FUNGAL ENZYMIC DEGRADATION OF TIMBER WITH PARTICULAR REFERENCE TO THE HEMICELLULOSES

A thesis presented in part fulfilment of the requirements for the degree of Doctor of Philosophy of the University of London

By

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ABSTRACT

The methods whereby selected species of wood-destroying fungi operate were investigated using two approaches, (i) biochemical techniques and (ii) the scanning electron microscope. Twenty strains of fungus, comprising four different species each representing a different type of wood-decaying action were used. Chaetomium globosum a soft rotter (9 strains), Poria monticola a brown rotter (8 strains), Phialophora fastigiata a stainer/soft rot (2 strains) and Polystictus versicolor a white rotter (1 strain) were cultured in a liquid nutrient medium with birch and spruce sawdust as sole C-source and their extracellular hemicellulases investigated. Limited studies of the growth of three of these species, (i.e., excluding P. versicolor) on hemicelluloses as sole C-source, were carried out. Selected strains and species of fungus were cultured on both soft and hardwoods and the patterns of behaviour investigated with the S.E.M. A technique of serial S.E.M. photographs found to be successful for easy visualisation of the attack of wood by fungi. Evidence is provided which throws some light on the role which hemicelluloses play in the fungal degradation of wood. The differences found in induction of galactoglucomannanases and xylanases appear to reflect any tendency for a fungus to inhabit either a soft or hardwood.

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General Introduction

In spite of the tremendous economic importance of wood and its long standing use, there is surprisingly little precise information available concerning the natural enemies of wood and its effective protection. This thesis is based upon two widely differing modes of attack on the problem of wood decay. On the one hand classical biochemical, chemical and botanical techniques were used to gain an insight into the physiology of fungi attacking wood, and on the other the scanning electron microscope (SEM) was employed to improve visualization of the same process.

In the introduction, which is divided into three sections: (1) Timber, (2) Enzymes and (3) Fungi (wood destroying), the author has endeavoured to cover these subjects from the view point of a biochemist, i.e., sections (1) and (3) have been written assuming the reader has only a limited knowledge of the field.

TIMBER

Introduction

Timber is one of mans' oldest raw materials. The use of timber, first as a source of warmth in the form of firewood, then as a structural material, dates from prehistoric times. Some of the many more recent uses of timber are in ships, buildings, railway sleepers, pit-props, fence posts, and for the manufacture of paper. Other materials can be and have been used for all these tasks, and indeed there has been a trend over the last half century to new materials. This is especially true in the packaging industry, where plastic containers have proved to be serious competitors to the traditional cellulose-based ones. It is envisaged that a return to the use of degradable garbage bags and packaging will take place in the next few years, applying even greater pressure to the need for timber as a raw material of the future. Thus in a world that is becoming increasingly over-populated, and where pollution and resource problems are becoming daily more significant, the increased and more efficient use of timber and its by-products is an attractive proposition, and may indeed be an essential one. Comparatively little research and development could result in far reaching changes in our way of life.

When viewed in this context, the need for efficient forest management is paramount. About one third of this planet's land area is now forested. Although quite a large fraction of this is at present not exploitable for geographic reasons,

in many regions only a small proportion of the total accessible forest is in use. As new roads cut down the percentage of inaccessible forest, and rising prices and demand make it economically feasible to harvest timber in locations with difficult access, a point is eventually going to be reached where the burden of meeting a constant rise in demand is going to fall upon the forester. The responsibility of producing increased yields per land area will lie with him.

The net rate of saw-timber growth (expressed as a percentage of timber volume) has been estimated in the U.S.A. to range from 6.7% in the South to 4.5% in the North. In the very far North (Alaska), however, it was only 0.8% (Browning, 1963). It can thus be seen that, for more effective use of the world's timber supply, efforts should be concentrated in the warmer regions.

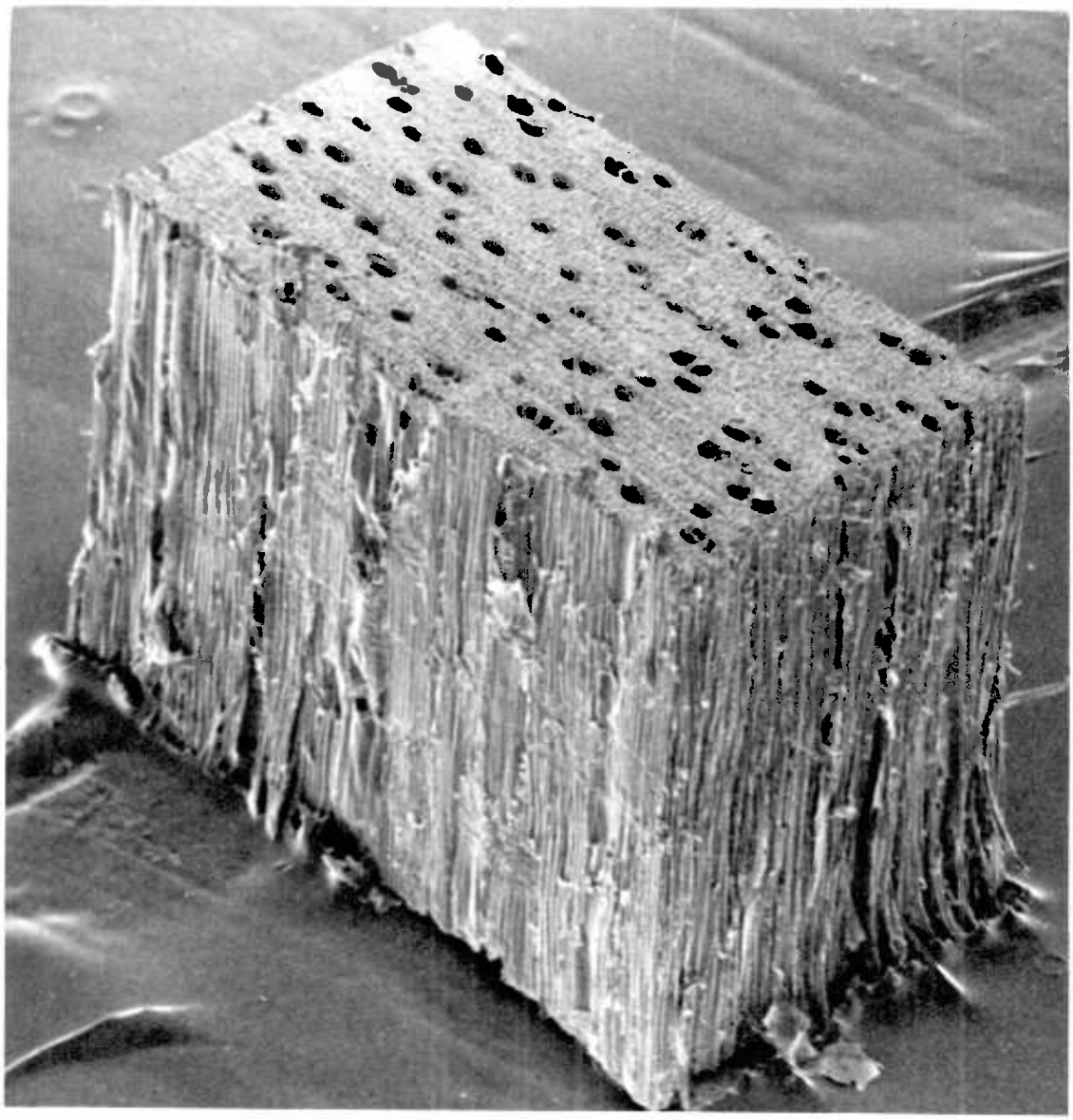
Structure

The plants from which wood is derived, the class of spermatophytes, may be subdivided into gymnosperms and angiosperms. The main group of the gymnosperms is the order of coniferales. These have been described as conifers, evergreens and softwoods. Many of these species bear cones as reproductive structures. Angiosperms are our typical flowering plants and can be subdivided into two classes, the monocotyledons and the dicotyledons. Examples of "woody" monocolyledons are the palms and bamboos. The dicotyledonous trees are our broad leafed, and are also known as deciduous or hard woods. The terms soft and hard do not necessarily relate to a physical degree of hardness, but were terms used in mediaeval times to distinguish pine from oak.

Wood is a heterogeneous material, composed of plant cell walls. These are mainly cellulose, lignin and hemicellulose, but some cells contain extraneous deposited materials. Most cells have their principal axis orientated parallel to that of the stem. The wood ray is unusual in that is principal direction of orientation is horizontal along the radius. This radial tissue is for transport of materials to and from the inner bark (phloem) to the woody (xylem) portion of the tree.

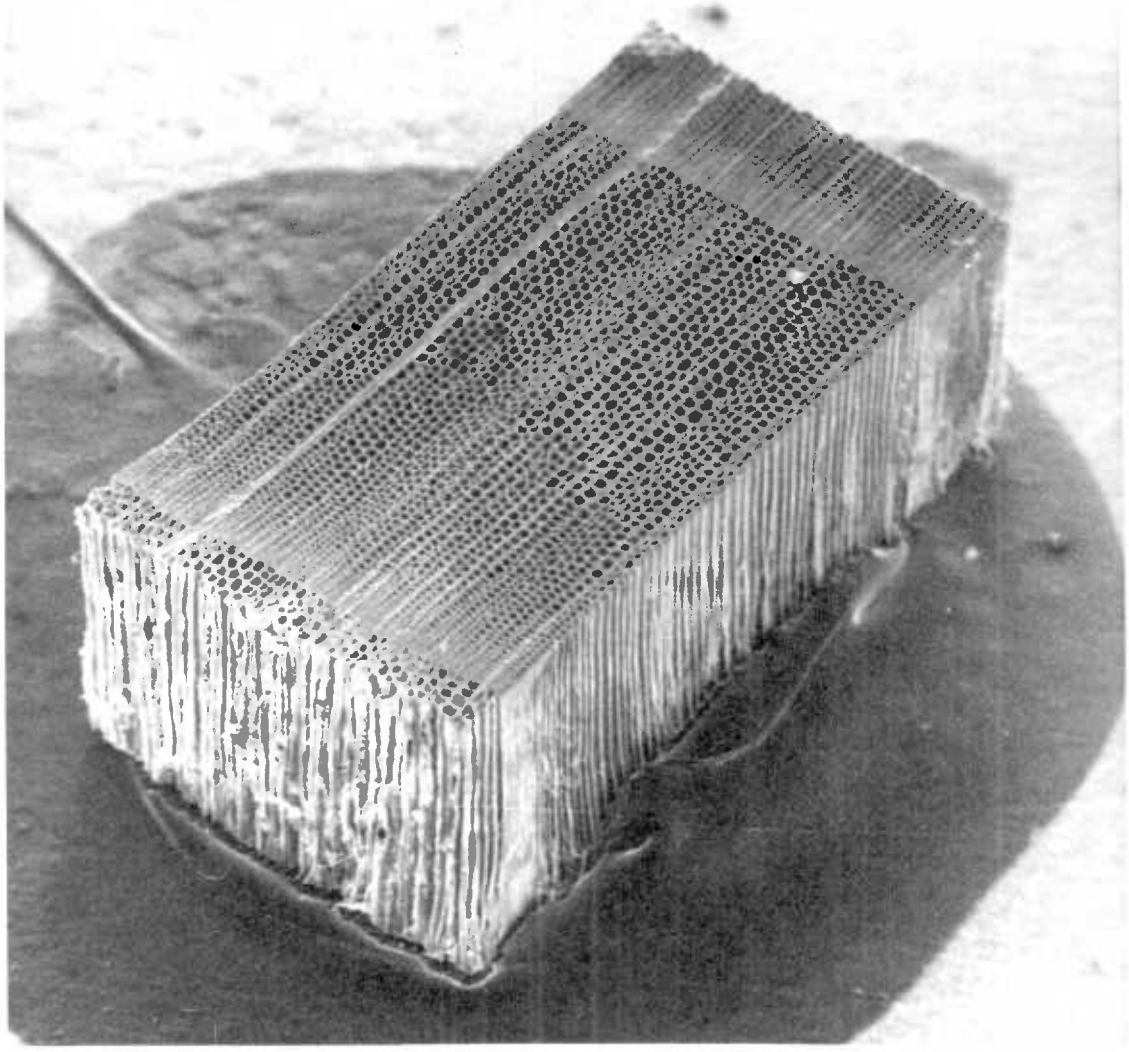
A stem has three planes of orientation. If a cut is made at right angles to the stem, it is termed a transverse section (T.S.); if a section is cut along a radius of the stem, the cut is in the longitudinal plane (R.L.S.); the last, also longitudinally cut, but perpendicular to the radius is called a tangential longitudinal section (T.L.S.).

On the following pages are shewn selected scanning electron micrographs which demonstrate the structure both of softwoods (Scots pine and Norway spruce) and of hardwoods (beech and birch). The arrangement of photographs and their numbering is fully explained in the SEM section (see page 245).



A birch block shewing T.S., R.L.S. and T.L.S. surfaces.

A.D. x 30.



The Norway spruce block shewing one complete annual ring, and the three types of section. The T.S. is the upper surface, the R.L.S. to the right side and the T.L.S. to the left side of the picture.

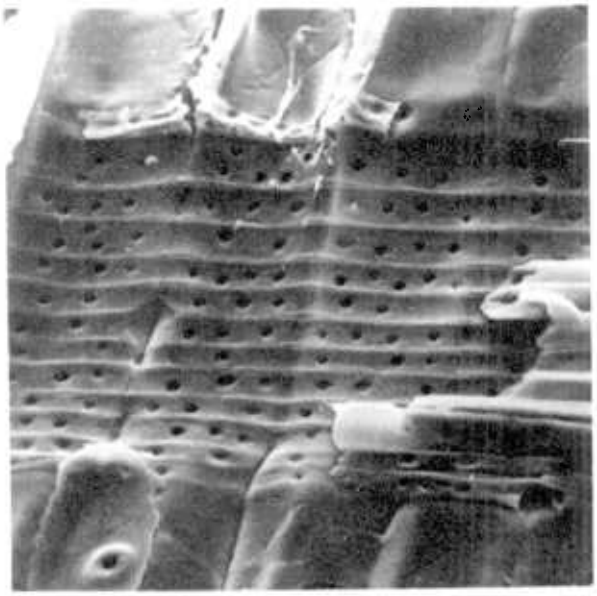
A.D. x 31.

STRUCTURAL

Plate A

No. 1 and 2 are R.L.S.'s of Norway spruce and show an area called the "cross field" where ray cells cross. In each of the rectangles caused by the interjunction of a single tracheid and ray cell there are three to five pits. These are piceoid. (See page 19 type number (3)). At the top and bottom of the position of each ray can be seen bordered pits, which originally would have connected with parenchyma cells in the ray. An example of this type of cell is observable in the bottom right hand corner of No. 1, where there are two parenchyma cells, or at least a part of them. The same is true at both the top and bottom of the ray material still present in No. 2. Photograph No. 3 is illuminating, since it shows many different layers. A very small fraction of "crossfield" similar in nature to No. 1 and 2 can be seen at the left hand side just below the centre line. Apart from this, the ray seems intact. All of the ray material in view is restricted to the top left hand quadrant, and pit openings connecting to the tracheid cell wall layer are apparent. Next is the tracheid cell wall material, which is seen partly stripped away in the top left hand side, but whole on the top right hand side. Down the centre of the photograph perpendicular to the ray can be seen a tracheid. On the upper surface five bordered pits are clearly visible. The top two are complete, in the sense that both halves of the pit are present, and the bottom three are shown with the cell wall of the adjacent tracheid stripped away. At the right hand side, in a central position, the line of demarcation between bordered pits (inter tracheid connection) and piceoid pit (tracheid to ray connection) is quite distinct. No. 4 is a low power photograph of Scots pine, showing ray cells in a R.L.S. No. 5 is a higher power picture of a ray clearly showing the fenestrate cross field pitting. (see page 19 number (1)). In the central part can be seen two tracheids with their concomitant intertracheid bordered pits. No. 6 shows ray cells under high power with the fenestrate pits complete with their membranes. At the top central part lies a small bordered pit which is connected to parenchyma cells, some of the remains of which are still present.

Plate A



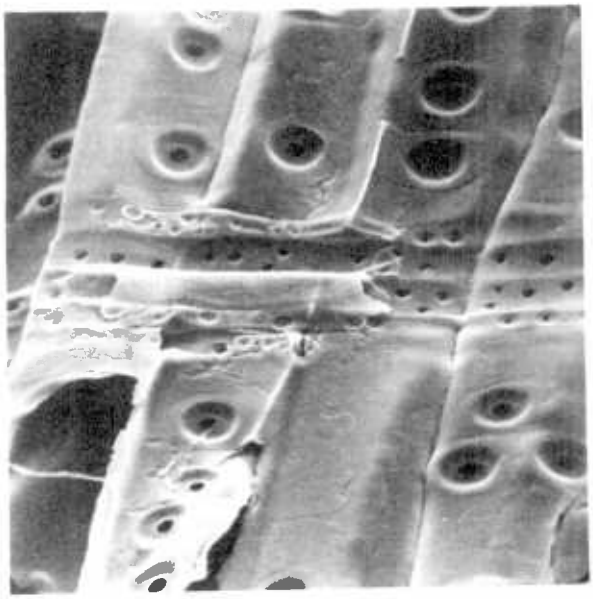
13352 P. monticola 280 x 381

Spruce R.L.S. A.D. 3 weeks



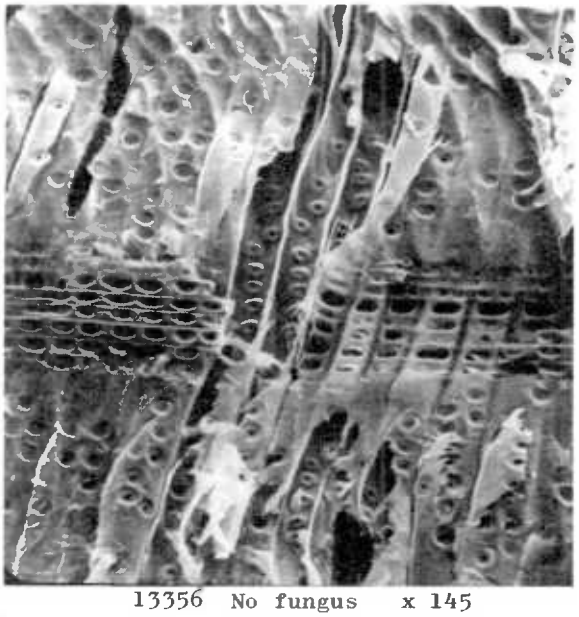
13353 No fungus x 37

Scots Pine R.L.S. A.D.



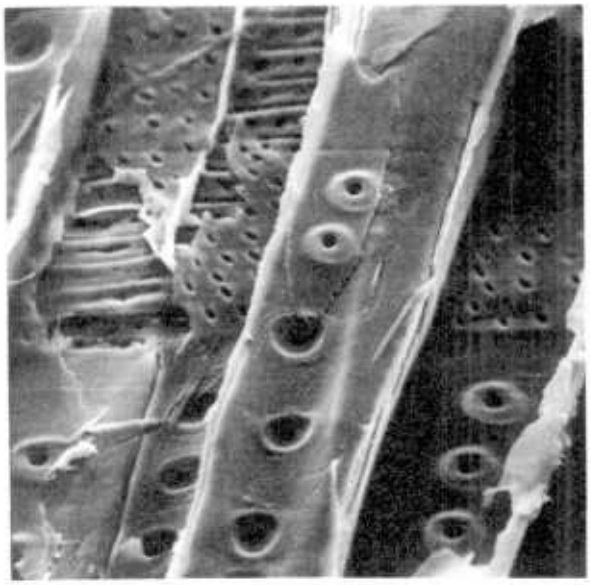
13349 P. monticola 280 x 381

Spruce R.L.S. A.D. 3 weeks



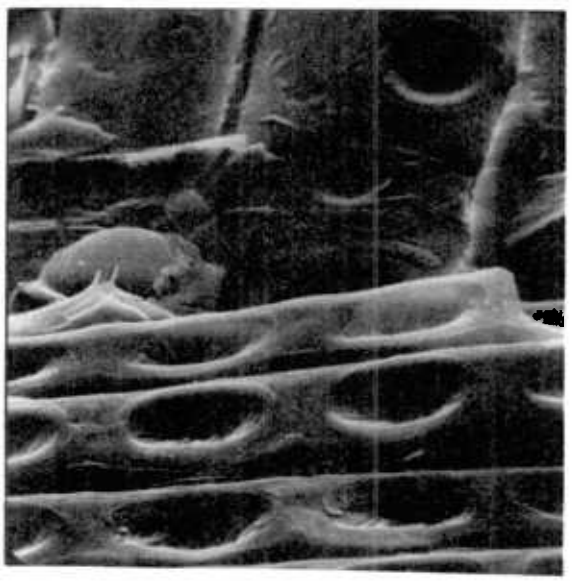
13356 No fungus x 145

Scots Pine R.L.S A.D.



13347 P. monticola 280 x 389

Spruce R.L.S. A.D. 3 weeks



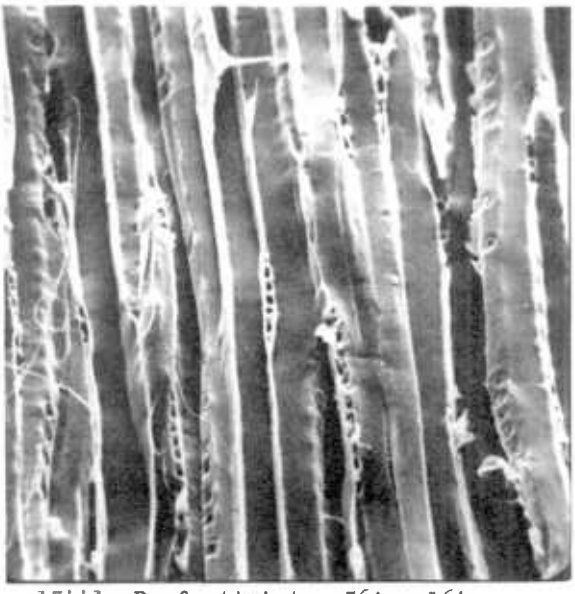
13117 No fungus x 890

Scots Pine R.L.S. A.D.

Plate B

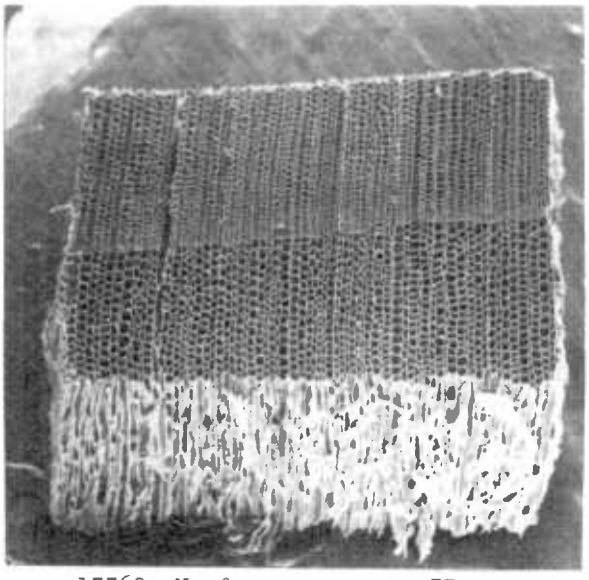
Nos. 1, 2 and 3 are T.L.S.'s and Nos. 5 and 6 are T.S.'s of Norway spruce. No. 1 shows ray cells in cross section and gives an impression of their size, numbers and distribution. No. 2 is a higher power picture of a single ray in cross section. Note that when wood is split in this way, ray cells are broken in tension parallel to the microfibrillar axis (the majority of the microfibrils having their orientation nearly parallel to the longitudinal axis of the ray) and this results in a characteristic messy fibrillar break. This is particularly true of No. 3, which is shown to demonstrate the position of a radial resin canal, seen in the centre of the photograph. No. 4 shows a T.S. of a complete spruce block. Horizontally across the centre lies the division between the springwood of one year and the summerwood of the next. The line of the rays is perpendicular to this and up and down the page as viewed. No. 5 is a similar block, showing two resin canals. These are situated in the centre of the summerwood. No. 6 is a high power magnification of one of these canals.

Plate B



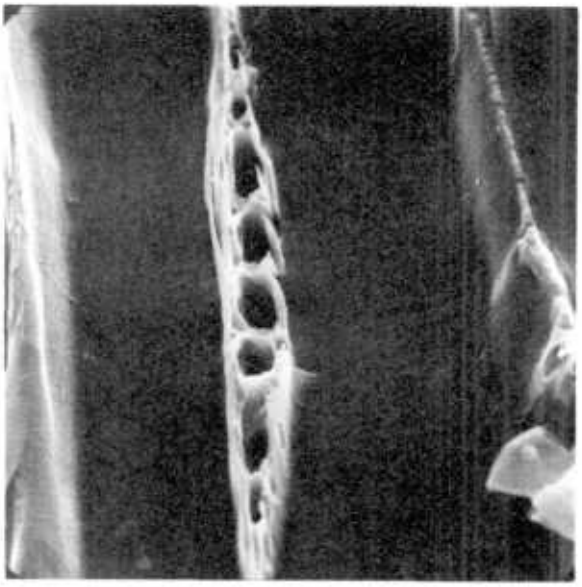
13441 P. fastigiata 56A x 164

Spruce T.L.S. A.D. 3 weeks



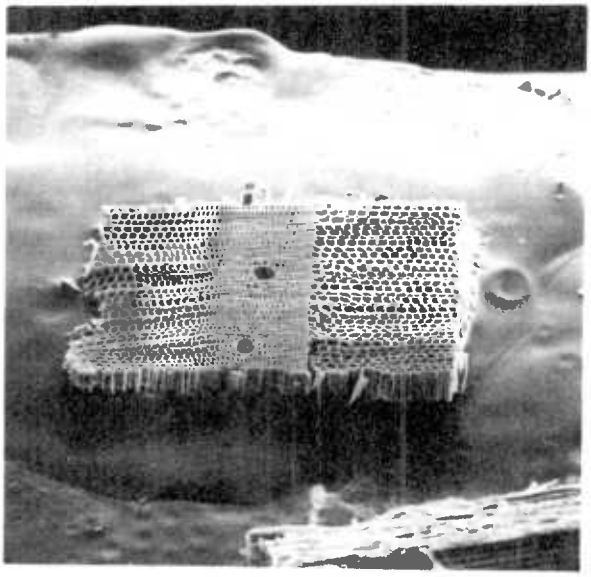
13360 No fungus x 37

Scots Pine general view A.D.



13443 P. fastigiata 56A x 815

Spruce T.L.S. A.D. 3 weeks



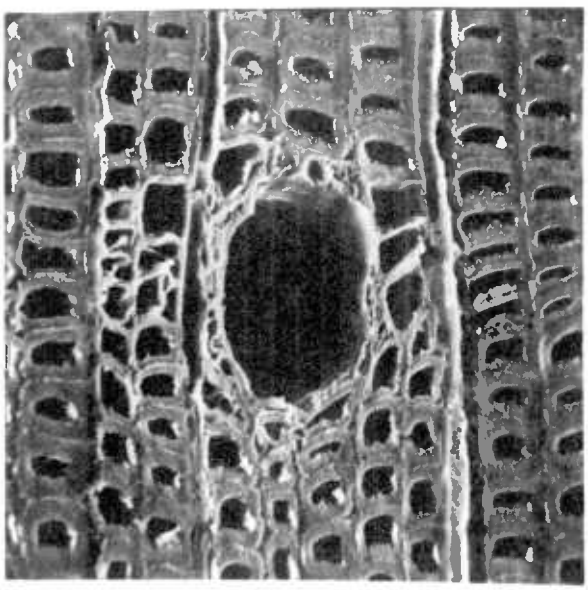
13450 P. fastigiata 56A x 34

Spruce T.S. A.D. 3 weeks



13445 P. fastigiata 56A x 400

Spruce T.L.S. A.D. 3 weeks



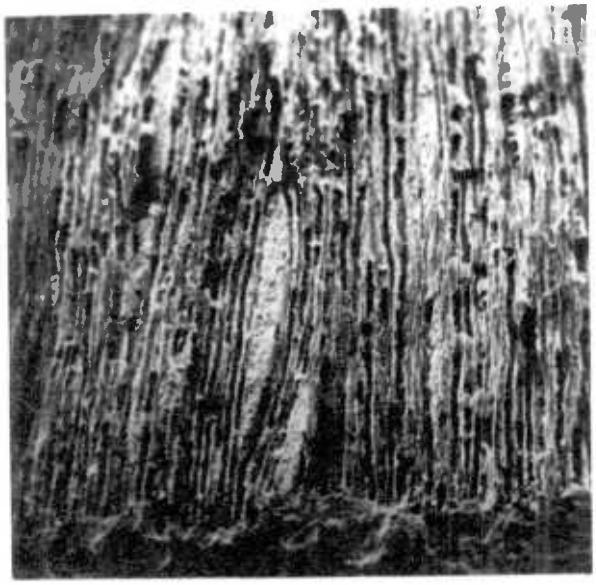
13452 P. fastigiata 56A x 339

Spruce T.S. A.D. 3 weeks

Plate C

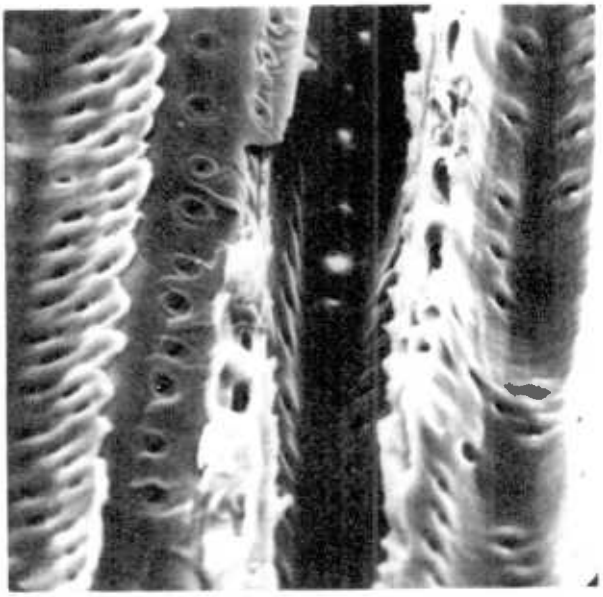
No. 1 is a photograph of beech wood in T.L.S. The very large rays are clearly visible even at this low magnification (17.5x) and are characteristic of beech wood. No. 2 shows this at a higher magnification. No. 3 shows the smaller uniserate rays also present in beech wood. No. 4 shows the well pitted nature of the fibres which is probably the reason for the rapid colonization of beech by fungi when the rays in this species are not colonized.

Plate C



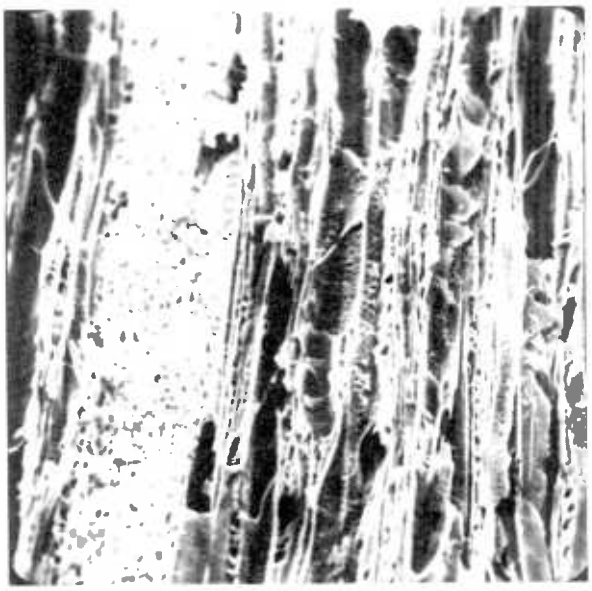
14305 No fungus x 17.5

Beech T.L.S. A.D.



14309 No fungus x 438

Beech T.L.S. A.D.



14306 No fungus x 89

Beech T.L.S. A.D.



14310 No fungus x 168

Beech T.L.S. A.D.

Coniferous Wood (soft wood)

The tracheid: these are very long cells of which the length to diameter ratio can be up to 75. In cross section they appear polygonal, arranged in radial rows. In the springwood they are large in width, rectangular or hexagonal, and tend to get smaller as the summerwood is approached.

The radial walls of tracheids have pits, most of which are complementary with pits in adjacent tracheids. These are doughnut shaped and are termed bordered pits. They are inter-tracheid pits. (See Figs. 1(a) + (b) for pit diagrams).

The liquid transport between rays and tracheids takes place through the cross field pits. The rays contain either ray parenchyma, ray tracheids, or both cell types. When the longitudinal and radial tracheids connect, small bordered pits are found. The pitting from ray parenchyma to the longitudinal tracheid is very characteristic and is of help in timber identification. (See Fig. 1 'b') (Phillips, 1948).

Classification of pitting is as follows:

(1) Fenestriform
(2) Pinoid
(3) Piceoid
(4) Taxodioid
(5) Cupressoid

Dicotyledonous Wood (hard wood)

This is orientated in the same way as coniferous timbers, but has a greater variety of cell types. The main difference

Fig. 1 'a' - Pit types.

A. Simple pit in vertical section a and surface view b.

B. Ramiform pits; in the half a, the cell wall is relatively thin and there are three groups of simple pits; in the half b, the pits of each group have coalesced, due to thickening of the wall, forming ramiform or branched pits.

C. Bordered pit in vertical section a and surface view b; the typical, somewhat rounded margin of the aperture of such a pit is shown (in section), at c.

D. Pit-pairs, such as might be found between a vessel element or tracheid and a parenchyma cell; a, both pits simple; b, one pit simple, the other bordered; c, both pits bordered.

E. Blind pits; the pits on contiguous walls do not form pairs.

F. Blind pits; portions of three contiguous cells surrounding an intercellular space; typical pit-pairs occur between adjacent cells, as well as blind pits, which abut on to the intercellular space; in one of these latter the middle lamella has disappeared.

G. a, a very elongate simple pit, surface view; b, showing its appearance in vertical section when the section passes along the short axis of the pit; c, in vertical section when the section passes parallel to the long axis.

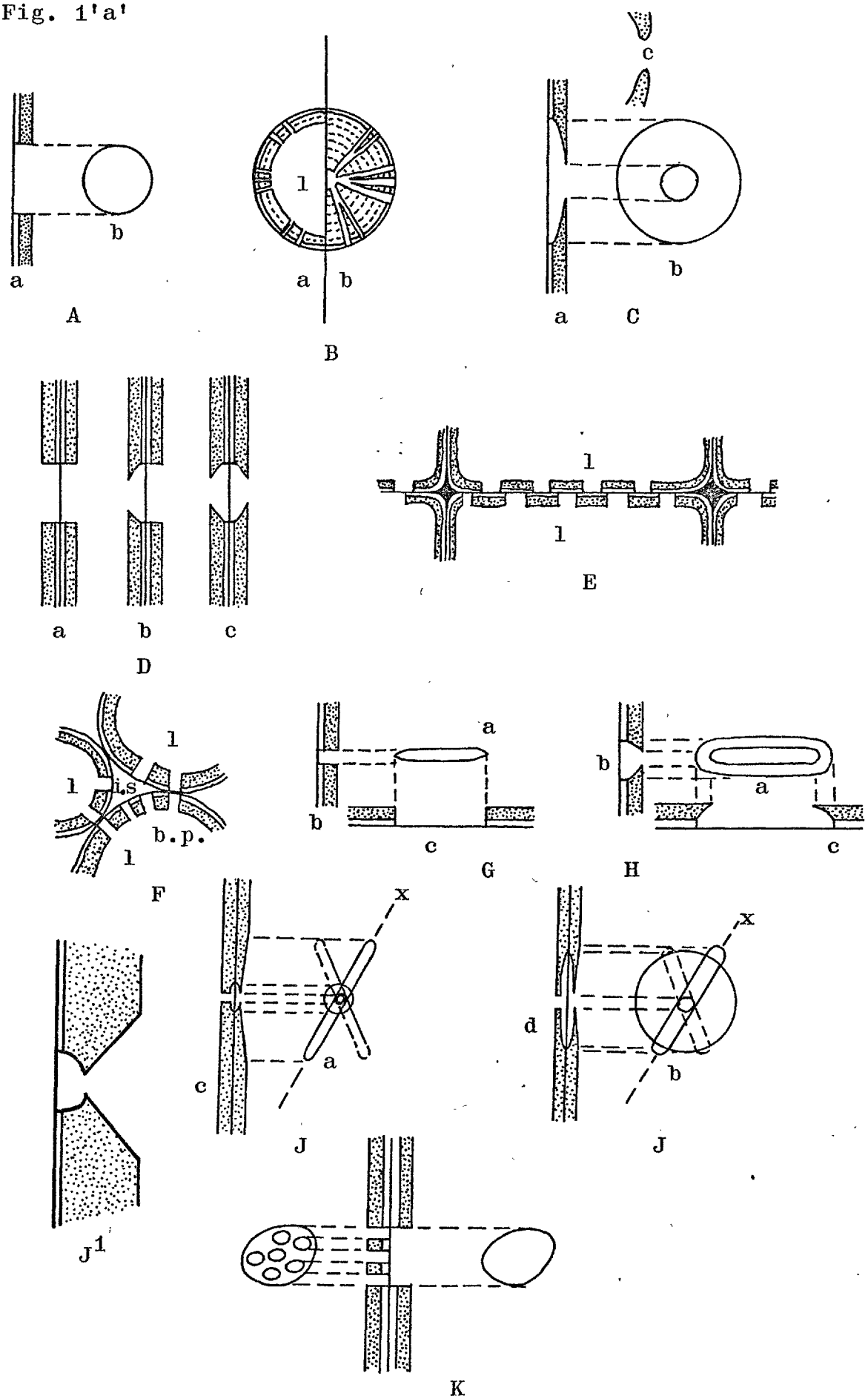
H. As G, but pit bordered.

J. Bordered pits with extended apertures; a and b in surface view, c and d in a vertical section along the plane marked x. J' is an enlarged view of the wall in J to show the primary and secondary layers.

K.Unilaterally compound pitting; on the right a single large simple pit has developed in the primary pit-field; on the left several small simple pits have developed in this field; b.p., blind pit; i.s., intercellular space; l., cell lumen. Where the wall is shown differentiated into layers, the middle lamella is shown as a black line, the primary wall white and the secondary wall stippled.

After Jane (1970)

Fig. 1'a'



After Jane (1970)

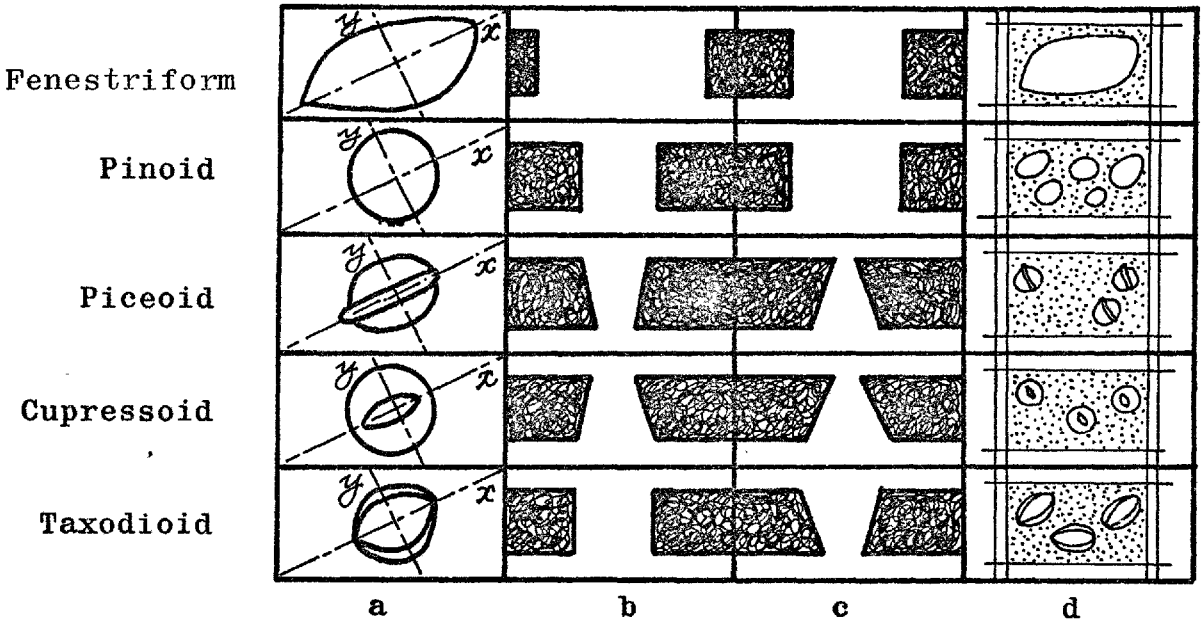


Fig. 1b. Diagrammatic representations of pitting on radial walls of ray parenchyma cells in coniferous wood. Column a, pits in surface view; column b, pits in section, cut along the axis marked x in column a; column c, pits in section, cut along the axis marked y in column a; column d, pits as seen in surface view in a cross-field (R.L.S.); the double vertical lines in d are the walls of an axial tracheid. N.B. - The pinoid pit may be slightly or partially bordered.

After Jane (1970)

is the presence of vessels, thus the term "porous woods". These longitudinal cells when viewed in cross-section have no radial alignment. If in the springwood, pores are more numerous than in the summerwood, an annular appearance results and the wood is termed ring porous. However, if the vessels are uniformly dispersed throughout the growth ring, it is a diffuse porous timber. In either case there will be a size gradient from spring to summerwood.

Vessel: This is a tube of indeterminate length composed of cells which coalesce end to end. At the junction between the vessel members the cell wall is dissolved to leave an opening. This can be either a single large one, known as a simple perforation, or ladder like in appearance, when it is termed scalariform.

Tracheids: These are of two types; the vascular and vasicentric. The vascular tracheid is a cell of great length to breadth ratio with pointed ends. It resembles a vessel but is smaller and does not have end wall perforations. The vasicentric is short, thus similar to a coniferous tracheid.

Fibre: There are two types, fibre tracheids and libriform fibres. They serve mainly as mechanical support, and are not heavily pitted. Libriform fibres exhibit simple pits which are usually smaller than the bordered pits of fibre tracheids, Libriform fibres generally have a smaller diameter and narrower lumen than tracheids.

The rays of hardwoods are more variable than softwoods both in height and width. Most species have rays more than one cell wide (multiserate). They are composed of parenchyma

cells with their long axes either radial or longitudinal, or a mixture of the two.

Tension wood and compression wood

When subjected to pressures such as compression or tension, the stems of trees form what is known as "reaction wood". Conifers will give rise solely to "compression wood" and angiosperms to "tension" wood, thus the location of reaction wood will vary with these timber groupings when the direction of the load is constant.

Sapwood and Heartwood

Sapwood is wood that is physiologically active and is almost always found in the outer portions of a tree stem next to the cambium. Young trees generally consist entirely of sapwood; as a tree ages, however, the inside tissues die forming a central core known as heartwood. Sapwood can vary from a few growth rings in some species to nearly 200 in others (Browning, 1963). In general heartwood appears to be a darker colour than sapwood, although some commonly used woods, such as spruce, are exceptions.

Timber structure (chemical)

Wood is a heterogeneous complex mixture of an interpenetrating system of high molecular weight polymers. At present the separation of any one of these components can only be achieved at the cost of the partial destruction of other components. Even then it is not certain that the compound isolated has not undergone some modification.

1. Carbohydrates: These are represented by the polysaccharides. Wood is 75% polysaccharide, the main polysaccharide component being cellulose (50%) with hemicellulose, starch, pectic substances, and water soluble polysaccharides in smaller quantities.

2. Phenolic substances: The major component is lignin. This is an aromatic material characterized by the presence of phenolic hydroxyl groups which are partly methylated. It has a high molecular weight and is insoluble in common solvents.

   There are also present other organic compounds such as terpenes, aliphatic acids, alcohols, and proteins.

3. Separation of Wood components: It is customary to differentiate between the cell wall components (lignin and polysaccharide) and the extraneous components. The latter are not considered part of the wood structure, and are extracted by neutral organic solvents. Wood which has been treated thus is designated extractive free. The following diagram represents the basis of wood structure. (Fig. 2)

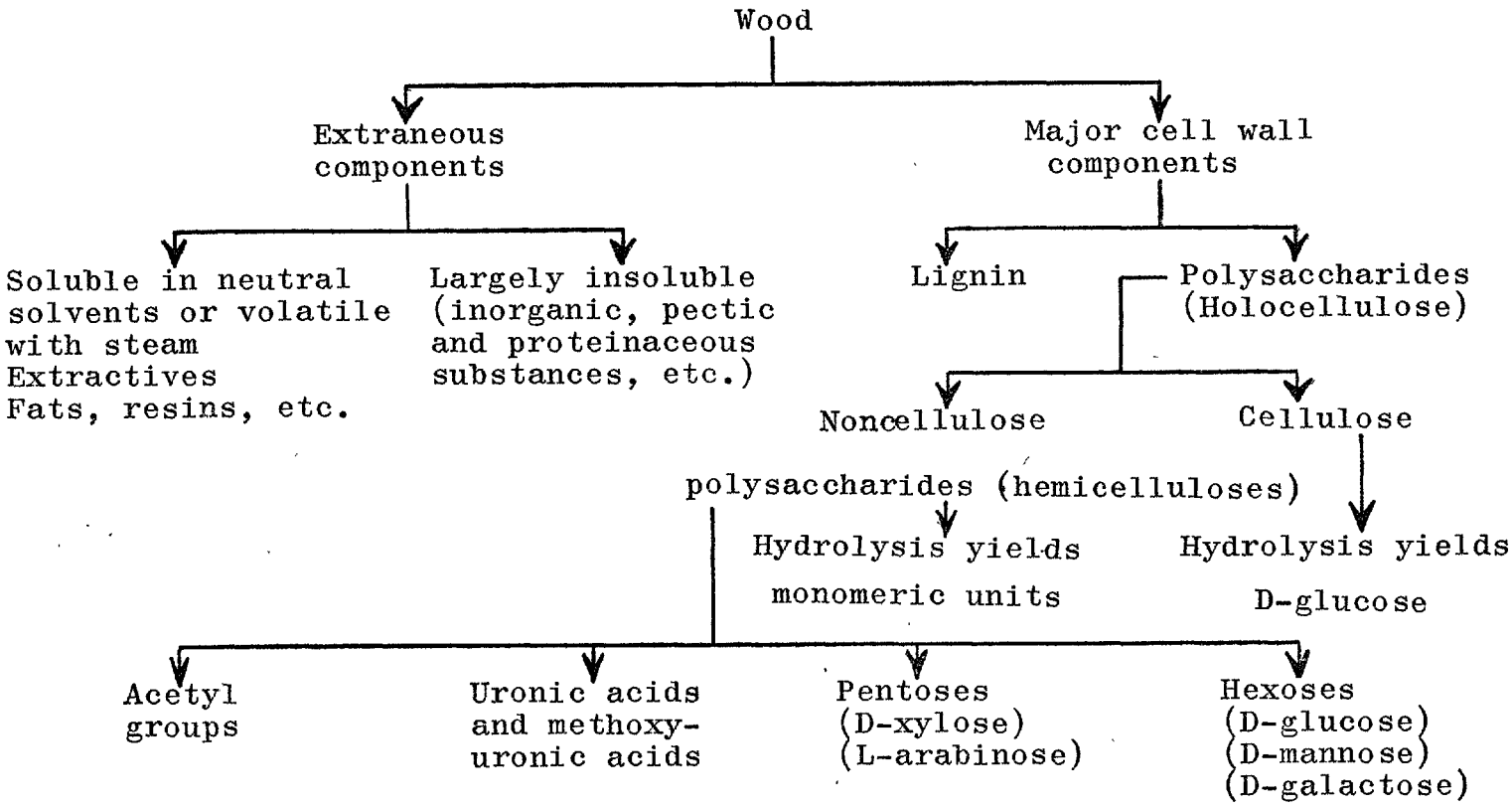


Fig. 2. Idealized outline of wood components.

After Browning (1963)

Cellulose yields D-glucose upon total hydrolysis, whereas the non-cellulosic polysaccharides yield other sugars as well. D-mannose, D-galactose, D-xylose, L-arabinose, and in some timbers L-rhamnose are to be found in hydrolysates of these materials. In addition to these sugar units one finds uronic acid and monomethyl uronic acid units. They are generally found attached to one or more sugar residues (usally D-xylose) when in hydrolysates.

The polysaccharide chains can generally be isolated into fractions with well defined sugar ratios, e.g. glucomannan, arabinogalactan, methyl glucuronoxylan. It is rare to find homopolymers in wood, with the exception of cellulose, but they are quite common in other plant sources e.g. esparto grass xylan and ivory nut mannan. (Aspinall et al. 1953 (a)&(b).)

Before successful extraction of these compounds can be achieved, it is generally necessary to remove the lignin, in such a way as to provide a minimal loss of carbohydrate. The product of this procedure is termed holocellulose (Ritter and Kurth, 1933). There are two main ways of producing holocellulose:

(1) alternate chlorination and extraction with an alcoholic solution of an organic base such as monoethanolamine (Van Beckum and Ritter, 1937)

(2) Treatment with a buffered aqueous solution of sodium chlorite to yield "chlorite holocelluloses" (Jayme and Grøgaard 1942, Wise 1945)

It has been found that the non-cellulosic polysaccharides are soluble in various compounds, the chief one being the alkalis. These polysaccharides were termed hemicelluloses by Schulze (1891). After removal of the hemicellulose the remaining substance is called alpha-cellulose, which upon hydrolysis yields other monosaccharides as well as D-glucose, some hemicellulose being impossible to remove.

Wood also contains acetyl (CH_3CO-) groups and these are combined as esters of acetic acid with non-cellulosic polysaccharides. They are removed during alkaline extraction as acetic acid. Dimethyl sulphoxide has been used as a solvent to extract holocelluloses without removal of these groups.

Lignin has yet to be isolated as a pure substance of definite composition.

Uniformity of chemical composition

The specialisation of a tree into roots, stem, and crown will of necessity involve a chemical non-uniformity. Indeed it is found that variety exists between samples taken at different heights along the stem and at different distances from the pith. (Browning, 1963). The sapwood and heartwood exhibit characteristic chemical differences, as does the summer and springwood. Within a single cell there are changes in composition across the cell wall.

It is thus very important when dealing with chemical analyses of wood to clearly define from which portion of the tree any particular sample has been obtained, wherever this is possible. The following charts give summative analyses of several different types of wood. (Figs. 3 and 4).

Fig. 3

Summative Analysis of Canadian Woods (all values in per cent)

Species	Soluble in alcohol-benzene	Soluble in hot water[a]	Ash	Acetyl	Lignin	Alpha-cellulose	Hemi-celluloses	Total
Red pine (Pinus resinosa)	9.7	1.8	0.2	1.9	23.4	47.8	15.1	99.9
Tamarack (Larix lariciana)	3.1	7.9	0.3	1.6	24.8	49.5	13.3	100.5
Basswood (Tilia americana)	6.3	1.3	0.6	5.9	17.2	45.0	22.9	99.2
White elm (Ulmus americana)	2.2	1.3	1.1	5.0	23.4	47.2	18.5	98.7
Black ash (Fraxinus nigra)	3.4	1.3	0.7	5.3	18.6	47.4	21.2	97.9
Yellow birch (Betula lutea)	3.4	0.7	0.3	5.4	18.8	42.6	26.6	97.8
Beech (Fagus gradifolia)	2.0	0.7	0.4	5.3	22.2	43.6	23.6	97.8
Sugar maple (Acer saccharum)	2.7	1.0	0.4	4.6	21.1	46.8	22.2	98.8

[a]After extraction with alcohol-benzene.

After Clermont and Schwartz (1952)

Fig. 4.

Summative Analysis of Woods (per cent of extractive-free dry wood)

Constituent	Douglas-fir	Western hemlock	Loblolly pine	Black spruce	Western red-cedar	Monterey pine	Southern red oak	Black tupelo
Ash	0.3	0.5	0.3	0.4	0.3	0.2	0.2	0.7
Acetyl	0.6	1.2	1.1	1.1	0.5	1.4	3.3	3.7
Lignin	28.4	30.4	29.5	28.0	32.5	26.5	25.2	25.8
				Summation A				
Alpha-cellulose	57.2	51.6	55.0	51.5	52.7	54.8	45.7	46.5
Hemicellulose	14.1	15.5	15.3	17.4	14.6	16.4	23.3	23.4
Total[a]	100.6	99.2	101.2	98.4	100.6	99.3	97.7	100.1
				Summation B				
Alpha-cellulose[b]	48.3	44.5	46.6	45.6	47.5	45.3	43.7	45.6
Mannan[c]	5.4	4.1	4.7	8.0	5.1	11.7		
Xylan	6.2	7.3	10.1	10.5	8.1	9.3	20.0	17.1
Uronic anhydride	2.8	5.0	3.8	4.1	4.2	3.4	4.5	4.7
CH_2[d]	0	0.2	0.2	0.2	0.2	0.1	0.6	0.9
Total[a]	92.0	93.2	96.3	97.9	98.4	97.9	97.5	98.5

[a]Including ash, acetyl, and lignin.

[b]Corrected for mannan, xylan, and uronic anhydride.

[c]By the phenylhydrazine method; the figures are probably low.

[d]Calculated from methoxyl not in lignin.

After Brasch and Wise (1956); Wise and Pickard (1955), Wise and Ratliff (1947)

Comparison between Softwood and Hardwoods.

Although there is considerable variation among species within either group, certain characteristic differences are consistent. In temperate zones softwoods contain higher quantities of extractives. The mannan and xylan fractions are striking in their difference. The mannan content of softwoods is in the range of 10 to 15%, but rarely exceeds 2 to 3% in hardwoods. On the other hand, the xylan content of hardwoods is from 12 to 20%, but in softwoods rarely exceeds 10%. There is a considerable difference in lignin content, the softwoods ranging from 23 to 33%, and the hardwoods from 16 to about 25%. Both woods contain approximately the same total quantity of methoxyl groups, and these are present in the lignin, thus the lignin in hardwoods has a higher concentration of methoxyl groups than that of softwoods.

The following tables serve to show some of these differences. Fig. 5.

Fig. 5

Composition of North American Woods (per cent of extractive-free wood)

Species	Glucan	Mannan	Galactan	Xylan	Arabinan	Uronic anhydride	Acetyl	Lignin	Ash
				Hardwoods					
Trembling aspen (Populus tremuloides)	57.3	2.3	0.8	16.0	0.4	3.3	3.4	16.3	0.2
Beech (Fagus grandifolia)	47.5	2.1	1.2	17.5	0.5	4.8	3.9	22.1	0.4
White birch (Betula papyrifera)	44.7	1.5	0.6	24.6	0.5	4.6	4.4	18.9	0.2
Yellow birch (Betula lutea)	46.7	3.6	0.9	20.1	0.6	4.2	3.3	21.3	0.3
Red maple (Acer rubrum)	46.6	3.5	0.6	17.3	0.5	3.5	3.8	24.0	0.2
Sugar maple (Acer saccharum)	51.7	2.3	0.1	14.8	0.8	4.4	2.9	22.7	0.3
Sweetgum (Liquidambar styraciflua)	39.4	3.1	0.8	17.5	0.3			23.7	0.2
White elm (Ulmus americana)	53.2	2.4	0.9	11.5	0.6	3.6	3.9	23.6	0.3
Southern red oak[a] (Quercus falcata	40.6	2.0	1.2	19.2	0.4	4.5	3.3	23.9	0.8

[a]Data supplied through the courtesy of the Forest Products Laboratory to Browning (1963); others from Timell (1957, 1958)

[b]Galactan included with glucan, and arabinan included with mannan.

Fig. 5 (continued)

Composition of North American Woods (per cent of extractive-free wood)

Species	Glucan	Mannan	Galactan	Xylan	Arabinan	Uronic anhydride	Acetyl	Lignin	Ash
				Softwoods					
Balsam fir (Abies balsamea)	46.8	12.4	1.0	4.8	0.5	3.4	1.5	29.4	0.2
Eastern white-cedar (Thuja occidentalis)	45.2	8.3	1.5	7.5	1.3	4.2	1.1	30.7	0.2
Eastern hemlock (Tsuga canadensis)	45.3	11.2	1.2	4.0	0.6	3.3	1.7	32.5	0.2
Jack pine (Pinus banksiana)	45.6	10.6	1.4	7.1	1.4	3.9	1.2	28.6	0.2
White pine (Pinus strobus)	44.5	10.6	2.5	6.3	1.2	4.0	1.3	29.3	0.2
Loblolly pine[a] (Pinus taeda)	45.0	11.2	2.3	6.8	1.7	3.8	1.1	27.7	0.3
Douglas-fir[a] (Pseudotsuga taxifolia)	43.5	10.8	4.7	2.8	2.7	2.8	0.8	31.5	0.4
Black spruce[a] (Picea mariana)	47.9	10.5	[b]	8.0	[b]	4.1	1.1	28.0	0.4
White spruce (Picea glauca)	46.5	11.6	1.2	6.8	1.6	3.6	1.3	27.1	0.3
Tamarack (Larix laricina)	46.1	13.1	2.3	4.3	1.0	2.9	1.5	28.6	0.2

[a]Data supplied through the courtesy of the Forest Products Laboratory to Browning (1963); others from Timell (1957, 1958)

[b]Galactan included with glucan, and arabinan included with mannan.

Cell Structure

The cell is composed of an outer (exterior) primary cell wall (P), upon which is deposited a stiffer secondary cell wall which is normally divided into three regions. The first (S_1), is a thin outer layer; the second (S_2), is relatively thick; and the third (S_3), is thin and is sometimes absent. The whole unit is separated from other adjacent cells by the compound middle lamella layer, composed largely of lignin and pectic materials.

These different layers are not homogeneous but in the main consist of components called microfibrils. (A microfibril is a very long thin string of cellulose, the detailed structure of which will be discussed at a later stage.) The microfibrils are arranged in an organised but different manner for each layer. In the middle lamella there are no microfibrils, however the primary wall microfibrils lie at approximately 90° to the longer cell axis. The orientation is not uniform and there are a few which definitely lie in the axial plane. In the S_1 the microfibrils are at a more definite angle, about 70°, to the longer cell axis. The majority lie in "S" helices though some occupy "Z" helices. In S_2 the angle to the axis is about 20° in a "Z" helix. Lastly the S_3 layer is composed of "S" helices at about 70° in a similar fashion to the bulk of the S_1 layer.

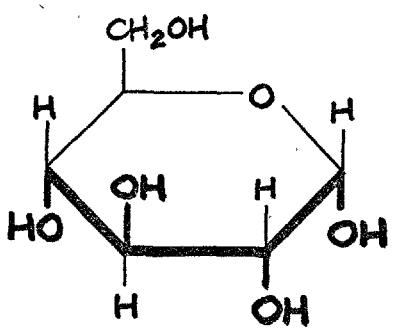
Fig. 8 shews a diagram of this complex orientation.

Microfibril structure.

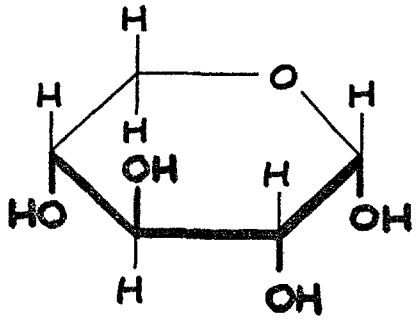
When wood holocellulose is hydrolysed, the bulk of the monosaccharides obtained are D glucose, D mannose, D xylose, D galactose, and L arabinose. About 50% is a polysaccharide called α cellulose which yields on hydrolysis mainly β-D glucose. Hence cellulose can be regarded as a polymer of β-D glucose. The chemical formulae for these compounds are shown in Figs. 6 & 7. When a glucose molecule is linked in the β 1-4 configuration to another glucose, a disaccharide is obtained called cellobiose. If this is repeated until a long chain results, cellulose will be obtained. It can be observed (see Figure 7) that each β glucose molecule is arranged at 180^{o} to its neighbour, so that strictly speaking each single repeating unit could be regarded as cellobiose. So cellulose can equally accurately be regarded as a polymer of cellobiose. Conveniently, however, the number of glucose molecules is used as a basis characterizing this type of molecule. Although it is open to discussion, most workers agree that the "degree of polymerisation" (D.P.) of cellulose is between 5000 and 10,000 glucose molecules. This is amazingly long for a single molecule. The size of glucose is about $5A^{o}$, which makes the cellulose molecule 50,000 A^{o}, or 5μ long.

The electron microscope has been the main instrument in resolving the ultimate structure of wood to our present state of knowledge. It is thus that cellulose molecules are known

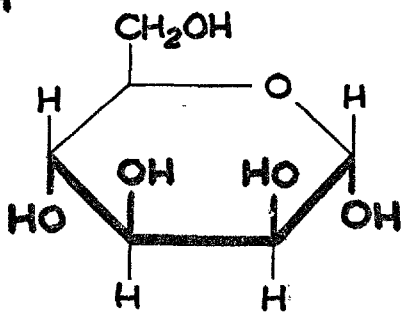
Fig. 6



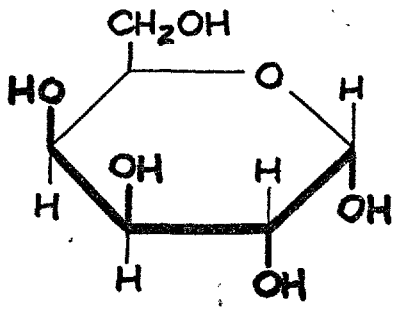
D-glucose



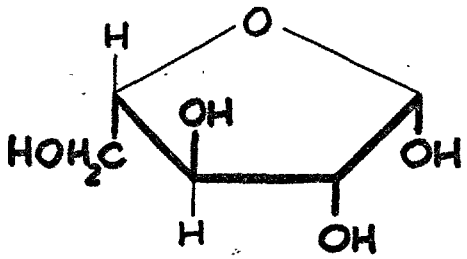
D-xylose



D-mannose

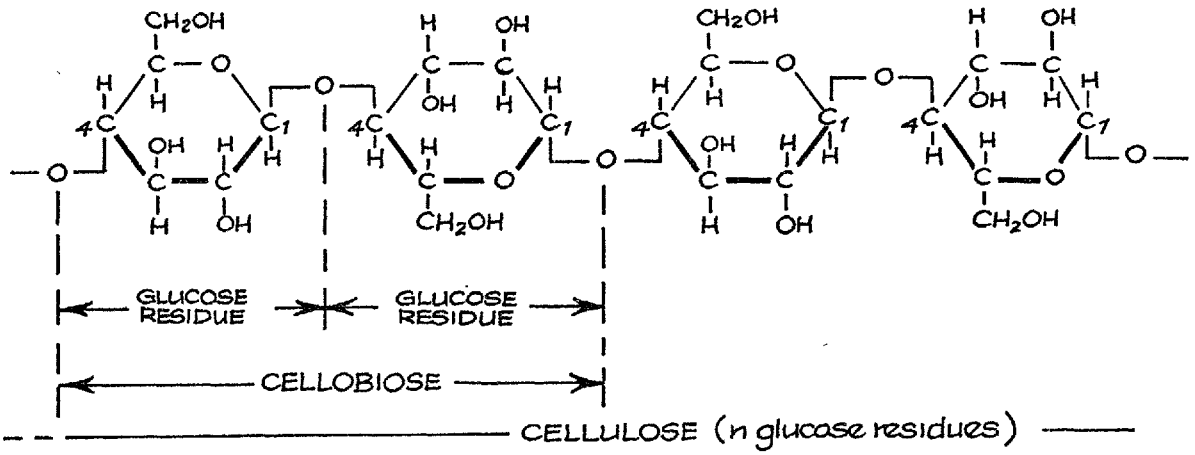


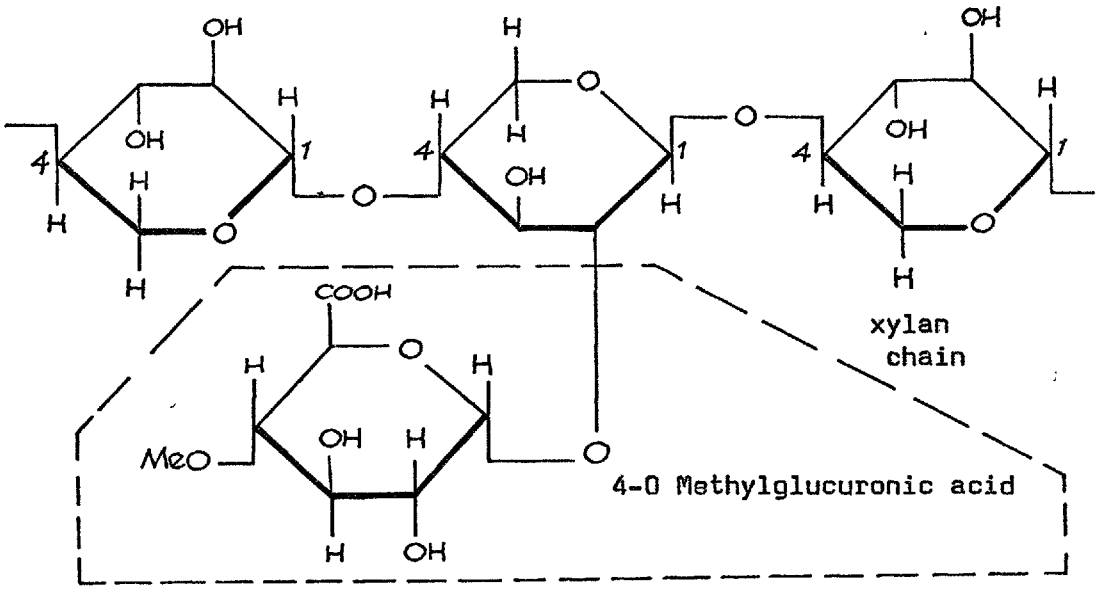
D-galactose

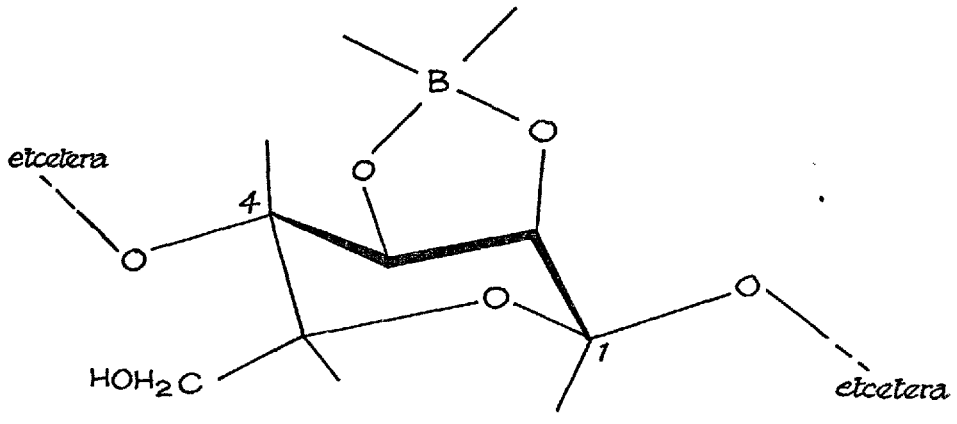


L-arabinose

Fig. 7.







The cis-hydroxyl groups of mannan combined with borate.

to be aggregated into bundles, or microfibrils of constant thickness, about 100A° in diameter and of indefinite length. From studies using X-ray diffraction and polarization microscopy, it is evident that cellulose is organized into discrete areas of crystallinity. These are termed micelles. Less organized or amorphous regions are also present, and since lignin and hemicellulose can be removed from the cell walls by chemical treatment without significantly altering the optical properties of cellulose, it has been assumed that these chemicals constitute the amorphous regions.

From X-ray diffraction data it is possible to estimate the dimensions of micelles in cell walls. The majority of workers agree that they are about 600A° long, 50-100A° wide, and perhaps half this distance in thickness. (Hengstenberg and Mark, 1928; Kratky and Sembach, 1955; Herzog, 1926; Kratky and Porod, 1949; Porod, 1952; and Heyn, 1955 a and b Manley, 1964 , however, has found that the average width of microfibrils is 30-40A°. It is significant to note the fact that when wood cellulose is subjected to heterogeneous hydrolysis microfibrils are degraded to cigar shaped rodlets of length 300-700A°. (Immergut and Rånby, 1956). It is this order of magnitude one would expect when preferential hydrolysis occurs in the amorphous regions leaving behind the highly crystalline and more resistant micelles.

From this evidence one would be justified in assuming that the cellulose microfibril consists of highly crystalline areas, interspersed with amorphous regions (most likely containing a high hemicellulose content). There is a continuity of cellulose

chains through these regions due to the length of the cellulose molecule (ca. 10,000A^{o}), which can thus stretch through from one crystalline region to another. The lignin in wood is considered to be deposited in the spaces between the microfibrils. The association between cellulose and lignin is thought to be physical rather than chemical. Thus it can be seen that wood is constructed in a manner analogous to that of ferro-concrete.

Microfibril orientation within the wood cell.

Within the wood cell microfibrils are mostly organised so that they form definite structural entities. These are digramatically represented in Fig. 8.

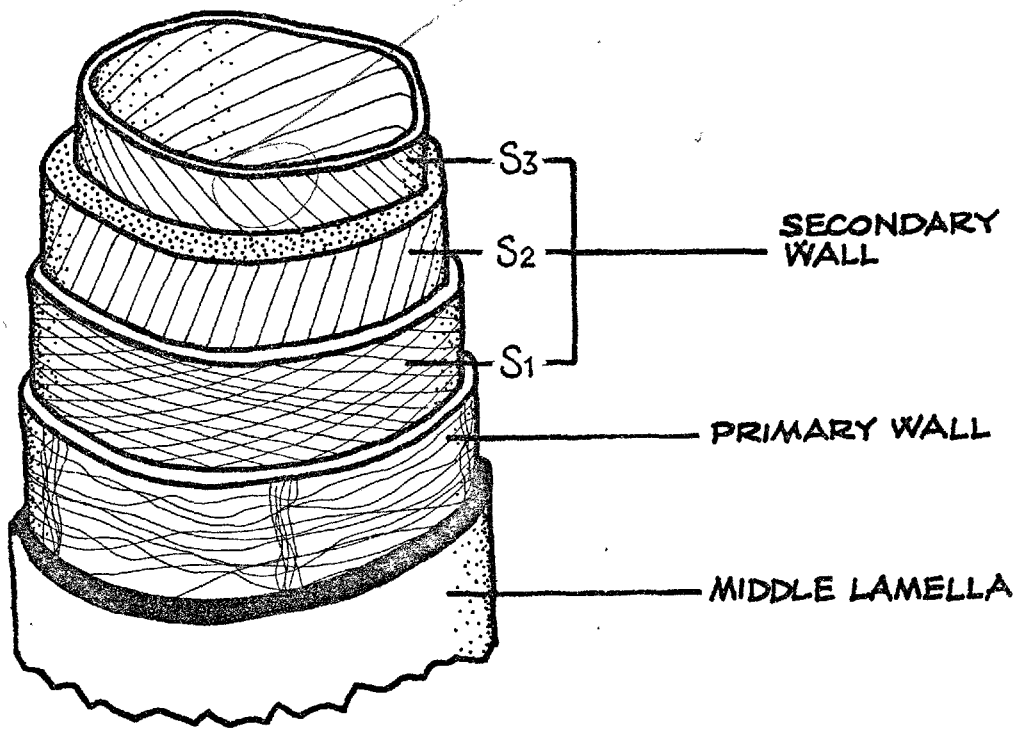


Fig. 8. - Diagram of part of a fibre or tracheid to shew the usual orientation of the microfibrils in the various layers of the wall. There are no microfibrils in the middle lamella. In P they are sparse, and their orientation is rather irregular, but generally approximating to the transverse plane, except that some may be nearly axial. In S_1 the microfibrils lie mainly in rather flat "S" helices, though there are some lying in much less conspicuous "Z" helices. In the S_2 the microfibrils lie in steeply pitched "Z" helices, while in S_3 they form flatter "S" helices. The transverse surface of the middle lamella is shewn black, and that of the S_2 is shaded, indicating their appearance as seen in the polarizing microscope. In contrast, P, S_1 and S_3 appear bright in transverse section.

(Because the terms "right-handed" and "left-handed", which have been used to define the direction of a helix, are ambiguous, the letters S and Z are often used instead. In an "S" helix the direction of the turns, on the side nearest the observer, is the same as the middle part of the capital S. A helix of the opposite sense is a "Z" helix.)

After Jane (1970).

Hemicelluloses

(1) Introduction

These are in general water insoluble, alkali soluble substances which are more readily hydrolysed by acid than is cellulose. They are an integral part of the plant cell wall, and cannot be extracted with great ease. That they may have a structural role, can be surmised from the fact that some stem tissues growing under unusual physical strain have abnormal amounts of certain hemicelluloses (Browning, 1963). Upon complete hydrolysis hemicelluloses yield D-Glucose, D-Mannose, D-Xylose, D-Galactose, L-Arabinose, and in the case of acidic hemicelluloses monomethyl glucuronic acid alone or oligosaccharides containing it. Unlike in some grasses and other herbaceous plant sources, the hemicelluloses of woody tissues are heteropolymers. None of the hypothetical homopolymers have been isolated (Perila and Bishop, 1961).

Although complex polysaccharide mixtures, hemicelluloses can be resolved into two distinct groups. One is composed of the glucuronoxylans, which consist of an essentially linear chain of xylose units to which methyl glucuronic acid units are attached as side chains. They are the chief hemicellulose of hardwoods, and a minor component in the softwoods. In softwoods arabinose units are part of the structure, as end units or side chains. The polymer is then termed an arabinoglucuronoxylan. Acetyl groups are combined as esters with sugar units in the xylans of hardwoods.

The other group consists of the glucomannans; they are the major part of the hemicellulose of softwoods and also are present

to a small degree in hardwoods. Galactose is very often present in the form of galactoglucomannan. The acetyl groups present in softwood are generally associated with glucomannan.

(2) Isolation.

Hemicelluloses are generally isolated from holocellulose that has been prepared from extractive free wood. Nelson and Schuerch, 1957, have shown that in the case of softwoods delignification is necessary. Occasionally they may be obtained directly from the wood; however, although this results in less structural degradation, there is also contamination with lignin and some extractives.

Extraction of holocellulose with aqueous alkalis will generally result in the removal of a large portion of the hemicellulose; however the remaining alpha-cellulose invariably retains xylose and mannose units. Recovery from the alkaline solution can be effected by precipitation in 2-4 volumes of alcohol, previously acidified with enough acid to neutralize the resultant mixture. Elimination of acetyl groups cannot be avoided when using an alkaline extraction. The effects of alkali can be kept to a minimum by excluding oxygen (a nitrogenous atmosphere will suffice), avoiding high temperatures, and employing the minimum of time necessary for optimum extraction.

(3) Alkaline solvents.

Aqueous potassium and sodium hydroxide have been found to be efficacious for extraction of hemicelluloses.

Potassium hydroxide has been found to be very favourable, because the potassium acetate formed on neutralization with acetic acid is more soluble after the addition of alcohol. Quite often it is desirable to perform a two step extraction, first with 5%, and then with 24% aqueous potassium hydroxide. This prevents the extreme swelling which occurs when holocellulose is directly treated with the concentrated solvent. It has been found that the xylans are readily solubilized by this treatment, but the glucomannans are only partially dissolved.

Sodium hydroxide is also used, a 5% solution removing predominantly the xylans. Stronger solutions can be used, for example 10% and 18%, but with increasing concentrations, the glucomannan content of the extract hemicellulose also rises. Alternate freezing and thawing will also increase the accessibility of the hemicellulose (Thomson and Kaustinen, 1964).

The relative dissolving power of sodium and potassium hydroxides depends on the nature of the hemicellulose, the temperature, and the concentration of the alkali. Generally speaking the xylans are equally extracted by either solvent, but the mannose-containing polymers require either successive treatments of 10 and 18% sodium hydroxide, or 45% Potassium hydroxide. (Hamilton and Quimby, 1957).

The addition of borates (Jones et al, 1956) markedly improves the extraction of mannose-containing polysaccharides, the structural requirement being the cis hydroxyl group

(see Fig. 7), whereby a cross-link with the borate ion $B(OH)_4^-$ may complex two adjacent molecular chains. Boric acid is generally used in 3-4% concentration, in conjunction with alkali.

(4) Selective successive extractions.

Quite good degrees of separation of hemicellulose components may be obtained by careful selection of a series of extractive solutions. When it is desired to retain acetyl groups, dimethyl sulphoxide (DMSO) and water are two excellent solvents; however, both are limited in the quantity of hemicellulose which they remove. (Hägglund et al, 1956).

Pine holocellulose has been extracted successively with dimethyl sulphoxide, boiling water, 14% potassium hydroxide, and 24% potassium hydroxide containing 3% boric acid (Croon et al, 1959).

In the extraction of western hemlock sulphite pulp, Hamilton and Quimby, 1957, recommended a primary extraction with 1.3N sodium hydroxide (ca. 5%), which gave a maximum yield of polysaccharides containing xylose, arabinose, galactose, rhamnose and uronic acids, followed by a series of extractions using 10-18% sodium hydroxide, which gave glucomannans. The glucomannans, however, are almost always contaminated with a minor quantity of xylan. It is only by repeated fractionations that the xylans can be removed.

Beelik et al (1965 & 7) have overcome this when using softwood holocelluloses. They impregnated the holocellulose with barium hydroxide prior to extraction with 10% potassium hydroxide. The Mannose-containing polymers were largely kept out of solution allowing the xylans to dissolve. After removal of the barium ions by dilute aqueous acetic acid the more soluble branched galactoglucomannans were then extracted with dilute sodium hydroxide, leaving behind the glucomannans to be extracted with 15% sodium hydroxide. The hemicelluloses were of such a standard of purity that further re-precipitation could be kept to a minimum.

(5) Purification.

The preparations obtained by alkaline extractions result in mixtures of polysaccharides, though generally with one predominant component. Further purification is tedious and can result in low yields. Several repetitions are generally necessary to achieve maximum purity. The most efficient methods involve reagents such as alkaline copper, barium hydroxide, and cetyl ammonium compounds.

(6) Copper complexes.

The anion $Cu(OH)_4^{=}$ will form insoluble complexes with a number of different polysaccharides. Timell and Tyminski (1957) utilized Fehling solution in the study of white spruce glucomannan. Fehling solution was added to an alkaline (+borate) extract of the wood. The thick blue precipitate was washed three times with distilled water and the washings

were then pooled. This fraction was then precipitated with ethyl alcohol. The main precipitate, now washed, was suspended in iced water prior to the addition of a mixture of acetic and hydrochloric acids to destroy the copper complex. The small amount of acid in soluble material was centrifuged off, and the glucomannan precipitated by addition of alcohol. The crude glucomannan was redissolved in alkali and the treatment repeated twice more, firstly with four water washes, and then with seven water washes. Each wash was of 2-3 hours duration. (See Fig. 9).

Fig. 9. Precipitation of some polysaccharides as metal complexes

Polysaccharide \ Precipitant	Fehling's solution	$Ba(OH)_2$ 0.03M	$Ba(OH)_2$ 0.15M	$Pb_2(OAc)_3OH$	$Pb(OAc)_2$
Galactomannan (guaran)	+	+	+	+	+
Glucomannan (softwood)	+	+	+	+	+
Galactan (compression wood)	-	(+)	+	+	+
Arabinoglucuronoxylan (softwood)	-	-	-	+	-
Glucuronoxylan (hardwood)	+	-	+	+	+
Acetylated glucuronoxylan (hardwood)	- → +	-	- → +	+	-
Arabinogalactan (larch)	-	-	-	-	-

After Meier from Lindberg (1961)

(7) Barium hydroxide.

In concentrations as low as 0.03M insoluble complexes with mannan-containing polysaccharides have been formed (Meier, 1958b). The vicinal cis 2-3 hydroxyl groups were probably responsible. The hemicelluloses were dissolved in water or dilute sodium hydroxide, and saturated barium hydroxide was then added. Any precipitate formed was centrifuged, dissolved in water and reprecipitated with barium hydroxide. The precipitate was then dissolved in 2N acetic acid, and the hemicellulose obtained by precipitation with ethanol. It was washed successively with ethanol-water, ethanol, and ether.

At higher concentrations acidic polysaccharides are also precipitated. Birch glucuronoxylan came down at 0.15M (Meier, 1958b) See Fig. 9.

(8) Cetyl ammonium compounds $C_{16}H_{31}N^{+}(CH_3)_3Br^{-}$

Cetyltrimethylammonium bromide has been used for fractionation of acidic polysaccharides (Jones, 1953, Swartz & Timell, 1963, Scott, 1955). Cetyltrimethylammonium hydroxide has been used by Kooiman and Adams (1961).

Crude hemicellulose (50g) was gradually added to water (2.5L) at $0^{o}C$ and stirred for three days. Undissolved material was removed by centrifuging and the supernatant made up to 4.5L with water. Addition of 2% aqueous Cevalon reagent (cetyltrimethyl-ammonium hydroxide) (1L) resulted in a precipitate which was allowed to settle overnight. The supernatent solution containing the galactoglucomannan and glucomannan was decanted leaving the glucuronoxylan precipitate.

Cetylpyridirium bromide, $C_{16}H_{31}N^{+}$  Br^{-}, can also be used in a similar way. (For a more comprehensive study of these compounds Lindberg, 1961 p. 72-73 is recommended).

(9) Characterization.

Hemicelluloses are characterized by specific rotation, hydrolysis and determination of constituent sugars by chromatography (Partridge, 1946), carbon dioxide yield from hexuronic acids, methoxy content, and behaviour in complexing solvents.

Of these methods the prime one used was the estimation of the constituent monosaccharides after hydrolysis. Hydrolysis is usually achieved by using mineral acids. Because of the easy removal of the acid as barium sulphate, sulphuric acid has found extensive application. Insoluble polysaccharides require a primary hydrolysis with strong acid to effect solution, followed by a secondary hydrolysis in weak acid at an elevated temperature. 72% sulphuric acid has been used as a first step, followed by 3-4% as a second. It is usually best to ensure that the reaction is followed by checking the reducing power of the hydrolysate until a maximum is reached. The reaction cannot be continued unduly for reversion reactions in acid solution destroy monosaccharides already formed. (See Appendix II).

Uronic acids present in the original polysaccharide are usually still linked to another sugar as an aldobiuronic acid (usually glucuronic acid and xylose). To determine the aldobiuronic acid structure, it is best to reduce their methylated esters to

the corresponding disaccharides which are readily hydrolysed to simple sugar derivatives (Whistler and Hough, 1953).

(10) Proof of Structure.

Once a polysaccharide is thought to be homogeneous and the individual sugars have been determined, the following information is needed: the number of free hydroxyl groups, the linkage between monomers, the kind of branching and distribution of branches in the structure, the molecular weight, the molecular weight distribution and the size and shape of the molecule.

The number of free hydroxyl groups is dependent on the nature of the constituent monosaccharides, and by complete methylation or acetylation (which can be checked by the absence of a hydroxyl peak in the infra red spectrum), followed by hydrolysis will reveal which bonds are used in linkages, and conversely which are normally hydroxyl groups.

Considerable information may be obtained by the use of glycol cleaving reagents such as sodium periodate or lead tetraacetate. For each glycol unit oxidized one atom of oxygen is required, e.g. 1 molecule of periodate is reduced to iodate (Bobbitt, 1956; Dyer, 1956; "Organic Reactions", 1944; Whistler and Smart, 1953 (b)). The two adjacent hydroxyl groups appear as free aldehydes; also oxidized are alpha-hydroxy-aldehydes, alpha-hydroxyketones, and alpha-hydroxy-hemiacetals. The acetal link is stable. Formaldehyde is the product of oxidation of a primary hydroxyl group, formic acid that from an aldehyde. From the comparison between the amount of periodate consumed and the

production of formaldehyde and formic acid much information can be obtained regarding the structure of the polysaccharide.

Much other information can be pieced together; for example the number of primary hydroxyl groups can be estimated by tritylation, cis hydroxyl groups cause gelation with borate solution, and adjacent free hydroxyl groups cause large rotations in cuprammonium solutions. Alpha and beta linkages can be determined by the direction of mutarotation during hydrolysis. Partial enzymic or acid hydrolysis will result in di-, tri-, and oligosaccharides that can be unequivocally identified with standards, using paper chromatographic separation techniques (Browning, 1963).

(11) Structure of Hemicelluloses.

(a) Xylans:

Much of the earlier studies of xylans were restricted to plant sources other than wood. Esparto grass xylan (Wethern, 1952, Haworth et al., 1929, Chanda et al., 1950, Aspinall et al. 1953a) was easily extracted and found to contain β 1;4 linked xylose units. L-Arabinose units were also found. Other plant sources of xylan were also found to contain arabinose in varying quantities, sometimes in high yields (Perlin, 1951 a & b).

The studies by Perlin on wheat flour xylan led to the conclusion that arabinose residues were attached to the xylan main chain either at the C-2 or C-3 position, or sometimes at both positions . Normally they are terminal units, but in the xylan of barley husks (Aspinall and Ferrier, 1957) side chains consisting of xylose linked β 1-2 to arabinose have been found, the probability being that the arabinose

is connected to the main chain in the usual way. Arabinose is only a minor constituent of angiosperms, but can be regarded as a significant portion of gymnosperms. For example it has been found in European larch in significant quantities. (Aspinall and McKay, 1958; Aspinall and Carter, 1956). Xylans containing arabinose have been reported in applewood (Gerhardt, 1929) and also in white spruce bark (Painter and Purves, 1959). In the applewood the xylose to arabinose ratio was found to be 7:1 though this ratio will vary considerably depending upon the source.

All the evidence to date suggests that xylan occurs almost universally as β1:4 linked anhydropyranose units either slightly branched, or in a linear chain. Aspenwood xylan has two or three branches per molecule at C3 (Zinbo and Timell, 1965). There are certain exceptions among the seaweeds where 1:3 links have been found (Björndal et al, 1965 Percival and Chanda, 1950).

In timber the xylan backbone is modified by the presence of other groups. Glucuronic acid, galacturonic acid and 4-O-Methylglucuronic acid are the most common substituents found in wood xylans. Rhamnose has been found closely associated with birch xylan, very probably connected by glycosidic linkage (Shimizu and Samuelson, 1973). The 4-O-Methylglucuronic acid group is predominant in hardwoods, usually linked via the C-2 position. Xylose to uronic acid ratios vary from 3:1 to 20:1 but mostly the values fall between 7:1 and 12:1. It is not known whether the groups are in random or strict sequence.

Shimizu and Samuelson have found galacturonic acid moieties to be integral parts of birch xylan, probably linked to the non-reducing terminal xylose units. The galacturonic : 4-O-Methyl-glucuronic acid ratio was 1:5, indicating the presence of three galacturonic residues per molecule of D.P.164. Since the galacturonic acid was C-4 linked to a xylose unit it was thought possible that the acid was linked to a non-reducing xylose moiety, thus providing strong circumstantial evidence to show that the xylan was slightly branched.

Xylans have been studied from the following wood sources; in birch, (Gustafsson et al, 1952, Timell, 1962, 1967a, Timell and Jahn, 1951, Anderson et al, 1940, Bouveng et al, 1960, Bouveng, 1961, Lindberg et al, 1973, Saarnio et al, 1954; Shimizu and Samuelson, 1973; in poplar (Jones et al, 1961, Zinbo and Timell, 1965; in larch, Aspinall and McKay, 1958); in white elm (Gillham and Timell, 1958 a and b); in Western hemlock (Hamilton and Thomson, 1957); in Norway spruce (Aspinall and Carter, 1956, Zinbo, 1968); in trembling aspen (Milks & Purves, 1956); in beech (Aspinall et al, 1954, Adams, 1957a); in applewood, (Dutton and Murata, 1961); in tamarac, (Hoffmann and Timell, 1972); in pine, (Harwood, 1972); in hornbeam, (Ebringerova et al, 1969). Springer and Zoch, 1968, concluded from studies after hydrolysis that the xylans from the four hardwoods Populus tremuloides, Betula papyrifera, Ulmus americana, and Acer rubrum, were all the characteristic xylan structure.

Evidence from studies on birch xylan extracted with DMSO (Bouveng, 1961, and Bouveng et al, 1960, Hägglund et al, 1956) showed 11.8% uronic acid groups. The O-acetyl content was 17%. 58% of the xylose residues were free of acetyl groups, 24% contained one acetyl group at the C-3 position, 12% at the C-2 position and 6% had acetyl groups upon both C-2 and C-3 positions. It appeared that acetylation was confined to the xylose backbone and did not extend to the uronic acid residues.

Lindberg et al, 1973, comprehensively modified the technique of Bouveng, 1961, and thus improved the reliability of the location of O-acetyl groups by reducing most of the O-acetyl migration to a thermodynamic equilibrium. They concluded that the earlier papers were in error, and that the correct distribution pattern was: non-acetylated xylose residues, 44%; acetylated in the C-2 position, 24%; C-3 position, 22% and; both C-2 and C-3 positions 10%. It was the opinion of these workers that in the biological state the C-2 position dominates.

Meier, 1961a, Koshijima, 1960, Annergren and Croon, 1961 have shown that pine arabinoglucuronoxylan differs from that of a hardwood in that it contains little if any acetyl groups.

It is interesting to note the effects that the presence of O-acetyl groups have on the solubility of a hardwood xylan. When subjected to Fehling's solution and 0.15M $Ba(OH)_2$, they change from soluble to insoluble as the hemicellulose is de-acetylated, but in dilute 0.03M barium hydroxide they remain in solution. Thus it can be seen that hemicelluloses, when in the naturally acetylated state, are considerably more soluble than the same compounds obtained by alkaline extraction.

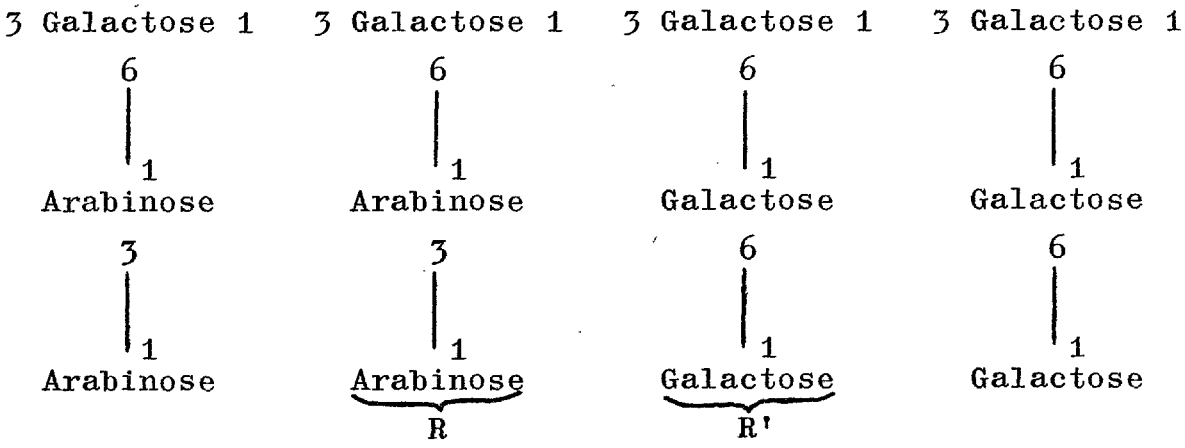
Glucuronic acid residues are normally linked to the C-2 position of a xylose unit (by an α link, Bishop, 1953 a + b) in the middle of a chain; however, it could be the case that uronic acid residues end up the xylan chain. It is thought that this is the case in oak heart wood (Hirst, 1961) See Fig. 7.

The length of the main xylan chain has been estimated as being between 50 and 300 xylose units. This is the degree of polymerization or D.P. It is generally considered that the higher values (about 200) are more likely due to the vigorous nature of the extraction procedure involved. There is evidence for a single branch point, both in esparto grass xylan and that of Lolloby Pine, where three xylose end groups are present per molecule. Similar branching may also occur in xylans from beech, jute, milkweed floss and kapok (Whistler, 1950), Rhamose has been found as a minor component in hardwood xylans. (Geerdes and Smith, 1955, Aspinall et al, 1954, Gorrod and Jones, 1954).

(b) Arabinogalactans:

Arabinogalactans represent an exception in softwood hemicelluloses, in that they may be extracted directly from the wood with water. Much work has thus been achieved. They have been found in spruce and fir (Thompson et al, 1953); in spruce (Bouveng and Meier, 1959, Adams, 1957b and 1958) in pine (Ball et al, 1956, Jones and Painter, 1957, Bishop, 1957, Brash and Jones, 1959, Wadmann et al, 1954, Meier, 1958(a));

in Japanese larch (larix leptolepis) (Aspinall et al, 1968) in both European and Western larch,(Adams, 1959, Aspinall et al, 1958a, Aspinall and Nicholson, 1960, Aspinall et al, 1959 Bishop, 1956, Bouveng, 1959 a + b, Bouveng and Lindberg, 1956, 1958, Campbell et al, 1948, Hirst, 1955, Jones, 1953, Peterson et al, 1940, Whistler and Smart, 1953a,White, 1942, a, b + c. The studies by the above lead to the conclusion that arabinogalactans are highly branched structures with the general features shown below.



The proportionsof R to R' are such that 1 in 12 of the galactose residues carried a side chain containing arabinose.

(c) Glucomannans and galactoglucomannans.

These are mainly found in the woods of the conifers, though they can be present to a lesser extent in hardwoods. As their name suggests these polymers contain mannose and glucose (Rebers and Smith, 1954, Smith and Srivastava, 1956); and Andrews et al, 1953, 1956).

Glucomannans have been studied in the following sources: in aspen wood, (Jones et al, 1957, Sharkov, 1970), in birch, (Timell, 1959, 1960), in cedar wood, (Hamilton and Partlow, 1958), in western hemlock, (Hamilton and Kircher, 1958, Hamilton et al, 1956); in larch, (Aspinall, 1959, Hashi et al, 1971, Aspinall et al 1962), in pine, (Anthis, 1956, Aspinall, et al, 1962, Bishop and Cooper, 1959, and 1960, Brash and Jones, 1959, Croon et al, 1959, Jones and Painter, 1957, 1959, Leech, 1952, Yundt, 1951, Gyaw and Timell, 1960, Meier, 1958 (a), and 1960a, Hoffmann and Timell, 1970, Hamilton et al, 1960); in spruce (Aspinall et al, 1957, Croon and Lindberg, 1958, Adams, 1957b, Dutton and Hunt, 1958, Hess and Lüdtke, 1928, Husemann, 1940, Merler and Wise, 1958, Timell and Tyminski, 1957, Lindberg and Meier, 1957); in tamarac, (Hoffmann and Timell, 1972, Kooiman and Adams, 1961); in Red maple, (Mian and Timell, 1960); in hornbeam (Ebringerová et al, 1972).

On hydrolysis glucomannans from conifers give glucose to mannose ratios, which vary between 1:1 and 1:4. They have been found to be linear molecules linked β1:4 as a random co-polymer. Blocks of similar residues may occur as well as single alternating residues. Oligosaccharides of two, three or more residues in many different orders have been isolated from partial digests of glucomannans. Where they have been studied in hardwoods e.g. pine, birch and maple (see above), the evidence suggests a similar structure.

Where galactose residues are present, they have been found after methylation and hydrolysis, as tetramethyl D-galacto-pyranose, thus indicating occurrence as end groups. When subjected to partial hydrolysis, Norway spruce hemicellulose (Croon and Lindberg, 1958, Lindberg and Meier, 1957 and Meier, 1960) gave rise to a disaccharide Gal α-1:6Mann β-1:4 Mann.

Hamilton and Partlow, 1958 found in red cedar wood that the glucomannan gave the following oligosaccharide fragments; glucose-mannose, mannose-mannose, mannose-glucose, glucose-glucose, mannose-mannose-mannose, connected by β-1:4 links.

Timell, 1961 has proposed that glucomannans contain varying amounts of galactose but are rarely ever free of this sugar. Meier, 1960 & Hägglund et al, 1956, report that in Norway spruce hemicellulose, the acetyl groups are attached to the glucomannan system and not the xylan. Branching occurs in glucomannans of some species; for example hemlock, Norway spruce, Scots pine and Loblolly pine, may have up to three branch points per 100 sugar units. The D.P. varies from 40 to 100, but most workers agree that the higher figure is more likely owing to the nature of the extraction methods.

(d) acidic glucans

Hoffmann and Timell, 1970, 1972, have studied the polysaccharides of tamarac compression wood. They isolated and characterized an acidic glucan which was termed 'laricinan', and comprised 2-4% of the wood. The evidence showed that the glucan consisted of a minimum of 200 β D-glucose residues most of which were linked by 1-3 glucosidic bonds. The remaining residues, ca. 6-7% of the total, were joined by 1-4 links. Glucuronic acid and a few galacturonic acid residues were also present but their exact number and mode of attachment to the glucan backbone was not determined. The main chain was branched in about eight places per macromolecule of 200 units. The size in the natural state was estimated to be about 300 residues. Larcinan was also found in the compression wood of red spruce and balsam fir and in smaller quantities in the normal wood of balsam fir. In normal wood it appears to be concentrated in the ray cells.

In the second paper of this series by the same authors, 1972b, isolation and determination of the structure of the compression wood xylan was effected. They concluded that the xylan was similar to normal larch xylan but possessed only half as many side chains.

In the third paper, 1972c, they established that the constitution of the compression wood galactoglucomannan was the same as that of normal wood.

(e) (acidic) galactans

Galactans comprise some 10-11% of compression wood. Compression wood galactans have been studied by; Hägglund and Larsen, 1937, Stockman and Hägglund, 1948, Bouveng and Meier, 1959, Schreuder et al, 1966, Coté et al, 1968.
Recently Jiang and Timell, 1972, in the fourth paper on tamarac compression wood, investigated the constitution of an acidic galactan. It was found that the usual techniques of hemicellulose extraction were obviated by simply recovering the material in the chlorite liquors. It was found to be a chain of 200-300 β1-4 linked galactose residues, occasionally branched at the C-6 position. One in twenty galactose units carried a single terminal β galacturonic acid residue attached to the C-6 position.

It is interesting to compare this with galactans isolated from tension wood. Meier, 1962, was the first to isolate and characterise a galactan from tension wood. He prepared from beech a complex product which was thought to be homogeneous. The relative neutral sugar compositions were; galactose 69.3%, rhamnose 20.5%, arabinose 6.0% and xylose 4.2%. The galactan was unique amongst the hemicelluloses isolated to date in that it was found to contain both β-1-4 and β-1-6 linked galactose residues.

Kuo and Timell, 1969, isolated a galactan from a different species of birch tension wood with a similar neutral sugar composition of galactose 62.4%, rhamnose 22.2% arabinose 9.7%, xylose 5.7%. Their studies showed that several structures

were possible, but it was suggested that the most probable consisted of a backbone of β-1-4 linked galactose residues, about half of which were substituted at C-6 with side chains of various kinds. The side chains were partly comprised of β-1-6 linked galactose residues, some of which were substituted at their C-6 end units with a terminal glucuronic or 4-O-Methylglucuronic acid unit. Other side chains were, either 1-4 linked L-rhamnose residues substituted at C-2 by an α link to galacturonic acid units, or 1-5 linked arabinose residues. Most of the arabinose was found to be terminal while some were substituted at C-5. The D.P. was 350, and it was highly branched.

Both tension and compression wood galactans are based on a main chain of β-1-4 linked galactose residues substituted at C-6 and both contain galacturonic acid. The difference lies in the constitution and complexity of the side chains, for example, the compression chains being linked β-1-4 and the tension β-1-6 linked.

Chemical distribution in the microstructure of wood.

Cowling, 1961, has stated: "A major justification for fundamental research in the field of wood deterioration is the increasing use of wood destroying organisms and isolated microbial enzymes in the studies of lignin and cellulose structure (Nord and Schubert, 1957; Pew, 1957) and wood anatomy (Asunmaa, 1955; Bailey and Vestal, 1937, Meier, 1955; Reese and Mandels, 1959). The specificity of microbial enzymes and the very mild conditions under which their reactions proceed, make them potentially ideal reagents for delicate studies of structure (Reese and Mandels, 1959). More complete characterization of the enzymes of wood-destroying fungi, and their effects during decay, would allow these methods to be applied with greater precision and confidence".

Within the plant cell wall the xylan distribution has been measured by allowing cell walls to react with bases that form coloured salts (Asunmaa and Lange, 1952). The bases react with carboxyl groups and the intensity of the coloured salt is seen across a cell wall. The colour was found to be high near the primary wall and decreased linearly across the wall to the lumen. In this study there was an assumption that the content of carboxyl groups was even throughout the hemicellulose.

When subjected to a suitable agent, wood fibres swell in a characteristic fashion. Certain portions of the outer layers of the fibres remain relatively intact causing restrictions, which can be separated in small quantities.

Upon hydrolysis and chromatography, these portions were predominantly found to contain mannose, with small amounts of glucose present. It appears therefore that mannan can be concentrated into small regions of the fibre. (Wise et al, 1949; Lewis et al, 1940).

Another approach to solving the problem of polysaccharide distribution has been that of comparing wood tracheids at various stages of development. (Lindberg and Meier, 1959). Mature cells contain M.L., P, S1, S2 and S3 layers (See Fig. 10). Immature tracheids contain M.L., and P.; some contain M.L., P, and S.; some contain M.L., P., S1 and S2. When separated thus and analysed for monosaccharides, it was found that araban, galactan, and pectic acids were concentrated in M.L. and P. Meier studied three types of wood, birch, spruce, and pine and found a marked difference in the arabinan content between the birch and the softwoods in the M + P layer. In birch it is half that of pine. When, however, the xylan content was analysed the reverse was found to be true. In birch the xylan concentration seems to be high in the S_1 and that part of the S_2 nearest the S_1, but fell slightly as the S_3 layer was approached. The cellulose content in birch was highest in the S_2 and S_3 layers. In pine the outer portion of the S_2 layer was richest in cellulose, and the glucomannan content increased steadily from the outside inwards. With pine and spruce the glucurono-arabinoxylan content of the S_3 and S_1 was high but was slightly lower in the S_2. Of note is the fact the

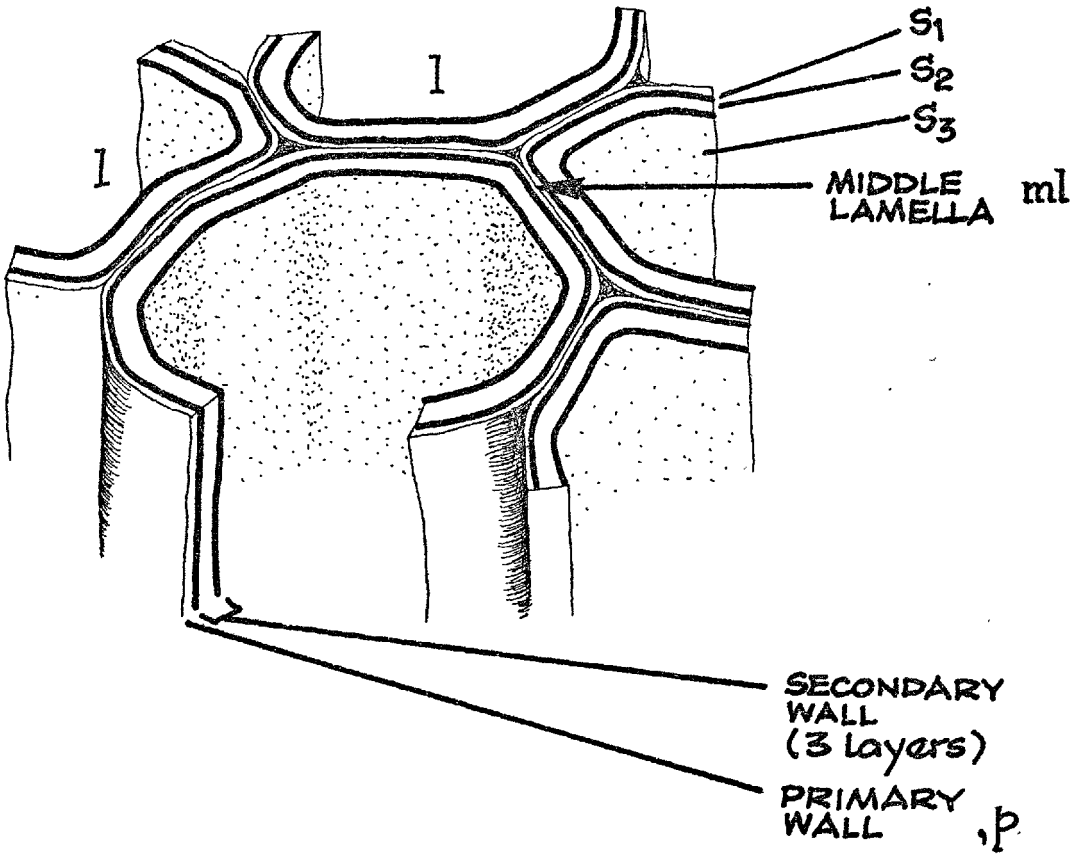


Fig. 10. Diagram of the layers of the cell wall of a woody cell. The middle lamella is shown in black, followed by the primary wall (white), the outer layer (s_1) of the secondary wall (black), the middle layer (s_2) of the secondary wall (white) and the inner layer (s_3) of the secondary wall (stippled), which surrounds the lumen of the cell. m.l., middle lamella; p., primary wall; s_1, s_2, s_3, successive layers of the secondary wall; l., lumen of cell.

arabinan content of the secondary walls always seems very low. Where galactoglucomannans are known to be a constituent hemicellulose, namely in the softwoods pine and spruce, galactan values for the S_1 layers are much higher than in birch. (See Fig. 11).

It was stressed that this was only correct if the polysaccharide content of each layer remains constant once it has been formed.

When compression wood was examined, it was found that an unusally high concentration of galactan was present. This was also true of the lignin concentration (Stockman and Hägglund, 1948; Bouveng and Meier, 1959).

As previously mentioned, there is an anatomical difference between springwood and summerwood. This difference is clearly reflected in the chemical composition as well. The main structural difference is the larger S_2 layer of the summer wood. The proportion of middle lamella region is larger in springwood, giving it a higher lignin content. Thus summerwood has a higher glucomannan and lower glucuronoarabinoxylan content than springwood.

Fig. 11. Percentages of polysaccharides in the different layers of the fibre wall.

Polysaccharide	M+P*	S_1	S_2 outer part	S_2 inner part + S_2
Birch				
Galactan	16.9	1.2	0.7	0.0
Cellulose	41.4	49.8	48.0	60.0
Glucomannan	3.1	2.8	2.1	5.1
Arabinan	13.4	1.9	1.5	0.0
Glucuronoxylan	25.2	44.1	47.7	35.1
Spruce				
Galactan	16.4	8.0	0.0	0.0
Cellulose	33.4	55.2	64.3	63.6
Glucomannan	7.9	18.1	24.4	23.7
Arabinan	29.3	1.1	0.8	0.0
Glucurono-arabinoxylan	13.0	17.6	10.7	12.7
Pine				
Galactan	20.1	5.2	1.6	3.2
Cellulose	35.5	61.5	66.5	47.5
Glucomannan	7.7	16.9	24.6	27.2
Arabinan	29.4	0.6	0.0	2.4
Glucurono-arabinoxylan	7.3	15.7	7.4	19.4

*Contains also a high percentage of pectic acid.

