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A DISEASE OF NORWAY SPRUCE (*PICEA EXCELSA* (LAM.) LINK.) ASSOCIATED WITH *STEREUM SANGUINOLENTUM* (A. et S.) FR. AND *PLEUROTUS MITIS* (PERS.) BERK.

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(With Plates I to VIII and 1 Text-figure)

Abstract

This study has been undertaken to provide information which would assist in evaluating the importance of a serious disease of Norway spruce growing in a mixed stand in Glentress forest, near Peebles, in Scotland. Two fungi, viz., *Stereum sanguinolentum* and *Pleurotus mitis* have been found to be the cause of the disease. They have been isolated from the internal rots of the same or different standing trees. Of these, *S. sanguinolentum* appeared more commonly during isolation.

The external and internal symptoms of the disease have been fully described, the essential features being pronounced fluting of the stem as a result of longitudinal depressions in the bark and its subsequent cracking with copious outflow of resin. The infected lesions are mostly restricted within the limits of pruning and occur round about the pruning wounds where strips of cambium have been killed due to the entry of the pathogens.

The isolates of *S. sanguinolentum* and *P. mitis* have been grown in culture. Their characteristic appearance and constant behaviour have been presented in potato-dextrose agar and malt agar. On these two media both the fungi behave very similarly as a rule. In all isolations of each fungus the cultures have started in the same way but the growth rates have been greater on potato-dextrose agar than on malt agar. They are both 'white rot' fungi since they show positive oxidation reactions.

Typical fructifications of *S. sanguinolentum* and *P. mitis* bearing normal basidia and basidiospores have been obtained from mycelia originating from the infected tissues of Norway spruce. Various devices adopted to induce the cultures to fructify have been fully described. A low range of temperature (5°C–15°C) has been found to be essential for the production of sporophores of *P. mitis*, below or above which fruit-bodies are not formed at all. *S. sanguinolentum*, on the other hand, can fructify well under ordinary conditions of light and temperature of the laboratory.

Fructifications of both *S. sanguinolentum* and *P. mitis*, that developed on sections of infected trees after felling, have been thoroughly described and their anatomical peculiarities noted.

Microscopic characters of the rots have been fully described. The rotted tissues show the presence of mycelium in all stages of decay, appearing most abundantly in the advanced stages. It is particularly plentiful in the medullary rays which seem to be the first element to be attacked. Closely interwoven mycelium often fills up many of the ray parenchyma cells, sometimes tracheids and associated with gum-like material giving the appearance of a zone-line. The mycelium ramified at first through the pits but later penetrates the cell-walls forming numerous bore-holes.

Chemical changes of the wood during the process of decay have been tested with micro-chemical stains in common use. The change consists mainly in the delignification of the highly lignified parts of the secondary walls of the wood-elements associated with digestion of cellulosic materials. It is also evident

that in the early stages of decay *S. sanguinolentum* can utilize more lignin than *P. mitis* while the latter digests more cellulose at the beginning.

The natural resistance to decay of spruce timber has been tested in the laboratory by exposing small sterilized test-pieces of the sound timber to the attack of *S. sanguinolentum* and *P. mitis* separately growing in pure culture under controlled conditions, and the amount of decay resulting from the fungal attack after 4 and 8 months' tests has been calculated by estimating the loss in dry weight. After the experiments it has been found that the spruce timber is 'non-resistant' to decay. It has been found that the loss in weight due to *P. mitis* is less than that caused by *S. sanguinolentum* both after 4 and 8 months' studies. But the interesting fact is that the loss in weight after 8 months is double of that after 4 months in both cases.

Inoculations have been made on cut twigs in the laboratory and also on trees in the field. It has been observed that when freshly cut surfaces of these twigs are inoculated with spore-suspensions of *S. sanguinolentum* and *P. mitis* in sterile water, the spores germinate readily on the surface within a short time and send down hyphae into the elements of the wood. Since these twigs cannot be kept in an actively growing condition, their invasion by *S. sanguinolentum* and *P. mitis* cannot be regarded as an evidence for their parasitic action on trees. Nevertheless, it can be said that if by chance the spores some how alight on the surface of the wound, they can germinate there under suitable conditions and pass down their hyphae into the elements of the wood. On the other hand, inoculation experiments on living trees carried out in the field have shown that both *S. sanguinolentum* and *P. mitis* can infect living trees through wounds in the bark, and from the results of inoculations they can be regarded as wound parasites on normal vigorous trees.

Introduction

It has been observed that about 20 per cent of forty to sixty years old Norway spruce trees (*Picea excelsa* (Lam.) Link.) growing in a dense, mixed, stand with Sitka spruce and Douglas fir at Glentress forest near Peebles in Scotland, have been for the last four or five years in a diseased condition and are gradually dying. The trees are badly shaped and in most cases there is considerable amount of resin flow from the bark on the lower trunk. The trees do not give any evidence of the presence of frost rings or cracks in their wood. This plantation occupies a hill-slope of moderate gradient, leading to the top of the hill (about 700–1000 feet above sea-level). On the whole it is a cool, damp and muddy situation and areas of bad drainage occur here and there. Since the economic value of Norway spruce as a timber-yielding plant is well known, it is thought that the information concerning the causal agent, and the amount of damage caused by it is desirable. With this end in view, the present investigation has been undertaken during the latter half of 1949. It is interesting to note here that during this investigation two fungi, viz., *Stereum sanguinolentum* and *Pleurotus mitis* have been found to be associated with the disease and these are capable of infecting living trees through wounds, eventually causing rot in the wood. It is apparent that this association of two pathogens has greatly enhanced the importance and complex nature of the problem. At the present moment it is difficult to estimate the relative importance of the two fungi in bringing about the decay in living trees and the nature of reactions existing between them. An investigation of this nature would necessarily require a longer period owing to the slow growth of the fungi and their effects on a living host. The data, so far obtained, have been given in the following pages based largely on studies in the laboratory as well as in the field.

Historical

Gäumann and Jaag (1937) have described a disease in spruce and fir causing severe damage in a re-afforestation area of the Emmentel in Switzerland. The symptoms described by them are somewhat similar to those observed in the present study and are attributed only to the virulent pathogen, *Pleurotus mitis*. Although this fungus has been known to cause heart-rot of conifers on the continent of Europe, yet up to the present it does not appear to have been recorded on living trees in Great Britain.

Recently, Day (1950) has reported a severe case of bark necrosis on Norway spruce in Drummond Hill Forest in Perthshire, Scotland. The injuries are diagnosed by him as having been caused by frost damage usually after pruning live branches. He has reported and figured cracking of the bark with consequent outflow of resin. The effect of the injuries on the wood of the tree, however, has not been fully investigated by him. He mentions that under such conditions some degree of fungal infection is inevitable although it has not yet been possible for him to say what types of fungi may have entered.

The same type of injury has been reported by Laing (1947) on Sitka spruce and the cause of such damage has been attributed to *Nectria cucurbitula*. He has found abundant mycelium and conidia of *N. cucurbitula* on the dead bark but he did neither actually isolate the fungus from the internal tissues nor carried out any infection experiments to prove its pathogenicity.

Although *Stereum sanguinolentum* has long been known to occur commonly on dead stumps and branches of coniferous trees in Great Britain and on the continents of Europe and America, there still exist contradictory statements in the description of the rot made by various workers from time to time. As such it will be evident that the external symptoms exhibited by the infected trees under consideration are somewhat different from those described by previous workers. In Great Britain very little is known about its parasitic activity although it is reported to cause a butt-rot of coniferous trees (Day and Peace, 1936) and timber-stain in Norway spruce (Cartwright, 1937). Cartwright and Findlay (1946) have isolated it from freshly felled poles of spruce and larch and incidentally referred to its sporadic occurrence in living trees. In the continent of Europe it occurs on various conifers in Denmark (Ferdinandsen and Jorgensen, 1938–39) and Germany (Bergenthal, 1933) and also as a wound-parasite in fir and pine (Rohmeder, 1937). In Russia, it causes a slow decay in fir and pine (Vakine, 1934). This fungus is also known to attack living spruce in Norway (Jorstard and Juul, 1939) and in Sweden (Lagerberg, 1919, 1923) where it gains entrance through top-breakage due to snow damage or through wounds. Robak (1936) has isolated it from heart-rot of living spruce and mentioned (Robak, 1942) that it is commonly found on dead coniferous wood in Sweden. In America, it causes a heart-rot of balsam fir (Faull and Mounce, 1924; McCallum, 1925, 1928), often causing a top-rot (Kaufert, 1935). It also induces a sap-rot causing much damage in firs, Douglas firs, larches, pines and spruces (Hubert, 1935; Baxter 1937).

It is a dangerous fungus in pruning wounds of northern white 'pine (Spaulding and Coll., 1935) and the principal agent causing 'slash' decay (Spaulding, 1929). Thus, it becomes event that the importance of the fungus has been gradually recognised although it is difficult at the present moment to estimate the amount of losses caused by this fungus. The indications are such that it may prove to be a serious pathogen in Great Britain and the losses caused by it may ultimately become very serious.

Symptoms

The symptoms of the disease are few and conspicuous. The infected trees show longitudinal depressions of the bark (Pl. I, figs. 1 & 2) which may often be ill-defined and somewhat flat. These extend from the butt upwards up to 6 feet, but occasionally up to a height of 10–12. feet, giving the trunk a somewhat fluted appearance. The bark of the sunken areas is often discoloured. Its underlying tissues die, become dessicated and eventually it splits lengthwise accompanied by copious outflow of resin (Pl. III, fig. 7). Gradually, these areas appear to sink deeply into the trunk owing to continued growth in thickness in the areas lying between them. The lesions usually occur round about pruned branches, run up and down the trunk and are restricted within the limits of pruning. They may sometimes extend towards the base of the trunk where fluting becomes most pronounced (Pl. I, fig. 2). The bark near the base of the trunk ultimately becomes loose and separates easily, particularly when the poles are cut and dried. A mass of whitish discoloured tissue and mycelium is often present in between the bark and the sapwood. The inner surface of the bark and the surface of the sapwood in contact with it show a white mottled appearance.

Cross sections of freshly felled poles in the early stages of decay show irregular to wedge-shaped areas (Pl. III, figs. 1, 2 & 4) at the periphery. These are light to dark brown, sometimes reddish-brown in colour. They gradually extend towards the centre of the wood into the base of the trunk and finally coalesce causing a butt rot (Pl. III, fig. 3). In such cases the course of infection appears to be from the bark to the centre of the stem. Close examination of the infected area reveals that the cambium is killed near the pruned branches. This is possibly due to the entry of the pathogens through dead branches or other injuries such as pruning cuts and after killing the cambium they spread from the periphery to the centre of the wood. The organisms seem to spread much more rapidly in a longitudinal direction than laterally. In this way, longitudinal strips of cambium are killed near the pruned branches and as a result further secondary growth cannot continue. In the areas between these dead cambial strips growth continues and the trunk ultimately shows prominent ridges. The transition from the normal to decayed wood is abrupt and there is no sign of any black line of decay. The discoloured areas, in an advanced stage, show small pockets which are somewhat whitish and hollow.

There was no sign of any fructification of the causal organisms on the surface of the infected trees but recently felled logs of such trees that had

lain on the forest floor were found with fructifications of *Stereum sanguinolentum* and *Pleurotus mitis* over the rotted areas on the surface of the cross sections (Pl. I, figs. 3 and Pl. II, fig. 5).

