STUDIA FORESTALIA SUECICA

NR 52 1967

Physiology of fungi attacking birch and aspen pulpwood

Fysiologi hos svampar som angriper björk- och aspmassaved

by

BJÖRN HENNINGSSON

SKOGSHÖGSKOLAN

ROYAL COLLEGE OF FORESTRY

STOCKHOLM

Printed in Sweden
by ESSELTE AB
STOCKHOLM

CONTENTS

1. Introduction

The present study is part of a comprehensive investigation of microbial attack on pulpwood of birch and aspen. The purpose of this study was to determine the general physiological requirements of the attacking decay fungi, and to compare these requirements with the physiological environment offered to the fungi within the wood. Special interest has been devoted to the carbon and nitrogen nutrition, since these two nutrients must be of extreme importance for the development of decay. The influence of other factors of major importance for decay, as, for instance, temperature and moisture content of the wood, have also been studied.

The chemical decomposition of the wood and the interactions between the different microbes have also been investigated and will be published in further papers (HENNINGSSON 1967c and 1967e). In evaluating the results obtained in physiological experiments with single cultures, and before any application of these results to the problem of the decomposition of pulpwood, it must be borne in mind that a number of interactions which occur between the micro-organisms in naturally infected wood are also of importance for the total wood decomposition.

II. Materials and methods

The choice of test organisms was based on results from field studies of stored pulpwood of birch *(Betula verrucosa* Ehrh. and *Betula pubescens* Ehrh.) and aspen *(Populus tremula* L.), cf. HENNINGSSON 1967 e. Species frequently observed forming sporophores on the wood and frequently identified as mycelia growing within the wood were selected for the present study. Amongst the strains used *Polyporus betulinus, Polyporus marginatus, Schizophyllum commune, Libertella betulina* and *Cytosporina sp. 2* were isolated by the author. The remaining strains were obtained from Dr. A. KÄÄRIK at the Royal College of Forestry in Stockholm, being part of the reference collection of wood-destroying fungi at the Dept. of Forest Products (KÄÄRIK 1963).

Strains used in the physiological experiments:

Fungus	Strain No.	Isol. date
Polyporus betulinus (Bull.) Fr.	B 6032	Oct. 1960
Polyporus marginatus Fr.	B 6115	May 1961
Polyporus hirsutus (Wulf.) Fr.	A 477	Oct. 1960
Polyporus versicolor (L.) Fr.	A 361	Oct. 1958
Polyporus zonatus (Nees) Fr.	A 360	Aug. 1960
Polyporus adustus (Willd.) Fr.	A 466	Feb. 1962
Lenzites betulina (L.) Fr.	A 365	Sept. 1960
Daedalia unicolor Fr.	A 520	Oct. 1962
Stereum hirsutum (Willd.) Fr.	A 373	Sept. 1958
Stereum purpureum Fr.	A 744	Oct. 1964
Corticium laeve Pers.	A 26	Oct. 1947
Corticium confluens Fr.	A 661 (from CBS, strain Boidin)	
Peniophora incarnata (Pers.) Karst.	A 507	Oct. 1962
Schizophyllum commune Fr.	B 64105	Oct. 1964
Libertella betulina Desm.	B 6320	Aug. 1963
Cytosporina sp. 2	B 6321	Aug. 1963

The two fungi first mentioned, *Polyporus betulinus* and *Polyporus marginatus,* attack living trees and should consequently be regarded

as parasites. *Polyporus betulinus* has been found exclusively on *Betula* species, whereas *Polyporus marginatus* occurs on several host-tree species, hardwoods as well as softwoods. The remaining fungi normally attack the wood saprophytically after felling. *Libertella betulina* has been found exclusively in birch wood and *Cytosporina* sp. 2 exclusively in aspen wood, while the other saprophytes have been observed in both birch and aspen.

The stock cultures were kept on malt agar in Pyrex culture tubes at +5°C. Before running the experiments, the mycelia were transferred to plastic petri dishes of 90 mm diameter, containing an agar substrate. The experiments were mainly carried out in 100 ml Erlenmeyer flasks or plastic petri dishes. Flasks used in experiments with fluid synthetic media were carefully cleaned with dichromatic-sulphuric acid solution and washed with hot tap water and redistilled water.

For culturing the fungi on wood, 100 ml Erlenmeyer flasks were used, containing 10 gm vermiculite (a mica material from AB Mataki, Sweden). To the vermiculite was added 20 ml distilled water or nutrient solution. The size of the wood blocks was 10 × 10 × 20 mm, the two 10 × 10 mm surfaces being perpendicular, and the four 10 × 20 mm surfaces being parallel to the long axis of the fibres of the wood. Usually three or four wood samples were laid beside one another in the flask. Depending on the purpose of the experiment, the following variations of the method were used:

1. The wood blocks were placed in or on the vermiculite before the flasks were autoclaved for 20 min. at 120°C. The inoculation employed a mycelial suspension or pieces from an agar culture.

2. The flasks were autoclaved separately and inoculated with the fungi. When a vigorous mycelium had developed in the vermiculite (usually within 10—14 days), the wood blocks were autoclaved in glass petri dishes and sterilely transferred to the flasks.

The incubation period varied between one and four months, depending on the purpose of the experiment. The fungal activity in the wood was determined in terms of the loss in dry weight during the incubation. The dry weights were determined after 24 hours at 105°C.

In experiments with liquid nutrient media, 20 ml nutrient solution per flask was used. After autoclaving for 20 min. at 120°C, the flasks were inoculated either with a mycelial suspension, for shake cultures, or with small pieces from an agar culture, for surface cultures. In most cases growth was more rapid in shake cultures than in surface cultures. However, certain species, e.g. *Stereum purpureum*, grew better stationary than on the shaker. The mycelial yields from liquid

media were determined as mycelial dry weight per flask by filtering off the nutrient solution in weighed glass crucibles (Jena duran G 3) or on circular filter papers, washing thoroughly with distilled water, drying for 20 hours at 105°C, cooling in a desiccator and weighing. The reported mycelial weights usually represent the mean of from three to five replicates. The standard errors very seldom exceeded 10 per cent of the mean values; if otherwise, this is indicated in the tables.

In some experiments with semi-solid agar media, growth was measured as the radial growth of the mycelium in millimetres per day. Plastic petri dishes inoculated at the edge of the dish were used. Growth measurements were not started until the mycelia had grown out from the inoculum and had become established in the medium. Except when the carbon, nitrogen or vitamin sources were varied, the following media were used:

Medium B

Glucose 20.0 gm; KH_2PO_4 1.0 gm; $MgSO_4 + 7\ H_2O$ 0.5 gm; Ammonium tartrate 2.5 gm; $CaCl_2 + 2\ H_2O$ 0.3 gm; NaCl 0.1 gm; $ZnSO_4$ 8.79 mg; $CuSO_4$ 0.393 mg; Fe-citrate 5.0 mg; H_3BO_3 0.057 mg; $(NH_4)_6MO_7O_{24}$ 0.368 mg; $MnSO_4 + 7\ H_2O$ 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; P-aminobenzoic acid 50 µg; Vitamin B12 4 µg; Redistilled water to 1000 ml.

Medium C

Malt extract (syrup) 25 gm; Distilled water to 1000 ml.

Medium D

Malt extract (syrup) 25 gm; Agar-agar 15 gm; Distilled water to 1000 ml.

Medium D 2

Agar-agar 15 gm; Medium B to 1000 ml.

Medium E

Per flask: Vermiculite (air dry) 10 gm; Medium C 20 ml; 3—4 wood blocks.

Medium F

Per flask: Vermiculite (air dry) 10 gm; Distilled water 20 ml; 3—4 wood blocks.

III. Carbon Nutrition

A. Introduction

Except for small amounts of organic substances in the cell lumina, the wood-destroying fungi must obtain the carbon needed for their metabolic activities from the carbon-rich polymers which make up the cell walls. Consequently, these polymers and their degradation products are of major interest in a physiological study. The principal cell wall constituents are cellulose, lignin and hemicelluloses.

Cellulose usually constitutes 40 to 50 per cent of the weight of the wood. Thus birch wood contains 40—43 and aspen wood 44—48 per cent cellulose (TRENDELENBURG 1955, TIMELL 1964, HENNINGSSON 1967 e). All layers of the cell wall contain cellulose, although most cellulose is contained in the two innermost layers, S_2 and S_3. The fact that cellulose makes up nearly half of the wood indicates the importance of this substance as an energy source for the decay fungi.

Angiosperm wood is generally not as rich in lignin as is gymnosperm wood. Birch and aspen, for instance, contain 19—21 per cent lignin, while pine and spruce (*Pinus sylvestris* L. and *Picea abies* (L). Karst.) contain 27—30 per cent (HENNINGSSON 1962, 1967 e, TRENDELENBURG 1955). Most of the decay fungi found in birch and aspen wood have proved capable of decomposing the lignin fraction in intact wood (cf. CAMPBELL 1930, 1931, 1932, SCHEFFER 1936, COWLING 1961, KUBIAK & KERNER 1963 and SEIFERT 1966). There is also evidence that certain decay fungi can utilise a purified lignin as their sole source of carbon (GARREN 1938, DAY et al. 1949, van VLIET 1955). As the chemical nature of lignin is not fully known, this substance has not been used in the present investigation.

In angiosperm wood from temperate zones, the hemicellulose fraction, as distinct from that of gymnosperm wood, is characterised by a high percentage of pentosans—xylans and minor amounts of arabans. In birch and aspen wood xylose makes up 39 and 30 per cent respectively of the carbohydrates. The corresponding figures for arabinose are 2.5 and 0.5—1.0 (GUSTAFSSON et al. 1951). The wood hemicelluloses are heteropolysaccharides, however, so the pentosans occur in polysaccharides such as glucuronoxylan and glucuronoarabinoxylan. The

degree of polymerisation (DP) of these polysaccharides usually ranges between 50 and 250. It might be noted that the xylan content of the various layers of a birch cell wall is three to four times as high as that of pine wood, and that in birch wood the xylan content of the S_1-layer and the outer part of the S_2-layer is especially high (MEIER 1962). In birch and aspen wood the pentosans make up some 27 and ten per cent respectively of the weight of the wood (cf. HENNINGSSON 1962 and TIMELL 1964). This indicates that the pentosans, if they can be utilised, are an important source of the carbon needed for energy and synthesis in the fungi growing within the wood.

The wood hemicelluloses also contain hexosans. A few per cent of the carbohydrate fraction in birch and aspen wood consist of galactans and mannans (GUSTAFSSON et al. 1951). The galactans are restricted almost exclusively to the primary wall and the middle lamella, whereas the mannans are more evenly distributed throughout the cell wall. The mannan content of gymnosperm wood is generally about ten times as high as that of angiosperm wood (MEIER 1962). Glucose, glucoronic acid, and galacturonic acid are also more or less common components of the hemicelluloses. Although the hexose polymers of the hemicelluloses make up only a minor part of the wood carbohydrates, they may be of importance from the physiological point of view (as nutrients, enzyme inducers etc.).

The effects on growth of various sugars, polysaccharides, sugar alcohols and organic acids have been studied in the present investigation. Amongst the carbon sources tested are many of those normally occurring in birch and aspen wood, either free or as various polymers, (TIMELL 1964). In experiments with water-soluble carbon sources, medium B was used, with glucose replaced by 10 gm per litre of the carbon source tested. The carbohydrate solutions were filter-sterilised and added sterilely to the autoclaved nutrient solution. The flasks were inoculated with one millilitre of a mycelial suspension and incubated at $+22 \pm 2°C$ in a shaker for ten days.

B. Growth on cellulose, cellobiose and glucose

Cellulose as the sole source of carbon was studied in several ways. The growth on filter-paper cellulose was studied by covering agar plates containing medium D_2 (glucose excluded) with round filter papers. After the inoculation of the fungi in the centre of the paper, radial growth and the thickness of the mycelium were estimated. Sometimes radial growth was faster in dishes lacking any carbon

Tab. 1. Relative mycelial dry weight of birch and aspen fungi grown in medium B with various carbon sources

Carbon source	Polyporus betulinus	Polyporus marginatus	Polyporus hirsutus	Polyporus versicolor	Polyporus zonatus	Polyporus adustus	Lenzites betulina	Stereum hirsutum	Stereum purpureum	Corticium laeve	Corticium confluens	Peniophora incarnata	Schizophyllum commune	Libertella betulina	Cytosporina sp 2	Average
D-arabinose	*18*	27	25	**5*	16	11	**6*	**3*	*8*	44	16	*4*	**5*	13	**5*	14
D-xylose	103	101	141	66	107	138	104	17	90	133	107	*6*	38	108	57	88
D-glucose	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
D-galactose	63	20	9	**6*	19	10	**9*	**3*	**4*	43	*4*	**12*	19	17	24	17
D-mannose	104	112	102	67	106	119	98	137	96	92	120	97	61	113	107	102
D-fructose	78	83	116	67	88	79	101	144	51	107	106	123	63	93	82	92
D-cellobiose	67	97	102	56	86	103	162	54	387	111	111	106	103	104	115	118
lactose	**17*	25	103	43	52	22	17	28	29	111	19	**8*	25	49	*4*	37
D-maltose	115	60	66	100	128	114	112	67	57	128	111	109	43	119	48	92
D-saccharose	50	43	138	65	105	*6*	111	114	78	26	13	108	96	151	59	77
raffinose	*16*	37	108	86	85	16	64	87	21	*4*	*2*	36	49	120	25	50
starch	77	111	116	100	98	122	68	89	126	129	108	83	64	123	97	101
inulin	—	42	17	12	13	27	17	20	57	15	*11*	123	52	*11*	*12*	31
glycerol	473	171	108	171	93	65	116	99	55	106	103	123	106	47	44	125
mannitol	128	98	100	201	95	59	99	94	151	104	103	87	91	96	42	103
sorbitol	124	63	94	186	92	65	97	89	55	109	103	83	108	99	32	93
no carbon	13	7	3	3	3	5	7	2	8	3	7	8	7	8	8	6

* Sparse growth occurred when $(NH_4)_2$-tartrate was replaced by casein hydrolyzate as the nitrogen source in the medium. In all the other combinations where no growth occurred with $(NH_4)_2$-tartrate as nitrogen source it was not possible to induce growth with casein hydrolyzate either.

source than in those with filter-paper cellulose. In dishes without cellulose there was, however, always a very thin, sparse mycelium, and the mycelial front was often irregular and indistinct. Single hyphae occasionally grew rapidly over the whole plate without branching. Consequently, the radial growth alone gave no information about the availability of the substrate. The most valuable indication of growth in these types of experiment is the thickness of the mycelium. It was shown that only the two brown rot fungi *Polyporus betulinus* and *Polyporus marginatus* were completely incapable of utilising filter-paper cellulose. *Corticium laeve* and *Stereum purpureum* produced a limited growth, whereas the rest of the fungi grew well with filter-paper cellulose as their sole source of carbon. The inability of the brown rot fungi to utilise cellulose in these types of experiments has been observed earlier (cf. HENNINGSSON 1965, JOHANSSON 1966). In the work of JOHANSSON (1966) it was further demonstrated that

Tab. 2. Cellulolytic activity (C_1) of birch and aspen fungi measured as the width of clear zones formed in a cellulose-agar medium

Fungus	Test method according to Bose			Test method employing Lugol's reagent and H_2SO_4			
	Radial growth in mm after	Width of clear zone in mm after*		Radial growth in mm after**		Width of non-coloured zone in mm after**	
	5 days*	5 days	8 days	3 days	6 days	3 days	6 days
Polyporus betulinus	13	11	17	4	11	10	16
Polyporus marginatus....	11	0	13	5	14	6	15
Polyporus hirsutus.....	20	13	45	12	32	14	34
Polyporus versicolor.....	29	27	76	16	39	13	39
Polyporus zonatus.....	27	28	70	14	38	13	38
Polyporus adustus.....	42	58	83	18	—	20	—
Lenzites betulina.....	16	19	45	8	27	12	22
Stereum hirsutum.....	20	11	53	8	34	10	25
Stereum purpureum.....	22	13	26	0	20	4	11
Corticium laeve.....	18	10	—	7	30	9	33

* inoculated at the edge of the petri dish.
** inoculated in the centre of the petri dish.

the C_1-activity, not the C_x-activity, was lacking in the brown rot fungi, and that none of the substances which normally induce cellulolytic activity in white rot fungi were effective in the case of the brown rot fungi.

