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Physiology and decay activity of the birch conk fungus *Polyporus betulinus* (Bull.) Fr.

Fysiologi och vednedbrytning hos björktickan Polyporus betulinus (Bull.) Fr.

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CONTENTS

Introduction

Sporophores of the birch fungus *Polyporus betulinus* have only been observed on species belonging to the Betula genus. Consequently the fungus is considered to show host specifity. According to Lohwag (59), however, the fungus has once been observed on *Carpinus betulus,* which is considered to have a close relationship to the Betula genus.

The fungus has been reported in the literature under the following synonyms: *Boletus suberosus* L., *Piptoporus betulinus* (Bull.) Karst., *Boletus betulinus* Bull., *Placodes betulinus* (Bull.) Quel., *Ungulina betulina* (Bull.) Pat., *Fomes betulinus* (Bull.) Maub., and *Piptoporus suberosus* (L.) Murr.

In Sweden sporophores of the *P. betulinus* are very common on dead standing or fallen birches. They are less commonly found on living birches, and if so, only on dead parts of the tree. Cartwright & Findlay (25) report observations of sporophores on living branches, although the author has never observed this phenomenon. Fresh spore-producing sporophores can be found in Sweden from the end of July till November or December. They are annual and are usually destroyed the year after their formation. Mostly the sporophores can be seen one or two together on the trunk several meters above the ground, but sometimes a dead tree can be crowded with sporophores—20 to 30 on the same tree. Sporophores also form on dead fallen birches, which is easily seen. Because of the geotropism they always form their pore-layers in the direction of the ground. The sporophores are always attacked by insects whose larvae live in the sporophore tissue or in the pore-layer. Larvae of the species *Diáperis boléti, Tetratóma fungorum* and *Cis boleti* are almost always found in the sporophores of *P. betulinus.*

Björkman et al. 1964 made an estimation of the frequency of heart rots in birch wood in Sweden. They based their figures on damaged pulp wood logs and found that only 2.2 % of the damage was caused by *P. betulinus.* This indicates that the fungus is not as common in birch as one would believe judging from the frequency of sporophores on trees in the forest. However, birches bearing sporophores are often so severely decayed that they will be rejected already in the forest.

Different authors have had different opinions as to whether the fun-

gus should be regarded as a parasite or not. Thus Kreisel (50) states that *P. betulinus* is the most important parasite on Betula in Germany. Peace (74), on the other hand, is not willing to admit that the fungus shows any parasitic behaviour. He even states that, if the fungus invades a tree which is still alive, it will only attack those parts of the wood which are already dead. In support of this opinion he mentions that Rozanova (86) and Macdonald (63) both failed, when they tried to infect sound living birches with *P. betulinus*. Surprisingly enough Macdonald himself regarded the fungus as a parasite, mostly because successful infections had already been carried out by Mayr in 1884 (64). He transferred increment cores, aseptically removed, from an infected tree to sound trees. Only two months after the infection he could observe that the mycelium had spread in the infected trees and that the colour of the attacked wood had become intensively brown during this period.

Mayr could also show that only the secondary walls of the wood cells were attacked. Consequently, at an advanced stage of decay only the middle-lamellae were left. The mycelium very easily penetrated the cell walls; the holes thus formed showed a shape, more or less like an hour-glass with a sharp restriction at the passage of the middle-lamella.

Wood analyses performed by Mayr on sound and decayed wood showed a decline in the specific dry weight from 74.39 to 30.02. In other words: as a result of the fungus attack, the dry density of the wood had been reduced by around 60 %. Chemical analyses showed a lower percentage of carbon, hydrogen and oxygen in the decayed wood than in the sound wood.

Decay experiments

General remarks

The rot caused by *P. betulinus* must be classed as a brown rot or more correctly as a destruction rot (shrink rot). This type of rot is characterized by brown discolouration and, in advanced stages, a more or less cuboidal cracking of the wood (14, 81). Chemically a brown-rotted wood has a much higher percentage of lignin and a correspondingly lower percentage of cellulose than the sound wood (2, 17, 28). Under the microscope the brown-rotted wood is distinguished by the secondary wall of the wood fibres being successively destroyed. Finally only the middle-lamella remains (65). Ultimately the fungi causing brown-rot are characterized by their inability to form polyphenol oxidases (6, 62, 68).

As seen above *P. betulinus* belongs to a group of wood-destroying fungi which is in several respects very limited. This group can be still more restricted, as it is very unusual for brown-rotting fungi to attack only angiosperm wood. According to Cowling (28), only 27 percent of the brown rotters are restricted to angiosperm wood, whereas as much as 58 percent occur only on gymnosperm wood. As for white rotters the opposite is the case and even more so—75 percent on angiosperm wood and only 18 percent on gymnosperm wood.

The decay experiments reported in this paper were mainly carried out in order to determine the changes occurring in the wood during an attack by *P. betulinus*. Comparative experiments were almost always carried out using *Polyporus marginatus* as a test organism. This fungus was chosen as a comparative organism because—like *P. betulinus*—it causes a typical destruction rot, but unlike the latter it attacks several different tree species—gymnosperms as well as angiosperms. An analysis of the activity of 39 presumptive birch rotters on sapwood from *Betula verrucosa* were also carried out. Finally, as far as *P. betulinus* and *P. marginatus* are concerned, their activities on wood species other than birch were investigated as well as their temperature requirements for their optimal decay activity on birch wood.

Experimental methods

Various methods of measuring the wood-destroying capacity of fungi have been described. Principally two main types of methods can

be distinguished. In the first type the fungi are allowed to attack intact pieces of wood, usually blocks of wood. These blocks may be placed so that they come in contact with a nutrious substrate (82) or without any such contact (28, 12). In the second type of method the fungi attack sawdust to which water and usually further nutrients have been added. Methods of the first type can be used when more or less natural conditions in the wood are desired. Methods of the second type are to be preferred when a rapid and homogeneous attack of the wood substrate is of great importance. The hitherto most used measure of the rot activity on wood is the loss of wood substance (weight loss) resulting from an attack. Usually, the loss is expressed

a

b

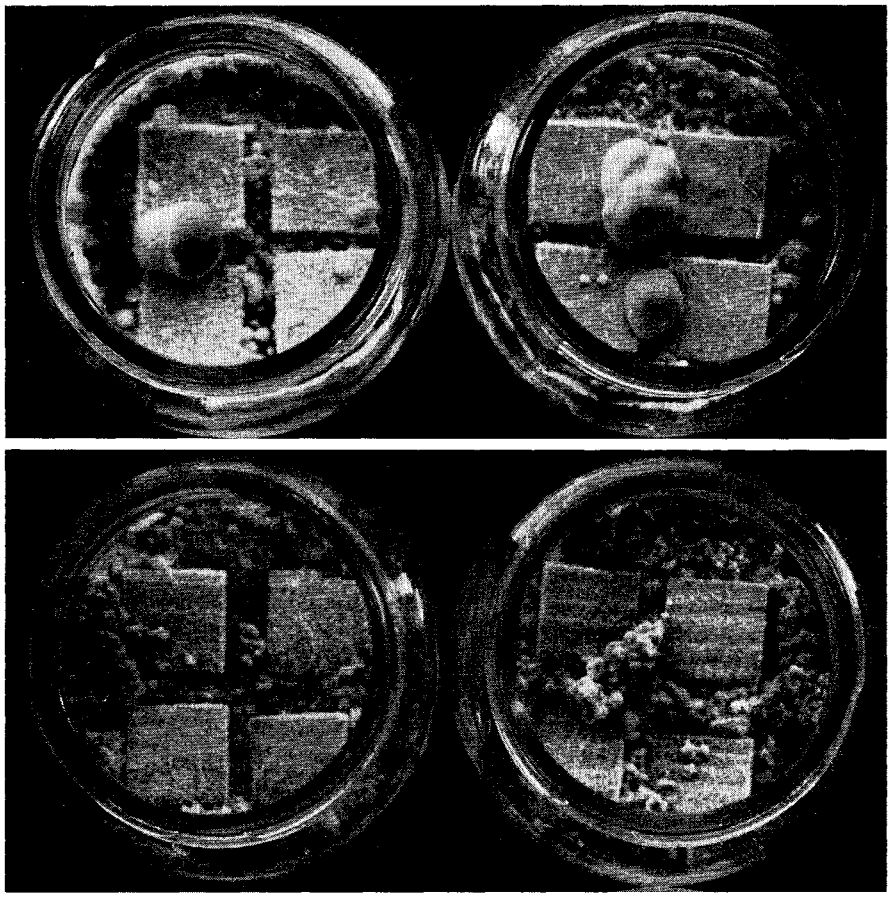

Fig. 1. Blocks of birch (a) and spruce (b) sapwood in an experiment with *P. betulinus* using the soil jar method. Note the formation of fruiting bodies on the blocks of birch sapwood.

as a percentage of the dry weight of the sound wood. However, methods basing their results on reduction in strength of wood have also been used.

In the experiments published in this paper two methods have been used, although in both cases whole pieces of wood have been exposed to attack from the fungi:

1. *The soil jar method* (fig. 1). This method which has been worked out and described by Rennerfelt (82), is somewhat related to the American soil block method (1), but differs greatly from the German DIN-method (30). The wood blocks (20 × 20 × 20 mm) are buried in the soil in glass jars which are autoclaved and inoculated with a macerated mycelium suspension of the desired fungus.

2. *The glass bench method* (fig. 2). This method has been used, when the attacked test blocks were to be chemically analyzed. In cubical wood blocks of 20 × 20 × 20 mm a hole was bored in a radial direction. The hole was made 5 mm in diameter and 15 mm in depth. After

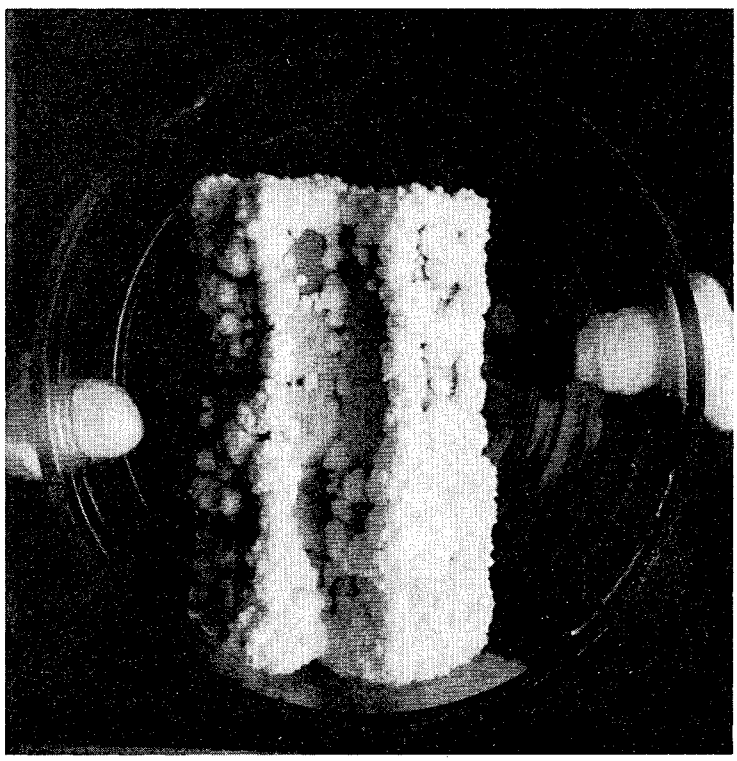

Fig. 2. Blocks of birch sapwood in an experiment with *P. betulinus* using the glass bench method.

having been weighed absolutely dry and autoclaved, the blocks were placed in sterile water in a vacuum. When all the blocks had sunk they were transferred (under sterile conditions) into glass-bowls, where they were placed on a bench made from a bent glass rod. To keep a high humidity in the bowls they were filled with water to a depth of 1 cm. The wood blocks were then inoculated in the holes with a mycelial suspension.

In both methods the glass jars were kept during the experiment at $22 \pm 1°$ C and at a relative humidity of about 85 %. The results of the attack were expressed as a percentage of the loss of weight and the weight of the original dry wood. The values in the tables are the average values of at least 5 wood blocks, and the standard deviation has been calculated using the formula $\varepsilon = \frac{\sum(m-M)^2}{n(n-1)}$; $\varepsilon =$ standard deviation, $m =$ original experimental value, $n =$ number of experimental values and $M =$ average of n experimental values. All the reported standard deviations have been calculated in the same manner.

A comparison between the rates of breakdown of the two methods described was made using *P. betulinus* B6062 as the test organism and sapwood from *Betula verrucosa* as the substrate. It is obvious from this test that method 2 gives a much slower breakdown of the wood than method 1. This is most probably a result of the very good supply of nutrients (especially nitrogen) in the soil.

Material. Cultures isolated from the tissue of sporophores assembled by the author have been used (see page 20) in most cases. In the experiment with different presumptive birch rotters a lot of the tested fungi have been kindly obtained from mrs dr Aino Käärik at the Royal College of Forestry, Department of Forest Products. The test blocks of birch were, unless otherwise stated, prepared from sapwood of *Betula verrucosa* from the Stockholm region.

Maximum degradation of birch wood

In table 1 and fig. 3 it is possible to follow closely the course of degradation in birch wood attacked by *P. betulinus* and *P. marginatus* (*Fomes pinicola*). The soil jar method was used. From the results it can clearly be seen that for the two test organisms the maximal loss of weight was just over 70 percent. Consequently, between 25 and 30 percent of the wood substance in birch wood was not accessible to these fungi—they lacked the enzymes for decomposing that part of the wood. It is also evident from fig. 3 that there was a rapid consumption

Tab. 1. Weight losses and moisture quotients of blocks of birch sapwood decayed by *P. betulinus and P. marginatus* according to the soil jar method.

Incubation time days	Polyporus betulinus			Polyporus marginatus		
	Weight loss %	Moisture quotient wood	soil	Weight loss %	Moisture quotient wood	soil
0	0	45	61	4.0	41	65
15	0.2	52	60	4.3±0.5	46	46
30	0.5±0.1	49	60	29.2±1.0	56	30
45	10.2±3.0	52	60	46.6±0.5	73	46
60	26.8±3.4	62	--	55.2±0.6	79	30
75	52.1±1.3	94	64	61.5±0.5	100	46
90	57.0±1.7	96	61	65.0±0.2	94	30
105	64.0±0.8	118	62	68.6±0.7	111	39
120	65.7±0.8	143	61	71.0±0.1	108	45
135	68.4±0.4	139	53	70.6±0.5	132	47
150	69.0±0.3	129	62	72.1±0.2	111	46
165	70.3±0.1	115	40	71.1±0.2	142	46
180	70.8±0.3	106	56	73.5±0.3	96	30
195	71.3±0.5	123	60	73.0±0.4	135	44
210	71.3±0.6	117	37	72.6±0.6	138	32

of wood substance between 15 and 90 days, when 65—70 % of the wood substance was consumed. The moisture content in the soil was fairly constant during most of the experimental time. Within the blocks, on the other hand, the moisture content was far from constant. It rose steeply during the phase of rapid decay, partly due to the accumulation of respiration water in the wood and partly due to the water in the growing mycelium itself. At least for *P. betulinus* a decrease of the moisture content in the wood was obvious during the latter part of the experiment, when the consumption of wood substance was almost nil. This was probably a result of the mycelium dying within the blocks, when the accessible part of the wood was metabolized. Consequently, the amount of water in the wood decreased as the respiration and the amount of living mycelium decreased.

Finally, changes in the hygroscopicity occurred during the decay. These changes act upon the adsorption as well as the desorption of water (28, 73), and therefore the moisture content of the wood can change independently of the direct fungal activity.

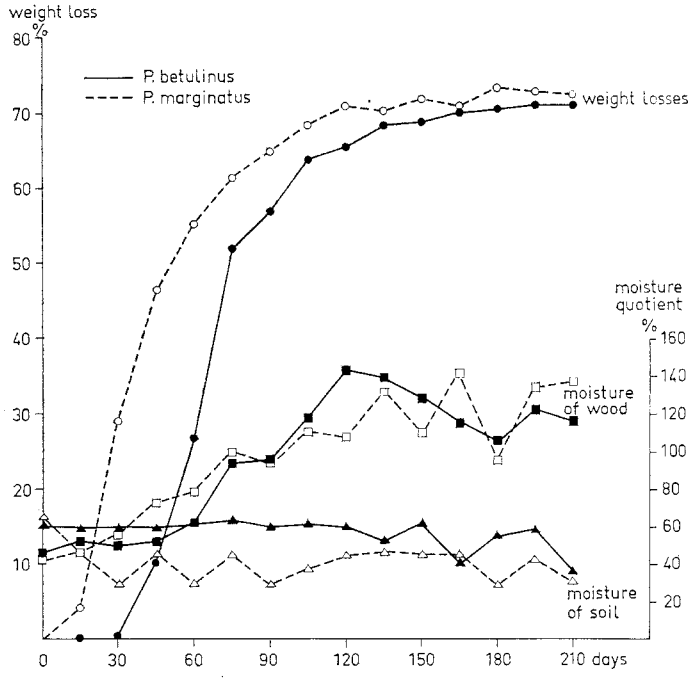

Fig. 3. Weight losses and moisture contents of wood and soil in an experiment with *P. betulinus* and *P. marginatus* using the soil jar method.

Comparative decay experiments with fungi attacking birch and other hardwoods

By using the soil jar method the activities of different fungi on birch wood were tested. Organisms were selected which occur or may occur as parasites or saprophytes in birch wood. The experimental period was 90 days. The experiment included six fungi which are not normally referred to as rot fungi, namely *Coryne sarcoides, Daldinia* sp., *Heterosporium* sp., *Stemphylium* sp., *Pseudeurotium zonatum* and *Telephora terrestris*. The first four of these fungi have been isolated from living birches. The strain of *Pseudeurotium zonatum* used here was isolated from a figurehead of limewood from the warship Wasa which sank in 1628. The fungus has also been found by Brewer (20) in slime accumulations in pulp and paper mills. *Telephora terrestris* finally is in most cases regarded as a litter decomposer but it can also act as a parasite on roots and young seedlings of pine and spruce.

Results of the decay experiments can be seen in tab. 2. The capacity of decay varied considerably for different species. Maximum weight

Tab. 2. Weight losses of blocks of birch sapwood decayed by a variety of presumptive birch *root fungi. The soil jar method was used with an incubation period of* 90 days.

Group	Organism	Isolated from	Maximal weight loss %	Average weight loss %
I. more than 75% weight loss	Fomes applanatus	Oak	78.4	72.4±1.8
	Lenzites betulina	Birch	91.8	85.9±2.6
	Polyporus hirsutus	Oak	88.1	83.1±1.3
	Polyporus versicolor	Beech	81.7	77.9±0.8
II. weight loss 50%-75%	Coniophora puteana	Birch	60.4	52.6±2.5
	Polyporus adustus	Birch	65.9	56.4±2.4
	Polyporus betulinus 1	Birch	65.1	57.4±2.4
	Polyporus betulinus 2	Birch	58.2	52.3±1.5
	Polyporus brumalis	Birch	61.6	50.0±2.4
	Polyporus cinnabarinus	?	53.7	46.9±1.5
	Polyporus frondosus	Oak	74.9	63.2±3.5
	Polyporus marginatus	Pine	70.2	66.1±0.9
	Polyporus marginatus	Birch	70.7	69.1±0.4
	Polyporus rutilans	Elm	63.1	53.6±2.2
	Polyporus sulphureus	Oak	74.1	66.2±2.4
	Polyporus zonatus	Birch	50.6	37.1±3.2
	Trametes serialis	Pine	61.9	55.5±1.7
III. weight loss 25%-50%	Fomes conchatus	?	29.0	25.7±0.9
	Fomes fomentarius	Birch	40.5	31.8±2.6
	Fomes igniarius	Birch	36.2	25.2±3.7
	Hymenochaete rubiginosa	Birch	26.1	21.1±0.9
	Pholiota heteroclita	Birch	29.9	13.8±2.0
	Schizophyllum commune	Aspen	24.6	22.4±0.6
	Stereum hirsutum	Birch	25.4	18.1±1.2
IV. weight loss 10%-25%	Daldinia sp.	Birch	24.9	22.7±0.8
	Daedalia confragosa	Birch	16.0	12.2±1.2
	Fomes connatus	Birch	13.4	11.5±0.6
	Hymenochaete tabacina	Aspen	15.7	9.2±0.9
	Lentinus tigrinus	Maple	21.5	9.1±3.2
	Polyporus dichrous	?	12.5	11.9±0.4
	Polyporus fumosus	Alder	13.3	6.6±0.9
	Poria ferrea	Spruce	18.6	12.5±0.9
	Poria obliqua	Birch	13.3	10.0±0.7
	Pseudeurotium zonatum	Lime	11.3	7.1±0.9
	Telephora terrestris	?	11.8	7.5±1.7
	Stereum purpureum	Birch	34.8 in 6 months	
V. weight loss below 10%	Coryne sarcoides	Spruce	7.5	5.2±0.4
	Stemphylium sp.	Birch	9.3	7.9±0.5
	Heterosporium sp.	Birch	6.0	4.6±0.2
	None		2.0	1.3±0.1

losses between 6.0 and 91.8 % could be observed. Among the true rot fungi (the Basidiomycetes), maximum weight losses ranged from 12.5 to 91.8 %.

