about 0.5 pH units in three weeks.

Since cellobiose is supposed to be the end product of fungal extracellular cellulose decomposition, the ability of two bacteria to utilise this carbon source was studied. The bacteria were cultured in medium B with D-glucose or D-cellobiose as the sole carbon source. Growth was measured photometrically and reported as the Z-value (cf. SUNDSTRÖM 1964). The results presented in Fig. 7 show that both bacteria could utilise glucose, but that only B 64 D was able to use D-cellobiose. Thus at least bacterium B 64 D might exist at the expense of decay fungi by utilising the extracellular cellulolytic end products.

Tab. 7. Effect of bacterium B 64 D on the decomposition of impregnated birch wood blocks by Stereum hirsutum and Polyporus zonatus in medium F.

Five concentrations of each chemical were tested. A positive effect is indicated by + and a negative by —. No visible effect is indicated by 0. Parantheses indicate a slight but not significant influence.

Substance	Effect of bact. B 64 D on the rate of decay caused by	
	Ster. hirs.	Pol. zon.
PCP	+	(—)
PC P-Na	0	0
o-Aminophenol	(+)	+
8-hydroxy quinoline	(+)	(+)
NaN_3	0	0
$HgCl_2$	0	0
$CuSO_4$	0	(+)

In plate tests, the growth of decay fungi from birch and aspen was checked completely by PCP-Na (sodium pentachlorophenolate) at a concentration of 5—50 ppm. Similar tests with bacteria isolated from the same wood showed that a concentration of 100—500 ppm was necessary to inhibit growth. Thus, the bacteria tolerated 2—100 times as much PCP-Na as did the fungi. Since the concentration required for the complete inhibition of several bacteria increased with the incubation time, it seems probable that these bacteria were able to detoxify the compound. It is known, for instance, that other bacteria are able to split the cyclic structure of phenols.

Seven substances, some of which have been shown to be especially toxic to birch and aspen fungi, were selected for study of the influence of bacterial action on wood impregnated with these toxicants. The substances were: PCP, PCP-Na, o-aminophenol, 8-hydroxyquinoline, NaN_3, $HgCl_2$ and $CuSO_4$. Small standard blocks of birch were impregnated with solutions of five concentrations of the toxicants. The vermiculite containing medium F was used (HENNINGSSON 1967 b) and the flasks were incubated with bacterium B 64 D in combination with *Stereum hirsutum* or *Polyporus zonatus*. The bacterial inoculation was performed 14 days after the fungal inoculation.

The results of the experiments are summarised in Tab. 7. These results show that the presence of bacterium B 64 D significantly accelerated only the decomposition (weight loss) in wood impregnated with o-aminophenol and attacked by *Polyporus zonatus* and in wood impregnated with PCP and attacked by *Stereum hirsutum*.

Tab. 8 and 9 show the results obtained with o-aminophenol and PCP respectively. From these results it is quite clear that bacterium

Tab. 8. Effect of bacterium B 64 D on the decomposition produced by Polyporus zonatus on birch wood blocks impregnated with o-aminophenol.

T-tests have been performed on the differences (number of observations 6—7). Conventional significance signs, viz:

* significance at the 5 per cent level
** " " " 1 " " "
*** " " " 0.1 " " "

Conc. of the solution %	Uptake mg/g wood (dry weight basis)	Weight loss per cent					
		buried blocks			superficial blocks		
		no bact.	bact.	diff.	no bact.	bact.	diff.
0	0.0	14.1	17.5	3.4	6.3	7.4	1.1
0.001	0.010	14.6	21.8	7.2*	5.3	7.0	1.7
0.01	0.11	17.8	20.5	2.7	4.6	6.6	2.0
0.1	1.1	12.7	18.5	5.8***	5.0	7.3	2.3*
1.0	10.4	8.7	15.2	6.5**	0.0	3.3	3.3**
2.0	x	4.1	9.2	5.1***	—	—	—

x compound not completely dissolved

B 64 D accelerated wood decomposition in impregnated blocks, especially in concentrations near the toxic limit.

Tab. 9. Effect of bacterium B 64 D on the decomposition produced by Stereum hirsutum on birch sapwood blocks impregnated with PCP.

Compound dissolved in ethanol. T-tests have been performed on the differences (see Tab. 8).