The causal agents

Materials for study

The materials were obtained in November, 1949, in the form of freshly felled infected trees from Glentress forest near Peebles in Scotland. The fructifications of *Stereum sanguinolentum* and *Pleurotus mitis* were also collected while growing in an excellent condition on the surface of the cross sections of recently felled Norway spruce poles lying on the floor of the forest near the infected trees. The diseased trees were carefully selected, felled and macroscopically examined in the field to note, as far as possible, the progress of decay in the wood. For further study, small cylindrical sections from different regions of the infected trunk were selected, brought into the laboratory, examined and studied while in a fresh condition. Microscopic examination of the rotted areas revealed the presence of abundant mycelium within the tissues of the host.

Isolation and identification of the causal organisms

The mycelium from the host tissues was isolated and studied. The isolation was made from the infected wood by using portions of isolated rotted tissues from selected regions of the same or different poles. Small pieces (about $2'' \times \frac{1}{2}'' \times \frac{1}{2}''$) of such wood blocks were washed several times with sterile distilled water, dipped quickly in rectified spirit and flamed in order to free the surface, as far as possible, from superficial contaminations. One side of the exterior portion of such a wood block was removed with a sterile scalpel. A small piece of wood was then carefully cut out from the freshly exposed surface near the edge of the decayed area and transferred aseptically to a tube containing 2% malt agar slant into which it was partially embedded. In this way several transfers were made. Cultures were also made from poles bearing fructification of *Stereum sanguinolentum* and *Pleurotus mitis* on their cross-section-ends. These were then incubated at 23°C. in darkness. In most cases the mycelia grew out within a week and sub-cultures were made from each of these in 2% malt agar tubes which were subsequently used as stock cultures. After close examination of all the isolates it was found that these cultures belonged to two different fungi and exhibited the characteristics of the cultures of *Stereum sanguinolentum* and *Pleurotus mitis*. These were compared with the polysporous cultures of *Stereum sanguinolentum* and *Pleurotus mitis* already made available for the purpose and found to be identical with the latter in both cultural and microscopic details. The identity of these isolates was further confirmed when these cultures subsequently produced typical fruiting bodies in artificial cultures (Pl. II, fig. 6 and Pl. IV, figs. 1 & 2).

Spore cultures of *Stereum sanguinolentum* and *Pleurotus mitis* were also made in the usual way. The spores germinated readily on 2% malt

agar slants within 12 to 24 hours and good mycelial growth was obtained in all the tubes within a fortnight. These were used as stock cultures for further study and comparison.

In a great majority of cases the isolates were those of *Stereum sanguinolentum* but nevertheless *Pleurotus mitis* was also isolated from separate regions of the same or different poles. In one instance both the organisms were not only isolated from the same pole but the respective rots were only a few centimetres apart from each other, being separated by healthy tissues. This statement was further corroborated by inducing the fungi to produce fructifications on the rotted areas at the cross-section-end of the infected pole from which isolations were made (Pl. III, fig. 6). From the number of isolations so far made, it was evident that *Stereum sanguinolentum* was more widely distributed within the host tissues than *Pleurotus mitis*. Further investigations in future with isolations made from large numbers of infected trees would possibly throw some light as to their relative distribution within the host plant.

The sporophores

1. *STEREUM SANGUINOLENTUM* (A. et S.) Fr. (Plate I, figs. 3 & 4).

Fructifications. Sessile, at first forming somewhat orbicular and resupinate patches, then narrowly reflexted, later becoming laterally confluent and forming broad continuous sheet, measuring 30—40 × 30—50 m.m. or more; reflexed portions about 2 m.m. deep from margin to back, often becoming densely imbricated and laterally confluent, somewhat folded; dimension of the resupinate position usually 10—15 m.m. but in younger specimens much less (2–3 × 3–5 m.m.): thin and leathery: margin thin, acute, usually white in colour. *Upper surface.* Villose: with somewhat satiny and radiately adpressed hairs: faintly zoned near the margin: pinkish buff* or pale olive buff to pale drab gray or pale smoke gray towards the margin. *Context.* Thin: coriaceous: whitish. *Hymenial surface.* Smooth, often with small isolated tubercles: slightly wrinkled at places: becoming irregularly cracked in the resupinate portion on drying; bleeding red when cut or bruised: pale olive buff, olive buff or fuscous, becoming avellaneous or wood brown on drying. *Basidia.* Cylindrical to clavate: dimension about 30–40(50) × 4–8μ; di- to tetrasterigmatic: sterigmata 4–5μ long (text-fig. 1, a). *Spores.* White in mass, otherwise hyaline: smooth; oblong or slightly curved: with an apiculus; dimension about 6–7.5(8.5) × 2–3μ (text-fig. 1, b). *Lactiferous cells.* Numerous: reddish-brown in colour: arising from the layer below the hymenium and extending into it; elongated but clavate near the apex; about 300–400 × 4–5μ but near the apex 5–7.5μ wide; bleeding when broken; when mounted in glycerine the contents break up into somewhat rectangular blocks. *Tissue differentiation.* Entire fructification about 300–400 (600)μ thick and the following regions can be differentiated : (*a*) a hymenial layer up to about 50μ thick: (*b*) an intermediate hyphal layer measuring 150–250μ thick.

* Ridgeway (1912).

composed of densely arranged hayline hyphae and from this layer coloured conducting organs originate and curve upwards into the hymenium; (*c*) a narrow golden yellow zone, about 50μ wide lying between the intermediate layer and the outermost hairy covering, composed of longitudinally arranged hyphae; and (*d*) the outermost hairy covering composed of densely matted hyphae, about 50–100 (200)μ thick. *Hyphal characteristics.* Two kinds of hyphae can be recognised : (1) thick-walled, matted or loosely interwoven hyaline hyphae of the hairy covering, more or less straight, sometimes slightly flexuous, mostly unbranched, distantly septate, lumen often almost obliterated, without clamp-connections, about 2.5—5(7.5)μ wide and sometimes with a few granular contents, and (2) comparatively thin-walled interwoven or longitudinally arranged hyphae of the intermediate layer and golden yellow zone, sparingly branched, distantly septate, without clamp-connections, sometimes pale yellow in colour, with granular contents, about 3—5μ wide.

The fructifications of this fungus develop readily on freshly felled poles of infected trees during the months of October to January. They are found either on the cross-section-ends over the rotted areas or on the cracked bark. They are commonly recognised in having thin, coriaceous and resupinate fruiting bodies and with a narrowly turned back margin. The hymenium, somewhat drab in colour, bleeds red when wounded and cracks irregularly and deeply on drying. Numerous coloured lactiferous cells in the hymenium and underlying tissues are conspicuous diagnostic characters by which the species can be readily identified. Owing to their small size they are difficult to recognise from a distance and as such, of little help as an indication of decay in standing trees in the forest. According to Burt (1920) and Rea (1922) there are no cystidia but recently Wakefield and Dennis (1950) have mentioned the presence of yellowish brown cystidia in the hymenium. They have however, not stated anything about the presence of numerous lactiferous cells so characteristic of the species and as such yellowish brown cystidia described by them are in reality the conducting organs of the previous workers. These organs bleed i.e., their contents come out when broken and this is the unique character of the conducting organs and not of cystidia as given by Overholts (1929).

2. *PLEUROTUS MITIS* (Pers.) Berk. (Plate II, figs. 1–3)

Pileus. Sub-sessile or shortly stipitate, continuous with the stipe in a more or less horizontal plane: thin, slightly fleshy, tough and fexible; sub-spathulate to somewhat reniform; about 5–15 (20) m.m. across. *Upper surface.* At first entirely white, later becoming slightly rufous or showing shades of salmon colour or flesh ochre near about the stipe; smooth, glabrous, often showing fine longitudinal striations near the margin; margin entire, involute when young or on drying. *Flesh.* Thin; sub-gelatinous below the cuticle; white; less than .5 m.m. thick. *Gills.* Very narrow, linear-lanceolate; crowded, adnate; white, becoming pale salmon colour with age or on drying; about 1–1.5 m.m. wide. *Stipe.* Short, lateral, widening into the cap; white or tinged rufous; somewhat compressed; covered all over with whitish, mealy squamules; about 1–3 m.m. long,

sometimes becoming much elongated (6–12–15 m.m.) when developed within cracks, about 1–1.5 m.m. thick. *Basidia.* More elongated than the immature ones: narrow clavate; with 2-4 strigmata (1.5–2μ long); dimension about 16–20–25$\times$3.5–4μ: immature basidia about 9.5–12.5$\times$3–3.5μ (text-fig. 1, i). *Spores.* Sausage-shaped; hyaline; smooth; dimension about 4–5$\times$1.5–2–2.5μ (text-fig. 1, h). *Tissue differentiation.* A vertical section through the upper fleshy portion of the pileus shows three distinct regions : (*a*) the outermost brownish cuticular layer, about 20–30μ thick, (*b*) an intermediate sub-gelatinous layer, about 180–200μ thick, with loose stainable hyphae embedded in a gelatinous matrix, and (*c*) a more or less brownish basal portion, about 80–100μ thick. In a cross section of the stipe three different regions can be distinguished, viz., (*a*) a dark compact and circular central zone, about 900–1200μ thick and consisting of densely interwoven hyphae, (*b*) an intermediate hyaline sub-gelatinous zone, about 200–300μ thick and with loosely interwoven hyphae and (*c*) the outer most compact zone of about 150–200μ in thickness. A transverse section of the gill shows a central sub-gelatinous trama, about 60–150μ wide, composed of compact parallel hyphae and its central axis loosening from the sub-hymenium and hymenium. *Hyphal characteristics.* Two kinds of hyphae appear to have entered into the composition of the fruit-body viz., (*i*) thin-walled, hyaline, wider hyphae, straight and more or less parallel, sometimes slightly flexuous, distantly branched and septate, with a few granular contents, sometimes empty, about 2.5–4(5)μ wide, without clamp-connections and with walls often partially gelatinised, and (*ii*) thin-walled, hyaline, narrow hyphae, about 1.5 to 2.5μ wide, flexuous, sparingly branched, with clamp-connections, full of granular contents and more or less intertwined.

The afore-said two kinds of hyphae are characteristic of the gills and the stipe. In the gill the wider hyphae are more numerous than the other type and appear to run more or less parallel within the trama. The narrower hyphae, on the other hand, are highly flexuous and embedded in a gelatinous matrix within the stipe. The fleshy portion of the pileus is entirely made up of this type of narrow fluxuous hyphae similarly embedded in a gelatinous matrix, the wider hyphae being unrecognisable as a result of gelatinisation.

This fungus, long called *Agaricus mitis* Pers., is well known in France, Switzerland as well as in all of Europe and is frequently found on dead twigs and stumps of coniferous trees. Berkeley (1860) mentions that it occurs on larch in Scotland and is abundantly found in Nottinghamshire. The writer has collected it growing on dead twigs and stumps of Norway spruce, Sitka spruce and Douglas fir from Peebles, Scotland. It has also been collected while growing saprophytically on Scots pine in this country. Josserand and Smith (1937) state that this species is identical with the American species *Panus bacillispora* Kauffman and suggest that these two names should fall into synonymy with *Panellus mitis* (Pers.) Singer. In order to avoid confusion, the writer has retained the older name *Pleurotus mitis* by which it is familiarly known in Great Britain and on the continent of Europe. *Pleurotus mitis* is easily recognised by its tiny, white fructifications which later become somewhat refuscent. Though not externally

recognised, its gelatinous flesh and trama and narrowly allantoid spores are distinctive characters. The fructiacations revive after a period of dessication and are gregarious.

