A STUDY OF POLYPORUS BETULINUS (BULL.) FRIES

by

James A. Macdonald, B.Sc.

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CONTENTS.

1. INTRODUCTION.

The common birch Polyporus - Polyporus betulinus (Bull.) Fries - has been made the subject of frequent investigations. By far the most extensive researches have been those of Mayr (54) who studied the mycelium in the infected wood and the structure of the sporophore, as well as undertaking infection experiments. Workers, subsequently, have tended to confine themselves largely to the questions of the type of rot produced and of the parasitism of the fungus. References, on the whole, are fragmentary. It seemed worth while, therefore, to reinvestigate these problems and to complete the study of the fungus in culture.

Polyporus betulinus occurs on Betula spp. throughout the temperate regions of the northern hemisphere. It has been recorded frequently from Asia, Europe and North America. In Great Britain it is very widespread. In Scotland it is the fungus which occurs most commonly on birch. It seems possible that it has become increasingly common during the last hundred years. In 1826 Greville (28) described the sporophore from birch in Rosslyn woods, adding the comment that it was certainly rather infrequent as it was absent from all the local floras

in his possession. Stevenson (71) in 1879 records it from all divisions of Scotland, except that which he designates "Sutherland." The present writer has found the fructifications abundantly wherever birch grows in Scotland, from the Dornoch firth to the Tweed in the East, and from Loch Carron to the Solway in the west.

The amount of the fungus present in Scotland is undoubtedly due to the general lack of attention given to our birch woods. Birch in pure woods occurs only in areas which are unfavourable to the growth of more economically important species, e.g., the exposed upper valleys in the Highlands, or the swampy lands at the sides of river beds. Seedlings appear also as weed birch in plantations on better ground. The slow-growing trees in these neglected areas, together with ringed weed birch in plantations, often bear fructifications of P. betulinus. The dismal unhealthy appearance of such trees has led to the prevalence of the idea that the fungus is only able to grow on dead or dying birch.

Various interesting uses have been found for the sporophores of the fungus or the wood of infected trees. According to Neger (59) the powdery wood produced is used as a polishing material in the Swiss watch industry. The same authority, together with numerous others, states that the sporophores, when

cut off in strips, were largely used as razor strops. The infected wood has been used also in the manufacture of charcoal crayons according to Gäumann (26). Small strips of the sporophore are useful for mounting entomological specimens after pinning.

Polyporus betulinus belongs to the family bearing the same name. The Polyporaceae are Basidiomycetous fungi placed in the Hymenomycetes by Fries (24) and other early systematists. In the more detailed classification adopted by Brooks (8) the family is put in the group designated Aphyllophorales, while even more recently Clements, Shear and Clements (19) have grouped it with the Agaricales.

As is usual with fungi, which early attracted the attention of botanists, the records show this species under a considerable number of names. Saccardo (67) notes the following synonyms - Boletus betulinus Bulliard (15), B. suberosus Linnaeus (48), B. sutorius Scopoli (70) and Polyporus albellus Peck (61). Murrill (58) uses the name Piptoporus suberosus (L.).

II. METHODS EMPLOYED.

For general culture work the fungus was grown on malt agar made up according to the formula given by Gwynne Vaughan and Barnes (30). The fixatives and stains employed were used as recommended by These authors, or by Chamberlain (20).

FIXATION. Material of wood and sporophores was fixed in Acetic Alcohol, Carnoy's Fluid, Chromo-Acetic Solution and Flemming's Fluid (strong or half strong). The last fixative (half strong) gives the best results with sporophore material as it casues little shrinkage. Films of mycelium on glass slides were fixed in half strong Flemming or Sass's special fixative (68), which has the advantage of hardening the film more and thus making subsequent handling easier.

The material fixed with Flemming's fluid was subsequently bleached with a solution of hydrogen peroxide in spirit.

EMBEDDING. Material for microtoming was embedded in wax of melting point 48°C., 50°C. or 52°C. according to the hardness of the material.

STAINING. Wood sections were stained with the following combinations - Haidenhain's Iron-Haematoxylin, Safranin and Haematoxylin, Safranin and Picro-Anilin Blue, Bismarck Brown and Methyl Violet, Greenacher's Borax Carmine alone and with Methyl Green, Cotton Blue and Lactic Acid. Sporophore material was stained with the following - Haidenhain's Iron - Haematoxylin alone and followed by Light Green, Safranin and Picro-Anilin Blue, Safranin, Gentian Violet and Orange G, and Safranin Polychrome Methylene Blue and Orange Tannin (Breinl's Stain). Mycelial films were treated with Magdala Red and Light Green, Breinl's Stain, Iron-Haematoxylin and Iron Haematoxylin with

Light Green. Iron-Haematoxylin with Light Green, and Breinl's Stain gave the best results with both sporophore material and mycelium. Hand sections of all classes of material were mounted and examined in an eosin and glycerine medium.

III. HOSTS AND PARASITISM.

HOSTS. The fungus has been recorded almost exclusively on Betula. The species is rarely mentioned. Tubeuf (73), however, gives B. verrucosa and B. pubescens as hosts. Hubert (37) mentions B. papyrifera. Lowe (50) gives the following common names which appear to correspond with the species (in brackets) in Boulger's "Wood"(5) - grey (populifolia) yellow (lutea), Sweet (lenta), white (papyrifera). It is widespread on the silver birch B. alba. Carleton Rea (18) and Ramsbottom (62) give beech as a second host.

PARASITISM. Rostrup (65) and Mayr (54) were the earliest investigators to describe the parasitism of this fungus. Mayr (54), by inserting a plug of naturally infected birch wood into a boring of similar size in a healthy trunk, produced the early stages of infection. By this means the fungus mycelium was induced to penetrate the healthy wood to a distance of $2\frac{1}{2}$ cm. in a period of just over fourteen weeks. Unfortunately the experiment was not carried on to sporophore formation, and this has not been attained by any subsequent worker.

Neger (59) states that Dohse (21) estimated, in one district of Mecklenburg, that about 2% of the birch trunks were destroyed by this fungus over a period of 4-5 years. This was in a stand of trees 50-60 years old. Vannine (74), working on birch in Russia, estimated that 15% of the stand was infected with Fomes fomentarius and Polyporus betulinus. Another Russian investigator, Rozanova (66), working on the birch woods in the Zvenigorod district, formed the opinion that these two fungi were growing largely as saprophytes. In support of this view she quoted the negative results obtained, after one year, when six healthy, thirty-year old birches were inoculated with P. betulinus. Other writers incline to this latter view, e.g., Rankin (63) "badly injured or weakened trees are attacked." During the present investigation, infection of adult birch trunks has been attempted after the manner indicated by Mayr(54). Two trees were inoculated in the autumn of 1932 and a further two in the autumn of 1933. In autumn and winter 1934-35 six trunks were inoculated. The inoculum used consisted of chopped up sporophore material mixed with infected wood and mycelium growing on malt agar slopes. These experiments have yielded negative results so far. Nevertheless, as the result of four years' observation of infected woods, during which thousands of healthy and infected trees have been periodically examined, the writer is convinced that the fungus is a parasite.

As evidence in support of this view it may be mentioned that sporophores are found on birch trees the trunks of which bear leafy branches above the visibly infected parts. The following example may also be quoted. A birch tree, which appeared perfectly healthy, was observed, during summer 1933, to have a split in the bark about midway up the stem. The crack was about three feet long and over ½in. wide in places. In late autumn of the same year a sporophore appeared in the split (Pl. 1.). As there were no broken branches and no other dead wood on the tree the fungus must have been able to attack the healthy tissues exposed by the cracking.

Two facts are probably largely responsible for the idea that P. betulinus grows mainly saprophytically; (1) when the slow rate of advance of the mycelium, as observed by Mayr (54), is considered, it seems that the fungus mycelium remains localised near the point of entry for some time. If that is the case it will bring about the death of the tissues which is occupies, thereby causing the interruption of the vascular system and the starvation and death of the upper parts of the stem. The tree thus weakened presents an unhealthy appearance, and when the fructifications appear they are taken as indicating a new attack on an already sickly tree; (2) crops of fructifications are produced year after year on dead trunks, whether standing (Pl. 2.) or

fallen (Pl. 3.), thus creating the impression of a purely saprophytic means of life.

After the appearance of the first crop of sporophores the area bearing them increases annually. Each subsequent year new fructifications arise among the remains of the previous year's crop;but they gradually decrease in size and numbers. In addition new areas of the attacked stem produce sporophores. The distance between the last formed fructification of one annual crop and the corresponding fruit body of the next season has been observed to vary from about 6in. to about 6ft. either up or down the trunk. In this way a rough estimate may be made of the rate of spread of the mycelium.

The fungus,does, of course, readily attack birch which is weakened by some other agency. The writer has frequently observed the fructifications on young birch injured by burning or on ringed trees in conifer plantations (Loch Ness). Neger (59) comments on the frequent occurrence of sporophores on young dead birch, presumably weakened by the severe weather, in alpine districts. They occur on young and old birch attached by Armillaria mellea (Dawyck, Loch Ness), on the same trunks as Fomes igniarius var. nigricans in many parts of the Highlands (Dunkeld, Kilmorack and Rogie falls, Loch Duntelchaig). The lower parts of trunks which the fungus occupies sometimes bear the fruit

bodies of Stereum hirsutum (Dawyck, Fala Toll). It has not been proved that the fungus will infect fallen birch timber, nor has it been found on the dead wood of any other genus in the forest. This is of interest as the mycelium can be grown in culture on a range of timbers. Leise (46) has grown it on beech, pine, alder and oak. Lutz (51) infected an oak block in which he had previously reduced the tannin content. In this case the fungus produced a sporophore. The present writer has grown the mycelium on blocks of birch, beech and Abies sp.. On all three woods sporophores were produced (Pl. 4.).

Various means by which the fungus might bring about infection have been suggested from time to time. Mayr (54) was of the opinion that the spores gained entry following on the fresh breaking of branches. Von Schrenk and Spaulding (69) stated that the fungus gained entry through the bark, probably through lenticels or wounds. The present writer is strongly of the opinion that the usual way in which the fungus enters is by wounded or severed branches. Almost all the trees examined during the present investigation, have shown, at the stage of infection characterised by the first appearance of the sporophores, portions of one or more broken branches associated with the infected region. Old trees such as are readily attacked invariably bear a number of these exposed pieces of dead wood. The

fungus spores are so numerous that it is more than probable that a number may alight on a single snag in one season. The time during which the spores are set free includes the period of the autumn and spring gales when numerous twigs and even considerable branches are severed. These must afford a ready means of entry. Young trees, apparently, are not attacked by P. betulinus except after some weakening influence has been at work (see p. 8.). Yet shading and mechanical breakage are responsible for the exposure of unprotected surfaces in young birch trees just as frequently as in adult specimens. Taking into consideration the spore measurements it is improbable that the size of the openings is of much importance. Mayr (54) has noted the production of cork layers by the cambium in the bark of an infected tree, in an unsuccessful attempt to prevent the advance of the fungus hyphae. It is probable, therefore, that in the young birch the activity of the cambium is sufficiently great to enable it to complete the cork barrier and thus check any infection in its early stages.

One or more years after infection has taken place the first crop of sporophores breaks out through the bark. The trees figured (Pls. 5-6) are characteristic of this state of affairs. The trunk in each is apparently sound and bears a large number of leafy branches, though the dead side branch shows sporophores of the fungus. Once the

trunk prpper is attacked it is often so weakened that a strong wind brings about its downfall. The tree illustrated (Pls. 7-8) has been broken over in this way. A sporophore of the previous year's crop is showing at (a) on the broken side branch, by which the fungus gained entry. Young sporophores are to be seen breaking through the bark at (b) on the upper portion of the trunk. The wood at the part where the splitting has taken place showed the early stages of the typical rot. From this time onwards the death of the attacked tree is a matter of only a few years. The mycelium spreads both upwards to the tip of the dead severed portion and downwards in the still - living trunk. The rate of spread is marked by the ever-increasing area on which the fructifications appear. As the wood becomes broken down these decrease in size and number on the first infected parts. In cases in which infection takes place near the top of the tree, and the growth of the mycelium is accordingly almost entirely earthwards, the course can be clearly followed (Pl. 2.). The tree illustrated was about 25ft. high and had been dead for several years when photographed. It bore sixteen of the current year's crop of sporophores. The smallest are towards the top and the largest near the ground. The mycelium had apparently spread through about 15ft. of the trunk. The wood towards the top was becoming exhausted with the result that the mycelium was dying out in that part.

As it appears that there is no point of maximum weakness in a normally developed tree, the height at which an attacked trunk may break over is probably connected with the original point of infection. The breaking takes place frequently about 10ft. above ground level, as recorded by Brooks (8). In the case of trees originally attacked at some considerable distance from the ground the trunk may break across on several different occasions as succeeding portions of the wood are weakened by the advance of the mycelium down the stem.

IV. TYPE OF ROT.

Due to the attack of this fungus birch wood gradually darkens in colour. The colour of the typical rot has been described as brown by Hubert(37) and red by Hartig (33). The type of rot produced is powdery according to Rankin (63) and Vannine (75), and cubical according to Hartig (33). Vannine (75) and Hemmi and Kurata (35) state that the sapwood is first attacked and a mixed type of rot produced by the irregular advance of the hyphae leaving sound patches of sapwood after the heartwood has been reached and attacked. Rankin (63) states that the sapwood is first attacked, but emphasises that the heartwood remains untouched as long as the tree is alive. His statement seems to be based on the supposition that the fungus does not enter through branch wounds or other injuries where heartwood is

exposed. It is difficult to understand why it should refrain from doing so when this means of entry is so frequently offered. Hubert (38) states that the rot develops rapidly in the sapwood and ultimately destroys all the heartwood. He classifies the fungus with those causing heartwood rots. The typical stage as observed by the writer is a reddish-brown, cubical rot attacking both sapwood and heartwood, though doubtless originating in the peripheral parts of the stem (Pl. 9.).

An infected limb 8ft. in length was cut from a tree, which appeared to be otherwise healthy. It carried two sporophores and bore a living branch on the opposite side to them and some 2 ft. further away from the trunk. The limb was sawn into short lengths for examination. In this way it was possible to trace the path of the fungus in the wood over a distance of approximately 6ft. The broken upper end of the branch showed the typical cubical rot. The entire cross section of the wood was attacked for a distance of about 3ft. from the broken end (Pl. 10.). The next 2ft. showed the rotted portion as an area spreading from the outside to the centre on that side of the branch which bore the sporophores (Pl. 11.). One fructification was produced on the completely attacked area and one on the partially attacked portion. Still nearer the main stem the infected area was restricted to the centre of the branch(Pl.12).

The infection extended 2½ft. beyond the lower sporophore. The infected wood was dark brown showing zones of lighter colour towards the white, unattacked portions. Some days after cutting, the white mycelium of the fungus grew out from the exposed wood on the infected area (Pl. 13.).

In Betula alba there is no colour differentiation between sapwood and heartwood but it may be accepted that hyphae penetrating the centre of a branch 3-4in. in diameter are attacking the heartwood. As the previous paragraph shows, the fungus mycelium, far from growing in the sapwood as long as the tree lives, has evinced a definite tendency to attack the heartwood first. Stone (72) states that there is a difference in appearance between heartwood and sapwood in Betula lenta. The writer has made no observations on this species and cannot therefore say by what steps the mycelium advances in this case.

The type of rot designated above (p.12.) as a mixed rot would be produced readily by the hyphae spreading down the wood of a branch, such as that just described, and penetrating and rotting the heartwood on the near side of the trunk, before those spreading round the circumference had attacked the whole of the sapwood.

A macroscopical examination of the attacked wood reveals thin white plates of mycelium under the bark and surrounding the cubical masses of wood. The

principal paths of advance for the mycelium are thus shown to be tangentially, in the position of the cambium, and between the annual rings of wood; radially, by means of the medullary ray tissues(Pl.14.). The fungus spreads both longitudinally and transversely by these means.

When microscopically examined, the wood shows the hyphae of the fungus ramifying in every direction. They penetrate the walls of the vessels by means of the passages provided by the pits, but also very largely by solution of the walls (Figs. 1-2.) In some sections they were so numerous as practically to block the passages of the containing vessels (Fig.3). It will readily be appreciated that mycelium occurring in such abundance must interfere considerably with the passage of water to the parts of the stem above the point of attack, and will bring about their death. The hyphae are branched and septated. They bear clamp connections (Fig. 4.). They appear colourless. The secretion of ferments, which is discussed later, is presumably responsible for the reduction in thickness and strength, and the ultimate penetration of the cell walls. / new paragraph. Hubert (37), in the course of a survey of the methods of wall penetration by wood-destroying fungi, brings out the two following facts. Firstly, from Hartig's work (33), he shows that hyphae may pass through cell walls with or without constriction. Constriction, where it occurs, takes

place in the case of the older hyphae. Secondly, he makes the point that the bore holes by which the fungi make their way from cell to cell may be small and regular-sided, in the case of young or older constricted hyphae, or irregular in outline in the case of unconstricted older hyphae. Hiley (36) attributed the bore holes of Fomes annosus in the wood of larch to enzyme action, and deduced that the enzymes were only secreted by the tips of the hyphae from the fact that once penetration was secured the hole ceased to enlarge. This seems entirely logical, and the complementary assumption, that those bore holes which are greater in diameter than the hyphae passing through them are produced by a more sustained secretion of enzymes, due to the activity of at least some of the other cells of the filament, follows from it. Mayr (54) describes and figures the hyphae of Polyporus betulinus as constricted when passing through cell walls. Lindroth (47), however, states that the hyphae are not constricted. In the observation of the writer, young hyphae may pass through bore holes which very nearly equal them in diameter, and, therefore, might be supposed to be slightly constricted; but the older hyphae are considerably smaller than the containing bore holes. The sides of the small bore holes are regular, while those of the larger ones may be irregular in outline. (Fig.1.). Hyphae pass both transversely and

and longitudinally in the wood. They penetrate the walls at right angles or obliquely, by means of the pits, or through the thickest portions of the walls, without making any attempt to select the easiest path for their advance (Figs.1-2.). The transverse walls of the elements show the most obvious signs of attack. They are reduced in thickness and the middle lamella stands out clearly, while the thickening layers show as small cubical masses separating the pits. These masses finally disappear. The middle lamella resists the fungus attack for a little longer but gives way in the end. All that remains of it is two peg-like structures attached to the longitudinal walls (Fig. 5.). The same steps are followed in the decomposition of these latter walls. This process is accompanied by the production of a brown fluid which collects in drops in the attacked elements. The droplets may accumulate to such an extent that parts of the elements are blocked by the fluid. The browning is most conspicuous in association with the transverse walls or their remains. The elements in the bark are attacked in the same way. The softer tissues are filled with masses of the hyphal threads passing in all directions. As Mayr (54) has observed, even the stone cells may have their walls penetrated by the hyphae and their cavities discoloured by the brown decomposition fluid (Fig.6.). As the decay advances the breakdown of the wood is

assisted further by the appearance of irregular cracks across the vessels. In this final condition the wood is reduced to a powdery consistency and is then easily rubbed between the fingers. The course of this disintegration has been traced on birch wood blocks in culture, and the typical stage of the rot produced (Pls. 15-16.). These changes are, naturally, accompanied by a very pronounced loss in weight. Mayr (54) estimated that 100 parts, by volume, of absolutely dry wood diminished in weight by approximately 60%, when totally decomposed by the fungus. The writer has obtained similar results. Hubert (37), in diagnosing wood decays caused by fungi, makes use of both the gross and microscopical characters exhibited by the wood, in the typical stages of the rots, in determining the organisms responsible. The microscopical characters which he employs in identification are given below. The words in brackets refer to Polyporus betulinus and are also taken from his table; (a) bore hole size (large), (b) presence or absence of spiral cracks (present), (c) old hyphae constricted or not in their passage through the cell wall (not), (d) buckles (not noted), (e) medallion hyphae (none), (f) hyphae beyond the incipient colour (not present). The writer's observations agree with those recorded above except in so far as they refer to buckles and medallion hyphae. From Hubert's drawings of these structures, found in other fungi,

they appear to be merely rather unusual forms of clamp connections. Similar irregular forms have been observed in the present investigations.

V. ENZYMES.

It has been suggested for many years that the ability of wood-destroying fungi to penetrate cell walls is due not only to mechanical pressure but also to the secretion of digestive ferments or enzymes. The number of these is, in the case of most fungi, still a matter for speculation. Those, however, which bring about the complete disintigration of a tree host must obviously contain ferments capable of attacking the lignin and cellulose of the woody walls. Buller (10), in his studies of Polyporus squamosus, has tested the sporophores of that fungus for a number of ferments and shown definitely, by means of chemical tests, that seven of these are present. Following roughly his methods, which were given in detail in a separate paper (9), the presence or absence of a number of ferments was ascertained in the case of Polyporus betulinus. Sporophore material was tested; but no attempt to carry out an exhaustive survey was made. The mycelium from the wood was not investigated at all. The enzymes present in the vegative hyphae may, however, be assumed to be as numerous as those in the sporophores, where their presence is more easily ascertained. The sporophore extract was prepared by pressing the fruit bodies,

which were previously cut into pieces, through a hand mincing machine. A quantity of juice was expressed and this was used in the experiments. In the case of old dried sporophore material it was still possible to obtain a reactive juice if the pieces of material were, first of all, thoroughly soaked in water, and the liquid was then extracted in the manner mentioned above. This enzyme activity was retained into April by material collected in the previous autumn. Adhering to the nomenclature employed by Buller (9), the following results were obtained by chemical test. Enzymes shown to be present: Diastase, rennetase, coalgulase, emulsin, lipase, protease, tyrosinase, laccase. Enzymes yielding negative results with the tests employed: Invertase, maltase, pectase, cytase, hydromase, trehalase. A comparison of this list with Gortner's (27) classification of the enzymes will show that those which have been found to be present belong to many groups with different functions. Owens (60) states that there are two general types of wood-destroying fungi based on their enzyme action. The first, or delignifying type, turns the wood white and fibrous, while the second, or carbonizing type, (cellulose attacking) makes the wood friable like charcoal and usually red or brown in colour. Cubical cracking and finally a complete powdering of the attacked wood are given as other common features of this second type of attack. From these descriptions it is clear that Polyporus betulinus belongs

to the second group, i.e. those which are supposed not to contain enzymes capable of attacking lignin. Hemmi and Kurata (35) have classified P. betulinus as belonging to the group of cellulose attackers by means of micro-chemical tests and by using Bavendamm's culture method (1). This latter method may be briefly summarised as follows. The wood-destroying fungi were found to fall into two groups, (1) the cellulose destroyers, which failed to produce a halo or oxidation ring when grown on meat extract agar containing added substances, among which tannic and gallic acid gave the best results; e.g., Coniophora cerebella and Merulius lacrymans: (2) the lignin destroyers which produced definite halos under the same circumstances; e.g., Trametes radiciperda and Stereum purpureum. The oxidation rings were attributed to the action on the tannins of oxydases or phenolases such as laccase and tyrosinase which were secreted by the hyphae.

The writer's work on the enzyme content of the sporophores has shown the presence of both laccase and tyrosinase. The culture method was, therefore, experimented with. Oxidation rings, similar to those illustrated by Bavendamm (1), were formed with all the strengths of tannic and gallic acid employed (Pls. 17-19.). This confirms the findings of the writer's chemical tests and is completely at variance with the ideas expressed by the workers mentioned above.

A table, showing the results of the culture work, is appended:

SET UP		EXAMINED			
2/2/35.	11/2/35.	18/2/35.		25/2/35.	
Tannic Acid	Halo	Colony Diam. in mm.	Halo	Colony Diam. in mm.	Halo	Colony Diam. in mm.
.5%	very dark	12	black	55	black	72
.25%	very dark	15	very dark	60	very dark	75
.1%	paler	18	dark	61	dark	74
.05%	pale	22	pale	58	very pale	80
Gallic Acid						
.5%	-	innoc. only	black	43	very black	59
.25%	-	innoc. only	very dark	45	very dark	61
.1%	very pale	8	dark	48	dark	74
.05%	darker	17	dark	50	dark	77

VI. CULTURAL CHARACTERS OF THE MYCELIUM.

Following on the lines of Fritz's (25) investigation on "The Cultural Criteria for the Distinction of Wood-destroying Fungi" the present fungus was grown in test tube culture on a range of agar media. The cultures were grown in series on slants. Each tube contained about 15cc. of medium and measured 6 x 4/5in. The inoculum in each case was a small piece of mycelium growing on malt agar. Six cultures were prepared on each medium. They were incubated at about 22°C. Cultures were examined periodically up to six months. The cultures studied in this way originated from (a) naturally formed sporophore tissue; (b) mycelium contained in diseased wood of silver birch (Betula alba); (c) a pure culture obtained from the Forest Products Research Laboratory, Princes Risborough. All three cultures have continued to exhibit the same cycle of characters throughout the longest period of subculturing - from early March, 1931, in the case of (a) and (b), till April, 1935. The agars selected for comparison were potato-dextrose, potato, malt, Czapek's Synthetic and Czapek's Synthetic modified. All were made up according to the formulae given by Fritz (25), although slight modifications appeared to have little effect on the growth produced.

A description of the cultures follows.

Potato-dextrose. The hyphae had advanced to the foot of the slant by the end of three weeks. They penetrated the agar. They covered the surface sparsely and were very little raised above the surface of the agar. There were distinct traces of zonation. At the end of a month the suferficial growth was still slight, being rather more conspicuous on the inoculum. The mat did not attain any density. There was an entire absence of moisture secretion up to one month.