Many experiments were carried out using cellulose powder suspended in medium B and D 2. Despite the difficulty of separating mycelium and undissolved carbohydrates at the end of the experiments, the following facts emerged, viz. that:

1. All the fungi tested produced at least some growth on cellulose.

2. Growth on cellulose was extremely slow in *Polyporus betulinus, Polyporus marginatus, Corticium laeve* and *Corticium confluens.*

3. Rapid growth on cellulose was exhibited by *Polyporus versicolor, Polyporus zonatus, Polyporus adustus, Lenzites betulina, Stereum hirsutum, Stereum purpureum, Schizophyllum commune, Libertella betulina* and *Cytosporina* sp. 2.

Tests for cellulolytic activity were also performed using a technique described by BOSE (1963) and a modified form of that technique employing a colour reaction obtained by adding Lugol's reagent and sulphuric acid to the plates. The results given in Tab. 2 show that the weakest cellulolytic activity was exhibited by the two brown rot fungi *Polyporus betulinus* and *Polyporus marginatus* and by *Stereum purpureum.*

The general conclusions that can be drawn from the cellulose experiments are as follows:

The brown rot fungi, in spite of their high cellulolytic activity in wood, exhibited a low cellulolytic activity and very sparse growth or no growth at all on cellulose given as the sole source of carbon. *Stereum purpureum, Corticium laeve* and *Corticium confluens,* which all are weak wood destroyers (see Fig. 5), also showed a low activity on cellulose when it was given as the sole source of carbon. Cellobiose, the extracellular end product in cellulose decomposition, proved to be an excellent carbon source for the birch and aspen-attacking fungi (Tab. 1). For the most part it was equal to and sometimes even superior to its monomer glucose. Cellobiose is also utilised by virtually all fungi hitherto tested (COCHRANE 1957), which indicates a widely distributed β-glucosidase activity among fungi.

Polysaccharides attacked by fungi are at first decomposed by extracellular enzymes, e.g. cellulases, xylanases and mannanases. These enzymes usually act as endoenzymes (NORKRANS & RÅNBY 1956, NORKRANS 1959, COWLING 1961 and 1963, AHLGREN et al. 1966). At the disaccharide stage, further breakdown is taken over by extracellular glucosidases, e.g. cellobiase, xylosidase and mannosidase. The cellulolytic enzymes may be surprisingly small. Thus PETTERSSON et al. (1963) isolated a cellulase from *Polyporus versicolor* with a molecular weight of only 11,400. It has further been shown for *Polyporus versicolor* and *Aspergillus spp* (AHLGREN et al. 1966 and PETTERSSON & PORATH 1963) that enzymes attacking the polysaccharides have smaller molecules than the glucosidases attacking the corresponding disaccharide. If this is general amongst fungi, it is of great importance, since the polysaccharide-splitting enzymes must diffuse considerable distances in the cell wall to reach their substrates. Consequently, a small molecule will be more efficient than a large one. For the glucosidases, diffusion is not necessary, since their substrates, the disaccharides, are water-soluble and thus can themselves diffuse.

Several experiments were carried out with the white rot fungi *Stereum hirsutum* and *Libertella betulina* to identify cellobiose and glucose as end products resulting from extracellular cellulose decomposition. The fungi were grown as shake cultures in medium B, where glucose was replaced by a two per cent cellulose suspension. After incubation periods varying between one and 65 days the mycelia and the remaining cellulose were filtered off. The culture filtrates were then analysed for glucose and cellobiose, using a thinlayer chromatographic method described by STAHL (1962). The method uses silica

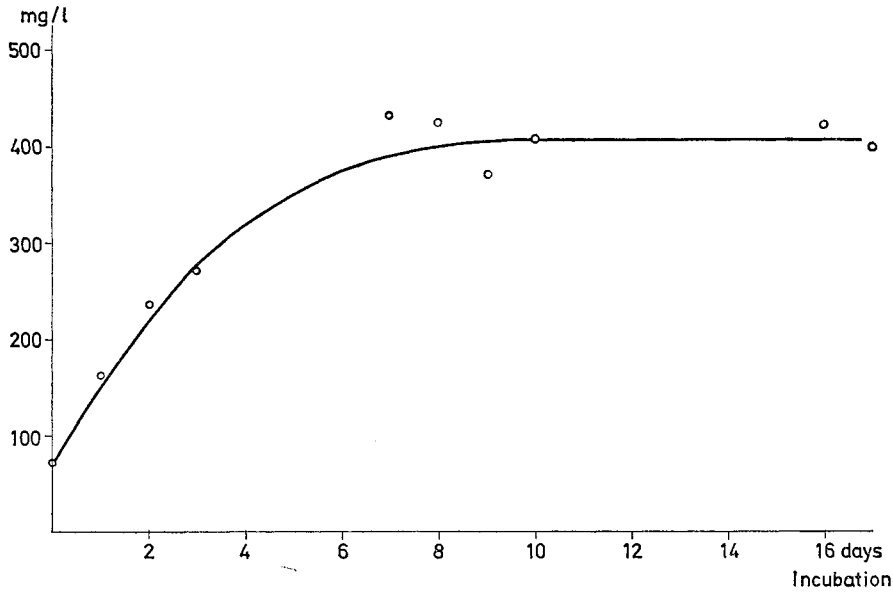


Fig. 1. Hydrolysable intermediates, derived from the extracellular cellulose decomposition, occurring in culture media of *Stereum hirsutum* when this is supplemented with cellulose as the sole carbon source. The hydrolysates were measured photometrically as glucose.

gel plates and a solvent system consisting of ethyl acetate, isopropyl alcohol and water. The chromatograms were developed by spraying them with benzidine dissolved in acetic acid and ethyl alcohol. The culture filtrates were also analysed for hydrolysable intermediates, measured as potential reducing substances. Samples taken from the filtrates were hydrolysed with three (w/v) per cent sulphuric acid (Cowling 1961), followed by neutralisation with NaOH and colorimetric determination of the glucose (Somogyi 1952).

The results showed that except for very faint spots of glucose during the first two days (emanating from the inoculum), neither glucose nor cellobiose could be discovered on the chromatograms. However, if the fungal uptake of glucose was blocked by the addition of merthiolate to the flasks containing the growing mycelia, or by subjecting the growing cultures to a temperature of +45°C, it was possible to obtain glucose but not cellobiose from the media. The addition of merthiolate and heating to +45°C did not significantly decrease the activity of cellulases and glucosidases (cf. Ascan & Norkrans 1953, Johansson 1966).

The results of the analyses for hydrolysable intermediates in the

culture filtrates are shown in Fig. 1. The amount of hydrolysable intermediates increased during the first week of incubation, after which a constant level of approximately 400 mg per litre of filtrate was reached. This value corresponds to about two per cent of the cellulose originally given to the cultures.

The following conclusions may be drawn from the chromatographic and photometric analyses of culture filtrates:

1. During the first week of the extracellular enzymatic decomposition of cellulose, the production of soluble intermediates with a degree of polymerisation (DP) above two was in excess of the further metabolism of these substances.

2. After one week's incubation the production of intermediates and the further metabolism proceeded at the same rate.

3. If the formation of cellobiose and the splitting of this substance into glucose molecules are extracellular processes, they must be synchronised with the uptake of the extracellular end product, glucose. Alternatively, the cellobiose may be taken up directly and transformed to glucose within the cells.

C. Growth on carbohydrates occurring in the hemicelluloses.

Growth on xylan was studied in the same way as was growth on powdered cellulose (p. 10). The results can be summarised as follows:

1. All the fungi tested exhibited at least some growth on xylan.

2. Growth on xylan was very slow in *Polyporus marginatus* and *Peniophora incarnata.*

3. Rapid growth on xylan was exhibited by *Polyporus versicolor, Polyporus zonatus, Polyporus adustus, Lenzites betulina, Stereum hirsutum, Stereum purpureum, Schizophyllum commune, Libertella betulina* and *Cytosporina* sp. 2.

Enzymatic attack on hemicelluloses is supposed to proceed similarly to that on cellulose, resulting in a final formation of dimers and monomers, which then enter the fungal cells. The effects on growth of various monomers of the hemicellulose are shown in Tab. 1. Of the two aldopentoses tested, D-xylose was generally an excellent carbon source. Only *Peniophora incarnata* failed to grow on xylose. Since xylans are so common in birch wood, an inability to metabolise xylan and xylose should make such organisms less competitive in birch wood. However, in spite of its weak ability to utilise xylose and xylan, *Peniophora incarnata* was one of the most common fungus species in birch pulpwood.

The further intracellular breakdown of pentoses in fungi is not very well known. However, since it has been demonstrated in the past decade that many fungi are able to use the hexose monophospate (HMP) pathway in their catabolism of glucose (BLUMENTHAL 1965), it is probable that after xylose has entered the cell, it is phosphorylated and introduced into the second part of the HMP pathway. Since there exist intermediates common to both the HMP pathway and to the general glycolytic Embden-Meyerhof (EM) pathway, the converted xylose molecule can easily be used in the energy-releasing and synthetic processes.

The general availability of xylose as a carbon source for the fungi found in birch and aspen wood might indicate the occurrence of a well-developed HMP pathway. However, the poor utilisation of xylose showed by *Stereum hirsutum* and *Peniophora incarnata* (Tab. 1), does not necessarily mean that the HMP pathway functions insufficiently in these organisms, since the inability to utilise the substance may equally well reflect a requirement for special inducing substances, or a lack of permeases needed in the transporting mechanisms; and it may be due to all of these factors.

Xylose has also proved to be a good carbon source for many other wood-destroying fungi. *Merulius lacrymans,* for instance, grew better on xylose than on glucose (GOKSØYR 1958). Other *Merulius* species, too, as well as *Fomes annosus,* could utilise xylose to the same degree as glucose (NORD & SCIARINI 1946).

Arabanase activity by fungi has been comparatively little studied, because arabans usually occur together with pectic substances or other polysaccharides in complexes, which are difficult to separate due to the arabans' being extremely susceptible to acid hydrolysis. However, FUCHS et al. (1965) demonstrated that some phytopathogenic and saprophytic fungi exhibited a considerable arabanase activity. The enzymes were induceable extracellular exo-enzymes "not cleaving the araban molecule randomly, but splitting off monosaccharide units". Arabinose, in contrast to xylose, was a very poor carbon source for the fungi tested in the present study. Only *Corticium laeve, Polyporus marginatus* and *Polyporus hirsutus* appeared to be capable of utilising arabinose. The L-isomer of arabinose is generally more available to fungi than the D-isomer (COCHRANE 1958). For *Verticillium albo-atrum,* L-arabinose was the best of several tested carbon sources (MALCA et al. 1966). Members of the genus *Ophiostoma (Ceratocystis)* also generally utilise this sugar (KÄÄRIK 1960 and GAGNON 1963), and in *Exobasidium* both isomers were utilised (SUNDSTRÖM 1964). In-

ability to utilise arabinose is, however, frequently reported, e.g. among *Coprinus* and *Phialophora* species (FRIES 1955 and BREWER 1959).

Since D-arabinose was utilised (to some degree at least) by certain of the birch and aspen-attacking fungi, there evidently exists a pathway through which the arabinose molecule can enter the metabolic systems. Very little is known about the breakdown of arabinose in fungi. A reductive pathway has, however, recently been found, in which L-arabinose is converted to xylulose-5-phospate (BLUMENTHAL 1965). This substance is also part of the HMP pathway, and thus the converted arabinose molecule can reach the glycolytic highways. As far as the present author can discover in the literature, nothing is known about the breakdown of D-arabinose. It might be not be too presumptuous to postulate the existence of an isomerase capable of converting D-arabinose to some of the intermediates in the two last-mentioned glycolytic pathways.

The hexoses mannose and galactose also occur in the hemicellulose fraction. In combination with each other or with glucose they build up, for instance, galactomannans, found in the middle lamella and the primary wall, and glucomannans, found in all layers of the cell wall (MEIER 1962). Mannose proved to be about equally as good a carbon source as glucose for the birch and aspen attacking fungi (Tab. 1). Galactose, however, was a poor source of carbon for most fungi. Only *Polyporus betulinus* and *Corticium laeve* could utilise D-galactose to some extent. The results indicate that parts of the middle lamella and the primary wall which contain galactans might be more resistant to attack by these fungi than the other cell wall layers.

Inability to utilise hexoses is usually attributed to impermeability or lack of the enzymes responsible for the phosphorylation of the free sugar, since the phosphorylated forms of glucose, mannose, fructose and galactose are easily transformed one into the other (COCHRANE 1957). Mannose is usually equivalent to glucose for the growth of fungi, whereas galactose gives varied results. Galactose was, for instance, a poor carbon source for *Coprinus* species (FRIES 1955). In the genus *Tricholoma,* however, the saprophytic species utilised D-galactose, while the mycorrhiza-forming species did not (NORKRANS 1950). NYMAN (1961) reports that among the fungi isolated from slime flux, *Rhodotorula spp* grew poorly on D-galactose, whereas *Phialophora sp* and *Dipodascus aggregatus* produced a good growth. Similarly, several species of *Ophiostoma* and *Exobasidium* (KÄÄRIK 1960, SUNDSTRÖM 1964) could utilise D-galactose. In *Ophiostoma multiannulatum* it was possible to obtain galactose-adapted strains by repeated transfer of

cells on galactose media (LINDBERG 1963). The above-mentioned results indicate that even if the decay fungi have difficulty in metabolising the galactose of the middle lamella and the primary wall, many other wood-inhabiting fungi have the power of utilising this galactose, and these fungi might thus be of importance in the complete decomposition of the cell walls.

D. Growth on carbon sources not occurring in the cell wall

Several monosaccharides and disaccharides occur in free form in the wood. Birch and aspen wood, for instance, contain glucose, fructose, sucrose, raffinose and stachyose. Aspen wood contains, in addition, free L-rhamnose (LINDBERG & SELLEBY 1958 and LARSSON & SELLEBY 1960). There is, however, a rapid decrease in free sugar content from the cambial zones inwards through the sapwood (DIETRICHS 1963). The availability of these sugars to the decay fungi attacking birch and aspen pulpwood can be seen in Tab. 1.

The only ketohexose tested, D-fructose, promoted rapid growth in all the fungi, although it was generally inferior to D-glucose (Tab. 1). Saccharose (sucrose) which is utilised variably amongst fungi, was also available to various extents in birch and aspen fungi. For most of the fungi tested, saccharose was a good carbon source. For *Polyporus hirsutus* and *Libertella betulina* this sugar was an even better carbon source than was glucose. *Corticium confluens,* however, grew only slowly and *Polyporus adustus* could not utilise saccharose at all. Since both of the last-mentioned fungi could use fructose and glucose, their inability to use saccharose was probably due to lack of invertase. The same explanation may be given for their poor growth on the trisaccharide raffinose. Invertase splits the raffinose molecule into fructose and melibiose. Complete assimilation of raffinose also requires both an α-galactosidase to split the melibiose molecule and an ability to assimilate galactose. Table 1 shows that only *Polyporus hirsutus* and *Libertella betulina* grew equally fast on raffinose as on glucose. It is interesting to note the similarities in useableness between saccharose and raffinose. Also, other decay fungi have proved capable of using raffinose as their sole carbon source (NORD & SCIARINI 1946). Starch and maltose were utilised by all the birch and aspen fungi, indicating a general distribution of the two amylases and maltase. Starch occurs as reserve food in the living cells of cambium and rays. In birch and aspen, however, the principal reserve food is of a fatty nature—primarily fatty acid esters of glycerol, sterols and terpenoid

alcohols (SELLEBY 1960 and LARSSON & SELLEBY 1960, PAASONEN 1966), so the starch content rarely exeeds one per cent.