Fungi in culture

The isolates of *S. sanguinolentum* and *P. mitis* from Norway spruce were grown in culture and their characteristics studied on two different media, viz., *potato-dextrose agar* and *malt agar*. Potato-dextrose agar was prepared according to the recipe of Fritz (1923). All cultures were kept in darkness in different incubators at 18°C. and 23°C. In order to maintain experimental conditions as constant as possible, small circular discs of inocula (about 3–4 m.m. in diameter) were taken from young cultures and placed with upside downwards upon the media in tubes and petri-dishes, each containing approximately 20 c.c. and 30 c.c. of the medium respectively. The pH value of each medium was adjusted and after sterilization was found to be 5.2. The colour exhibited by the mycelium has been expressed according to Ridgeway (1912) and the terms used to describe the texture of the mat are those proposed by Long and Harsch (1918) and Nobles (1948).

In culture *S. sanguinolentum* was studied repeatedly by various workers such as Lagerberg (1924), Bavendamm (1928), Bergenthal (1933), Cartwright and Findlay (1938) and others but their descriptions were widely divergent. Robak (1942) made an extensive study of the different strains of the fungus and divided them into three main types. It has been found that the present isolate does not strictly fall entirely to any of the groups as given by Robak (1942). Very little is known about the detailed cultural characteristics of *P. mitis* although Gaumann and Jaag (1937) determined the maximum, optimum and minimum temperatures for the development of the fungus on malt agar.

Oxidase tests

There are evidences in support that the intensity of attack on lignin is directly dependent upon the oxidase activity of the white rot fungi and this can be detected by the method described by Bavandamm (1928). This oxidase test was made by growing the fungi in Petri dishes on 2.5% malt agar containing 0.5 per cent of gallic or tannic acid and kept at a constant temperature of 23°C. in darkness. Dark brown rings appeared within 24 hours around the inocula of both the fungi indicating the presence of oxidase. This positive reaction indicate that both the isolates of *S. sanguinolentum* and *P. mitis* were white rot fungi but their intensity of reaction showed some variation. On gallic acid medium within 24 hours after inoculation *P. mitis* showed very intense reaction and the diameter of the ring was 20–25 m.m., whereas the diameter of the same produced by *S. sanguinolentum* was only 10 m.m. and the reaction was less intense than the other. On tannic acid medium the reactions were intense to very intense in both cases. Another method to distinguish white rot fungi from brown rot ones (Preston and McLennan, 1948) was successfully

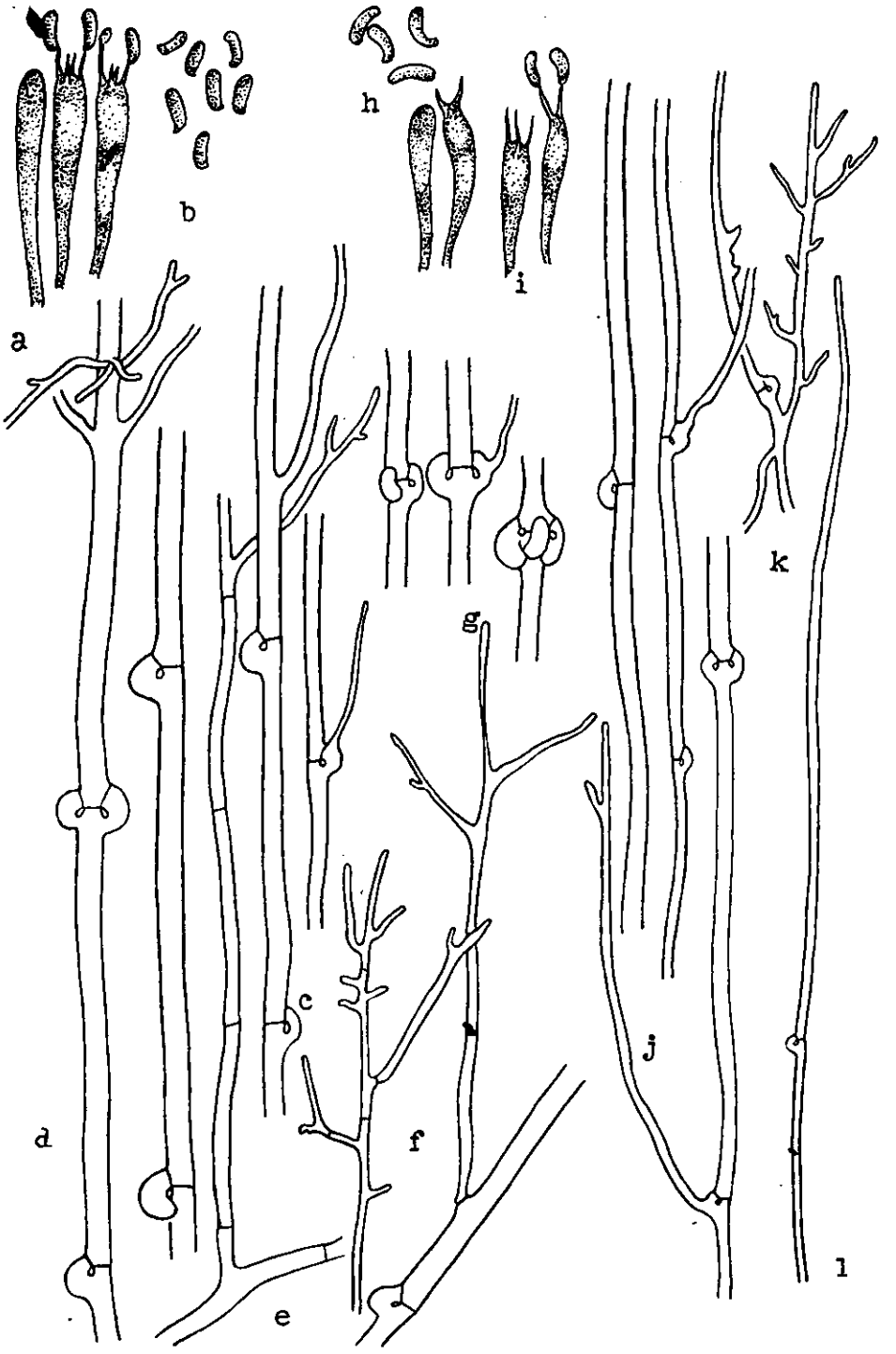
tried by growing both the fungi on 2.5% malt agar containing 0.007 per cent gentian violet. Being white rot fungi, the violet colour of the medium was bleached slowly by them but about 6 to 8 weeks incubation was necessary before positive results could be obtained. According to Preston and McLennan (1948) complete decolorization of the dye would take place only when the oxygen concentration above the medium was greater than 6% and this discolouration was probably due to the production by the white rot fungi, of an extracellular oxidase system.

Cultural characteristics

A. *STEREUM SANGUINOLENTUM* (A. et S.) Fr.

(*i*) Habit of growth (Pl. IV, figs. 3, 5 & 6). On *potato-dextrose agar* at 18°C, the growth of the mycelium started with moderate rapidity. It was initially thin and arachnoid with an even advancing zone. During the first seven days after inoculation it remained in this condition excepting on the transplant where a shaggy ball was most often present. As the growth advanced, the mat proper, at first glabrous, gradually became denser and covered the whole surface of the slant with soft, downy-woolly mycelium, somewhat thin, appressed and fringed at the margin. In about 12 days after inoculation the mycelium tended to fill the base of the tube with a compact felty mat which in the older part, was depressed and somewhat opaque. The new growth was downy to cottony-woolly and somewhat translucent, particularly towards the upper end of the tube. The colour of the medium changed to yellowish with a pinkish tinge. Yellowish glistening drops of liquid appeared over the inoculum and on the mat in about three weeks time but later these disappeared. At 23°C, the growth was more or less the same but the condensation of the mat seemed to be more pronounced. On *malt agar* under identical conditions the growth was rather slow, thin and arachnoid at the beginning but somewhat cottony to felty over the inoculum and not a shaggy ball. As the growth advanced the mat became felty, patchy and in about a month, formed a tough skin-like mat, difficult to tear. In general, growth and subsequent condensation of the mycelium to form a thick mat was less pronounced in malt agar than that in potato-dextrose agar.

(*ii*) Colour. On *potato-dextrose agar* at 18°C, colouration appeared first after a week's growth over the inoculum as a patch of apricot buff which later deepened to buckthorn brown or mummy brown. Within a fortnight after inoculation, pigmentation of the mat at 23°C, was confined to the area just around the inoculum and included shades of antimony yellow, buckthorn brown and mummy brown, while the major portion of the mat remained white. Within a week after this, diffused antimony yellow colour appeared over the whole mat but it was more pronounced towards the lower end of the slant which later deepened to patches of ochraceous orange. Although pigmentation started early at 23°C., later it was more pronounced in cultures kept at 18°C. On *malt agar* at 18°C, colouration of the inoculum and the surrounding mat started in about 12 days after inoculation and was pinkish buff in colour. Later



Text-fig. 1. Basidia, basidiopores and aerial and submerged hyphae of *S. sanguinolentum* (*a–g*) and *P. mitis* (*h–l*) (*c–g*, *j–l*, × 650). See text.

other shades such as warm buff, antimony yellow and ochraceous orange developed on the mat. These were restricted to limited areas of depressed mycelium of a denser texture. At 18°C. colouration of the mat seemed to be more pronounced than that at 23°C. and also more pronounced than that at 18°C. and 23°C. on potato dextrose agar.

(*iii*) Rate of growth. The increment in diameter of Petri dish cultures on 2.5% *malt agar* at 23°C. in darkness were determined as follows :—

Days	2	4	6	8	10	12
Average diameter of growth (m.m.).	15	30	45	60	75	90

These figures indicate that the fungus grows with moderate rapidity, with an average daily increment in diameter of 15 m.m. At 18°C. on the same medium the growth was slightly slower with an average daily increment of growth of 14 m.m. On *potato-dextrose agar*, on the other hand, the rate of growth at 23°C. was much more rapid than that on malt agar with an average daily increment in diameter of 17 m.m.

(*iv*) Hyphal characters (text-fig. 1, c–g; Pl. V, figs. 1, 2 & 4).

Advancing zone. Hyphae hyaline, straight or flexuous, sparingly branched, usually distantly septate, with frequent large, simple clamp-connections, sometimes in opposite pairs or whorled, about 3–6(7)μ wide, with conspicuous granular contents, sometimes pale yellow in colour. *Aerial mycelium*. Three types of hyphae can be recognised, viz., (1) wider thin-walled hyphae, more or less straight or slightly flexuous, usually distantly septate, with a few simple or paired, arched clamp-connections, very sparingly branched, usually 5.5–6.5μ wide but may be up to 7.5–8μ broad, sometimes with yellowish-brown contents, often giving rise to finer hyphae from the sides which may be clustered at the septa (text-fig. 1, c–g; Pl. V, figs. 2 & 4); (2) thin-walled narrower hyphae, springly branched, distantly septate, rarely with simple clamp-connections which may grow out to form new hyphae, about 2.5–4.5μ wide, often spirally coiled (Pl. V, fig. 1), with or without granular contents, hyaline or brownish in colour (text-fig. 1, c, e); (3) very narrow, thin-walled, hyaline, much branched hyphae originating from the wider or intermediate types, often flexuous, with dense, granular contents, without clamp-connections (text-fig. 1, f). *Submerged mycelium*. The same three types of hyphae can be recognised but with slight differences as follows—(1) wider hyphae very numerous, hyaline, mostly with granular contents, about 5.5–6.5μ wide, often slightly constricted at the septa, without clamp-connections; (2) intermediate type fairly numerous, sparingly branched, branches flexuous, with or without granular contents, hyaline or faintly coloured, distantly septate, rarely spirally coiled; (3) finer hyphae very few. Crystals large, numerous.