Old cultures. Drops of moisture were common. The liquid was often amber-coloured. The mat was very similar to that produced in the young cultures but slightly thicker (Pl. 20.). Dark lines were of frequent occurrence (Pls. 21-22.). After three months bulbils or sclerotia were common (Pl. 23.), as well as structures, which were regarded as abortive sporophores.

Potato. Early growth on potato was very similar to that on potato-dextrose. The mat in some cases, however, was so slight as to readily pass unnoticed. For this reason, probably, there was little sign of zonation.

Old cultures. These showed very little change, except that the mycelium grew sparsely on the glass of the tube (Pl. 24.).

Malt. The growth was much more conspicuous than on potato-dextrose. It spread with about the same

rapidity. By the end of a month the surface was covered completely. The aerial mats were composed of hyphae having a fine woolly appearance. These readily spread over the surface of the glass.

Old cultures. After two months the growth was very much more obvious than on potato-dextrose. There was less moisture present, and an entire absence of dark lines (Pl. 25.). At ten weeks and upwards many of the cultures showed drops of clear or pale amber liquid. Attempts at sporophore formation were quite frequent.

Czapek's Synthetic (modified). The growth was slightly less extended than on potato-dextrose, and much more dense than on either potato-dextrose or malt. There was a faint margin of spreading aerial hyphae followed by this dense mat. After about three weeks dark lines developed in the medium in some cases. The hyphae grew readily on the glass, as in malt. The surface exhibited slight undulations corresponding with a ringing or zonation of the aerial growth. Drops of moisture were present.

Old cultures. After two months the growth was similar to that on malt but woollier (Pl. 26.). The raised hyphae were often so abundant as to completely block the base of the tube with a cotton wool-like mass (Pl. 27.).

Czapek's Synthetic. This medium closely resembles potato in its effect on the growth of the fungus.

In some cases white, irregular patches showed early near the inoculum.

Old cultures. After two months the growth compared favourably with potato. There were no drops of moisture present. Dark lines had appeared on the parts of the cultures which earlier showed white patches (Pl. 28.). After three months a stalk-like structure appeared in one tube, which had no dark lines.

Colour. The young mycelium, on all media experimented with, is colourless. Colour begins to develop earliest on Czapek's Synthetic (modified) agar, being quite obvious from about three weeks onward. As a rule, on the standard medium used for general culture work (malt agar), cultures are at least six weeks old before colouring appears. The colouring in the early stages is so slight that there is no doubt that the fungus falls into Fritz's class of those in which the mycelium is white during a month's growth. The colour of the mat is a light yellow or, very occasionally, pinkish-brown. It deepens slightly with age till, in very old cultures (six months and upwards), there may be a brown, crusted appearance. The colour appears quite irregularly; but usually first in the drier upper part of the tube near the inoculum. This brown colouring is not to be confused with the dark lines mentioned above. Reference is made to these elsewhere. Mats on potato-dextrose, potato and Czapek's Synthetic were completely

colourless after one month's growth.

Rate of growth. In all cultures the mycelium established itself below the surface first of all. Aerial spread was most rapid on malt, potato-dextrose and Czapek's Synthetic (modified) in that order. Spread on the peptone-containing Czapek and on potato was slower and the amount of mycelium produced very much less. The surfaces were covered in every case at the end of a months, but the mats were very sparse in these two latter cases.

Light. Cultures were grown in light and in darkness. The rate of growth appeared to be slightly slower in light; but the general characters of the mycelium produced were not apparently affected.

Temperature. The best growth rate was obtained using a temperature of 25°C. as compared with 15°C., 20°C., 22°C. and 30°C. The rate at 22°C. was very satisfactory for general work and, as this temperature could be conveniently maintained, it was, as a rule, employed. It was found that the mycelium could survive being kept at just below 0°C. for twenty-four hours on end. Hemmi and Kurata (35), who carefully compared the growth obtained at various temperatures, found the optimum rate at about 28°C., while a little growth was obtained both at about 4°C. and about 36°C.

Microscopic features. The general characters were as follows. The hyphae at the edge of any colony were delicate, hyaline, branched, septated threads. Clamp connections were present. They were small. The

hyphae varied in width from about 1-4μ in the apical cells. Submerged, advancing hyphae were, as a rule, very similar to the above. They tended to be not quite so fine. Old submerged and surface hyphae showed an increase in diameter ranging up to about 7μ. Clamp connections were much larger on the older threads. The angle of branching is not uniform, varying from a very acute angle to an obtuse angle in some cases. Cell contents frequently included a large number of refractive oily drops. Vacuoles of very varying size were common. Empty cells and portions of hyphae were found. The hyphae were sometimes constricted at the cross walls. In the submerged mycelium, hyphae of irregular outline were a common feature (Fig. 7.). The surface mycelium tended in the more luxuriant cultures to be overgrown by a raised mass of hyphae, largely composed of fine hyaline fibre-like threads with thickened walls about half the breadth of their lumina. These fibre hyphae were without clamps or obvious contents, sparingly branched and with very few or no septa. They were sometimes undulating in outline.

A few points of interest, exhibited by mycelia growing on particular media are given below.

Potato-dextrose. Fibre hyphae were relatively few in number.

Malt. Clamp connections were not readily observed on the young hyphae.

Czapek's Synthetic (modified). Clamp connections

were particularly large and numerous on the young hyphae.

Czapek's Synthetic and Potato. There was an absence of fibres due to the paucity of the surface growth.

In the case of the present fungus, malt agar was as good a general purpose medium for growth as potato-dextrose. These two were superior to the other media employed. For the sake of conformity with Fritz's(25) work, the growth obtained on the latter medium is, however, taken as the standard. The fungus then fits into the first part of her key (a) to the identification of wood-destroying fungi as follows:

(a) Key

(i) Mats white or faintly yellow during a month's growth.

(ii) Mats faintly pink.

(iii) Mats variously and often deeply coloured, not as above

(i) Mats radially furrowed
---10 Polystictus abietinus

Mats not furrowed
Chlamydospores present
---7 Polyporus borealis.

Chlamydospores absent

Fibres present
Fibres sparingly branched

Fibres uniform
---12 Poria subacida

Polyporus betulinus

Fibres with expansions
---11 Polystictus versicolor

Fibres much branched
---15 Balsam rot Type B (2).

Fibres absent

(b) Summary of diagnostic characters. Mats at first thin, soon cotton-wool like, diffused, or in luxuriant cultures, overgrowing the glass and tending to fill the lower part of the tube with a dense plug. Drops of clear to amber-coloured liquid frequently excreted. Flat pore surfaces (Pl. 29.) and roughly rounded sporophores develop on drier parts of the medium. Sclerotia or bulbils are formed. Mycelium colour white often becoming pale brown later. Dark lines common. Spread fairly rapid, slants covered in three weeks, mats thick. Submerged mycelium: (1) Hyphae variable, fine, 1-7μ thick, branched, septated, sometimes constricted, clamp connections single. Walls of hyphae firm, colourless, refractive. Refractive drops frequent in old hyphae.
(2) Chlamydospores absent.
Aerial mycelium: (1) Advancing zone and appressed mat similar to above. (2) Chlamydospores absent. (3) Fibre-like hyphae constitute a large part of the cotton-wool mass in old cultures. They have thick walls, narrow lumina, few branches, very few septa, no clamp connections.

A comparison of the available data is necessary to separate further the two fungi brought together by the key (a). The rate of growth of Poria subacida appears to be more rapid. The type of surface mycelium, too, is much more uniform. Fritz (24) states that the fibres of Poria subacida are without septa. Those occurring in the fibres of Polyporus

betulinus are sufficiently rare to make this difference of little value in diagnosis. The presence of heavy deposits of crystals encrusting the submerged hyphae on Czapek's Synthetic agar is noted by Fritz (25). No crystals were observed round the hyphal strands on any of the media employed in the case of P. betulinus. Yellowing of the medium occurs with P. subacida; but there is no mention of the production of dark lines. Bulbils, too, appear to be present only in P. betulinus.

In lacking chlamydospores P. betulinus differs from all four Polypori examined by Fritz (P. borealis, P. sulphureus, P. balsameus and P. schweinitzii). In having colourless mycelium it agrees with P. borealis and P. balsameus. P. borealis and P. sulphureus also have fibre hyphae.

VII. ZONE LINES.

In some cultures on wood blocks, zone lines developed on the surfact and in the mycelial mass towards the base of the tube (Pl. 30.). Black lines have been observed in a naturally infected birch trunk in the open. Attempts to obtain cultures of P. betulinus from the black line were, however, unsuccessful. Hubert (37) has noted the appearance of a black line on the cut end of infected birch timber (B.papyrifera) after drying in the laboratory. Black zone lines have been reported as occurring in various timbers infected by other species of Polyporus,

e.g. P. nigricans (Lindroth 47), P. adustus (Hubert 37)

Pure cultures of P. betulinus on nutrient agars frequently showed zoned darkening of the mycelium and the medium (Pls. 21-22.). Other writers have observed that mixed rots of timber, involving species of Polyporus, may show zone lines between the rots. e.g. Polyporus anceps and Lenzites separaria in Picea canadensis (Hubert 37).

Hemmi and Kurata (35) have illustrated that in mixed cultures of P. betulinus with Fomes ulmarius or Lenzites tenuis, under certain circumstances, dark zone lines were produced at the lines of contact of the two mycelia. In the case of P. japonicus the dark lines were formed with the same fungi and in addition with Fomes applanatus. The writer has attempted to induce the formation of zone lines in mixed culture, at the junction of colonies of P. betulinus and Fomes fomentarius. These attempts have not been successful.

Hartig (33), on examining the zone lines of Armillaria mellea, found them to be composed of swollen bladder-like hyphae, which were brown in colour. They occupied the lumina of the tracheids just as tylosis do in the wood of deciduous trees. Campbell (16)(17), dealing with the zone lines caused by Xylaria polymorpha and A. mellea, described the development of the zone lines from normal hyaline hyphae by a process of swelling, accompanied by

close septation, and the development of a brown colour. The more or less regular row of densely packed hyphae thus formed corresponded, he suggested, with the wall of a true sclerotium. The name entostromata was applied to those areas of infected wood which were bounded by the zone lines in the case of X. polymorpha, while the term pseudosclerotium was used to describe similar areas of the wood together with the rhizomorphs, in the case ofA.mellea. An examination of the zone lines of P. betulinus produced in pure culture on wood blocks, has shown them to be composed of dark-brown-walled hyphae. The walls were also thinkened, particularly at the ends, so that the apices of the cells showed a crescent-shaped thickening when the hyphae were teased out on a slide (Fig. 8.). This is strongly reminiscent of the appearance of the hyphal tips which form the wall of the young sclerotium in Typhula gyrans.(53). It appears, therefore, that the black lines may be correctly regarded in this case also as representing the wall of a somewhat modified resting body. As confirmatory evidence of this (1) it may be recalled that true sclerotia are present in several members of the genus Polyporus:- P. Tuberaster, P. Berkeleyi P. umbellatus, P. frondosus, P. sacer, P. Goetzii, P. Sapurema, P. rhinocerotis, P. basilapidiodes and P. Mylittae are all listed by Gäumann (26).

Some of these sclerotia attain a great size, e.g. those of P. Sapurema may weigh up to 20kg. (Harshberger 32), while those of P. umbelatus are branched and may reach a size of 1sq. metre (Bommer 3.) (2) During the present study, small light-brown, roughly spherical, sclerotium-like bodies have formed in culture. These may be termed bulbils.

The word bulbil was used by Lyman (52) to describe small bodies, sclerotioid in character; but which did not show any differentiation of the limiting layer from the medullary hyphae and which, therefore, he considered, could not properly be termed sclerotia. Gäumann (26) employs the term to describe the structures produced in basidiomycetous fungi when "occasionally they (sclerotia) are formed in large numbers and reach only a small size." The bulbils of P. betulinus belong to this latter class. They are composed of closely intertwined hyphae. The medulla is uniform, having much the appearance of the parenchymatous tissues of the higher plants. There is no hollow centre. The outer branches of the hyphae are placed so that their apical cells form a continuous layer enclosing the medulla. The outermost walls show a very pronouncedly thickened layer. These "epidermal" cells resemble the bladder hyphae found in the zone lines, being much swollen as compared with the normal hyphae (Fig. 9.). The body of the bulbil contains numbers of irregular crystals.

Larger sized crystals cover the outside of the bulbil (Figs. 9-10.). The close relationship between bulbil and black line is further emphasised by their regular association in culture. Agar cultures, which show dark lines in the medium, frequently bear bulbils on the surface above these lines (Pls. 31-32.).

VIII. SPOROPHORES.

In Nature. These are freely formed. There may be an annual crop of any number up to 25 on a single tree. They appear on dead or, less commonly, on living branches and trunks, and continue to inhabit fallen birch timber for some years after it is severed from the standing stem. They are soft and fleshy, rarely remaining attached to the stems for more than one year. Bulliard (15) stated that they might be biennial. If this is the case, it must be a very rare occurrence indeed. When dried they are leathery and keep well. They make their appearance from the last fortnight in August onward till late in November, breaking out through the bark as round white structures which resemble firm puff balls. Mayr (54) suggested that they emerged through lenticels or through the bore holes of insects. There is frequently an obvious connection between their point of appearance and a lenticel in the case of those which are produced on young branches. This is by no means invariable however. Plate 33. shows at (a) the preliminary swelling of the bark due to the massing

of the hyphae underneath it. At (b) the bark has ruptured and the loose flap has been pushed up by the emerging sporophore. The lenticels are not being utilised in this case. The growth rate varies. In some cases the maximum size is attained in about six weeks. When mature the fruit bodies measure from 5cm. in breadth upwards. Some of those emerging late in the year do not get beyond the "puff ball" stage. The largest specimens observed by the writer were about 10in. across. Other observers have recorded sizes up to 12in(69). By more rapid growth of the tissues towards the upper side, the round shape is gradually lost and the sporophore is transformed into a small bell- or hoof-shaped structure. This may show a very short stalk or be stalkless. The upper surface is more or less sloping and is covered with a thin tough whitish skin. It is convex. The edge curves over the lower surface projecting about 1cm. as a sterile rim. The rim is often waved. The lower surface, which ultimately produces the hymenium, may be slightly concave or, more usually, very slightly convex. It is sterile at first; but sooner or later the pore layer develops, completely covering it. This layer makes its appearance in some cases by mid September. By this time the pellicle on the upper side has assumed a brownish colour and is easily cracked by handling. The fertile area consists of a continuous layer of small pores which are circular to angular in outline. They

average 3-4 per mm. though sometimes unusually large ones over 1mm. in diameter are encountered. They are white turning brown with age. Part or all of the hymenial surface may at times be covered by a mass of hyphae which grows down the pores, choking their cavities and emerging to spread over the surface. The mature pore layer varies in depth from about $\frac{1}{2}$in. in the oldest parts to little over $\frac{1}{4}$in. at the rims of the sporophore. In the mature fructification this layer is readily separated from the ground tissue above it as a complete sheet. The sporophores have a pungent acid smell which is more marked when they are broken open. Murrill (58) has noted that a pink colour may develop on the exposure of the internal tissues. The sporophores are remarkably uniform in shape; but, occasionally, unusual structures make their appearance. The upper surface may be very much humped (Pl. 34.). A number may issue from the bark at the same point or at least so close together as to form a solid mass when broken away from the tree trunk (Pl. 35.). Very occasionally the writer has found on fallen branches fructifications which were flat against the wood, round in shape and more or less centrally attached (Pl. 36.). This is doubtless due to formation taking place on the under side of a sloping branch or fallen stem. On one occasion a fructification was observed growing from the north side of the lower of two fallen

branches. It was very poorly illuminated. The structure was finger-shaped, with the stalk laterally placed at the base. The fertile pore region was borne on a number of folds at the head of the sporophore (Pl. 37.). The abnormal sporophores in plates 38 and 39 were formed on a branch while still attached. In this case they grew in a well-lighted position. The atmosphere was very smokey. No other reason can be advanced to account for the abnormal shape. In the one case the pore layer points downward as usual though the shape of the fruit body is far from typical. In the second, not only is the shape even more monstrous, but the pore surface appears in two places (a) and (b) one facing vertically downwards and the other facing horizontally. In cases in which the direction of a fallen branch has been altered, normal, attached fructifications, if still in active growth, have orientated themselves so that the pore layer was turned downwards.

The pores are lined by the hymenium, which consists of a layer of basidia formed from the slightly swollen ends of the branches of the medullary hyphae. These latter form an interwoven mass, the individual strands of which run, in general in a longitudinal direction. The free ends turn outwards to form the basidia (Figs. 11-12.). The basidia average 4μ in diameter. This figure includes young, mature and old, collapsed specimens. The young basidium is filled with a

dense cytoplasm. As it matures it protrudes slightly into the pore. The cytoplasm becomes vacuolate. Sharply pointed sterigmata, usually four in number, appear on the top end. These ultimately bear the allantoid spores (Fig. 13-14.). It seems feasible that the vacuolation of the basidium is connected with a pressure mechamism for driving their contents into the spore. This has been suggested by Buller(13) when writing of the Hymenomycetes generally. The nuclear phenomena observed are in accordance with the state of affairs generally supposed to exist throughout fungi of this type. In the young basidium two nuclei are present. These, first of all, are in the lower part of the structure. They move towards the top of the cell and there fuse. The fusion nucleus divides, and each daughter nucleus probably divides again so that four nuclei are often discernible in the mature basidium. Mayr (54) has stated that the basidia sometimes only have three sterigmata and spores. Sometimes the material investigated has had very much this appearance, though it is very possible that they way in which the basidia were lying might have been responsible for creating such an impression. Basidia are observed, too, in which only three nuclei are present. This may very well be due to the delayed division of one of the two daughter nuclei; but the possibility does exist that one may fail to divide altogether and in this way the three-spored

basidium would be accounted for. The nuclei pass into the spores. These, when shed, are uninucleate and sometimes vacuolate. They are curved with their concave surfaces facing inwards. The point of attachment is terminal. The old basidium now collapses returning to the general level of the hymenium. All these stages described above are figured in the following drawings (Figs. 11-13). The spores measure from 4.4-5.5 X 1.4-2μ. These figures agree with those given by most authors except Brooks (8) and Carleton Rea (18) who give the size as 5-7 X 2μ. Morphologically all appear identical.

Laboratory experiments were carried out to determine the period over which spore discharge takes place. Sporophores were carefully watched as they matured in the open and, when the pore layer made its appearance, they were transferred to the laboratory. There they were placed above pieces of brown paper, which were examined and changed daily. When spore production commenced in bulk the white spores formed casts similar to those observed by Brooks (7) and Buller (10) in the case of Polyporus squamosus. No spore cloud was discernible, however, in this case. Sometimes an appreciable cast was obtained within a few hours of the start of the experiment. In other cases as long as 7 days elapsed before it appeared. In the case of any individual the longest period over

which spore discharge was discernible was 15 days, in the dry air of the laboratory. Sporophores covered by bell jars produced spore casts for periods up to three weeks. The length of the period of discharge diminished as the season advanced. Casts were obtained from mid September till the end of March, while viable spores were obtainable from some sporophores until well on into April. The appearance of the fructifications proved to be quite unreliable as a guids to the spore-producing potentialities. Very brown specimens sometimes gave good casts due, no doubt, to the production of spores from the basidia higher up the pores; while in many cases white healthy looking specimens failed altogether to form casts. Completely saturated fruit bodies required a few days to dry before spore production commenced. Those which were frozen quite hard yielded to the warmer indoor temperature within 48 hours. In general the temperature employed varied about 20°C. If it dropped as low as 15°C there was a marked falling off in the number of spores produced. The discharge appears to take place uninfluenced by the time of day or the presence or absence of light. It begins in the older middle parts of the fruit body and gradually spreads to the edges, so that for a time the process is taking place simultaneously over the whole surface, i.e., the basidia towards the tops of the tubes in the older part produce their crop concurrently with those towards the foot of the pores

situated nearer the edges of the fructification. Finally the cast disappears from the middle part and a rim of spores only is left. Many of the browned fruit bodies collected in spring show this last state of affairs. Experiments with sporophores placed under bell jars showed that the spores do not fall perpendicularly to the ground in a still air chamber. After some time the upper surfaces of the sporophores and the inside walls of the bell jars became coated with a white layer of spores. This observation corresponds with the findings of Falck (22). Buller (11) has shown that this is due to convection currents. For this reason spore casts collected on glass slides, smeared with egg albumin, showed a uniform distribution of the spores. There is no grouping to correspond with particular pores, and, to begin with, there is no overlying. The majority of spores come to rest on their sides; but some few remain on their backs.

By early spring the vast majority of the sporophores are riddled by the ravages of other species of fungi, and of insects. They hang as mere shells disfiguring infected trees till the following autumn. The attack on the fructifications by adult and larval insect forms begins almost as soon as they appear in autumn. In the Tweed valley the larvae are usually species of Fungus Gnats (Mycetophyllidae), while a rather rare fungus-eating Melandryid beetle Tetratoma fungorum was remarkably common in the adult condition.

The basidia are sparingly interspersed with cystidia. These structures were recorded by Mayr (54); but some subsequent workers have failed to confirm his observation, e.g., Lowe (50). The medulla between the pores consists of a mixture of ordinary and fibre hyphae. The expanded ends of some of these latter form the cystidia. They show little or no contents, are thick-walled and lanceolate with bluntish points. They are usually solitary but sometimes occur several together. In some cases they exhibit marked spiral twisting. (Figs. 19-20.). They protrude very considerably into the pore space and, although they do not reach the basidial layer on the other side of the pore in mature fruit bodies, their function, in developing sporophores, is probably to keep neighbouring pore surfaces apart and to assist in preserving the general shape of the hymenial layers. This function was originally suggested for the cystidia of certain species of Coprinus by Micheli (55). Buller (13) has found them functioning similarly in several other species of the same genus. Those of Coprinus Micaceus, however, he regards as functionless pegs. It may be that the cystidia of Polyporus betulinus belong to this latter class.

In culture. When cultures were kept in practically continuous darkness sporophores were readily formed on wood blocks and on agar plates and slopes. They most frequently formed on the drier parts of the cultures. They were usually roughly rounded and

tended to produce pores on all exposed suffaces without discrimination (Pl. 4.). Occasionally they appeared as flat pore layers on the surface of wood blocks or on agar cultures (Pl. 29.). Falck (23) has illustrated them in this form. The rounded specimens measured up to 3/5in. in diameter. The appearance of these fructifications took place from ten weeks after inoculation onwards. In no case did they show the form of normal fructifications. Brefeld (6), Buller (11) and Fritz (25) also have suggested the importance of light in this connection. Accordingly a series of malt agar cultures was grown in normal daylight. These failed to produce fruit bodies of any sort. As is mentioned below, sporophores which began development in darkness continued when transferred to day and night conditions. This state of affairs most nearly approaches that occurring naturally.

Sporophores were unresponsive to the direction of light. They made very little attempt to orientate themselves with respect to gravity as Buller (11) has suggested such sporophores do. In form they seem to resemble the fructification of Polystictus cinnabarinus which Hasselbring (34) produced by eliminating the action of gravity with a clinostat. Naturally forming sporophores do, however, alter their position in response to the combined action of light and gravity (Pl. 3.).

In other cases bodies appeared which strongly resembled the rounded sporophores described above. They grew to a similar size; but failed to produce pores. Many became overgrown by the vegetative mycelium. Microscopic examination revealed that they were composed of a limiting layer and parenchymatous medulla, as in the case of the bulbils.

In addition to the sporophore forms mentioned above, more unusual structures were produced from time to time in agar cultures. In certain cases yellow projections appeared on the inoculum after about one month. In the early stages they resemble bulbils forming. One of these increased in size till at the end of a week it was 4/5in. long by $\frac{1}{8}$-$\frac{1}{4}$in. broad. The broadest part formed a swollen head. At this stage it collapsed onto the surface of the plate. A drop of pale amber liquid was secreted at the tip (Pl. 40.). This ultimately reached $\frac{3}{8}$in. in diameter. A second similar but smaller structure was produced from the same point. The head was more expanded. Buller (11) has recorded that spprophores of Polyporus squamosus failed to produce pilei in continuous darkness. These structures, then, appear to be similar and correspond with the finger-shaped fruit body produced naturally under reduced light conditions (Pl. 37.). In construction, however, they correspond exactly with the bulbils produced in the same and other cultures. Both are closely connected with the dark zone lines (Pls. 31-32.).

A consideration of the various abnormal forms produced in culture leads to a realization of how difficult it is to say definitely whether a body is sclerotioid or sporophore in nature, and how gradually the one form seems to pass into the other. The structure in each appears to be the same, viz. a medulla of densely woven hyphae bearing at the surface a more or less continuous row of terminal hyphal cells which are considerably swollen. If these cells remain thin-walled the structure is recognisable as a sporophore. If they become thickened, then a resting sclerotial stage is formed. Whether it be the readily recognised bulbil, the abortive mycelium-covered ball, or the wood mycelium bounded by a zone line, all are, the writer is convinced, essentially resting sclerotia. This fundamental sameness of sporophore and sclerotium has been demonstrated already in the case of Typhula gyrans (53).

IX. LIFE CYCLE IN CULTURE.

The life history of the fungus has been carried through from spore to spore in culture. In mid-October, 1934 spores were sown out on malt agar slopes. These gave rise to the ordinary vegetative mycelium and, after 9-10 weeks, in some cases sporophore formation commenced. The cultures were then transferred to a moist chamber and placed horizontally so that the sporophore lay above a clean glass surface. At the end of three months (mid-January) slight spore

casts were discernible on the glass of the lower side of the tubes. Examination showed these to be composed of the characteristic allantoid spores of P. betulinus. They corresponded also in size with spored produced from typical sporophores. A microscopic examination of these culture fruit bodies showed that the pores were lined, as in the naturally formed fructifications, by a layer of basidia sparingly interspersed with cystidia. The layer seemed to be slightly less regular. Mature and developing spores were observed.