% PCP	Weight loss per cent					
	1 month's incubation			2 months' incubation		
	no bact.	bact.	diff.	no bact.	bact.	diff.
0	8.3	5.9	2.4	22.5	23.9	1.4
0.01	5.4	9.2	4.4*	12.7	21.8	9.1**
0.1	0.0	1.2	1.2	8.5	10.5	2.0

IV. Discussion

In 1911, HARDER experimented with mixed cultures of various decay and other fungi, and demonstrated that the mycelia usually inhibited each other's development. Inhibition frequently occurred before mycelial contact was established between the fungi. Harder suggested that this was caused by factors which the fungi exuded into the substrate and which were diffusable. Sometimes one mycelium overgrew the other, which was structurally changed or even killed. In continuing Harder's work, ZELLER & SCHMITZ (1919) stated that mixed cultures with various fungi resulted most frequently in the inhibition of one of the mycelia before or after contact. Only one combination resulted in growth stimulation by the action of diffusable factors. The growth rate, however, often increased in a dominating mycelium, when this overgrew a weaker mycelium.

BÄRLUND (1950) studied the influence on wood decomposition of the introduction, after certain periods of incubation, of a second decay fungus. The most frequent result was that the second fungus could not become established at all. In cases in which establishment occurred, dark zones developed between mycelia. The competitive ability of the species did not seem to be proportional to its decomposing activity, and the difficulties of the second fungus in invading the wood were attributed to exhaustion of the medium and not to toxic factors.

A comprehensive study of the inter-relationships between a number of decay fungi and between these fungi and bacteria was published by OPPERMANN (1951). In this the results of Harder and Zeller & Schmitz were verified; namely, that antibiotic activities are the most frequent phenomena in mixed cultures of decay fungi on agar. Similar inhibitions of growth as those on agar plates were also obtained with culture filtrates from growing fungi.

Recently, a number of wood-inhabiting non-Basidiomycetes have been shown to have a strong inhibitory influence on decay fungi—see p. 5. Usually, the inhibiting fungi seem to be those which occur early in the succession of invading fungi—for instance, certain mould fungi—and which are highly dependent on soluble carbohydrates. When the soluble carbohydrates are exhausted, these fungi probably have to make room for others with a more pronounced ability to attack

the polymeric carbohydrates of the wood. However, from the point of view of wood preservation, these early-occurring fungi are of the utmost interest, owing to their strong antagonistic activity against decay fungi. They may be regarded as a base for future research into the biological protection of, for instance, stored pulpwood (cf. SHIELDS & ATWELL, 1963 and BERGMAN & NILSSON, 1967). In Canada, much interest has recently been devoted to certain *Ascomycetes* with antibiotic activity against decay fungi (ETHERIDGE, 1957; BOURCHIER, 1961; WHITTAKER, 1962; BASHAM, 1966 and STILLWELL, 1966). Compared with the mould fungi, the *Ascomycetes* cause greater destruction of wood but they also more effectively penetrate the wood, and may thus be useful for biological control of decay fungi.

As was pointed out earlier and as is apparent from the present study also, the decay fungi influence each other's activities. Fungi which occur early in pulpwood, e.g. *Corticium laeve,* are easily defeated by more competitive species. Complete lysis of the weaker mycelium is not unusual (see Tab. 2). It is most probable that many wood-decaying fungi act as necrotrophic parasites in certain situations (BARNETT, 1963). Antibiotic phenomena between various decay fungi occur so frequently (see Tab. 2) that they must be of great importance in the total course of decay under natural conditions. Since the temperature factor has been shown to be decisive for many antibiotic and dominance phenomena (see Tab. 2), it seems probable that the interrelations of dominating and dominated decay fungi in naturally infected wood may be changed completely during a day with pronounced temperature variations or from season to season during the year. The moisture content of the wood certainly influences the interactions, too. Thus the results of BIER (1966) indicate that the development of decay fungi in wet wood may be at a disadvantage in relation to that of other microbes.

During the past few years, increasing evidence has been presented to demonstrate that certain wood-inhabiting bacteria can attack the cell walls of wood (KNUTH & MCCOY, 1962; HARMSEN & NISSEN, 1965 and GREAVES, 1966). However, bacterial attack has been demonstrated only in extremely wet—water-saturated—wood and the decomposition of the structural elements of the cell wall proceeds extremely slowly. From the point of view of wood preservation, bacterial action on the cell wall may thus be regarded as a factor of minor importance.

However, wood-inhabiting bacteria may be of great indirect importance because of their ability to influence more aggressive wood-destroying micro-organisms. Bacteria occur in growing trees (SHIGO, 1965)

as well as in wood after felling (see Tab. 1). Several bacteria have been reported to inhibit the wood-destroying fungi (cf. ASANTE & NEAL, 1964; SHIGO, 1965) by exuding diffusable antibiotic substances into the substrate. This is also demonstrated in Fig. 3 of the present paper. However, the bacteria may also stimulate some activities of the decay fungus—as, for instance, by increasing the formation of pigment in decay fungi (SHIGO, 1965), or by increasing the growth of a decay fungus by producing vitamins or other essential compounds (see Tab. 6, Fig. 6 and FRIES, 1938). Similar effects are also known from mixed cultures of fungi (cf. EVELEIGH & BREWER, 1965).