B. *PLEUROTUS MITIS* (Pers.) Berk.

(*i*) Habit of growth (Pl. IV, figs. 4 & 7). On *potato-dextrose agar* at 18°C. (Pl. IV, fig. 3a) the growth was at first slow being only confined to the inoculum as a dense, white and felty covering. As the growth

advanced, the mycelium condensed over it forming a felty ball while on the agar surface it remained entirely appressed, sodden and colourless with indistinctly fimbriated margin. In about 10 days after inoculation, the mat proper became differentiated into a broad central, white and felty zone around the inoculum and a distinct appressed and colourless advancing zone, about 2–3 m.m. wide. In cultures about 20-days-old the mat presented the appearance of a dense, white and smooth felt while the region just behind the advancing zone tended to become somewhat raised and sub-felty. In old cultures both the central and advancing zones became felty but were separated from each other by a narrow sub-felty area and eventually a thin skin was formed. Considerable discolouration of the medium was noted in cultures 10-days-old and it was pale yellow. At 23°C. (Pl. IV, fig. 3b) on the other hand, the habit of growth was more or less the same but the rate of growth was rather slow and the mat was more compact. On *malt agar*, both at 18°C. and 23°C. (Pl. IV, fig. 3c, d) the growth was poor and the mat proper remained mostly appressed and colourless for a considerable length of time. Towards the end of the second week after inoculation a felty to pulverulent central zone was differentiated and an outer appressed and colourless zone of variable width (2–8 m.m.) could be clearly recognised.

(*ii*) Colour. On both the media all cultures remained white throughout and no other pigment developed even in cultures 2 to 3-months old.

(*iii*) Rate of growth. The rate of growth on both the media, under identical conditions of light and temperature as stated before, was rather slow to start with but after a week it increased and the daily increment in diameter of Petridish cultures were determined. The radial growth in all cases was unequal and as such average measurements were taken. On malt agar both at 18°C. and 23°C. (Pl. V, fig. 7) the daily increment of growth in diameter varied between 13 and 14 m.m. while on potato-dextrose agar, it varied between 10 and 14 m.m.

(*iv*) Hyphal characters (Text-fig. 1, j–l; Pl. V, fig. 3).

Aerial mycelium. Hyphae hyaline, more or less straight or slightly flexuous, remaining unbranched for a considerable distance (text-fig. 1,j) but often becoming very flexuous and closely branched (text-fig. 1, k), usually with simple clamp-connections at every septum, paired clamp-connections few, often developing into new hyphae, mostly 1.5 to 3μ sometimes 3.5–4.5μ wide, with dense to sparsely granular contents, sometimes empty. *Submerged mycelium.* Hyphae hyaline, sparingly branched, usually flexuous, simple clamp-connections (text-fig. 1, b) frequent but not so numerous as those in aerial hyphae, distantly septate, mostly 3.3.5μ sometimes 1.5–2.5μ wide; a few prominent flexuous usually unbranched hyphae with densely granular, highly refractive contents present, without clamp-connections, about 5–7.5μ occasionally 3.5μ wide; on being mounted in lectophenol its contents become more or less homogeneous and the walls of the hyphae bulge out at places to form lateral swellings (Pl. V, fig. 3).

Production of fruit-bodies

It has already been stated that the two fungi isolated from the infected trees were identified as *S. sanguinolentum* and *P. mitis* respectively, by studying and comparing their cultures with the polysporous cultures of the same already made available for the purpose. Since the identification of unknown cultures largely depends upon their forming characteristic fructifications, various attempts were made to obtain these in pure cultures of the two isolates so that they could be easily recognised. Both the isolates, however, did not form fructifications on various media tried such as potato-dextrose agar, malt agar and oatmeal agar which were subjected to various conditions of moisture, light and temperature within the laboratory. Only *S. sanguinolentum* formed small resupinate fructifications on sterlized wood blocks of Norway spruce within 2 to 3 months after inoculation (Pl. IV, fig. 2). These wood block cultures were kept in darkness and at a constant temperature of 23°C. in an incubator. Similar wood block cultures of *P. mitis* did, however, remain sterile. Eventually the medium for developing sporopheres recommended by Badcock (1941) was tried with considerable success. Dry sawdust of readily decaying wood such as beech and spruce were separately mixed with Badcock's 'accelerator' in various proportions (5% to 40% by weight), the principal ingredients being maize-meal and bone-meal. The mixtures, after being soaked in tap water, were put in Petri dishes, test tubes (20×4 c.m. and 15×2 c.m.) and Badcock's apparatus (1943) and the various devices described by him for obtaining an atmosphere of relatively high humidity around the developing sporophore, were tried. This was found to be necessary as Badcock rightly pointed out that no single method could be expected to induce fructifications in all species of wood-rotting fungi equally, for they behave very differently under natural conditions. After sterilization the media were inoculated with the isolates and the cultures were kept in darkness at room temperature (18°C. to 21°C.) inside a closed chamber in which a moderately high humidity was maintained. Subsequently, when the mycelia entirely covered the surface of the medium or reached the ends of the tubes, all the cultures were removed and placed in strong diffused light on a table about 8 ft. from a large window of the laboratory. Both the fungi produced exceptionally rapid and luxuriant growth on the sawdust medium, particularly on those containing 30% or 40% by weight of the 'accelerator'.

In all cases, *S. sanguinolentum* began to form either resupinate or typical effuso-reflexed fructifications within 2 to 3 months after inoculation (Pl. IV, fig. 1). When the medium in large tubes was completely permeated with compact mycelium, the cotton plugs were removed and each was supported in an inclined position between two Petri dish lids, the lower being filled with water. A thick pad of absorbent cotton wool was placed within the mouth of the tube and was never allowed to dry out. Within a few days, when vigorous surface growth developed, the pad was gently pushed down so as to touch the medium. The tube was then placed in such a position so that light from the window could fall directly on its open mouth. In this way several effuse-reflexed fructifications were obtained in about 5 to 6 months after inoculation. Although there was

risk of contamination, this method proved to be very successful. In Badcock's apparatus small resupinate fruiting bodies developed near the rim of the test tube within the flask but the growth was very slow. Cultures in Petri dishes in an advanced stage of growth were exposed by removing the lids to humid atmosphere and these also fructified and produced resupinate fruiting bodies within 2 to 3 months after inoculation, particularly near the edge of the lid. It was noted that in case of *S. sanguinolentum* the medium containing 5% 'accelerator' was found to be most satisfactory for the production of sporophores. The higher the percentage of the 'accelerator' the greater was the vegetative growth with consequent delay in producing fruting bodies.

Great difficulty was, however, experienced in obtaining fruit-bodies of *P. mitis* although various devices recommended by Badcock were tried. Under laboratory conditions of light and temperature the mycelium covered the surface of the medium with white and compact growth and crept over the rim of the lower lid of the Petri dish forming somewhat compact, tough, irregular and skin-like mat. At this stage the upper lids were carefully removed and the cultures were kept inside a bell-jar in which a relatively high humidity was maintained by keeping a dish of water exposed within it. Within a fortnight short stalks began to develop near the edge but these did not expand further to form pilei. About the middle of October, 1950, some of these cultures (about 4–5 months old) with rudimentary stalks were then kept in the open outside the laboratory, under a moist bell-jar below a shade, in order to avoid direct sunlight. A wad of moist absorbent cotton wool was placed on the culttre in close contact with the undeveloped stalks and never allowed to dry out. The temperature was daily recorded and it varied between 5°C. and 15°C. till the middle of November. Within a fortnight numerous stalks began to develop on the outer edge of the skin-like mat and within a month their tips expanded and formed pilei with rudimentary gills on their under surface (Pl. II, fig. 6). In this way several crops of fructifications of *P. mitis* were obtained. It was found that temperature of the laboratory (18°C.—21°C.) and incubators had a retarding influence on the production of sporophores. Given all other necessary conditions, a range of temperature varying between 8°C. and 15°C. was found suitable for their development. When the temperature fell below 8°C. development of fructifications was gradually stopped. *P. mitis* appeared to be very sensitive to temperature conditions and it was also necessary to increase the amount of 'accelerator' to 40% by weight for ready development of the sporophores. Fructifications of both the fungi when sectioned showed well-developed hymenia with normal basidia and basidiospores.

Fructifications of both *S. sanguinolentum* and *P. mitis* began to develop readily when small sections of freshly felled infected trees were brought into the laboratory and kept outside in the open, during the months of November to January (Pl. I, fig. 4). Occasional showers kept the poles moist and a comparatively low temperature was found to be suitable for their development. In this respect *P. mitis* appeared to be very sensitive to outside temperature for it was also observed that a range of temperature between 8°C. and 15°C. was found favourable for their development.

During the rest of the months the fungi lived well in the moist wood but did not fructify at all. Similar infected poles were also kept moist in diffused light and temperature (18°C.—21°C.) of the laboratory. *P. mitis* formed dense strands of white mycelium on the surface of the wood (Pl. II, fig. 4), but did not produce any fruit-body. Small resupinate fructifications of *S. sanguinolentum*, however, developed at the cross-section-ends of the poles but their growth appeared to be very slow.

Decay of Spruce

Microscopic details of the rot

In order to find out the extent and effects of the mycelium within the host, pieces of wood in various stages of decay were sectioned both free hand and with the microtome. Transverse, radial and tangential sections of the wood, about 15–20μ thick, were cut without any special treatment, from freshly felled poles. For ordinary purposes free hand sections were found to answer the requirements well to ascertain the presence of mycelium within the host tissue. Differential stains such as those recommended by Hubert (1922), Cartwright (1929) and others were tried but the method proposed by Cartwright was found to be very satisfactory in rendering the hyphae visible and easy to manipulate.

In an early stage of decay caused by *S. sanguinolentum*, the mycelium was often scanty but later it was distributed throughout all the elements (Pl. V, fig. 6) being particularly plentiful in the medullary rays which seemed to be the first element of the wood to be attacked. The hyphae, sometimes filling entire cavities of the elements were of two kinds,—firstly, rather fine, profusely branched, hyaline, about 1–2μ across and secondly, a coarser hyphae (Pl. VI, figs. 1–3) of about 3–5 (6)μ wide. The larger hyphae were hyaline or pale yellow in colour, occasionally with reddish-brown contents and usually bearing large, simple or double clamp-connections (Pl. VI, figs. 2 & 3). Similar double clamp-connections on coarser hyphae were recorded in two samples of spruce by Jorstad and Juul (1931). Clamp-connections were rare on the finer hyphae. The finer hyphae appeared to be more numerous and probably were the first to invade the uninfected parts. These could be definitely demonstrated in properly stained preparations even in an incipient stage of decay in which the coarser hyphae with double clamp-connections had not been observed. The hyphae at first ramified through the pits but later directly through the cell-walls forming numerous bore-holes (Pl. VII, fig. 4). The much branched finer hyphae in passing through the cell-walls exhibited little or no diminution in diameter while the larger hyphae when passing through the cell-walls, became markedly attenuated or constricted towards the apex. The photograph (Pl. VI, fig. 4) submitted in evidence shows clearly the bore-holes slightly wider in diameter than the contained hyphae; these bore-holes were formed in advance by enzyme action at the point of contact of the hyphae. The bore-holes were usually circular or somewhat cylindrical and plentiful in an advanced stage of decay. Many of the ray parenchyma cells and resin canals (Pl. V. fig. 5) were filled with

closely interwoven mycelium associated with a yellowish gum-like substance. These were also occasionally present in the tracheids. Similar plugs of gum-like materials giving the appearance of a zone-line had also been observed by Cartwright and Findlay (1946). In passing through the bordered pits the hyphae destroyed the closing membranes, thus enlarging the opening. In an advanced stage of decay the walls of the bordered pits were dissolved by enzyme action as shown by the smooth contours of their surfaces and their circular form. The wood in an advanced stage of decay (Pl. VI, fig. 6) showed that the secondary walls of the tracheids had been gradually dissolved outwards towards the middle lamella and this appeared to be a slow process.