After Meier (1961) (b)

Liang et al, 1960, have used polarized infra red spectrometry to identify xylans in the cystalline state.

Boutelje, et al, 1971 treated spruce holocellulose with a purified xylanase enzyme. The extent of hydrolysis of the xylan was determined by chemical analysis and also followed by interference microscopy. It was shown that 20-30% of the xylan fraction of the holocellulose was specifically hydrolysed and removed from the fibre wall. The interference microscopy revealed the mass losses occurring at different positions on the fibre cross section. Most of the loss occurred in the S_2 layer.

Perila, 1961, studied the ray cells of spruce, pine and birch woods. He found that in the softwoods the rays contained about the same amount of hemicellulose as the tracheids, but that the latter contained more mannan and less xylan. In birch wood however, he found startling differences in composition. The xylan content in the vessels was found to be about two thirds that of the cellulose; however, in the rays there was four times more xylan than cellulose.

The chemical composition of reaction wood is very different from the norm, as might be suggested by the large morphological differences observed. In pine the lignin content of compression wood can be as high as 40%. In the table overleaf (Meier, 1961b), differences in carbohydrate composition can be seen, that of galactan being most prominent. It has been found that the spruce galactan is β-1:4 linked into a linear chain (Bouveng and Meier, 1959). This is very similar to the composition of pectin.

Carbohydrate composition of normal wood and compression wood from spruce (Picea abies).

	Galactose	Glucose	Mannose	Arabinose	Xylose
Normal wood	2.1	70.7	17.2	1.3	8.7
Compression wood	19.6	55.6	11.5	2.6	10.7

The composition of tension wood fibres has been studied (Meier, 1961b) and is given below. There is also a lower lignin concentration.

Carbohydrate composition of normal wood and tension wood from beech (Fagus Silvatica L.)

	Galactose	Glucose	Mannose	Arabinose	Xylose
Normal Wood	1.6	55.4	4.9	1.0	35.1
Te sion Wood	6.6	73.5	-	2.6	17.3

The higher cellulose, the lower lignin and the lower xylan content can be rationalized by examining microscopically sections of tension wood. It is then seen that there is a gelatinous layer

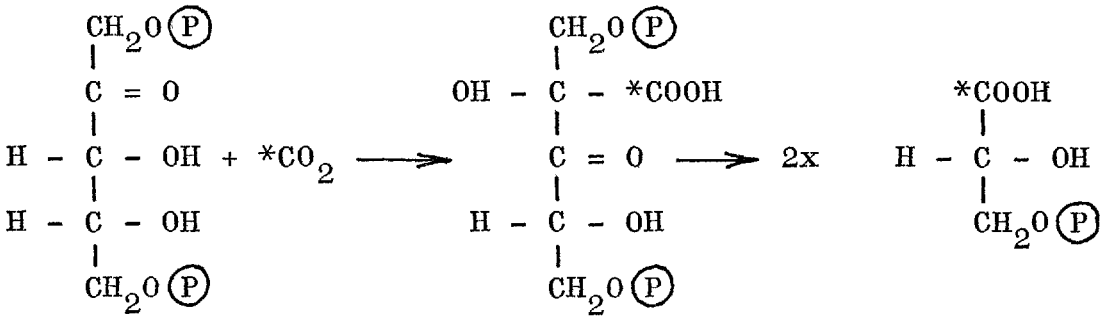
bordering the lumen almost entirely constructed of cellulose. Presumably this considerably increases the tensile strength.

The distribution of lignin throughout the fibre has been exhaustively studied. Staining reagents, dissolution of non-lign ified material, microdissection with chemical analysis, ultraviolet, and fluorescence microscopy have all been used. 60 to 90% of the lignin is in the M.L. + P. region. In soft woods it is nearer 60% and in hardwood nearer 90%. One interesting way of obtaining a visual picture of lignin distribution is that of subjecting the wood to a brown rot fungus. This type of fungal attack will remove most of the cellulose and hemicellulose leaving behind a lignin skeleton, which may be seen in sections of the decayed material.

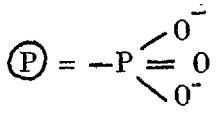
Metabolism of carbohydrates in wood.

Carbohydrate metabolism in trees has not been extensively studied, no doubt due to the practical difficulties involved with the trees' size. However, metabolic pathways in smaller plants and in fungi have received considerable attention, thus giving a good indication of possible pathways in trees. It is known that D-glucose, D-mannose, D-galactose, 3-O methyl galactose, D-fructose, L-arabinose, D-xylose, 2-O methyl xylose, L-rhamnose, L-fucose, 2-O methyl fucose, glucuronic acid, 4-O methyl, glucuronic acid, and galacturonic acid, are all found combined in tree polysaccharides. In the free form glucose, arabinose, fructose, rhamnose, ascorbic acid, hexitols, heptitols, octitols, sucrose, and sucrose derivatives, are all found.

3-Phosphoglyceric acid (P.G.A.) has been shown by Calvin (1962) to be one of the first stable products of photosynthesis in Chlorella;



ribulose diphosphate — erithro pentulose diphosphate — 3 phospho glyceric acid (P.G.A.)



From the equation above it can be seen that P.G.A. can be converted to the aldehyde, and thus to hexose diphosphate. The diphosphate can be dephosphorylated to give fructose phosphate. Pathways to the other hexoses, to organic acids, to fatty acids, and toamino acids are then available.

Mahler and Cordes, 1966 have summarized the various different reactions involved in the carbohydrate metabolism of carbon dioxide assimilation. (See Fig. 12).

Higher plants are sometimes confronted with the problem of converting fats or 2-carbon metabolites (and hence Acetyl Co A) into carbohydrates and cell constituents, e.g. in seed germination. This is done by means of co-ordinated reaction described by Kornberg, 1965. There are two key enzymes. (i) isocitrase, (ii) malate synthetase, which are absent from animal tissues. Also required are three enzymes from the citric acid cycle: (i) citrate synthase, (ii) aconitase,

Fig. 12. The overall scheme, or "Calvin-Bassham cycle", is shown below. It is thought to be made up of the following reactions (names of enzymes are shown in italics, carbon skeleton rearrangements are indicated in brackets):

6-Ribulose-1,5-diP+6CO_2+6H_2O → 6(intermediate) → 12 glycerate-3-P
($6C_5$+$6C_1$ = $12C_3$) carboxydismutase (CD)

12 Glycerate-3-P+12ATP ⇌ 12 glycerate-1,3-diP+12ADP
($12C_3$ - $12C_3^!$) phosphoglycerate kinase

12 Glycerate-1,3-diP+12NADPH ⇌ 12 glyceraldehyde-3-P
+12$NADP^+$+12Pi
($12C_3^!$ - $12C_3^{!'}$) D-glyceraldehyde phosphate dehydrogenase (NADP specific)

5 Glyceraldehyde-3-P ⇌ 5 dihydroxyacetone-P
($5C_3^{!'}$= $5C_3^{!''}$) triosephosphate isomerase

3 Glyceraldehyde-3-P + 3 dihydroxyacetone-P → 3 fructose-1,6-diP
($3C_3^{!'}$+ $3C_3^{!''}$= $3C_6$) aldolase

3 Fructose-1,6-diP+3H_2O → 3 fructose-6-P+3Pi
($3C_6$ = $3C_6^!$) phosphatase (specific?)

2 Fructose-6-P+2 glyceralde-3-P ⇌ 2 erythrose-4-P + 2 xylulose-5-P
($2C_6^!$ + $2C_3^{!'}$ = $2C_4$ + $2C_5$) transketolase (TK)

2 Erythrose-4-P + 2 dihydroxyacetone-P ⇌ 2 sedoheptulose-1,7-diP
($2C_4$+$2C_3^{!''}$= $2C_7$)

2 Sedoheptulose-1,7-diP+2H_2O → 2 sedoheptulose-7-P+2Pi
($2C_7$ = $2C_7^!$) phosphatase (specific??)

2 Sedoheptulose-7-P+2 glyceraldehyde-3-P ⇌ 2 ribose-5-P
+ 2 xylulose-5-P
($2C_7^!$ + $2C_3^{!'}$ = $2C_5^!$ + $2C_5^{!'}$) transketolase (TK)

2 Ribose-5-P $\rightleftharpoons$ 2 ribulose-5-P
($2C_5^{I} = 2C_5^{I''}$) phosphopentose isomerase

4 Xylulose-5-P $\rightleftharpoons$ 4 ribulose-5-P
($4C_5^{I'} = 4C_5^{I''}$) phosphoketopentose epimerase

6 ribulose-5-P+6ATP $\rightarrow$6 ribulose-5-diP+6ADP
($6C_5^{I''} = 6C_5$) phosphopentokinase

SUM

6 Ribulose-1,5-diP+$6CO_2$+18ATP+12NADPH $\rightarrow$
6 ribulose-1,5-diP+fructose-6-P+18ADP+17Pi+12NADP
($6C_5+6C_1 = 6C_5+C_6$) H_2O not shown)

or, after hydrolysis of the hexose phosphate, schematically,

$CO_2+3ATP^{4-}+2NADPH+3H_2O \rightarrow$
1/6 hexose+$3ADP^{3-}+3Pi^{=}+2NADP^{+}+H^{+}$

After Mahler and Cordes (1966)

(iii) malate dehydrogenase, and the electron transport chain for the oxidation of NADH by molecular oxygen. Two molecules of acetyl CoA yield one molecule of succinate simultaneously removing two reducing equivalents. Succinate thus produced may be converted to carbohydrate. (See Fig. 13).

Some work on pine seedings by Krotkov et al (1961) shows that sucrose is the main water soluble product, when the seedings are allowed to photosynthesize in a $^{14}CO_2$ atmosphere for eight hours. (See Fig. 14).

Sucrose is a major component of the sieve tube fluid with some incidence of higher molecular weight sugars such as raffinose, stachyose, and verbascose. These are derivatives of sucrose and contain alpha linked galactose residues; however free galactose does not appear.

Fructose $\overset{\beta\alpha}{2:1}$ Glucose $\overset{\alpha}{6:1}$ Galactose $\overset{\alpha}{6:1}$ Galactose $\overset{\alpha}{6:1}$ Galactose

Sucrose

Raffinose

Stachyose

Verbascose

It is of interest to note the similarity in links with that of galactoglucomannan.

The synthesis of various sugars from carbon dioxide can be demonstrated and followed by ^{14}C labelling. Andrews and Hough, 1958, examined plum leaves which had been kept in the dark for 24 hours, then allowed to photosynthesize in the presence of

Fig. 13

The Glyoxylate Cycles.

Acetyl CoA+oxaloacetate+H_2O	$\rightarrow$ citrate+CoASH	$\Delta G^{o'}$=-7.5kcal/mole
Citrate	$\rightleftharpoons$ D_s-threoisocitrate	$\Delta G^{o'}$ _0
D_s-threoisocitrate	$\rightleftharpoons$ succinate+glyoxylate	$\Delta G^{o'}$=2.11 kcal/mole
Acetyl CoA+glyoxylate+H_2O	$\rightarrow$ malate+CoASH	$\Delta G^{o'}$=-10.63 kcal/mole
Malate+NAD^+	$\rightleftharpoons$ oxaloacetate+ NADH+H^+	$\Delta G^{o'}$=6.69 kcal/mole
NADH+$\frac{1}{2}O_2$+H^++(3ADP+3P_i)	$\rightarrow$ NADH+H_2O+ (3ATP)	$\Delta G^{o'}$=-54.4 (or -29) kcal/mole

---

2AcetylCoA+$\frac{1}{2}O_2$+(3ADP+3P_i)	$\rightarrow$ succinate+2CoASH+(3ATP)
Succinate+$\frac{1}{2}O_2$+2P_i+2ATP	$\rightarrow$ fumarate+H_2O+2ATP
Fumarate+H_2O	$\rightleftharpoons$ malate
Malate+NAD^+	$\rightleftharpoons$ oxaloacetate+NADH+H^+
Oxaloacetate+ATP	$\rightleftharpoons$ phosphoenolpyruvate+ADP+CO_2
Phosphoenolpyruvate+NADH +ATP	$\rightarrow$ $\frac{1}{2}$hexose+NAD^++ADP+2P_i

---

Net: 2acetylCoA+O_2+H_2O $\rightarrow$ $\frac{1}{2}$hexose+CO_2+CO_2+2CoASH

or, from acetate,

2 acetate+O_2+H_2O $\rightarrow$ $\frac{1}{2}$hexose+CO_2+2H_2O

Fig. 13 After Mahler and Cordes (1966)

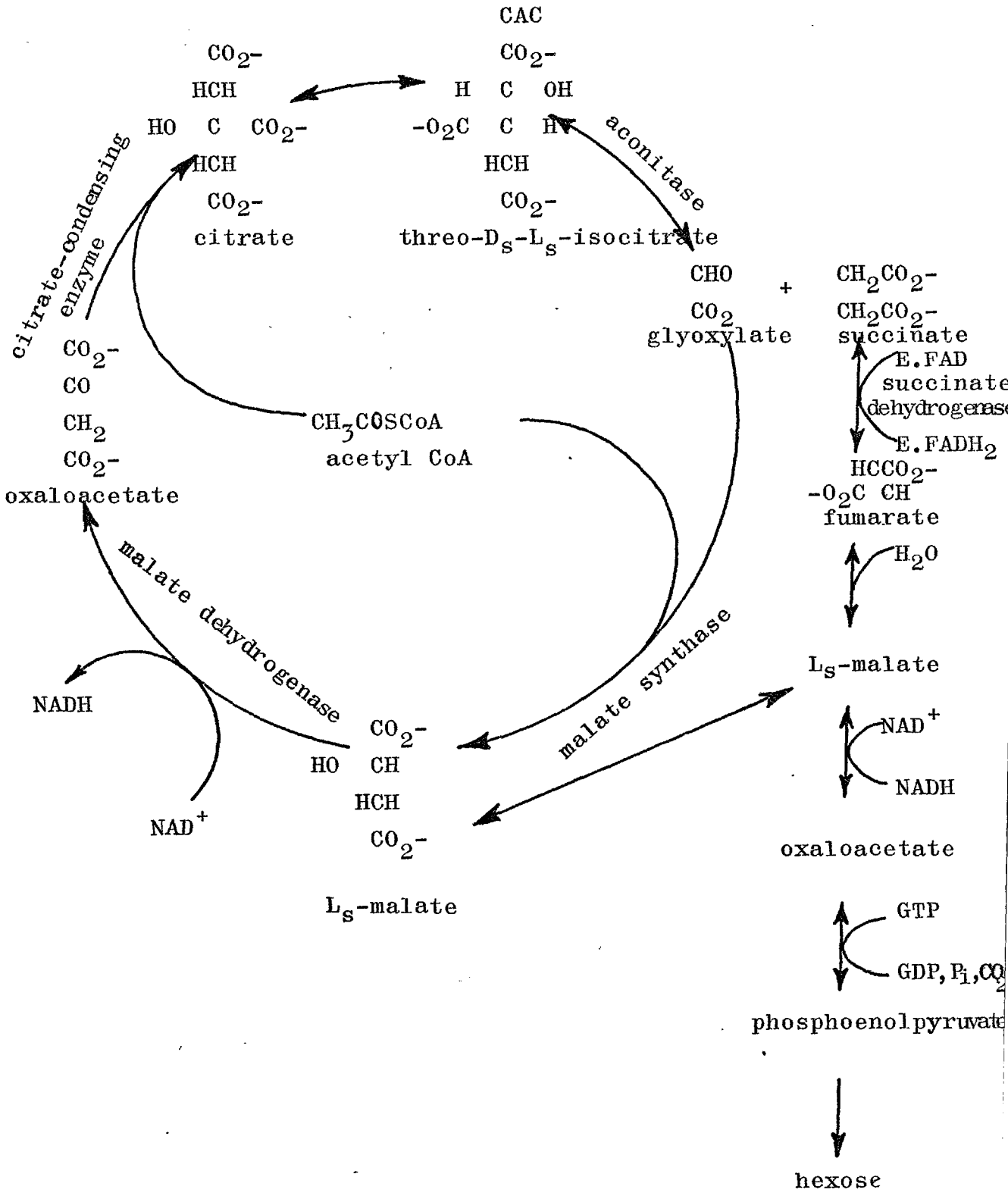


Fig. 14.2500 FT-c photosynthesis

	Shoot (%)	Stem (%)	Root (%)
Pentose	-	-	-
Fructose	11.2	1.5	1.8
Glucose	10.0	1.4	2.3
Sucrose	74.3	94.6	94.2
Dissacharide	0.9	0.8	-
Raffinose	1.2	1.7	1.7
Trisaccharide	0.1	-	-
Neutral substance	1.8	-	-
Organic acid	0.5	-	-
Total	100.0	100.0	100.0

After Jones (1961)

Fig. 15. Leaf fractions as percentage of ^{14}C supplied in $^{14}CO_2$

Period of photosynthesis (h)	1	1.5	3	6	15.5	24	48
Activity:							
in leaf extract	18.4	24.5	53.8	58.4	56.3	52.3	49.0
in soluble leaf residue	6.2	9.5	25.0	39.9	40.7	38.7	32.3
Total	24.6	34.0	78.8	98.3	97.0	91.0	81.3

Relative distribution of ^{14}C between leaf extracts

Leaf extract	75	72	68	59.6	58	57.5	60
Insoluble leaf residue	25	28	32	40.5	42	42.5	40

After Jones (1961)

$^{14}CO_2$. After five hours, a switch was made to $^{12}CO_2$. Sample leaves were removed and examined at intervals. (See Fig. 15).

Total activity in leaf residues was less than that of the leaf extracts, showing that the rate of assimilation of labelled CO_2 into metabolically inert polysaccharides is slow.

Fig. 16 shows the percentage of labelled sugars in three extracted fractions, the water soluble, the soluble in cold N. NaOH, and the soluble in hot 2.5N. NaOh. It is interesting to note that the rate of conversion of D-glucose to D-xylose is low because starch is the major product. This suggested that xylan was not an active metabolite. Whistler and Young (1960).

Figs. 17 and 18 show the changes in radioactive polysaccharides during photosynthesis. Cellulose metabolism was not reversible and intermediates were slowly converted to cellulose.

Fig. 16 Polysaccharide sugar-carbon (%) originating from $^{14}CO_2$ after 48 hours photosynthesis.

	Water soluble	Soluble in cold N NaOH	Soluble in hot 2.5 N NaOH
D-Glucose	-	21.6	18.5
D-Galactose	1.66	-	1.72
L-Rhamnose	0.94	-	1.12
L-Arabinose	1.58	0.90	0.74
D-Xylose	0.21	0.30	0.49
D-Galacturonic acid	0.08	-	-

After Jones (1961)

Fig. 17 48h Plum leaves

Monosaccharides from polysaccharides which are	Monosaccharide constituent	Weight isolated	Specific activity of undiluted sugar (μc/milli-atom-equiv.of C)
Water soluble	D-Galactose	16.0	0.338
	D-Galacturonic Acid	9.3	0.017
	L-Arabinose	14.0	0.320
	D-Xylose	2.6	0.043
	L-Rhamnose	5.5	0.190
Soluble in cold n NaOH	L-Arabinose	5.4	0.182
	D-Glucose	41.0	4.40
	D-Xylose	8.0	0.062
Soluble in hot 2.5N NaOH	D-Galactose	19.7	0.350
	L-Arabinose	31.0	0.149
	D-Glucose	45.3	3.76
	D-Xylose	26.5	0.100
	L-Rhamnose	8.1	0.227

After Andrews and Hough (1958)

Fig. 18 Incorporation of 1-(^{14}C)-D-glucose into plum leaf polysaccharides

Period of incorporation (h)	Polysaccharide fraction	Monosaccharide constituent	Weight isolated (mg)	Specific activity of sugar carbon (μc/mA)	Distribution of ^{14}C along carbon chain of monosaccharide					
					C_1	C_2	C_3	C_4	C_5	C_6
5	Hot water soluble	D-Glucose	9.6	0.53	64.6		13.7			21.7
		D-Galactose	11.6	0.031	77.3		6.7			16.5
		L-Arabinose	15.4	0.027	91.4		6.2		2.4	
		D-Xylose	2.9	0.020	65.0		28.8		6.2	
		L-Rhamnose	3.7	0.022	56.4		7.8		1.9	33.9
5	Hot alkali soluble	D-Glucose	40.3	0.815	59.9		12.8			27.3
		D-Galactose	26.0	0.035	68.5		12.9			18.7
		L-Arabinose	95.3	0.017	85.0		9.1		7.1	
		D-Xylose	42.1	0.016	89.0		7.0		4.0	
		L-Rhamnose	19.0	0.022	78.5		3.0		1.1	17.4
5	Cellulose	D-Glucose	138.6	0.0094	71.8		6.6			21.6
5	Hot water soluble	D-Glucose	24.1	0.21	28.6		1.8			69.5
		D-Galactose	19.9	0.024	21.6		3.2			75.2
		L-Arabinose	16.4	0.0029[a]	66.6		24.9		8.3	
		D-Xylose	4.9	0.0058[a]	78.0		32.0		0.0	
		L-Rhamnose	7.2	0.017	20.0		4.0		2.9	73
5	Cellulose	D-Glucose	46.8	0.0034	23.1		1.9			75.0
20	Cellulose	D-Glucose	116.6	0.0065	21.5		6.0			72.5

[a]Activities determined to standard deviation of 4%
[b]Activities determined to standard deviation of 10%
[c]C_3 lost during preparation of sample.

After Jones (1961)

Wolfrom et al, 1959, studied the biosynthesis and fragmentation of ^{14}C labelled cellulose from the cotton plant. Their work was on the maturing 21 day old cotton boll. Having developed a convenient chemical method for the determination of the label distribution on glucose they found that C^2 labelled glucose when incorporated into the plant structure was retained to the extent of 58%. 25% of the label was found at the C^5 position. They also examined the label distribution in seed oil, and these combined data indicated that the metabolism of glucose took place through the Embden-Meyerhoff pathway, though this was not extensive.

In a study on the role of hemicelluloses in the oat plant, Whistler and Young (1960) surrounded actively growing plants by an atmosphere containing $^{14}CO_2$ for 4 hours. Subsequently they took samples of the stem and leaves at several day intervals until maturity. Cellulose, hemicellulose A (mainly xylan) and hemicellulose B were isolated and their specific activities determined. The total activities rose during the first five days after labelling, and remained constant thereafter. This clearly indicated that hemicelluloses were stable end products of metabolism and were not part of a dynamic system. They concluded that the hemicelluloses of the oat plant were structural components and not acting as food reserves under these normal conditions of growth.

Neish (1955), and Altermatt and Neish (1956) studied the biosynthesis of cell wall carbohydrates, in particular cellulose and xylan, in the wheat plant. The labelled monosaccharides

D-glucose $^{14}C^{1}$ and C^{2}, D-xylose $^{14}C^{1}$ and C^{5}, D-arabinose $^{14}C^{1}$, D-gluconolactone $^{14}C^{1}$, D-mannitol $^{14}C^{1}$, D-arabitol $^{14}C^{1}$ and C^{5}, were administered to wheat plants. The cellulose and xylan in the cell walls were isolated after varying periods of 2 to 23 hours. They found that mannitol and arabitol were not converted to either cell wall polysaccharide. Arabinose was utilized only slightly. (These workers used D- instead of L-arabinose which might have affected their results with this particular monosaccharide). All the other compounds gave rise to both labelled cellulose and xylan. The glucose and xylose obtained from their respective cell wall polysaccharides were degraded by fermentation with Leuconostoc mesenteroides.

Glucose and gluconolactone were equally good precursors of xylan and were superior to the other compounds tried. They appeared to give rise to other units for xylan formation by loss of the C^{6} atom. Free xylose was converted to xylan, but only after extensive re-arrangement of the carbon skeleton, such as occurred in the conversion of xylose to cellulose units. Figs. 19 and 20 show the individual results obtained by these workers. From this and other data they postulated a hypothetical outline of polysaccharide synthesis involving uridine diphosphate D-glucose (U.D.P.-glucose) as a central intermediate, that comes very close to explaining the facts. See below:

Fig. 19 Incorporation of C^{14} into Cellulose and Xylan by Wheat Plants* metabolizing labelled Monosaccharides.

Expt. No.	Compound fed	Dose per gm. of dry plant material		Duration of treatment hr.	% of C^{14} fed found in holocellulose	mμc./mM. of carbon		Relative specific activity (x 10,000)	
		μM. of compound	mμc. of C^{14}			Cellulose	Xylan	Cellulose	Xylan
1	D-Arabitol-1-C^{14}	39.0	912	20	Nil	-	-	-	-
2	D-Arabitol-5-C^{14}	40.7	666	20	Nil	-	-	-	-
3	D-Mannitol-1-C^{14}	39.8	3,790	20	Nil	-	-	-	-
4	D-Glucitol-1-C^{14}	39.5	618	20	4.03	1.37	1.94	5.24	7.39
5	D-Arabinose-1-C^{14}	60.6	3,650	5	0.89	0.24	2.85	0.20	2.37
6	D-Xylose-2-C^{14}	87.5	11.010	5	3.40	1.64	2.26	7.10	10.2
7	D-Xylose-5-C^{14}	58.6	2,110	5	3.03	2.59	3.35	3.60	4.67
8	D-Glucose-1-C^{14}	30.0	1,700	2	7.38	8.62	7.02	8.85	7.20
9	D-Glucose-1-C^{14}	32.2	1,820	20	19.1	21.4	24.1	21.8	24.7
10	D-Glucose-2-C^{14}	55.9	2,340	5	10.7	15.7	16.1	22.6	23.1
11	D-Glucose-1-C^{14}	54.6	765	23	16.9	7.73	10.1	33.6	43.8
12	D-Glucuronolactone-1-C^{14}	46.0	865	23	11.1	2.23	12.4	7.15	39.7

*Khapli wheat was used in Experiments 11 and 12 and Thatcher wheat in the other experiments.

Specific activity of carbon dioxide obtained on complete combustion of the polysaccharide divided by by the specific activity of the carbon dioxide obtained by combustion of the original labeled monosaccharide. This gives the specific activity of the polysaccharide relative to that of the compound fed.

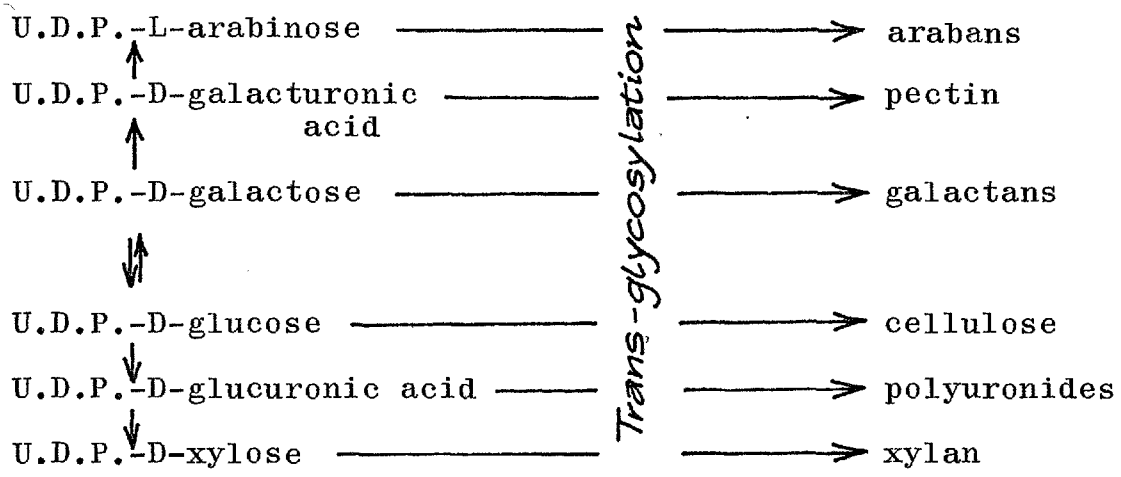
After Altermatt and Neish (1956).

Fig. 20 Distribution of C^{14} in Glucose and Xylose isolated from the Cell Wall Polysaccharides of Wheat Plants.

Expt. No.*	Compound fed	Compound isolated	C^{14} as % of total in glucose of xylose molecule					
			Carbon-1	Carbon-2	Carbon-3	Carbon-4	Carbon-5	Carbon-6
4	D-Glucitol-1-C^{14}	Glucose	47.0	16.7	5.1	3.3	6.8	21.0
		Xylose	70.0	8.2	9.7	5.1	7.0	-
6	D-Xylose-2-C^{14}	Glucose	5.6	47.0	20.2	5.6	16.0	5.6
		Xylose	21.5	40.1	11.5	9.3	17.6	-
7	D-Xylose-5-C^{14}	Glucose	25.6	9.2	6.9	3.4	3.1	51.4
		Xylose	28.6	18.1	6.9	12.8	33.6	-
8	D-Glucose-1-C^{14}	Glucose	78.2	7.8	1.9	1.2	0.7	10.2
		Xylose	91.1	1.2	2.6	1.3	3.8	-
9	D-Glucose-1-C^{14}	Glucose	73.0	8.7	2.7	1.6	1.0	13.0
		Xylose	84.5	1.8	3.3	2.0	8.4	-
10	D-Glucose-2-C^{14}	Glucose	4.2	69.3	11.3	1.4	11.6	2.2
		Xylose	4.7	86.7	4.4	0.5	3.7	-
11	D-Glucose-1-C^{14}	Glucose	74.0	7.2	2.7	2.0	1.5	12.6
		Xylose	86.1	2.9	3.9	3.0	4.1	-
12	D-Glucuronolactone-1-C^{14}	Glucose	13.8	64.6	3.3	2.9	6.1	9.3
		Xylose	88.0	1.9	2.9	2.0	5.2	-

*Experiment number the same as in Table I.

After Altermatt and Neish (1956)



In an investigation to determine the site of deposition of new cell wall material Ray (1967) used segments cut from growing oat coleoptiles and also pea stems. It was suggested that growth of the primary cell wall occurs from within or at the wall surface (apposition) or a combination of the two. Segments were allowed to metabolize in the presence of glucose-^{3}H, with the growth hormone indole acetic acid (I.A.A.), for varying periods of time. By means of electron microscope radioautography the deposition of new cell wall material was closely followed. Selective extraction procedures prior to radioautography showed that a large percentage of the material deposited was hemicellulose This was confirmed by hydrolysis and chromatography of the extracted material. Quantitative profiles for the distribution and incorporation with position through the thickness of the wall were obtained for the thick outer wall of epidermal cells. Deposition of hemicellulose occurred mainly in the internal manner, while cellulose was deposited almost entirely by apposition He gave a table with the distribution of activity among cell wall components of the labelled (^{3}H) oat coleoptiles. See next page.

Distribution of Activity among Cell Wall Components of ^{3}H-labelled Oat Coleoptiles.

Coleoptile segments had been incubated 8 hrs.in glucose-^{3}H plus IAA and fixed with OsO_4.

Extract	Activity in extract as per cent of total	Activity in components of hydrolysate, as per cent of total recovered*.								
		Glc	Gal	Man	Ara	Xyl	GX	MGX	Gal-U	other UA
Pectinase	8.7	2.9	3.3	1.1	2.0	2.9	6.3	4.2	43.0	7.6
0.05N H_2SO_4	58.8	14.7	2.8	0.6	20.1	20.4	13.3	3.8	1.9	7.3
4N KOH	3.4	26.9	18.9	3.3	16.1	24.1	1.0	0.9	2.0	1.1
72% H_2SO_4	29.1	92.6	0	0.3	0	2.8	0	0	0	0

*Following abbreviations are used: GX, glucuronoxylxylose; MGX, 4-O-methylglucuronosylxylose; other UA, other aldobiouronic acids and galacturonic acid oligomers; Glc; glucose; Gal, galactose; Man, mannose; Ara, arabinose; Xyl, xylose; Gal U, galacturonic acid. Activity of components is given as per cent of the total activity that was recovered from chromatograms of each hydrolysate, including unidentified activity at the origin and elsewhere. After Ray, 1967.

Note that 29.1% of the radioactivity is present in the cellulose (removed by 72% sulphuric acid), and most of the rest is present as xylan or glucurono-xylan (extracted with 0.05N H_2SO_4). Obviously the role of glucomannans in oat coleoptiles is considerably more limited than in mature trees.

Concept of minor linkages in the hemicelluloses.

The discussion of hemicelluloses has so far treated them as if they were completely independent molecules. This is not universally regarded as being true. Many inter and intra polymeric bonds have been postulated. Most of the discussion has been concerned with the conceptsof a lignin-carbohydrate bond, cellulose-hemicellulose links, and cellulose crosslinks.

Yaku and Koshijima, 1971, formed a complex hydrogen bond between the carbonyl group in lignin and a hydroxyl group in acetyl glucomannan of the Björkman lignin carbohydrate complex (L.C.C.). The resultant complex was soluble in water, thus providing proof that acetyl glucomannan from L.C.C. renders lignin soluble in water by bonding it. Simonson, 1971, took finely ground (Björkman) birch wood and using electrophoresis and permeation chromatography techniques suggested the presence of a hemicellulose-lignin bond. Shakov et al, 1971, also used electrophoresis (paper) to demonstrate the absence of lignin-carbohydrate complexes in the wood of Pinus sibirica, and in sunflower and cotton husks. The Björkman isolation of lignin involves ball-milling wood for considerable periods in non-swelling solvents such as dioxane and toluene. Milling times are in terms of days or weeks, and lignin yields are about 50%. The product is soluble.

Initially, because of the difficulty found in removing all traces of extraneous sugars from alpha cellulose, it was thought that covalent bonding existed. However, techniques have now improved to the extent that not very much non-glucose monosaccharide is now found. The question, therefore, of this type of link becomes less significant.

Hydrolysis of wood under acid conditions will in fact release hydroxyl groups in lignin and make them available for methylation (Brauns, 1952). It was thought that this indicated a lignin-carbohydrate bond, but since then penolic hydroxyls have been found in carbohydrate-free lignin treated similarly (Hägglund and Richtzenhaun, 1952, Sarkanen and Schuerch, 1957). Thus the above cannot be regarded as evidence of this type of bond.

Evidence gleaned from the differing solubility of pentosans at various concentrations and temperatures of alkali, leads to the conclusion that it is a physical restraint rather than a covalent bond which governs the dissolution of pentosans. This does not necessarily rule out some kind of covalent linkage, but indicates that the bulk of the xylan is not bound in this way.

Reports of lignin-carbohydrate complexes such as by Hägglund, 1951, are almost invariably preceded by drastic chemical or physical pre-treatments. This could well result in artifacts.

ENZYMES

Introduction

Proteins are complex substances of high molecular weight which contain nitrogen as well as carbon, hydrogen and oxygen. When hydrolysed, they yield a mixture of simple nitrogen - containing sub units termed amino acids. These are limited in number to about twenty. They are joined to each other by a bond between a carboxyl group and an amino group called a peptide bond.

Enzymes from natural sources are made of protein (as was established by Sumner in 1926) and as such have the same properties. Their function is to change the rate of a chemical reaction in a similar way to that of a chemical catalyst. They are effective in very small quantities and are exceedingly efficient. The turnover number, or the number of moles of substrate converted into product by one mole of enzyme per minute, varies between one hundred and three million. This is 10^8 to 10^{11} times faster than non-enzymic catalysis. This is all the more amazing when one finds that enzymic catalysis occurs at near neutral pH and at physiological temperatures. Most enzymes are very specific in terms of the substrate. Enzymes will also catalyse a very wide spectrum of reactions, for example, oxidation-reductions, hydrolyses, polymerizations, and free radical reactions. One of the most important characteristics of enzymes in the cell, is that a great deal of control can be exercised over their activity. Their rate of synthesis and final concentration can be under genetic control and can also be influenced by small molecules

such as the products of the reaction which the enzyme is catalysing. Enzymes may be present both in active and inactive forms and the rate of reaction can be influenced by the conversion from inactive to active, which in turn is affected by the environment at that moment. Nearly all biological reactions are enzyme catalysed.

Active Site

With the recent increase in knowledge gained from studies on protein structure, it has become apparent that a certain portion of the enzyme molecule is essential for activity. Any damage to the three-dimensional structure of this part of the protein results in reduced, or more likely total inactivation. When one considers that the majority of substrate molecules are very small compared with an enzyme, it becomes apparent that only a few amino acids in the protein can be in contact with the substrate. From this realisation, the concept of an "active site" has been derived. It includes all those side chains and peptide bonds which are in contact with the substrate, or perform a direct function in the catalytic process (Koshland 1960). Certain amino acids have regularly been found associated with the active sites of which serine, histidine, lysine, alanine and glycine are examples.

Enzymes can be studied both in vivo and in vitro, but in the case of the extracellular hemicellulases it is possible to operate in vitro very close to the natural state. Growth of the hyphae in a culture medium containing wood sawdust as sole carbon

source results in an extracellular enzyme system which can be extensively studied and yet is relatively close to the state which a wood-destroying fungus would occupy in natural conditions.

Cellulases in relation to hemicellulases

Most of the work on fungal extracellular hydrolases has been concentrated on those enzymes which degrade cellulose. (B 1:4 glucan glucano hydrolases). Despite the fact that hemicellulose is the second most abundant organic compound, very little work has been done on the enzymes which destroy it. Because of the similarity in substrate structure much information on cellulose - degrading enzymes is a useful indication for hemicellulases. It is important to remember that conditions such as the pH, temperature, ionic strength, substrate concentration and nutrient balance are all artificially maintained at those levels which are most favourable for the organisms concerned. These are not necessarily the same conditions which produce the highest enzymic activity.

Cowling (1958) in his review on the enzymatic degradation of cellulose and wood gave nine ways in which the extent of hydrolysis of cellulose might be determined

(1) Increase in reducing sugar value.
(2) Decrease in viscosity of soluble cellulose derivatives or purified natural celluloses.
(3) Decrease in the tensile strength of cotton fibre, or the bursting strength of a cellophane film.
(4) Loss in weight of an insoluble substrate, such as cotton, wood or rayon.
(5) Chromatographic separation and identification of end products such as cellobiose or glucose.
(6) Increase in susceptibility of the substrate to another enzyme of known specific activity.
(7) Increase in swelling on exposure to dilute alkali.

(8) Increase in dye uptake.

(9) Decrease in opacity of an agar medium containing an insoluble cellulosic material.

The above or similar methods can also be used in hemicellulase detection.

It is of value to review the present knowledge of the mechanism whereby fungi degrade wood cellulose. It is considered that extracellular enzymes secreted by the hyphae diffuse to the wood surface which is then hydrolysed. Water is necessary for this diffusion to occur, thus fungal decay will not occur in dry wood. Cellobiose is the main product of this reaction which in turn diffuses into the hyphae, whereupon it is further hydrolysed to glucose prior to entering known areas of glucose metabolism. The hydrolysis is completed in a series of distinct steps described by Cowling (1958) as follows:

"(1) The wood cellulose may be digested by an unknown factor 'X', presumably enzymatic, possessed only by fungi adapted to this type of substrate. Absence of this factor accounts for the inability of non-wood-destroying fungi to utilize wood for food.

(2) The laterally bonded cellulose chains occurring in cotton are apparently separated from one another by an enzyme designated 'C1' Organisms which do not possess this enzyme or the factor 'X' are unable to utilize natural celluloses for food and must therefore be considered noncellulolytic.

(3) The linear anhydroglucose chains produced by 'C1'are hydrolysed by a series of enzymes designated Cx's such as Cx_A,

Cx_B, Cx_C, to give cellobiose. The Cx enzymes are active not only on chains liberated by C1,but also on those provided artificially by the delignification of wood in the pulping process, or by the regeneration or substitution of cotton or wood cellulose.

(4) Cellobiose is hydrolysed to glucose by the intracellular-enzyme β glucosidase. This enzyme is partly reversible and may be capable of regenerating saccharides containing 3 or 4 or perhaps more glucose units. β glucosidase is produced by many fungi which bear little relation to cellulose digestion in the usual sense of the term.

The enzymatic degradation of whole wood, is less well understood owing to its complex nature. It is considered that the presence of lignin is the chief limiting factor in the case of a large number of cellulolytic fungi which cannot utilize wood as food. The 'X' factor is necessary before the 'lignin barrier' can be overcome".

Individual Enzyme Systems

One method of obtaining an insight into the mechanisms by which fungi degrade wood is to study the individual enzymes in vitro. Much work has been done on these lines for 'cellulases', but similar studies on hemicellulases have been severely restricted by the difficulty in obtaining substrates. Hemicellulose substrates are tedious to extract, and it requires a great deal of work to characterise them. Even after this has been achieved one is not certain that the substrate is in the same state as it

was when in the original wood. With our present knowledge, extracts with D.M.S.O. represent the nearest condition to the natural state that hemicellulose can be obtained. Procedures involving delignification are bound to result in minor changes of the structure; despite this, much useful information has been obtained by using alkaline extracts.

(a) Coniophora cerebella - (General Enzyme System)

In one such study, King (1966) studied the extracellular enzymes of Coniophora cerebella, a brown rot causing widespread destruction of wood in the U.K. This type of fungus predominantly attacks the polysaccharides of the cell wall. It was found that enzymes which degraded cellulose derivatives, hemicelluloses, starch,laminarin, pectin and several oligosaccharides could be present, but the type of activity depended on the carbon source. When Norway spruce sawdust (100 mesh) was used as sole carbon source, some activity was detected when spruce α-cellulose, C.M.C., cellobiose, laminarin, maltose, spruce xylan, spruce glucomannan and larch arabinogalactan were used as substrates in a reducing sugar assay. There was no pectinase found. All activities were weak except when α-cellulose or hemicellulose was used as the sole carbon source. The enzymes, (with the exception of laminarinase) were found to be inducible.

It was also found that acid production was a characteristic of this fungus. Peak activity occurred in the 25 - 45 day of growth region. The temperature optima were generally very high. Cellulase and hemicellulase were both 50°C. Thermal stability was also found to be very high, some activity being present after heating at 65°C. for ten minutes.

(b) Coniophora cerebella xylanase

The xylanase system of C. cerebella has been studied in more detail (King and Fuller, 1968).

When grown on poplar 4-O- methyl glucuronoxylan as carbon source the enzyme system induced degraded the poplar xylan to xylose, acidic and neutral oligosaccharides, and an enzyme-resistant polymer. The culture filtrate was purified by cold ethanol fractionation. This resulted in a separation of two types of activity. One predominantly produced xylose and xylobiose and the other produced xylobiose, xylotriose and xylotetrose. Full separation of these types of activity was achieved. It was concluded that one was a xylanase (β1:4 xylan xylanohydrolase, E.C. 3.2.1.8.) which hydrolysed xylan in an essentially random manner, giving little xylose but large quantities of oligosaccharides. The other was a β xylosidase (β D xylose xylohydrolase, E.C. 3.2.1.37.) releasing xylose from xylobiose and these oligosaccharides, but having very little activity on the long chain xylan. This extra cellular β-xylosidase is unusual compared with most β glucosidases in being extra rather than intracellular. It was noted that free 4-O-methyl-glucuronic acid was not produced, and so it was thought that the fungus was unable to produce an enzyme catalysing the removal of these units from the xylan chain. Owing to the low mobility on chromatography of the acidic oligosaccharides, it was thought by these workers that the 4-O-methylglucuronic acid residues afforded some protection to the main chain from the random action of the enzymes, by stopping hydrolysis occurring at the xylose units close to the uronic acid groups.

Xylanases

(1) General Introduction.

In 1889 Hoppe-Seyler first demonstrated the biological decomposition of a wood xylan. A suspension of beech wood xylan was inoculated with river mud and the gaseous development studied. Using a preparation from snails, Ehrenstein (1926) studied xylanase activity upon wheat straw xylan. He found that after 16 hours half the xylan was decomposed. After two weeks only 68% had been used. He identified xylose as an end product. Grassman et al. (1933) used fungal mycelium as an enzyme source and partly separated cellulase and xylanase activities. Voss and Butter(1938), used enzyme sources from snails, malt and fungi, to study the decomposition of xylan from beechwood. Sorensen (1952, 1953, 1955, 1956, & 1957) intensively studied the decomposition of xylan from wheat straw. He studied six test microorganisms selected for their ability to grow on xylan. These were the fungi Penicillium janthinellum, Chaetomium globosum; the actinomycetes Streptomyces albus , Micromonospora chalcea; and the bacteria Cellvibrio fulvus , and Chlostridium sp.The temperature optima for the extracellular xylanase of the fungi was 37^{o}C., somewhat lower than has been found by most other workers. The fungal enzymes were found to be adaptive, with the pH optima in the 5.9 to 6.5 region. Studies of the reaction products led to the conclusion that the extracellular xylanase attacked the xylan chain at random along its length, yielding a mixture of shorter or longer chain fragments.The part of the main chain devoid of side chains gave rise to oligosaccharides of xylose units, and

the part of the main chain carrying the side groups yielded mixed oligosaccharides. These oligosaccharides were further degraded, xylobiose being the shortest one attacked by the extracellular xylanase. The end products were: xylose, arabinose, xylobiose, uronic acid or uronic acid xylose oligosaccharides.

The main yield of the extracellular enzyme was xylobiose. When xylobiose was incubated for 10 days with the enzyme, no activity was found. However, dried cell wall material of the test fungi was capable of splitting this, thus indicating the presence of β-xylosidase activity. Similarity between the structures of xylan and cellulose led Sorensen to investigate the possibility that one enzyme system was responsible for both activities. In order to study this, he selected the cellulose destroying bacterium Sporocytophaga myxococcoides which is unable to grow on xylan or xylose. He found that when grown on cellulose, an extracellular enzyme was produced which could hydrolyse xylan; the products, however, were not metabolised and remained in the extracellular medium. Its production was independent of the presence of xylan as co-inducer, when grown both on glucose or cellulose. Other experiments comparing extracellular cellulase and xylanase of the test fungi, induced with cellulose and xylan, supported the concept of a cellulase with partial activity on xylan, and a xylanase with partial activity on cellulose.

(2) Chrysosporium lignorum

The production of extracellular hydrolytic enzymes from the fungus Chrysosporium lignorum has been studied by Eriksson

and Rzedowski(1969 a&b)This is a thermophilic fungus causing serious damage to wood chip piles with an optimum growth rate at 40°C. It attacks both cellulose and lignin. Cultivation for 20 days, using cellulose as a sole carbon source was found to produce the following enzymes: - cellulase, xylanase, mannanase,β glucosidase and aryl β-glucosidase. When xylan was the inducer, cellulase and mannanase were absent and the glucosidases very low in concentration. With mannan, however, traces of all the enzymes were found but in low concentration. It is of interest that the pH for cellulose and xylan decreases rapidly and then remains relatively constant; with mannan a rise in pH is observed after about the fourth day.

These proteins were concentrated successfully by ultra-filtration and dialysis. Isoelectric focusing was used to determine the change in the protein content from the sixth to the twentieth day of culture. All showed a shift to a higher isoelectric pH and an increase in heterogeneity.

The fact that xylanase appeared when cellulose was the sole carbon source indicated that this was a constitutive enzyme, however, since xylan is known to occur in some fungal mycelia (Bjorndal,1969) it is quite possible that xylanase might be self-induced. Care should be taken when interpreting the results of experiments on hemicellulases, when the hemicelluloses used are not extracted from timber. In the case of the above work esparto grass xylan, and guar powder mannan were used. For work concerned with timber it seems correct to employ only those hemicelluloses isolated from timber.

Evidence for these cellulases being glycoproteins has resulted from further studies on C. lignorum. After five days dialysis, constant sugar levels were found in these fractions separated on Biogel and DEAE-sephadex. Analysis of the carbohydrate moiety showed mannose with traces of galactose. The cellulase from Aspergillus niger was also found by Ikeda et al. (1967) to be a glycoprotein. It is possible, therefore, that the xylanase may also fit into this category.

It is of interest that the unpurified culture filtrate released sugar at a much higher rate than any of the three purified fractions or combinations of the fractions. This strongly indicates the presence of a C_1 enzyme.

(3) Aspergillus species

Whistler and Mazak (1955) in an early study on the xylanase from Aspergillus foetidus were able to separate it into two components. After growth on corn cob xylan the mycelial extract was separated into two fractions on a cation exchange column; a) one that would hydrolyse xylan and xylo-oligosaccharides to xylose without any intermediates and b) one that would hydrolyse xylan and xylo-oligosaccharides to xylose and a mixture of many intermediate oligosaccharides. Fractionation of the extract on a celite column gave a fraction that did not hydrolyse xylan but that would attack 'hemicellulose B' (i.e. glucomannan).

Ahlgren *et al* (1967) Part I, have used the techniques of isoelectric focusing, gel filtration and zone electrophoresis to study the enzymes of *Aspergillus*. They used a commercial preparation "Cellulase 36" and detected cellulase, mannanase, xylanase, β glucosidase, aryl β glucosidase, mannosidase and xylosidase. The reducing sugar test for xylanase activity was upon xylo-oligosaccharides D.P. 5 - 7. Mannanase and cellulase were determined viscometrically. They first investigated the molecular sizes of the enzymes and concluded mainly from gel filtration studies, that two groups of molecules were present. The extracellular endoenzymes cellulase, mannanase and xylanase were all found to be of low molecular weight, whereas the corresponding disaccharide decomposing enzymes (e.g. β-glucosidase) were of a larger size. This was a logical find because the substrate of the former is an insoluble long chain molecule needing a small enzyme for easy access, whereas the latter substrate is soluble and can thus move towards the larger enzyme. The isoelectric points were found to be in the range 3.9 - 4.7 and some heterogeneity was evident from the broad peaks; this was especially evident with mannanase. The xylanase and cellulase were more homogeneous. Zone electro-phoresis confirmed this conclusion. In this method,because the size of the molecule is one of the factors affecting mobility, the peaks were broader than in the isoelectric focusing.

In the second paper of this series (Ahlgren and Eriksson II 1967) three other fungi were tested in a similar way. *Stereum sanguinolentum*, *Fomes annosus*, and *Chrysoporium lignorum*, had

with few exceptions enzymes whose isoelectric points were within the range 3.6 - 4.7. Again the extracellular endoenzymes were small, and the corresponding exoenzymes were large. The xylanase appeared (upon filtration with Sephadex) to possess a single peak in Stereum and Aspergillus, but had a double peak in Fomes and Chrysosporium.

Isoelectric focusing revealed startling differences in the enzymic distributions between the three different species of fungus. Neither in sequence nor in number of peaks were there any similarities. It was of particular interest that the xylanase of Fomes and one of the peaks of Chrysosporium xylanase were of very high pI (above 6.0). Although it fell within the average range, the xylanase of Stereum was the highest of the extracellular enzymes for that particular fungus.

It was apparent that the quantities of xylosidase and mannosidase were low and in some cases they were absent. This indicated that these are more likely to be intracellular or located on the cell membrane. This was also found by King and Fuller (1968). From the fact that the pI values are also very close to the optimum pH's for most of these enzymes it was concluded that the most active configurations for these enzymes were when they were in the neutral state.

(4) Stereum sanguinolentum

The xylanase from Stereum sanguinolentum was chosen for further study. (Eriksson and Pettersson,1971).

Induction was on cellulose and after three different filtration steps the xylanase was almost completely separated from the cellulase by zone electrophoresis. As in previous

studies the xylanase activity was measured as a reducing power after incubation with xylo-oligosaccharides D.P. 5-7 obtained from espartograss. Cellulase was determined viscometrically. After separation the molecular weights were determined by ultracentrifugation, giving a result of 21,600 for the xylanase and 23,200 for the cellulase. The molar extinction coefficients were then found to be 6.25×10^4 and 8×10^4 respectively. Upon analysis of the amino acids both were found to be fairly similar, containing abundant proportions of acidic and aromatic amino acids. In the cellulase, aromatic amino acids were more plentiful, as was confirmed by the higher molar extinction coefficient. Calculations of the molar activity gave a result at the best of 3 bonds broken per second. A similar figure for the cellulase from the same culture was 13 bonds per second.

(5) Chaetomium globosum

In a study on the utilization of various carbohydrates by the soft rot fungus Chaetomium globosum, Takahashi and Nishimoto (1973) looked at the potential of wood hemicellulose components as carbon sources. They used D-Arabinose, xylose, glucose, mannose, galactose, glucuronic acid, galacturonic acid, maltose, C.M.C and a crude beech xylan. It should be emphasized that in woods L - Arabinose is the commonly occurring isomer (Browning, 1967) and also that the glucuronic acid groups are methylated. It was found that xylose, mannose, glucose, galactose, and maltose were available to the fungus. Xylose and mannose produced the highest mycelial weight closely followed by glucose.

Galactose resulted in the slowest growth, but the end point was very close to glucose. D-Arabinose was not metabolised. Surprisingly, glucuronic acid and galacturonic acid were also not used; however, the pH values of the starting media were low in the case of these acids, and that could have contributed to lack of growth. It was also found that growth on C.M.C. was poor, a finding directly conflicting with that of Fuller (1970). It could be that the difference in the type and degrees of the substitution of the C.M.C. used caused this variance in results.

Growth on xylan was also tested with two other fungi, Coriolus versicolor and Tyromyces palustris, respectively white and brown rot fungi. The Chaetomium grew well on xylan, the maximum weight of hyphal material being attained in 5 days. The other fungi were comparable but achieved slower growth rates. Under these conditions, tailing off in the growth was observed in all three fungi after 12 - 15 days.

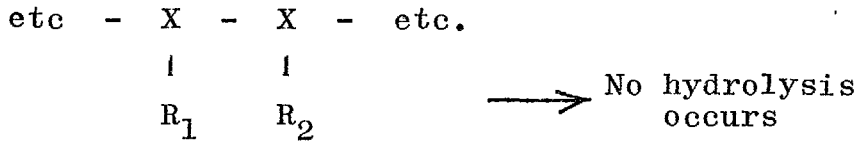
It was noted that a pronounced drop in pH occurred around the sixth to the twelfth day; it rose again to the original level or higher later on in the culture's life. This has also been observed in this thesis in wood cultures. In the xylose and xylan media this effect was very much more prominent. In this work it was found that the pH drop was greater for hardwood than softwood.

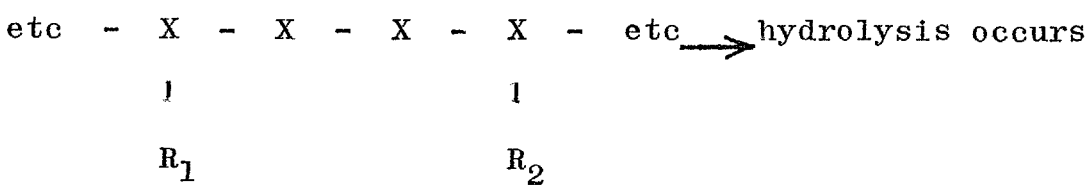
(6) A Bacterial Enzyme

While working with wheat-flour arabino xylan Perlin and Reese (1963) established definite limits to the active site of an enzyme. The polysaccharide material was a β 1,4 linked xylan chain with about two in every five units serving as attachments for arabinose chains; these were in the order of

two or three units long. The enzyme was obtained from a bacterium, Streptomyces sp. QM B814

The polysaccharide was partly degraded into oligosaccharides, xylobiose and xylotriose. From the data obtained they showed that the enzyme could not hydrolyse xylan with adjacent branch chains present. The minimum spatial requirement for the active site was found to be two xylose units free of side chains. See below.





Fungal Enzymes Considered as Proteins

1. Penicillium notatum

Considerable progress in the characterization of cellulases as proteins has illuminated the path for future work on hemicellulases. This could result in increasing knowledge in the comparative and the absolute sense with cellulase.

Studies using proteolytic enzymes on the cellulase from P. notatum (Eriksson and Pettersson,1968) are an example. When the cellulase was incubated with carboxypeptidase and leucine aminopeptidase,no decrease in cellulase activity could be detected. These enzymes are responsible for removing the C - terminal and N - terminal amino acids from the protein chain.

No such amino acids were detected. When, under the same conditions, cellulase was incubated with the endopeptidases, subtilisin, pronase and trypsin, the activity decreased to 50% within a few hours. The degradation with serum albumen was twice as fast, indicating that the cellulase molecular configuration is fairly resistant to endopeptidases, and totally resistant to exopeptidases.

These workers also investigated the possibility of obtaining a fragment of the enzyme retaining activity. Cellulase was incubated with subtilisin until 50% of the original activity was present; the mixture was then separated on a Sephadex column. Unfortunately the elution pattern showed that activity was still only present with the main protein peak in an identical place to that of whole cellulase. Amino acid compositions were also identical with the intact enzyme.

The observation that very small concentrations of mercuric ions were highly inhibitory led to the study of difference spectra of the enzyme and of tryptophan. Mercuric acetate was used in the buffer and spectra were read 30 minutes after addition of the mercury ions. The similarity in spectra differences given by the enzyme and tryptophan when contaminated by mercuric ions was found to be quite striking.

Difference spectra obtained at acid pH's indicate that a peak found at 290 mμ is due to changes in the environment around tryptophan residues, while the peak found at 284 mμ is probably due to changes involving Histidine. Similar spectra obtained from heat-denatured cellulase have maxima at 285 and 292mμ considerably larger than those produced by acid. Thus it was

concluded that upon heat denaturation striking structural differences in the environment of tryptophan residues occurred, making it seem probable that in the intact enzyme tryptophan lies buried in the interior of the molecule.

Pettersson and Eaker (1968) have used another technique, that of free-zone electrophoresis, to study and purify the P. notatum cellulase. They considered the product to be homogeneous by chemical and physicochemical criteria. Their determination by the Archibald method gave a molecular weight of 34,500, which compared favourably with an amino acid analysis value of 35,500. They found the molecule to be rich in aromatic and acidic amino acids with the N - terminal being, and the C terminal suspected of being alanine. The molecule consisted of a single polypeptide chain cross-linked at one point by a disulphide bridge. They did not find any carbohydrate covalently linked to the protein.

An earlier study by Pettersson (1968 b) had previously shown the enzyme to produce a single band when subjected to polyacrylamide gel electrophoresis.

Pettersson and Andersson (1968) have studied the P. notatum cellulase using potentiometric titration and optical rotatory dispersion. With the former the relation between the hydrogen ion activity and the state of particular titratable groups can be determined, as well as the mean net charge on the protein molecule. The titration curves produced with proteins are complex, and this causes difficulty in their interpretation. Because there are usually a large number of titratable groups present in each protein molecule, 50 to 60 per 100,000 molecular weight, the

pKa for each of the groups may differ from that of the simple amino acid by several pH units. This can be attributed to three factors (a) the electrostatic effects resulting from ionization of other groups on the protein (b) effects in the medium due to the presence of hydrophobic residues (c) hydrogen bonding. In general the majority of the groups are on the outside of a protein; however, some irreversible unfolding occurs at extremes of pH. It was found best to perform the titrations quickly to avoid complication by these internal groups. The cellulase gave results indicating a compact molecule with a spherical shape, with a molecular unfolding or swelling at low pH. At pH 5.8 it was found that the steepness of the curve sharply increased and this was attributed either to some local conformational changes, or cations binding to the protein molecule.

From optical rotatory dispersion studies it was concluded that there was 20 - 30% α helix structure in the protein and that this remained even in quite strong alkali. In 6M quanidine chloride, however, no α helix properties were apparent.

Tetranitro methane (T.N.M.) is a mild specific reagent for nitration of tyrosyl groups (Horinishi, 1964). Results with the P. notatum cellulase showed that only five of the fifteen available tyrosines were nitrated. This increased to 11-12 when the cellulase had been heat denatured prior to the titration. About 35% of initial activity was retained when five histidine groups were thus modified. Both heat denatured and whole enzyme which had been subjected to treatment with T.N.M.were hydrolysed and

analysed for amino acid content. 3 - nitro tyrosine appeared as a new peak for the active enzyme, whilst the tyrosine peak disappeared totally in the denatured enzyme analysis, indicating that all the tyrosine residues had been modified. The reduction of the active enzyme tyrosine peak corresponded to five residues, thus agreeing with the titration results.

The same workers have introduced diazonium - 1 - H tetrazole (D.H.T.) for determination of histidine residues. Sokolovski and Vallee (1966) modified the procedure. The cellulase completely lost its activity at very low concentrations of this reagent. Criticism can be levelled at the conclusion that histidine is integral with activity, on the grounds that D.H.T. is not very specific. Nevertheless it is probable that inhibition is due to interference with histidyl residues, which might have been changed to monoazohistidyl derivatives.

Upon succinylation with succinic anhydride, some 60% of the amino groups were modified without any deleterious effect on the activity.

Inhibition by riboflavin - 5 - phosphate has been found but is somewhat restricted in effect. 20mM concentrations gave approximately a 30% reduction in activity. Histamine had a similar effect. This is very unlike the effect which these inhibitors had on lysosyme, which was inactivated. Shinitsky et al. (1966).

By chemical modification of selected groups in the protein structure it might be deduced from any possible change in activity, what role that particular type of group played in the

enzyme function. Tryptophan residues can be selectively oxidised with N - bromo succinimide (N.B.S) (Spande and Witkop 1967), or with 2-hydroxy-5 nitrobenzyl bromide (Horton and Koshland 1965).

When Pettersson (1968a)titrated the cellulase with N.B.S. it was found that complete inactivation occurred, after accounting for only two tryptophan residues. When a similar titration was carried out in 8 M. urea, a denaturing medium, 12 out of the 13 available tryptophan residues were oxidised. This is evidence for the limited accessibility of tryptophan in the cellulase molecule.

Substitution of the tryptophyl residues with 2-hydroxy-5 nitrobenzylbromide, gave a degree of modification of 4.6 residnes per mole of cellulase. Activity determinations showed that the cellulase retained 60% of its activity. It is probable that N.B.S. causes a greater change in structure to the active site than does the introduction of a benzene ring.

(2) Trichoderma viride

The specific modes of action which hemicellulose destroying enzymes employ have yet to be seriously investigated. Most of the work on the molecular action of β1-4 linked carbohydrate destroying enzymes has been limited to purified cellulases. Because of the similarity in substrate composition it is a valid exercise to consult selective papers on the mechanism of cellulase action, in the realistic hope that hemicellulose is broken down by similar mechanisms. Li et al.(1965) demonstrated that the truly cellulolytic fungus Trichoderma viride produced culture

filtrates containing three distinct types of enzymic activity towards cellulose (1) an enzyme removing successive glucosyl moieties from the non-reducing end of the substrate (an exo-glucanase) (2) an enzyme attacking internal bonds (an endo-glucanase) and (3) a component capable of attacking crystalline cellulose (C_1). From their results a reasonably clear theory of action of the complete culture filtrate could be developed. Attack on crystalline cellulose regions was attributed to the C 1 enzyme, the main product being cellobiose. The endoglucanase was assigned the role of initiating destruction of the amorphous regions, the main products being oligosaccharides of small to medium chain length. The exoglucanase was then credited with the hydrolysis of the products of the other two components. It would seem likely that hemicellulose being considerably smaller in chain length and restricted, it is thought, to the amorphous regions, would not require an enzyme analagous to the C1 found in T. viride.

(3) Myrothecium verrucaria

One of the first ideas on the possible size and shape of cellulase was proposed by Whitaker et al. in 1953. From certain physical properties of the cellulase from Myrothecium verrucaria, the molecular weight was estimated as 63,000 ± 1600, and the shape thought to be an elipsoid with an axial ratio of between five and ten. Most recent workers believe that enzymes are more likely to be globular, for example lysozyme (Perutz 1967, Blake et al, 1967).

The specific method of cleavage of cellotriose has been closely studied for this enzyme (Whitaker and Merler 1956). The distribution

of ^{14}C in the enzymic products of ^{14}C labelled cellotriose (the glucose unit at the reducing end) was studied, and it was found that the linkage adjacent to the non-reducing end was preferentially hydrolysed. The rate of hydrolysis was five times that of the other bond. Whitaker (1954) found the rate of cleavage of the central glucosidic linkage in cellotetraose was twice that of the two terminal links. This was also true for the non-terminal links in cellopentaose, but hydrolysis of cellohexaose showed no evidence for preferential attack at any one link. In 1956, Whitaker investigated the hydrolysis of a cellodextrin with a number average D.P. of 24, in the presence of ^{14}C labelled cellobiose.

The specific activities of cellotriose and cellotetraose isolated were less than 20% that of cellobiose. He concluded that transfer reactions with cellobiose contribute little towards the production of fragments of DP 3 and 4. The ratio of cellotriose to cellobiose formed was 0.7, and for cellotetraose was 0.4. The results he obtained were consistent with a degradation by random attack.

When working with Penicillium cellulase Pettersson (1969), tested nine α or β linked substrates and found that lichenin (a mixed β1:4, β1:3 glucan) was the only one attacked apart from cellulose. Mannan and xylan were amongst the compounds which he tested. He found that the enzyme very slowly degraded cellobiose and that the half lives of the members of the series at constant enzyme concentration were 3700, 50 and 3 minutes respectively for cellobiose, cellotetraose and cellopentaose. From studies of the products it was found that the central links were

preferentially attacked. From observations of the Km values for cellopentaose and celloteraose it was thought that the specificity region of the enzyme was for five glucose units.

A similar pattern has been found with Myrothecium verrucaria cellulase (Whitaker 1959) where the relative rates were $[G]_5$ 500 > $[G]_4$ 83 > $[G]_3$ 16 > $[G]_2$ 1.2.

Heat Stability and Metal Ions

One of the most striking feature of cellulases and hemicellulases derived from fungi is their quite remarkable heat stability. In most cases the temperature optima are around 50 C and it is not exceptional to find some at 60°C. In some cases activity has been found even after boiling; the only truly effective method of heat denaturation on these occasions has been found to be autoclaving. Thus, it is interesting to note, that the complete molecular structure of an enzyme called thermolysin has recently been determined. (Colman et al. 1970) Thermolysin is a protein degrading enzyme secreted by a bacterium which is found to be quite stable at 65°C and can be exposed to 80°C without undergoing immediate denaturation. It has been shown that thermolysin activity is dependent on one Zn^{++} and four Ca^{++} ions per molecule. The Zn^{++} ion is essential for activity, whereas the Ca^{++} ions do not affect the activity, but removal does seriously reduce stability.

Upon X-ray analysis roles of these ions are revealed. The enzyme consists of two globular sections of protein joined by a narrow strip, resulting in a deep trench in the middle of the molecule. In the centre of this groove is situated the Zn^{++} ion, bound to three amino acid residues of the protein. It is reasonable to assume that the protein substrate to be split, is

bound in the groove and is degraded by a mechanism involving the Zn^{++} ion. This would explain why Zn^{++} is necessary for catalytic activity. Zn^{++} ions are used in a precisely identical fashion in the enzyme carboxy-peptidase, the only difference being that the protein is split not in the middle, but at a terminal amino acid. It is thus not surprising that the Zn^{++} ion in carboxy-peptidase has been found in a notch in the enzyme rather than in a deep long trench.

The role of Ca^{++} ions is also revealed by the X-ray studies. These are strongly bound to negative centres in the enzyme such as carboxylate or carbonyl groups. These form a network of electrostatic bonds, which are remarkable as they are bound into the interior of the enzyme protein, so that the bond network is out of contact with the external water. In such a non-polar environment the electrostatic bonds become extremely strong. No doubt the extraordinary thermal stability of this enzyme is dependent on the Ca^{++} ions' ability to participate in this polar network in a non-polar environment.

In the light of the recent discussion on the effect of metal ions on thermolysin, it is interesting to investigate work done on the trace elements required both in cellulase production (Mandels and Reese, 1957), and cellulase activation and inhibition (King,1961) In the former supplementary calcium at 0.03% greatly increases the yield of cellulase, though it is found that magnesium at a low concentration is always necessary. Strontium can partly replace this calcium, but barium has no effect. The optimum calcium concentration was found to be 0.02% (or 72 p.p.m.). When other trace elements were tested for optimum cellulase production, it

was found that best yields were obtained in the presence of Fe, Mn, Zn and Co. Omission of Zn reduced the yield, whereas omission of any of the others had little effect. Further experiments showed that any combination of Fe^{++} or Mn^{++} with Zn^{++} or Co^{++} gave a reasonable enzyme yield. Cobalt was found to be the only element capable of action on its own. The optimum concentration for cobalt was around 10 ppm, considerably less than the optimum for calcium.

Stimulation of the enzymic activity itself has been reported for the cellulase from Aspergillus orizae by Fe^{+++} (Jermyn 1952, a and b). Fe^{+++} reversed the inhibitory effect of chelating agents. Norkrans (1950), observed a calcium phosphate activation, King (1959) an iron and cobalt activation and Sison and Schubert (1958) a manganese and cobalt activation.

It seems possible, if not probable, that cellulases and hemicellulases possess a similar dependence on metal ions as has thermolysin. It is likely that, as they are not quite so stable as thermolysin to heat, the lattice work is not so well defined.

This is not the only way in which stability may be conferred upon a protein structure. Most work on the detailed protein structure in the crystal state has been on a single chain of amino acids. Most enzymes are oligomers, consisting of more than one chain. It has been a point of interest to know whether or not the individual sub-units possess catalytic activity; or whether this is a property of the intact composite. The problem inherent in answering the question is that conditions breaking up the oligomer (strong acids or concentrated urea) are those which one would expect to destroy activity. If this is done and

the sub-units are transferred to a milder environment, where their individual activity could be studied, they promptly reassociate. Chan and Mawer (1973) neatly overcame this hurdle when studying the enzyme aldolase. After dissociation, in urea, aldolase was placed in porous beads of sepharose, which had been chemically treated to provide a limited number of sites reacting with protein. The end result was that the individual sub-units were spaced out and fixed in a sepharose matrix. This was then treated with a large volume of buffer to return the sub-units to their active state. As a control oligomers were bound to sepharose to determine any possible effects of the link with sepharose. It was found that the sub-units were just as active catalytically, but were considerably more fragile than the tetramer. They were inactivated by treatments with mild alkali which had no effect on the whole enzyme. It seems, therefore, that the association of four sub-units provides a very stable conformation, but does not increase catalytic activity per se.

Synzyme

With their remarkable properties especially in terms of speed, selectivity, and efficiency, it would seem unlikely that man can improve on nature. In a recent report(Kiefer, 1972) Klotz, and his co-workers have achieved just this. They called their product a synzyme. In an attempt to discover how chymotrypsin catalysis occurred, Klotz prepared polymeric molecules which mimicked the enzymes activity. He initially fabricated a molecular framework which would bind the substrate and then introduced catalytic groups to effect the reaction. Their framework consisted of polyethyleneimine, with imidazole side chains for catalysis. The surprising finding was that this

was able to break down nitrophenol esters at a fast rate with kinetics of a nature similar to an enzyme. The imidazole group has a secondary role in the chymotrypsin mechanism; when, however, a reaction was known to involve histidine with its imidazole group, as a primary catalytic agent, the results were very remarkable. The genuine enzyme was responsible for the breakdown of sulphate esters, and it was found that the synzyme was some 100 fold faster than the natural protein. Again the kinetics were enzyme-like, as was the globular shape of the synzyme. If at the first attempt man can improve on nature 100-fold, and if this is achieved in the cellulase and hemicellulase field, the economic and ecological prospects are significant.

Mannanases

From the same "Cellulase 36" source as has been previously mentioned, Eriksson and Winell (1968) have isolated and purified a β - Mannanase. They used a pure mannan, guaran, for the substrate so the work cannot be directly correlated to galactoglucomannanases. However, the substrates are similar enough to warrant a close look at the work. These workers used ammonium sulphate precipitation, gel filtration, ion exchange chromatography and zone electrophoresis to purify the mannanase. There were still traces of cellulase and laminarinase activity. When ultrafiltration studies were carried out a molecular weight of 42,000 $\pm$ 2000 was found. This was about twice the figure for the xylanase. Amino acid analysis, though not absolute because of the slight contamination, showed the same general trend as other related enzymes of high content of aromatic and acidic amino acids. This was also confirmed by the low value for the

isoelectric point, found to be 4.1. The pH activity curve showed two optima at pH 3.0 and 3.8 and this was an unexpected result. Thermal stability and temperature optima were both very high indeed, the latter being 65°C. This is quite often also a characteristic of other fungal cellulases and hemicellulases.

Bouveng et al. (1963) with a view to increasing knowledge of the structure, studied glucomannan from Norway spruce which contained glucose (27%) mannose (72%) and galactose (1%),and used a commercial enzyme preparation from the fungi Penicillium chrysogenium and P. notatum . They examined the hydrolysis products by paper and carbon column chromatography. Unfortunately, the enzyme preparation was not specific for β-1:4, links and so the purpose of their experiment was not attained.

Perila and Bishop (1961) studied the enzymic hydrolysis of a glucomannan from Jack pine (P. banksiana). The substrate was comprised of glucose, mannose, galactose and xylose in the following ratio 10:25:1:1. They used a commercial hemicellulase preparation (which are generally not very highly purified) to degrade the glucomannan and collected the enzymic products by continuous dialysis during the incubation. Several different types of oligosaccharides resulted. These, and the quantities in which they were produced are given below:

Oligosaccharides.	* % of original glucomannan
M M	18.5
G M	1.4
M G	0.7
X G	0.5
M M M	6.5
M G G	0.7
X G G	0.1
M M G	0.04
G M M	0.03
Ar↜G↜M ?	0.01
Ar↜Ar↜Gal?	0.01

*all β-1:4 links.

The last two listed were trisaccharides with the given composition but no established sequence.

The cellulase activity was determined on this enzyme and proved to be non-existent, also when a purified cellulase (Whitaker 1953) was applied, it had no effect on the glucomannan of this wood. Likewise pectinase yielded only the odd monosaccharide.

Courtois et al (1958) investigating takadiastase found an enzyme which hydrolysed luzerne galactomannan. They found that galactose was first liberated, then mannose and finally a disaccharide which was isolated and found to be β-1:4 linked mannobiose.

Reese and Shibata (1965) in an exhaustive search for mannanase activity tested over 290 fungal cultures and 60 commercial preparations. They found that activity was induced, yields generally being 10 to 100 fold greater when grown on mannan. All the enzymes found were random endopolysaccharases, with no activity on mannobiose or mannotriose. The most active organisms were Penicillium wortmanni, Aspergillus giganteus and Paecilomyces varioti. A general survey of the properties of mannanases revealed that most pH activity curves showed peaks around 6.0 to 8.0. However, below pH 4.5 there were two distinct groups. One was characterized by A. giganteus and was relatively inactive below pH 4.5 and the other group by Penicillium verruculosum, which possessed relatively high activity even at pH 3.0. From the shapes of the curves these workers thought that the presence of two distinct mannases was likely.

Thermal stability was also a characteristic feature of most of these enzymes, whilst maximum activity was in the 60^{o} to 70^{o}C. range.

In conclusion, it can be said that very little work has been done on mannanases, particularly in the mannanases degrading wood. Information obtained from xylanase and cellulase research can give a useful indication as to what might be found in future investigation of mannanases.

FUNGAL DECAY

Introduction

Fungal decay in temperate countries is the most important cause of timber loss. Fungi are parasites or saprophytes. They do not possess chlorophyll, and are therefore unable to photosynthesize, but live on either animal or plant material. Wood destroying fungi are in the main "higher fungi" or Basidiomycetes. The name is derived from the fact that the fungus reproduces by the production of spores on clublike structures called basidia. The great majority of brown rot and white rot fungi are Basidiomycetes. Some, however, belong to the other main classes the Ascomycetes, whose spores are kept in a cylindrical container called an ascus, and those whose perfect (or sexual) stage of reproduction is not known and are called the Fungi Imperfecti. Soft rot and staining fungi are usually members of these two groups.

Wood inhabiting fungi have been classified according to the following scheme (Levy, 1969).

(i) Moulds
(ii) Stainers
(iii) Stainer soft rots
(iv) Soft rots
(v) Brown rots
(vi) White rots

The dividing line is sometimes difficult to draw and the classification of a particular fungus may be dependent upon the nature of its substrate. It is possible for an organism to

display one type of attack on a hardwood and another on a softwood. For example, Phialophora fastigiata can act as a staining fungus in softwood but as a soft rot and stainer in hardwood.

(i) Moulds

These may belong to any of the main fungal classifications, but in particular Phycomycetes, Ascomycetes, or Fungi Imperfecti. They seem to have little or no effect on the basic structure of the wood but primarily feed on the food substances stored in the wood (e.g. starch), leaving the timber structure unattacked to form their habitat. Moulds may be regarded as inhabiting only the surface of the wood, and disfigure by means of the pigments in their conidia. Where mould hyphae penetrate into the surface layers of wood it is generally through the existing pits, or holes produced by other organisms. They normally feed on food reserves of cut cells on the surface. Mould fungi assume a more important role than their intrinsic one in that they are almost invariably the natural precursors of more vigorous wood destroying fungi. It is possible that the wood is left in a more vulnerable state as a result of their activity, or the mould mycelium could be used as food, thus allowing colonization to take place. This would be impossible on wood which has not been subjected to mould growth (Banerjee, 1969).

Nilsson (1973) tested several non-identified species of Penicillium and found that some of them caused a slight attack of the erosion type in birchwood. No soft rot cavity formation was found. This contrasts with the finding by Levy (1969) that Penicillium cyclopium produced soft rot cavities in birch and beech

(ii) Staining fungi

These are similar to moulds in that they use stored materials for their food rather than the wood structure; staining fungi are quite capable of very active penetration into the wood particularly into the parenchyma of the rays and, as their name suggests, are responsible for severe discoloration and the consequent economic loss. Infection can occur either by insect borne or by air borne spores. Little if any structural loss of strength results from decay by staining fungi although parenchyma cells seem to be susceptible to stainers. Liese and Schmid (1961) have observed destroyed cell walls in the ray parenchyma, and resin canal parenchyma has also been destroyed (Scheffer and Lindgren, 1940). Bending and compressive strengths remain relatively intact but shock resistance is slightly lowered. (Findlay and Pettifor, 1939) It is more usual to find staining fungi inhabiting the sapwood, where a supply of nutriment is present; some are found in the heartwood although the reason for this is obscure (Etheridge and Pomerleau, 1961). Increased heartwood attack may ensue where the heartwood is in close physical contact with infected sapwood. Rennerfelt, 1945, inhibited stainer growth in sapwood by prior soaking in phenolic extractives previously contained in the heartwood. The sapwood of pines is more susceptible to stainers than the sapwood of spruces (Findlay, 1959).

Hyphae may pass from cell to cell via the pits, but can also penetrate by small bore holes, resulting in the characteristic staining fungus "transpressorium", or swelling of the hypha

prior to the formation of the fine constricted hypha through the wall. It is most common to find the rays heavily colonized by these fungi. Seifert (1964) studied the chemical changes effected in wood components by the blue-stain fungus Pullularia pullulans. The different constituents of pine wood were determined by chemical analyses both before and after infection. It was found to cause a depolymerization of the carbohydrates to the extent that 7% cellulose and 3 to 4% hemicellulose (measured as pentosans) were lost during the test period. Lignin was not metabolised. Upon chromatographic analysis he found that the cellulase component responsible for splitting micelles into individual filamentary molecules was virtually undeveloped though polysaccharide chains were degraded, perhaps where present in the amorphous regions.

(iii) Stainer soft rots

Krapivina (1960) first suggested that staining fungi could also cause soft rot. Karkanis (1966) examined eight species of staining fungus and found two, Ceratocystis piceae and Phialophora fastigiata capable of causing soft rot in beech and birch. No attack of softwoods was observed using malt agar medium for growth, and he came to the conclusion that these fungi did not produce soft rot cavities. Nilsson (1973) has shown that cavity formation can be induced by using a different medium though even under his conditions cavity formation appeared only after 29 weeks' incubation. Levy (1967) listed a number of species capable of causing soft rot.

Umezurike (1969) investigated the staining fungus isolated from Bombax wood and found it to be Botryodiplodia theobromae. Upon examination it was found to produce elongated cavities in a manner similar to that of a soft rot. Starch and other "free" substrates were however, preferentially used prior to cavity formation.

Various reports of staining fungi producing soft rot cavities under special conditions, together with staining fungi, which given certain culture conditions produce soft rot decay only when in certain wood species, result in an intermediate class of fungi which has yet to be well defined. Liese (1964) has observed formation of lysis zones in conifer wood cell walls caused by Alternaria sp. Liese and v. Pechman (1959), Courtois (1963)a, Merrill (1965) Levy (1967, 1969) and Merrill and French (1965) observed shaped cavities in the cell walls of wood attacked by the mould Trichoderma viride. Many workers however, have not yet observed, or been able to reproduce these results. Bearing in mind the experiment by Banerjee (1969) (see later), investigating the effect of the prior culture media, it is possible that soft rot depends not only on the substrate wood, but also upon the enzymes induced in stock culture.

(iv) Soft rots

Savory, 1954 (a) and (b), stated that the serious deterioration of the preserved timber in water cooling towers was attributable to fungi. He suggested the term "soft rot" for the particular pattern of decay found. He established the

following five points concerning soft rot fungi;

(i) they were species of Ascomycetes and Fungi Imperfecti.

(ii) they tended to run spirally in the S_2 layer giving rise to cavities with pointed ends.

(iii) they decomposed cellulose, and initiated attack in the less heavily lignified parts of the cell wall.

(iv) the rate of attack was increased when organic nutrient salts were added to the wood.

(v) a gradient of attack was established with the most heavily decayed wood on the exterior, and the least attacked towards the centre.

The second characteristic enumerated above, namely the decay pattern observable under the microscope, is most typical. Chains of cavities follow the microfibrillar axis (see Fig. 8) in the S_2 layer. This has been termed Type 1 decay. Lumen erosion or Type 2 decay is very often present. (Corbett, 1965) No satisfactory explanation for the shape of cavities has yet been given. The factors responsible must be;

either (a) the wood microstructure

or (b) the influence of the fungus.

These will be dealt with separately although a combination of both is quite possible

(a) <u>the wood microstructure</u>.

This can be further subdivided into,

I Physical,

and II Chemical.

I Physical

Observations as early as 1850 by Schacht, have shown that the lines of soft rot cavities assume peculiar and definite orientations. Bailey and Vestal (1937) described these cavities in some detail and isolated a number of organisms. The lines of cavities formed were parallel to the plane of orientation of the cellulose microfibrils. Thus they found that the direction of longitudinal fungal growth was along these axes. Lateral enzymic activity, which was responsible for formation of the cavities, was restricted to certain planes. These they measured and found to be 20-25° to the main axis, and concluded that it was the molecular conformation of cellulose in the microfibrils which caused the shaped cavities.

This concept was further enlarged by Frey-Wyssling (1938) who used the now disputed Meyer and Misch (1937) model for the molecular structure of cellulose, to point out that the $41\bar{4}$, $43\bar{4}$ and $45\bar{4}$ planes could be sites for preferential hydrolysis. The angle which the $45\bar{4}$ plane makes to the primary axis is approximately 23°, agreeing very well with the 20-25° angles measured by Bailey and Vestal.

Wardrop and Jutte (1968) observed conical ends (with angles of similar degree to Bailey) on the microfibrils from Valonia ventricosa when they were subjected to a snail cellulose (Helix pomatia). They performed similar experiments on wood fibres (Jutte and Wardrop, 1970) and found residual areas of birefringence tapered to an angle of 24°. Thus they concluded that the shape of the soft rot is dependent on the physical nature of the cellulose.

II Chemical

The lignin concentration has been suggested as being a vital factor in the interaction of a fungus with wood. Barghoorn(1949) attributed the fact that soft rot fungi start degradation in the S_2 layer, to differential lignin concentrations. Olson et al (1937) Waksman and Cordon (1938), and Virtanen et al (1938) all claim to have demonstrated a correlation between lignin content and decay of cellulose-lignin materials though not necessarily by soft rot organisms. Levi and Preston (1965) proposed that the rate of decomposition was limited by lignin, and consequently that the rate of decomposition of cellulose was limited by the rate of "modification" of lignin by an enzymic mechanism. They added that at the amorphous regions of cellulose this "modifying" enzyme was capable of lateral penetration.Bailey et al (1968), Courtois (1963b) and Novertné (1968) have shown that susceptibility to soft rot fungi is much greater after partial delignification. It was observed during the present work that holocellulose was more subject to fungal degradation than sawdust.

Findlay (1970) found in his work on rubbery apple wood, where it is thought that lignin is deposited as a series of monomeric units rather than as a polymeric whole (Sondheimer and Simpson, 1962; Scurfield and Bland, 1963), that no cavity formation occurred after attack by Phialophora fastigiata. Breakdown of the wood structure occurred more in a random dissolution with the absence of cavities, similar in the manner to the brown or white rots. He concluded that it was not the

presence of the individual monomeric units of lignin which 'protects' the cellulose, but the polymerised lignin and its possible linkage with cellulose.

He went on to propose, subject to certain assumptions, that the degradation of an individual microfibril by a limited amount of enzyme complex would stop at a region of higher lignin concentration. Simple diffusion proceeds spherically, but in wood a solid ellipse would be formed owing to the differential rates of diffusion along two axes, one parallel and one perpendicular to the microfibril. The shape which is finally determined is due to differential lignin concentration stopping hydrolysis along individual microfibrils, but giving a smooth appearance at higher magnifications than the microfibril level. It appears to the author that it is unlikely for lignin distribution to be so ordered as to cause this shape, but seems more probably a property of the cellulose. This is substantiated by a fact already demonstrated that cavities can be formed in cotton fibres, where there is no lignin, and in jute, where the lignin concentration is small.

It is of importance to note that most of the soft rot organisms tested, when grown in culture, will produce a fluid of pH 6 or more. This is in direct contrast with other types of rot such as brown or white which are characterised by conditions of low pH (ca. 2-3). It is possible that the difference observed in the methods, whereby these fungi solubilize cellulose, is a result of varying pH levels.

The greater susceptibility of hardwoods to soft-rot attack indicates that a chemical difference (between soft and hard woods) may be regarded as having a bearing on the formation or non-formation of cavities. During this work it was observed that the soft rot stainer Phialophora fastigiata can attack hardwoods causing typical soft-rot decay patterns and cavities, but when in softwood it merely penetrates the wood in the manner of a stainer and causes no weight loss.

There are two main chemical differences in the wood, firstly, the lignin concentration is much higher in softwoods; secondly, in the hemicellulose of softwoods the glucomannan predominates, whereas in hardwoods it is the xylan. To date most workers have opted for the former as an explanation for the behaviour of the fungus; in this present work, however, the role of the hemicelluloses and the enzymes which degrade them have been more thoroughly investigated; and it is believed that this may be a significant contributory factor in the difference of attack shown by one fungus to soft and hardwoods.

Jutte and Wardrop (1970) found that artificial "lignification" according to Solberg and Higginbottom (1957) resulted in a protection from attack by 'helicase' cellulose for as long as six months. They artificially lignified several strains of wood using isolated fibres, and also found that control fibres which had not been lignified decayed quite easily.

(b) The influence of the fungus

Roelofsen (1956) attributed cavity formation to the fungus rather than the timber microstructure. He questioned the plausibility of planes of hydrolysis on any more than the

crystalline level. He doubted the validity of these planes on a microfibrillar or macrofibrillar level. Instead he cited the work of Galloway (1931), who found that hyphae grown on woven fabrics grow in perfectly square or rhombic shapes. It was suggested that the hyphae preferred to grow along the direction of cellulose in the warp and weft, rather than in a random fashion. He proposed that the rhombic shape of cavities in wood derived from fungal hydrolytic enzymes diffusing preferentially along the microfibrils at right angles to the microfibrillar axis rather than at any particular angle. To do this he assumed "that the rate of corrosion was limited by cellulose decomposition and not by lignin decomposition", and also that cellulose was bound or adsorbed onto cellulose and could not freely diffuse. He also had to assume that cellulase could diffuse through intermicrofibrillar points of contact. These assumptions are all possible, but in the light of present opinions it seems unlikely that all three are either true or significant as a cause of the cavity shape.

It is possible that there is some effect on the shape of cavities due to the fungal culture medium. Banerjee (1969) used C. globosum, which had been subcultured four successive times on Abrams' cellulose agar, and obtained pronounced diamond shaped cavities in addition to the normal elongated soft rot type. It seems possible therefore that the high concentration of polysaccharide destroying enzyme, induced when C. globosum is thus grown, may be responsible for an enhanced lateral penetration for the same axial penetration. The increase in weight loss of test blocks was very marked when Abrams' cellulose was used

instead of malt. In birch the increase was from 0.7% to 27.2%, and in Scots pine from 0 to 2.6%. The degree of attack as seen under the microscope closely reflected these figures.

Bailey et al (1968) point out that "it is important to remember that these cavities result from the activities of living organisms, and their origin should not therefore be sought solely in terms of the structure of the substrate". They go on further to postulate a "pre-cellulolytic" system, which fungi use when attacking wood and which is in some way deficient in the case of soft-rots. They then go on to explain the shape of the cavities on the basis of differential diffusion rates, the lateral diffusion rate being hindered by a lack in the pre-cellulolytic system.

In perhaps the most comprehensive of recent work in this field Nilsson (1973) studied 160 different species of microfungi. Most were wood inhabiting and belonged to the moulds, stainer soft-rot, and soft-rot categories. These fungi were tested for their decay pattern on birch. Five different types of sap wood, aspen, beech, birch, pine and spruce were used to investigate the decay capability of 51 selected fungal species. Cellulolytic activity was determined for all 160 fungi by the Rautella and Cowling (1966) method of clearing zones of Walseth cellulose agar in test tubes. In addition some 44 selected fungi were tested in a modified Bravery (1968) medium, from which almost all alternative carbon sources apart from cellulose had been eliminated He found that 109 species gave positive cellulase activity by the first method, and of the 44 species tested on both media, some four species gave positive results on the Bravery medium, which

had previously proved to be negative on the other. The conclusion drawn was that small quantities of alternative carbon sources (present in the Rautella and Cowling method) could influence the result of a cellulolytic activity determination. Fungi were classified into three groups with respect to their activity on birch wood: (1) species giving only Type 1 decay (cavity formation) (2) species giving Type 2 decay (lumen erosion) and (3) species giving both Type 1 and 2.

One of the major discoveries was that all the species giving Type 2 or Type 1 + 2 decay showed cellulolytic activity, but fifteen out of the twenty-three species which gave only Type 1 decay showed no clearing of cellulose agar. It was observed that Type 1 decay fungi, which gave a negative result with the cellulose test and yet were capable of wood decay, grew sparsely on cellulose agar and vigorously on malt agar. Cavity formation was considerably enhanced when malt agar was used instead of cellulose agar. This difference was not observed when Type 1 fungi gave a positive cellulolytic result, most of the fungi in groups (2) and (3) gave equal or more growth on cellulose that on malt agar.

The first visible attack on the softwoods was always found in the S_2 layer adjacent to the S_3, and a considerable amount of S_2 layer could be removed before any visible degradation of the S_3 became apparent. This confirmed a similar finding by Bravery (1972). Degradation of the softwood S_3 layer was observed only with very few species. Cavity formation in the two softwoods was very much slower than in birch, though all species which formed cavities in birchwood eventually did so in the softwoods. This

was a suprising finding contrasting with the present work and will be fully discussed at a later stage.

(V) Brown rots

As implied by the name, the wood residue left when a brown rot has been responsible for decay is often brown coloured, owing to the high residual lignin content. When brown rotted wood is dried it tends to shrink considerably, and this in turn often gives rise to a cubical pattern of cracks very characteristic of certain brown rot species. Under the microscope the specificity of brown rot decay quickly becomes apparent. Boreholes are formed probably in the later stages of decay by the fungus passing from cell to cell, and are enzymic rather than mechanical in origin. Apart from these holes there is very little early evidence of destruction owing to the residual lignin framework upholding the structure of the wood. The early strength loss, however, is considerable. At an advanced stage of decay the cell wall collapses causing decreased cell size and wall thickness. In a detailed study using the electron microscope Meier (1955) observed the attack of brown rot on spruce and birch. He found that in both cases the S_2 layer was attacked first; this was then progressively decomposed towards the middle lamella. The S_3 was found to be attacked in direct contradiction with Bravery's work (1972). The brown rot decay did not appear to be uniform in the sense that some cells would be decayed while the next cell could be whole. Cowling (1961) has made an extensive study of the biochemistry of the decay of sweetgum sapwood by the brown rot

fungus Poria monticola. He observed the characteristic lack of utilization of lignin, and a very rapid depolymerization of α-cellulose suggesting an endo-action enzyme. The lignin, although not appreciably metabolised, was modified as was shown by a greater solubility in water and 1% sodium hydroxide. This could be due to removal of methoxy groups. Brown rots tend to be associated with gymnosperms (Hartley, 1958).

(VI) White rots

This type of fungus does as its name implies impart a white or bleached hue to the timber which it infects. This is not due to the preferential utilization of lignin, but as a result of the modification of chromogens in the timber (Campbell, 1952). White rotted timber shows normal shrinkage properties, in direct contrast to brown and soft-rotted timber. Under the light microscope pit enlargement is evident and so is uniform erosion or gradual thinning of the cell wall from the lumen to the middle lamella. The electron microscope revealed that this uniformity was suspect, and small holes were in fact found in the secondary wall and middle lamella prior to general thinning (Cowling, 1961; Meier, 1955). Bravery (1972) has shown scanning electron micrographs where deep grooves are associated with the fungal hyphae, almost certainly as a result of the diffusion of enzymes, and consequent solubilization of the cell wall material. Lignin is metabolised though not preferentially. Wilcox (1968) observed that lignin decomposition appeared to precede cellulose decomposition in lignin-poor regions and was more even near the lignin-rich middle lamella. An exception to this generalization is the fungus

Peniophora sp. (Henningsson et al, 1972) which is being developed with a view to delignification in the pulp industry. White rot fungi tend to destroy angiosperms (Hartley, 1958), although they can cause damage to gymnosperms under laboratory conditions (Cowling, 1961). From viscosity studies Cowling was able to establish that Polyporus versicolor produced only a gradual decrease in D.P. of the cellulose in the attacked wood. He attributed this to the fungus selectively attacking only small portions of the holocellulose at a time, so that the average D.P. of the remaining molecules was not greatly affected.

Materials

Glassware and General

Pickle jars, for decay capability tests, were of square cross-section (60 x 60 mm.), 250 mm. high and a round screw top (48 mm.) pierced with one hole (3-4 mm.) packed with non-absorbent cotton-wool plug.

Assay tubes were 20 mm. x 150 mm. (Pyrex) Medical flats, obtained from Beatson, Clark & Co., Rotherham, Yorks, were 160 x 45 x 80 mm and had a volume of 475 mls.

90 mm. single-vent petri dishes were from Sterilin, Richmond, Surrey. (The depth was 15 mm.)

Poly-stoppered specimen tubes were flat bottomed soda glass and of two sizes, 2" x $\frac{1}{2}$" and 3" x $\frac{1}{2}$".
Plastic netting was from Transatlantic Plastics, and had about 7 holes / cm^2.

Chemicals

Oxoid agar No. 3 was from Oxoid, London. Sodium carboxy-methyl cellulose "Cellofas B" Grade 50 was a kind gift from I.C.I.
Amberlite resin 1R - 120 standard grade was from B.D.H.
All standard chemicals used such as NaOH, KCl, etc. were of "Analar" grade unless otherwise stated.
N.J.K. - Spruce GGM - was a kind gift of Dr. N.J. King, P.R.L.
All other hemicelluloses were prepared as described in the Appendices I and II. Cellulose powder was Whatman's CF 12 (Fibrous).

Equipment

The Laminar Flow Bench was Model 64T by W.H.S. (Pathfinder) Ltd., Havant, Hampshire. This is now marketed by Microflow Ltd., Fleet, Hants.

The Pipetman 1000 was from Gilson, Ltd.

The Zipette (10 mls and 50 mls) was from Jencons, Hemel Hempstead.

The Freeze-drier was an Edwards Model 30 P.I.T. Serial No. 115 with a shelf temperature of - 40°C.

Introduction and Comment on the METHODS Section

Wherever it was considered to result in greater clarity of presentation, experimental detail has been confined to an Appendix. The methods section therefore sometimes contains only a brief indication of the procedure used. Appendix I is a general collection of the various detailed experimental methods used, whilst Appendix II is confined to describing the production of hemicelluloses. It is of interest to note that the latter was the most laborious part of this work and took some two years to complete. Recent work in this laboratory has indicated that for xylan, at least, there is a commercial source available, which results in very similar results for the hemicellulase monitoring experiments. However, there was no such availability of a glucommanan. (Lewis and George, 1973).

For most of the test methods there are alternative procedures, for example, reducing sugars may be measured by a variety of methods other than the Nelson-somogyi, however the adopted methods were generally chosen because other workers in the laboratory were familiar with them, thus reducing the learning time involved. Simplicity and speed of operation was a significant criterion in assessing the potential of a new or unfamiliar method.

METHODS

Weight Loss Tests.

These were carried out using 1 cm. cubes on a mycelial mat growing on wood sawdust agar. For experimental details see Appendix I. Owing to various initial difficulties, for example contamination, a variety of different experimental weight loss tests were performed.

Enzyme Assays.

These were achieved by determining the increase in the reducing sugars produced after incubation with a substrate solution. Substrates were in solution/suspension at pH 5.0 in acetate buffer (0.2M.) at a concentration of 0.1%. The method used was that of Somogyi modified by Nelson (Nelson, 1944; Somogyi, 1952.) The results were in terms of monosaccharide equivalents, (e.g. glucose, xylose, mannose, arabinose, galactose) or, in the case of a complex substrate like galactoglucomannan, they were calculated proportionately to the individual components (see Appendix II; standard curves). Incubation time was generally 60 minutes. In the case of insoluble substrates under test it was 7 or 40 days.

Lignin Estimation.

This was a modification of the Klaison (1906) procedure. (See Appendix I).

Degree of Rotation.

The degree of rotation of plane polarized light was determined on a Bellington and Stanley polarimeter for birch; xylan, D.M.S.O. extract, glucomannan, α-cellulose, and for Norway spruce; xylan, D.M.S.O. extract, galactoglucomannan, and α-cellulose.

Investigation of growth and production of hemicellulases by fungi in liquid culture on wood termed hereafter "Hemicellulase monitoring experiment"

Nineteen strains of fungi were used comprising three species as listed in Appendix I. They were grown in small scale stationary culture in medical flats with wood as the sole carbon source. Duplicate flats were prepared for both birch and Norway spruce woods resulting in 80 bottles including controls. After inoculation the growth at 25°C was monitored by extracting samples from the liquid culture in a sterile manner. This was done at 0, 3, 6, 9, 12, 15, 20, 25, 32, 40, 55 and 70 days. (In the case of the repeat experiment with P. monticola and P. fastigiata samples were taken at 0, 9, 16, 23, 30, 37, 51, 65 and 80 days). The pH was measured prior to addition of 0.2M acetate buffer pH 5.0 and sodium azide. Storage was in polythene stoppered specimen bottles at 2°C. With the exception of the pH, the following tests were carried out on the modified liquid culture samples.

I pH (on the initial culture filtrate).

II C.M.C.-ase (i.e.'cellulase' activity)

III Protein

IV(a) Spruce galactoglucomannanase (the G.G.M. given by N.J. King) referred to as N.J.K. Spruce GGM-ase.

IV(b) Spruce galactoglucomannanase (GGM extracted from Norway spruce) refered to as Spruce GGM-ase.

V Xylanase (xylan extracted from birch wood)

VI Birch D.M.S.O. hemicellulase activity.

VII Spruce D.M.S.O. hemicellulase activity.

Substrates involved in reducing sugar assays were at 0.1% w/v concentration, and were tested under their respective specified conditions of assay.

After calculation of the above activities in terms of µgms per tube, graphs of activity were plotted against the time taken for growth. See results section.(See page 161)

Reproducibility Experiment (to test the reproducibility of the hemicellulase monitoring experiments with different operators)

In order to test the procedure devised on the hemicellulase monitoring experiments under normal working conditions, a modified version of the hemicellulase monitoring experiment was set up in an undergraduate class of seven operators. For economic reasons wood of mesh size 85 upwards was used, although it is recommended that 40-80 mesh be used. Four test fungi were chosen to represent a spectrum of wood-destroying activity. They were;

1. Chaetomium globosum S70B..... a soft rot.
2. Phialophora fastigiata 56A..... a soft rot stainer.
3. Poria monticola 304B..... a brown rot.
4. Polystictus versicolor IMI 79126.... a white rot.

The sawdust and mineral medium was incubated with the test fungi as for the hemicellulase monitoring experiment described in Appendix I. Norway spruce and birch woods were used, each fungal species was inoculated in duplicate medical flats and with both types of wood. Controls consisting of un-inoculated mineral medium and sawdust were also set up. Growth was limited to 40 days at 25°C, a period of high yield indicated by previous data. At approximately weekly intervals the flats were gently shaken and the developing hyphae redistributed through the medium.

After extraction of the culture filtrate, its activity was determined in terms of reducing sugars on three substrates. This gave rise to carboxymethylcellulase, (CMC-ase), galactoglucomannase (GGM-ase) and xylanase (XYL-ase) activities. Nelson-Somogyi reducing sugar tests on buffer were used as blanks for the calculation of final reducing sugar values.

Long Term Enzyme Digests.

Reducing sugars were estimated in samples from digests after seven days and forty days.(see Appendix I.)The enzymes were from culture filtrates of C. globosum S 70B, P. fastigiata 56A, P. monticola 304B and P. versicolor IMI 79126, grown on both birch and Norway spruce (60-80) sawdust for forty days. The same culture filtrates were employed in the viscosity and cellobiase assay, but they had no connection with the hemicellulase monitoring experiment culture filtrates.

Cellobiase assay. (after Fuller, 1970.)

Activity was determined by using cellobiose (40 mg./l.) in a 0.2 M. acetate buffer pH 5.0 as a substrate in the 60 minute incubation reducing sugar assay.

pH measurement.

This was on a Pye Unicam 292 pH meter.

Protein estimation.

This was by a modification of the Lowry et al.(1951) method. See Appendix I.

Culture medium.

This was a Fuller (1970) modification of Abram's (1948) medium. See Appendix I.

Hemicellulose preparation.

See Appendix II.

Growth on hemicellulose

This experiment was designed to determine the effect of a particular hemicellulose being used as the sole carbon source in a culture on the resultant enzymic production. Birch XYL and Norway spruce GGM were used to grow fungi in exactly the same way as the wood culture bottles. Several bottles were incubated for each fungal strain. (see page 326 Appendix I). The scope of the experiment was broadened by using inocula from three different sources within one fungal strain. For example P. fastigiata 56A was grown on spruce agar, birch agar, and malt agar on McCartney slopes, and all three were used in inoculation. Growth was for 31 days when birch and spruce agar were used, and for 24 days when malt agar was employed. The temperature of incubation was $25^{o}C$.

Harvesting of the cultures of fungi grown on Hemicellulose

Medical flats were combined where inoculation and substrate were the same prior to centrifuging off the dense growth of mycelium. A record was kept of the volume, and pH of the resultant culture centrifugate. This enzyme solution was made up to pH 5.0 with 4N NaOH or 1 N.HCl as was necessary. The volume of acid or base used was also noted Reducing sugar assays were determined in triplicate on enzyme at pH 5.0, after prior addition of sodium azide (ca. $120g/l^{-1}$) so as to make the final concentration 0.31 $g.l^{-1}$

See Fig. 32, p.240.

Viscometric assay.

This was based on the method of Almin and Eriksson (1967) and Almin et al.(1967). The substrate was C.M.C. (2.0 gm./lit.) A No. 2 suspended level viscometer of the following specifications was used;

Efflux volume.....7 mls.

Capillary length.....9 cms.

Capillary diameter.....1 mm.

Efflux time for 0.2 M. sodium acetate buffer at pH 5.0 and 40°C.....70 seconds.

All assays were at 40°C. Substrate solution(15 mls.) was introduced into the viscometer and allowed to equilibrate to that temperature . The efflux time was then noted for substrate without enzyme, after which 250 µl. of preincubated enzyme solution was added and mixed, concurrent with the starting of a stopclock. Measurement of efflux time was as soon as possible after this mixing, and successive efflux times were noted until the time taken stopped decreasing. This was usually near the figure of 70 seconds required for the buffer alone, and in general the reaction took 30 minutes. 40 day old birch and spruce culture filtrates of four test fungi were assayed for activity by viscometry. The fungi used were C. globosum S 70 B, P. fastigiata 56 A, P. monticola 304 B and P.versicolor IMI 79126. Graphs were plotted of Log.$\int \mu \int$against the time in seconds, according to Fuller (1970), and arbitrary units of enzymic activity were determined from the resultant curves.

Scanning Electron Microscopy

Specimen preparation

Samples were of two types; whole timber, which was used as a control, and timber which had been attacked by fungi. In the former the sample was either a small block (ca. 1.5 x 0.75 x 0.75mm.) or was a section, and in the latter the sample was always in the form of a section. Section sizes varied depending upon the nature of the centimetre cube from which they were cut.

The sections were approximately 7 x 5 x 1mm. for radial and tangential longitudinal sections (R.L.S. & T.L.S.). These were produced by splitting the orientated test block with a scalpel. It was convenient to mount both the R.L.S. and T.L.S. of any particular centimetre cube on the same stub. Durofix glue was used to attach the sample to the stub and then a thin layer of Silverdag (a conducting glue-like material) was applied to the sides of the wood in such a way as to provide a conducting layer between the wood and the stub. This was not applied to any surface which had been an outside edge of the original cm^3.

Considerable technique and practice was required to produce good transverse sections. A piece of wood approximately 10 x 2 x 0.75mm. was cut from the centimetre cube in such a way as to provide at least one "outside" surface from the original cube. This was then held under a binocular microscope and sections cut off as thin as was possible using a new section of "Gillette" razor blade for each cut. As a section was cut it would stay in place on the razor blade (possibly by electrostatic attraction) and could thus be directly transferred to an already stub. About ten to fifteen sections were cut per sample and

placed on the same stub. R.L.S. T.L.S. and T.S. were produced for each of the timbers under test. In the majority of cases the samples were freeze dried; some however were oven dried at 105°C.

Stub Preparation

The stub consisted of a metal disc 12mm. in diameter with a small holder on the underside. The top surface was polished with fine grade emery paper prior to addition of the wood.

Specimen Coating

A thin layer of carbon or metal (usually silver or gold/palladium), is generally used to suppress charging and increase electron emission. However, despite being very effective, the method has limitations. The material will be deposited unevenly, giving rise to a situation whereby a particular topographical feature is unnaturally enhanced. The prepared stub was therefore spun through as many axes as possible during the coating procedure, and also the conducting layer was kept to the minimum thickness consistent with good results. In this work most of the wood samples were coated twice in gold/palladium, each application being with about 2.5 cm. of wire.

Aperture

Three aperture sizes were available (50,100 and 200μ). These were termed Nos. 3, 2 and 1 respectively. No. 1 was used in all the photographs shewn.