X. HETEROTHALLISM.

The study of the problems connected with heterothallism in the fungi has been going on for many years; but the bringing forward of statistical evidence in determining the number and origin of such heterothallic strains is a much more recent development. Towards the end of the nineteenth centure the nuclear phenomena in certain rusts and in Armillaria mucida were studied by Rosen (64) who demonstrated, in the case of the latter fungus, that, with the approach of maturity, the nuclei present in each basidium were reduced in number till only one remained. It is now generally accepted that throughout the autobasidiomycetes the young basidium, as a rule, contains two nuclei which fuse as the structure matures, the fusion nucleus dividing twice to give one nucleus for each of the four basidiospores. The vegetative hyphae

have been found to contain nuclei in conjugate pairs. These phenomena are regarded by many people as being part of a reduced sexual process, the haploid condition being maintained from the division of the fusion nucleus till the nuclei conjugate in the vegetative hyphae, the diploid phase lasting from the moment of association till the nucleus, formed by the delayed fusion in the young basidium, divides. The origin of the paired vegetative nuclei was first investigated by Kniep (39), (40) and (41). Bensaude(2) working with two monospore cultures of Coprinus fimetarius (lagopus) found that these monospore cultures remained in the "primary" condition when maintained separately, i.e., each cell showed a single nucleus, no clamp connections were formed, branching was irregular and no sporophores were produced. When grown together the united mycelia exhibited what she regarded as the secondary characteristics,i.e. paired nuclei, clamp connections, regular branching and perfect fruit bodies. The paired nuclei divided conjugately, each division of the dicaryon being associated with the formation of a septum with a clamp connection. It appeared, therefore, that the two nuclei which fuse in each basidium are derived originally from a pair of nuclei coming from uninucleate spores of opposite type or "sex." Among other workers, Kniep (42) with Schizophyllum commune and Bose (4) with Polyporus ostreiformis and Polystictus hirsutus have shown that monospore cultures of certain

fungi are capable of producing fertile fruit bodies, though the spores formed are always of only one kind. The appearance of sporophores in monospore cultures does not, therefore, preclude the possibility of a species being heterothallic.

Theories attempting to show how diploidisation takes place and, incidentally, to account for the presence of the clamp connections, have been brought forward by Bensaude (2), Lehfeld (45), Kniep (42) and Buller (14). Buller (14) regards the clamp connections as a means for readier passage of cell protoplasm and an an unnecessary adjunct to the paired nuclear divisions, which they invariably accompany.

The accumulated evidence seems to point to the fact that the only readily observable and trustworthy criterion of the diploid condition is the presence of clamp connections. The presence of these on monospore mycelia is taken to indicate that the species is homothallic, and, their absence, that it is heterothallic. Mounce (56 and 57), working with species in the genus Coprinus, has demonstrated both these conditions. Following up her work on Coprinus lagopus, which indicated that this fungus produced more than two types of spores, Hanna (31), in an exhaustive study of "sex" in this fungus, has shown that the spores belong to four different types (as demonstrated by pairing a number of monospore cultures in every possible way), and that the factors determining these groups segregate out according to

Mendelian principles, as was recorded by Kniep (43) for Schiziphyllum commune. The reduction of the chromosome number may take place in the basidium of any fungus, either at the first or second division of the fusion nucleus resulting in the production of two or four spore types.

Spores of Polyporus betulinus were examined in hanging drop cultures. They germinated sparingly from five days onwards. The germ tubes were usually single and most commonly terminal. They sometimes appeared from the lateral walls. Occasionally two tubes were produced at the same time (Fig. 18.). The best germination was secured using 5% glucose solutions at a temperature of 20°C.-22°C. A range of temperatures from 29°C. to 0°C. was experimented with. No germination was secured at either extreme. Germination was very slow at 25°C. and 15°C. Germination is also slow on the solid agar media. Ten days elapsed before the appearance of colonies on malt agar. Once established the colonies grew rapidly.

Monospore cultures were obtained by the dilution method of Gwynne Vaughan and Barnes (30), by the capillary tube syphon devised by Gupta (29), and by smearing the surface of agar plates with a dilute spore suspension, and cutting out the resulting colonies as soon as they appeared. All three methods required considerable care. The second is probably

only really satisfactory for large spored forms, while the apparatus is difficult to make. The last was probably rendered practicable only on account of the poor germination rates obtained.

The colonies produced from single spores grew strongly on agar or on birch blocks, though less vigorously than mycelia originating from multi spore cultures. They retained their vitality over equally long periods.

Gelatin agar films were prepared as recommended by Sass (68) and used to study the growth of the fungus mycelium in situ. Latterly these were replaced by films of potato-dextrose agar which also gave satisfactory results.

No attempt has been made in the present work to go into great detail in the problems of heterothallism and diploidisation referred to above. The writer has confined himself to showing the existence of the heterothallic condition in P. betulinus, and the number of the spore types. Monospore mycelia failed to produce clamp connections though kept under observation in tubes for periods up to six months. Their nuclei were single with the exception of certain apical cells in which, due to the rapid growth of the filament, the production of cross walls had not kept pace with the division of the nuclei (Fig. 21.).

The pairing of a dozen monospore strains obtained from a single sporophore was carried out in every

possible way. The table given below indicates that clamp connections were produced only in certain cases, while the spores fell into two groups indicating that the reduction division in the basidium takes place at the first division of the fusion nucleus.

	1	2	3	4	5	8	11	12	6	7	9	10
1	0	0	0	0	0	0	0	0	X	X	X	X
2	0	0	0	0	0	0	0	0	X	X	X	X
3	0	0	0	0	0	0	0	0	X	X	X	X
4	0	0	0	0	0	0	0	0	X	X	X	X
5	0	0	0	0	0	0	0	0	X	X	X	X
8	0	0	0	0	0	0	0	0	X	X	X	X
11	0	0	0	0	0	0	0	0	X	X	X	X
12	0	0	0	0	0	0	0	0	X	X	X	X
6	X	X	X	X	X	X	X	X	0	0	0	0
7	X	X	X	X	X	X	X	X	0	0	0	0
9	X	X	X	X	X	X	X	X	0	0	0	0
10	X	X	X	X	X	X	X	X	0	0	0	0

0 = clamp connections absent after 14 days
X = clamp connections present after 14 days.

Paired monospore mycelia unite uniformly or exhibit "space of aversion" or a heaping of the hyphae (Pl. 41.), without regard to from which spore type they originate. There was no evidence of the different angle of branching between monospore and heterospore colonies, which Bose (4) observed in both Polyporus ostreiformis and Polystictus hirsutus.

There is no sudden onset of sporophore formation when the two types are brought together; but anastomoses are frequent and clamp connection formation is free. The nuclei appear associated in pairs as they are in multi-spore cultures, or those produced from infected wood and fruit bodies (Fig. 22.). The division of the pairs of conjugate nuclei takes place in relation to the clamp connections. As with Typhula gyrans (53) the absence of the cross-walls in the early stages of clamp connection formation is a noticeable feature (Figs 23-25.). It seems that one of the nuclei passes out into the bulge of the forming clamp. Both then divide, or the nucleus in the main hypha may divide slightly beforehand. In the former case the cross walls in the hypha and in the clamp probably form simultaneously, and, in the latter, the cross - wall in the hypha may be completed before that in the clamp has begun to form (Figs. 23-25). At no time have nuclei been observed forcing their way through pores in the cross walls, and these have not been observed except under circumstances which made it possible to interpret them as incomplete septa in the normal course of formation. These findings agree substantially with the observations of Kniep (44) and Bensaude (2).

XI. SUMMARY.

1. An introductory survey is made of the distribution, uses, synonyms and ststematic position of Polyporus betulinus (Bull) Fries.

2. Parasitism is discussed. Negative results have been obtained from infection experiments; but field observations force the writer to the opinion that the fungus is a parasite.

3. From observations in Nature and in culture the type of breakdown produced in the attacked wood is classified as a red-brown cubical rot. The microscopic characters are listed following Hubert's method. The stages in decay are illustrated.

4. A brief survey of the enzyme content is made, following Buller's methods. The fungus is shown to contain a variety of ferments capable of attacking the woody tissues of the birch. It appears to belong to both the lignin-destroying and the cellulose-destroying groups of wood-attacking fungi.

5. The culture characters of the mycelium are investigated and the fungus classified according to Fritz's work.

6. Black lines are formed in culture. These are related by position with bulbils (sclerotia). It is suggested that here, as elsewhere, both are resting stages.

7. Sporophores are described in Nature and from culture. The hymenium consists of pores lined by

basidia and sparingly interspersed with cystidia. These latter appear to be modified fibre hyphae. The sequence of nuclear phenomena in the basidium is traced. The period of spore discharge is determined for the species and for individual sporophores.

8. From an examination of the structure of sporophores, bulbils and zone lines it is suggested that all three are constructed on the same fundamental plan.

9. The life history of the fungus has been carried through from spore to spore in culture, in a space of three months.

10. Monospore cultures were obtained. The fungus is heterothallic. The spores were shown to fall into two groups by pairing 12 monospore colonies in every possible way. Monosporous mycelia show single nuclei and no clamp connections. The normal hyphae contain paired nuclei and bear clamp connections.

11. A bibliography of 75 titles is appended.

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XIII. EXPLANATION OF PLATES AND FIGURES.

Plates.

1. Sporophore emerging from split in bark of actively growing tree.
2. Dead birch with sporophore crop. The largest fructifications are towards the foot of the trunk.
3. Fallen trunk bearing sporophores for the second year. Note orientation in respect of changed position.
4. Mycelium on sterilised birch block. The sporophore is producing pores on all exposed surfaces.
5. - 6. Birch in full leaf showing infected branches at (a).
7. - 8. Broken birch showing last year's sporophore at (a), this season's at (b).
9. Infected timber illustration the cubical rot.
10. Infected branch cross section of upper completely attacked part.
11. Infected branch cross section of middle portion showing attack, spreading from one side.
12. Infected branch cross section of lower part showing attack localised in the middle.
13. Mycelium growing from attacked wood of branch illustrated in Plates 10-12.
14. Infected wood showing mycelial plates.
15. Sterilised birch wood block inoculated with P. betulinus mycelium showing the cubical rot after 3 months.

16. As Pl. 15. showing normal and rotted wood.

17. Oxidation ring caused by the growth of the mycelium of P. betulinus on a culture plate containing .25% of Tannic Acid.

18. As Pl. 17. but the culture contains .5% of Gallic Acid.

19. As Pl. 18. under side of Petri dish.

20. Test tube culture on potato-dextrose agar 2 months old.

21. As Pl. 20. Dark lines in the mycelium at (a). Drops of liquid at (b).

22. As Pl. 20. Dark lines in the medium.

23. As Pl. 20 but 3 months old. Two small groups of bulbils are shown.

24. Test tube culture on potato agar 2 months old.

25. " " " " Malt agar " " "

26. " " " " Czapek's Synthetic (modified) agar, 2 months old.

27. As Pl. 26 showing cotton wool-like mass of hyphae at base of tube

28. Test tube culture on Czapek's Synthetic agar. 2 months old.

29. Test tube culture on Potato-Dextrose agar showing flat pore surface.

30. Mycelium on sterilised birch block showing black lines.

31. Malt agar Petri dish culture viewed from under side. Note black lines.

32. As Pl. 31. seen from above. Note abortive aporophore at (a) and ring of sclerotia at (b) corresponding with the dark line in the previous plate.

33. Infected birch branch. (a) Swelling of the bark due to massing hyphae. (b) Ruptured bark with emerging sporophores.

34. Hump-backed sporophore on an infected trunk.

35. Firmly united mass of three sporophores.

36. Clock-face shaped sporophore flat on fallen branch.

37. Finger-shaped sporophore. Fertile region at (a)

38. Abnormal fruit body. " " " (a)

39. " " " Fertile regions at (a) and (b).

40. Abortive sporophores (c.f. Pl. 32.). Note large drop of liquid on one of the swollen heads (a).

41. Union of monospore cultures showing (a) space of aversion (b) uniform mixing (c) heaping of the hyphae.

Figures.

1. Hyphae in L.S. of infected birch wood. Iron-Haematoxylin x 2000.
2. As in Fig. 1. Younger hyphae.
3. Hyphae in T.S. of infected birch wood. Safranin and Picro-Anilin Blue x 850.
4. Hyphae from wood. Iron-Haematoxylin and Light Green x 1150.
5. (a)-(d) Stages in the breakdown of the wood elements. Greenacher's borax Carmine x 1250.
6. Stone cells from infected bast showing (a) hyphae penetrating the walls, (b) gumming of the cavities. Iron-Haematoxylin x 1250.
7. Hyphae from culture showing (a) oil drops, (b) empty cells, (c) vacuoles and irregular outline (d) constriction at cross walls. Mounting medium x 1500
8. Black line hyphae showing crescent-shaped thickening. Mounting medium x 1200.
9. T.S. Bulbil showing modified limiting layer (a); parenchymatous cells, (b); and crystals (c). Mounting medium x 1250.
10. Crystals from surface of sclerotium. Mounting medium x 1250.
11. Young basidia showing paired nuclei. Breinl x 3000.
12. Basidia showing (a) paired nuclei, (b) the fusion nucleus and (c) the pairs of nuclei produced by the division of the fusion nucleus.

13. Basidia showing (a) vacuolation, (b) nuclear stages. Breinl x 2300.

14. Basidia showing (a) 4 nuclei, (b) sterigmata. Breinl x 1600.

15. Basidia showing the first and second divisions of the fusion nucleus. Iron-Haematoxylin and Light Green x 3000.

16. Part of basidial layer showing nuclei and at (a) base of a cystidium. Iron-Haematoxylin and Light Green x 2000.

17. Spores showing nuclei and vacuoles. Iron-Haematoxylin and Light Green x 2500.

18. Spores showing stages in germination. Hanging Drop Culture x 1300.

19. Cystidium showing spiral twisting. Mounting medium x 1250.

20. Paired cystidia. Breinl x 1500.

21. Monospore hyphae. (a) Single nuclei, (b) multinucleate apical cell. Iron-Haematoxylin and Light Green x 2000.

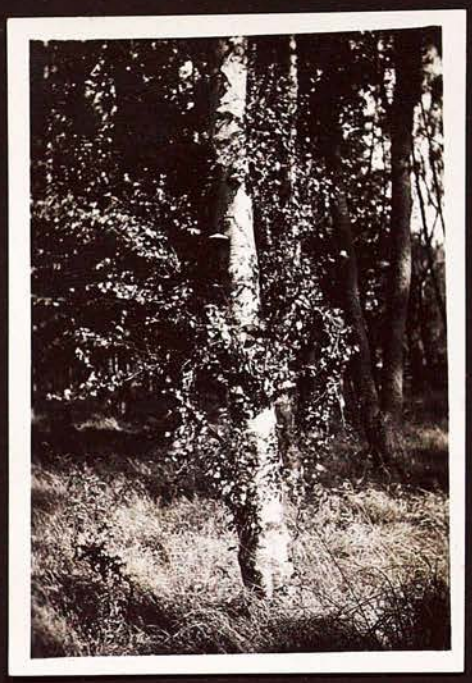
22. Hyphae showing paired nuclei. Iron-Haematoxylin and Light Green x 2000.

23. Hypha showing clamp connection forming. No septa present. Breinl x 3000.

24. Hypha with clamp showing completed division of the nuclei. Both cross walls are complete. Breinl x 2000.

25. Hyphae showing stages in clamp formation (c), (a), (b) and (d), successive stages in the division of

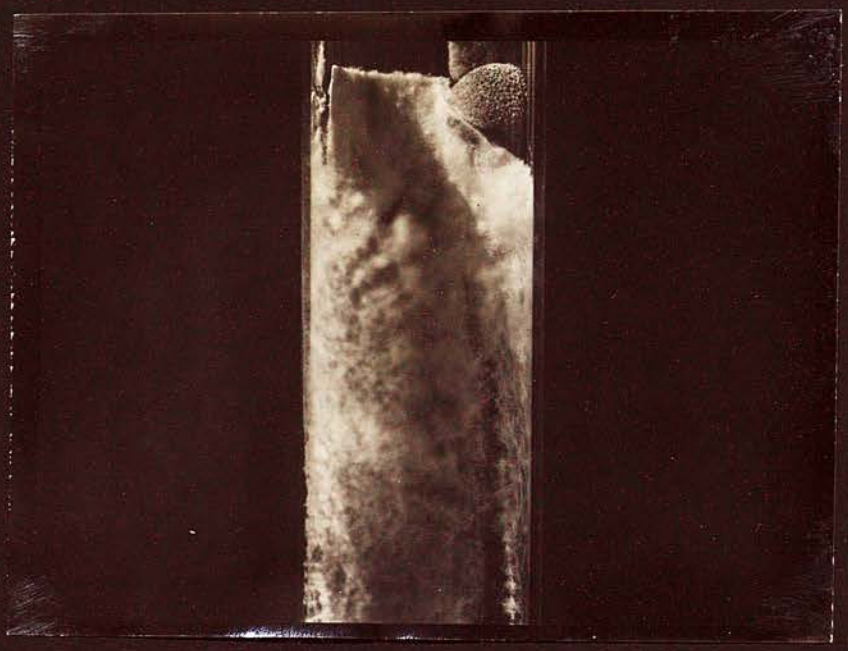
the paired nuclei. Iron Haematoxylin and Light Green x 2500.



Pl. 1.

Pl. 3

Pl. 2



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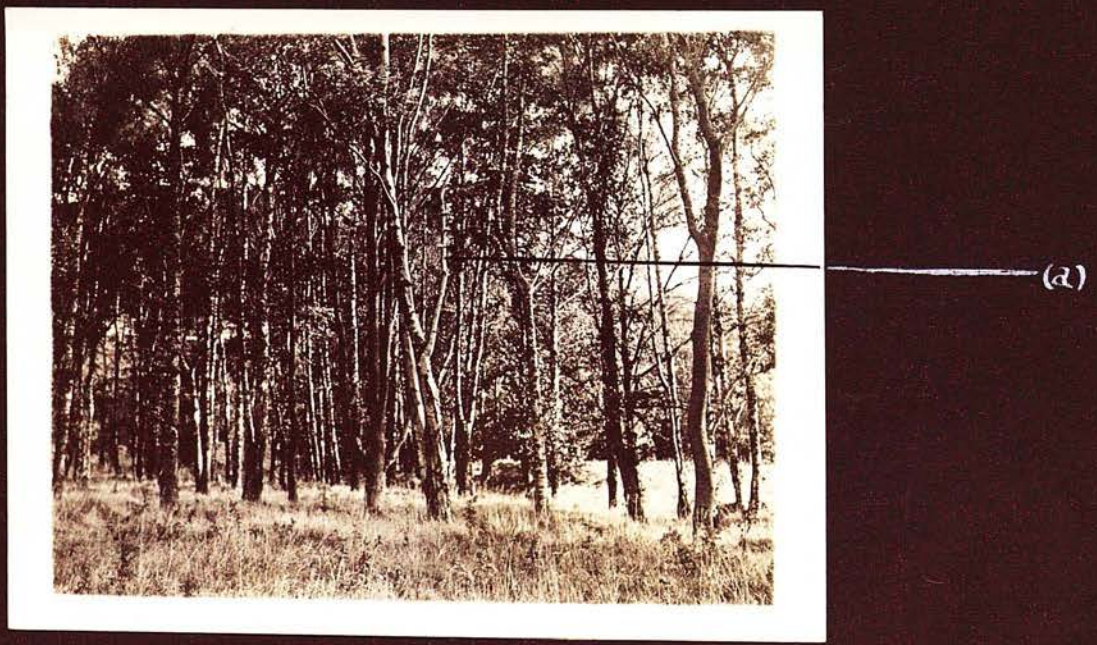

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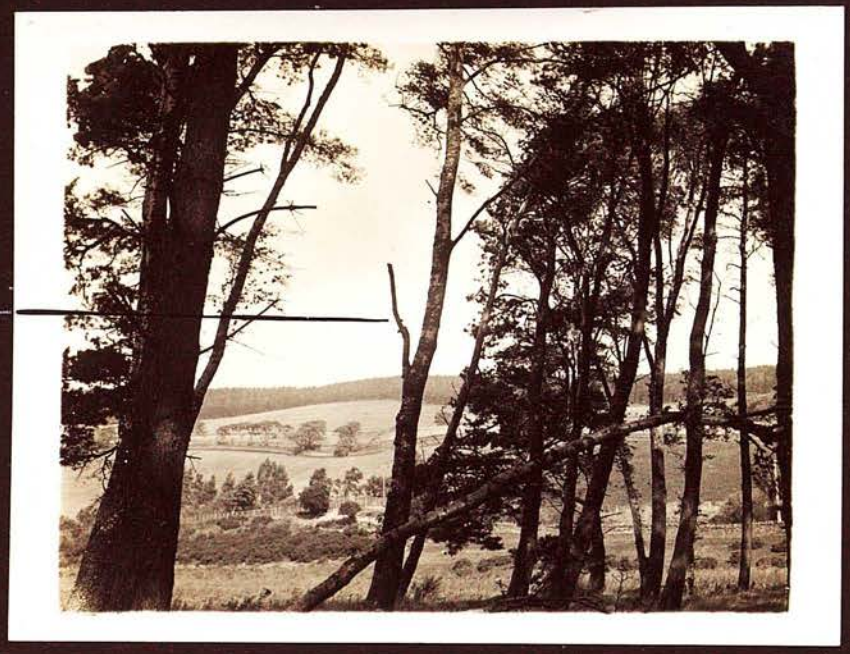
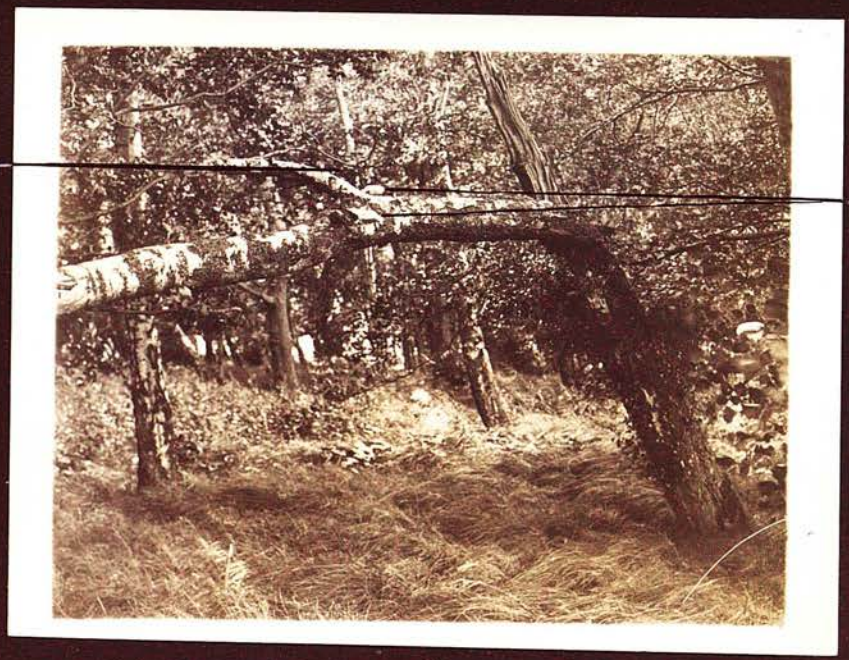


Pl. 6



Pl. 7

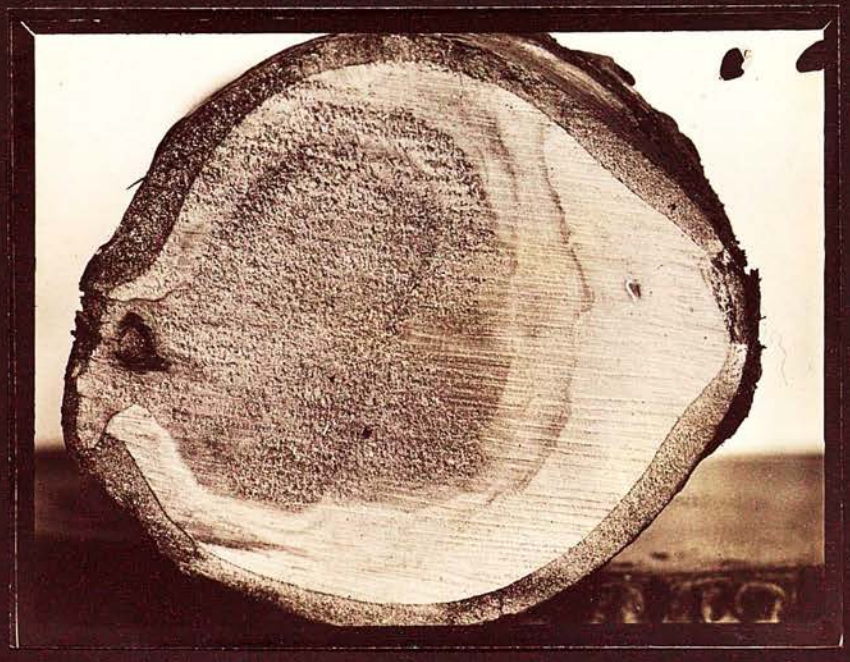
(b)

(a)