The microscopic details of the rot caused by *P. mitis* were more or less similar to those caused by *S. sanguinolentum* except for the following differences. The hyphae were exceedingly narrow, much branched, hyaline, about 1–1.5μ across and plentiful in the decayed wood (Pl. VII, figs. 1 & 2) but slightly wider hyphae up to 3μ wide were also present. Simple clamp-connections (Pl. VII, fig. 2) were very numerous, almost at every septum; the modes of hyphal penetration were through the pits as well as through the cell-walls (Pl. VII, fig. 2). The bore-holes (Pl. VII, fig. 2), exceedingly small, more or less circular in outline, were very numerous. The walls of the bordered pits (Pl. VII, fig. 3) were broken down early. Entangled masses of hyphae were quite common in the tracheids. The ray parenchyma cells were filled with interwoven hyphae and yellowish or brownish gummy materials but the latter were rare in the tracheids.

Microchemical studies

Microchemical tests for lignin and cellulose in the sound and partly decayed wood have been recorded. This was done in the usual way by staining the sections of wood with phloroglucin and hydrochloric acid for lignin and with chlor-zinc-iodine for cellulose. The combination stain, safranin and fast green which differentiates lignified (red staining) and unlignified (green staining) structures and has superseded the old safranin and haematoxylin combination, was tried. Various other microchemical stains were also employed, most of which agreed closely with the changes indicated by the use of above-mentioned stains. It is ,however, not wished to draw any definite conclusion from these stain-reactions alone, since it has been recently shown (Harlow 1928; Hirt 1927) that these microchemical stains are not infalliable indicators of the chemical changes that take place during the process of decay in the wood. Nevertheless, these stains are still used in such work to indicate the presence of lignin and cellulose.

In the normal wood the middle lamellae of all the elements stained red. The secondary walls of the wood tracheids were partly light red and partly green but sometimes entirely green. The secondary walls of the ray cells were however, occasionally partly green. The secondary walls of the epithelial cells of the resin canals were so thin that possibly

the red tinge came from heavily stained middle lamellae. The bordered pits were mostly pinkish but with a faintly greenish tinge near the aperture.

In the decayed wood a light red to pink colour was more noticeable over the entire sections. The middle lamellae remained red but they were light red to somewhat pinkish in the way and epithelial cells of the resin canals. The secondary walls of all the elements were predominently pinkish but occasionally light red, and faintly greenish to almost colourless surrounding the lumen. The bordered pits were often entirely greenish in colour.

Table 1. *Results of staining with safranin and fast green for cellulose and lignin in normal and partially decayed wood of* Norway spruce (*Picea excelsa*)

		CELLULOSE (Fast green)	
Elements	Normal Wood	Wood decayed by *S. sanguinolentum*	Wood decayed by *P. mitis*
Wood tracheids	Secondary walls surrounding the lumen either green or partly green and extending up to middle-lamella, occasionally almost entirely green; bordered pits often faintly green surrounding the aperture.	Secondary walls round the lumen faintly green, often partly light green, and extending up to middle lamella.	Secondary walls in the summer wood, occasionally partly pale green; spring wood mostly pale green round the lumen; bordered pits often greenish.
Ray cells	Secondary walls often partly greenish.	Occasionally faintly green or colourless.	Occasionally colourless.
Resin ducts	No reaction	No reaction	No reaction
		LIGNIN (Safranin)	
Elements	Normal Wood	Wood decayed by *S. sanguinolentum*	Wood decayed by *P. mitis*
Wood tracheids	Primary walls deep red; secondary walls lighter red; bordered pits mostly pink.	Primary walls red; secondary walls mostly pink towards the middle lamella.	Primary walls red; secondary walls almost wholly light red to pink, occasionally partly so; bordered pits often pinkish.
Ray cells	Primary walls red; secondary walls light red.	Primary and secondary walls mostly pink.	Primary walls red or light red; secondary walls mostly light red to pink.
Resin ducts	Red	Pink	Light red.

Certain investigators in using safranin and fast green as a combination stain state that the red colour indicates the presence of lignin and that the unlignified tissues are stained green. Accordingly, it can be broadly

interpreted that the fungi in question gradually delignify all the elements but also utilize to a great extent the cellulosic materials of the wood. It would be hardly wise to draw any definite conclusion from the above staining reactions, since when other stain combinations such as gentian violet and Bismarck brown are used the partly brownish colour of the epithelial cells do not necessarily indicate lignin.

Table 2. *Results of staining for cellulose and lignin in normal and partly decayed wood of* Norway spruce (*Picea excelsa*) *with chlor-zinc-iodine and phloroghicin-HCl respectively*

		CELLULOSE (Chlor-zinc-iodine)	
Elements	Normal wood	Wood decayed by *S. sanguinolentum*	Wood decayed by *P. mitis*
Wood tracheids	Secondary walls near the lumen mostly blue, sometimes light blue, in summer wood less distinct; primary walls occasionally faint blue; bordered pits partly faint blue in most cases.	Secondary walls near the lumen light blue mostly, particularly in the spring wood; primary walls occasionally partly light blue; this reaction more conspicuous than that in normal wood; bordered pits faint blue at places.	Secondary walls partly faint blue at places; reaction more feeble than that in normal wood; primary walls at places faint blue in spring wood.
Ray cells	Secondary walls partly faint blue, particularly of the ray tracheids.	Secondary walls partly light blue.	Secondary walls mostly colourless.
Resin ducts	Partly faint blue	No reaction	No reaction
		LIGNIN (Phloroghicin-HCl)	
Elements	Normal wood	Wood decayed by *S. sanguinolentum*	Wood decayed by *P. mitis*
Wood tracheids	Primary walls deep red; secondary walls lighter red near the middle lamella to pink near the lumen; often entirely light red or pink; bordered pits pink.	Primary walls red or pink; secondary walls mostly pink; light pink particularly near the rays; bordered pits pink or colourless.	Primary walls red or pink; secondary walls light red or pink, occasionally faint pink; bordered pits often colourless near the aperture but mostly pink.
Ray cells	Primary walls red; secondary walls pink.	Primary walls pink; secondary walls pink; occasionally both colourless.	Primary walls pink; secondary walls light pink; occasionally both faintly pink.
Resin ducts	Mostly red	Pink, occasionally red	Mostly red

Phloroglucin–HCl.—In normal wood the middle lamellae of all the elements stained a deep red. The secondary walls changed from light red near the lamellae to somewhat pink near the lumen. Occasionally, they were entirely light red or pink. The secondary walls of the ray cells

and the walls of the bordered pits were pink. The epithelial cells of the resin canals remained red.

In the decayed wood the middle lamellae of the wood tracheids remained red or became pink and those of the ray cells mostly pink. The secondary walls of the wood tracheids and ray cells mostly became entirely pink or lighter in colour but occasionally remained light red. The middle lamellae and secondary walls of some of the ray cells became colourless. The walls of some of the epithelial cells turned pink.

Chlor-zinc-iodine.—In normal wood the middle lamellae seldom showed faint blue colouration. The secondary walls of the wood tracheids were mostly blue or light blue and those of the ray cells, faint blue. This was particularly the case with the ray tracheids. In the summer wood this reaction was less distinct. The walls of the epithelial cells were partly faint blue.

In the decayed wood occasionally the middle lamellae remained partly faint blue. In wood decayed by *S. sanguinolentum* the secondary walls of the wood tracheids showed intense cellulose reaction. They were mostly light blue, particularly in the spring wood and partly light blue in ray cells. The bordered pits were faint blue, particularly in the spring wood. In wood decayed by *P. mitis*, on the other hand, the secondary walls of the wood tracheids showed feeble cellulose reaction, being partly faint blue at places. The secondary walls of the ray cells were mostly colourless. The bordered pits remained colourless in both cases.

The pink colouration of the secondary walls of the wood tracheids and ray cells when treated with phloroglucin-HCl and the blue or light blue colouration in response to chlor-zinc-iodine, indicate that they are only moderately lignified. The chemical changes indicated by these staining reactions clearly show that there was delignification of the lignified parts of the secondary walls associated with the digestion of cellulosic materials of the wood. In wood decayed by *S. sanguinolentum* some of the secondary walls showed only pale pink colouration but mostly stained light blue throughout, suggesting partial removal of lignin from those walls. This reaction was more conspicuous than that in the normal wood. In case of *P. mitis* there was least cellulose reaction and the walls turned red or pink, suggesting the presence of more lignin in these walls.

Sulphuric acid test.—Sections of normal and decayed wood corresponding to those used for microchemical stains were placed upon slides and treated with 72% sulphuric acid as suggested by Ritter (1925). Immediately, in sections of normal wood, the cells swelled up rapidly and broke apart into fragments in the spring wood. The secondary walls of the wood tracheids became transluscent and partly dissolved while the network of the middle lamellae remained intact in the summer wood but torn apart in the spring wood. The ray elements remained completely unaffected but their secondary walls became swollen and partly dissolved. The violent action during the process was due to the presence of considerable amount of cellulose in the cell-walls of the sound wood.

The swelling of cellulose preceeding dissolution forced all the elements apart in the spring wood.

In sections of wood decayed by both *S. sanguinolentum* and *P. mitis* fragments of secondary walls of the wood tracheids were dissolved leaving the network of middle lamellae and wood elements in place on the slide. In both cases the secondary walls of the tracheids became swollen, partly dissolved and semi-transparent. The network of middle lamellae mostly remained in tact though broken off at places in the spring wood. There was much less distortion of the elements in spring wood than those in the normal wood. The ray elements mostly remained unaffected and portions of their secondary walls were the only parts dissolved.

It may be concluded that the less violent action and consequently less distortion of the wood elements in the decayed wood were due to the presence of less cellulose than that in the sound wood. This is particularly true in case of wood decayed by *P. mitis*. Though not conclusive in itself, this test at least indicates the presence of less cellulose in the wood decayed by *S. sanguinolentum* and *P. mitis* respectively than that in the normal wood.

Decay resistance tests

In order to determine the natural resistance and progress of decay of spruce samples caused by *S. sanguinolentum* and *P. mitis* separately, under controlled condition in the laboratory, wood-block cultures of these fungi were made in the following way. In general, the method recommended by Baxter (1925) was employed with slight modification.

Small bits of actively growing mycelia were transferred to sterile 2 per cent malt agar slants in 1000 c.c. Erlenmeyer flasks. Each flask contained 250 c.c. of the medium which was slanted after sterilization. After inoculation the flasks were incubated at 23°C. in darkness. Within a week the mycelia covered the surface of the slants and were ready to receive the wood blocks.