Beam Voltage

This was 20kV.

Tilt

The angle of tilt can be varied from 0 - 90° with optimum resolution at 45°. Most electron micrographs were taken at the latter angle, and when this was not the case the tile angle was defined.

Operation

This was according to the manufactures instructions.

RESULTS and DISCUSSIONS.

The Results and Discussions section is concerned with the work done on ten different experiments. These are listed as follows:-

1. Weight Loss Tests
2. Lignin Estimation
3. Degrees of Rotation Estimation
4. The Hemicellulase Monitoring Experiment
5. The Reproducibility Experiment
6. Long Term Enzyme Digests
7. Cellobiase Assay
8. Activity Measurement by Viscosity
9. Fungal growth on Hemicellulose
10. S.E.M.

The results for each experiment have been tabulated or presented as concisely as possible and each is immediately followed by a discussion/conclusion concerned with that particular section of results. A final discussion for the whole thesis is presented later.

The weight loss tests are a series of six different experiments, the variables for each experiment (i.e., type of wood block, agar, duration of test, etc.) are shewn in tabular form before the results and it is labelled "Summarised Experimental Details of a Series of Weight Loss Tests". This table is an amalgum of the many different experimental attempts to find satisfactory conditions for weight loss estimation, and although the variation of the components of the test is planned, some are repeated more than others. For example 1(a), (b) and (c) are testing the same combination of fungus and substrate, but 1(d) and (e) vary the type of wood block and wood agar.

Lignin estimation is a necessary check for determining the degree of delignification of the holocellulose, and thus the degree of accessibility of the hemicellulose to be extracted.

The degrees of rotation estimation is a standard method of characterising hemicelluloses.

The hemicellulose monitoring experiment was complex, in principle it involved culturing a fungus in liquid medium containing nutrient salts and wood sawdust as sole carbon source. Some of the resultant enzyme activities induced were then assayed. These included the hemicellulases GGM-ase and xylanase.

Long term enzyme digests were made to compare the present results with those obtained by Fuller in 1970. This was also true of the cellobiase assay, and in addition it was to confirm that the cellobiase had a minimal contribution to the activities shewn in the hemicellulase monitoring experiment.

Viscometric assays have been recommended as being the method closest to the ideal standards set by the Enzyme Commission for the measurement of cellulase activity. A convenient, relatively quick method of measurement of activities by viscometry was therefore investigated for comparison with the reducing sugar assay. In the table of results xylanase and GGM-ase activities are also shewn.

The growth of wood destroying fungi, with hemicellulose as sole carbon source instead of wood, was recorded by colour photography. The filtrates were harvested their pH and volume recorded prior to adjusting them to pH 5.0. Each culture filtrate was then assayed for cellulase, xylanase and GGM-ase activities.

The SEM section consists of approximately 120 photomicrographs shewing a variety of aspects of the behaviour of fungi in wood.

Summarised Experimental details of a Series of Weight Loss Tests Fig.21

Exp. No.	Type of wood	Type of agar.	Fungal species and strain.	No. of tests	No. of $cm.^3$ per test.	Duration of test.	Type of drying
1(a)	Birch	Birch	C. globosum S70 to RLB	3	3	8 weeks.	A.D.
1(b)	Birch	Birch	"	6	1	8 weeks.	A.D.
1(c)	Birch	Birch	"	6	1	8 weeks.	A.D.
1(d)	Spruce	Spruce	"	3	6	8 months.	A.D.
1(e)	Spruce	Birch	"	1	3	8 months.	A.D.
2(a)	Birch	Birch	P. monticola 304B to 280.	2	3	8 weeks.	A.D.
2(b)	Spruce	Spruce	"	2	3	8 weeks.	A.D.
2(c)	Spruce	Birch	"	2	3	8 weeks.	A.D.
3(a)	Birch	Birch	P. fastigiata 56A and Bann 1	2	3	8 weeks.	A.D.
3(b)	Spruce	Spruce	"	2	3	8 weeks.	A.D.
4(a)	Birch	Birch	P. monticola 304B to 280	1	10	5 months.	F.D.
4(b)	Spruce	Spruce	"	1	10	5 months.	F.D.
5(a)	Birch	Birch	P. fastigiata 56A and Bann 1	1	10	5 months	F.D.
5(b)	Spruce	Spruce	"	1	10	5 months.	F.D.
5(c)	Spruce	Birch	"	1	10	5 months.	F.D.
6(a)	Beech	Malt	C. globosum S70B	7	3	4 weeks.	A.D.
6(b)	Beech	Malt	P.monticola 304B	7	3	4 weeks.	A.D.
6(c)	Beech	Malt	P. fastigiata56A	7	3	4 weeks.	A.D.
6(d)	Beech	Malt	P. versicolor	7	3	4 weeks.	A.D.
6(e)	S. pine	Malt	C. globosum S70B	7	3	4 weeks.	A.D.
6(f)	S. pine	Malt	P. monticola304B	7	3	4 weeks.	A.D.
6(g)	S. pine	Malt	P. fastigiata56A	7	3	4 weeks.	A.D.
6(h)	S. pine	Malt	P. versicolor	7	3	4 weeks.	A.D.

A.D. = air dried, F.D. = freeze dried. at 105 C. Expt. No. = number of experiment

RESULTS

Weight loss tests. (See "Summarised Experimental Details")

		Test 1		Test 2		Test 3		Average		Average
		O.W.	W.L.	O.W.	W.L.	O.W.	W.L.	O.W.	W.L.	% W.L.
1(a) C. globosum.										
S 70	BIRCH	4.69	0.61	4.73	0.42	4.55	1.15	4.66	0.727	15.6
S 70 B	BIRCH	4.72	0.76	4.64	0.35	4.75	0.49	4.70	0.53	11.3
S 70 E	BIRCH	4.89	1.06	4.66	0.30	4.67	0.61	4.74	0.657	13.9
S 70 G	BIRCH	4.68	0.70	4.70	0.50	4.70	0.70	4.69	0.63	13.4
S 70 K	BIRCH	4.76	0.47	4.71	0.30	4.50	0.55	4.66	0.44	9.7
S 70 L	BIRCH	4.79	0.52	4.62	0.53	4.66	0.89	4.69	0.647	13.8
S 70 M	BIRCH	4.74	0.88	4.71	0.64	4.47	0.38	4.64	0.65	14.0
S 70 N	BIRCH	4.70	0.82	4.69	0.82	4.77	0.71	4.72	0.783	16.6
RLB	BIRCH	4.59	0.69	4.80	0.28	4.75	0.32	4.71	0.43	9.2
Control	BIRCH	4.86	0.04	4.71	+0.06	4.79	0.11	4.79	0.032	0.69

1(b)		Test 1	Test 2	Test 3	Test 4	Test 5	Test 6	Average %
S 70	BIRCH	4.60	6.47	9.70	6.89	6.32	5.01	6.5
S 70 B	BIRCH	6.40	12.1	6.50	2.42	15.3	1.22	9.15
S 70 E	BIRCH	9.32	11.1	8.46	8.74	12.3	9.41	8.69
S 70 G	BIRCH	1.46*	1.42*	1.38*	2.07"	2.14"	1.49"	-
S 70 K	BIRCH	11.7	6.55	7.28	9.44	8.28	10.1	8.89
S 70 L	BIRCH	8.82	5.22	4.42	6.63	7.07	15.4	7.93
S 70 M	BIRCH	9.35	8.96	8.24	8.03	11.1	8.07	8.95
S 70 N	BIRCH	4.94	9.30	6.24	6.22	5.59	5.67	5.59
RLB	BIRCH	8.71	5.50	5.91	7.77	5.99	9.63	7.30
Control	BIRCH	1.04	1.53	1.45	1.43	1.40	1.55	1.40
1(c)								
S 70	BIRCH	24.3	19.5	15.0	15.5	14.7	16.3	17.5
S 70 B	BIRCH	13.4	14.2	18.2	15.5	19.1	-	16,1
S 70 E	BIRCH	16.7	18.1	16.3	18.5	20.5	16.3	17.0
S 70 G	BIRCH	22.1	27.1	18.3	11.9	16.4	26.6	20.4
S 70 K	BIRCH	15.3	15.3	22.5	19.3	16.2	15.5	17.4
S 70 L	BIRCH	20.3	21.0	12.9	13.3	15.7	14.4	16.3
S 70 M	BIRCH	18.6	15.2	24.6	24.3	17.9	14.1	18.6
S 70 N	BIRCH	19.4	22.6	18.5	18.7	17.8	15.7	18.8
RLB	BIRCH	18.6	17.7	14.4	18.0	17.1	21.6	17.9
Control	BIRCH	0.97	0.88	1.43	1.26	1.14	1.30	1.16

O.W. = original weight, W.L. = weight loss, + = weight gain

* = no growth at all, " = only sparse growth observed.

Weight loss tests.(continued).

		Test 1		Test 2		Test 3		Average		Average
		O.W.	W.L.	O.W.	W.L.	O.W.	O.W.	O.W.	W.L.	% W.L.
1(d)C.globosum.										
S 70		1.99	0.04	1.97	0.01	1.93	0.00	1.96	0.02	1.0
S 70 B	SPRUCE	1.95	+0.04	2.03	+0.02	2.10	+0.07	2.03	+0.04	+2.0
S 70 E		2.06	0.08	1.94	+0.01	1.99	0.01	1.99	0.03	1.5
S 70 G		2.00	0.03	2.05	0.05	2.19	0.04	2.08	0.04	2.0
S 70 K		2.08	+0.04	2.03	+0.02	2.09	+0.01	2.07	+0.02	+1.0
S 70 L		1.95	0.01	1.89	0.02	1.95	0.01	1.93	0.01	0.5
S 70 M		2.05	0.04	1.98	0.01	2.02	0.01	2.02	0.02	1.0
S 70 N		1.97	0.01	1.99	0.03	1.96	0.03	1.91	0.02	1.0
RLB		1.97	0.01	1.94	0.01	1.64	0.00	1.86	0.01	0.5
Control		2.00	0.01	1.97	0.02	2.07	0.01	1.96	0.01	0.5
1(e)										
S 70	SPRUCE	2.06	0.02	2.17	0.04			2.12	0.03	1.4
S 70 B		2.03	0.02	2.00	0.00			2.02	0.01	0.5
S 70 E		2.11	0.00	-	-			2.11	0.00	0.0
S 70 G		1.96	0.01	2.03	0.03			2.00	0.02	1.0
S 70 K		2.05	0.01	2.01	0.01			2.03	0.01	0.5
S 70 L		2.05	0.01	2.03	0.01			2.04	0.01	0.5
S 70 M		2.04	0.00	1.92	0.00			1.98	0.00	0.0
S 70 N		2.01	+0.02	2.03	0.00			2.02	+0.01	+0.5
RLB		2.08	0.01	2.14	0.03			2.11	0.02	0.9
Control		2.03	0.03	2.10	0.01			2.07	0.02	0.9

+ = weight gain.

		Test 1		Test 2		Average		Average
		O.W.	W.L.	O.W.	W.L.	O.W.	W.L.	%W.L.
2(a) *P. monticola.*								
304 B	BIRCH	3.58	0.05	3.71	0.03	3.65	0.04	1.10
304 C		3.70	0.10	3.45	0.12	3.58	0.11	3.09
304 D		3.71	0.03	3.57	0.07	3.64	0.05	1.38
304 E		3.71	0.33	3.71	0.36	3.71	0.35	9.30
304 F		3.74	0.20	3.66	0.28	3.70	0.24	3.86
304 G *		3.64	0.02	3.55	0.06	3.60	0.04	1.10
304 H		3.57	0.20	3.64	0.17	3.60	0.19	5.14
280		3.64	0.51	3.43	0.09	3.53	0.30	8.31
2(b)								
304 B	SPRUCE	1.03	0.10	1.05	0.24	1.04	0.17	16.4
304 C		1.09	0.07	1.02	0.16	1.06	0.12	11.3
304 D		0.95	*	1.02	*	-	-	-
304 E		1.02	0.13	1.00	0.15	1.01	0.14	13.9
304 F		0.95	0.17	1.07	0.26	1.01	0.22	21.8
304 G		1.01	0.06	0.96	+0.02	0.99	0.03	3.0
304 H		0.94	0.29	0.99	+0.07	0.97	0.15	15.5
280		0.99	0.33	1.02	0.41	1.00	0.37	37.0
2(c)								
304 B	SPRUCE	3.07	0.28	3.07	0.31	3.07	0.30	9.6
304 C		2.42	0.16	2.65	0.27	2.54	0.22	8.4
304 D		2.65	0.03	2.72	0.04	2.64	0.04	1.2
304 E		2.81	0.40	2.65	0.44	2.73	0.42	15.4
304 F		2.53	0.20	2.62	0.26	2.08	0.23	8.9
304 G		2.52	0.03	2.55	0.04	2.54	0.04	1.4
304 H		2.74	0.39	2.45	0.28	2.59	0.34	12.8
280		2.57	0.19	2.52	0.36	2.55	0.28	10.8

3(a) and (b) No satisfactory growth. * = contaminated, Penicillium sp.

+ = weight gain.

	O.W.	W.L.	% W.L.	
4(a)				
P.monticola				
304B	6.78	0.81	11.9	
304C BIRCH	6,90	0.25	3.6	
304D	6.76	+0.06	+0.9	only sparse growth
304E	6,85	0.89	13.0	
304F	6.71	0.55	8.2	
304G	6.77	+0.09	+1.3	" " "
304H	6.90	0.40	5.8	
280	6.80	0.30	4.4	
4(b)				
304B SPRUCE	4.85	1.27	28.2	
304C	4.36	0.97	22.2	
304D	4.41	0.18	4.1*	
304E	4.59	1.34	29.2	
304F	4.63	1.08	23.3	
304G	4.41	0.15	3.4*	
304H	4.64	1.32	28.4	
280	4.64	0.55	11.9	

These test blocks were checked because the results seemed too low. On microscopic examination of the wood, they were found to be Scots pine. An examination of every single block in this series of tests shewed that only the ones thus indicated were Scots pine, and that all the others were Norway spruce. += weight gain.

5(a)			
P fastigiata			
56A BIRCH	6.70	0.03	0.44
Bann1	6.99	+0.01	+0.14
5(b) SPRUCE			
56A and Bann1	No growth.		
5(c)			
56A SPRUCE	4.57	0.01	0.22
Bann1	4.59	0.01	0.22
Birch control.	6.77	+0.06	+0.88
Spruce control.	4.22	contaminated.	

Fungus	% Weight Losses.							
	Test1	Test2	Test3	Test4	Test5	Test6	Test7	Av.%
6(a) S 70B C.globosum.	6.3	5.7	4.6	7.3	6.3	6.3	6.9	6.4
(b) 304 B P.monticola.	4.6	7.8	9.7	4.3	14.8	6.9	7.8	7.8
(c) 56 A P.fastigiata.	No result . Desiccation of plate and slow growth.							
(d) P.versicolor.	25.0	12.8	22.0	33.7	19.3	22.0	19.2	22.0
(e) S 70B C.globosum.	6.7	5.6	8.4	8.5	5.4	6.1	13.3	7.7
(f) 304B P.monticola.	5.7	10.5	14.8	3.3	12.0	13.3	10.5	10.5
(g) 56A P.fastigiata.	No result.							
(h) P.versicolor.	6.3	13.0	9.4	9.6	6.8	9.4	14.2	9.4

Type of wood blocks: 6(a) to (d) = beech

6(e) to (h) = S. pine

Discussion of Weight Loss Tests.

Difficulty was experienced in the experiments with contamination and, in the case of the two fungi P. monticola and P. fastigiata, in obtaining optimum growth. The latter was probably a result of uncontrolled humidity, which is known to be an important factor in the growth of P. monticola (Gay, 1973). The format of the tests was varied; for example, in the number of blocks used, the growth medium, and the growth container. This gave rise to a rash of different tests as indicated in the Methods section. At the time these experiments were performed the equipment available was limited. It has been concluded since then, that the following improvements should be seriously considered.

(1) The use of a laminar flow bench.

(2) The introduction of deeper square petri dishes as used by Bravery, 1972.

(3) The possible incorporation of a minimal quantity of glucose in the wood sawdust medium to aid the initial stages of fungal growth, but not enough to affect the later stages. (This could be compared to the cell contents in wood, and is thus not deviating far from the idea of using wood as sole C-source).

(4) The introduction of a relative humidity control into the incubation chamber. (P. monticola is particularly susceptible to moisture conditions).

(5) The possible impregnation of the blocks with a mixture of mineral salts, such as was used by Nilsson, 1973. This would overcome the shortage of nitrogen in wood and thus the possible limitation of growth due to this factor.

Discussion of Weight Loss Test Results.

As a result of the factors just discussed, the significance of the weight loss results is limited. Therefore only general trends are considered.

When C. globosum attacked birch wood, a 10-15% weight loss occurred in 8 weeks. On spruce wood no significant weight losses were observed in 8 months. Scanning electron microscopy revealed that the birch was attacked, with cavities evident in the S2 layers, whilst spruce was only colonised, giving rise to the occasional weight gain. This behaviour is not typical of C.globosum in softwood. Other workers have obtained small but appreciable weight losses in spruce, concomitant with cavity formation. The different behaviour of the fungus in the present work may be attributed to the restriction of the C-source (i.e., wood only) in the growth medium.

P. monticola gave more varied results, and this variation seemed to be correlated to the visible growth of the fungus. Under the conditions of test the growth was highly variable, sometimes intense like cotton wool, and sometimes very sparse. Where good growth was present, high weight losses were found (ca. 10-15%) on both birch and spruce. Of note was strain No. 304G in experiment 2(a) which was contaminated by a Penicillium sp. antagonistic to the growth of P. monticola. Despite the limited nature of the experiment, there was a definite tendency for the spruce to be attacked to a greater extent than the birch, under the conditions of experiment No. 2. The same trend was seen in experiment No. 4, the duration of which was 5 months. Scots pine, included by accident, was seen to be more resistant to decay than spruce. With only two fungal strains involved, however, this finding needs confirmation.

P.fastigiata gave no significant results.

An experiment to investigate the action on spruce blocks of fungi grown on birch agar was also set up. (Nos.1(e) and 5(c)) Although growth on birch agar was more prolific than on spruce no differences in weight losses were found.

When malt agar was used as the growth medium, beech and Scots pine were the woods under test. The experiment was originally a class demonstration, but is included because it demonstrated the increased speed of decay when malt agar was used. Weight losses were nearly 10% in four weeks. A fungus not previously used, Polystictus versicolor, gave a high weight loss of 22%. P. versicolor is a typical white rot fungus.

Lignin Estimation

In sawdust: Birch (as % of extractive free)

original weight of wood = 1.0112g

weight of lignin residue = 0.2070g

∴ lignin content 20.7%

Spruce (as % of extractive free)

original weight of wood = 1.0101g

weight of lignin residue = 0.2790g

∴ lignin content 27.9%

If one assumes that the percentage of extractives present is in the region of 5%, these figures become approximately,

Birch	19.7%
Spruce	26.5%

expressed in terms of the whole wood.

These figures are in accordance with the findings of other workers.

In holocellulose

There were three separate productions of holocellulose, two Norway spruce and one birch.

(1) spruce	10.2%
(2) spruce	8.3%
(3) birch	4.7%

These figures are expressed in terms of holocellulose.

Assuming the above lignin concentrations the respective values in terms of extractive free wood are:

(1) 7.2%

(2) 5.9%

(3) 3.8%

Hemicelluloses were satisfactorily extracted from holocellulose with these lignin concentrations.

Results of Degrees of Rotation Estimation

from	Birch wood		from Norway Spruce Wood	
	Xylan	-73^{o}	Xylan	-73^{o}
	DMSO extract	-52^{o}	DMSO extract	-36^{o}
*	"Glucomannan"	-104^{o}	Galactoglucomannan	-105^{o}
	α cellulose	-22^{o}	α cellulose	-16^{o}

Also tested was a Larch xylan -61^{o} (commercial).

These figures correspond closely with those of other workers, for example, King and Fuller (1968) obtained an $[\alpha]_D^{22}$ of -72^{o} for poplar xylan and Zinbo and Timell obtained -76.5^{o} for the same substrate.

* Note the percentage monosaccharide composition of this "glucomannan" in Appendix II.

The Results of the Hemicellulase Monitoring

Experiment

The results are expressed in the form of graphs; four to each page. On the left hand side are shewn the activities of cultures grown on birch sawdust, and on the right hand side the activities on spruce sawdust. For each wood type eight activities were tested; on the upper graph, (1) pH (11) CMC-ase activity and (111) protein; and on the lower graph, (iv) a N.J.K. Spruce GGM-ase (1V)b. Spruce GGM-ase (V) Xylanase (V1) Spruce D.M.S.O. activity and (V11) birch D.M.S.O. activity. (The labels refer to the substrates used in a standardized Nelson-Somogyi reducing sugar assay). The left hand ordinate axis is in micrograms and either refers to µgm of protein, or µgm of reducing sugar equivalents. The right hand ordinate axis is pH units. The abscissa is graduated in days and refers to the age of the fungal culture on the wood. Each fungal strain was grown in duplicate and termed Birch No. 1/Spruce No. 1 or Birch No. 2/Spruce No. 2.

With all strains of C.globosum samples of culture fluid were taken on days 3, 6, 9, 12, 15, 20, 25, 32, 40, 55 and 70. With the other species, samples were removed on days 9, 16, 23, 30, 37, 51, 65 and 80 except P.fastigiata which had an additional sample taken on day 12.

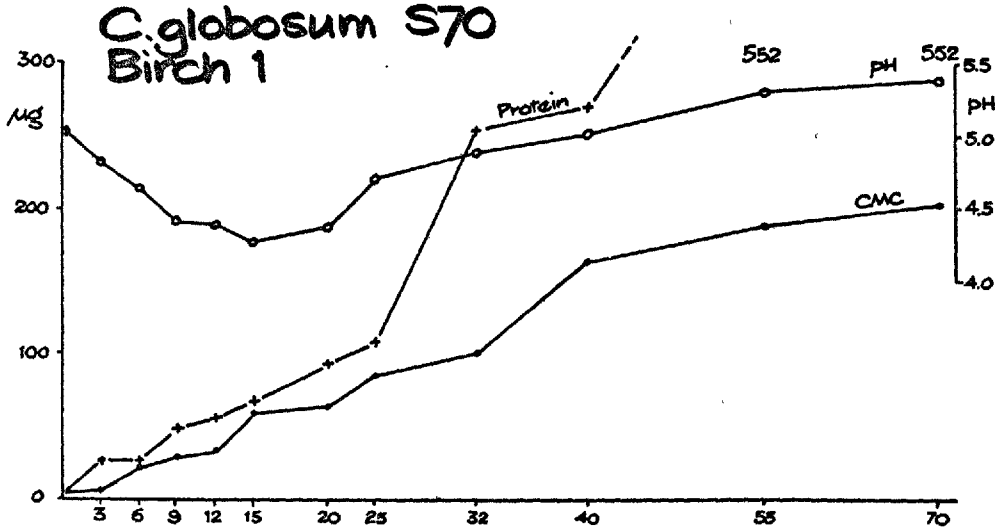
The days for taking samples were chosen on the basis of prior pilot experiments with an example of each fungal species. It was noted that P.monticola and P.fastigiata grew very slowly, and so for them approximately weekly intervals were chosen. For C.globosum it was necessary to increase the number of samples in the initial growth phase to one every three days.This was then phased out and longer intervals between sampling was introduced.

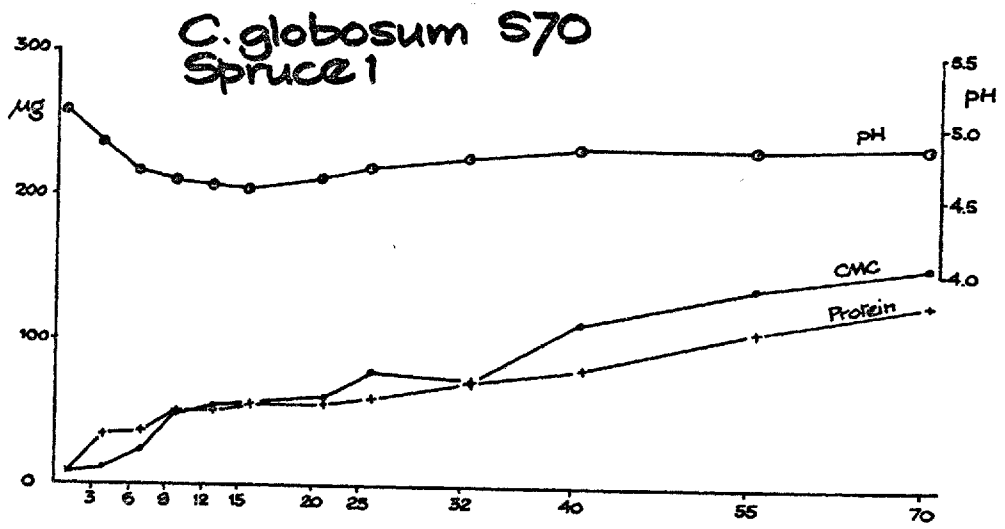
Any sharp jagged change in shape of the curves is likely to be an error. Where graphs have been discontinued, it was either due to desication or contamination.

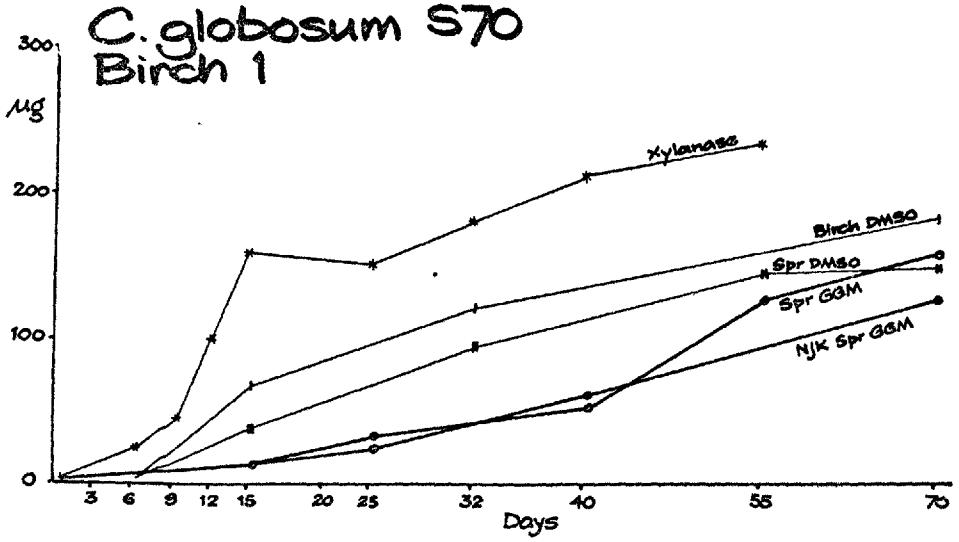
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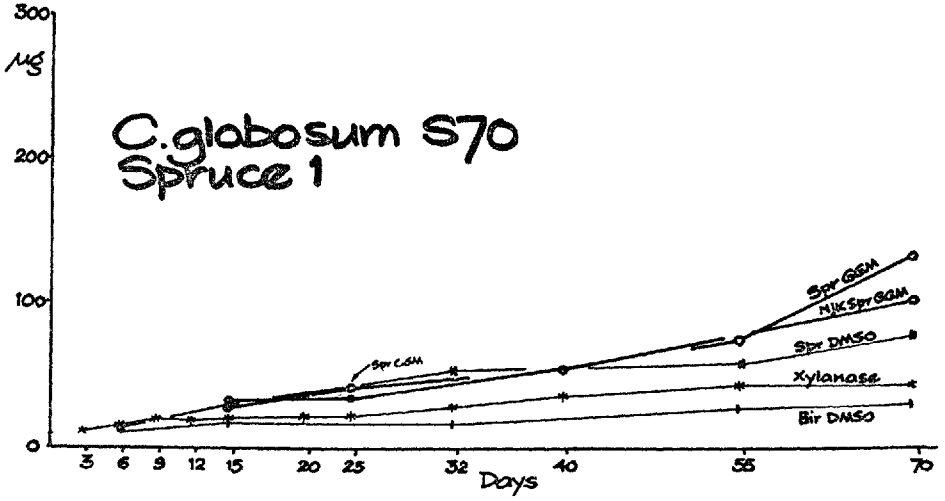
o—o	= pH	+—+	= Birch DMSO
+—+	= Protein	‖—‖	= Spruce DMSO
●—●	= CMC	o—o	= Spruce GGM
—	= Xylanase	o—o	= NJK Spruce GGM

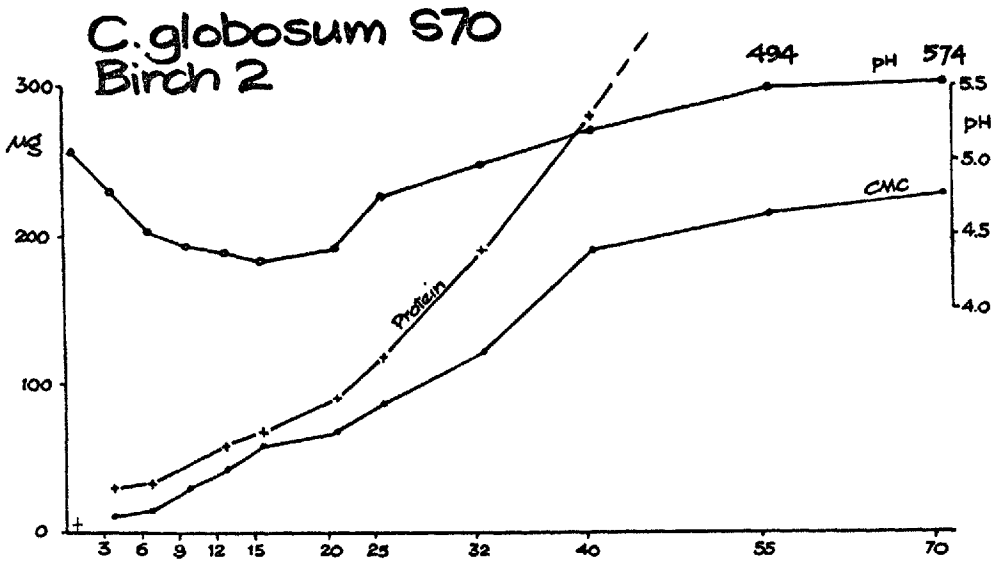
The numerical results of reducing sugar assays are listed in Appendix III.

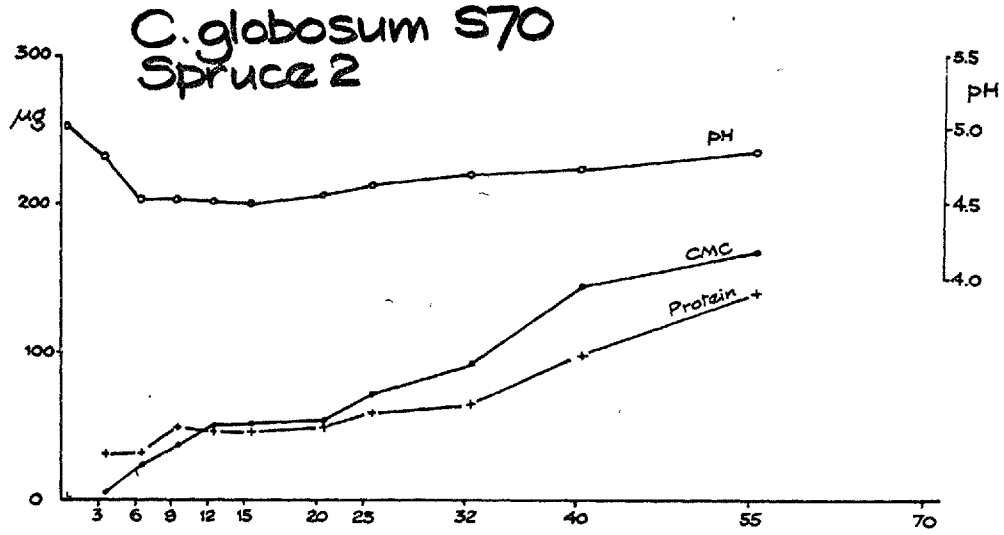

C. globosum S70
Birch 1
552
pH
552
Protein
CMC
µg
pH
300
200
100
0
5.5
5.0
4.5
4.0
3 6 9 12 15 20 25 32 40 55 70

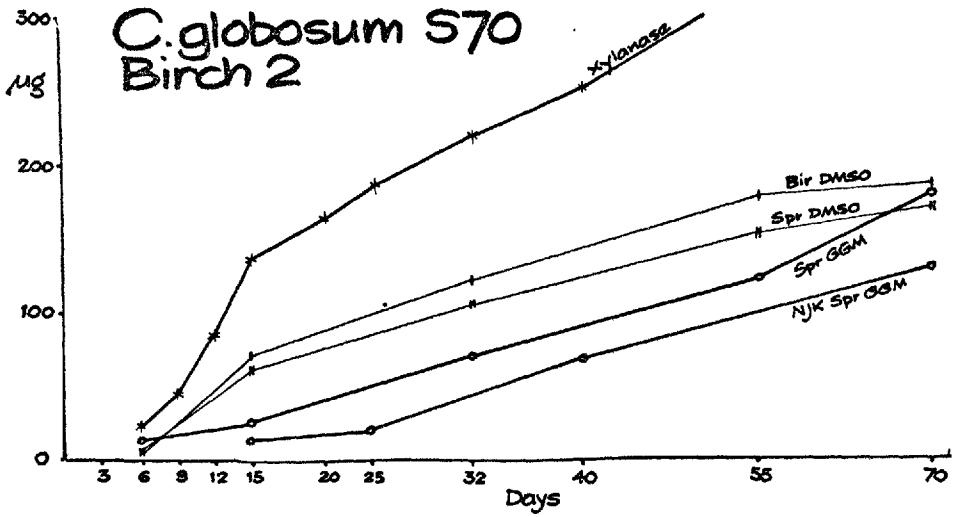

C. globosum S70
Spruce 1
µg
pH
CMC
Protein
300
200
100
0
5.5
5.0
4.5
4.0
3 6 9 12 15 20 25 32 40 55 70

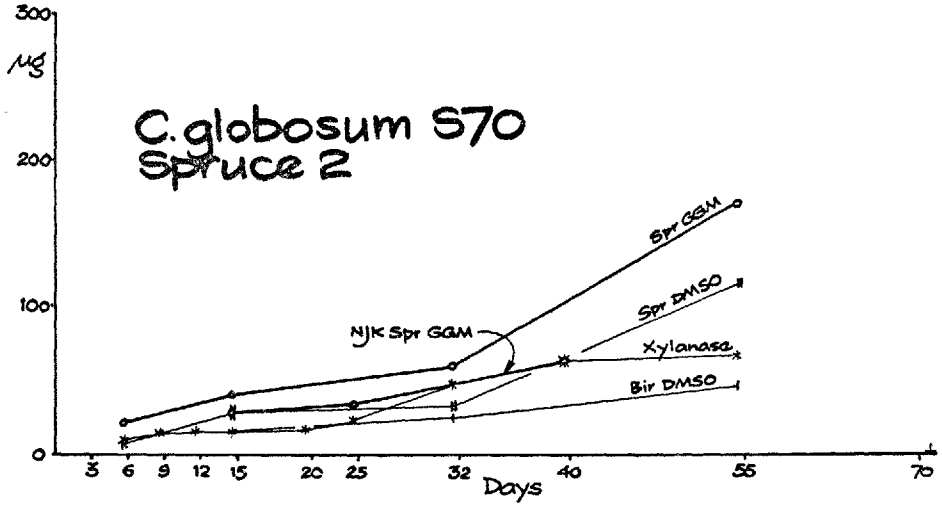

C. globosum S70
Birch 1
µg
Xylanase
Birch DMSO
Spr DMSO
Spr GGM
NJK Spr GGM
300
200
100
0
3 6 9 12 15 20 25 32 40 55 70
Days

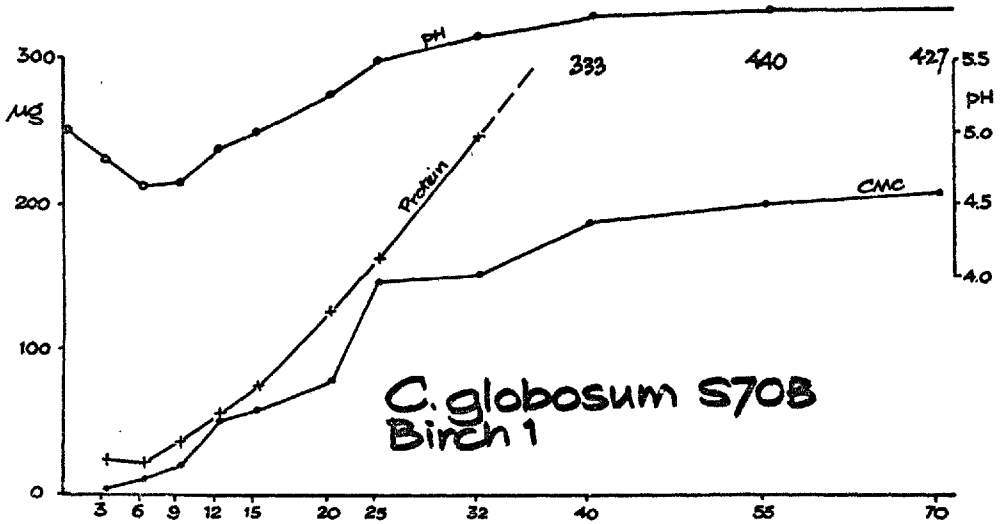

C. globosum S70
Spruce 1
µg
Spr GGM
NJK Spr GGM
Spr DMSO
Xylanase
Bir DMSO
300
200
100
0
3 6 9 12 15 20 25 32 40 55 70
Days

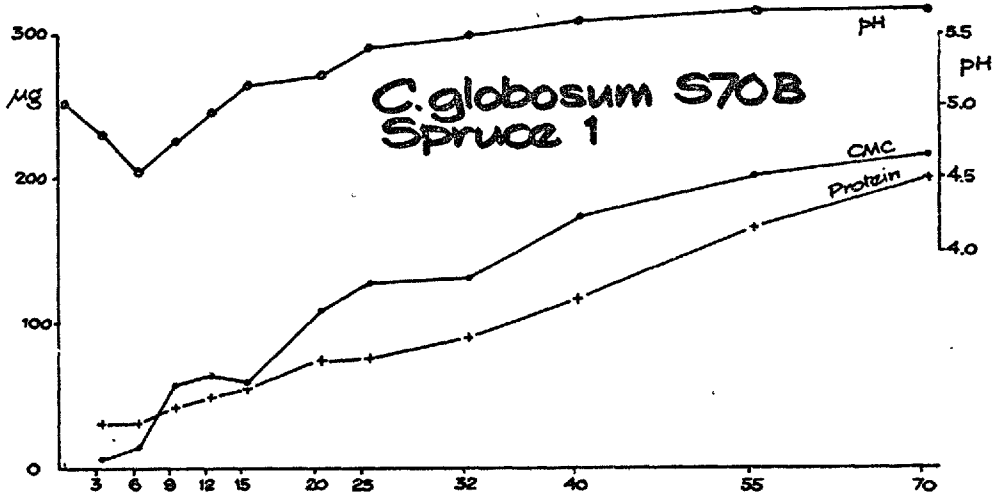

C. globosum S70
Birch 2
494
pH
574
300
µg
200
100
0
5.5
pH
5.0
4.5
4.0
CMC
Protein
3
6
9
12
15
20
25
32
40
55
70

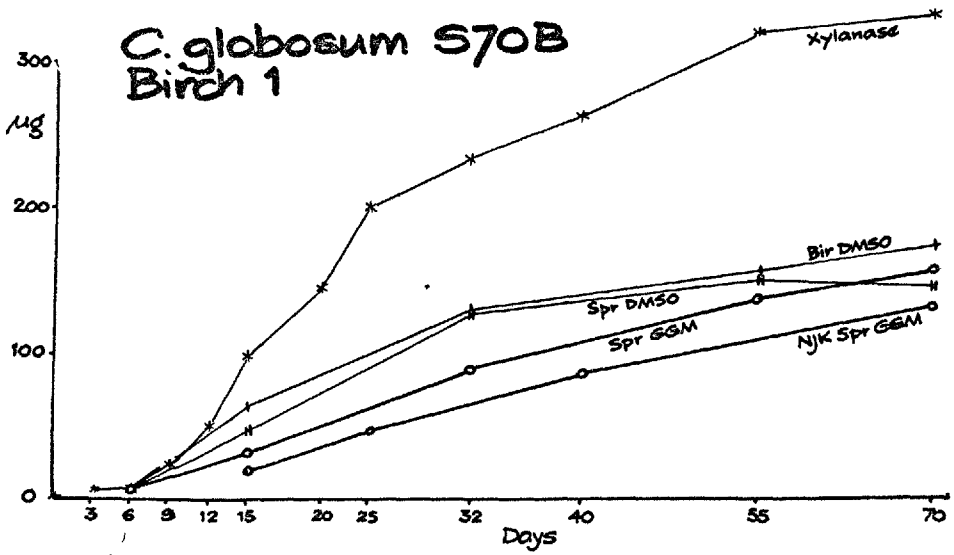

C. globosum S70
Spruce 2
300
µg
200
100
0
5.5
pH
5.0
4.5
4.0
pH
CMC
Protein
3
6
9
12
15
20
25
32
40
55
70

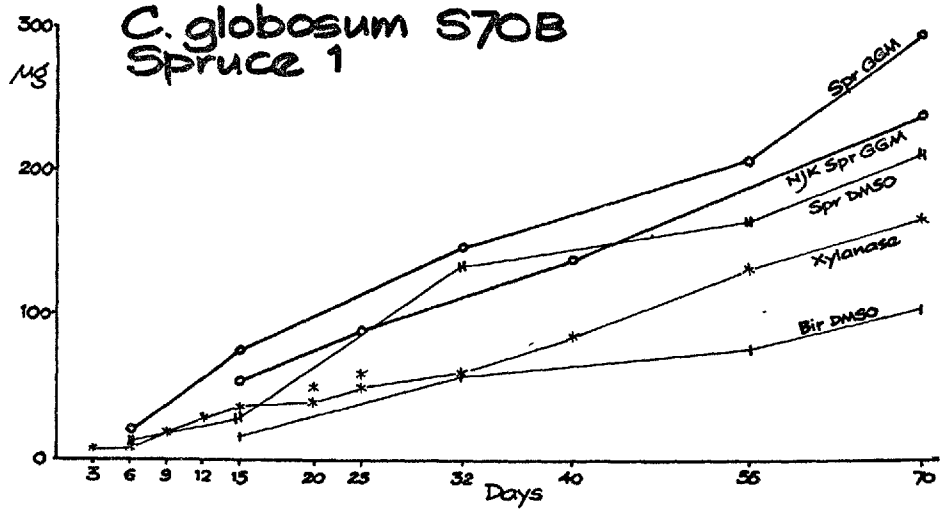

C. globosum S70
Birch 2
300
µg
200
100
0
Xylanase
Bir DMSO
Spr DMSO
Spr GGM
NJK Spr GGM
3
6
9
12
15
20
25
32
40
55
70
Days

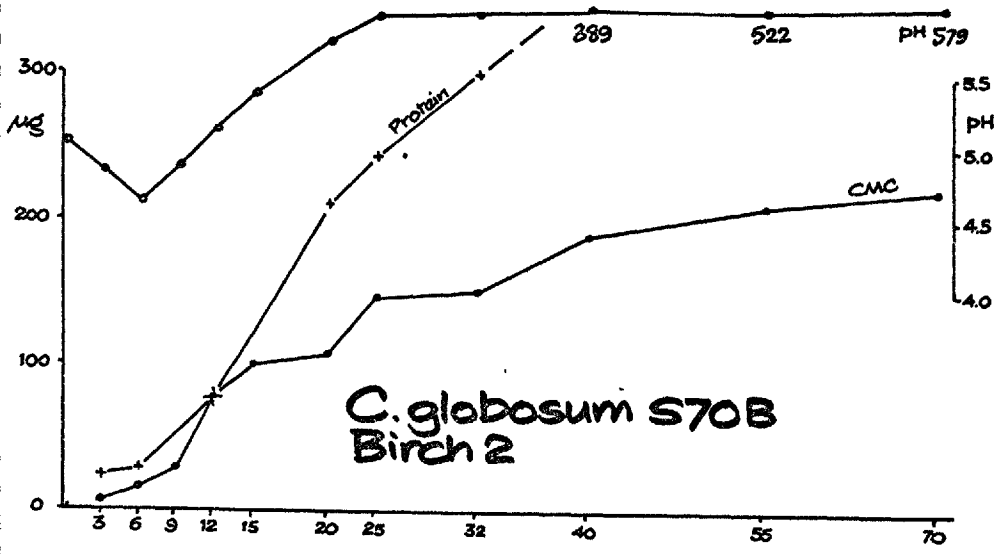

300
µg
200
100
0
C. globosum S70
Spruce 2
Spr GGM
Spr DMSO
NJK Spr GGM
Xylanase
Bir DMSO
3
6
9
12
15
20
25
32
40
55
70
Days

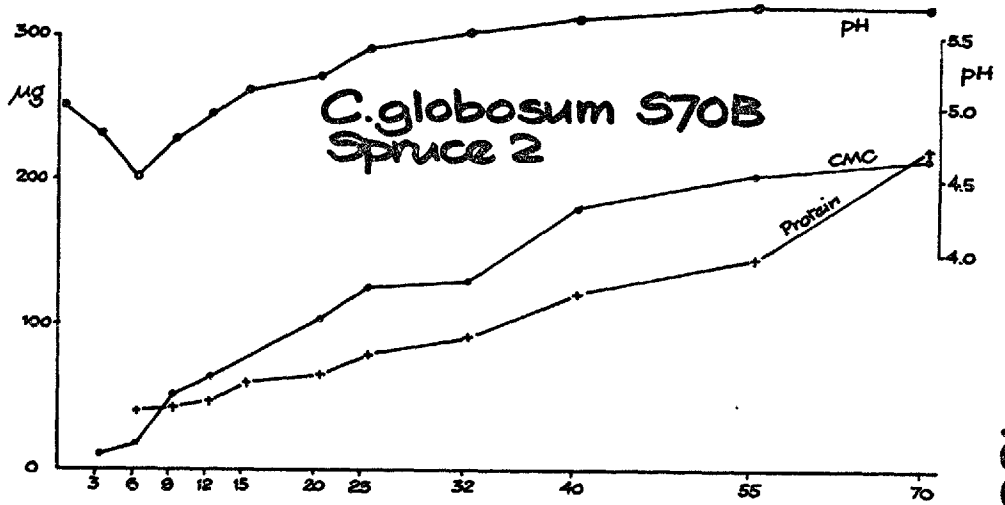

pH
333
440
427
Protein
CMC
300
µg
200
100
0
5.5
pH
5.0
4.5
4.0
3 6 9 12 15 20 25 32 40 55 70
C. globosum S70B
Birch 1

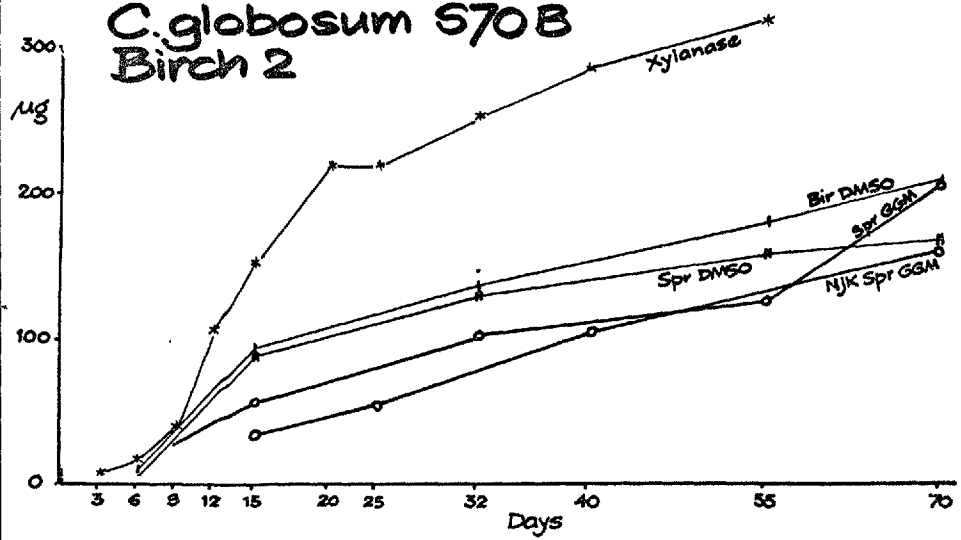

pH
C. globosum S70B
Spruce 1
CMC
Protein
300
µg
200
100
0
5.5
pH
5.0
4.5
4.0
3 6 9 12 15 20 25 32 40 55 70

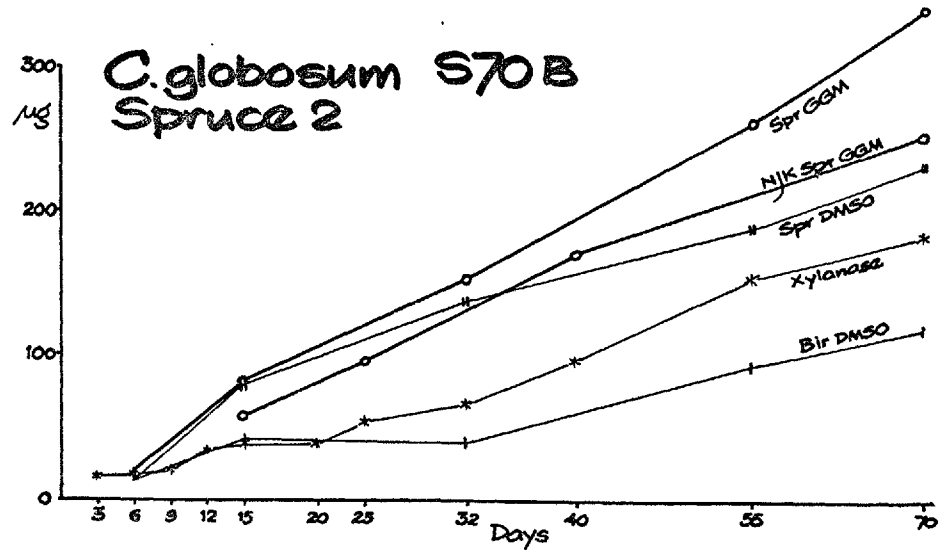

C. globosum S70B
Birch 1
Xylanase
Bir DMSO
Spr DMSO
Spr GGM
NJK Spr GGM
300
µg
200
100
0
3 6 9 12 15 20 25 32 40 55 70
Days

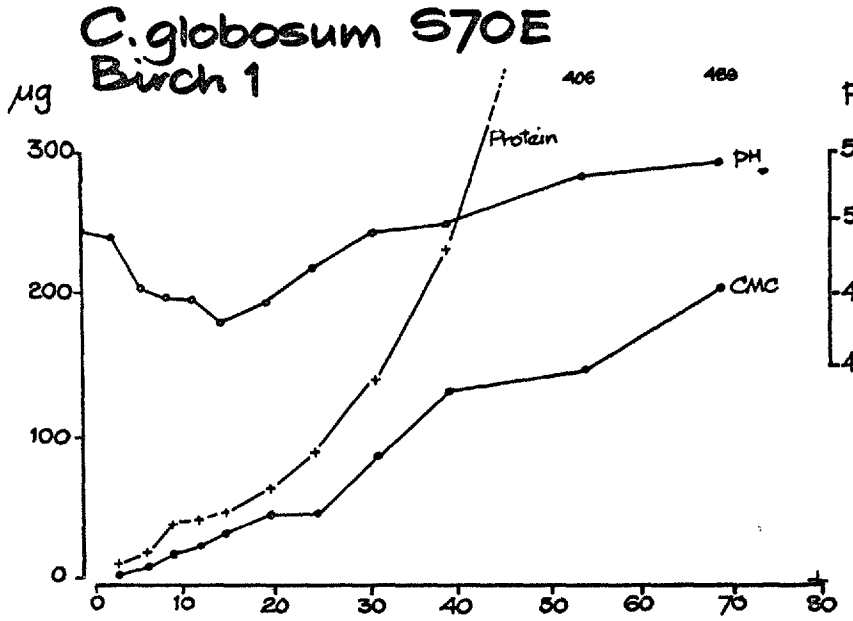

C. globosum S70B
Spruce 1
Spr GGM
NJK Spr GGM
Spr DMSO
Xylanase
Bir DMSO
300
µg
200
100
0
3 6 9 12 15 20 25 32 40 56 70
Days

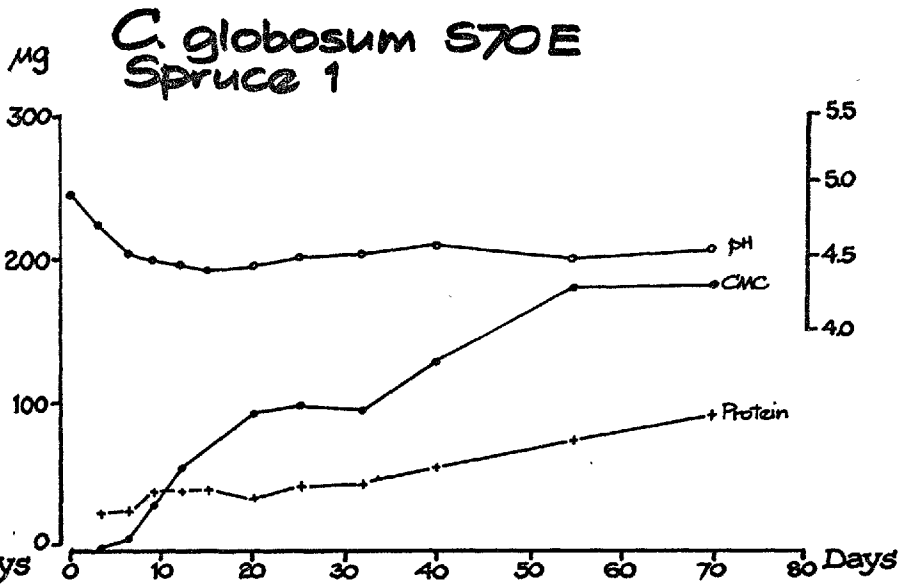

389
522
pH 579
300
µg
200
100
0
Protein
CMC
8.5
pH
5.0
4.5
4.0
C. globosum S70B
Birch 2
3 6 9 12 15 20 25 32 40 55 70

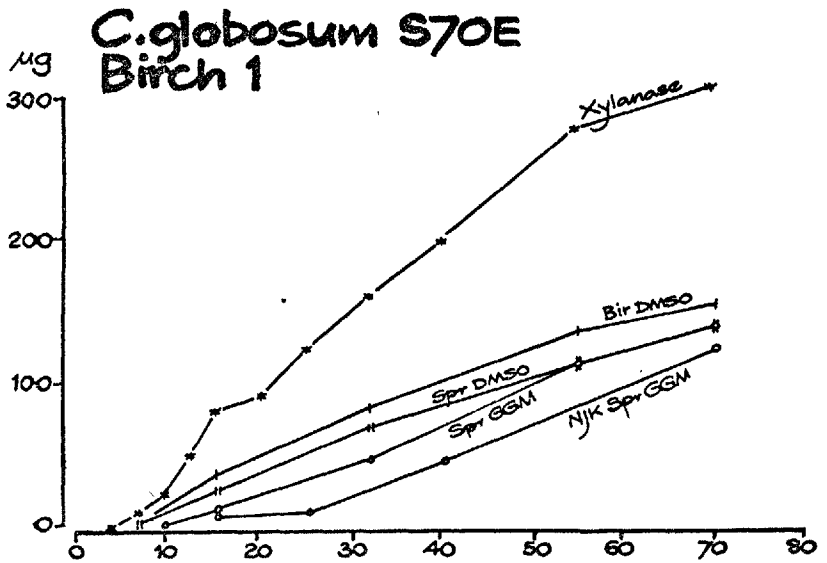

pH
300
µg
200
100
0
C. globosum S70B
Spruce 2
CMC
Protein
8.5
pH
5.0
4.5
4.0
3 6 9 12 15 20 25 32 40 55 70

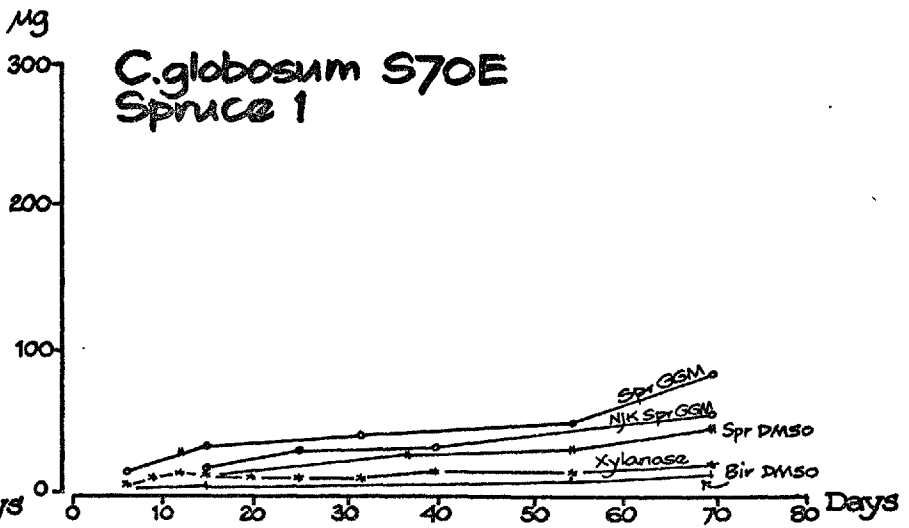

C. globosum S70B
Birch 2
300
µg
200
100
0
Xylanase
Bir DMSO
Spr GGM
Spr DMSO
NJK Spr GGM
3 6 9 12 15 20 25 32 40 55 70
Days

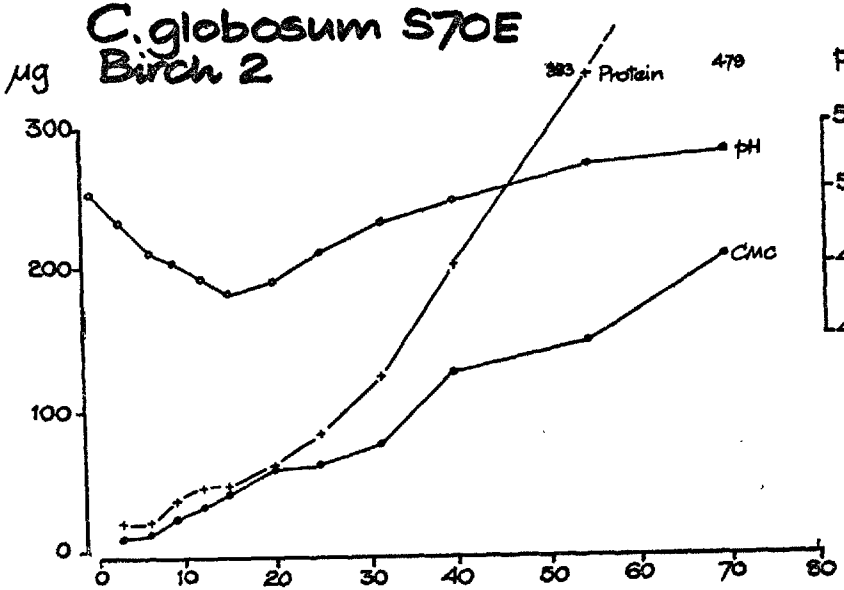

C. globosum S70B
Spruce 2
300
µg
200
100
0
Spr GGM
NJK Spr GGM
Spr DMSO
Xylanase
Bir DMSO
3 6 9 12 15 20 25 32 40 55 70
Days

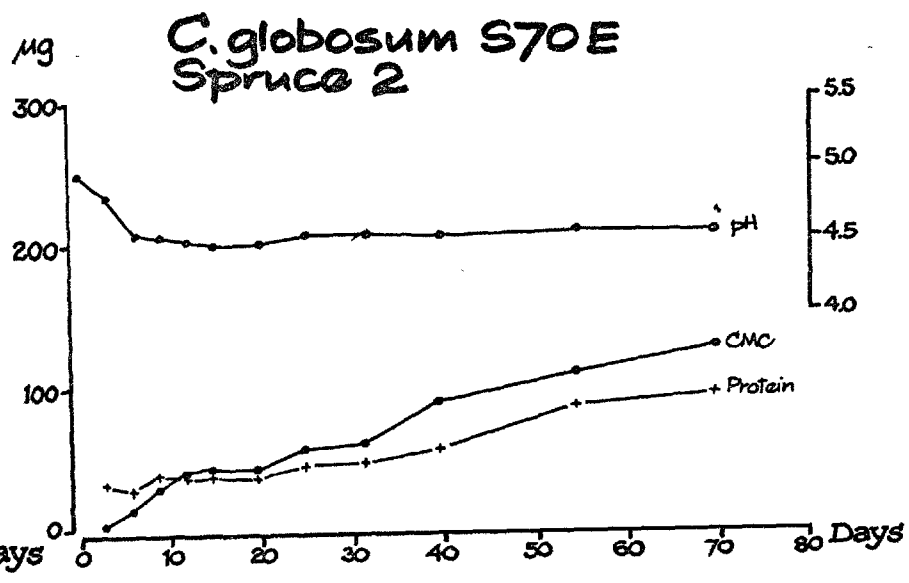

C. globosum S70E
Birch 1
µg
pH
406
466
Protein
pH
CMC
300
200
100
0
5.5
5.0
4.5
4.0
0 10 20 30 40 50 60 70 80
Days

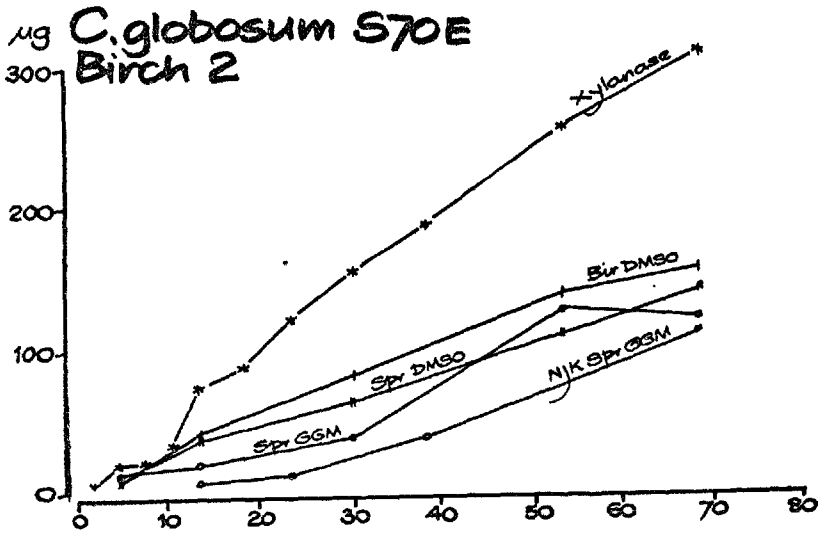

C. globosum S70E
Spruce 1
µg
pH
pH
CMC
Protein
300
200
100
0
5.5
5.0
4.5
4.0
0 10 20 30 40 50 60 70 80
Days

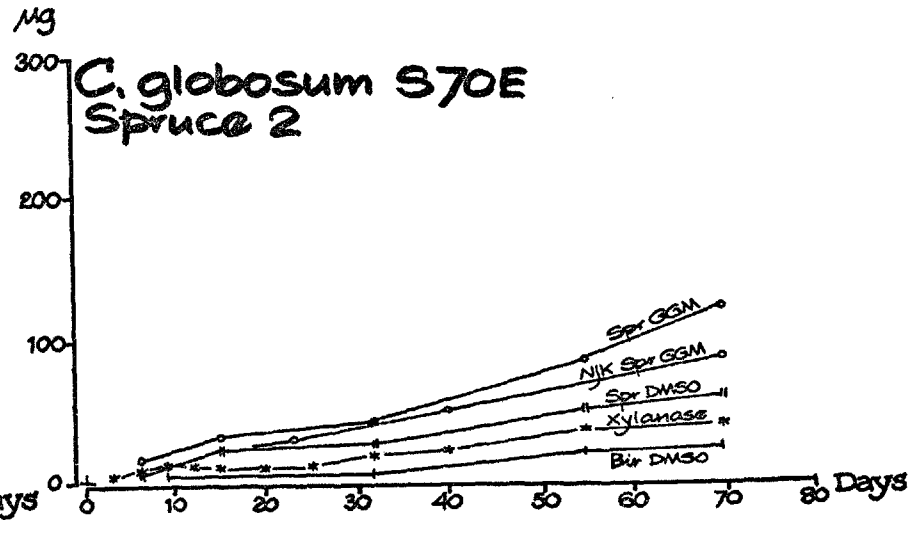

C. globosum S70E
Birch 1
µg
Xylanase
Bir DMSO
Spr DMSO
Spr GGM
NJK Spr GGM
300
200
100
0
0 10 20 30 40 50 60 70 80
Days

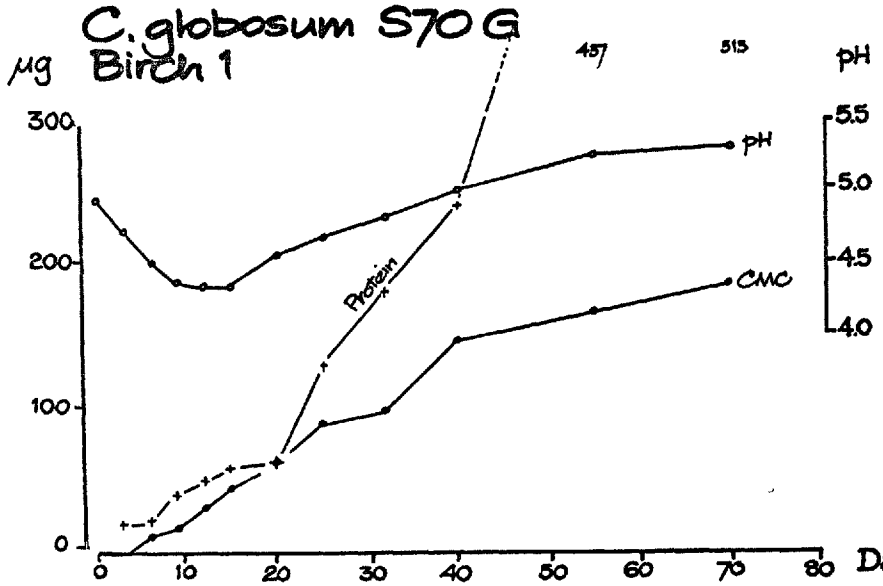

C. globosum S70E
Spruce 1
µg
Spr GGM
NJK Spr GGM
Spr DMSO
Xylanase
Bir DMSO
300
200
100
0
0 10 20 30 40 50 60 70 80
Days

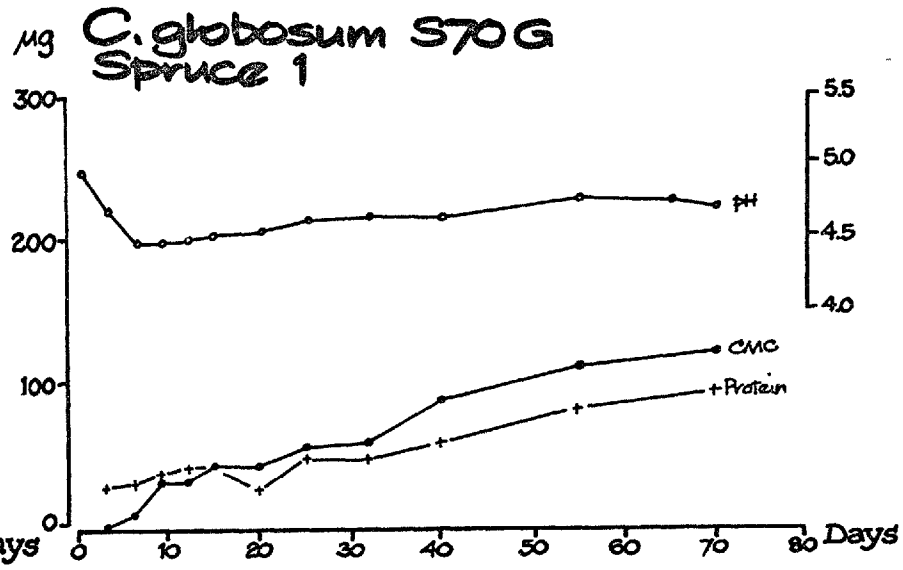

C. globosum S70E
Birch 2
µg
383 + Protein
479
pH
CMC
300
200
100
0
5.5
5.0
4.5
4.0
0 10 20 30 40 50 60 70 80
Days

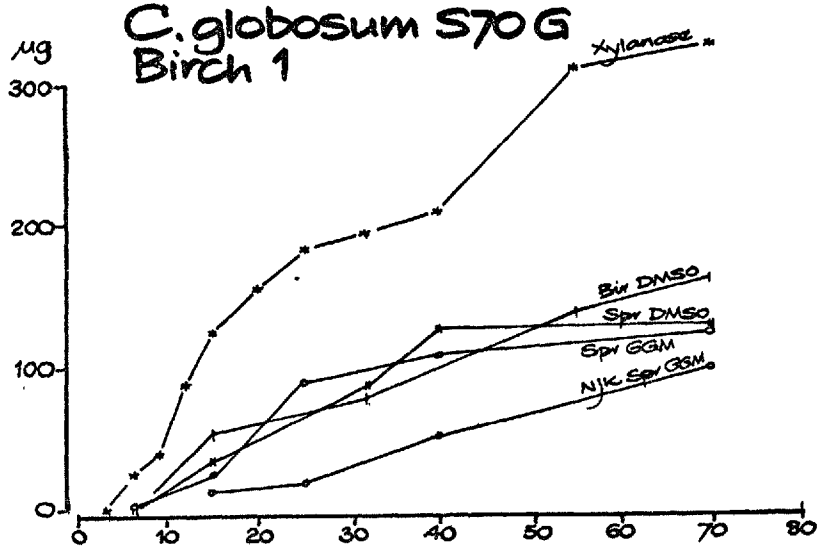

C. globosum S70E
Spruce 2
µg
pH
CMC
+ Protein
300
200
100
0
5.5
5.0
4.5
4.0
0 10 20 30 40 50 60 70 80
Days

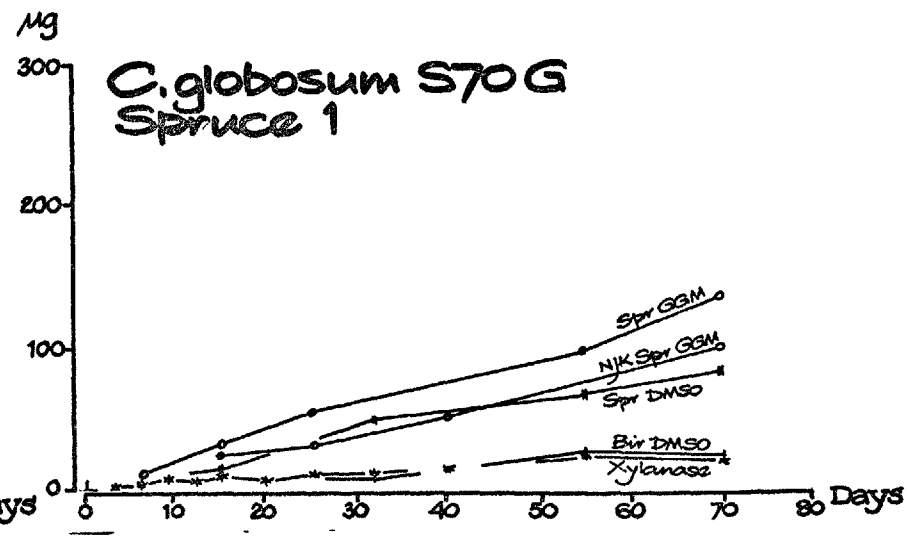

µg
C. globosum S70E
Birch 2
Xylanase
Bir DMSO
Spr DMSO
Spr GGM
NJK Spr GGM
300
200
100
0
0 10 20 30 40 50 60 70 80
Days

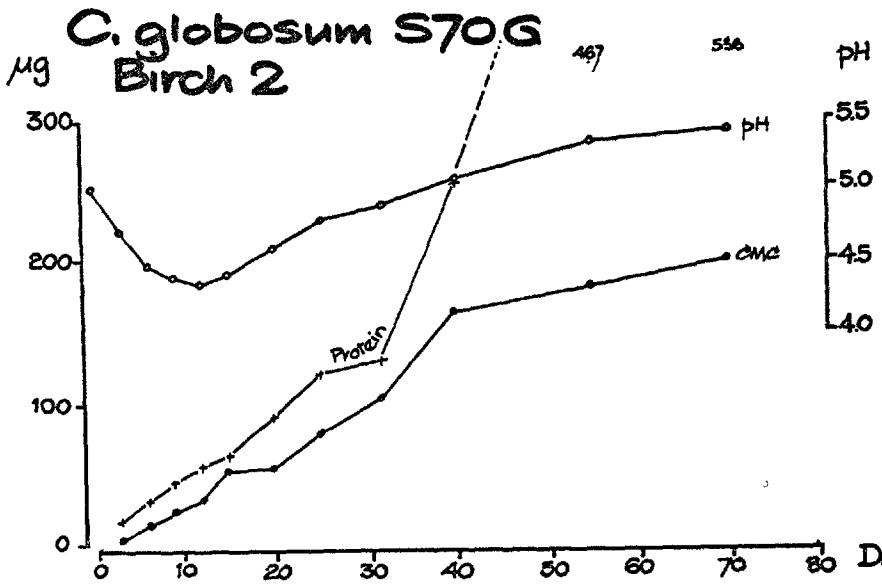

µg
C. globosum S70E
Spruce 2
Spr GGM
NJK Spr GGM
Spr DMSO
Xylanase
Bir DMSO
300
200
100
0
0 10 20 30 40 50 60 70 80
Days

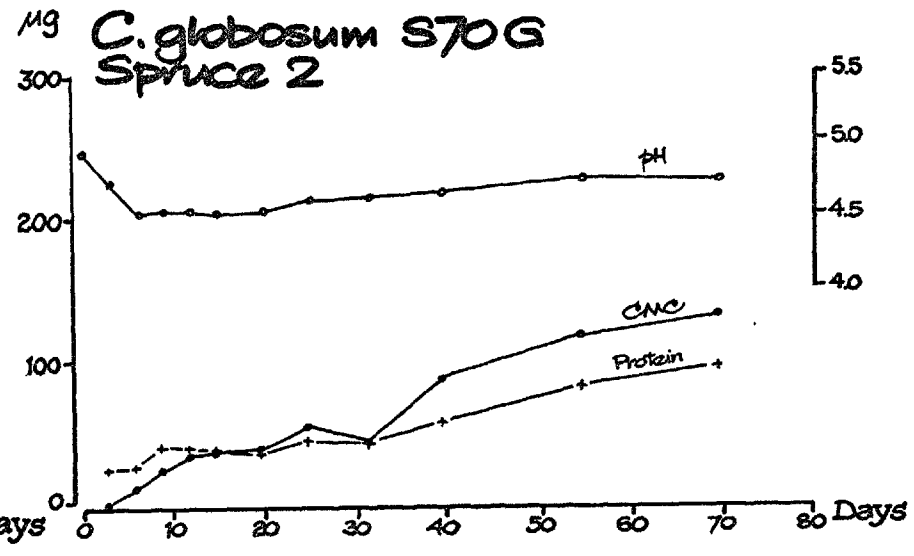

C. globosum S70 G
Birch 1
µg
pH
457
515
300
200
100
0
5.5
5.0
4.5
4.0
pH
CMC
Protein
0 10 20 30 40 50 60 70 80
Days

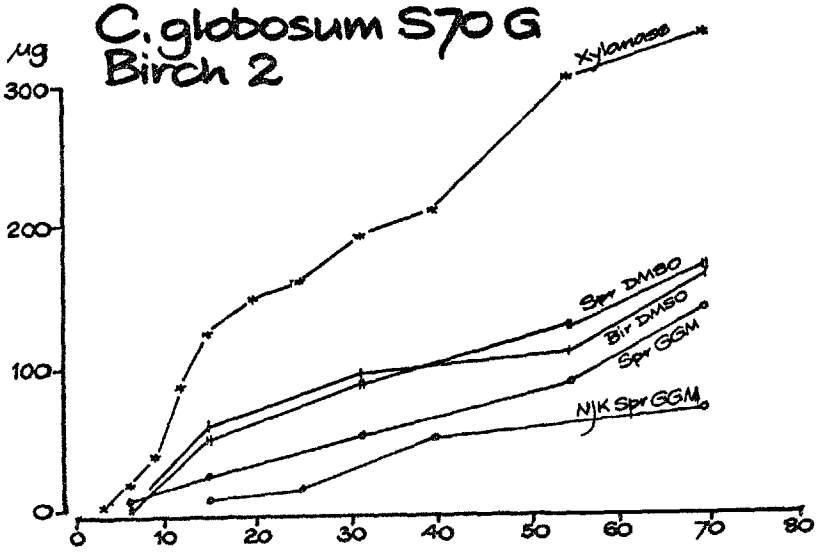

µg
C. globosum S70 G
Spruce 1
300
200
100
0
5.5
5.0
4.5
4.0
pH
CMC
+Protein
0 10 20 30 40 50 60 70 80
Days

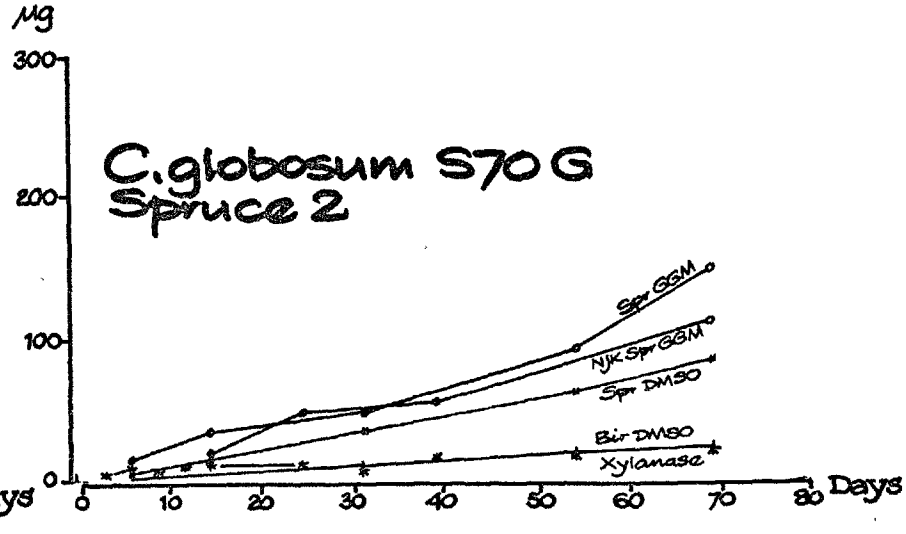

µg
C. globosum S70 G
Birch 1
Xylanase
300
200
100
0
Bir DMSO
Spr DMSO
Spr GGM
NJK Spr GGM
0 10 20 30 40 50 60 70 80
Days

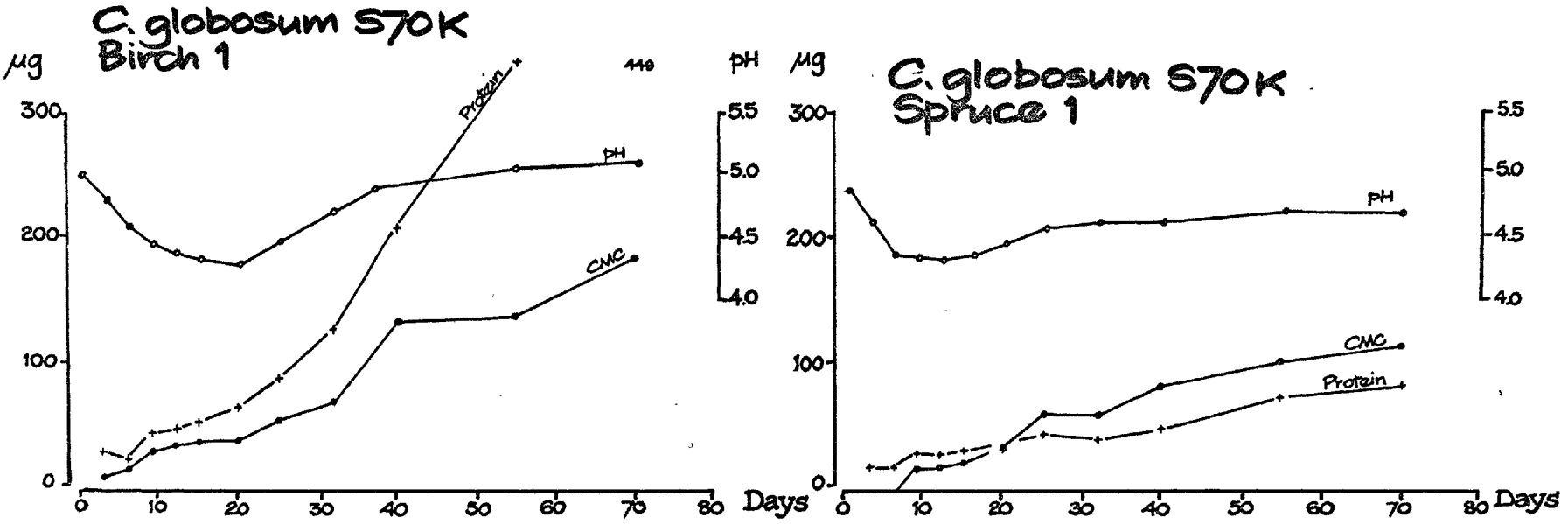

µg
C. globosum S70 G
Spruce 1
300
200
100
0
Spr GGM
NJK Spr GGM
Spr DMSO
Bir DMSO
Xylanase
0 10 20 30 40 50 60 70 80
Days


C. globosum S70G
Birch 2
µg
pH
467
556
300
200
100
0
5.5
5.0
4.5
4.0
pH
CMC
Protein
0 10 20 30 40 50 60 70 80
Days

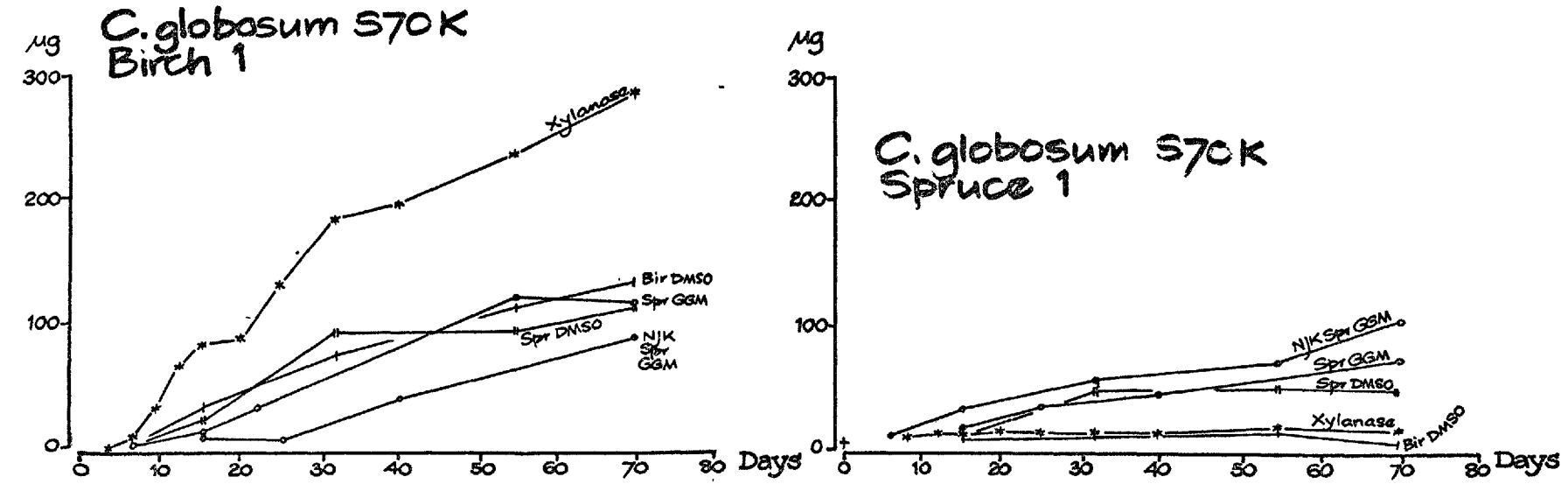

µg
C. globosum S70G
Spruce 2
300
200
100
0
5.5
5.0
4.5
4.0
pH
CMC
Protein
0 10 20 30 40 50 60 70 80
Days


µg
C. globosum S70G
Birch 2
300
200
100
0
Xylanase
Spr DM30
Bir DM30
Spr GGM
NJK Spr GGM
0 10 20 30 40 50 60 70 80
Days

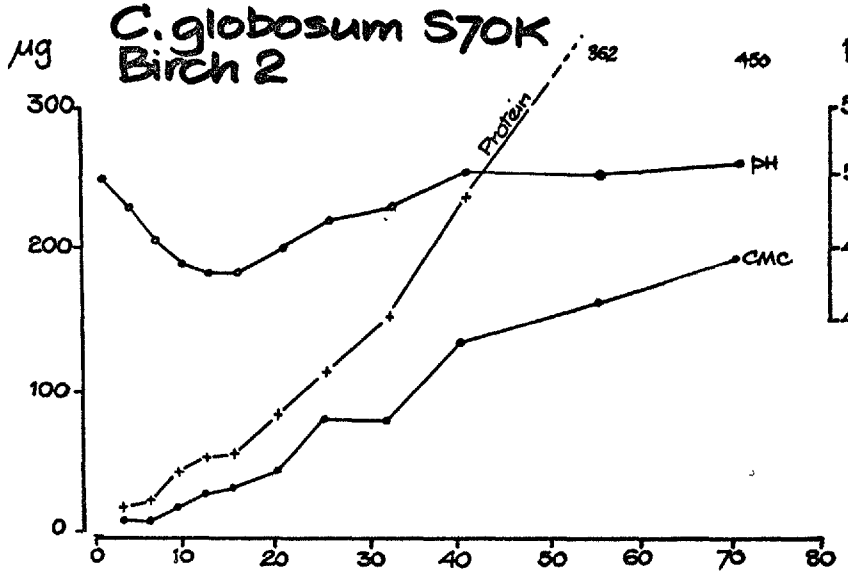

µg
300
200
100
0
C. globosum S70G
Spruce 2
Spr GGM
NJK Spr GGM
Spr DM30
Bir DM30
Xylanase
0 10 20 30 40 50 60 70 80
Days

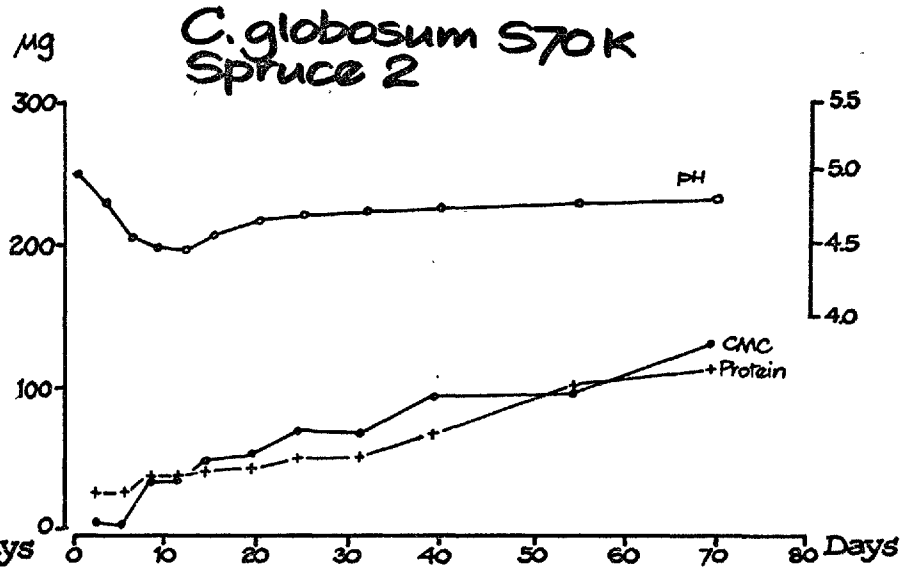

C. globosum S70K
Birch 1
µg
pH
300
200
100
0
5.5
5.0
4.5
4.0
Protein
pH
CMC
0 10 20 30 40 50 60 70 80
Days

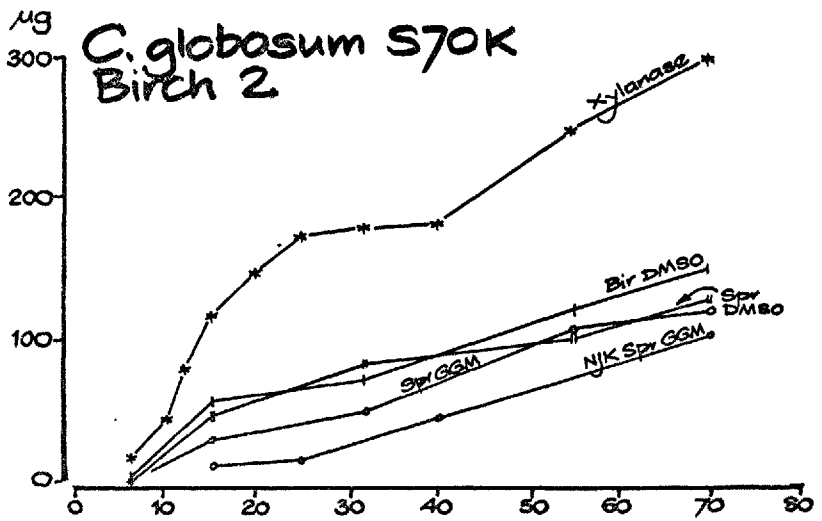
C. globosum S70K
Spruce 1
µg
pH
300
200
100
0
5.5
5.0
4.5
4.0
pH
CMC
Protein
0 10 20 30 40 50 60 70 80
Days

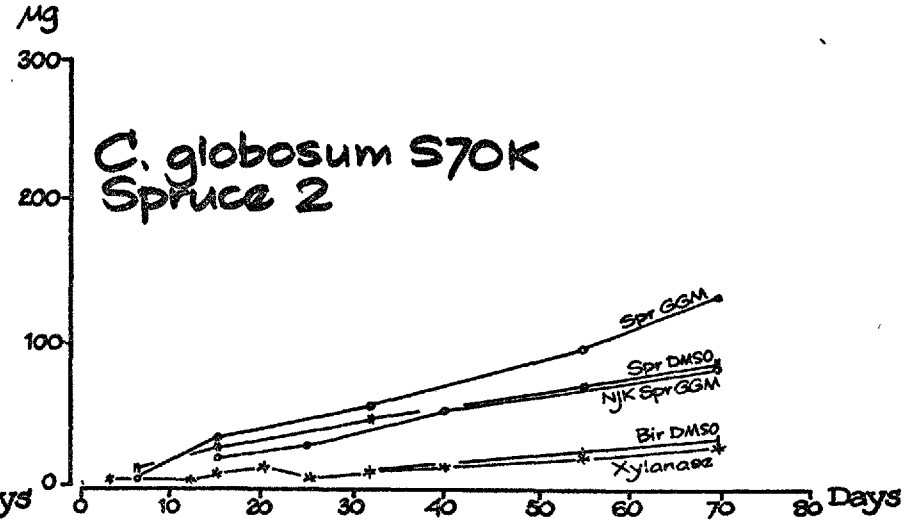

C. globosum S70K
Birch 1
µg
300
200
100
0
Xylanase
Bir DMSO
Spr GGM
Spr DMSO
NJK Spr GGM
0 10 20 30 40 50 60 70 80
Days

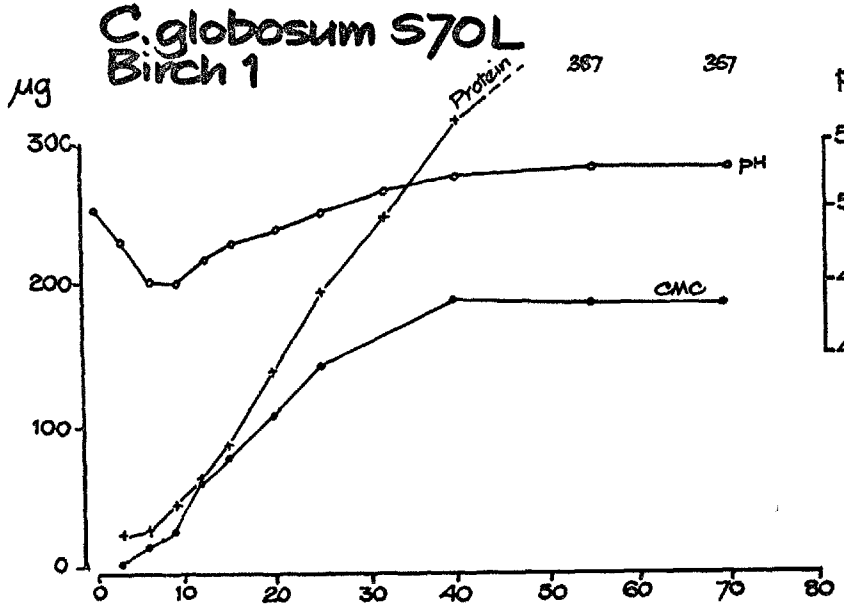
C. globosum S70K
Spruce 1
µg
300
200
100
0
NJK Spr GGM
Spr GGM
Spr DMSO
Xylanase
Bir DMSO
0 10 20 30 40 50 60 70 80
Days

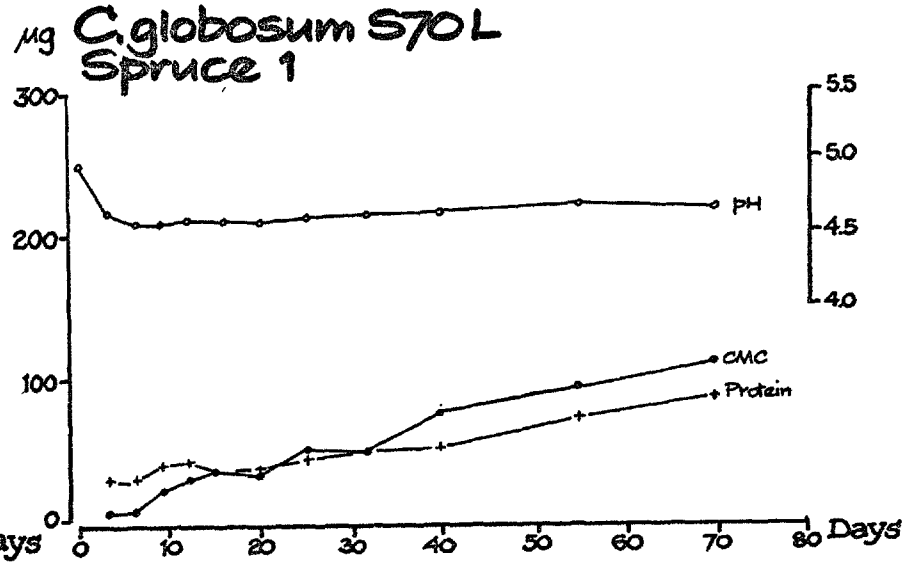

C. globosum S70K
Birch 2
µg
pH
362
450
Protein
pH
CMC
300
200
100
0
5.5
5.0
4.5
4.0
0 10 20 30 40 50 60 70 80
Days

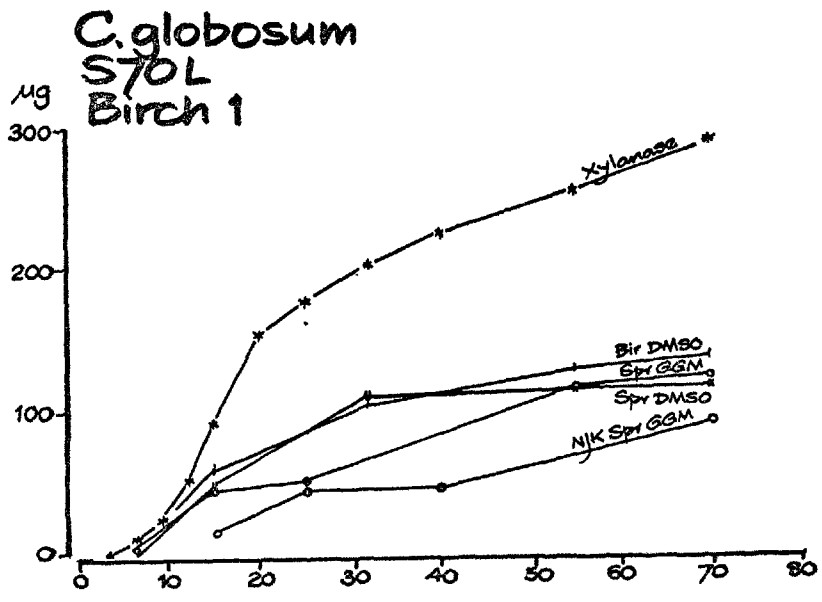

C. globosum S70K
Spruce 2
µg
pH
CMC
Protein
300
200
100
0
5.5
5.0
4.5
4.0
0 10 20 30 40 50 60 70 80
Days

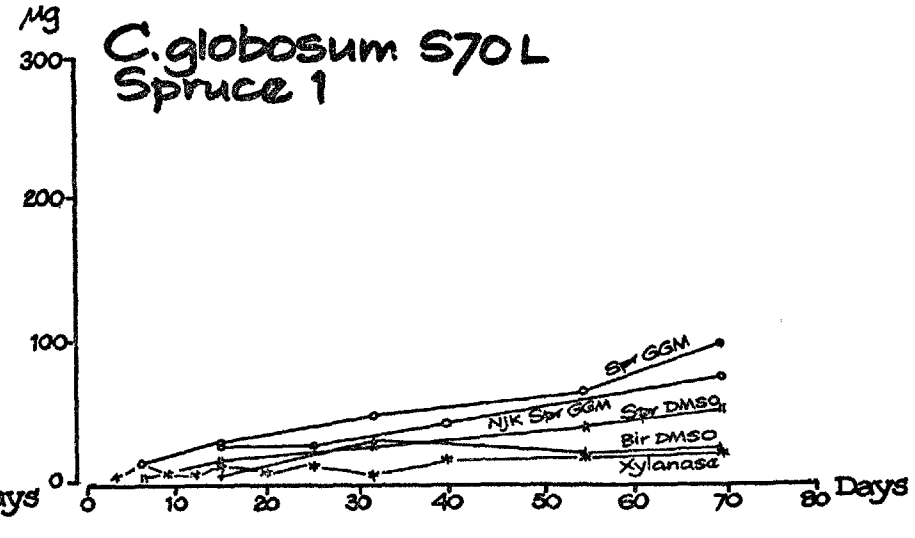

C. globosum S70K
Birch 2
µg
Xylanase
Bir DMSO
Spr DMSO
Spr GGM
NJK Spr GGM
300
200
100
0
0 10 20 30 40 50 60 70 80
Days

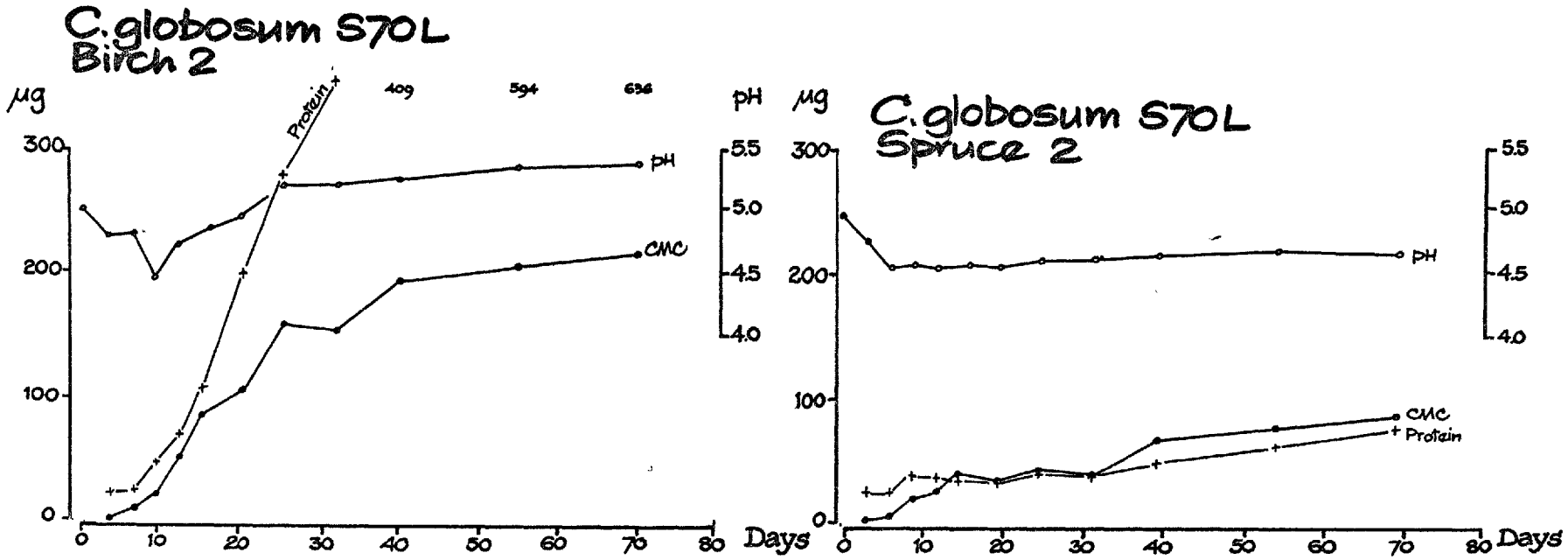

C. globosum S70K
Spruce 2
µg
Spr GGM
Spr DMSO
NJK Spr GGM
Bir DMSO
Xylanase
300
200
100
0
0 10 20 30 40 50 60 70 80
Days


C.globosum S70L
Birch 1
µg
pH
Protein
CMC
Days

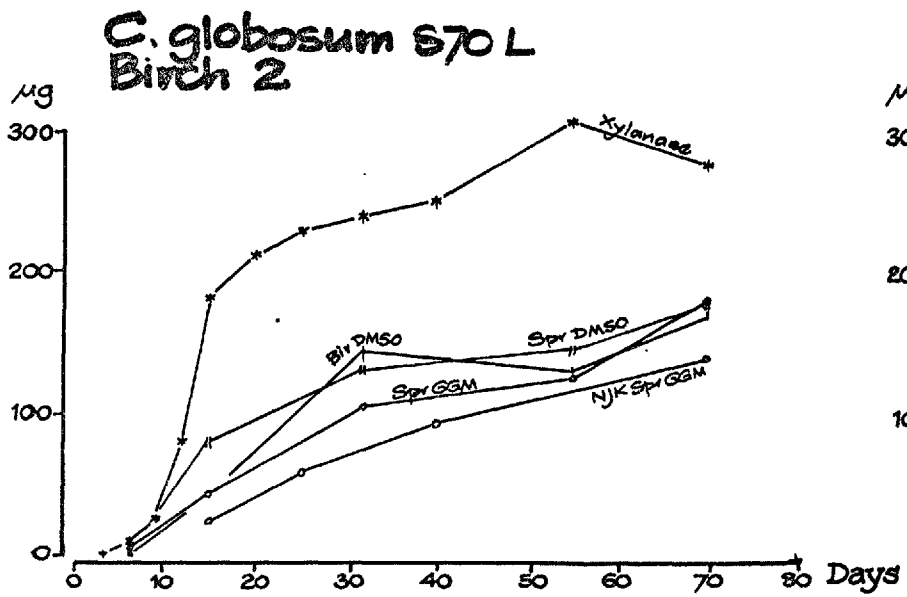

µg
C.globosum S70L
Spruce 1
pH
CMC
Protein
Days

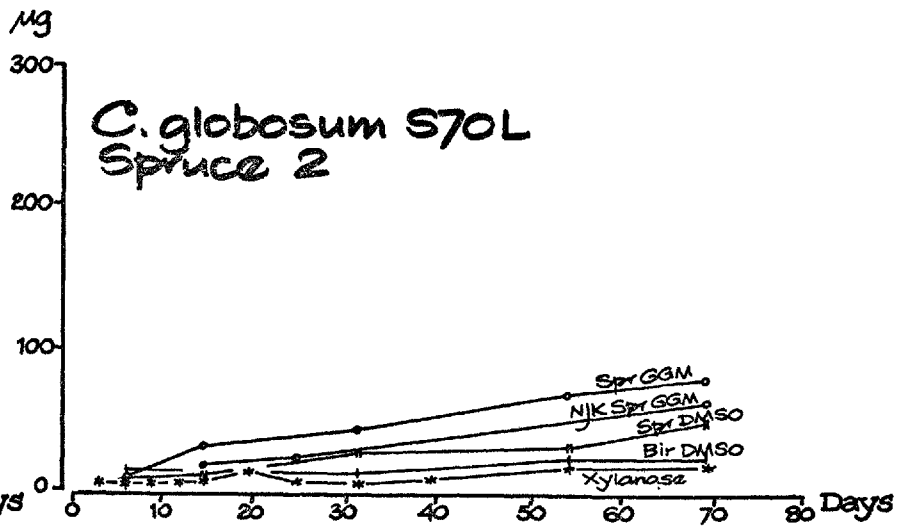

C.globosum
S70L
Birch 1
µg
Xylanase
Bir DMSO
Spr GGM
Spr DMSO
NJK Spr GGM
Days

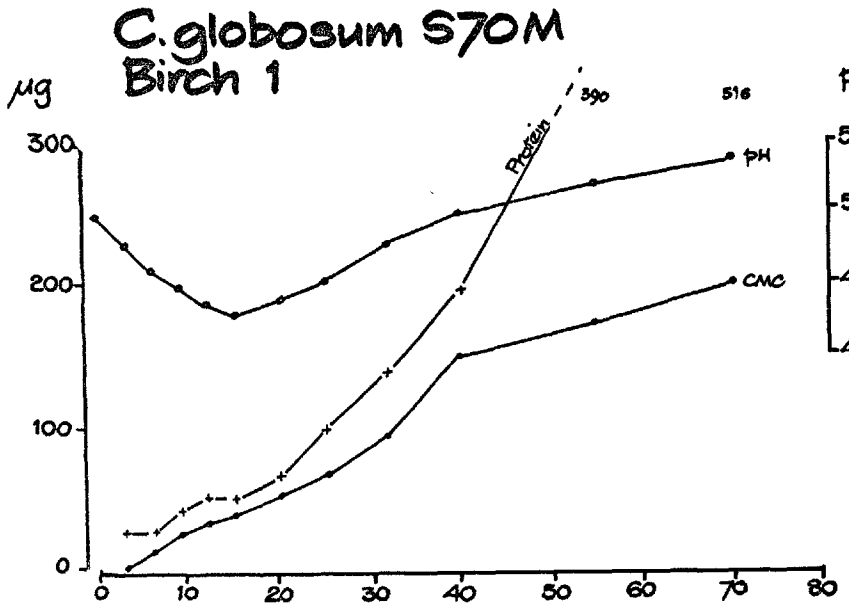

µg
C.globosum S70L
Spruce 1
Spr GGM
NJK Spr GGM
Spr DMSO
Bir DMSO
Xylanase
Days

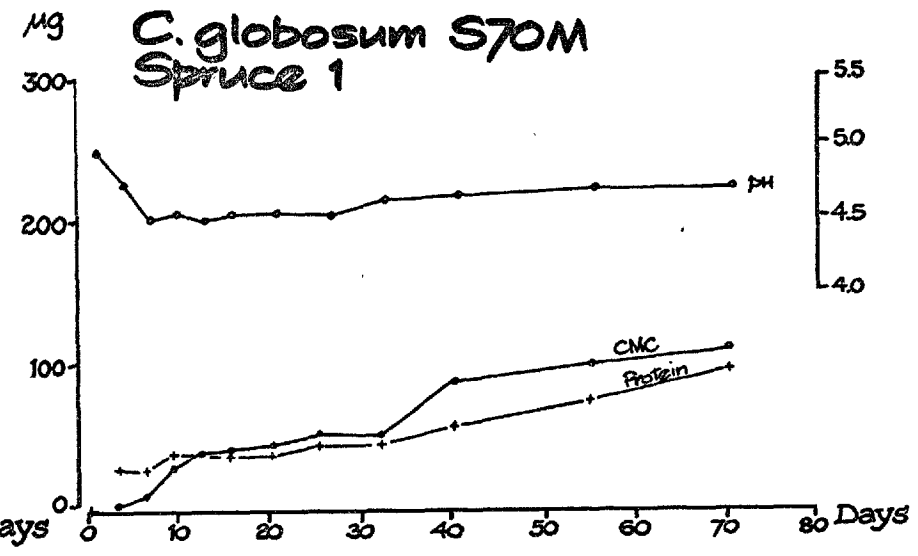

C. globosum S70L
Birch 2
µg
pH
409
594
636
Protein
pH
CMC
300
200
100
0
5.5
5.0
4.5
4.0
0 10 20 30 40 50 60 70 80
Days

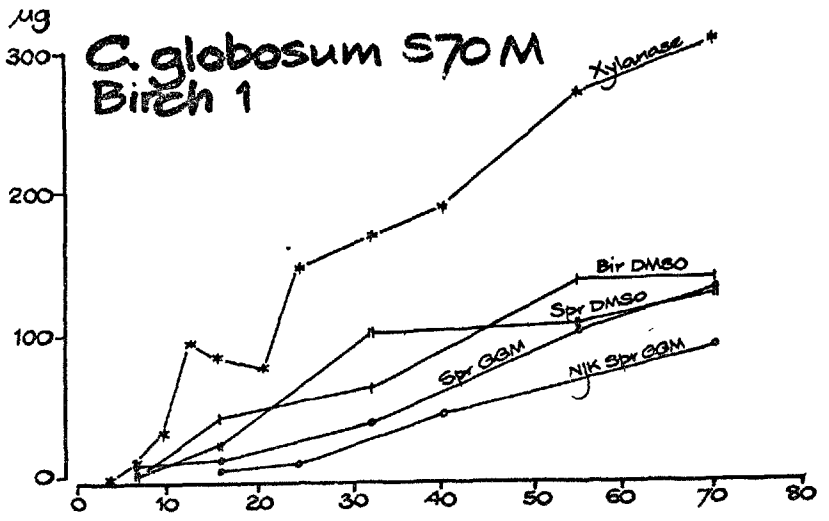
µg
C. globosum S70L
Spruce 2
300
200
100
0
pH
5.5
5.0
4.5
4.0
CMC
Protein
0 10 20 30 40 50 60 70 80
Days