Based on decay capacities a division of the tested fungi was performed, in which the figures representing maximum weight losses constituted the basis for subdivision. The three common rot fungi on birch wood, *Lenzites betulina, Polyporus hirsutus,* and *Polyporus versicolor,* which caused weight losses of more than 75 % in 3 months, were placed in group I. These three fungi are more or less typical white rotters and consequently they consume lignin as well as the carbohydrates of wood (13, 14, 24, 28, 68). According to Cowling (27), the white rotters are characterized by a uniform decomposition of the main wood components. This means that the proportions between lignin, cellulose and pentosans are practically the same in heavily decayed wood as in sound wood. It also means that the so-called white rot fungi could, under suitable conditions, cause weight losses of nearly 100 %.

P. betulinus and the other brown rotters are found in group II, characterized by weight losses of 50—75 % during a period of 3 months. As brown rotters on the whole are considered incapable of consuming lignin, these fungi cannot break down all the wood substance. The lignin, constituting about 20 % of the birch wood, will be left. More or less pronounced white rotters as *Polyporus adustus, Polyporus cinnabarinus* and *Polyporus zonatus,* commonly causing storage rot or stump rot, also belong to group II.

Group III (25—50 % weight loss) includes *Fomes fomentarius* and *Fomes igniarius,* both parasitic rot fungi frequently occurring on birches. Storage rot fungi like *Stereum hirsutum* and *Schizophyllum commune* also belong to group III.

In group IV (10—25 % weight loss) we find another exclusive rot fungus on birches—*Poria obliqua,* together with some rot fungi, which occur on oak, elm and maple.

The three very slow wood decomposers in group V (less than 10 % weight loss) do not belong to the true rot fungi. All the tested non-basidiomycetes are found in groups IV and V. Nevertheless, it is obvious that the ascomycetes *Coryne sarcoides, Daldinia* sp., and *Pseudeurotium zonatum,* and also the Fungi Imperfecti, *Heterosporium* sp., and *Stemphylium* sp. were all capable of decomposing wood.

Microscopical studies of decayed wood

Birch sapwood, decayed to known weight losses by *P. betulinus,* was examined in microscope. The wood pieces were boiled in a mixture of glycerol and water (1 : 3) in order to make them soft. Sections, 10—15 μ thick, were then prepared in a microtome. The

sections were stained in safranin and picro aniline blue (25), washed and dehydrated in alcohol, cleared in clove oil, washed in cedar wood oil and xylol, and finally mounted in Canada balsam. The method was useful in the early stages of decay. However, the more decayed were the wood pieces, the more difficult it became to cut out acceptable sections, and therefore some embedding technique is here to be recommended.

It was noticed that the mycelium rapidly infiltrated the wood, and that hyphae occurred in abundance at first in and around the ray cells and later it was found in ample supply all over the wood. The hyphae penetrated the cell walls in all directions, whilst within the cells the hyphae primarily ran in a longitudinal direction. The secondary walls were dissolved during the course of decay. In the most decayed wood they almost completely vanished.

Meier (65) shows with electron micrographs how the cell wall structure in the wood fibres of birch are dissolved as a result of an attack by *P. betulinus*. The secondary wall is dissolved from the cell lumen outwards. The tertiary wall on the other hand remains fairly intact even in advanced stages of decay and thus, in a cross section of heavily decayed wood, the tertiary wall can be seen like a separate ring lying in the cell cavity. The middle lamella which is rich in lignin stays unattacked in the very advanced stages of decay, even for instance at weight losses of about 68 %.

Activity on various kinds of wood

Several authors have observed that, in spite of its naturally occurring specifity, *P. betulinus* is able to decompose wood from other trees than Betulas. Thus, Lutz showed already in 1929 (61) that the fungus in sterile laboratory tests could develop on Betula, Populus, Fagus and Pinus but not at all on Quercus. The first adequate measurements of the rot activity of *P. betulinus* on birch wood were carried out by Liese 1928 (55). He measured the weight losses of the wood. Later Meier (68) and Lohwag (59, 60) showed that, in accordance with German DIN-method, the fungus was able to cause substantial weight losses in several different kinds of wood—in most cases different hard-woods. However, it was not possible to procure an attack on heart wood of *Quercus sessiflora* and *Pinus silvestris*.

Table 3 shows the results of decaying experiments by the soil jar method. From *Pinus silvestris* and *Picea abies* both sapwood and heart wood were used in the experiments. From *Fagus silvatica* and *Betula verrucosa* only sapwood was used. The experiments were run

Tab. 3. Weight losses of wood blocks from various kinds of wood decayed by *P. betulinus* and *P. marginatus* using the soil jar method.

Kind of wood	Polyporus betulinus weight loss		Polyporus marginatus weight loss	
	g	%	g	%
Betula verrucosa sapwood	2.58	52.8±3.1	3.38	68.7±0.2
Fagus silvatica sapwood	3.20	56.7±4.2	3.54	62.8±2.0
Pinus silvestris sapwood	0.22	5.5±3.6	2.81	67.4±0.6
Pinus silvestris heartwood	0.46	4.6	1.93	30.1±1.3
Picea abies sapwood	2.04	60.7±3.0	2.28	67.0±1.4
Picea abies heartwood	2.10	62.7±3.3	2.28	66.4±2.4

for 75 days at 20—23° C. Because of their high content of volatile substances the test blocks of pine heart wood were dried in an desiccator over phosphopentoxide instead of being dried at + 105° C.

It is obvious from the results that *P. marginatus* was able to attack all kinds of wood. The weight loss figures ranged approximately from 63 to 68 % except for heart wood of pine, which lost about 30 % of its original weight. The resistance of pine heart wood is partly caused by its high content of pinosylvin and the fungicidic substances related to it (78). *P. betulinus* caused only a small loss of weight in the pine wood, but the other kinds of wood were heavily attacked.

Thus, once again it was demonstrated that in the laboratory *P. betulinus* is able forcibly to attack wood from other tree species than the Betulas. In these experiments, however, an interesting observation was made. The irregular fruiting bodies, which occurred frequently in cultures of the fungus, never formed in the experimental jars containing blocks of pine and spruce. This is shown in fig. 1. Thus, these woods may have an obstructing or retarding influence on the formation of fruiting bodies.

Temperature requirements

The soil jar method was used. After inoculation the jars were allowed to stand at room temperature until mycelial growth was observed in all the jars. This mostly occurred after three days. The jars were then placed at temperatures from + 5 to + 35° C. Experimental time for *P. betulinus* was 105 days and for *P. marginatus* 75 days.

From fig. 4 it is obvious that the minimum temperature for the ability to attack birch wood for both fungi lies between + 7 and + 9° C. Optimum temperature for *P. betulinus* seems to be around + 25° and

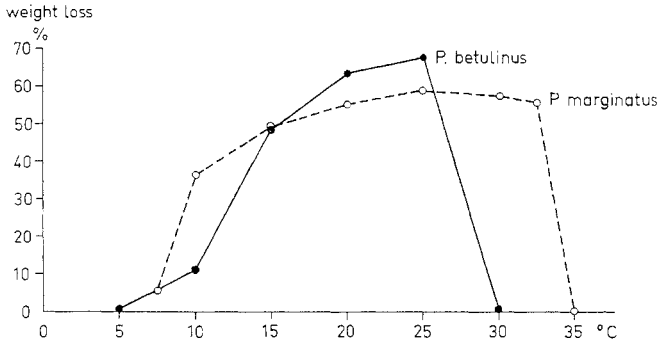

Fig. 4. Weight losses of blocks of birch sapwood decayed by *P. betulinus* and *P. marginatus* at different temperatures.

for *P. marginatus* between + 25 and + 30° C. The former does not attack wood at temperatures above 30° while the latter has its maximum between 32.5 and 35° C. *P. marginatus* obviously has a somewhat broader temperature tolerance than *P. betulinus*.

Changes in wood-pH resulting from decay

Blocks of birch wood decayed to known weight losses were ground to pass a 1 mm sieve. 1 g of wood meal and 20 ml of distilled water were put in a 50 ml Erlenmeyer flask. The flasks were shaken in dark-

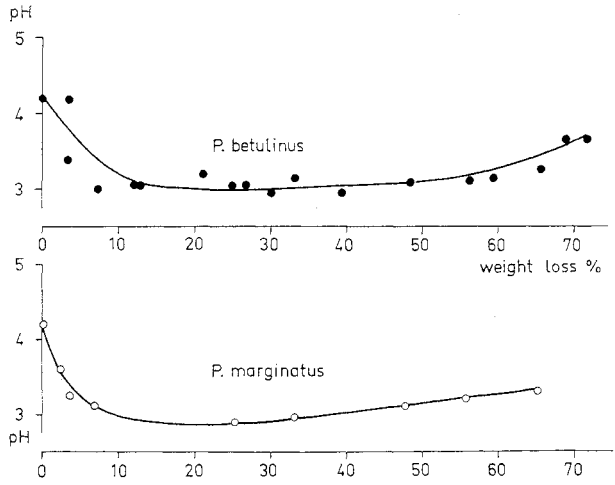

Fig. 5. pH of cold water extracts from birch sapwood decayed to different weight losses by *P. betulinus* and *P. marginatus*.

ness for 20 hours at room temperature. The wood meal was carefully filtered away, and the pH of the fluid was measured.

The results are shown in fig. 5. The pH of the wood meal extract decreased during the course of decay until the advanced stages, when the pH again increased. This was true for both fungi. A decrease of the pH in brown-rotted wood has also been observed by Robak (83) and Cowling (28). The pH drop is largely supposed to be a result of a substantial formation of organic acids, which are actively secreted by fungal hyphae (11, 70).

Changes in the chemical composition of birch wood after an attack by P. betulinus and P. marginatus

For these studies groups of 3—5 woodblocks with approximately the same losses in weight were put together. The reported weight loss for each group is the average weight loss for these wood blocks.

Two different decaying methods were used. The glass bench method was used for tests with *P. betulinus* up to 40.5 % weight loss. *P. betulinus* with higher weight losses and for all tests with *P. marginatus* the soil jar method was used.

The wood blocks were ground in a Whiley Mill to pass a 2 mm sieve. Analyses were then made for fats and resins (extracts), lignin and pentosanes. The hexosans were then theoretically calculated as being the remainder of the wood substance, including, of course, hexosans other than the glucosans, such as mannans, galactans, and partly degraded polysaccharid products. Furthermore, small amounts of inorganic ash substances are included. The ash content, on the other hand, is very low—in sound birch wood about 0.3 % and in heavily decayed birch wood about 0.6 % (88).

The content of fats and resins was determined by extraction with dichoromethane and alcohol, followed by the weighing of the dry extracts (92). The lignin content was determined as sulphuric acid lignin (93). Finally the pentosans were determined by distillation with hydrochloric acid followed by colorimetric determination with orcinol (94).

In table 4 and in fig. 6, 7 and 8 the results of the chemical analyses are shown. It is obvious that the course of decay was almost identical for the two species *P. betulinus* and *P. marginatus*.

Fig. 6 shows that the lignin content grew with increasing losses in weight. At the maximum weight loss, for instance, lignin constituted about two thirds of the weight of the rotten wood. At a weight loss of 61.5 % for wood decomposed by *P. betulinus* the content of extracts

Tab. 4. Chemical composition of blocks of birch sapwood decayed by *P. betulinus* and *P. marginatus* C = hexosans, P = pentosans, L = lignin, E = extracts, x = sample boiled over.

	Weight loss %	Content in % of weight of decayed wood				Content in per cent of weight of sound wood				Content in per cent of original content = relative content			
		C	P	L	E	C	P	L	E	C	P	L	E
Polyporus betulinus	0.0	53.3	26.0	19.6	1.10	53.3	26.0	19.6	1.10	100.0	100.0	100.0	100.0
	5.1	55.3	22.7	20.9	1.10	52.5	21.5	19.8	1.04	98.5	82.8	101.1	94.9
	8.3	56.3	20.8	21.8	1.19	51.6	19.1	19.9	1.09	96.8	73.4	101.8	99.2
	13.3	58.5	18.0	22.2	1.29	50.7	15.6	19.3	1.12	95.2	60.0	98.3	101.7
	21.2	56.8	17.2	24.6	1.43	44.7	13.6	19.4	1.13	83.9	52.1	98.9	102.4
	30.3	56.7	14.6	27.2	1.50	39.5	10.2	18.9	1.05	74.2	39.1	96.6	95.1
	40.5	52.4	12.3	33.6	1.72	31.2	7.3	20.0	1.02	58.5	28.2	102.0	93.0
	51.8	43.4	11.3	42.7	2.55	20.9	5.5	20.6	1.23	39.3	21.0	105.0	111.7
	61.1	39.4	9.1	52.6	3.85	15.3	3.6	20.5	1.50	28.8	13.7	104.4	136.2
	71.0	25.8	4.6	66.9	(2.71)[x]	7.5	1.3	19.4	(0.79)[x]	14.1	5.1	98.9	(71.5)[x]
Polyporus marginatus	4.5	55.1	23.0	21.0	0.96	52.6	21.9	20.1	0.92	98.7	84.3	102.4	83.4
	29.6	55.4	15.1	28.0	1.45	39.0	10.7	19.7	1.02	73.2	41.0	100.6	92.8
	45.6	49.5	11.6	36.7	2.17	26.9	6.3	20.0	1.18	50.5	24.3	102.0	107.3
	54.4	44.2	10.1	43.1	2.57	20.2	4.6	19.7	1.17	37.8	17.7	100.3	106.5
	65.3	31.7	7.7	56.8	3.85	11.0	2.6	19.7	1.34	20.7	10.3	100.5	121.5

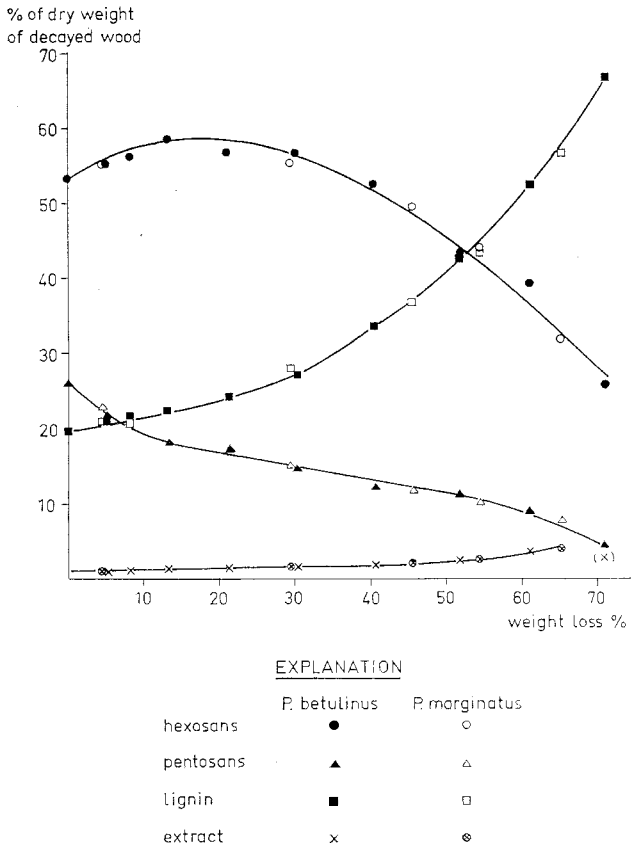

Fig. 6. Composition of birch sapwood in progressive stages of decay by *P. betulinus* and *P. marginatus*. The percentages are based on weight of decayed wood.

was about 3.5 times of the sound wood. The pentosan content decreased with increasing weight losses, whereas the hexosans increased a little in the early stages. At 13.3 % weight loss the hexosans constituted 58.5 % of the weight of rotted wood. Then the hexosan content dropped rapidly.

From fig. 7 and 8 it is obvious that no registrable consumption of lignin occurred during the whole course of decay. At the same time the fungi metabolized both hexosans and pentosans, resulting in the substantial accumulation of lignin, shown in fig. 6. Thus, it is evident that these two fungi were not able to consume lignin. The consumption of hexosans proceeded in early stages less rapidly than the consumption of pentosans, but later on, it proceeded more rapidly than the consumption of pentosans.

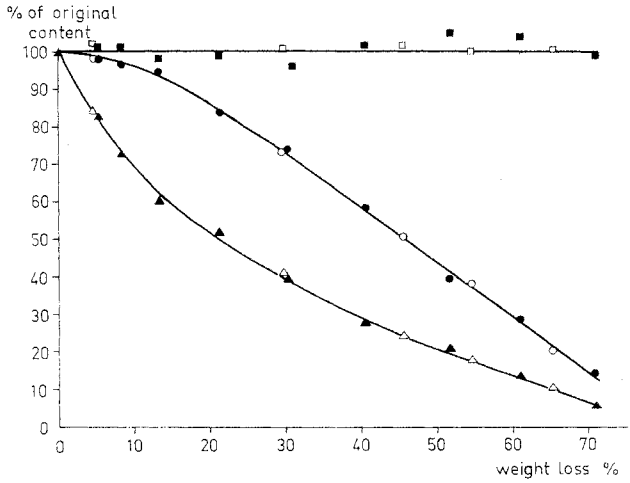

Fig. 7. Composition of birch sapwood in progressive stages of decay by *P. betulinus* and *P. marginatus*. The percentages are based on original amounts of the analyzed substances. Explanation of symbols is found in fig. 6.

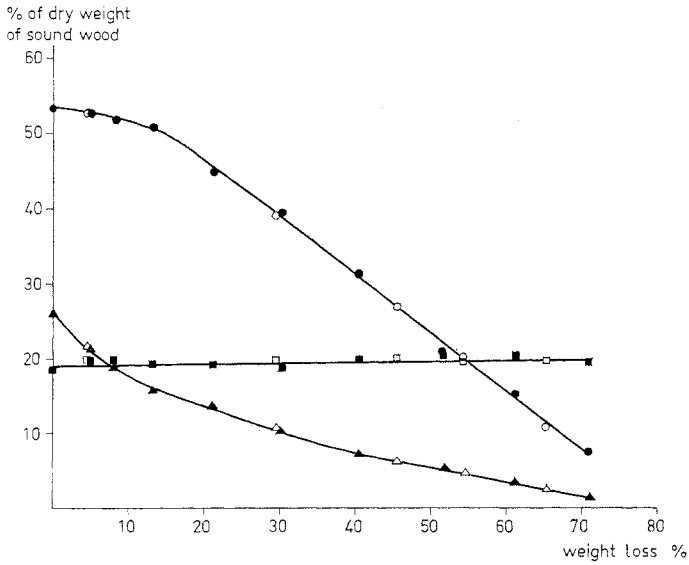

Fig. 8. Composition of birch sapwood in progressive stages of decay by *P. betulinus* and *P. marginatus*. The percentages are based on weight of sound wood. Explanation of symbols is found in fig. 6.

At maximum level of decay, when no further loss in weight could be noted, 14.1 % of the original amount of hexosans and 5.5 % of the original amount of pentosans remained. Together they constituted 8.8 % of the original weight of wood. This means that about 9 of the original 80 % of carbohydrates were not accessible to these fungi. There may be two explanations for this phenomenon. These carbohydrates occurred either in a form which was not accessible to the fungi, or they were very firmly bound to or completely surrounded by lignin.

The methods of analysis which were used here do not give any information about changes in the structure of the lignin which may have occurred as a result of fungal action. However, it is obvious that the lignin of brown-rotted wood is in several respects different from the lignin of sound wood (2, 28, 40). Rytjkova (88) analyzed birch wood which had been decayed by *P. betulinus* and found that the methoxyl content in sound wood was 5.78 % and in decayed wood 7.98 %. As the lignin content in the decayed wood was twice that of the sound wood, a loss of methoxyl groups must have occurred as a result of the decay. Thus it is obvious that even if no registrable loss of lignin occurs after an attack by *P. betulinus*, a change in the lignin itself does probably occur.

Infection of living trees

Sterilized wooden plugs of beech were placed on malt agar cultures of *P. betulinus* and *P. marginatus*. The plugs soon became invaded by the mycelia. After about one month the plugs were transferred to sterile test tubes with water agar in the bottom.

At Bogesund, a forest experimental area north-east of Stockholm, a number of birches (*Betula verrucosa*) and aspens (*Populus tremula*) were selected. An increment borer sterilized with alcohol was used to make sterile holes at about breast height. By studying the removed increment cores, it was ascertained that the trees were sound. The infected beech wood plugs were then transferred very carefully from the test tubes into the holes to avoid contamination from other fungi. The holes were then plugged with grafting-wax.

The infection was carried out in September. The next May it could be observed that the test fungi had infected birches as well as aspens. Discolouration of the wood was discernible both above and below the place of infection even if it had advanced further upwards (30—40 cm) than downwards (10—15 cm). In aspen the discolouration was less spread than in birch. The fungi could easily be re-isolated and identified by placing small, discoloured wood pieces on malt agar.

Thus, it was possible to infect not only living birches but also aspens with *Polyporus betulinus*. This indicates that Mayr (64) and Macdonald (63) were right when they considered *P. betulinus* to be a parasite. The fungus is able to continue its development in the living tree if its mycelium is brought there. Furthermore, because of the successful infections of aspen the host specifity of *P. betulinus* is questionable.