The wood blocks measuring $2'' \times \frac{1}{2}'' \times \frac{1}{2}''$ were obtained from sound wood of Norway spruce. These were planed and serially numbered on all faces and dried to a constant weight in an oven maintaining a temperature of 60°C. A comparatively low temperature was preferred to avoid the harmful effects of rather high temperatures on the composition of the wood blocks. Before sterilization these test-pieces were thoroughly soaked in distilled water, three such pieces were put in each Roux tube having distilled water at the bottom, plugged and sterilized in an autoclave at 15 lbs. pressure for 10 minutes. The idea of sterilizing the wood blocks in this way was to keep the atmosphere around them more or less saturated during the process so that little loss of water could take place from the wood blocks and consequently lesser time was necessary for sterilization as pointed out by Chidester (1937, 1939). The test-pieces were then taken out individually from the Roux tubes by a sterile forceps and exposed to fungal action by placing them on cultures of *S. sanguinolentum* and *P.*

mitis (Pl. VIII, figs. 1,2). These flasks, each containing six samples of wood were kept at a constant temperature of 23°C. in darkness.

In order to obtain a significant loss in weight the periods of exposure to fungal attack were 4 months and 8 months. After completion of the tests the flasks were opened, the superficial mycelium was carefully removed without damaging the wood-blocks (Pl. VIII, figs. 3 & 4) weighed and again dried to a constant weight in an oven at 60°C. The final dry weight subtracted from the original dry weight gave the loss in weight of the wood blocks due to decay in 4 and 8 months. The results are presented in Table 3.

The average moisture-content of the wood blocks before they were exposed to fungal action was determined by weighing another set of 12 blocks, soaked and sterilized in the same way. The final dry weights when subtracted from these gave the initial moisture-content of the wood-blocks before the experiment. This was necessary to keep the moisture-content of the wood-blocks much above the 'fibre-saturation-ponint' which was necessary for the activity of the wood-rotting fungi. In this way the moisture-content of the inoculated wood-blocks after the tests was also determined. The initial moisture-content of the wood blocks before the experiment averaged 158%. After both 4 and 8 months' test it was found to lie between 52–62%.

Table 3. *t-test of comparison of loss in dry weight of wood blocks of* Norway spruce *exposed to* S. sanguinolentum *and* P. mitis *after* 4 *and* 8 *months.*

Test Fungus	Months	Number of Replicates	Mean loss in dry wt. (%)	S.E.	Comparison between Means	*t*	Probability
S. sanguinolentum	4	12	14.6 A	±1.25	A and B	14.23	<.01
	8	12	29.0 B	±3.11	C and D	51.89	<.01
P. mitis	4	12	12.5 C	±1.21	A and C	3.12	<.01
	8	12	23.8 D	±2.16	B and D	5.40	<.01

It is evident from the *t* values of Table 3 that the loss in weight due to *P. mitis* is significantly less then that caused by *S. sanguinolentum*. The loss in weight after 8 months is double of that after 4 months in each case, and is highly significant. It can, therefore, be concluded that the wood has low resistance to decay. Accordingly, spruce wood falls under Findlay's (1938) 'non-resistant group as this class undergoes average losses from 10–30% during four months' test.

Field inoculations

Inoculation experiments were carried out with young twigs in the laboratory and also with trees in the field. In the former case the method described by Brooks and Moore (1923) was followed. In this experiment, 2 to 3-years-old twigs of Norway spruce, about 4″ along, were kept with their ends in water in the laboratory, their upper ends were cut off and spore-suspensions of *S. sanguinolentum* and *P. mitis* in sterile water were applied separately to the freshly exposed surface and never allowed to dry out. These were placed under a bell-jar in which a relatively high humidity was maintained. Longitudinal sections through the inoculated ends of the twigs were made at intervals of 24, 48, 72 and 96 hours after the spores had been added. Sections cut after a period of 24 to 48 hours showed that the majority of the spores had germinated on the surface and had sent hyphae down into the elements of the wood. A few spores were, however, found to enter through the cut ends of the tracheids and had germinated there. Sections cut at intervals of 72 a d 96 hours indicated that the spores had formed vigorous mycelia and invaded all the elements of the twig. Since these twigs could not be kept in an actively growing condition, their invasion by *S. sanguinolentum* and *P. mitis* could not be regarded as an evidence for their parasitic action on trees. Nevertheless, it can be said that if by chance the spores some how alight on the surface of the wound, they can germinate under suitable conditions producing germ-tubes which in some way pass down into the elements of the wood.

Field inoculations on living trees were started in early March, 1950, at Glentress forest. Two inoculations were made on 50 to 60-years-old vigorously growing Norway spruce with each fungus and with equal number of controls below. The operation involved was to make a cross-incision through the bark on the trunk and the trees were inoculated by transferring actively growing mycelium into the wound by carefully lifting a flap of the bark still attached to it.

Prior to inoculation the surface of the bark was wiped out with absolute alochol and subsequently washed with sterile distilled water in order to free the surface from contamination as far as practicable. The flap was then covered with moist, absorbent, sterile cotton wool with a piece of thick paper over it in order to prevent drying out and firmly bound with a string. Towards the end of March the trees were examined. After removing the string and the cotton wool it was found that in both cases the trees seemed to have taken the infection. The inoculated areas were distinctly bulged out with conspicuous secretions of resin. The controls, on the other hand, remained flat, rather depressed and there was little resin flow. These trees were again inspected in July and it was observed that in both cases the controls remained flat and were healing up satisfactorily. There was very slight resin secretion within the cut. In case of *S. sanguinolentum* the wounds were gaping open, looked more or less swollen, somewhat spindle-shaped and there was copious secretion of resin running down the stem (Pl. VIII, fig. 6). From the appearance of the bark it looked as if the infection had spread on the left side about

1½" from the point of infection. Infections with *P. mitis* also looked somewhat oval and the resin was running down the stem (Pl. VIII, fig. 5). The infection appeared to have spread about an inch from the point of infection. In November, after 8 months, the trees were examined and the progress of infection was noted. All controls healed up. In case of *S. sanguinolentum* the bark was gradually sinking and there was more resin flow from the infected wounds than those caused by *P. mitis*. From the outward indications it could be said that both the fungi had infected the living trees through wounds, although *S. sanguinolentum* seemed to be more active of the two pathogens and produced more resin which ran down the stem. At this time no fruiting-bodies of the fungi were observed and this was only to be expected since both the fungi was slow growers within the tissues of the host.

In March, 1951, the trees were examined and one of them felled. The internal conditions of the inoculated areas were microscopically examined; mycelia of both *Stereum sanguinolentum* and *Pleurotus mitis* respectively were present in the elements of the wood. Copious gum formation had taken place within the medullary rays.

Microscopic examination of the infection by *Stereum sanguinolentum* showed characteristic reddish-brown rot in the sapwood. The mycelium had spread approximately eight inches both above and below the point of infection. Spread in the transverse direction was about half an inch. In the infection with *Pleurotus mitis* the yellowish brown rot is confined to the sapwood and extended about two inches both above and below the point of inoculation; the spread in the radical direction is about one quarter of an inch. Subsequently re-isolations of the two pathogens from infected tissues were made and these agreed with the original cultures of *S. sanguinolentum* and *P. mitis* in all essential details.

Discussion

This study has been undertaken to obtain information of a serious disease causing bark necrosis on the trunk on Norway spruce at Glentress forest, near Peebles, in Scotland. The injuries are restricted to the stem mostly within the pruning limits and have been diagnosed as having arisen from the attack of *Stereum sanguinolentum* and *Pleurotus mitis*. These fungi have entered through the dead branches or other injuries such as pruning cuts that might have taken place at the beginning or the end of the growing season. Although similar injuries on Norway spruce have been attributed by Day (1950) as entirely due to frost damage, there is no such evidence on trees under consideration. Gäumann (1950) has described similar injuries in Switzerland on the bark of Norway spruce and silver fir, in a situation exposed to frequent hailstorms and strong electrical discharges which killed strips of cambium; infection with *P. mitis* followed. Careful examination of the infected trees and the anatomy of the injuries did neither reveal the presence of frost cracks and frost rings in their wood nor was there any sign of bark injury due to electrical discharges. In addition, during the past few years there is no evidence

that frost injury has really occurred in the crop as a whole. On the other hand, close examination of the wounds reveals that the cambium has been killed near the pruned branches and the indications are that the causal agents after entering through the wound in the dead branches or pruning cuts, have killed the cambium and spread from the periphery to the centre of the wood, thereby rendering the wood unfit for commercial purposes. In the absence of any other evidence, it may be taken that these wounds have served as initial portals of entry. Both the fungi are common in the plantation as saprophytes on fallen branches lying on the floor of the forest during the colder months of autumn and the early part of winter.

Low air-temperatures appear to be favourable for the development of the fruiting bodies and during the spells of mild weather, i.e., late autumn and early spring when the trees are in a dormant condition, the spores after being carried away by the wind alight on the cut surfaces of the pruned branches where they germinate and penetrate the wood. It has been experimentally proved that a comparatively low temperature is necessary for the development of the fruit-bodies, particularly in case of *P. mitis*, and that the spores can germinate within a short time on the cut surfaces and infect the twigs under moist conditions. Inoculation experiments on living trees carried out in the field during early spring have shown that both the pathogens can infect standing trees through wounds in the bark and from the results of inoculation they can be regarded as wound parasites on normal vigorous trees. Although no infection experiments have been carried out with dead branches, it can be assumed that presumably they can also infect them as they can live well in the wood of the fallen branches. As long as the trees are in sap and the air-temperatures are high, the fructifications cannot develop and consequently there is comparatively less chance of any infection taking place during the period. In summer, the fungi thrive well in the dead branches in a moist cool situation. Experiments with infected trunks point to the conclusion that the fungi can live within the wood throughout the year and begin to fructify only during the months of October and November, when the outside temperature is low and favourable for their development.

Both *S. sanguinolentum* and *P. mitis* have been isolated from standing trees and found to be the cause of the disease. Previous investigators of spruce have failed to record that *P. mitis* can cause an important decay in wood of living trees in Great Britain. In transverse sections of the infected poles, the general appearance of the rots in the early stages of decay caused by *S. sanguinolentum* and *P. mitis*, is very similar and in this study both decays were at first thought to be caused by the former fungus until cultural and microscopic investigations proved differently. This has emphasized the importance of conducting cultural studies in support of decay determinations which are often based on gross appearance of the rots. It has, however, not been possible to find out whether both the pathogens have infected the trees at about the same time or whether one of them has entered first and weakened the tree, thus preparing the ground for a secondary infection with the other fungus. At the present moment it is difficult to estimate the relative importance of the two fungi

in bringing about infection and decay in living trees and the nature of interactions existing between them but this association of two pathogens has no doubt greatly enhanced the importance and complex nature of the problem. However, both the fungi have been isolated not only from the same pole but the respective rots were only a few centimetres apart from each other, being separated by healthy tissues. From the number of isolations so far made, it is apparent that *S. sanguinolentum* is more widely distributed within the host tissues than *P. mitis*. Further investigations in future with large number of infected trees would possibly throw some light as to their distribution within the host plant. In consequence, the amount of damage in the field caused by the pathogens could not be determined. But experiments on durability tests on spruce timber carried out in the laboratory have shown that spruce timber is 'non-resistant' to decay caused by *S. sanguinolentum* and *P. mitis*. There is no doubt, however, that such injuries caused by these fungi, will cause serious deterioration in the value of timber and the damages caused by them may become very serious.