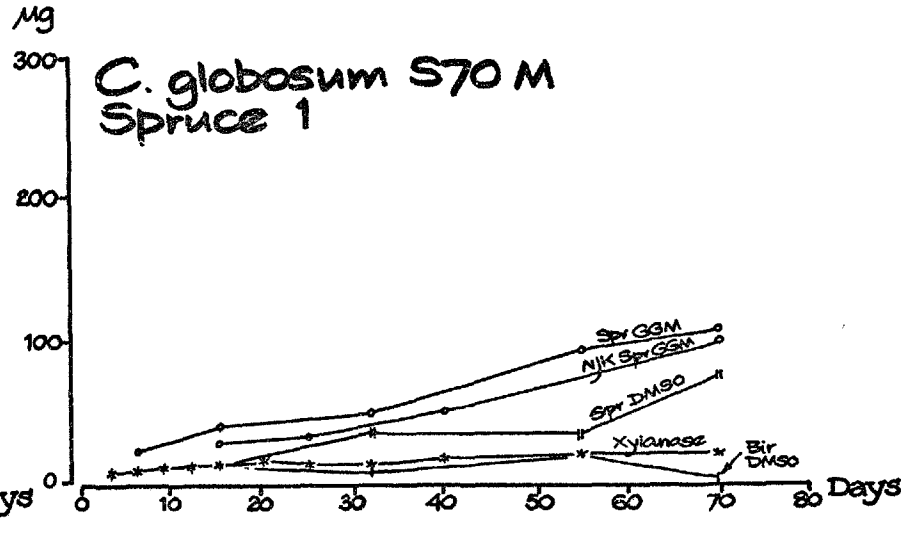

C. globosum S70 L
Birch 2
µg
300
200
100
0
Xylanase
Bir DMSO
Spr DMSO
Spr GGM
NJK Spr GGM
0 10 20 30 40 50 60 70 80
Days

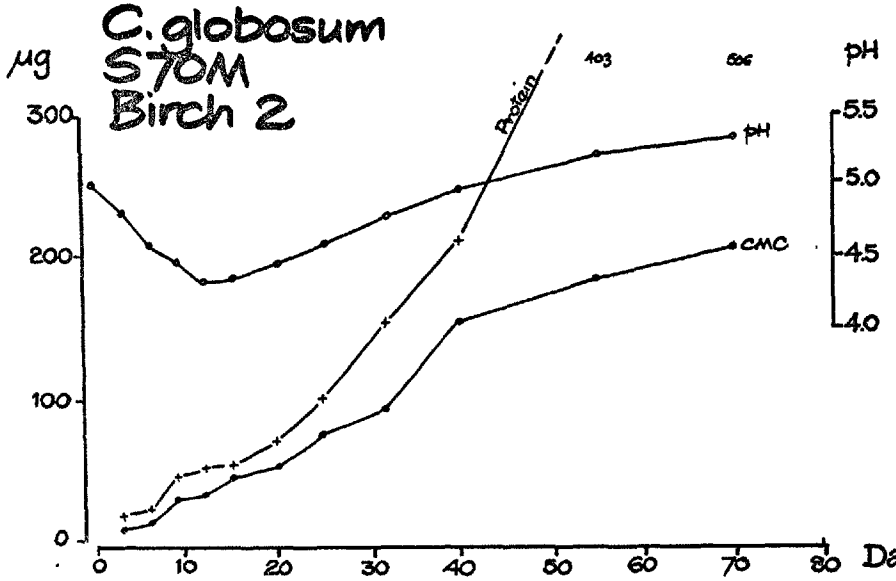

µg
C. globosum S70L
Spruce 2
300
200
100
0
Spr GGM
NJK Spr GGM
Spr DMSO
Bir DMSO
Xylanase
0 10 20 30 40 50 60 70 80
Days

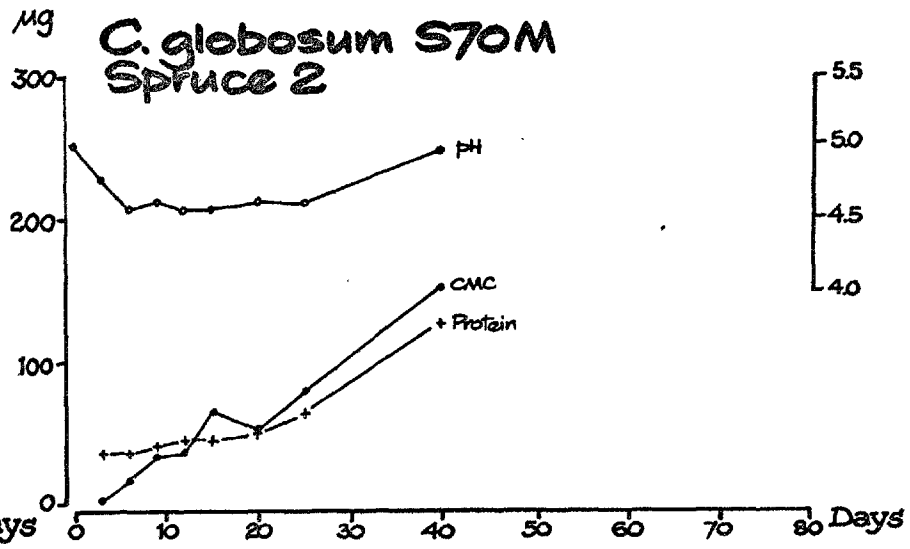

C. globosum S70M
Birch 1
µg
pH
Protein
390
516
pH
CMC
300
200
100
0
5.5
5.0
4.5
4.0
0 10 20 30 40 50 60 70 80
Days

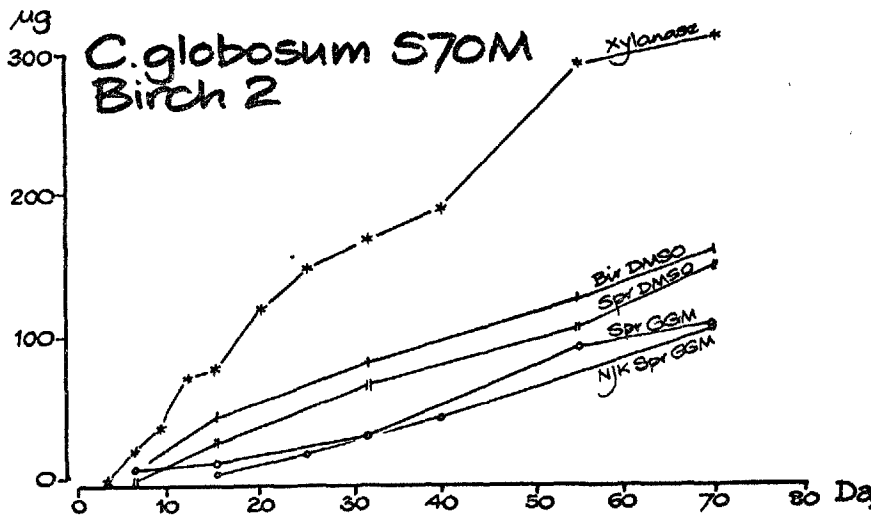

C. globosum S70M
Spruce 1
µg
pH
CMC
Protein
300
200
100
0
5.5
5.0
4.5
4.0
0 10 20 30 40 50 60 70 80
Days

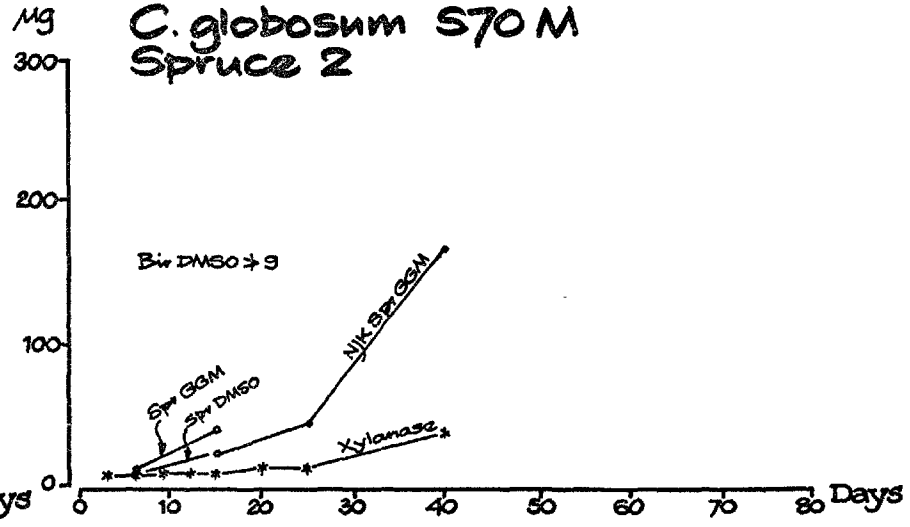

C. globosum S70M
Birch 1
µg
Xylanase
Bir DMSO
Spr DMSO
Spr GGM
NJK Spr GGM
300
200
100
0
0 10 20 30 40 50 60 70 80
Days

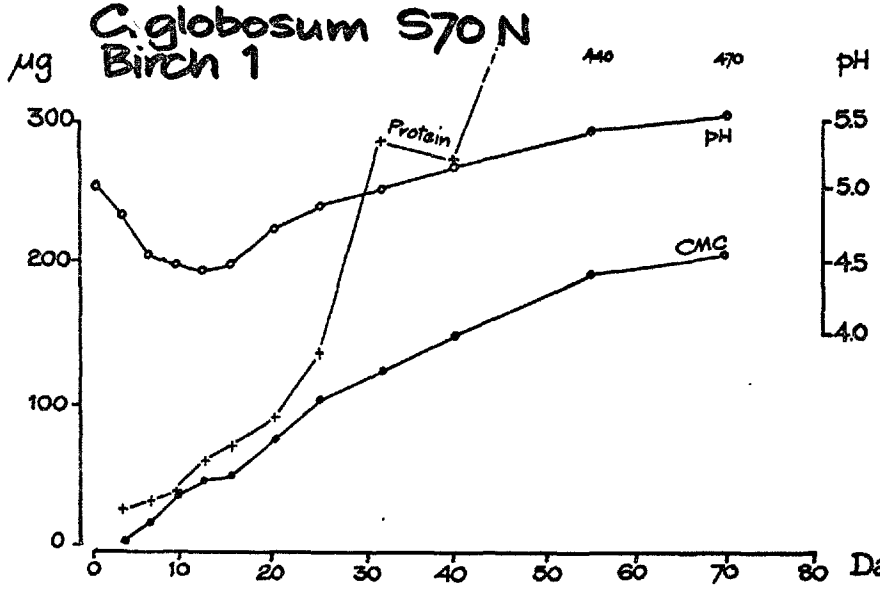

C. globosum S70M
Spruce 1
µg
Spr GGM
NJK Spr GGM
Spr DMSO
Xylanase
Bir DMSO
300
200
100
0
0 10 20 30 40 50 60 70 80
Days

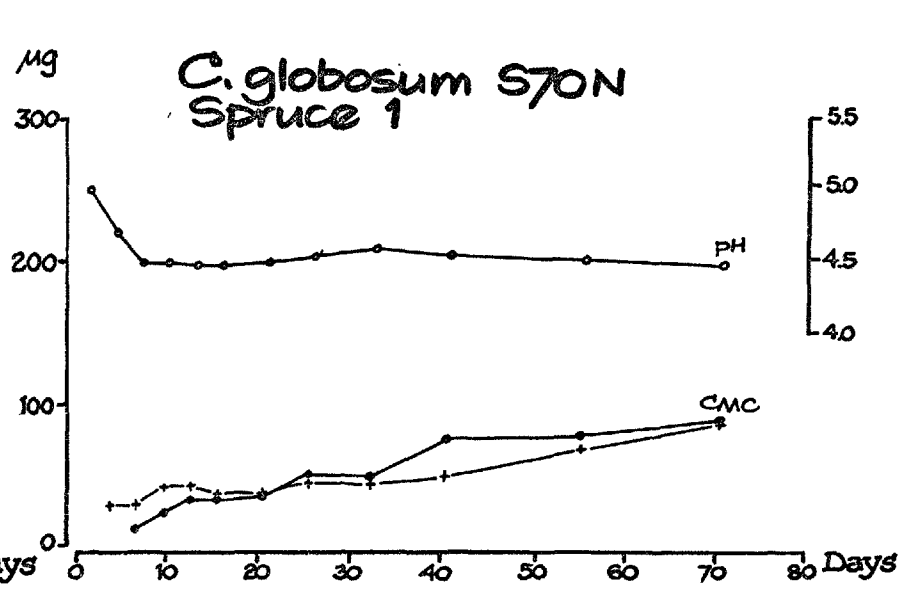

C. globosum
S70M
Birch 2
µg
pH
Protein
pH
CMC
300
200
100
0
5.5
5.0
4.5
4.0
0 10 20 30 40 50 60 70 80
Days

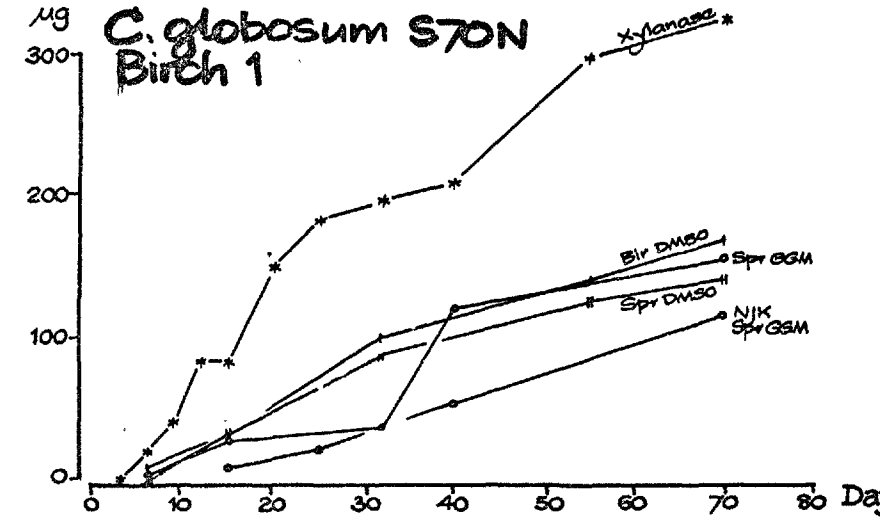

µg
C. globosum S70M
Spruce 2
pH
CMC
+ Protein
300
200
100
0
5.5
5.0
4.5
4.0
0 10 20 30 40 50 60 70 80
Days

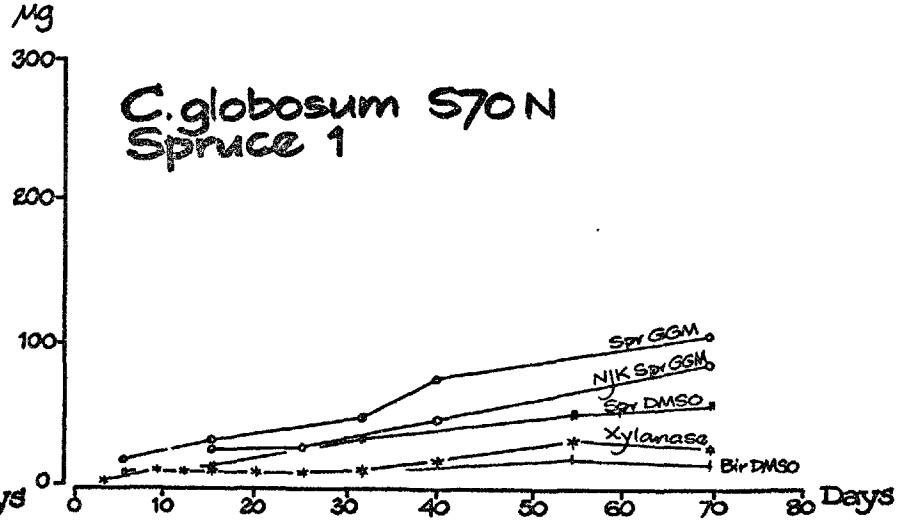

µg
C. globosum S70M
Birch 2
Xylanase
Bir DMSO
Spr DMSO
Spr GGM
NJK Spr GGM
300
200
100
0
0 10 20 30 40 50 60 70 80
Days

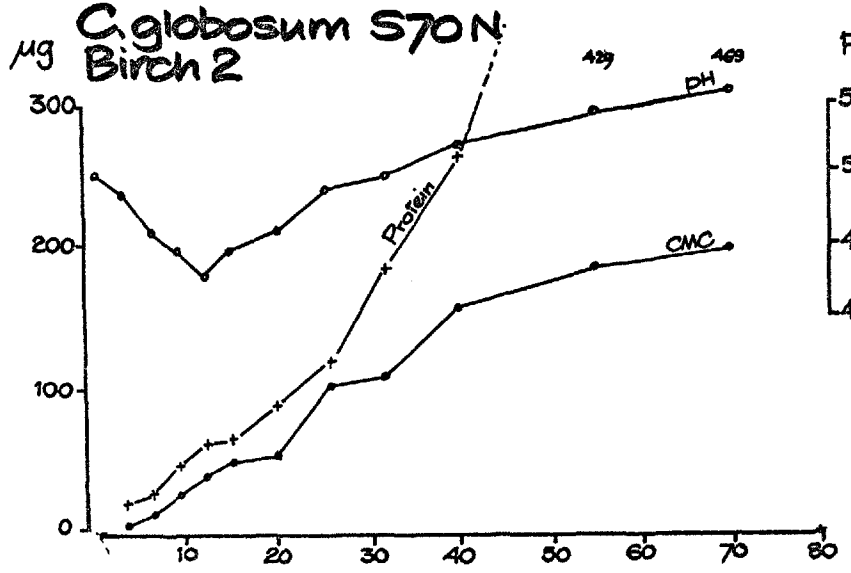

µg
C. globosum S70M
Spruce 2
Bir DMSO ÷ 9
NJK Spr GGM
Spr GGM
Spr DMSO
Xylanase
300
200
100
0
0 10 20 30 40 50 60 70 80
Days

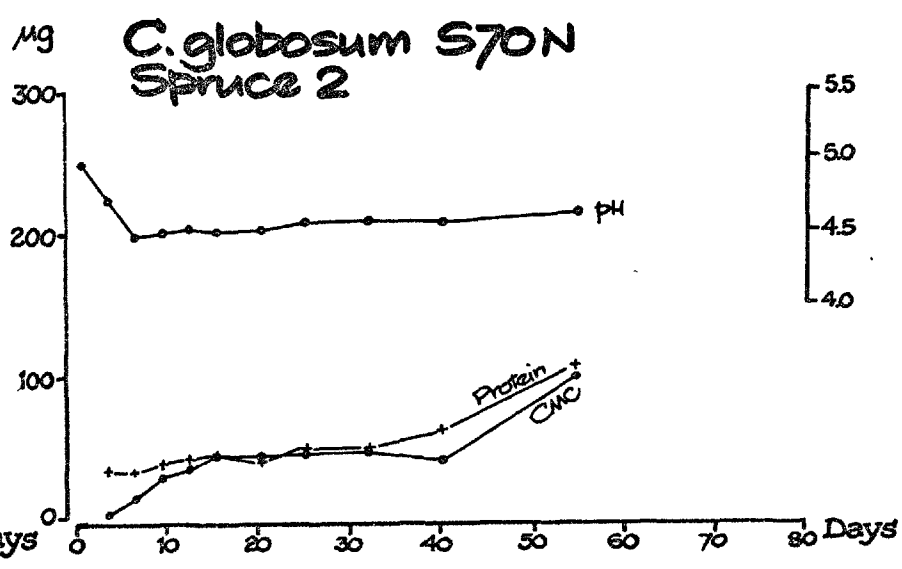

C. globosum S70N
Birch 1
µg
pH
440
470
Protein
pH
CMC
300
200
100
0
5.5
5.0
4.5
4.0
0 10 20 30 40 50 60 70 80
Days

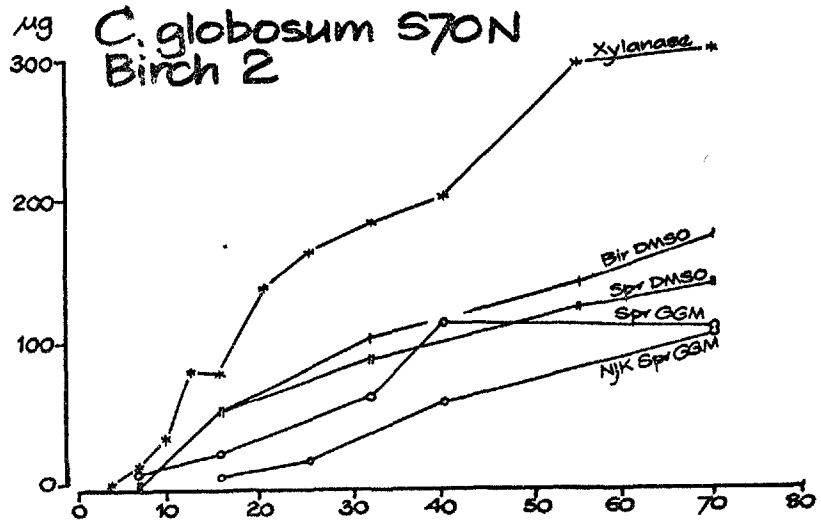

C. globosum S70N
Spruce 1
µg
pH
pH
CMC
300
200
100
0
5.5
5.0
4.5
4.0
0 10 20 30 40 50 60 70 80
Days

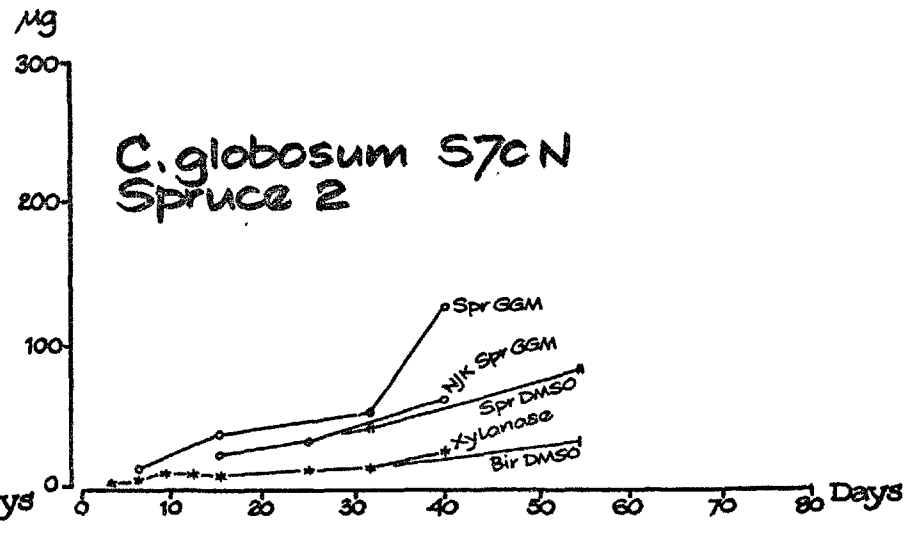

µg
C. globosum S70N
Birch 1
Xylanase
Bir DMSO
Spr GGM
Spr DMSO
NJK Spr GGM
300
200
100
0
0 10 20 30 40 50 60 70 80
Days

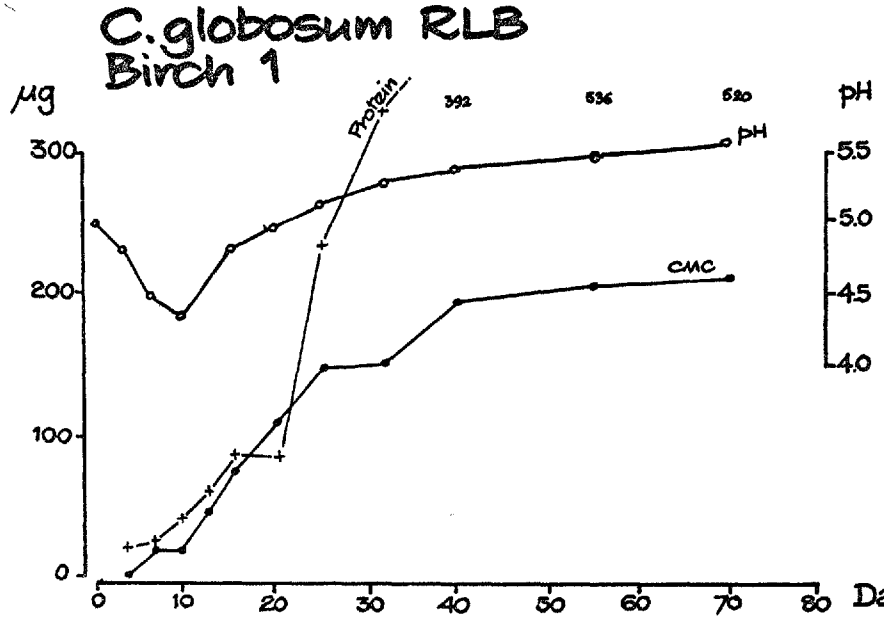

µg
C. globosum S70N
Spruce 1
Spr GGM
NJK Spr GGM
Spr DMSO
Xylanase
Bir DMSO
300
200
100
0
0 10 20 30 40 50 60 70 80
Days

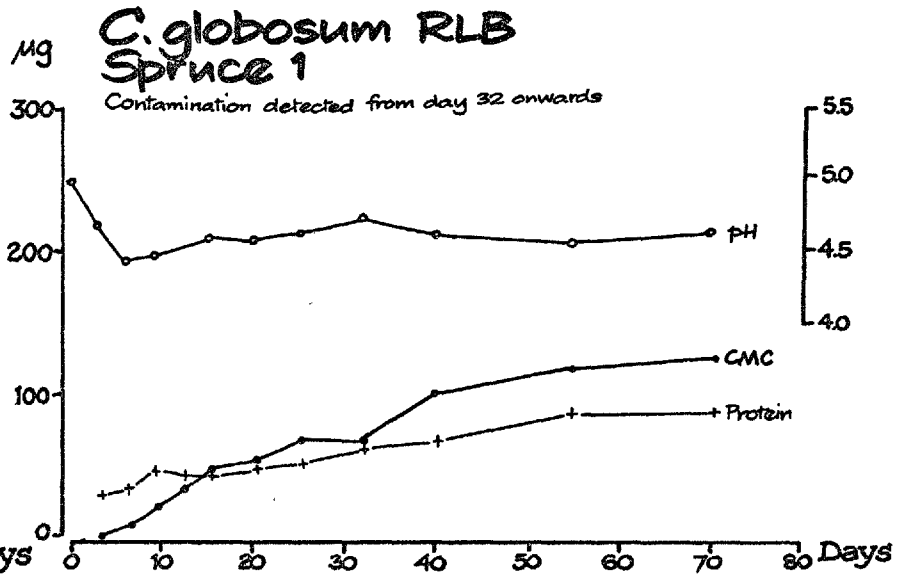

C. globosum S70N
Birch 2
µg
pH
429
469
300
200
100
0
5.5
5.0
4.5
4.0
Protein
CMC
10 20 30 40 50 60 70 80
Days

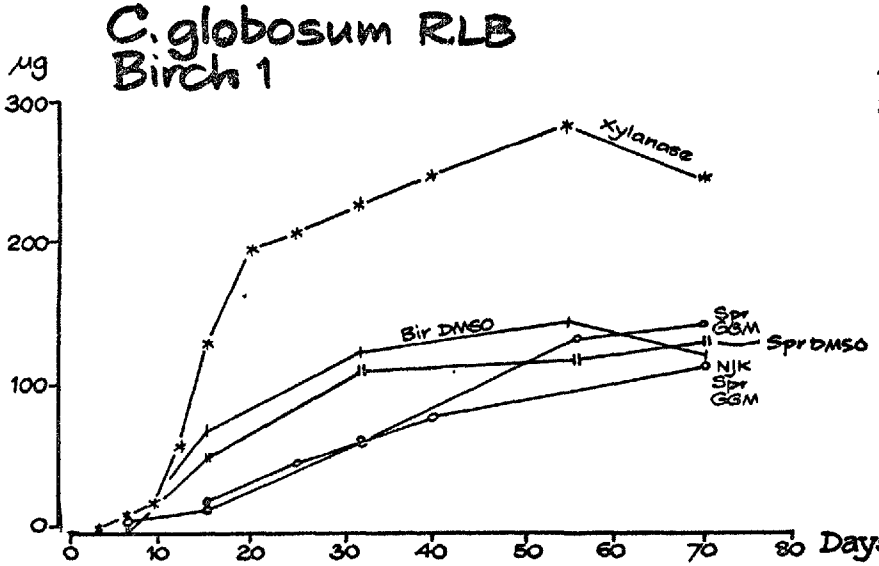

C. globosum S70N
Spruce 2
µg
pH
300
200
100
0
5.5
5.0
4.5
4.0
Protein
CMC
10 20 30 40 50 60 70 80
Days

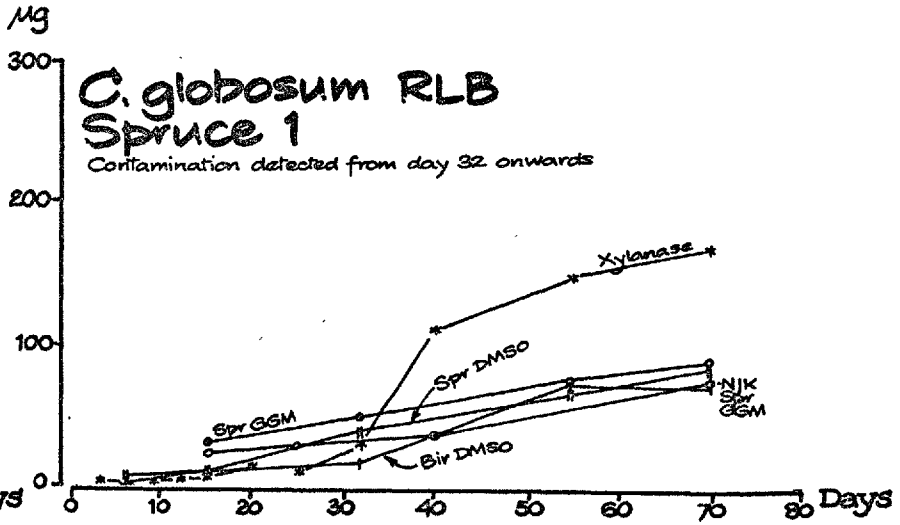

C. globosum S70N
Birch 2
µg
300
200
100
0
Xylanase
Bir DMSO
Spr DMSO
Spr GGM
NJK Spr GGM
10 20 30 40 50 60 70 80
Days

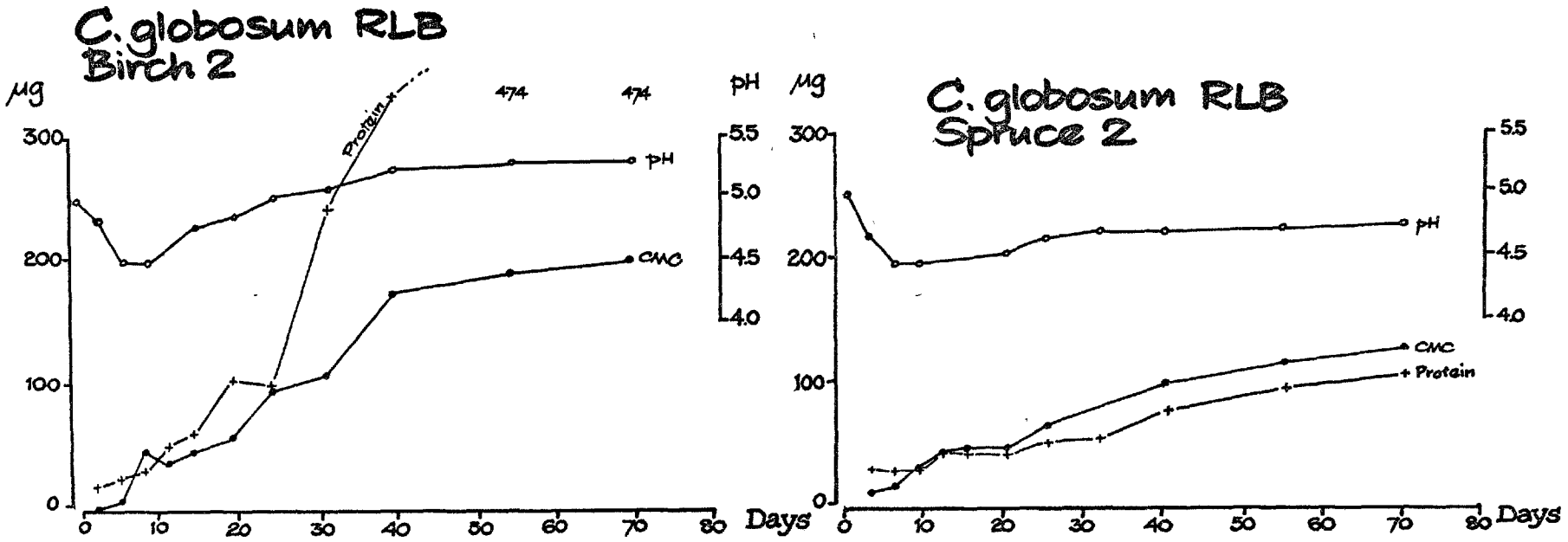

µg
300
200
100
0
C. globosum S70N
Spruce 2
Spr GGM
NJK Spr GGM
Spr DMSO
Xylanase
Bir DMSO
10 20 30 40 50 60 70 80
Days


C. globosum RLB
Birch 1
µg
pH
Protein
392
536
520
pH
CMC
300
200
100
0
5.5
5.0
4.5
4.0
0 10 20 30 40 50 60 70 80
Days

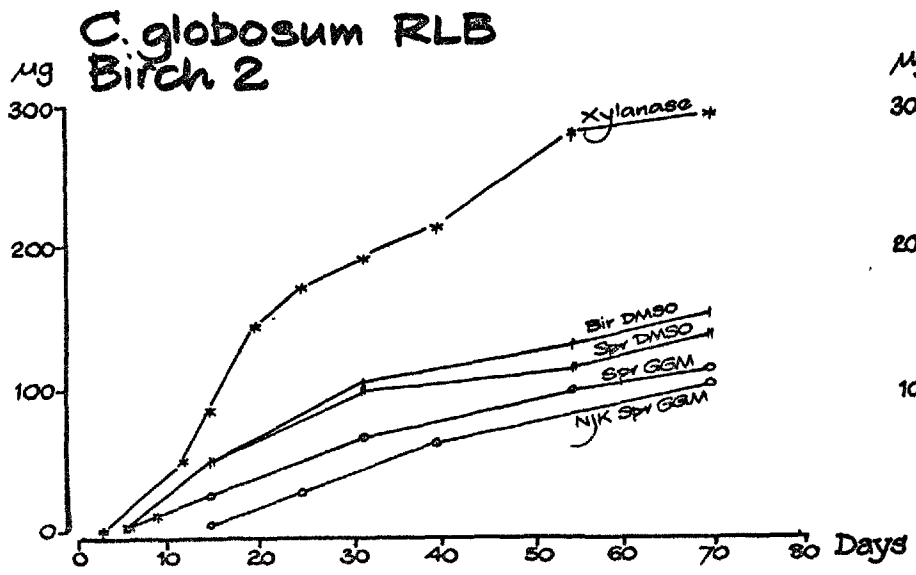

C. globosum RLB
Spruce 1
Contamination detected from day 32 onwards
µg
pH
CMC
Protein
300
200
100
0
5.5
5.0
4.5
4.0
0 10 20 30 40 50 60 70 80
Days

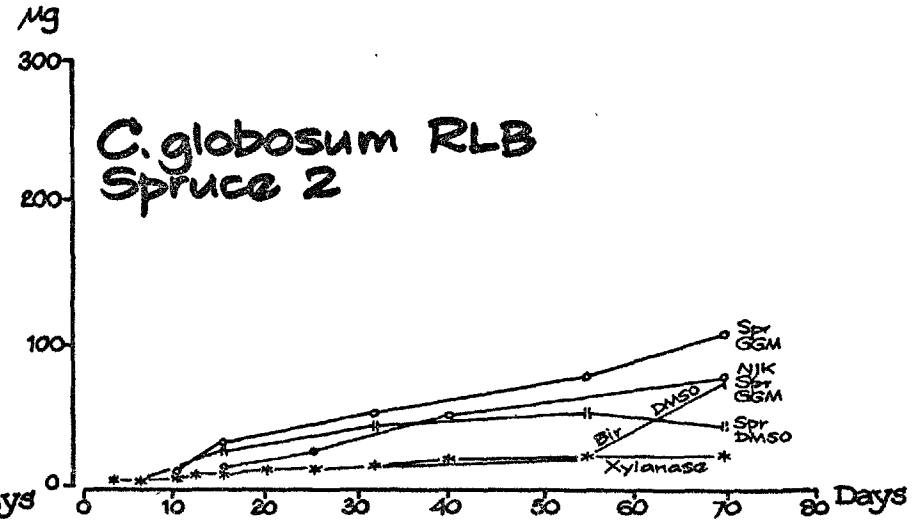

C. globosum RLB
Birch 1
µg
Xylanase
Bir DMSO
Spr GGM
Spr DMSO
NJK Spr GGM
300
200
100
0
0 10 20 30 40 50 60 70 80
Days

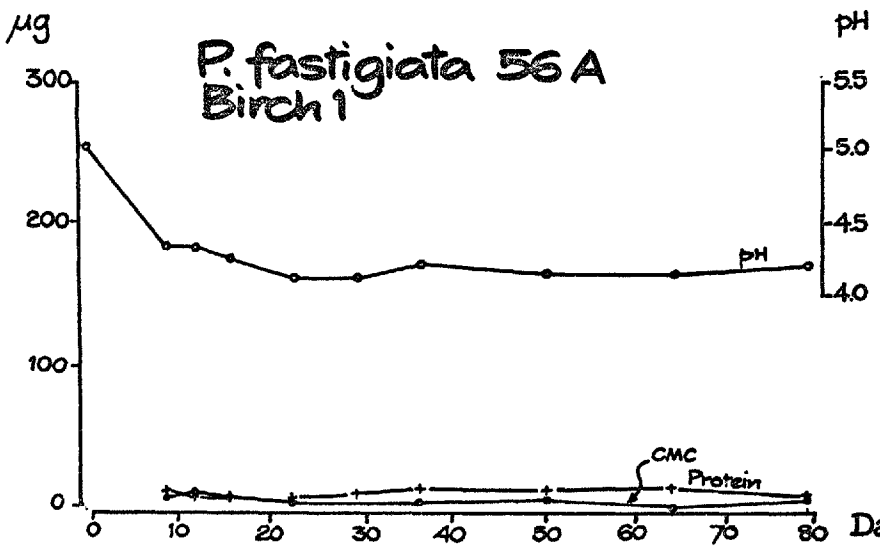

µg
C. globosum RLB
Spruce 1
Contamination detected from day 32 onwards
Xylanase
Spr DMSO
Spr GGM
NJK Spr GGM
Bir DMSO
300
200
100
0
0 10 20 30 40 50 60 70 80
Days

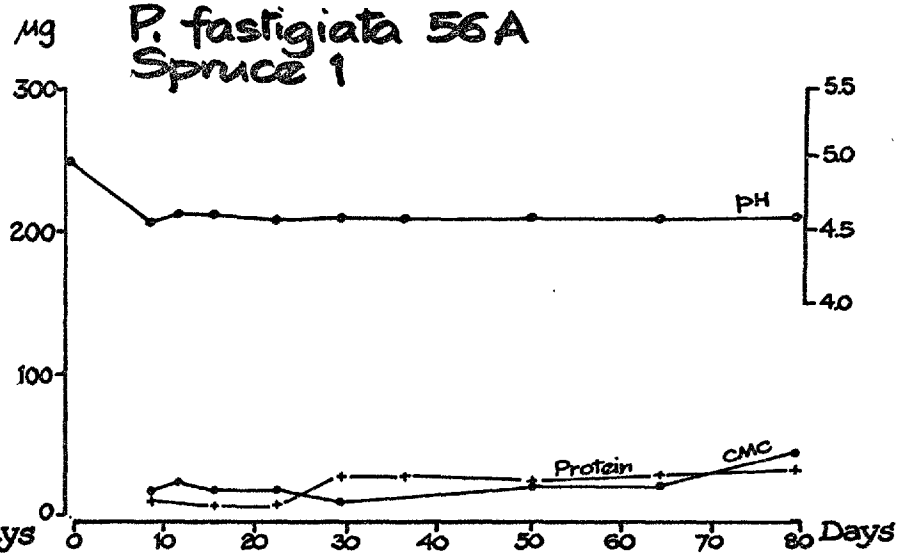

C. globosum RLB
Birch 2
µg
pH
474
474
Protein
pH
CMC
300
200
100
0
5.5
5.0
4.5
4.0
0 10 20 30 40 50 60 70 80
Days

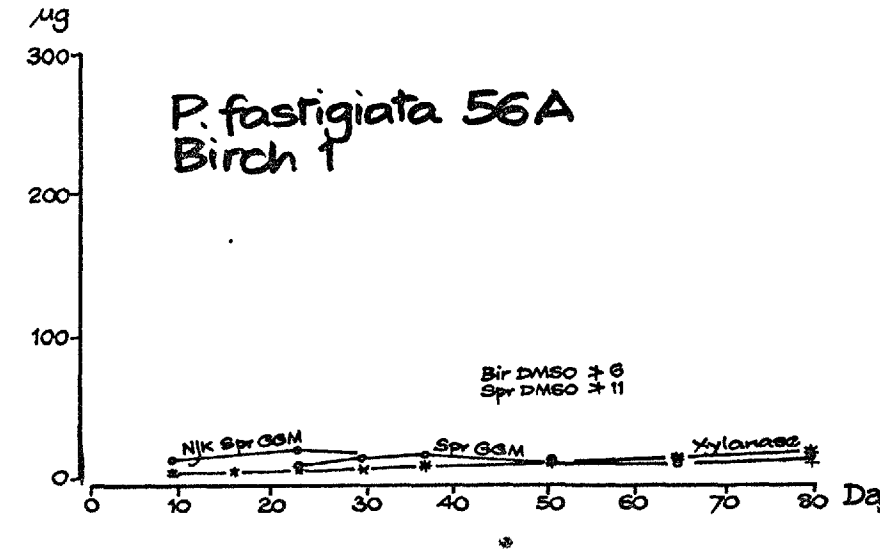
C. globosum RLB
Spruce 2
µg
pH
CMC
+ Protein
300
200
100
0
5.5
5.0
4.5
4.0
0 10 20 30 40 50 60 70 80
Days

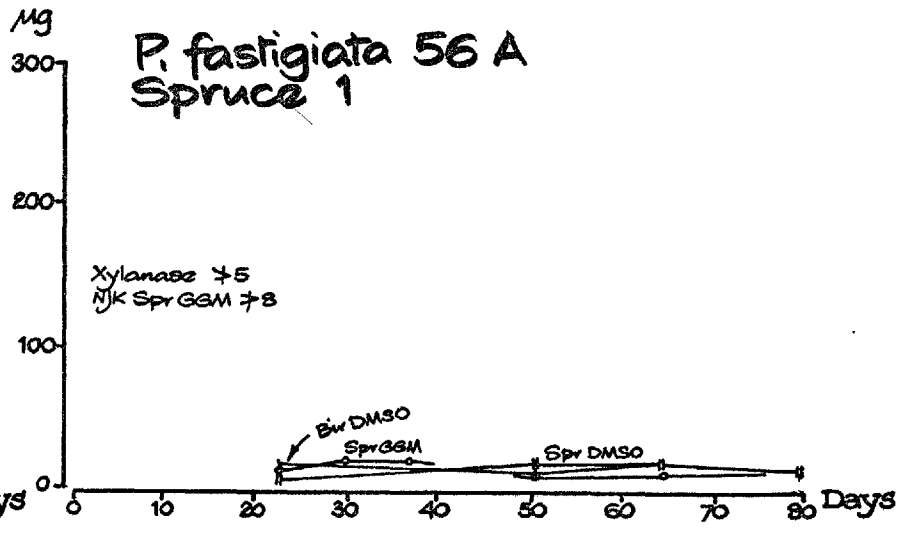

C. globosum RLB
Birch 2
µg
Xylanase
Bir DMSO
Spr DMSO
Spr GGM
NJK Spr GGM
300
200
100
0
0 10 20 30 40 50 60 70 80
Days

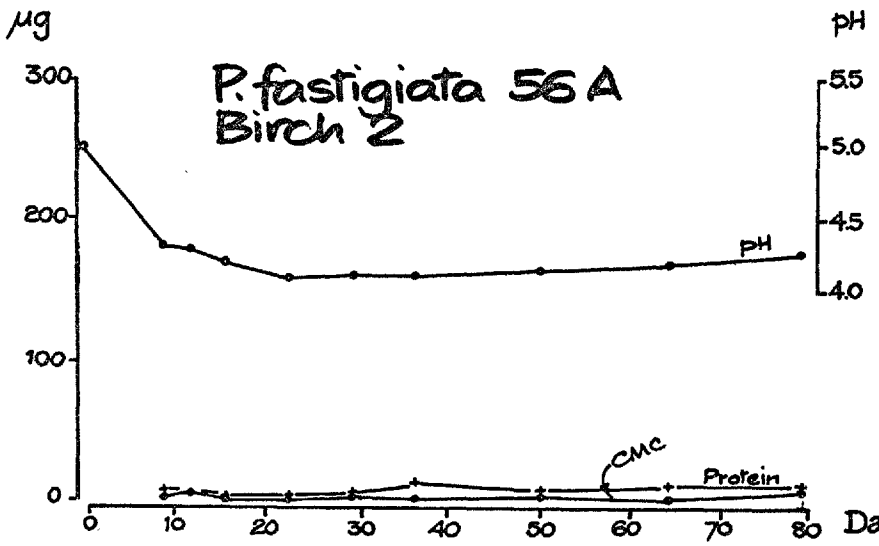

µg
C. globosum RLB
Spruce 2
Spr GGM
NJK Spr GGM
DMSO
Bir
Spr DMSO
Xylanase
300
200
100
0
0 10 20 30 40 50 60 70 80
Days

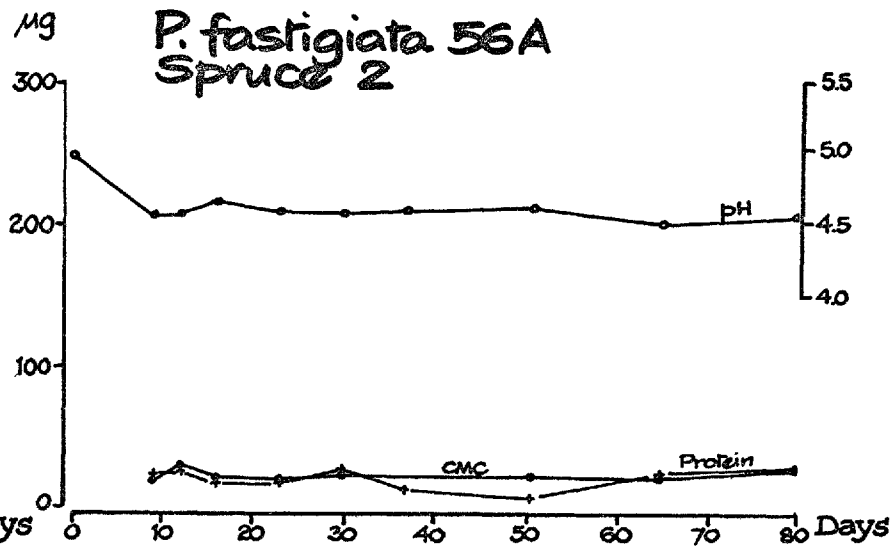

µg
pH
P. fastigiata 56 A
Birch 1
300
200
100
0
5.5
5.0
4.5
4.0
pH
CMC
Protein
0 10 20 30 40 50 60 70 80
Days

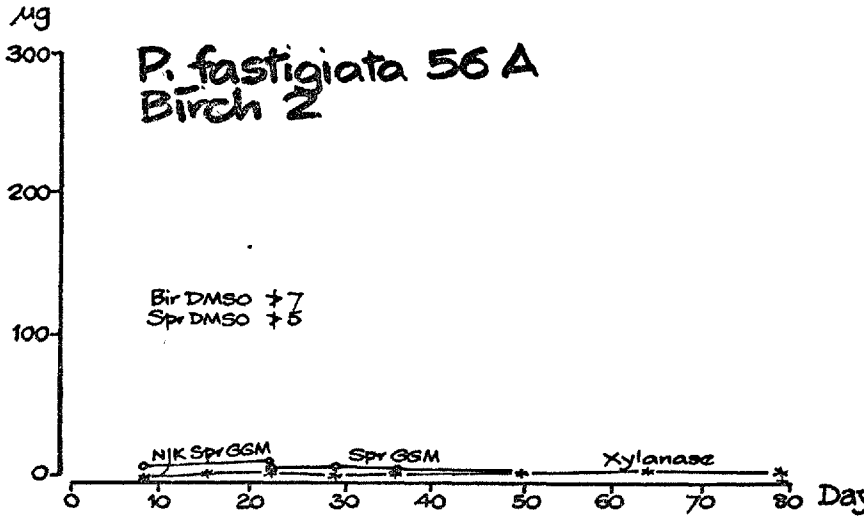

µg
P. fastigiata 56 A
Spruce 1
300
200
100
0
5.5
5.0
4.5
4.0
pH
Protein
CMC
0 10 20 30 40 50 60 70 80
Days

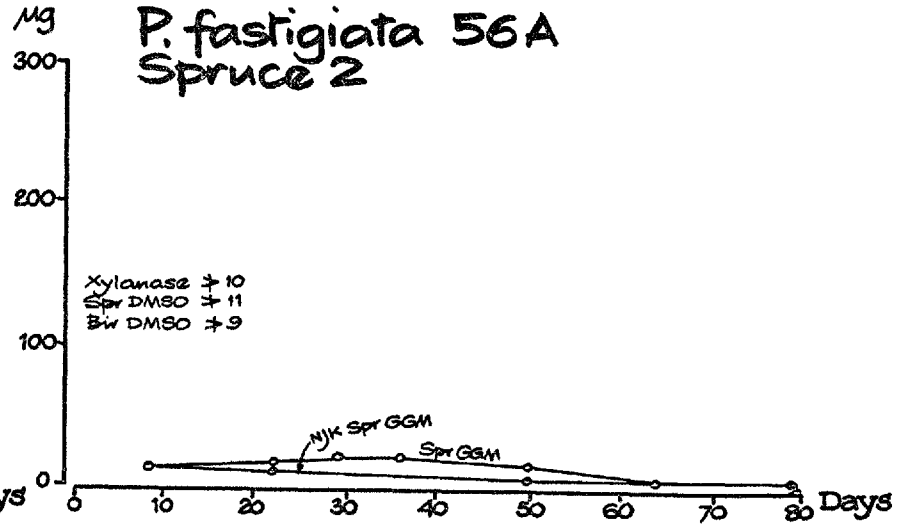

µg
P. fastigiata 56A
Birch 1
300
200
100
0
Bir DMSO ≠ 6
Spr DMSO ≠ 11
NJK Spr GGM
Spr GGM
Xylanase
0 10 20 30 40 50 60 70 80
Days

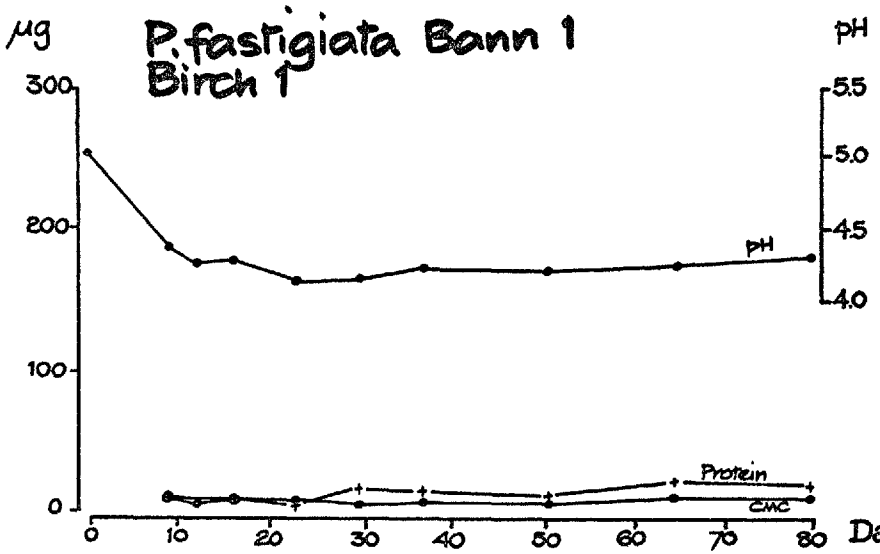

µg
P. fastigiata 56 A
Spruce 1
300
200
100
0
Xylanase ≠ 5
NJK Spr GGM ≠ 8
Bir DMSO
Spr GGM
Spr DMSO
0 10 20 30 40 50 60 70 80
Days

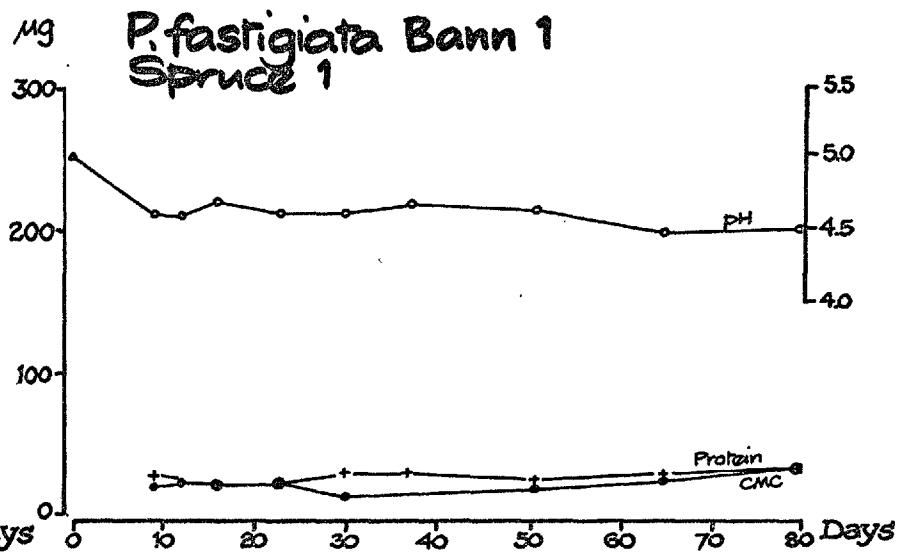

µg
pH
P. fastigiata 56A
Birch 2
300
200
100
0
5.5
5.0
4.5
4.0
pH
CMC
Protein
0 10 20 30 40 50 60 70 80
Days

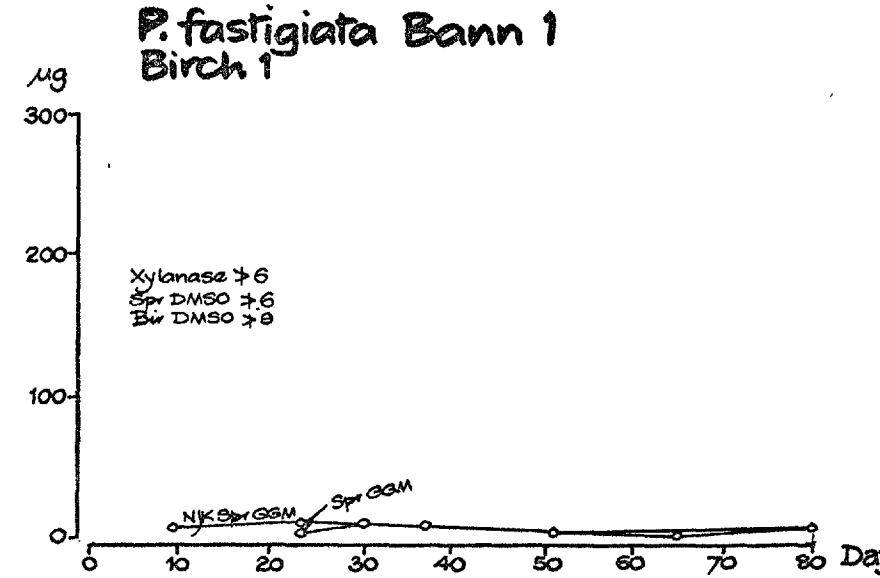

µg
P. fastigiata 56A
Spruce 2
300
200
100
0
5.5
5.0
4.5
4.0
pH
CMC
Protein
0 10 20 30 40 50 60 70 80
Days

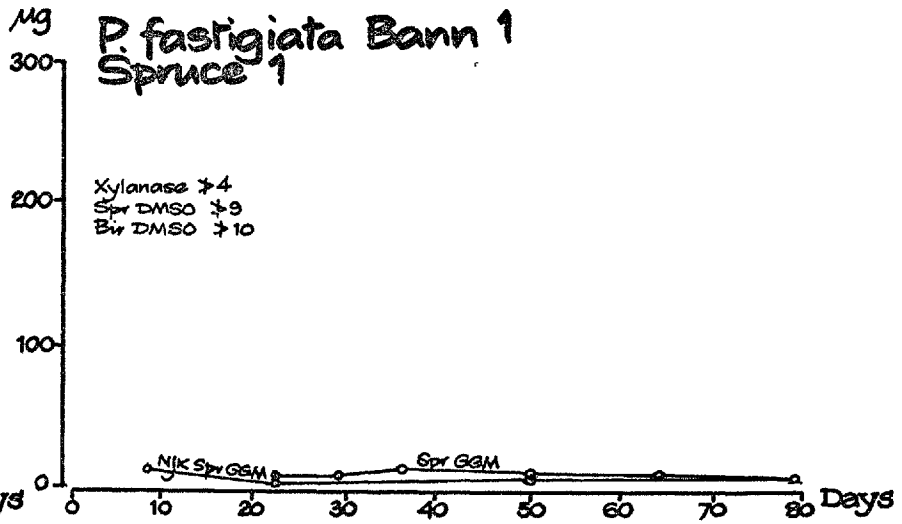

µg
P. fastigiata 56A
Birch 2
300
200
100
0
Bir DMSO + 7
Spr DMSO + 5
N|K Spr GGM
Spr GGM
Xylanase
0 10 20 30 40 50 60 70 80
Days

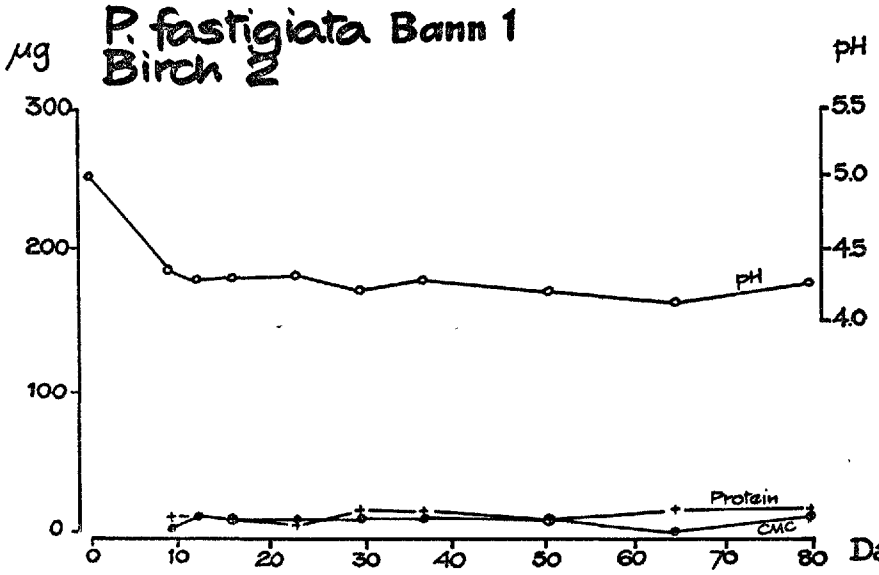

µg
P. fastigiata 56A
Spruce 2
300
200
100
0
Xylanase + 10
Spr DMSO + 11
Bir DMSO + 9
N|K Spr GGM
Spr GGM
0 10 20 30 40 50 60 70 80
Days

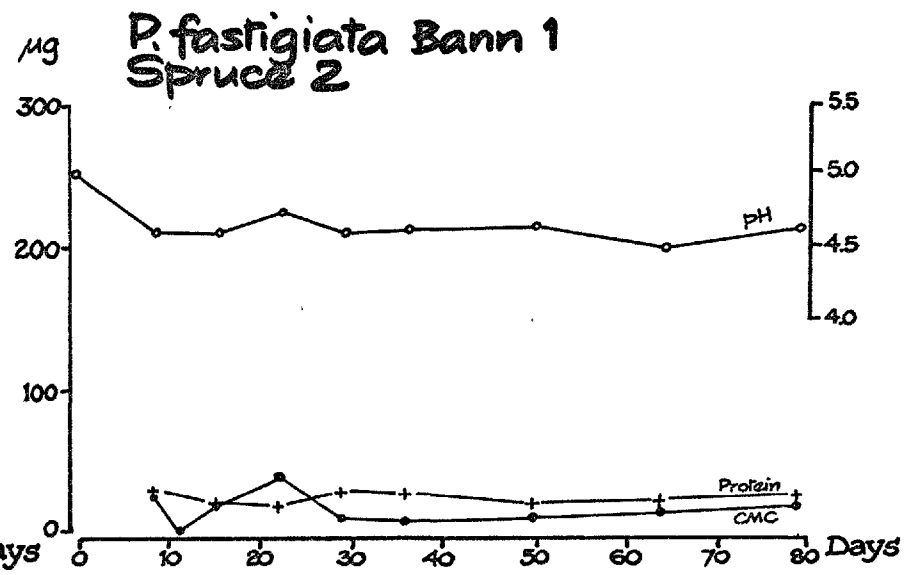

µg
P. fastigiata Bann 1
Birch 1
pH
300
200
100
0
5.5
5.0
4.5
4.0
pH
Protein
CMC
0 10 20 30 40 50 60 70 80
Days

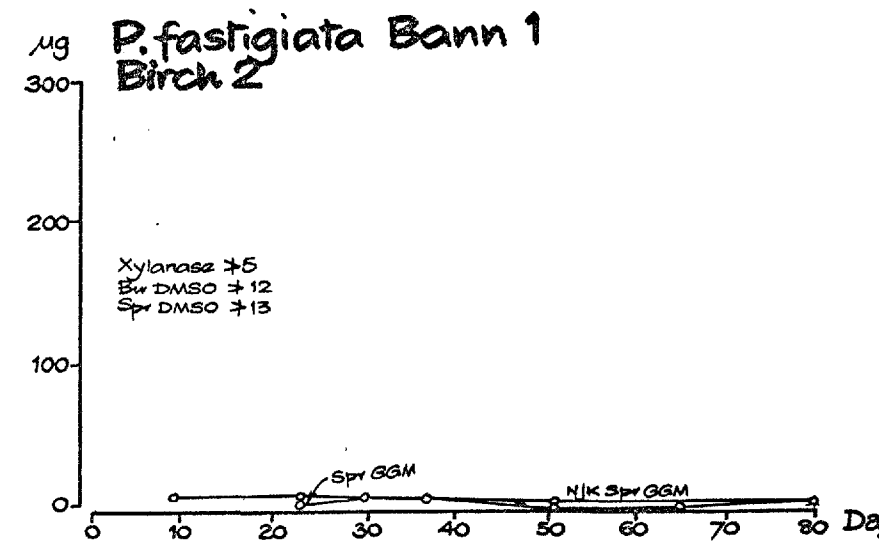

µg
P. fastigiata Bann 1
Spruce 1
300
200
100
0
5.5
5.0
4.5
4.0
pH
Protein
CMC
0 10 20 30 40 50 60 70 80
Days

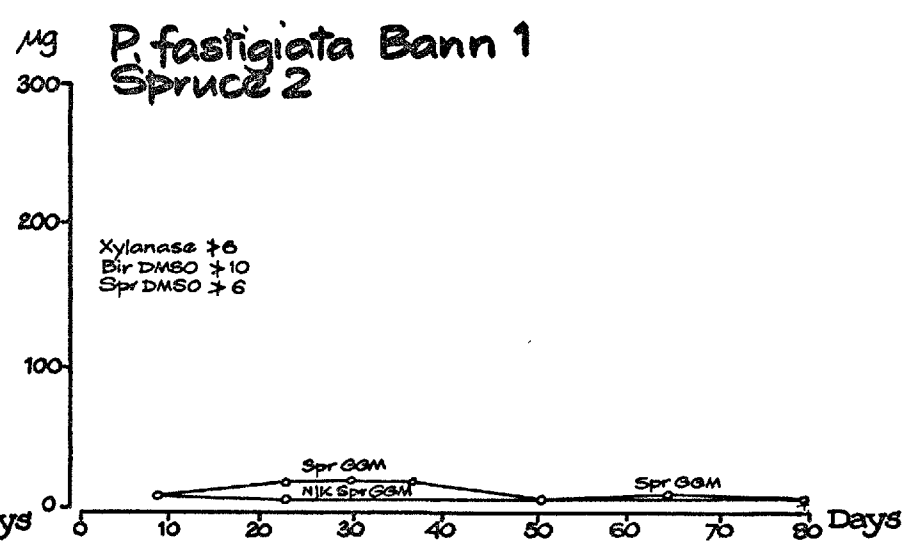

P. fastigiata Bann 1
Birch 1
µg
300
200
100
0
Xylanase ⊁6
Spr DMSO ⊁6
Bir DMSO ⊁8
NJK Spr GGM
Spr GGM
0 10 20 30 40 50 60 70 80
Days

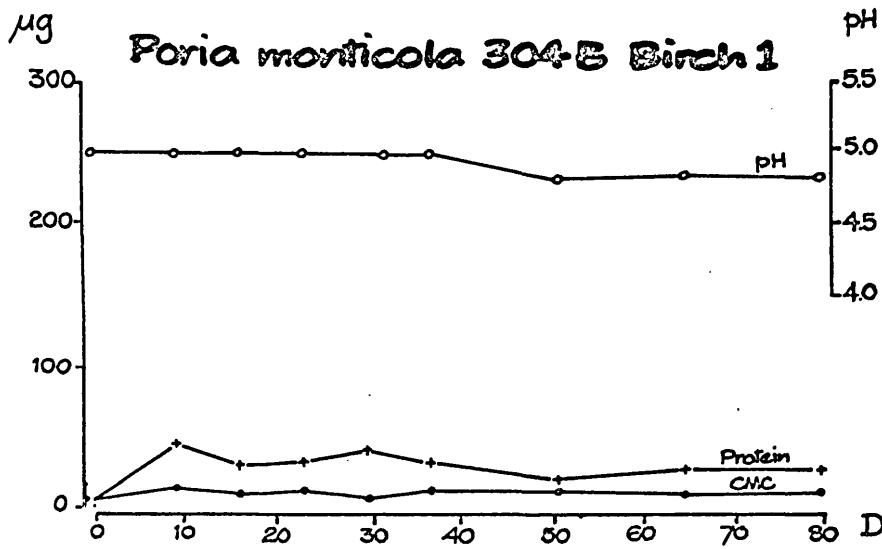

µg
P. fastigiata Bann 1
Spruce 1
300
200
100
0
Xylanase ⊁4
Spr DMSO ⊁9
Bir DMSO ⊁10
NJK Spr GGM
Spr GGM
0 10 20 30 40 50 60 70 80
Days

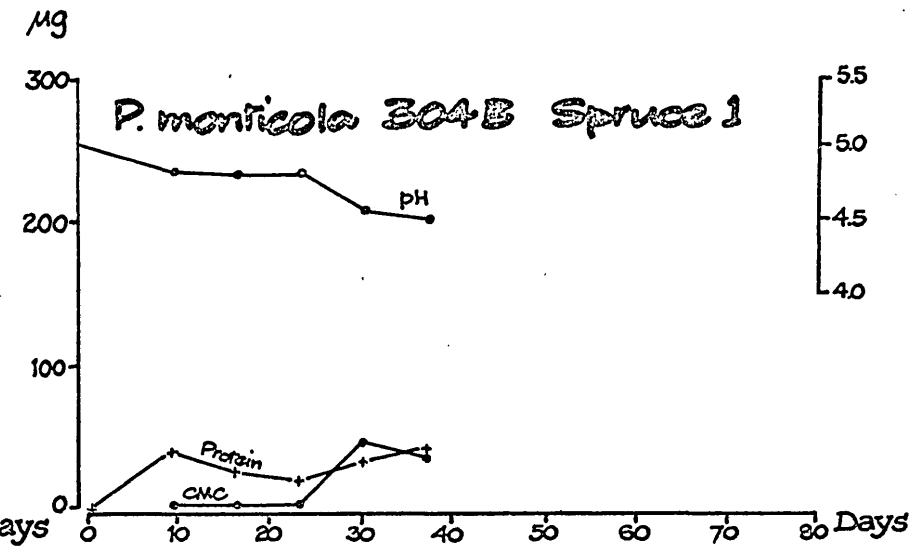

P. fastigiata Bann 1
Birch 2
µg
pH
300
200
100
0
5.5
5.0
4.5
4.0
pH
Protein
CMC
0 10 20 30 40 50 60 70 80
Days

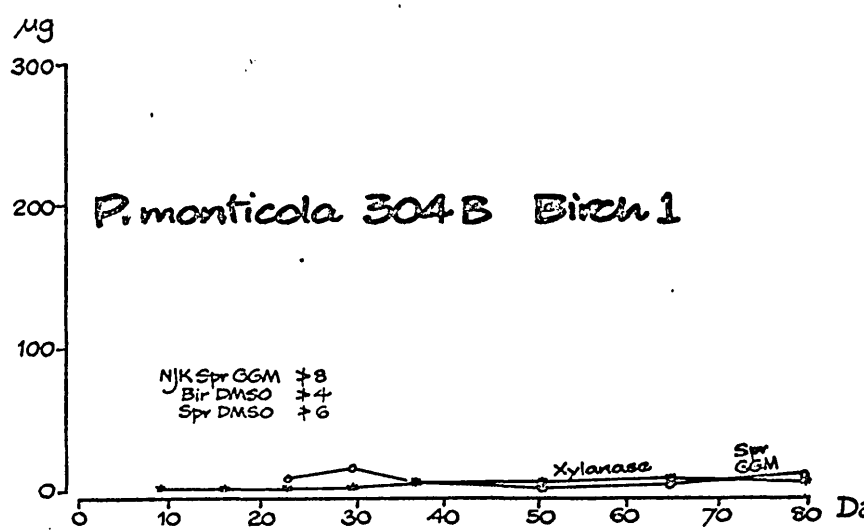

P. fastigiata Bann 1
Spruce 2
µg
300
200
100
0
5.5
5.0
4.5
4.0
pH
Protein
CMC
0 10 20 30 40 50 60 70 80
Days

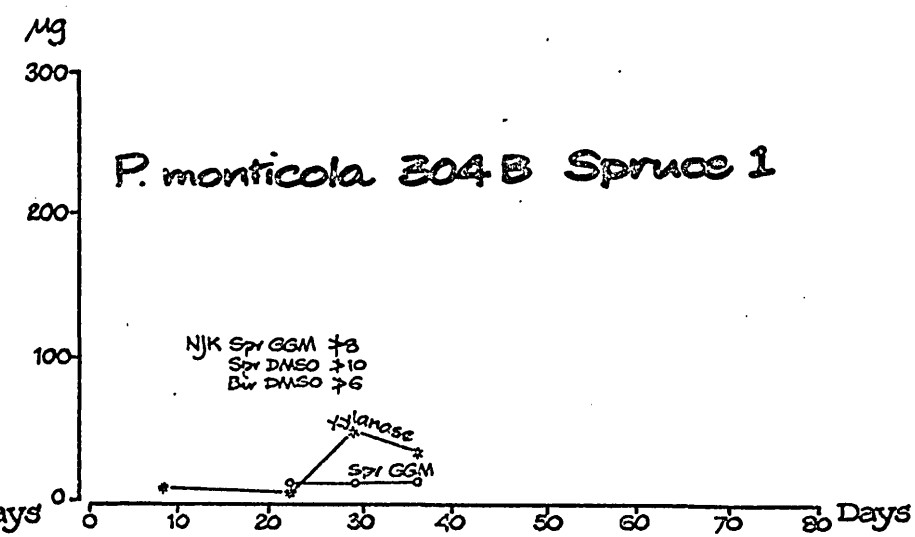

P. fastigiata Bann 1
Birch 2
µg
300
200
100
0
Xylanase ✝5
Bir DMSO ✝12
Spr DMSO ✝13
Spr GGM
N|K Spr GGM
0 10 20 30 40 50 60 70 80
Days

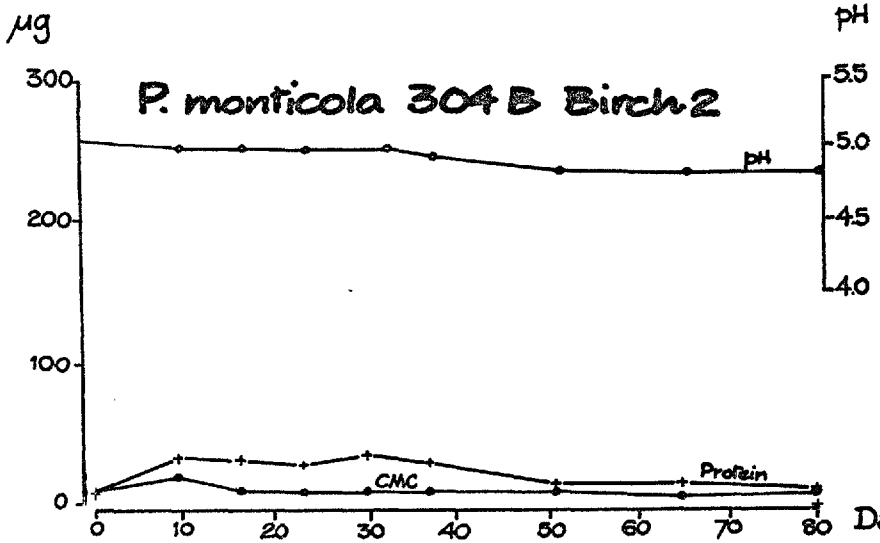

P. fastigiata Bann 1
Spruce 2
µg
300
200
100
0
Xylanase ✝6
Bir DMSO ✝10
Spr DMSO ✝6
Spr GGM
N|K Spr GGM
Spr GGM
0 10 20 30 40 50 60 70 80
Days

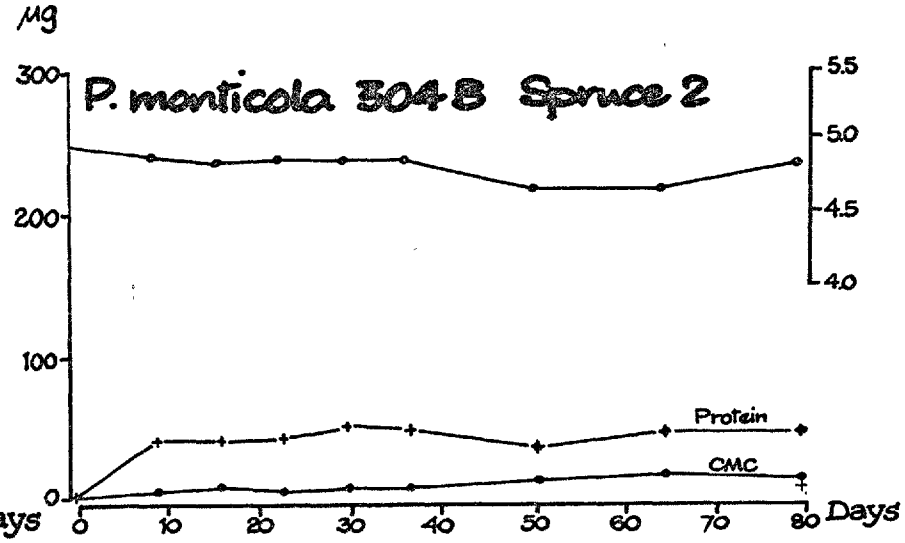

µg
pH
Poria monticola 304B Birch 1
300
200
100
0
5.5
5.0
4.5
4.0
pH
Protein
CMC
0 10 20 30 40 50 60 70 80
Days

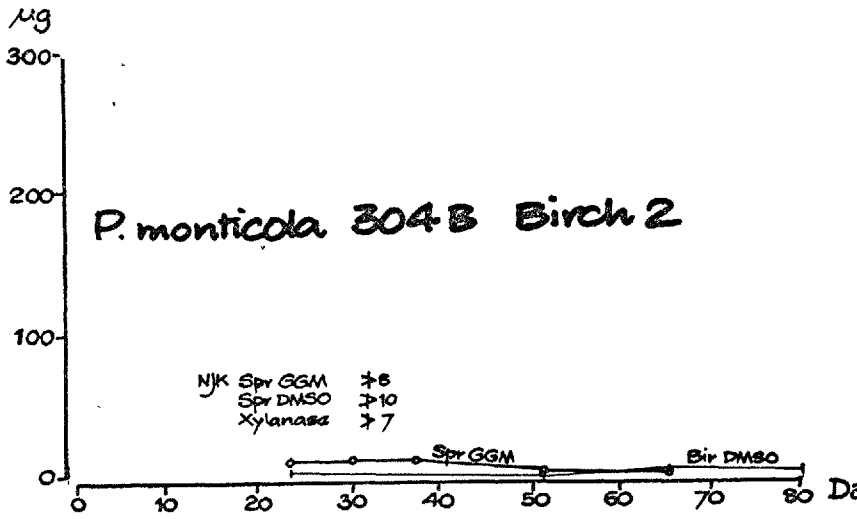

µg
P. monticola 304B Spruce 1
300
200
100
0
5.5
5.0
4.5
4.0
pH
Protein
CMC
0 10 20 30 40 50 60 70 80
Days

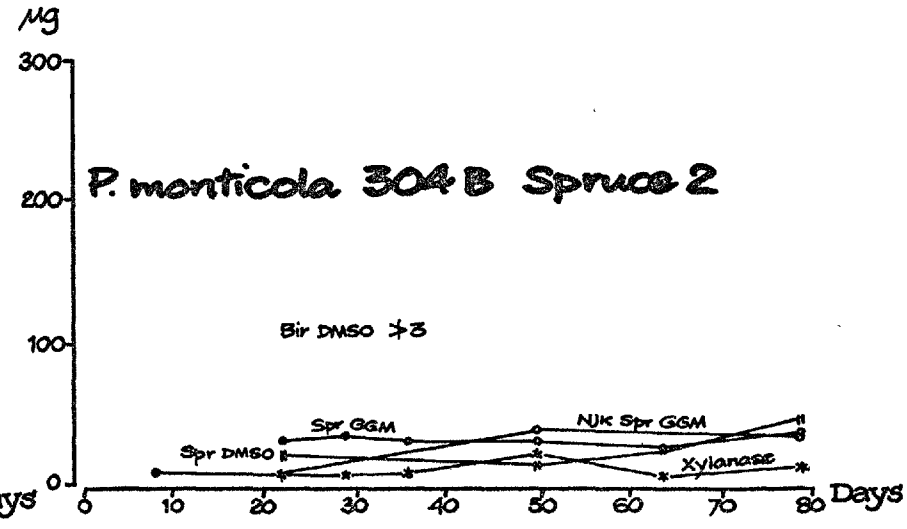

µg
P. monticola 304B Birch 1
300
200
100
0
NJK Spr GGM ⊹8
Bir DMSO ⊹4
Spr DMSO ⊹6
Xylanase
Spr GGM
0 10 20 30 40 50 60 70 80
Days

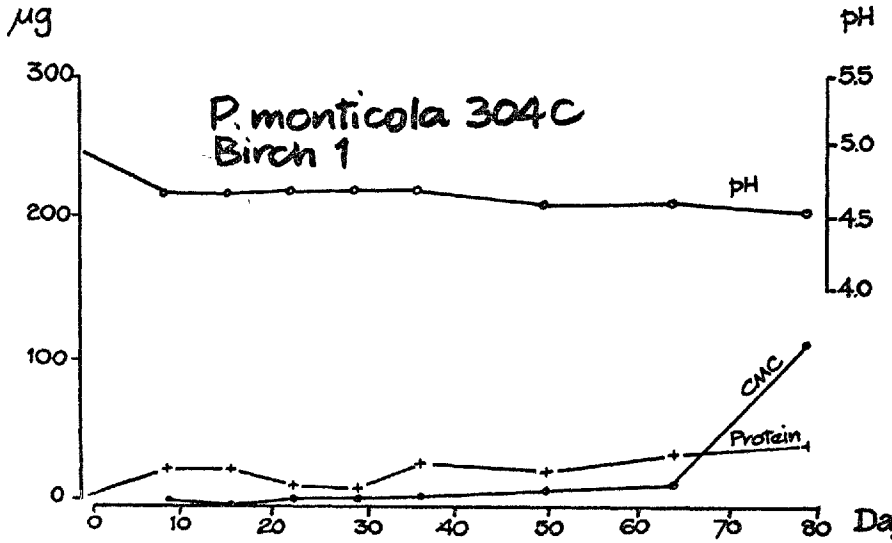

µg
P. monticola 304B Spruce 1
300
200
100
0
NJK Spr GGM ⊹8
Spr DMSO ⊹10
Bir DMSO ⊹6
Xylanase
Spr GGM
0 10 20 30 40 50 60 70 80
Days

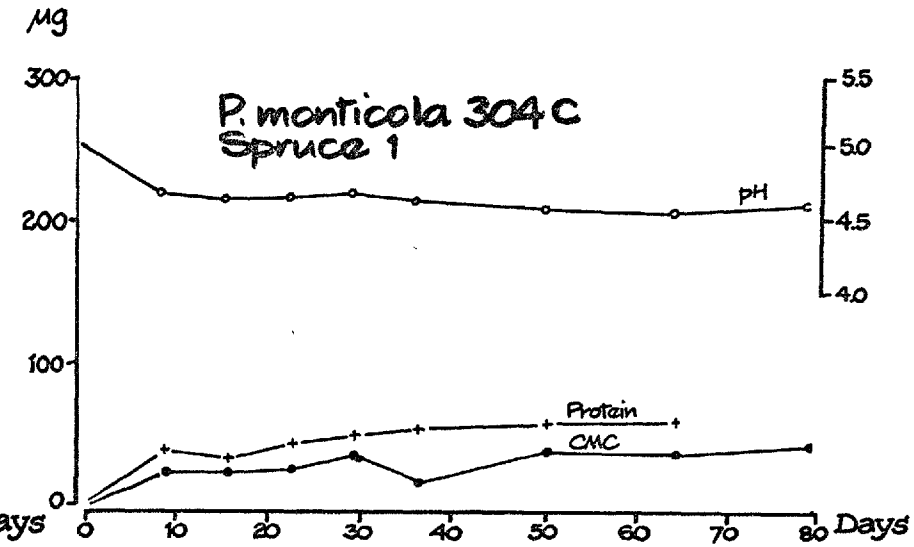

µg
pH
P. monticola 304 B Birch 2
300
200
100
0
5.5
5.0
4.5
4.0
pH
CMC
Protein
0 10 20 30 40 50 60 70 80
Days

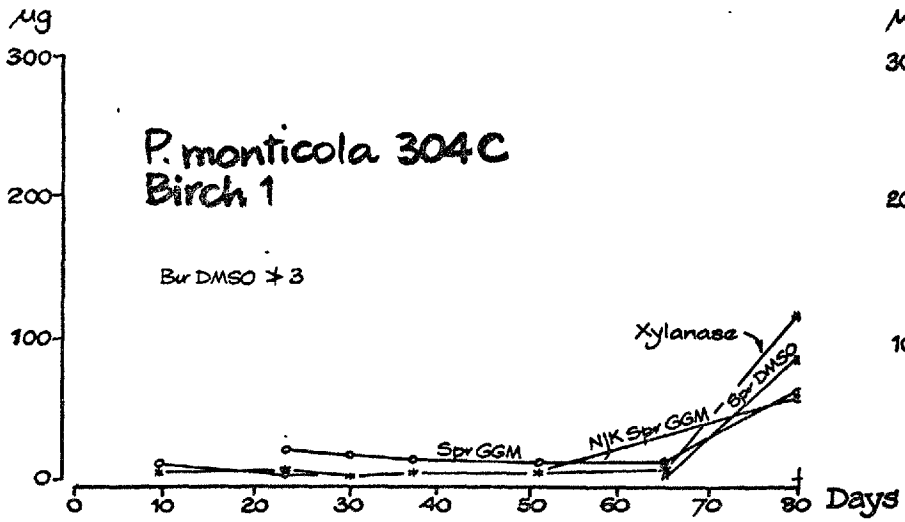

µg
P. monticola 304 B Spruce 2
300
200
100
0
5.5
5.0
4.5
4.0
Protein
CMC
0 10 20 30 40 50 60 70 80
Days

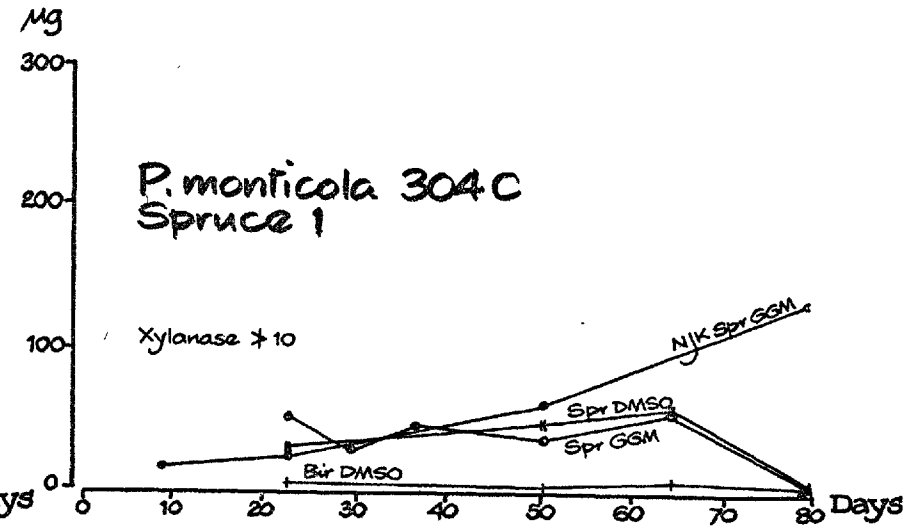

µg
P. monticola 304 B Birch 2
300
200
100
0
NJK Spr GGM
Spr DMSO
Xylanase
Spr GGM
Bir DMSO
0 10 20 30 40 50 60 70 80
Days

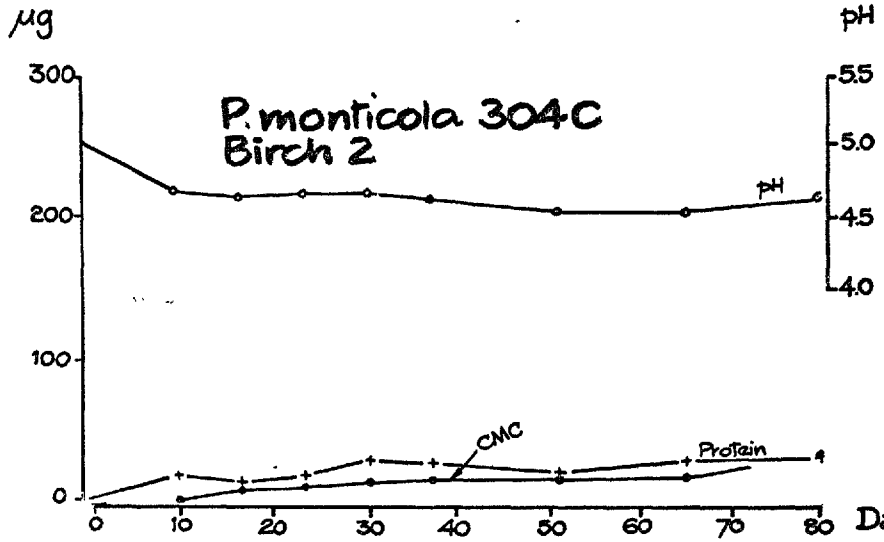

µg
P. monticola 304 B Spruce 2
300
200
100
0
Bir DMSO
Spr GGM
NJK Spr GGM
Spr DMSO
Xylanase
0 10 20 30 40 50 60 70 80
Days

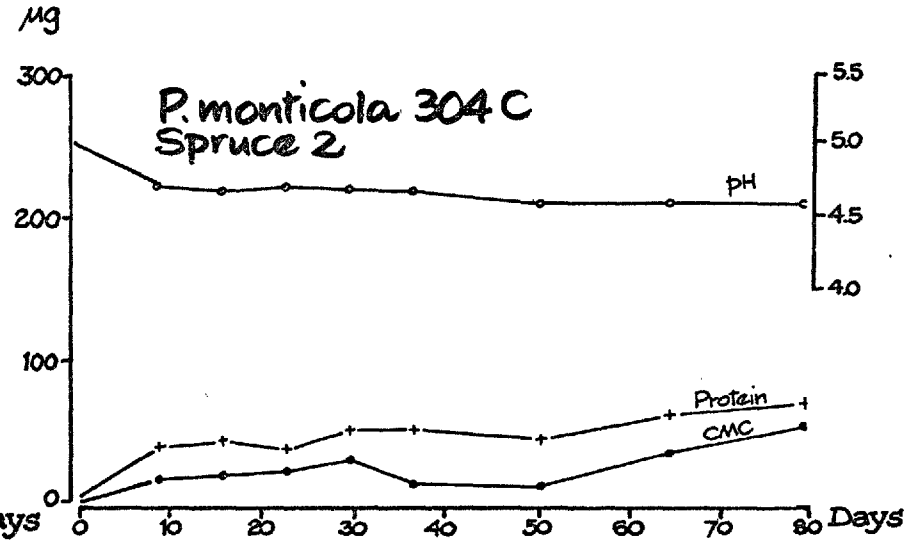

µg
pH
300
200
100
0
5.5
5.0
4.5
4.0
P. monticola 304C
Birch 1
pH
CMC
Protein
0 10 20 30 40 50 60 70 80
Days

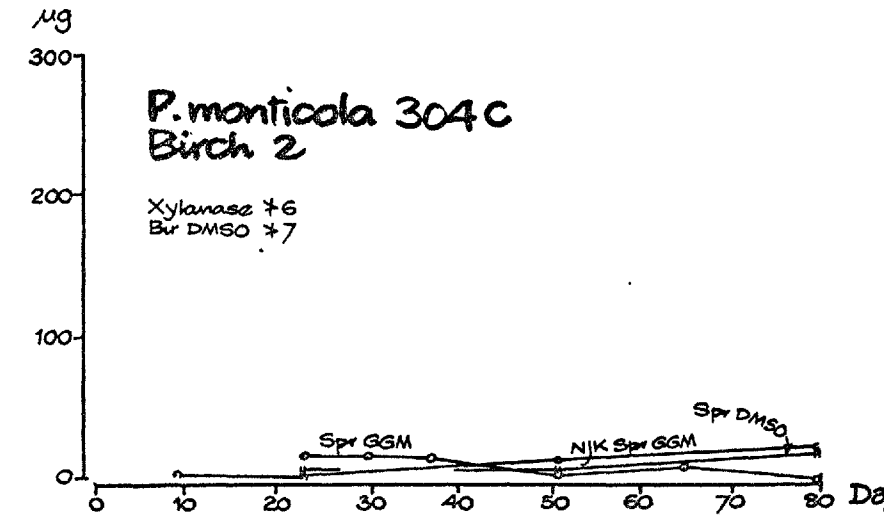

µg
300
200
100
0
5.5
5.0
4.5
4.0
P. monticola 304C
Spruce 1
pH
Protein
CMC
0 10 20 30 40 50 60 70 80
Days

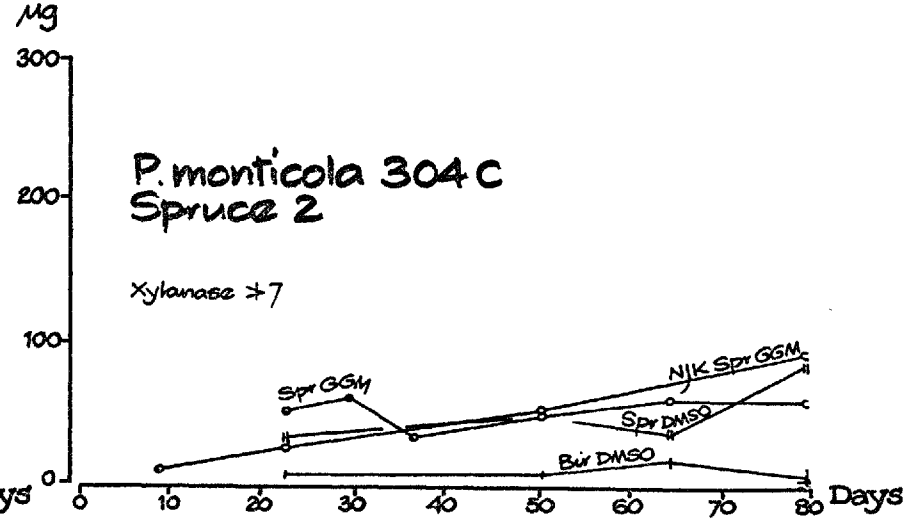

µg
300
200
100
0
P. monticola 304C
Birch 1
Bir DMSO * 3
Xylanase
N/K Spr GGM - Spr DMSO
Spr GGM
0 10 20 30 40 50 60 70 80
Days

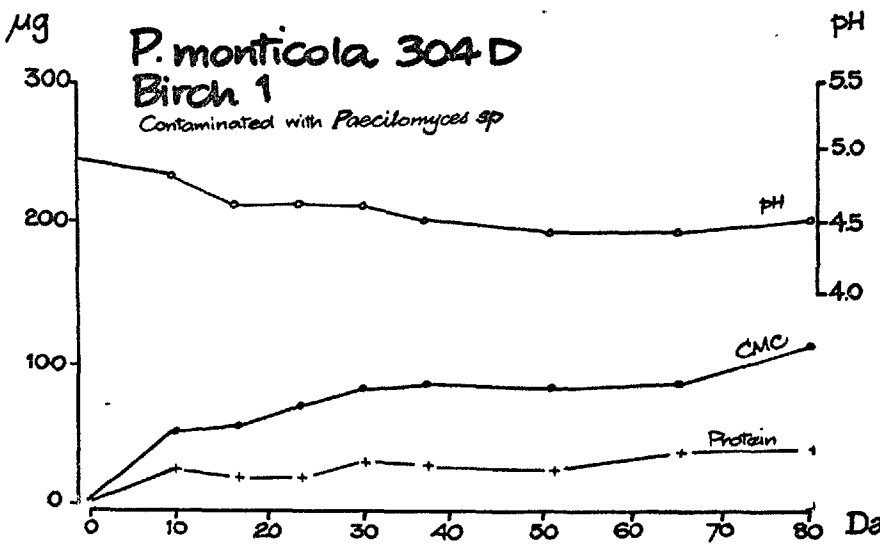

µg
300
200
100
0
P. monticola 304C
Spruce 1
Xylanase * 10
N/K Spr GGM
Spr DMSO
Spr GGM
Bir DMSO
0 10 20 30 40 50 60 70 80
Days

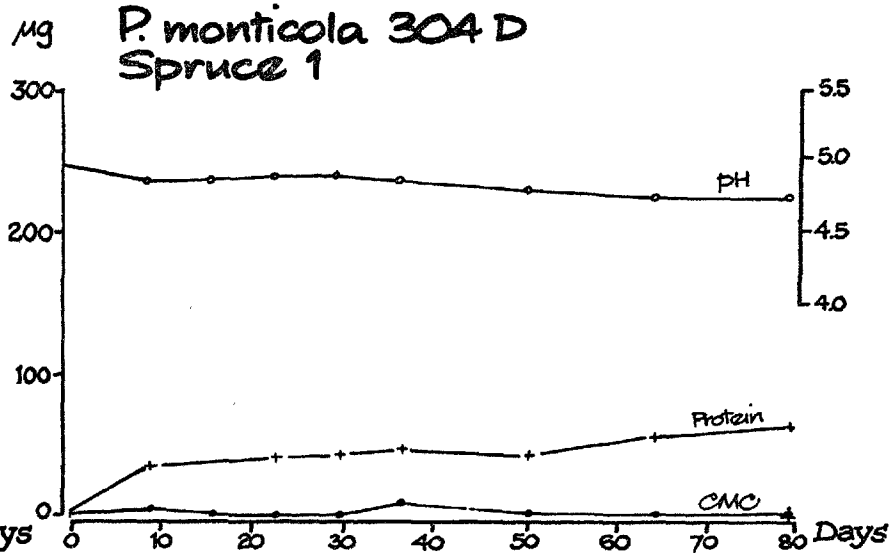

µg
pH
P. monticola 304C
Birch 2
300
200
100
0
5.5
5.0
4.5
4.0
pH
CMC
Protein
0 10 20 30 40 50 60 70 80
Days

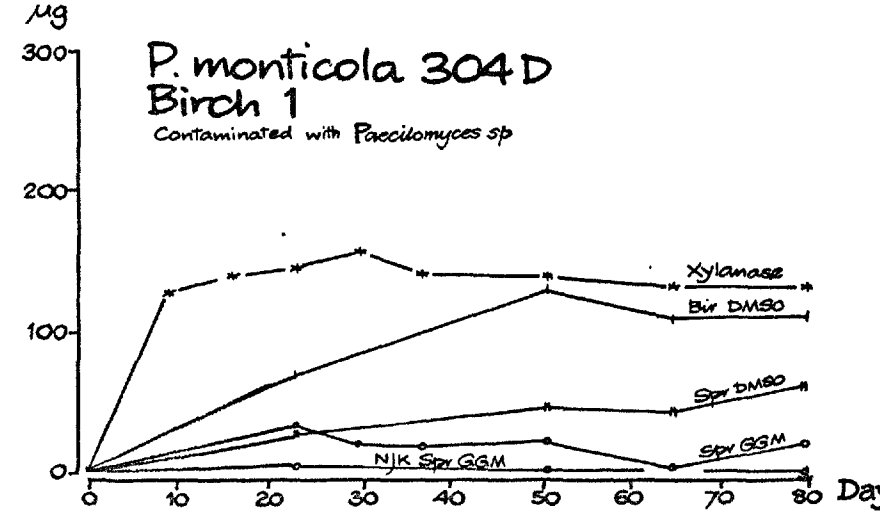

µg
P. monticola 304C
Spruce 2
300
200
100
0
5.5
5.0
4.5
4.0
pH
Protein
CMC
0 10 20 30 40 50 60 70 80
Days

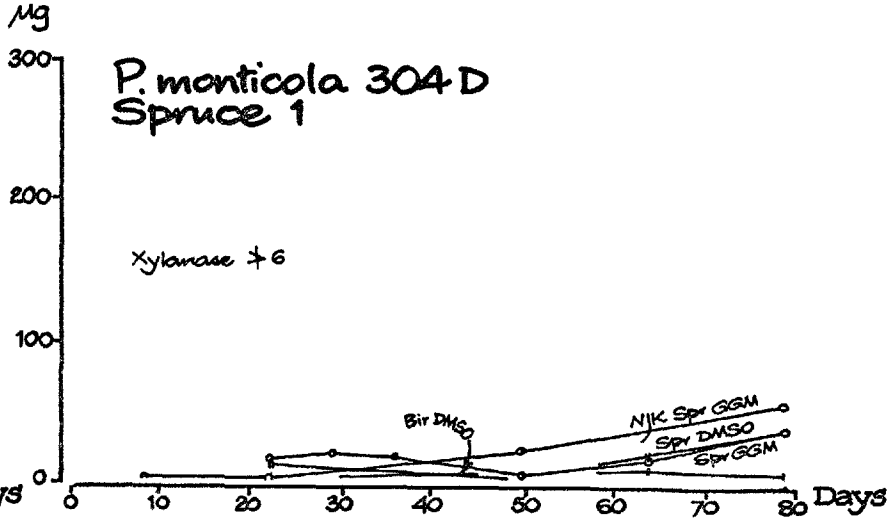

µg
P. monticola 304C
Birch 2
Xylanase ×6
Bir DMSO ×7
300
200
100
0
Spr GGM
NJK Spr GGM
Spr DMSO
0 10 20 30 40 50 60 70 80
Days

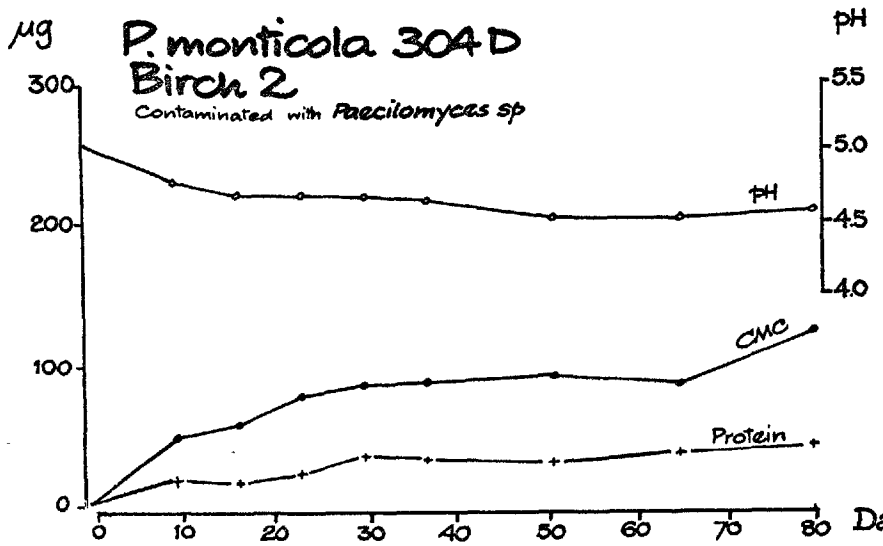

µg
P. monticola 304C
Spruce 2
Xylanase ×7
300
200
100
0
Spr GGM
NJK Spr GGM
Spr DMSO
Bir DMSO
0 10 20 30 40 50 60 70 80
Days

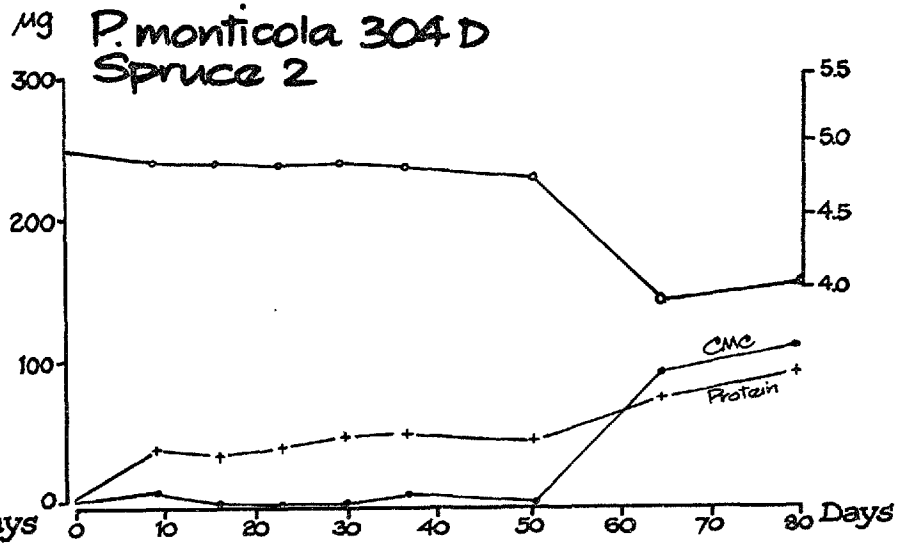

µg
pH
P. monticola 304 D
Birch 1
Contaminated with Paecilomyces sp
300
200
100
0
5.5
5.0
4.5
4.0
pH
CMC
Protein
0 10 20 30 40 50 60 70 80
Days

