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1

STUDIES ON DECAYS OF FELLED TIMBER—II. A ROT OF SĀL CAUSED BY *DAEDALEA FLAVIDA* LÉV.

By

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(With Plates I to VI and 1 Text-Figure)

This investigation has been undertaken to study decay in stacked timber of Sāl (*Shorea robusta* Gaertn.f.) due to *Daedalea flavida* Lév.

The external morphology of the fungus growing on sāl logs has been fully described.

The mycelium from the infected logs has been isolated, grown in culture and compared with the original cultures of *D. flavida*, and found to be identical in every respect.

The gross characteristics of the rot have been described. The decay is mainly confined to the sapwood and with no apparent effect on the heartwood. The decayed sapwood shows characteristics of the 'stringy rot'.

Microscopic examination of the decayed wood shows that in an advanced stage of decay all the wood elements are attacked by thin-walled, hyaline hyphae with frequent clamp-connexions. The vessels are severely affected and often clogged by a mass of interwoven mycelial weft within their lumina. The gummy substances of the medullary rays are gradually dissolved and eventually completely digested. Wood parenchyma also disintegrates and finally cracks appear within the wood.

Microchemical studies show the chemical changes in the wood during the process of decay. Both lignin and cellulosic materials are destroyed. Primarily *D. flavida* is a lignin-destroyer, but in later stages of decay it also consumes the cellulosic materials.

Decay resistance tests in the laboratory show that if the moisture-content of the host-wood is maintained above the 'fibre-saturation-point', *D. flavida* can cause considerable damage to both sapwood and heartwood. The sapwood is 'perishable' and the heartwood is 'non-resistant' to the attack of the fungus. Furthermore, the tests also indicate that the secondary mycelium has greater decaying capacity than the primary one.

Toxicity tests following wood-block method have been performed with zinc chloride, ascu 'A' and borax. Observations indicate that secondary mycelium has the least tolerance for toxic substances, and ascu 'A' is the best preservative in comparison with the other two.

Primary mycelium has been obtained from a single spore and by subsequent pairing experiments with several primary mycelia, secondary mycelium is produced.

Basidiospores from fresh fructifications have been found to germinate on various media of which the media containing malt-extract have been found to be best suited for profuse germination within a comparatively short time.

D. flavida, being a 'white-rot' fungus, gives 'strong' oxidase reactions when grown on *malt-agar* containing 0.5% gallic or tannic acid.

Cultural characteristics of the isolate and secondary mycelium on 2.5% *malt-agar* under ordinary conditions of light and temperature of the laboratory have been described.

The secondary mycelium and the isolates from the decayed wood have been found to fructify in wood-block cultures under controlled conditions in the laboratory.

Introduction

Sāl (*Shorea robusta* Gaertn.f.) is one of the important timber-producing hardwood species in India, and forms an integral factor in the national economy. Recently, demand for sāl logs has greatly been increased due to various developmental schemes in this country. As a result, problems inherent to the utilization of sāl wood have become evident. One of the difficult problems arises due to decay of logs, posts, lumbers, etc., by the wood-rotting fungi. In conformity with the general neglect of Forestry and Timber Pathology in this country, little attention was paid in the past to the decays of felled timbers. A series of investigations concerning the destruction and disintegration of stacked timbers caused by fungi have already been undertaken in this laboratory. The present paper forms a part of the series and reports on the decay of sāl caused by *Daedalea flavida* Lév.

Daedalea flavida Lév. is one of the common wood-rotting tropical polypores in India. In Bengal its fructifications are abundantly produced during the rainy season (July to October), but new fructifications can appear even up to December, provided the logs on which they grow get regular supply of water. The severity of damage of stacked logs of sāl due to the attack of the fungus can be witnessed in any timber-yard of Calcutta and its suburbs, especially during the wet months of the year. It has been estimated that about 20–30% of the logs are infected with the fungus and thus become non-merchantable imparting a heavy toll to the timbermen.

Daedalea flavida was first reported and described by Léveille (1844)*. Like many other fungi it exhibits considerable variation in morphological characters, and, as such, the fungus appears in mycological literature under a variety of names. Berkeley described the species as *Daedalea hobsoni* (Saccardo, 1888). Lloyd (1911), while working on collections of the Polyporoid types at Leiden, mentions that the type collection of *Daedalea flavida* Lév. has been labelled as synonym of *Polystictus lenziteus* Lév., and according to him it is quite correct. He thinks that it is also synonymous with *Lenzites ochroleuca* Lév. and *Daedalea hobsoni* Berk. Bose (1920) at first described the fungus from Bengal as *D. hobsoni* Berk., but later publishes his researches (1938) on this species under the name *Daedalea flavida* Lév. He (1920-21) also thinks that fructifications of *Daedalea microzona* Lév., having regular pore-mouths and thinner context is a synonym of *Daedalea flavida* Lév., Presumably, *Trametes flavida* and *Lenzites flavida* (Lloyd, 1915) are also similar to the same fungus (Butler and Bisby, 1931). The host-range and geographical distribution of the fungus have recently been recorded by the writers in a previous communication (1956).

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* Cited by Saccardo (1888).

Source of material

The materials were collected during the rainy months of July to September, 1956, in the form of short, cylindrical sections of the infected logs of *Shorea robusta*, from the timber-yards of Calcutta and suburbs. The logs showing various stages of decay and with fructifications of *D. flavida* were selected and macroscopically examined in the field to note their characteristics as far as practicable. The cross-section-ends of the infected logs showed the presence of numerous, irregular rotted areas mainly in the sapwood. The demarcating line between the decayed sapwood and heartwood though being very sharp in most cases, no definite conclusion could be drawn in the field as regards the nature of the rot. For further detailed study, those decayed sections of the logs with fructifications were brought into the laboratory. Microscopic examinations of the sections from the rotted areas exhibited the presence of numerous, much-branched, septate hyphae with frequent clamp-connexions within the different wood-elements. The fruit-body of *D. flavida* has been found to be perennial and can resume growth under favourable conditions. In order to get regular supply of fresh fructifications, during the entire period of investigation, some of the logs with fructifications were kept moist in the laboratory. Moreover, the dried fructifications of the fungus when soaked with water readily revived and discharged viable spores copiously within a few hours.