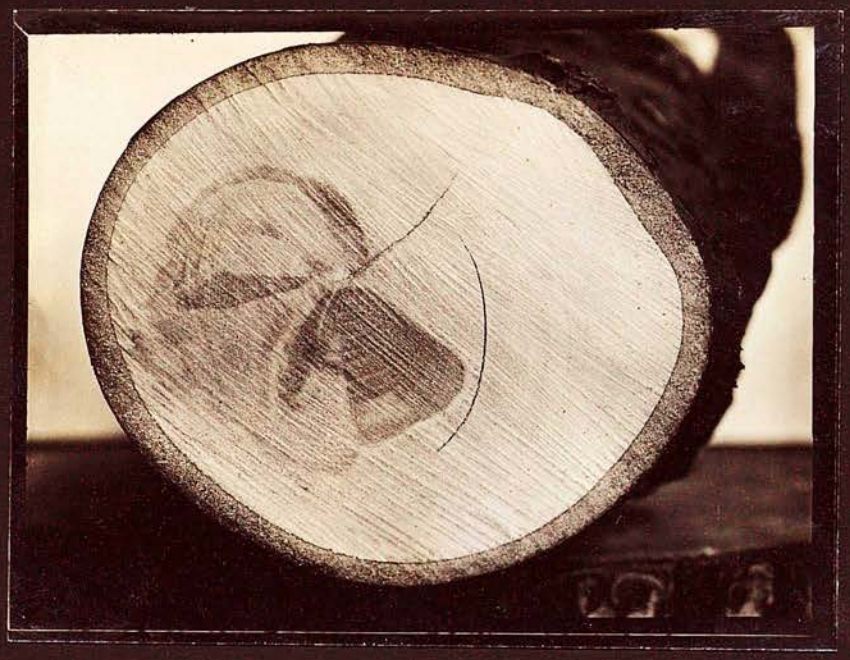
Pl. 8

Pl. 9

Pl. 10

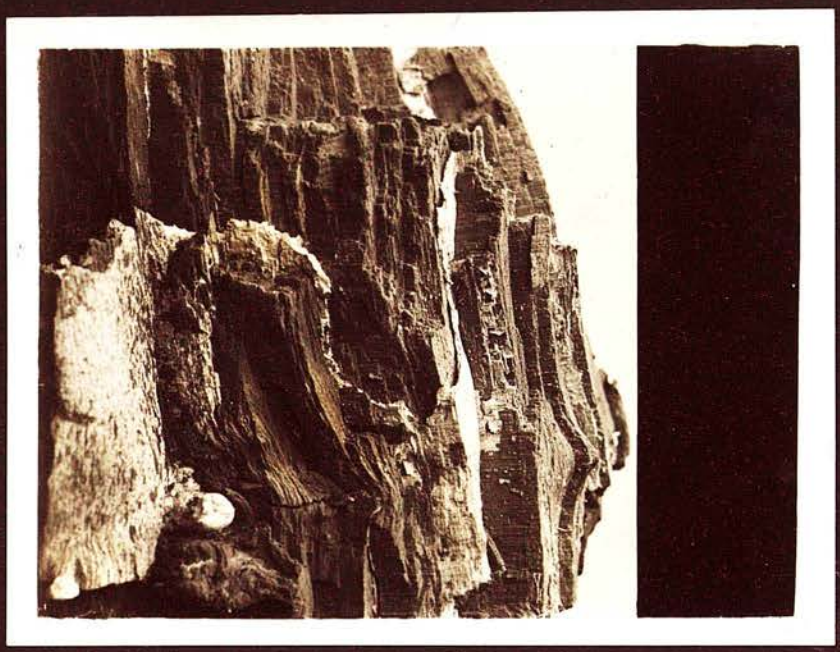


Pl. 11



Pl. 12

Pl. 13



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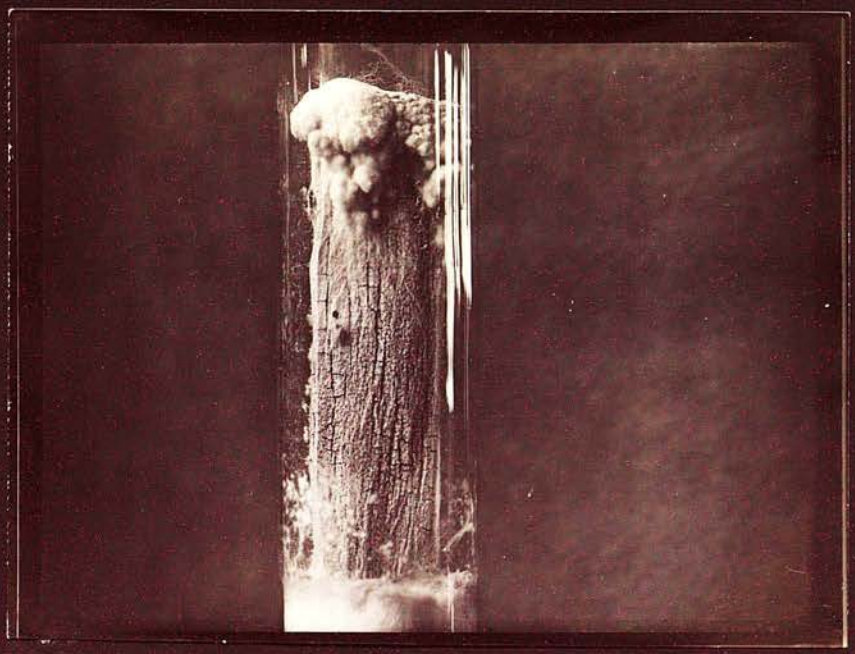

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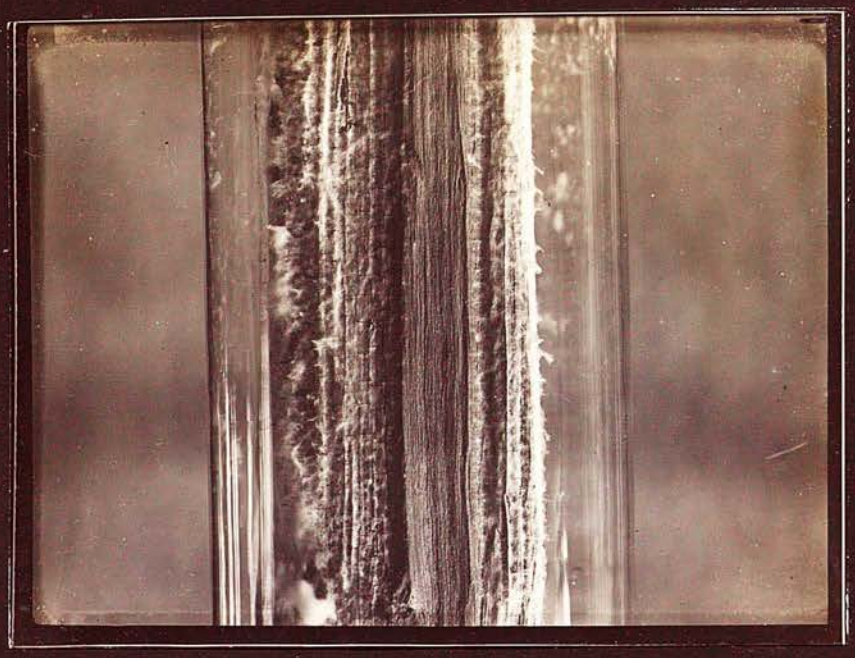
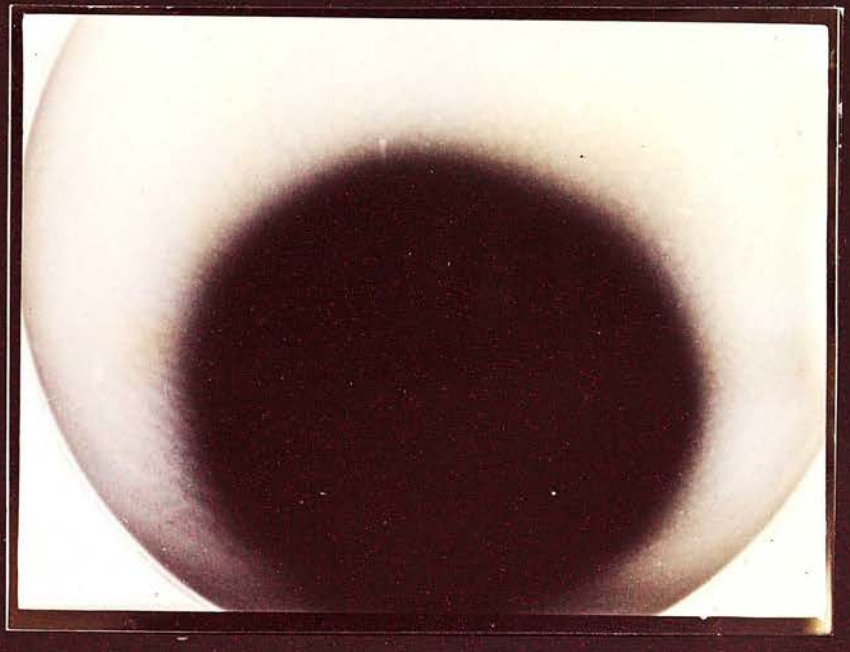


Pl. 16

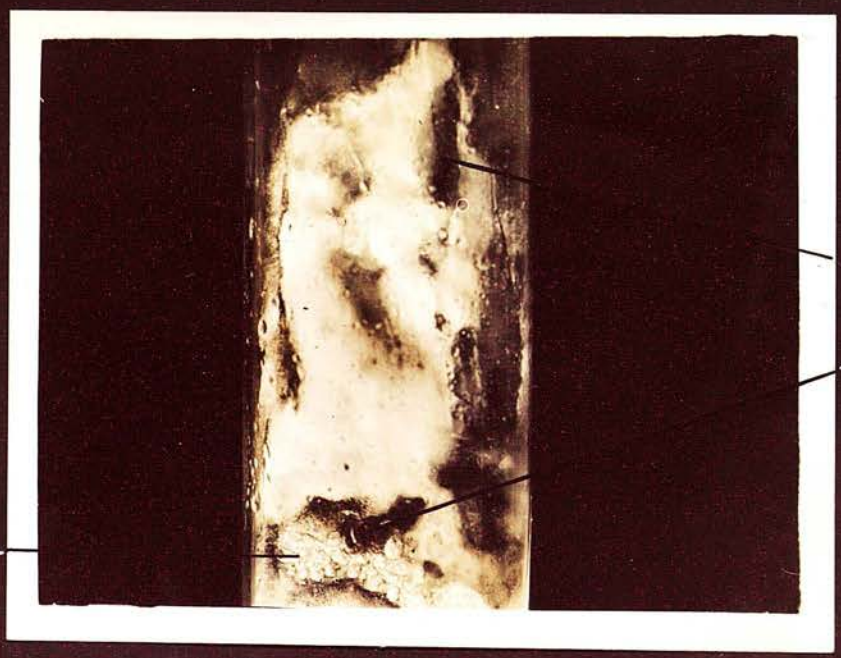
Pl. 17

Pl. 18



Pl. 19

Pl. 20



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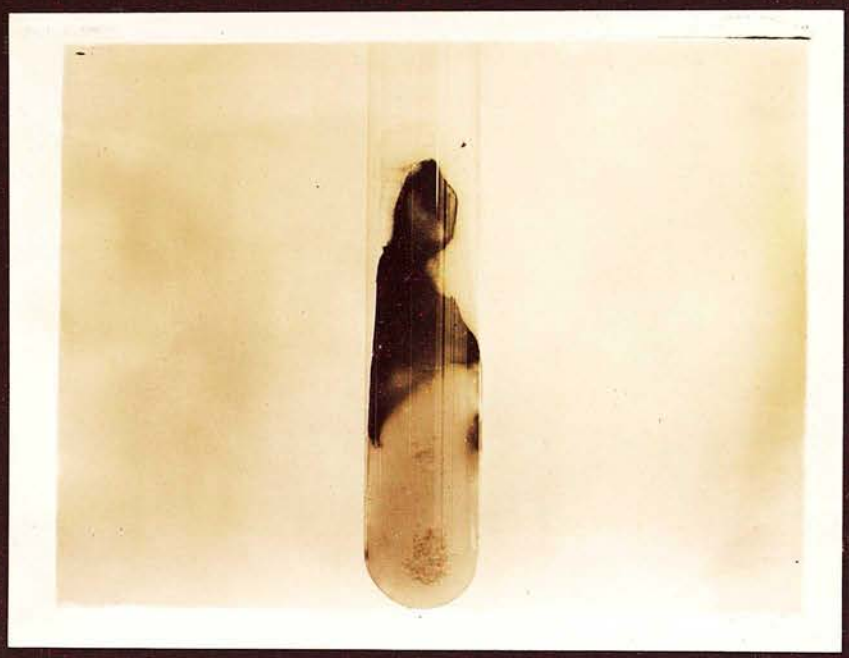

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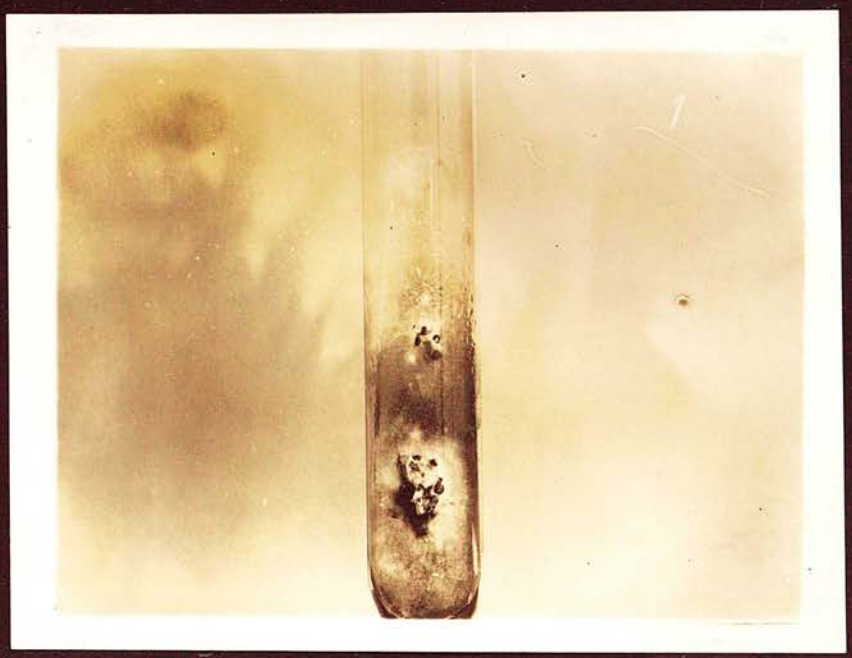

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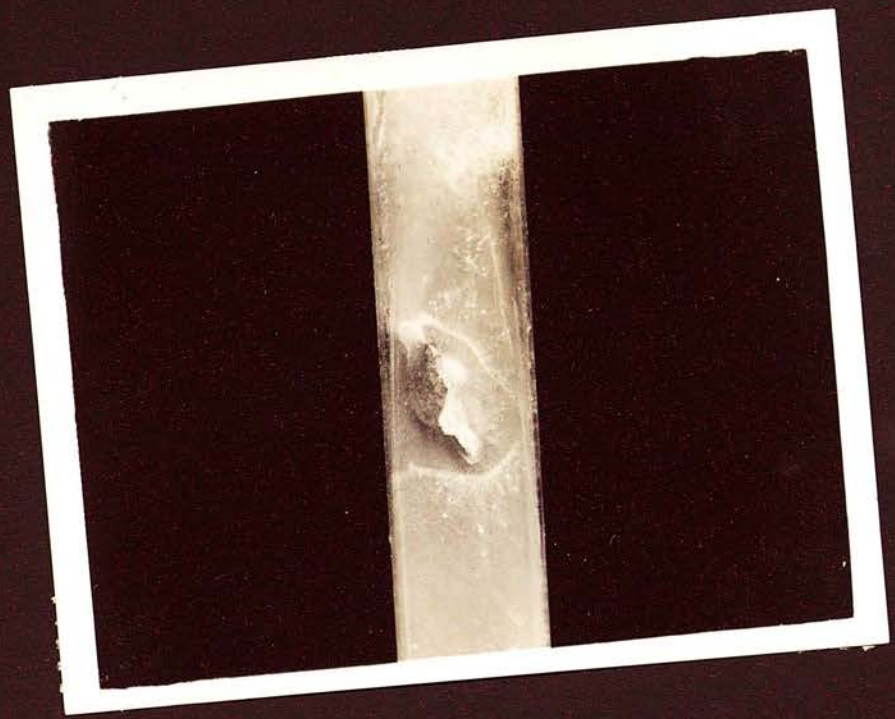
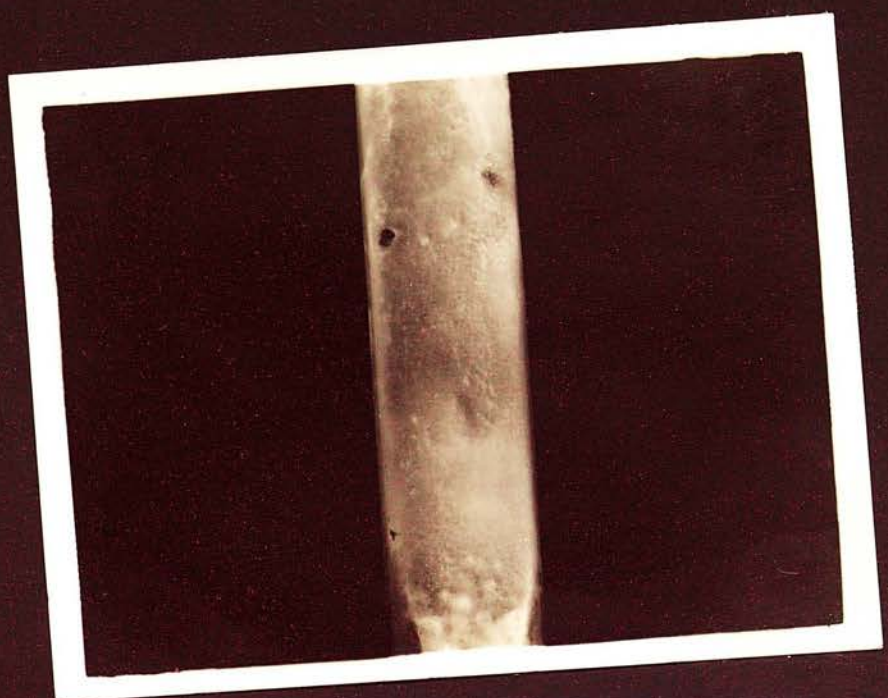


Pl. 24



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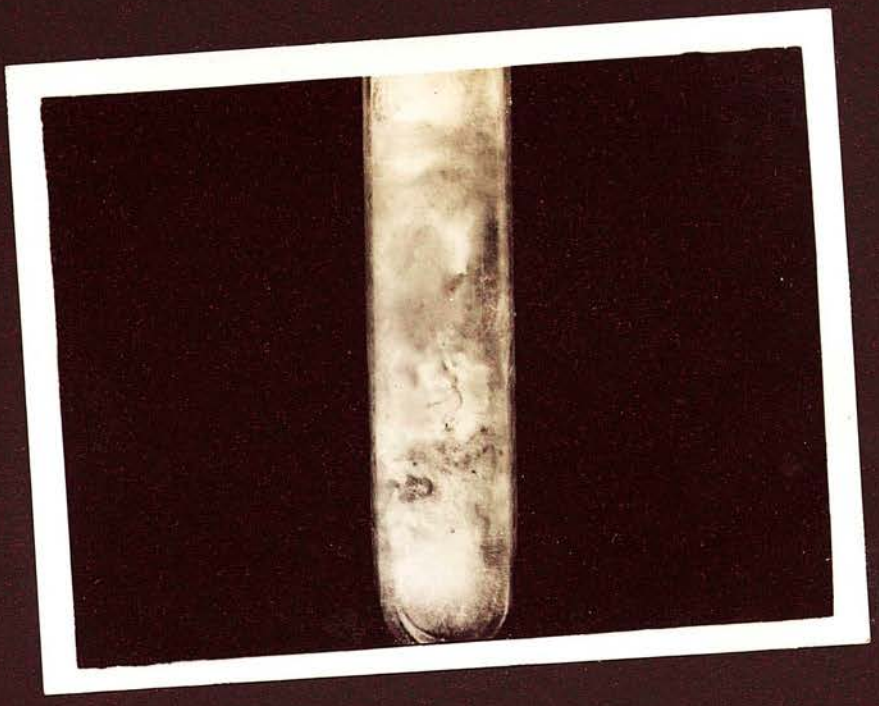

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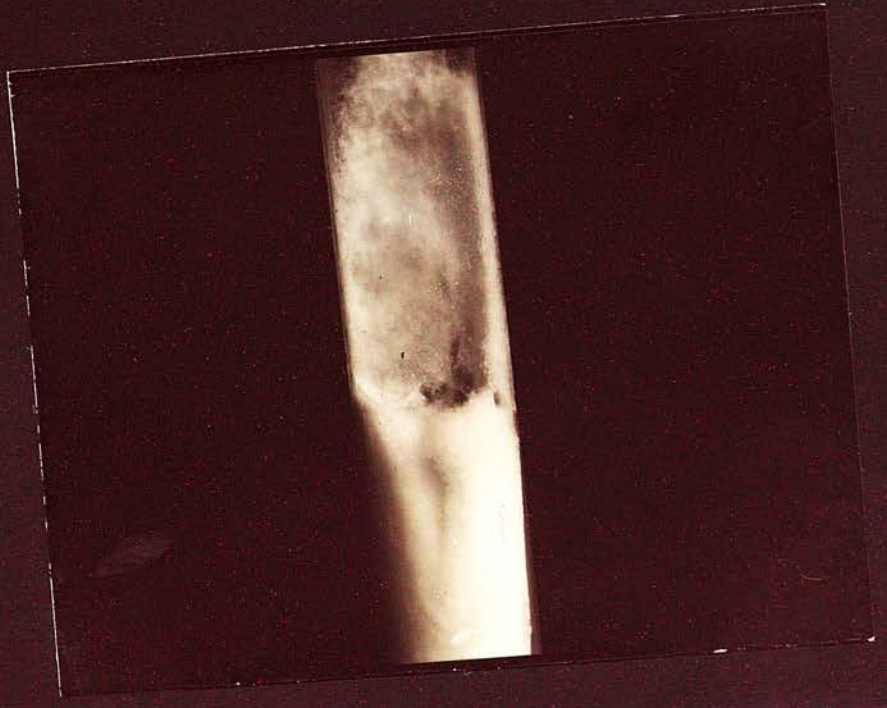

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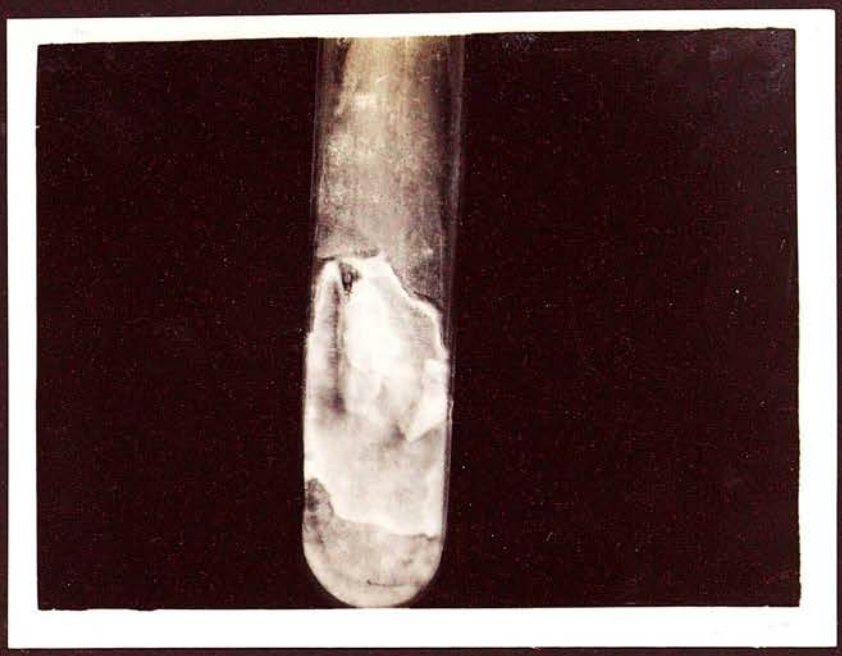

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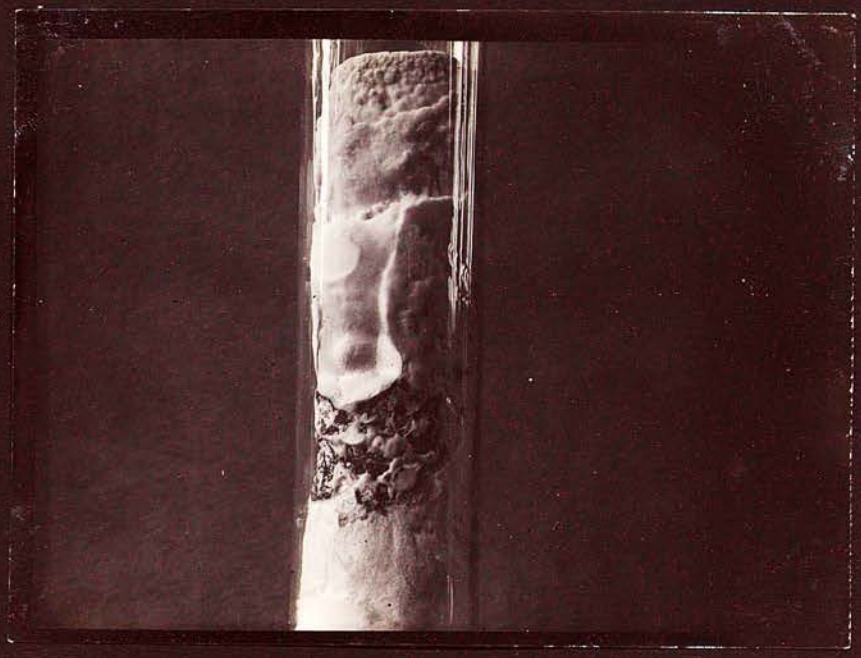
Pl. 29