Physiological experiments

General remarks

Publications on the physiology of *P. betulinus* are not very frequent, and mostly rather old. La Fuze published in 1937 (54) a comparative nutritional study of the three rot fungi *P. betulinus, Fomes pinicola* (= *P. marginatus*) and *P. versicolor,* the results of which indicated that, among various sources of energy, polysaccharides gave the best mycelial growth. Pentoses were not utilized as efficiently as hexoses. La Fuze also showed that nitrogen in amino or ammonium form was better utilized than when it was in the form of nitrates, or nitrites. The best growth was observed when proteins with high contents of glutamic acid and tryptophane were added. Amino acids with phenyl and disulfide radicals checked growth. La Fuze also studied the influence on the growth of various wood extracts. He found that, out of warm water extracts from nine different tree species, only the extracts from White birch and Black birch promoted growth. However, it must be remembered that La Fuze used highly concentrated extracts, which might in lower concentrations have had positive effects on growth.

Studies concerning cultural characteristics, growth, temperature relations etc. for *P. betulinus* and *P. marginatus* have been carried out by —among others—Fritz 1923 (38), Bavendamm 1936 (7), Liese 1928 (55), Mounce 1929 (66), and Hemmi & Kurata 1933 (44). From these studies we know that both fungi can be regarded as rapid growers in culture. Mycelial growth should occur at temperatures between + 5 and + 35°C. Optimum temperature for growth has been ascertained to be 27—29°C. However, it is noticeable that all the reported calculations of growth including those of La Fuze were based on measurements of radial growth on an agar substrate.

Macdonald, 1937 (63), in his monograph on *P. betulinus* also described some enzymatic in-vitro tests with sporophore tissue. It was suggested that the fungus lacked maltase and trehalase.

Fries (36) demonstrated in 1938 that *Fomes pinicola* (*P. marginatus*) showed aneurin heterotrophy to a certain extent. However, no information about the vitamin requirements of *P. betulinus* have been found.

Nutrient media

In the physiological experiments both fluid and semi-solid nutrient media were used. Semi-solid media (agar media) were not used to a very great extent in the experiments themselves but were often used for preparing inoculas. Experiments dealing with the effect on the growth of various nutrients were always carried out on well-defined fluid nutrient media, chemicals of the purest possible quality being used.

The following nutrient media were used:

Medium A:

Glucose, 20.0 g; KH_2PO_4, 1.0 g; $MgSO_4 + 7H_2O$, 0.5 g; $(NH_4)_2SO_4$, 0.5 g; $CaCl_2 + 2H_2O$, 0.3 g; NaCl, 0.1 g; $ZnSO_4$ (Zn-konc. 1/500), 0.5 ml; Ferric citrate (1 % soln.), 0.5 ml; Thiamine HCl, 100 µg; Distilled water to 1000 ml.

Medium B:

Glucose, 20.0 g; KH_2PO_4, 1.0 g; $MgSO_4 + 7H_2O$, 0.5 g; Casamino acids (vitamin free), 7.0 g; $CaCl_2 + 2H_2O$, 0.3 g; NaCl, 0.1 g; $ZnSO_4$, 8.79 mg; $FeSO_4 + 7H_2O$, 0.9955 mg; $CuSO_4$, 0.393 mg; H_3Bo_3, 0.057 mg; $(NH_4)_6Mo_7O_{24}$, 0.368 mg; $MnSO_4 + 7H_2O$, 0.061 mg; Thiamine HCl, 100 µg; Biotin, 10 µg; Nicotinic acid, 100 µg; Riboflavin, 100 µg; Calcium pantothenate, 100 µg; Pyridoxine, 100 µg; Folic acid, 100 µg; Para-aminobenzoic acid, 50 µg; Vitamin B_{12}, 4 µg; Distilled water to 1000 ml.

Medium C:

Malt extract (syrup), 20.0 g; Distilled water to 1000 ml.

Medium D (maltagar):

Malt extract (syrup), 25.0 g; Agar-agar, 15.0 g; Distilled water to 1000 ml.

Methods

In experiments with fluid nutrient media only 100 ml Erlenmeyer flasks were used. The flasks, carefully cleaned with dichromatic sulfuric acid and washed in tap water and distilled water, contained 20 ml of nutrient solution. Inoculation was made either with 1 ml of mycelium suspension or with small pieces of an agar culture. Flasks inoculated with suspension were then either shaken or allowed to stand still during the experimental period resulting in "shake cultures"

and "submerse cultures". Flasks inoculated with pieces of agar culture were left standing, resulting in "floating cultures".

Inoculas for "floating cultures" were prepared by cutting out square pieces (3 × 3 mm) from a two-week-old petri dish culture of the fungus. In most cases 10 ml of medium D was used for a substrate in the dishes. Inoculas were taken in a zone 10—20 mm from the edge of the dish.

Mycelium suspensions were prepared by homogenizing 10—15 floating cultures of the fungus, about two weeks old, and filling up a maximum of 100 ml with sterile distilled water. Each experimental flask was then inoculated with 1 ml of the suspension thus prepared.

At the end of the experiment mycelia were filtered away in glass crucibles, which were then dried, one day at room temperature and one day at + 105°C. After having been cooled in a desiccator over phosphopentoxide the crucibles were then weighed.

The pH of the nutrient solution was determined at the beginning as well as at the end of the experiments.

Usually the experiments were run with five equally treated flasks. The standard deviation was calculated by the formula on page 10.

Material

The cultures used in the experiments were isolated in 1960—1961. They were tissue cultures from fresh sporophores collected in the forest. The stock cultures were kept in test tubes under paraffin oil.

Isolation B 6032 was selected as representative of *P. betulinus*. Unless otherwise stated, this isolation was used in the experiments. B 6032 was isolated from a fresh sporophore, growing on a dead standing birch near Kosta in southern Sweden. The birch was growing in a mixed stand of spruce and birch. A great deal of the birches in that stand had been attacked and killed by *P. betulinus*.

In several experiments *P. marginatus* and *Fomes fomentarius* were used as comparative organisms. The isolations B 6115 and 6116 were used. Both were isolated from sporophores, growing on dead standing birches at Märsta, north of Stockholm. In this stand, practically all the birches were attacked by the three fungi mentioned above.

Cultural characteristics

When cultured *P. betulinus* grows very well on most substrates. On malt agar its aerial mycelium looks loose at first. After about three weeks the aerial mycelium collapses, at least in older parts of the culture, and small clusters of more compact mycelium form. After two

Tab. 5. Radial growth at room temperature of malt agar cultures of 8 strains of *P. betuliuns* and of *P. marginatus* and *F. fomentarius*

Strain tested	Average radial growth mm/day
Polyporus betulinus B6029	4.7
Polyporus betulinus B6030	5.7
Polyporus betulinus B6032	6.7
Polyporus betulinus B6033	7.2
Polyporus betulinus B6034	6.6
Polyporus betulinus B6064	7.0
Polyporus betulinus B6065	6.4
Polyporus betulinus B6102	5.4
Polyporus marginatus	6.0
Fomes fomentarius	9.1

to three months some cultures form irregular nodules (fruiting bodies), 10—30 mm in size, along the edge of the petri dish. On these nodules a pore layer may form producing fully germinative spores.

A Bavendamm test (6) on gallic and tannic acids gave no zones. Consequently, the mycelium produces no polyphenol oxidases indicating a fungus that causes a destructive type of rot.

The rate of growth measured as the radial growth was very different for various isolates. Radial growth on malt agar in growth tubes varied at room temperature (20—22°C) between 4.7 and 7.2 mm per day for 8 different isolates of *P. betulinus* (see tab. 5). Among the other tested fungi, *P. marginatus* had a rate of growth comparable to that of the *P. betulinus* isolates whereas *Fomes fomentarius* grew much more rapidly. According to Bavendamm (7) all three belong to the rapidly growing rot fungi. A variation in the rate of growth measured as the dry weight of produced mycelium also occurred. This can clearly be seen in fig. 9. However, *P. marginatus* and four of the five *P. betulinus* isolates showed closely comparable growth rates during the first three weeks. The divergence of the growth curves which then occurred was probably due to the production of fruiting bodies, the time needed for formation of which varied for various isolates. However, when the formation had once started the dry matter production seemed to be equally rapid.

Variation in the rate of growth measured as radial growth was not in accordance with growth measured as dry matter production. For instance, isolate B 6033, which showed the greatest radial growth,

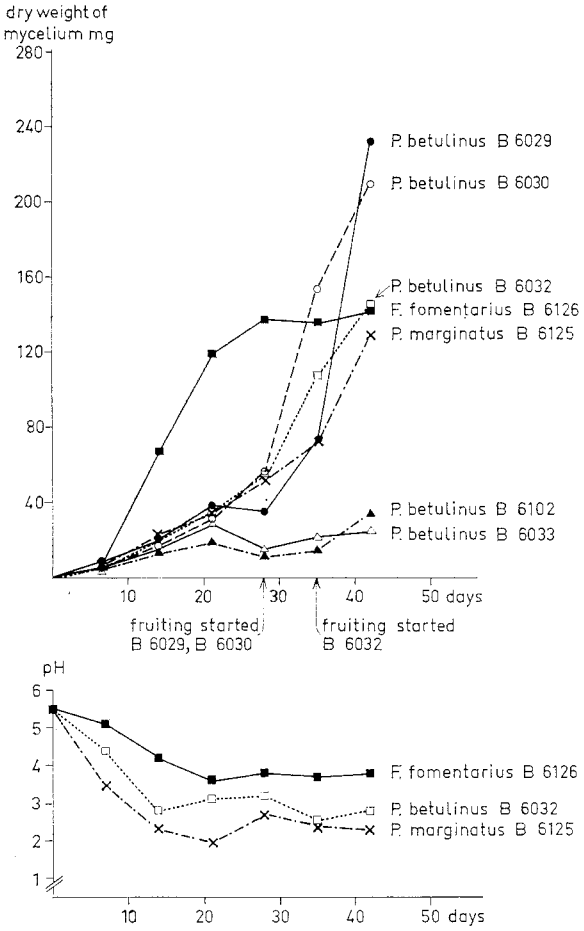

Fig. 9. Dry weight production and pH of the nutrient solution of *Fomes fomentarius*, *P. marginatus* and 5 strains of *P. betulinus* grown as floating cultures on medium C.

actually had a slow mycelial production. Isolate B 6029, on the other hand, had the slowest radial growth but the most rapid mycelial production.

The growth of the fungi resulted in a pH-drop in the nutrient solution. The tested isolates of *P. betulinus* were usually half way between *F. fomentarius* and *P. marginatus*, which caused the greatest pH-drop.

Temperature and growth

From fig. 10 we can see the growth effects at different temperatures. *P. betulinus*, *P. marginatus* and *F. fomentarius* were grown as floating cultures on medium C.

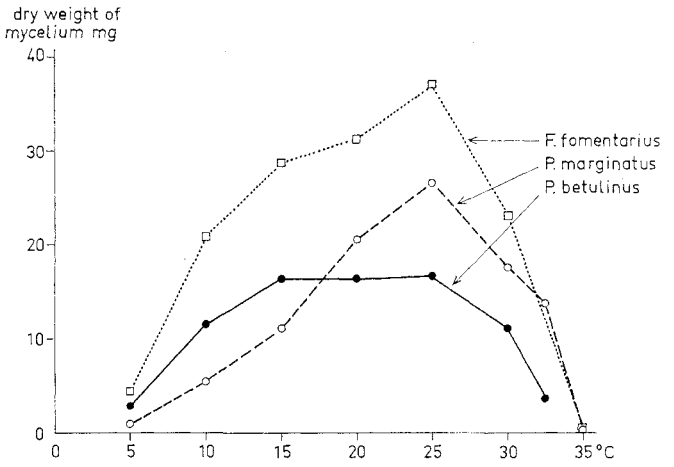

Fig. 10. Dry weight production of *P. betulinus*, *P. marginatus* and *F. fomentarius* grown as floating cultures on medium C at different temperatures.

P. betulinus has a symmetric temperature curve around an optimum of about 20° C, whilst the other two have asymmetric curves skewed to the right and with optima between 25 and 30°C, which is the most common temperature curve among fungi (27).

Maximum temperature for growth seems to be around 35°C for all the three fungi. Minimum temperature for *P. marginatus* is obviously about + 5°C and for the other two a little lower.

According to Humphrey and Siggers (47) *P. betulinus* should be placed in a group of rot fungi with a growth optimum below + 24° C. In this group there is also to be found *Merulius lacrymans, Coniphora cerebella* and *Stereum sanguinolentum* (12). Likewise, the other two tested fungi belong to a group of rot fungi with optima between + 24 and + 32°C. Rennerfelt (79) investigated temperature relations for several fungi living in ground wood pulp. The temperature optima for growth were then determined for three rot fungi: *Pholiota mutabilis* 22° C, *Polystictus versicolor* 27° C and *Polyporus hirsutus* 32° C. Generally the optimum temperatures for most rot fungi are between + 25 and + 30°C (25). Temperature optima for some other fungi and groups of fungi may be mentioned for comparison: *Psaliota bispora* 24°C (97), *Tricholoma* spp. 17.5—27° C (72), *Phacidium infestans* 15° C (76), bluening fungi 22—32° C and *Torulopsis* spp. 22—27° C (79).

For mycelia growing on malt agar the resistance to low temperatures was studied. Four-day old agar plate cultures were transferred from room temperature (+ 22°C) to — 30 ± 1°C. Daily (later at longer intervals) 3 plates were retransferred to room temperature. The daily

radial growth was then measured. After 42 days at — 30°C the mycelium of *P. betulinus* was no longer alive and no further growth occurred, when the mycelium was retransferred to room temperature. On the other hand, the mycelium of *P. marginatus* was still alive even after 84 days at — 30° C. The lag phase, before growth occurred again after the plates had been retransferred to room temperature, was extended the longer the mycelia had been kept at the very low temperature. Thus the lag phase for *P. betulinus* after freezing for 1 day was 3 days and after freezing for 35 days 9 days. For *P. marginatus* the corresponding figures were 4 days lag after freezing for 1 day, 6 days lag after freezing for 35 days and 10 days lag after freezing for 84 days.

pH and growth

The pH requirements for growth were determined in two experiments. In the first experiment buffer solutions of varying pH values were prepared by mixing 1/15 M KH_2PO_4, 1/15 M Na_2HPO_4 and 1/10 N HCl. The culture flasks contained 10 ml of the buffer solution and 10 ml of a nutrient solution identical with that recommended by Lindeberg (58), except that asparagine was replaced by ammoniumtartrate as the nitrogen source.

When the mycelium was growing in nutrient solutions with pH ranging from 3.7 to 6.0 there was a very rapid drop in pH (tab. 6 and fig. 11). After only two weeks a substantial pH drop was noticeable. After two weeks the growth seemed to have an optimum in the medium with a staring pH of 5.5. After six weeks the optimum starting pH was about 5.0, and there appeared a good growth even at pH 2.1. The maximum seemed to be around pH 7.0.

Tab. 6. Dry weight production of *P. betulinus* grown at varying pH values in buffered nutrient solutions according to Lindeberg 1944 (56). However, asparagine was replaced by ammoniumtartrate as the nitrogen source.

Incubation time weeks		Initial pH					
		2.1	3.7	4.7	5.5	6.0	6.9
2	dry weight mg	1.2	3.5	5.3	6.1	5.9	0.2
	final pH	2.0	3.4	3.6	3.6	4.5	6.8
4	dry weight mg	4.8	6.0	7.9	8.8	7.1	2.0
	final pH	2.0	3.5	3.6	3.4	3.5	6.8
6	dry weight mg	12.0	16.4	18.2	17.9	14.0	4.6
	final pH	1.9	3.0	3.1	3.1	3.2	6.5

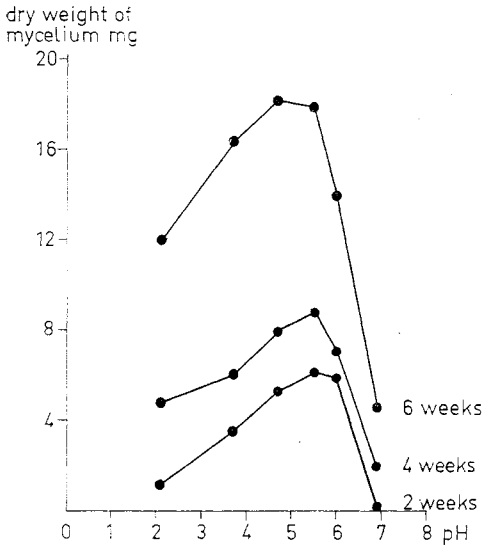

Fig. 11 a. Dry weight production of *P. betulinus* grown for 2, 4, and 6 weeks as floating culture on buffered nutrient solutions according to Lindeberg (58).

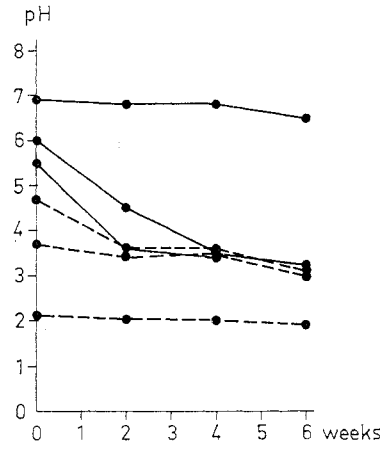

Fig. 11 b. Changes of pH of the nutrient solutions during growth of *P. betulinus*.

In the other experiment various buffer systems were used with pK-values appropriate for the different pH intervals. Medium B (with 3.5 g casamino acids per liter) was used and 10 ml of the doubly concentrated nutrient solution was poured into the culture flask with 10 ml of the buffer solution.

The following buffer systems were used:

buffer substances	pH of buffer	pH of buffer + nutrient solution after autoclaving
0.2 M KCl + 0.2 N HCl	1.4	1.6
0.1 M citric acid + 0.2 M Na_2HPO_4	3.7—5.4	3.7—5.0
0.1 M KH_2PO_4 + 0.1 M NaOH	5.8—7.6	5.4—7.0

Also with these buffer systems the pH of the nutrient solution decreased drastically in 13 days (tab. 7). Its optimal pH in this solution seemed to be very close to 5.0 (fig. 12).

Wood-destroying fungi usually grow between pH 2 and pH 7, i.e. at an acid pH. Furthermore, they produce acids, which acidify the medium. Thereby brown rotters cause a greater acidification than white rotters (11). Wolpert (99) found that rot fungi had a very wide pH

Tab. 7. Dry weight production of *P. betulinus* grown on medium B (casamino acids 3.5 g/l) at varying pH. Buffer systems used: see page 24.

Initial pH	Dry weight mg	Final pH	Initial pH	Dry weight mg	Final pH
1.6	2.8	1.6	5.4	16.0	3.3
3.7	13.8	3.3	6.0	11.2	5.3
4.1	16.2	3.5	6.8	6.4	6.7
4.5	16.0	3.6	7.0	4.7	6.8
5.0	17.9	3.8	K(4.7)	15.7	3.2

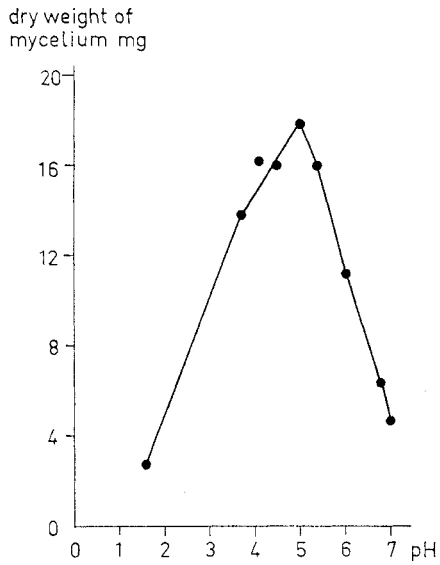

Fig. 12. Dry weight production of *P. betulinus* grown as floating culture on medium B with different pH. Three buffer systems were used.

tolerance in the acid region. Thus, *Lenzites sepiaria* was able to grow at all tested pH values from 2.5 to 8.0. *P. betulinus* was studied by Rumbold, who found that the fungus showed the best growth in acid media, some slight growth in a neutral medium and no growth at all in alkaline media.

Effect of minerals and vitamins on growth

The following components from medium A constituted the basic nutrient solution: Glucose 20.0 g; KH_2PO_4 1.0 g; $MgSO_4 + 7H_2O$ 0.5 g; $(NH_4)_2SO_4$ 0.5 g and Fe-citrate (1 % soln.) 0.5 ml.

Tab. 8. Dry weight production of *P. betulinus* grown on media containing a basic nutrient solution and various minerals and vitamins.

Medium containing							Dry weight mg/flask
Basic nutrient Solution	$ZnSO_4$	Thiamine	NaCl	$CaCl_2$	Micro elements	Vitamin mixture	
x							5.1
x	x						5.2
x		x					17.7
x	x	x					17.6
x	x	x	x				18.2
x	x	x		x			19.6
x	x	x	x	x			22.9
x	x	x	x	x	x		21.0
x	x	x			x		17.3
x	x	x				x	20.0
x	x	x	x			x	19.3
x	x	x	x	x		x	28.2
x	x	x	x	x	x	x	27.3
x	x		x	x	x	x	9.5

To this basic solution the following nutrients and growth substances were added, alone or in various ways mixed with each other; the same amounts and concentrations as in media A and B (see page 25) were used: 1. Zinc sulphate; 2. Sodium chloride; 3. Calcium chloride; 4. Micro elements containing Copper sulphate, Boric acid and Ammoniumparamolybdate; 5. Thiamine hydrochloride; 6. Vitamin mixture containing Biotin, Niacin, Riboflavin, Ca-pantothenate, Pyridoxine, Folic acid, Inositol, P-aminobenzoic acid, Vitamin B_{12}.