Lactose was a poor source of carbon for most of the birch and aspen fungi (Tab. 1). Only in the case of *Polyporus hirsutus* and *Corticium laeve* was lactose as good a carbon source as D-glucose. *Peniophora incarnata* and *Cytosporina* sp. 2 could not utilise lactose at all. Lactose, a carbohydrate not normally occurring in higher plants, is generally a poor carbon source among fungi. An inability to grow on lactose has been demonstrated, e.g. in *Dipodascus aggregatus, Rhodotorula, Exobasidium, Tricholoma* and *Coprinus* species (NYMAN 1961, SUNDSTRÖM 1964, NORKRANS 1950 and FRIES 1955). Growth after a prolonged period of standstill, indicating an adaptive enzyme system, has been shown in several fungi, e.g. *Verticillium albo-atrum* (MALCA et al. 1966). In cases in which lactose was utilised much better than galactose, cf. *Polyporus hirsutus, Polyporus zonatus* and *Libertella betulina* in Tab. 1, there is evidently a high β-glucosidase activity, resulting in the liberation of glucose monomers sufficient for growth. It is also possible that the liberated glucose induces the formation of enzymes necessary for galactose assimilation. In the case of *Polyporus betulinus,* where growth occurred on galactose but not on lactose, a lack of the specific β-glucosidase may be the cause.

Inulin has been studied as an example of a fructosan. Fructosans usually do not occur in wood, but since some types of fructosan are formed by bacteria, this type of compound may occur in wood attacked by bacteria (BONNER 1950). Inulin was not very available to birch and aspen fungi, cf. Tab. 1. However, an enzyme system capable of splitting inulin into fructose monomers to an extent sufficient to allow full growth on the D-fructose thus liberated, seems to exist in *Stereum purpureum, Peniophora incarnata* and *Schizophyllum commune.* Inulin is also poorly utilised by other groups of fungi. For instance, *Ophiostoma* species grew poorly and *Coprinus* and *Exobasidium* species did not grow at all on inulin (KÄÄRIK 1960, FRIES 1955, SUNDSTRÖM 1964).

Three sugar alcohols were tested (Tab. 1). Glycerol in the form of glycerol esters is a substantial part of the neutral ethyl extractives from birch wood (PAASONEN 1966). Mannitol and sorbitol are also frequently found in higher plants. Sorbitol is especially common in rosaceous plants (BONNER 1950). The formation of sugar alcohols is not unusual among bacteria and fungi (COCHRANE 1958). Thus *Aspergillus* and *Byssochlamys* species have been reported to exude mannitol into the culture medium, indicating that sugar alcohols may be formed

Tab. 3. Relative mycelial dry weights of birch and aspen fungi grown in medium B with citric and tartaric acids as carbon sources.

Fungus	Citric acid			Tartaric acid		
	Per cent acid giving best growth	Relative dry weight of mycelium in % of growth on 1 % glucose	pH after growth	Per cent acid giving best growth	Relative dry weight of mycelium in % of growth on 1 % glucose	pH after growth
Polyporus betulinus.....	0.1	46.2	3.6	1.0	59.8	2.4
Polyporus marginatus.....	0.1	28.8	3.6	1.0	30.8	2.4
Polyporus hirsutus.....	0.1	31.2	5.3	0.5	26.8	2.7
Polyporus versicolor.....	0.1	21.2	5.9	0.5	14.8	2.7
Polyporus zonatus.....	0.5	17.8	2.9	0.5	13.2	2.7
Polyporus adustus.....	0.1	33.3	3.8	0.5	30.7	2.7
Lenzites betulina.....	0.1	14.3	5.0	0.5	11.8	2.7
Stereum hirsutum.....	0.1	15.3	4.0	0.5	15.1	2.8
Stereum purpureum.....	0.1	12.5	3.8	0.1	7.8	3.4
Corticium laeve.....	0.1	27.3	5.3	0.1	18.0	3.5
Corticium confluens.....	0.1	21.2	4.9	0.1	21.6	3.5
Peniophora incarnata.....	0.1	14.4	5.4	0.1	15.6	3.4
Schizophyllum commune..	0.1	17.3	4.1	0.1	13.0	3.5
Libertella betulina.....	0.1	26.4	—	0.1	18.0	3.6

Starting pH Citric acid: 1 % = 2.5
0.5 % = 2.8
0.1 % = 3.2

Tartaric acid: 1 % = 2.4
0.5 % = 2.6
0.1 % = 2.9

in wood by micro-organisms after invasion. Glycerol, mannitol and sorbitol yield on oxidation glyceraldehyde (or dihydroxyaceton), fructose and glucose respectively, and in these forms they should easily enter the glycolytic pathways. All of these were excellent carbon sources for the birch and aspen fungi. Growth was especially increased in *Polyporus betulinus* and *Polyporus versicolor*. From the average figures in Tab. 1 it appears that glycerol was a better carbon source than the other two alcohols tested; its accessability varied, however, between different fungi. Relatively low mycelial weights were obtained for *Stereum purpureum, Libertella betulina* and *Cytosporina* sp. 2, whereas *Polyporus betulinus* was greatly stimulated by glycerol.

Citric and tartaric acids were tested as carbon sources, mainly because the formation and exudation of organic acids is very common amongst fungi. The formation of citric acid has been demonstrated in e.g., *Mucor, Aspergillus, Fusarium, Penicillium, Merulius, Coniphora* and *Polyporus betulinus,* (COCHRANE 1958, BIRKINSHAW et al. 1940 and HENNINGSSON 1965). *Fusarium* spp have been shown to produce tartaric acid. It is thus quite probable that besides the production of small

amounts of Krebs cycle acids in the cambium and ray parenchyma, several organic acids are produced in the wood as soon as it is invaded by micro-organisms. Growth on citric and tartaric acids is shown in Tab. 3. The relative mycelial dry weights were calculated in per cent of the growth on one per cent glucose. Three concentrations of the acids were used, viz. 1.0, 0.5 and 0.1 per cent. The figures in Table 3 refer to the optimum concentration of each acid. Despite the low pH-values in some of the nutrient solutions, all the fungi were able to utilise the two acids to some extent. Impermeability at the pH needed and chelating effects on metallic ions in the media are the main obstacles preventing the effective use by fungi of many organic acids as carbon sources. For instance, *Ophiostoma* species and several *Exobasidium* species could not grow with citrate as their sole source of carbon (KÄÄRIK 1960 and SUNDSTRÖM 1964). However, once the citric acid has entered the cell it should easily be metabolised in the tricaboxylic acid cycle.

IV. Nitrogen nutrition

A. Introduction

The nitrogen nutrition of wood-destroying fungi is especially interesting, since the nitrogen content in pure wood is very low. It rarely exceeds 0.3 per cent of the dry weight of the wood (MERRILL & COWLING 1966 a). Recently performed analyses of stem wood (whole debarked disks) of *Betula pubescens* in Sweden yielded 0.08—0.14 per cent nitrogen (HOLMEN, personal communication 1967). However, in the bark (outer and inner bark together) the N content was three to eight times as high, viz. 0.43—0.63 per cent. Similarly, MERRILL & COWLING (1966 a), when analysing *Populus grandidentata,* found the N content of the bark to be three to eight times as high as that of the whole wood.

Within the wood cylinder there is also a non-uniform distribution of nitrogen. This was clearly demonstrated by MERRILL & COWLING (1966 a). The N content was highest in the cambial zones and in the pith region, and between these two regions it decreased, being at its lowest just outside the pith region. In heartwood-forming species there was usually an abrupt decrease at the heartwood limit. The decrease in N content was attributed successively to dilution, because of the deposition of secondary cell wall material; then to the death and rapid loss of cytoplasm in maturing wood fibre cells; to the elution of nitrogen compounds by the action of the transpiration stream; and finally to the death and loss of cytoplasm in the parenchyma cells. MERRILL & COWLING (1966 a) also found that early wood was richer in nitrogen than the late wood, that the correlation between parenchyma volume and N-content was good, and probably, that most of the nitrogen was not in the cell lumina.

The wood's nitrogen occurs in many forms. The cambium and the rays, for instance, contain proteins, peptides, nucleic acid and other typical cytoplasmic nitrogen compounds. The wood fibres and the dead parenchyma cells may also contain protoplasmic residues. It is also known that the young cell wall, at least, contains proteins, for instance, in certain oxidative enzymes functioning in the middle la-

mella and the primary wall. A protein rich in hydroxyproline has also been found in the primary walls (LAMPORT & NORTHCOTE, 1960).

MERRILL & COWLING (1966 a) demonstrated that various extracts from aspen wood *(Populus grandidentata)* contained aspartic acid, glutamic acid, serine, glycine, alanine, valine, leucine—isoleucine and threonine. Threonine was found in the heartwood only. In addition to the mostly protinaceous nitrogen found in the cells, nitrogen in the form of various organic compounds is found in the transpiration and assimilation streams. The amount and composition of the nitrogen compounds in these streams is far from constant and varies with tree species, part of the tree, season and time of the day (TRENDELENBURG 1955). Encrustations of special and rather complicated nitrogen compounds, e.g. various alkaloids, have been reported from several tree species.

From this it may be concluded that fungi growing in the wood must, to meet their nitrogen demand, be able to utilise a variety of nitrogen sources.

B. Results

The nitrogen nutrition of fungi attacking birch and aspen wood was studied in three separate experimental series.

a. A comparison between some inorganic and organic nitrogen sources given as the sole source of nitrogen.

b. A comparison between 19 amino acids, normally found in plant protein, each given as the sole source of nitrogen.

c. The effect on decay of artificial addition of nitrogen to the wood.

a. *Growth on inorganic and organic nitrogen.*

The results of the first series of experiments with nitrogen nutrition are shown in Tab. 4 and Fig. 2. The growth data are represented by relative growth figures calculated as being the mycelial dry weights in per cent of the mycelial dry weight obtained using the middle concentration of ammonium tartrate. By this operation all growth figures in the table can be quantitatively compared directly.

Tab. 4 clearly demonstrates that nitrogen given in the form of ammonium compounds is utilised by all fungi tested. Ammonium tartrate, ammonium sulphate and ammonium chloride were utilised with about equal efficiency at the lowest concentration (15.2 mg N per litre). Higher amounts of ammonium tartrate (76 and 380 mg N per litre) resulted in a two to fourfold increase in growth. With ammonium

Tab. 4. Relative mycelial dry weights of birch and aspen fungi grown in medium B with various nitrogen sources

Nitrogen source	Nitrogen conc. mg per litre	Polyporus betulinus	Polyporus marginatus	Polyporus hirsutus	Polyporus versicolor	Polyporus zonatus	Polyporus adustus	Lenzites betulina	Stereum hirsutum	Stereum purpureum	Corticium laeve	Corticium confluens	Peniophora incarnata	Schizophyllum commune	Libertella betulina	Cytosporina sp. 2.
$(NH_4)_2$--Tartrate	380	181	109	95	315	251	253	141	245	80						
»	76	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
»	15.2	50	57	32	31	36	33	34	28	134						
$(NH_4)_2SO_4$	380	157	118	91	76	83	55	104	122	78						
»	76	100	95	66	68	81	48	96	87	102	53	38	81	91	29	73
»	15.2	53	51	29	26	21	28	26	23	134						
NH_4Cl	380	91	87	74	70	91	57	88	111	88						
»	76	77	92	76	70	90	63	100	84	92	75	40	95	54	29	67
»	15.2	59	61	18	28	26	28	29	24	108						
KNO_3	380	*7*	*6*	*2*	*8*	*4*	54	*0*	*1*	*15*						
»	76	*11*	11	*3*	*1*	*3*	24	*7*	*2*	*17*	10	*6*	*5*	*5*	133	*12*
»	15.2	24	16	*3*	*1*	*2*	6	*7*	*1*	*9*						
NH_4NO_3	380	60	77	31	45	43	276	76	79	72						
»	76	78	73	46	47	58	90	79	60	54	64	39	55	68	172	70
»	15.2	52	35	16	13	18	25	22	13	52						
L-Glutamine	380	161	120	182	189	201	170	173	251	96						
»	76	100	93	60	56	63	52	86	63	72	114	63	68	69	100	108
»	15.2	42	36	21	24	23	15	25	18	58						
L-Glutamic acid	380	228	350	157	96	221	252	78	254	*5*						
»	76	139	115	105	85	123	89	131	60	126	154	95	79	90	93	91
»	15.2	56	43	21	36	32	28	41	24	116						
DL-Asparagine	380	44	60	420	290	258	337	179	267	158						
»	76	74	60	58	109	143	112	125	91	271	225	71	104	149	154	130
»	15.2	58	25	12	37	49	34	41	21	185						
DL-Aspartic acid	380	206	349	238	184	258	183	112	184	33						
»	76	193	151	93	204	171	97	177	60	170	97	102	72	74	53	53
»	15.2	67	63	15	21	24	30	51	18	160						
Casein hydrolyzate	380	415	280	189	263	201	211	153	228	49						
»	76	216	94	67	79	148	84	115	56	25	54	104	60	73	104	85
»	15.2	78	37	23	13	28	22	45	16	*4*						
Urea*	380	65	153	409	360	228	366	273	246	158						
»	76	44	47	93	188	147	106	76	102	118						
»	15.2	37	41	24	28	46	28	43	33	52						
No nitrogen	0	15	4	5	8	1	1	13	2	17	4	4	12	3	1	11

* filter sterilised

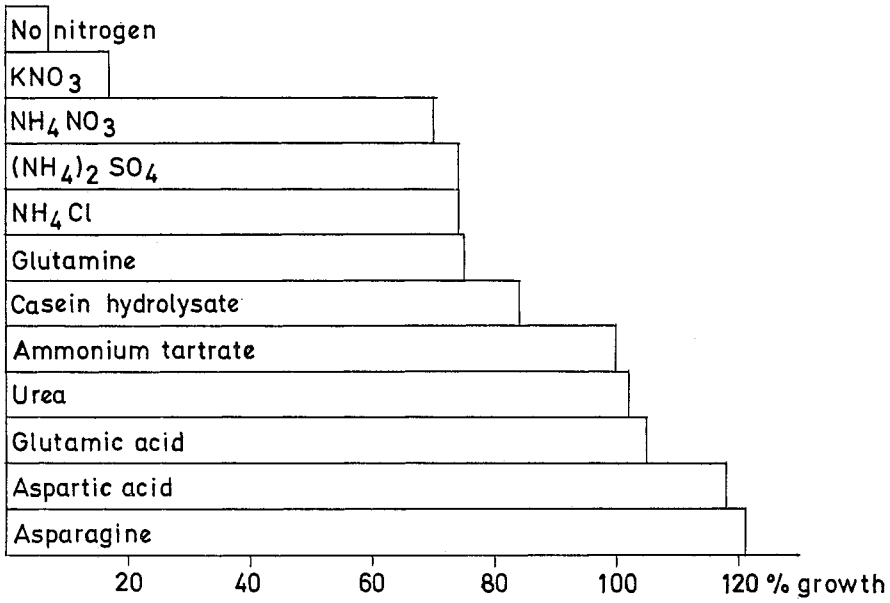


Fig. 2. The average relative mycelial growth of 15 birch and aspen fungi in medium B with varying nitrogen sources given in amounts corresponding to 76 mg N per litre. Growth on ammonium tartrate was assumed to be 100 per cent.

sulphate and ammonium chloride, however, there was only a small increase in growth, and sometimes the growth was inferior when the concentration was increased from 76 to 380 mg N per litre. This would be explicable if the rate of uptake were strongly limited by the low pH. Usually, the pH fall was more pronounced in the higher than in the lower concentrations. The unilateral uptake of cations and the exudation of organic acids in combination probably caused the fall in pH in media containing ammonium compounds. Many wood-destroying fungi, especially brown rot fungi, have been shown to exude substantial quantities of various organic acids into the medium (NORD & SCIARINI 1946, NORD & VITTUCCI 1947, RALPH & ROBERTSON 1950, BIRKINSHAW et al. 1940, HIURA & NAGATA 1955, HENNINGSSON 1965). The two brown rot fungi *Polyporus betulinus* and *Polyporus marginatus* caused a greater fall in pH in all media than did the white rot fungi.