Control measures

As wounds on the stem appear to be closely associated with the disease, the treatment should be preventive and not curative. Although further infection experiments on living spruce by these fungi, following pruning of living branches, are necessary, control measures based on general principles can be suggested and these may be put into effect in the affected areas in order to prevent spreading of the disease. Great care should be taken during pruning operation and thinning in order to avoid injuries caused by careless work. Pruning should be done close to the stem but not so close as to damage the bark and no projecting stubs should be left. The pruned surface should be smooth and covered with paint in order to protect the surface from moisture and to prevent penetration of germ-tubes from germinating spores. An alternative method is to apply a fungicide to the cut surface to kill any superficial spore. Removal of all fructifications from the dead branches, as often suggested for preventing dissemination of spores, seem to be an impractiacable proposition, since, tiny fructifications like those of *S. sanguinolentum* would invariably be overlooked during the search. Up to the present the effect on the trees of pruning has been limited almost entirely to the extent of healing process. The evidence which has now been brought forward concerning the infection by fungi as the result of wounding must now be seriously considered.

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EXPLANATION OF PLATES I—VIII

Plate I

Fig. 1. A portion of the plantation at Glentress forest showing infected Norway spruce trees characterised by ill-defined, flat and longitudinal depressions of the bark extending from the butt upwards.

Fig. 2. A longitudinal crack in the depressed bark accompanied by an outflow of resin.

Fig. 3. Fructifications of *Stereum sanguinolentum* on the cross-section-end of a freshly felled trunk of Norway spruce lying on the floor of the forest.

Fig. 4. Effuso-reflexed fructifications of *S. sanguinolentum* have developed on the rotted areas at the cross-section-end of a Norway spruce pole.

PLATE II

Fig. 1. Fructifications of *Pleurotus mitis* on dead branch of Scots pine.

Figs. 2. & 3. Typical fructifications of *P. mitis* on dead twigs of spruce showing various stages of development.

Fig. 4. Mycelial strands of *P. mitis* developed on the surface of the infected pole under moist conditions.

Fig. 5. Fructifications of *P. mitis* with abnormally elongated stipes and mycelial strands formed within the cracks of spruce poles.

Fig. 6. Fructifications of *P. mitis* in culture. (×4).

PLATE III

Figs. 1–4. Cross sections of Norway spruce poles showing various stages of decay; characteristic light to dark irregular or wedge-slaped areas can be seen from the periphery to the centre of the mature wood.

Fig. 5. Longitudinal section of the wood showing the rotted areas.

Fig. 6. Fructifications of both *S. sanguinolentum* and *P. mitis* have developed on respective rots at the cross-section-end of the infested pole under experimental conditions.

Fig. 7. A longitudinal crack in the bark with copious outflow of resin.

PLATE IV

Fig. 1. Effuso-reflexed fructifications of *S. sanguinolentum* formed within test-tubes in artificial culture.

Fig. 2. A resupinate fructification of *S. sanguinolentum* formed on wood-blocks of Norway spruce in culture.

Fig. 3. Culture of *S. sanguinolentum*, 21-days-old, on *potato-dextrose agar* and *malt agar* in darkness at 18°C. (a, c) and 23°C. (b, d) respectively.

Fig. 4. Cultures of *P. mitis*, 21-days-old, on *potato-dextrose agar* and *malt agar* in darkness at 18°C. (a, c) and 23°C. (b, d) respectively.

Fig. 5. Culture of *S. sanguinolentum*, 21-days-old, on *malt agar* at 23°C. in darkness.

Fig. 6. Culture of *S. sanguinolentum*, 21-days-old, on *potato-dextrose agar* at 23°C. in darkness.

Fig. 7. Culture of *P. mitis*, 21-days-old, on *potato-dextrose agar* at 23°C. in darkness.

PLATE V

Fig. 1. Helicoid hyphae of the aerial mycelium of *S. sanguinolentum*. (×400).

Figs. 2 & 4. Aerial hyphae with double clamp connections of *S. sanguinolentum* in culture. (×900).

Fig. 3. Specialized, usually unbranched, flexuous hyphae with densely granular contents; characteristic of the submerged mycelium of *P. mitis*.

Fig. 5. Part of the radial section of the infected wood showing masses of hyphae of *S. sanguinolentum* within the horizontal resin canal. (×100).

Fig. 6. Distribution of the hyphae of *S. sanguinolentum* within the sections of decayed wood; the hyphae are abundant within the tracheids and they have passed mainly through the bordered pits. (×200).

PLATE VI

Figs. 1–3. Hyphae with simple and double clamp connections of *S. sanguinolentum* within the tracheids. (×450).

Fig. 4. Penetration of the cell-wall by the hyphae of *S. sanguinolentum;* the bore-hole is visibly larger than the penetrating hyphae. (×900).

Fig. 5. Part of a *t.s.* showing decay of Norway spruce caused by *P. mitis;* the spring-wood is the most affected part of the section. (×100).

Fig. 6. Part of a *t.s.* showing decay of Norway spruce caused by *S. sanguinolentum;* the elements of the spring-wood are badly damaged.

Plate VII

Fig. 1. Distribution of the hyphae of *P. mitis* within the sections of decayed wood; the hyphae have ramified through the bordered pits and also through the walls forming bore-holes. (×450).

Fig. 2. Hyphae of *P. mitis* within the tracheid showing simple clamp connections; the bore-holes can be clearly seen. (×900).

Fig. 3. Eroded bordered pits of the tracheids due to the activity of *P. mitis.* (×900).

Fig. 4. Hyphae of *S. sanguinolentum* passing through the walls of the bordered 'pits.

Plate VIII

Figs. 1 & 2. Wood-block cultures of *S. sanguinolentum* and *P. mitis* for testing decay resistance in the laboratory; photographed after 45 and 60 days respectively.

Figs. 3 & 4. Wood-blocks of Norway spruce showing decay after 4 months' exposure to *S. sanguinolentum* and *P. mitis* respectively.

Figs. 5–6. Infections on trunk of Norway spruce with *P. mitis* and *S. sanguinolentum* respectively, 5 months after inoculation.

A disease of Norway spruce S. N. Banerjee

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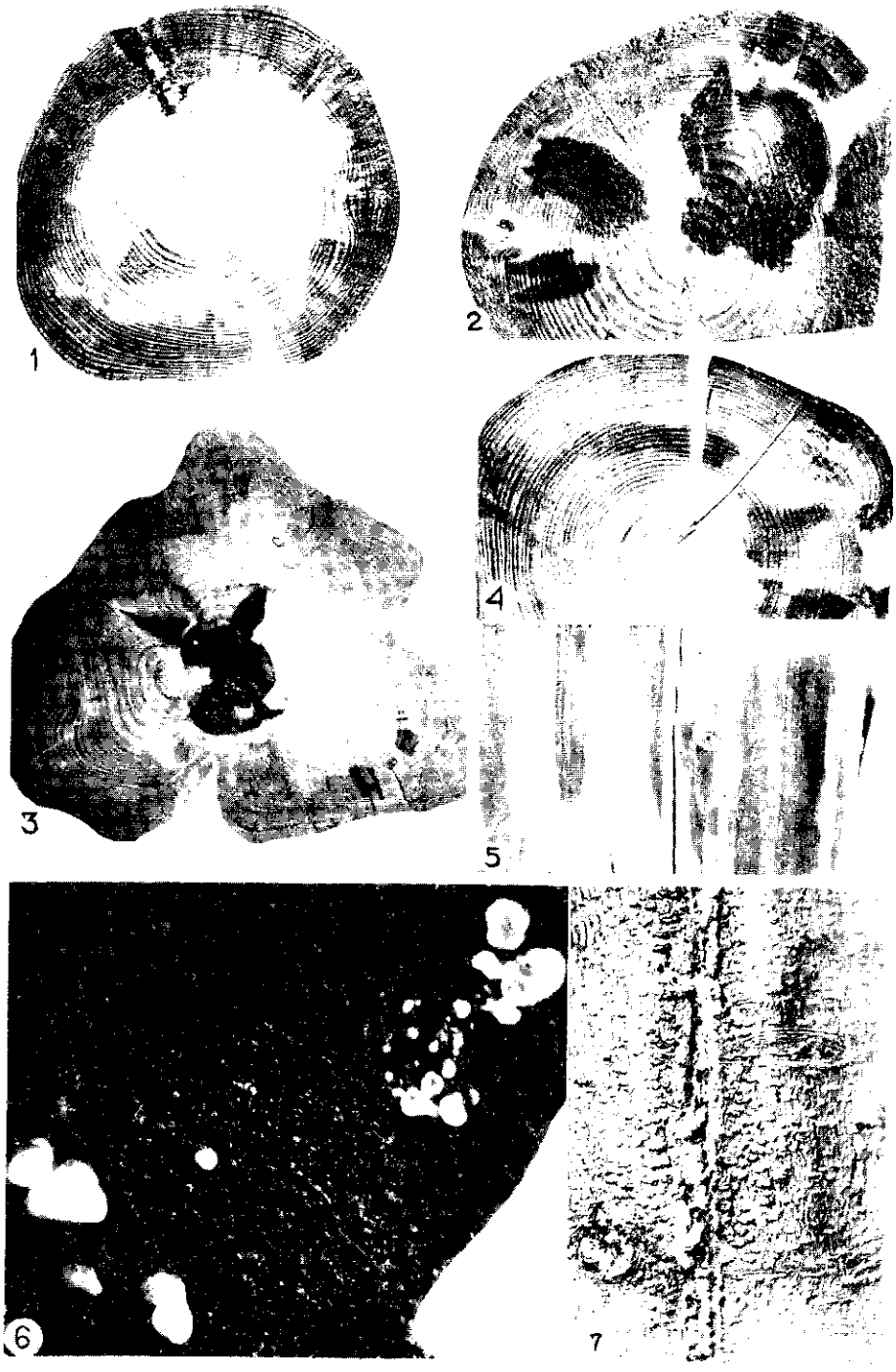
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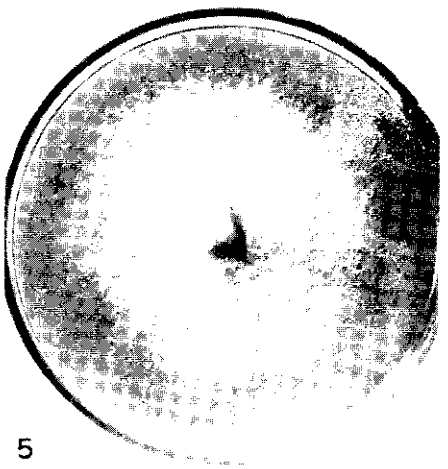

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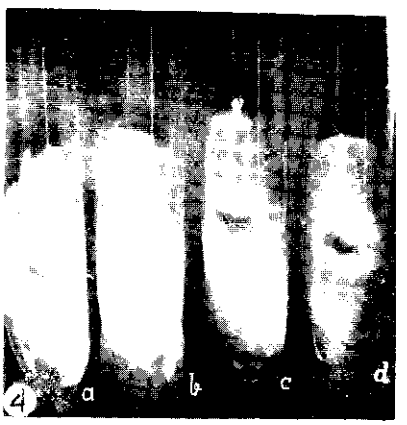

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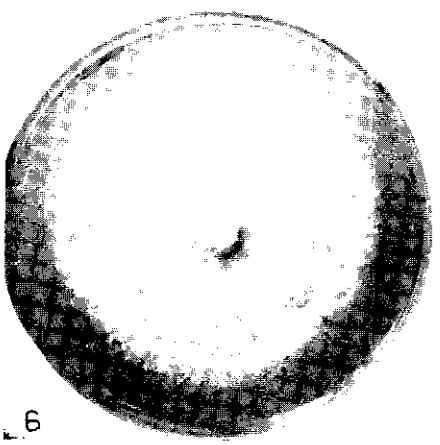