The basidiocarp*

(Plates I & II, figs. 1 to 4)

Fructifications sessile; usually dimidiate and attached to the substratum by its comparatively narrow base, sometimes somewhat reniform, and often encircling the substratum from which it grows, rarely more or less circular in outline and attached to the upper surface of the woody substratum by its central base, sometimes partly resupinate; applanate; solitary, rarely imbricate and laterally confluent; tough, coriaceous to corky, often woody on drying; dimension very variable, 12–22 cm. (30–45 cm.) across, 8–15 cm. deep and 1·5–2·5 cm. in thickness. Margin thin, usually entire sometimes broadly lobed, white in colour. *Upper surface* smooth, rugulose or uneven; marked with concentric, raised or depressed, narrow or broad zones; colour when fresh Cinnamon-Buff or Clay colour, sometimes Zinc-Orange or Cinnamon-Orange with a few narrow Cinnamon-Brown rings towards the periphery, zonations becoming Light-Buff, Warm-Buff or Ochraceous-Buff and occasionally with a few Tawny-Olive rings towards the margin on drying. *Context* coriaceous to corky; about 5–12 mm. in thickness; white when fresh becoming concolourous with the hymenium on drying. *Hymenial surface* smooth; Milk-White when fresh, but changing to Light-Buff, Light-Ochraceous-Buff or Ochraceous-Buff on drying; pore-mouths typically labyrinthiform, in part at least in some

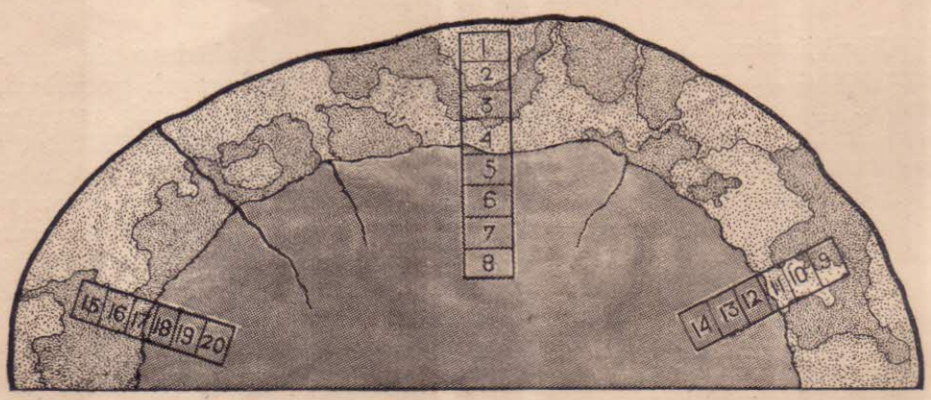
* Colour according to Ridgway (1912).

poroid at first, often becoming partly lamellate which anastomose with one another by thick dissepiments having obtuse edges, transitional stages from poroid to lamellate types quite common, sometimes torn into teeth; a narrow sterile zone at the margin always present; without any shining lustre; pore-tubes wide, deeping unequally into the substance of the context and not forming a distinct layer, about 8–15 mm. long. *Basidia* elongated and elevated; tetrasterigmatic and quadrisporous; dimensions about 12.5–20 × 1.5–5.5μ; with simple clamp-connexions at the base. *Basidiospores* hyaline, more or less oval but flattened at one side and with an excentric apiculus; thin-walled; smooth; dimensions 4–6 × 1.5–2.5μ.

Decay of Sāl

Isolation and identification of the causal organism of the rot

In order to study the causal organism in culture, the mycelia from the rotted tissues of the infected logs were isolated. Isolations were made from different portions of the rotted areas of the same and different logs which bore externally large number of fructifications of *D. flavida*. Following the method of Banerjee (1955), small pieces of infected wood were obtained from carefully selected regions of the logs and serially numbered (Text-fig. 1). After surface sterilization, small pieces of internal tissues from each block were aseptically transferred to sterile 2% *malt-agar* slants. The cultures were then kept under ordinary conditions of temperature (28–32°C.), humidity (62%–67%) and diffused light of the laboratory.



Text-fig. 1. Part of a cross-section of a decayed log of *S. robusta* showing the areas from which isolations were made (Diagrammatic).

Within a week mycelia grew out from the inocula obtained from the areas 1, 2, 3, 4, 9, 10, 11, 15, 16 and 17 and spread over the surface of the slants, while, the inocula from areas 6, 7, 8, 13, 14, 19 and 20 failed to produce any mycelium. It can, therefore, be concluded that the central core of the heartwood is entirely free from fungal hyphae, and is definitely in a

healthy condition while, the areas 5, 12 and 18 lying in contact with the sapwood show the presence of mycelia in them, although they appear to be sound macroscopically.

From these isolates several subcultures were made in 2% *malt-agar* slants which later served as stock cultures for further study. When critically examined, all the isolates exhibited identical macroscopic and microscopic characteristics and resembled one another in essential details. These were then compared from all aspects with the polysporus mycelium of *D. flavida* already made available for the purpose and found to be identical with the latter. The identity was further confirmed later when the isolates produced typical fruit-bodies of *D. flavida* under cultural conditions (PLATE VI, FIG. 26).