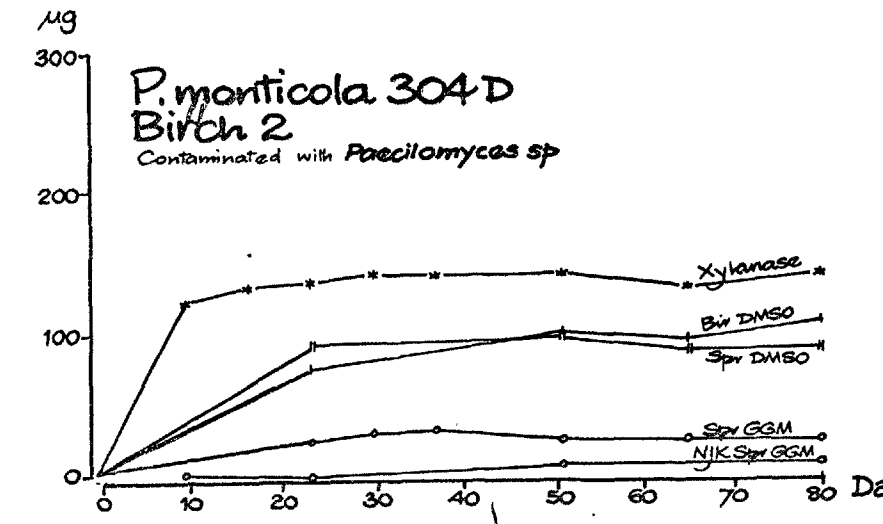

µg
P. monticola 304 D
Spruce 1
300
200
100
0
5.5
5.0
4.5
4.0
pH
Protein
CMC
0 10 20 30 40 50 60 70 80
Days

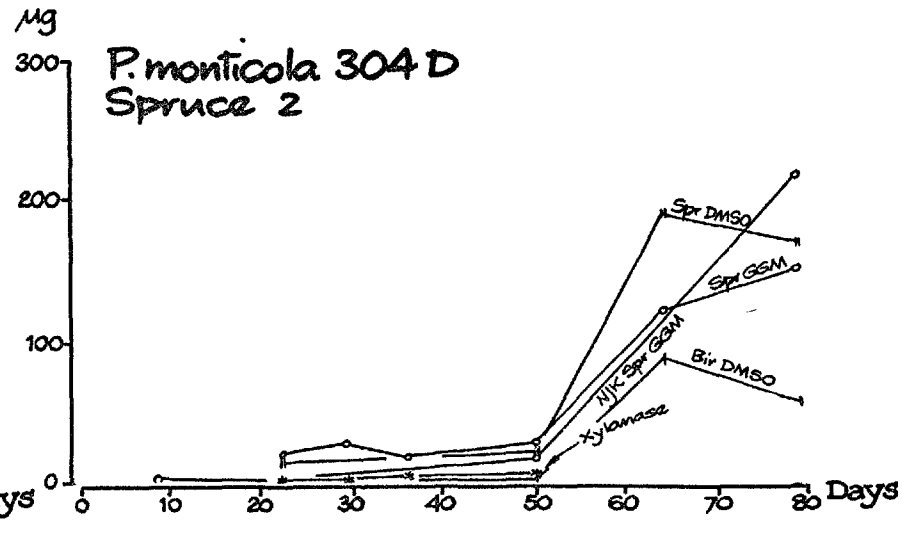

µg
P. monticola 304 D
Birch 1
Contaminated with Paecilomyces sp
300
200
100
0
Xylanase
Bir DMSO
Spr DMSO
Spr GGM
NJK Spr GGM
0 10 20 30 40 50 60 70 80
Days

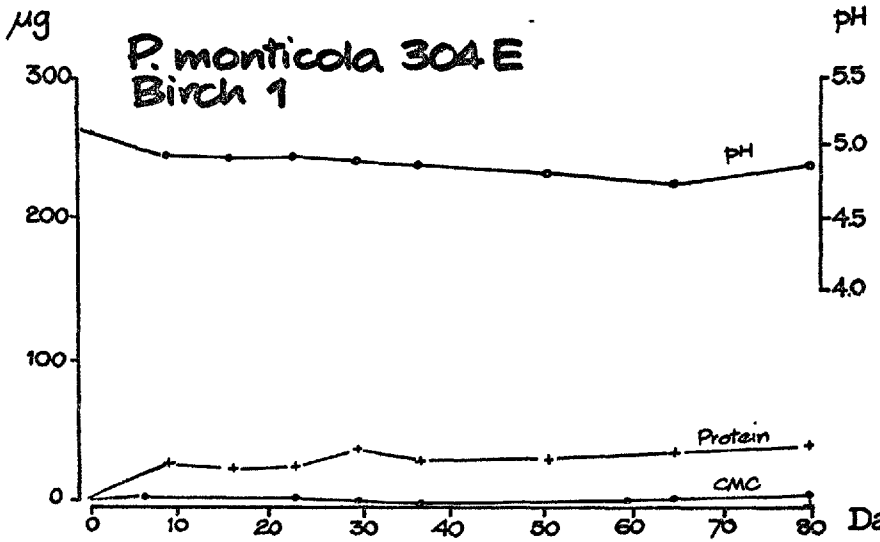

µg
P. monticola 304 D
Spruce 1
300
200
100
0
Xylanase ÷ 6
Bir DMSO
NJK Spr GGM
Spr DMSO
Spr GGM
0 10 20 30 40 50 60 70 80
Days

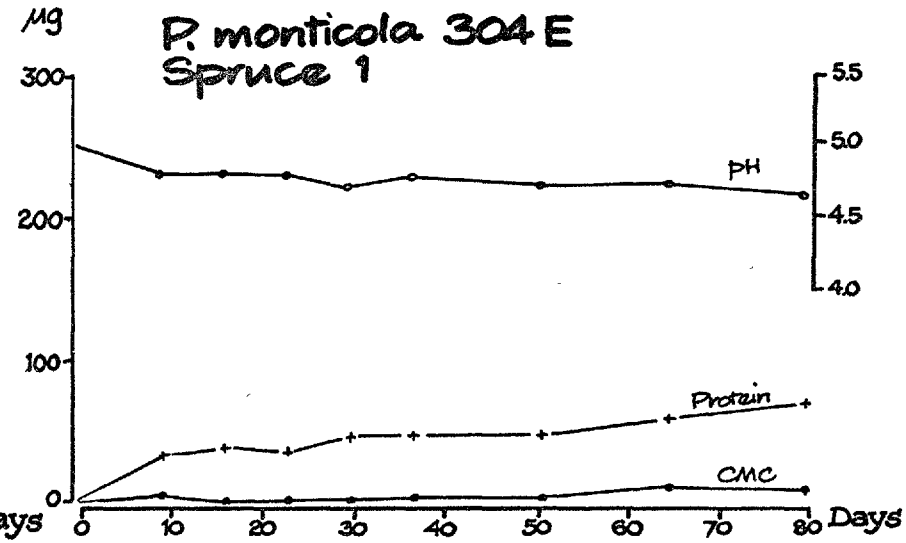

µg
P. monticola 304 D
Birch 2
Contaminated with Paecilomyces sp
pH
300
200
100
0
5.5
5.0
4.5
4.0
pH
CMC
Protein
0 10 20 30 40 50 60 70 80
Days

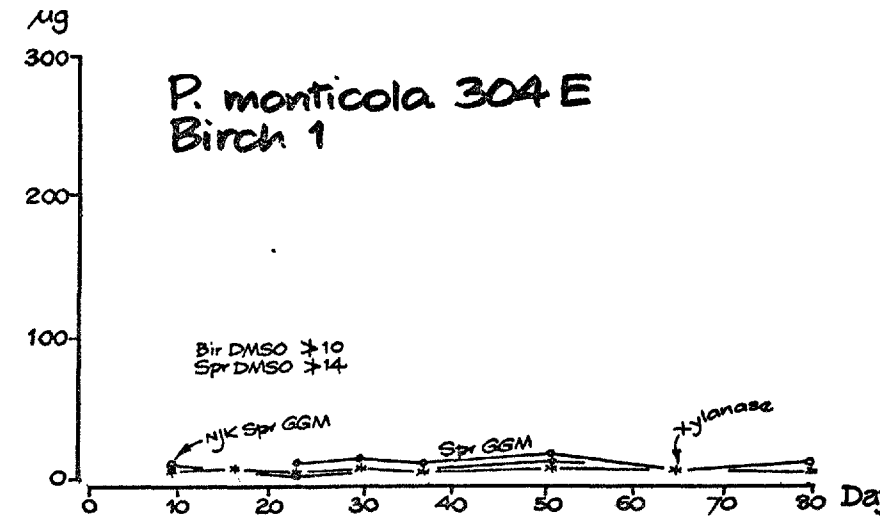

µg
P. monticola 304 D
Spruce 2
300
200
100
0
5.5
5.0
4.5
4.0
CMC
Protein
0 10 20 30 40 50 60 70 80
Days

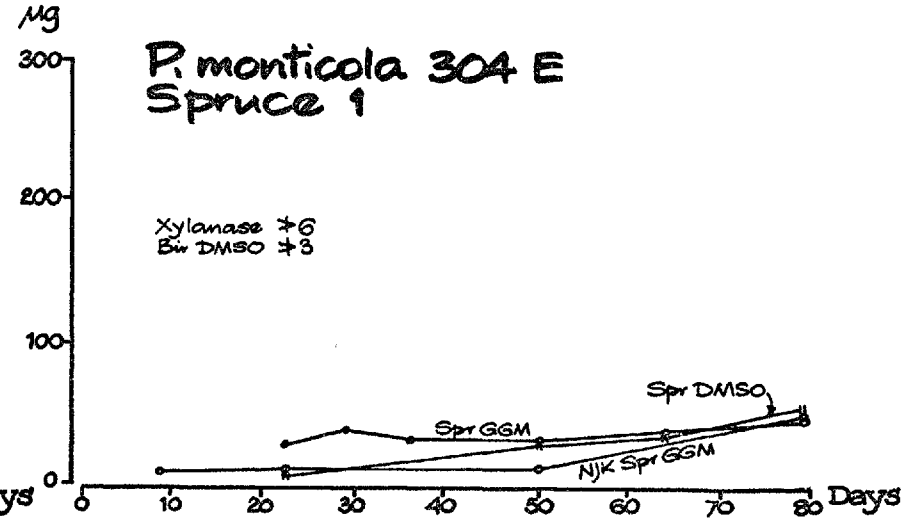

µg
P. monticola 304 D
Birch 2
Contaminated with Paecilomyces sp
300
200
100
0
Xylanase
Bir DMSO
Spr DMSO
Spr GGM
NJK Spr GGM
0 10 20 30 40 50 60 70 80
Days

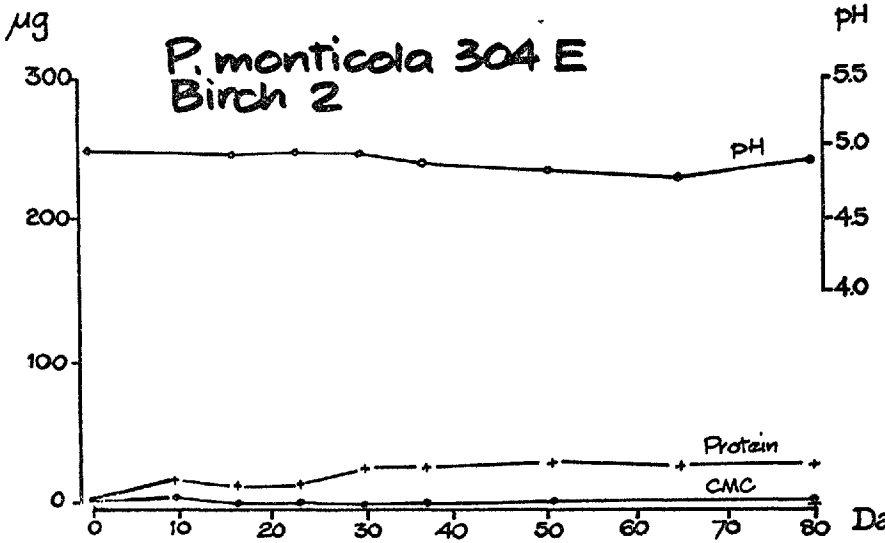

µg
P. monticola 304 D
Spruce 2
300
200
100
0
Spr DMSO
Spr GGM
NJK Spr GGM
Bir DMSO
Xylanase
0 10 20 30 40 50 60 70 80
Days

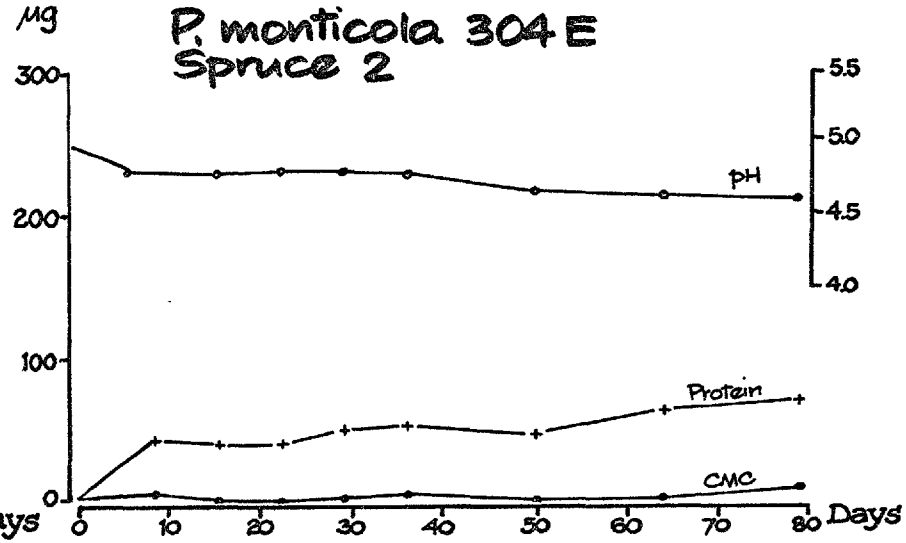

µg
pH
P. monticola 304 E
Birch 1
300
200
100
0
5.5
5.0
4.5
4.0
pH
Protein
CMC
0 10 20 30 40 50 60 70 80
Days

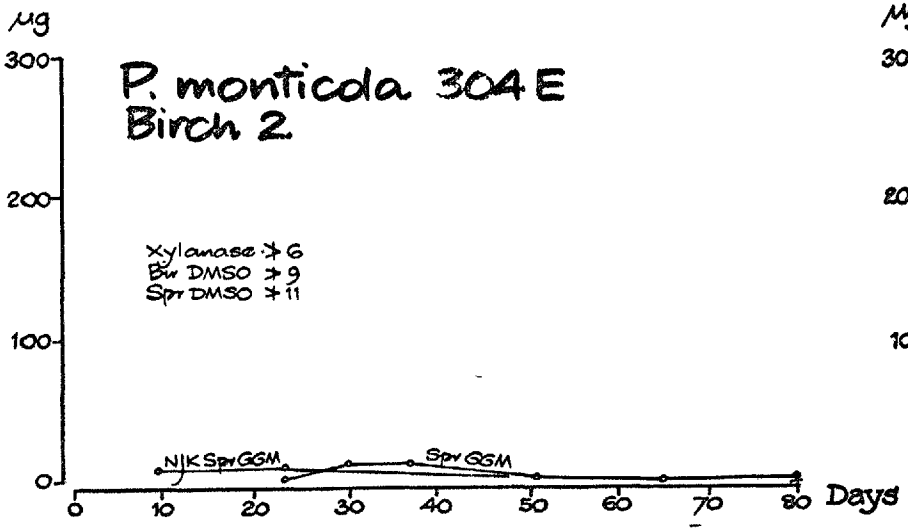

µg
P. monticola 304 E
Spruce 1
300
200
100
0
5.5
5.0
4.5
4.0
pH
Protein
CMC
0 10 20 30 40 50 60 70 80
Days

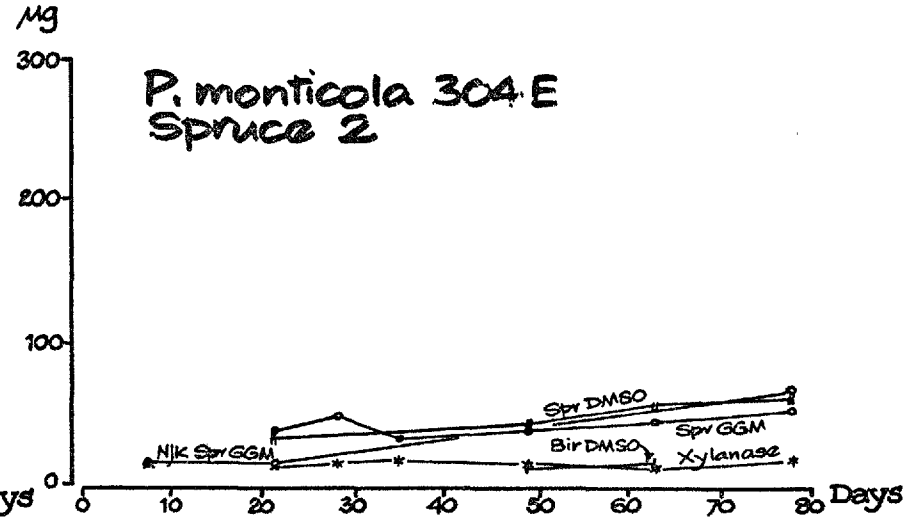

µg
P. monticola 304 E
Birch 1
300
200
100
0
Bir DMSO ✳10
Spr DMSO ✳14
NJK Spr GGM
Spr GGM
✳ylanase
0 10 20 30 40 50 60 70 80
Days

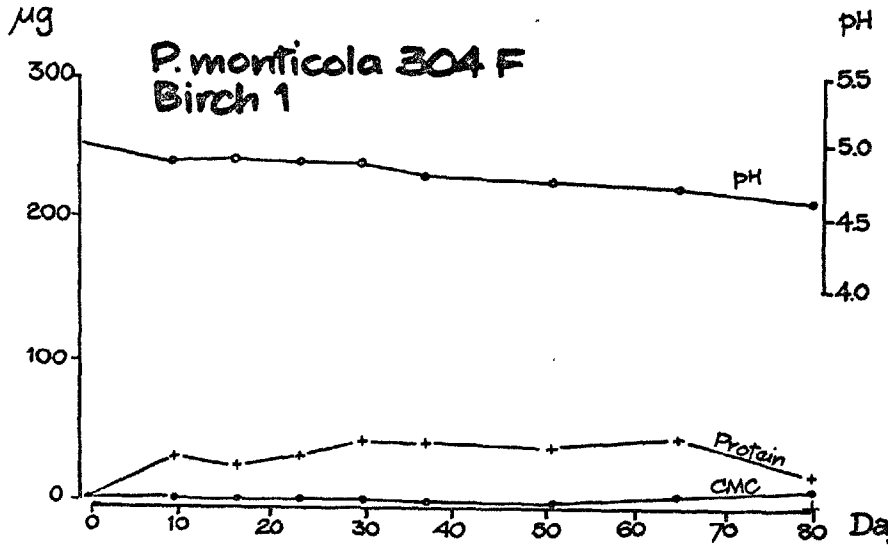

µg
P. monticola 304 E
Spruce 1
300
200
100
0
Xylanase ✳6
Bir DMSO ✳3
Spr DMSO
Spr GGM
NJK Spr GGM
0 10 20 30 40 50 60 70 80
Days

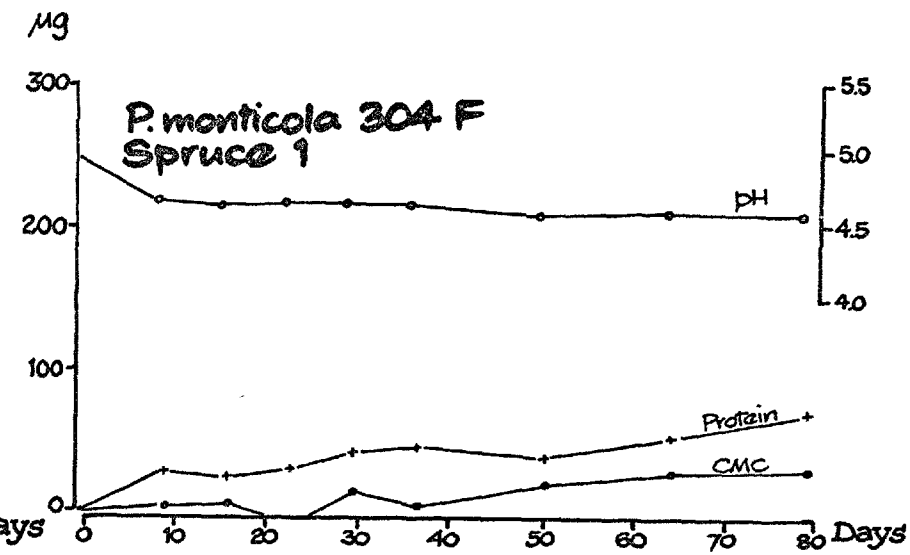

µg
pH
P. monticola 304 E
Birch 2
300
200
100
0
5.5
5.0
4.5
4.0
pH
Protein
CMC
0 10 20 30 40 50 60 70 80
Days

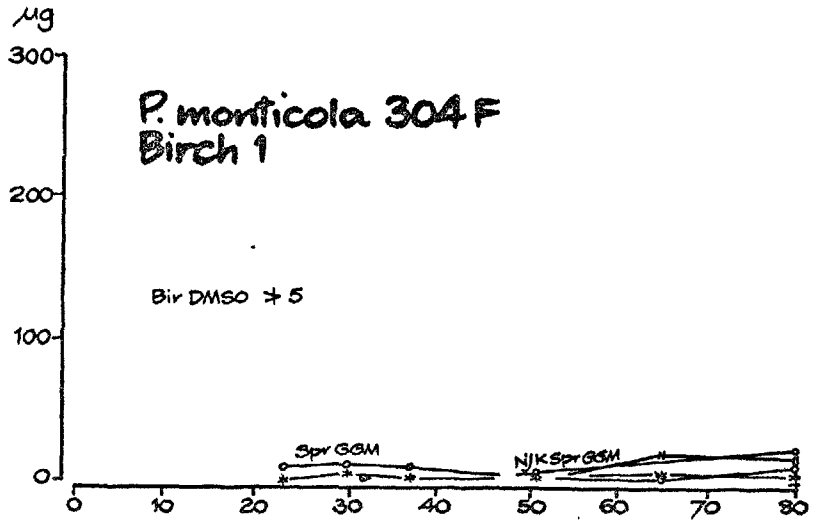

µg
P. monticola 304 E
Spruce 2
300
200
100
0
5.5
5.0
4.5
4.0
pH
Protein
CMC
0 10 20 30 40 50 60 70 80
Days

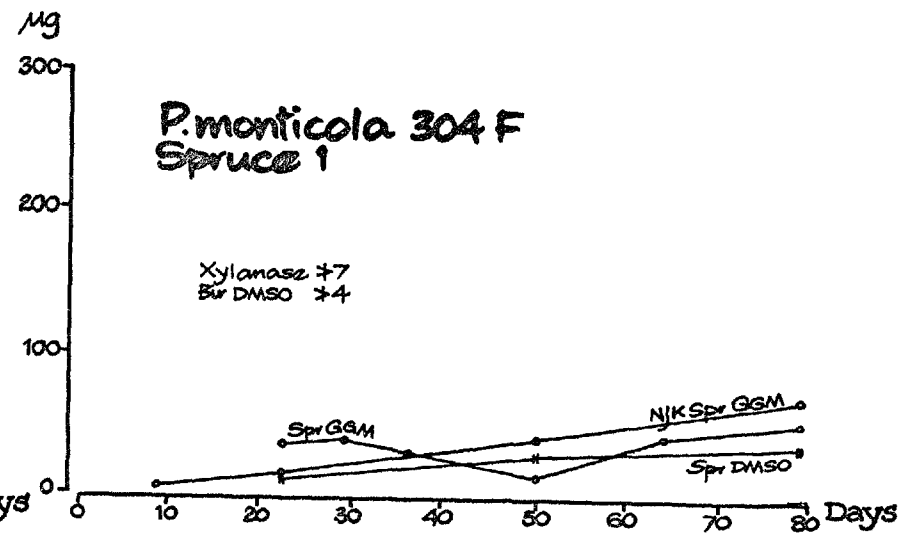

µg
P. monticola 304 E
Birch 2
300
200
100
0
Xylanase → 6
Bir DMSO → 9
Spr DMSO → 11
N|K Spr GGM
Spr GGM
0 10 20 30 40 50 60 70 80
Days

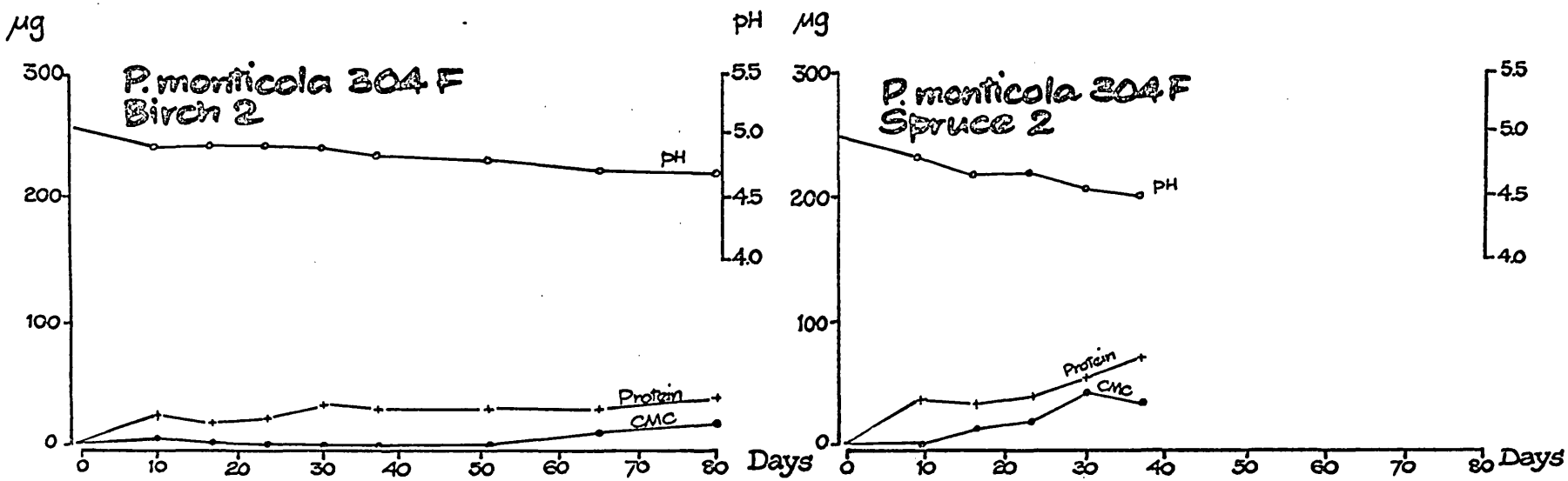

µg
P. monticola 304 E
Spruce 2
300
200
100
0
Spr DMSO
Spr GGM
Bir DMSO
Xylanase
N|K Spr GGM
0 10 20 30 40 50 60 70 80
Days


µg
pH
P. monticola 304 F
Birch 1
300
200
100
0
5.5
5.0
4.5
4.0
pH
Protein
CMC
0 10 20 30 40 50 60 70 80
Days

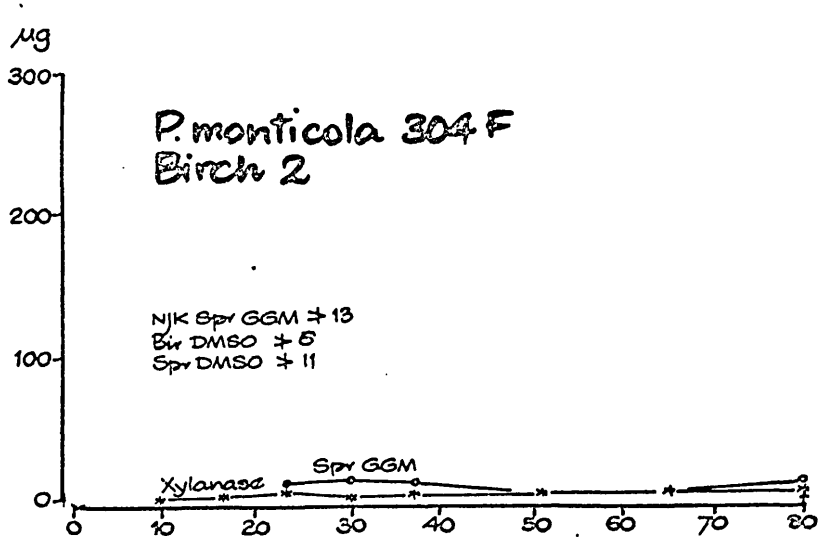

µg
P. monticola 304 F
Spruce 1
300
200
100
0
5.5
5.0
4.5
4.0
pH
Protein
CMC
0 10 20 30 40 50 60 70 80
Days

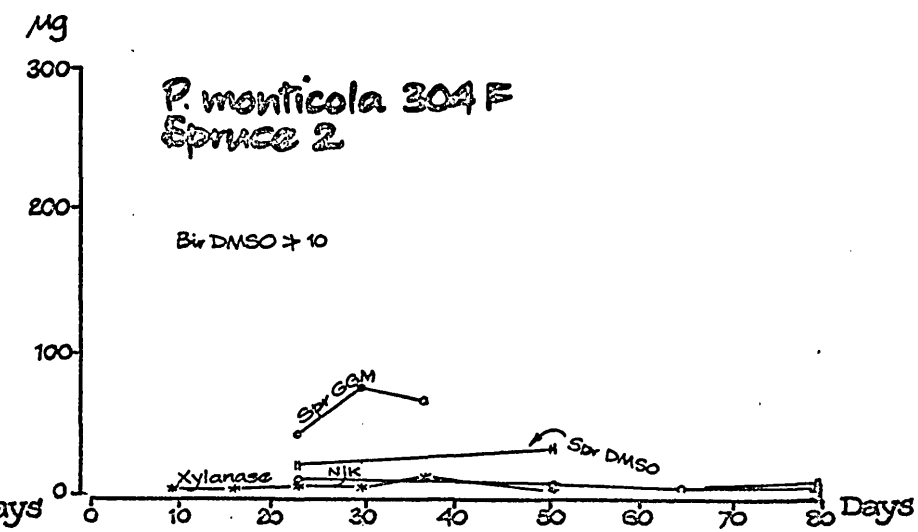

µg
P. monticola 304 F
Birch 1
300
200
100
0
Bir DMSO ✳5
Spr GGM
N/K Spr GGM
0 10 20 30 40 50 60 70 80
Days

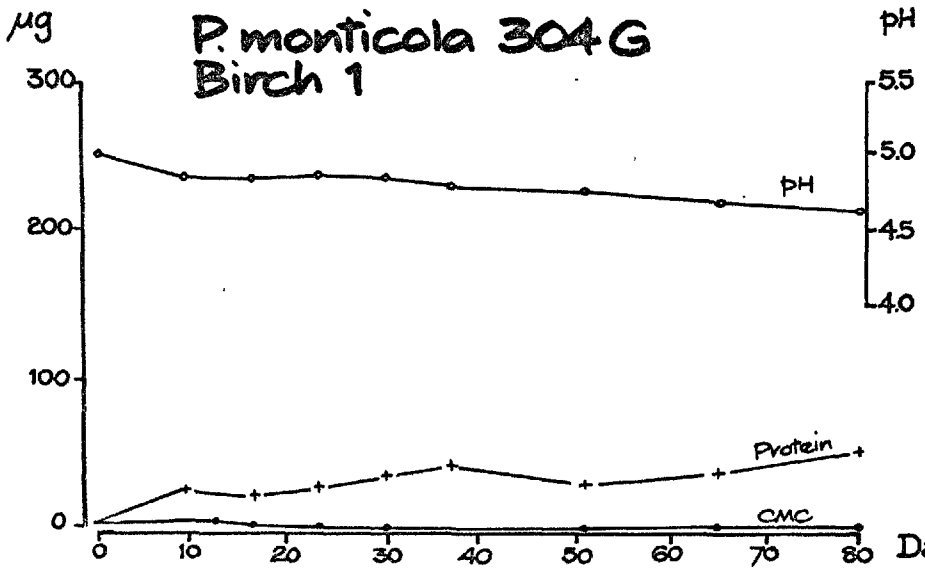

µg
P. monticola 304 F
Spruce 1
300
200
100
0
Xylanase ✳7
Bir DMSO ✳4
Spr GGM
N/K Spr GGM
Spr DMSO
0 10 20 30 40 50 60 70 80
Days

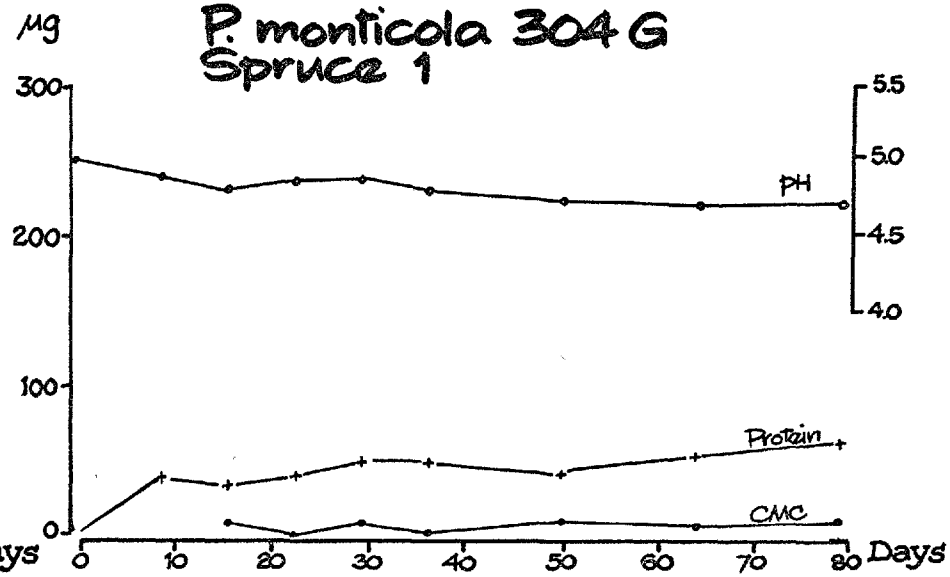

µg
pH
P. monticola 304 F
Birch 2
300
200
100
0
5.5
5.0
4.5
4.0
pH
Protein
CMC
0 10 20 30 40 50 60 70 80
Days

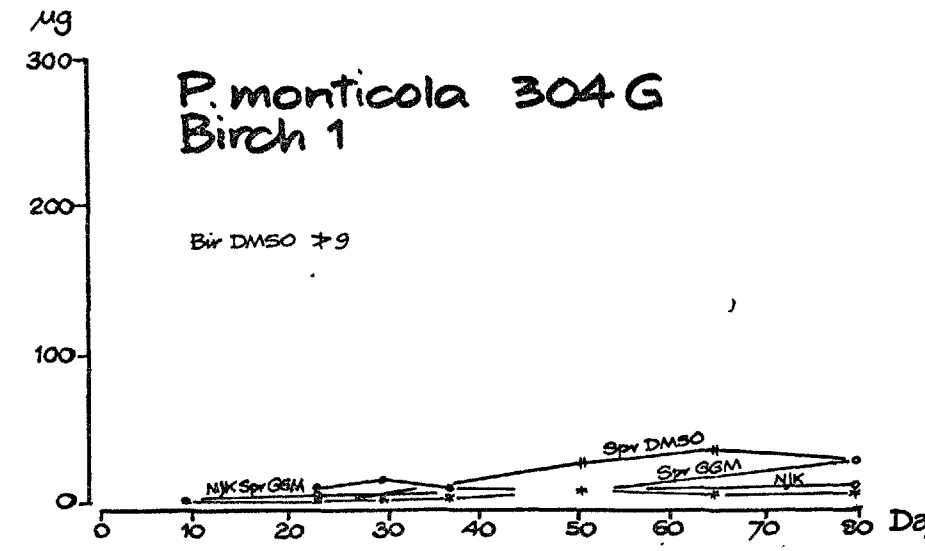
µg
P. monticola 304 F
Spruce 2
300
200
100
0
5.5
5.0
4.5
4.0
pH
Protein
CMC
0 10 20 30 40 50 60 70 80
Days

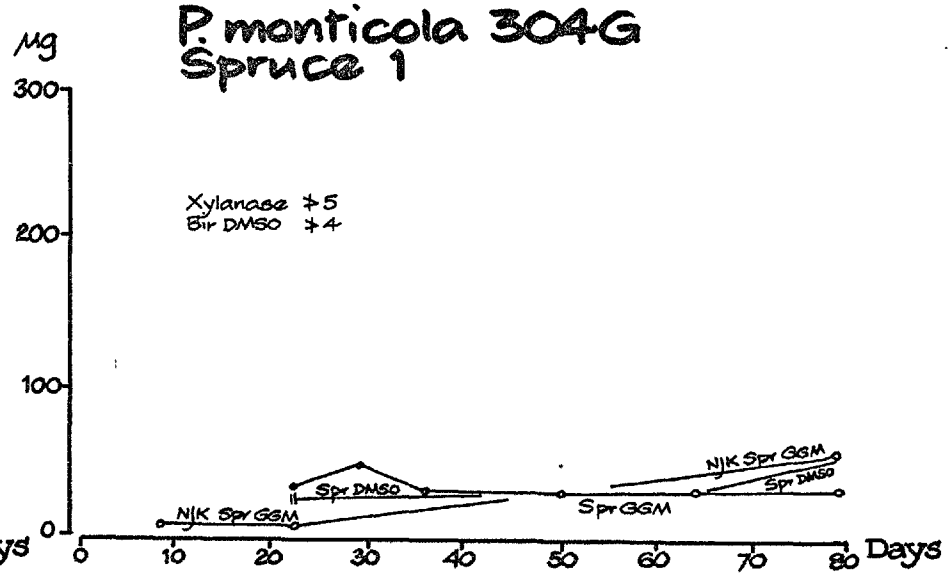

µg
P. monticola 304 F
Birch 2
300
200
100
0
NJK Spr GGM ± 13
Bir DMSO ± 6
Spr DMSO ± 11
Xylanase
Spr GGM
0 10 20 30 40 50 60 70 80
Days

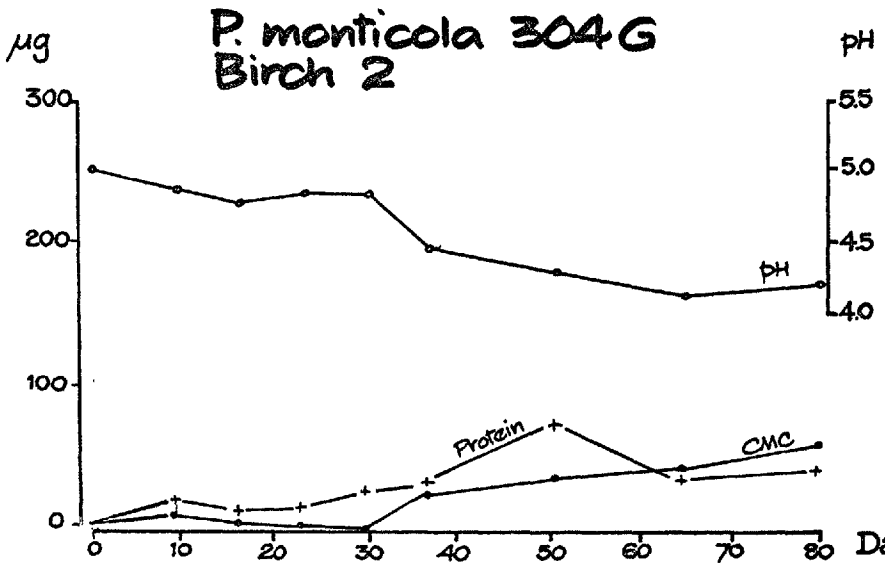

µg
P. monticola 304 F
Spruce 2
300
200
100
0
Bir DMSO ± 10
Spr GGM
Spr DMSO
NJK
Xylanase
0 10 20 30 40 50 60 70 80
Days

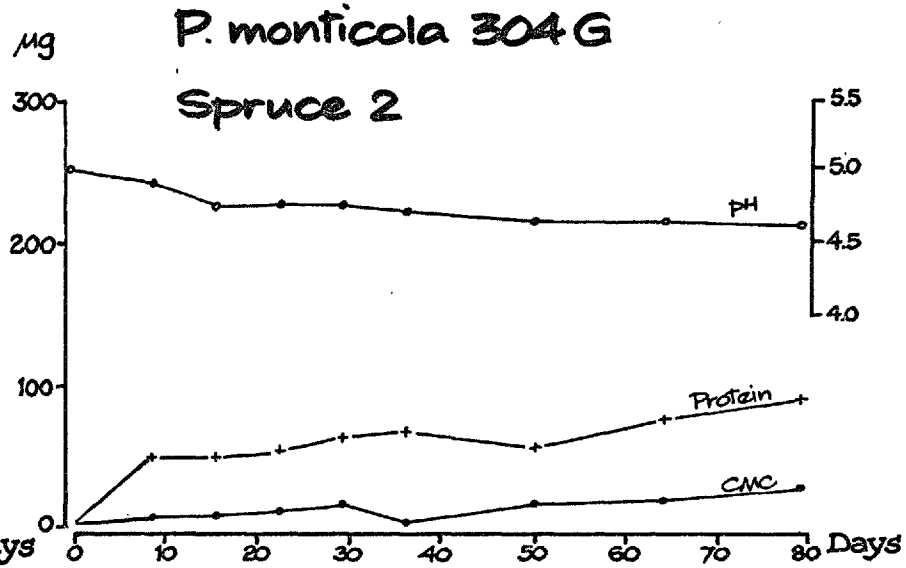

µg
P. monticola 304 G
Birch 1
pH
300
200
100
0
5.5
5.0
4.5
4.0
pH
Protein
CMC
0 10 20 30 40 50 60 70 80
Days

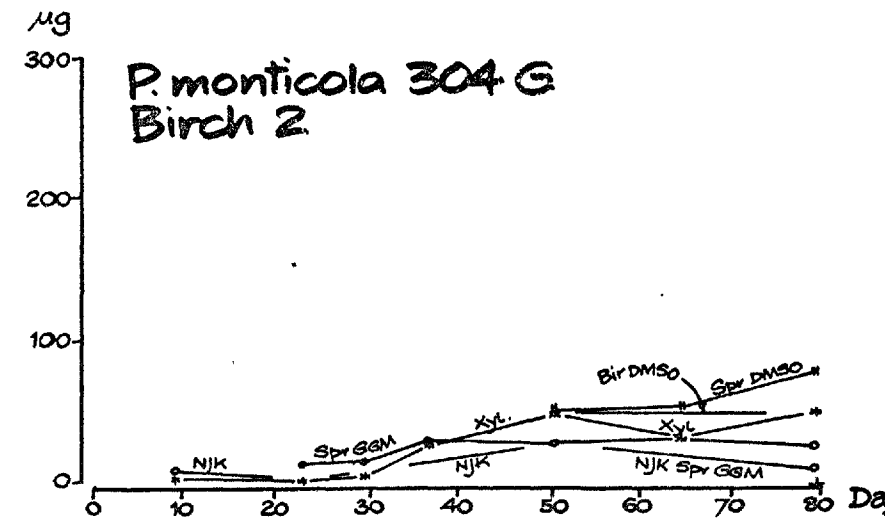

µg
P. monticola 304 G
Spruce 1
300
200
100
0
5.5
5.0
4.5
4.0
pH
Protein
CMC
0 10 20 30 40 50 60 70 80
Days

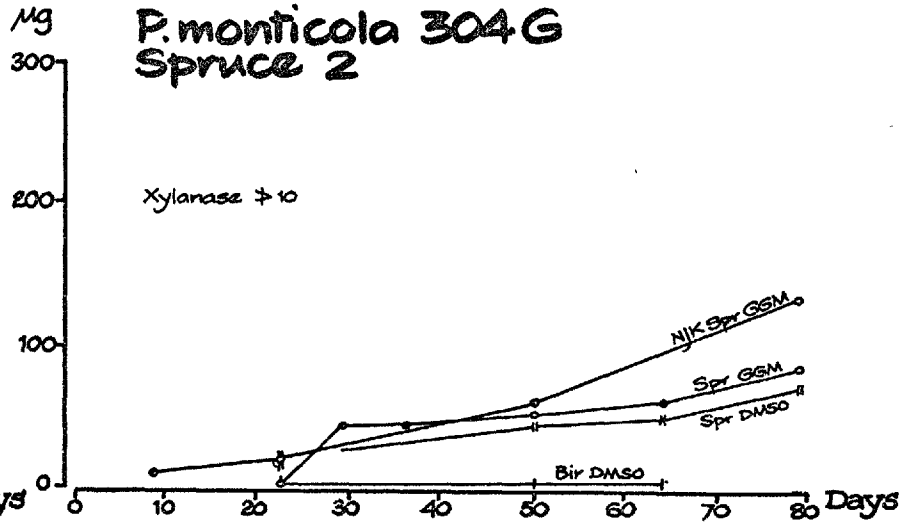

µg
P. monticola 304 G
Birch 1
Bir DMSO ∓9
300
200
100
0
Spr DMSO
Spr GGM
NJK Spr GGM
NJK
0 10 20 30 40 50 60 70 80
Days

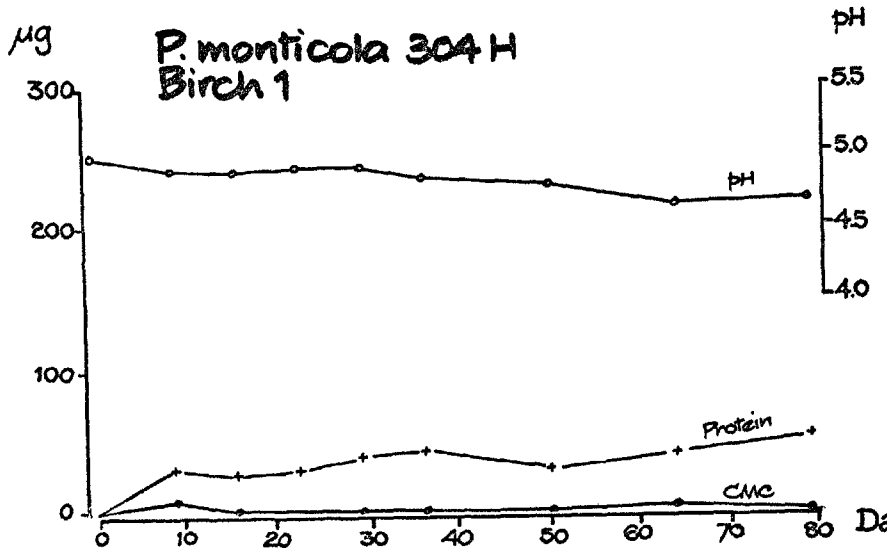

µg
P. monticola 304G
Spruce 1
Xylanase ∓5
Bir DMSO ∓4
300
200
100
0
Spr DMSO
NJK Spr GGM
Spr GGM
NJK Spr GGM
Spr DMSO
0 10 20 30 40 50 60 70 80
Days

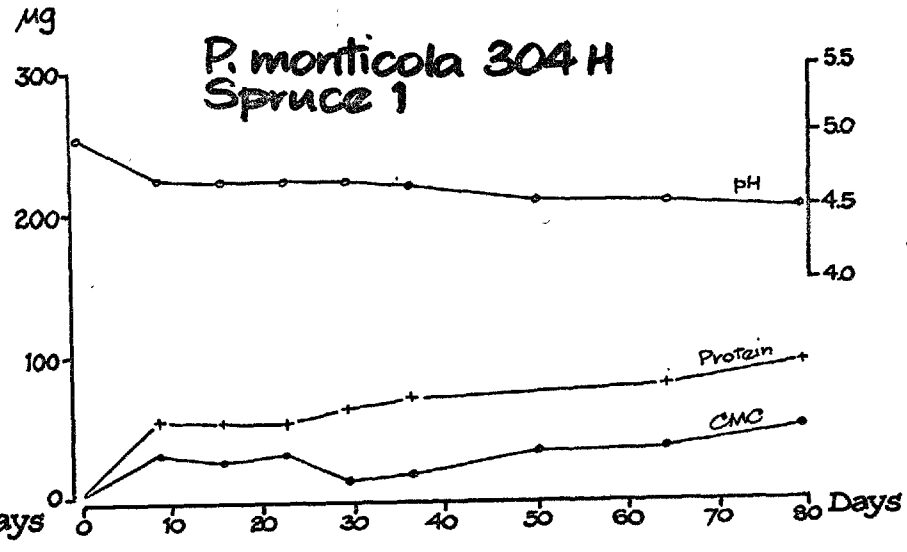

P. monticola 304 G
Birch 2
µg
pH
300
200
100
0
5.5
5.0
4.5
4.0
pH
Protein
CMC
0 10 20 30 40 50 60 70 80
Days

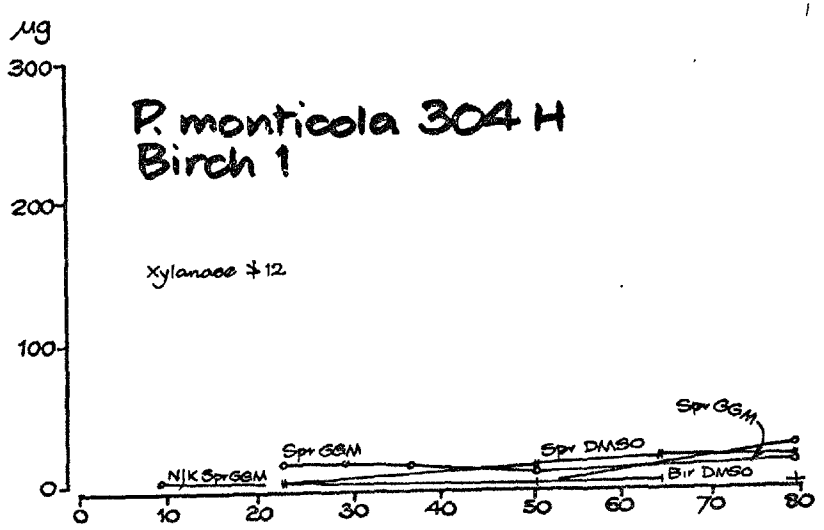

P. monticola 304 G
Spruce 2
µg
300
200
100
0
5.5
5.0
4.5
4.0
pH
Protein
CMC
0 10 20 30 40 50 60 70 80
Days

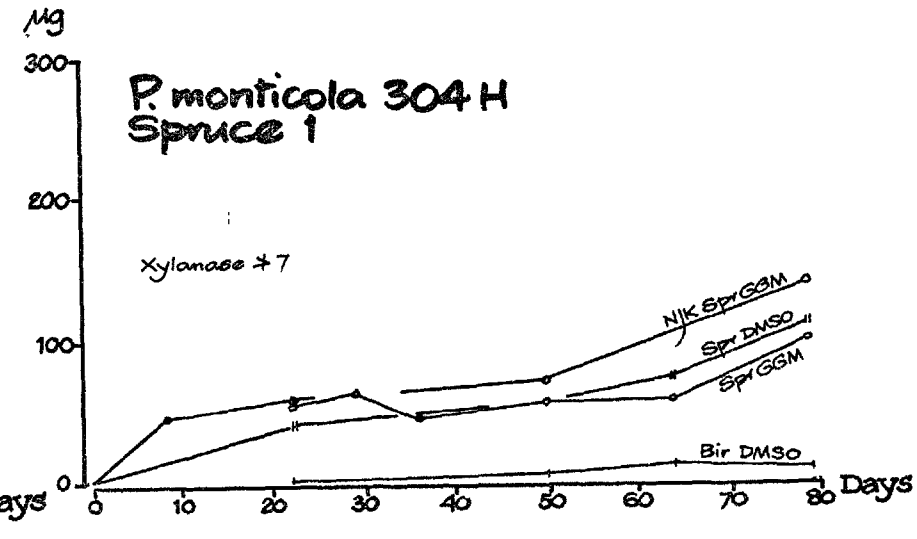

µg
P. monticola 304 G
Birch 2
300
200
100
0
Bir DMSO
Spr DMSO
Xyl.
Xyl.
Spr GGM
NJK
NJK
NJK Spr GGM
0 10 20 30 40 50 60 70 80
Days

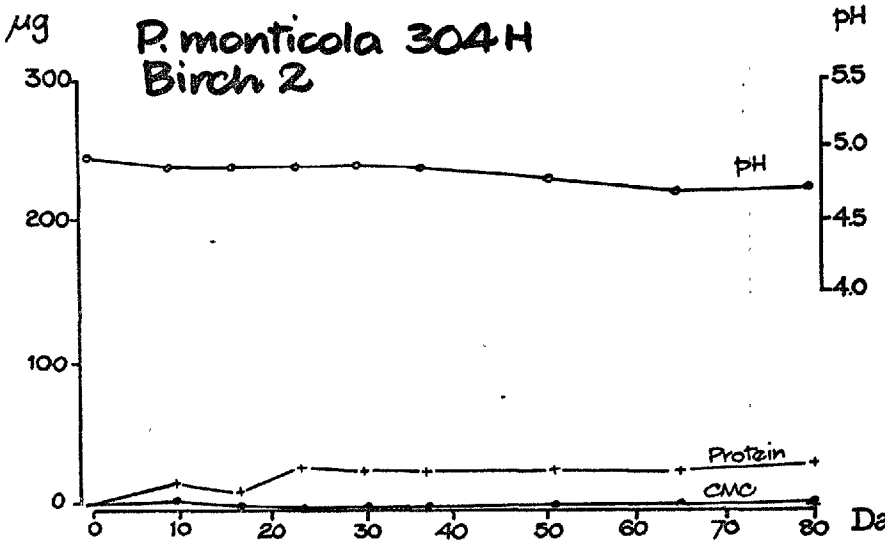

µg
P. monticola 304 G
Spruce 2
300
200
100
0
Xylanase ÷ 10
NJK Spr GGM
Spr GGM
Spr DMSO
Bir DMSO
0 10 20 30 40 50 60 70 80
Days

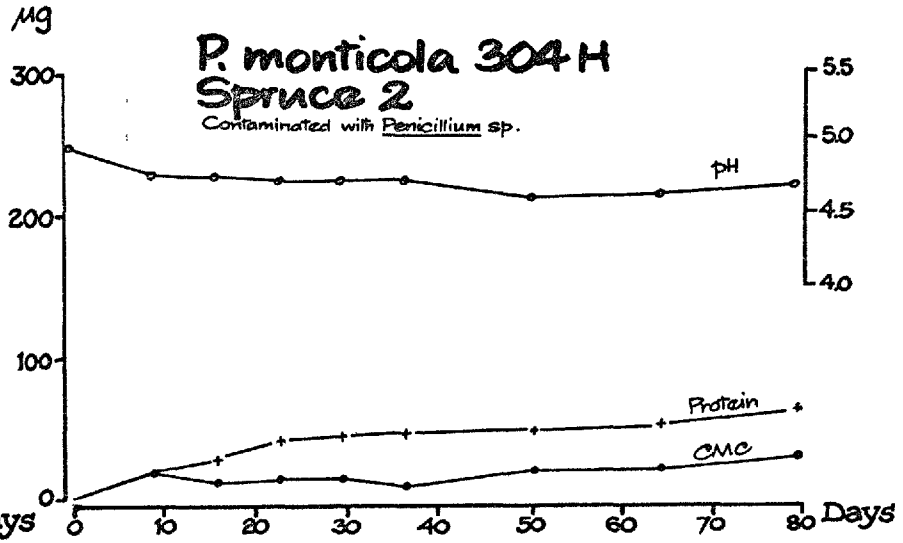

µg
P. monticola 304 H
Birch 1
pH
300
200
100
0
5.5
5.0
4.5
4.0
pH
Protein
CMC
0 10 20 30 40 50 60 70 80
Days

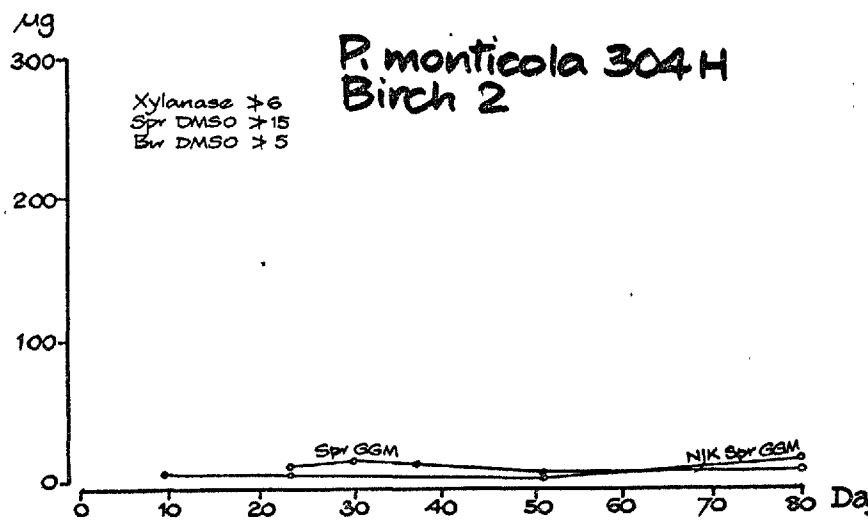

µg
P. monticola 304 H
Spruce 1
pH
300
200
100
0
5.5
5.0
4.5
4.0
pH
Protein
CMC
0 10 20 30 40 50 60 70 80
Days

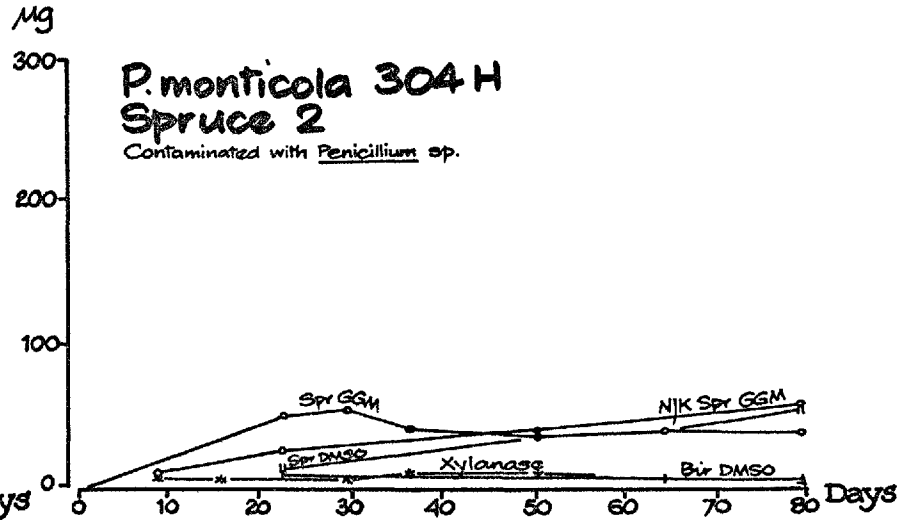

µg
P. monticola 304 H
Birch 1
Xylanase ÷ 12
300
200
100
0
N/K Spr GGM
Spr GGM
Spr DMSO
Spr GGM
Bir DMSO
0 10 20 30 40 50 60 70 80
Days

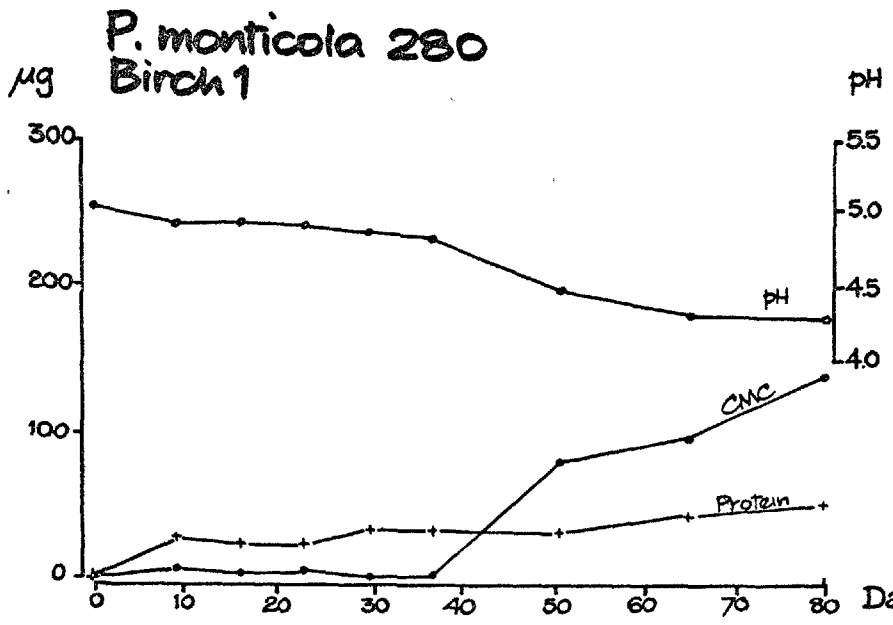

µg
P. monticola 304 H
Spruce 1
Xylanase ÷ 7
300
200
100
0
N/K Spr GGM
Spr DMSO
Spr GGM
Bir DMSO
0 10 20 30 40 50 60 70 80
Days

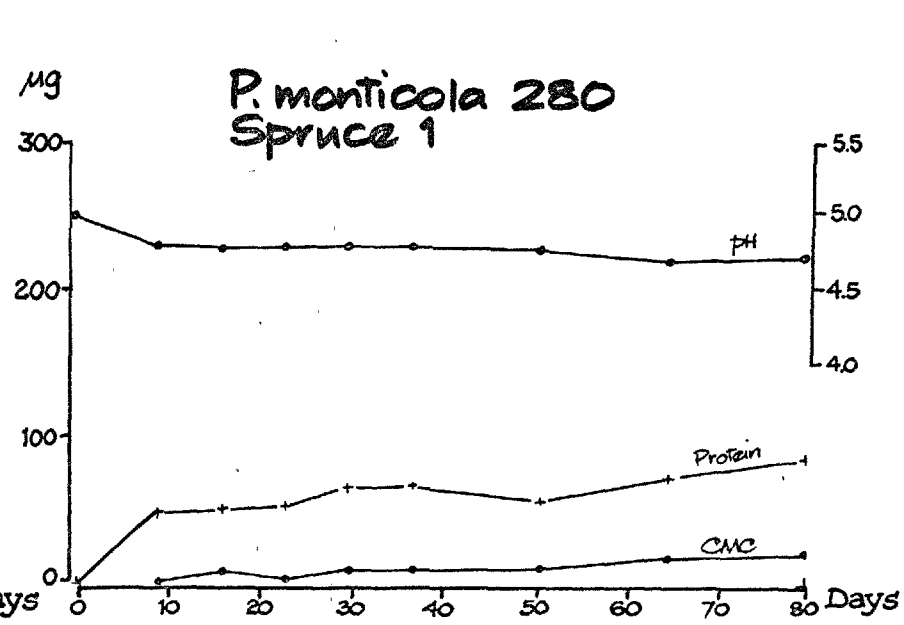

µg
pH
P. monticola 304 H
Birch 2
300
200
100
0
5.5
5.0
4.5
4.0
pH
Protein
CMC
0 10 20 30 40 50 60 70 80
Days

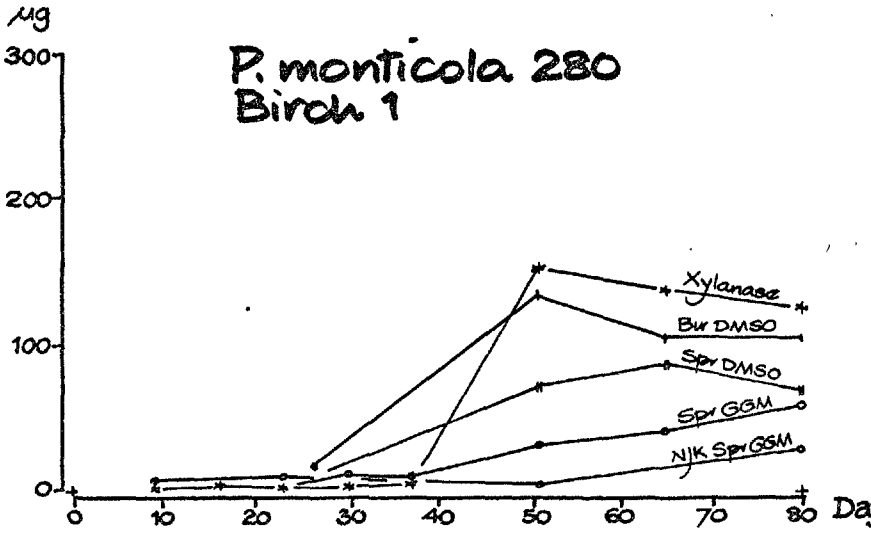

µg
P. monticola 304 H
Spruce 2
Contaminated with Penicillium sp.
300
200
100
0
5.5
5.0
4.5
4.0
pH
Protein
CMC
0 10 20 30 40 50 60 70 80
Days

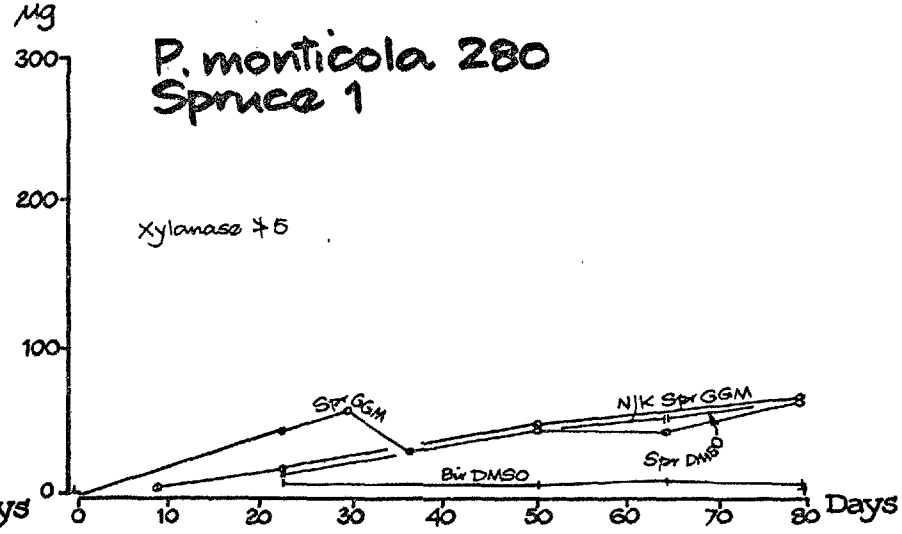

µg
P. monticola 304 H
Birch 2
Xylanase ✗6
Spr DMSO ✗15
Bir DMSO ✗5
300
200
100
0
Spr GGM
NIK Spr GGM
0 10 20 30 40 50 60 70 80
Days

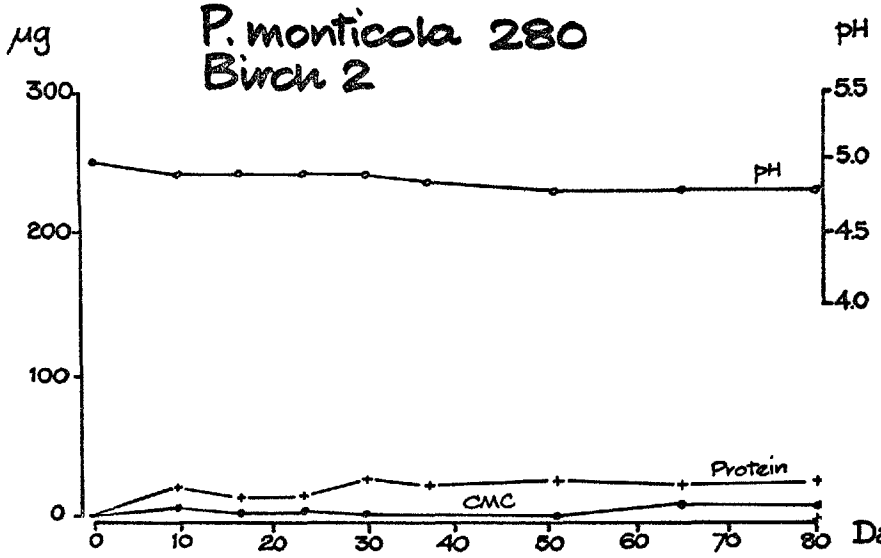

µg
P. monticola 304 H
Spruce 2
Contaminated with Penicillium sp.
300
200
100
0
Spr GGM
NIK Spr GGM
Spr DMSO
Xylanase
Bir DMSO
0 10 20 30 40 50 60 70 80
Days

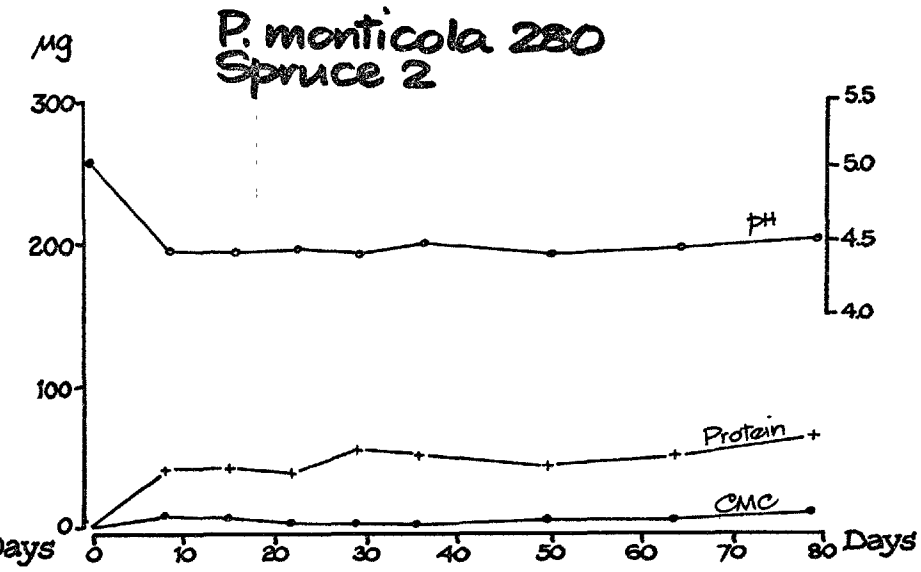

P. monticola 280
Birch 1
µg
pH
300
200
100
0
5.5
5.0
4.5
4.0
pH
CMC
Protein
0 10 20 30 40 50 60 70 80
Days

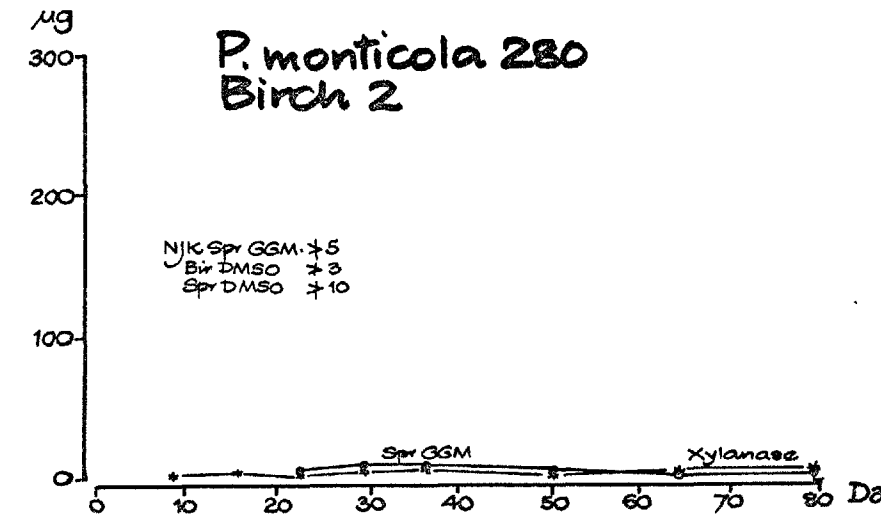

µg
P. monticola 280
Spruce 1
300
200
100
0
5.5
5.0
4.5
4.0
pH
Protein
CMC
0 10 20 30 40 50 60 70 80
Days

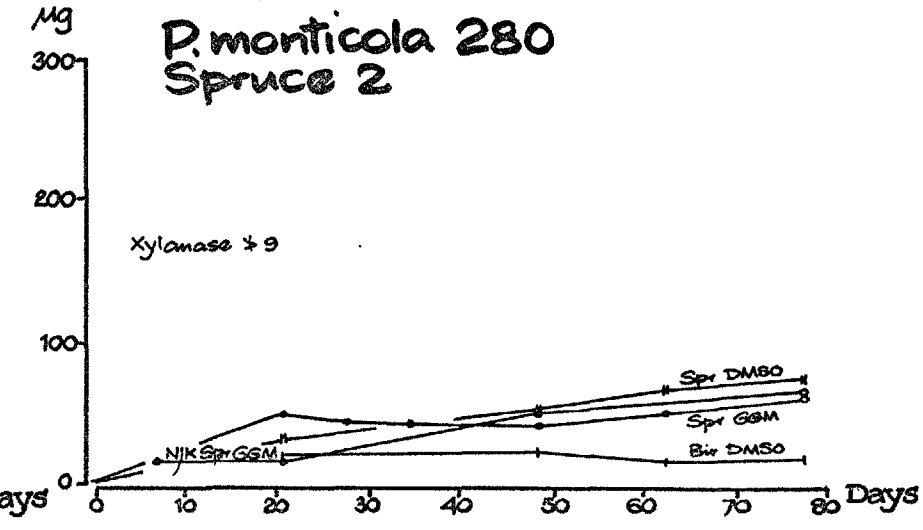

µg
P. monticola 280
Birch 1
300
200
100
0
Xylanase
Bir DMSO
Spr DMSO
Spr GGM
NJK Spr GGM
0 10 20 30 40 50 60 70 80
Days


µg
P. monticola 280
Spruce 1
300
200
100
0
Xylanase ÷5
Spr GGM
NJK Spr GGM
Spr DMSO
Bir DMSO
0 10 20 30 40 50 60 70 80
Days


P. monticola 280
Birch 2
µg
pH
300
200
100
0
5.5
5.0
4.5
4.0
pH
Protein
CMC
0 10 20 30 40 50 60 70 80
Days


P. monticola 280
Spruce 2
µg
pH
300
200
100
0
5.5
5.0
4.5
4.0
pH
Protein
CMC
0 10 20 30 40 50 60 70 80
Days


P. monticola 280
Birch 2
µg
300
200
100
0
NJK Spr GGM ÷5
Bir DMSO ÷3
Spr DMSO ÷10
Spr GGM
Xylanase
0 10 20 30 40 50 60 70 80
Days


P. monticola 280
Spruce 2
µg
300
200
100
0
Xylanase ÷9
Spr DMSO
Spr GGM
Bir DMSO
NJK Spr GGM
0 10 20 30 40 50 60 70 80
Days

Discussion

One of the major objects at the commencement of this work was to compare different strains of a fungus. The result found was that fungal strains of the same species shewed the same trends and any differences were in general only those of degree. These were relatively small. Instead of discussing individual results of each strain, it would be more profitable to restrict discussion to the trends shewn.

C.globosum (9 strains)

(1) pH

The pH dropped until about day 10 or 15 and then gradually rose throughout the rest of the experiment. In the case of the birch wood this rise exceeded the initial pH value and continued into the alkaline side. The spruce pH values rose very little and only in one case did they give similar values to the typical birch wood results. This was S70B, where the pH profile was reminiscent of the more active birch.

Of note, were the pH curves of S70B on birch. In both duplicates the pH rise started on day 6 and continued to the highest alkaline values recorded by any strain. The lowest terminal (Day 70) pH value given on birch wood was by both duplicates of strain S70K. These graphs barely crossed the initial starting point of 5.0.

(11) C.M.C.-ase activity.

All day 70 birch C.M.C.-ase activities were consistently close to a value of 200µgms. With spruce there was a greater spread, most values lying around 100 µgms. S70 No.1 and No.2 were about 150 µgms, S70E (No.1) and both S70B duplicates were in the 200 µgms region. In general, the spruce values were about half that of birch.

(111) Protein.

The difference in protein production between the birch and spruce cultures was marked. In birch culture the 300 µgm level was reached near day 40 (with the single exception of S70L duplicate No.2) which corresponded to an actual concentration of 600 µg/ml. of culture filtrate, since a 0.5 ml. sample volume was used for the assay. In spruce cultures the protein values were generally about 100 µgms, and always below 222 µgms. S70B, was exceptionally high with duplicate values of 200, and 222. This disparity between spruce and birch protein values is only partly reflected in other enzymic activities. There must, therefore, be some other major protein production occurring in the hardwood cultures the activity of which has not been monitored in this experiment.

(1V)a N.J.K. Spruce GGM-ase and (1V)b Spruce GGM-ase.

(Substrates were both samples of galactoglucomannan. One was supplied by Dr. N.J. King and is prefixed accordingly, the other was prepared for this work.)

In birch culture 'N.J.K. spruce GGM-ase' activity consistently gave the lowest hemicellulase values. In general 'spruce GGM-ase' values were 10-20% higher. Day 70 values for the former activity were in the region of 100 µgms.

The spruce induced cultures were the opposite in that with one exception (S70K No.1) the 'Spruce GGM-ase' was the largest hemicellulase activity to be found. This was closely followed by 'N.J.K. spruce GGM-ase' activity, about 10-20% below it. The values were about the same as when cultured on birch sawdust, (ca. 100 µgms). An exception was S70B No.1 and 2 which both exceeded 300 µgms.

(V) Xylanase.

In birch cultures the xylanase activities were consistent with varying strains. The curve rapidly reached about 150 µgms, generally by day 12 or 15. In most cases by day 70 it was in excess of 300 µgms. S70M No.1 shews irregularities with days 12 and 20. This is almost certainly experimental error, the true shape of the curve probably being a line drawn through the points at day 9, 15 and 25. These xylanase activities were the highest recorded for any hemicellulase.

By contrast the xylanase values for spruce, with the exception of S70B (No.1 and 2), were uniformly lower. The values were between 25 and 50 µgms.

The discrepancies after 70 days of culture between the xylanase activities of birch and spruce cultures were large. The relative proportions after 25 days were even greater, xylanase in birch generally being over 150 µgms, whereas in spruce the corresponding values were about 10-20 µgms.

(V1) Spruce D.M.S.O. activity.

Chromatographic analysis revealed that this polysaccharide was a complex mixture containing all five monosaccharides for which it was tested. (See Appendix II). All day 70 birch cultures gave between 120 and 160 µgms. This represented middle of the range values for hemicellulase activity.

In spruce culture, 'spruce D.M.S.O.' activity again shewed that activities were in the mid-range of the hemicellulases.S70B No.1 and No.2 were above 200 µgms. The other strains were between 50 and 75 µgms.

(V11) Birch D.M.S.O. activity.

When cultured on birch sawdust 'birch D.M.S.O.activity' with two exceptions (S70G No.2 and RLB No.1) consistently gave the second greatest activities.

Both duplicates of S70 and S70B were in the 200 µgms region by day 70, whereas most of the others were ca. 150 µgms.

When cultured on spruce sawdust however, the birch D.M.S.O. was more often than not the lowest hemicellulase activity. Where this was not true it was the second lowest by a very small margin to the xylanase (except RLB No.2 which may possibly be an exaggerated result.) Values were generally in the 10-20 µgm region with the exception of S70B No.1 and No.2 (ca. 100 µgms.) and RLB No.1 and 2 (ca. 75 µgms.)

These findings are not surprising since the composition of 'birch D.M.S.O.' extract is very similar to xylan. It contained glucose and galactose residues, in addition to xylose. (See the percentage composition of hemicelluloses in Appendix II). p. 379

P. fastigiata (2 strains)

This was the second attempt at monitoring hemicellulase activities of this species of fungus. The growth was for 80 days, and sampling days were modified. (See methods). Growth was definately present but enzymic activities were low.

(1) pH

In birch culture this dropped to 4.2 or 4.3 most of the fall occurring in the first 10 days. With spruce the fall was less pronounced, in general 4.5 to 4.6.

(11) CMC-ase

In both woods this was very small. The spruce was higher than the birch.

(111) Protein

This gave similar results to CMC-ase activity.

Hemicellulases (1V) to (V11)

No significant differences were observable since all values were very low. Growth on birch and spruce sawdust by P.fastigiata 56A was repeated in a later experiment with more sucessful results (See the experiment testing the reproducibility of assay page218).

P.monticola. (8 strains)

This was a repetition of an earlier experiment which failed owing to lack of growth and contamination problems. As with P. fastigiata the sampling days have been modified.

Contamination has been indicated when and where it was detected. Some graphs, however, rise suddenly for no apparent reason. Examples of this are 304C No.1 and 280 No.1 on birch sawdust. Two possible explanations are that (1) undetected contamination has occured (despite stringent checks to isolate any contaminants on malt agar plates four times during the 80 day incubation period) (11) during the process of incubation the conditions in the medical flat in some way optimised for the growth of P. monticola . (It may be possible that the evaporation of water led to an optimal concentration of nutrient salts, or the relative humidity in the medical flat fluctuated in such a way as to encourage above the surface mycelial growth). Neither of these possibilities are to be happily tolerated. Protection against contamination would improve with better laboratory facilities, such as a constant temperature room free from other cultures. This was not possible in the present work. A constant humidity device would be useful in the growth of this fungus, as it is particularly susceptible to changes of relative humidity (Gay , 1973). It is probable that the former explanation is most likely.

(1) pH.

When cultured on birch the pH dropped slightly over the 80 day range. The fall was, in general, to between pH 4.6 and 4.8. There were two exceptions; (1) strain 280 No.1 and (11) 304G No.2. These both fell to 4.30.

On spruce the values were similar. Strains 304B No. 2, 304C No. 1 and 280 No. 2 all shewed a marginal rise. It is difficult to establish whether such small differences are significant or a result of some artifact. In the case of 304D No. 2, a marked drop was observed; this was closely associated with a sudden rise in enzymic activities.

(II) CMC-ase

When grown in birch culture the values for CMC-ase activity were generally low (ca. 10-15 µgms). The exceptions were 304C No. 1 which rose to over 100 µgms between day 65 and 80, 304G No. 2 which shewed a gradual rise after day 30 to reach ca. 60 µgms by day 80, and 280 No. 1 which reached 160 µgms over a similar period.

In spruce culture the results were similar, the exception being 304D No. 2.

(III) Protein

The birch culture filtrates gave very little protein (ca. 30 to 50 µgms). Strain 304G No. 2 day 51 is almost certainly in error, but because the volume of culture filtrate sample was small, repeat protein estimations were not feasible, since the volume needed for an assay was large (0.5 ml) compared to that required for hemicellulase assays (0.1 ml.). The same is true for day 80 304F No. 1.

In spruce culture, protein values were twice as much (ca. 60 to 100 µgms).

Hemicellulase assays

(IV)a N.J.K. Spruce GGM-ase and (IV)b Spruce GGM-ase

In birch culture most were low (ca. 10-20 µgms). 304C No. 1 was an exception.

In spruce culture 'N.J.K. spruce GGM-ase' activity was in most cases the most active hemicellulase present with spruce

GGM-ase in the second or third place. Occasionally this order was reversed (e.g. 304C No. 1), and often spruce D.M.S.O. activity interpenetrated. (e.g. 304C No. 2, 304D No. 1 and 2, 304E No. 1 and 2, 304H No. 1 and 280 No. 2).

The day 80 result of spruce GGM-ase is likely to be in error.

(V) Xylanase activity

In birch culture filtrates xylanase activity was low except 304C No. 1 and 304G No. 2.

In spruce culture filtrates no significant xylanase activities were found in any 80 day cultures.

(Vl) Spruce D.M.S.O. activity

In birch culture the 80 day activity was below 10 µgms except in 304C No. 1 and No. 2, 304H No. 1, 280 No. 1 and 304G Nos. 1 and 2. In the 304G cultures the spruce D.M.S.O. activity was the highest hemicellulase present.

In spruce culture 'spruce D.M.S.O.' activity was, in general the highest activity recorded. Values varied and reflected the overall activity of the culture. In most cases they lay between 40 and 70 µgms; 304D No. 2 and 304H No. 1 were in excess of 100 µgms.

With 304C No. 1 the day 80 result is likely to be in error.

(VII)Birch D.M.S.O. activity

In birch culture activities were either low or not significant with the exception of 304C No. 1 and 304G No. 2.

In spruce culture filtrates activities were low or not significant excepting 304D No. 2.

General discussion of C.globosum hemicellulases

By monitoring the hemicellulase activities of the culture filtrates, clear differentiation can be seen in the mode of attack on birch and spruce woods, by the fungus C. globosum. All nine strains tested gave similar results, but one particular strain, S70B, was exceptional in its enzymic activities on both types of wood. (This was not reflected in the weight loss tests).

The major differences can be enumerated as follows (1) xylanase activity was considerably greater on birch than on spruce (11) GGM-ase activity represented a much larger proportion of the hemicellulase activity on spruce than on birch (111) birch wood gave greater yields of hemicellulase (and protein) than spruce wood (1V) with C. globosum a rise in pH was associated with greater activity (V) S70B was an exceptionally active strain. (V1) from these results it would appear that xylan is a far more susceptible polysaccharide to C. globosum than galactoglucomannan (V11) there was a general trend of the spruce induced hemicellulases to "catch up" with the birch induced after about days 20 to 30, i.e. the relative differences between the enzymic activities induced by the two woods was greatest before days 20 to 30 and thereafter gradually declined.

General discussion of P. fastigiata hemicellulases

The results from this experiment were disappointing in that there was very little growth of the fungus under these conditions. Further work to determine the optimum conditions for growth, and the method of inoculation most likely to result in good growth should be carried out.

General discussion of P. monticola hemicellulases

These were only marginally better than P. fastigiata. The birch cultures were less active than the spruce. In the latter it was only 304B No.1, and 304F No.2 that shewed inadequate growth. 304D No.2 was suspect in that from day 50 to 80 activity increased in a suspicious manner. These patterns of sudden increase have been mentioned earlier. Despite thorough checks, contamination would appear to be the most likely explanation, but it is possible that this may represent true growth of P. monticola

A pattern whereby spruce cultures induced relatively high activities of both types of 'spruce GGM-ase' and also of 'spruce D.M.S.O.' activity was observed. It was noted that the xylanase did not represent a large proportion of the hemicellulase either in spruce or in birch cultures. In general lower pH values were associated with higher activities of enzyme.

Contamination was detected in 304D No. 1 and No. 2 when grown on birch, and this was identified as a species of Paecilomyces. The behaviour of this was interesting. Xylanase, birch D.M.S.O. activity and CMC-ase were active (in excess of 100 µgms). Spruce D.M.S.O. was intermediate and both types of GGM-ase were low.

Discussion

This proved to be the most interesting experiment undertaken. The idea that the hemicelluloses in wood, and the hemicellulases which fungi produce when grown on wood, might play a more significant role than has so far been attributed to them, was proposed by Takahashi and Nishimoto in 1968. They said that "considering ..... the ability of C.globosum to utilise xylose and xylan, this soft rot fungus has possibly an active pathway to metabolize these mono - and polysaccharides, indicating the possible reference tothe greater susceptibility of hardwoods to soft rot fungi". Their work was only published in a Western journal in 1973, at a time when the present work was nearly completed. Keilich et al in 1970, after the present work had been started published results of an investigation into "The enzymatic degradation of cellulose, cellulose derivatives and hemicelluloses in relation to the fungal decay of wood." They tried to compare the enzymes from a typical brown rot fungus (Polyporus schweinitzii) and a soft rot fungus (C. globosum) in order to see whether any characteristic differences existed which might explain;

" (a) the different micromorphological changes produced and
(b) the predominant occurrence of brown rots and soft rots in softwoods and hardwoods respectively"
(the present work had similar aims, but the scope of the experiment was extended to include at first three and then four species of fungus, comprising the brown, white, soft and stainer soft rot types).

Criteria of comparison for the enzymes were their molecular weights, stabilities, pH and temperature optima and substrate specificities; for this purification procedures were established and used on each fungal culture. Assay for enzymic activity was by a viscometric method on artificial substrates (C.M.C., water soluble Tubera salep mannan and wheat straw xylan). The present work has shewn that an assay method based upon reducing sugar estimation has given suprisingly similar results to a viscometric method when assaying for CMC-ase activity. (See viscometry experiment). The reducing sugar method has the advantage that substrates actually derived from the wood (and therefore much closer to the true state in vivo) may be used, which is not possible in the viscometric determination. Both "artificial" substrates are linear homopolymers, a state in which hemicelluloses have yet to be found in wood. It would therefore appear to be more acceptable to use only hemicelluloses derived from wood as substrates, until it has been shewn that homopolymeric substrates are a satisfactory alternative. (In the case of cellulase estimation, for this work it was decided that the advantages of using a soluble derivative of cellulose (CMC) outweighed the disadvantages.) Limited assays using hemicelluloses derived from Pinus strobus were performed by Keilich et al., who used paper chromatographic techniques to establish the presence or absence of small molecular weight soluble sugars after incubation of the hemicellulose with an enzyme solution. No quantitative data were given, but there appeared to be an indication that the brown rot was more active against the soft wood type of hemicellulose (GM) and the soft rot against the hard wood type of hemicellulose (XYL). (It should be noted that this xylan was in fact extracted from Pinus strobus and merely represents the type of hemicellulose, normally found in abundance in hard woods).

It was tentatively suggested that these data might correspond to the predominant occurrence of brown rot and soft rot on soft woods and hardwoods respectively. No conclusive correlation was claimed.

In the hemicellulase monitoring experiment considerable quantitative evidence is presented which supports this theory. It was indeed independently postulated in this laboratory prior to reading the Keilich et al. publication. Examination of the results of the hemicellulase monitoring experiment reveals a definite trend for the wood destroying fungus to try to produce an enzyme system which reflects the composition of the medium upon which it is grown. Thus C. globosum, when cultured on birch sawdust (which is rich in xylan) would give rise to high xylanase activity, whilst on spruce sawdust (which contains proportionately greater amounts of GGM than xylan) GGM-ase was the most active hemicellulase. The quantity of the enzymes produced was not equal, the xylanase on hard wood being far more abundant than the GGM-ase on soft wood. It is suggested that this enzymic production pattern is a possible explanation of the known tendency for hardwoods to be preferentially attacked by soft rot fungi, and softwoods to be preferentially attacked by brown rot fungi.

Previous workers (e.g., Findlay, 1970, Fuller 1970, Bailey et al 1968), have suggested that lignin concentration is the prime factor in explaining these observed preferences of attack. It is postulated that hemicellulose composition plays a far more significant role than has been hitherto suggested.

Enzyme production under the conditions of culture of the hemicellulase monitoring experiment was high for C. globosum but disappointingly low for P. monticola and virtually non-existent for P.fastigiata. A criticism of this experiment could be that the total enzyme yields did not correspond to the known decay potential of the fungus as measured by weight loss tests. For example, it was not uncommon to find an enzymic activity in the 200 µgm region with C. globosum but with P.monticola 50 µgs would be characteristic, yet both gave similar weight losses when cultured on agar. This technique of investigation therefore needs considerable work to be done on the investigation of culture conditions, to determine the inadequacies of the present culture medium responsible for the sub-optimal growth with P. fastigiata and P. monticola. The results of the hemicellulase monitoring experiment when looked at from the point of view of an individual fungal species gave no such anomalies with the theory postulated concerning the substrate preferences of these fungi.

Lyr,(1963) shewed that four different strains of Merilius silvester grown on cellulose, exhibited only small differences in growth and production of several enzymes,and so he concluded that the results obtained from one strain of wood-roting fungus were not far from being representative of the species. In the present work nineteen strains comprising three different species were used in this experiment to gain an insight into the variation of their enzymic activity within a single species. This aspect of the experiment was mainly restricted to C. globosum with nine strains and P. monticola with eight, owing to difficulties

experienced in obtaining a large number of strains of P. fastigiata. In general where protein production was high, enzymic activities were also high; this could therefore be used as a rough guide to categorize the strains. There was a marked tendency for the less productive strains to catch up with the more productive from day 40 to day 70. A list, therefore, of the strains of C. globosum in increasing order of activity on birch sawdust (measured as protein production) would read as follows:-

for day 40	Approximate * N° µg.	and for day 70	Approximate* N° µg
S70 M	205	S70K	450
S70E	215	S70N	470
S70K	225	S70E	485
S70G	255	RLB	495
S70N	265	S70B	500
S70	270	S70M	510
RLB	315	S70L	520
S70B)	360	S70G	525
S70L)	360	S70	560

(* Average of Birch 1 and 2 protein results rounded up to the nearest 5 units).

Of note is the larger spread at day 40 than at day 70, and that the most active fungi at day 40 are not most active at day 70. No correlation was found between the spread of protein activity either at day 40 or day 70, and the results of the weight loss tests (birch wood blocks on birch agar 1(a), (b) and (c) when placed in increasing order of weight loss as is shewn below.

1(a)	1(b)	1(c)
RLB	(No result for S70G)	S70B
S70K	S70N	S70L
S70B	S70	S70E
S70G	RLB	S70K
S70E	S70L	S70
S70L	S70E	RLB
S70M	S70K	S70M
S70	S70M	S70N
S70N	S70B	S70G

One of the aims of this work was to find out if there was a correlation between high enzyme production (represented by protein production), and the destructive power of the fungal strain (represented by weight loss testing). It can be seen that this was not achieved. Comparison of the results of 1(a), (b) and (c) will shew that it was not even possible to obtain reproducible results with weight loss tests under these conditions. However, with experimental conditions under stricter control along the lines suggested in the discussion of the weight loss experiment, a pattern of decay capability might

emerge. In conclusion it can be said that in the present work, although some strains were more active than others in the hemicellulase monitoring experiment, this was not reflected in the results of the weight loss experiment.

The pH profile of C. globosum for all of the strains tested shewed a rise after an initial drop. The rise was pronounced in birch culture and minimal in spruce. It would be interesting to establish whether this is a cause or effect, in the light of the known preference of this fungus for the hardwood. P. monticola and P. fastigiata exhibited contrasting behaviour, in that the pH of all strains of their cultures dropped and shewed no pattern of rise. Whether this difference in behaviour is significant is open to question in view of the general inadequacy of growth of these two fungal species.

The interrelationship of the pH, the activity of enzymes produced, and the types of wood preferentially attacked by wood-destroying fungi, is a field ripe for further study. In particular the following questions should be asked:- Which comes first, a change in pH, or the onset of enzymic production? Why does C. globosum cause a rise, and the other two fungi cause a lowering of pH? Is this difference in any way connected with their modes of attack on soft and hardwoods? What would be the effect on enzymic production, if the pH was stabilized in culture? At what pH should it be stabilized, and what would be the effect of varying this? From what metabolic process does the alkalinity or acidity arise, when these fungi are cultured in a liquid medium? What is the extent of acid or alkali production of a wood

destroying fungus in wood? Fuller (1970) has shewn, that under the conditions of culture he used, three staining fungi selected produced cellulases which had much lower pH optima than typical soft rot fungi. As a consequence of the difficulty in working with CMC at low pH values (the carboxyl groups are undissociated and there is a possibility of complexing with proteins), the experimental data below pH 4.0 were limited. It would be interesting to amplify this work and to determine any significance and possible connections with the answers to the questions posed above.

The use of hemicelluloses as a substrate to assay for hemicellulases has provided strong evidence that the role which hemicelluloses play in the fungal decay of wood is more extensive than has been thought previously. The differences found between the GGM-ases (the main enzyme component in spruce cultures) and xylanases (the main component in birch cultures) appeared to reflect any tendency of a wood-destroying fungus to grow either on soft or hardwood. It is suggested that this difference in enzyme induction could provide a sensitive screening test to investigate and clarify the action of a variety of wood destroying fungi.

Fig. 22

Results of the Reproducibility Experiment. (µgm. reducing sugars.)

Enzyme	CMC-ase		XYL-ase		GGM-ase	
Culture C-source	birch	spruce	birch	spruce	birch	spruce
C.globosum S70B	131	114	267	70	76	52
duplicate No.1.	134	108	266	83	91	59
	115	88	264	75	79	51
	130	115	282	93	99	51
	124	105	273	96	67	48
	157	122	276	93	102	57
	122	87	242	80	74	51
duplicate No.2.	144	119	341	115	79	76
	149	108	317	106	92	95
	120	90	280	91	79	77
	156	128	349	115	109	99
	121	101	453	110	87	76
	168	138	311	100	102	104
	164	101	264	108	85	57
P.fastigiata56A	15	0	20	2	4	3
duplicate No.1.	21	7	20	3	1	1
	8	-3	20	6	5	11
	24	3	19	5	5	3
	13	1	16	-2	-7	-8
	41	13	20	0	3	-4
	8	0	19	5	1	1
duplicate No.2	19	-2	22	10	2	1
	20	5	22	8	1	0
	9	-2	25	7	3	3
	25	-9	19	13	8	3
	12	2	17	1	-9	-2
	30	20?	9	-2	-2	-9
	13	-21?	24	6	1	7

Enzyme	CMC-ase		XYL-ase		GGM-ase	
Culture C-source	birch	spruce	birch	spruce	birch	spruce
P.monticola 304B	15	30	-2	-7	17	18
duplicate No.1.	20	30	0	-1	23	30
	9	22	1	2	18	25
	19	36	4	-2	35	21
	13	20	-1	-4	27	18
	26	33	-3	-4	22	11
	22	16	-2	-5	17	7
duplicate No.2.	12	87*	-5	10*	13	91*
	15	92*	-2	20*	23	119*
	3	71*	0	27*	15	95*
	13	100*	-2	23*	28	150*
	0	79*	-5	21*	19	95*
	33	103*	-3	-2*?	21	-7*?
	14	66*	-5	19*	13	88*
* contaminated with Aspergillus sp.						
P.versicolor	27	3	39	5	22	14
duplicate No.1.	29	2	40	10	25	12
	27	2	42	1	22	14
	48	4	45	13	29	2
	26	5	75	22	21	13
	45	9	39	3	-	9
	19	1	39	9	19	9
duplicate No.2.	27	-1	27	8	23	13
	37	4	33	10	19	10
	22	-11	32	7	18	16
	53	3	39	9	28	13
	22	-1	37	8	19	-2?
	42	10	22	4	7	-3?
	26	-7	30	4	17	1?

Reproducibility Experiment, Mean values. Fig.23

	dupl.No.	CMC-ase		XYL-ase		GGM-ase	
		birch	spruce	birch	spruce	birch	spruce
C. globosum S70B	1	129	106	267	84	84	53
S70B	2	146	112	331	106	90	86
Average		138	109	299	95	87	68
P. fastigiata 56A	1	19	3	19	0	1	1
56A	2	16	-3	21	6	-1	1
Average		17.5	0	20	3	0	1
P. monticola 304B	1	17	26	0	-3	23	16
304B	2	11	85*	-3	20*	19	106*
Average		14	26	-1.5	-3	21	16
P.versicolor	1	32	4	45	9	23	9
	2	33	-1	30	7	20	7
Average		32.5	1.5	37.5	8	21.5	8

* contaminated with Aspergillus sp.

Standard Deviations (σ) Calculated from the results of the Preproducibility Experiment Figure 24.

Datum: $= \sqrt{\frac{x^2 - (\sum x)^2/n}{n - 1}}$

Enzyme ——		CMC-ase		XYL-ase		GGM-ase	
Culture C-Source —		birch	spruce	birch	spruce	birch	spruce
Fungus & Duplicate No.							
C.glob.S70B	No.1	13.4	13.5	12.8	10.0	13.4	3.9
" "	No.2	19.3	16.9	61.9	8.6	11.4	16.6
P.fast.56A	No.1	11.6	5.4	1.5	2.9	4.2	6.0
" "	No.2	7.5	12.6	5.5	5.1	5.2	5.0
P.mont.304B	No.1	5.8	7.4	2.4	2.9	6.6	7.9
" "	No.2	10.6	14.1*	2.0	9.8*	5.6	4.8*
P.vers.IMI.79126	No.1	10.7	2.7	13.2	7.1	3.2	4.3
" "	No.2	10.7	7.0	5.8	2.3	6.4	8.0

and calculated using all fourteen results from duplicates 1 and 2.

C.glob. S70B	17.8	15.1	54.1	14.6	12.4	19.7
P.fast. 56A	10.0	9.5	3.9	4.4	4.6	5.3
P.mont. 304B	8.6	7.4	2.5	2.9	6.2	7.9
P. vers. IMI.79126	10.8	5.5	12.2	5.2	5.5	6.4

* Contaminated with an Aspergillus sp.

Discussion of the Reproducibility Experiment

The object was to determine whether the procedure evolved for growth and assaying in the hemicellulase monitoring experiment was sufficiently reproducible to be reliable. Also it was considered desirable to gain an insight as to the quantity of the error involved with this technique. Seven different operators (a"third year option" class at Imperial College, Botany Department) were provided with identical solutions of substrate and enzyme. Each was then instructed to assay for reducing sugars in the manner described for the hemicellulase monitoring experiment.

Individual and mean values are both shewn. There are two main differences between this and the hemicellulase monitoring experiment. Firstly, the wood C-source was 80 mesh, and secondly P. versicolor was included. The general trend of the results seemed to be unaffected by the change in mesh size. This conclusion is, however, tentative in view of the limited nature of the experiment. P. versicolor, a typical white rot, is capable of heavy degradation, measured as weight loss. (see Weight Loss Tests). However when cultured in this liquid medium under these conditions gave low enzymic activities in a similar fashion to P. monticola. It was however, better in this respect than P. fastigiata.

Standard deviations are also shewn and it can be seen by comparison with the mean values that the coefficients of variation for C. globosum are relatively good (ca. 10-15%) but for the other three fungi are not good (ca. 30% or quite often higher).

The conclusion that can be drawn from this reproducibility experiment, is that with a group of different workers, for C. globosum reasonably acceptable reproducibility can be achieved. A less satisfactory standard can be obtained for P. monticola and P. versicolor, but P. fastigiata was unsatisfactory. Much of the reason for this state of affairs can be attributed to the lack of growth (and consequent low assay figures) on the last three mentioned fungi.

Fig.25

Results of Long Term Enzyme Digests.(expressed as µgms. of reducing sugar equivalents per 0.5ml. of digest.)*

Substrate(0.5%)	CELLULOSE POWDER				SPRUCE HOLOCELLULOSE			
Enzyme	from birch culture		from spruce culture		from birch culture		from spruce culture	
Fungus	day7	day40	day7	day40	day7	day40	day7	day40
C.globosum	0.9	50.6	0.1	24.2	5.7	223	-1.8	109
P.fastigiata	-0.7	3.3	-0.7	6,9	13.7	0	0.8	114
P.monticola	-1.4	4.3	-1.7	11.5	7.3	258	1.3	239
P.versicolor	-3.0	2.7	-0.8	18.8	23.8	304	-7.2	198
Substrate(0.5%)	BIRCH SAWDUST				SPRUCE SAWDUST			
C.globosum	-2.8	7.4	0.6	4.6	-2.6	20.4	-1.7	13.1
P.fastigiata	-2.2	-4.2	-1.8	7.0	-1.1	3.0	-2.2	4.2
P.monticola	-1.9	-4.4	-1.2	-5.7	-2.1	7.1	-0.9	-2.3
P.versicolor	-3.5	13.1	-1.7	4.5	-1.3	14.3	-0.8	14.5

* see"Factor for conversion to Fuller's units" (Appendix I)

The value for day 40 of P. fastigiata (when the enzyme was obtained from a birch culture) incubated on spruce holocellulose, appeared nonsensical. At a value of 0.µgms. It was suspected that enzyme had been accidentally left out of the incubation mixture, or that some such similar error had occurred. Therefore day 40 was repeated, and a value of 71 µgms obtained.

(cf. point "4" in the discussion on the next page)

Discussion of the Results of Long Term Enzyme Digests (See fig. 25)

From the figures shown in the Results of the Long Term Enzyme Digests the following points emerge:

1. There was an order of increasing enzymic breakdown by culture filtrates as follows: birch and spruce sawdust, cellulose powder, and spruce holocellulose.

2. Very little attack occurred in 7 days.

3. On cellulose powder C. globosum was most active, the birch culture giving double the activity of the spruce. In the other three fungal species the spruce culture filtrates were more active than birch. This was especially the case for P.versicolor where activities differed by a factor of 7.

4. Spruce holocellulose gave comparatively large results after 40 days incubation, C. globosum and P.versicolor birch culture filtrates were more active than their spruce conterparts, P. monticola had roughly equal activities, whilst P.fastigiata gave an anomalous result with no activity indicated in the birch filtrate. This was checked at a later date.

5. Both types of wood substrates were barely attacked by the enzymes. All 7 day incubations, with one exception, gave negative results. The same was true for

P. monticola enzymes, the one exception being the birch culture filtrate when incubated with spruce sawdust.

6. In view of the general preference of C. globosum for birch, it was surprising to observe that the spruce sawdust substrate gave consistently greater values than birch. However, the birch induced enzymes were in both cases more active than the spruce induced enzymes.

7. On both wood substrates P. fastigiata enzymes were virtually inactive, but P.versicolor enzymes gave low but appreciable activities.

7. On the birch substrate P.fastigiata gave a positive activity only with the 40 day spruce induced enzyme. This result was surprising. On the spruce substrate both 40 day culture filtrates gave very low but positive activities.

8. P.versicolor 40 day enzymes gave appreciable activities with all except the birch substrate spruce induced enzyme.

The general trends can thus be briefly summarized.

The holocellulose was much more suceptible to decay than any of the other substrates.

The birch induced enzymes were marginally more active than spruce induced enzymes. C.globosum generally appeared to yield the most active enzymes, closely followed by P.versicolor. The other two fungal species showed less activity.

Discussion.

Fuller (1970) carried out similar long term digests. The differences between his and the present work were;

(i) his enzyme solution was induced by cellulose as carbon source in the culture medium;

(ii) the enzyme had been partially purified and concentrated;

(iii) he had only one incubation time of 28 days;

(iv) the only fungi common to the two tests were P. fastigiata and C. globosum .