Nitrate as the sole source of nitrogen could be utilised by only two of the fungi tested. These fungi, *Polyporus adustus* and *Libertella betulina,* also grew better than any of the other fungi on ammonium nitrate. Utilisation of nitrate led to an increase of pH in the medium.
The changes in pH of media in which *Polyporus adustus* had grown

on ammonium nitrate indicates that the ammonium ion was taken up before the nitrate ion.

Amino and amide nitrogen were good nitrogen sources for all the fungi tested (Tab. 4). On the average, asparagine and aspartic acid were the best nitrogen sources of all tested (Fig. 2), even though growth on aspartic acid as well as on glutamic acid was somewhat checked in the highest concentrations by the low pH. The amino acids were usually better utilised than the corresponding amides.

Casein hydrolysate, which should contain most of the amino acids occurring in proteins, also brought about good growth. Here, the growth response to increasing amounts of the substance was more pronounced than for any other nitrogen compound tested. *Stereum purpureum* was the only fungus which did not grow well on this source of nitrogen. Urea, which was filter-sterilised, proved to be an excellent nitrogen source—comparable, on the average, with ammonium tartrate and glutamic acid.

b. *Growth on different amino acids.*

In the second series of experiments with nitrogen nutrition, 19 different amino acids normally occurring in plant proteins were tested for their effect on growth when they were given as the sole source of nitrogen. The amino acids were supplied in amounts equivalent to 38 mg N per litre. This low nitrogen content was chosen to ensure that nitrogen was the growth-limiting factor in the experiments. The results are shown in Tab. 5 and Fig. 3.

The following amino acids could be used by all fungi tested: glycine, alanine, valine, leucine, serine, threonine, arginine, aspartic acid, glutamic acid, methionine, phenylalanine and tyrosine. The three last-mentioned acids generally caused less growth than the others. Furthermore, methionine was very poorly utilised by *Peniophora incarnata*.

Three amino acids (isoleucine, cystine and proline) were available to all except one of the fungi tested. For instance, isoleucine could not be utilised by *Polyporus betulinus* and caused only sparse growth in *Polyporus marginatus* and *Polyporus versicolor*. Cystine was not available to *Peniophora incarnata* and was poorly utilised by *Stereum purpureum* and *Libertella betulina*. Proline was unavailable to *Polyporus betulinus* and poorly utilised by *Stereum purpureum*.

Lysine, tryptophan and hydroxyproline failed to induce growth in two of the fungi tested. *Schizophyllum commune* and *Libertella betulina* did not grow on lysine. This amino acid was also poorly

Tab. 5. Relative mycelial dry weights of birch and aspen fungi grown in medium B with various amino acids as the nitrogen source.

Nitrogen source	Polyporus betulinus	Polyporus marginatus	Polyporus hirsutus	Polyporus versicolor	Polyporus zonatus	Polyporus adustus	Lenzites betulina	Stereum hirsutum	Stereum purpureum	Corticium laeve	Peniphora incarnata	Schizophyllum commune	Libertella betulina	Cytosporina sp 2.
Glycine	136	53	79	165	117	115	60	96	153	106	75	139	96	147
DL-Alamine	46	43	148	194	128	94	75	—	188	110	85	144	134	164
DL-Valine	36	36	88	90	79	95	28	90	105	39	69	86	108	—
L-Leucine	61	37	136	97	96	98	79	116	149	99	56	96	87	131
DL-Isoleucine	10	11	83	11	27	77	26	116	45	53	31	72	75	80
DL-Serine	171	54	112	156	106	87	51	134	134	91	94	146	103	135
DL-Threonine	97	54	87	99	106	83	60	92	80	53	65	108	98	82
L(+)-Lysine	22	14	59	10	8	21	11	72	11	41	16	6	*5*	22
L(+)-Arginine	147	48	114	175	127	94	86	101	153	100	70	133	106	91
DL-Aspartic acid	135	61	104	150	90	105	97	96	103	102	61	155	57	70
L-Glutamic acid	107	59	81	82	56	77	60	88	109	54	29	86	58	87
L-Cystine	34	43	50	30	30	47	33	35	15	33	*5*	58	18	35
DL-Methionine	32	23	83	90	62	87	36	87	40	58	11	100	63	74
DL-Phenylalanine	33	15	96	81	136	59	40	43	45	51	52	100	81	78
L-Tyrosine	35	74	49	23	76	66	20	17	22	41	23	32	42	52
L-Tryptophane	*12*	6	62	17	30	—	10	27	71	32	24	16	15	49
L(—)-Histidine	*4*	38	37	7	*1*	17	*5*	26	23	38	*7*	23	34	70
L(—)-Proline	*0*	31	113	70	23	27	66	37	13	127	24	53	104	168
L(—)-Hydroxyproline	*18*	16	16	6	12	20	11	36	10	122	*9*	45	31	76
Ammonium tartrate	100	100	100	100	100	100	100	100	100	100	100	100	100	100
No nitrogen	18	2	8	2	2	12	6	2	4	2	8	5	5	4

utilised by most fungi except *Polyporus hirsutus, Stereum hirsutum* and *Corticium laeve*. Tryptophan was not utilised at all by the brown rot fungi *Polyporus betulinus* and *Polyporus marginatus* and except for *Polyporus hirsutus, Stereum purpureum* and *Cytosporina* 2, was generally a poor nitrogen source. Hydroxyproline was not utilised by *Polyporus betulinus* and *Peniophora incarnata*. Very poor utilisation of this compound was shown also by *Polyporus versicolor, Lenzites betulina* and *Polyporus hirsutus,* while it was a surprisingly good nitrogen source for *Corticium laeve*.

Histidine, finally, was not at all available to four fungi, *Polyporus betulinus, Polyporus zonatus, Lenzites betulina* and *Peniophora incarnata,* and was a very poor nitrogen source for *Polyporus versicolor* and *Polyporus adustus*. Only *Cytosporina* sp. 2 utilised histidine efficiently.

Considering the individual fungi, it seems that *Polyporus betulinus* and *Peniophora incarnata* were especially sensitive to changes of the

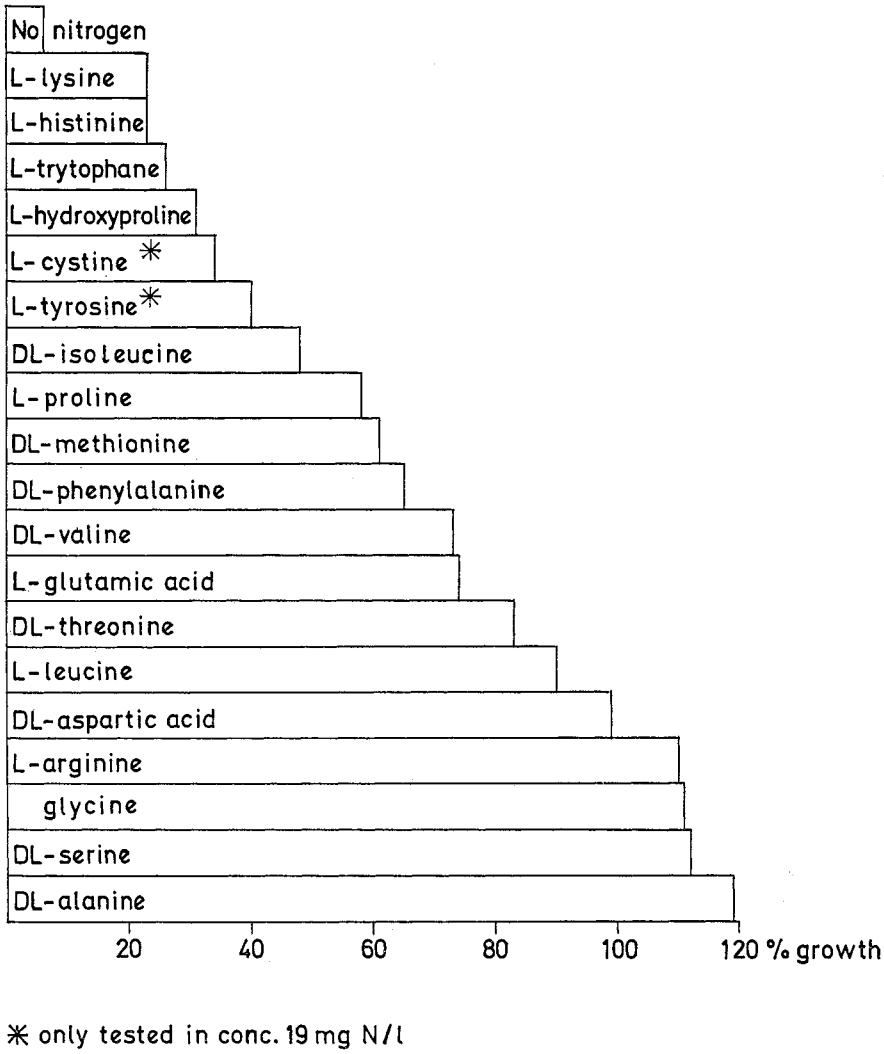


Fig. 3. The average relative mycelial growth of 14 birch and aspen fungi in medium B with varying amino acids given in amounts corresponding to 38 mg N per litre. Growth on ammonium tartrate was assumed to be 100 per cent.

amino acids. Least sensitive to such changes seemed to be *Polyporus hirsutus, Stereum purpureum* and *Cytosporina* sp. 2.

Fig. 3, which shows the average growth of all the fungi tested on the various amino acids, demonstrates that there were no clear correlations between groups of chemically related amino acids and the availability of these to the fungi tested. However, it seems that the monoamino monocarboxy acids (glycine, alanine, leucine and valine),

Tab. 6. Weight losses of blocks of birch sapwood impregnated with various nitrogen compounds and decayed by *Polyporus zonatus* and *Stereum hirsutum*

Nitrogen compound	Conc. of impregnation solution mg/l	Uptake of nitrogen in wood samples mg N/gm wood	Polyporus zonatus			Stereum hirsutum		
			Percentage weight loss after			Percentage weight loss after		
			30 days	60 days	90 days	30 days	60 days	90 days
Casein hydrolysate	1160	0.33	10.9	25.3	49.8	10.9	29.5	48.8
	580	0.19	10.4	19.8	42.9	11.4	23.6	46.2
Asparagine	720	0.38	6.7	11.5	11.8	8.2	22.0	32.6
	360	0.20	4.3	7.9	14.8	7.5	20.9	30.8
$(NH_4)_2$-tartrate	1000	0.39	4.4	7.5	12.3	3.0	18.6	29.4
	500	0.20	6.4	11.0	15.5	7.7	19.8	33.0
NH_4Cl	580	0.38	3.5	10.0	12.8	6.1	11.9	25.3
	290	0.19	5.1	7.2	12.5	7.2	17.8	27.4
No nitrogen	0	0.0	3.8	12.1	24.9	4.5	9.3	17.0

the sour amino acids (aspartic and glutamic acids) and the hydroxyaminoacids (serine, threonine) were easily assimilated. Further, it seems that the aromatic amino acids were generally not as easily assimilated as were the aliphatic acids.

c. *Effect on decay of artificial addition of nitrogen to the wood.*

In the third experimental series with nitrogen, sapwood samples of birch were impregnated with different nitrogen compounds in order to increase the nitrogen content of the wood. The impregnated wood blocks were placed in 100 ml Erlenmeyer flasks containing medium F, inoculated with *Polyporus zonatus* and *Stereum hirsutum* and incubated at room temperature for 30, 60 or 90 days.

The results shown in Tab. 6 indicate that an increased nitrogen content of the wood influences the decay rate differently. *Stereum hirsutum*'s reaction was very simple. Independently of the nitrogen compound chosen, any increase in nitrogen content resulted in an increased weight loss of the attacked wood. The increase in weight loss was especially high when nitrogen was added in the form of hydrolysed casein. *Polyporus zonatus* reacted quite differently and in a more complicated way. In the earlier stages of decay, after 30 days' incubation, the loss in weight of nitrogen-enriched samples was greater than or about equal to that of samples without additional nitrogen. How-

ever, after 60 days' and more pronouncedly after 90 days' incubation, there was a lower weight loss in nitrogen-enriched samples, except where the nitrogen compound added was casein hydrolysate. Broadly speaking, in the case of *Polyporus zonatus,* casein hydrolysate was the only nitrogen compound capable of stimulating the metobolism when this organism was growing in birch wood.

C. Discussion

Considering the experimental results shown in Tab. 4 and 5, it seems clear that the birch and aspen fungi have a wide tolerance of changes in the nitrogen composition of their substrate. They can utilise a variety of nitrogen sources.

As has been found for most *Basidiomycetes* (cf. NORKRANS 1950, FRIES 1955, JENNISON et al. 1955 and HENNINGSSON 1965) and for fungi in general (cf. KÄÄRIK 1960, MALCA et al. 1966, BREWER 1959), ammonium nitrogen in various forms was also easily utilised by the fungi found in birch and aspen wood—see Tab. 4. Failure to utilise ammonium has been found only amongst a very few lower *Phycomycetes* (COCHRANE 1958). However, most *Phycomycetes* also grow well on ammonium, e.g. *Aphanomyces astaci* (UNESTAM 1965).

Nitrate proved to be a poor source of nitrogen for most of the fungi attacking birch and aspen. An inability to utilise nitrate is especially common amongst *Basidiomycetes* (cf. NORKRANS 1950, FRIES 1955, HACKSKAYLO et al. 1954, JENNISON et al. 1955, HENNINGSSON 1965), *Saprolegniaceae* and *Blastocladiales* (NICHOLAS 1965 and COCHRANE 1958). Certain *Basidiomycetes,* however, (SUNDSTRÖM 1964) and many other groups of fungi (KÄÄRIK 1960, BREWER 1959, MALCA 1966) can utilise nitrate as their sole source of nitrogen. In media containing both ammonium and nitrate ions, preferential utilisation of ammonium seems to be the rule amongst fungi. There is even a general suppression of the nitrate assimilation, caused by the presence of ammonium ions, as was demonstrated by MORTON & MACMILLAN (1954). This suppression is caused by a feed-back mechanism in which the ammonium ions, being the end-product of nitrate reduction, inhibit the production of nitrate reductase (KINSKY 1961). The changes in the substrate's pH, where *Polyporus adustus* (a nitrate-utilising birch fungus) had grown on ammonium nitrate, indicate that this feed-back mechanism functioned also in a *Basidiomycete.*

The general availability of aspartic and glutamic acid, of their corresponding amides and of casein hydrolysate, to the birch and as-

pen-attacking fungi is in agreement with the finding that these nitrogen compounds are utilised by almost every fungus tested (COCHRANE 1958). The amino acid and amide metabolism in fungi is very similar to that in other organisms. There is, however, also evidence for their direct incorporation into proteins, without previous degradation to ammonia (NICHOLAS 1965). Specific amino acid requirements in fungi are found almost exclusively in induced mutants. An inability to utilise certain amino acids is, however, not unusual among fungi. Inhibition of growth caused by certain amino acids has also been demonstrated (cf. LINDEBERG & LINDEBERG 1964).