Macroscopic characters of the rot

The first externally visible symptom in the field is the formation of typical brackets of *D. flavida* of different sizes on the surface of the logs (PLATE III, FIG. 5). The cross-section-ends of the logs, however, show considerable sign of decay in the wood, and this appears to be very distinctive in character. In the early stages the demarcating line between the sapwood and heartwood is more prominent than that in the normal wood and this seems to be due to some gross changes in the appearance of the sapwood. The rot at this stage is confined entirely to the sapwood, which forms a conspicuous decayed zone surrounding a central practically unaffected heartwood.

In the first stage of decay the sapwood becomes pale brown in colour, which appears to be lighter than that of normal sapwood. With the gradual advancement of decay, the colour turns to pale yellow and finally to yellowish white, and this shows that considerable bleaching of the sapwood has gradually taken place. At first, the rotted areas appear at the periphery as irregular pale brown coloured areas, which are often separated from one another by broad or narrow brownish black zones (PLATE III, FIGS. 6 & 7). Gradually, as the decay proceeds, these rotted areas extend inwards upto the heartwood and also spread longitudinally and laterally as evidenced in both transverse and longitudinal sections (PLATE III, FIGS. 6–8). Eventually the rots coalesce to form larger areas and the individuality of the blackish brown regions in the sapwood becomes partially lost. Numerous rot-pockets become conspicuous at this stage (PLATE III, FIG. 6). Macroscopically the heartwood appears to remain unaffected.

In the later stages of decay, the texture of the sapwood loses its toughness and becomes soft, light and somewhat spongy. The wood also appears to be highly bleached, compressible, full of numerous rot-pockets consisting of masses of yellowish white-coloured fibres and form a stringy rot (PLATE III, FIGS. 6 & 7). The pockets are large, hollow and pale yellow to yellowish white in colour. No fungal hyphae have been externally recognized in the pockets. The reddish brown heartwood, on the other hand, remains apparently unaffected except for somewhat intensification of the colour in the regions directly abutting the decayed sapwood (PLATE III, FIG. 8). The fungus has been mentioned to cause a white spongy rot in

Shorea robusta by Bagchee, Puri and Bakshi (1955) and in other literature*, but the nature seems to be rather stringy than spongy. The stringy nature of the rot becomes obvious as the decayed portion of wood cannot be polished, as its fibres break off in minute needles over the sand paper owing to the 'brashy' nature of the decayed wood.

Microscopic details of the rot

Infected logs collected from the timber yards and bearing fructifications of the fungus were found to be unsuitable for obtaining satisfactory sections, although the internal mycelium was in good conditions for the study. This is due to the fact that the rotted samples of wood were already in an advanced stage of decay, and therefore, they had become rather 'brash'. As the actual age of the samples of wood collected from the timber-yards could not be determined, owing to the non-availability of necessary data, they were selected for studying the latter stages of decay only. The early stages of decay were studied by following the method proposed by Banerjee and Sinha (1954). Sound, rectangular sapwood blocks ($2'' \times 1'' \times \frac{1}{2}''$) were exposed aseptically to the attack of *D. flavida* growing on 2% *malt agar* slants in Kolle flasks, as has been described in details in a later chapter on 'decay-resistance tests', and kept under controlled conditions for a period of four months. Four sets of test-samples infected in this way were taken out in batches, one set at the end of each month in order to obtain representative materials for observing gradual process of attack and consequent progress of decay in early stages. These test-samples, as well as samples from decayed wood obtained from nature, were then fixed and preserved in Formal-Acetic-Alcohol (70% Alcohol 85 c.c., Gl. acetic acid 5 c.c., and Formalin 10 c.c.) for future use.

Before sectioning, the test-samples, in almost all cases, were softened by special treatment, as recommended and followed in the Forest Products Research Laboratory, Princes Risborough, England. The wood-pieces were kept immersed in a vessel of gently boiling water until they became completely water-logged and sank. This process was found to be quite satisfactory for the wood showing late stages of decay, but sectioning of wood with early stages of decay required further softening. This was done by transferring the water-logged wood blocks to a mixture of equal volumes of glycerine and methylated spirit, and left as such for 2 to 3 days before sectioning. Sound wood-samples were also treated in a similar manner, and these served as controls. Transverse, radial-longitudinal and tangential-longitudinal sections were cut both by free hand and with the aid of a microtome. Microtome sections about 15–20μ thick were cut, but in general, free hand sections were found to be more suitable in rendering the internal mycelium visible without much distortion. Of the various differential staining methods, the combination stains Saffranin and Picro-Aniline blue, as suggested by Cartwright (1929) proved to be very suitable for detecting the hyphae in the woody tissues. In the resulting prepara-

* List of Common names of Indian Plant Diseases compiled by the Plant Pathology Committee of the Indian Council of Agricultural Research (1950).

tions the lignified cell-walls of the wood elements showed various shades of red, and the fungal hyphae appeared in deep blue colour.