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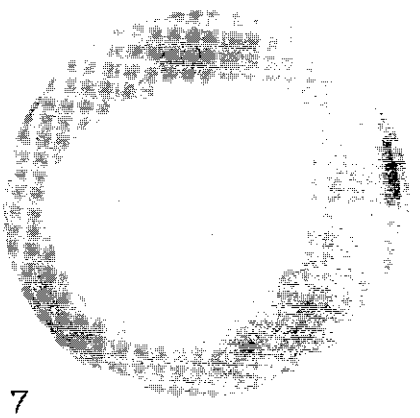

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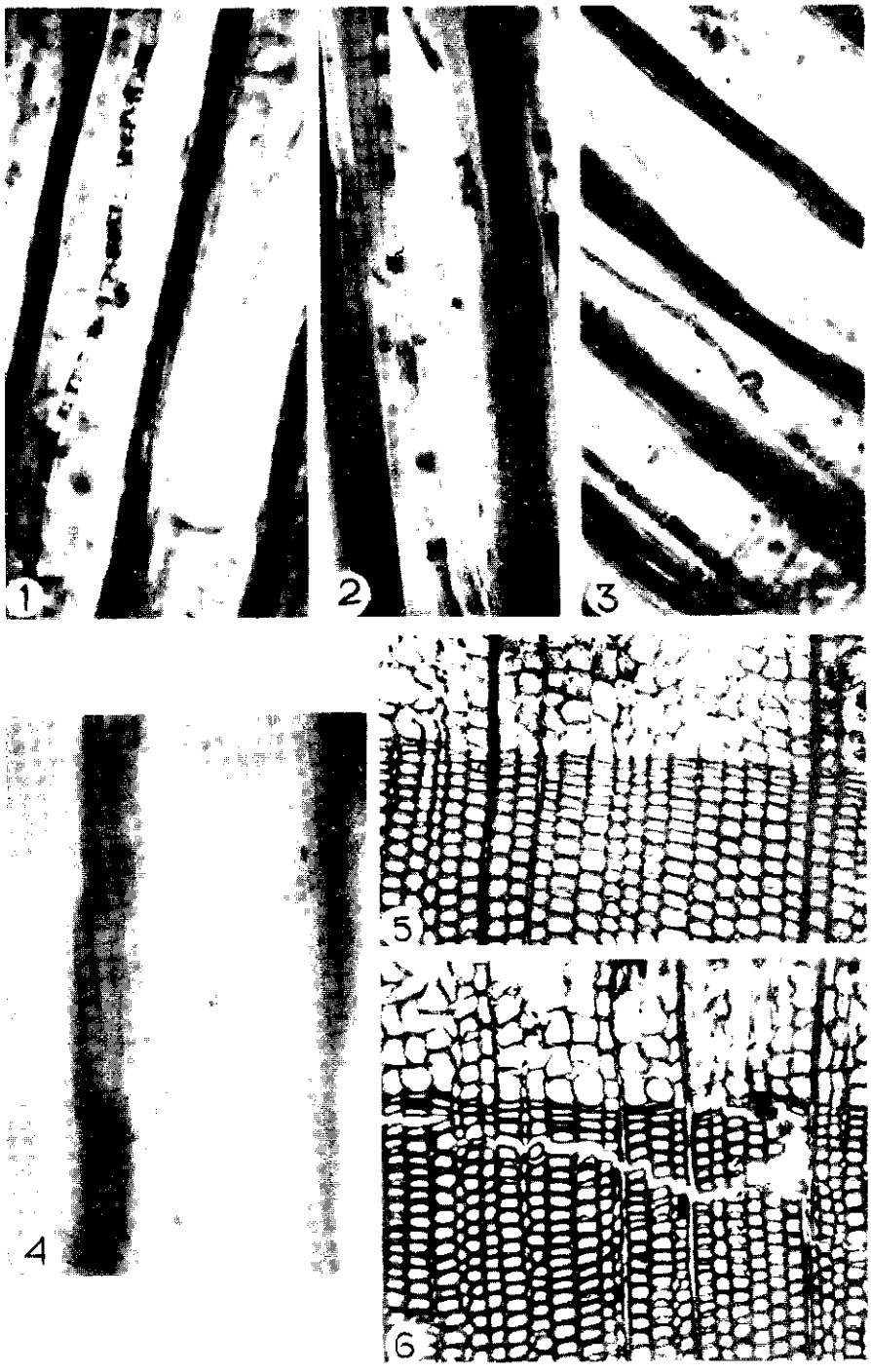
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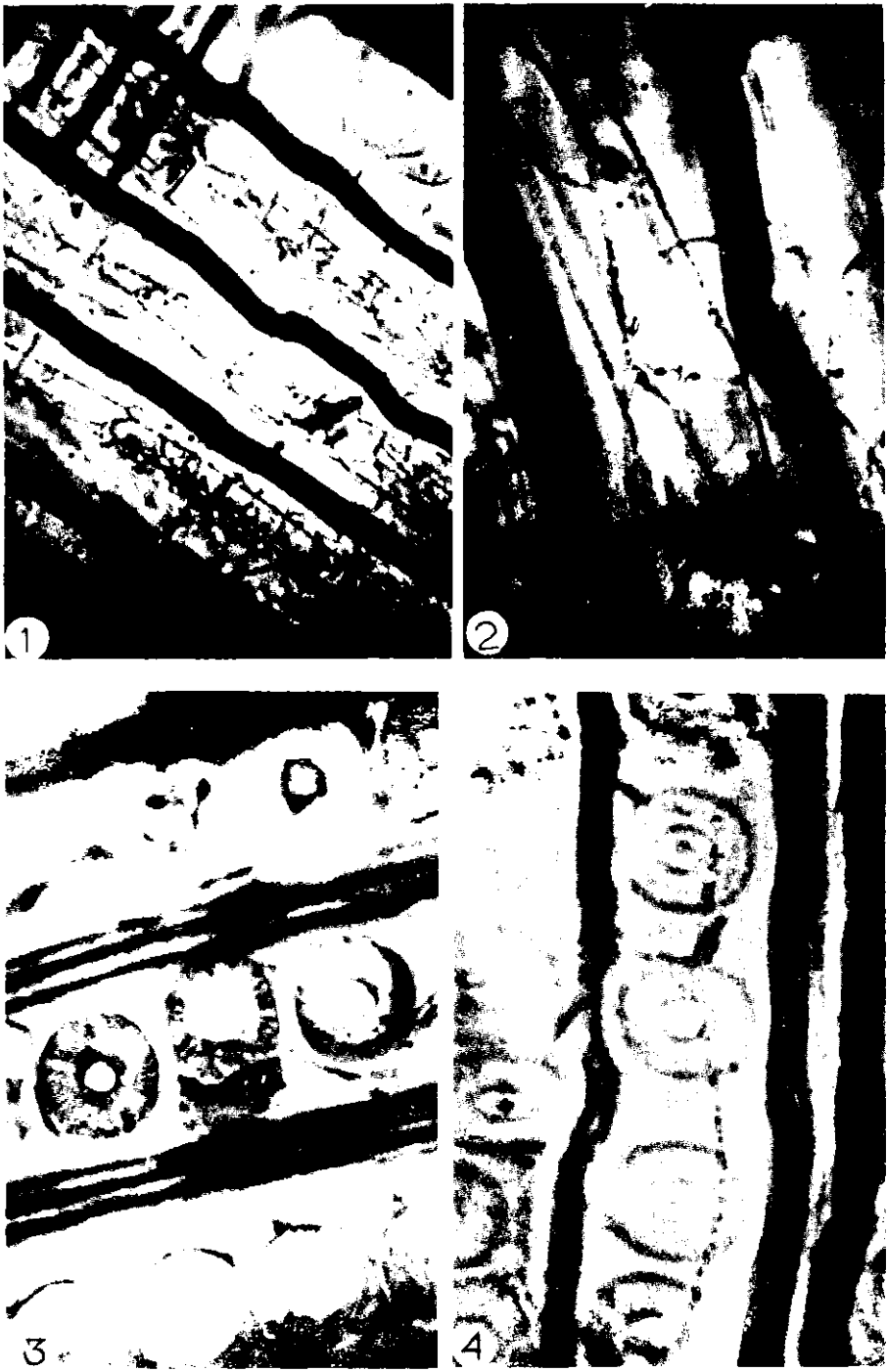
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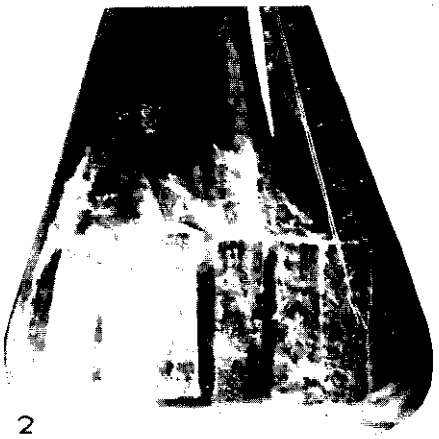

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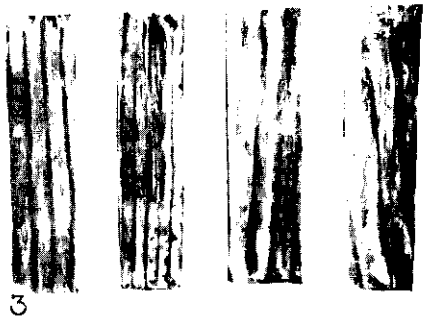

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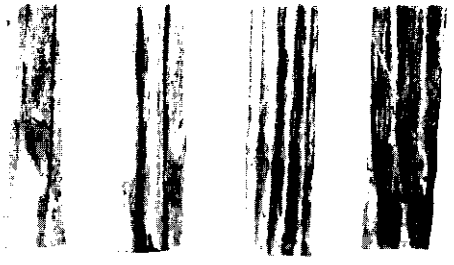

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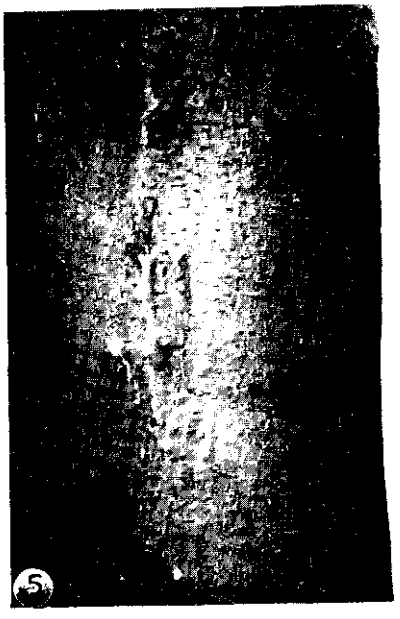
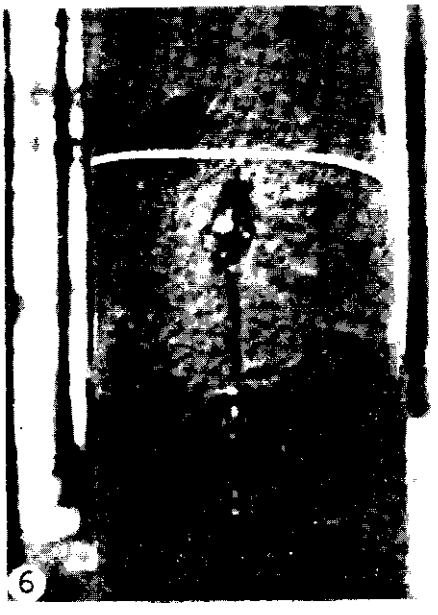


5



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