Wood-rotting *Basidiomycetes* have been shown to utilise most of the amino acids normally occurring in proteins (JENNISON et al. 1955); but when alanine and aspartic acid were tested, only the naturally-occurring L-forms were available (JENNISON & PERRITT 1960). As much of the nitrogen in wood occurs either as free amino acids or as bound acids in proteins and peptides, it is of importance for the wood-attacking fungi to be able to utilise amino acid nitrogen. It is interesting to note that all the amino acids found by MERRILL & COWLING (1966 a) in *Populus grandidentata* (see p. 23) could be utilised by all of the birch and aspen-attacking fungi tested in the present investigation.

When carrying out decay experiments on annual rings of *Populus grandidentata* containing different amounts of nitrogen, MERRILL & COWLING (1966 b) demonstrated that there was a good correlation between the nitrogen content of the wood and the rate of decay. As the nitrogen content is so low in the wood, this might be suspected of containing suboptimal quantities of the nutrient. If this were true, an addition to the wood of nitrogen in a suitable form should result in an increasing rate of decay. This suggestion was supported by experiments carried out by FINDLAY (1934). It is also possible to increase the rate of decay caused by soft rot fungi if nitrogen is added (DUNCAN 1960). In investigations by SCHMITZ & KAUFERT (1936) and KAUFERT & BEHR (1942), some contradictory results were obtained. From these results and the results shown in Tab. 6 it is evident that it is impossible to find a uniform pattern. The results are influenced by fungus species, wood species and by the nitrogen compound added. Organic nitrogen compounds, however, seem generally to have the most positive effect on the rate of decay. In mixed cultures moulds and bacteria seem to be more favoured than decay fungi by an increase of the nitrogen in wood (MANGENOT 1963).

Fixation of atmospheric nitrogen, which is known from certain bacteria and yeasts, has once been indicated in filamentous fungi—in,

amongst others, *Pullularia pullulans* (SCHANDERL 1942). In wood-destroying *Basidiomycetes,* however, nitrogen fixation has never been demonstrated, although this has been attempted (cf. KLINGSTRÖM & OXBJERG 1964). Considering the theoretical calculations presented by COWLING & MERRILL (1966) and MERRILL & COWLING (1966 c), the fixation of nitrogen by decay fungi, in the sporulating stage at least, might be one explanation of the problems concerning the nitrogen nutrition of wood-decaying fungi. It is also possible that other micro-organisms, like bacteria, yeasts or, for instance, the above-mentioned *Pullularia pullulans,* which have frequently been isolated from decayed wood, may be able, by their fixation of atmospheric nitrogen, to increase the nitrogen content of the wood, thus increasing the rate of wood decomposition by decay fungi.

V. Thiamine Requirements

Growth in synthetic media with or without the addition of thiamine was studied. To this end, fragments of aerial mycelia taken from malt agar cultures of the fungi were used as inocula. To avoid carrying over any material from the agar substrate to the nutrient solutions, the mycelia were removed very carefully and washed several times in sterile distilled water. Erlenmeyer flasks (100 ml) were used, each containing 20 ml of medium B from which either all vitamins or all vitamins except thiamine were excluded. The flasks were incubated in a shaker.

The results in Tab. 7 show that, except for *Libertella betulina*, all the fungi tested were more or less auxoheterotrophic for thiamine, although restricted growth was produced on the medium lacking thiamine by all the fungi, particularly by *Stereum purpureum* and the brown rot parasitic fungi *Polyporus betulinus* and *Polyporus marginatus*. Whether this was due to impurities in the nutrients or to a real partial deficiency is uncertain.

The thiamine concentrations needed for obtaining maximal growth rate in medium B were also studied. Thus the fungi were grown as stationary floating cultures in medium B with all vitamins excluded, being supplemented only with thiamine hydrochloride in varying concentrations. As inocula were used small pieces of cultures grown on medium D_2. Before inoculation the inocula were soaked for three days in sterile redistilled water.

The results showed that a thiamine concentration of 1—100 μg/l was sufficient to give the maximal growth rate on medium B. The fungi examined could be divided into three groups, as follows:

1. 1 μg thiamine per litre sufficient for maximal growth rate: *Stereum purpureum*.

2. 10 μg thiamine per litre sufficient for maximal growth rate: *Polyporus betulinus, Polyporus marginatus, Polyporus hirsutus, Polyporus versicolor, Polyporus zonatus, Polyporus adustus* and *Corticium laeve*.

3. 100 μg thiamine per litre sufficient for maximal growth rate: *Lenzites betulina* and *Stereum hirsutum*.

Tab. 7. Mycelial dry weights of birch and aspen fungi grown in medium B with and without thiamine

Fungus	Incubation time days	Dry weight of mycelium mg/flask	
		with thiamine	without thiamine
Polyporus betulinus	23	18.7	6.0
Polyporus marginatus	23	21.2	6.8
Polyporus hirsutus	14	45.3	4.4
Polyporus versicolor	16	38.5	4.0
Polyporus zonatus	14	50.8	4.8
Polyporus adustus	14	49.5	2.0
Lenzites betulina	23	36.4	4.2
Stereum hirsutum	14	72.6	4.8
Stereum purpureum	23	32.4	8.3
Corticium laeve	14	46.6	2.7
Libertella betulina	21	44.3	42.7

Growth curves for one representative fungus of each of the three groups are presented in Fig. 4.

Thiamine heterotrophy was first demonstrated in fungi in 1934 by SCHOPFER (1934) and BURGEFF (1934). Since then an increasing number of fungi—*Basidiomycetes* in particular—have been shown to exhibit heterotrophy for thiamine. Wood-destroying *Hymenomycytes* belonging to a variety of genera are thiamine heterotrophic, cf. FRIES (1938), SCHOPFER & BLUMER (1940), NOECKER (1938), ROBBINS & MA (1943), RENNERFELT (1944), YUSEF (1953) and HENNINGSSON (1965). Comprehensive information concerning vitamin requirements is given by COCHRANE (1958) and FRIES (1965).

Since thiamine, in the form of pyrophosphate, functions as the coenzyme of many enzymes, for instance decarboxylase and transketolase, the substance occurs in all living cells. Even if the number of living cells is low in wood, it is most probable that the protoplasmic residues of these cells contain thiamine sufficient to support growth of the wood-destroying fungi. And, as indicated above (p. 33), the thiamine requirements of fungi found in birch and aspen wood are extremely low.

Thiamine may also be produced and exuded into the wood by wood-inhabiting auxoautotrophic micro-organisms (bacteria, yeasts, fungi) in quantities sufficient to stimulate growth of the auxoheterotrophic fungi. Such examples were demonstrated by FRIES (1938) with bacteria and wood-destroying fungi, and in a future paper, experiments with birch and aspen micro-organisms in syncultures indicate that

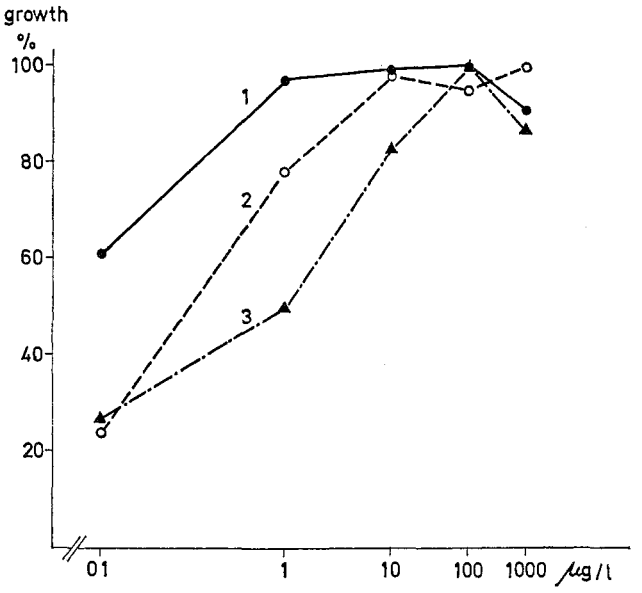


Fig. 4. Relative growth of surface cultures in medium B with varying concentrations of thiamine. The curves represent: 1. *Stereum purpureum*, 2. *Polyporus adustus* and 3. *Lenzites betulina*.

syntrophism between bacteria and fungi may occur in pulpwood of birch and aspen (HENNINGSSON 1967 c, in prep.).

Vitamin deficiency may be due to the culture conditions (conditioned deficiency), which it is of great importance to remember when generalising from results obtained in physiological experiments. Several examples have been given by SUNDSTRÖM (1964), in his investigation of the physiology of *Exobasidium*.

VI. pH and growth

When birch and aspen pulpwood are decayed under natural conditions, the pH factor probably plays a subordinate role. The pH of water extracts from such pulpwood stored for three summers showed only slight changes in the range of pH 4—5.

The influence of the substrate's pH on the radial growth of birch and aspen-attacking fungi was studied on medium D in petri dishes. pH-values from pH 1.5—8.0 in the agar medium were obtained by adding HCl or NaOH. The results are shown in Tab. 8. Optimal growth was reached at pH 6 for *Stereum purpureum, Corticium laeve* and *Polyporus marginatus* and at pH 5 for the rest of the fungi tested. The brown rot fungi *Polyporus betulinus* and *Polyporus marginatus* were especially tolerant of an acid substrate and grew substantially even at pH 1.5. On the other hand, these two fungi were more sensitive to higher pH than the other fungi. Most sensitive to an acid substrate was *Corticium laeve,* the growth of which was almost completely checked at pH 3.

It has been demonstrated by several authors that the wood-destroying fungi usually have a wide pH-tolerance in the acid region. One of the most comprehensive studies in this field was performed by WOLPERT (1924). He found that with small variations, depending on the composition of the medium, the wood-destroying fungi grew between

Tab. 8. Daily radial growth of birch and aspen fungi grown on medium D at various pH levels

Fungus	Daily growth in mm at various hydrogen ion concentrations of the substrate							
	pH 1.5	pH 2.0	pH 3.0	pH 4.0	pH 5.0	pH 6.1	pH 7.2	pH 8.3
Polyporus betulinus.....	2.8	3.8	4.6	5.5	5.8	5.5	0.2	0.0
Polyporus marginatus.....	2.1	3.8	4.5	5.1	5.3	5.6	2.0	0.0
Polyporus hirsutus.....	0.0	0.0	2.1	5.0	6.2	6.1	5.2	5.3
Polyporus versicolor.....	0.0	0.0	2.8	7.2	9.3	8.0	6.3	4.5
Polyporus zonatus.....	0.0	0.0	2.6	7.8	9.3	8.7	5.8	5.0
Polyporus adustus.....	0.0	0.0	3.0	8.5	11.3	10.6	8.0	7.8
Lenzites betulina.....	0.0	0.5	3.7	6.1	6.5	5.8	4.4	3.9
Stereum hirsutum.....	0.0	1.6	4.3	5.3	7.1	6.6	4.8	2.3
Stereum purpureum.....	0.0	0.0	1.8	8.9	9.8	11.3	10.4	9.3
Corticium laeve.....	0.0	0.0	0.3	4.4	6.4	6.8	6.2	5.5

pH 2 and pH 8. The results of the present investigation confirm the results found by WOLPERT and those found recently by MADHOSINGH (1962) and HENNINGSSON (1965) for *Polyporus versicolor, Polyporus adustus* and *Polyporus betulinus* respectively.

Decay fungi are known to acidify the substrate by their production of acids. Brown rot fungi cause a greater acidification than do the white rot fungi (BIRKINSHAW et al. 1940). Even strongly buffered media may be rapidly acidified (HENNINGSSON 1965) by growing brown rot fungi. This shift in the substrate's pH should be continuously adjusted when determining the growth at various pH levels.

VII. Temperature relations

The fungal activity at various temperatures was studied either by measuring daily radial growth in petri dishes on medium D or by calculating the average weight loss of wood samples decayed on medium E in 100 ml Erlenmeyer flasks incubated for two months. The flasks were exposed to the various temperatures immediately after inoculation. The temperature intervals were 5°C in the decay experiments. The curves for daily growth reported in Fig. 5 are syntheses of four repeated experiments where the series of temperature intervals were changed between 5, 10, 15 . . . etc., and 7.5, 12.5, 17.5 . . . etc. Growth at +2°C was also measured for some of the fungi.

The results (Fig. 5) show that the optimal temperatures for growth and decay of birch and aspen attacking fungi vary between 20—22.5°C and 32.5—35°C. The maximum temperature at which growth and decay can go on exceeds 35°C for three fungi only. All the fungi which were tested at +2°C, were able to grow at that low temperature, and some of them can probably grow at still lower temperatures. It was, however, not possible to establish that growth had occurred at -2°C, even after two months' incubation on medium D.

For several fungi the temperature for optimal radial growth seemed to be somewhat higher than that for optimal decay activity—cf. *Polyporus adustus, Polyporus versicolor* and *Polyporus zonatus.*

According to their temperature requirements for growth and decay, the tested fungi may be divided into three groups:

1. Low-temperature fungi, with optimal growth and decay activity below +25°C. To this group belong *Corticium laeve, Corticium confluens* and *Peniophora incarnata.*

2. High-temperature fungi, with optimal growth and decay activity above +30°C. *Polyporus hirsutus, Lenzites betulina* and *Schizophyllum commune* may be referred to this group.

3. Intermediate-temperature fungi, with optimal growth and decay activity between +25 and +30°C. Here the rest of the fungi tested are found.

Fig. 5 also shows that the low-temperature fungi have a comparatively low decay activity even at their optimal temperatures, and that many fungi with higher temperature optima are more active at low tem-

peratures (5—10°) than the low temperature fungi themselves. This does not necessarily indicate that the low-temperature fungi or any fungus with low decay activity is unimportant from the practical point of view. *Corticium laeve,* for instance, is perhaps the most common early species on stored birch pulpwood together with *Libertella betulina.* By their immediate and general attack on the pulpwood, these fungi may act on the wood for a longer period than other more active fungi. It is also possible that these early-invading micro-organisms prepare the wood substrate in such a way that it becomes more susceptible to attack by other fungi.

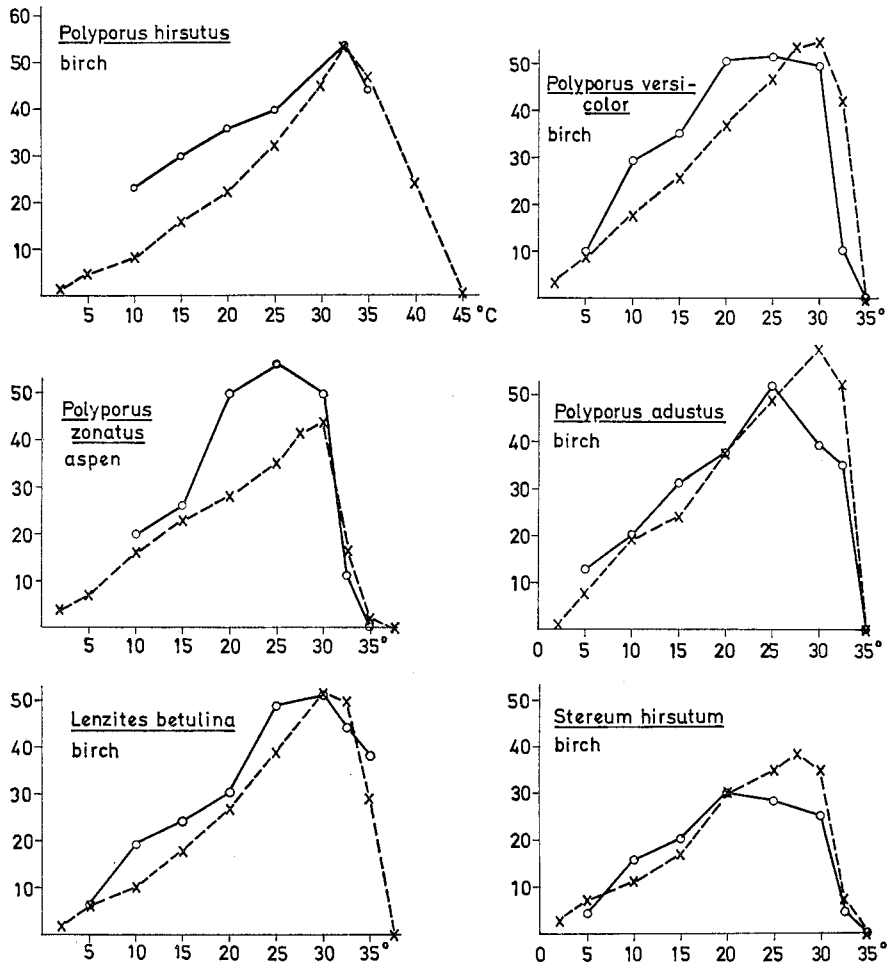
The temperature relations for growth and decay by rot fungi have been studied intensively in the past by e.g. HUMPHREY & SIGGERS (1933) and BJÖRKMAN (1946). These earlier results are summarised in the comprehensive study by CARTWRIGHT & FINDLAY (1958). The optimal and maximal temperatures for the fungi reported there are in good agreement with those of the present investigation.