In the early stages of decay fungal hyphae are usually confined to the vessels and wood parenchyma, but the distribution of the hyphae is not uniform throughout. A few hyphae, however, are noticeable in the medullary ray cells. The hyphae are thin-walled, hyaline, sparsely branched, distantly septate, and with frequent clamp-connexions, almost at each septum. These hyphae can be easily distinguished into two types. Hyphae of comparatively broader diameter (2.5–4.5μ) are very frequent in the lumina of the vessels and wood parenchyma, and are deeply stainable. They pass longitudinally through the wood elements, and produce conspicuously narrow branches (1–1.5μ in diameter). These branches are found predominantly in other wood elements, and penetrate the vertical walls of the elements in transverse direction. Both the types of hyphae are full of granular protoplasmic contents which take up the stain deeply. As the decay proceeds, the mycelium gradually ramifies, and in the lumina of the vessels loosely interwoven mycelial weft is formed (PLATES IV, FIGS. 10–12). It is very probable that in the initial stages of attack the hyphae of broader diameter simply pass, without any resistance, in the longitudinal direction through the wide lumina of the vessel (PLATE IV, FIG. 11). Gradually, however, the wood parenchyma and medullary ray cells are invaded by their branches. The tracheids and wood fibres, except a few, are the least affected elements at these stages. Radial penetration and consequent invasion of the neighbouring elements by the narrow branches are mainly through the pits of the thick-walled vessels. In the later stages of decay, the pits of different wood elements become very much enlarged in their diameter due to dissolution of the walls by fungal action, so that the hyphae pass loosely through them. The hyphae may also penetrate the cell walls directly at right angles to them forming circular to oval bore-holes. The smooth and moulded contours and not ruptured or splintered walls of the bore holes and pits through which hyphae pass radially, support Proctor's (1941) theory of cell-wall penetration due to enzymic activity of the fungus.

As the decay advances, cell-walls of groups of parenchyma cells are rendered very thin and ultimately disintegrated, forming spongy areas between the wood fibres. As a consequence, the compactness of the wood is gradually lost, and in later stages of decay cracks appear in the wood, mostly between the groups of fibres, splitting them into several irregular strands (PLATE IV, FIGS. 10 & 18). Dark brown gummy materials of the medullary ray cells (PLATE IV, FIG. 13) are gradually removed and the cell-cavities become filled with masses of hyphae (PLATE IV, FIG. 12). Gradually these cells are completely bleached and are without gummy substances (PLATE IV, FIGS. 14 & 16). In still later stages of decay the walls become comparatively thin, and ultimately break down forming conspicuous gaps in between the fibres and occupying the positions of the medullary ray cells (PLATE IV, FIGS. 15 & 17). This is in harmony with the complete bleaching of the wood-blocks as has been observed during decay-resistance tests.

Microchemical studies

In order to gather preliminary informations about the chemical changes that take place with the progress of decay microchemical tests, that are generally employed, for lignin and cellulose in sound and partially decayed wood, had been performed. The usual practice of staining the sections of the wood with phloroglucin-HCl and chlorzinc-iodine to detect the presence of lignin and cellulose respectively had been employed. Phloroglucin–HCl turns lignified walls red, while chlor-zinc-iodine turns cellulosic walls blue. The results obtained by staining with phloroglucin-HCl were further verified by "Maule treatment" in which the sections were treated with 1% aqueous potassium permanganate solution for 10–30 minutes washed in distilled water, placed in dilute HCl (sp.gr. 1.06) for about 3–4 minutes until the colour disappeared and washed again. On mounting the sections in ammonium hydroxide solution, lignified walls took red stain. The results indicated by chlor-zinc-iodine had also been verified by treating the sections with iodine-potassium-iodide and sulphuric acid (65%). As a corroborative evidence of the findings obtained by employing the above tests, the combination stain Gentian violet and Bismarck brown, differentiating lignified (violet staining) from non-lignified (brown staining) structures had been used. The results of these staining reactions are given in Tables 1 to 5.

Table 1. *Results of staining for lignin in normal and partially decayed wood of* Shorea robusta *with phloroglucin–HCl.*

Elements	Normal wood	Partially decayed wood
Vessels	Primary walls deep red; secondary walls light red. Tyloses almost colourless.	Primary walls light red; secondary walls pink to almost colourless towards the lumen. Fragments of heavily-decayed vessels almost colourless.
Wood parenchyma	Primary walls red; secondary walls light red.	Primary walls faintly pinkish; secondary walls pale pink to colourless.
Tracheids	Primary walls deep red; secondary walls light red to pinkish red.	Primary walls light-red; secondary walls pink to almost colourless.
Wood fibres	Primary walls deep red; secondary walls faintly red or pink.	Primary walls pink; secondary walls pale pink in patches to colourless.
Ray cells	Primary walls light red; secondary walls pinkish.	Primary walls light red; secondary walls almost colourless.

Table 2. *Results of staining for lignin in normal and partially decayed wood of* Shorea robusta *with 'Maule treatment'* ($KMnO_4$–HCl–NH_4OH)

Elements	Normal wood	Partially decayed wood
Vessels	Primary walls red; secondary walls pinkish to almost colourless.	Primary walls pale pink; secondary walls colourless.
Wood parenchyma	Primary walls light red; secondary walls pale pink.	Primary walls pale pink; secondary walls pale pink in patches to colourless.
Tracheids	Primary walls red; secondary walls somewhat pinkish towards the lumen.	Primary walls pale pinkish red; secondary walls colourless towards the lumen.
Wood fibres	Primary walls bright red; secondary walls paler red and pinkish towards the lumen.	Primary walls dirty red; secondary walls pinkish red to almost colourless towards the lumen.
Ray cells	Pale orange red in patches.	Pale pinkish to orange red in patches.

Table 3. *Results of staining for cellulosic materials in normal and partially decayed wood of* Shorea robusta *with chlor-zinc-iodine.*

Elements	Normal wood	Partially decayed wood
Vessels	Secondary walls pale blue in patches.	Secondary walls almost col urless.
Wood parenchyma	Secondary walls pale blue.	Secondary walls faint blue to colourless.
Tracheids	Some secondary walls faintly blue.	Secondary walls bluish to almost colourless.
Wood fibres	Secondary walls light blue to colourless.	Secondary walls almost colourless; in some cases faintly blue.
Ray cells	Light bluish green	Light bluish green.