Despite these differences the present results, although smaller, are in the same order of magnitude. It is apparent from a study of both sets of results that the relative activities of spruce holocellulose, powdered cellulose and birch sawdust were in the same order , whether wood or cellulose were used as the carbon source in the initial culture medium.

Cellobiase Assay in µgms reducing sugars.

Fig.26

	Birch induced enzyme (40 day culture filtrate)*	Spruce induced enzyme (40 day culture filtrate)*
C.globosum S70B	3.1	6.8
P.fastigiata 56A	1.5	10.0
P.monticola 304B	- 4.9	5.3
P.versicolor IMI 79126	7.9	12.3

* The same culture filtrates as were used in the long term enzyme digests and viscosity experiments.

Discussion.

These results confirm the findings of previous workers that cellobiase is predominantly an intracellular enzyme.

(e.g. Reese et al., 1950; Cowling, 1958)

Comparison of activity measured by viscosity with activities of CMC-ase, XYL-ase and GGM-ase.

Fig.27

Culture C-source	BIRCH				SPRUCE			
Fungus	V	CMC	XYL	GGM	V	CMC	XYL	GGM
C. globosumS70B	238	178	279	166	119	145	125	181
P. fastigiata56A	11	7	9	-1	67	38	15	13
P. monticola304B	23	20	-1	24	72	56	2	57
P. versicolor	41	47	59	20	89	86	70	70

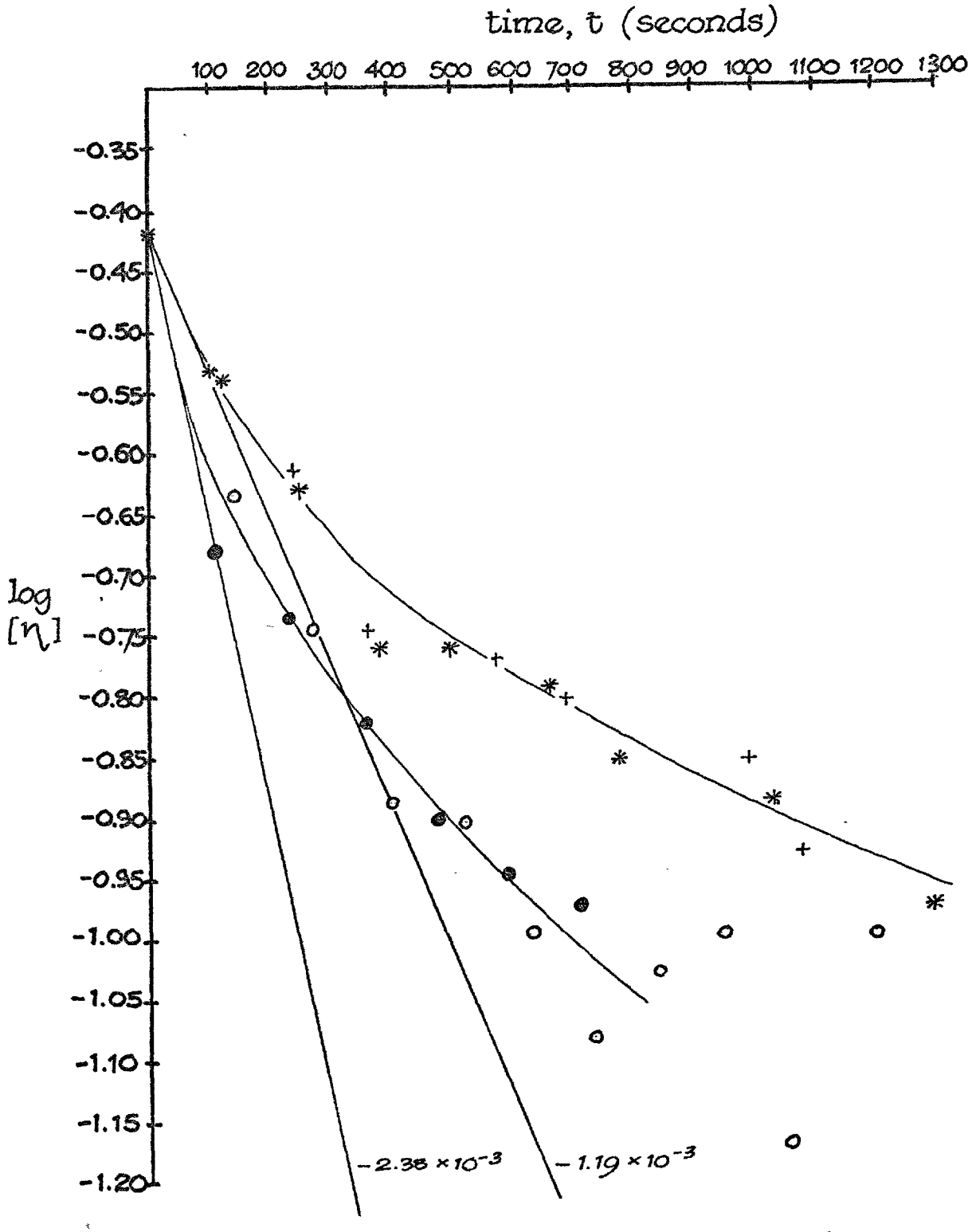
The reducing sugar activities were the result of duplicate assays.(in µgms.).

The viscosity,V, is expressed in purely arbitrary units. The slopes of the tangents to the initial curves have been made positive and multiplied by a factor of 1×10^5 resulting in numbers of the same order as the reducing sugar assays. (See Figs 28 to 31.)

The assay is a sensitive measure of the 'endo' activity. When grown on birch the proportions of 'V' activity between the four fungi were similar to those obtained with CMC-ase, except C. globosum which was larger. On spruce the relative proportions were again similar, but C. globosum was slightly lower and P. monticola slightly higher. It would appear that the 60 minute incubation reducing sugar test gives relative results remarkably close to those obtained by the superior viscosity assay, thus justifying the use of the former method for the work involving large numbers of assays.

(See also data in Appendix IV.)

Chaetomium globosum S70 B



Spruce * Birch o

Spruce + Birch ●

(duplicate assays.)

Fig.28

Phialophora fastigiata 56A

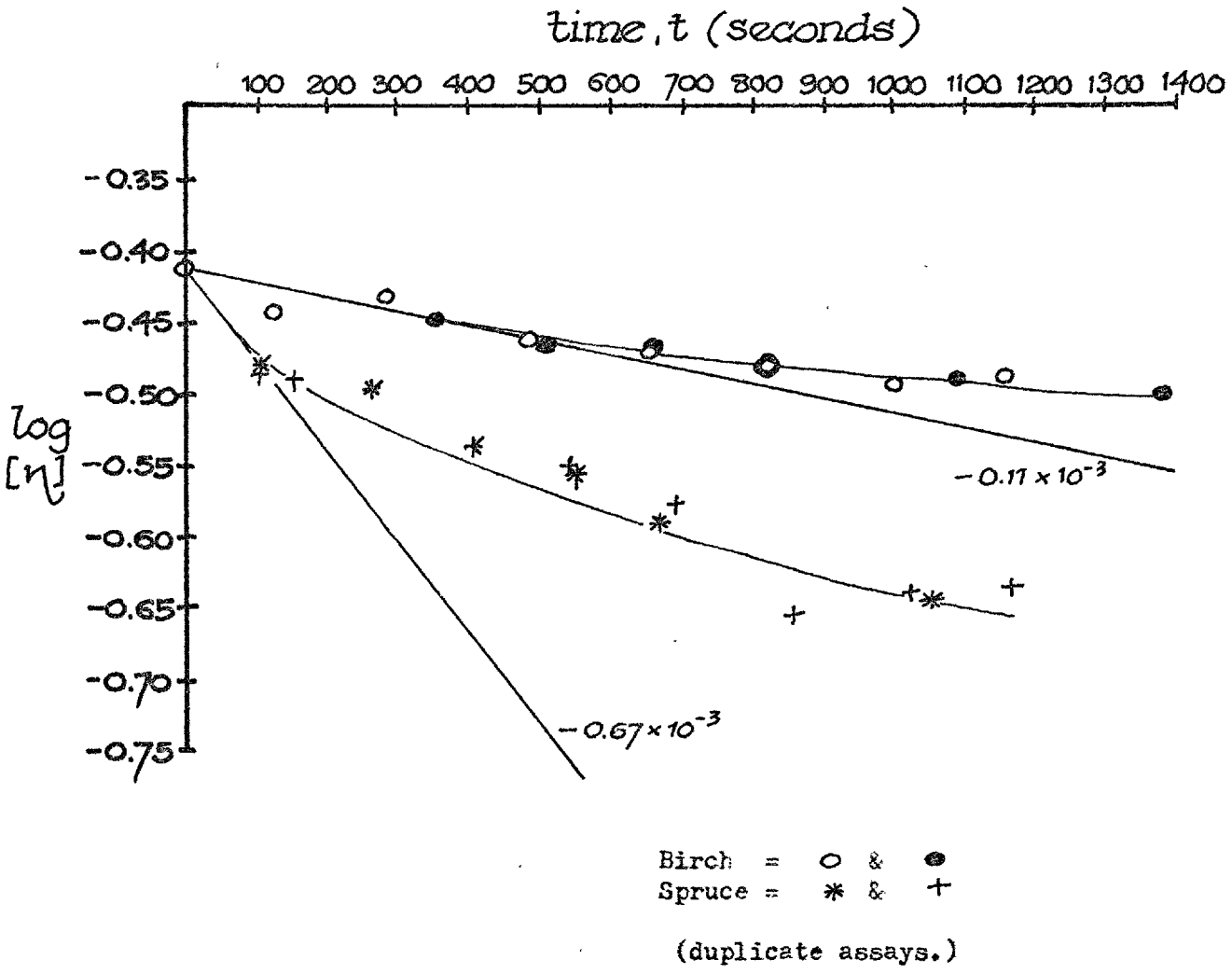
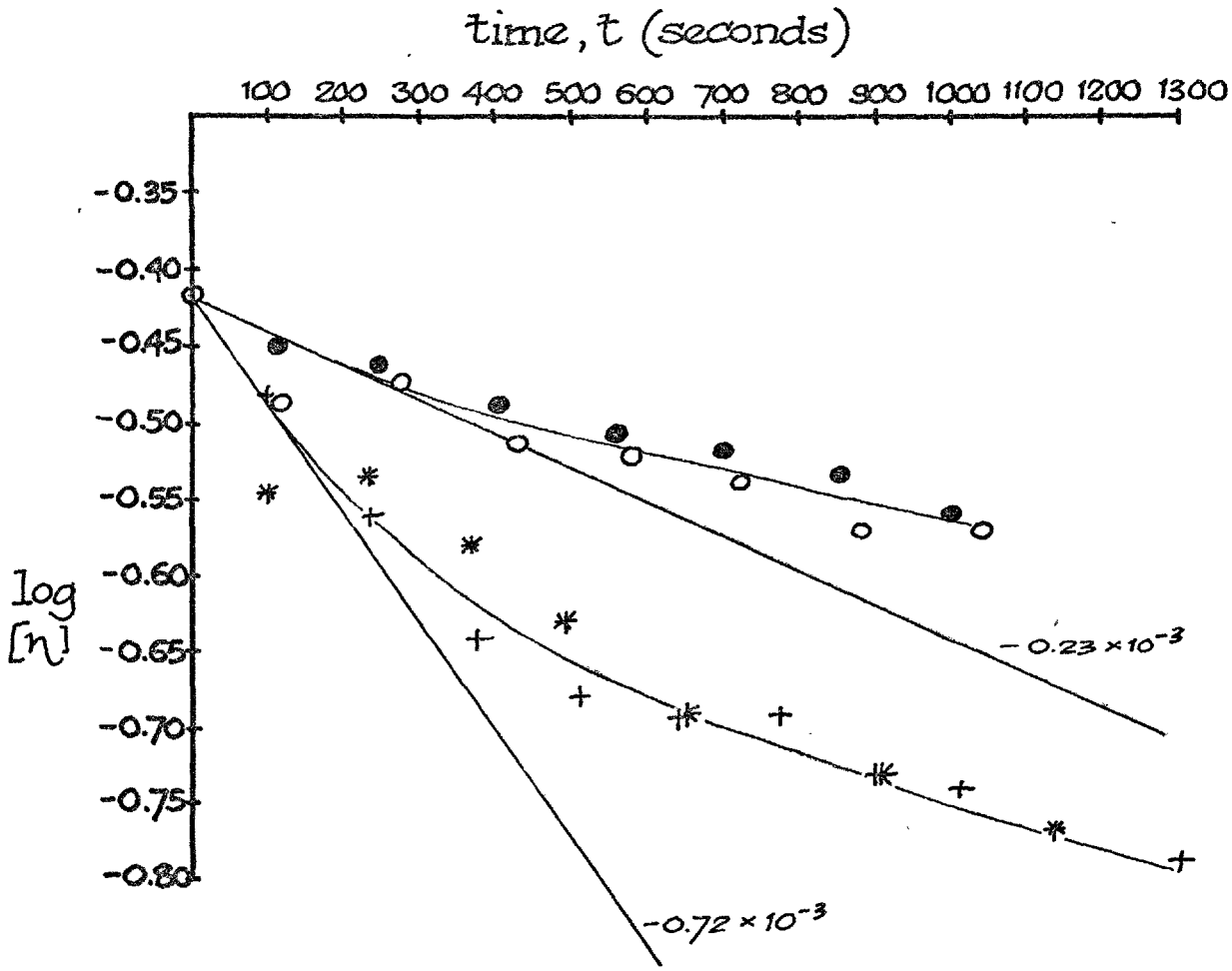


Fig.29

Poria monticola 304 B



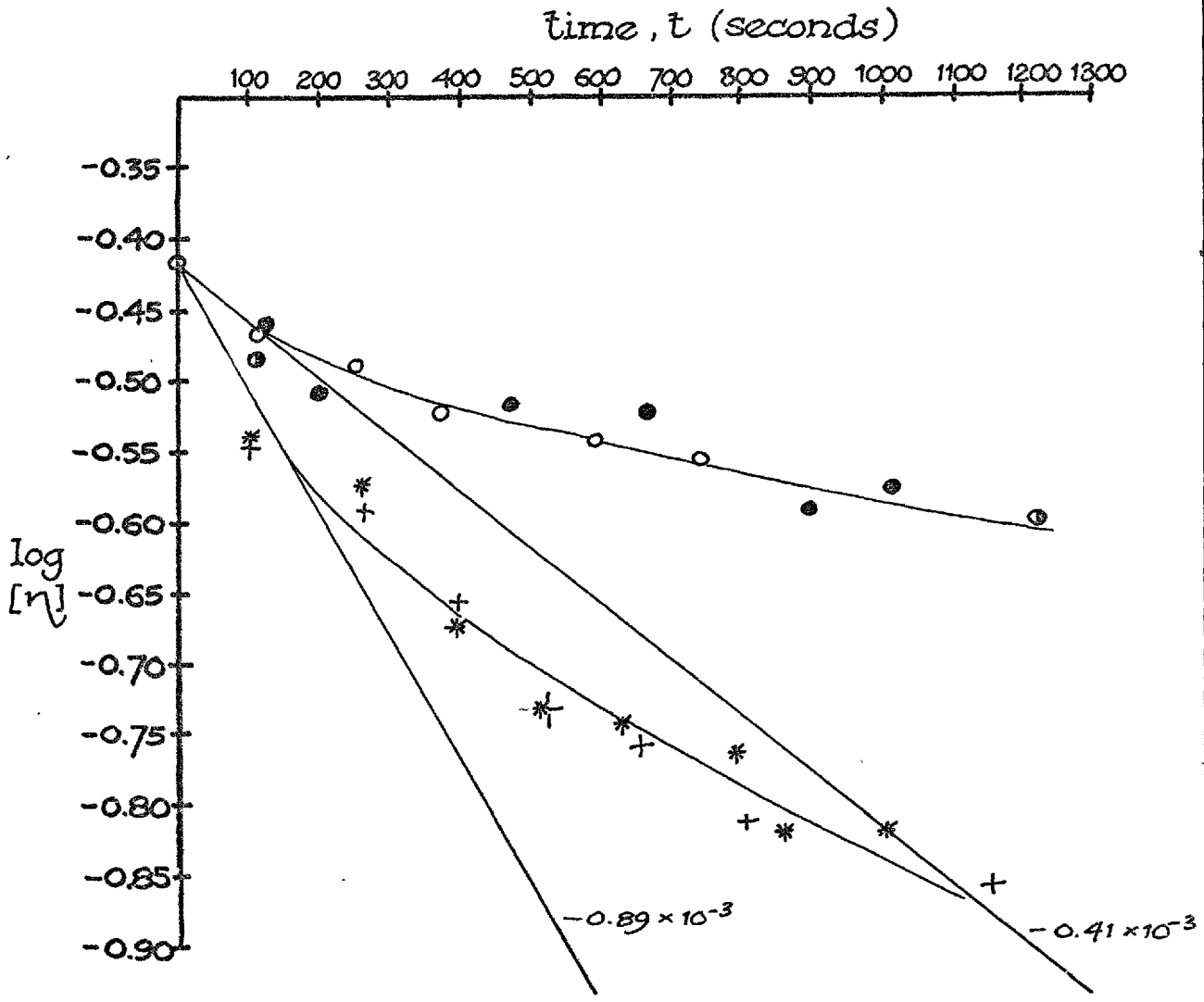
Birch = o & ●

Spruce = + & *

(duplicate assays.)

Fig. 30

Polystictus versicolor



Birch = ○ ● ◑
Spruce = ✱ & +

(Triplicate and duplicate assays.)

Fig. 31

Hemicellulose Cultures

Prior to harvesting the culture filtrates photographs of representative samples were taken and these are shown in Plates D to F.

Discussion of observation of results (Growth)

No. 1

Fungus	*Chaetomium globosum* S70B				
C-source	XYL	XYL	XYL	GGM	GGM
Inoculum type of agar	SPRUCE	BIRCH	MALT	SPRUCE	BIRCH

No. 1 The effect of various types of inoculi were investigated using the fungus *C.globosum* S70B with both xylan and galactoglucomannan as sole C-sources.

On xylan there was an increase in intensity of sporulation from spruce birch malt agar as inoculi. This was particularly pronounced with malt agar as it had been growing for seven days less than the others (31 days). Sporulation and growth was considerably less with glucommannan as sole C-source, the birch agar inoculated fungus only showing marginally more sporulation than the spruce, and the same order of growth.

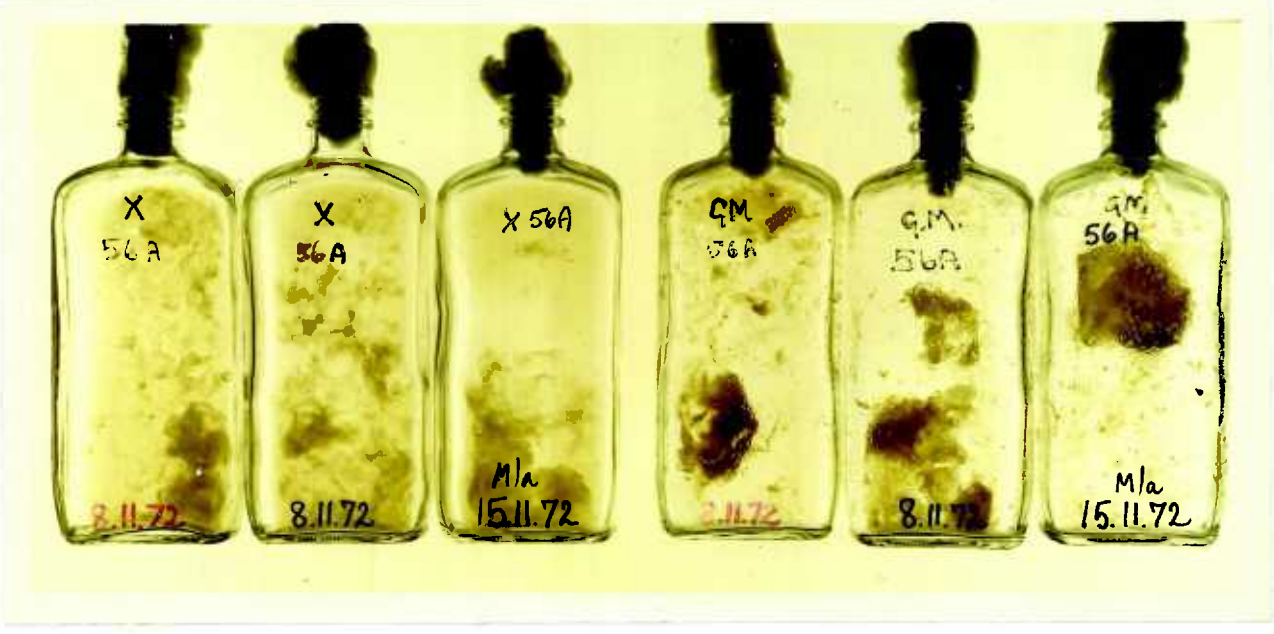
No. 2

Fungus	*Phialophora fastigiata* 56A					
C-source	XYL	XYL	XYL	GGM	GGM	GGM
Inoculum type of agar	SPRUCE	BIRCH	MALT	SPRUCE	BIRCH	MALT

No. 2. This was a similar experiment to No. 1, except that the fungus was *P.fastigiata* 56A. Growth was fairly evenly distributed regardless of the type of inoculum; it was apparent that GGM as sole C-source resulted in slightly more pigmentation.

Plate D


X
S70B
8.11.72
X
S70B
8.11.72
S70B
X
15.11.72
GM
S70B
8.11.72
GM
S70B
8.11.72


X
56A
8.11.72
X
56A
8.11.72
X 56A
M/a
15.11.72
GM
56A
8.11.72
G.M.
56A
8.11.72
GM
56A
M/a
15.11.72

No. 3

fungus	Chaetomium Globosum				
	S70M	S70M	S70B	S70M	S70M
C-source	XYL	XYL	GGM	GGM	GGM
Inoculum type of agar	BIRCH	MALT	SPRUCE	BIRCH	MALT

No. 3 As in No. 1, this experiment tested C.globosum but the strain was different, namely S70M. (An exception was the GGM C-source spruce agar inoculated bottle which was S70B). The results were similar to No. 1, the malt agar inoculated sample showing particularly great sporulation.

No. 4

fungus	Poria monticola 304B				Controls, no fungus	
C-source	XYL	XYL	GGM	GGM	XYL	GGM
Inoculum type of agar	BIRCH	MALT	BIRCH	MALT	-	-

Growth: Birch and Spruce Agar inoculated - 31 days
Malt Agar inoculated - 24 days

No. 4. This was the same as the previous experiments, except that P.monticola 304B was the fungus under observation. Growth was equal between the two C-sources and two un-inoculated controls were included as a means of comparison.

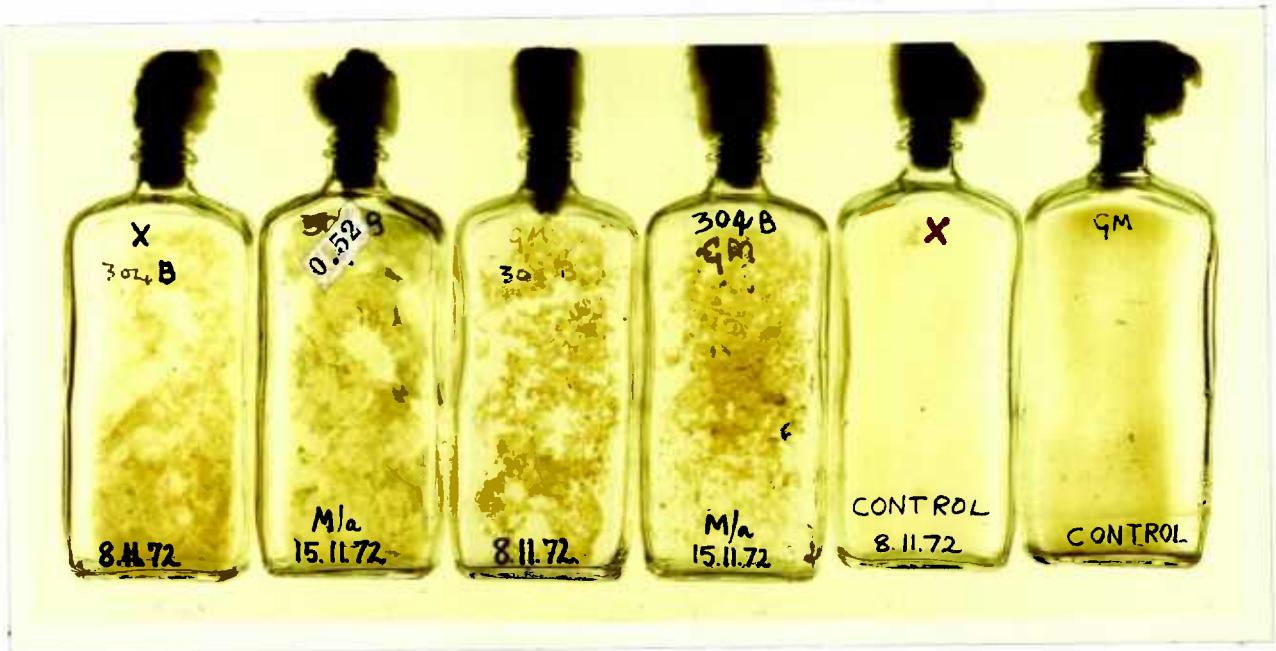
No. 5

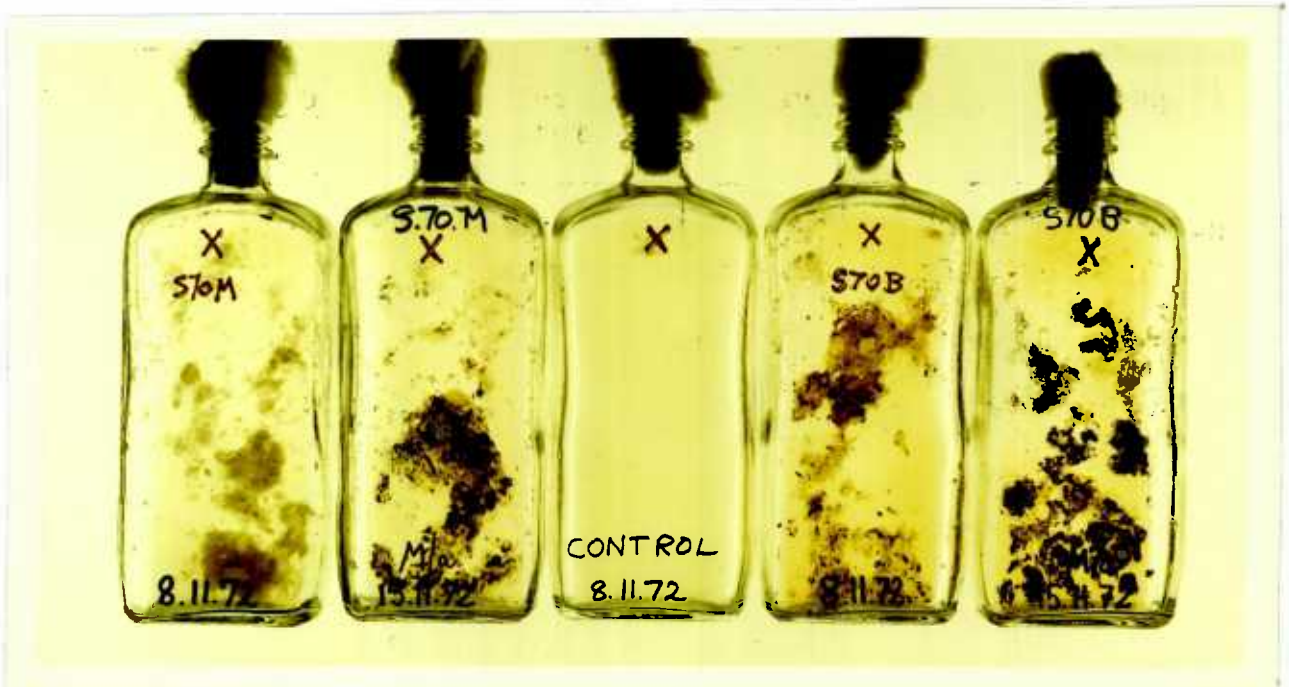
fungus	C. globosum S70M		Control no fungus	C.globosum S70B	
C-source	XYL	XYL	XYL	XYL	XYL
Inoculum type of agar	BIRCH	MALT	-	BIRCH	MALT

No. 5 This was a comparison between two strains of the same fungus C.globosum. S70M and S70B were used with xylan at the sole C-source. It was apparent with both strains that malt agar inoculi gave much more sporulation. S70B was more heavily sporulated than S70M with both types of inoculum. A control bottle in the centre shows no fungal growth.

Plate E


X
570M
570M
X
M/a
8.11.72
15.11.72
GM
570B
GM
570M
570M
GM
8.11.72
8.11.72
M/a
15.11.72


X
304B
0.52B
304B
GM
X
GM
8.11.72
M/a
15.11.72
8.11.72
M/a
15.11.72
CONTROL
8.11.72
CONTROL


X
570M
570M
X
X
X
570B
570B
X
8.11.72
M/a
15.11.72
CONTROL
8.11.72
8.11.72
M/a
15.11.72

No. 6.

fungus	P.fastigiata 56A	P.monticola 304B	C.globosum S70M	C.globosum S70M	No Fungus Control
C-source	XYL	XYL	XYL	XYL	XYL
Inoculum type of agar	BIRCH	BIRCH	BIRCH	BIRCH	-

No. 6.

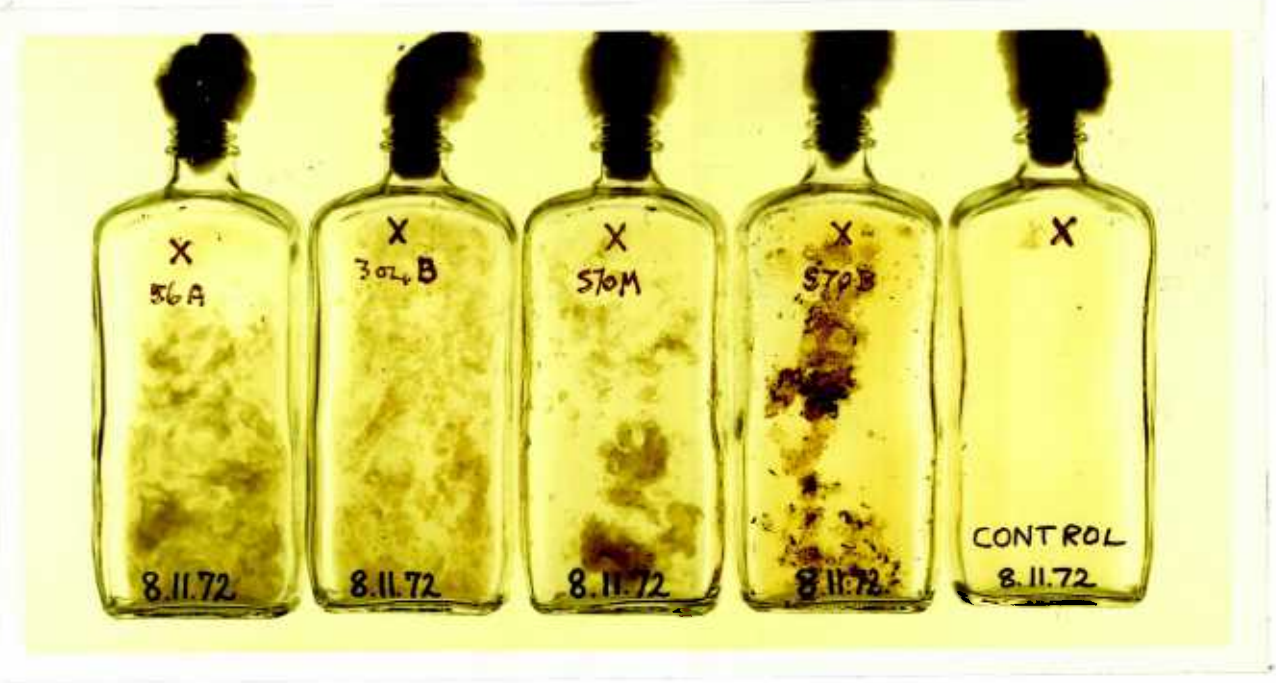
This was with xylan as sole C-source, and birch agar as inoculum. Samples of four fungal strains were represented. P.fastigiata 56A. P.monticola 304B, C.globosum S70M and S70B. The fifth bottle is a control.

No. 7

fungus	P.fastigiata 56A	P.monticola 304B	C.globosum S70M	C.globosum S70B	No Fungus Control
C-source	GGM	GGM	GGM	GGM	GGM
Inoculum type of agar	BIRCH	BIRCH	BIRCH	BIRCH	-

This is similar to No. 6 in all respects except that GGM is used as sole C-source instead of xylan. Growth seemed less pronounced than on xylan. C.globosum hardly sporulated at all. The other two species were not similarly affected, growth being of equivalent magnitude on either xylan or GGM.

Plate F


X
56A
8.11.72
X
304B
8.11.72
X
570M
8.11.72
X
570B
8.11.72
X
CONTROL
8.11.72


GM
56A
8.11.72
GM
304-3
8.11.72
GM
570M
8.11.72
GM
570B
8.11.72
GM
CONTROL

Hemicellulose Growth Experiment Fig. 32.

Table shewing the final pH, and the number of µls. of reagent required to obtain pH 5.0 (hemicellulose cultures.)

Fungus	Carbon source GGM				Carbon source XYL			
Inoculum type	No. of flats	No. of µls. acid/base a & b	Total vol.	pH	No. of flats	No. of µls. acid/base a & b	Total vol.	pH
C. globosum S70B			mls.				mls.	
Spruce agar	2	140 b	45	4.73	5	no result	-	5.69
Birch agar	3	195 a	65	5.67	5	220 a	70	5.72
Malt agar	1	lost during incubation			1	no result	-	5.69
P. fastigiata 56A								
Spruce agar	2	140 b	45	2.91	5	140 b	45	3.93
Birch agar	3	165 b	65	3.01	5	140 b	45	4.16
Malt agar	1	33 b	10	2.95	1	30 b	10	3.91
C. globosum S70M								
Spruce agar	2	140 b	45	4.85	5	140 a	45	5.54
Birch agar	3	contaminated			5	contaminated		
Malt agar	2	110 a	35	5.42	4	180 a	58	5.62
P. monticola 304B								
Birch agar	2	140 b	45	2.97	4	195 b	65	4.68
Malt agar	4	263 b	85	2.91	4	195 b	65	3.00

a = acid, 1N hydrochloric

b = base, 4N sodium hydroxide.

The hemicellulose culture filtrates were tested for reducing sugar activity with three different substrates; C.M.C., XYL and GGM using the 60 minute incubation period assay. The results obtained with both GGM induced and XYL induced culture filtrates are given below.

Enzymic activities of hemicellulose cultures. Fig. 33

		Hemicellulose used as C-source in liquid culture					
		GGM induced			XYL induced		
Fungus	Type of inoculum	CMC-ase µgms.	XYL-ase µgms.	GGM-ase µgms.	CMC-ase µgms.	XYL-ase µgms	GGM-ase µgms
C. globosum S70B	Spruce agar	45	2	25	161	157	230
	Birch agar	35	3	35	194	177	209
	No malt agar experiment						
P. fastigiata 56A	Spruce agar	-4	1	-4	12	18	35
	Birch agar	-3	3	14	16	20	43
	Malt agar	2	7	11	15	28	44
C. globosum S70M	No spruce agar experiment						
	Birch agar	34	2	27	142	148	159
	Malt agar	20	4	34	151	193	229
P. monticola 304B	No spruce agar experiment						
	Birch agar	120	41	186	122	64	312
	Malt agar	126	43	196	126	64	260

The following points were noted:

1. Without exception, the XYL - induced culture filtrates were more active than their GGM induced counterparts.

2. In the GGM induced filtrates P.monticola was considerably more active than the other three fungi.

3. In the XYL induced filtrates P.fastigiata was considerably less active than the other three fungi.

4. The differences between C.globosum S70M and S70B both on XYL and GGM were not significant with the exception of birch agar inoculated, XYL induced, S 70B having slightly higher values than S70M.

5. In all the GGM induced cultures, activity did not vary much with the types of inocula. The same was true for XYL induced filtrates of P.monticola and P.fastigiata. However, with the C.globosum filtrates, activity showed an increasing trend from spruce agar, to birch agar, to malt agar inocula.

6. XYL-ase activities were consistently low when the fungi were grown on GGM ; cellulase and GGM-ase seemed,however, to be equally induced.

7. GGM-ase activities were consistently and surprisingly high; when the fungi were grown on XYL, they were without exception greater than both the cellulase and xylanase activities.

8. P. fastigiata grown on GGM produced very little of any enzymic activity, the greatest being when the inoculum used was grown on malt agar. On XYL activities were much higher all round, although still not large compared with C. globosum.

9. P. monticola consistently produced high cellulase and GGM - ase and very low XYL - ase activities, with both types of C-source.

10. pH changes were more pronounced than when the fungi were cultured on wood, but the trends were similar.(i.e.C. globosum rose, the other fungi fell.)

11. To the naked eye, growth of P. fastigiata on GGM was equal to the other fungi, and yet it gave low results for the three enzymes screened. The question that must be asked is, on what is P. fastigiata living when it is in culture on GGM? Further tests to establish this point might prove useful.

S.E.M.

General Introduction

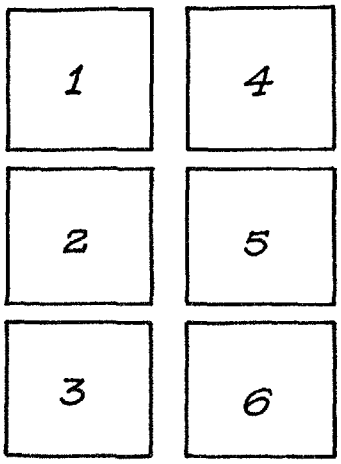
Although the initial objective of obtaining a correlation between biochemical changes and observed micromorphological patterns of decay, was not attained, the S.E.M. photographs have consolidated and expanded the present fund of knowledge in this field. In particular the technique of serial photography using the SEM, as demonstrated in Plate 19, has proved to be a useful form of visualizing larger areas of wood sections at high magnifications.

Plates 1 to 9 deal specifically with the colonisation of wood by the fungus C. globosum. Plates 10-13 are concerned with P. monticola, whilst Plates 14 and 15 deal with P. fastigiata. Plates 16 - 19 deal with general aspects not covered in the previous photographs, and may not be specifically restricted to any one fungus.

Scanning Electron Microscopy

Discussion of Pictures

The following notation will be used in respect to the arrangement of scanning electron micrographs.



i.e. The top L.H.S. photograph is termed No. 1 and the bottom R.H.S. photograph is termed No. 6.

Captions are at the bottom of their respective photographs, and discussion for each individual plate is on the opposite facing page. Magnifications are approximate in as much as the exact figure given represents a mean. It is probable that there is a $\pm$ 5% variation. The tilt angle of the specimen is 45° unless otherwise stipulated.

L.H.S. means: Left hand side

R.H.S. means: Right hand side

A.D. =Air dried.

F.D. = Freeze dried.

All photographs were taken at 20 kV.

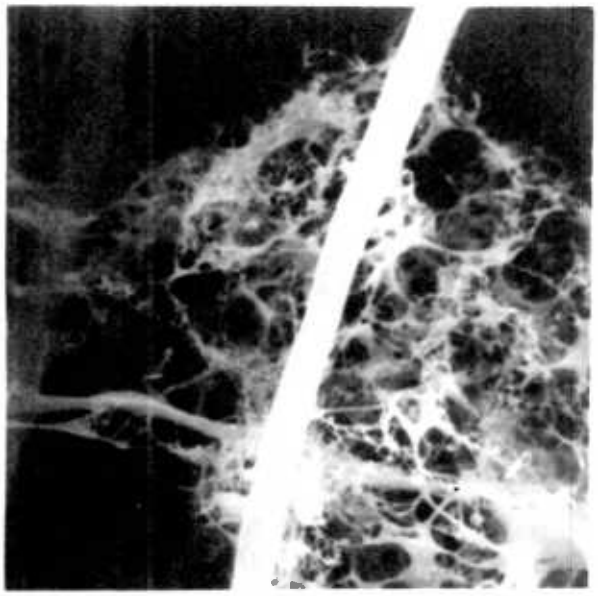
Plate 1

This a series of six photographs of the same strain of fungus C.globosum RLB exhibiting different shapes of individual hyphae. All are birch wood except No. 2 which is beech. No. 1 is an example of the large bubbly hyphal type, which throughout this work has only been found on the exterior of a wood block. No. 2 is representative of a normal single filament type of foraging hypha, with the proviso that the small thread-like attachments to the cell wall have not been seen before. No. 3 is an example of "sparse diffuse" hyphae, whilst No.4 is of "compact diffuse" or "cotton wool - like" hyphae. Photograph No. 5 contains both a small section of "bubbly" hyphae and an example of an intermediate shape between the filamentous and diffuse hyphae. Stub No. 6 is a highly magnified view of a small vesicle in the hyphal material, of which the function is unknown. It is to be emphasized that all these photographs were derived from the same pure strain of R.L.B. Although better examples of the characteristic morphological types could be found in other strains it was decided to present these six photographs of a single strain to demonstrate this point.



781-13 C. globosum RLB x 7696

Birch R.L.S. F.D. 5 months



784-9 C. globosum RLB x 3810

Birch T.S. F.D. 5 months



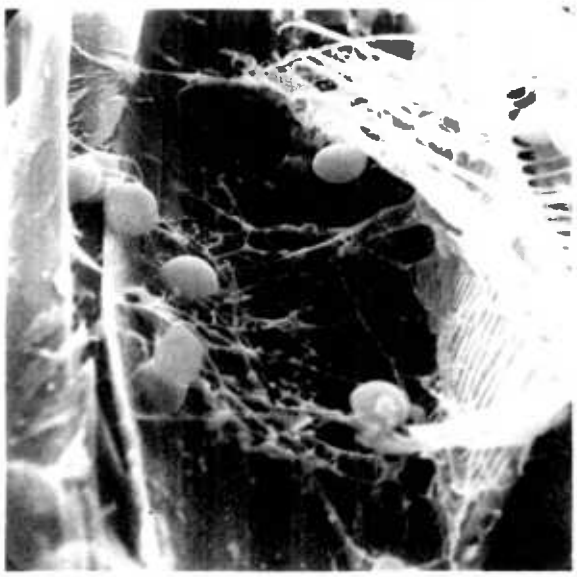
14546 C. globosum R B x 1882

Beech R.L.S. F.D. 3 weeks



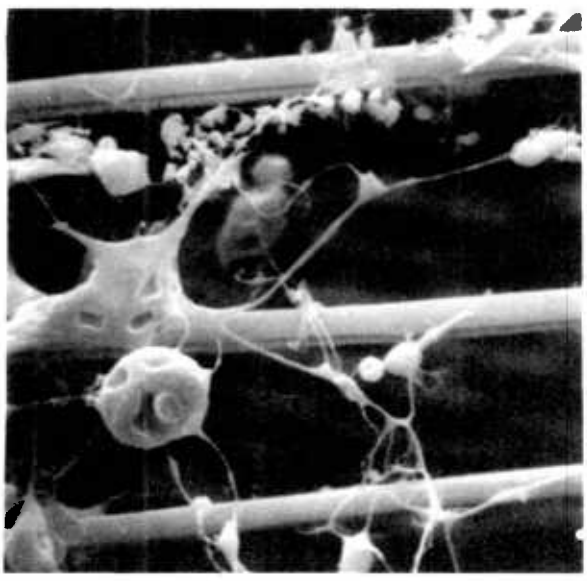
781-8 C. globosum RLB x 1676

Birch R.L.S. F.D. 5 months



250-18 C. globosum RLB x 991

Birch R.L.S. F.D. 3 weeks



780-24 C. globosum R.B x 3848

Birch R.L.S. F.D. 5 months

Plate 2.

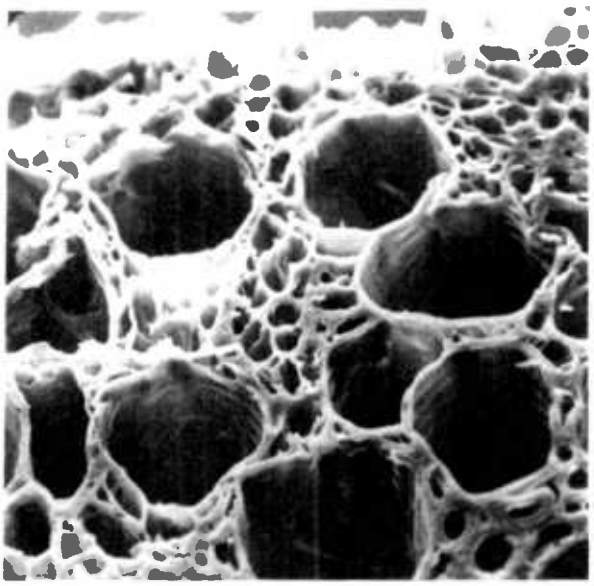
These are all beech wood in T.S. having been attacked by C. globosum S70 for 8 weeks. All were taken at 45° tilt except the bottom R.H.S. picture No. 6.

No. 1 shows a general view of a beech sample on a stub. Note that the section from which the T.S. was cut is particularly thin in the radial dimension, so as to enable the resultant T.S. to be cut with a single stroke of the razor blade (see preparation of S.E.M. stubs). The glue-like material covering the central portion of the wood sample is a conductor "silverdag", which enhances the electrical contact between the specimen and the stub. No. 2 shows the left hand portion of the specimen in more detail. The striking point of interest is the large ray which is a characteristic of beech wood. No. 3 shows an area which seems to be unaffected by the fungus and is characteristic of healthy wood. The first three photographs form a sequence of increasing magnification. Nos. 4, 5 and 6 are another sequence, showing increasing colonization and degradation by the fungus. Of particular interest is No. 6 where great numbers of hyphae can be seen in the vessels. Visualization of this is aided by a specimen tilt angle of 0°. (i.e. electron beam normal to the specimen).



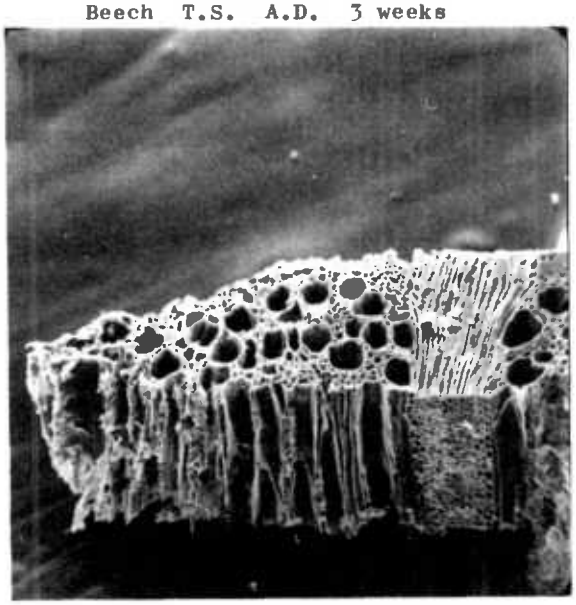
13537 C. globosum S70 x 18

Beech T.S. A.D. 3 weeks



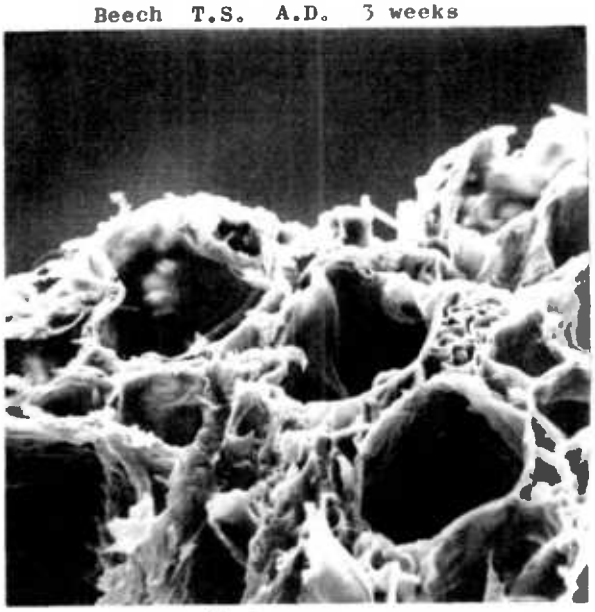
13540 C. globosum S70 x 465

Beech T.S. A.D. 3 weeks



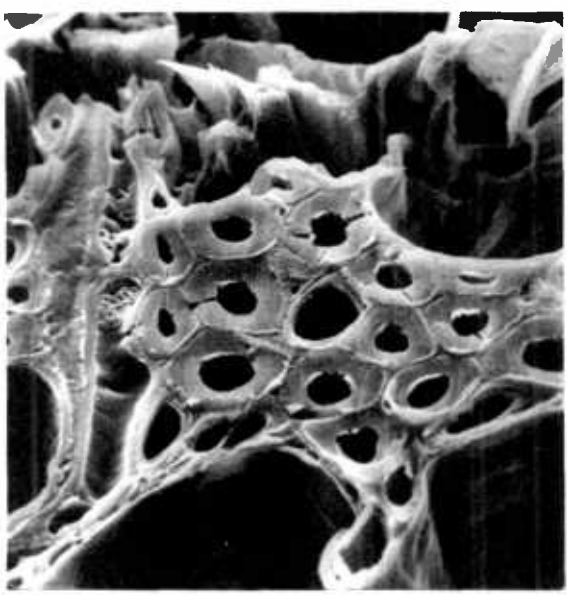
13538 C. globosum S70 x 92

Beech T.S. A.D. 3 weeks



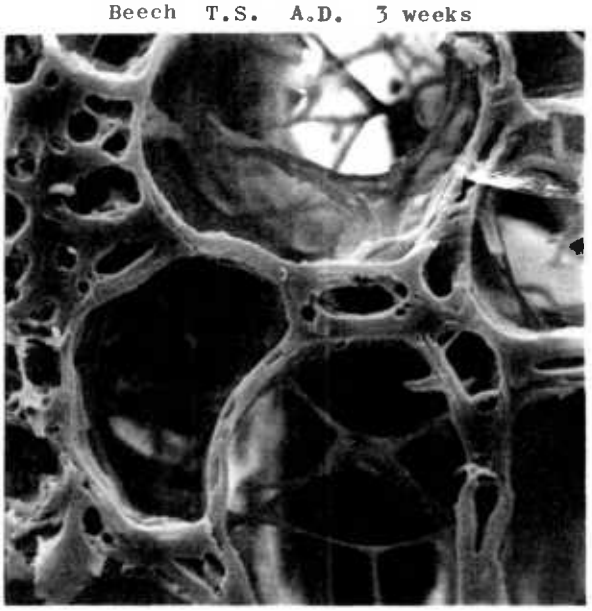
13539 C. globosum S70 x 465

Beech T.S. A.D. 3 weeks



13539 C. globosum S70 x 937

Beech T.S. A.D. 3 weeks



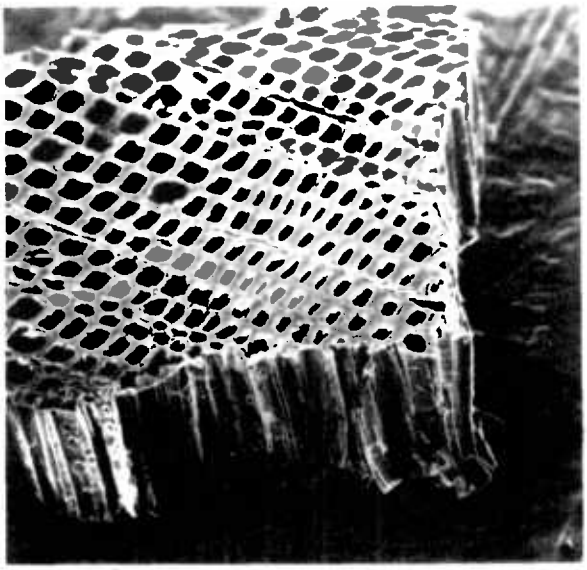
13543 C. globosum S70 x 846

Beech T.S. 0^{o} tilt A.D. 3 weeks

Plate 3.

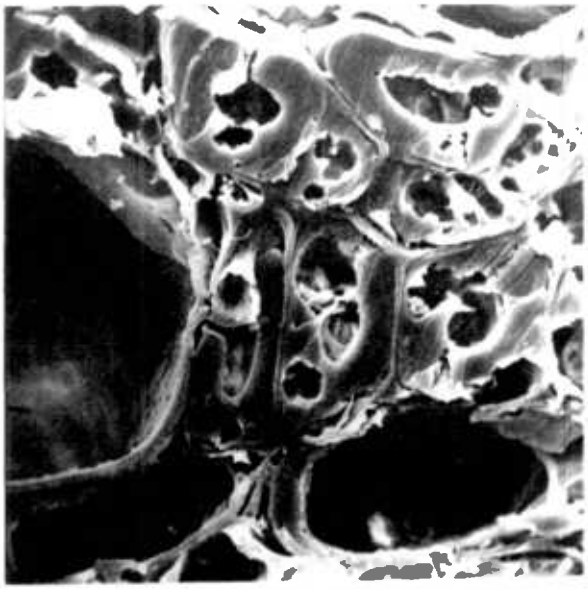
This again shows soft rot attack by C. globosum in T.S. Nos. 1, 2 and 3 are Norway spruce, whilst Nos. 4, 5 and 6 are beech. The woods were subjected to fungal decay for three weeks and then freeze dried. In the softwood three different strains of C. globosum are shown, S70L, S70N and S70B respectively. In all cases the wood appears quite healthy and undegraded. Although not visible in these photographs, the fungus has been observed to inhabit the softwood sparsely in the lumen and in greater amounts on the outside surface of the block. No visible degradation in the S_2 layer was apparent. This contrasts with the hardwood series all of which are the S70M strain. Nos. 4, 5 and 6 show an increase in attack and are all viewed at 0^{o} tilt.

Decay has occurred mainly in the S_2 layer and eventually has resulted in a skeletal middle lamellar region, as can be seen in No. 6 and parts of No. 5. The specimens for these three photographs were taken from a single block which had been subjected to fungal decay for three weeks. Towards the centre of the block there is very little decay and the intensity of attack increased as samples were taken from progressively nearer the surface. One of the reasons for this was probably a function of the time taken by the fungus to reach a central location. The sequence shows this trend. Bravery (1972) has observed similar attack "gradients".



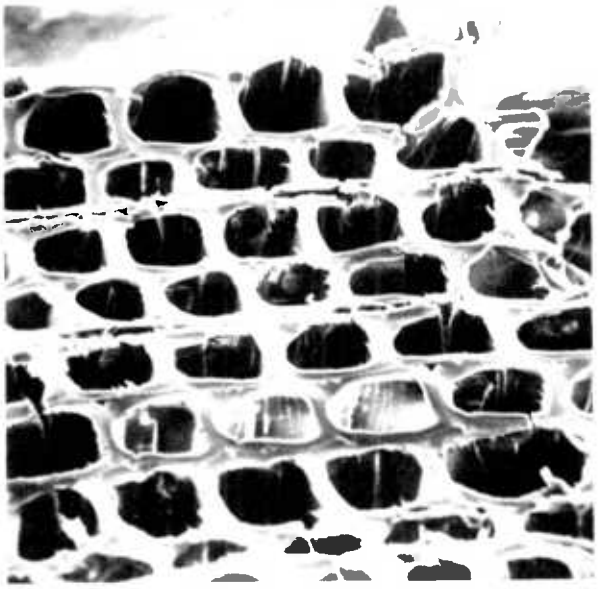
756-5 C. globosum S70L x 156

Spruce T.S. F.D. 3 weeks



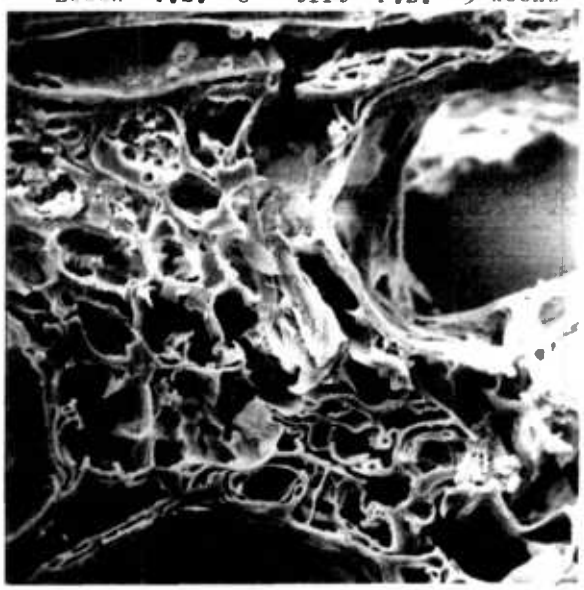
14453 C. globosum S70M x 1600

Beech T.S. 0° tilt F.D. 3 weeks



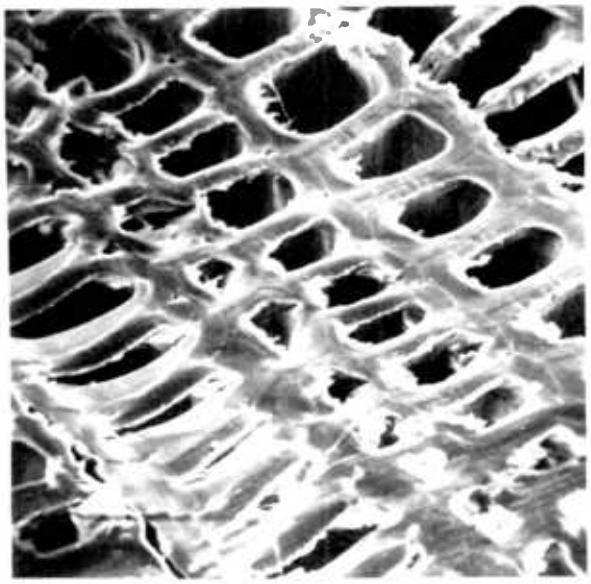
756-11 C. globosum S70N x 419

Spruce T.S. F.D. 3 weeks



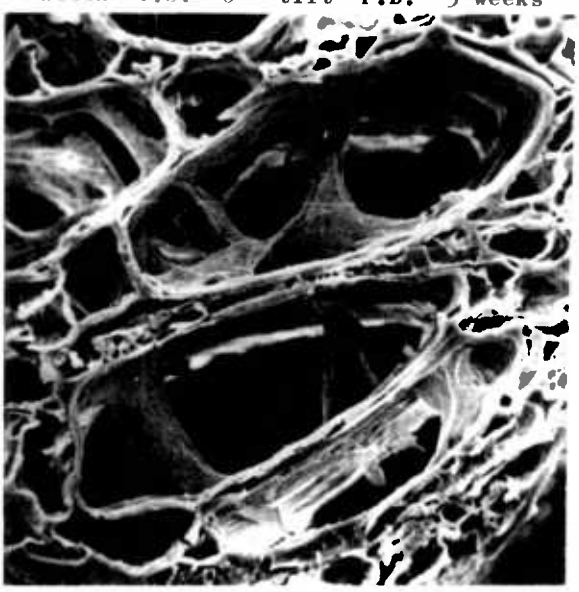
14455 C. globosum S70M x 1600

Beech T.S. 0° tilt F.D. 3 weeks



754-21 C. globosum S70B x 1288

Spruce T.S. F.D. 3 weeks



14457 C. globosum S70M x 800

Beech T.S. 0° tilt F.D. 3 weeks

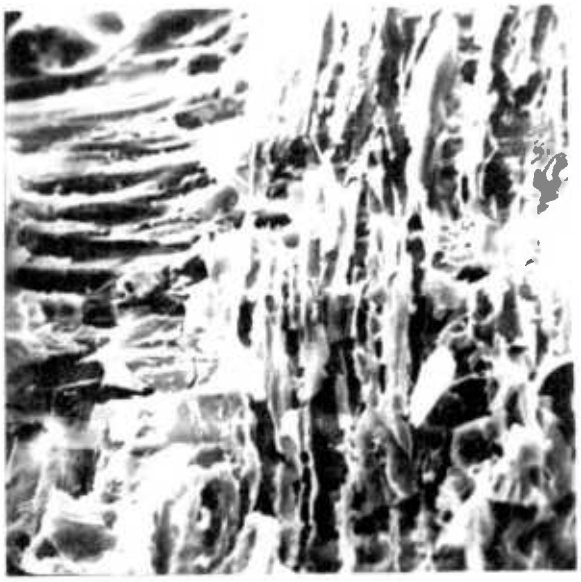
Plate 4.

This demonstrates in R.L.S. the action of C.globosum on beech wood for three weeks. No. 1, 2 and 3 are strain S70, whereas No. 4, 5 and 6 are strains S70L, S70 and S70B respectively. No. 1 shows a general view of beech R.L.S., in the centre of which is a large ray. Note that small rays are also present. Nos. 2 and 3 are higher power magnifications of this ray area, showing the abnormal way in which the ray material has ruptured after fungal attack and also the small holes which are possibly enlarged pits. Nos. 4 and 5 show similar damage to fibre material, and in No. 6 hyphae can be clearly seen.



13496 C. globosum S70 x 44

Beech R.L.S. A.D. 3 weeks



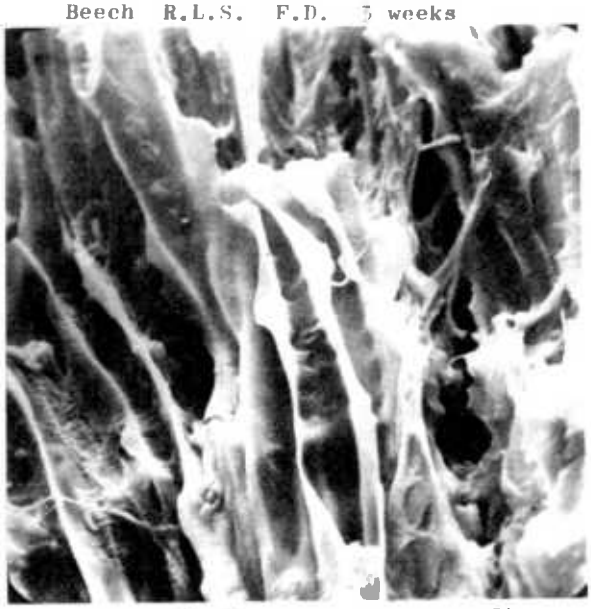
14115 C. globosum S70L x 472

Beech R.L.S. F.D. 3 weeks



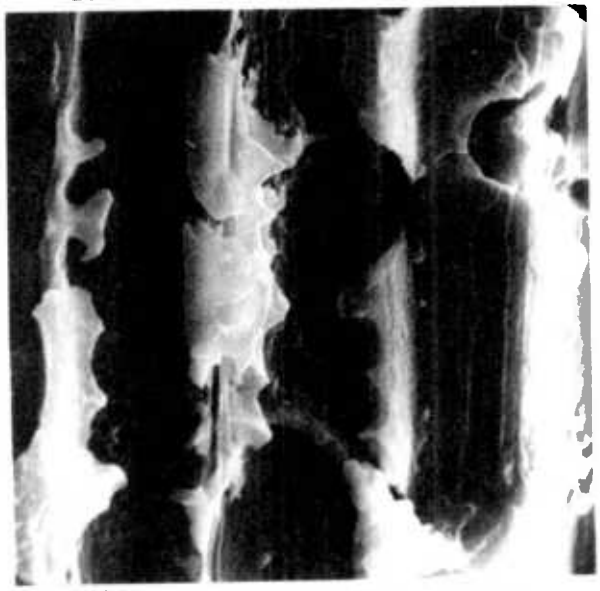
13500 C. globosum S70 x 884

Beech R.L.S. A.D. 3 weeks



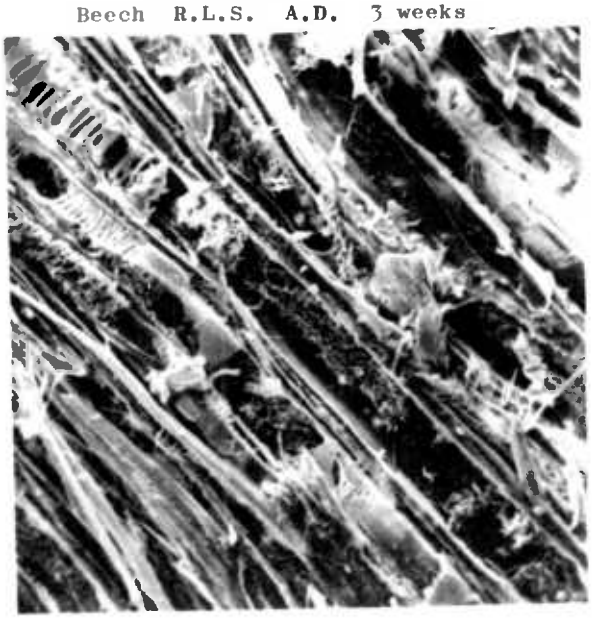
13505 C. globosum S70 x 884

Beech R.L.S. A.D. 3 weeks



13499 C. globosum S70 x 1733

Beech R.L.S. A.D. 3 weeks



10-25 C. globosum S70B x 167

Beech R.L.S. 0° III F.D. 3 weeks

Plate 5

This plate is an example of the action of several strains of C. globosum on birch wood. (Split in L.S.) No. 1 is S70M (T.L.S.) after 5 months of decay; the section was taken from the centre of the block because the outside was very brittle after such long exposure to the fungus. Hyphae are abundant in the lumena, but it is difficult to gauge the attack to the S_2 layer in this type of section. Spores are visible on the section, although it is more probable that these were deposited during the preparation. No. 2 is the cross-field area of a ray showing the debris remaining after five months' attack. No. 3 is strain S70B looking filamentous and like cotton-wool in a birch vessel, behind an undegraded scalariform end plate. The typical resistance of vessel wall material even after five months decay is characteristic. No. 4 (S70N) shows a single strand foraging hypha whilst No. 5 (S70), though no hypha is present, shows signs of severe degradation. No. 6 (S70E) is a lower power view of a similar scene of attack to No. 2. There is more debris present and the hyphal remains can be seen, particularly at the R.H.S.



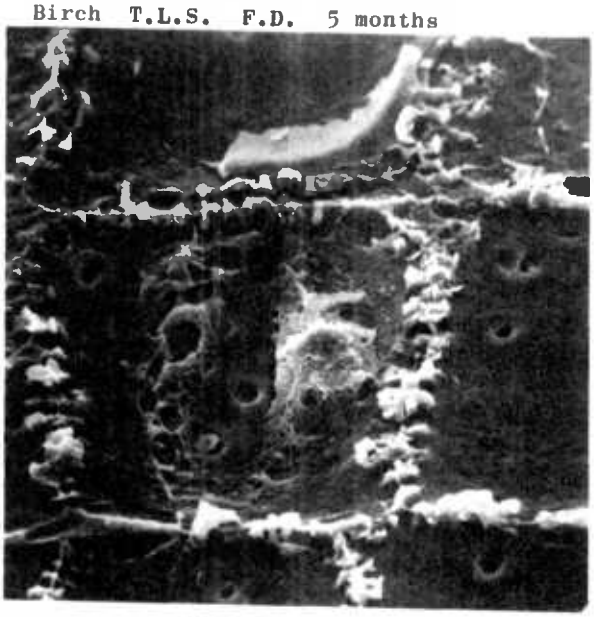
215-5 C. globosum S70M x 518

Birch T.L.S. F.D. 5 months



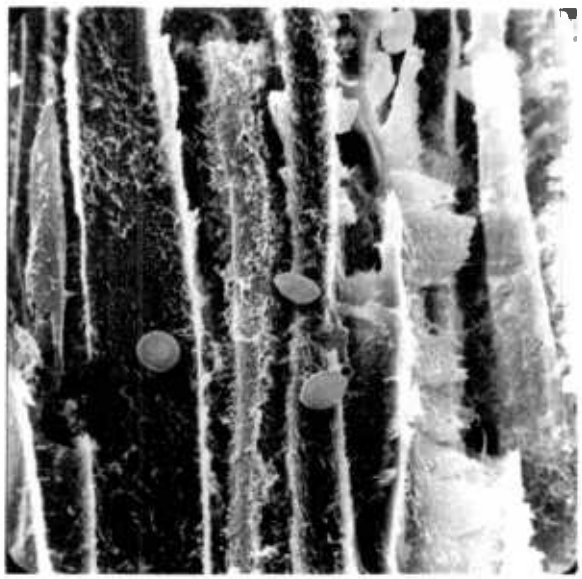
246-3 C. globosum S70N x 838

Birch T.L.S. F.D. 5 months



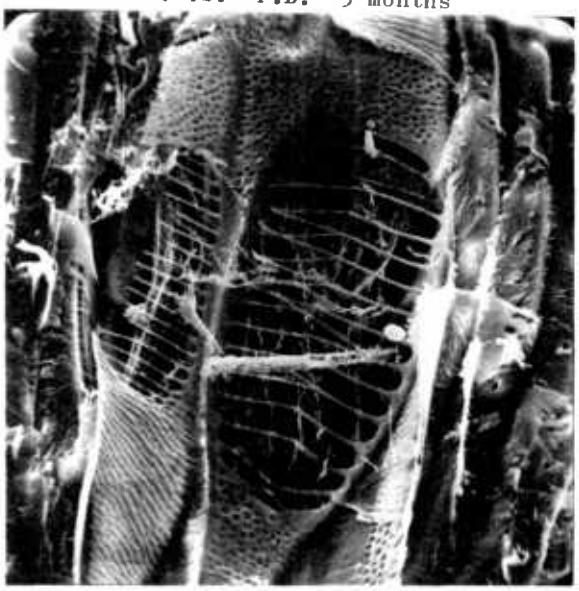
781-2 C. globosum RLB x 1524

Birch R.L.S. F.D. 5 months



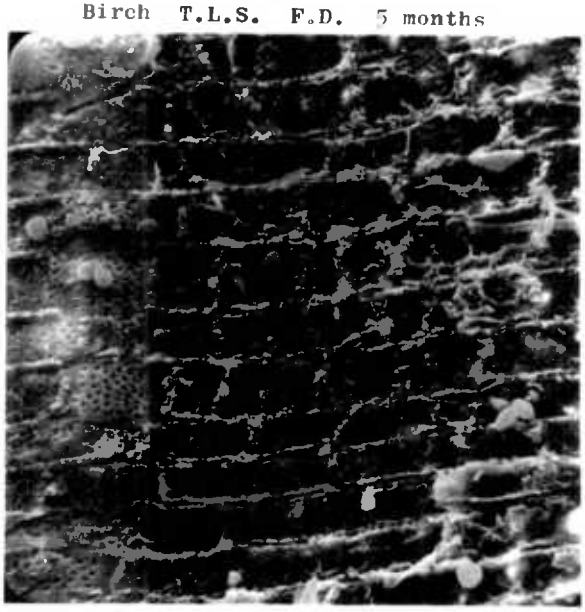
204-2 C. globosum S70 x 419

Birch T.L.S. F.D. 5 months



206-20 C. globosum S70B x 396

Birch R.L.S. F.D. 5 months



204-13 C. globosum S70E x 454

Birch R.L.S. F.D. 5 months

Plate 6

This shows C. globosum on Norway spruce in L.S. attacked for 5 months. No. 1 (S70) is a T.L.S. and the hyphae are plentiful. Note that the cotton wool texture is quite unlike the long foraging filaments usually characteristic of hyphal structure. No. 2 (S70E) shows a slightly different shape which the hyphae can possess. Note the webbed and branched appearance. No. 3 (S70G) is similar. No. 4 (S70K) is like No. 1, but looks as though the fungal infestation is even greater. This contrasts strongly with No. 5 (S70M), which is an example of a "normal" single filament shape. A higher magnification photograph of the "cotton wool" or diffuse hyphae is shown in No. 6 (S70N). During this work it became apparent that many different forms of hyphal shape could exist in the same block of wood. In spruce wood, however, the diffuse type appeared to predominate. Although the hyphae were present in reasonable quantities it was clear, both from the state of the block when subjected to splitting and from the appearance of the wood under the microscope, that very little damage had occurred to the soft wood when subjected to any strain of C. globosum. This finding was true for C. globosum grown either on birch or spruce agar. Almost all workers in this field, for example Nilsson (1973) and Fuller (1970), have been able to obtain appreciable, and in some cases severe (Banerjee, 1969, Chou, 1971) attacks of softwoods by C. globosum. In all such cases the fungus was cultured on a nutrient medium such as malt agar, or Abram's cellulose agar. This appears to be the only different factor between their work and the present experiments. It appears conclusive that the culture medium plays a significant role in determining the type and extent of attack when this fungus inhabits wood. Banerjee (1969) found that the nature of the nutrient agar used had a profound effect on the cavity formation caused by C. globosum.



204-6 C. globosum S70 x 412

Spruce T.L.S. F.D. 5 months



214-7 C. globosum S70K x 454

Spruce T.L.S. F.D. 5 months



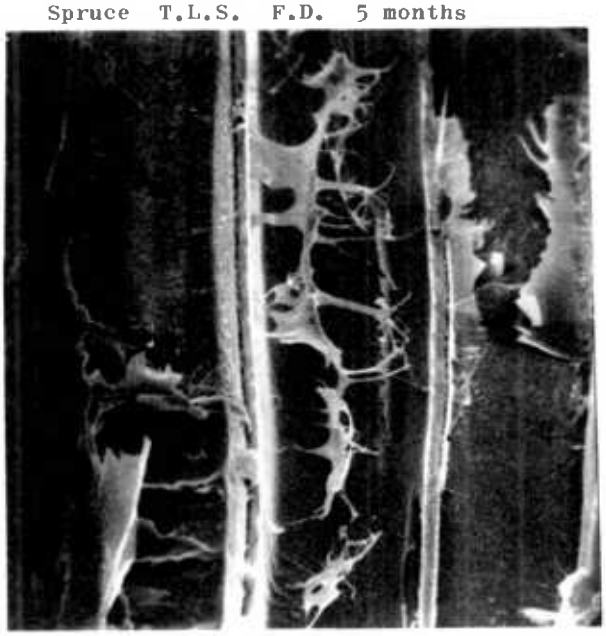
794-10 C. globosum S70E x 762

Spruce T.L.S. F.D. 5 months



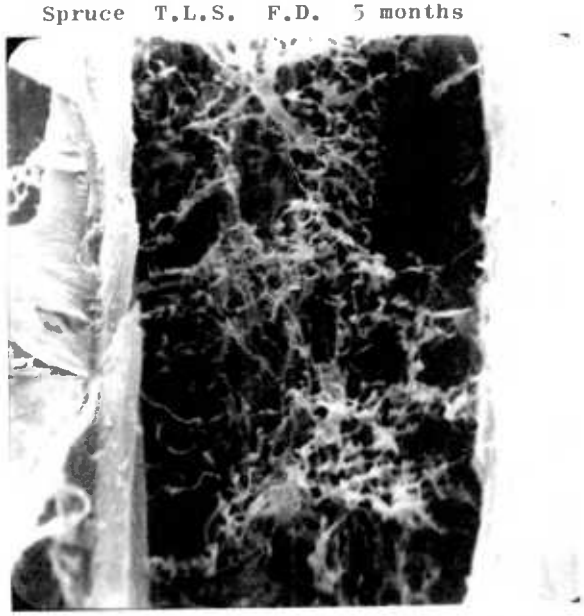
245-24 C. globosum S70M x 876

Spruce T.L.S. F.D. 5 months



796-15 C. globosum S706 x 705

Spruce R.L.S. F.D. 5 months

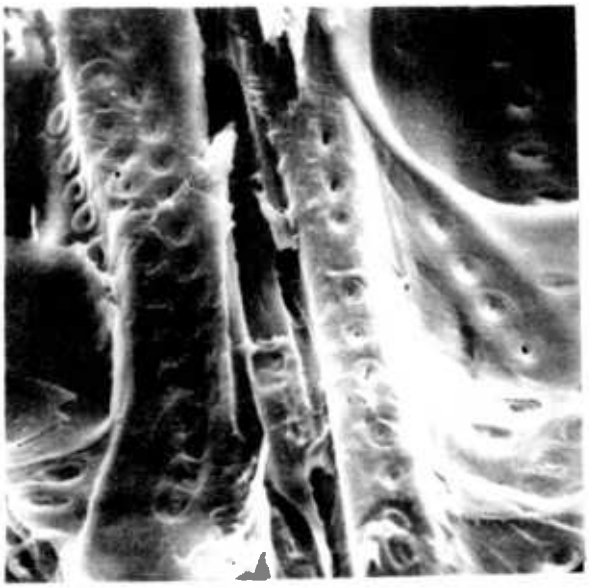


246-13 C. globosum S70N x 1676

Spruce R.L.S. F.D. 5 months

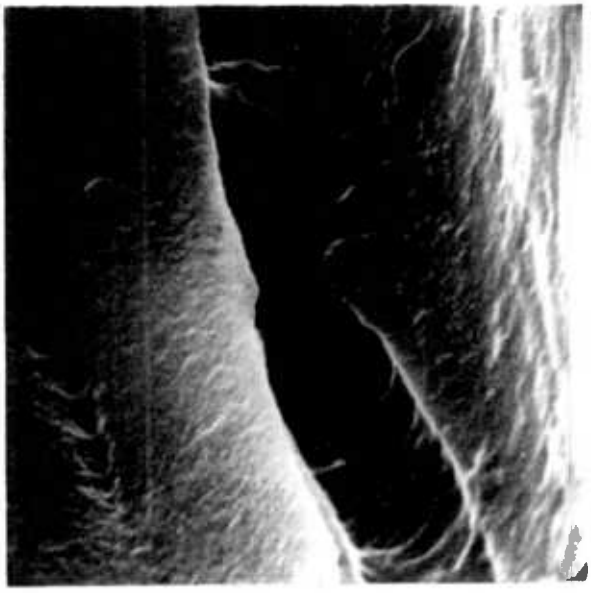
Plate 7

This is C. globosum (S70B) on beech wood after decay for three weeks. A sequence of increasing magnification is shown of a groove or tunnel caused by fungal action. Note that the angle to the longitudinal axis of the cell is about 10^{o} or 15^{o}, and follows the axis of the microfibrils. No hyphal material is apparent, but this could well be due to the air drying used on this specimen. To the L.H.S. of the groove a 'cave-in' or weakness is visible, which possibly indicates the presence of another, hidden tunnel. This kind of degradation is one characteristic of soft-rotted wood and, although easily visible in the light microscope and in T.S., has rarely been seen in longitudinal splits prepared in this manner.



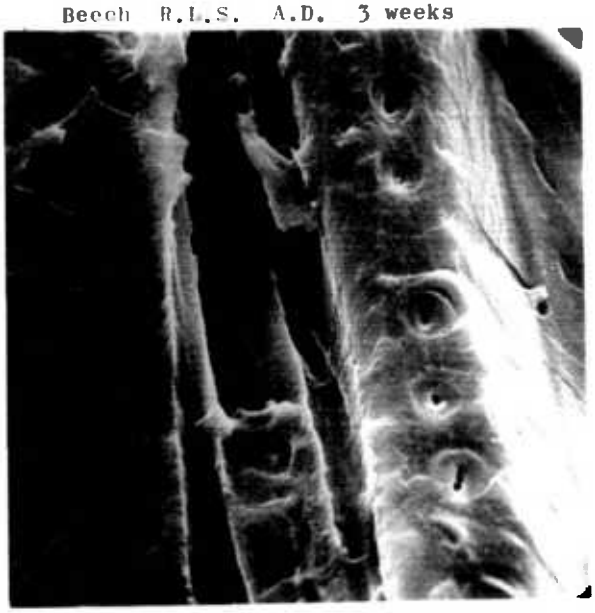
13521 C. globosum S70B x 853

Beech R.L.S. A.D. 3 weeks



13524 C. globosum S70B x 8382

Beech R.L.S. A.D. 3 weeks



13522 C. globosum S70B x 1692

Beech R.L.S. A.D. 3 weeks



13525 C. globosum S70B x 4267

Beech R.L.S. A.D. 3 weeks

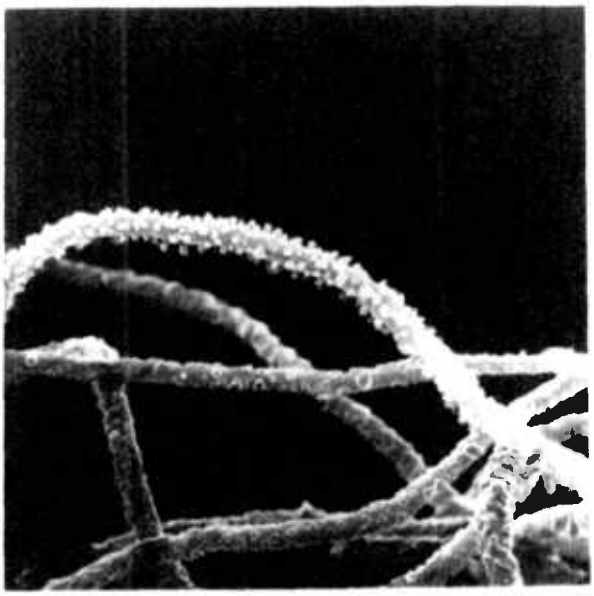
Plate 8

This is the freeze-dried hyphal material of C. globosum RLB scraped off a hardwood block. An increase in magnification from 14.5x to 7,400x is displayed over No. 1 to No. 6. Above magnification of about 1000x it is apparent that the hyphae are covered with small protuberances or 'bubbles'. On certain hyphae these are missing and circular disc-like remains are left. As far as is known the only similar photograph to have been published is by Locci (1971) whilst investigating the fungal diseases of rice, including colonization by C. globosum. His photographs show the fungus on the exterior of a rice grain and no picture of any sections of the interior were present. Most of the hyphae were smooth, but one photograph termed by this worker as "an encrusted hair" was identical with the photographs opposite.



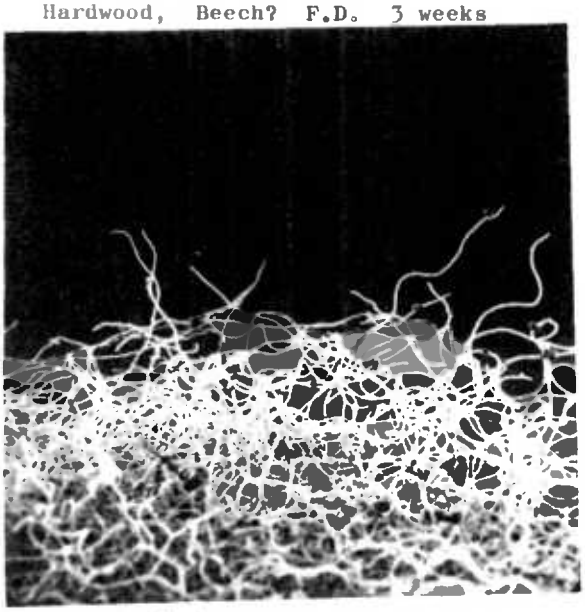
14420 C. globosum RLB x 15

Hardwood, Beech? F.D. 3 weeks



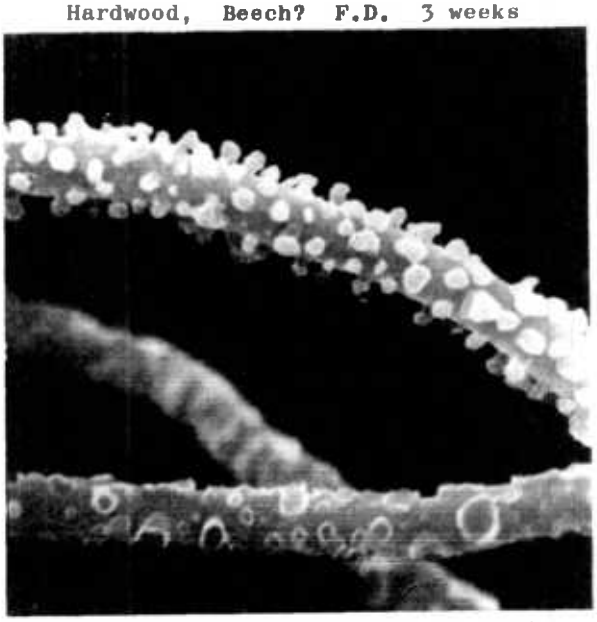
14424 C. globosum RLB x 1486

Hardwood, Beech? F.D. 3 weeks



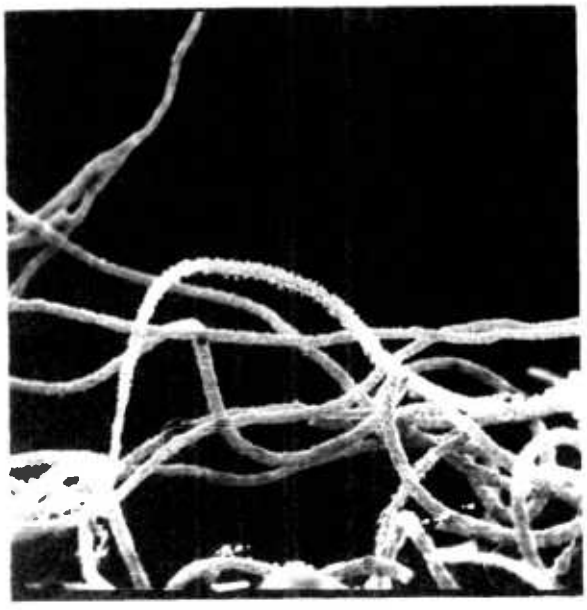
14421 C. globosum RLB x 145

Hardwood, Beech? F.D. 3 weeks



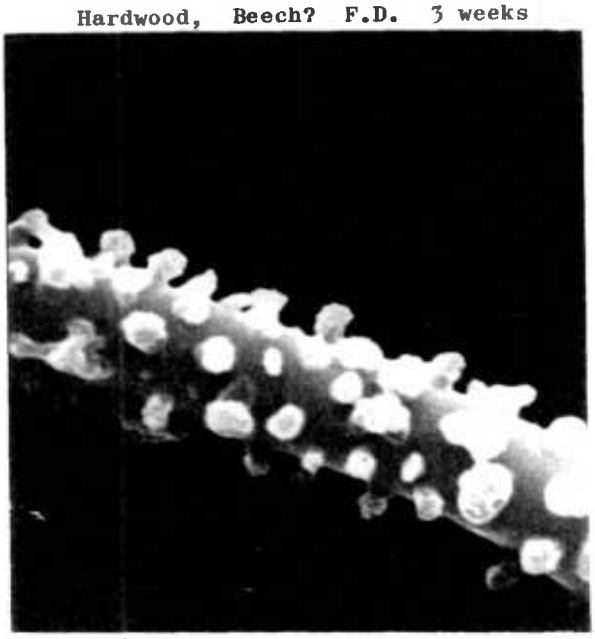
14425 C. globosum RLB x 3734

Hardwood, Beech? F.D. 3 weeks



14423 C. globosum RLB x 747

Hardwood, Beech? F.D. 3 weeks

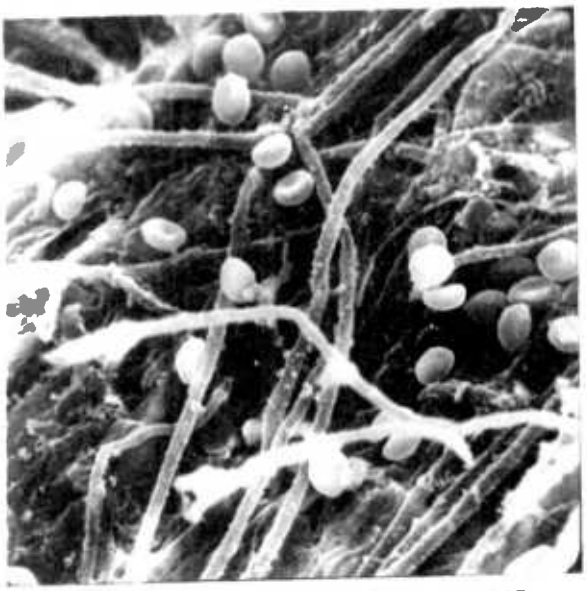


14426 C. globosum RLB x 7391

Hardwood, Beech? F.D. 3 weeks

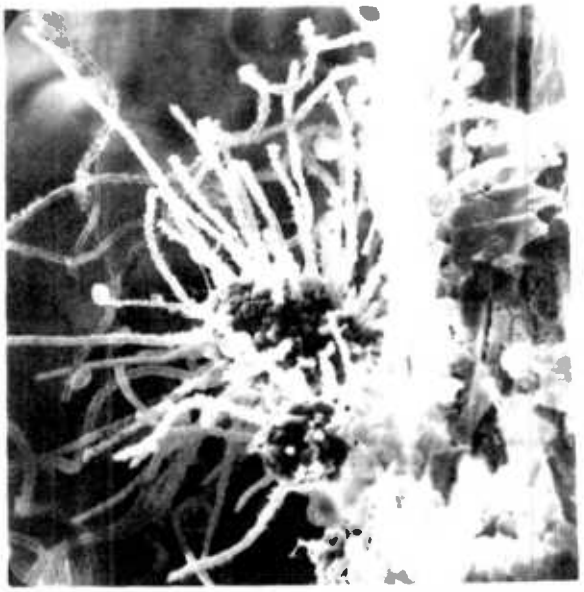
Plate 9.

Six different strains of C. globosum are shown. All were taken from the outside of a hardwood block, and are characterized by a 'bubbly' appearance. Nos. 1-6 are S70B, S70E, S70G, S70K, S70L and R.L.B. respectively. Of particular interest are the facts that:- (i) the "bubbles" on No. 2 appear to be larger, closer together and more translucent; (ii) No. 3 has a film presumably of hyphal material as well as bubbly hyphae; (iii) in No. 4 the hyphae appear to be from a bunch of germinating spores.



10-20 C. globosum S70B x 823

Beech R.L.S. A.D. 3 weeks



213-14 C. globosum S70K x 427

Birch R.L.S. F.D. 3 weeks



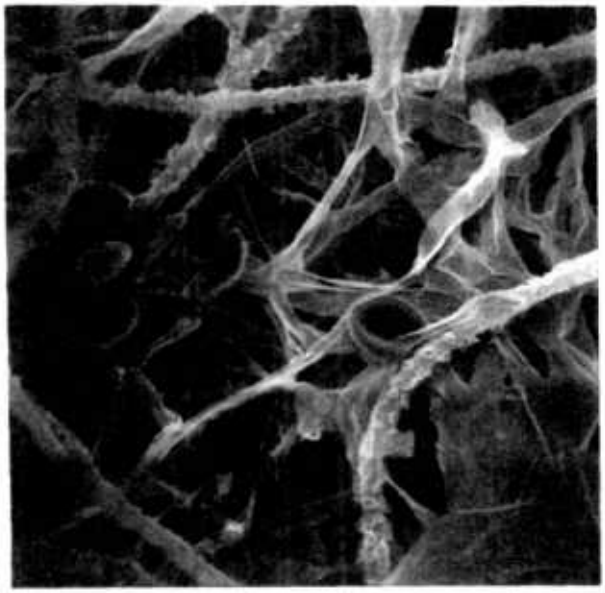
204-14 C. globosum S70E x 434

Birch F.D. 3 weeks



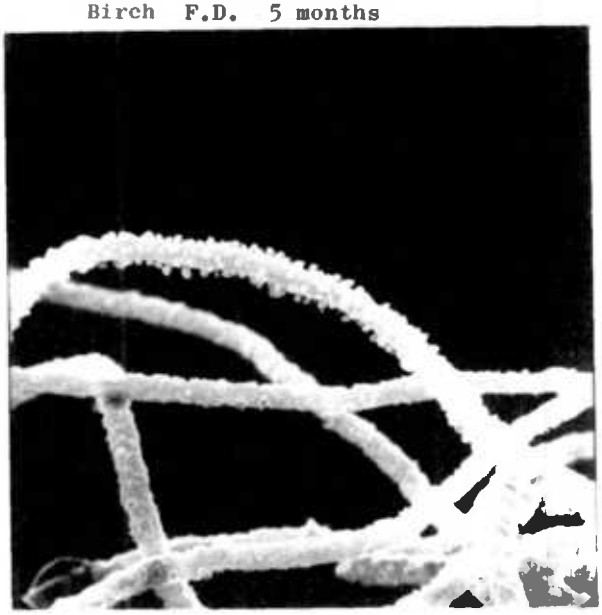
797-11 C. globosum S70L x 686

Birch F.D. 5 months



794-19 C. globosum S70G x 1572

Birch F.D. 5 months



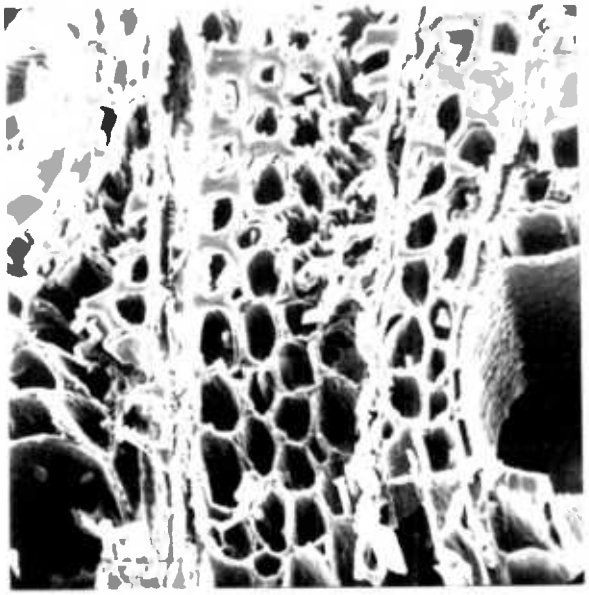
1424 C. globosum S70L x 1456

Hardwood, Beech? F.D. 3 weeks

Plate 10

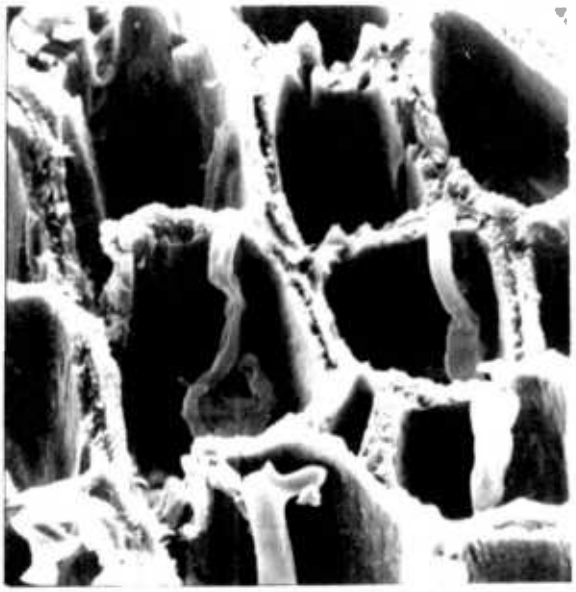
The effect of brown rot fungal decay is clearly shown in this series of photographs. Nos. 1-4 are P. monticola 304F in birch wood. Nos. 5 and 6 in Norway spruce wood are strains 304E and 280 respectively. All have been subjected to decay for five months. No. 1 shows a portion in the top third of the photograph where degradation is not so great. This contrasts strongly with the area below. The effect of the brown rot is to thin the cell wall from the lumen. This results in skeletal remains around the middle lamellar region. As can be seen from an examination of Nos. 2, 3 and 4 the cell wall material becomes thin and brittle. This is presumably due to the rapid depolymerizing action of the enzyme as found by Cowling (1961). Occasionally fungi can be observed in the cell lumen preserved by freeze-drying. Small checks or splits are apparent especially in No. 3, which are presumably due to the shrinking caused by enzymic removal of cell wall material. Nos. 5 and 6 show the action on softwood. This is more difficult to visualize; however weakening and consequent crumbling of the cell wall is seen in No. 5. Hyphae can also be detected at the bottom of the picture. The effects of a brown rot fungus on softwoods and hardwoods as seen under the electron microscope are essentially similar. The fungus inhabits the lumen and secretes an enzyme system, which gradually dissolves a large portion of the available cell wall.

Observation of No. 1 would perhaps give rise to a conclusion different from that of Bravery (1972) who suggested that enzyme diffused through an intact S_3 layer to degrade the S_2. At no point was this observed. It should be emphasized, however, that the medium was different, and this might well result in slight modifications in the pattern of decay. Until weight loss tests are standardized at an optimum for each fungal species variations in patterns of decay are inevitable.



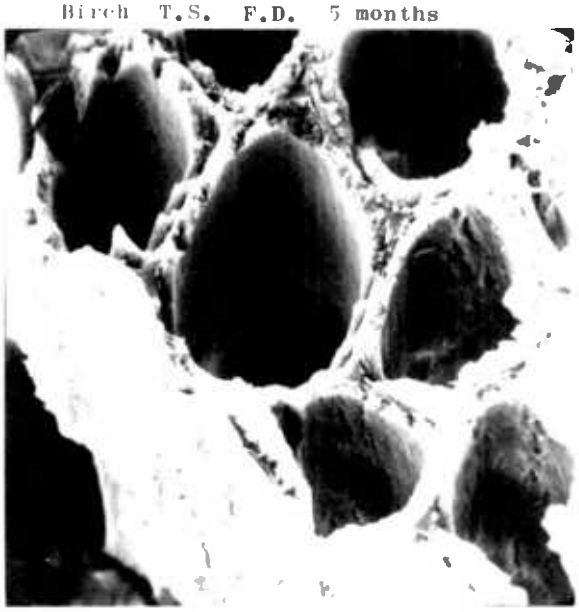
376-8 P. Monticola 304F x 450

Birch T.S. F.D. 5 months



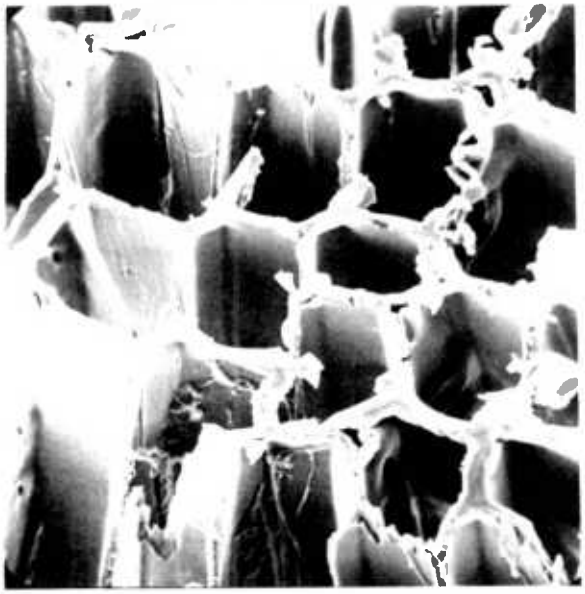
376-9 P. monticola 304F x 1585

Birch T.S. F.D. 5 months



376-10 P. monticola 304F x 1753

Birch T.S. F.D. 5 months



347-16 P. monticola 304E x 589

Spruce T.S. F.D. 5 months



550-18 P. monticola 304F x 991

Birch T.S. F.D. 5 months



764-11 P. monticola 280 x 362

Spruce T.S. F.D. 5 months

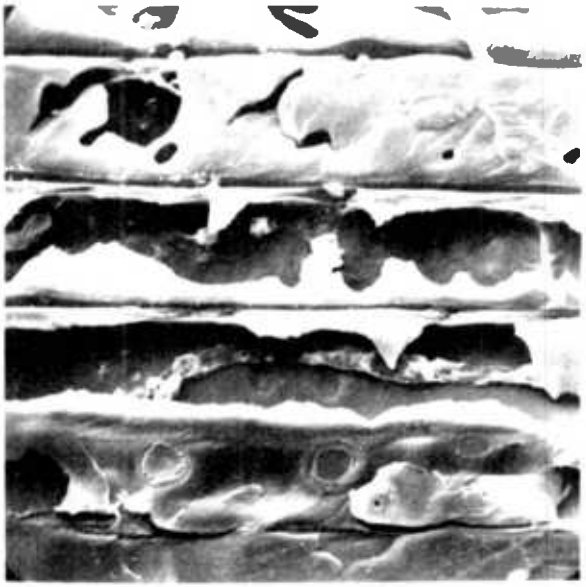
Plate 11

This shows the action of P. monticola in a softwood L.S. after five months' decay. No. 1 (304E) is a general view, including some very brittle ray cells and fungal infestation of tracheids on the R.H.S. The action of the enzyme system secreted has caused the ray cells to break in an uncharacteristic way. Nos. 2 and 3 (also 304E) are typical of the type of mycelium generally found. The L.H.S. of No. 2 shows that autolysis has set in. Hyphae are confined to the lumen and no cavities are apparent. Nos. 4 and 5 are details from ray cells of 304E and 304F respectively. 304E shows the remains of hyphae in the lumen. No. 6 (304H) has a slightly less bulbous appearance than 304E, and small elongated holes are present in the radial wall, through one of which has penetrated some fungus. These holes may have been derived from the enzymic action on pits or small fissures existing in the cell wall prior to infestation.



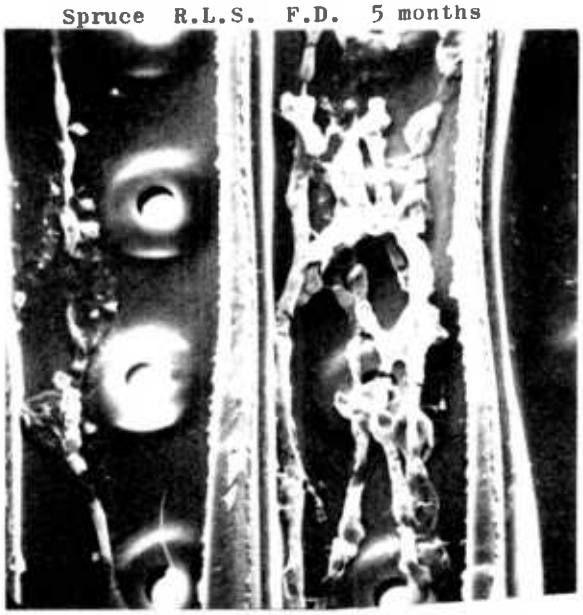
337-20 P. monticola 304E x 188

Spruce R.L.S. F.D. 5 months



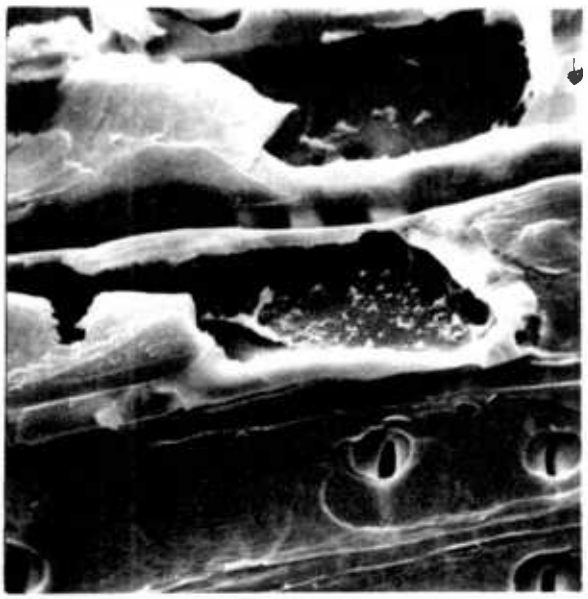
337-21 P. monticola 304E x 945

Spruce R.L.S. F.D. 5 months



337-19 P. monticola 304E x 930

Spruce R.L.S. 0^{o} tilt F.D. 5 months



766-3 P. monticola 304H x 1755

Spruce R.L.S. F.D. 5 months



337-16 P. monticola 304E x 1209

Spruce R.L.S. F.D. 5 months



766-15 P. monticola 304H x 1755

Spruce R.L.S. F.D. 5 months

Plate 12

This is a continuation of plate 11. All are R.L.S. of birch wood after 5 months' decay. Nos. 1 to 4 show fibre degradation. In No. 1 (304F) note the well preserved hyphae. Also to be seen are small unidentified vesicles of similar consistency to hyphal material. Large cracks are obvious with orientation 10^{o} to 15^{o} to the longitudinal axis. (Note the similarity of the angle to the micro-fibrilar axis of the S_2 layer shown in Fig. 8 page 40). Pits are also elongated in this direction. In No. 2 (304F) there is, in addition to fibres, a large proportion of severely degraded ray. Boreholes are large, especially towards the top of the picture. Pit enlargement has also occurred at the junction between two ray cells and a peculiar characteristic pattern of pit decay is apparent. This takes the shape of a series of minute holes at regularly spaced intervals, giving a brick-like appearance to the material between the holes. No. 3 (304F) is an example of extreme degradation. The remnants of hyphal material are apparent to the left of the picture. Checking and splitting of the internal wall of the lumen are extensive. No. 4 (304D) shows an earlier stage of attack, where both hypha and cell wall are relatively intact.

At the R.H.S. note the different way in which the relatively unattacked wood has split, leaving stringy fibrillar debris. The attacked wood has a much shorter and cleaner break. No. 5 (304F) is another example of degraded ray, showing the remains of a hypha, the "brick-like" effect previously mentioned and general debris from destroyed ray cells. No. 6 (304D) shows in lower power the very peculiar colonization of vessels by this fungus. The whole of the vessel appears to be filled with hyphae, in a series of "plugs", It is debatable whether this compartmentalization is an artifact of the freeze-drying process (the wood block was cooled by immersion in liquid nitrogen), or is in reality a natural feature of the fungus. From the general appearance of the breaks the former seems more probable.



359-17 P. monticola 304F x 1448

Birch R.L.S. F.D. 5 months



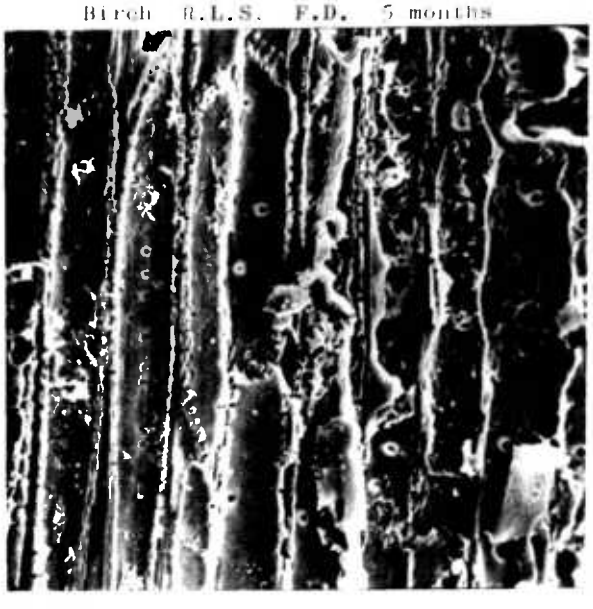
329-3 P. monticola 304D x 1882

Birch R.L.S. F.D. 5 months



359-11 P. monticola 304F x 732

Birch R.L.S. F.D. 5 months



359-15 P. monticola 304F x 732

Birch R.L.S. F.D. 5 months



359-10 P. monticola 304F x 732

Birch R.L.S. F.D. 5 months



329-5 P. monticola 304D x 143

Birch R.L.S. F.D. 5 months

Plate 13

This plate continues from the last two in being P. monticola. All six photographs are T.L.S. on Norway spruce wood subjected to decay for 5 months. With the exception of No. 3, which is strain 304E, all are strain 304H. Nos. 1 and 2 were respectively low and high power magnifications of a hypha leaving a ray cell and penetrating a fibre via a pit. It is possible that the reverse is occurring, but that is less likely, because penetration through the wood is achieved by use of the rays as pathways of easy access. Note that in No. 2 the ray cell near the bottom also contains hyphal matter. Nos. 3, 4 and 5 demonstrate clearly the characteristic way in which ray cells break after being attacked by this brown rot fungus. The break is clean and there are no stringy fibrils. (in No. 5 the material to the right hand side of the ray has a fibrous nature to its breaking, and does not appear to have been affected to the same degree by the hydrolytic enzymes). For this ray cell, which has been broken in tension very nearly parallel to the microfibrillar axis, the absence of a "fibrillar" tear probably demonstrates the presence of a depolymerizing enzyme system. No. 6 shows a resin canal in a ray in which the cells have been degraded. Hyphae are visible in the fibre cells to the left of the canal.