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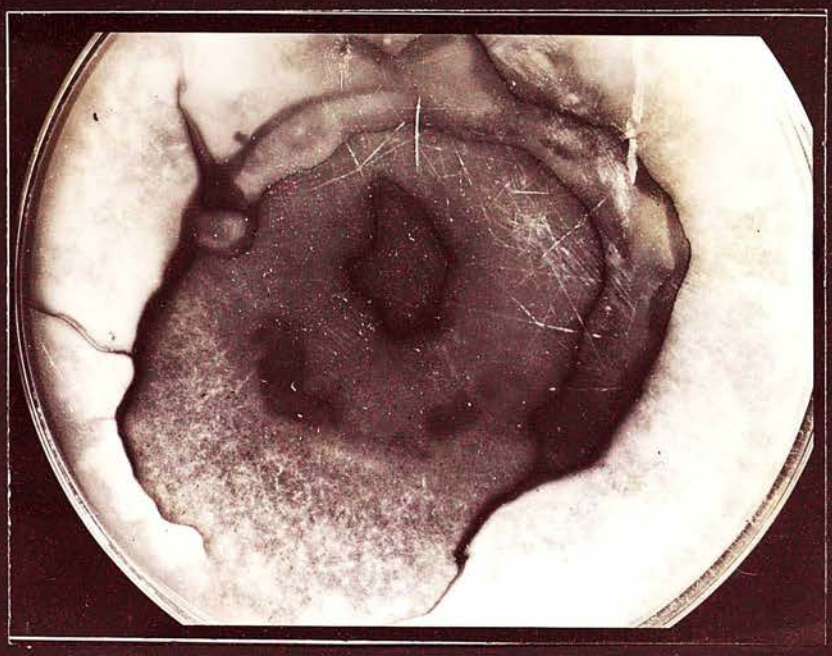

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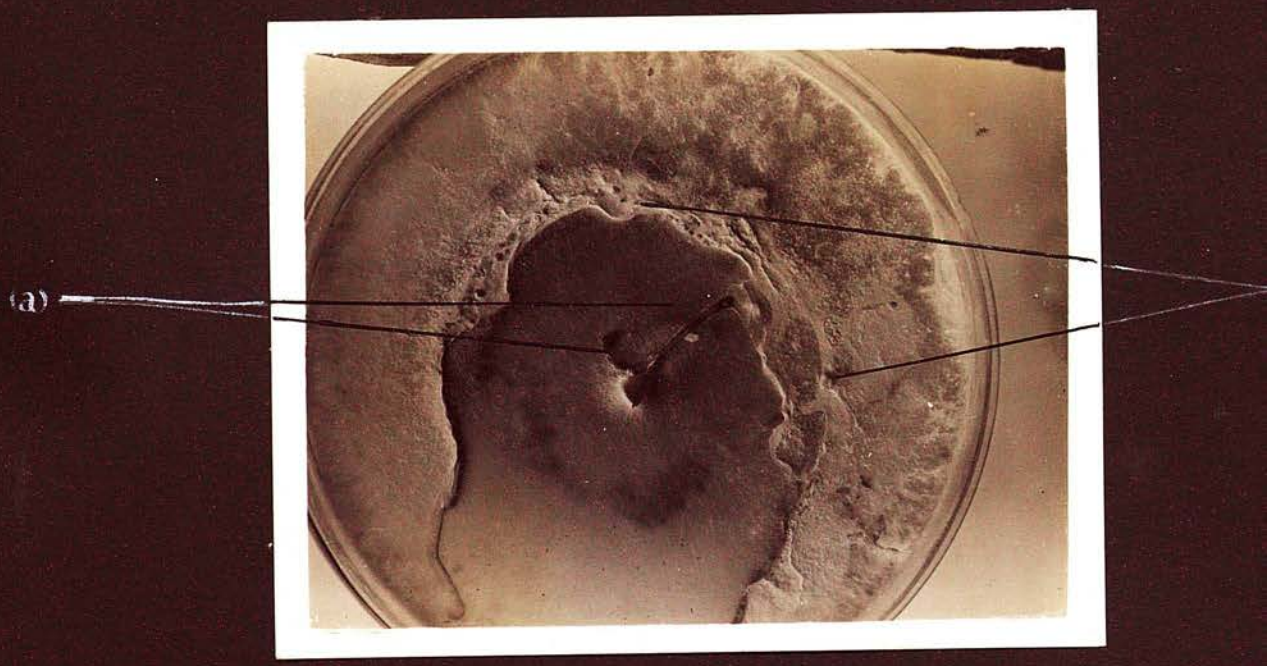

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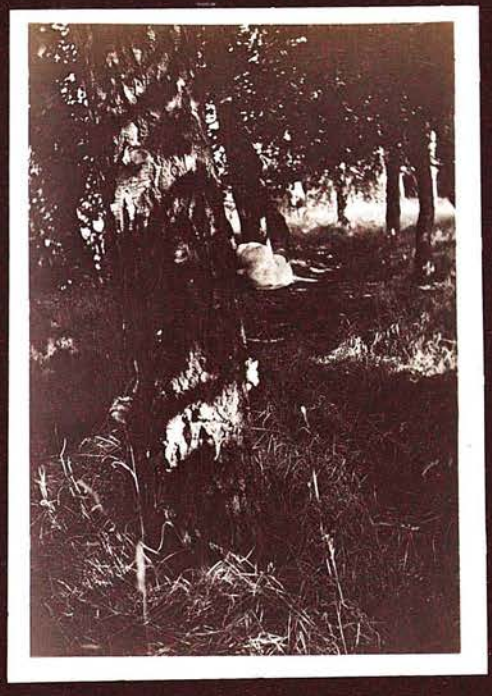
Pl. 33



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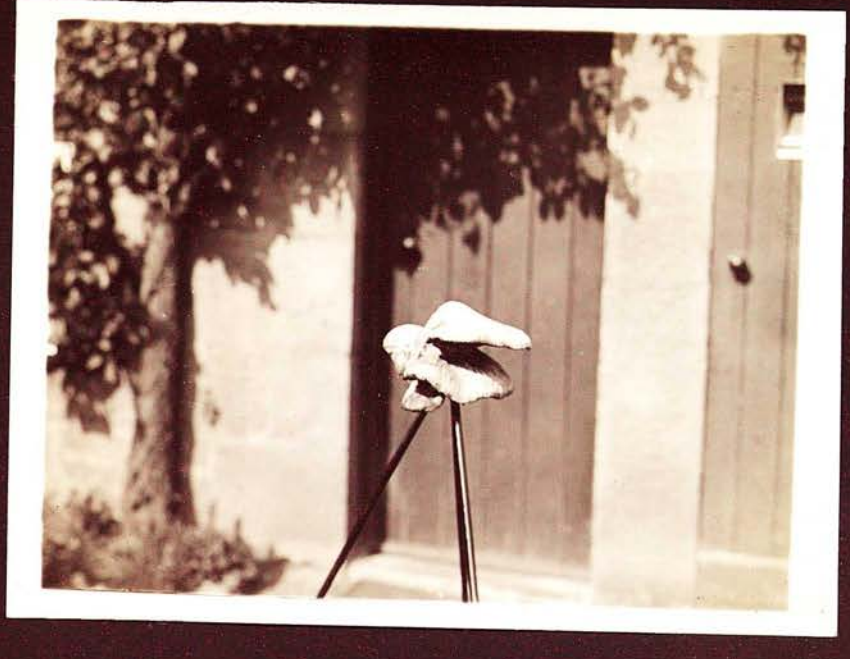
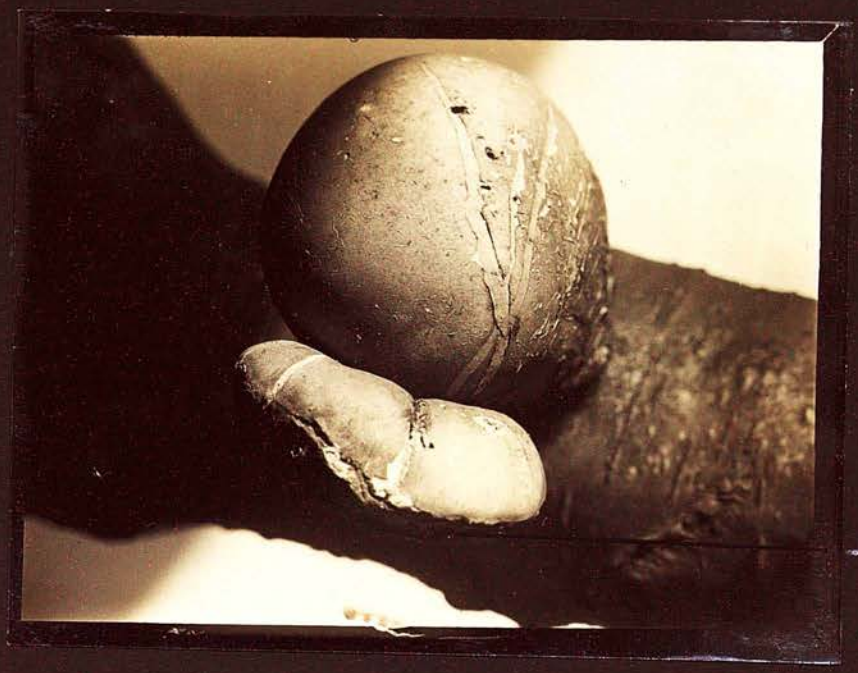


Pl. 35

Pl. 36

(a)

Pl. 37



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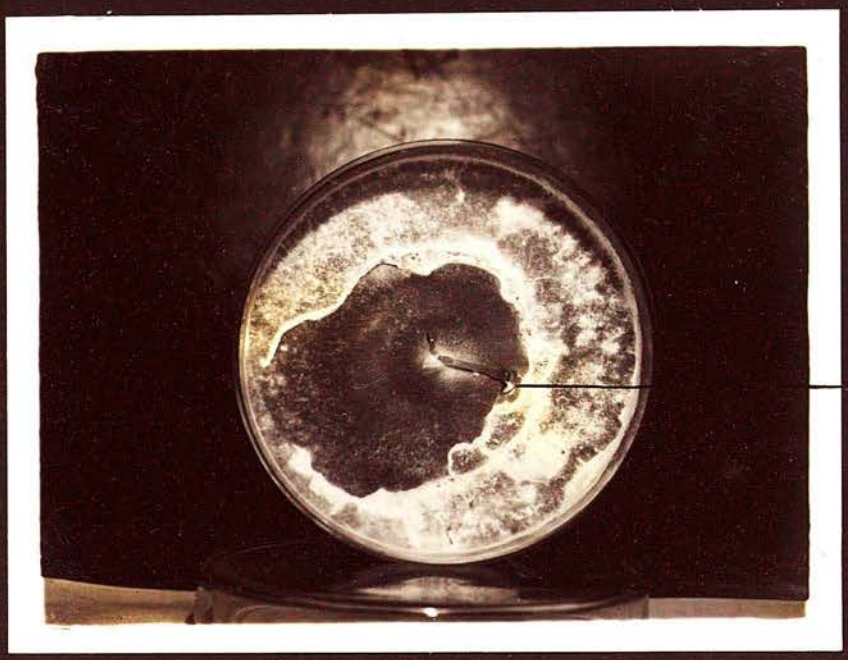
Pl. 39



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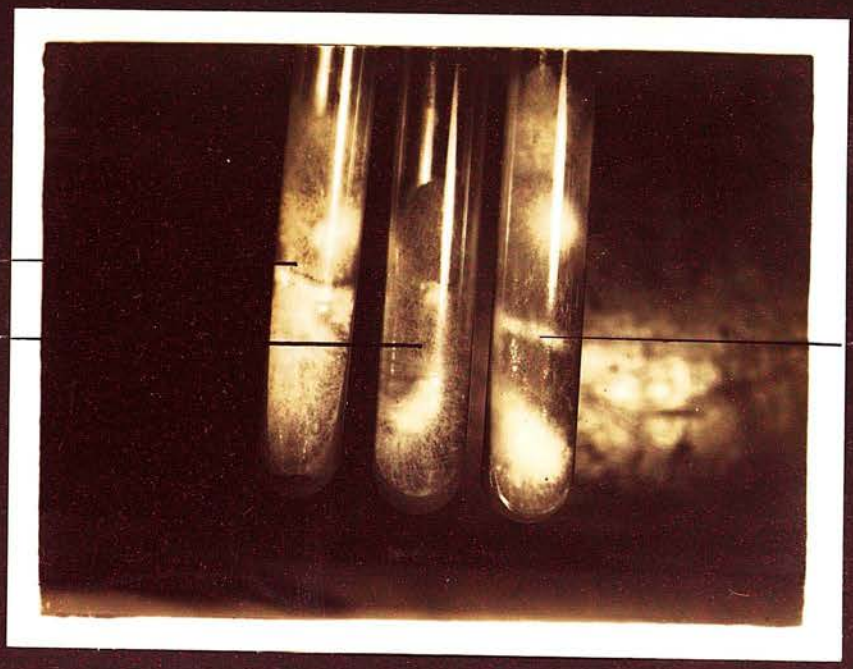

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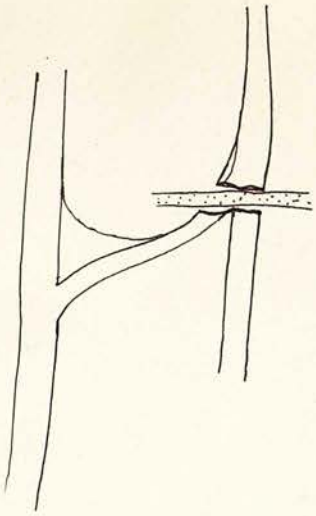
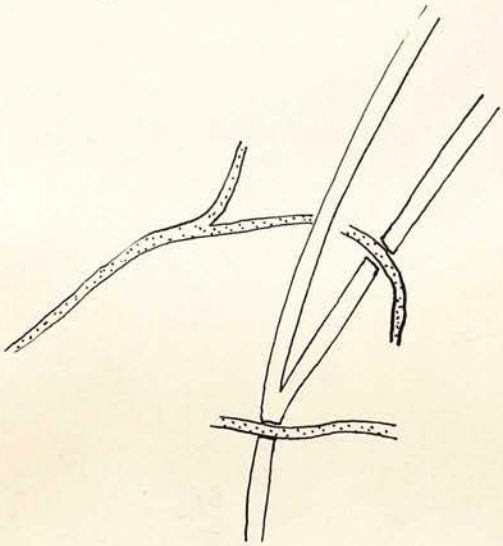
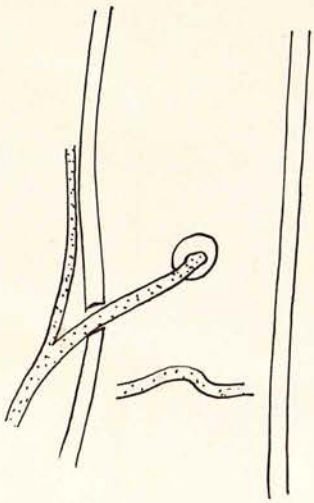






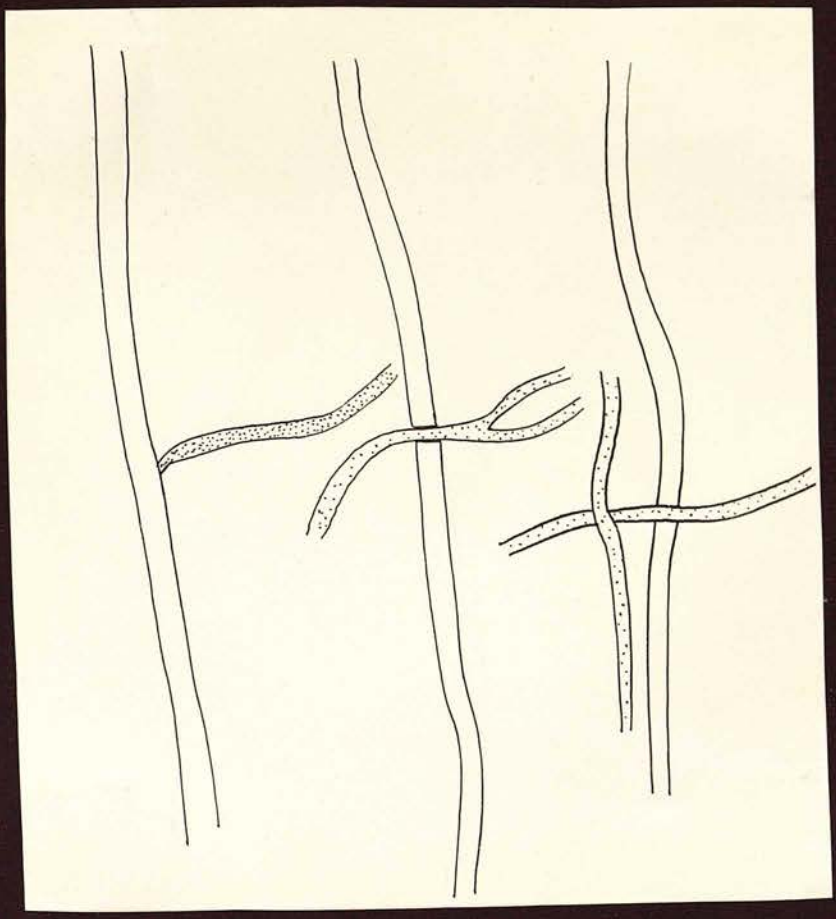
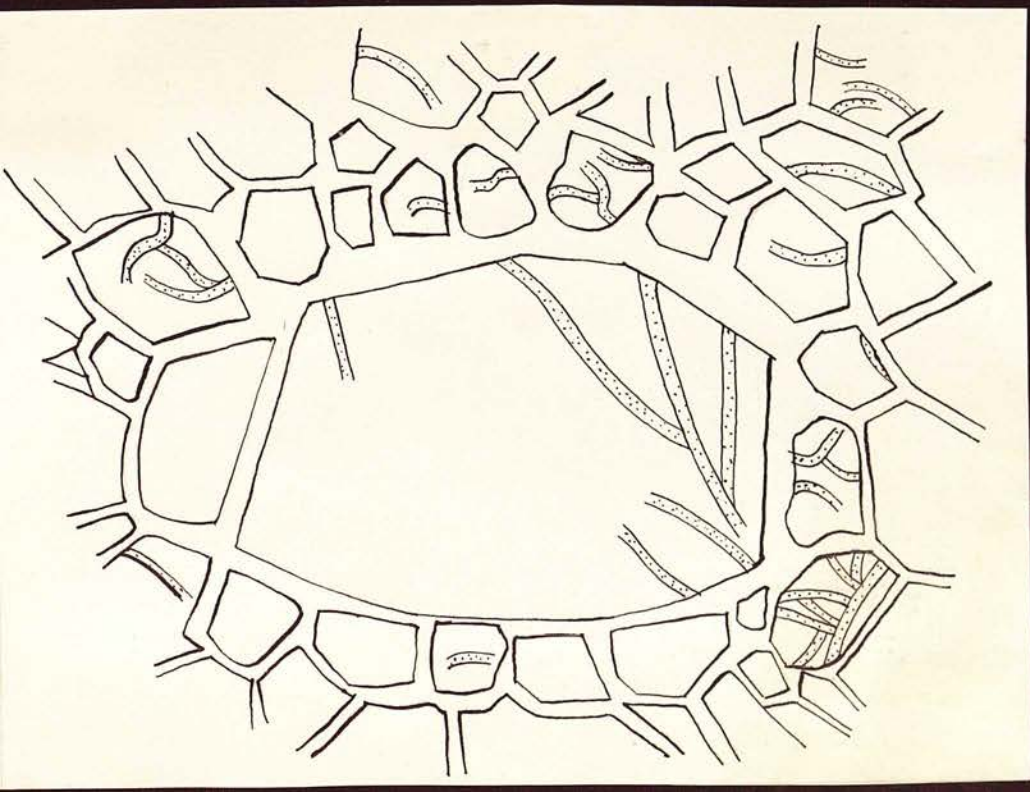
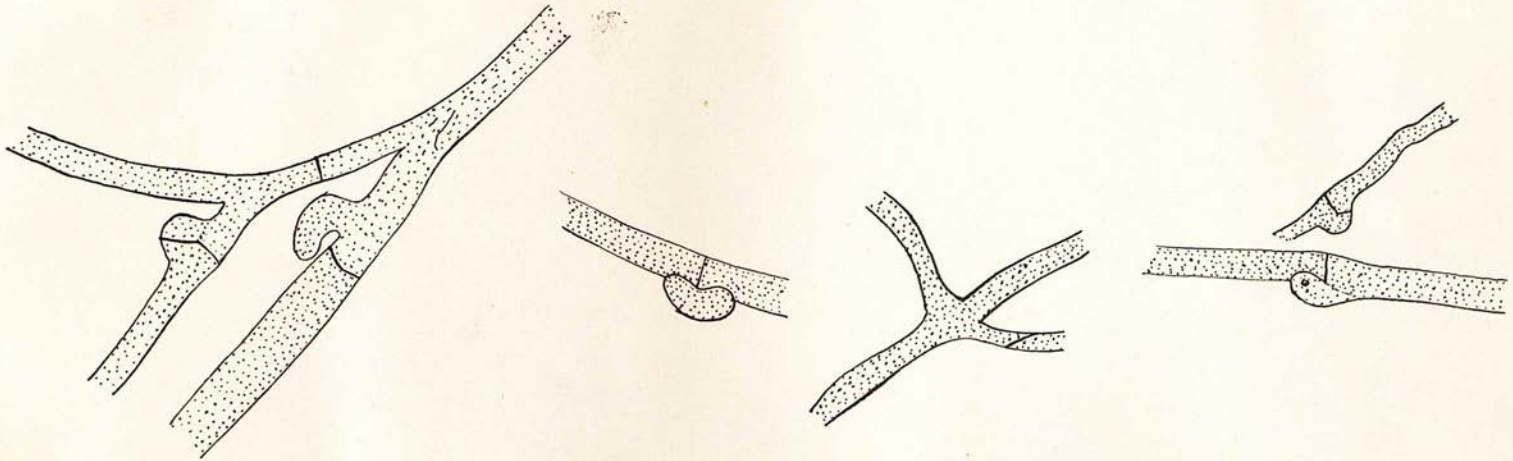
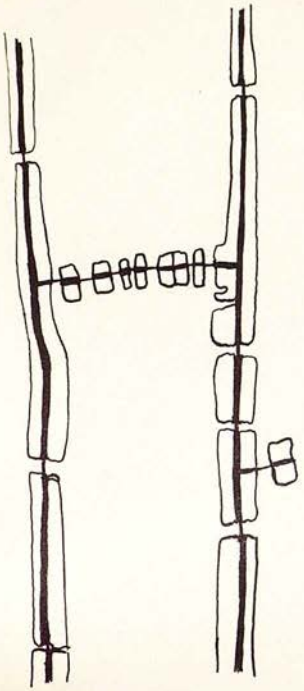


Fig. 2







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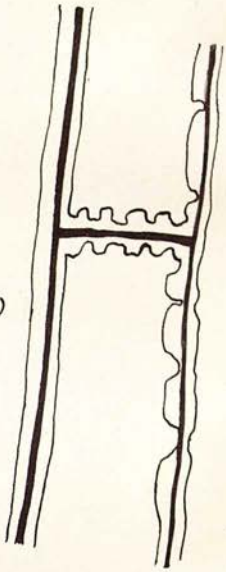

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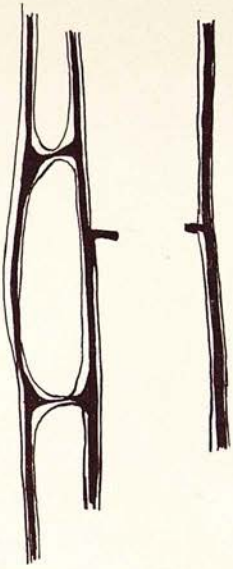

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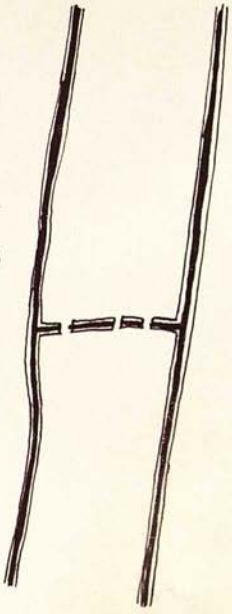