Table 4. *Results of staining for cellulosic materials in normal and partially decayed wood of* Shorea robusta *with iodine-potassium-iodide and sulphuric acid.*

Elements	Normal wood	Partially decayed wood
Vessels	Primary walls golden brown; secondary walls faintly greenish blue.	Primary walls yellowish; secondary walls yellowish green.
Wood parenchyma	Primary walls deep yellowish brown; secondary walls faintly blue.	Primary walls yellowish; secondary walls faintly blue.
Tracheids	Primary walls pale brown; secondary walls mostly faint blue.	Primary walls greyish yellow; secondary walls pale greenish yellow in patches.
Wood fibres	Primary walls deep brown; secondary walls blue.	Primary walls greyish brown; secondary walls greenish yellow to faint blue.
Ray cells	Primary walls yellow; secondary walls pale bluish green.	Primary walls yellowish; secondary walls faint bluish green.

Table 5. *Results of staining for lignin and cellulosic materials in normal and partially decayed wood of* Shorea robusta *with gentian violet and bismarck brown*

Elements	Normal wood	Partially decayed wood
Vessels	Primary walls deep violet; secondary walls light violet. Tyloses dirty brown.	Primary walls light violet; secondary walls yellowish brown to colourless. Tyloses dirty brown.
Wood parenchyma	Primary walls deep violet; secondary walls light violet to brownish yellow.	Primary walls light violet; secondary walls greyish brown to colourless.
Tracheids	Primary walls violet; secondary walls light violet but brownish around the lumen.	Primary walls light violet; secondary walls yellowish brown in patches.
Wood fibres	Primary walls violet; secondary walls in golden brown patches near the lumen; violetish near the middle lamella.	Primary walls light violet; secondary walls pale violet in patches to yellowish brown.
Ray cells	Primary walls violet; secondary walls light violet in patches; mostly yellowish brown.	Primary walls lightviolet; secondary walls greyish brown.

From Tables 1 and 2 the presence of lignin in both primary and secondary walls of the normal wood elements is evident. But the relative concentration in the primary walls is greater than that in the secondary walls. In partially decayed wood, on the other hand, the intensity of staining reactions of both primary and secondary walls of all the wood elements is variable, and indicates a gradual delignification of the lignified parts. The pink, pale pink to almost colourless conditions of the secondary walls of all the wood elements indicate delignification to a remarkable extent. There is also considerable delignification in the primary walls of all the wood elements excepting in the ray cells as indicated by their lighter colour reactions than that in the normal wood. It is clearly evident that the secondary walls suffered greater delignification than the primary walls.

From Tables 3 and 4 it is revealed that the cellulosic materials have also undergone digestion during the process of decay, but the destruction is much less than that suffered by the lignified structures. It is also clear that cellulose is consumed from the secondary walls of all the wood elements, such as vessels, wood parenchyma, tracheids and wood fibres. Walls of ray parenchyma remain bluish green as in the normal wood, and this indicates that no appreciable loss of cellulosic materials has taken place at least, in the early stages of decay. Staining with Gentian violet and Bismarck brown also supports these findings. It is also revealed by these staining reactions that the decay of wood is due mainly to loss of lignin present in the primary and secondary walls. It appears that digestion of cellulosic materials also goes on simultaneously but at a much slower rate in the early stages of decay, when the wood can withstand sectioning. No further comment can be made as to whether the fungus in its later

stages of decay can consume cellulosic materials at a much higher rate than that in the earlier stages, since the wood at these stages is useless for sectioning, as it has become 'brash'.

Sulphuric acid test.—Following Ritter (1925), sections of natural and partially decayed wood, corresponding to those used for microchemical tests were treated with 72% sulphuric acid on slides. The cells of the sections of normal wood swell immediately after the acid treatment, break apart and dissolution of the secondary walls occurs, leaving on the slides a mixture of floating lamellae, vessels, ray cells and wood parenchyma. Sections of the partially decayed wood, on the other hand, when treated similarly give the same reaction as in the case of normal, but there the action of sulphuric acid is much less vigorous and less rapid. The violent action in the case of normal wood is due to the presence of large quantities of cellulosic substances in it which in partially decayed wood are present in a somewhat lower concentration, as indicated by less vigorous action. Though this test is not conclusive in itself, it may be concluded at least, that cellulosic materials are present in the partially decayed wood in comparatively lower concentrations than those in the sound wood.

All the above microchemical staining reactions indicate that *Daedalea flavida* can also consume some cellulose in addition to its great preference for lignin destruction in the early stages of decay. The most extreme decay will probably show the digestion of cellulose in comparatively much greater proportion.

Decay resistance

In the past the natural resistance of various timbers to the attack of wood-rotting fungi has been tested in the laboratory, and various methods of decay resistance were suggested by a number of investigators.

In all cases the fundamental principle underlying the technique is to expose small samples of sterilized test-pieces of sound wood to the attack of pure cultures of wood-rotting fungi under controlled conditions. The amount of decay resulting from fungal attack is calculated in terms of final loss in dry weight of the test-pieces. For the present investigation the writer had mainly followed the method suggested by Banerjee (1955) with some modifications as follows :—

Small rectangular, sound sapwood and heartwood blocks ($2'' \times 1'' \times \frac{1}{2}''$) were cut from a sāl log (*S. robusta*) of moderate age (12″ in diameter) and planed. These test-blocks were then serially numbered, and were dried in an oven at 60°C. to a constant weight. They were then soaked in distilled water under reduced atmospheric pressure till the average moisture-content of the individual wood-block rose much above the 'fibre-saturation-point'. Eight such blocks were put on a moist cotton pad in a Kolle flask, plugged, and sterilized in an autoclave for 10 minutes at 15 lbs. pressure.

The sterilized wood-blocks were then exposed to primary and secondary mycelia of the test fungus already grown on sterile 2% *malt-agar* slants in Kolle flasks. These flasks, each containing four samples of wood, were

kept under ordinary conditions of temperature (28–32°C.) and diffused light of the laboratory.