Differences between various isolates of the same species as regards their temperature relations were thoroughly investigated by COWLING & KELMAN (1964) on *Fomes annosus.* There were no significant differences in temperature optima between 94 isolates, including dicaryotic as well as monocaryotic mycelia from various geographical areas. This indicates that the temperature relations for one isolate are probably representative of all isolates of the same species.

An important effect of temperature is the differentiation of the mycoflora within a log or a pile. Thus LOMAN (1962) found a correlation between the fungus flora and the extreme temperature variations in lodgepole pine logging slash. In chip piles, where the temperature in some parts can reach more than +60°C, there exists a typical microflora for each temperature zone. Decay fungi are usually found only in the outer, colder parts of the piles (NILSSON 1965, BERGMAN & NILSSON 1966). For the birch and aspen pulpwood, similar temperature effects on the distribution of the decay fungi were observed by the author. In the upper layer of the piles, where the temperatures on sunny days were high and the temperature variations were great, *Polyporus hirsutus* often predominated on birch and *Schizophyllum commune* on aspen. This is in good agreement with the results in Fig. 5, showing these two fungi to be high-temperature fungi with a wide temperature tolerance.

While much research has been done to determine the optimal temperature for growth and decay, relatively little research has been devoted to studying the activities at low temperatures. Recently, how-

ever, VON PECHMANN et al. (1964) showed that several blue stain fungi were capable of growing and producing spores even at temperatures below freezing point. However, there exists no evidence that decay fungi are active at temperatures below 0°C, even if many decay fungi show activity at temperatures just above 0°C. This was, for instance demonstrated by AMMER (1964) for *Lenzites abietinus* and *Stereum sanguinolentum* growing on spruce wood. On examination of Fig. 5, it becomes apparent that several of the birch and aspen-attacking fungi can develop at temperatures near 0°C. This indicates that the decomposition of the wood can continue throughout the year as soon as the temperature is above 0°C.



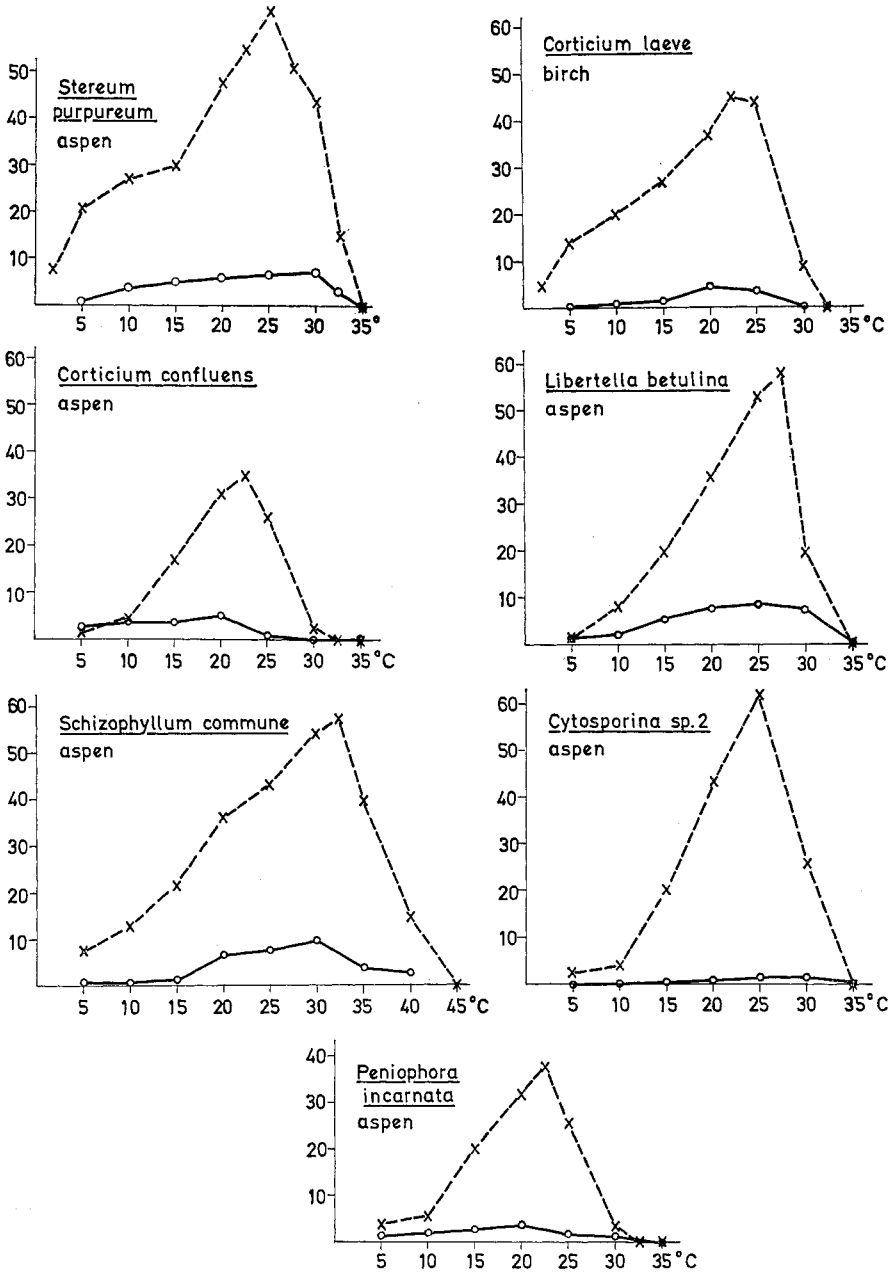


Fig. 5. Daily radial growth in mm (broken lines) and percentage weight loss of wood samples (unbroken lines) decayed in medium E at various temperatures.

VIII. Decay resistance of birch and aspen wood

It is quite clear from storage experiments with roundwood for pulping that birch is decomposed much more rapidly than aspen (BJÖRKMAN 1953, HENNINGSSON 1967 e). Some laboratory experiments were carried out to see if there existed an inherent resistance to decay in aspen wood, which could explain the field observations. Sapwood of birch and aspen was decayed in 100 ml Erlenmeyer flasks in medium F. The flasks were incubated at 20—23°C. At intervals from 15 to 150 days the weight losses were investigated. Fungi which may occur on aspen as well as birch pulpwood were used. The results are shown in Fig. 6. In the early stages *Polyporus hirsutus, Polyporus zonatus* and *Polyporus adustus* decomposed the aspen sapwood equally or even more rapidly than they did the birch sapwood. Later (above 15 per cent weight loss) the birch was decomposed much more rapidly than the aspen. *Stereum hirsutum* and *Stereum purpureum*—the former only rarely and the latter very frequently found in aspen pulpwood—both decomposed birch faster than aspen during the whole course of decay. Only *Corticium laeve* caused a greater weight loss in aspen than in birch.

When comparing the decay resistance of sapwood and heartwood of aspen, four fungi commonly found in aspen pulpwood were used. These organisms were allowed to attack the test specimens in medium E for three months at 20—23°C. The results in Tab. 9 show that heartwood was more resistant to decay than sapwood. This was especially pronounced when using *Stereum purpureum* and *Schizophyllum commune* as test organisms.

Tab. 9. Weight losses of blocks of aspen sap and heartwood decayed by various aspen fungi

Fungus	Sapwood		Heartwood	
	Weight loss %	S.E.	Weight loss %	S.E.
Stereum purpureum.....	4.4	0.4	2.5	0.3
Schizophyllum commune....	11.8	0.6	5.8	0.6
Polyporus zonatus.....	49.2	2.1	46.1	1.6
Cytosporina sp. 2.....	1.4	0.2	0.8	0.2

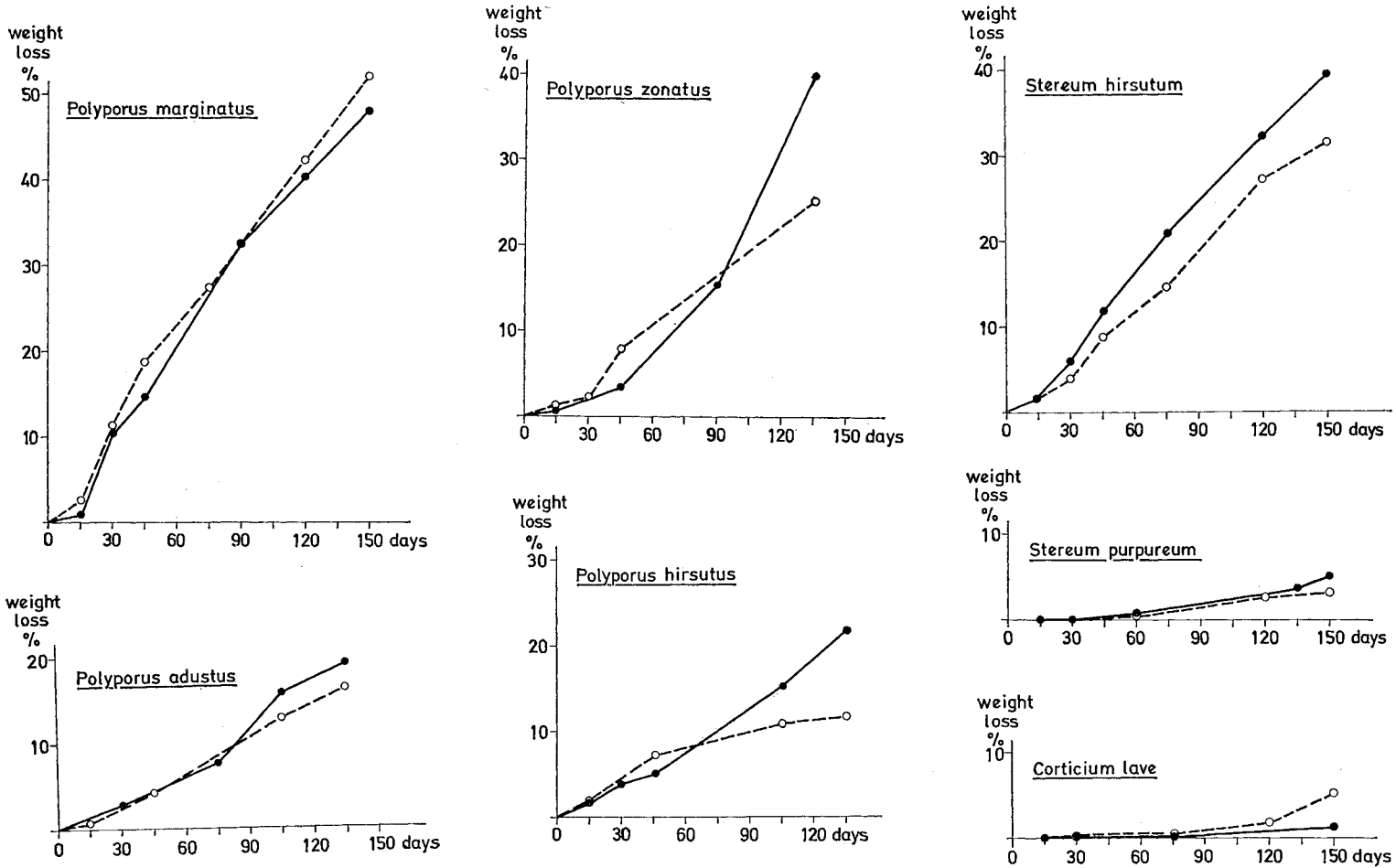


Fig. 6. Percentage weight loss of wood samples in medium F during course of decay by seven fungi occurring in both birch and aspen pulpwood. Broken lines represent aspen, and unbroken lines birch, sapwood.

Tab. 10. Relative mycelial dry weights of aspen fungi grown in medium B supplemented with water extracts from sapwood and heartwood of aspen

Fungus	Extract	Relative mycelial dry weight in per cent of growth in medium B without any wood extract added					
		Extract from sapwood			Extract from heartwood		
		10 ml/flask	1 ml/flask	0.1 ml/flask	10 ml/flask	1 ml/flask	0.1 ml/flask
Polyporus zonatus	C	150	100	92	137	105	100
	H	150	116	114	138	121	104
Stereum purpureum	C	116	95	97	127	103	99
	H	117	98	106	98	102	102
Corticium laeve	C	131	136	134	109	99	102
	H	106	107	107	112	110	112
Corticium confluens	C	175	168	162	92	92	111
	H	74	97*	141*	61	81	120
Peniophora incarnata	C	135	139	129	114	99	97
	H	86	101	95	85	106	—
Schizophyllum commune	C	121	111	100	130	112	105
	H	123	110	116	128	111	113

C = cold water extract, H = hot water extract
* = standard error exceeds 10 % of the mean value

The influence of two types of water extract on the growth of six aspen fungi was also studied. Cold water extracts were prepared by shaking 15 gm of wood meal and 300 ml redistilled water for 20 hours. Hot extracts were prepared by autoclaving 15 gm of wood meal and 300 ml redistilled water for one hour. Ten ml of the filter-sterilised extracts were added to 10 ml doubly concentrated medium B in 100 ml Erlenmeyer flasks. Stationary floating cultures were used, the incubation time being 29 days. The figures reported in Tab. 10 are the average dry weights in per cent of the dry weights in medium B.

The results show that the fungi reacted in different ways. A simple reaction was exhibited by *Polyporus zonatus* and *Schizophyllum commune,* which were stimulated by all four extracts tested. This indicates that the partial resistance of aspen heartwood to attack by *Schizophyllum commune* (Tab. 10) is probably not caused by a water-soluble factor in the wood. The growth of *Stereum purpureum* was slightly stimulated by the extracts from sapwood and by the cold water extracts from heartwood. The hot water extract from heartwood, however, did not increase growth. In *Corticium laeve,* the cold water extract from

sapwood increased growth, while the other extracts stimulated growth only slightly. *Corticium confluens* and *Peniophora incarnata* showed a similar reaction, growth being stimulated by the cold water extract from sapwood and reduced by the two hot water extracts. It may be concluded from these results that it was not possible by water extraction to remove any factors responsible for the partial decay resistance of aspen heartwood.

The results of the present investigation show that sapwood from aspen was generally more resistant to decay than sapwood from birch and that aspen heartwood is more resistant to decay by certain fungi than is aspen sapwood. This resistance can partly explain why aspen pulpwood is decomposed at a much slower rate than birch pulpwood under natural conditions. However, a decisive influence is certainly exerted by other factors, such as the composition of the microbial flora and the protective influence of the bark, which remained alive for long periods after felling (HENNINGSSON 1967 e).

IX. The influence of the moisture content on the rate of decay

By mixing various amounts of distilled water and vermiculite and embedding the wood samples there, various moisture contents of the wood were obtained after autoclaving. The moisture contents of the wood samples proved to be fairly constant for each water: vermiculite ratio. This was ascertained in several preliminary experiments.

The test samples, which were placed three in each flask just below the surface of the vermiculite, were inoculated by introducing a heavily infected wood stick into the covering layer of vermiculite. The flasks were incubated for three months at 20—23°C.