The total weight loss of wood attacked by a decay fungus may increase when a bacterium, which is not itself capable of decomposing the wood, is added (see Tab. 5). In this case malt extract was added, so the stimulation probably did not result from the production of vitamins by the bacterium. However, this bacterium species easily utilised cellobiose as the carbon source; and since a feedback mechanism is known in fungi, relating the cellobiose concentration of the substrate and the production of cellulases, an increased decomposition of cellulose by the fungus may result from the continuous removal of cellobiose by the bacterium.

In a publication by DUNCAN & DEVERALL (1964) it was shown that various wood-inhabiting *Ascomycetes* and *Fungi imperfecti* were able to detoxify certain chemical compounds used in wood-preservatives—for instance, pentachlorophenol. Even decay fungi which produce laccases have been shown to reduce the poisonous effect of pentachlorophenol (LYR, 1963 and MADHOSINGH, 1961).

Among bacteria, the ability to split phenols is widely distributed. The result shown in Tab. 8 was therefore not surprising. However, it indicates the necessity for considering not only the activities of wood-decomposing microbes, but also the activities of other micro-organisms found in wood, when judging the effectiveness of wood preservatives.

V. Summary

The microbial invasion of unpeeled birch pulpwood during outside storage was studied. It was shown that the wood was invaded by a number of different micro-organisms—especially by fungi. Fungi with a minor wood-destroying capacity were found early in the wood, but later disappeared, being unable to compete with more pronounced wood-destroyers.

When various decay fungi were cultured together, most frequently the mycelium of one of the two species finally suppressed or destroyed that of the other. *Corticium laeve*, for instance, which occurs in the wood at an early stage, was suppressed and its mycelium lysed by most of the other decay fungi tested.

Temperature had a decisive effect on the development of interaction phenomena between decay fungi.

Coloured contact-zones between two different mycelia commonly occurred in culture.

Interactions between bacteria isolated from birch and aspen pulpwood and decay fungi found in the same wood were studied. If the bacteria were transferred to the substrate before or at the same time as the decay fungi, the development of the latter was almost always reduced. If, on the contrary, the bacterial inoculation was performed after the fungal inoculation, the inhibiting effect often did not occur; a stimulation of the activity of the fungi even resulted.

The bacteria produced diffusable substances, which could reduce or inhibit the growth of the decay fungi at considerable distance from the bacterial colony.

The temperature had a substantial influence on the antagonistic activity of the bacteria on agar as well as in wood.

Small amounts of culture filtrate from bacteria could completely replace thiamine in a nutrient solution for thiamine-heterotrophic decay fungi.

One wood bacterium species greatly accelerated the decomposition caused by decay fungi on wood impregnated with o-aminophenol or sodium pentachlorophenate.

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VII. Sammanfattning

Samspelet mellan mikroorganismer från björk- och aspmassaved

Den mikrobiella invasionen i obarkad björkmassaved under utomhuslagring har studerats. Därvid framkom att veden invaderades av en mängd olika mikroorganismer — främst svampar. Svampar med liten veddestruerande förmåga påträffades tidigt i veden men utkonkurrerades senare av mera utpräglade vedförstörare.

Vid parvis samodling av olika rötsvampar från björk- och aspmassaved tog oftast den ena artens mycel överhanden. Den i veden tidigt uppträdande arten *Corticium laeve* t. ex. undertrycktes och dess mycel lyserades av nästan alla andra testade svampar.

Temperaturen hade en avgörande effekt på utvecklingen av samspelsfenomen mellan rötsvampar.

Färgade kontaktzoner mellan två olika mycel var mycket vanliga i kultur.

Samspelet mellan bakterier isolerade från björk- och aspmassaved och rötsvampar funna i samma ved studerades. Om bakterierna tillfördes substratet före eller samtidigt som rötsvamparna, hämmades nästan alltid de senares utveckling. Om däremot bakterieympningen skedde efter svampympningen, uteblev hämningseffekten ofta helt; en stimulation av svamparnas aktivitet kunde t. o. m. erhållas.

Bakterierna producerade diffunderbara substanser, som kunde hämma rötsvamparnas tillväxt på ett avsevärt avstånd från bakteriokolonien.

Odlingstemperaturen hade stort inflytande på bakteriernas antagonistiska aktivitet såväl på agar som i ved.

Små mängder kulturfiltrat från bakterier kunde helt ersätta thiamin i en närlösning för thiaminheterotrofa rötsvampar.

En vedbakterie kunde avsevärt påskynda rötsvampars nedbrytning av ved, som impregnerats med o-aminofenol eller natriumpentaklorofenolat.