From the results in tab. 8 it is obvious that *P. betulinus* shows thiamin heterotrophy. Na + Ca and especially Na + Ca + vitamin mixture also gave positive growth effects. Calcium has been observed to give stimulating effects on mycelial growth of basidiomycetes. Thus, Fries found in 1956 (35), that for *Coprinus ephemerus* a concentration of 1.2 mg Ca per liter nutrient solution was sufficient to give maximal growth.

Adding zinc or micro elements, however, did not give further growth effects. Probably impurities in chemicals and glassware were sufficient to cover the requirements for these substances. A vitamin mixture together with all the other nutrients could not replace thiamine.

Thiamine heterotrophy has often been reported among wood-destroying Hymenomycetes and it has been demonstrated by, among others, Fries (36), Robbins (84), and Yussef (100). Also other wood-inhabiting fungi show more or less absolute thiamine requirements like most *Ophiostoma* species (51).

Carbon nutrition

The influence of five simple sugars of various concentrations on two isolates of *P. betulinus* (6032, 6065) was examined in a preliminary test. The sugars were those which usually occur in a polymerized form in angiosperm wood (28, 41). Medium A without the glucose and with ammonium-tartrate as nitrogen source was used. The sugars were added to the nutrient solutions before autoclaving. The solutions were buffered by adding phosphate buffer in accordance with Lindeberg (58).

Tab. 9. Dry weight production of two different strains of *P. betulinus* grown on medium A containing 5 different simple sugars. Nutrient solution and sugars were authoclaved together.

Carbon source tested	Conc.	Polyporus betulinus B6032			Polyporus betulinus B6065		
		Dry weight	pH		Dry weight	pH	
	g/l	mg/flask	Initial	Final	mg/flask	Initial	Final
Glucose	40	24.3	5.0	3.1	26.8	5.0	3.1
	20	14.5	5.0	3.0	20.3	5.0	3.1
	5	8.1	5.0	3.0	5.7	5.0	3.3
Mannose	40	11.8	4.6	3.3	10.0	4.6	3.3
	20	7.9	5.0	3.3	7.0	5.0	3.4
	5	6.9	5.0	3.2	2.9	5.0	3.4
Galactose	40	13.1	5.0	3.1	6.5	5.0	3.3
	20	10.0	5.0	3.2	3.7	5.0	3.3
	5	2.1	5.0	3.3	2.0	5.0	3.4
Xylose	40	4.1	4.8	3.4	4.0	4.8	3.3
	20	3.1	5.0	3.4	2.9	5.0	3.5
	5	1.9	5.0	3.3	1.5	5.0	3.6
Arabinose	40	2.0	4.8	3.3	1.8	4.8	3.4
	20	0.4	5.0	3.8	0.3	5.0	3.6
	5	0.7	5.0	4.5	0.7	5.0	4.5

Tab. 10. Dry weight production of *P. betulinus* grown on medium A containing various carbohydrates.

Carbon source tested	Initial pH	Dry weights and pH after various incubation times													
		14 days		21 days		28 days		35 days		42 days		49 days		56 days	
		mg/flask	pH	mg/flask	pH	mg/flask	pH	mg/flask	pH	mg/flask	pH	mg/flask	pH	mg/flask	pH
None	4.3	1.2±0.1	4.0	1.6±0.1	4.0	2.1±0.1	3.9	0.9±0.2	3.8	1.2±0.1	3.7	1.0±0.1	3.7		
D-arabinose	4.2	2.1±0.3	3.4			2.4±0.1	3.4			2.7±0.2	3.0			4.5±0.1	2.8
D-xylose	4.0	2.6±0.3	2.9	4.5±1.1	2.9	10.9±2.4	2.9	12.8±2.1	2.9						
L-rhamnose	4.3	0.9±0.2	3.1	0.9±0.1	3.1	2.6±0.2	3.0	2.6±0.2	2.9	2.1±0.2	2.6	2.1±0.1	2.7		
D-ribose	4.1	0.5±0.1	3.3	1.1±0.1	3.2	2.3±0.2	3.1	1.7±0.4	2.9	1.1±0.5	2.7	1.8±0.3	2.7		
D-glucose	4.1	1.8±0.2	3.1	5.6±1.5	2.8	16.0±1.1	2.5	20.4±1.7	2.5	22.9±1.2	2.2				
D-galactose	4.0	2.4±0.6	2.9	2.5±0.2	2.8	7.4±1.9	2.6	8.5±1.6	2.7						
D-mannose	4.0	4.1±0.7	2.9	8.2±0.7	2.8	12.2±2.4	2.7	13.8±2.8	2.6						
D-fructose	4.1	1.5±0.2	3.0	4.3±0.7	3.0	5.8±0.6	2.9	6.2±0.2	2.6	6.9±0.8	2.5	9.3±2.2	2.6		
L-sorbose	4.0	0.5±0.2	3.7	0.7±0.1	3.5	2.6±0.2	3.4	1.5±0.5	3.2	1.1±0.1	3.0	1.1±0.1	2.9		
D-cellobiose	4.1	10.3±1.0	2.9	25.6±1.5	2.6	32.6±1.3	2.5	50.8±3.6	2.5	58.4±7.2	2.2				
lactose	4.0	2.3±0.5	3.1	3.8±0.2	2.8	6.1±0.6	2.6	10.2±1.4	2.4	18.8±2.6	2.4				
D-maltose	4.0	3.3±0.6	3.0	4.7±0.4	2.9	11.3±0.9	2.7	12.7±1.4	2.8	18.4±1.8	2.6				
D-sacharose	4.1	2.2±0.3	2.9	4.8±0.9	2.8	10.0±1.6	2.7	11.8±2.8	2.6	12.6±2.4	2.4				
D-trehalose	4.2	1.1±0.1	3.2	4.2±0.3	2.9	7.3±0.7	2.7	8.3±1.3	2.8	10.4±2.5	2.7				

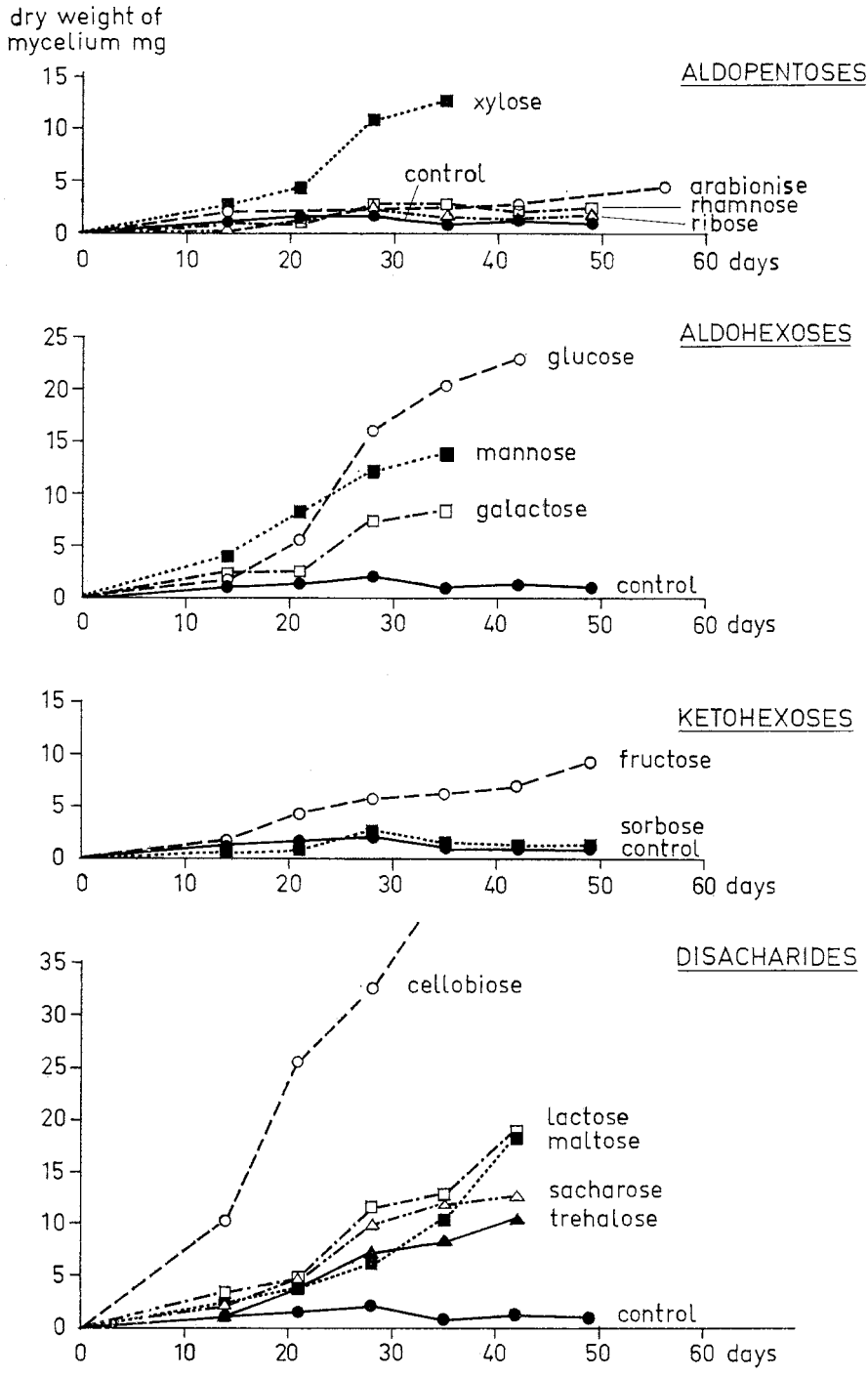

Fig. 13. Dry weight production of *P. betulinus* grown as floating culture on medium A containing different sugars as the sole sources of carbon.

Both isolates produced best growth on glucose. Galactose and mannose were about equally effective, while arabinose and xylose were very poor carbon sources. Arabinose was probably not at all accessible. (See tab. 9.)

This very poor utilization of certain sugars may depend on changes which have occurred during the autoclaving procedure. For instance, it is known that furfural can form when xylose is autoclaved in contact with phosphates or amino acids (27).

Furfural formation may also occur when monosaccharids of the hexose type are autoclaved in the nutrient medium (56). Therefore, in the following experiments the carbon source was always autoclaved separately and then aseptically added to the nutrient solution. Xylose and maltose, which have been observed as being instable when heated, were filter-sterilized and aseptically added to the experimental flasks.

The results can be seen in tab. 10 and fig. 13. Among the tested aldopentoses xylose was utilized quite well, whereas arabinose, ribose and rhamnose were utilized very poorly, or not at all. It is possible that by enzymatic adaptation a small utilization of arabinose occurred after a very long time. Among the aldohexoses glucose constituted the best source of energy. Mannose was better than galactose. Two ketohexoses were tested. Among these, fructose could be utilized but not sorbose. All the tested disaccharides were accessible. Cellobios stimulated growth to a high degree. Lactose and maltose seemed to be better carbon sources than saccharose and trehalose.

A comparison between *P. betulinus* and *P. marginatus* is shown in tab. 11. Here also the trisaccharide raffinose and the polysaccharides glycogen and inulin were tested. It is evident that during the experiment arabinose, rhamnose, ribose, and sorbose were not utilized by any one of the fungi. Good sources of energy for both fungi were glucose, mannose and cellobiose. The rest of the tested carbohydrates showed a varying degree of accessibility. It may be worth mentioning that *P. marginatus* made a good growth on glycogen and that *P. betulinus* did not grow on inulin given as the sole source of carbon.

The cellobiose used in the experiments was showed by control tests to contain substances, which to a certain degree could replace thiamine in the nutrient solution. This and other possible impurities in the cellobiose, which was of the highest purity available, may perhaps explain the growth effects of *P. betulinus*.

A test of growth with cellulose as the sole carbon source was made. Thereby the fungi were allowed to attack circular filter papers, which, after dry weight determination were placed on glass triangles in petri

Tab. 11. Comparison between relative growths of *P. betulins* and *P. marginatus* growing in medium A with varying carbon sources.

Carbon source tested	Relative dry weight	
	Polyporus betulinus	Polyporus marginatus
None	13	4
D-arabinose	15	7
D-xylose	68	43
L-rhamnose	16	3
D-ribose	14	11
D-glucose	100	100
D-galactose	46	5
D-mannose	76	88
D-fructose (Laevulose)	36	90
L-sorbose	16	4
D-cellobiose	204	108
lactose	38	74
D-maltose	71	41
D-sacharose	63	50
D-trehalose	46	101
D-raffinose	18	52
glycogen	36	132
inulin	12	31

dishes with medium A (carbon source excluded). A radial strip was cut in the filter papers and bent down into the nutrient medium. When nutrient solution had soaked up through the strips and spread in the filter papers, these were inoculated with pieces from an agar culture. After 42 days at room temperature the filter papers were taken out of the dishes, and after removing the surface mycelium they were thoroughly washed in distilled water, dried and weighed. The differences in the weights of the filter papers before and after the fungus attack were calculated and used as a measure of growth.

From the results in table 12 it is obvious that the two brown-rot fungi *P. betulinus* and *P. marginatus* caused a comparatively small decomposition of cellulose when this was given as the sole carbon source in the form of filter paper. As both fungi easily utilized cellobiose it must be the production of cellulases and not the synthesis of cellobiase that is poorly developed. White-rot fungi in general seem to be able to decompose filter paper cellulose given as the sole source

Tab. 12. Weight losses of filter papers resulting from attacks of various root fungi. Medium A was used.

Organism	Type of rot	Weight loss mg/paper
Peniophora gigantea	White	20.7
Lenzites betulina	White	13.7
Polyporus zonatus	White	11.4
Polyporus rutilans	White	9.0
Polyporus hirsutus	White	8.2
Fomes fomentarius	White	7.3
Poria corticola	White	4.6
Pseudeurotium zonatum x)	?	4.5
Polyporus betulinus	Brown	3.9
Fomes igniarius	White	3.4
Polyporus marginatus	Brown	2.0

*) According to Brewer (19) found as slime fungus in white water from pulp industries.

of carbon. However, under natural circumstances cellulose is rapidly decomposed by brown-rot fungi like *P. betulinus* and *P. marginatus* (88). In the laboratory these two fungi and many other brown-rot fungi have also proved able to decompose cellulose in wood (2, 28, 73). A richer synthesis of cellulase may be induced by other carbohydrates in the complex wood substrate.

Among the hexoses, glucose, mannose and also fructose are regarded as equally good carbon sources for fungi. Thus, several species of the genera *Tricholoma* (72), *Coprinus* (34) and *Ophiostoma* (51) and also *Stereum gausapatum* (45) showed about equally good rates of growth on these three hexoses. This tendency is also clear from an outline presented by Lilly & Barnett (56), where 21 species belonging to 17 different genera were tested.

Galactose is utilized by certain fungi e.g. *Ophiostoma* species (51) and *Psaliota bispora* (97), while other groups of fungi like *Coprinus* (34) and *Tricholoma* (72) grow very badly on this sugar. Among Fungi Imperfecti about 1/5 of 57 tested species were unable to utilize D-galactose (27). Enzymatic adaptation is known for galactose but in certain cases toxicity has also been shown (27, 72).

Among the pentoses, xylose, arabinose and frequently also rhamnose are good whereas ribose is a poor carbon source for *Ophiostoma* species, according to Käärik 1961 (51). Xylose often gives a good growth for *Coprinus* spp., *Lentinus lepideus* and *Psaliota bispora* (34, 71, 97)

whereas on the other hand, most *Tricholoma* species grow very poorly on xylose. Arabinose, which is not at all utilized by fungi like *Coprinus* spp. and *Phialophora fastigiata* (21), has given adaptive growth by certain fungi (52). The conclusion must be that pentoses as the sole source of carbon are utilized very differently by fungi.

Among disaccharides the α-glucoside maltose and the β-glucoside cellobiose are the best carbon sources for fungi (27). Trehalose and saccharose are available to most fungi, whereas lactose (β-galactosidoglucose) can be utilized by a number of fungi smaller than the four disaccharides mentioned above. As in the case of galactose an adaptive growth is not unusual on lactose. This is on the whole confirmed by most investigations, e.g. for cellulose decomposing *Tricholoma* species (72), *Ophiostoma* species (51) and *Coprinus* species (34). However, saccharose is not at all available to the genus *Coprinus*. Parallel with *P. betulinus,* it is interesting to notice that cellobiose has a great growth-promoting effect on *Coprinus narcoticus*. According to Fries (34) cellobiose gave a growth which was almost twice that of its single component, glucose. Furthermore, it is not unusual for cellobiose to give a better growth than glucose. This is the case with e.g. *Venturia inaequalis* (33), *Ophiostoma albidum* and *Ophiostoma gaeliformis* (51).

The trisaccharide raffinose as a carbon source can be utilized by many fungi, but there are great variations even within genera (51). According to Nord & Sciarini 1946 (70) *Fomes annosus* and three *Merulius* species can utilize raffinose. *Venturia inaequalis* is also reported to afford better growth on raffinose than on glucose.

Among the tested polysaccharides, glycogen, which is chemically almost identical to the amylopectin fraction in starch, can with a certain amount of difficulty be utilized by fungi. The polyfructoside inulin is a poor carbon source for *Ophiostoma* species (51) and is not at all available to *Coprinus* species (34). According to Cochrane (27) limited data indicate, however, that inulin is a good carbon source for fungi.

Because of its insolubility in water, cellulose causes difficulties when growth experiments of the conventional type are made. Thus, the methods used to test microbial growth on cellulose have been various and sometimes, perhaps, not quite adequate. As a rule use has been made of losses in strength or weight of the cellulose material after microbial attack (90). Soluble cellulose derivatives like carboxymethyl cellulose added to a nutrient solution have also been used. Another method is to measure the total nitrogen or protein content of the

mixture of mycelium and more or less degraded cellulose which is formed when there is a growth of fungus on cellulose (72).

Cellulose is very poorly utilized by *Ophiostoma* species. Among the *Tricholoma* species there are types, however, with good cellulolytic activity (72) and among the *Coprinus* most species are able to utilize cellulose. All wood-decomposing fungi attack the cellulose part of the wood.

Nitrogen nutrition

In these experiments "floating cultures" as well as "submerse cultures" and "shake cultures" were used. Both medium A and B with varying nitrogen sources were tested. The following sole N-sources were tried: inorganic ammonium and nitrate nitrogen, the amino acids aspartic acid and glutamic acid with corresponding amides, casein hydrolyzate, acid glucose amine, and finally urea. Three nitrogen concentrations were tested.

The results can be seen in tab. 13 and 14. Among the inorganic nitrogen sources the ammonium nitrogen was well utilized by both *P. betulinus* and *P. marginatus*. Nitrate seemed to be very poorly or not at all available. Among the organic nitrogen sources asparagine appeared to be a surprisingly poor nitrogen source, when the culture was floating or shaken. On the whole, the amides were poorer nitrogen sources than the corresponding amino acids. Urea was an excellent nitrogen sources while acid glucose amine was not at all available. Where the nitrogen sources were easily utilized the highest concentration generally gave the best growth.

Among basidiomycetes, inability to utilize nitrate is very common. Out of eight tested *Tricholoma* species, only one could grow on nitrate (72). Hackskaylo et al. (42) found a slow utilization of nitrate by a few basidiomycetes. 14 of 18 *Coprinus* species could not grow on nitrate as the sole nitrogen source (34). The same is true of *Coprinus heptemerus* (10), *Psaliota bispora* (97) and *Marasmius* spp. (58). Jennison et al. (48) did not succeed in growing any one of 15 wood-destroying basidiomycetes on nitrate. As an example of another group of wood-inhabiting fungi mention may be made of the genus *Ophiostoma,* where nitrate is a good nitrogen source. As a conclusion it seems as though utilization of nitrate is very rare among higher fungi.

The inability of *P. betulinus* and *P. marginatus* to grow on nitrate is not caused by molybdenum deficiency, since medium B, which contains molybdate, was used in several of the experiments. A test with *P. betulinus* showed that nitrite (KNO_2) could be utilized as the ni-

Tab. 13. Dry weight production of cultures of *P. betulinus* grown in various ways in media A and B containing varying nitrogen sources and concentrations. Concentration N contained 76.065 mg nitrogen per liter. 5N and N/5 were five times and one fifth respectively of the N concentration.