766-25 P. monticola 304H x 419

Spruce T.L.S. F.D. 5 months



766-20 P. monticola 304H x 419

Spruce T.L.S. F.D. 5 months



766-24 P. monticola 304H x 1676

Spruce T.L.S. F.D. 5 months



766-19 P. monticola 304H x 1676

Spruce T.L.S. F.D. 5 months



766-19 P. monticola 304E x 4572

Spruce T.L.S. F.D. 5 months

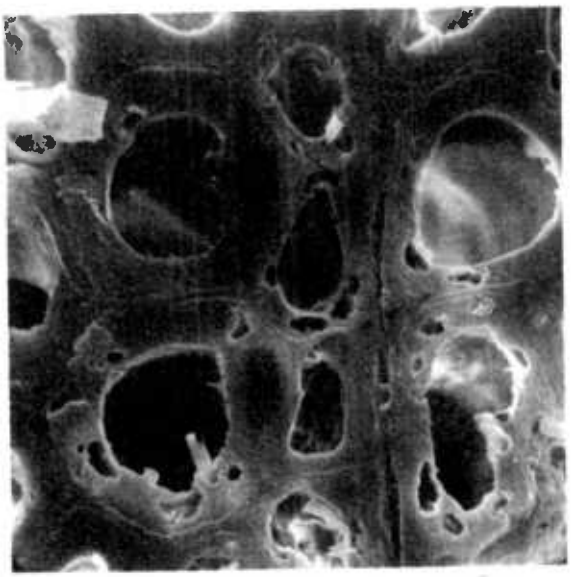


766-23 P. monticola 304H x 858

Spruce T.L.S. F.D. 5 months

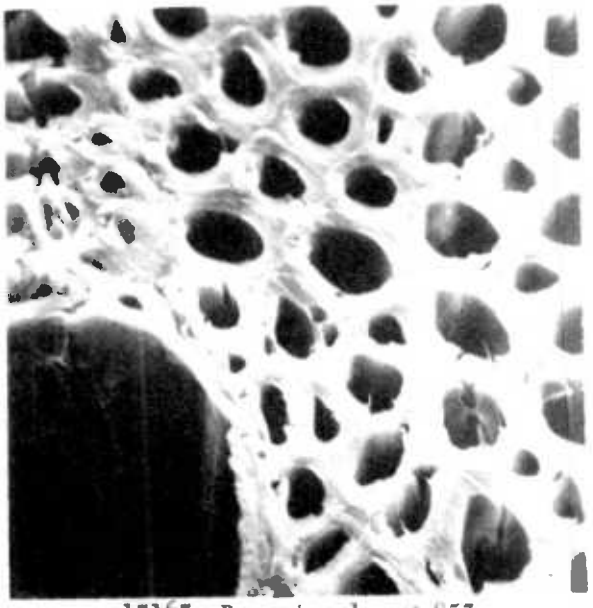
Plate 14

This shows the action of P. fastigiata (Bann 1) in a T.S. of birch wood after 3 weeks' attack. Nos. 1 and 2 are photographed at 0^{o}, the remainder are at 45^{o} tilt. Nos. 1 and 2 show clear evidence of soft rot attack with cavities in abundance. They appear to be localized and for the most part roughly circular or oval, but occasionally half-moon shaped cavities are present. In No. 2 three cavities are shown very close to one another; these would be very likely to coalesce causing one big semicircular cavity. The S3 layer is hardly ever ruptured through to the lumen. No. 3 shows a single small cavity clearly confined to the S2 layer. The small flap of material observable to the R.H.S. of the hole is an artifact of the razor cutting used in the preparation. The same is true of the very small grooves observed. Nos. 4, 5 and 6 are a consecutive series of increasing magnification.



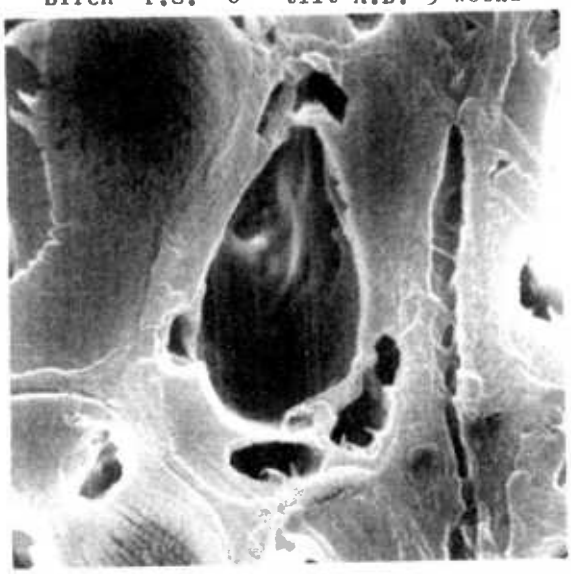
13160 Banerjee 1 x 1661

Birch T.S. 0^{o} tilt A.D. 3 weeks



13163 Banerjee 1 x 853

Birch T.S. A.D. 3 weeks



13161 Banerjee 1 x 4153

Birch T.S. 0^{o} tilt A.D. 3 weeks



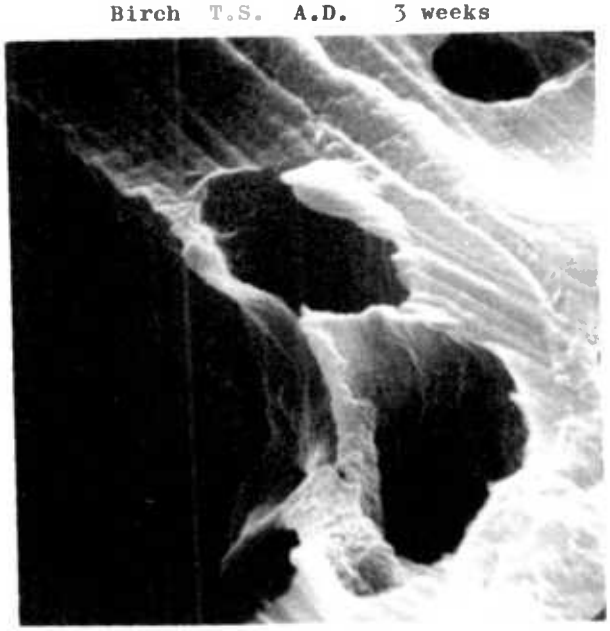
13165 Banerjee 1 x 4267

Birch T.S. A.D. 3 weeks



13169 Banerjee 1 x 4267

Birch T.S. A.D. 3 weeks

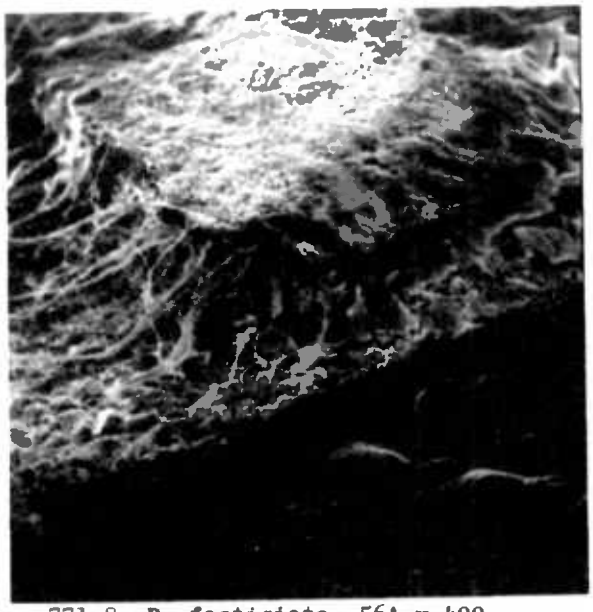


13166 Banerjee 1 x 7711

Birch T.S. A.D. 3 weeks

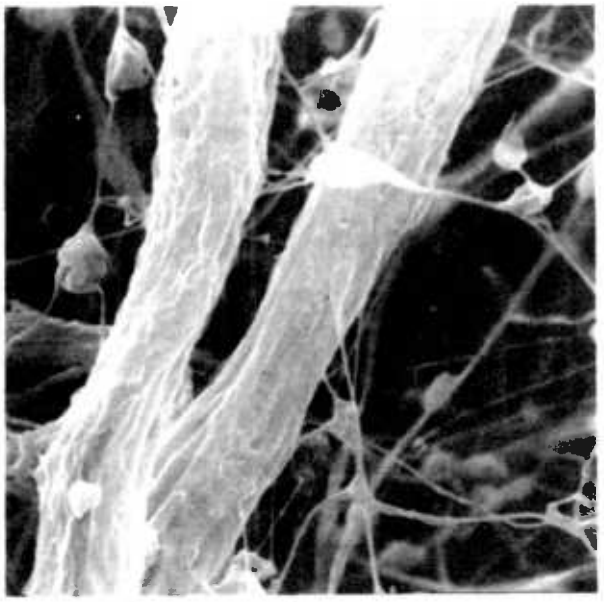
Plate 15

Shows P. fastigiata (56A) on the surface of a block of birch wood. No. 1 is a general low power view of a scraping of the fungal growth from the top of a birch block. The hyphae seem to be divided into three types, a main very thick "trunk-like" type, thinner offshoots comprising the bulk of the hyphal material and a vesicular type with two to four thin conecting hyphae. The first type is seen clearly in No. 2, whilst No. 3 is predominantly the second type, although to the L.H.S. the first type is present. Photograph No. 4 has all three present, whilst Nos. 5 and 6 are a low and high magnification of the vesicular hyphae.



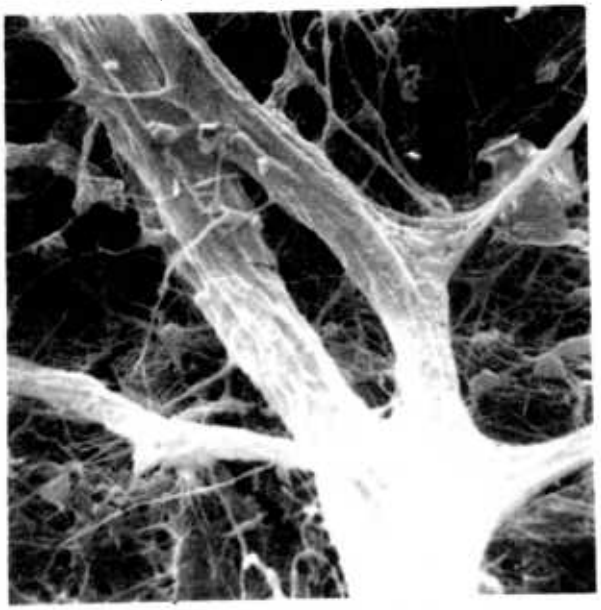
771-8 **P. fastigiata** 56A x 400

Birch F.D. 8 weeks



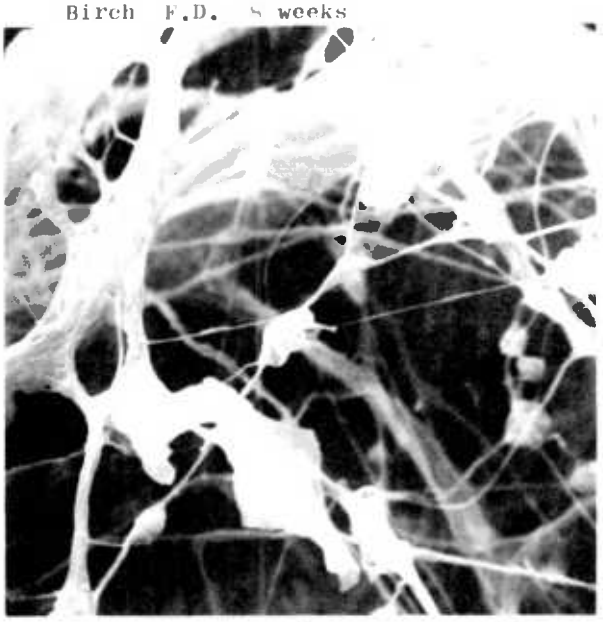
771-6 P. fastigiata 56A x 838

Birch F.D. 8 weeks



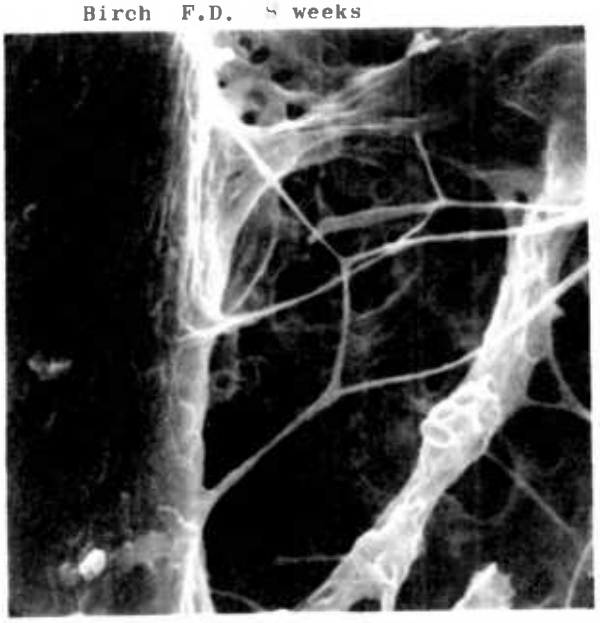
771-7 P. fastigiata 56A x 419

Birch F.D. 8 weeks



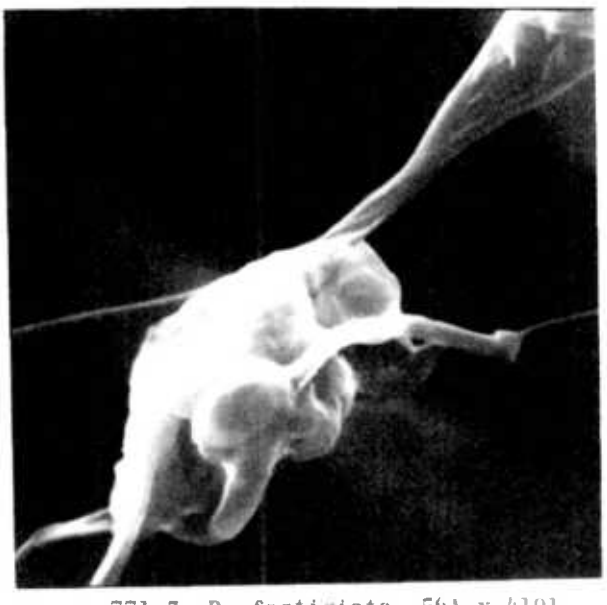
771-5 P. fastigiata 56A x 838

Birch F.D. 8 weeks



771-11 P. fastigiata 56A x 1600

Birch F.D. 8 weeks



771-3 P. fastigiata 56A x 4191

Birch F.D. 8 weeks

Plate 16

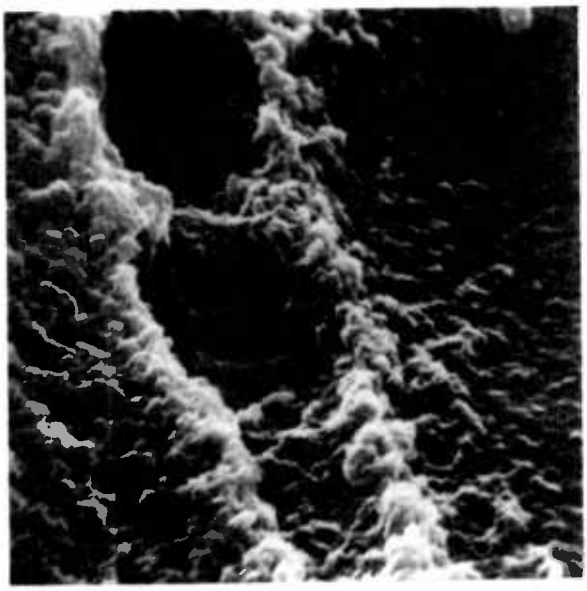
This is a series of photographs showing debris left after infection by C. globosum S70L in beech wood decayed for three weeks. No. 1 is a very small inexplicable circular etching with a piece of unidentified material in the centre. No reason could be seen for this type of behaviour, although it is just possible that it could be the remains of a very large pit. The dimensions of the etched material would tend to make this a less likely explanation than that it occurred as a result of unusual enzymic action.

Nos. 2 and 5 are respectively low and higher magnifications of hyphae which have undergone some form of destruction, possibly autolysis. The remains of the entry into a crossfield pit are still visible. Note that even at this early stage of destruction the central portion has collapsed, leaving the outside edges of the hyphae to form a shape similar to two rails running parallel to one another. Nos. 4 and 3 show what is possibly an extension of this, where in the centre of the photograph No. 3 there is actual separation into two strands. No. 4 shows this in much closer detail.



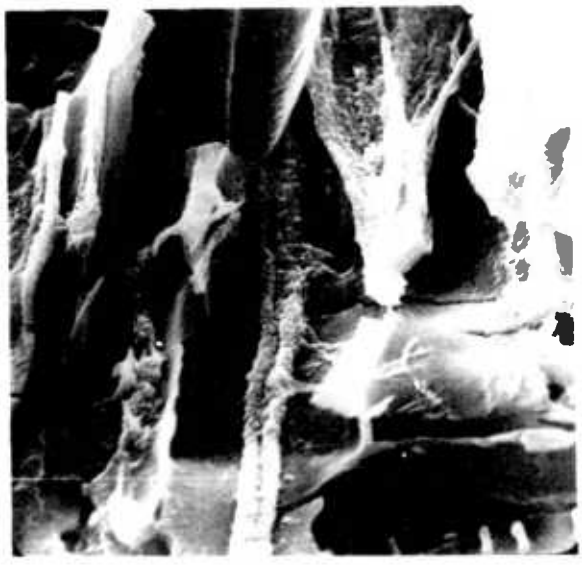
14199 C. globosum S70L x 8153

Beech R.L.S. F.D. 3 weeks



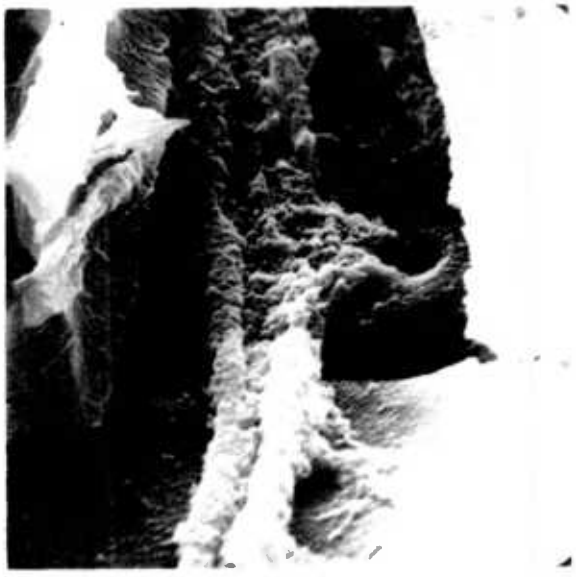
14114 C. globosum S70L x 9220

Beech R.L.S. F.D. 3 weeks



14119 C. globosum S70L x 1890

Beech R.L.S. F.D. 3 weeks



14120 C. globosum S70L x 4724

Beech R.L.S. F.D. 3 weeks

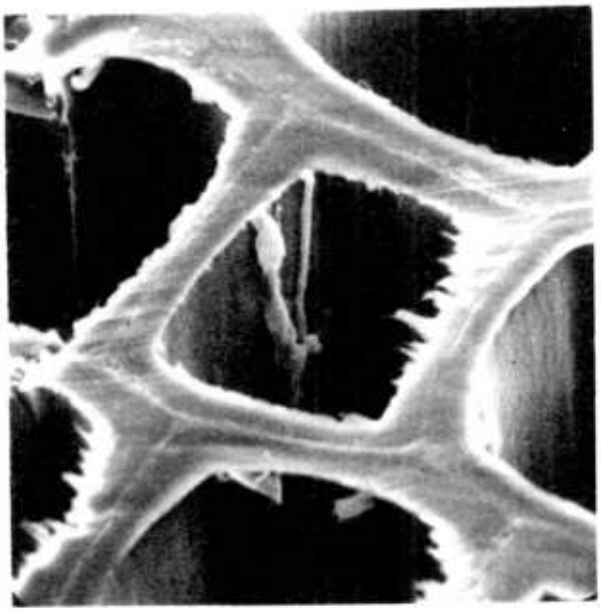


14112 C. globosum S70L x 1844

Beech R.L.S. F.D. 3 weeks

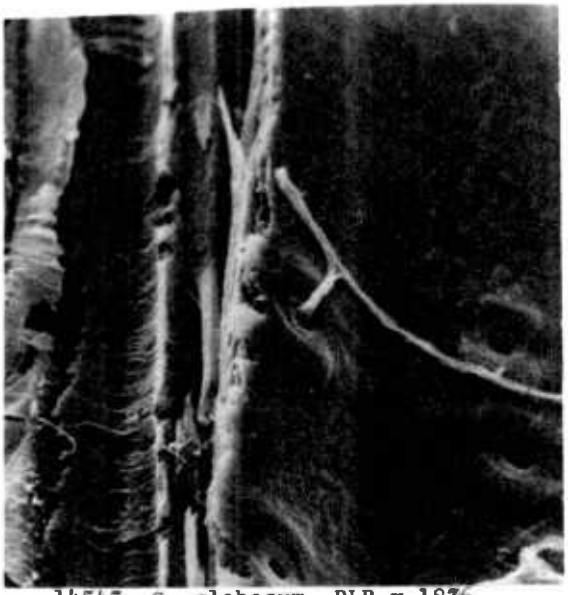
Plate 17

These photographs are a few examples of many available and demonstrate points concerning the pathways which fungi take. No. 1 shows P. monticola 304H in the lumen of Norway spruce. No. 2 is strain 304B of the same fungus also in spruce. Here the two branches of hyphae are clearly seen emanating from a ray cell in T.L.S. No. 3 shows a similar but more complicated picture. C. globosum S70M in a spruce T.L.S. has subdivided into three portions, and is seen emerging from the ray cell area. Note also the presence of some diffuse hyphae. No. 4 is the same fungus, strain R.L.B., in beech and shows entry into or depart from a pit No. 5, strain S70B, is an example of this fungus boring through cell wall material. In the centre of the photograph can be seen a hypha emerging from the solid cell wall. This strain is again shown in No. 6 emerging from a pit in L.S. It can be seen that natural pathways open to the fungus, such as pits, are used wherever possible.



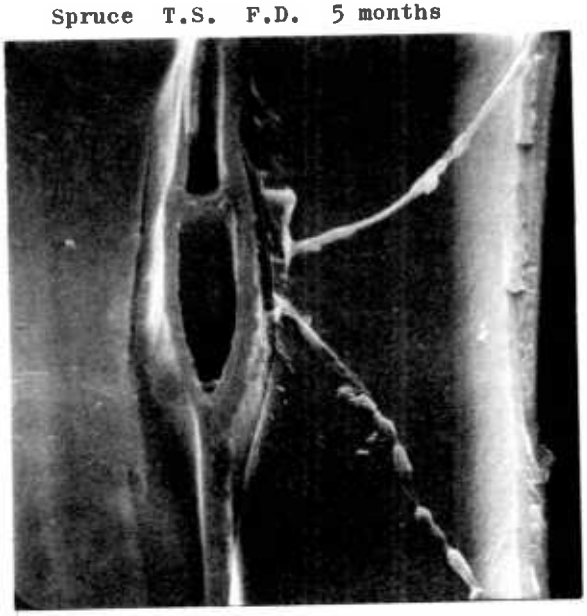
767-13 P. monticola 304H x 1600

Spruce T.S. F.D. 5 months



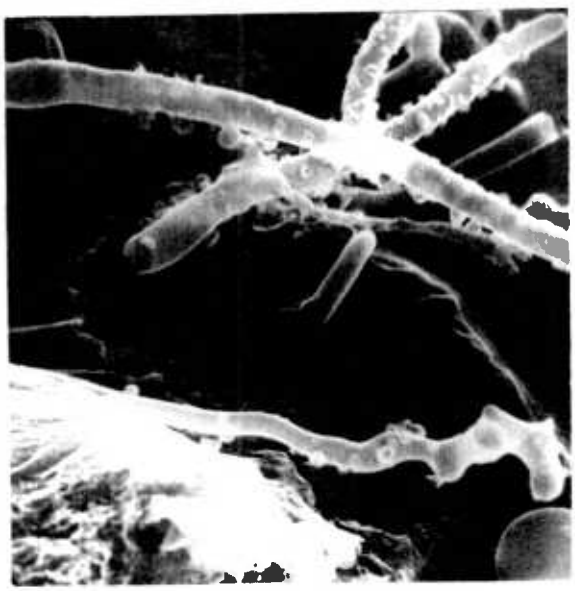
14543 C. globosum RLB x 1836

Beech R.L.S. A.D. 3 weeks



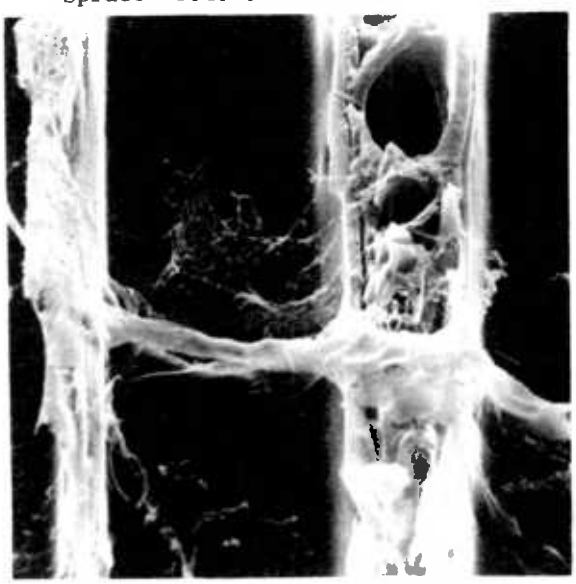
770-20 P. monticola 304B x 1524

Spruce T.L.S. F.D. 5 months



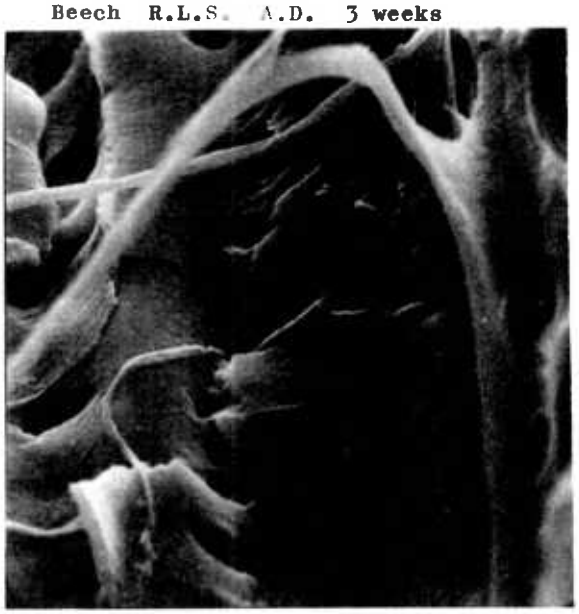
2-12 C. globosum S70B x 1615

Beech R.L.S. A.D. 3 weeks



245-23 C. globosum S70M x 1600

Spruce T.L.S. F.D. 3 weeks

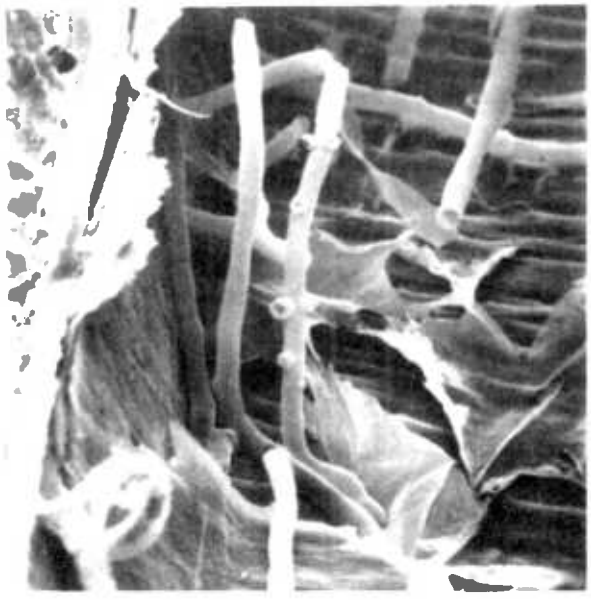


13528 C. globosum S70B x 1791

Birch R.L.S. A.D. 3 weeks

Plate 18

This plate consists of photographs of phenomena not considered to be in any particular bracket. No. 1 is a photograph of C.globosum S70 in birch wood after 5 months' decay; inside a vessel lie some hyphae, to one of which about six vesicles are attached. These vesicles are punctured, showing that they are clearly hollow. This was the only indication of the possible nature of the "bubbles" on the "bubbly" hyphae. No. 2 is a higher power photograph of hyphae from the same region. What is particularly interesting is that the diameter and general appearance of the hyphae, which are situated in the centre of the block, are the same as are found on the outside, with the exception that no "bubbles" were present. Hyphae in C. globosum were not usually this shape and size in the centre of the block. No. 3 is inexplicable. Four "cakes" of material are visible on the tangential wall of a spruce tracheid in a block, which had been degraded by P. monticola 304E. The nature of the cakes and the method whereby they were deposited are unknown. No. 6 shows a similar example in 304H, alongside a hypha .
No. 4 is a photograph of the exterior of a birch block attacked by C. globosum S70. The interest lies in the hyphae behind the normal outside "bubbly" hyphae; it is apparent that they are smooth and without many, if any, vesicles. In the centre can be seen a section of material which had once contained vesicles. All that is left are annulate remains, which it is suggested are the base or supporting structure for a vesicle. No. 5 is an example of the "hairy" hyphae which are occasionally found when C. globosum attacks wood. This is strain R.L.B. in birch wood and the "hairs" are clearly visible connecting the lower part of the fungus with its substrate. Similar but better defined organelles have been observed with the fungus Verticillium albo-atrium when destroying a tomato stem. (Cooper, 1973)



796-21 C. globosum S70K x 1448

Birch R.L.S. F.D. 5 months



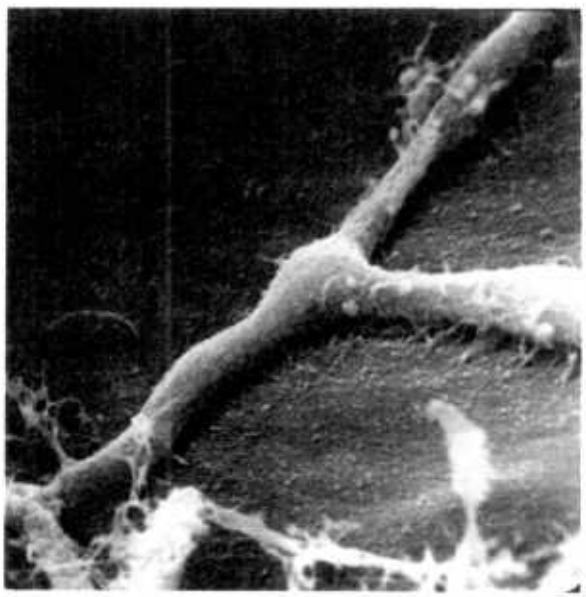
797-12 C. globosum S70L x 3429

Birch F.D. 5 months



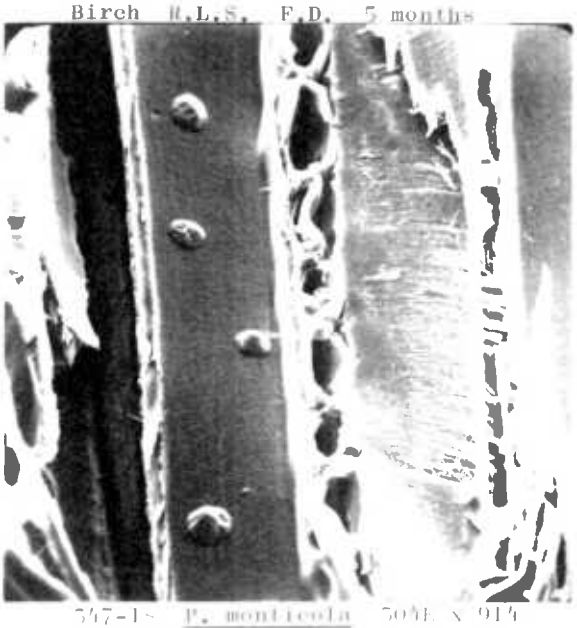
797-5 C. globosum S70K x 4001

Birch R.L.S. F.D. 5 months



14547 C. globosum RLB x 4724

Birch R.L.S. F.D. 3 weeks



547-15 P. monticola 504E x 914

Spruce [illegible] 5 months



766-6 P. monticola 504H x 4382

Spruce R.L.S. F.D. 3 months

Plate 19

This plate is a photograph of a series of photographs built up by piecing together several sequential scanning electron micrographs and dry mounting them on a large board. The end result is a picture shewing more small detail than is possible by photographic enlargement. The difficulties involved in the production of this serial SEM work for a thesis are considerable, because of the large size of the final product, and so the picture opposite is a small one and is included only to give an indication of the potential of this method.

The beech block has been incubated for 8 weeks with C. globosum S70M. This has resulted in a definite area of attack shewn in this photograph. The R.H.S. of the picture shews a marked thinning of the fibre cell walls as a result of fungal decay. This can be compared with the thickness of fibre cell walls elsewhere, even in the early part of the growth ring on the top left of the block. Beech rays were unaffected confirming an observation of Bravery, 1972. The angle of tilt and thiness of the specimen section both contribute towards a very clear picture of hyphae in vessels and the general degradation caused, particularly at the bottom R.H.S.

Legend: C. globosum S70M x 135

Beech. T.S. A.D. 8 weeks.

0^{o} Tilt.

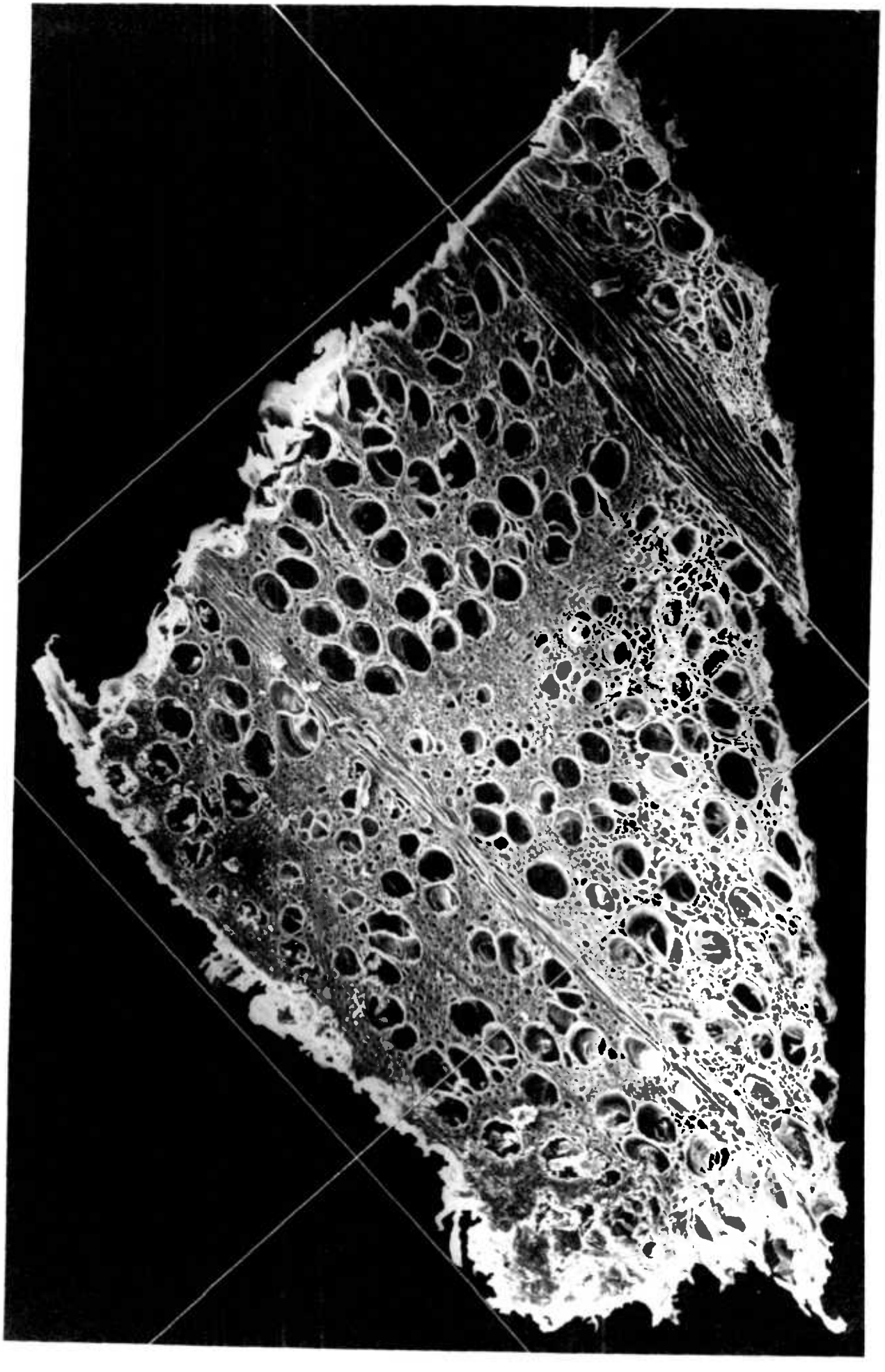


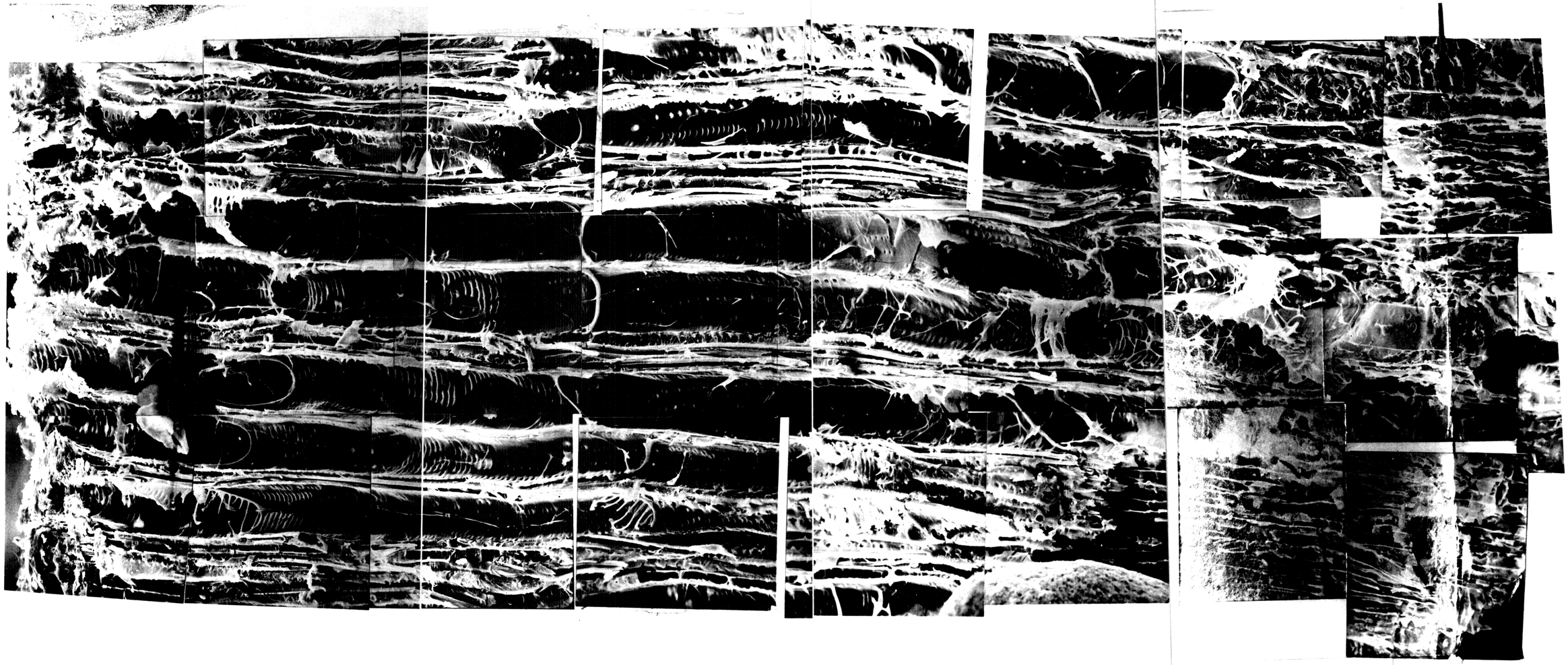
Plate 19a.

This plate shews a L.S. built up from a number of smaller photographs in the same way as in plate 19. Visualisation of the mode of entry, location and type of attack is much improved over(a) a section under a light microscope, (b) a single S.E.M. photograph and (c) the direct electron microscope (transmission) This technique has the unique advantage of presenting a more complete picture of the pattern of attack.

Note the lack of decay of the vessel wall, which the hyphae have used primarily as a pathway for access, and the degraded nature of the fibres into which the hyphae have passed.

Legend.

A.D. x 700
Beech. C. globosum S 70 M.
Approx. 6 weeks.



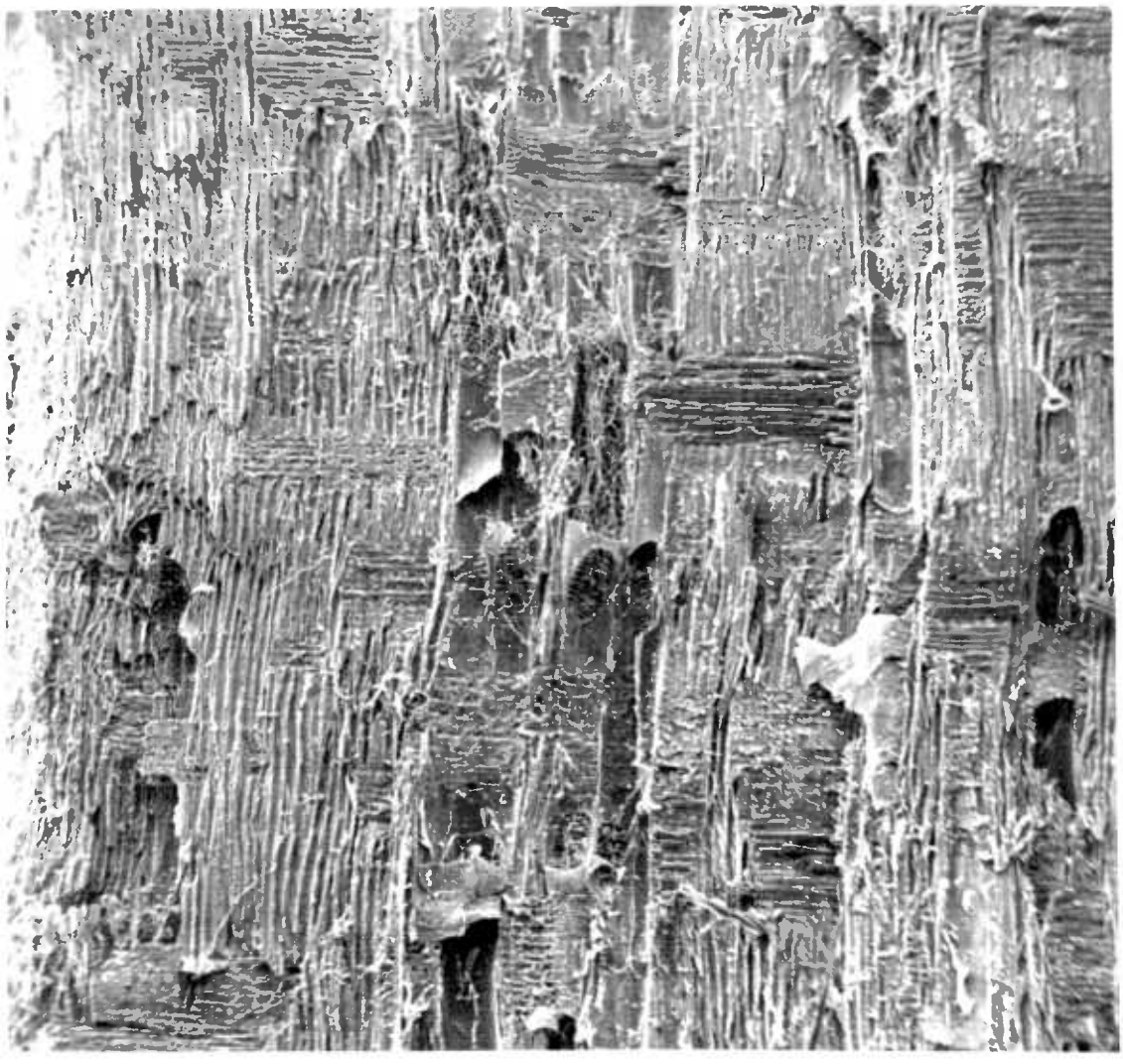


Plate 20 This shews a general low power view of birch wood minimally attacked by P. monticola. The area shewn exhibits no visible signs of fungal decay, and is presented as a typical example of a specimen split in longitudinal section.

Strain No. 304F Photo No. 360-1

X 75 F.D.

General discussion and conclusions.

Both the longitudinal and transverse sections of wood exposed to soft rot fungi showed no signs of lumen erosion (Type 2 decay). This type of decay has been observed to form a portion of the destructive process of the same species of fungus in beech and birch even when exposed for considerably smaller incubation times (Bravery, 1972). He found lumen erosion both of the fibres and the vessels, although it was occasionally difficult to differentiate between lumen erosion and the breakthrough of cavities into the lumen (possibly during the "splitting" process in preparation). Birch wood appeared to be more susceptible to lumen erosion than beech possibly because of the difference in their S_3 layers. In the present work it seems possible that this absence of lumen erosion even after several months exposure is a consequence of using birch mineral agar rather than the malt agar used by Bravery.

The soft rot fungi colonized Norway spruce predominantly in the late wood but prolonged searching did not reveal any cavity formation or lumen erosion. No weight loss was observed. Nilsson, 1973, when subjecting spruce wood to decay by C. globosum under optimum conditions for several months, only obtained a weight loss of 0.6%.

The conditions of growth in these experiments did not result in maximal decay. One objective in using birch agar as the sole carbon source was to determine whether there was any difference in

decay pattern between it and malt agar. It was also thought desirable to eliminate any induction of enzyme systems not directly concerned with wood decay.

The great variation in morphology and size of the fungal hyphae is difficult to understand. The hyphae of P. fastigiata varied between0.25μ and 15μ, whilst those of C. globosum were between 0.5μ and2.5μ. In P. monticola this wide divergence of size was not apparent. The last mentioned fungus caused erosion from the lumen, whilst the first two mentioned were responsible for decay in the S_2 layer. It may be that the enzyme system of P. monticola is free to move further,or is capable of greater diffusion than those of the other fungal types.

It is possible that when the fungus reaches a suitable area within the wood microstructure a change of shape and size results in certain advantages. A small hypha would be better than the larger foraging hypha due to the increased surface area per unit volume. Thus it is possible that the smaller hyphae are responsible for a much larger and more effective production of polysaccharide and lignin degrading enzymes than the larger. The stimulus which causes a change of this nature would be highly complex, involving reorganization of the genetic coding as a consequence, perhaps, of the ingestion of key or trigger molecules, much in the same way as induction and repression of enzyme systems are known to occur.

The type, nature and quantity of decay depends to a very great degree upon the agar growth medium. Mineral salts in the agar enhance attack, (Savory, 1954 (b)) and cellulose incorporated

into the medium results in higher weight losses than when malt alone is used. (Banerjee, 1969). Sawdust (40-80 mesh) included with malt agar also results in more pronounced attack (Zainal, 1973). Nilsson, 1973, has even impregnated nutrient salts into wood blocks prior to subjecting them to fungal decay. In the present work only mineral salts, agar and sawdust were used for culturing fungi in weight loss determinations. An experiment to determine the effect of agar as a carbon source showed that it played little if any role in the growth of C. globosum. The fungus was repeatedly subcultured on mineral agar with no carbon source added to the medium. Growth was very sparse, individual hyphae being easily distinguished and distributed very thinly around the plate. The whole plate was evenly covered, apparently because the hyphae were "searching" for suitable nutrient. A gradient of decay such as was found by Bravery (1972) was also apparent when hardwoods were attacked by soft rot and stainer soft rot fungi. It seems possible that nitrogen limitation is the cause of this gradient since Nilsson (1973) did not report any similar gradation in blocks, which had been impregnated with nitrogen containing salts prior to the test.

The original purpose of using the scanning electron microscope, to endeavour to correlate decay patterns with the biochemical information obtained, was not achieved. A more fruitful approach would be to use mutant fungi lacking cellulases but possessing hemicellulases and to investigate the resultant decay pattern, However, there might be limitations in the feasibility of mutant production. (See discussion of recent literature re. Eriksson and Goodel).

DISCUSSION

Discussion of the results in the context of a survey of the recent literature.

The use of weight loss tests for measurement of the capacity of a fungus to degrade wood is open to some criticism. The medium used to grow the fungus can be varied and this may affect the result. The shape and size of the wood block and consequently the surface area to volume ratio, is another variable. In the present work one-centimetre cubes have been used, and this is consistent with previous work from this laboratory; however Nilsson (1973 and 1974 (a)and (b)) is one of the many workers who have used different sized blocks of wood. Thus weight losses are only relative within one experimental technique and comparisons with other techniques are of limited value. Nilsson, 1973, has advocated the use of blocks of the size 5 x 5 x 10mm. for "the determination of the type of wood decay" and 10 x 20 x 20mm. "for decay capacity tests". When comparing data of decay capability tests of brown rot, white rot, and soft rot fungi, the tendency of the last mentioned to attack predominantly the outer surfaces, leaving the inside relatively whole, should be noted. Thus soft rot fungi may give rise to weight losses which are smaller than the other two types of decay fungi which are capable of attacking the whole block.

The general pattern which has been found by numerous previous workers (e.g., Findlay, 1970, Bailey et al. 1968), namely that softwoods are more resistant to decay than some specifically chosen hardwoods under these test conditions, was confirmed. Spruce wood was in general more resistant than birch and this was particularly true of the fungus C. globosum,

which was not attacked at all under the conditions used in this work. The question must therefore be asked: what is the stimulus for change from passive to active penetration that was present in birch wood but absent in spruce wood, bearing in mind that spruce blocks were cultured not only on spruce agar but also on birch agar?

The fact that spruce blocks were not decayed by C.globosum even when cultured upon birch agar, a medium upon which the fungus readily grows, indicates that it is the nature of the spruce wood which makes it resistant to cavity formation. It is possible that with further time (i.e., in excess of 5 months) under these conditions cavity formation would occur, but this does seem less likely. Cavities caused by C.globosum have been found in spruce by Banerjee, 1969, Findlay 1970, and in pine and spruce by Nilsson, 1973. With the first two workers malt extract was in the culture medium, whilst Nilsson used glucose in his medium. It would therefore appear that the culture conditions and the composition of the growth medium, particularly with respect to small sugars, are both factors which need to be further investigated when considering the results of decay capability tests on soft rot fungi in the future.

Nilsson 1973, when testing six soft rot monocultures of fungi, obtained a decreasing gradient of susceptibility of the following wood species: aspen, beech, birch, pine and spruce. In the present work when Scots pine was used by accident instead of spruce, the brown rot P. monticola gave rise to much lower weight losses. With only two strains tested, however, this needs confirmation.

It is a necessary condition for the fungal decay of wood that extracellular hydrolytic enzymes are present. It would therefore be an attractive idea to monitor the production of the major wood degrading enzymes and determine whether there is any correlation with weight loss test results. The hemicellulase monitoring experiment was designed with this as one of the objectives. Recent work by Nilsson, 1974 (a), in this field has placed the results and discussion of this experiment in perspective. Several factors affecting the results of an investigation of extracellular enzymes must first be discussed.

The conditions and composition of a liquid culture medium vary considerably from that of the fungus in the 'natural' state. Different workers each favour their own particular ideas of the salt composition in a liquid medium. One of the most important differences observed has been in the use of trace elements. In the present work and in Highley 1973, trace elements are incorporated into the medium, but Nilsson in his recent series of five papers does not use them. This difference, which will be discussed at a later stage, could be an explanation of some of Nilsson's unexpected observations. The lack of certain metal ions may be a limiting factor in the production of extracellular enzymes. Other similar enzymes are known to depend upon metal ions for their function (Mandels and Reese, 1957).

It may be argued that when the fungus secretes extracellular hydrolytic enzymes in a culture medium, it represents a degree of inefficiency. A fungus, for example which produces soft

rot cavities in wood (i.e., production of enzyme only at the site of action), and yet which gives rise to little or no enzyme in the culture filtrate could be regarded as highly efficient. Fungi which fit into this category pose a considerable problem for the research worker in this field. Nilsson, 1974a, incubated four cellulolytic soft rot fungi in liquid cultures on birch meal for 42 days without obtaining either a weight loss or production of cellulase and xylanase activities. It is the opinion of the author that this anomalous result might be the consequence of the lack of trace elements in Nilsson's medium. Other factors such as the degree of aeration and the concentration of carbon source could also be contributory. The size of the wood particles used is one such consideration. Nilsson used wood particles ground to pass an 80 mesh, but in the present work 40-80 mesh is used. This also might give rise to differences in enzyme production. Highley, 1973, found an increasing order of growth and sometimes enzyme production with both brown and white rot fungi from wood meal to holocellulose to ball-milled wood. Basu and Ghose, 1960, were unable to produce significant cellulase in liquid culture with C.globosum, a finding in direct contradiction to the present work.

Nilsson, 1974b, incubated whole cotton and jute fibres in liquid culture with five wood destroying soft rot fungi including a species of Phialophora. The last mentioned did not give rise to a weight loss, to enzyme production or to any visible microscopic degradation. The previously mentioned differences in culture conditions regarding trace elements could cause this lack of activity or alternatively the degree of

saturation of the cotton or jute fibres may be significant.

In the discussion of weight loss tests, a suggestion (No. (3)) was made that "incorporation of a minimal quantity of glucose in the wood sawdust medium might aid the initial growth of the fungus", and this has since been performed by Nilsson, 1974 a. He shewed that the addition of a range of concentrations of" start-glucose" (0.005% to 0.1%) did not stimulate Bispora betullina, Ceratocystis albida or Phialocephala sp.A to produce cellulase or xylanase. In fact increasing quantities of glucose inhibited the production of these extracellular enzymes for some species of fungus.

The role of small molecules such as glucose has been noted by several workers. Bravery, 1968, shewed that small quantities of alternative carbon sources, such as asparagine and yeast extract, inhibited cellulase production, measured as the clearing of cellulose agar, of certain soft rot fungi. Highley, 1973, shewed that the production of enzymes from white rot fungi was repressed by simple sugars. Glucose (0.5 to 1%) added after 7 days not only stopped production but caused a considerable drop in the cellulase activity by day 21. This could be either as a result of some specific direct form of degradation or simply that a dynamic equilibrium existed between enzyme production and degradation (due to heat, pH, etc.) which had been disturbed by lack of production. The effect of glucose on brown rots was the opposite, enhanced production being obtained. In fact, Highley could not obtain enzyme yields without it.

The addition of glucose, and especially cellobiose, gave rise to greatly enhanced cellulase (C_x) activity with brown

rots, but yeast, peptone and asparagine merely increased the growth with a slight reduction in Cx activity. Nilsson 1973, coined the term "non-cellulolytic" to describe certain soft rot fungi which were capable of degrading wood, and yet gave no positive cellulase assays by clearing zones in cellulose agar. These "non-cellulolytic" soft rot fungi were found,Nilsson1974b, not to yield extracellular enzymes, regardless of the presence of alternative carbon sources such as glucose. These "non-cellulolytic" fungi are able to degrade cellulose (in wood) and so are really fungi "in which it is difficult, at present, to demonstrate cellulase in vitro". Small quantities of glucose present in the cellulose agar medium of cultures of "non-cellulolytic" fungi did however inhibit the degradation of cellophane, a substrate normally attacked by these fungi. The cellulolytic soft rot fungi (i.e., those which cleared cellulose agar) exhibited no such repression, and indeed in some cases were found to shew enhanced activity.

With so many factors capable of influencing the extracellular enzyme production of wood destroying fungi, great care should be taken in drawing conclusions from work of this nature. It has been shewn by Nilsson, 1973, that it is desirable to use more than one method when screening for cellulolytic fungi to maximise detection. The hemicellulase monitoring experiment does, however, provide a very useful indication of the enzymes which are possibly used in wood destruction by fungi. Some quite distinctive behaviour particularly in the comparison of soft and hard wood, was observed with C. globosum in this experiment. There was

thus a clear differentiation in enzyme production between birch and spruce cultures, and therefore it is assumed, in the mode of attack of the fungus on these woods. This was expressed mainly in the xylanase and galactoglucommananase activities, but the enzyme yield *in toto* was greater on birch than on spruce wood. The enzyme xylanase appeared to be induced at greater levels than GGM-ase. Does this mean that under these conditions xylan is much more accessible, or susceptible to decay than galactoglucommanan? With *P. monticola* the converse effect was observed. GGM-ase activities were uniformly the highest, whilst the xylanase were in general, the lowest. Similarly, the same questions should be asked this time concerning the accessibility of GGM.

Consultation of the graphs of results shew that a change in pH (a rise for *C. globosum* and a lowering for the other species) is always associated with an increase in activity. This is in agreement with many previous workers, however it was unusual to find a rise in pH. Fuller, 1970, obtained variable terminal pH's for cultures of *C. globosum* on cellulose in the same liquid medium. With the Imperial College strain he found pH's of 5.0 and 2.7, and with S70K, 6.2, 6.8 and rather surprisingly 4.5. It is possible that this last culture was in some way retarded and held in the "acid production phase" where the pH drops. This generally only lasted one to two weeks on sawdust cultures in the present work, whilst Fuller's cultures were harvested after five weeks. Contamination seems a strong possibility to explain this anomaly. The relative failure of *P. fastigiata* and *P. monticola* in liquid culture under conditions of

test was disappointing, however future work in investigation of suitable culture condition could change this. Nilsson, 1974a, has said that "the fact that one or more of the three cell wall degrading enzymes could not be detected in all of the wood-degrading fungi is considered to be a result of deficiencies in the test methods employed, and not the inability of the fungus to produce the enzyme."

Implicit in this statement is the assumption that all three enzymes cellulase, mannanase and xylanase are produced, but that the limitations of the assay methods result in their lack of detection. Lack of detection in a culture filtrate could be for two reasons (1) the enzyme is not produced, or (2) the enzyme is very closely bound to the fungal hyphae. Nilsson, 1974a, discusses the work of Eriksson and Goodell (in press) who conclude that the wood-rotting fungus Polyporus adustus has the induction of cellulase, xylanase and mannanase controlled by a single regulator gene. In the present work large quantitative differences were found between the production of xylanase and galactoglucomannanase of C. globosum, especially in the early stages of growth. Fuller, 1970, found that the Imperial College strain of C. globosum when cultured on cellulose in a liquid medium gave rise to cellulase but no xylanase. It would be interesting to see whether this is compatible with the theories of Eriksson and Goodell when they are published.

Using a clearing zone method of detection, based on that of Rautella and Cowling (1966), Nilsson, 1974a, shewed that in a group of 33 wood-destroying microfungi, 20 gave rise to all three cell wall polysaccharases,

(cellulase, xylanase and mannanase), 5 produced two types of enzyme, and for 3 species only one type was found. It would be interesting to obtain subcultures of these 33 species and to compare their enzyme productions under the conditions of the hemicellulase monitoring experiment. The action of these fungi was further investigated on birch wood and it was found that 4 out of the above 20 fungi gave rise to type 1 decay patterns and the other 16 gave rise to type 2. Is it possible that measurement of activities induced would shew biochemical differences between type 1 and type 2 decay patterns?

After investigating many different methods of enzyme estimation Nilsson, 1974a, shewed that the modification of the Rautella and Cowling, 1966, technique by using the medium VII of Bravery, 1968, gave rise to the highest number of positive results. A criticism of the present work is that CMC is used as the sole substrate for the estimation of 'cellulase' activity. In retrospect this is unsatisfactorily indirect in that the structure of CMC is too far removed from that of wood to give more than a general estimate of wood cellulose destroying ability. A combination of several different assays, though more tedious, would give rise to a far better understanding of the action of wood destroying fungi on wood cellulose. In particular it would have been desirable to have included some form of C_1 activity determination. Although involving more work, one of the most attractive methods would be by microchemical analysis of the woody material left by the fungus. It

would be worthwhile investigating procedures whereby this microanalysis could be set up on a semi-automatic basis, much as amino acid analysis is routinely performed today.

One of the other methods used was most interesting. It was an assay for cellulase and xylanase actually present in wood blocks attacked by the test organisms. Sections of decayed wood (up to 1mm. thick) were placed on test plates prepared according to Stranks and Bieniada (1971). Plates consisted of twin layers of agar with a thin monolayer of substrate (0.25%) at the interface of the layers. Sodium azide (0.005%) was used to inhibit fungal growth. Formation of clearing zones indicated activity. Although barely semi-quantitative, this assay is attractive for it is a direct measurement of enzymes found in the wood. Comparisons with the observed type and extent of attack in wood also can be made. The sensitivity was such that several fungi were demonstrated to have cellulase, which had given negative results with all the previous tests. There were, however, still some species of fungus which degraded wood and yet shewed no clearing by this method. Nilsson advanced two explanations for this fact: " (1) the enzymes were produced in only minute amounts in the wood, adequate for wood degradation but not sufficient for diffusion in the wood agar: and (2) the enzymes are firmly bound either to the wood substrate and/or the mycelium, and are thus not able to diffuse freely out into the test agar. " The first explanation appears to be less likely. If a section is cut through the wood, some hyphae and cavities are going to be directly exposed to the agar gel and if diffusable enzyme

is present even in small quantities, it appears to the author that a clearing zone will result. If clearing zones arise from one piece of degraded timber and do not from another degraded to the same extent, it would be reasonable to assume that both will have produced approximately the same quantity of enzyme, and therefore that binding of the enzyme is more likely. This binding could either be to the outside of the fungal hypha or to the substrate in the immediate area of the fungus. Fuller, 1970, found that washing the mixture of fungus and cellulose (obtained by filtration of the culture) with a solution of C.M.C. extracted large amounts of apparently "bound" enzyme and thus gave rise to an increased yield. The enzyme may be held loosely by some form of low energy bond, or alternatively a full covalent bond might be present. A disulphide bond is a possibility, in view of the evidence of Barash and Klein (1969) who found that the release of polygalacturonase from Geotrichum candidum was greatly increased by treatment with mercaptoethanol, a reagent responsible for the breakdown of disulphide bonds. Highley, 1973, checked for cellulase activity the mycelial mats left behind after filtration of the liquid medium. Very little (ca. 0.5%) C_x activity remained with either Poria monticola or Polyporus versicolor, but about 20% of the C_1 activity of the white rot was detected in the mycelial suspension. In the light of this work it would appear that some form of substrate binding or affinity is a more likely explanation for Nilsson's inability to demonstrate the presence of enzymes with some active wood-

destroying fungi when cultured either in wood or on cellulosic substrates in a liquid medium.

This relationship between enzyme production (and estimation) and the actual destruction of wood substrate is further complicated by the fact that the converse to the above phenomenen has also been found, namely that enzyme production and detection is not always correlated with a significant weight loss. Nilsson, 1974a, found three species which fell into this category. He does however say that "since great errors are involved in the procedure of determining weight losses of the cellulose substrates, only weight losses above 10 percent are regarded as significant". This degree of accuracy is not sufficient definitely to exclude weight losses with the above three species of fungi.

In the present work, no correlation could be found between decay capacity tests and the enzymes detected in a culture filtrate. One reason for this is the great difficulty in obtaining reproducible results for weight loss tests. The factors governing this and suggestions for modification of the techniques used, are fully discussed in the section on "weight loss tests". Another factor may be simply that there this is no correlation - i.e., that enzymic activity induced does not always represent "weight loss". Occasionally contamination (e.g. Penicillium sp.) was detected by fungi that are thought to be unable to degrade wood sufficiently to give rise to a weight loss, and yet great cellulase and xylanase activities were found. (See hemicellulase monitoring experiment). Further investigation of the action on wood of those contaminating fungi would be most interesting.

Thus it is possible that a particular combination of enzymes are necessary before weight losses occur. It should be noted in this context, that these enzymes, when tested by long term incubation with spruce holocellulose, gave rise to very small "weight losses" measured in terms of an increase in soluble sugars (Nelson-Somogyi). Thus they are capable, under the correct circumstances, of producing an attack of sorts on wood.

Fungal-free enzyme solutions, obtained by induction using birch and spruce sawdust as sole C-source, have been tested for their ability to destroy various substrates. Birch and spruce sawdust, cellulose powder, and spruce holocellulose was the increasing order of susceptibility to attack by culture filtrates of any of the four fungi tested. The most active fungal species was C. globosum S70B (only one strain of each fungus was tested), followed by P. versicolor, after which came P. monticola and P. fastigiata. Not surprisingly in view of the results of the hemicellulase monitoring experiment, the birch induced culture filtrates were, in general, more active that the spruce induced. In retrospect it would have been a good idea to have checked the wood particles, both by use of the light and S.E.M., and by microchemical analysis. For example, with the soft rot fungus some idea of the type of degradation causing the production of reducing sugars would be useful. Was there any Type 1 or 2 decay or anything similar, in the absence of hyphae?

This experiment backs up the concept that enzyme solutions that have been obtained by "induction by wood as sole C-source", when incubated with cellulosic substrates (for

example, spruce holocellulose), will give rise to definite enzymic destruction.

From microscopic studies of the behaviour of wood-destroying fungi a decreasing order can be established with respect to their ability to "act at a distance", the converse of "their degree of being bound". Starting with the type of fungal decay that seems to be able to act at the furthest distance this order is as follows: brown rot, white rot, soft rot and stainer. The last mentioned has a very minimal effect on the wood.

The enzymes produced by white and brown rots have been studied recently by Nilsson (in press) and Highley, 1973. The following comparisons emerge from their results, and those of previous workers. Brown rot fungi were, in general, limited in their ability to clear a zone in cellulose agar plates, i.e., the majority do not possess a C_1 activity. Nilsson (in press) tested 31 different species and only seven gave rise to clearing zones, thus brown rot fungi can be subdivided into two types: those which can, and those which cannot give rise to C_1 activity when cultured on cellulose. In a similar study of 31 different white rots all 31 gave clearing zones indicative of C_1 activity. This is, in a sense, an artificial classification, because all brown rot fungi must possess some form of C_1 modifying factor in order to degrade wood cellulose.

Cowling (1961) showed that with the white rot fungus Polyporus versicolor, both the amorphous and crystalline regions of wood cellulose were locally degraded, and no rapid depolymerization took place. This was the opposite for the

brown rot Poria monticola, where depolymerization of the cellulose was rapid and extended throughout the cell wall. Recent photomicrographs of Bravery (1973) show the typical limited 'groove' attack of white rot fungi.

Nilsson, in another experiment from the same paper, grew nine selected brown rot species on birch blocks, and assayed for cellulase and xylanase clearing zones by placing wood sections upon suitable 'twin agar' plates. It was found that xylanase was present in all nine but that cellulase was limited to three species. (These three were common to the group of seven species that had previously cleared cellulose, i.e., possessed C_1 activity).

When cotton fibres (supported in a 'wood-sandwich' to prevent complete saturation) were incubated by Nilsson with brown rot fungi, five out of six showed no visible signs of decay with microscopic examination (the sixth one being restricted to marginal lumen erosion and small helical cracks). Although no outward signs of degradation were marked, the tensile strength of the cotton was severely reduced. Thus some'depolymerizing' factor was able to diffuse out of the wood and affect the cotton cellulose. It would be interesting to determine the average length for the cellulose molecule for this 'depolymerized' or 'tensile strength' reduced cotton compared with whole cotton, in order to find out more about the action of this factor. Does it, for example, merely break down very long chains of 5 - 10,000 units of glucose into smaller chains of ca.100 units? Or is it perhaps restricted to the separation of the bonds which hold microfibril to microfibril laterally, i.e., does it affect the 'sliding

shear' of cellulose? Degradation into water soluble oligosaccharides would have resulted in observable changes under the microscope.

The question remains as to what is the nature of the factor which accomplishes the dissolution of wood cellulose by brown rot fungi. Nilsson says that it "might also be explained by an extremely efficient binding of the cellulase to the wood substance", but this explanation seems less plausible . He goes on to postulate that "(1) cellulase of the C_1 type is produced only in lignified tissue and (2) the cellulase produced is most active against lignified cellulose. The activity against pure cellulose is restricted to a limited cleaving of the cellulose chains". No reasons are given for assuming that the enzyme-substrate binding is a "less plausible" theory. The possibility of binding has been fully discussed at an earlier stage.

Eriksson and Pettersson (1972) investigating the fungus Chrysosporium lignorum, responsible for damage to chip piles in Sweden, used a liquid culture on cellulose to investigate its enzyme components. Five C_x enzymes were obtained with a high degree of purity. They were associated with mannose-containing carbohydrate moieties. One C_1 enzyme, measured as its ability to liberate reducing sugars from dewaxed cotton fibres, was found to be free of carbohydrate and was separated from the C_x components. This had a molecular weight of ca.50,000. Amino acid analyses showed the presence of 7 - 8 disulphide bridges, indicating a very solid well bound structure. (A normal figure would be 2 or 3 bridges). He found this component to be an exo-enzyme

removing glucose or cellobiose units from cellodextrins or Avicel. He explained the reason for the synergistic effect between C_1 and C_x as being a consequence of the C_x providing the substrate, i.e., the products of an endo-type reaction, for C_1. This is a reversal of the theory proposed by Reese et al (1950).

Cowling, 1958, suggested that another factor be applied to wood or lignocellulosic materials, for the preparative stage prior to cellulolysis. He postulated that the cellulose of these materials "may be digested by an unknown factor "X", presumably enzymatic, possessed only by fungi adapted to this type of substrate. Absence of this factor accounts for the inability of nonwood-destroying fungi to utilize wood for food". His postulated concept of the role of C_1 was that it separated the lateral bonds in cellulose chains, to provide substrate for C_x enzymes.

Fuller, 1970, also concludes that "the possession of C_1 type enzymes by a fungus is not necessary for the attack of wood" and continues further to postulate that "the rate of attack of wood is controlled by the so-called X-factor, which must consist of lignin modifying or cellulose releasing enzymes".

Koenigs (1972) suggested the possibility that the factor might be non-proteinaceous, having greater ability to diffuse through the wood leaving the lignin skeleton behind.

Highley, 1973, whilst working with three brown rot and three white rot fungi, also found that the former lacked and the latter possessed C_1 activity. He also concluded from repression studies with simple sugars that the C_x

activity of white rots was inducible and that of the brown rots constitutive. Addition of simple sugars to cultures of brown rots on complex cellulose substrates in some cases even enhanced enzymic activity. This behaviour fits well with the observation by Cowling (1961) that in a brown rot (P. monticola) rapid depolymerization (and consequent production and accumulation of simple sugars) occurred in the initial stages. Thus cellulases of brown rots in their natural environment are required to work in a significant concentration of simple sugars. This is not true of white rots where the products of the reaction act as repressors. Further biochemical work on this question of induction and repression in brown and white rots would be required before conclusions can be definite.

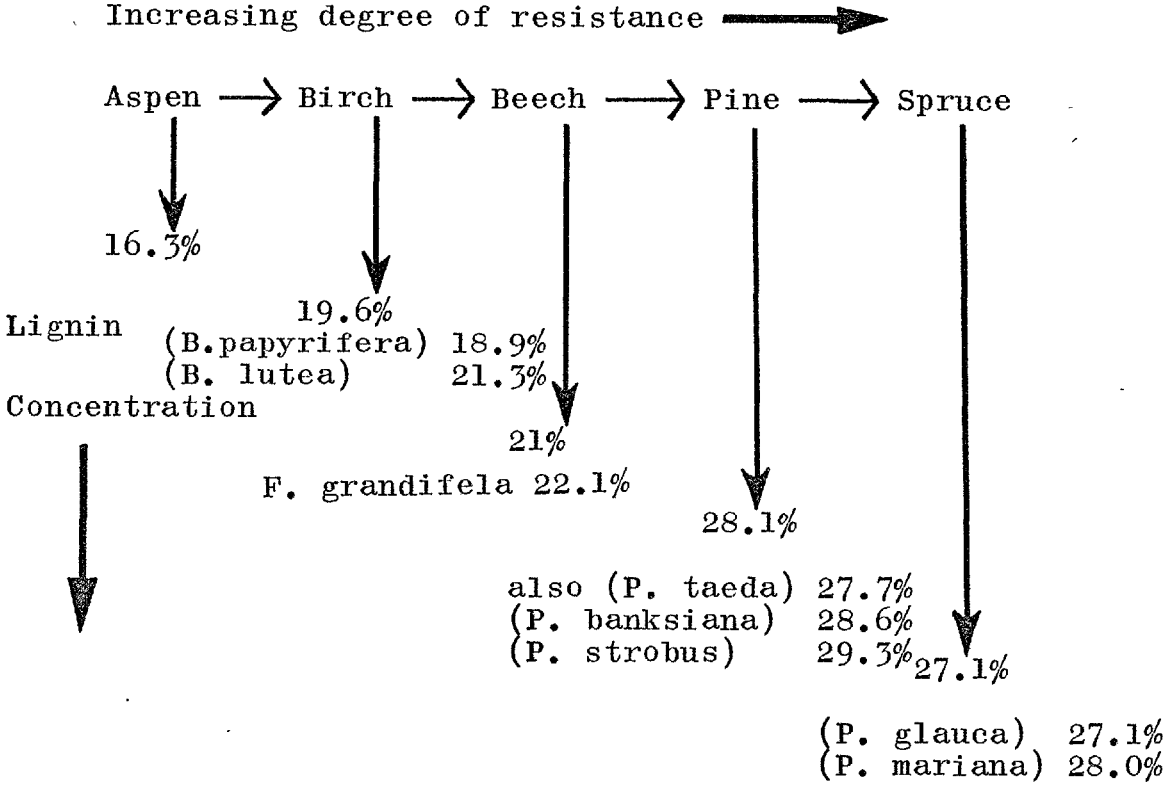
Cowling and Brown (1969) gave eight factors which were of importance in the susceptibility of a cellulosic fibre to enzymic degradation. They were (1) the moisture content; (2) the size and diffusibility of the enzyme molecules in relation to the "microspaces" in the fibre structure; (3) the degree of crystallinity of the cellulose; (4) its unit cell dimensions; (5) the conformation and steric rigidity of the anhydroglucose units; (6) the degree of polymerization of the cellulose; (7) the nature of the substances with which the cellulose is associated; and (8) the nature, concentration and distribution of substituent groups. Of particular signifi - cance in wood decay by fungal enzymes is (7), where hemi- cellulose and lignin play a significant role.

The role of lignin has been investigated particularly in the case of soft rot fungi and their cavity forming

method of decay. Findlay, 1970, found that soft rot cavities did not form after delignification of the wood. Courtois, 1963(b), Nouvertne, 1968, Bailey et al., 1968, and Findlay, 1970, all found increased attack by soft rot fungi after delignification of softwoods. Nilsson, 1974(b), found that "delignification (of jute) led to an increased amount of erosion-type attack, but the cavity formation decreased considerably". Findlay, 1970, does not indicate an erosion increase in wood, but merely a decrease in cavity formation. Perhaps the same phenomenon which occurred in jute with Nilsson went undetected with Findlay? Nilsson, 1974(b), has shown that certain fungi can degrade lignified jute fibres (concentration ca.10% lignin) but are unable to degrade cotton. Upon investigation of his culture medium, it was found that no trace elements had been included. Could it be that the natural fibre of jute, with its lignin present, contains sufficient quantities of these trace elements for cavity formation to occur, whilst the highly pure cotton does not? A possible mechanism for inhibition of cavity formation could be that metal ions, known to be necessary for this type of enzymic action, are not present in sufficient quantities to give rise to the correct molecular conformation for activity. Nilsson, 1973, demonstrated a decreasing order of susceptibility to soft rot fungi with the species "aspen (Populus tremula L.), beech (Fagus silvatica L.), birch (Betula verrucosa Ehrh.), pine (Pinus silvestris L.) and spruce (Picea abies (L.) H. Karst)".

Using Nilsson's data on lignin concentrations and also

from Browning, 1963, an interesting pattern of concentrations emerge. The lignin concentrations of all of the species which were used by Nilsson are shown, and also some examples of other species are given in parentheses.



For comparative purposes it can be noted that in the present work lignin concentrations were estimated as being 20.7% for birch and 27.9% for spruce, expressed in terms of extractive free whole wood. It can be seen that the general trend of increasing resistance to fungal decay is paralleled by the rise in lignin concentration. Basu and Ghose, 1960, found that delignified jute fibres were more susceptible than lignified jute fibres. Nilsson, 1974(b), concludes that increasing lignin concentration enhances cavity (Type 1) decay, and the converse is true for lumen erosion (Type 2) decay. Also he unsuccessfully endeavoured to use artificially lignified cellophane to investigate further the role of lignin.

It would appear that this line of approach would be worth expanding in such a way that more than one lignin concentration is tried, and also more than one type of lignin (i.e., from different sources and by different extraction methods).

Findlay, 1970 and Levy, 1969, observed that cavity formation did not occur with some 'soft rot stainers' in certain softwoods under their particular growth conditions. Nilsson, 1973, was able to obtain cavities in softwood with any fungus capable of producing them in hardwood. He did, however, observe a considerably greater degree of resistance in the softwoods tested. The questions must therefore be asked, is lignin more closely bound with GGM in the softwoods tested, and is glucuronoxylan freer from lignin in the hardwoods tested? This, perhaps, may be an answer to the relative roles played by lignin and hemicellulose, postulated by Cowling and Brown as one of the "eight factors" relating to enzymic destruction of a cellulosic fibre.

One of the most distinctive features of soft rot decay is the regular shape and orientation of a cavity. This has been a source of controversy amongst research workers in this field, some attributing the shape to the physical or chemical constitution of cellulose and its associated components, and some to the nature of the fungus itself. A definite sequence of events in the formation of cavities (i.e., Type 1 decay) has emerged in recent years, major contributions being from work by Levy, 1967, 1969, and Nilsson, 1974(e). The first step is passive penetration into a wood block using the natural pathways open to the fungus such as rays, vessels and pit openings. Next an

active penetration occurs at right angles to the microfibril axis. There are several interesting features concerning this stage. The diameter of the hypha is very small (ca.0.1μ - from Levy, 1967, a photomicrograph (Fig.1) ; the immediate area of wood around the penetrating hypha is not subjected to any lysis visible under the light microscope; the hypha can then do one of four things (a) pass straight through and into the lumen of the adjacent cell, (b) form a 'T' branch as described by Levy, 1967, (c) form a "cross-road" as described by Nilsson, 1974(e),(d) perform a right-angled turn. The third stage is this hyphal alignment parallel to the microfibrillar axis. The fourth stage involves enzyme production and the consequent lysis of cell wall with a simultaneous elongation of the hyphae along the long axis of the microfibril. The lysis is not uniform, chains of cavities being formed with periodic constrictions present. Where the orientation of cellulose microfibrils differs from the general pattern as shown in Fig.8, as for example around a pit, then the chains of cavities may follow this new line of orientation (Nilsson, 1974(e)). Type 2 decay, occurs in a completely different way, as a relatively uncomplicated erosion from the lumen.

Where cell wall thickness varies, for example, as between a vessel cell wall and the S_2 layer of a fibre, then the size of the chain of cavities also varies (Nilsson 1974(e)). In fact with kapok fibres, with an average thickness of 1μ (one third that of the cell wall of an earlywood pine tracheid) Nilsson, 1974(b), found that many species of fungus known to be capable of cavity degradation in jute and sisal, were unable to form cavities. Weak erosion and bore

holes were evident only after prolonged incubation.

The theory that cavity formation may be dependant upon the presence of lignin (e.g., Fuller, 1970) and for hemicellulose appears untenable in view of the fact that Nilsson 1974(e), has obtained cavities in cellulosic substrates (e.g., Avicel, cotton and spruce holocellulose) with little or none of these substances present. In this paper he tested numerous cellulosic 'fibrous' substances but restricted his studies to one fungus, Humicola alopallonella. Thus it should be borne in mind when interpreting his results on cavity formation that it is possible that all soft rot fungi do not operate in the same manner. From his studies both in 1974(b) and (e) much new information has emerged. Cavity formation was more abundant and quicker in naturally lignified fibres, and also for some reason cavity formation was not good when the fibre was saturated, such as in a liquid medium. After testing many culture conditions, it was found that the cellulosic materials (e.g. Avicel, cotton) were degraded by cavity formation at the fastest rate when placed upon birch wood blocks so that they had an average moisture content of ca.50%.

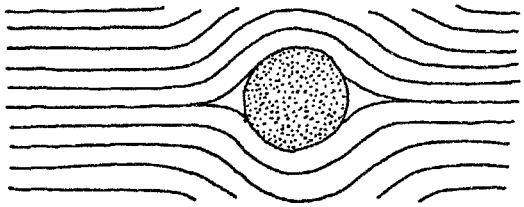
A liquid medium with vermiculite was used to achieve this, and it may be that the reason the author was unable to obtain cavities in spruce with C. globosum could possibly be attributed to moisture conditions in the blocks on wood agar. Type 2 decay was always found to be greater in the hardwoods tested (deliberately chosen for their ease of degradation) than in the softwoods. Type 1 was 'repressed' by use of liquid media, whereas the effect of liquid on

Type 2 decay was variable, with some species enhancing and with some repressing decay. With many fungal species tested only type 2 decay could be found. Cavity formation was "an inherited characteristic which was restricted to certain species. The cavity-forming ability varied among different species". Four basic shapes of the cavities obtained were shown (Nilsson 1974(b)).

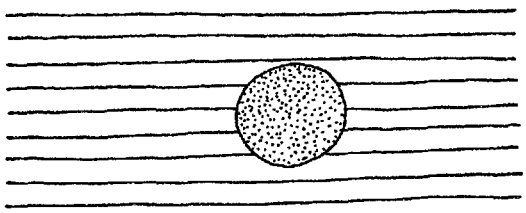
Several questions still remain. Why these shapes? What is the nature of the stimulus, connected with the need for alignment along the microfibrillar axis, that starts cavity formation when none was apparent with the fungus penetrating at right angles to the long axis? If this stimulus is biochemical in origin would experiments with substituted and artificial celluloses not further our knowledge in much the same way as our knowledge of the chemical structure of a "substrate inhibitor" has increased our learning on enzyme mechanisms? What is the original stimulus for entry? Which, if either, is correct, Levy (1967) who postulates a plasmadesmata theory, or Nilsson (1974(e)) who postulates a random mechanism?

What is the difference in culture conditions between the work of Levy and co-workers, and Nilsson, 1973, that allows the latter to obtain Type 1 decay in softwoods, which was not obtained by the former workers? Did these conditions directly affect the capacity of the fungus to produce an enzyme system parallel to the microfibrils, or was it merely a general repression of growth? In what way are these fungi related to the "true" staining fungi which are known (under present conditions) to be unable to form cavities? Is the

action of a penetrating soft rot fungus and a true staining fungus at right-angles to the microfibrillar axis , really mechanical as was suggested by Levy, 1967, or does a very restricted enzymic action take place? It would seem to the author that if the action was purely mechanical, hyphae even if only 0.1μ in diameter would tend to split the microfibrils in such a way that cracks, visible to the direct electron microscope, would be present ahead of the fungus. (This can be best visualised by considering the progress of an axe or nail through wood). Have any such cracks been seen? Has the orientation of microfibrils seen in a T.S. of the fungus gone around the hypha, as would be necessary if mechanical pressure was the means of entry? See below:



Or alternatively, are the microfibrils cut off neatly as if by enzymic action.



From electron micrographs by Findlay, 1970, and unpublished work by Zainal, 1974, no such splitting has ever been observed. It is therefore tentatively postulated that this pattern of wood decay has some form of chemical modification of the wood microfibrils, most likely enzymic, to allow penetration of the hypha.

Why are these discrete cavities in the form of chains rather than one continuous tube? Fuller, 1970, postulated build up of phenolic compounds cyclically repressing the enzymic activity, however Nilsson, 1974(e), has obtained similar chains of cavities in lignin-free materials where obviously there is no build up of polyphenols. Various other explanations were reviewed by Nilsson and he concluded by suggesting, "(1) the inhibition of enzyme synthesis could be due to catabolic repression by cellobiose or glucose released during the lysis of the cell wall around the hyphae. (2) It might be possible that the enzymes are produced only when a hypha in the cell wall is growing exactly parallel to the microfibrils. Even a slight deviation of the hypha would make production cease. As the hyphal tip growths further in the wall it later resumes its orientation parallel with the microfibrils and a new cavity is formed."

Siu, 1951, tabulates a detailed chemical analysis of the cotton fibres. The composition includes pectic substances (0.9%), organic acids , (0.8%) and some sugars (0.3%) including arabinose and xylose. Thus it can be seen that the so-called pure cellulose fibres does contain very small traces of other monosaccharides than glucose - such as xylose and mannose. The last two mentioned are abundant in wood. Perhaps the "key" to initiation of cavity formation, and possibly to the formation of chains of cavities, lies in the fungal enzyme finding a specific sequence of monosaccharides for it to "fit the lock" and start lysis. From a study of a similar hydrolytic enzyme (see Section II on "Enzymes") it is known that a sequence of six monosaccharides fits into a cleft in the lysozyme molecule (Perutz, 1967).

It is possible that the 'key' may consist not of a molecular arrangement of monosaccharides but with the concentration of nitrogen, and its redistribution and organizational use by the fungus. Is it possible that with the very small quantities of nitrogen present in wood that this has to be constantly "reutilized and re-located", thus, giving rise to discrete chains of cavities? One clue that may help answer this question lies in the observation that the S_3 layer is resistant to the formation of cavities and indeed to Type 2 decay. Comparison of the chemical composition with the S_2 layer may shew differences that possibly could explain this behaviour. Any of the above factors combined with the specific structure of cellulose may be the cause of the phenomenon of Type 1 decay in wood.

One of the questions that has remained unanswered when working with the decay of wood by fungi has been why is there a predominant occurrence of brown rot fungi on softwoods and white and soft rot on hard? Some obvious chemical differences in the substrates exist. The softwoods tested in this work have a higher lignin content than the hardwoods. Hardwoods have xylan as their major hemicellulose, whereas with softwoods it is glucomannan. Highly, 1973, says that "C_x and C_1 activity of the white rotters was no greater on hardwood sawdust - intact and modified by ball-milling and removing lignin (holocellulose) - than on softwood sawdust". Thus, since both types of wood contain similar quantities of cellulose in a similar pattern of distribution, it is probable that the answer does not lie with the cellulase production. In the present work cellulase

was induced on both birch and spruce, with the latter in somewhat smaller quantities.

Keilich et al., 1970, obtained indications that the brown rot Polyporus schweinitzii was more active against glucomannan, and the soft rot C. globosum was more active against xylan. This has been extended and confirmed in the present studies in the hemicellulase monitoring experiment. Nilsson, 1974(a), touches on the problem when he says, "A simultaneous production of xylanase and mannanase is most probable since production of only one of the enzymes would restrict the degradation ability to either softwoods or hardwoods". The picture, as seen by the author, is not so clear cut. It is considered, from the results of the hemicellulase monitoring experiment, that variable and differing quantities of these enzymes are produced, rather than either a positive or a negative situation as described by Nilsson. This worker's tests indicated presence or absence of an enzyme, and did not give more than a general idea of a quantitative estimation of their activity. Thus it can be seen that the hemicellulase monitoring experiment, the major part of this thesis, has provided strong indications of the role of hemicelluloses in fungal timber decay.

As a result of this work the following recommendations are made for further studies:-

(1) Investigate the culture conditions suitable for growth of P. monticola, P. fastigiata and P. versicolor with wood as sole C-source.

(2) Maximize the reproducibility of decay capability tests.

(3) Further investigate the behaviour of P. fastigiata in decay capability tests upon different media.

(4) Establish whether the resistance of Scots pine to decay by P. monticola, compared with spruce, was a genuine finding or an artifact.

(5) Investigate further the relationship of pH and culture activity, particularly with the differences between birch and spruce.

(6) Further study the possibility of using commercially prepared larch xylan, instead of the laborious preparation of the present work.

(7) Establish techniques of microchemical analysis of decayed wood on a semi-automatic basis.

(8) Investigate the effect of carbon coating of wood in the preparation for the S.E.M.

(9) Investigate any possible relationship between the size of wood particle in culture, the type of decay observed under the microscope, (e.g., Type 1 or 2 in the case of soft rot) and the enzymes formed.

(10) Study of the enzymes formed when growth is on the hemicelluloses xylan and GGM.

(11) Endeavour to purify and crystallize these enzymes with a view to computer programmed 'X'-ray crystallography to elucidate their three-dimensional structures.

APPENDIX I

1. Weight loss tests

(a) Preparation

1 centimetre cubes were used, orientated so that two opposite surfaces were in the transverse plane. Prior to use all blocks were examined to ensure that they were free from knots and blemishes which might affect the result. For each test the number of individual blocks varied between 3 and 10. For the first few tests the container used was a plastic petri dish inside a polythene bag (to prevent dessication of the agar), but this was discontinued at a later date. The modification was to use pickle jars containing a large slope of mineral agar which retained moisture for many months. When petri dishes were used, they were filled to the desired level solely with wood agar. When pickle jars were in operation a slope was made with mineral salts agar and then a laminate of wood agar (of about 5mm.) was placed on the surface of the slope.

The centimetre cubes were weighed when dry and sterilized by twice autoclaving at 120°C and 15 p.s.i. for twenty minutes separated by a three day interval. They were then dipped into sterile water prior to the start of the test.

The test plates were inoculated from cultures which were kept on wood agar slopes in McCartney bottles. When the fungal mycelium had covered most of the dish a sterile plastic mesh

(see Materials page 135) was put over the mycelial mat prior to the introduction of the sterile test blocks.

(b) Operation

The petri dishes or pickle jars were incubated at 25°C ± 1° usually for a standard 8 weeks, but in some cases the test was prolonged for several months. Checks were made at approximately weekly intervals, for contamination which was usually Penicillium sp. and Aspergillus sp. At the end of the test period one of two procedures would be carried out either

(i) with the petri dishes, excess mycelium was carefully scrapped off the block so as not to remove any woody material, and the blocks were dried in an oven at 105°C until constant weight (usually 24 hours).

(ii) The blocks were placed in a refrigerator at 1°C overnight, and then frozen to -90°C by immersion in liquid nitrogen. They were then freeze-dried, and weighed and stored in a desicator for future use in scanning electron microscope studies.

The results were expressed in terms of percentage weight loss. Mycelial weight was ignored.

2. Wood agar preparation

By experimentation it was found that a concentration of 10% w/v for birch and 7.0% w/v for Norway spruce 40-80 mesh

sawdust provided slurries of the correct consistency. This was due to the difference in the nature of the wood particles. Agar was used at 2% w/v concentration. The mineral medium was the same as was required for liquid culture.
(See Culture Techniques. page 332).

When constructing a wood agar slope in a pickle jar, for example, the following procedure was carried out. A slope of mineral agar was made in the normal fashion in such a way as to use about 90% of the available space. The pickle jar was then cooled to -20°C prior to filling the remaining 10% space with wood agar at 40°C. The gel, consequently, set rapidly and there was no settling of the wood suspension.

Slopes made in McCartney bottles were composed entirely of wood agar rather than layered mineral agar. The extra cost involved in using wood agar was justified by the reduction in labour time achieved, compared with layering technique.

3. Enzyme assays.

(a) Short term

0.1% Substrate solution which was contained in a "Zipette" dispensor, set to deliver 2.0 mls., was pre-incubated at 40°C. Test tubes containing enzyme solution (250 µl) were placed in a water bath at 40°C until enough time had elapsed for equilibration (c^a.5 mins.), then at suitably timed intervals, the substrate solution (2 mls. per tube) was added. The reaction was stopped after one hour by the addition of Somogyi reagent (2 mls.). The test tubes were removed from the 40°C water bath and immersed in one at 100°C for 30 ± 2 minutes. They were then allowed to cool, whereupon a small deposit of red copper oxide could be

seen in the tubes containing the more active enzymes. Nelson reagent (2 mls.) was added, and when the effervescence had died down the contents of the tube were swirled and shaken by means of a Whirlimixer.

Distilled water (15 mls.) was added from a Zipette in sucb a way as to produce an even mixture with the original contents of the tube. The intensity of the colour formed was read using an Eel colorimeter with an "OGRI" green filter distilled water as a blank. The organisation was such, that approximately three to four hundred test-tubes could be accommodated into one session. The limiting factor was that after one hour the first test tube needed to be stopped, so in practice only about 40 minutes was spent initiating assays before preparation to terminate them started.

When insoluble solid substrates were used in an assay, the incubation time was increased to seven or over forty days.

(b) Long Term Enzyme Digests

The following substrates were made up as 0.5% suspensions in 0.2M acetate buffer at pH 5.0.

(a) Cellulose powder.

(b) Birch sawdust, 60-80 mesh, acetone washed.

(c) Spruce sawdust, 60-80 mesh, acetone washed.

(d) Never dried spruce holocellulose. (The concentration of the stock suspension was determined by drying a known volume to constant weight. A 0.5% suspension was then prepared in acetate buffer and preserved with toluene.)

An 8 ml. aliquot of each suspension was taken and incubated with 0.25 ml. enzyme preparation at 40°C in covered test tubes. The holocellulose was found to be susceptible to drying out and required preservation from desiccation by a thin layer of toluene. Duplicates were set up for each enzyme.

After incubation, three 1 ml. aliquots were removed from each tube, made up to 2 mls. with distilled water, and the reducing sugars produced estimated by the Nelson modification of the Somogyi method (Nelson 1944, Somogyi 1952). Controls without enzyme were set up for each substrate in duplicate, and the values obtained subtracted from the experimental results. All absorbencies were obtained against a distilled water blank and are expressed in terms of reducing sugar equivalents. For readings with these substrates, care had to be exercised to ensure that no solid particles were allowed into the colorimeter cell. An Eel colorimeter with an OGRI filter was used.

3 (c) Factor for conversion to Fuller's units

Fuller's assay (1970)

This was 6.0 mls. of substrate and 0.2 mls. enzyme (E)

Total volume was therefore 6.2 mls.

0.5 mls. of this mixture was made up to 2 ml. and used in the standard assay procedure.

$\therefore$ 0.5 mls. contains $\frac{0.5 \times 0.2}{6.2} = 0.016$ mls. of E solution.

i.e. each Fuller reducing sugar equivalent (in μgms.) refers to 0.016 mls. of E. (His enzyme was partly purified).

In the present assay were used;

8.0 mls. of substrate and 0.25 mls. E

Total volume was therefore 8.25 mls.

1.0 mls. of this mixture was made up to 2 mls. and used in the standard assay procedure.

$\therefore$ 1.0 mls. contains $\frac{0.25}{8.25} \times 1.0 = 0.030$ mls. E.

Thus each of the present assays refers to 0.030 mls. E. (i.e. of culture filtrate, not purified enzyme).

In order to convert the present units into Fuller's units the reducing sugar results were multiplied by a factor of

$$F = \frac{0.016}{0.030} = 0.533$$

4. pH measurement.

pH determinations were carried out using a Pye Unicam 292 pH meter with a combined E.J. 702 electrode. The meter was standardized with potassium hydrogen phthalate (10.21 gm. lit^{-1}) at pH 4.00 and 25°C. Care was taken to check this before use or every twenty four hours during continuous use. The usual precautions were taken against scratching the glass electrode, desiccation and loss of saturated KCL solution.

5. Protein estimation.

(a) Method

The reagents were made up as follows:

A 2% Na_2CO_3 in 0.1N NaOH

B_1 1% $CuSO_4$

B_2 2% Na/K tartrate

C A (100 mls.) plus B_1 (1 ml.) plus B_2 (1 ml.)

D Folin-Ciocalteau reagent. (1N)
(diluted 1:1 to make it 1N)
Standard stock solution of Bovine Serum Albumen at 1 mg. ml^{-1}.

To a test tube (15 m m. x 150 m m.) containing the protein (0.5 mls.), reagent C was added (2.5 mls.), mixed well and allowed to stand for ten minutes at room temperature. Folin-Ciocalteau reagent (250 µl) was then added so that mixing took place within

a second, following this distilled water (7.0 mls.) was introduced. The intensity of the blue colour formed was estimated on an Eel colorimeter using a red Eel 608 filter.

(b) Standard curve

Protein standard solutions were made up in such strength that 0.5 mls. (the experimental volume) gave concentrations of 25,50, 100, 125, 200, and 250 µgms. per assay tube. A standard curve was constructed using an average of triplicate assays for each concentration. As a consequence of the varying nature of the reagents involved in this test a triplicate standard of 100 µgms. per tube was included into every protein assay session. If the average absorbency of this fell far out from the standard curve valued for 100 µgms., then a factor was incorporated into the calculation such that any error due to chemical variance was corrected. The standard curve was linear only in the 0 - 50 µgm. range and so values for absorbance had to be read off the curve individually if they were above this range. All results were multiplied by a factor of 1.428 to account for the dilution of the original culture filtrate sample from 3.5 ml. to 5.0 ml. (see Paragraph entitled "Dilution Factor" in section 9, page 329)

6. Measurement of small volumes.

Throughout this work all volumes of 100 to 500 µl were measured accurately on a Pipetteman 1000 variable dispensor. The accuracy and reproducibility was checked prior to use by weighing the accumulation of 100 aliquots of distilled water at 25°C, over the range indicated.

7. Fungi under test.

Stocks of the following fungal species and strains were maintained throughout this work on both malt and wood agar slopes.

Species 1. Chaetomium globosum

Strain Nos.	Source
S70, S70B, S70E, S70G, S70K, S70L, S70M, S70N	Forest Products Research Laboratory now Princes Risborough Laboratory of the "Building Research Establishment"
RLB ⟶	Dr. D.J. Dickinson: Rentokil Research Laboratories

Species 2. Phialophora fastigiata

Strain Nos.	
56A *	F.P.R.L.
Bann 1	I.C. strain isolated from a fence post by A.K. Banerjee (1968).

Species 3. Poria monticola

Strain Nos.	
304B, 304C, 304D, 304E, 304F, 304G, 304H	F.P.R.L.
280	(P. vaporaria)

*Erratum. This should read "S 6A".

Original cultures were maintained on malt agar slopes in McCartney bottles. For the purpose of inoculation of wood, these were subcultured several time onto wood agar. The wood agar was either hard or soft wood, and in general corresponded to the species of wood block being degraded.

After inoculation of a fungus onto a wood agar slope, growth was allowed to take place until the mycelium completely covered the slope (ca. 1 - 3 weeks) and then the bottle was stored at -20°C until wanted. Stocks were sub-cultured at about nine month intervals. All work on subculturing and inoculation was performed under sterile air conditions in a laminar flow bench.

In the late stages of this work a white rot fungus Polystictus versicolor strain No. IMI 79126 was introduced to widen the scope of the experimentation. Stocks were cultured solely on 2% malt agar slopes and were obtained from the Princes Risborough Laboratory.

8. Standard Curves for Monosaccharides.

Under the conditions of assay stipulated, standard curves for arabinose, glucose, mannose, galactose, and xylose were constructed. These were over the range of 0 to 250 µgms., and were linear. Solutions were made so that the sample volume of 2 ml. contained 25, 50, 75, 100, 150, 200 and 250 µgms. of sugar. Assays were carried out on three different occasions in time, and in triplicate for each point. Different solutions were tested with a view to checking the

reproducibility and reliability of the assay. The spread of results was not large and the slope of the line drawn through the points was noted for each sugar. Great care was taken in the weighing-out of the monosaccharides which had been kept in a vacuum desiccator for 48 hours prior to use. The results are shown below.

Sugar	Absorbance at 100 µgms.	No. of µgms. for absorbency of 1.0	Factor for conversion into µgms.
Glucose	4.47	22.3	22.346
Mannose	4.27	23.4	23.529
Xylose	4.49	22.3	22.340
Arabinose	3.62	28	27.871
Galactose	3.47	28.4	28.694

When calculating the number of µgms. of reducing sugar in a particular test tube all that was necessary was to multiply the absorbency obtained from the Eel colorimeter by the suitable factor or "combination of factors" to obtain the number of micrograms present.

9. Calculations involving complex substrates such as galactoglucomannan and 4-O Methyl glucuronoxylan.

From the results of the chromatographic analysis, (Appendix II it was possible to determine the percentage of individual components of monosaccharide. Thus it was assumed that the

chromagen for each sugar was produced on a strictly proportional basis, and a factor was accordingly calculated. It was realized that this was merely a close approximation to the real situation but was considered the most accurate method in the circumstances.

Dilution Factor: When converting a given figure from Eel absorbence units into micrograms, in addition to the factor required given above each figure was also multiplied by 1.428, obtained by dividing 10 by 7. This was because the initial enzyme solution from the culture fluid was diluted in one of the following ways:

	Either	Or
Sample from culture filtrate	7.0 mls.	3.5 mls.
Acetate Buffer pH 5.0 (0.2M.).....	2.0 mls.	1.0 ml.
Sodium azide (3.1 gm. l^{-1}) in acetate buffer)...	1.0 ml.	0.5 mls.
Total vol.	10.0 mls.	5.0 mls.

This assumed that at this strength the enzyme solution was proportional in its activity with the concentration. Since the culture filtrates were very dilute this was a valid assumption. (see also "Sampling Technique" Section 14 page 334).

10. Example of Calculation of "Overall Factor" for a Complex Substrate, e.g. G.G.M.

The table below showsa figure for the combined dilution factor and absorbence conversion factor for each of the five monosaccharides present in G.G.M. Also shown is the percentage composition of G.G.M. (See Appendix II).

	Combined Factor	% Composition
Galactose	41.004	5
Glucose	31.932	27
Mannose	33.623	61
Arabinose	38.828	2
Xylose	31.932	5

Therefore using the above information the following equation can be formulated for the calculation of an "overall factor" for G.G.M.

$$\frac{\left(\begin{matrix}\text{Factor}\\ \text{Gal.}\end{matrix}\right)5 + \left(\begin{matrix}\text{Factor}\\ \text{Gluc.}\end{matrix}\right)27 + \left(\begin{matrix}\text{Factor}\\ \text{Man.}\end{matrix}\right)61 + \left(\begin{matrix}\text{Factor}\\ \text{Arab.}\end{matrix}\right)2 + \left(\begin{matrix}\text{Factor}\\ \text{Xyl.}\end{matrix}\right)5}{100} =$$

$$\frac{(41.004)5 + (31.932)27 + (33.623)61 + (38.828)2 + (31.932)5}{100}$$

Therefore Overall Factor for G.G.M. is 33.555.

In a similar fashion the overall factors for the other hemicellulose substrates were calculated. They are as follows:

	Birch D.M.S.O.	32.476	
	Spruce D.M.S.O.	33.414	
	Birch xylan	31.932	(based on 100% xylan)*
and	Spruce G.G.M.	33.555	

*This was based on xylan although it was assumed that the 4-O-methyl glucuronoxylan contained an unknown but appreciable amount of 4-O-methylglucuronic acid (e.g. 10%), the absorbence conversion factors for glucose and xylose are almost identical, thus the error was minimal.

11. Solubilization of substrates.

(a) Reducing sugar assay

Hemicelluloses which had been freeze dried were mostly in the form of aggregates of dried spongy material about one inch cube in shape and of a similar consistency to expanded polystyrene. It was thus necessary to grind this material to a very fine uniform powder using a mortar and pestle with the hemicellulose cooled in liquid nitrogen. Assiduous grinding resulted in a powder of a fairly large grain. This was reduced to over 60 mesh in a Wiley mill at room temperature. Some of the resultant powder (2 gms.) was placed piecemeal into a 1M solution of sodium hydroxide (100 mls.) and was stirred magnetically overnight in an atmosphere of O_2 free nitrogen gas. When dissolution was complete, 1M. acetic acid was added slowly with continuous stirring until the pH of the solution/suspension was 5.0. The mixture was then made up to 2 L. with distilled water to give a final concentration of 0.1%. The pH was once more checked, adjusted if necessary and finally the mixture was steamed at 100°C for 30 minutes before storage in a sealed container at 2°C. C.M.C. used in the reducing sugar assay was solubilized as described below in (b), but the concentration was 0.1% w/v.

(b) Viscosity test

Carboxymethylcellulose (C.M.C.) was directly dissolved into 0.2M acetate buffer (pH 5.0) in the following manner. C.M.C. (4 gms.)

was added piecemeal into buffer (1 Litre), which had been heated to 95°C, with continuous blending using a high speed blendor (Vortexmixers). After checking for the absence of any lumps or solid, the rest of the buffer (1 Litre) was added, to result in a final concentration of 2 gms. lit.$^{-1}$.

12. Culture techniques.

These were based on Fuller's (1970) method. The basic mineral medium contained:

Ammonium nitrate NH_4NO_3	3.0 g
Potassium dihydrogen phosphate KH_2PO_4	4.5 g
Magnesium sulphate hydrated $MgSO_4.7H_2O$	2.0 g
Calcium chloride $CaCl_2$	0.1 g
Thiamine hydrochloride	1.0 mg

Trace elements;

Boric acid H_3BO_3	570 µg
Ferric chloride $FeCl_3$	145 µg
Cobalt chloride $CoCl_2.6H_2O$	40 µg
Copper sulphate $CuSo_4.5H_2O$	60 µg
Manganese Chloride $MnCl_2.4H_2O$	30 µg
Ammonium molybdate $(NH_4)_6Mo_7O_{24}.4H_2O$	20 µg
Zinc sulphate $ZnSo_4.7H_2O$	310 µg

These chemicals were dissolved in 1 litre of distilled water and the pH was adjusted to 5.0 with 4M Sodium hydroxide. Fuller has quoted this medium as being a slight modification

of that of Abrams (1948). Trace metals are made up in a concentrated stock solution (x 100), 10 mls. of which were added per litre of final medium.