In order to determine the average moisture content of the wood-blocks just before their exposure to fungal action, another set of test-pieces of both sapwood and heartwood, soaked and sterilized in the same way, were carefully weighed. The initial moisture-contents of the test-pieces were expressed in percentage and calculated by following the relationship* pointed out by Savory (1954).

Following the recommendation of Cartwright and Findlay (1946), a period of four months' exposure of the blocks to fungal attack, for obtaining significant results in decay-resistance tests, was employed. After the test period the blocks were taken out, and after careful scraping of the superficial mycelium, weighed in order to find out their moisture-content at this stage. The test-blocks were then examined to record external manifestation of decay and dried in an oven at 60°C. to a constant weight. The final dry weights of test-pieces when substracted from their original dry weights gave the loss in weight due to fungal attack. The results thus obtained are given in Table 6.

Table 6. *t-test of comparison of loss in dry weight* (%) *of sapwood and heartwood of* Shorea robusta *exposed to primary and secondary mycelia of* Daedalea flavida

Type of wood	Kind of mycelia	Number of replicates	Range	Mean	S.E.	t
Sapwood	Primary	12	23.50—35.56	29.34	$\pm$1.47	4.8
	Secondary	12	29.98—50.06	40.41	$\pm$1.76	
Heartwood	Primary	12	2.03—11.34	6.34	$\pm$1.01	6.1
	Secondary	12	9.66—17.95	13.93	$\pm$0.73	

Table 6 clearly indicates that the loss in weight of heartwood is less than that in the sapwood on the exposure to either primary or secondary mycelium. The mean loss in case of sapwood during the test period is 29.3 ± 1.47 in case of primary mycelium, and 40.41 ± 1.76 in case of secondary mycelium; the same in heartwood are 6.33 ± 1.01 and 13.93 ± 0.73 respectively. Further in the comparison of means of primary and secondary for sapwood, the value of $t = 4.8$ for 22 degrees of freedom is highly significant showing that there is a real difference in the activity of two types of mycelia. A similar significant difference is also clear from the $t = 6.1$ for heartwood under similar conditions.

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* Moisture Content $= \frac{\text{Wt. of water present in the wood} \times 100}{\text{Oven dry wt. of the wood}}$

Following Findlay's (1946) classification, the sapwood of *S. robusta* exposed to secondary mycelium may be classed as 'perishable', and the same when exposed to primary mycelium is 'non-resistant'. On the other hand, the heartwood blocks exposed to primary and secondary mycelia fall under 'moderately resistant' and 'non-resistant' groups respectively. The controls in all the cases, which were free from fungal mycelium, show no appreciable loss in weight of the test-blocks. Bagchee, Puri and Bakshi (1955), carrying out similar experiments, concluded that the fungus caused a 10% loss in weight of sāl sapwood in four months. As detailed informations regarding the conditions of their experiment such as, temperature, light, the size of the test-pieces and the types of mycelia they used are lacking, the writers will refrain from making any comment on this very wide variation in the two results.

The foregoing results clearly exhibit the fact that *D. flavida* attacks both sapwood and heartwood under controlled conditions in the laboratory, and there exists a definite relationship between the types of mycelia and the nature of the wood. The severity of decay is extreme in the sapwood, and in the heartwood region it is moderate.

The initial moisture-content of the sapwood and heartwood blocks before the experiments were on the average about 80% and 60% respectively, which can be regarded as optimum moisture-content as pointed out by Kaufert (1936). After the experimental period, the sapwood blocks attacked by the primary and secondary mycelia retained the average moisture-content of about 72% and 68% respectively, while that of heartwood blocks under identical conditions were 49% and 46% respectively. As no control measure had been adopted to maintain the exact moisture-content of the wood blocks, they were not constant during the experimental period as evidenced from above. But it is clear that the normal activity of the fungus did not suffer in any way, as the moisture-contents are always found to be much above the 'fibre-saturation point'.

The test-blocks at the end of the experimental period show macroscopic manifestations of decay. The sapwood blocks become light, soft, and porous. Areas which have undergone severe damage, have rough and loose fibrous texture, and crumble into 'brashy' needles of wood elements, when a rubbing pressure is applied. That the sapwood has become 'brash' is evident when tested with a knife. The fibres break short over the top of the knife owing to their loss of toughness. The sapwood blocks exposed to secondary mycelium, in most cases, have undergone complete bleaching covering the entire wood-block and all its surfaces (PLATE III, FIG. 9b), while those exposed to primary mycelium show variable extents of bleached areas indicating that the bleaching is but partial (PLATE III, FIG. c–g). The bleached areas have no sharp outline and are not streaked, spotted or banded by darker and lighter colours.

The heartwood blocks show similar external manifestations of decay, but the bleaching is not so prominent as in the sapwood. As a result of bleaching, the wood blocks become yellowish white in colour, while the normal colour of sapwood and heartwood is pale brown and brown to reddish-brown respectively.

Toxicity test

Though hundreds of chemicals and various combination mixtures of different chemicals have been suggested as wood preservatives, the ideal preservative, according to Cartwright and Findlay (1946), should possess the most important character that it should have a high toxicitity towards wood-rotting organisms. Of the various preservatives, three different water soluble preservatives, *viz.*, Zinc chloride, Ascu 'A' and Borax were selected. All the three preservatives possess the characteristics, as stated by Cartwright and Findlay, of ideal preservatives.