The results are shown in Tab. 11 and Fig. 7. In Tab. 11 the minimum, the average and the maximum weight losses of the six test samples decayed at each moisture content are shown, as well as the average moisture content of the samples at the end of the incubation period. The moisture contents are calculated in per cent of the oven dry weight of the samples. The following conclusions can be drawn from the results:

1. Generally, the maximum rate of decay was obtained at initial moisture contents ranging from 60 to 120 per cent in birch and 60 to 100 per cent in aspen.

2. The fungi were able to decompose wood within a wide range of moisture content—from 35 to 160 per cent.

3. The variation in weight loss of test samples within the same series was sometimes great enough to conceal the differences between the series.

4. The fungi, by their own activity, changed the original moisture content, sometimes drastically.

5. Since the moisture content of fresh birch and aspen wood ranges between 80 and 100 per cent (HENNINGSSON 1967 e), all the fungi tested should be capable of decomposing such pulpwood immediately after felling, provided that the infection can take place.

The influence of the moisture content on the rate of decay is not yet sufficiently known, although many studies have been performed in this field. The great problem in such investigations is, as pointed out in paragraph 4 above, that the fungi actively change the moisture

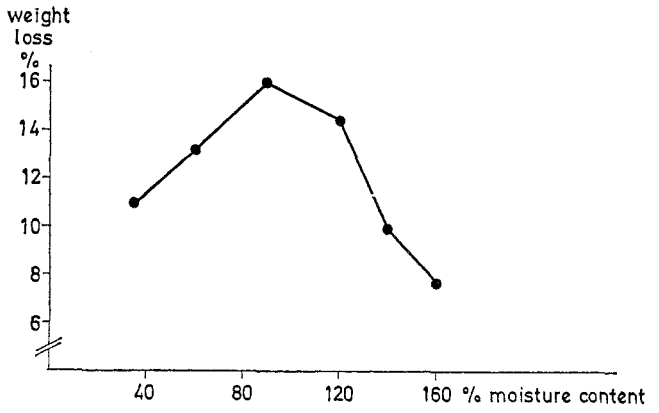


Fig. 7. Weight loss of birch wood samples with various moisture contents. The results are the average for 15 fungi (see Tab. 11).

content of the wood during the course of decay. This continuous drift in moisture is a combined result of the production of fungal respiration water, of the active uptake or emission of water by the mycelium and of changes in the hygroscopicity of the wood as decay proceeds. The change in hygroscopic behaviour is especially pronounced in wood decayed by brown rot fungi (AMMER 1963).

An extensive investigation concerning the influence of the moisture content on the activity of several common decay fungi was carried out by BJÖRKMAN (1946). Even if the methods, the fungi and the wood species in Björkman's experiments differed from those of the present investigation, the results are very similar, indicating that decay fungi can decompose wood within a wide range of moisture content. Similar results were also obtained by AMMER (1964 a) although he used still other methods and fungi. Ammer found that decomposition occurred between 30—31 per cent and 180 to 210 per cent moisture content. In another publication, AMMER (1964 b) demonstrated elegantly that the moisture content not only influences the rate of decomposition but also its response to low temperatures.

Tab. 11. Weight losses of blocks of birch and aspen sapwood decayed by birch and aspen fungi at various moisture contents of the wood

Fungus	Weight loss of birch sapwood at various moisture contents					
	35 %	60 %	90 %	120 %	140 %	160 %
Polyporus betulinus	14.1—*16.1*—19.0 (64)	19.9—*23.7*—27.0 (96)	25.0—*31.1*—37.9 (178)	23.6—*27.5*—30.1 (164)	1.6—*2.1*—2.5 (133)	8.9—*9.1*—9.3 (116)
Polyporus marginatus	26.1—*28.1*—30.0 (76)	24.6—*27.4*—30.4 (145)	29.1—*31.8*—33.3 (161)	27.5—*31.5*—37.9 (181)	26.7—*31.3*—40.2 (172)	24.6—*25.7*—27.0 (184)
Polyporus hirsutus	10.9—*13.5*—17.9 (47)	14.4—*16.8*—21.0 (103)	11.3—*13.2*—16.2 (122)	10.4—*19.2*—25.9 (170)	11.3—*12.8*—13.6 (162)	15.0—*16.5*—18.0 (146)
Polyporus versicolor	17.8—25.0—33.3 (44)	27.5—30.1—31.6 (68)	22.6—28.3—34.7 (115)	19.4—32.8—40.4 (183)	19.8—26.7—32.4 (125)	8.3—9.3—10.3 (153)
Polyporus zonatus	16.0—*20.2*—28.4 (45)	34.5—*35.2*—35.6 (68)	31.1—*44.7*—54.0 (93)	34.0—*41.0*—51.3 (171)	12.3—*14.1*—17.4 (163)	— —
Polyporus adustus	10.2—*11.5*—13.5 (44)	8.3—*12.8*—16.1 (85)	7.4—*14.1*—24.5 (134)	9.8—*14.4*—16.7 (149)	12.8—*14.0*—15.2 (171)	8.2—*9.2*—10.3 (161)
Lenzites betulina	17.3—*18.7*—20.7 (46)	10.5—*13.9*—16.8 (98)	18.4—*21.1*—24.5 (135)	6.9—*12.5*—21.8 (154)	5.1—*6.8*—8.2 (142)	5.8—7.2—8.8 (153)
Stereum hirsutum	20.9—*23.4*—27.6 (81)	25.0—*26.2*—28.6 (110)	31.8—*35.3*—40.4 (193)	20.3—*22.7*—26.1 (177)	19.3—*22.4*—25.9 (174)	15.2—*17.9*—20.2 (158)
Stereum purpureum	0.7—*1.4*—2.6 (41)	1.7—*3.1*—3.9 (76)	0.8—*4.5*—9.8 (94)	1.9—*2.7*—3.7 (128)	2.1—*2.6*—2.9 (141)	2.5—*2.7*—3.0 (145)
Corticium laeve	1.0—*2.1*—3.2 (58)	1.7—*1.8*—1.9 (90)	1.7—*1.8*—2.0 (120)	0.8—*1.6*—2.0 (137)	1.7—*2.3*—3.2 (138)	1.6—*2.0*—2.5 (140)
Corticium confluens	0.8—*2.0*—2.9 (47)	0.8—*1.2*—2.0 (85)	1.8—*1.9*—2.0 (92)	1.6—*1.7*—1.8 (118)	1.5—*2.0*—2.4 (132)	1.7—*2.1*—2.5 (145)
Peniophora incarnata	1.5—*1.7*—2.1 (39)	2.1—*4.0*—6.7 (70)	1.6—*3.3*—5.6 (88)	1.7—*1.8*—1.9 (142)	1.5—*2.1*—2.8 (142)	1.8—*1.9*—2.1 (143)
Schizophyllum commune	2.0—*2.6*—3.7 (46)	0.9—*2.5*—3.9 (76)	2.4—*2.8*—3.4 (90)	0.9—*2.1*—3.3 (123)	2.2—*2.6*—2.9 (142)	1.5—*1.9*—2.5 (139)
Daedalia unicolor	4.1—*5.5*—7.5 (43)	5.3—*7.8*—9.2 (90)	11.9—*18.4*—23.6 (143)	10.1—*15.3*—19.5 (151)	9.2—*11.7*—14.3 (153)	5.5—*7.9*—11.3 (143)
Libertella betulina	1.7—*1.8*—1.8 (39)	2.5—*3.3*—3.8 (76)	1.8—*2.2*—2.4 (109)	1.8—*2.2*—2.8 (117)	2.3—*2.5*—2.9 (127)	0.8—*0.9*—0.9 (142)
Cytosporina sp 2						

Fungus	Weight loss of aspen sapwood at various moisture contents					
	40 %	60 %	80 %	100 %	120 %	160 %
Polyporus marginatus	14.6—*18.6*—22.6 (64)	17.1—*22.9*—27.6 (117)	22.0—*24.0*—26.9 (113)	25.5—*28.8*—23.7 (172)	12.0—*25.0*—36.3 (183)	21.8—*25.1*—*28.4* (178)
Polyporus hirsutus	2.9—*8.7*—18.5 (48)	—*15.1*— (82)	5.0—*8.4*—11.3 (82)	5.7—*6.3*—7.1 (119)	8.1—*8.5*—8.8 (131)	6.4—*6.9*—7.3 (126)
Polyporus versicolor	2.1—*9.4*—19.4 (40)	9.1—*18.1*—27.1 (71)	17.0—*17.6*—18.2 (93)	13.4—*14.4*—15.6 (124)	14.2—*14.5*—15.1 (123)	13.3—*15.0*—18.2 (177)
Polyporus zonatus	9.7—*12.7*—15.8 (46)	22.9—*27.7*—30.2 (80)	27.2—*28.2*—29.1 (124)	23.2—*27.5*—29.8 (154)	18.3—*20.5*—22.6 (147)	17.2—*20.0*—21.4 (185)
Polyporus adustus	4.9—*7.2*—9.8 (43)	4.6—*7.1*—8.5 (68)	5.5—*10.8*—15.1 (91)	5.1—*7.5*—9.9 (132)	3.9—*5.5*—7.4 (146)	4.7—*4.9*—5.0 (131)
Stereum hirsutum	11.3—*12.4*—13.6 (62)	12.2—*12.4*—*12.8* (83)	11.8—*13.0*—14.3 (95)	14.0—*15.2*—16.7 (141)	8.9—*14.7*—19.4 (138)	9.3—*11.7*—14.0 (136)
Stereum purpureum	1.0—*1.6*—2.0 (37)	2.1—*2.2*—2.4 (64)	1.1—*1.6*—1.8 (76)	2.3—*2.5*—3.0 (110)	0.9—*1.2*—1.7 (99)	0.0—*0.3*—1.0 (128)
Corticium laeve	0.9—*1.0*—1.2 (35)	1.0—*1.0*—1.1 (64)	1.2—*1.7*—2.0 (77)	2.0—*2.6*—3.1 (108)	0.0—*0.3*—0.9 (106)	0.0—*0.0*—0.0 (174)
Corticium confluens	2.1—*2.3*—2.4 (37)	0.8—*1.3*—2.1 (74)	0.9—*1.3*—2.0 (71)	1.1—*1.9*—2.3 (91)	0.9—*0.9*—1.0 (102)	0.0—*0.0*—0.0 (122)
Peniophora incarnata	0.9—*1.6*—2.0 (35)	1.0—*1.7*—2.0 (57)	0.7—*2.2*—3.4 (76)	1.1—*1.4*—1.9 (102)	0.0—*0.4*—1.1 (104)	*0.0*—*0.0*—0.0 (103)
Schizophyllum commune	3.0—*3.2*—3.6 (49)		1.7—*2.3*—2.9 (65)	1.9—*2.0*—2.1 (105)	1.1—*1.4*—1.9 (146)	1.7—*1.8*—2.0 (117)
Daedalia unicolor	6.1—*6.8*—8.3 (41)	7.9—*9.6*—10.7 (63)	5.1—*5.9*—6.6 (75)	5.5—*6.0*—6.4 (102)	4.1—*6.0*—7.4 (123)	5.4—*5.8*—6.4 (118)
Cytosporina sp 2	0.0—*0.7*—1.0 (36)	0.9—*1.2*—*1.8* (59)	1.1—*1.3*—1.8 (72)	0.9—*1.4*—2.3 (98)	0.0—*0.6*—1.0 (107)	—*0.2*— (114)

Summary

The general physiological requirements of decay fungi normally occurring in pulpwood of birch and aspen have been described. Thorough studies have *inter alia* been performed on the ability of the fungi to utilise carbohydrates occurring in the wood. It was established that with the exception of D-galactose and D-arabinose, these carbon sources were assimilated without significant difficulties. It was also shown that the birch and aspen-attacking fungi could metabolise certain polysaccharides, sugar alcohols, and organic acids, which are occasionally produced by other wood-inhabiting microbes.

As the very small nitrogen supply in pure wood is regarded as being a limiting factor for the activity of wood-destroying fungi, much importance has been attached to an analysis of the ability to utilise various nitrogen compounds. With the exception of nitrate, which was useable only by two of the fungi tested, most of the nitrogen compounds occurring in birch and aspen wood were assimilated. Increasing the amount of nitrogen in the wood by impregnating it with various nitrogenous compounds resulted only occasionally in increased wood decomposition.

Of the fungi tested, all except *Libertella betulina* were heterotrophic for thiamine. About 1—100 μg per litre of thiamine in the nutrient solution was sufficient for ensuring the maximal growth rate.

Optimal growth generally took place at pH 5—6. Brown rot fungi were able to grew even at substrate pH as low as 1.5, while growth of the white rot fungi stopped at pH 2—3. On the other hand, the white rot fungi tolerated a higher substrate pH than did the brown rot fungi.

Most of the fungi tested had their optimal growth and decay activity at a temperature of 25—30°C. However, three fungi had their temperature optima above +30°C, and three fungi had temperature optima below +25°C. All the fungi tested grew at +2 but not at —2°C. The fungi were able to decompose wood with moisture contents ranging from 35 to 160 per cent. The optimal moisture contents varied between 60 and 120 per cent.

Generally, the aspen wood was somewhat more resistant to attack by these fungi than was the birch wood. Furthermore, the heartwood of aspen was more resistant than was the sapwood. The factors determining the higher resistance of the heartwood were not extractable in water.

REFERENCES

AHLGREN, E., ERIKSSON, K-E. and VESTERBERG, O.: Characterization of cellulases and related enzymes by isoelectric focusing gel filtration and zone electrophoresis. I. Studies on Aspergillus enzymes — Acta Chem. Scand., 1967 (in press).

AMMER, U.: Untersuchungen über die Sorption pilzbefallenen Holzes. — Holz als Roh.-u. Werkstoff, 21, 1963.

— Über die Holzzerstörung durch Rotstreife-Pilze im Bereich niedriger Temperaturen. — Angew. Botanik, XXXVIII: 5, 1964 a.

— Über den Zusammenhang zwischen Holzfeuchtigkeit und Holzzerstörung durch Pilze. — Holz als Roh- u. Werkstoff, 22, 1964 b.

ASCHAN, K. and NORKRANS, B.: A study in the cellulolytic variation for wildtypes and mutants of *Collybia velutipes*. I.—Physiol. Plant., 6, 1953.

BERGMAN, Ö. and NILSSON, T.: On outside storage of pine chips at Lövholmens paper mill. — R. Coll. For., Dep. For. Prod., Res. Notes R 53, Stockholm 1965.

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.

BJÖRKMAN, E.: Om lagringsröta i massavedgårdar och dess förebyggande. — Medd Statens Skogsforskn., 35: 1, 1946.

— The occurrence and significance of storage decay in birch and aspen wood with special reference to experimental preventie measures. — Skogshögskolans Skr., 16, 1953.

BLUMENTHAL, H. J.: Carbohydrate Metabolism. I. Glycolysis — The Fungi, vol. I, New York, 1965.

BONNER, J.: Plant Biochemistry — New York 1950.

BOSE, R. G.: A modified cellulosic medium for the isolation of cellulotic fungi from infected materials and soils. — Nature, 198, 505, 1963.

BREWER, D.: Studies on slime accumulations in pulp and paper mills. II. Physiological studies of *Phialophora fastigiata* and *P. riccardsis*. — Can. J. Bot., 37: 3, 1959.

BURGEFF, H.: Pflanzliche Avitaminose und ihre Behebung durch Vitaminzufuhr. Vorläufige Mitteilung. — Ber. Deut. Botan. Ges., 52, 1934.

CAMPBELL, W. G.: The chemistry of the white rots of wood. I. The effect on wood substance of *Polystictus versicolor* (Linn.) Fr. — Biochem. J., 24, 1930.

— The chemistry of the white rots of wood. II. The effect on wood substance of *Armillaria mellea* (Vahl.) Fr., *Polyporus hispidus* (Bull) Fr. and *Stereum hirsutum* Fr. — Biochem. J., 25, 1931.