Nitrogen source tested	Medium A, floating cultures Dry weights in mg/flask after 36 days incubation time			Medium A, submerse cultures Dry weights in mg/flask after 36 days incubation time			Medium B, shake cultures Dry weights in mg/flask after 10 days incubation time			Medium B, shake cultures Dry weights in mg/flask after 15 days incubation time		
	5N	N	N/5	5N	N	N/5	5N	N	N/5	5N	N	N/5
Ammonium tartrate	-	19.1±0.4	14.5±0.6	26.9±0.5	23.2±1.8	11.6±0.3	34.2±1.3	20.8±1.0	12.5±0.4	54.4	30.7	12.1
$(NH_4)_2\ SO_4$	23.3±1.3	18.3±1.1	21.5±0.4	13.5±0.1	12.5±0.6	8.5±0.3	30.3±3.1	20.8±2.1	12.9±0.8	45.0	30.1	12.8
$NH_4\ Cl$	24.4±1.8	22.7±1.2	13.4±1.2	13.3±0.6	12.6±0.4	8.9±0.9	19.2±2.1	17.0±0.9	13.9±0.2	39.1	27.3	14.3
KNO_3	0	0	0	7.7±0.4	7.8±0.4	5.3±0.2	5.3±0.5	5.9±0.6	8.1±0.4	1.4	5.9	6.2
$NH_4\ NO_3$	19.9±0.9	26.1±1.4	10.9±1.1	9.8±0.3	9.9±0.6	6.3±0.1	14.2±1.1	17.4±1.5	12.8±0.5	24.5	20.5	10.8
Glutamine	1.3±0.4	14.5±0.6	8.2±1.7	11.9±0.2	10.2±0.2	7.5±0.3	30.9±2.6	20.8±1.3	11.2±0.2	55.7	24.8	10.8
Glutamic acid	28.5±4.5	19.1±2.4	7.1±0.6	8.7±0.7	11.3±0.4	10.8±0.3	42.1±5.2	27.2±0.9	13.5±0.2			
Asparagine	4.9±0.1	6.8±0.2	7.4±0.7	23.4±1.0	17.2±0.9	11.0±0.3	11.5±0.7	16.5±2.0	13.8±0.2			
Aspartic acid	25.7±2.4	15.3±0.9	8.3±1.0	10.4±0.1	14.4±0.6	11.8±0.4	38.4±3.7	36.2±2.4	15.3±0.3			
Casein hydrolyzate	30.3±5.6	9.6±1.1	3.7±2.5	29.3±2.5	13.6±0.3	8.2±0.3	29.9±0.5	12.1±0.5	8.5±0.2			
Glucose amine-HCl	0	0	0	6.8±0.3	7.1±0.2	5.8±0.2	-	-	-			
Urea	-	-	-	18.6±1.0	14.1±0.4	10.5±0.3	14.9±0.9	11.4±0.7	10.3±0.3			
None					6.5±0.3			6.8±0.7			5.1	

Tab. 14. Dry weight productiin in floating and shake cultures of *P. marginatus* grown on medium A and medium B respectively. Three nitrogen concentrations were used.

Nitrogen source tested	Medium A, floating cultures Dry weights in mg/flask after 25 days incubation time			Medium B, shake cultures Dry weights in mg/flask after 8 days incubation time		
	5N	N	N/5	5N	N	N/5
Ammonium tartrate	30.4±2.1	23.2±1.3	18.0±2.6	23.3±0.8	21.6±1.1	13.6±0.5
$(NH_4)_2\ SO_4$	33.1±1.6	24.4±2.5	25.3±1.5	24.0±1.1	20.6±0.6	12.5±0.1
$NH_4\ Cl$	23.3±1.7	24.5±0.7	12.1±1.1	19.2±0.5	20.1±1.5	14.5±0.4
KNO_3	0	0	0	4.4±0.4	5.3±0.1	6.3±0.2
$NH_4\ NO_3$	21.9±1.1	21.9±1.3	12.5±0.8	17.3±0.6	16.7±0.4	9.7±0.8
Glutamine	16.5±1.5	21.3±1.3	12.6±0.7	25.2±1.0	20.4±0.7	9.9±0.8
Glutamic acid	17.2±1.1	28.1±0.8	15.1±0.8	67.5±3.5	24.3±0.3	11.1±0.5
Asparagine	1.9±0.6	5.7±0.4	2.1±0.4	14.2±0.6	14.2±0.4	7.9±0.2
Aspartic acid	23.2±1.9	15.4±1.2	22.0±1.1	67.4±1.4	31.0±0.5	14.7±0.6
Casein hydrolyzate	28.9±1.4	9.2±1.9	+	21.7±1.0	8.7±0.4	6.6±0.1
Glucose amine-HCl	0	0	0	-	-	-
Urea	-	-	-	31.5±2.2	11.9±0.4	10.8±0.6
None					4.0±0.4	

trogen source. Furthermore, as we have seen from tab. 13, various ammonium salts were excellent nitrogen sources. Thus, it is most probable that the first step in the generally accepted hypothetical reduction series (reduction of nitrate to nitrite) is blocked, e.g. though its inability to synthetize nitrate reductase.

In spite of the great and rapid pH drop, which, inter alia because of the unilateral uptake of cations occurred in nutrient solutions containing ammonium salts, the latter were easily assimilated by *P. betulinus* and *P. marginatus*. Ammonium nitrogen is also readily utilized by most other fungi. Only certain lower phycomycetes show a real inability to grow with ammonium as the sole source of nitrogen (27). It may be mentioned that among higher fungi *Coprinus* species, *Tricholoma* species and wood-destroying fungi generally utilize ammonium (34, 72, 48).

Aspartic acid, glutamic acid and corresponding amides are generally good nitrogen sources for higher fungi. Amide nitrogen usually surpasses amino nitrogen. However, it is not unusual to find fungi, which produce a poorer growth on asparagine than on aspartic acid (34, 42). Jennison et al. (48) found the following list of precedence for 15 rot fungi: casein hydrolyzate > glutamine > aspartic acid, glutamic acid and asparagine.

Glucose amine was tested as a source of nitrogen because 2-aminoglucose in polymerized form appears as chitin in cell walls of most fungi, and, as nitrogen must be regarded as a limiting factor of growth in wood, it might be possible for young mycelium to utilize older autolyzed mycelium as a nitrogen source. However, neither *P. betulinus* nor *P. marginatus* could grow on glucose amine.

It is already mentioned that pure wood contains a very small amount of nitrogen. Normally the nitrogen content rarely exceeds 0.3 % (24). However, Vorreiter (98) reports that birch wood (unspecified part of the wood) contains about 0.34 % nitrogen. At all events the nitrogen content in wood is small enough to make it a possible growth-limiting factor. This suggestion is supported by the fact that artificial addition of nitrogen—especially in organic form—to the wood will greatly increase the rate of decomposition (31). If, when the N/C-quotient for birch wood is calculated, and the nitrogen content 0.3 % and the carbon content 30 %,—found by Rytjkova (88)—are used, we find that this quotient is about the same as in medium A. Thus, the two nutrients occur in proportional amounts in birch wood and in medium A. Generally, as an increase in the total nitrogen content in wood leads to a more rapid decomposition, an increase in the

Tab. 15. Contents of nitrogen and phosphorus in mycelia of *P. betulinus* grown in medium A with varying nitrogen sources and concentrations.

Nitrogen source tested	Conc.	Mycelial N %	Mycelial P %	Nitrogen source tested	Conc.	Mycelial N %	Mycelial P %
	5N	5.54	0.76		5N	4.86	0.72
Ammonium tartrate	N	6.13	0.61	Glutamine	N	4.90	0.70
	N/5	3.90	0.50		N/5	4.00	0.60[x)]
	5N	6.76	1.04		5N	6.91	1.13
$(NH_4)_2\ SO_4$	N	6.82	0.83	Glutamic acid	N	6.45[x)]	0.93[x)]
	N/5	4.92	0.79[x)]		N/5	1.95[x)]	0.54[x)]
	5N	6.67	0.96		5N	5.04	1.06[x)]
$NH_4\ Cl$	N	6.32	0.87	Asparagine	N	4.21[x)]	-
	N/5	5.06	0.74		N/5	3.68	0.52
	5N	2.05[x)]	-		5N	6.89	0.92[x)]
$KN\ O_3$	N	2.68[x)]	-	Aspartic acid	N	5.73	1.04
	N/5	2.24[x)]	-		N/5	3.82	0.58[x)]
	5N	6.66	0.86[x)]		5N	4.49	0.68
$NH_4\ NO_3$	N	6.37	0.90	Casein hydrolyzate	N	1.73[x)]	-
	N/5	4.04	0.58		N/5	-	-
	5N	4.92[x)]	0.74[x)]		5N	3.64[x)]	-
Urea	N	3.12[x)]	0.64[x)]	Glucose amine - HCl	N	-	-
	N/5	1.53[x)]	-		N/5	3.46[x)]	0.45[x)]

*) Simple analyzes only.

nitrogen content of the nutrient solution also gives a more rapid mycelial production. See tab. 13. On the other hand, there are indications that the nitrogen of wood is not consumed at the same rate as other more amply occurring nutrients and that rot fungi have a metabolism with the ability to economize with the nitrogen available (24). Fixation of air nitrogen has not been observed for any rot fungi (49).

The nitrogen content in fungus mycelium varies with both species and growth conditions. Thus Norkrans (72) found that in a nutrient medium containing ammonium tartrate, the nitrogen content of

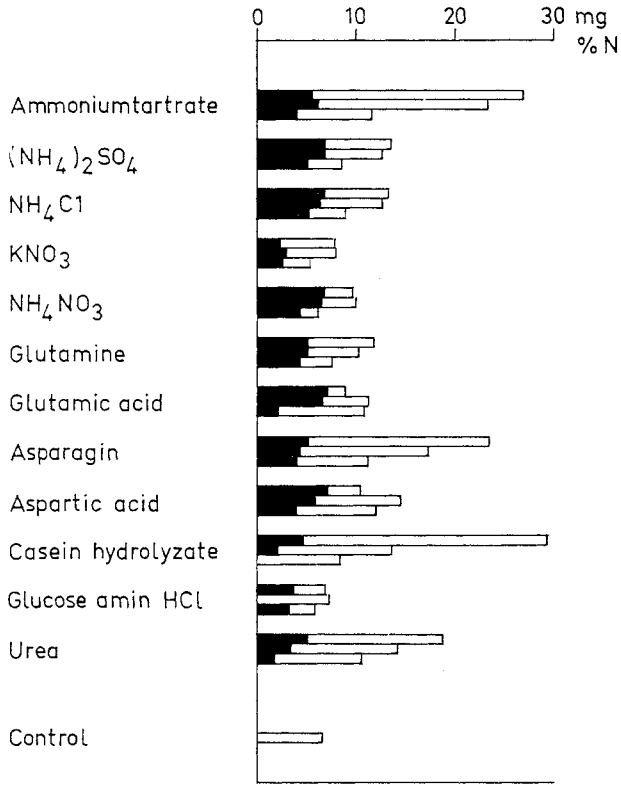

Fig. 14. Dry weight production and nitrogen contents of mycelia of *P. betulinus* grown in medium A. The black parts of the columns indicate the nitrogen content.

mycelia of various *Tricholoma* species ranged between 3.1 and 7.7 %. For *Tricholoma gambosum* the nitrogen content, when grown on various amino acids and amides, varied between 5.3 and 7.5 %. Furthermore, the nitrogen content was higher in the young mycelium than in old mycelia. Finally, the nitrogen content of the mycelia was usually proportional to the nitrogen content of the nutrient solution (27).

Analyses were made to find out variations in the contents of nitrogen and phosphorus in mycelia of *P. betulinus* grown on various nitrogen sources in varying concentrations. Thereby the total nitrogen was determined by the micro Kjeldahl method and the phosphorus content by a photometrical method described by Tamm in 1953 (95).

The results are shown in tab. 15 and fig. 14. Hence it is clear that the contents of both nitrogen and phosphorus in the mycelium increased with increasing nitrogen concentration of the nutrient solution. High contents of nitrogen were found in mycelia grown on ammonium

or amino nitrogen—5.73 to 6.82 % in the middle concentration. The amides glutamine and asparagine gave lower contents—4.90 and 4.21 % in the middle concentration. Casein hydrolyzate gave a nitrogen content of only 1.73 % in middle concentration and 4.49 in the highest concentrations. Norkrans (72) showed that with *Tricholoma fumosum*, an addition of casein hydrolyzate resulted in a substantially lower nitrogen content in the mycelium. However, the species differed in reaction; *Tricholoma vaccinum* obtained a much higher content of mycelial nitrogen when casein hydrolyzate was added to the medium.

Wood extracts and growth

Both cold water extracts and warm water extracts were prepared from sound wood meal. The wood was ground to pass a 2 mm sieve.

A cold water extract was prepared by shaking wood meal and distilled water in the proportions 1 : 20 (w/v). Shaking proceeded for 20 hours at room temperature. After filtration and centrifugation, the extracts were filter-sterilized. The extracts were used by sterile addition to a concentrated autoclaved nutrient solution.

Warm water extracts were prepared from the wood meal which had been air-dried and from which the cold water extract had been removed. 5 grams of that wood meal was extracted with 200 ml of distilled water for 4 hours in a Soxhlet apparatus. Cellulose extraction thimbles of the highest quality were used. The extracts were then filtered, centrifugated and filter-sterilized before being used.

In a preliminary experiment medium A was used together with various cold water extracts. 10 ml of the extracts were added to the experimental flasks. Besides the inner bark, the extract concentrations used were: undiluted extract (N), extracts diluted to one tenth (N/10), and extract diluted to one hundredth (N/100). The extracts from the inner bark appeared to contain about eight times more dry substance than the extracts from other parts of the trunk. Therefore this extract was diluted to one eighth of the original concentration.

The results in tab. 16 show that, when this meagre nutrient solution was used, clearly positive growth effects were obtained with the two higher concentrations. The variations between parallels within the same series were, however, great.

By using "shake cultures" and the more complete medium B the variations between parallels were reduced to an acceptable level. See tab. 17 and fig. 15. The "shake cultures" were inoculated with 0.5 ml of a very thin mycelium suspension prepared from "floating cultures" grown on medium A; thus the growth curves for "floating cultures"

Tab. 16. Dry weight production in floating cultures of *P. betulinus* grown on medium A with various types of cold water extracts from birch (Betula verrucosa) added. N is the undiluted extract and N/10 and N/100 are one tenth and one hundredth of N respectively.

Extract added	Conc.	Dry weight mg/flask	pH	
			Initial	Final
Outer bark	N	36.5±8.0	4.0	2.5
Outer bark	N/10	22.7±2.5	4.0	2.8
Outer bark	N/100	8.4±2.0	4.0	2.8
Inner bark	N	21.8±3.7	4.1	2.6
Inner bark	N/10	10.0±1.1	4.1	2.9
Inner bark	N/100	25.5±7.8	4.0	2.7
Outer sapwood	N	51.7±16.1	4.3	2.6
Outer sapwood	N/10	25.1±7.1	4.0	2.9
Outer sapwood	N/100	12.2±4.2	4.0	3.0
Inner sapwood	N	41.0±11.4	4.1	2.6
Inner sapwood	N/10	22.4±2.7	4.0	2.7
Inner sapwood	N/100	11.1±1.6	4.0	3.0
Central wood	N	48.9±7.6	4.3	2.7
Central wood	N/10	15.2±3.5	4.0	2.8
Central wood	N/100	12.3±4.5	4.0	3.0
None		12.3±1.4	4.0	3.0

and "shake cultures" could more readily be compared. It is also obvious that the addition of wood extracts gave a lower promotion of growth in the more complete medium B than in the poor medium A. Fruiting bodies formed after 40—50 days in "floating cultures" with wood extracts and after 45—55 days in "floating cultures" without extracts whereas they never formed at all in the "shake cultures". When the formation of the fruiting bodies started, the dry weight production increased rapidly, and therefore a shelf developed on the growth curve. Formation of fruiting bodies started after greatly varying times in the flasks, which resulted in a great variation of mycelial dry weights within the series (tab. 17). For instance, in cultures grown on merely nutrient solution, the dry weight production after 57 days

Tab. 17. Dry weight production in shake cultures and floating cultures of *P. betulinus* grown in medium B with or without the addition of cold water extract (conc. N/10) of birch sapwood.

Incubation time days	Shake culture				Floating culture			
	Extract added		No extrakt		Extract added		No extract	
	Dry weight mg/flask	pH	Dry weight mg/flask	pH	Dry weight mg/flask	pH	Dry weight mg/flask	pH
0	0.0	5.1	0.0	4.8	0.0	5.1	0.0	4.8
7	0.5±0.1	4.7	0.2±0.1	4.5	5.0±1.0	4.6	4.1±2.1	4.6
13	11.4±0.4	3.1	8.6±1.4	3.6	31.6±1.6	3.3	35.4±0.9	3.3
20	42.1±4.0	2.0	31.2±2.9	2.3	44.1±4.9	2.9	45.6±2.4	3.1
27	56.3±3.6	2.1	43.5±2.9	2.3	62.8±11.6	2.8	60.9±7.0	3.0
34	38.8±3.8	2.0	33.2±2.1	2.1	59.1±8.8	2.7	61.8±6.3	2.7
41	35.6±2.1	2.1	33.4±1.3	2.0	68.1±3.2	2.7	64.5±2.4	2.8
50	27.4±2.1	2.0	37.4±2.8	1.9	108±4.8	2.8	71.8±5.5	2.8
57	28.8±1.2	2.0	29.5±1.6	2.0	180±35.3	2.8	145.5±35.2	-
64	25.8±3.7	2.0	22.5±2.4	2.0	194±25.0	3.2	189±26.0	3.2

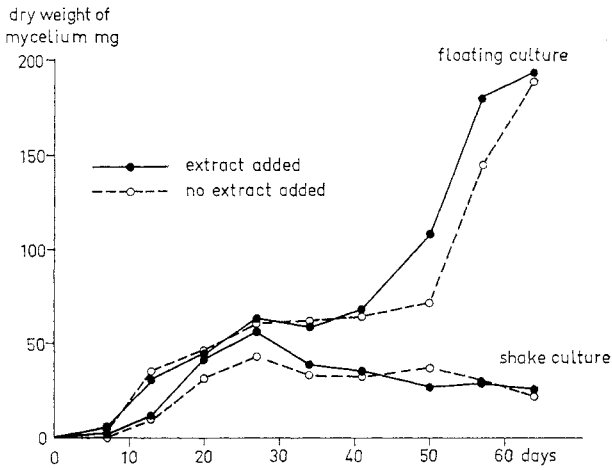

Fig. 15. Dry weight production of *P. betulinus* grown as floating culture and shake culture in medium B to which cold water extract (N/10) of birch sapwood had been added.

Tab. 18. Dry weight production in shake cultures of *P. betulinus* and *P. marginatus* grown in medium B with cold water extracts of different kinds of wood. Conc. N/5 was used.

Extract added	Initial pH	Polyporus betulinus		Polyporus marginatus	
		Dry weight mg/flask	Final pH	Dry weight mg/flask	Final pH
Birch sapwood	4.7	50.1±2.7	1.7	67.8±4.3	1.9
Birch central wood	4.4	39.1±3.3	2.1	71.3±2.8	1.9
Birch outer bark	4.6	63.9±2.9	1.7	67.5±1.1	2.0
Alder sapwood	4.7	47.1±2.6	1.7	61.1±2.7	1.9
Aspen sapwood	4.6	58.4±4.1	1.8	58.3±1.6	1.9
Pine sapwood	4.7	51.3±4.3	1.7	61.3±0.3	1.9
None	4.8	44.9±2.7	1.7	53.2±1.2	1.9

in two flasks without fruiting was 71.4 and 56.1 mg respectively, whereas the dry weight in flasks where fruiting bodies had formed were 238.0, 169.7 and 192.3 mg respectively.

One ml of cold water extract from the outer bark of a birch proved able to substitute part of the thiamine in the nutrient solution. However, there was also a growth-promoting effect in addition to this thiamine effect.

From tab. 18 it is obvious that cold water extracts from several kinds of wood act to promote the growth of *P. betulinus* grown on medium B. In fact, all the tested extracts promoted growth except for the central birch wood extract, and this negative effect is not significant.

The growth-promoting effects in various concentrations of sapwood extract were studied. A concentrated extract (2N) was prepared by altering the wood-water proportions before shaking. Results are shown in tab. 19 and fig. 16. Even if medium B is a very rich nutrient medium all the tested extracts promoted growth within the concentration interval N to N/50, but an increase of the extract concentration to 2N retarded growth in all the tested cases. As in all the other experiments with extracts, 10 ml of the extracts were added to 10 ml of a doubly-concentrated nutrient solution in Erlenmeyer flasks.

The evaporation of cold water extracts from birch and aspen sapwood on water bath and resolvation resulted in growth retardation for *P. betulinus* at as low a concentration as N/5. *P. marginatus* did not react in that way.

The effects of various warm water extracts were also studied. The

Tab. 19. Dry weight production in shake cultures of *P. betulinus* grown on medium B. Cold water extract of varying concentration having been added.

Extract added	Dry weights in mg/flask at various concentrations				
	2N	N	N/5	N/10	N/50
Birch sapwood	7.9±0.6	60.2±5.5	71.5±6.6	67.3±3.8	62.7±2.2
Alder sapwood	11.6±1.3	53.0±3.1	63.7±2.3	57.1±5.6	58.1±3.3
Aspen sapwood	0.8±0.0	55.8±7.1	-	77.8±7.9	59.5±5.5
Pine sapwood	17.2±1.0	72.5±7.1	61.9±5.8	62.0±5.5	59.6±1.6
None		39.0±2.1			

concentration 2N was prepared by dissolving the chemicals constituting medium B directly in the extracts. For the other cases 10 ml of the extracts were added to 10 ml of doubly-concentrated nutrient solution in Erlenmeyer flasks. The results are summarized in tab. 20. The mycelial weights are reported in relation to those of the control. Only in one case was a clearly positive effect observed, namely when an addition of N/5 concentration of birch sapwood extract was made. Extracts from central birch wood reduced growth even in that concentration. The rest of the extracts had no effect in the lowest concentration and clearly negative effects in the two higher concentrations.