The wood cultures were in 400 ml. medical flats containing extractive free 40-80 sawdust (0.5 gms.), and mineral medium (50 mls.). The medical flat was stoppered with a non-absorbent cotton plug to allow easy access of atmospheric oxygen. The flats were laid on their sides to increase the surface area of the liquid, and after inoculation were incubated for the growth period at $25^{o}C \pm 1^{o}C$.

13. Inoculation of hemicellulase monitoring experiment.

Two weeks prior to the planned start of the main hemicellulase monitoring experiment, the growth of the inoculation flasks was initiated. 40 medical flats with the appropriate mineral medium, 20 containing birch sawdust and 20 containing Norway spruce sawdust, were plugged, autoclaved, cooled, and inoculated. The inoculum consisted of a small plug of mycelium approximately 4 mm. in diameter from a wood agar slope. Birch slopes were used for birch wood culture medium and spruce slopes for spruce wood culture medium.

After two weeks' growth at $25^{o}C$ the culture vessels were ready to act as a source of inoculum for the main experiment. A "Kenwood liquidizer" was sterilized by autoclaving and then by flaming well with alcohol. The contents of a medical flat

were then introduced into the liquidizer and homogenized for 30 seconds. It was then left for a moment to allow the larger wood particles to sink to the bottom. The liquid (1 ml.) containing homogenized hyphae was introduced in a sterile manner into each of the two medical flats prepared for the hemicellulase monitoring experiment. It was thus hoped that each individual flask contained, as closely as was possible, an identical inoculum at the start of the experiment. Great care was taken to sterilize the Kenwood liquidizer in between inoculating different fungal strains and fresh sterile pipettes were used for each stage of the operation.

Although satisfactory in theory this method of inoculation eventually proved to be more troublesome than it was worth owing to contamination problems and so any future worker, given the same working conditions, would be recommended to inoculate directly, if somewhat unevenly, from the wood agar slopes.

14. Sampling technique to determine the enzyme levels in a culture filtrate.

This was performed at various different time intervals during the growth of the fungus in liquid culture. Initially (for the first two samples day 3 and 6) 7 mls. of culture fluid were taken, but this proved to be more than was necessary and was consequently reduced to 3.5 mls. Pipettes used in the operation were sterilized by heating to 350^{o}F for 12 hours, and contained small cotton wool plugs in the mouth orifice.

After measurement of the pH, 0.2M acetate buffer pH 5.0 (1.0 mls.), and sodium azide 3.1 gm. lit. (0.5 ml.) were added (where the initial volume was 3.5 mls.).

15. Determination of the Degree of Rotation.

(a) Method

Solutions of the substrates to be tested were dissolved/suspended in 10% w/v sodium hydroxide solution, at a known concentration in the region of 2g. per 100 ml. In an initial experiment 1g. per 100 ml. (as used by King, 1968) was found to be unsatisfactory because it gave too low a value for accurate readings. When dissolved, the solutions were stored overnight at 3°C, then allowed to equilibrate to room temperature (25°C) before use. A photoelectric polarimeter (Bellington and Stanley PL 70/1) was warmed up for thirty minutes before use.

Solutions were made of the following compounds

From birch wood:	From Norway spruce wood:
4-O-methylglucuronoxylan	4-O-methylglucuronoxylan
birch D.M.S.O. extract	D.M.S.O. extract
"glucomannan"	galactoglucomannan
α-cellulose	α-cellulose

The polarimeter was zeroed with air and each of the solutions was placed in turn in the cell and the degree of rotation measured. A blank containing only sodium hydroxide was also measured and the value obtained used as a correction factor on the other results

Before each reading about three minutes was allowed to elapse to bring the temperature of the cell into equilibrium with the chamber, which was $25^{o}C$.

Duplicate samples of the substrate solutions were dried to determine the true weight of carbohydrate in solution. To do this an aliquot (10 mls.) of each solution was introduced into a preweighed dry crucible. Sodium hydroxide blanks were also included. The solutions were then evaporated and dried in an oven at $105^{o}C$ for 48 hours prior to a redetermination of their weights. The weight per 100 ml. could thus be calculated and corrected for the dry weight of sodium hydroxide.

(b) Theory

$$(\alpha)_{D}^{t} = \text{Specific rotation for D line (Sodium light) at } t^{o}C$$

$$= \frac{100\alpha}{lc}$$

where; α = measured rotation
l = length of the cell in dm. (1 dm.= 10 cm.)
c = concentration in gm./100 ml.

Calculation

An example of a typical calculation is as follows:

Air blank	-	0.101^{o}	These should be similar - the solvent blank value was used.
Sodium hydroxide blank	-	0.111^{o}	
Birch xylan	-	1.210^{o}	

Therefore birch xylan $\alpha^{25} = -1.099$

The residual weight after drying at 105°C, of 10 mls. of birch xylan solution was 1.19g. *

The residual weight after drying at 105°C of 10 mls. of sodium hydroxide was 1.04g. *

therefore extrapolating to 100 mls. the weight of 100 mls. xylan solution = 11.9 g.

and the weight of 100 mls. of sodium hydroxide solution = 10.4 g

therefore weight of hemicellulose = 1.5 g. per 100 mls.

$$(\alpha)_D^{25} = \frac{100 \times (-1,099)}{1 \times 1.5} = -73.27^\circ$$

$$(\alpha)_D^{25} = -73^\circ$$

16. Lignin estimation

The principle involved in one estimation of lignin is to remove all non-lignaceous materials by extraction and hydrolysis and determine the weight of the residue. Klaison (1906) was the first to use sulphuric acid for this purpose. The method used is a modification of his procedure and the product is termed "Klaison Lignin".

*Average of duplicate values.

Sawdust was placed into a thimble in a Soxhlet extraction apparatus, and extraction with ethanol:benzene (1:2) allowed to take place for 12 hours. The solvent was then removed from the wood-meal by allowing a stream of air to pass over it for twenty-four hours whilst in a fume cabinet. After drying a known weight (approximately 1g.) was transferred to a conical flask (250 mls.). 95% ethanol was added to the extractive free sawdust in such a quantity as just to wet it, whereupon 66% w/v sulphuric acid (50 mls.) was added very slowly and with stirring. This was allowed to stand at room temperature (ca. 23^{o}C) for 24 hours. After this time concentrated sulphuric acid was added in small aliquots until such time as the suspension was a clear brown solution, or very nearly so. The final concentration required was generally 101% w/v (or 55% v/v). The flask was left for 24 hours at room temperature prior to dilution with distilled water to the capacity of the container (250 mls.). The resultant mixture was filtered on a fine porosity pre-weighed sintered glass suction funnel, and washed well with distilled water. The sinter containing the mixture of carbohydrate and lignin was placed in a beaker of 0.5% hydrochloric acid, with a large watchglass covering the top of the beaker in such a way as to act as a condenser. The whole was placed in an autoclave and subjected to 120^{o}C and 15 p.s.i. for three to four hours.

The level of hydrochloric acid in the beaker was such that the mixture in the sinter remained in its location during autoclaving.

The product was washed well with distilled water and then dried at 75°C for several hours prior to subjecting it to 105°C for one hour. It was cooled in a desiccator and reweighed. The difference in weight between this reading and the original wood meal was considered to be the weight of lignin.

For holocellulose, a sample from the acid chlorite delignification (never dried) was washed in acetic acid (2N), acetone, and Soxhlet extracted with 95% ethanol for 4 hours, washed in ice water and dried in a stream of air, prior to the determination of lignin.

APPENDIX II

Details of Birch Hemicellulose Production

Extractive free sawdust

1000g. of birch 40-80 sawdust were placed in a muslin bag, which in turn was introduced into a Soxhlet apparatus. Ethanol: benzene 1 : 2 (15L.) was used as a solvent to extract the sawdust overnight. The muslin bag was then allowed to drain in a fume cupboard. Care was taken that benzene fumes came into contact with the operator as little as possible. The contents of the muslin bag were spread on large filter papers (30 cms. diameter), so that the depth of the sawdust was about 1 cm. Removal of the last traces of solvent was effected by allowing fresh air to be drawn over them while still in a fume cabinet. An extracted 40-80 mesh birch sawdust weighing approximately 900g. was the end product. This procedure was repeated once, yielding approximately 1800g.

Delignification

This was achieved by the acid chlorite method. 1000g. of the extractive free 40-80 birch sawdust was placed in the delignification apparatus (see Fig.34). Enough distilled water was added to enabled a stirrable slurry to form (ca. 7-8L.) Reagent grade glacial acetic acid (200 mls.)

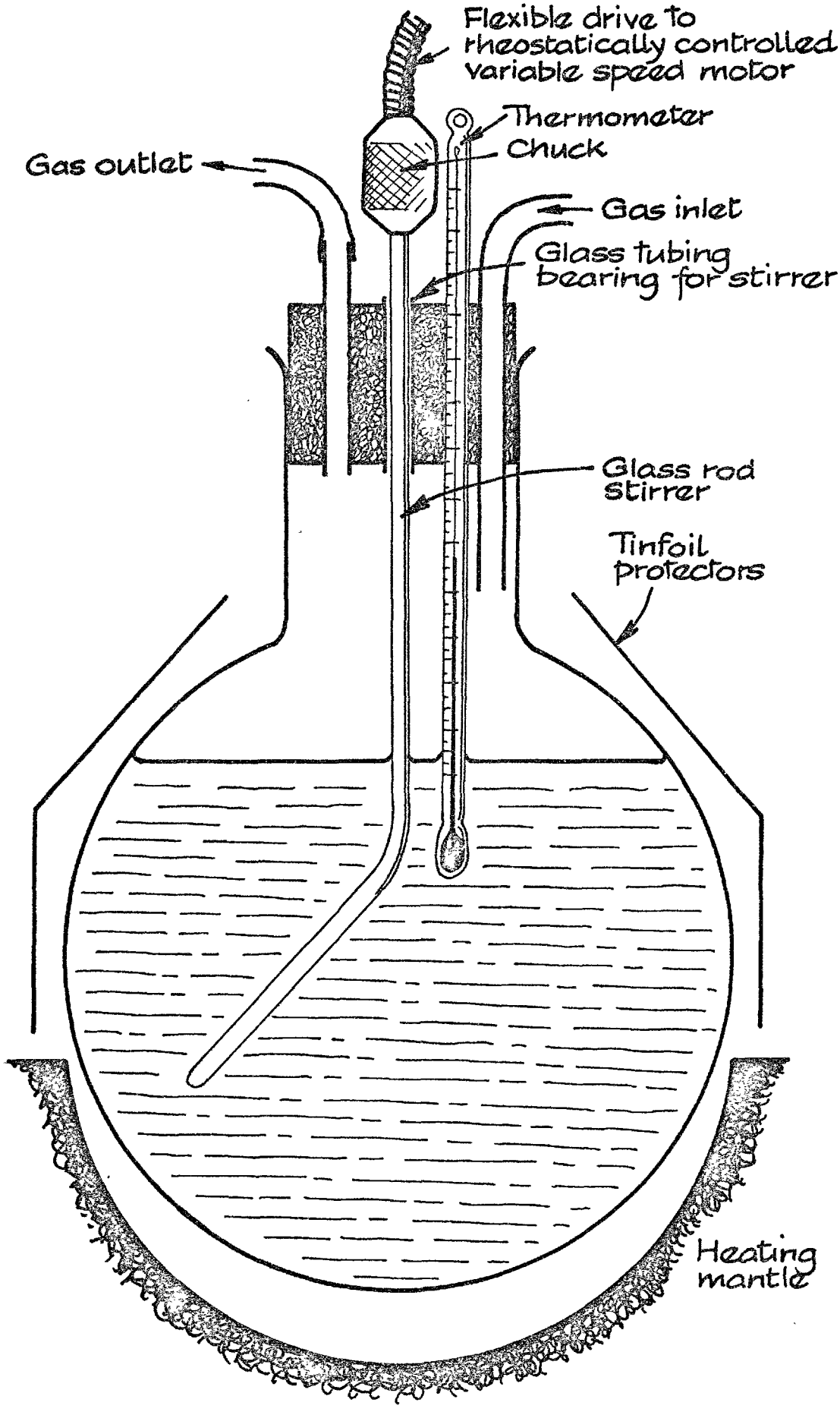


Fig. 34 Delignification/Extraction Apparatus

was added prior to sodium acetate (246g.). Then sodium chlorite (198g.), the last of which had previously been dissolved in the minimum quantity of water, was slowly added. Continuous stirring was employed throughout the additions, and octan-2-ol was used to control any foaming which occurred. The temperature was raised to between 70^{o} and 80^{o}C, trying to keep nearer the former figure. At hourly intervals for three hours (four additions altogether) similar quantities of reagents were added to the slurry, maintaining the same order of addition. The apparatus was set up in a fume cabinet with shields to prevent access to direct sunlight, as chlorine dioxide, which is produced in the reaction, is explosive under those conditions.

At the end of four hours the container was cooled with tap water, and then placed in a cold room (ca. 3^{o}C) until its temperature was below 10^{o}C. The light yellow bleached chlorite holocellulose was then washed in a minimum quantity of iced distilled water until the colour and smell of the chlorine dioxide was removed. This was mainly done by decantation, then finally by use of a muslin filter. The mass of holocellulose was not allowed to dry out but stored wet. The combined chlorite liquors were kept stored in the cold room, and small samples (5-10g.) of the holocellulose were taken for future analysis of lignin content. (see later).

Chlorite liquors.

These were evaporated from about 15L to 1L using a vacuum rotary evaporator at 40°C. They were then dialysed against distilled water leaving plenty of room in a large length of wide bore Visking tubing. After several changes of water the contents of the tubing were again evaporated, until solids had begun to precipitate out of solution. The procedure was repeated several times until the dialysate was free of small molecular weight contaminants.

First extraction of birch holocellulose.

This was to obtain hemicellulose containing acetyl groups. The birch holocellulose was squeezed free of most excess water in a muslin filter, and the "cake" of wood meal introduced into the same apparatus which was used for the delignification. The air in this container was displaced by O_2 free N_2 gas, and dimethyl sulphoxide (5L.) was added. The mixture was gently stirred overnight with a small but continuous stream of nitrogen passing through the vessel. The total volume of the slurry was ca. 8L. Room temperature was 23 $\pm$ 1°C.

The suspension was then filtered, using muslin, and the filtrate stored at 3°C. The residue was replaced in the extraction apparatus and distilled water was added until the volume of the slurry reached 8L. The temperature was raised until the slurry started to boil, whereupon the extraction was left to continue overnight without any more

application of heat. (At this stage the whole apparatus was stored at 3°C under N_2 gas for three days, owing to some technical difficulties in obtaining reagents necessary for the next operation. Ideally the extraction should be as continuous as possible). The slurry was then filtered, and the two filtrates combined (9L.). They were cooled to 3°C, and precipitated in $2\frac{1}{2}$ volumes of cold (3°C) ethanol (95%) acidified with reagent grade glacial acetic acid (ca. 500 mls.). The precipitate was left in the cold to aggregate overnight, after which it was collected by decanting off the supernatant fluids prior to centrifuging off most of the excess fluid. The hemicellulose solid, of the same consistency as 'papier-maché', was stored at -20°C.

Primary alkaline extraction

This was to obtain hemicellulose rich in glucuronoxylan. To the residue obtained from the previous water extract, potassium hydroxide solution was added to make the final volume of the slurry (8L.) a concentration of 7% w/v (ca. 560 gms. were used). As previously, the air was displaced by nitrogen and the extraction allowed to continue overnight. The suspension was then filtered. To do this another person apart from the operator stood by, playing a jet of nitrogen gas in such a way as to minimise the contact of the reagents with oxygen in the atmosphere. The filtrate was stored under

nitrogen at 3°C, whilst the residue was re-extracted under similar conditions for 5 hours. After this time the holocellulose suspension was again filtered and the two filtrates pooled. The resultant solution (ca. 10L.) was then cooled to 3°C and added to acidified ethanol (at 3°C) composed of the following: 95% ethanol (25L.), 75% ethanol (20L.), reagent grade glacial acetic acid (1650 mls.). The glacial acetic acid was in sufficient quantity to neutralize the potassium hydroxide and provide a very slight excess of acetic acid. Ethanol at 75% concentration had been reclaimed from a previous precipitation and was used for economic reasons. The total concentration of ethanol was enough to raise the ethanol aqueous ratio above 2. The precipitate formed was allowed to stand overnight before being recovered and stored in a similar fashion to the dimethyl sulphoxide extract.

Secondary alkali extraction

This was to obtain a product rich in glucomannans and galactoglucomannans. The residue obtained from the previous extraction was immediately replaced in the apparatus under nitrogen and the extracting solvent was added to make a final volume of 8L. This was a solution of sodium hydroxide (reagent grade 1440 g.) containing boric acid (320 g. reagent grade) to make a final concentration of 18% and 4% respectively.

The slurry was stirred overnight under a continuous stream of nitrogen at room temperature, after which it was filtered and the residue re-extracted with an identical solution for 5 hours. This in turn was filtered and the two filtrates combined (ca. 10L.). The filtrates were then precipitated in suitably acidified ethanol as described in the 7% KOH extraction.

Birch α cellulose

The residue, from which most of the hemicelluloses had been extracted, was exhaustively washed with distilled water, until the pH of the washings was neutral. It was then washed in reagent grade acetone, and allowed to air dry in a laminar flow bench for 48 hours.

Spruce Hemicellulose Production

Extractive free Norway spruce sawdust was produced in exactly the same way as birch. Production of spruce holocellulose was also the same except that five treatments of acid chlorite reagents were needed instead of four. The spruce sawdust was less dense than the birch and therefore was divided into two portions, which were delignified separately. This was necessary because the size of the container was insufficient for 1000 gms. of spruce 40-80 sawdust slurry. The holocellulose produced from these separate treatments was then combined prior to extracting

with DMSO, hot water, 7% potassium hydroxide, and 18% NaOH + 4% borate, in exactly the same way as the birch holocellulose. An exception was that the DMSO/water extract (11L.) was rotary-evaporated under reduced pressure at 40°C to give a volume of 6L. prior to precipitation with acidified ethanol. Water evaporating off was checked from time to time with a hygrometer to ensure that no DMSO was present. This procedure significantly reduced the volume of ethanol needed and thus was of economic importance. The total volume of the spruce extraction was 9L. All hemicelluloses were stored at - 20°C.

Dialysis and Freeze Drying

Each of the three extracts, (DMSO/water, 7% KOH, 18% NaOH + 4% borate) from both soft and hardwoods were removed from storage at -20°C, allowed to warm to room temperature and then exhaustively dialysed (ca. 5 times each) against a large volume of distilled water (10L.). Care was taken to ensure that plenty of room was left in the Visking tubing to allow for osmotic expansion. The supernatant fluid thus obtained was rotary-evaporated at 40°C under a vacuum, in between each dialysis. The resultant hemicelluloses were then frozen in metal trays, and freeze dried (at P.R.L.). The resultant powder was then weighed and stored in glass jars in a desiccator.

Analysis of hemicelluloses and α cellulose

The hemicellulose sample (2-3g.) was cooled in liquid nitrogen and ground with a pestle and mortar into a fine powder. Any large fragments remaining were removed with a 120 mesh sieve, prior to hydrolysis with sulphuric acid.

Hydrolysis

After much experimentation with the hydrolysis conditions, the following procedure was found to be satisfactory (based upon that of Saeman et al, 1954).

Spruce glucomannan-rich hemicellulose

Hemicellulose (250 mgs.) was placed in a conical flask (50 mls.) equipped with a well rounded glass rod. Absolute alcohol (300-500 µl) was added until the whole of the powder had been wetted, and the flask was left open to the atmosphere until any excess alcohol had evaporated. 72% sulphuric acid (3 mls)was added in small aliquots with stirring in between additions. (sulphuric acid 72% : made up by 1278 ml. concentrated acid to 780 ml. distilled water). The powder was ground until well mixed with the acid, then incubated at 30°C for one hour, with occasional stirring. The mixture was then cooled to 3°C and kept for two days, after which the contents of the 50 mls. flask was quantitatively transferred to a 500 ml conical flask using distilled water (87 mls.). The diluted acid solution was autoclaved at 15 p.s.i. for one hour.

All Birch hemicelluloses, spruce DMSO extract, and xylan

It was found necessary to add another aliquot of 72% sulphuric acid (3mls.) after two days at 3°C. The birch glucomannan and xylan now dissolved, but it was found that both birch and spruce DMSO extracts and spruce xylan needed another hour's incubation at 30°C. After this treatment the spruce xylan dissolved, leaving only the two types of DMSO extract slightly cloudy. To these were added small quantities of concentrated sulphuric acid (ca. 500µl.) until dissolution was effected. Water (174mls.) was added and the mixture autoclaved as before.

This treatment resulted in a small sediment forming, which was filtered off in a preweighed sinter. The constant weights obtained after drying at 105°C were as follows:-

Spruce:	DMSO	: 10.8mgs.	Birch:	DMSO	: 1.8mgs.
	Xylan	: 23.0 "		Xylan	: 0 "
	Glucomannan:	5.6 "		Glucommannan:	0 "

Even in the case of spruce xylan, which was the highest weight, the percentage of solids was below 10%. This was still not considered good enough to continue with the analysis, and so the spruce hydrolyses were repeated until the weight of solids was below 5mgs. Barium carbonate solid was added in small quantities(ca. 250mg.) to the hydrolysate, which was shaken at low revolutions on a Griffin and George Shaker. The additions were continued every five to ten minutes until the solution was pH 6. The barium sulphate

thus formed was removed by centrifuging, and the clear supernatant and washings were passed through an IR120 Cation exchange resin column (20 x 1.5cm.) to remove any remaining barium ions. The eluate and washings (distilled water) were combined and reduced to 5mls. using a rotary evaporator under vacuum at 40°C. These solutions were then stored at 3°C prior to paper chromatographic analysis.

Paper chromatography

Whatman's No. I paper (9.5 x 22.5 ms.) was cut at the bottom edge with pinking shears to produce a serrated end. Throughout this work the same batch of paper was used. Chromatography was carried out in a descending apparatus at 25°C ± 0.5° in a position well shielded from draughts. The solvent used was ethylacetate-pyridine-water (8 : 2 : 1, by volume) (Jermyn and Isherwood,1949).

Each chromatogram was washed in distilled water for 12 hours in the chromatography apparatus, then dried before application of the sugars. The origin line was drawn in pencil 6cms. from the non-serrated end, and two 90° folds at 2 and 5cms. respectively produced a "U" shape suitable for placing in the chromatography tank. Six spots were drawn on the origin line evenly distributed over the width of the paper allowing room for spread at the edges. Samples and standards were placed on the chromatogram using an Arnould microapplicator. This machine was set to deliver

0.25µl aliquots, which when spread out on dry paper attained a diameter of approximately 2-3mm. Drying was speeded by a blast of cold air from a fan directed underneath the chromatogram. Samples varied between 2½µl (10 x 0.25 aliquots) to 25µl (100 x 0.25 aliquots). All monosaccharide standards were 2µl (8 aliquots) except xylose which was 2½µl. Standard solutions of five monosaccharides were made by dissolving 250mgs. of distilled water (10mls.); thus each µl contained 25µg. of mono-saccharide. Standards were prepared of the following sugars; glucose, mannose, xylose, arabinose, and galactose.

When a chromatogram had been suitably prepared, it was placed in the developing tank in an atmosphere of the solvent to be used, then allowed to equilibrate for several hours, after which the developing solvent mixture was added to the trough, via a small hole in the glass lid which was in turn covered by a microscope slide. The developing time was 20 hours when the tank was occupied by two chromatograms and 16 hours when one was present. After this time the sheets were removed and air dried in a fume cupboard until all traces of solvent had evaporated (ca. 4 hrs.).

The chromatograms were developed with aniline phthalate reagent (Partridge, 1946) made up as follows. Phthalic anhydride (8.3g) was dissolved in water-saturated butanol (500mls.). Before use aniline (1ml.) was added to 100mls. of

the solution. After spraying evenly, the chromatogram was allowed to air dry a few minutes and then placed in an oven at 105°C for exactly 5 minutes. Pentosans gave red spots, aldohexoses gave brown spots, and the background was a very slight yellow colour.

Elution of the developed chromatogram

All traces of the aniline phthalate reagent were allowed to evaporate in a fume cupboard. A record of both the length of spot and distance travelled from the origin was taken. This was related to glucose, giving R-glucose values. Using a standard perspex rectangle of suitable dimensions, spots were marked out with a pencil in such a way that every spot was marked on a piece of paper of uniform size. The size of the rectangle was chosen so that the largest spot was just accommodated. The rectangles of paper containing the coloured spots were then cut out and placed into labelled test tubes containing eluting medium (4mls.). This was 0.7N HCl in 80% ethanol v/v (Partridge 1946). After allowing 12 hours for elution of the colour, the absorbence at 480 mμ gave the relative concentration of monosaccharide. Standards from the chromatogram were used to calculate the concentration in μgms. Owing to the inherent variation possible in the many steps of this test, standards were necessary for each chromatogram, and the results of one could not be used for

calculation of another. Three blank papers of identical size were used for zero values and their absorbence subtracted from the experimentals.

Experimental

Experimental chromatograms were run for each type of hemicellulose hydrolysate to obtain a rough idea of their relative concentrations of monosaccharides. From these chromatograms it was possible to establish whether it was necessary to lengthen the autoclaving step. Hydrolysates that resulted in a long trail and much material still on the origin were subjected to longer hydrolysis periods, when the hydrolysis was repeated with a fresh sample of material.

From the results of these chromatograms it was decided that the following further chromatograms would give accurate estimations of monosaccharide content. They are labelled A - I. Experimental spots varied between 2.5 - 10µl and standards were all 1.5µl. After all the chromatograms were run, they were stored prior to being developed and eluted in one large batch. The results from these follow later.

Chromatogram

Name						
A	G	Bir	X	G	Gal	Bir
		DMSO 10 µl	Bir DMSO			DMSO 10 µl
B	X	Bir		X	Bir	X
		Xyl 2.5 µl	2.5 µl		Xyl 2.5 µl	
C	X	Spr	G	Spr	M	Gal
		Xyl 2.5 µl		Xyl 2.5 µl		
D	G	Bir	Gal	A	G	Bir
		Xyl 10 µl				Xyl 10 µl
E	G	Bir	Gal	A	G	Bir
		GM 5 µl				GM 10 µl
F	Spr	Spr	M	Spr	G	X
	GGM 5 µl	GGM 5 µl		GGM 10 µl		
G	Spr	Spr *	M	Spr	G	X
	DMSO 5 µl	DMSO 10 µl		DMSO 10 µl		
H	Spr	G	Spr	M	Gal	G
	α cell 2.5 µl		α cell 10 µl			
I	Bir	G	Bir	M	Gal	G
	α cell 2.5 µl		α cell 10 µl			

KEY:

G = Glucose
Gal= Galactose
A = Arabinose
M = Mannose
X = Xylose

Bir = Birch
Spr = Spruce
DMSO = Dimethyl sulphoxide extract
Xyl = Xylan
GM = Glucomannan
GGM = Galactoglucomannan

All standards were 1.5 µl (6 x 0.25 µl) containing 37.5 µg

* On developing it was found that this spot had been forgotten.

Chromatogram Results

"A"		R. Glucose	Abs. 480 mμ (corrected)	μgms ‡
Std. G		1.000	0.035	37.5
Bir. DMSO	10 μl			
	Gal ?	0.817	0.017	17 *
	G ?	0.996	0.046	495
	A ?	1.424	trace	-
	X ?	1.766	0.274	194
Std. X		1.847	0.053	37.5
Std. G		1.000	0.045	37.5
Std. Gal.		0.828	0.050	37.5
Bir. DMSO	10 μl			
	Gal ?	0.785	0.027	17 *
	G ?	0.945	0.059	44
	A ?	1.385	trace	-
	X ?	1.614	0.249	175
Average for	3 Blanks		0.033	

* Average result

‡ These results calculated using the nearest standard on chromatogram.

Average Bir. DMSO Gal ?	for 10 μl	17
" " " " ?	" "	45.5
" " " " ?	" "	185

Chromatogram Results

"B"		R. Glucose	Abs. 480 mμ (corrected)	μgms
Std. X		1.700	0.063	37.5
Bir. Xyl.	2.5 μl			
	X ?	1.758	0.067	40
Bir. DMSO	2.5 μl			
	Gal ?	0.856	0.005	4
	G ?	1.000	0.010	9.5
	X ?	1.700	0.093	55.5
Std. X		1.895	0.056	37.5
Bir. Xyl	2.5 μl			
	X	1.903	0.045	31
Bir. DMSO	2.5 μl			
	Gal ?	0.856	0.010	8
	G ?	1.000	0.010	13.5
	X ?	1.859	0.066	44.5
Average Blanks			0.030	
Average Bir DMSO Gal? for 2.5 μl				6
" " " G ? "				11.5
" " " X ? "				49.5
" Bir.Xyl X ? "				35.5

"C"		R. Glucose	Abs. 480mμ (corrected)	μgms
Std. X		1.956	(0.020) 060 *	37.5
Spr. Xyl.	2.5 μl			
	Gal ?	0.826	0.004	3
	G ?	1.019	0.016	15
	M ?	1.288	0.014	11
	A ?	1.586	0.006	3
	X ?	1.971	0.032	24
Std. G		1.000	(0.065) 040 *	
Spr. Xyl	10 μl			
	Gal ?	0.073	0.032	24
	G ?	0.875	0.067	63
	M ?	1.149	0.049	41
	A ?	1.432	0.043	23
	X ?	1.807	0.233	146
Std. M		1.288	0.048	37.5
Std. Gal		0.788	(0.038) 050 *	37.5
Average Blanks value			0.030	

Used Std-Arabinose Value 0.072 *

These results were repeated at a later stage owing to the fact that the standards were incorrect.

Results in parenthesis ignored.

* Standard values assumed on basis of other chromatograms.

"D"			R. Glucose	Abs. 480 mμ (corrected)	μgms
Std.	G		1.000	0.030	37.5
Bir.	Xyl	10 μl			
"Trail"		X ?	-	0.067	42
"Actual Spot"		X ?	1.733	0.287	180
Std.	Gal		0.820	0.042	37.5
Std.	A		1.471	0.072	37.5
Std.	G		1.000	0.060	37.5
Bir.	Xyl	10 μl			
"Trail"		X ?	-	0.085	53.5
Spot		X ?	1.534	0.313	196.5
Average	Blanks			0.040	

Used an assumed standard xylose value 0.060

This was repeated after complete hydrolysis.

"E"					
Std.	G		1.000	0.025	37.5
Bir.	GM	5 μl			
		G ?	0.956	0.010	8.5
		M ?	1.165	0.019	16
		X ?	1.806	0.352	220.5
Std.	Gal		0.835	0.016	37.5
Std.	A		1.534	0.065	37.5
Std.	G		1.000	0.041	37.5
Bir.	GM	10 μl			
		G ?	0.956	0.028	17.5
		M ?	1.165	0.032	26.5
		X ?	1.694	0.587	375
Average	Blanks			0.038	
Assumed	Std.	M		0.045	
"	"	X		0.060	

"F"		R. Glucose	Abs. 480 mμ (corrected)	μgms
Spr. G.G.M.	5 μl			
	G ?	0.986	0.030	34
	M ?	1.259	0.150	123
	X ?	1.918	0.018	12
Spr. G.G.M.	5 μl			* Average result
	G ?	1.005	0.028	
	M ?	1.300	0.143	
	X ?	1.977	0.023	
Std. M.		1.264	0.045	37.5
Spr. G.G.M.	10 μl			
	Gal ?	0.750	0.008	6
	G ?	0.923	0.069	65
	M ?	1.214	0.253	211
	X ?	1.895	0.058	36
Std. G		1.000	0.073	37.5
Std. X		1.918	0.088	37.5
	Average	Blank	0.035	
	Assumed	Std. G	0.040	
		" Gal	0.050	
Average result		Sp. GGM		
" "		G ?	for 5 μl	34
		M ?	"	123
		X ?	"	12

Average result using both sets of readings calculated for 5 μl

G ?		33.5
M ?	"	117
X ?	"	14
Result for Gal ?	"	3

"G"		R. Glucose	Abs 480 mμ (corrected)	μgms
Spr. DMSO	5 μl			
	Gal ?	0.807	0.019	17.5
	G ?	0.960	0.035	37.5
	M ?	1.250	0.113	94
	A ?	1.580	0.015	7.5
	X ?	1.972	0.047	29.5
Std. M		1.278	0.057	37.5
Spr. DMSO	10 μl			
	Gal ?	0.710	0.019	17.5
	G ?	0.898	0.061	65.5
	M ?	1.188	0.155	130
	A ?	1.506	0.021	10.5
	X ?	1.898	0.071	44.5
Std. G		1.000	0.037	37.5
Std. X		1.983	0.056	37.5
	Average	Blanks	0.037	
	Assumed	Std. Gal	0.040	
	"	" M	0.045	
	"	" A	0.072	

The second spruce DMSO 10 μl spot, was not present.

"H"				
Spr. α cell	2.5 μl			
	G ?	0.987	0.116	154.5
	M ?	1.268	0.006	3.0
Std. G		1.000	0.080 (correct)	37.5
Spr. α cell	10 μl			
	G ?	0.874	0.500	233.5
	M ?	1.176	0.040	21
Std. M		1.273	0.072	37.5
Std. Gal		0.798	0.079	37.5
Std. G		1.000	0.126 (wrong)	-
	Average	Blank	0.051	
	Result spr α	cell G ?	for 2.5 μl	154.5
	" "	" M ?	" 10 μl	21

"I"		R. Glucose	Abs. 480 mμ (corrected)	μgms
Bir α cell	2.5 μl			
	X ?	1.966	0.096	45
Std. G		1.000	0.066	37.5
Bir α cell	10 μl			
	Gal ?	0.766	trace	
	G ?	0.933	trace	
	X ?	1.966	0.475	225
Std. M		1.272	0.060	37.5
Std. Gal		0.816	0.047	37.5
Std. G		1.000	0.019 (wrong)	-
	Average	Blanks	0.045	
	Assumed	Std. X	0.080	

From the results of chromatograms A to I, it became apparent that more chromatograms were necessary to provide a clearer picture of the monosaccharide content of these hemicelluloses. These were constructed as described on the following page and were labelled J to X.

J	G	Spr α cell 2.5 µl	M	Spr α cell 10 µl	G	M
K	X	Spr α cell 2.5 µl	X	Bir α cell 2.5 µl	Bir GM 2.5 µl	X
L	Gal	Bir α cell 10 µl	Gal	G	G	G
M	Gal	G	Bir 10 µl DMSO	Gal	Bir DMSO 10 µl	G
N	X	Bir DMSO 2.5 µl	X	Bir 2.5 µl DMSO	Spr DMSO 5 µl	X
O	G	Bir GM 10 µl	M	Bir GM 10 µl	G	M
P	Gal	Spr DMSO 12.5 µl	A	Spr DMSO 12.5 µl	Gal	A
Q	G	Spr DMSO 5 µl	M	Spr DMSO 5 µl	G	M
R	X	Spr Xyl 2.5 µl	X	Spr Xyl 2.5 µl	Spr GGM 10 µl	X
S	X	Bir GM 2.5 µl	X	Spr DMSO 5 µl	X	
T	Gal	Spr Xyl 10 µl	M	Spr Xyl 10 µl	Gal	M
U	A	Spr Xyl 10 µl	G	Spr Xyl 10 µl	A	G
V	X	Spr GGM 10 µl	X	Gal	Spr GGM 15 µl	Gal
W	G	Spr GGM 2.5 µl	M	Spr GGM 10 µl	G	M
X	G	Gal	G	M	A	X

All standards contained 2 µl (50 µg) except xylose which had 2.5 µl (62.5 µg)

"J"		R. Glucose	Abs. 480 mμ (corrected)	μgms	
Std. G		1.000	0.114	50	
Spr α cell	2.5 μl				
	G ?	1.013	0.141	66	"more likely to be accurate "
	M ?	too faint			
Std. M		1.250	0.105	50	
Spr. α cell	10 μl				
	G ?	1.067	0.497	233	"less accurate"
	M ?	1.164	0.062	27	
Std. G		1.000	0.100	50	
Std. M		1.175	0.122	50	
	Average 3	Blanks	0.118		
	" Std.	Mannose	0.114	50	
	" "	Glucose	0.107	50	
"K"					
Std. X_A		2.146	0.139	62.5	
Spr. α cell	2.5 μl				
	G ?	1.000	0.181	N.S.A.	
	M ?	1.280	0.015		
Std. X_B		2.150	0.146	62.5	
Bir α cell	2.5 μl				
	X ?	2.071	0.131	59.3	
Bir. GM	2.5 μl				
	G ?	1.000	0.012	N.S.A.	
	M ?	1.320	0.014	N.S.A.	
	X ?	2.119	0.153	69.3	
Std. X_C		1.889	0.130	62.5	

NSA = No standard available.

Average result for Std. X

A, B & C 0.138 62.5

"L"		R. Glucose		Abs. 480 mμ (corrected)	μgms
Std. Gal		0.864		0.119	50
Bir α cell	10 μl				
	Gal ?	0.852		0.053	24.1
	X ?	1.894		0.496	N.S.A.
Std. Gal		0.852		0.147	50
Std. G		1.000		0.100	50
Std. G		1.000		(0.179)	
Std. G		1.000		0.129	50
	Average	Blanks		0.189	
	Average	Std.	Galactose	0.110	50
	Average	"	Glucose	0.115	50
"M"					
Std. Gal		0.861		0.081	50
Std. G		1.000		0.094	50
Bir. DMSO	10 μl				
	Gal ?	0.788		0.022	13.5
	G ?	0.972		0.071	42.3
	A ?	1.778		0.002	N.S.A.
	X ?	2.361		0.406	N.S.A.
Std. Gal		0.800		0.081	50
Bir. DMSO	10 μl				
	Gal ?	0.722		0.027	16.7
	G ?	0.928		0.054	32.1
	A ?	1.778		0.011	N.S.A.
	X ?	2.189		0.411	N.S.A.
Std. G		1.000		0.074	50
	Average	Blanks		0.042	
	"	Std. Galactose		0.081	50
	"	"	Glucose	0.084	50
	"	Bir. DMSO Gal ?		for 10 μl	15.1
		" " G ?		" "	37.2

"N"		R. Glucose	Abs. 480 mμ (corrected)	μgms
Std. X		2.017	0.109	62.5
Bir. DMSO	2.5 μl			
	Gal ?	0.844	trace	NSA
	G ?	1.000	trace	NSA
	X ?	2.042	0.081	44.8
Std. X		1.998	0.127	62.5
Bir. DMSO	2.5 μl			
	Gal ?	0.818	trace	NSA
	G ?	1.000	0.006	NSA
	X ?	2.004	0.089	49.2
Spr. DMSO	5 μl			
	Gal ?	0.774	0.028	NSA
	G ?	1.000	0.057	NSA
	M ?	1.255	0.108	NSA
	X ?	1.973	0.033	18.3
Std. X		1.992	0.103	62.5
	Average	Blanks	0.103	
	"	Std. Xylose	0.113	62.5
	"	Bir. DMSO X ?	for 2.5 μl	47
	Spr.	DMSO X ?	" 5 μl	18.3

"O"		R. Glucose	Abs. 480 mμ (corrected)	μgms
Std. G		1.000	0.101	50
Bir. GM	10 μl			
	Gal ?	0.787	trace	-
	G ?	0.994	0.029	15.9
	M ?	1.288	0.041	26.6
	A ?	1.550	trace	-
	X ?	2.044	0.721	NSA
Std. M		1.324	0.086	50
Bir. GM	10 μl			
	Gal ?	0.791	0.002	NSA
	G ?	0.970	0.026	14.3
	M ?	1.268	0.056	36.4
	A ?	1.550	trace	-
	X ?	2.015	0.715	NSA
Std. G		1.000	0.081	50
Std. M		1.308	0.067	50
	Average	Blanks	0.085	
	"	Std. Glucose	0.091	50
	"	Std. Mannose	0.077	50
	"	Bir. GM G ?	for 10 μl	15.1
	"	" " M ?	" "	31.5

"p"			R. Glucose	Abs. 480 mμ (corrected)	μgms
Std.	Gal		0.928	0.075	50
Spr.	DMSO	12.5 μl			
		Gal ?	0.826	0.020	11.8
		G ?	1.000	0.110	NSA
		M ?	1.381	0.186	NSA
		A ?	1.765	trace	-
		X ?	2.224	trace	-
Std.	A		1.840	0.074	50
Spr.	DMSO	12.5 μl			
		Gal ?	0.725	0.073	42.9
		G ?	1.000	0.091	NSA
		M ?	1.301	0.207	NSA
		A ?	1.653	0.020	13.2
		X ?	2.107	trace	-
Std.	Gal		0.880	0.095	50
Std.	A		1.696	0.077	50
		Average	Blanks	0.062	
		"	Std. Galactose	0.085	50
		"	" Arabinose	0.075	50
		"	Spr. DMSO Gal ?	for 12.5 μl	27.3
		"	" DMSO A ?	" "	13.2

"Q"		R. Glucose	Abs. 480 mμ (corrected)	μgms
Std. G		1.000	0.085	50
Spr. DMSO	5 μl			
	Gal ?	0.763	trace	-
	G ?	0.961	0.026	17.8
	M ?	1.256	0.104	60.8
	X ?	1.980	trace	-
Std. M		1.270	0.093	
Spr. DMSO	5 μl			
	Gal ?	0.763	0.010	NSA
	G ?	0.980	0.027	18.5
	M ?	1.280	0.084	49.1
	X ?	2.043	trace	-
Std. G		1.000	0.061	50
Std. M		1.217	0.078	50
	Average	Blanks	0.064	
	"	Std. Glucose	0.073	50
	"	Std. Mannose	0.085	50
	"	Spr. DMSO G ?	for 5 μl	18.2
	"	" " M ?	for 5 μl	54.8

"R"		R. Glucose	Abe. 480 mμ (corrected)	μgms
Std. X_A		2.241	0.130	62.5
Spr. XyL	2.5 μl			
	Gal ?	0.814	0.003	NSA
	G ?	1.000	0.018	NSA
	M ?	1.315	trace	-
	A ?	1.708	trace	-
	X ?	2.214	0.060	28.6
Std. X_B		2.220	0.114	
Spr. Xyl	2.5 μl			
	Gal ?	0.819	0.005	NSA
	G ?	1.000	0.016	NSA
	M ?	1.358	trace	-
	A ?	1.697	trace	-
	X ?	2.188	0.057	27.2
Spr. GGM	10 μl			
	Gal ?	0.781	Not done	
	G ?	1.000	0.134	NSA
	M ?	1.315	0.268	NSA
	A ?	1.670	trace	-
	X ?	2.155	0.039	18.6
Std. X_C		2.117	0.148	62.5
	Average	Blanks	0.025	
	Average	of the		
xylose	standards	A B C	0.131	62.5
	Average	Spr. XyL X ?	2.5 μl	27.9
	"	" GGM X ?	10 μl	18.6

"S"			R. Glucose	Abs. 480 mμ (corrected)	μgms
Std.	X		2.230	0.091	62.5
Bir.	GM	2.5 μl			
		Gal ?	7.886	0.002	NSA
		G ?	1.000	trace	-
		M ?	1.299	0.004	NSA
		X ?	2.206	0.128	75.5
Std.	X			0.122	62.5
Spr.	DMSO	5 μl			
		Gal ?	0.785	0.001	NSA
		G ?	1.000	0.030	NSA
		M ?	1.311	0.062	NSA
		A ?	1.736	trace	-
		X ?	2.229	(0.012)	(7.1)
Std.	X		2.480	0.104	62.5
Spr.	DMSO	5 μl			
		Gal ?	0.817	trace	-
		G ?	2.229	0.014	NSA
		M ?	1.424	0.053	NSA
		A ?	1.900	trace	-
		X ?	2.363	(0.006)	(3.5)
		Average	Blanks	0.020	
		"	Std. Xylose	0.106	62.5
"K" + "S"		"	Bir. GM ?	for 2.5 μl	72.4 see also "K"
		"	Spr. DMSO X ?	" "	5.3 - unreliable so repeated.

"T"

This was found to possess unreliable standards and so the whole chromatogram was repeated. Using standards from averaging all the other chromatograms, the following "estimated" results were obtained.

Spr.	Xyl	Gal ?	10 μl	25.4
"	"	M ?	"	40

These are only rough estimates. See repeated $c/_{gm.}$ for accurate result.

"U"		R. Glucose	Abs. 480 mμ (corrected)	μgms
Std. Ar.		1.676	0.086	50
Spr. Xyl.	10 μl			
	Gal ?	0.806	0.018	NSA
	G ?	1.014	0.061	59.2
	M ?	1.315	0.048	NSA
	A ?	1.685	0.016	9.5
	X ?	2.102	0.233	NSA
Std. G		1.000	0.050	50
Spr. Xyl.	10 μl			
	Gal ?	0.792	0.017	NSA
	G ?	0.977	0.048	46.6
	M ?	1.269	0.036	NSA
	A ?	1.602	0.013	7.7
	X ?	2.056	0.215	NSA
Std. A		1.755	0.083	50
Std. G		1.000	0.053	50
	Average	Blanks	0.046	
	"	Std. A	0.085	50
	"	" G	0.052	50
	"	Spr. Xyl A ?	for 10 μl	8.6
	"	" G ?	" "	52.9

"V"		R. Glucose	Abs. 480 mμ (corrected)	μgms	
Std. X		2.199	0.093	62.5	
Spr. GM	10 μl				
	Gal ?	0.803	trace	-	
	G ?	1.000	0.071	NSA	
	M ?	1.341	0.279	NSA	
	A ?	1.762	trace	-	
	X ?	2.172	0.027	15.3	See "R" also
Std. X		2.194	0.127	62.5	
Std. Gal		0.864	0.071	50	
Spr. GM.	15 μl				
	Gal ?	0.825	0.007	5.4	See "α" also
	G ?	1.000	0.093	NSA	
	M ?	1.363	0.277	NSA	
	A ?	1.773	0.003	1.8 *	
	X ?	2.211	(0.027)	(15.3)	too low
Std. Gal		0.848	0.058	50	
	Average	Blanks	0.059		
	"	Std. Xylose	0.110	62.5	
	"	" Galactose	0.065	50	
* Assumed standard Arabinose			0.084	50	

"W"		R. Glucose	Abs. 480 mμ (corrected)	μgms
Std. G		1.000	0.052	50
Spr. GGM	2.5 μl			
	G ?	0.918	0.030	25.2(x4=100.4)≠
	M ?	1.432	0.079	56 (x4=226) *
	X ?	2.393	0.013	(7.1) less accurate
Std. M		1.290	0.076	50
Spr. GGM	10 μl			
	G ?	0.802 low result	0.110	92.4 ≠
	M ?	1.148	0.295	209.4 *
	X ?	2.142	0.027	14.7(÷4 ≙4)
Std. G		1.000	0.067	50
Std. M		1.246	0.065	50
	Average	Blanks	0.069	
	"	Std. Glucose	0.060	50
		" Mannose	0.070	50
Results used:	Spr. GGM	G ?	for 2.5 μl	25.2
	" "	M ?	for "	56
	" "	X ?	for 10 μl	14.7

"X"				
Std. G		1.000	0.105	50
Std. Gal		0.795	0.147	50
Std. G		1.000	0.144	50
Std. M		1.258	0.149	50
Std. A		1.652	0.181	50
Std. X		2.068	0.202	62.5
	Average	Blanks	0.141	

After chromatograms J - X had been interpreted, it was found necessary to repeat some and to enlarge on others. The following were therefore set up.

"T" repeated	Gal.	Spr.	M	Gal	Spr.Xyl	M
		Xyl 10 µl			10 µl	
α	X→ A	Spr. GGM	X→ A	Gal	Spr. GGM	Gal
		25 µl			25 µl	
β	A→ X	Spr. GGM	A→ X	G	Spr. GGM	G
		25 µl			25 µl	
γ	X→ A	Bir.	X→ A		Bir.	X→A
		Xyl 2.5 µl			Xyl 2.5 µl	
δ	A→ X	Bir.	G	Bir Xyl	G	A→X
		Xyl 20 µl		20 µl		

A mistake was made in the application of standards resulting in "α" and "γ" Xyloses being in fact arabinose, and "δ" and "β" arabinoses being Xylose. The results were thus interpreted with each others standards.

Chromatogram T was also repeated. For results see next page.

"T" repeated		R. Glucose	Abs. 480 mμ (corrected)	μgms
Std. Gal		0.867	0.032	50
Spr. Xyl	10 μl			
	Gal ?	0.828	0.009	12.3 See "U" also
	G ?	1.000	0.047	NSA
	M ?	1.236	0.020	37.7 See "U" also
	A ?	1.483	0.019	NSA
	X ?	1.773	(0.118)	NSA
Std. M		1.236	0.021	50
Std. Gal		0.828	0.041	50
Spr. Xyl	10 μl			
	Gal ?	0.793	0.030	41 See "U" also
	G ?	1.000	0.046	NSA
	M ?	1.217	0.037	69.8 See "U" also
	A ?	1.448	0.022	NSA
	X ?	1.744	0.180	NSA
Std. M		1.212	0.032	50
	Average	Blanks	0.043	
	"	Std. Galactose	0.037	50
	"	" Mannose	0.027	50
* Average Spr. Xyl. Gal ?			for 10 μl	25
* Average " " M ?			" "	61

* Includes results calculated from chromatogram "U"

"α"		R. Glucose	Abs. 480 mμ (corrected)	μgms
Std. A		1.509	0.033	50
Spr. GGM	25 μl			
	Gal ?	0.810	0.034	49.3
	G ?	1.000	0.265	NSA
	M ?	1.292	0.689	NSA
	A ?	1.570	0.019	26.8
	X ?	1.969	0.090	NSA
Std. A		1.682	0.038	50
Std. Gal		0.887	0.043	50
Spr. GGM	25 μl			
	Gal ?	0.807	0.033	47.8
	G ?	1.000	0.151	NSA
	M ?	1.299	0.504	NSA
	A ?	1.562	0.008	11.3
	X ?	1.962	0.058	NSA
Std. Gal		0.899	0.026	50
Traces also of di/trisaccharide(?) unknowns	(a)	0.415		
	(b)	0.520		
	Average	Blanks	0.061	
	Average	Std. Galactose	0.035	
	"	Std. Arabinose	0.036	
	Average	Spr. GGM Gal ?	for 10 μl	19.4
	"	" " A ?	" "	7.6

"β"		R. Glucose	Abs. 480 mμ (corrected)	μgms
Std. X		1.773	0.057	50
Spr. GGM	25 μl			
	Gal ?	0.702	0.056	NSA
	G ?	0.874	0.263	298.9
	M ?	1.150	0.838	NSA
	A ?	1.408	0.042	NSA
	X ?	1.709	0.133	88.1
Std. X		1.871	0.094	50
Std. G		1.000	0.039	50
Spr. GGM	25 μl			
	Gal ?	0.712	0.050	NSA
	G ?	0.877	0.193	219.3
	M ?	1.158	0.690	NSA
	A ?	1.411	0.036	NSA
	X ?	1.767	0.087	57.6
Std. G		1.000	0.049	50

<u>Traces</u> also
of di-trisaccharides

unknowns	(a)	0.340		
	(b)	0.442		
	Average	Blanks	0.052	
	Average	Std. Glucose	0.044	
	"	" Xylose	0.076	
	Average	Spr. GGM X ?	for <u>10 μl</u>	29

"γ"		R. Glucose	Abs. 480 mμ (corrected)	μgms
Std. A		1.650	0.024	50
Bir. Xyl	2.5 μl			
	X ?	2.092	0.197	137
Std. A		1.653	0.058	50
Bir. Xyl	2.5 μl			
	X ?	2.023	0.186	137
Std. G		1.000	0.050	50
Std. A		1.584	0.026	50
	Average	Blanks	0.078	
	Average	Std. A	0.025	50
	"	" G	0.054	50
"Assumed"	"	" X	0.070	50
	Average	Bir. Xyl X ?	per 2.5 μl	137
"δ"				
Std. X			no result	-
Bir. Xyl	20 μl			
	Gal ?	0.807	0.036	NSA
	G ?	0.952	0.024	37.5
	X ?	1.994	Not done	-
Std. G		1.000	0.031	50
Bir. Xyl	20 μl			
	Gal ?	0.813	0.021	NSA
	G ?	0.955	0.014	21.9
	X ?	1.973	Not done	-
Std. G		1.000	0.033	50
Std. X		1.922	0.028	50
	Average	Blanks	0.067	
	Average	Std. G	0.032	
	Average	Bir. Xyl G?	per 20 μl	29.6

Conclusions of Chromatogram Results

		Monosaccharides	µgms	Monosaccharides Percentage Composition
Birch DMSO	10 µl	Gal	15.1	6.2
		G	37.2	15.5
		M	None	-
		A	None	-
		X	188	78.2
		Total	240.3	
Birch Xylan	10 µl	Gal	trace	
		G	14.8	2.6
		M	None	-
		A	None	-
		X	547	97.4
		Total	561.8	
Birch Glucomannan	10 µl	Gal	trace	< 0.5
		G	15.1	4.5
		M	31.5	9.4
		A	None	-
		X	289.6	86.1
		Total	336.2	
Spruce DMSO	10 µl	Gal	21.8	17
		G	36.3	10.2
		M	109.6	4.9
		A	10.6	16.8
		X	36.6	50.9
		Total	214.9	
Spruce Xylan	10 µl	Gal	24.6	9.4
		G	52.9	20.1
		M	60.6	23.1
		A	8.6	3.3
		X	116	44.1
		Total	262.7	
Spruce GGM	10 µl	Gal	19.4	5.2
		G	100.4	27.2
		M	224	60.9
		A	1.8	2.1
		X	16.7	4.5
		Total	368	

		Monosaccharides	µgms	Percentage
Birch α Cellulose	10 µl	Gal	24.1	9.2
		G	None	-
		M	None	-
		A	None	-
		X	237.2	90.8
		Total	261.3	
Spruce α Cellulose	10 µl	Gal	None	-
		G	264	90.7
		M	27.2	9.3
		A	None	-
		X	None	-
		Total	291.2	

Solubilization of the hemimcelluloses.

Hemicellulose (2 gms.), which had been ground into a very fine powder under liquid nitrogen, was added to 1M. sodium hydroxide solution (200 mls.) with magnetic stirring. The addition was performed over two or three hours in a series of very small quantities, the whole solution being under nitrogen gas. After 12 hours stirring, acetic acid (1M) was added slowly from a burette until the pH of the solution/suspension was 5.0. The hemicellulose was then steamed at 100°C for twenty minutes on two successive days. It was also steamed after use. Storage was at 3°C.

APPENDIX III

Hemicellulase Monitoring Experiment

Numerical results expressed in the graphs were as follows:-

I. pH

	C. globosum S70				C. globosum S70B			
Day	Birch 1	Birch 2	Spruce 1	Spruce 2	Birch 1	Birch 2	Spruce 1	Spruce 2
0	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
3	4.80	4.73	4.78	4.80	4.81	4.80	4.79	4.80
6	4.62	4.47	4.58	4.51	4.62	4.60	4.54	4.51
9	4.40	4.37	4.51	4.52	4.65	4.83	4.75	4.77
12	4.38	4.31	4.49	4.50	4.88	5.10	4.95	4.95
15	4.25	4.26	4.46	4.49	5.00	5.35	5.14	5.11
20	4.35	4.34	4.52	4.54	5.25	5.70	5.21	5.21
25	4.68	4.68	4.60	4.60	5.50	5.89	5.40	5.40
32	4.87	4.89	4.66	4.67	5.66	5.92	5.50	5.52
40	5.06	5.11	4.71	4.70	5.80	5.96	5.59	5.62
55	5.32	5.40	4.70	4.81	5.84	5.94	5.65	5.70
70	5.41	5.43	4.71	-	5.87	5.99	5.66	5.70
	C. globosum S70E				C. globosum S70G			
0	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
3	4.85	4.80	4.79	4.85	4.79	4.70	4.74	4.79
6	4.60	4.59	4.59	4.57	4.57	4.47	4.51	4.58
9	4.53	4.51	4.55	4.57	4.44	4.39	4.51	4.60
12	4.51	4.41	4.52	4.54	4.42	4.34	4.53	4.60
15	4.35	4.31	4.49	4.51	4.42	4.41	4.51	4.59
20	4.48	4.40	4.51	4.54	4.64	4.60	4.60	4.62
25	4.72	4.60	4.57	4.60	4.78	4.78	4.69	4.69
32	4.87	4.81	4.58	4.61	4.90	4.90	4.71	4.72
40	5.01	4.95	4.65	4.60	5.10	5.09	4.71	4.77
55	5.33	5.21	4.56	4.65	5.37	5.35	4.85	4.86
70	5.42	5.30	4.61	4.64	5.42	5.43	4.81	4.86
	C. globosum S70K				C. globosum S70L			
0	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
3	4.80	4.80	4.76	4.80	4.78	4.79	4.67	4.79
6	4.58	4.58	4.50	4.57	4.50	4.81	4.59	4.59
9	4.45	4.41	4.48	4.50	4.50	4.47	4.59	4.61
12	4.37	4.35	4.47	4.50	4.67	4.72	4.62	4.59
15	4.35	4.89	4.51	4.61	4.78	4.85	4.62	4.61
20	4.31	4.56	4.61	4.71	4.89	4.95	5.62	4.60
25	4.50	4.75	4.73	4.76	5.02	5.19	4.66	4.66
32	4.73	4.85	4.79	4.80	5.16	5.19	4.69	4.65
40	4.91	5.12	4.80	4.85	5.27	5.25	4.70	4.70
55	5.11	5.12	4.90	4.91	5.35	5.34	4.78	4.75
70	5.18	5.23	4.90	4.99	5.36	5.38	4.75	4.73

pH

Day	Poria Monticola 304 B Birch 1	Birch 2	Spruce 1	Spruce 2	Poria Monticola 304 C Birch 1	Birch 2	Spruce 1	Spruce 2
0	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
9	4.91	4.91	4.86	4.75	4.70	4.69	4.70	4.74
16	4.93	4.93	4.83	4.70	4.70	4.65	4.66	4.70
23	4.90	4.92	4.85	4.72	4.71	4.68	4.67	4.73
30	4.91	4.93	4.59	4.70	4.71	4.66	4.68	4.72
37	4.90	4.89	4.53	4.70	4.72	4.61	4.63	4.69
51	4.73	4.80	-	4.61	4.61	4.52	4.56	4.60
65	4.79	4.81	-	4.60	4.62	4.52	4.51	4.60
80	4.75	4.83	-	4.75	4.55	4.61	4.55	4.60

Day	Poria Monticola 304 D Birch 1	Birch 2	Spruce 1	Spruce 2	Poria Monticola 304 E Birch 1	Birch 2	Spruce 1	Spruce 2
0	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
9	4.74	4.80	4.89	4.90	4.94	5.00	4.80	4.80
16	4.65	4.72	4.89	4.90	4.92	4.97	4.80	4.79
23	4.67	4.70	4.90	4.89	4.94	4.98	4.79	4.80
30	4.65	4.69	4.90	4.90	4.91	4.97	4.70	4.80
37	4.54	4.67	4.87	4.86	4.87	4.89	4.77	4.79
51	4.46	4.55	4.79	4.79	4.80	4.83	4.69	4.67
65	4.45	4.58	4.74	3.90	4.75	4.78	4.68	4.63
80	4.51	4.62	4.74	4.05	4.86	4.90	4.61	4.60

Contaminated with Paecilomyces sp.

Day	Poria Monticola 304 F Birch 1	Birch 2	Spruce 1	Spruce 2	Poria Monticola 304 G Birch 1	Birch 2	Spruce 1	Spruce 2
0	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
9	4.89	4.90	4.80	4.83	4.85	4.85	4.89	4.79
16	4.91	4.91	4.75	4.79	4.83	4.78	4.82	4.73
23	4.89	4.91	4.77	4.71	4.84	4.85	4.85	4.75
30	4.87	4.91	4.75	4.58	4.83	4.84	4.86	4.75
37	4.80	4.84	4.72	4.50	4.79	4.49	4.81	4.70
51	4.75	4.80	4.63	-	4.77	4.33	4.75	4.63
65	4.70	4.70	4.62	-	4.69	4.30	4.71	4.64
80	4.61	4.69	4.59	-	4.65	4.39	4.73	4.61

Day	Poria Monticola 304 H Birch 1	Birch 2	Spruce 1	Spruce 2	Poria Monticola 280 Birch 1	Birch 2	Spruce 1	Spruce 2
0	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
9	4.90	4.91	4.71	4.80	4.89	4.90	4.31	4.40
16	4.90	4.91	4.69	4.78	4.91	4.91	4.29	4.40
23	4.91	4.91	4.70	4.75	4.89	4.90	4.30	4.44
30	4.91	4.91	4.70	4.75	4.85	4.90	4.30	4.42
37	4.85	4.87	4.65	4.75	4.81	4.84	4.30	4.50
51	4.80	4.80	4.58	4.62	4.47	4.78	4.28	4.44
65	4.65	4.70	4.55	4.63	4.31	4.80	4.20	4.52
80	4.68	4.71	4.51	4.68 *	4.30	4.80	4.23	4.61

* Contaminated with Penicillium sp.

pH

	C. globosum S70M				C. globosum S70N			
Day	Birch 1	Birch 2	Spruce 1	Spruce 2	Birch 1	Birch 2	Spruce 1	Spruce 2
0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
3	4.80	4.80	4.76	4.76	4.80	4.84	4.73	4.73
6	4.61	4.57	4.55	4.55	4.52	4.59	4.50	4.49
9	4.50	4.46	4.58	4.61	4.45	4.46	4.51	4.52
12	4.39	4.34	4.54	4.54	4.41	4.39	4.50	4.53
15	4.33	4.37	4.59	4.57	4.45	4.46	4.51	4.52
20	4.41	4.45	4.60	4.60	4.70	4.61	4.54	4.54
25	4.55	4.59	4.59	4.60	4.85	4.89	4.59	4.60
32	4.78	4.79	4.70	-	4.99	5.00	4.65	4.60
40	5.01	4.98	4.75	4.96	5.15	5.20	4.62	4.60
55	5.23	5.22	4.81	-	5.40	5.44	4.62	4.68
70	5.39	5.34	4.85	-	5.51	5.59	4.61	-

	C. globosum RLB			
Day	Birch 1	Birch 2	Spruce 1	Spruce 2
0	5.0	5.0	5.0	5.0
3	4.82	4.83	4.69	4.67
6	4.50	4.54	4.43	4.43
9	4.50	4.51	4.49	4.46
12	-	-	-	-
15	4.81	4.79	4.58	4.54
20	4.95	4.87	4.56	4.56
25	5.13	5.05	4.62	4.65
32	5.25	5.15	4.73	4.70
40	5.35	5.28	4.60	4.70
55	5.44	5.35	4.53	4.72
70	5.51	5.40	4.60	4.78

	P. fastigiata 56 A				P. fastigiata BANN 1			
Day	Birch 1	Birch 2	Spruce 1	Spruce 2	Birch 1	Birch 2	Spruce 1	Spruce 2
0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
9	4.32	4.32	4.57	4.58	4.39	4.39	4.60	4.60
12	4.30	4.30	4.65	4.59	4.24	4.29	4.60	4.61
16	4.23	4.21	4.65	4.68	4.25	4.31	4.68	4.76
23	4.10	4.10	4.60	4.61	4.11	4.21	4.61	4.61
30	4.10	4.11	4.62	4.62	4.14	4.21	4.62	4.65
37	4.20	4.20	4.61	4.63	4.21	4.27	4.65	4.66
51	4.15	4.15	4.62	4.65	4.20	4.20	4.62	4.69
65	4.17	4.19	4.60	4.55	4.24	4.14	4.49	4.55
80	4.23	4.26	4.61	4.60	4.31	3.27	4.52	4.68

II. C.M.C. - ase *

C. globosum	S70				C. globosum	S70 B		
Day	Birch 1	Birch 2	Spruce 1	Spruce 2	Birch 1	Birch 2	Spruce 1	Spruce 2
0	-	-	-	-	-	-	-	
3	4	4	3	3	3	3	5	9
6	17	9	15	21	11	13	13	22
9	25	24	41	35	20	26	58	51
12	30	37	46	49	51	78	60	64
15	58	51	N.T.T.	50	58	99	58	N. .
20	60	60	52	50	79	107	109	103
25	84	84	70	70	146	145	127	126
32	99	113	61	77	152	150	130	130
40	163	181	100	141	188	189	173	180
55	189	205	125	163	201	210	201	204
70	203	217	138	N.T.T.	209	222	216	217

C. globosum	S70 E				C. globosum	S70 G		
0	-	-	-	-	-	-	-	-
3	11	9	5	5	2	5	2	6
6	16	12	11	17	17	16	11	17
9	23	23	35	31	22	24	34	28
12	30	32	32	42	36	33	34	38
15	38	40	43	44	50	53	47	42
20	52	33	50	46	68	55	47	44
25	51	62	53	60	96	80	60	61
32	92	76	51	65	105	106	64	51
40	135	127	82	94	155	166	94	97
55	149	149	84	115	176	183	118	127
70	204	209	86	133	197	203	130	142

C. globosum	S70 K				C. globosum	S70 L		
0	-	-	-	-	-	-	-	-
3	8	12	5	7	3	3	7	8
6	15	11	7	6	16	11	8	11
9	28	21	30	36	26	22	22	24
12	33	32	31	38	60	54	32	33
15	35	36	35	54	79	88	45	44
20	39	49	48	59	109	107	42	38
25	57	86	75	76	143	158	52	48
32	72	87	75	76	147	153	51	43
40	137	141	100	104	191	193	79	73
55	141	173	120	108	191	207	96	84
70	190	206	135	145	193	216	114	95

* in µgms of reducing sugar equivalents.
NTT = No test taken

C.M.C. - ase

C. globosum	S70 M				C. globosum	S70 N		
Day	Birch 1	Birch 2	Spruce 1	Spruce 2	Birch 1	Birch 2	Spruce 1	Spruce 2
0	-	-	-	-	-	-	-	-
3	3	9	3	3	3	3	-	6
6	15	14	10	17	15	11	14	18
9	26	30	30	32	29	25	27	30
12	35	33	40	37	42	37	35	37
15	40	45	42	65	48	49	37	45
20	54	55	49	53	74	53	39	45
25	68	76	58	79	102	102	57	47
32	96	94	58	-	121	109	57	48
40	151	156	95	151	171	156	84	91
55	175	186	113	-	190	185	90	104
70	203	209	123	-	203	200	104	-

C. globosum	RLB			
Day	Birch 1	Birch 2	Spruce 1	Spruce 2
0	-	-	-	-
3	3	2	1	8
6	21	9	12	16
9	21	48	22	33
12	48	39	34	40
15	76	48	49	46
20	110	61	56	46
25	148	100	69	65
32	151	125	67	56
40	194	178	106	100
55	204	198	130	116
70	209	209	142	127

P. fastigiata	56 A				P. fastigiata	BANN 1		
Day	Birch 1	Birch 2	Spruce 1	Spruce 2	Birch 1	Birch 2	Spruce 1	Spruce 2
0	-	-	-	-	-	-	-	-
9	5	5	23	21	8	4	18	25
12	9	9	26	31	-3	11	22	-1
16	6	3	20	24	8	7	20	18
23	2	82 ?	21	23	4	10	21	40
30	-	-6	15	25	5	10	12	23
37	3	3	-	8	6	10	3 ?	10
51	6	5	23	26	6	9	19	14
65	3	-3	23	25	12	2	25	19
80	9	8	47	32	14	15	35	25

C.M.C. - ase

Poria Monticola 304 B					Poria Monticola 304 C			
Day	Birch 1	Birch 2	Spruce 1	Spruce 2	Birch 1	Birch 2	Spruce 1	Spruce 2
0	-	-	-	-	-	-	-	-
9	-8	-10	1	-3	6	1	25	18
16	-3	- 1	3	6	2	9	24	21
23	-6	- 1	3	2	-6	10	26	24
30	-1	- 3	49	3	5	13	36	32
37	-7	- 4	36	-3	7	15	17	15
51	-6	- 5	-	-7	11	15	36	14
65	-4	- 4	1	9	15	16	33	36
80	-7	-11	-1	6	113	30	38	57

Poria Monticola 304 D					Poria Monticola 304 E			
Day	Birch 1	Birch 2	Spruce 1	Spruce 2	Birch 1	Birch 2	Spruce 1	Spruce 2
0	-	-	-	-	-	-	-	-
9	58	51	-8	-10	- 8	- 8	- 7	- 7
16	60	60	-4	- 2	-	- 4	- 1	1
23	74	80	-5	1	- 5	- 4	- 3	- 1
30	86	88	-4	2	- 3	- 2	1	2
37	88	91	-12	- 7	- 2	- 4	- 4	- 6
51	85	95	- 4	- 1	-	- 3	- 1	1
65	88	90	2	92	4	-	9	3
80	115	127	3	109	7	- 4	7	9

Contaminated with Paecilomyces sp.

Poria Monticola 304 F					Poria Monticola 304 G			
Day	Birch 1	Birch 2	Spruce 1	Spruce 2	Birch 1	Birch 2	Spruce 1	Spruce 2
0	-	-	-	-	-	-	-	-
9	- 4	-7	16	4	-6	-8	-	6
16	- 3	-3	18	16	-3	-4	8	8
23	3	-2	- 1	21	-2	-3	1	12
30	3	1	23	44	-1	-2	10	17
37	2	1	13	36	-	24	3	3
51	1	1	25	-	2	38	11	18
65	6	11	32	3	3	47	9	18
80	10	19 *	32	1	4	66	14	28

* Contaminated pennicillium sp.

C.M.C. - ase

Poria Monticola 304 H					Poria Monticola 280			
Day	Birch 1	Birch 2	Spruce 1	Spruce 2	Birch 1	Birch 2	Spruce 1	Spruce 2
0	-	-	-	-	-	-	-	-
9	-8	-8	29	22	-6	-7	-1	-5
16	-2	-4	22	15	-4	-3	9	3
23	-	-2	27	17	-6	-6	3	-1
30	-1	-2	10	17	-1	-3	10	2
37	1	-2	15	11	-3	-	10	-3
51	1	-1	31	22	81	2	11	9
65	5	-1	34	23	99	11	19	11
80	2	-2	48	31	140	8	22	18

III. Protein (in µgms)

C. Globosum S70					C. globosum S70B			
Day	Birch 1	Birch 2	Spruce 1	Spruce 2	Birch 1	Birch 2	Spruce 1	Spruce 2
0	-	-	-	-	-	-	-	-
3	23	23	27	29	24	22	30	-
6	29	26	29	30	22	26	30	29
9	47	-	43	47	37	-	41	42
12	54	51	43	43	57	75	49	46
15	65	61	47	43	75	-	57	59
20	91	83	46	46	126	210	73	65
25	107	111	50	56	164	243	74	79
32	252	182	61	61	247	300	88	91
40	269	270	69	94	333	389	116	121
55	456	494	94	136	440	522	166	146
70	552	574	114	-	427	579	200	222

C. globosum S70 E					C. globosum S70 G			
0	-	-	-	-	-	-	-	-
3	19	20	29	34	24	19	31	29
6	26	21	31	30	29	32	33	31
9	41	37	43	40	44	45	40	45
12	49	44	43	39	56	57	44	44
15	53	46	43	40	64	64	44	43
20	69	62	39	39	68	91	30	42
25	94	83	49	49	136	121	51	51
32	144	123	50	51	189	181	52	50
40	234	201	61	62	250	259	64	65
55	406	383	79	94	437	467	89	91
70	489	479	96	102	513	536	103	106

C. globosum S70 K					C. globosum S70 L			
0	-	-	-	-	-	-	-	-
3	29	21	31	29	23	24	30	31
6	24	26	31	29	27	26	31	30
9	43	46	41	41	46	49	40	41
12	49	57	41	42	66	71	43	40
15	54	62	44	46	89	109	37	37
20	66	89	45	49	141	200	39	36
25	90	119	59	58	196	280	46	46
32	129	159	56	59	248	354	51	43
40	211	243	65	77	317	409	54	56
55	344	362	93	116	387	594	75	69
70	449	450	103	129	367	636	89	84

Protein (in µgms)

C. globosum S70 M					C. globosum S70 N			
Day	Birch 1	Birch 2	Spruce 1	Spruce 2	Birch 1	Birch 2	Spruce 1	Spruce 2
0	-	-	-	-	-	-	-	-
3	29	19	31	34	25	19	31	34
6	28	23	31	36	31	26	34	34
9	44	45	40	41	39	45	43	40
12	54	51	44	45	59	61	45	44
15	53	56	41	44	69	65	39	44
20	69	73	40	51	88	88	40	41
25	101	101	49	64	133	119	51	50
32	139	153	51	-	281	184	51	51
40	198	211	63	126	269	264	58	65
55	390	403	84	-	440	429	80	111
70	516	506	106	-	470	469	102	-

C. globosum RLB				
Day	Birch 1	Birch 2	Spruce 1	Spruce 2
0	-	-	-	-
3	24	18	29	29
6	29	26	34	29
9	44	33	46	30
12	63	54	43	39
15	88	64	43	43
20	87	109	49	40
25	234	104	51	52
32	327	247	61	54
40	392	337	67	69
55	536	474	84	91
70	520	474	86	103

P. fastigiata 56 A					P. fastigiata BA NN 1			
Day	Birch 1	Birch 2	Spruce 1	Spruce 2	Birch 1	Birch 2	Spruce 1	Spruce 2
0	-	-	-	-	-	-	-	-
9	10	11	26	26	9	11	26	27
12	6	-	-	27	-	-	-	-
16	6	6	20	20	7	13	20	20
23	6	7	20	21	7	7	20	19
30	9	9	30	30	17	17	28	29
37	14	16	30	29	16	16	29	29
51	13	11	26	23	14	13	23	23
65	16	14	31	29	21	19	27	27
80	14	14	34	33	19	20	34	31

Protein (in µgms)

Poria Monticola	304 B				Poria Monticola	304 C		
Day	Birch 1	Birch 2	Spruce 1	Spruce 2	Birch 1	Birch 2	Spruce 1	Spruce 2
0	-	-	-	-	-	-	-	-
9	39	24	39	39	26	20	40	41
16	24	23	26	40	26	16	34	47
23	26	20	20	41	16	19	43	40
30	34	29	33	49	13	29	50	54
37	27	23	54	44	30	26	54	54
51	14	11	-	31	24	19	57	46
65	21	16	4	40	36	27	57	64
80	21	13	-	39	40	29	-	70

Poria Monticola	304 D				Poria Monticola	304 E		
0	-	-	-	-	-	-	-	-
9	30	23	39	40	30	19	36	43
16	24	19	-	36	26	16	39	40
23	23	26	44	41	27	17	37	41
30	34	39	46	49	39	26	46	51
37	31	36	49	50	33	26	47	53
51	27	33	44	44	31	29	46	47
65	39	41	56	74	37	26	57	64
80	41	46	64	90	41	27	67	70

Poria Monticola	304 F				Poria Monticola	304 G		
0	-	-	-	-	-	-	-	-
9	33	26	40	39	27	19	39	49
16	27	19	36	36	23	14	34	49
23	34	24	40	41	29	16	40	53
30	44	33	51	57	37	27	50	63
37	43	30	53	71	44	33	50	67
51	39	31	44	-	31	77	43	57
65	46	31	57	3	40	36	56	76
80	20	40*	71	-	54	46	64	90

Poria Monticola	304 H				Poria Monticola	280		
0	-	-	-	-	-	-	-	-
9	30	20	53	23	27	20	49	36
16	26	14	50	31	23	13	51	39
23	29	30	53	44	24	16	53	37
30	40	27	61	47	34	27	66	54
37	43	26	69	49	33	21	67	51
51	30	26	-	49	33	26	56	46
65	41	24	77	54	44	24	73	57
80	53	30	93	64	53	26	86	71

Contaminated with Penicillum sp

IV (a)
N.J.K. G.G. M- ase*

	C. globosum		S70		C. globosum		S70 B	
Day	Birch 1	Birch 2	Spruce 1	Spruce 2	Birch 1	Birch 2	Spruce 1	Spruce 2
0	-	-	-	-	-	-	-	-
15	10	8	24	23	17	29	50	50
25	19	16	27	29	44	49	85	88
40	56	65	48	59	83	100	135	162
70	120	128	100	-	130	159	238	244
IV (b)			SPRUCE - GGM - ase					
6	8	9	18	17	4	9	16	10
15	28	21	34	36	29	52	72	72
32	47	67	49	56	85	99	143	144
55	120	120	71	167	135	122	204	254
70	152	178	131	-	156	204	292	332

IV (a) N.J.K. G.G.M. - ase

	C. globosum		S70 E		C. globosum		S70 G	
0	-	-	-	-	-	-	-	-
15	9	4	18	19	14	9	24	19
25	11	9	29	27	21	16	30	48
40	46	36	32	50	54	52	50	55
70	124	114	56	87	101	121	98	112
IV (b)			SPRUCE - GGM - ase					
6	4	7	13	16	6	9	11	15
15	14	16	31	32	27	27	31	33
32	47	36	40	38	91	54	54	48
55	114	126	49	83	110	90	94	91
70	138	121	84	121	127	141	131	149

IV (a) N.J.K. G.G.M. - ase

	C. globosum		S70 K		C. globosum		S70 L	
0	-	-	-		-	-	-	-
15	9	12	16	23	17	23	24	18
25	10	17	31	30	42	59	24	23
40	40	42	41	54	44	92	38	35
70	91	106	73	84	92	141	70	60
IV (b)			SPRUCE - GGM - ase					
6	4	3	7	6	4	6	12	9
15	15	28	26	36	42	44	25	32
32	37	50	53	58	51	107	43	41
55	122	110	69	100	117	127	62	66
70	117	121	103	134	124	182	94	77

* in µgms of reducing sugar equivalents.

IV (a)
N.J.K. G.G.M. - ase

C. globosum			S70 M		C. globosum		S70 N	
Day	Birch 1	Birch 2	Spruce 1	Spruce 2	Birch 1	Birch 2	Spruce 1	Spruce 2
0	-	-	-	-	-	-	-	-
15	7	7	24	22	12	7	23	20
25	11	21	28	42	24	19	25	28
40	47	48	47	167	58	60	44	58
70	94	111	96	-	121	111	83	-
IV (b)			SPRUCE	G.G.M. - ase				
6	8	7	18	10	6	9	16	15
15	14	15	35	37	29	24	30	35
32	41	32	44	-	40	64	46	50
55	104	99	92	-	124	116	73	123
70	137	114	104		137	114	104	-

IV (a) N.J.K. G.G.M. - ase

C. globosum	RLB			
	Birch 1	Birch 2	Spruce 1	Spruce 2
0	-	-	-	-
15	20	10	20	12
25	48	32	26	22
40	80	67	33	45
70	117	109	70	70
IV (b)	SPRUCE	G.G.M. - ase		
6	7	6	11	9
15	14	28	29	29
32	63	70	47	47
55	134	104	72	72
70	144	119	85	100

- - - - - - - -

IV (a) N.J.K. G.G.M. - ase

	Poria Monticola		56 A		Poria Monticola		BANN 1	
Day	Birch 1	Birch 2	Spruce 1	Spruce 2	Birch 1	Birch 2	Spruce 1	Spruce 2
0	-	-	-	-	-	-	-	-
9	-10	- 9	-17	- 5	- 9	-10	13	- 4
23	-16	-13	- 5	- 3	-13	-10	- 1	- 2
51	- 4	-	- 2	2	- 5	3	4	- 2
80	- 4	-	8	4	- 7	- 4	2	3

IV (b) SPRUCE G.G.M. - ase

0	-	-	-	-	-	-	-	-
23	5	8	12	12	3	4	7	15
30	9	9	18	17	11	8	7	16
37	10	7	16	17	10	9	14	13
51	3	6	- 4	12	5	1	7	- 2
65	- 1	- 1	4	4	-3	- 1	5	4
80	-	- 2	7	- 2	-10	- 1	- 1	- 1

IV (a) N.J.K. - G.G.M. - ase

	Poria Monticola		304 B		Poria Monticola		304 C	
0	-	-	-	-	-	-	-	-
9	- 8	- 4	- 4	4	-11	1	14	5
23	- 6	- 2	1	3	- 4	- 1	21	21
51	8	4	7	33	3	16	59	49
80	1	8	- 1	32	62	26	131	89

IV (b) SPRUCE G.G.M. - ase

0	-	-	-	-	-	-	-	-
23	9	8	13	27	20	16	50	46
30	17	-10	51	29	17	18	26	57
37	5	11	47	25	13	18	44	29
51	- 2	5	- 2	26	12	5	34	44
65	3	2	- 4	22	11	11	56	56
80	11	-	13	33	62	5	- 4	65

IV (a) N.J.K. G.G.M. - ase

	Poria Monticola 304 D				Poria Monticola 304 E			
Day	Birch 1	Birch 2	Spruce 1	Spruce 2	Birch 1	Birch 2	Spruce 1	Spruce 2
0	-	-	-	-	-	-	-	-
9	-	-5	-2	-2	-8	-9	10	-4
23	5 *	4 *	2	-3	-3	-11	12	4
51	4	11	20	15	8	-5	11	27
80	5	14	52	213	-4	-4	46	58

IV (b) SPRUCE G.G.M. - ase

0	-	-	-	-	-	-	-	-
23	34	27	16	19	11	3	28	26
30	22	34	20	26	13	13	36	37
37	20	35	17	17	10	13	31	20
51	24 *	27 *	4	26	17	4	30	27
65	6	28	14	119	3	1	36	35
80	23	28	35	149	8	4	42	42

	Poria Monticola 304 F				Poria Monticola 304 G			
IV (a)	N.J.K. G.G.M. - ase							
0	-	-	-	-	-	-	-	-
9	-	-13	5	-	-4	-7	8	12
23	-3	- 6	16	10	-7	-3	7	20
51	5	- 1	40	-	10	30	27	61
80	24	6	69	2	15	12	56	133
IV (b)	SPRUCE G.G.M. - ase							
0	-	-	-	-	-	-	-	-
23	12	8	37	41	12	13	33	5
30	13	14	39	73	18	16	47	43
37	12	9	31	65	10	27	30	44
51	3	6	14	-	10	27	28	52
65	3	2	42	-	-	31	30	60
80	11	14	52	-	31	26	30	83

* Contaminated with Paecilomyces sp.

IV (a) N.J.K. G.G.M. - ase*

Poria Monticola 304 H					Poria Monticola 280			
Day	Birch 1	Birch 2	Spruce 1	Spruce 2	Birch 1	Birch 2	Spruce 1	Spruce 2
0	-	-	-	-	-	-	-	-
9	-5	-4	21	6	-8	-5	3	1
23	-	-3	31	23	-10	-3	18	2
51	1	7	68	37	-3	3	46	35
80	27	18	135	54	-29	-4	62	56

IV (b) SPRUCE G.G.M. - ase

0	-	-	-	-	-	-	-	-
23	17	10	58	47	3	6	41	37
30	18	13	61	50	12	10	56	30
37	12	12	45	36	10	9	27	28
51	9	8	56	31	32	8	40	26
65	-1	36	56	35	41	5	38	35
80	17	12	95	35	58	6	61	45

* in µgms of reducing sugar equivalents.

V. XYLANASE *

	C. globosum S70				C. globosum S70B			
Day	Birch 1	Birch 2	Spruce 1	Spruce 2	Birch 1	Birch 2	Spruce 1	Spruce 2
0	-	-	-	-	-	-	-	-
3	-1	-	1	-	4	3	3	7
6	22	18	6	6	4	11	5	8
9	42	39	10	11	22	34	15	12
12	97	80	9	12	59	101	25	25
15	155	132	12	12	96	170	33	33
20	146	159	13	13	142	216	35	32
25	177	182	14	19	199	214	45	46
32	207	216	20	44	231	248	56	58
40	236	250	30	61	262	281	82	88
55	328	322	39	63	319	315	129	146
70	346	346	40	-	331	353	164	175

	C. globosum S70E				C. globosum S70G			
0	-	-	-	-	-	-	-	-
3	-2	-1	-	3	1	4	1	3
6	12	14	4	6	26	19	3	8
9	25	16	9	9	40	40	7	5
12	51	28	12	10	89	88	6	8
15	82	70	10	9	126	127	8	11
20	92	85	9	10	157	150	6	3
25	125	118	10	11	183	162	10	11
32	162	152	10	18	197	194	11	7
40	199	185	16	21	211	211	14	14
55	277	255	15	36	312	303	20	17
70	305	309	20	40	328	335	17	21

	C. globosum S70K				C. globosum S70L			
0	-	-	-	-	-	-	-	-
3	2	-	1	5	-1	1	3	5
6	10	18	-	5	12	10	16	4
9	33	42	6	-	24	27	6	4
12	67	80	9	5	77	82	4	6
15	82	118	7	10	92	181	9	6
20	89	147	11	14	154	211	5	12
25	132	173	11	7	177	228	9	6
32	183	177	12	11	201	240	4	4
40	195	182	13	14	224	252	13	7
55	236	247	15	20	252	309	14	15
70	287	298	14	26	287	276	17	15

* in µgms of reducing sugar equivalents.

XYLANASE

	C. globosum S70M				C. globosum S70N			
Day	Birch 1	Birch 2	Spruce 1	Spruce 2	Birch 1	Birch 2	Spruce 1	Spruce 2
0	-	-	-	-	-	-	-	-
3	2	2	3	5	2	1	2	2
6	19	21	4	5	22	15	4	3
9	34	38	6	6	42	34	9	8
12	96	74	6	6	87	82	8	7
15	86	81	8	7	86	81	8	7
20	79	122	11	13	151	140	7	-
25	149	151	9	14	184	165	7	8
32	170	173	9	-2	198	185	13	11
40	191	194	14	37	211	204	16	22
55	272	296	17	2	300	299	28	67
70	311	317	18	-	328	309	23	-

	C. globosum			RLB
0	-	-	-	-
3	-1	-3	3	3
6	11	6	2	2
9	18	16	6	4
12	59	53	5	7
15	131	89	10	7
20	195	148	12	10
25	209	174	10	8
32	229	196	33)	12
40	249	217	110) *	14
55	284	284	146)	15
70	248	298	167)	15

* contaminated

	P. fastigiata 56A				P. fastigiata BANN 1			
0	-	-	-	-	-	-	-	-
9	1	1	1	1	-2	1	-3	-1
16	2	3	-	1	-2	1	-	-
23	-2	5	1	3	2	5	-2	-1
30	1	-1	2	1	-	-	1	2
37	4	-4	-1	-6	-3	-2	-3	-2
51	-3	-3	-1	-3	-2	1	-2	-3
65	-5	-5	-5	-7	-4	-4	-4	-6
80	-8	-5	-5	-2	-6	-4	3	-6

XYLANASE

	Poria Monticola 304B				Poria Monticola 304C			
Day	Birch 1	Birch 2	Spruce 1	Spruce 2	Birch 1	Birch 2	Spruce 1	Spruce 2
0	-	-	-	-	-	-	-	-
9	-1	-3	-4	-	-4	-2	1	-1
16	1	-3	-	-	-	-1	-	-
23	-2	-2	-2	-2	7	-2	-1	-3
30	-2	3	45	1	-2	-	10	4
37	-5	-7	31	-3	-4	-6	-2	-1
51	-6	4	5	17	-3	-1	-4	-4
65	-8	1	1	-2	-7	5	-5	-4
80	-7	-3	1	-7	115 *	-4	-3	-7

* contaminated

	Poria Monticola 304D				Poria Monticola 304E			
0	-	-	-	-	-	-	-	-
9	126	125	-2	-	4	1	1	2
16	138	135	-	-	7	3	3	-
23	145	139	-2	-1	4	6	-2	-2
30	157	145	-1	-1	7	3	1	5
37	141	144	-5	-4	3	2	-4	-6
51	139	146	-5	-5	6	3	6	-5
65	133	137	-6	86 ?	-2	-2	-3	-3
80	133	147	-3	38 ?	-1	-3	-4	-9

* Contaminated with Paecilomyces sp.

	Poria Monticola 304F				Poria Monticola 304 G			
0	-	-	-	-	-	-	-	-
9	-	-2	-2	-4	-2	-4	-2	-2
16	-	-3	-2	-3	-	-	-2	-1
23	2	-6	-4	-6	-2	-2	-2	-
30	7	3	1	5	-3	5	-2	2
37	-3	-5	-4	9	-4	26	-3	-3
51	-3	-3	-7	-1	-9	49	-5	-5
65	-7	-3	-6	-2	-8	32	-5	-6
80	-4	3	-6	-	-7	52	-5	-10

XYLANASE

	Poria Monticola 304H				Poria Monticola 280			
Day	Birch 1	Birch 2	Spruce 1	Spruce 2	Birch 1	Birch 2	Spruce 1	Spruce 2
0	-	-	-	-	-	-	-	-
9	-2	-1	-1	2	-1	-2	-1	1
16	-2	-3	-	1	-3	-3	-	1
23	-2	-2	-3	6	-2	-2	-1	-1
30	-3	-1	5	1	-3	5	-	9
37	-6	-6	-7	-6	-5	-6	-4	-4
51	-12	-4	-5	-5	152	-2	-3	-18 ?
65	- 6	-6	-3	-5	137	-6	-4	-1
80	- 6	-6	-5	-7	124	-7	-5	-

VI. SPRUCE D.M.S.O. Activity *

	C. globosum S70				C. globosum S70B			
Day	Birch 1	Birch 2	Spruce 1	Spruce 2	Birch 1	Birch 2	Spruce 1	Spruce 2
0	-	-	-	-	-	-	-	-
6	1	1	6	4	-3	1	9	4
15	35	57	22	25	44	85	12	70
32	90	101	46	29	124	125	130	128
55	139	151	53	113	148	155	162	180
70	142	169	76	-	142	165	209	223

	C. globosum S70E				C. globosum S70G			
0	-	-	-	-	-	-	-	-
6	-4	-1	-3	3	3	-1	7	5
15	27	32	11	21	37	51	13	14
32	69	61	26	25	89	88	49	35
55	115	110	31	50	128	129	64	61
70	137	142	48	60	132	171	79	83

	C. globosum S70K				C. globosum S70L			
0	-	-	-	-	-	-	-	-
6	-4	-1	1	-13	-1	-2	1	-7
15	24	48	15	28	48	80	15	8
32	93	82	43	49	110	131	22	30
55	96	102	49	69	115	144	36	28
70	116	128	47	88	117	180	49	45

	C. globosum S70M				C. globosum S70N			
0	-	-	-	-	-	-	-	-
6	-3	2	3	3	2	1	6	3
15	24	29	12	20	35	54	8	21
32	104	69	31	-	92	90	32	39
55	112	112	31	-1	129	126	48	78
70	135	154	72	-	145	144	55	-

* in µgms of reducing sugar equivalents.

VI SPRUCE D.M.S.O. Activity *

	C. globosum				RLB			
Day	Birch 1	Birch 2	Spruce 1	Spruce 2	Birch 1	Birch 2	Spruce 1	Spruce 2
0	-	-	-	-	-	-	-	-
6	-1	-4	4	-1				
15	52	54	8	23				
32	116	103	37	38				
55	122	119	66	45				
70	130	142	79	35				

	P. fastigiata 56A				P. fastigiata BANN 1			
0	-	-	-	-	-	-	-	-
23	-	4	12	7	9	-1	7	10
51	6	1	8	7	2	7	4	3
65	6	7	10	9	6	12	10	7
80	4	3	4	3	7	7	3	4

	Poria Monticola 304B				Poria Monticola 304C			
0	-	-	-	-	-	-	-	-
23	4	1	-4	1	1	2	4	1
51	-1	-2	-	-3	-	1	1	3
65	2	5	4	-1	3	2	5	14
80	-	6	2	-3	2	7	1	2

	Poria Monticola 304D				Poria Monticola 304E			
0	-	-	-	-	-	-	-	-
23	70	79	1	3	-2	-11	7	20
51	131	106	-	-1	7	-	32	31
65	112	101	7	86	8	6	35	47
80	114	114	4	54	14	-3	52	52

* Contaminated by Paecilomyces sp.

* in µgms of reducing sugar equivalents.

VI . SPRUCE D.M.S.O. Activity*

	Poria Monticola 304F				Poria Monticola 304G			
Day	Birch 1	Birch 2	Spruce 1	Spruce 2	Birch 1	Birch 2	Spruce 1	Spruce 2
0	-	-	-	-	-	-	-	-
23	-7	-11	15	20	1	1	25	22
51	1	6	29	30	10	49	22	44
65	20	-	53	-1	13	29	22	49
80	18	4	37	3	10	48	57	69

	Poria Monticola 304H				Poria Monticola 280			
0	-	-	-	-	-	-	-	-
23	3	-6	40	8	5	4	17	19
51	13	5	54	39	70	-10	47	36
65	19	-5	69	42	86	8	49	53
80	18	15	107	51	68	-3	55	60

* in µgms of reducing sugar equivalents.

VII. BIRCH D.M.S.O. Activity*

	C. globosum S70				C. globosum S70B			
Day	Birch 1	Birch 2	Spruce 1	Spruce 2	Birch 1	Birch 2	Spruce 1	Spruce 2
0	-	-	-	-	-	-	-	-
6	3	3	1	-	-3	4	-	-3
15	64	66	11	11	61	89	24	34
32	116	118	9	21	127	132	54	32
55	-3	177	24	44	153	176	76	86
70	176	185	29	-	172	207	103	110

	C. globosum S70E				C. globosum S70G			
0	-	-	-	-	-	-	-	-
6	5	1	4	-4	8	7	1	-2
15	37	34	3	-4	56	61	8	-12
32	83	80	-	6	77	99	10	11
55	137	138	11	20	140	111	24	20
70	154	157	15	23	163	168	21	24

	C. globosum S70K				C. globosum S70L			
0	-	-	-	-	-	-	-	-
6	4	4	1	-4	6	-2	1	-3
15	34	57	6	8	62	46	6	6
32	75	72	14	15	109	144	26	11
55	116	121	17	26	130	131	15	20
70	133	150	6	34	137	173	20	20

	C. globosum S70M				C. globosum S70N			
0	-	-	-	-	-	-	-	-
6	4	8	1	-1	10	4	4	1
15	44	48	11	9	61	55	7	5
32	66	88	6	-1	103	108	8	9
55	140	132	19	-	143	143	17	28
70	141	167	1	5	171	178	13	-

* in µgms of reducing sugar equivalents.

VII. BIRCH D.M.S.O. Activity*

	C. globosum RLB							
Day	Birch 1	Birch 2	Spruce 1	Spruce 2	Birch 1	Birch 2	Spruce 1	Spruce 2
0	-	-	-	-	-	-	-	-
6	4	-	1	-3				
15	73	55	10	7				
32	125	109	16	11				
55	146	137	70	19				
70	124	158	68	67				

	P. fastigiata 56A				P. fastigiata BANN 1			
0	-	-	-	-	-	-	-	-
23	4	5	-1	-3	-5	-8	-4	-3
51	-11	-22	-10	11	-2	-8	-1	6
65	1	4	9	7	2	-13	2	-6
80	-	5	4	-	6	6	9	-6

	Poria Monticola 304B				Poria Monticola 304C			
0	-	-	-	-	-	-	-	-
23	2	2	10	16	-5	3	27	29
51	6	-2	-2	9	-5	8	45	44
65	-6	-6	-5	22	1	-	57	32
80	-3	10	5	42	83	24	6	78

	Poria Monticola 304D				Poria Monticola 304E			
0	-	-	-	-	-	-	-	-
23	28	48	11	13	-2	-11	7	20
51	49	53	1	20	7	-	32	31
65	47	42	16	185	8	6	35	47
80	64	45	35	167	14	-3	52	52

* Contaminated with Paecilomyces sp.

* in µgms of reducing sugar equivalents.

VII. BIRCH D.M.S.O. Activity*

	Poria Monticola 304F				Poria Monticola 304G			
Day	Birch 1	Birch 2	Spruce 1	Spruce 2	Birch 1	Birch 2	Spruce 1	Spruce 2
0	-	-	-	-	-	-	-	-
23	1	-	4	1	1	-	4	1
51	1	-1	-	1	3	53	4	4
65	5		1	2	3	57	3	4
80	1	6	-4	-10	-	63	-2	-

	Poria Monticola 304H				Poria Monticola 280			
	-	-	-	-	-	-	-	-
23	-3	1	1	4	3	3	7	6
51	-1	-2	6	-	133	-2	4	10
65	-1	5	8	3	106	1	7	2
80	-	1	5	-2	105	-1	1	4

* in µgms of reducing sugar equivalents.

APPENDIX IV

Results of Viscometric Assay

Substrate: CMC, c_s = 2.0g/l. Symbols: t_1, time from start of reaction to start of measurement; T, efflux time of viscometer; T_o, efflux time of solvent (70 secs); $t = t_1 + T/2$, reaction time ($t_1 > 0$); $[\eta]$ calculated from $[\eta] = \frac{8}{c_s}\left(\sqrt[8]{\frac{T}{T_o}} - 1\right)$.

t_1 (sec)	T (sec)	t (sec)	$\sqrt[8]{T}$	$\sqrt[8]{\frac{T}{T_o}}$	$[\eta]$ (1/g)	log $[\eta]$
FIRST ASSAY						
0	144	0	1.862	1.095	0.381	-0.419
90	106	143	1.800	1.059	0.235	-0.629
221	99	271	1.776	1.045	0.180	-0.745
361	90	406	1.755	1.032	0.130	-0.886
476	89	521	1.753	1.031	0.125	-0.903
593	85	636	1.743	1.025	0.101	-0.996
699	82	740	1.735	1.021	0.082	-1.084
807	84	849	1.740	1.024	0.094	-1.027
912	82	953	1.743	1.025	0.101	-0.996
1025	80	1065	1.730	1.018	0.070	-1.152
1262	82	1303	1.743	1.025	0.101	-0.996
SECOND ASSAY						
0	144	0	1.862	1.095	0.381	-0.419
59	105	112	1.789	1.052	0.210	-0.678
187	100	237	1.778	1.046	0.184	-0.735
314	94	361	1.764	1.038	0.150	-0.824
436	89	481	1.753	1.031	0.125	-0.903
555	87	599	1.748	1.028	0.113	-0.947
671	86	714	1.745	1.027	0.106	-0.975

Chaetomium globosum (570B) cultured on birch sawdust.

t_1 (sec)	T (sec)	t (sec)	$\sqrt[8]{T}$	$\sqrt[8]{\frac{T}{T_o}}$	$[\eta](1/g)$	log $[\eta]$
FIRST ASSAY						
0	144	0	1.862	1.095	0.381	-0.419
53	139	123	1.853	1.090	0.360	-0.444
215	140	285	1.855	1.091	0.365	-0.434
422	135	490	1.846	1.086	0.344	-0.463
591	133	658	1.843	1.084	0.336	-0.474
762	131	828	1.840	1.082	0.330	-0.482
936	129	1001	1.835	1.079	0.318	-0.498
1097	130	1162	1.838	1.081	0.324	-0.490
SECOND ASSAY						
286	138	355	1.851	1.089	0.355	-0.450
446	134	513	1.845	1.085	0.340	-0.469
600	133	667	1.843	1.084	0.336	-0.472
760	131	826	1.840	1.082	0.330	-0.482
1028	130	1093	1.838	1.081	0.324	-0.490
1324	128	1388	1.834	1.079	0.315	-0.502

Phialophora fastigiata (56A) cultured on birch sawdust.

t_1 (sec)	T (sec)	t (sec)	$\sqrt[8]{T}$	$\sqrt[8]{\frac{T}{T_o}}$	$[\eta](1/g)$	$\log[\eta]$
FIRST ASSAY						
0	144	0	1.862	1.095	0.381	-0.419
33	137	102	1.849	1.088	0.325	-0.488
192	130	257	1.838	1.081	0.324	-0.490
345	122	406	1.823	1.072	0.290	-0.538
489	120	549	1.820	1.071	0.282	-0.550
639	116	697	1.811	1.066	0.264	-0.578
804	107	858	1.794	1.055	0.221	-0.656
973	108	1027	1.796	1.056	0.226	-0.646
1115	109	1170	1.798	1.058	0.230	-0.638
SECOND ASSAY						
35	136	103	1.848	1.087	0.348	-0.458
202	129	267	1.835	1.079	0.318	-0.498
350	122	411	1.823	1.072	0.290	-0.538
494	119	554	1.818	1.069	0.276	-0.559
618	115	676	1.809	1.064	0.256	-0.592
1000	108	1054	1.796	1.056	0.226	-0.646

Phialophora fastigiata (56A) cultured on spruce sawdust.

t_1 (sec)	T (sec)	t (sec)	$\sqrt[8]{T}$	$\sqrt[8]{\frac{T}{To}}$	$[\eta](1/g)$	log $[\eta]$
FIRST ASSAY						
0	144	0	1.862	1.095	0.381	-0.419
54	137	123	1.849	1.088	0.325	-0.488
214	133	281	1.843	1.084	0.336	-0.474
369	126	432	1.831	1.076	0.304	-0.517
520	124	582	1.827	1.075	0.300	-0.523
672	121	733	1.822	1.072	0.288	-0.541
830	117	889	1.813	1.067	0.266	-0.575
989	117	1048	1.813	1.067	0.266	-0.575
SECOND ASSAY						
50	138	119	1.851	1.089	0.355	-0.450
200	135	268	1.846	1.086	0.344	-0.463
350	130	415	1.838	1.081	0.324	-0.490
502	127	566	1.831	1.077	0.308	-0.511
642	125	705	1.828	1.075	0.301	-0.521
796	122	857	1.823	1.072	0.290	-0.538
954	118	1013	1.816	1.068	0.273	-0.564

Poria monticola (3048) cultured on birch sawdust.

t_1 (sec)	T (sec)	t (sec)	$8/\sqrt{T}$	$8/\sqrt{\frac{T}{To}}$	$[n](1/g)$	log $[n]$
FIRST ASSAY						
0	144	0	1.862	1.095	0.381	-0.419
40	131	106	1.840	1.082	0.330	-0.482
189	118	248	1.816	1.068	0.273	-0.564
326	115	384	1.809	1.064	0.256	-0.592
468	105	521	1.789	1.052	0.210	-0.678
592	104	644	1.786	1.051	0.202	-0.695
734	104	780	1.786	1.051	0.202	-0.695
858	100	908	1.778	1.046	0.184	-0.735
976	99	1026	1.776	1.045	0.180	-0.745
1262	96	13.0	1.769	1.041	0.162	-0.791
SECOND ASSAY						
36	132	102	1.841	1.083	0.332	-0.479
186	122	247	1.823	1.072	0.290	-0.538
318	116	376	1.811	1.066	0.264	-0.578
444	106	497	1.800	1.059	0.235	-0.629
606	104	658	1.786	1.051	0.203	-0.693
862	100	912	1.778	1.046	0.184	-0.735
1098	97	1145	1.772	1.042	0.170	-0.770

Poria monticola (304B) cultured on spruce sawdust.

t_1 (sec)	T (sec)	t (sec)	$\sqrt[8]{T}$	$\sqrt[8]{\frac{T}{T_o}}$	$[\eta](1/g)$	log $[\eta]$
FIRST ASSAY						
0	144	0	1.862	1.095	0.381	-0.419
52	134	119	1.845	1.085	0.340	-0.469
187	130	252	1.838	1.081	0.324	-0.490
312	124	374	1.827	1.075	0.300	-0.523
533	121	594	1.822	1.072	0.288	-0.541
685	119	745	1.818	1.069	0.276	-0.559
SECOND ASSAY						
60	135	128	1.846	1.086	0.344	-0.463
233	127	297	1.831	1.077	0.308	-0.511
415	126	478	1.831	1.076	0.304	-0.517
606	124	668	1.827	1.075	0.300	-0.523
840	119	899	1.818	1.069	0.276	-0.559
1088	116	1146	1.811	1.066	0.264	-0.578
THIRD ASSAY						
43	137	112	1.849	1.088	0.325	-0.488
1167	114	1224	1.807	1.063	0.252	-0.599

Polystictus versicolor cultured on birch sawdust.

t_1 (sec)	T (sec)	t (sec)	$\sqrt[8]{T}$	$\sqrt[8]{\frac{T}{T_o}}$	$[\eta](1/g)$	log $[\eta]$
FIRST ASSAY						
0	144	0	1.862	1.095	0.381	-0.419
41	121	102	1.822	1.072	0.288	-0.541
207	117	266	1.813	1.067	0.266	-0.575
342	105	395	1.789	1.052	0.210	-0.678
465	100	515	1.778	1.046	0.184	-0.735
586	99	636	1.776	1.045	0.180	-0.745
706	96	754	1.769	1.041	0.162	-0.791
820	94	867	1.764	1.038	0.150	-0.824
957	94	1004	1.764	1.038	0.150	-0.824
SECOND ASSAY						
43	120	103	1.820	1.071	0.282	-0.550
210	115	268	1.809	1.064	0.256	-0.592
346	107	400	1.794	1.055	0.221	-0.656
478	100	528	1.778	1.046	0.184	-0.735
604	98	653	1.774	1.044	0.174	-0.760
768	95	816	1.767	1.039	0.158	-0.801
1109	92	1155	1.760	1.035	0.141	-0.851
1464	90	1509	1.755	1.032	0.130	-0.886

Polystictus versicolor cultured on spruce sawdust.

t_1 (sec)	T (sec)	t (sec)	$\sqrt[8]{T}$	$\sqrt[8]{\frac{T}{T_o}}$	$[\eta](1/g)$	log $[\eta]$
FIRST ASSAY						
0	144	0	1.862	1.095	0.381	-0.419
42	123	104	1.825	1.074	0.294	-0.532
65	122	126	1.823	1.072	0.290	-0.538
195	110	250	1.800	1.059	0.235	-0.629
334	98	383	1.774	1.044	0.174	-0.760
457	98	500	1.774	1.044	0.174	-0.760
618	96	666	1.769	1.041	0.162	-0.791
734	92	780	1.760	1.035	0.141	-0.851
988	90	1033	1.755	1.032	0.130	-0.886
1257	86	1300	1.745	1.027	0.106	-0.975
SECOND ASSAY						
0	144	0	1.862	1.095	0.381	-0.419
51	123	113	1.825	1.074	0.294	-0.532
189	112	245	1.804	1.061	0.245	-0.611
316	99	366	1.776	1.045	0.180	-0.745
528	97	577	1.772	1.042	0.170	-0.770
650	95	698	1.767	1.039	0.158	-0.801
846	92	892	1.760	1.035	0.141	-0.851
1038	88	1082	1.750	1.029	0.117	-0.928

Chaetomium globosum [5708] cultured on spruce sawdust.

ADDENDUM

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