Weighed wood blocks soaked and impregnated with a graded series of concentrations of the three preservatives were subjected to the attack of both primary and secondary mycelia grown on 2·5% *malt-agar*. Since the sapwood of *S. robusta* is 'non-resistant' and 'perishable' to the attack of *D. flavida*, it had been selected for the preparation of test-pieces of toxicity test. The blocks (5 cm. × 2.5 cm. × 1.5 cm.) were dried and sterilized by heating in a drying oven at 100°C. for about 18 hours. The initial oven-dry weights were recorded. The concentrations used were different for different preservatives and in each case were determined beforehand by growing the two types of mycelia on 2% *malt-agar* containing different concentrations of the preservative. The blocks were impregnated with the preservatives by immersing them in the solution at laboratory temperature (23°C.) and then exposed to reduced atmospheric pressure, by connecting the vessels containing the solution to a suction pump. When the blocks sank down the pressure was released, and they were allowed to remain submerged in the solution for about 2 hours, after which they were completely saturated. After wiping off the superficial liquid they were weighed again. The amount of preservative absorbed was then calculated on the basis of the solvent absorbed, and the concentration of the solution expressed in percentage. The actual quantity of the preservative present in the wood was then calculated as kilograms per cubic metre of wood. At each concentration four blocks were treated and the results were averaged on these four blocks. A period of four months' test in ordinary temperature, humidity and diffused light of the laboratory had been employed in order to obtain significant results. After four months the final oven dry weights of the test-blocks and the usual manifestation of decay, if any, were recorded. The final dry weights of the test-pieces when subtracted from their original dry weights, gave the loss in weight of the wood-blocks due to fungal action for four months. The results are given in the Tables 7—12.

It is to be noted from Tables 7 to 12 that of the three preservatives, ascu 'A' is the most powerful, since both the types of mycelia have least tolerance for it. The sapwood treated with ascu 'A' is resistant' to the attack of both primary (PLATE V, FIG. 25) and secondary (PLATE V, FIG. 24) mycelia, when approximately 0.40–0.45 kilogram of the preservative is present per cubic metre of the wood. The 'very resistant' nature of the sapwood exposed to primary mycelium is probably due to the presence of increased quantity (0.55 kilogram/metre cube approx.) of the preservative together with the less vigorous activity of the primary mycelium, as already indicated in decay-resistant tests. Zinc chloride is less effective

Table 7. *Toxicity test with sapwood blocks of* Shorea robusta *treated with zinc chloride and exposed to mycelium primary of* Daedalea flavida *for a period of 4 months at 28–32°C.*

Conc. of treating solution	Serial No.	Initial oven dry weight (gms.)	Wt. after treatment (gms.)	Absorption Kg/m³	Final dry wt. (gms.)	Loss in dry wt. (gms.)	Loss%	Condition of samples after test
	1.	12.455	21.125	0.46	10.780	1.67	13.42	
0.1%	2.	12.435	20.555	0.43	10.720	1.71	13.95	Partially
	3.	11.695	20.050	0.71	10.300	1.39	11.89	decayed
	4.	13.395	20.735	0.58	11.205	2.19	16.36	
				Average loss in dry weight = 13.9%				
	1.	12.525	20.795	0.44	11.755	0.77	6.15	
0.15%	2.	11.250	19.025	0.41	10.350	0.90	8.00	Partially
	3.	12.355	19.555	0.62	11.825	0.53	4.29	decayed
	4.	11.755	20.355	0.69	11.015	0.74	6.29	
				Average loss in dry weight = 6.18%				
	1.	11.315	19.525	0.88	11.075	0.24	2.12	
0.2%	2.	12.315	19.550	0.77	12.150	0.16	1.30	Sound
	3.	12.525	21.125	0.91	12.375	0.15	1.20	
	4.	12.295	19.515	12.74	12.105	0.19	1.55	
				Average loss in dry weight = 1.29%				

Table 8. *Toxicity test with sapwood blocks of* Shorea robusta *treated with zinc chloride and exposed to secondary mycelium of* Daedalea flavida *for a period of 4 months at 28–32°C.*

Conc. of treating solution	Serial No.	Initial dry wt.(gms.)	Wt. after treatment (gms.)	Absorption Kg/m³	Final dry wt.(gms.)	Loss in dry wt.(gms.)	Loss%	Condition of samples after test
	1.	12.195	20.235	0.22	10.250	1.94	15.91	
0.05%	2.	11.855	20.095	0.22	9.585	2.17	19.99	Partially
	3.	12.475	20.755	0.21	10.560	2.9	15.24	decayed
	4.	12.035	20.055	0.21	9.820	2.21	18.37	
				Average loss in dry weight = 17.38%				
	1.	11.775	18.175	0.34	10.725	1.05	8.92	
0.1%	2.	12.985	18.455	0.33	11.015	1.27	9.76	Partially
	3.	12.445	21.855	0.50	11.195	1.25	10.04	decayed
	4.	11.275	20.445	0.49	10.200	1.07	9.49	
				Average loss in dry weight = 9.55%				
	1.	11.815	19.055	0.52	11.115	0.7	5.93	
0.15%	2.	11.835	20.550	0.52	11.350	0.58	4.90	Partially
	3.	11.930	20.450	0.69	11.375	0.56	4.69	decayed
	4.	11.640	20.175	0.69	11.015	0.65	5.68	
				Average loss in dry weight = 5.30%				

Table 9. *Toxicity test with sapwood blocks of* Shorea robusta *treated with ascu 'A' and exposed to primary mycelium of* Daedalea flavida *for a period of 4 months at 28–32°C.*