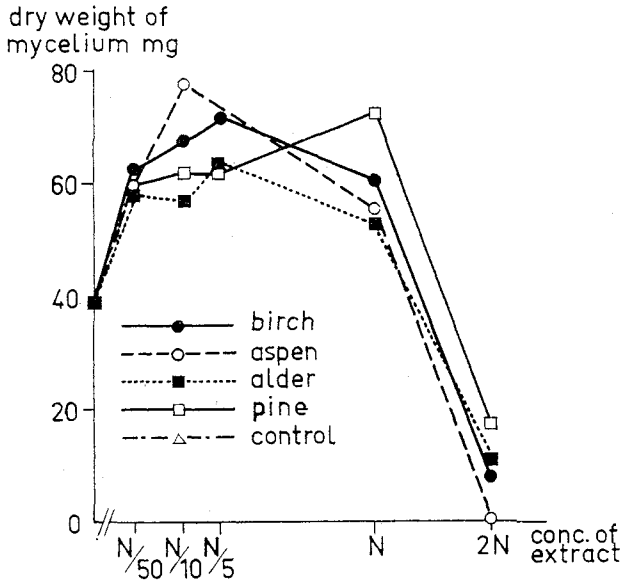

Fig. 16. Dry weight production of *P. betulinus* grown as shake culture in medium B containing cold water extracts of various wood species in different concentrations.

Tab. 20. Relative dry weight production in shake cultures of *P. betulinus* grown in medium B with warm water extracts from different kinds of wood.

Warm water extract added	Relative dry weights at various concentrations 2N	N	N/5
Birch sapwood	8.3	103	253
Birch central wood	-	56	84
Alder sapwood	-	98	106
Aspen sapwood	9.1	77	115
Pine sapwood	11	60	109
None	100	100	100

Growth-promoting effects of various extracts of organic origin have previously been reported several times. Fries (36) received positive effects on the growth of several wood-destroying fungi after adding malt extracts, yeast extract or culture filtrates from wood bacteria. Sometimes a similar growth promotion was obtained by the addition of aneurin. Sometimes, however, the aneurin effect was inferior to those of the extracts. Yussef (100) reported that about one half of the tested hymenomycetes produced a better growth on malt extract than on a complete basal nutrient medium containing salts, glucose, asparagine, casein-hydrolyzate and most of the vitamins B. In one case he could identify the malt effect as caused by adenin, which occurred in the malt extract added. In most cases, this malt effect could not be identified and it could not be classified among e.g. vitamins B, purines, pyrimidines, tryptophane or hydrolyzed nucleic acid.

Robbins and Hervey (85) found that malt extract, tomato extract and especially wood extract (autoclaved beech-wood extract) had a growth-promoting effect on rot fungi growing in a complete nutrient solution. This complete medium contained mineral salts, glucose, casein-hydrolyzate, B-vitamines, purines and pyrimidines. After addition of wood extract, a great number of the tested fungi produced a mycelial dry weight twice that produced on basal medium alone. Stimulation of growth occurred for 72 of the 82 tested fungi. Generally, the best growth occurred when the highest extract concentration was used. Malt extract was the least effective of the three tested extracts. Robbins and Hervey suggest that extracts from malt, tomato, and wood contain unidentified growth substances, that can satisfy certain deficiencies from which higher fungi suffer. Brewer (21) showed that recirculating white water from pulp industries contained substances, which stimulate the growth of the slime-producing fungi *Phialophora*

fastigiata and *Phoma* sp. Naturally he presumed that these substances emanate from the wood. In a later publication (22) Brewer also demonstrated that the growth of *Phialophora fastigiata,* when grown in a complete basal nutrient solution, was stimulated by water and acetone extracts from various Picea and Abies species. One of the stimulating substances was identified as biotin.

As *P. betulinus* gave a rather unspecific positive response to an addition of cold water extracts from various kinds of wood, it is probable that some general unidentified growth substances occur in most kinds of wood. Brewer (22) also found stimulating growth effects from all tested water and acetone extracts. In a similar way, Robbins and Hervey (85) obtained great stimulating effects from beech wood extract on varying types of rot fungi—from conifer rotters to specific rotters on broad-leaved trees, such as *Polyporus radiatus.*

The chemical composition of the wood extracts was not analyzed very closely. By using paper chromatography the extracts were shown to contain various sugars and amino acids. Analyses of sap from birch are reported by Trendelenburg (96). The sap contained amongst other things 0,0007—0,0033 % of protein and 0,0029—0,114 % ash components. A great number of possible growth-stimulating substances may exist in the protein and ash contents.

Spore germination and wood extracts

The spore material was obtained from a fresh sporophore, which was placed in a pre-sterilized petri dish. After having dropped spores for 24 hours at room temperature the sporophore was taken away. Following the method of Liss (52) a doubly-concentrated nutrient solution was pipetted into the petri dish, so that a very dense spore suspension was obtained. Immediately, mixtures were made in equal proportions between cold water extracts from various woods and the dense spore suspension, and instead of extracts distilled water was used in the control. The wood extracts were prepared by shaking 5 grams of wood meal and 100 ml of distilled water for 20 hours at room temperature. After filtration and centrifugation the extracts were filter-sterilized. Drops of the mixtures were placed on microscope slides, which were then kept at 20—22° C in a moist chamber. Every four hours some slides were taken out and one drop of 2.5 % $HgCl_2$ solution was added to each mixture drop, thus killing the spores immediately.

By using a lanameter (projection microscope from Reichert, Wien) in which the frosted glass disk was replaced by a clear glass disk, the microscopical picture was directly transferred to a photographic paper.

This method saves time as no negative is necessary. With the greatest possible enlargement (630×) a spore of *P. betulinus* which had not swelled reached a size of 3—4 mm on the paper.

As spores from *P. betulinus* are cylindrical and curved, and mostly produce their germ tubes from one end, it proved difficult to decide where the spore ended and where the germ tube started. Furthermore, the spore wall changed when swelling, thus becoming more diffuse. At the registration of the frequency of germinated spores, a spore was regarded as germinated, when spore and germ tube together constituted twice the original spore length, i.e. 7 mm on the photograph.

It proved difficult to find sporophores free from insect larvae. These larvae crept out of the sporophore during the collection of spores, leaving tracks in the fallen layer of spores. This has been observed with many fungi, e.g. *Polyporus fomentarius* (23). Yeasts and bacteria from the intestinal ducts of these larvae were very troublesome. Because of their rapid multiplication and growth, many experiments were spoiled until a spore suspension, satisfactorily free from infections, was obtained.

The results of the spore germination experiments are shown in tab. 21 and 22 and in fig. 17 and 18. It is obvious that the percentage of germinated spores increased most rapidly when hard wood extracts were added and—surprisingly enough—more rapidly by adding alder or aspen extracts than by adding birch extracts. The final percentage of germination was 70 % for the three hard wood extracts. Addition

Tab. 21. Germination percentage of spores of *P. betulinus* after adding cold water extracts of different sapwoods. Germination percentages are approximated to the nearest 5 %.

Germination time hours	Percentage germinated spores in media containing extracts				
	Birch	Aspen	Alder	Pine	None
20	2	5	1	0	0
24	5	15	15	0	0
28	10	25	20	0	10
32	15	30	30	2	15
36	25	45	50	5	20
40	-	55	55	15	30
44	50	65	65	20	30
48	60	70	70	30	25
52	70	70	70	25	30
65	70			30	

Tab. 22. Average length of spore + germ tube of *P. betulinus* after varying germination time. Cold water extracts of different sapwoods having been added. Length is expressed in mm and measured directly on the photo at an enlargement of 600x.

Germination time hours	Average length of spore + germ tube in media containing extracts				
	Birch	Aspen	Alder	Pine	None
20		7.0	7.0		
24	7.0	7.7	7.1		7.0
28	7.7	8.3	8.0		8.0
32	7.4	8.5	8.6	7.0	9.1
36	10.7	10.3	10.9	7.5	12.3
40	11.7	12.9	(14.6)	7.1	15.6
44	13.9			10.0	
48				12.6	

of pine wood extract had a retarding effect as compared with the control. The lag phase was 12 hours longer than for the hard wood extracts and the final percentage of germination was only 30 %.

When calculating the average length of germinated spores after varying intervals of time, a somewhat different picture of germination is to be found (tab. 22 and fig. 18). It appears that the germ tubes of

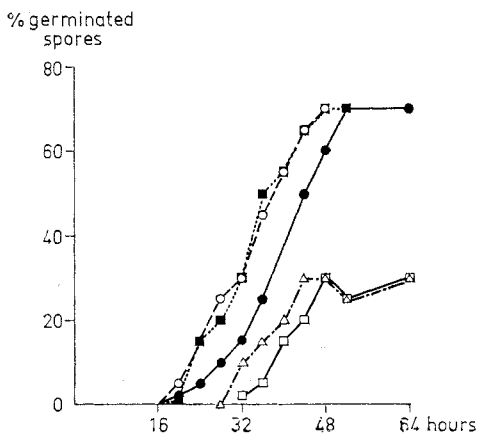

Fig. 17. Percentage germinated spores of *P. betulinus* following addition of different wood extracts (cold water extracts) to a nutrient solution according to Liss. Explanation of symbols is found in fig. 16.

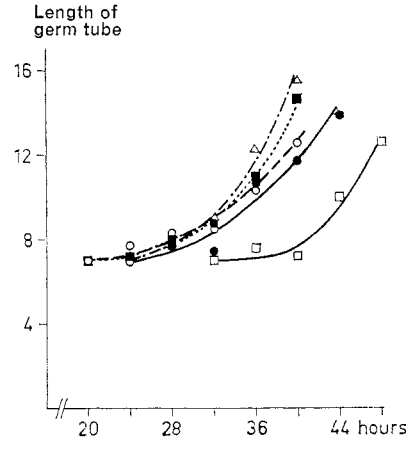

Fig. 18. Average length of spore + germ tube in a nutrient solution according to Liss following addition of different wood extracts (cold water extracts). Explanation of symbols is found in fig. 16.

actually germinated spores grew at the same rate in sole nutrient solution as in solutions with hard wood extracts. However, pine wood extract retarded the growth of the germ tubes. The following conclusion concerning the effect of various wood extracts on germination of *P. betulinus* can be made:

Germination character	Influence of cold water extracts of			
	birch	aspen	alder	pine
germination percentage	+	+	+	0
growth of germ tube	0	0	0	—
lag phase	+	+	+	—

+ = positive influence; – = negative influence 0 = no influence

The spore germination of basidiomycetes has been studied by many authors. Spores of wood-destroying fungi are considered to require an acid substrate for germination (25). This is especially true in the case of *Merulius lacrymans*. Spores of most other fungi also germinate best of all at an acid pH. Addition of plant materials or organic extracts mostly promote germination, but they can also retard or inhibit germination (27). Yet the presence of other organisms may stimulate growth. The yeast *Torulopsis sanguinea,* for example, stimulates germination of mykorrhiza fungi and litter-decomposing fungi (37). Rumbold showed as early as in 1908 (87) that spores of *P. betulinus* germinated easily on neutral and acid agar substrates. However, they did not germinate at all on alkaline substrate or in water.

Formation of fruiting bodies

As already mentioned on page 27 the formation of fruiting bodies constituted an irrational and disturbing factor in the determination of mycelial dry weights. This formation apparently started accidentally in floating cultures after varying intervals of time. Differences in fruiting time between flasks belonging to the same series varied considerably. Consequently, as dry weight production rises rapidly at the formation of fruiting, great variations in dry weights occurred in the same series. Fruiting bodies formed in "floating cultures" and on agar plates after 1—2 months. However, fruiting bodies never occurred in "shake cultures". The irregularly bulb-shaped, yellow-white fruiting bodies, like naturally formed sporophores, appeared corky after drying.

A comparison was made between fungal development in "shake cultures" and "floating cultures". Medium B was used, and nutrient solution and mycelia (including fruitings) were analyzed for nitrogen

Tab. 23. Mycelial production, glucose consumption, nitrogen and phosphorus states of mycelia and nutrient solutions in floating and shake cultures of *P. betulinus* grown on medium B.

Type of culture	Incubation time days	Final pH of medium	Dry weight mg/flask	Glucose		Nitrogen			Phosphorus		
				in medium	consumed	in medium	in mycelium		in medium	in mycelium	
				mg/flask	mg/flask	mg/flask	mg/flask	%	mg/flask	mg/flask	%
Floating	0	4.9	0.0	360	0	10.6	0.00	0.00	4.68	0.00	0.00
	7	4.3	2.9	333	27	10.3	0.14	4.82	4.66	0.02	0.71
	14	3.3	7.1	277	83	10.3	0.33	4.62	4.63	0.05	0.74
	21	3.1	10.2	269	91	9.8	0.63	6.19	4.59	0.09	0.83
	28	2.9	23.6	255	105	9.0	1.49	6.32	4.48	0.22	0.91
	35	2.8	26.4	234	126	8.9	1.46	5.56	4.42	0.23	0.89
	42	2.9	54.4	210	150	7.8	2.12	3.89	4.29	0.34	0.63
	50	3.0	133.9	125	235	6.6	3.24	2.35	3.96	0.73	0.54
	56	3.3	189.8	43	317	5.4	4.44	2.34	3.83	0.86	0.46
	63	3.8	174.2	47	313	5.9	4.32	2.48	3.86	0.81	0.46
Shake	0	4.9	0.0	360	0	10.6	0.00	0.00	4.68	0.00	0.00
	5	2.7	13.4	336	24	9.5	0.97	7.20	4.50	0.16	1.16
	9	1.7	40.0	226	134	6.7	3.76	9.40	4.10	0.51	1.27
	14	1.6	55.6	131	229	5.9	4.42	7.94	3.78	0.68	1.22
	21	1.6	50.3	75	285	7.0	3.04	6.04	3.98	0.54	1.07
	28	1.7	21.2	53	307	9.2	1.02	4.83	4.45	0.21	0.99
	35	1.7	19.1	35	325	9.5	0.84	4.38	4.51	0.14	0.72
	42	1.7	15.0	32	328	10.0	0.47	3.14	4.52	0.08	0.53
	50	1.8	14.9	24	336	9.8	0.51	3.43	4.55	0.09	0.63
	56	1.7	15.1	32	328	9.9	0.59	3.88	4.69	0.11	0.71
	63	1.7	15.1	31	329	9.9	0.56	3.73	4.68	0.09	0.58

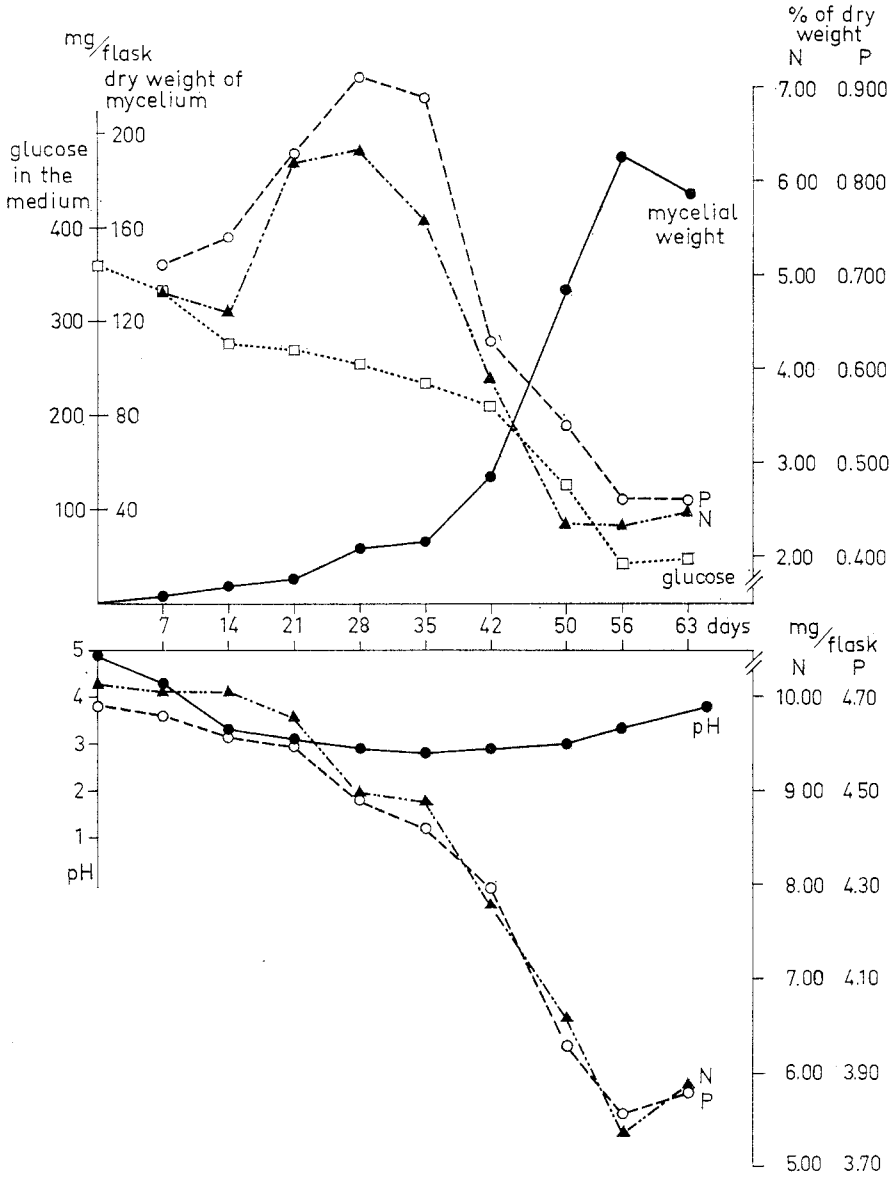

Fig. 19. Mycelial production, glucose consumption, nitrogen and phosphorus states of mycelia and nutrient solutions in floating and shake cultures of *P. betulinus* grown on medium B.

and phosphorus content (page 46). Determination of the glucose content was also made, using an enzymatic method described by Lindberg in 1963 (57). According to this method, glucose oxidase is allowed to

catalyze the transformation of glucose into gluconic acid. With water and oxygen present, this transformation occurs resulting in the formation of hydrogen peroxide which is split by the action of added peroxidase. The free oxygen thus formed then oxidizes a hydrogen donnor (O-anisidin) during the formation of water and a coloured substance. The amount of this coloured substance produced is proportional to the original amount of glucose and is easily determined photometrically (9).

Results are shown in tab. 23 and fig. 19. The first fruiting body formed in a "floating culture" after 35 days. From that time fruiting occurred here and there in the flasks and after 56 days fruiting bodies at various stages of development occurred in all the flasks. The pH of the nutrient solution was at its lowest level after 35 days, i.e. when the first fruiting bodies formed. Then the pH rose again. The great leap of growth occurred between the 35th and 56th day, i.e. when fruiting bodies were produced. On the whole the glucose consumption followed the mycelial production. However, just about one half of the available nitrogen and not more than one quarter of the available phosphorus of the nutrient solution had been consumed at the maximum dry weight production after 56 days. Consumption of nitrogen and phosphorus as well as glucose consumption grew with the increasing mycelial production. The nutrient state within the fungus, however, varied in a way much divergent from the mycelial production. In the beginning, both N and P contents increased, reaching a maximum after 28 days. They then decreased rapidly, apparently reaching a constant level after 50—60 days. Thus the mycelial contents of nitrogen and phosphorus decreased rapidly, when the formation of fruiting bodies started. Altogether, the mycelial content of nitrogen varied between 2.34 and 6.32 % and the content of phosphorus between 0.46 and 0.91 %.

The "shake cultures" showed a maximum mycelial weight and a minimum pH of the nutrient solution after only 14 days. Then about two thirds of the available glucose had been consumed. Obviously the minimum pH of growth was then passed and the mycelia simply died. This resulted in autolysis and the leakage of mycelial nitrogen from the hyphae to the solution and a reduction of the mycelial dry weight down to almost one quarter of its maximum. Mycelia from "shake cultures" reached a higher content of nitrogen and phosphorus than mycelia from "floating cultures".

In "floating cultures" great variations in phosphorus content, and especially in nitrogen content occurred. Therefore analyses were made

Tab. 24. Nitrogen content of mycelia and fruiting bodies of *P. betulinus* belonging to the same flasks and nitrogen content of a fresh sporophore.

Age of culture days	Analyzed part of fungus	Nitrogen content %
50	mycelium	4.93
	fruiting body	1.72
56	mycelium	5.63
	fruiting body	1.45
63	mycelium	4.09
	fruiting body	1.57
	sporophore	0.89

separately on mycelia and on the fruiting bodies. Tissue from a fresh sporophore was additionally analyzed. The results, shown in tab. 24, clearly demonstrate that the nitrogen content of the fruiting bodies was much lower than that of the mycelium—generally only one third or one quarter. The nitrogen content of the normal sporophore was lower still—about half that of the fruiting bodies.

The effects of various environmental factors on the formation of fruiting bodies were studied. In agar plates fruitings often formed along the edge of the dish. If a piece was cut away from a maltagar culture, fruiting bodies often formed along the cut edge. Also, by damaging the mycelium on a plate with a needle or a knife one could stimulate the production of aerial mycelium and—later—of fruiting bodies.