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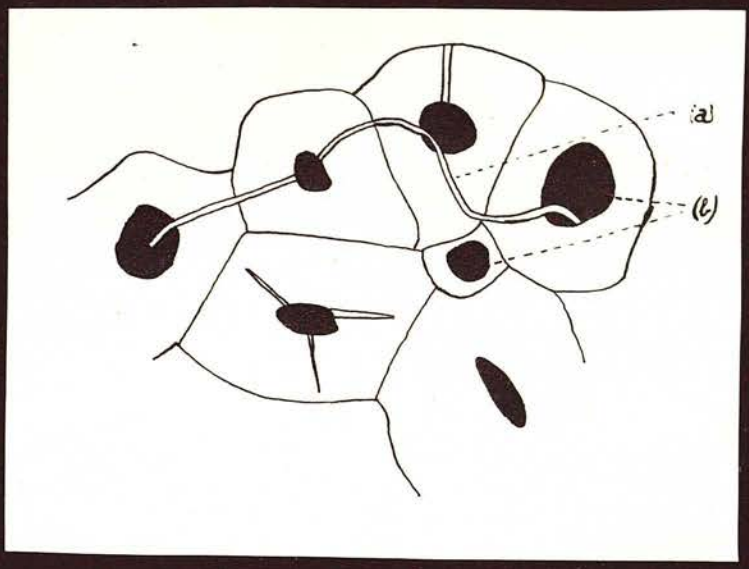
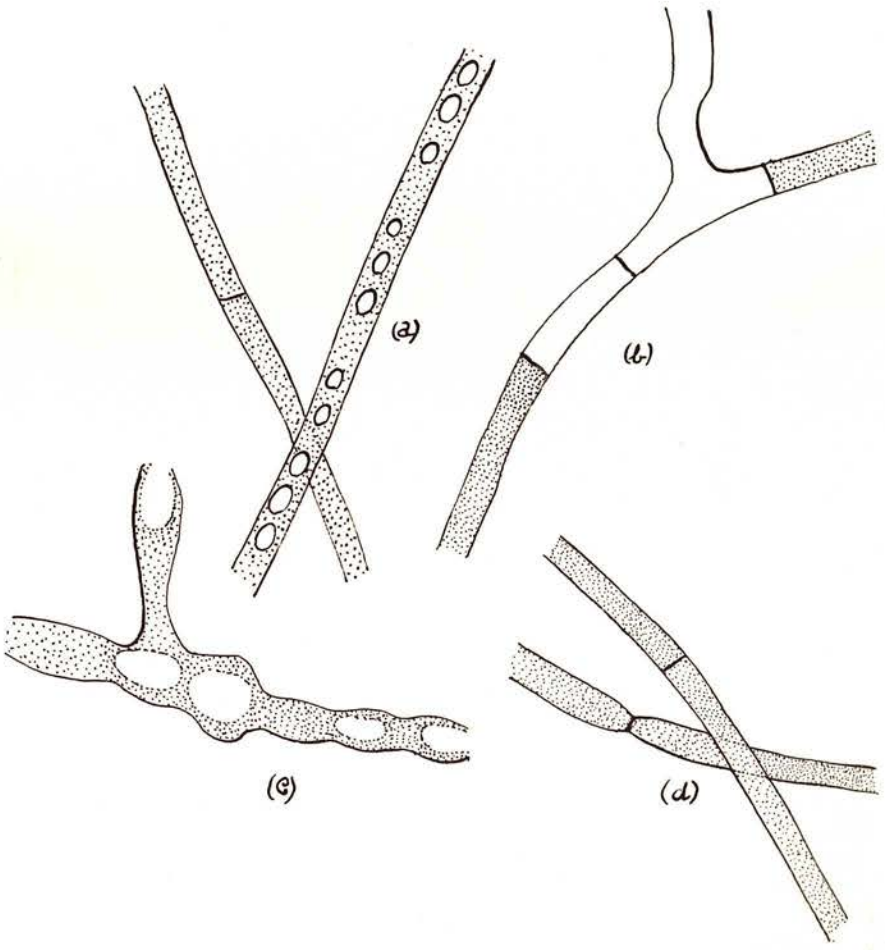


Fig. 6



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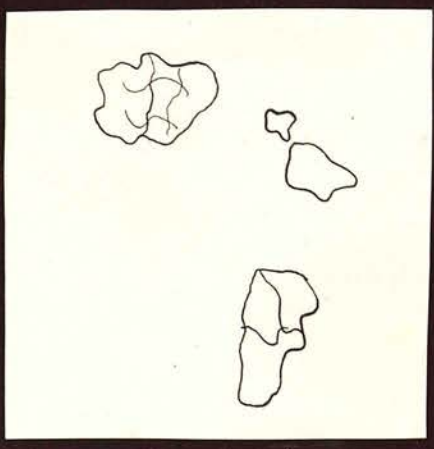

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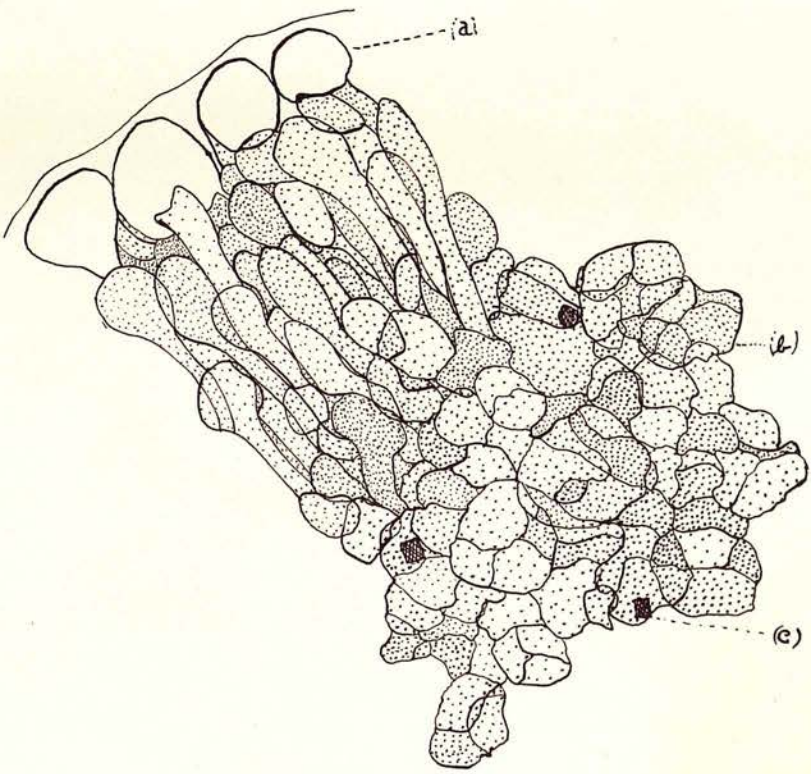

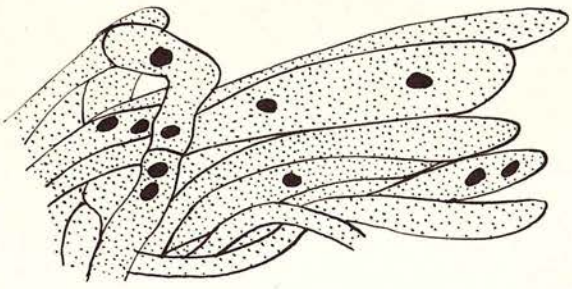

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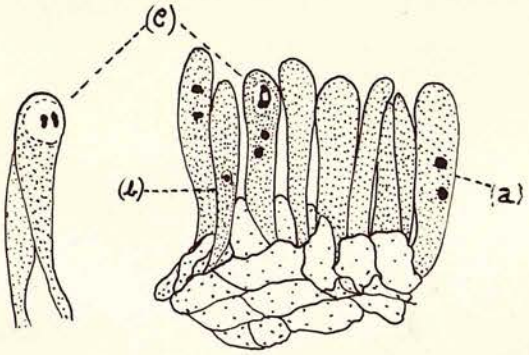

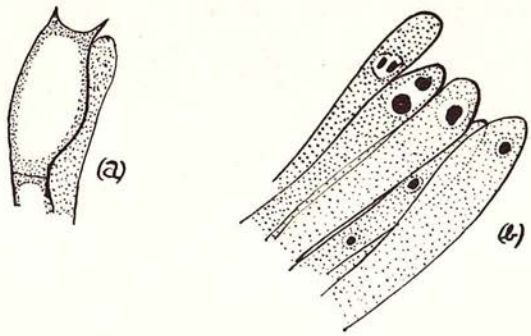

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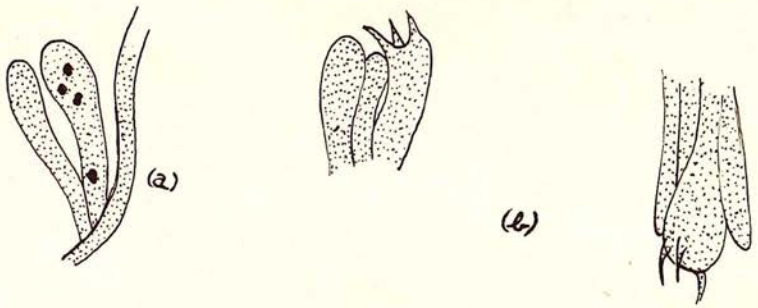

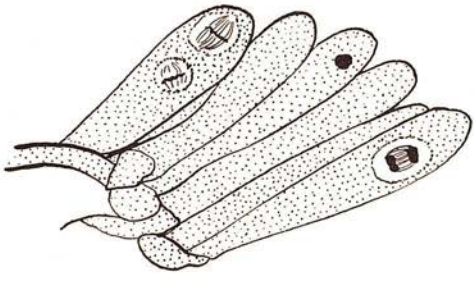

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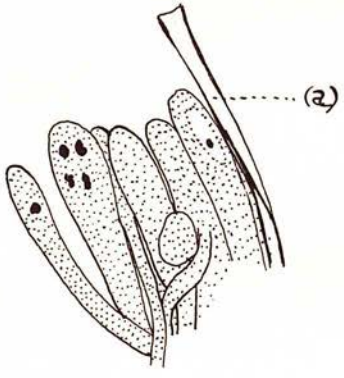


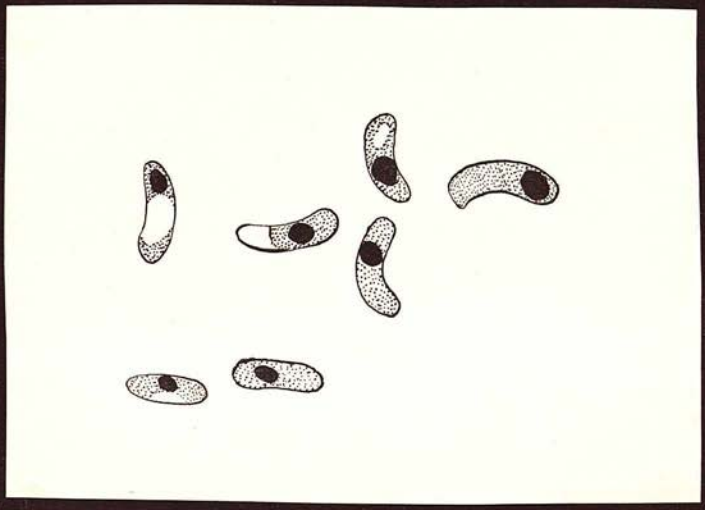
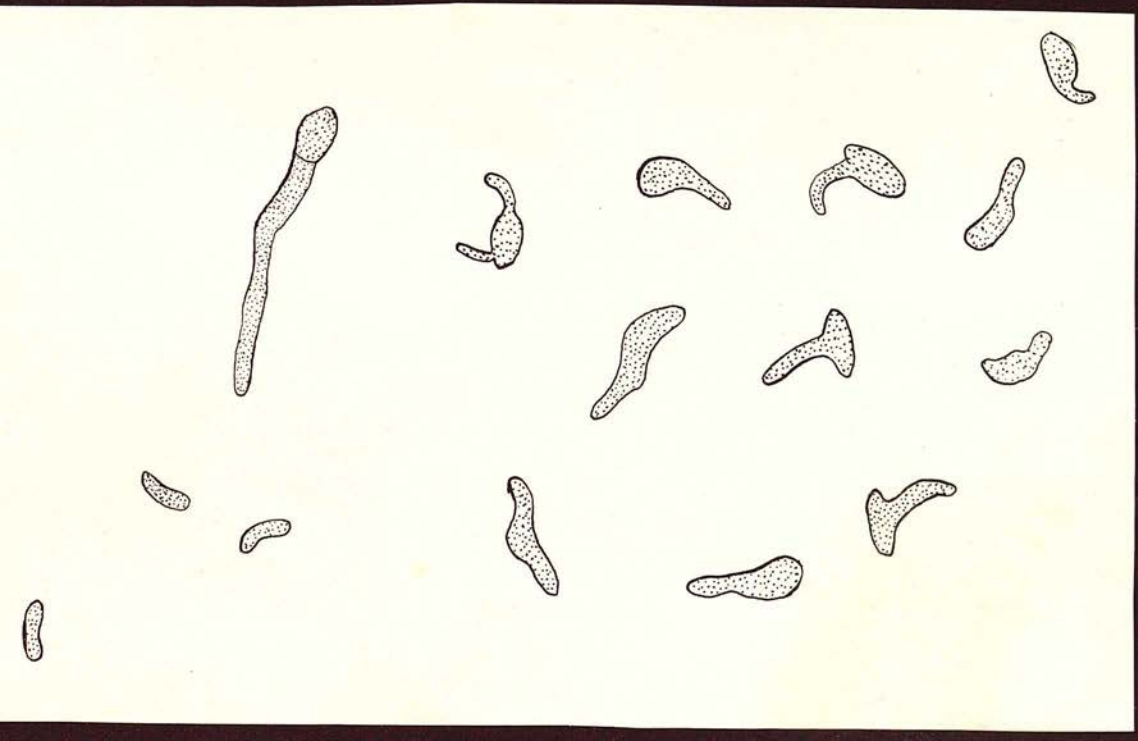


Fig. 17



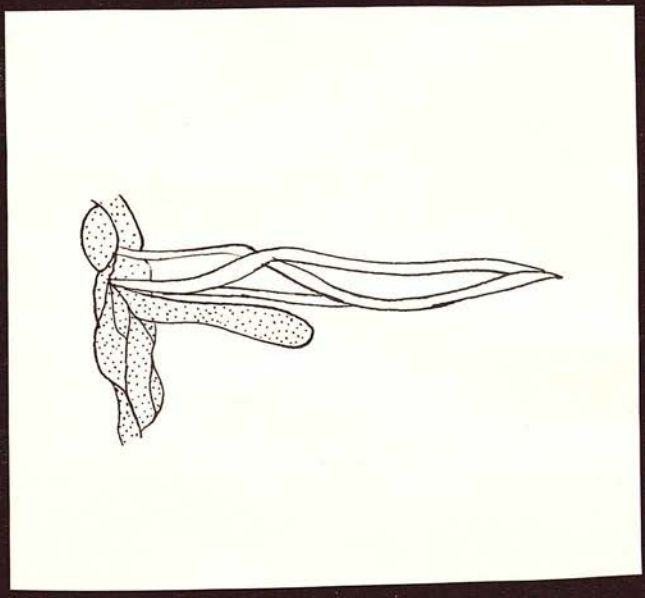
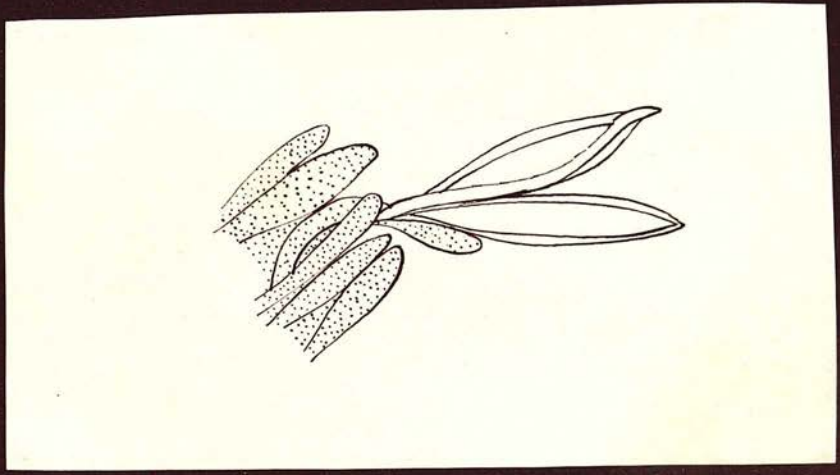
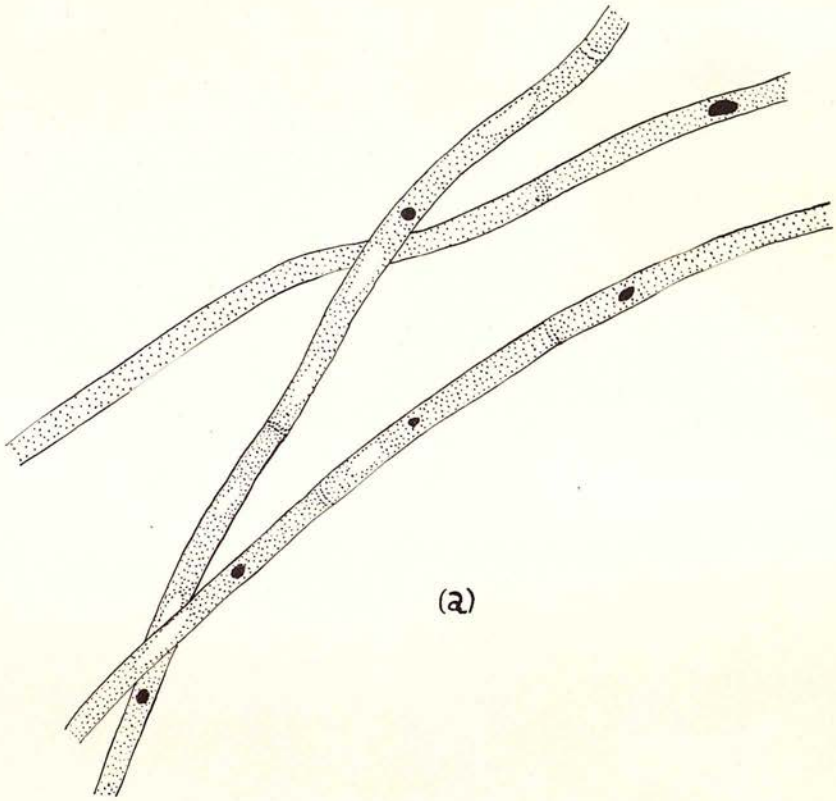


Fig. 19





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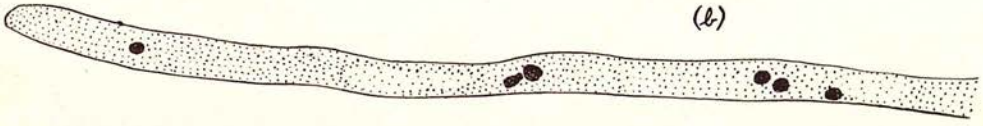

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Fig. 21

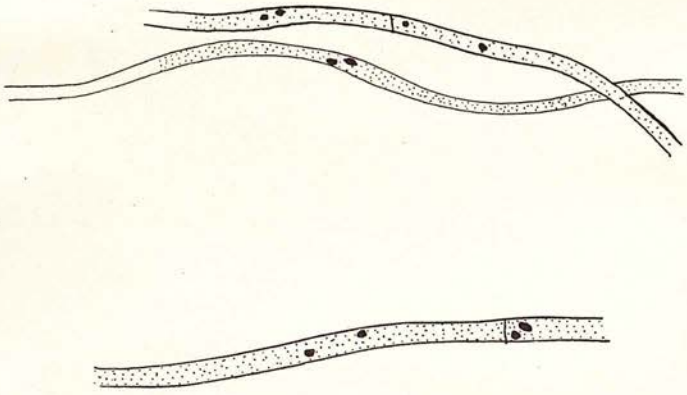
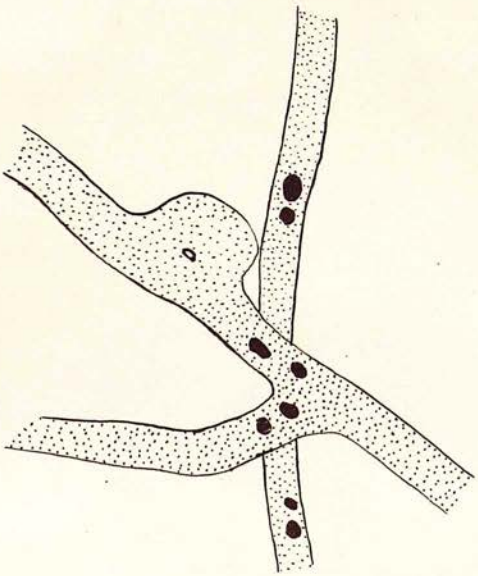
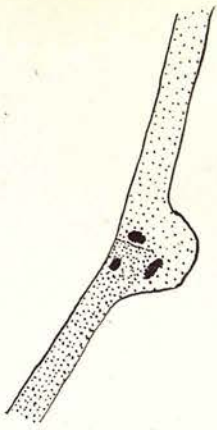


Fig. 22





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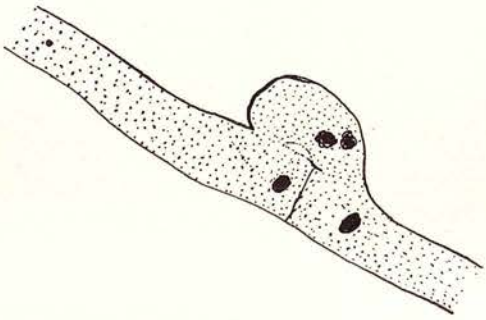

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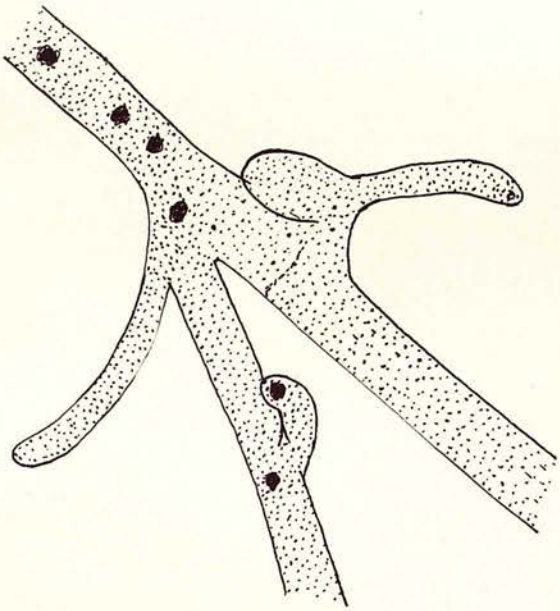

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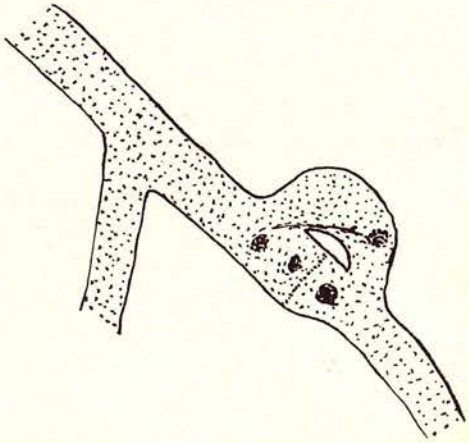

(d)

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THE LIFE HISTORY AND CULTURAL CHARACTERISTICS OF *TYPHULA GYRANS* (BATSCH) FRIES

By J. A. MACDONALD, B.Sc.

(*From the Mycology Department, University of Edinburgh.*)

(With Plates XXIX and XXX and 34 Text-figures.)

CONTENTS.

I. Introduction.

This investigation of the life history of *Typhula gyrans* was undertaken primarily with a view to determine the degree of pathogenicity of the fungus. The material which formed the basis of the work was obtained from Eastriggs, Dumfriesshire. It consisted of four sclerotia attached to a piece of swede turnip petiole. This was found in a field crop in early January. Subsequent work has shown that the fungus is in all probability only a saprophyte in this country. As this became increasingly clear more attention was paid to the cultural characteristics and to special points, *e.g.* the development of the sclerotium, and the constancy of the characters generally used in determining the genera of the Clavariaceae.

Typhula gyrans (Batsch) Fries is one of a genus placed in the Clavariaceae by most authors. The systematic position of the family differs in many classifications. It is placed in the *Agaricales* by Clements and Shear and Clements (13).

The fungus was first recorded by Bergius (4), who in 1765 observed the sclerotial stage and named it *Lycoperdon Brassicae*. Many other botanists writing towards the close of the eighteenth century have mentioned the sclerotial stage, using a wide variety of names. Batsch (3) recorded the sclerotial and sporophore stages together as *Clavaria gyrans* in 1786. Under this species he included the *Sphaeria Brassicae* of Dickson (15) which comprised ten forms including *Lycoperdon Brassicae* of Bergius. As late as 1860, however, Berkley (5) still recorded the sclerotial stage alone under the name of *Sclerotium semen*. Fries (16) was responsible for the formation of the genus *Typhula*. In 1821 (17) he described eight species including *T. gyrans*. He placed *Clavaria gyrans* Batsch, and *Cl. granulata* Willdenow (40) in this new species. In establishing the genus Fries used fungi from several genera—*Himantia*, *Chordostylum*, and *Clavaria*—and in grouping them together admitted that he was more struck by the general resemblance of their habit than by any particular characters. This same difficulty in defining the genus has been experienced by later writers. Saccardo (34*a*) and Rabenhorst (29*a*) were both struck by the easy gradation of forms from *Clavaria* through *Typhula* to *Pistillaria*. Saccardo (34*a*) gives as the distinguishing character between these two last that *Typhula* has two- or four-spored basidia, while *Pistillaria* has two-spored only. Massee (25) and others are inclined to make use of the separation of the sporophore into a definite head and a stalk—"separated from *Clavaria* and *Pistillaria* by having the stem distinct from the hymenium." Absence of branching in the sporophore is another point on which stress has been laid. Under cultural conditions *Typhula gyrans* has been found to produce sporophores which show no separation into stalk and hymenium, and others which branch freely. This would seem to indicate that the generic differences in the Clavariaceae are not very fundamental in nature.