— The chemistry of the white rots of wood. III. The effect on wood substance of *Ganoderma applanatum* (Pers.) Pat., *Fomes fomentarius* (Linn.) Fr., *Polyporus adustus* (Willd.) Fr., *Pleurotus ostreatus* (Jacq.) Fr., *Armillaria mellea* (Vahl.) Fr., *Trametes pini* (Brot.) Fr., and *Polystictus abietinus* (Dicks.) Fr. — Biochem. J., 26, 1932.

CARTWRIGHT, K. St, G and FINDLAY, W. P. K.: Decay of timber and its prevention. — London 1958.

COCHRANE, V. W.: Physiology of Fungi. — New York, 1958.

COWLING, E. B.: Comparative biochemistry of the decay of sweetgum sapwood by the white-rot and brown-rot fungi. — U.S. Dep. Agric. Tech. Bull., 1258, 1961.

— Structural features of cellulose that influence its susceptibility to enzymatic hydrolysis. — Advances in Enzymatic Hydrolysis of Cellulose and Related Materials, New York, 1963.

— and KELMAN, A.: Influence of temperature on growth of *Fomes annosus* isolates. — Phytopathology, 54, 1964.

— and MERRILL, W.: Nitrogen in wood and its role in wood deterioration. — Can. J. Bot., 44, 1966.

DAVIES, D. D., GIOVANELLI, J. and REES, T. A.: Plant Biochemistry — Oxford, 1964.

DAY, W. C., PELCZAR, M. J. and GOTTLIEB, S.: The biological degradation of lignin. I. Utilization of lignin by fungi. — Arch. Biochem. Biophys. 23, 1949.

DIETRICHS, H. H.: Das Verhalten von Kohlenhydraten bei der Holzverkernung. — Holzforschung, 17: 2, 1963.

DUNCAN, C. G.: Wood-attacking capacities and physiology of soft-rot fungi. — U.S. Forest Prod. Lab. Rept. No. 2173, 1960.
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., 48, 1934.
FRIES, L.: Studies in the physiology of Coprinus. I. Growth substance, nitrogen and carbon requirements. — Svensk Bot. Tidskr., 49: 4, 1955.
FRIES, N.: Über die Bedeutung von Wuchsstoffen für das Wachstum verschiedener Pilze. — Symb. Bot. Upsaliensis, III: 2, 1938.
— Vitamins and other organic growth factors. — The Fungi, vol. I., New York 1965.
FUCHS, A., JOBSEN, J. A. and WOUTS, W. M.: Arabanases in phytopathogenic Fungi. — Nature, 206: 714, 1965.
GAGNON, C.: Growth of *Ceratocystis ulmi* (Buisman) C. Moreau in liquid culture media. — Can. J. Bot., 39, 1961.
GARREN, K. H.: Studies on *Polyporus abietinus*. II. The utilization of cellulose and lignin by the fungus. — Phytopathology, 28, 1938.
GOKSØYR, J.: Studies on the metabolism of *Merulius lacrymans* (Jacq) Fr. I. Carbohydrate consumption, respiration and acid production in surface cultures. — Physiol. Plant., II, 1958.
GUSTAFSSON, C., SUNDMAN, J.: PETTERSSON, S. and LIND, T.: The carbohydrates in some species of wood — Papper och Trä, 33: 10, 1951.
HACKSKAYLO, J., LILLY, V. G. and BARNETT, H. L.: Growth of Fungi on three sources of nitrogen. — Mycologia, 46, 1954.
HENNINGSSON, B.: Studies on fungal decomposition of pine, spruce and birch pulpwood. — Medd. Statens Skogsforskn. Inst., 52: 3, 1962.
— Physiology and decay activity of the birch conk fungus *Polyporus betulinus* (Bull.) Fr. — Studia Forestalia Suecica, 34, 1965.
— Interactions between microorganisms found in birch and aspen pulpwood. — Studia Forestalia Suecica, 53, 1967 c.
— Microbial decomposition of unpeeled birch and aspen pulpwood during storage. — Studia Forestalia Suecica, 54, 1967 e.
HIURA, M. and NAGATA, Y.: Studies on the metabolic products of *Corticium centrifugum* (Lev.) Bres. Part 1. On the organic acids. — Research Bull. Fac. Agr. Gifu Univ., 3, 1954.
HOLMEN, H.: Personal communications, 1967.
HUMPREY, C. J. and SIGGERS, P. V.: Temperature relations of wood - destroying fungi. — J. Agric. Res., 47, 1933.
JENNISON, M. W., NEWCOMB, M. D. and HENDERSON, R.: Physiology of the woodrotting Basidiomycetes. I. Growth and nutrition in submerged culture in synthetic media. — Mycologia, XLVII: 3, 1955.
— and PERRITT, A. M.: Physiology of wood-rotting Basidiomycetes. III Studies on the utilization of optical isomers of amino acids. — Mycologia 52, 1960.
JOHANSSON, M.: A comparison between the cellulolytic activity of white and brown rot fungi. I. The activity on insoluble cellulose. — Physiol. Plant., 19, 1966.
KÄÄRIK, A.: Growth and sporulation of *Ophiostoma*. — Symb. Bot. Upsaliensis XVI: 3, 1960.
— Reference collection of pure cultures of wood-destroying and other fungi. — R. Coll. For., Dep. For. Prod., Res. Notes R 44, Stockholm 1963.
KAUFERT, F. H. and BEHR, E. A.: Susceptibility of wood to decay. Effect of urea and other nitrogen compounds. — Ind. Eng. Chem., 34, 1942.
KINSKY, S. C.: Induction and repression of nitrate reductase in *Neurospora crassa* — J. Bacteriol., 82, 1961.
KLINGSTRÖM, A. and OXBJERG, E.: Nitrogen source in fungal decomposition of wood. — Nature, 197: 97, 1963.
KUBIAK, M. und KERNER, G.: Die Veränderungen einiger physikalischen Eigenschaften, der Druckfestigkeit und der chemischen Zusammensetzung des Buchenholzes im Anfangsstadium des Holzabbaues durch *Coniophora cerebella* Pers. und *Stereum hirsutum* Willd. — Drev. Vyskum, 4, 1963.
LAMPORT, D. T. A. and NORTHCOTE, D. N.: Hydroxyproline in primary cell walls of higher plants. — Nature, 188: 665, 1960.
LARSSON, S. E. and SELLEBY, L.: Aspen Wood Constituents — Svensk Papperstidn., 63: 18, 1960.

LINDBERG, B. and SELLEBY, L.: Birch Wood Constituents. Part. 1. — Acta Chem. Scand., 12, 1958.

LINDBERG, M.: Galactose as a carbon source for the growth of *Ophiostoma multiannulatum*. — Physiol. Plant., 16: 3, 1963.

LINDEBERG, G. and LINDEBERG, M.: The effect of pyridoxine and amino acids on the growth of *Marasmius perforans* Fr. — Arch. Mikrobiol., 49, 1964.

LOMAN, A. A.: The influence of temperature on the location and development of decay fungi in lodgepole pine logging slash. — Can. J. Bot., 40, 1962.

MADOSINGH, C.: pH and decay studies on wood-inhabiting organisms. — Can. Plant. Dis. Survey, 42: 3, 1962.

MALCA, I., ERWIN, D. C., MOJE, W. and JONES, B.: Effect of pH and carbon and nitrogen sources on the growth of *Verticillium albo-atrum*. — Phytopathology, 56, 1966.

MANGENOT, F.: Influence de quelques sources de carbone et d'azote sur la manifestation de pourritures "blanches" en présence de populations du sol. — Holzzerstörung durch Pilze, Berlin 1963.

MEIER, H.: Chemical and morphological aspects of the fine structure of wood. — Pure Appl. Chem., 5, 1962.

MERRILL, W. and COWLING, E. B.: Role of nitrogen in wood deterioration: Amounts and distribution of nitrogen in tree stems. — Can. J. Bot., 44, 1966 a.

— and COWLING, E. B.: Role of nitrogen in wood deterioration. IV. Relationship of natural variation in nitrogen content of wood to its susceptibility to decay. — Phytopathology, 56: 11, 1966 b.

— and COWLING, E. B.: Role of nitrogen in wood deterioration: Amount and distribution of nitrogen in fungi. — Phytopathology, 56: 9, 1966 c.

MORTON, A. G. and MACMILLAN, A.: The assimilation of nitrogen from ammonium salts and nitrate by fungi. — Journ. Exptl. Botany, 5, 1954.

NICHOLAS, D. J. D.: Utilization of inorganic nitroganic nitrogen compounds and amino acids by fungi. — The Fungi, vol. I., New York 1965.

NILSSON, T.: Mikroorganismer i flisstackar. — Svensk Papperstidn., 68: 15, 1965.

NOECKER, N. L.: Vitamine B_1 in the nutrition of four species of wood-destroying fungi. — Am. J. Botany, 25, 1938.

NORD, F. F., and SCIARINI, L. J.: On the mechanism of enzyme action. Part 27. The action of certain wood-destroying fungi on glucose, xylose, raffinose and cellulose. — Arch. Biochem., 9, 1946.

— and VITUCCI, 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.

NORKRANS, B.: Studies in growth and cellulolytic enzymes of *Tricholoma*. — Symb. Bot. Upsaliensis, XI: 1, 1950.

— Influence of cellulolytic enzymes from Hymenomycetes on cellulose preparations of different crystallinity. — Physiol. Plant., 3, 1950.

— On cellulose and β-glucoside-splitting activity in enzyme preparations from some Hymenomycetes. — Marine Boring and Fouling Organisms, Seattle, 1959.

— and RÅNBY, B. G.: Studies of the enzymatic degradation of cellulose. — Physiol. Plant., 9, 1956.

NYMAN, B.: Physiological studies on fungi isolated from slime flux. — Svensk. Bot. Tidskr., 55: 1, 1961.

PAASONEN, P.: On the non-volatile ethyl ether extractives of birch wood and the changes in composition effected by aging and sulphate pulping. — Helsinki 1966.

VON PECHMANN, H., GRAESSLE, E. and WUTZ, A.: Untersuchungen über Bläuepilze an Kiefernholz. — Fortswiss. Cbl., 83, 1964.

PETTERSSON, G., COWLING, E. B. and PORATH, J.: Studies on cellulolytic enzymes. I. Isolation of a low-molecular-weight cellulase from *Polyporus versicolor*. — Biochem. Biophys. Acta, 67, 1963.

— and PORATH, J.: Studies on cellulolytic enzymes. I. Multiplicity of the cellulolytic enzymes of *Polyporus versicolor*. — Biochem. Biophys. Acta, 67, 1963.

RALPH, B. J. and ROBERTSON, A.: The chemistry of fungi, Part XIV. 2: 4: 5 — Trihydroxyphenylglyoxylic acid from *Polyporus tumulosus* Cooke. — J. Chem. Soc., 4, 1950.

RENNERFELT, E.: Die Entwicklung von *Fomes annosus* Fr. bei Zusatz von Aneurin und verschiedenen Extrakten. — Svensk Bot. Tidskr., 38, 1944.

ROBBINS, W. J. and MA, R.: The relation of certain fungi to thiamine. — Bull. Torrey Bot. Club, 70, 1943.
SCHANDERL, H.: Der elementare Luftstickstoff im Stickstoffhaushalt der Hefe. — Wschr. Brau., LIX: 13, 1942.
SCHEFFER, T. C.: Progressive effects of *Polyporus versicolor* on the physical and chemical properties of red gum sapwood. — U.S. Dep. Agric. Tech. Bull., 527, 1936.
SCHMITZ, H. and KAUFERT, F.: The effect of certain nitrogenous compounds on the rate of decay of wood. — Am J. Botany, 23, 1936.
SCHOPFER, W. H.: Versuche über die Wirkung von reinen kristallierten Vitaminen B auf *Phycomyces*. — Deut. Botan. Ges., 52, 1934.
— and BLUMER, S.: Recherches sur la repartition de l'hétérotrophie par rapport à l'aneurine chez les champignons. — Arch. Microbiol., 11, 1940.
SEIFERT, K.: Chemischer Abbau der Buchenholz-Zellwand durch den Weissfäulepilz *Polystictus versicolor* (Linn.) Fr. — Holz als Roh- u. Werkstoff, 24, 1966.
SELLEBY, L.: Birch wood constituents. Part 2. The ether extract. — Svensk Pappers-tidn., 63: 4, 1960.
SOMOGYI, M.: Notes on sugar determination. — J. Biol. Chem.,195, 1952.
STAHL, E.: Dünnschicht-Chromatographie — Berlin, 1962.
SUNDSTRÖM, K-R.: Studies of the physiology, morphology and serology of *Exobasidium* — Symb. Bot. Upsaliensis, XVIII: 3, 1964.
TIMELL, T. E.: Wood hemicelluloses: Part. I — Advances in Carbohydrate Chemistry, Vol. 19, 1964.
TRENDELENBURG, R./MAYER-WEGELIN, H.: Das Holz als Rohstoff. — München 1955.
UNESTAM, T.: Studies on the crayfish plauge fungus *Aphanomyces astaci*. I. Some factors affecting growth in vitro. — Physiol. Plant., 18: 2, 1965.
VAN VLIET, W. F.: The enzymatic oxidation of lignin. — Biochim. Biophys. Acta, 15, 1955.
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.
YUSEF, H. M.: The requirements of some Hymenomycetes for essential metabolites. — Bull. Torrey. Bot. Club, 80: 1, 1953.

Sammanfattning

Fysiologi hos svampar som angriper björk- och aspmassaved

De allmänna fysiologiska kraven hos ett antal rötsvampar, vilka under normala betingelser angriper massaved av björk och asp, har beskrivits. Bl. a. har svamparnas förmåga att metaboliskt kunna tillgodogöra sig i veden förekommande kolkällor ingående undersökts. Därvid har framkommit, att med undantag för D-galaktos och D-arabinos, assimilerades dessa kolkällor utan större svårigheter. Det har också visat sig, att de undersökta björk- och aspsvamparna kunde metabolisera vissa polysaccharider, sockeralkoholer och organiska syror, vilka stundom produceras av andra vedbeboende mikrober.

Eftersom den ringa kvävetillgången i ren ved anses utgöra en begränsande faktor för de vedförstörande svamparnas aktivitet, har stor vikt lagts vid en analys av förmågan att tillgodogöra sig olika kväveföreningar. Med undantag för nitratkväve, som endast kunde utnyttjas av två av de testade svamparna, assimilerades med lätthet de flesta i björk- och aspved förekommande kväveföreningarna. En ökning av vedens kvävehalt genom tillsats av olika kväveföreningar resulterade endast i vissa fall i en ökad nedbrytning.

Av de undersökta svamparna var alla utom *Libertella betulina* thiaminheterotrofa. En thiaminmängd av 1—100 μg/l i närlösningen var tillräcklig för att ge maximal tillväxthastighet.

Optimal tillväxt erhölls i allmänhet vid pH 5—6. Brunrötesvamparna kunde växa ännu vid ett så lågt substrat-pH som 1.5, medan vitrötesvamparna upphörde att växa vid pH 2—3. Å andra sidan tolererade vitrötesvamparna ett högre substrat-pH än brunrötesvamparna.

De flesta svamparna hade optimal tillväxt och rötförmåga vid en temperatur av 25—30°C. Dock hade tre svampar sina temperaturoptima över +30°C och tre under +25°C. Alla undersökta svampar kunde växa vid +2°C men inte vid —2°C.

Svamparna förmådde angripa och nedbryta ved med fuktkvoter varierande mellan 35 och 160 %. De optimala fuktkvoterna varierade för olika svamparter mellan 60 och 120 %.

I allmänhet var aspveden något resistentare än björkveden mot angrepp av dessa svampar. Dessutom var aspens kärna motståndskraftigare än dess splintdel. De faktorer, som bestämmer kärnvedens högre resistens, kunde inte extraheras i vatten.