It is known that the accumulation of CO_2 which occurs when sporulating fungi are cultured in more or less closed culture vessels, may stop sporulation (27). Cauchon in 1963 (26) also demonstrated that hypocultures of certain rot fungi produced fruiting bodies more rapidly than epicultures. This effect was ascribed to the culture orientation itself, but it might also be attributed to the accumulation of CO_2 over the epiculture.

An experiment was made to find out whether the removal of carbon dioxide from the atmosphere over a floating culture had any influence on the formation of fruiting bodies in *P. betulinus*. The experiment was carried out with floating cultures in 300 ml Erlenmeyer flasks containing 40 ml of medium B (only 3 grams of casamino acids per liter was used). A small beaker with 2 ml of 20 % KOH was hung in the experimental flask, about 1 cm above the surface. A folded filter paper was then placed in the beaker to increase the CO_2-absorbing surface.

More aerial mycelium was produced in flasks containing KOH as the absorbing medium for CO_2. The fruiting time—the time that elapsed from inoculation till the formation of the first fruiting body—varied for KOH cultures between 21 and 28 days (average 25 days) and for cultures without KOH between 23 and 34 days (average 30 days). The formation of fruiting bodies was evidently somewhat stimulated by the removal of the carbon dioxide produced from the atmosphere round the mycelium. After 34 days, when fruiting bodies had formed in all the flasks, the dry weight of mycelium was higher, and the pH of nutrient solution lower for KOH-cultures than for cultures without KOH.

Generally, the formation of reproductive organs in fungi is supposed to occur, when a rapidly-growing mycelium has impoverished the substrate. This is true, especially for carbon and nitrogen (56). However, production of fruiting bodies starts, as far as *P. betulinus* is concerned, even though C, N and P are still present in considerable amounts in the nutrient solution (tab. 23).

The nitrogen concentration of the medium apparently influenced the formation of fruiting bodies. In tab. 25 the results of culturing the fungus on a medium, containing various concentrations of casamino acids are presented. As early as 15 to 20 days after the inoculation, a clear difference in the manner of growth was discernible. At the highest nitrogen concentration, there was very little aerial mycelium, whereas in flasks with lower N-concentrations, aerial mycelium with mycelial clusters was abundant. Some of these clusters later developed into fruiting bodies. The formation of fruiting bodies was quickest of the two middle nitrogen concentrations. The highest concentrations resulted in a considerable retardation. When the first fruiting body was discernible at the highest concentration, all the flasks of the

Tab. 25. Formation of fruiting bodies and dry weight production in floating cultures of *P. betulinus* grown on medium B. The concentration of casamino acids was varied. Fruiting time is the time from inoculation until fruiting bodies occurred in the flasks.

Casamino acids g/l	Fruiting time days	Average fruiting time days	Total dry weight in mg/flask after		
			13 days	26 days	40 days
7	36 - 54	43	12.5	24.4	31.6
4	26 - 38	32	12.0	31.1	66.7
2	26 - 40	36	-	33.8	58.8
1	34 - 54	40	12.5	28.0	65.3

Tab. 26. Dry weights of mycelia and fruiting bodies in floating cultures of *P. betulinus* grown for 28 days on medium B. F = Fruiting bodies had been formed.

Casamino-acids g/l	Total dry weight mg	Dry weight of fruiting body mg	Dry weight of mycelium mg
4	25.9	0.0	25.9
4	29.1	0.0	29.1
4	30.4 F	1.6	28.8
4	44.3 F	12.4	31.9
4	26.0	0.0	26.0
2	33.6 F	1.8	31.8
2	28.9	0.0	28.9
2	49.6 F	17.0	32.6
2	25.8	0.0	25.8
2	31.2	0.0	31.2

second lowest concentration had produced fruiting bodies. Until fruiting started, the dry weight production was approximately the same in all series. Differences in dry weights within each series depended almost exclusively on fruiting, which became quite clear if the total dry weight was reduced with the dry weight of the corresponding fruiting body. The mycelial weight calculated in that way was about the same as the mycelial weight of flasks where no fruiting had occurred. See tab. 26.

The influence of sugar concentration on fruiting was studied on medium B (half nitrogen concentration was used). After no more than 2 to 3 weeks, it was obvious that the production of aerial mycelium was less at the highest sugar concentration than at the other glucose concentrations. From the results in tab. 27 it is evident that, in spite of a rapid mycelial production, a high sugar concentration retarded fruiting, and that fruiting bodies were not produced at glucose concentrations below 1 %.

Nutrient solution from cultures growing on medium B was filtered away at the time of fruiting. In various concentrations and after sterile filtration this "old" nutrient solution was added to young floating cultures of the fungus. No stimulating effect of the "old" solution on the formation of fruiting bodies was observed. Nor could any differences in fruiting time be registered when various types of inoculas were used; e.g. mycelia, which were just about to fruit, pieces from young fruiting bodies, or maltagar cultures of varying ages.

Tab. 27. Formation of fruiting bodies in floating cultures of *P. betulinus* grown on medium B (casamino acids 3.5 g/l), with varying concentrations of glucose.

Glucose conc. of the medium	Fruiting time days	Average fruiting time days	Total dry weight mg/flask
4 %	43 - 61	56	38.9
2 %	26 - 43	30	28.7
1 %	26 - 48	31	24.4
0.5 %	fruiting started in some flasks but was never completed		25.7
0.25 %	no fruiting		16.0
None	"		1.2

The pH-drop of the medium, which the growing fungus probably caused in an active way, aroused a suspicion that the mycelium produced some organic acid inducing formation of fruiting bodies. It is well known that several fungi produce organic acids. These acids often constitute intermediate products in their carbohydrate metabolism, and consequently they occur only for shorter periods. However, it is possible to fixate such acids as salts by adding alkali to the media. In that way lactic, oxalic, acetic, formic, fumaric, succinic, citric and itaconic acids amongst others have been shown in culture filtrates from fungi (32). Wood-destroying fungi have been shown to produce relatively large amounts of organic acids. Birkinshaw et al. (11) found citric acid in wood decayed by *Coniophora cerebella*. Oxalic, acetic and succinic acids were formed by *Merulius confluens, Merulius niveus, Merulius tremellosus,* and *Fomes annosus* (69, 70), oxalic acid by *Merulius lacrymans, Marasmius chordalis, Polyporus tumulosus,* and *Corticium centrifugum* (78, 91), gluconic acid by *Polyporus vaporarius* (89), and so on.

In order to identify the organic acids produced by *P. betulinus* present in the medium when fruiting started, paper chromatography was performed on the nutrient solutions. To this end, a method described by Blundstone (16) was used. The chromatograms were run in a mixture of n-butylformate, formic acid, and water, in the proportions 10 : 4 : 1. Sodium formate was added and also as an indicator, bromophenole blue. In that way the chromatograms became self-developing. After the chromatograms had been dried, the acids were descernible as distinct yellow spots against the violet-blue alkaline background. This method also has the advantage that no ester

Tab. 28. Formation of fruiting bodies in floating cultures of *P. betulinus* grown on medium B (casamino acids 3.5 g/l). Varying amounts of succinic, citric, and tartaric acids have been added. Incubation time was 32 days. The fruiting bodies were then 2—4 days old.

Acids added	Initial pH	Fruiting time days	Average fruiting time days	Total dry weight mg/flask	Final pH
Succinic acid 1 %	2.8	30 - 40	34	56.0	2.5
" " 0.1 %	3.6	26 - 37	30	57.4	2.8
" " 0.01 %	4.2	26 - 39	30	46.5	2.8
" " 0.001 %	4.6	26 - 39	30	62.7	3.0
Citric acid 1 %	2.4	48 -	> 66	27.2	2.3
" " 0.1 %	3.3	36 - 48	42	42.9	3.0
" " 0.01 %	4.0	26 - 36	33	49.6	3.0
" " 0.001 %	4.5	26 - 42	33	53.2	3.2
Tartaric acid 1 %	2.3		> 66	30.4	2.3
" " 0.1 %	3.0	36 - 53	48	37.5	2.7
" " 0.01 %	4.0	26 - 42	34	50.6	2.8
" " 0.001 %	4.5	29 - 36	33	46.2	2.8
None	4.7	26 - 39	30	51.2	2.9

formation occurs in the solution, which can thus be used for a long time. Nutrient solutions were used directly without purification. Ascending chromatography for 8 or 16 hours proved suitable. By this method two or possibly three organic acids proved to be present in detectable amounts in the medium at the time of fruiting. They were identified as succinic acid and citric acid. The identity of the third acid was uncertain; it was probably tartaric acid. In an experiment, the effect of these three organic acids on the fruiting of *P. betulinus* was studied. Medium B (3.5 grams of casamino acids was used) containing four concentrations (1 %, 0.1 %, 0.01 %, and 0.001 %) of the acids were used. The results are shown in tab. 28. A retarding influence on fruiting occurred at the higher concentrations. In the flasks containing tartaric acid and citric acid no fruiting was descernible even after 66 days. Here the pH of the medium was so low, however, that growth was greatly reduced. It is also most probable that all retardations in fruiting time were pH-effects rather than direct effects from the acids. During experiments with buffered media (page 24) it appeared that within the pH interval 3.7—6.5 no differences in

fruiting time occurred. On media of a higher and a lower pH, however, the formation of fruiting bodies had not started even after 61 days as a result of a too slow growth.

The following conclusions can be made concerning the fruiting of *P. betulinus:* Fruiting bodies are produced on solid, semi-solid or fluid media provided that they are standing still and not shaken. In floating cultures, fruiting starts before the medium is impoverished. Dry weight production increases rapidly when the fruiting bodies are being produced. Consumption of C, N and P greatly follows dry weight production. Mycelial nitrogen and phosphorus decrease rapidly on the formation of fruiting bodies. There is a much lower nitrogen concentration in the fruiting bodies than in the mycelium. In a natural sporophore, the nitrogen content is still much lower. The pH of the nutrient medium reaches its lowest value when fruiting commences. The age and origin of inoculas is without importance for the formation of fruiting bodies. Too high concentrations of carbon or nitrogen, as well as the accumulation of carbon dioxide in the atmosphere around the mycelium delay the formation of fruit bodies. Within the pH-intervals of the nutrient medium where growth is good, fruiting is generally not influenced by changes in pH. Additions to the nutrient solution of such organic acids, which are exudated by the mycelium, as well as the additions of the nutrient medium in which the fungus has grown, does not influence fruiting, provided that pH is not changed to such a degree that growth is retarded to any extent. Using the soil jar method fruiting bodies form within 3 months when the fungus grows on birch or beech wood, but not on spruce or pine wood. The formation of fruiting bodies is stimulated by damaging agar plate cultures.

The formation of fruiting bodies of wood-inhabiting fungi has been studied by e.g. Aschan-Åberg (3, 4, 5) on *Collybia velutipes* and Handke (43) on *Trametes versicolor, Polyporus brumalis* and *Panus tigrinus.* The latter made a special study of the influence of light on fruiting. He found that light has a stimulating effect on the production of immature fruiting bodies, which Aschan-Åberg could not find for *Collybia.* On the other hand, both authors consider light as being absolutely necessary for the formation of normal fruiting bodies—sporophores. *P. betulinus,* which in all the experiments has been cultured in dark, did never produce normal sporophores, only irregular, abnormal fruiting bodies.

Aschan-Åberg further shows that high concentrations of casein hydrolyzate or malt extract retarded fruiting (3). This was also true

for high concentrations of glucose and also for ammoniumtartrate even if less pronounced (4). Glucose medium was better than saccharose or maltose. Mn, Zn, Na and Ca had a high stimulating effect on fruiting, and this occurred within a broad pH-interval—4.6 to 7.1 (3). The amounts of mycelial nitrogen and phosphorus varied between 4.2 and 6.4 % and 1.3 and 3.7 % respectively. A drop in nitrogen content appeared when growth was most rapid (5). The results of Aschan-Åbergs experiments with *Collybia velutipes* correspond very closely to those found in the experiments concerning the fruiting of *P. betulinus*.

Summary

The destructive activity on wood and the physiological behaviour of *Polyporus betulinus* have been studied.

When attacking birch wood under favourable conditions *P. betulinus* proved able to cause a maximal weight loss of about 71 %. This was also true *for Polyporus marginatus* which was chosen as a comparative organism.

Wood moisture increased steeply during the attack, at its peak reaching more than three times the moisture at the start. *P. betulinus* and *P. marginatus* did not cause so great a weight loss on birch sapwood as the most destructive white rotters.

When attacking the wood the hyphae penetrated all the cells in all directions.

P. betulinus caused substantial weight losses on sapwood of birch, beech and spruce wheras both sap and heart of pine were very resistant to any attack.

Optimal temperature for decay was about 25° C. The attacked wood became more and more acid as weight losses increased. At the most advanced stages of decay, pH increased again.

The changes of the chemical composition of wood during an attack proceeded almost identically for *P. betulinus* and the comparative fungus *P. marginatus*. At the beginning of an attack the hexosan consumption proceeded more slowly than the pentosan consumption. Later the opposite was true. There was no registrable loss of lignin during the whole course of decay, which resulted in a substantial accumulation of that substance. 14 % of the original hexosan content and 5 % of the original pentosans remained when decay reached its maximum. Together they constituted about 9 % of the original weight of wood. Thus 9 % out of nearly 80 % of the original carbohydrates were not available to these fungi. The substances extractable in alcohol and dichloromethane increased during decay. At late stages of decay they reached 3.5 times their percentage in undecayed wood.

Living birches and aspens were infected with *P. betulinus* and *P. marginatus*. Half a year later discolouration was discernible above as well as below the infected points. Birches and aspens alike were attacked by both fungi, although the discolouration did not proceed as far in aspens as in birches. Thus, it proved possible to infect not only living birches but also living aspens with *P. betulinus*.

When in culture, *P. betulinus* was able to grow on most substrates generally used for culturing fungi. Bavendamm tests gave negative results indicating the inability to produce polyphenole oxidases.

The radial growth on malt agar for different strains varied between 4.7 and 7.2 mm/day. However, there was no correlation between radial growth and mycelial production measured by weight.

P. betulinus showed a broad optimum temperature range around 20° C. Maximum and minimum temperatures was about 35 and 5° C respectively. Growing on maltagar the mycelium survived 35, but not 42, days at — 30° C.

pH-optimum for growth proved to be around pH 5, and maximum at about pH 7. The mycelium could stand rather acid media and produced good growth even at pH 2.1.

P. betulinus showed thiamine heterotrophy. A complete vitamin- and micro-element mixture could not replace thiamine.

Addition of Na and Ca together, or better a mixture of Na + Ca + vitamin to a basic nutrient medium containing sugar, nitrogen, salts and thiamine promoted growth.

Among aldo-pentoses, xylose proved to be a good source of carbon. Arabinose, ribose and rhamnose were not accessible. Glucose was the best carbon source among the aldo-hexoses. Out of the two tested keto-hexoses, fructose could be utilized but not sorbose. The tested disaccharides cellobiose, lactose, maltose, trehalose and saccharose were all available. Cellobiose promoted growth to a great extent. Cellulose in the form of filter paper was a very poor carbon source for *P. betulinus* and *P. marginatus*. Several white-rot fungi, on the other hand, easily utilized cellulose in the form of filter paper. The polysaccharides glycogen and inulin were tested. Only the former was accessible to *P. betulinus*. *P. marginatus* was able to use both glycogen and inulin and also the trisaccharide raffinose, which was not accessible to *P. betulinus*.

Ammonium proved the best of several tested inorganic nitrogen sources. Nitrate was not available. As nitrite could be utilized, the synthesis of nitrate reductase is probably blocked. Among the tested organic nitrogen sources, the amides asparagine and glutamine were not as good as their corresponding amino-acids. Three concentrations of the nitrogen sources were used. At the middle concentration the N/C quotient was about the same in the nutrient solution as in fresh wood. Nitrogen content is considered to be one limiting factor for fungal growth in wood, and in the same way as an addition of nitrogen in suitable forms to wood speeds up the decomposition rate, a higher

nitrogen content in the nutrient solution generally increases mycelial growth.

Mycelial nitrogen and phosphorus increased with the increasing nitrogen content of the nutrient solution. Mycelia grown on media containing ammonium, and amino nitrogen, had a higher nitrogen content than mycelia growing on glutamine, asparagine or casein-hydrolyzate.

On a poor medium the mycelial growth of *P. betulinus* was greatly stimulated by the addition of cold water extracts from birch as well as from sapwood of alder (*Alnus glutinosa*), aspen (*Populus tremula*) and pine (*Pinus silvestris*). Among the warm water extracts, only that from sapwood of birch really stimulated growth. High concentrations of warm water extracts retarded growth.

Addition of wood extracts to spores from *P. betulinus* influenced germination in different ways. The percentage of germinated spores increased after the addition of extracts from birch, aspen and alder, while an extract from pine sapwood had no effect. Growth of germ tube was not affected by extracts from birch, aspen and alder. Pine extract, on the other hand, retarded growth of the germ tube. The lag phase until spore germination occurred, decreased when birch, aspen or alder extracts were added, whereas pine extract delayed germination.

Formation of fruiting bodies in culture was studied. Fruiting bodies formed on standing fluid or semi-solid media. Dry weight production increased rapidly when the formation of fruiting bodies started, which occurred before the nutrient medium was exhausted. The pH of nutrient solution was at its lowest when fruiting started. Consumption of C, N and P increased when dry weight production increased. The mycelial contents of N and P decreased when fruiting started. Fruiting bodies and natural sporophores had a much lower nitrogen content than the mycelium. The age and origin of inoculas did not influence the fruiting. Too high contents of C and N in the medium as well as any accumulation of CO_2 in the flasks retarded the formation of fruiting bodies. Within pH-intervals, where growth was good, changes in pH of the medium did not influence fruiting. The addition of some organic acids, normally exudated by mycelium, or, the addition of "old" nutrient solution, did not influence fruiting. Fruiting bodies formed when the fungus was grown on birch or beech wood, but not on spruce or pine wood.

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REFERENCES

1. AMERICAN STANDARD METHODS: Standard Method of Testing Wood Preservatives by Laboratory Soil Block Cultures. — ASTM, D 1413—61.
2. APENITIS, A., ERDTMAN, H., and LEOPOLD, B.: Studies on Lignin V. The decay of spruce wood by brown rotting fungi. — Svensk Kem. Tidskr., 63, 1951.
3. ASCHAN, K.: The Production of Fruit Bodies in *Collybia velutipes*. I Influence of Different Culture Conditions. — Physiol. Plant., 7: 3, 1954.
4. ASCHAN-ÅBERG, K.: The Production of Fruit Bodies in *Collybia velutipes*. II Further Studies on the Influence of Different Culture Conditions. — Physiol. Plant., 11: 2, 1958.
5. — Studies on Mono — and Di-Caryotic Mycelia of *Collybia velutipes*. — Physiol. Plant., 13: 2, 1960.
6. BAVENDAMM, W.: Über das Vorkommen und den Nachweis von Oxydasen bei holzzerstörenden Pilzen. — Z. Pfanzenkrank. Pflanzenschutz, 38, 1928.
7. — Erkennen, Nachweis und Kultur der holzzerfärbenden und holzzersetzenden. Pilze. — Handbuch der biologischen Arbeitsmethoden. Abt. XII, 2: 7, Berlin, 1936.
8. BAXTER, D. V.: Pathology in Forest Practice. — New York 1943.
9. BERGMEYER, H-U.: Methoden der enzymatischen Analyse. — Weinheim/Bergstr., 1962.
10. BILLE-HANSEN, E.: The Nitrogen Requirements of *Coprinus heptemerus* M. Lange & Smith. — Friesia V, 3—5, 1956.
11. BIRKINSHAW, J. H., FINDLAY, W. P. K., and WEBB, R. A.: Biochemistry of the Wood-Rotting Fungi. 2. A Study of the acids produced by *Coniophora Cerebella* Pers. — Biochem. J., 34, 1940.
12. BJÖRKMAN, E.: Om lagringsröta i massavedgårdar och dess förebyggande. — Medd. Statens Skogsforskningsinst., 35: 1, 1946.
13. — The Occurrence and Significance of Storage Decay in Birch and Aspen Wood with Special Reference to Experimental Preventive Measures. — Kungl. Skogshögskolans skrifter, 16, 1953.
14. — Lagringsröta och blånad i skogslagrad barr- och lövmassaved. — Kungl. Skogshögskolans skrifter 29, 1958.
15. BJÖRKMAN, E., FORSBLAD, L-E., REGESTAD, S. O., RINGSTRÖM, E. and RYDHOLM, S.: The use of decayed wood from some conifers and broadleaf trees for chemical pulping purposes. — Studia Forestalia Suecica, 21, 1964.
16. BLUNDSTONE, H. A. W.: Paper Chromatography of Organic Acids. — Nature, Jan., 1963.
17. BRAY, M. W., and ANDREWS, T. M.: Chemical Changes of Ground-wood during Decay. — Ind. Eng. Chem. 16, 1924.
18. BOURDOT, H. et GALZIN, A.: 1. Hymenomycetes de France. — Contribution á la Flore Mycologique de la France, Paris, 1927.
19. BOYCE, J. S.: Forest Pathology — New York 1961.
20. BREWER, D.: Studies on slime accumulations in pulp and paper mills. II. Physiological studies of *Phialophora fastigiata* and *P. richardsie*. — Canad. J. Bot., 37: 3, 1959.
21. — Studies on slime accumulations in pulp and paper mills. III. The stimulation of growth by »white water». — Canad. J. Bot., 37: 4, 1959.
22. — Studies on slime accumulatons in pulp and paper mills. V. Preliminary observations on the effect of extracts of Spruce and Fir on the growth of *Phialophora fastigiata*. — Canad. J. Bot., 41: 6, 1961.
23. BUCHWALD, N. F.: Om sporeproduktionens størrelse hos tøndersvampen. *Polyporus fomentarius* (L) Fr. — Friesia, II: 1, 1938.
24. CAMPBELL, W. G.: The Biological Decomposition of Wood. — Wood Chemistry (Wise & Jahn), New York, 1951.