Saccardo (35) lists sixty-four species of *Typhula*. Spore size has been determined for relatively few of these, and the criteria used in establishing the species are either those of host or habit. The range of hosts on which the species *gyrans* alone has been recorded (pp. 591–2), and the wide variety of forms in which it has appeared in culture, show the slight importance which can be attached to either of these in diagnosis. Spore size may turn out to be the only stable character in this group.

Typhula gyrans has been recorded in Germany (7*b*), Denmark (24), Norway (22), France (10), England (12), Scotland (38), and the United States of America (34*b*). Rostrup (32, 33) only has recorded it as a parasite, on cruciferous crops in Denmark. Lind (24) quotes Mortensen (26) as recording

the fungus on turnips in the pits. Withering (41) also records it on turnip roots. Dickson (15) and Withering (41) observed the fungus on the roots of parsnips. Jørstad (22) describes it on cabbage, carrot, and celery.

In all other cases there seems to have been no question of the fungus doing any damage. Most writers merely state that it is to be found among dead leaves. Bolton (6), Dickson (15), Rebentisch (30), de Candolle (11), Berkley (5), and Lind (24), however, record the fungus on dead leaves of Brassicae or more specifically of cabbage. Greville (20) and others describe the sclerotia on dead potato stalks; while still other hosts are listed by de Schweinitz (36) "in caulibus Liliorum," Massee (25), and Lind (24) dead stems of grasses, Lind (24) the pods of *Pisum sativum*, and on *Scorzonera hispanica*. It must be emphasised that in some of these cases at least sclerotia only were observed, and the possibility of error must be kept in mind.

II. Infection experiments.

In view of Rostrup's record the pathogenicity of the fungus was investigated in a series of experiments. Attempts were made to infect turnips in all stages of development with all stages of the fungus. The results of these experiments, which are given below, were entirely negative.

(1) In April 1932 swede turnips of the previous year's crop were inoculated with mycelium. Pieces were cut out of the roots and petioles, and the mycelium was inserted. The turnips remained apparently healthy. They were allowed to flower, and the seed was collected and sown out. It germinated in January 1933 giving perfectly healthy seedlings.

(2) In May 1932 attempts were made to infect seedling turnips.

A. Large pieces of mycelium were placed on the surface of the soil in pots containing turnip seedlings at the cotyledon or first-leaf stage. The pots were kept fairly moist and the mycelium spread considerably. The seedlings, however, were never visibly affected, and had formed perfectly healthy plants when last examined in late December 1932.

B. At the same time pots of seeds were set up which contained ten swede seeds each, with, in addition, twenty mature sclerotia. These latter germinated readily producing about one dozen sporophores when the seedlings were at the first-leaf stage, *i.e.* about a fortnight after sowing (Plate XXX, fig. 6). In spite of this exposure to a massed attack the plants produced all remained healthy.

(3) The first experiment was repeated using a variety of white turnip. In some cases sclerotia were inserted instead of pieces of mycelium. No infection was produced.

(4) In April 1933 another attempt was made to bring about infection, this time under more natural conditions. Two drills, each three yards long, were sown in open ground, with a mixture of swede seed and sclerotia. This experiment was duplicated at the Royal Botanic Gardens, Edinburgh, and at Peebles. One hundred sclerotia were included in each sowing. This was a particularly dry, warm period in Edinburgh, and the seeds germinated rapidly. No sporophores were observed at this time, however, or subsequently during weekly inspections. In the case of the Peebles experiment the soil was damper and cooler. The seedlings took a fortnight to reach the cotyledon stage, and by that time twelve spore-bearing sporophores had appeared. From May 9th till October 5th there were always sporophores observed on weekly examination, except for one period of one week at the beginning of July. Apparently the damper climate suited the fungus better. So far, however (May 1934), there have been no signs of sclerotia, sporophores, or mycelium on the living, or on the now abundant dead turnip tissues.

Nearby rows of cabbage and brussels sprout remained unaffected.

III. METHODS.

The preparation of the agars used for general culture work was based on the formulae given by Gwynne Vaughan and Barnes (21 *c*). The fixatives and stains employed were used as recommended by these authors.

Fixation and embedding. Undisturbed mycelium for examination was fixed in 50 per cent. spirit. In the case of sclerotia and sporophores acetic alcohol, strong and weak chromacetic mixtures, Fleming's fluid (strong), and Fleming's fluid ($\frac{1}{2}$ strong) were used. All except the last-mentioned were found to shrink the material rather drastically. This was more obvious in the case of the sporophores. Where embedding was to follow fixation, material was transferred, first of all, by the usual steps to absolute alcohol, xylol, and wax. Latterly, however, it was taken from absolute alcohol to tubes which contained alcohol resting on a layer of cedar-wood oil. After the material had sunk into the oily layer it was transferred to wax. It was frequently necessary to change the wax twice before the oil was completely removed. By this method it was found that material, particularly of sclerotia, was rendered very much less brittle.

A combination of the cedar-wood oil method with the use of wax of melting point 50° C. gave the most satisfactory results.

Staining. Mycelium was stained with erythrosin glycerine, an eosin mounting medium, safranin and light green, or iron alum haematoxylin, and mounted in glycerine jelly.

Embedded material was cut at 2μ or 5μ, usually the latter, and stained with iron alum haematoxylin, or Breinl's triple stain; cleared in cedar-wood oil, and mounted in canada balsam. Preparations of spores were obtained by smearing the fresh sporophores on slides treated with egg albumin, and staining as above.

Examination of fresh material of the fungus in all stages was carried out in mounting medium.

IV. Mycelium.

The type of mycelium produced by *Typhula gyrans* in culture varies considerably, depending on humidity, temperature, and the medium employed. Under the most favourable conditions for growth an entirely white spreading mycelium is produced. The hyphal filaments appear as fine threads radiating out from the point of inoculation (Plate XXIX, figs. 2 and 8).

To determine which medium gave the most satisfactory type of growth for general culture the following were experimented with under the same uniform conditions:

(1) Agars: malt, oat, turnip, prune, potato, soil extract.

(2) Sterilised and untreated material from petioles, leaves, and roots of swede and white turnips.

(3) Sterilised soil.

Malt agar proved to be by far the most satisfactory medium for obtaining a clean, rapid growth, and has accordingly been generally employed. A strong clean growth was always readily obtained. Sclerotia and sporophores formed freely. The hyphae were very little raised above the surface of the agar in the first-formed mycelium. The hyphae show as radiating lines spreading out from the centre of inoculation. The mycelium on the other agars varies in abundance, but is otherwise very similar. The growth produced on sterilised soil was sparse. The growth produced on sterilised turnip material was remarkable for the abnormal structures which made their appearance. These are discussed under sporophores and sclerotia. Growth produced on the unsterilised material exhibited no special feature of interest.

Soil survival. The following additional facts were ascertained relative to soil survival under laboratory conditions:

(1) Where sufficient water was provided, masses of sclerotia sown in large dishes germinated, producing both sporophores and mycelium. After seven months these sclerotia were still capable of producing fresh mycelium and sporophores.

(2) Sclerotia were frequently germinated in soil to produce sporophores for fixing and embedding. Six weeks after planting, in one case, mycelium growing from these sporophores over the soil surface produced small white sclerotia which matured in the usual way.

Judging from this evidence it seems probable that, under the more favourable conditions occurring naturally, the fungus may survive for long periods in soil which contains a reasonable amount of organic matter.

The mycelium on any of the agars shows a faint but none the less definite zonation. This takes the form of one to several rings with the point of inoculation as their centre. This phenomenon is not controlled by a light factor, or by temperature. Large numbers of agar plates were exposed to different fixed or varying temperatures; while still others were exposed to continuous light or continuous darkness, or alternating day and night. Zonation was produced in every case. Where cultures are made in tubes on agar slopes the zones appear as bars across the medium. Zonation is widespread among the fungi, and negative results, similar to those mentioned above, have been obtained by Stevens and Hall (37). Gwynne Vaughan and Barnes (21*a*) suggest that it is a chaemotropic reaction due to the reduction in growth produced by the deposit of katabolic substances in front of the advancing hyphae, followed by a renewal of active growth when a few hyphae break through the "staled" region and form a "new ring of richly branched mycelium."

As the mycelium grows older, usually from about three weeks onward, it becomes a little more raised above the surface of the medium and is woollier in appearance (Plate XXIX, fig. 2). There is no microscopical difference in the size or construction of these later-formed hyphae.

In some of the earlier cultures a dense felt of velvety brown mycelium made its appearance. This was very slow-growing (Plate XXIX, fig. 7). The mycelium was uniform in appearance, and it was not possible to distinguish individual threads without a microscope. Subcultures frequently gave rise to normal white growth. These early cultures were made either from entire sclerotia or from parts of them. It therefore

appeared possible that the colour of the hyphae might have been communicated to them in some way from the dark rind of the resting bodies. Later observations showed that ordinary white mycelium when subcultured sometimes gave rise to cultures composed entirely, or in part, of brown hyphae. This led to the checking up of the variable conditions, namely temperature and humidity. It was found that the brown mycelium made its appearance on new media only if the cultures were incubated at 20° C. or over. It appeared when old medium, which was very dry, was used, even at lower temperatures. These facts suggested that the brown mycelium was produced in response to dry conditions of growth. This was shown to be the case by growing the fungus at 25° C. in a very moist atmosphere. The normal white mycelium only was produced.

The optimum temperature for growth of the fungus in culture was also investigated. The maximum growth of mycelium was obtained at 15–17° C. The best temperature for sporophore formation was found to be slightly higher, namely 17–20° C., that for sclerotia slightly lower at 13–15° C.

During December 1933 and early January 1934 agar plates inoculated with mycelium were exposed to outside weather conditions. Though no very severe frost was experienced the average minimum temperature over the period was 0·5° C., and several degrees of frost were usually registered at night. In all cases the fungus not only remained alive, but grew a considerable amount, producing some sclerotia.

These temperature experiments show that the range within which the fungus may be expected to survive in the form of mycelium is from 25° C. to below 0° C. No doubt survival in the resistant sclerotial form is possible over a considerably greater range.

The most striking feature of the mycelium, when it is examined microscopically, is the presence on the hyphae of numerous clamp connections. These are formed in the following manner. A hooked or beaked projection appears on any part of the hypha, and, curving away from the growing point of the thread, unites with the same filament at a point further removed from the apex. The clamp connection is always associated with a cross-septum in the hypha forming it. This transverse wall is usually present before formation of the clamp begins (Text-fig. 2). In some cases it is not completed until after the protuberance has appeared (Text-fig. 1). Rarely it is only produced after the clamp connection has fused with the parent hypha (Text-fig. 4). No matter when the cross-wall is formed the clamp invariably has its origin above it, and rejoins

the hypha below it. Brefeld observed this phenomenon in the case of *Coprinus*; and caused de Bary (2*a*) to speculate whether or not this type of formation would be found to be the invariable rule.

The wall of the tip of the clamp disappears, making a temporary passage between the two hyphal cells (Text-fig. 3). Very shortly afterwards a septum appears in the clamp itself, usually in its basal portion, and in this way the passage is apparently closed (Text-fig. 5).

The cytoplasm of the vegetative hyphae is granular in character, containing particles of varying size scattered throughout. There is a tendency, in rapidly growing mycelia, towards concentration of the cytoplasm in the tips of the hyphae.

The mycelium branches freely and is many-celled. The length of the cells varies considerably. Many of the septa between hyphal cells are not associated with clamp connections (Text-fig. 15). Branches frequently, but not always, arise from cells which show clamp connections. Where the latter do occur connected with branching, the sequence of events leading to formation is as follows: A clamp connection forms in the way already described. Afterwards a bulge appears on the hypha (Text-fig. 8), or on the clamp (Text-fig. 9), or on both at the same time (Text-fig. 10). In any case the swelling is just below the cross-wall. It develops into the new branch. Sometimes another clamp appears on the base of the branch (Text-fig. 11).

When a branch has just begun to form there is sometimes difficulty in distinguishing it from a clamp in process of formation (Text-fig. 12). This led to some confusion at first. Careful observation, however, has always shown that if a protuberance appears on the side of a cross-wall further removed from the apex of a hypha it does not fuse with the hypha producing it.

The function of these clamp connections must still be regarded as not completely clear. Until recently they were assumed to provide the only means of communication between adjacent cells in a hyphal filament, and the idea expressed by many writers was that by their means nuclear migrations took place from cell to cell. The publication of the latest volume of Buller's *Researches* (9) has brought forward the view that they are of secondary importance; the primary means of communication between cells being provided by pores in the septa. These pores allow of the movement of nuclei from cell to cell, and preserve the continuity of the protoplasm. The function of the clamp connections is said to be to provide an additional pore in time of rapid cytoplasm movement, *e.g.* before sporophore formation commences. The writer has

frequently observed, in *Typhula gyrans* mycelium, septa with pores. These pores are more or less centrally placed. They have been assumed to be stages in the development of a complete septum whose formation began at the outside walls, progressing inwards till the pore was blocked, and communication between adjacent cells was prevented. Why clamp formation should begin, in some cases, before the cross-septum is complete, or even obvious, remains obscure.

Anastomoses occur between adjacent hyphae of the same mycelium. In some of these cases at least, the entire cytoplasmic content of one cell passes through the connecting tube into the cell of the other hypha (Text-fig. 14).

So far no staining method employed has made it possible to distinguish the vegetative nuclei with certainty. Particular attention has been paid to clamp connections and the tips of rapidly growing hyphae in an effort to clear up this important point.

Hyphae vary in breadth from a minimum of 3μ at the tips to a maximum of 7μ in some of the older parts. The average breadth of apical cells is 4μ, and that of cells on parts of the mycelium where growth has ceased 5μ.

The hyphae composing the brown felt of mycelium vary very little in breadth from those of the white mycelium. The cells are much shorter than those normally produced, and the walls are slightly thicker (Text-fig. 13). The cytoplasm is granular, as in the normal hyphae. The brown colour is due to an oily substance present in the cytoplasm and cell walls. This oiliness is also a feature of the rind of the sclerotium. As growth is invariably very slow—an increase in diameter of an inch in a month being the maximum observed in colonies on agar plates—it seems reasonable to regard these hyphae as forming a modified resting mycelium intermediate between the normal hyphae and the completely resting sclerotial stage. Normal hyphae are rapidly produced on the return of conditions favourable to growth. The brown mycelium would, therefore, appear to be capable of serving the same purpose as the persistent brown mycelium in *Sphaerotheca mors-uvae*, or the resistant cells in *Uncinula necator* and *Microsphaera Alni extensa* described by Appel (1) and Petri (28) respectively.

Hyphae which are massing together in the earliest stages of sclerotium formation, do not differ much in breadth from the ordinary hyphae. They are, however, very much interlaced and knotted (Text-fig. 15).

Cell contents do not appear to be any more abundant in sclerotia-forming hyphae, or in the cells of the brown mycelium, than in normal cells.

No oidia, such as are recorded by Brefeld ((7*b*), Tafel 8, Fig. 2) for *Typhula variabilis*, have been observed on mycelia of *T. gyrans*.

V. SCLEROTIUM.

The time of appearance of the sclerotia has been variously described as autumn, autumn and winter, winter, winter and spring. It has been found that sclerotia are readily formed all the year round under laboratory conditions.

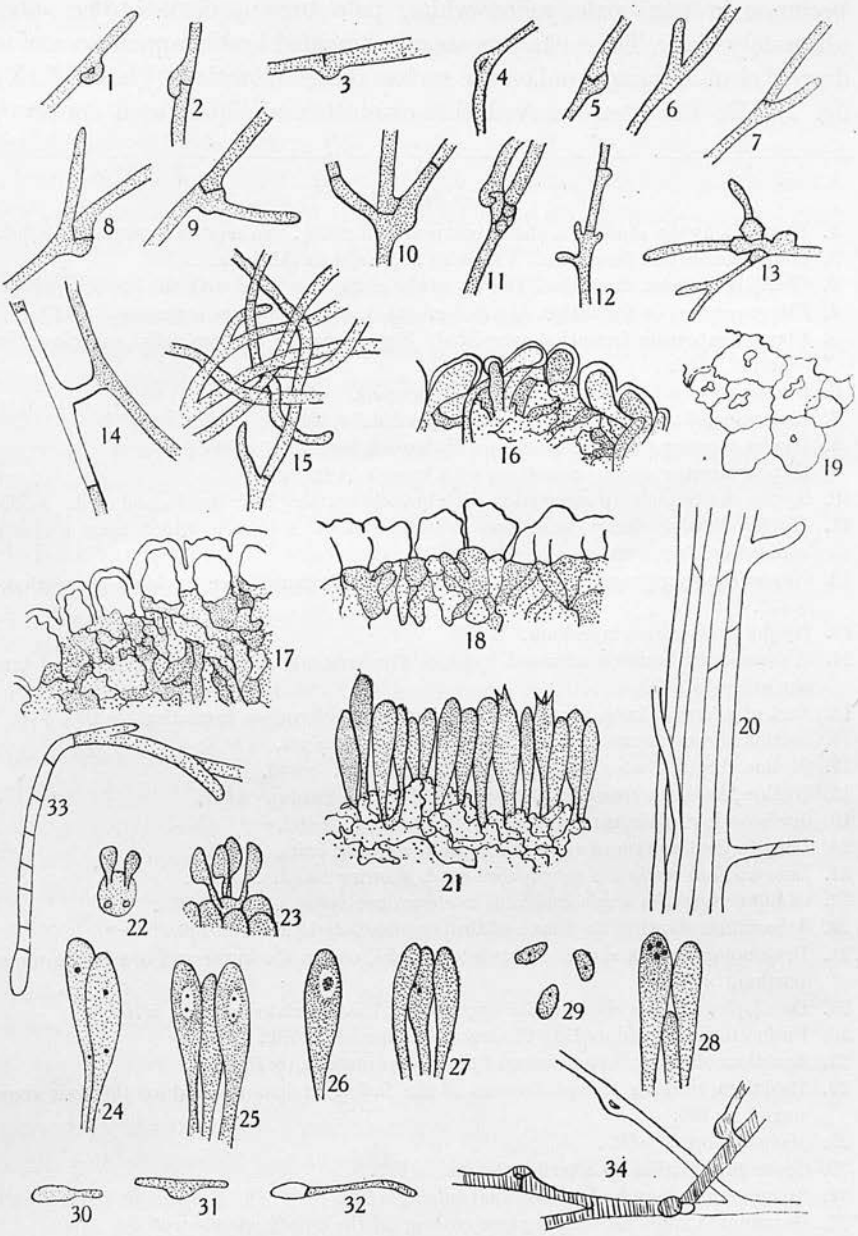
There is considerable variation in size, shape, and even colour of the sclerotia produced in culture. Checks in development are not infrequent, which result in sclerotia ceasing growth at one or other of the immature stages described below. These facts are reflected in the number of names under which the various forms went, particularly before the connection between the sporophore and sclerotial stages was established, and in many of the confusing differences in the descriptions of the earlier writers.

Mature, *i.e.* black sclerotia vary in size roughly from 1 to 3 mm. in diameter (Plate XXIX, fig. 1). Sometimes sclerotia join together to form large masses as recorded by Dickson (15); or even crusts as mentioned by de Bary (2*c*). The outline of the individuals can be made out more or less distinctly. In some cases they can be separated as complete sclerotia by very gently rubbing the mass between the fingers. In other cases they become firmly fixed together under a common rind with no separating walls. The size of these aggregate masses ranges up to 1 cm. in diameter. Complete drying reduces the size of the sclerotia by about one-third.

Single mature sclerotia are surrounded by a dark rind, which is dry and pitted. They are roughly rounded, and slightly depressed underneath. This depression marks the point of attachment of the sclerotium to the substratum.

The course of development has been traced in culture.

The first sign of sclerotium formation is the appearance of a very minute, dead white point on the surface of the mycelium (Plate XXIX, fig. 2). Brefeld (7*a*) states that the primordium of a sclerotium is a single branch of a mycelial filament which has rapidly produced a tuft of many branchlets. In this case, however, it appears to be composed of an interlaced mass of branched hyphae produced by many filaments uniting (Text-fig. 15). This denser point next assumes the form of a very small white mass resting on the substratum, to which it is attached by a number of hyphal threads. The "stalk" is too short to be measured. Increase in size takes place accompanied by changes in the colour of the rind. It



Text-figs. 1–34.

becomes in turn pale yellow-white, pale brown, dark brown, and ultimately black. These changes are accompanied by the appearance of a drop of clear-shining liquid on the surface of the sclerotium (Plate XXIX, fig. 2). De Bary(2c) observed this exudation of liquid as a constant

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Text-figs. 1–34.

1. Part of a hypha showing a clamp connection forming. No septum is present. ×595.
2. Clamp connection formation. A septum is present in this case. ×595.
3. Clamp connection formation. The tip of the clamp has fused with the hypha. ×595.
4. Clamp connection formation. As in Text-fig. 3, but no septum is present. ×480.
5. Clamp connection formation completed. Septa separate the two cells, and close the clamp. ×595.
6. Branching of a hypha. No cross-wall is present. ×595.
7. Branching of a hypha. A cross-wall is present. ×595.
8. Hypha showing clamp connection with branch beside it. ×595.
9. Hypha showing clamp connection with branch on it. ×595.
10. Hypha showing clamp connection with branches arising both beside, and on it. ×595.
11. Hypha showing clamp connection in surface view. A branch, which bears a clamp connection, is present on one side. ×595.
12. Hypha showing young branches to illustrate their resemblance to clamp connections. ×450.
13. Hypha from brown mycelium. ×450.
14. Anastomoses between adjacent hyphae. The contents of one cell have passed into the other. ×760.
15. Part of a hyphal knot, marking the beginning of sclerotium formation. ×480.
16. Section through outer part of a young white sclerotium. ×445.
17. Section through outer part of a brown sclerotium. ×445.
18. Section through outer part of a mature black sclerotium. ×480.
19. Surface view of the rind of a mature sclerotium. ×480.
20. Longitudinal section of sporophore stalk, showing hairs. ×595.
21. Longitudinal section of sporophore head, showing basidia. ×595.
22. Oblique view of a single basidium to show developing spores. ×760.
23. A basidium showing the four basidiospores and sterigmata. ×760.
24. Developing basidia showing associated nuclei, one in the lower and one in the upper portion. ×760.
25. Developing basidia showing the approach of the associated nuclei. ×760.
26. Fusion nucleus produced by the associated nuclei. ×760.
27. Basidium showing first division of the fusion nucleus. ×760.
28. Basidium showing second division of the fusion nucleus to produce the four spore nuclei. ×760.
29. Mature spores. ×595.
30. Spore germination by a terminal tube. ×650.
31. Spore germination by two terminal tubes. ×650.
32. Germinated spore showing septum cutting off the empty spore. ×650.
33. Young mycelium showing empty spore and seven empty cells. Two branches are already forming. ×595.
34. Fusion between hyphae of two three-day-old monospore cultures. Clamp connections are forming on both hyphae. ×595.

feature of sclerotium formation, and attributed it to the maturation processes going on inside these bodies. Increase in size of the sclerotium ceases when the rind is a uniform brown colour all over. The drop of liquid gradually disappears when the sclerotium has turned black. The surface is left glossy; but this glossiness gradually disappears as the sclerotium becomes older. When dry the sclerotium becomes gritty and carbonaceous in appearance (Plate XXIX, fig. 3). Bolton (6) states that the sclerotia become hollow as decay approaches; but sections through the present material at all stages of development have shown them to be composed of a limiting layer, a white medulla, and a hollow centre. The medulla has approximately the same appearance at all stages of development. The hyphae are much intertwined. Towards the outside they are densely packed, with few or no air spaces. This close packing gives way gradually towards the centre to a looser texture with numerous air spaces. The appearance of the individual hyphae under the microscope varies considerably, and is determined by the direction of the section. In general the medulla suggests a parenchymatous cell tissue. The walls of the hyphae are not conspicuously thickened. The hyphal cells contain large numbers of dark-staining granules (Text-figs. 16, 17 and 18).

The appearance of the rind changes considerably during development. Once the sclerotium has assumed its spherical form a limiting layer is already present. Certain branches of the hyphae composing the hyphal knot grow in such a way that their ends appear free on the outside of the mass. The free ends of these hyphae are swollen in the same manner as the terminal cells which form the basidia on the sporophores, although they are shorter (cf. Text-fig. 21). They form a practically continuous layer (Text-fig. 16). Thickening of this layer takes place early. The outer walls in particular increase in thickness; but the lateral and inner walls are also strengthened. In this way a strong wall without interstices is produced. The "cells" composing it lose their contents and deepen in colour to a very dark brown (Text-fig. 17). The rounded ends of the hyphae are responsible for the rough appearance of the outer surface. The extreme tips of the "cells" are, in many cases, less thickened than the rest of the sloping outer walls. This makes the rind appear to be composed of several layers, and accounts for the roughly concentric rings seen in surface view (Text-fig. 19). The sclerotium increases in size for some time after the outer layer is complete. This is probably sufficiently allowed for by the expansion of the rind produced due to the thickening of the lateral walls. If such were not sufficient it would be an easy matter for new hyphal branches to grow out into any part where

the rind cells had been forced apart by the pressure of the medullary hyphae. Sclerotia examined by means of a binocular microscope have always shown a complete surface.