25. CARTWRIGHT, K. ST. G. and FINDLAY, W. P. K.: Decay of Timber and its Prevention. — London, 1958.
26. CAUCHON, R.: Influence of slant orientation on fruiting in cultures of hymenomycetes. — Canad. J. Bot., 41, 1963.
27. COCHRANE, V. W.: Physiology of Fungi. — New York, 1958.
28. COWLING, E. B.: Comparative Biochemistry of the Decay of Sweetgum Sapwood by White-Rot and Brown-Rot Fungi. — U.S. Dept. Agr. Tech. Bull., 1258, 1961.
29. — Structural features of Cellulose that Influence its susceptibility to enzymatic hydrolysis. — Advances in Ezymatic Hydrolysis of Cellulose and Related Materials, New York, 1963.
30. DEUTSCHE NORMEN: Prüfung von Holtzschutzmitteln, Mykologische Kurzprüfung (Klötzchen — Verfahren). — DIN 52 176, Blatt 1, 1939.
31. FINDLAY, W. P. K.: Studies in the Physiology of Wood-destroying Fungi. I The Effect of Nitrogen Content upon the Rate of Decay of Timber. — Ann. Bot., XLVIII, 1934.
32. FOSTER, J. W.: Chemical Activities of Fungi. — New York, 1949.
33. FOTHERGILL, P. G. and ASHCROFT, R.: The Nutritional Requirements of *Venturia inaequalis*. — J. Gen. Mikrobiol., 12, 1955.
34. FRIES, L.: Studies in the Physiology of Coprinus. I. Growth Substance, Nitrogen and Carbon Requirements. — Svensk Bot. Tidskr., 49: 4, 1955.
35. — Studies in the Physiology of Coprinus. II. Influence of pH, Metal Factors and Temperature. — Svensk Bot. Tidskr., 50: 1, 1956.
36. FRIES, N.: Über die Bedeutung von Wuchstoffen für das Wachstum verschiedener Pilze. — Symb. Bot. Upsaliensis, III, 1938.
37. — Untersuchungen über Sporenkeimung und Mycelentwicklung bodenbevohnenden Hymenomyceten. — Symb. Bot. Upsaliensis, VI: 4, 1943.
38. FRITZ, C.: Cultural Criteria for the Distinction of Wood-Destroying Fungi. — Trans. roy. Soc. Can., V, 1923.
39. FÅHREUS, G., NILSSON, R. and NILSSON, G.: Studies in the Decomposition of Wood by Means of Some White Rot Fungi. — Svensk. Bot. Tidskr. 43, 1949.
40. GADD, O.: Wood Decay Resulting from Rot Fungi. — Paper and Timber, 39: 8, 1957.
41. GUSTAFSSON, C., SUNDMAN, J., PETTERSSON, S. and LIND, T.: The Carbohydrates in Some Species of Wood. — Papper och Trä, 33: 10, 1951.
42. HACKSKAYLO, J., LILLY, V. G. and BARNETT, H. L.: Growth of fungi on three sources of nitrogen. — Mycologia, 46, 1954.
43. HANDKE, H. H.: Zur Fruchtkörperbildung holzvewohnender Basidiomyceten in Kultur. — Holzzerstörung durch Pilze, Intern. Symposium, Eberswalde, Berlin, 1962.
44. HEMMI, T. and KURATA, S.: Pathological Studies on *Polyporus betulinus* (Bull.) Fr. — Forsch. Pflkr. Tokyo, I, 1931. Cit.fr. Biol. abstr., 7 (4), 7664, 1933.
45. HERRICK, J. A.: The Carbon and Nitrogen Metabolism of *Stereum gausapatum* Fries. — Ohio Jour. Sci., 40, 1940.
46. HIURA, M. and NAGATA, Y.: Studies on the Metabolic Products of *Corticium centrifugum* (Lev) Bres. Part 1. On the Organic Acids. — Res. bull. Fac. Agr. Gifu. Univ., 3, 1954.
47. HUMPHREY, C. J. and SIGGERS, P. V.: Temperature Relations of Wood-Destroying Fungi. — J. Agric. Res., 47, 1933.
48. JENNISON, M. W., NEWCOMB, M. D. and HENDERSON, R.: Physiology of the Wood-Rotting Basidiomycetes. I. Growth and Nutrition in Submerged Culture in Synthetic Media. — Mycologia, XLVII: 3, 1955.
49. KLINGSTRÖM, A., and OKSBJERG, E.: Nitrogen Source in Fungal Decomposition of Wood. — Nature, Jan., 1963.
50. KREISEL, H.: Die phytopathogenen Grosspilze Deutschlands. — Jena 1961.
51. KÄÄRIK, A.: Growth and Sporulation of Ophiostoma. — Symb. Bot. Upsaliensis, XVI: 3, 1960.
— Keimung und lebensdauer der Sporen einiger Bläuepilze bei verschiedener Luftfeuchtigkeit. — Ann. Soc. Litt. Est. Svecia, III, 1960.
53. KÖSTLER, J., und SCHMUCKER, T.: Silvae Orbis, Bd 4 B. — Berlin-Wannsee, 1941.
54. LAFUZE, H. H.: Nutritional Characteristics of Certain Wood-Destroying Fungi,

Polyporus betulinus, Fr., *Fomes pinicola* (Fr.) Cooke and *Polystictus versicolor* Fr. — Plant. Physiol., 12, 1937.
55. Liese, J.: Verhalten holzzerstörender Pilze gegenüber verschiedenen Holzarten und Giftstoffen. — Angew. Botanik, 10, 1928.
56. Lilly, B. G. and Barnett, H. L.: Physiology of the Fungi. — New York, 1951.
57. Lindberg, M.: Galactose as a carbon source for the growth of *Ophiostoma multiannulatum*. — Physiol. Plant., 16: 3, 1963.
58. Lindeberg, G.: Über di Physiologie ligninabbauender Bodenhymenomyzeten. — Symb. Bot. Upsaliensis, VIII: 2, 1944.
59. Lohwag, K.: Zur abbanintensität holzzerstörender Pilze. — Sydowia Ann. Myc., IX, 1955.
60. — Über die Abbauintensität des *Polyporus betulinus* (Bull.) Fr. — Sydowia Ann. Myc. Beiheft I. Festschrift für Franz Petrak.
61. Lutz, L.: Sur la spécifité de quelques Hyménomycetes. — Bull. Soc. Mycol. France, XLI, 1925.
62. Lyr, H.: Über den Nachweis von Oxydasen und Peroxydasen bei höheren Pilzen und die Bedeutung dieser Enzyme für die Bavendamm-Reaktion. — Planta, 50, 1958.
63. MacDonald, J. A.: A study of *Polyporus betulinus* (Bull.) Fries — Ann. Appl. Biol., 24, 1937.
64. Mayr, H.: Zwei Parasiten der Birke, *Polyporus betulinus* Bull. und *Polyporus laevigatus* Fries. — Bot. Centralbl., Bd XIX, 5, 1884.
65. Meier, H.: Über den Zellwandabbau durch Holzvermorschungs-pilze und die submikroskopische struktur von Fichtentracheiden und Birkenholzfasern. — Holz als Roh- und Werkstoff, 13, 1955.
66. Mounce, I.: Studies in Forest Pathology. II. Biology of *Fomes pinicola* (SW) Cooke. — Bull. Dep. Agric. Can., III, 1929.
67. Munsell book of color. — Munsell Color Company, Baltimore 1960.
68. Nobles, M. K.: Studies in Forest Pathology. VI. Identification of Cultures of Wood-rotting Fungi. — Can. J. Res., C. 26, 1948.
69. Nord, F. F.: and Sciarini, L. J.: On the Mechanism of Enzyme Action. 27. The Action of Certain Wood-Destroying Fungi on Glukose, Xylose, Raffinose and Cellulose. — Arch. Biochem., 9, 1946.
70. Nord, F. F. and Vittucci, J. C.: On the Mechanism of Enzyme Action. XXIX. The Acetate Metabolism of Certain Wood-Destroying Molds and the Mechanism of Wood Decay. — Arch. Biochem., 14, 1947.
71. — and Vitucci, J. C.: On the Mechanism of Enzyme Action. XXX. The Formation of Methyl-p-Methoxycinnamate by the Action of *Lentinus lepideus* on Glucose and Xylose. — Arch. Biochem., 14, 1947.
72. Norkrans, B.: Studies in Growth and Cellulolytic Enzymes of Tricholoma. — Symb. Bot. Upsaliensis, XI: I, 1950.
73. Nylinder, P. och Rennerfelt, E.: Undersökningar över rötskador i den helbarkade sulfitveden under olika huggnings- och lagringsförhållanden. — Medd. Statens Skogsforskningsinst., 44: 10, 1954.
74. Peace, T. R.: Pathology of Trees and Schrubs. — Oxford 1962.
75. Pechmann, H. V. and Schaile, O.: Changes in the Dynamic Strength and Chemical Composition of Wood by the Attack of Wood-destroying Fungi. — Forstwiss. Zbl., 69, 1950.
76. Pehrson, S. O.: Growth Physiology of *Phacidium infestans* Karst. — Physiol. Plant., 1: 1, 1948.
77. Pilat, A.: Polyporaceae I. — Atlas des Champignons de l'Europe, Tome III, Praha 1936.
78. Ralph, B. J. and Robertson, A.: The Chemistry of Fungi. XIV. 2: 4: 5-Trihydroxyphenyl-glyoxylic Acid from *Polyporus tumulosus* Cooke. — Chem. Soc., 4, 1950.
79. Rennerfelt, E.: Das Wachstum einiger Pilze aus Holzschliff bei verschiedener Temperatur. — Arch. Mikrobiol., 12: 1, 1941.
80. — Några undersökningar över olika rötsvampars förmåga att angripa splint- och kärnved hos tall. — Medd. Statens Skogsforskningsist., 36: 9, 1947.
81. — Träskydd. — utgiven av Svenska Skogsvårdsföreningen, Stockholm 1962.
82. — A Comparison between Swedish Field Tests and Laboratory Experiments with some Wood Preservatives. — Am. Wood-Preservers' Ass., 59, 1963.

83. Robak, H.: Cultural Studies in some Norwegian Wood-Destroying Fungi. — Medd. Vest. Forstl. Forsøksstasion, 25, 1942.
84. Robbins, W. J.: A survey of the Growth Requirements of some Basidiomycetes. — Mycologia, 42, 1950.
85. — and Hervey, A.: Wood, Tomato and Malt Extracts and Growth of Some Basidiomycetes. — Mycologia, 50, 1958.
86. Rozanova, M. A.: O rasprostranenii *Polyporus betulinus* Fr., *Fomes fomentarius* Fr., i *Fomes igniarius* Fr. v berezovyh roscah Zvenigorodskogo uezda Moskovskoj gubernii. — Zascita Rastenij ot Vreditelej, 2, R.A.M., 1926. Cit. fr. Peace: Pathology of Trees and Shrubs.
87. Rumbold, C.: Beiträge zur Kenntnis der Biologie holzzerstörender Pilze. — Naturw. Z. Forst u. Landw., 1908.
88. Rytjkova, A. G.: Ochimitjeskom sostave guiloj drevesing berezy i osiny. — Zurnal prikladnoj Chimii, 2: 31, 1958.
89. Schreyer, R.: Vergleichende Untersuchungen über die Bildung von Gluconsäure durch Schimmelpilze. — Biochem. Z., 240, 1931.
90. Siu, R. G. H.: Microbial Decomposition of Cellulose. — New York, 1951.
91. Smith, V. M.: On the Mechanism of Enzyme Action. XXXVI. Dehydrogenation Studies on *Merulius lacrymans* and *Marasmius chordalis*, and the Mechanism of Oxalic Acid Formation. — Arch. Biochem, 22, 1949.
92. Swedish Assosiation of Pulp and Paper Engineers: Determination of Resins and Fats in Pulp. — CCA 2, 1940.
93. — Determination of Lignin in Wood and unbleached Pulp. — CCA 5, 1940.
94. — Colorimetric Determination of Pentosans in Pulp. — CCA 24: 57, 1957.
95. Tamm, C. O.: Growth, Yield and Nutrition in Carpets of a Forest Moss (Hylocomium splendens). — Medd. Statens Skogsforskningsinst., 43: 1, 1953.
96. Trendlenburg, R./Mayer-Wegelin, H.: Das Holz als Rohstoff. — München, 1955.
97. Treschow, C.: Nutrition of the Cultivated Mushroom. — Dansk Bot. Arkiv, 11: 6, 1944.
98. Vorreiter, L.: Holztechnologisches Handbuch. — Wien 1949.
99. Wolpert, H.: Studies in the Physiology of the Fungi. The growth of Certain Wood-Destroying Fungi in Relation to the H-ion Concentration of the Media. — Ann. Mo. Bot. Gdn., 11, 1924.
100. Yusef, H. M.: The requirements of some hymenomycetes for essential metabolites. — Bull. Torrey Bot. Club, 80: 1, 1953.

Sammanfattning

Fysiologi och vednedbrytning hos björktickan Polyporus betulinus (Bull) Fr.

Björktickans, *Polyporus betulinus*, fysiologi och förmåga att under olika betingelser bryta ned ved har studerats.

Under optimala betingelser kunde *P. betulinus* hos björkved orsaka en maximal viktförlust av ca 71 %. Detsamma gäller också för klibbtickan, *Polyporus marginatus*, vilken valdes som jämförelseorganism.

Vedfuktigheten ökade kraftigt under rötförloppet och uppnådde som högst tre gånger begynnelsefuktigheten. *P. betulinus* och *P. marginatus* orsakade inte så stora viktförluster hos splintved av björk som de mest aktiva vitrötesvamparna.

Vid ett angrepp på veden kunde hyferna först iakttagas i och omkring märgstrålcellerna. Senare penetrerade hyferna alla celler i alla riktningar.

P. betulinus orsakade kraftiga viktförluster hos splintved av björk, bok och gran, medan både splintved och kärna hos tall var mycket motståndskraftiga mot angrepp.

Optimaltemperaturen för rötaktiviteten var omkring 25° C. Den angripna veden blev mer och mer sur vid ökande viktförluster. I de längst framskridna stadierna av röta steg vedens pH igen.

Ändringarna i vedens kemiska sammansättning under rötförloppet fortskred i det närmaste identiskt för *P. betulinus* och jämförelsesvampen *P. marginatus*. I början av ett angrepp förlöpte hexosankonsumtionen långsammare än pentosankonsumtionen. Senare var förhållandena omkastade. Ingen registrerbar ligninförlust inträffade under rötförloppet, vilket resulterade i en kraftig ackumulation av denna substans. 14 % av det ursprungliga hexosaninnehållet och 5 % av det ursprungliga pentosaninnehållet återstod vid maximal rötning. Tillsammans utgjorde de ungefär 9 % av den ursprungliga vedmängden. Sålunda var 9 av de ursprungliga 80 % kolhydrater otillgängliga för dessa svampar. De i alkohol och diklormetan extraherbara substanserna ökade under rötningen. I sena rötstadier utgjorde dessa 3,5 gånger halten i den friska veden.

Levande björkar och aspar infekterades med *P. betulinus* och *P. marginatus*. Efter ett halvår kunde missfärgningar i veden urskiljas såväl ovanför som nedanför infektionspunkterna. Svamparna kunde

återisoleras från denna missfärgade ved. Missfärgningen spreds inte lika snabbt i asparna som i björkarna. Det var sålunda möjligt att infektera inte bara levande björkar utan även aspar med mycel av *P. betulinus*.

P. betulinus växte i kultur på de flesta näringssubstrat, som används för svampodlingar. Bavendamm-test gav negativt resultat indikerande oförmåga att producera polyfenoloxidaser.

Olika stammars radialtillväxt på maltagar vid rumstemperatur varierade mellan 4,7 och 7,2 mm per dag. Det fanns emellertid inte någon korrelation mellan radialtillväxt och mycelproduktion mätt som mycelets torrvikt.

P. betulinus uppvisade ett brett temperaturoptimum för tillväxt kring 20—25° C. Maximum- och minimumtemperaturerna var omkring 35 resp. 5° C. På maltagar överlevde mycelet 35 men icke 42 dagars förvaring vid — 30° C.

pH-optimum för tillväxt visade sig ligga i närheten av pH 5 och maximum omkring pH 7. Mycelet förmådde växa i media med pH ända ner till 2.1.

P. betulinus uppvisade thiaminheterotrofi. En komplett vitamin- och mikroelementblandning kunde icke ersätta thiaminet. Tillsats av Na och Ca tillsammans eller ännu bättre Na + Ca + vitaminblandning till en basnärlösning innehållande socker, kväve, salter och thiamin stimulerade tillväxten.

Bland aldopentoserna var xylos en god kolkälla, medan arabinos, ribos och rhamnos inte kunde utnyttjas. Glukos utgjorde den bästa kolkällan bland aldohexoserna. Av de två testade ketohexoserna kunde fruktos, men inte sorbos utnyttjas. De testade disackariderna cellobios, laktos, maltos, trehalos och sackaros, kunde alla utnyttjas som energikällor. Cellobios stimulerade kraftigt tillväxten. Cellulosa i form av filtrerpapper utgjorde en mycket dålig kolkälla för *P. betulinus* och *P. marginatus*. Många vitrötesvampar kunde å andra sidan lätt använda filtrerpappercellulosa. Polysackariderna glykogen och inulin testades. Av dessa kunde endast den förra användas av *P. betulinus*, medan *P. marginatus* kunde utnyttja båda, och dessutom trisackariden raffinos, vilken inte kunde assimileras av *P. betulinus*.

Bland de testade oorganiska kvävekällorna var ammoniumföreningarna bäst. Nitrat kunde inte utnyttjas. Eftersom nitrit däremot kunde användas förefaller det vara produktionen av nitratreduktas, som är blockerad. Amiderna asparagin och glutamin var inte lika goda kvävekällor som sina motsvarande aminosyror. Kvävekällorna

testades i tre olika koncentrationer. Medelkoncentrationen hade ungefär samma N/C-kvot som i färsk ved. Kväve anses vara *en* begränsande faktor för svamptillväxt i ved, och på samma sätt som en tillsats av kväve i lämplig form till ved stimulerar nedbrytningen, så stimuleras myceltillväxten av en ytterligare tillsats av kväve till närlösningen.

Mycelets kväveinnehåll ökade med stigande kvävehalt i närlösningen. Mycel, som vuxit i närlösningar innehållande ammonium- eller aminokväve, hade en högre kvävehalt än mycel, som vuxit i närlösningar med glutamin, asparagin eller kaseinhydrolysat som kvävekällor.

I ett fattigt medium stimuleras myceltillväxten hos *P. betulinus* kraftigt vid tillsats av kallvattenextrakt från björkved samt från splintved av al, asp och tall. Bland varmvattenextrakten hade endast extraktet från splintved av björk stimulerande effekt på tillväxten. Höga koncentrationer av varmvattenextrakt hämmade tillväxten.

Tillsats av vedextrakt påverkade sporgroningen hos *P. betulinus* på olika sätt. Andelen grodda sporer ökade efter tillsats av extrakt från björk, asp och al, medan extrakt från tallsplintved inte hade någon inverkan. Groddslangens tillväxt påverkades inte av extrakt från björk, asp eller al. Tallvedsextrakt hämmade däremot groddslangens tillväxt. Lagfasen före sporgroningen minskade, när extrakt från björk, asp eller al tillsattes, medan tallvedsextrakt försenade groningen.

Fruktkroppsbildningen i kultur studerades. Fruktkroppsanlag bildades på stillastående, flytande eller halvfasta media. Torrviktsproduktionen ökade snabbt, när fruktkroppsbildningen började, vilket inträffade innan näringsmediet var utarmat. Konsumtionen av C, N och P ökade, när torrviktsproduktionen ökade. Halterna av N och P i mycelet minskade, när fruktkroppsbildningen igångsattes. Fruktkroppar och naturligt bildade tickor hade en avsevärt lägre halt av kväve än mycelet. Ålder och ursprung hos ympmaterialet påverkade inte fruktkroppsbildningen.

Alltför hög halt av C och N i substratet liksom ackumulering av CO_2 i försökskolvarna hämmade fruktkroppsbildningen. Inom pH-intervall, där tillväxten var god, influerade inte pH-ändringar i mediet fruktkroppsbildningen. Tillsats av tre organiska syror, vilka normalt exuderas av mycelet, eller tillsats av »gammal» närlösning påverkade inte fruktkroppsbildningen. Fruktkroppar bildades, när svampen växte på björk- eller bokved, men inte på gran- eller tallved