This sclerotium development has been described by Brefeld (7 *a*) and discussed by de Bary (2 *b*) in considerable detail.

In the mature stage the rind is easily cracked, the "cells" splitting away from each other (Text-fig. 18). When the sclerotium germinates it is through one of these cracks that the mass of hyphae pass to the outside. The rind does not take any part in this renewed growth. Some few threads turn downwards into the soil, giving the root-like appearance figured by Bolton (6). The rest of the mass grows upwards to form the young sporophore.

The time taken for the development of the sclerotium, from its first appearance to the mature stage at which the drop of liquid disappears, varies from nine or ten days upwards.

De Bary (2 *d*) states that mature sclerotia pass into a resting stage the duration of which varies with the species, and, in the individual, with internal and external causes. He gives the period of rest for the sclerotia of *Typhula gyrans* as summer. Sclerotia in culture have been germinated immediately on reaching maturity, or at any time up to twelve months afterwards (the longest period tried). Sclerotia sown outside germinated readily during summer, as has been noted under Infection Experiments. Individual sclerotia, however, showed wide variation. Germination has been secured over a period ranging from eight days to five months.

De Bary (2 *e*) also states that the sclerotia of Typhulae, on account of the differentiation of their tissues, do not appear to be able to regenerate rind, or to produce mycelium from rind cells. This is the case for *T. gyrans*. In some cases a drop of liquid was produced on the cut surface of the sclerotium. The only function of the rind, therefore, appears to be protection.

Attention has been drawn already to the zonation of the mycelium. Formation of sclerotia occurs singly or in groups on the hyphae (Plate XXIX, fig. 1). The sclerotia lie on the circumference of one or more rough circles centring at the point of inoculation of the petri dish (Plate XXIX, fig. 1), or in rows on the slopes (Plate XXX, fig. 7). The completeness and number of the circles or rows varies between cultures; but there is obviously a correspondence between the zones on the mycelium and the place of appearance of the sclerotia.

Hyphae frequently grow over the surface of the glass of the culture vessels. Sclerotia occasionally form on the glass.

VI. Sporophores.

The term sporophores is used to include all outgrowths from the mycelium of the fungus, other than sclerotia, whether they actually produce spores or not. The reasons for so doing are given later.

The time of appearance of sporophores in nature is given by most authorities as in autumn or in October, and, in one case, May. Normally sporophores arise from sclerotia. Very frequently in agar cultures structures identical with those produced by sclerotia in the laboratory arise directly from the surface of the mycelium without any kind of sclerotial structure at the base (Plate XXIX, figs. 6 and 7). Swartz (39), Fries (18) and others have noted that sporophores sometimes arise in nature without sclerotia being present.

All sporophores appear as rapidly growing masses of hyphae. They grow vertically upwards, and attain their full size in from one to four days normally. The typical sporophore is a simple unbranched club separable into definite stalk and head portions (Plate XXIX, fig. 5; Plate XXX, fig. 6). The stalk is greyish white and covered by short hairs of varying abundance, which give it a rough appearance to the naked eye. It is clearly marked off from the head by its colour and often by its diameter. The head is completely smooth and dead waxy white in colour. It tapers in some cases to a fairly fine point, in others the apex is blunt. When mature the entire head is covered with spores—the pollen of Batsch (3). The head and stalk are separated as if by a line drawn round the sporophore. The size of the head varies roughly from one-quarter to one-half the total length of the sporophore. Under outside conditions the largest sporophore produced was $2\frac{1}{2}$ cm. from the sclerotium to the apex. The head was 1 cm. long. In pots in the laboratory sporophores reached sizes up to 5 cm., and none were less than 2 cm. No matter from what type of sporophore they are produced spores always vary in size within the same limits.

All sporophores are very susceptible to drying and, when they are exposed to the air of the laboratory, exhibit the curving movements which caused Batsch ((3), f. 164) to give the fungus its specific name. Less than 5 minutes' exposure is sufficient to shrivel them up completely. This process always follows approximately the same steps. The head begins to droop sideways on the stalk. This movement is accompanied by a pronounced lateral corkscrew twisting of the neck portion, until the head is hanging upside down on the end of the wilting stalk. Finally the whole sporophore presents a brownish, shrivelled appearance, and

collapses on to the surface of the soil (Plate XXX, figs. 1 and 2). Many mature sporophores show a quite pronounced corkscrew marking on the upper part of the stalk when in a fresh condition. This has been observed and figured by Bolton (6).

The external differentiation into a head and stalk is reflected in the structure observed in microscopic sections. The stalk consists of a number of hyphae, closely packed together, running mainly vertically, but with branches passing among the parallel strands and holding them together (Text-fig. 20). Some of those towards the outside of the bundle have their ends running almost horizontally. These are free from the stalk and form the hairs (Text-fig. 20). The stalk cells are without obvious nuclei, and practically without contents. The length of the cells is variable.

In the head the hymenium completely covers the outside. It is formed by the ends of the hyphae turning outwards, vertical to the surface, and forming a continuous layer of swollen, round-ended cells. These are the young basidia (Text-fig. 21). Apparently every terminal cell is a potential basidium. According to Brefeld (7*a*) the basidium is capable of producing more than one crop of spores. No evidence of this has been forthcoming during the present work. The basidia do not all ripen at the same time, and the life of the average sporophore is so short that a renewal process seems unnecessary.

There do not appear to be any cistidia.

Both the stalk and the head are hollow. This makes a pronounced resemblance between sporophore and sclerotium in section. The fundamental plan of construction seems to be the same in both.

Various nuclear conditions were observed; and the maturation processes in the basidium probably take place by the following steps. When the swollen ends of the hyphae in the head have formed the young basidia they apparently contain two nuclei. One of these is in the lower, and one in the upper part of the structure (Text-figs. 24 and 25). These approach and fuse, usually in the upper part (Text-fig. 26). The single nucleus thus formed divides twice giving four nuclei (Text-figs. 27 and 28). Owing to the small size of the nuclei it has not been possible to distinguish chromosomes; but it will be shown later that a reduction to the haploid number probably takes place at one of these divisions. About this stage of development four sharp points appear at the upper end of the basidium. They form the corners of a rough square. They grow in length, forming stalks, and a swelling is produced at the tip of each (Text-fig. 22). These latter are the young spores. One nucleus passes nto each. It has been found impossible to keep spores attached to the

basidia during fixation and embedding. For this reason it is very difficult to say when exactly the nuclei pass in. Basidia bearing fully formed sterigmata have not been observed to contain nuclei (Text-fig. 21). It seems probable then that the nuclei pass into the developing spores. The stalks vary in length, being often as long as the spores themselves.

The average spore measurement calculated from fifty spores was $5{\cdot}6 \times 4{\cdot}4\,\mu$. These figures do not correspond with the size given by Patouillard (27)—$5–6 \times 2\,\mu$. They more nearly approach Massee's figures (25) of $5 \times 6\,\mu$. The present measurements are for fresh spores in mounting medium. Fixed and stained specimens gave measurements much closer to those found by Patouillard. The spores are longer than broad. They are usually rather boat-shaped, tending to be flattened on their inner and rounded on their outer sides (Text-fig. 29). They germinate readily in weak sugar solutions, or on malt agar. A germ tube is produced from either end, or from both ends at once (Text-figs. 30 and 31). The hyphae produced quickly become septate and branch readily. The cytoplasm tends to aggregate in the tips of the hyphae, with the result that the spore (Text-fig. 32), and a number of the cells nearest to it, are left without contents, often within twenty-four hours of germination (Text-fig. 33).

The definite separation of the sporophore into a head and stalk has broken down in culture. Under laboratory conditions long sporophore-like outgrowths are produced from the surface of the mycelium (Plate XXX, fig. 3). These attain lengths up to 12 cm. They are produced in circles or rows in the same way as the sclerotia (Plate XXIX, fig. 6). When young they resemble normal sporophores in colour and shape; but as they grow older they assume diverse forms varying considerably in diameter, and frequently showing a rope-like structure. Some authors have apparently doubted that these structures are true sporophores. Greville (20) describes a similar condition in *Phacorhiza filiformis* (*Typhula Phacorhiza* Fries). He mentions "abortive plants—frequently reaching 5–6 in.—often branched—in diameter scarcely exceeding a human hair. This change appears to be analogous to that in *Agaricus androsaceus*—and which has been described as a *Rhizomorpha*." In a recent paper on *Typhula graminum*, Remsberg and Hungerford (31) have recorded similar structures, regarding them apparently as in no way connected with the sporophore stage of that fungus, and calling them rhizomorphs. Comparison of photographs leaves no doubt that these structures are produced in the same way as those found in *T. gyrans*. Tubes containing these

elongated sporophores were placed in a damp, cool greenhouse. After a month they were completely covered by a white waxy bloom. On examination this proved to be composed of masses of basidiospores, produced under the more natural conditions. These corresponded in measurements with spores produced on typical sporophores, and on culturing gave rise to the usual mycelium.

The simple character of the sporophore is not a constant feature either. Short branches have been observed on the sporophores when growing out of doors. These give a stag's-horn-like appearance to the sporophores. In the case of sporophores growing under glass in pots this tendency to branch is more pronounced. The spurs often attain some size, and may even branch again. Side branches only appear when growth has ceased in the original branch. Occasionally a simple sporophore, which has ceased growth and has produced its spores, will start growing again, producing a fresh crop on the new hymenium. Side branches, too, bear the usual large numbers of spores. Branching takes place much more freely on culture media. In extreme cases as many as seven main branches have been counted on a single fructification, together with numerous tertiary spurs. A sporophore typical of this kind is illustrated (Plate XXX, fig. 8). These culture sporophores are hairy all over. When branched they have a stag's-horn or seaweed-like appearance, according to the extent of the branching. The tips are composed, as in young normal sporophores, of a large number of ends of hyphae curved over to form the rounded growing point. In some cases these terminal hyphae have been observed to feather out on coming in contact with the surface of the culture medium, or the glass of the containing vessel. This was also seen sometimes in pot cultures of sporophores. After spore production had ceased and the sporophore had bent over towards the soil, fine threads of mycelium grew out from the surface of the hymenium and stalk. Possibly by this means in nature the fungus can increase its chance of survival.

From these facts it appears that it is a comparatively easy matter for the fungus to pass from the vegetative to the reproductive stage and back again without the intervention of the resting sclerotia.

VII. ABNORMAL STRUCTURES.

Various curious intermediate stages between sporophore and sclerotium have been observed.

Many culture sporophores, which do not arise from definite sclerotia, are early observed to have slight white swellings at their bases. These

develop a darker colour as the sporophore gets older. Although they have never been observed to become black they are obviously modified sclerotial forms.

Certain cultures on turnip slices in tubes produced short, thick, white sporophores. These developed round swollen tops, which became brown in colour (Plate XXIX, fig. 3). In another case in which sporophores were developing, black tops appeared at their apices, above slight constrictions (Plate XXIX, fig. 4). Microscopic examination showed both the above abnormalities to have a sclerotium-like structure.

On some turnip agar plates thin sporophores of varying length developed. After a time these began to straggle over the surface of the agar. There some of them developed mature sclerotia at their tips (Plate XXX, fig. 4). In one case the "stalk" was over $\frac{3}{4}$ in. long.

These facts show that there is no essential difference between the hyphae going to form a sclerotium and those composing a sporophore. Under the uniform conditions of the laboratory the normal sequence of sclerotium—rest—sporophore is upset, and sclerotia, or sporophores directly, arise in the same culture, together with all intermediate stages between the two.

General observation of cultures has shown the sporophores to be negatively geotropic, and positively heliotropic. Their region of growth is apical.

VIII. Monospore cultures and heterothallism.

(1) Attempts to obtain monospore cultures were made first of all by rubbing an inoculating needle gently against a sporophore, and then drawing the needle across an agar plate. After twenty-four hours the young colonies were cut out and subcultured. Ten days later the subcultures were examined microscopically. Some contained clamp connections while others did not. Within three weeks all those which had shown clamps produced sporophores or sclerotia. Those without clamps did not. The results are tabulated on the opposite page.

From the cultures without clamp connections four agar plates were inoculated. Two different mycelia were placed on each, a little distance apart. After twenty days the mycelia mixed in one case. Sporophores were present, and the culture contained clamp connections. In the three other cases the mycelia approached but did not mix. No sporophores or clamp connections were present. These three cultures were kept under observation for three months. No change took place.

Date of subculturing 27. ix. 33.

	Date of examination								
	7. x. 33			21. x. 33			20. xi. 33		
Culture No.	Sp.	Sc.	CC.	Sp.	Sc.	CC.	Sp.	Sc.	CC.
A 1	–	–	–	–	–	–	–	–	–
A 2	–	–	×	×	×	×	×	×	×
B 1	–	–	–	–	–	–	–	–	–
B 2	–	–	–	–	–	–	–	–	–
B 3	–	–	–	–	–	–	–	–	–
B 4	–	–	–	–	–	–	–	–	–
C 1	–	–	–	–	–	–	–	–	–
C 2	–	–	×	×	–	×	×	–	×
C 3	–	–	×	×	–	×	×	–	×
C 4	–	–	–	–	–	–	–	–	–

Sp. = sporophores. CC. = clamp connections.
Sc. = sclerotia. – = absent, × = present.

The method used to obtain monospore cultures was obviously unsatisfactory; but a number of interesting points stood out. All the cultures did not behave in the same way. Excluding those "monospore" cultures which produced clamps, etc. (these have never appeared when such cultures were made by any recognised method), some pairs of monospore mycelia mixed and fused readily with each other, while in other cases apparently no mixing, and certainly no fusions, took place. The cultures in which the mycelia showed fusions produced clamp connections and, subsequently, sporophores or sclerotia or both. This strongly suggested that the two spores concerned were complementary to each other, while those the mycelia of which would not mix were of the same kind and in some way antagonistic to each other. Such a heterothallic condition with like and unlike monospore mycelia is present among the Rusts and Basidiomycetes generally.

(2) Monospore cultures were then obtained by the dilution method (Gwynne Vaughan and Barnes(21 *b*)). They always produced the pure white mycelium typical of *T. gyrans*; but, although they were examined at intervals over a period of three months, they failed to show clamp connections, sclerotia, or sporophores (Plate XXIX, fig. 8).

(3) Another set of monospore cultures was prepared in the same way at the same time. They were subcultured on eight agar plates in pairs, in the manner already described in Exp. 1. The original cultures were preserved. On two other plates pairs of mycelia were mixed on inoculation. After three weeks three out of the eight plates showed sporophores (Plate XXX, fig. 5). In these the mycelia showed fusions, and clamp connections were present. Sclerotia were subsequently produced. In the other five no fusions had taken place. There were no clamp con-

nections, sclerotia, or sporophores present. These were not produced subsequently (Plate XXX, fig. 9). One of the two plates on which the mycelia had been mixed at inoculation produced sclerotia. It contained clamp connections (Plate XXIX, fig. 2). The other showed mycelium only. There were no clamps present. None of the original cultures contained sclerotia, sporophores, or clamp connections. These latter, along with all cultures which gave negative results, were kept under observation for a period of five months. No change occurred in any of them.

A drawing of a fusion between two monospore colonies grown on filtered agar is included (Text-fig. 34). The colonies were three days old.

These anastomoses were not as a rule accompanied by the passage of the contents of one of the cells into the other. Obviously, however, some nuclear change must take place to affect the resulting mycelia so profoundly.

A similar union of monospore strains has been described by Lehfeld (23) for *T. erythropus*. He was of the opinion that the nucleus from one of the uniting cells passed into the other filament and there divided; one daughter nucleus associated with the nucleus already present to form a binucleate cell, while the other passed along the filament, rapidly dividing and bringing about the binucleate condition in every cell. The appearance of clamp connections was associated with these fusions. Buller (8) at first accepted Lehfeld's interpretation and applied it to those phenomena observed in certain other Basidiomycetes (*Coprinus lagopus*, etc.). This theory does account for the rapid appearance of clamp connections on the mycelia at points distant from the original union. The view expressed by Colson (14) in the case of a heterothallic strain of *Neurospora tetrasperma* seems just as applicable, however. The diploidisation of haploid mycelia in *Neurospora* is attributed to the rapid mixing of the hyphae of the two monospore strains. It is suggested that numerous fusions take place throughout the culture, and are all accompanied by the transference of nuclei.

It is reasonable to suppose that in *Typhula gyrans* the nuclei remain associated in pairs till the basidia have formed, when a pair passes into each. Their probable fusion there, with subsequent division to form four haploid spore nuclei, has been suggested in the part of this paper dealing with the sporophores.

IX. SUMMARY.

1. A description is given of the life history and cultural characteristics of *Typhula gyrans* (Batsch) Fries.

2. The parasitism of the fungus was investigated. Infection experiments yielded entirely negative results.

3. The fungus forms resting sclerotia. The mycelium is either pure white and rapidly growing or, under adverse conditions, dark brown and slow-growing. The sporophore consists typically of a stalk and club-shaped head. The hymenium is borne all over the surface of the head. The sporophore usually arises from a sclerotium.

4. The mycelium contains numerous clamp connections. Their function is doubtful.

5. The development of the sclerotium is traced with special reference to the limiting layer.

6. The sequence of nuclear changes in the basidium is suggested.

7. Various abnormal forms, intermediate in construction between sclerotium and sporophore, are described. It is suggested that a consideration of these, together with the resemblance in structure between normal sporophore and normal sclerotium, proves these latter to be fundamentally the same. The proportion of each formed at any time is governed by external conditions.

8. The fungus is heterothallic. Monospore strains fail to produce clamp connections, sclerotia, or sporophores. When monospore cultures are grown in pairs, in some cases mixing and fusion of the hyphae take place, with subsequent production of clamps, sclerotia, and sporophores; in others no fusions take place.

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EXPLANATION OF PLATES XXIX AND XXX.

PLATE XXIX.

Fig. 1. Culture showing two rings of sclerotia, which correspond with zones on the mycelium. Straggling sporophores are present in the middle of the plate. $\times \frac{2}{3}$.
Fig. 2. Two monospore strains mixed at inoculation. (*a*) Primordium of a sclerotium. (*b*) Maturing sclerotium showing exuded drop of liquid. $\times \frac{2}{3}$.
Fig. 3. Culture on turnip. (*a*) Mature sclerotia showing the wrinkled rind. (*b*) Abnormal structures with brown sclerotium-like heads. (*c*) White sclerotia. (*d*) Mature, dry, carbonaceous sclerotia. $\times 1\frac{1}{2}$.
Fig. 4. Culture on turnip. Abnormal structures bearing black heads. $\times 1\frac{1}{2}$.
Fig. 5. Two mature sporophores arising from a sclerotial mass. $\times 1$.
Fig. 6. Three groups of sporophores corresponding with zones on the mycelium. $\times 1\frac{1}{4}$.
Fig. 7. Brown mycelium has formed on the dry upper part of the slope. Numerous straggling sporophores are present. $\times 1$.
Fig. 8. Three-months-old monospore culture, showing mycelium only. $\times \frac{2}{3}$.

PLATE XXX.

Fig. 1. Three stages in the collapse of a sporophore on drying. $\times 1$.
Fig. 2. Two later stages of Fig. 1. $\times 1$.
Fig. 3. Slope showing prolific production of sporophores, which vary greatly in diameter. $\times 1\frac{1}{4}$.
Fig. 4. Mature sclerotium produced at the tip of a straggling sporophore. $\times 10$.
Fig. 5. Three-weeks-old culture of two monospore strains. The hyphae have mixed, and numerous fusions have taken place, with subsequent production of sporophores. $\times \frac{2}{3}$.
Fig. 6. Pot of swede seedlings with sporophores which arise from sclerotia beneath the soil surface. The difference in colour and diameter between the stalk and head of the sporophore is well shown. $\times \frac{1}{2}$.
Fig. 7. Slope culture with three groups of sclerotia corresponding with bars on the mycelium. $\times \frac{1}{2}$.
Fig. 8. Much-branched sporophore from an agar slope. $\times 2$.
Fig. 9. Three-months-old culture of two monospore strains. The mycelia have failed to unite. $\times \frac{2}{3}$.

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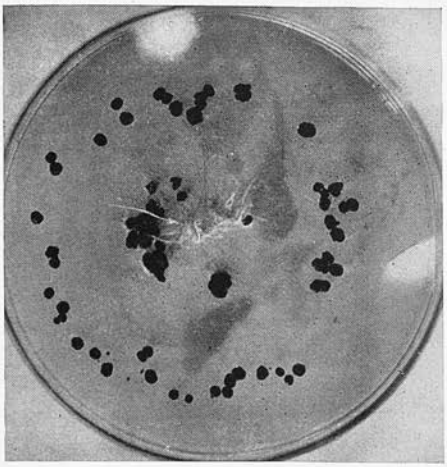
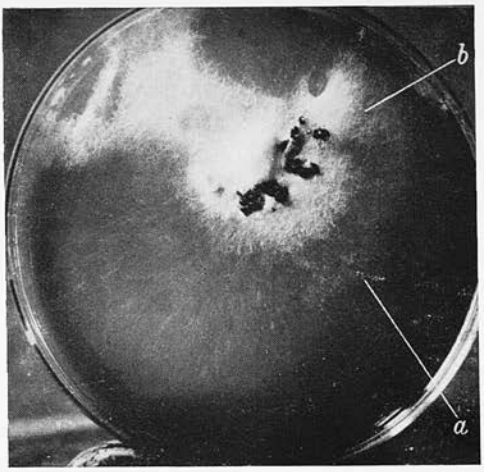


Fig. 1.



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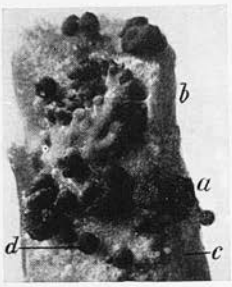

Fig. 3.

Fig. 4.

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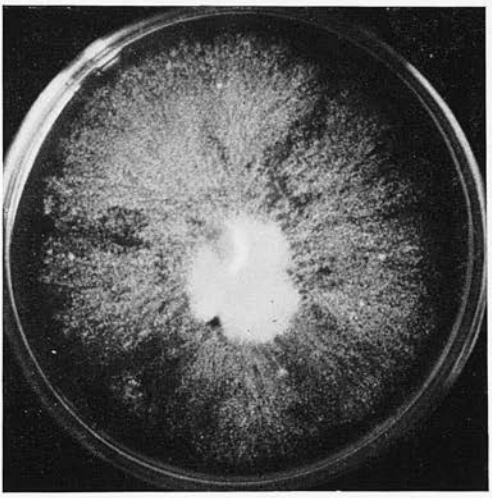
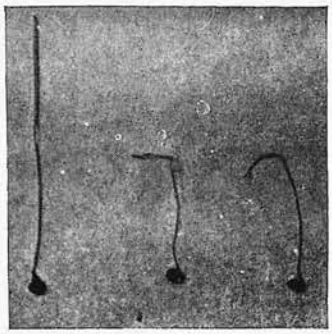


Fig. 8.

MACDONALD.—The Life History and Cultural Characteristics of *Typhula gyrans* (Batsch) Fries (pp. 590–613).



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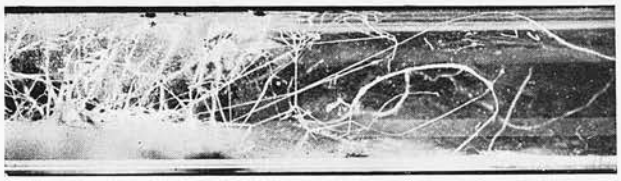

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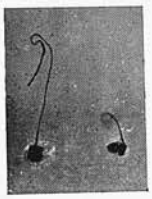

Fig. 2.

Fig. 4.

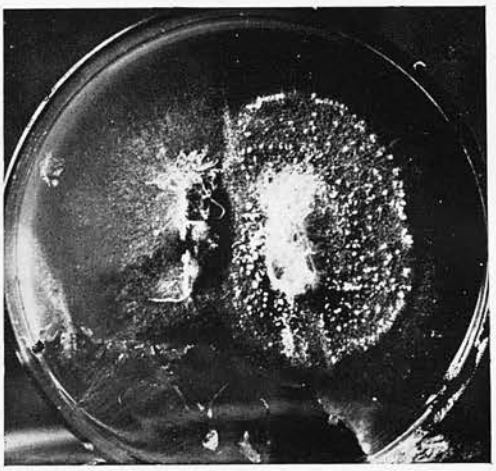

Fig. 5.

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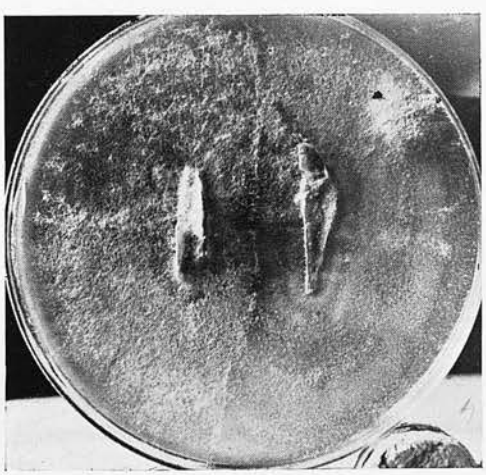

Fig. 9.

MACDONALD.—The Life History and Cultural Characteristics of *Typhula gyrans* (Batsch) Fries (pp. 590–613).