Conc. of treating solution	Serial No.	Initial dry wt.(gms.)	Wt. after treatment (gms.)	Absorption Kg/m³	Final dry wt.(gms.)	Loss in dry wt.(gms.)	Loss%	Condition of samples after test
0.03%	1.	12.125	20.610	0.13	11.103	1.02	8.41	Partially decayed
	2.	12.145	19.075	0.12	10.985	1.16	9.55	
	3.	12.435	19.795	0.11	11.120	1.31	10.85	
	4.	12.145	20.045	0.12	10.775	1.37	11.28	
				Average loss in dry weight = 10.04%				
0.08%	1.	12.723	22.065	0.39	11.925	0.90	7.07	Partially decayed
	2.	11.625	19.160	0.32	10.900	0.72	6.23	
	3.	12.795	21.785	0.37	11.885	0.91	7.11	
	4.	12.550	19.765	0.30	11.660	0.89	7.09	
				Average loss in dry weight = 6.87%				
0.13%	1.	11.325	19.925	0.62	11.095	0.23	2.03	Almost sound
	2.	11.555	19.870	0.57	11.185	0.17	1.47	
	3.	10.895	18.075	0.50	10.725	0.17	1.56	
	4.	11.905	20.350	0.58	11.765	0.14	1.17	
				Average loss in dry weight = 1.56%				

Table 10. *Toxicity test with sapwood blocks of* Shorea robusta *treated with ascu 'A' and exposed to secondary mycelium of* Daedalea flavida *for a period of 4 months at 28–32°C.*

Conc. of treating solution	Serial No.	Initial dry wt.(gms.)	Wt. after treatment (gms.)	Absorption Kg/m³	Final dry wt. (gms.)	Loss in dry wt. (gms.)	Loss%	Condition of samples after test
0.01%	1.	11.245	19.145	0.04	10.105	1.14	10.14	Partially decayed
	2.	12.425	19.600	0.03	11.375	1.05	5.45	
	3.	11.435	19.125	0.04	10.050	1.38	12.11	
	4.	11.175	18.565	0.03	10.100	1.07	9.62	
				Average loss in dry weight = 10.08%				
0.05%	1.	11.095	19.030	0.21	10.145	0.95	8.56	Partially decayed
	2.	12.325	19.965	0.20	11.520	0.80	6.53	
	3.	11.055	17.685	0.17	10.300	0.75	6.83	
	4.	12.235	21.355	0.24	11.925	0.31	2.53	
				Average loss in dry weight = 6.11%				
0.1%	1.	11.355	18.310	0.37	11.025	0.33	2.91	Almost sound
	2.	12.295	20.550	0.44	11.963	0.33	2.68	
	3.	12.850	21.355	0.45	12.605	0.25	1.95	
	4.	10.955	18.905	0.42	10.680	0.28	2.55	
				Average loss in dry weight = 2.52%				

Table 11. *Toxicity test with sapwood blocks of* Shorea robusta *treated with borax and exposed to primary mycelium of* Daedalea flavida *for a period of 4 months at 28–32°C.*

Conc. of treating solution	Serial No.	Initial dry wt. (gms.)	Wt. after treatment (gms.)	Absorption Kg/m³	Final dry wt. (gms.)	Loss in dry wt. (gms.)	Loss%	Condition of samples after test
0.1%	1.	12.320	19.575	0.37	10.355	1.96	15.90	Partially decayed
	2.	12.650	21.370	0.46	10.725	1.93	15.25	
	3.	11.695	20.070	0.45	9.810	1.89	16.16	
	4.	11.025	18.925	0.42	9.195	1.83	16.59	
			Average loss in dry weight = 15.75%					
0.15%	1.	11.760	19.725	0.63	11.105	0.65	5.53	Partially decayed
	2.	12.500	19.525	0.64	11.950	0.55	4.40	
	3.	11.750	20.750	0.72	11.290	0.46	3.91	
	4.	10.620	17.370	0.54	9.985	0.64	6.03	
			Average loss in dry weight = 4.96%					
0.2%	1.	11.165	18.820	0.81	10.755	0.41	3.67	Partially decayed
	2.	11.690	20.050	0.89	10.985	0.70	5.98	
	3.	13.025	20.725	0.82	12.175	0.85	6.52	
	4.	11.705	20.150	0.89	11.055	0.65	5.55	
			Average loss in dry weight = 5.43%					

Table 12. *Toxicity test with sapwood blocks of* Shorea robusta *treated with borax and exposed to secondary mycelium of* Daedalea flavida *for a period of 4 months at 28–32°C.*

Conc. of treating solution	Serial No.	Initial dry wt. (gms.)	Wt. after treatment (gms.)	Absorption Kg/m³	Final dry wt. (gms.)	Loss in dry wt. (gms.)	Loss%	Condition of samples after test
0.05%	1.	12.745	22.455	0.26	10.150	2.59	20.32	Partially decayed
	2.	12.675	21.940	0.24	10.305	2.37	18.69	
	3.	11.625	19.615	0.21	10.020	1.61	13.79	
	4.	12.575	20.350	0.19	10.075	2.50	20.00	
			Average loss in dry weight = 18.20%					
0.1%	1.	10.395	17.365	0.37	9.010	1.38	13.59	Partially decayed
	2.	12.155	20.950	0.46	10.875	1.28	10.65	
	3.	11.455	19.670	0.43	9.185	2.27	19.13	
	4.	12.350	19.420	0.39	10.435	1.91	15.32	
			Average loss in dry weight = 14.67%					
0.15%	1.	12.300	20.650	0.65	11.725	0.57	4.63	Partially decayed
	2.	11.955	19.775	0.63	11.205	0.75	6.30	
	3.	12.595	19.785	0.59	11.785	0.81	6.42	
	4.	12.175	20.870	0.68	11.505	0.67	5.49	
			Average loss in dry weight = 5.71%					

than ascu 'A' since for each cubic metre, approximately 0.60–0.70 kilograms of the preservative are required, to make the wood 'resistant' to the attack of the fungus. Borax appears to be the least effective, and a quantity as high as 0.60–0.70 kilograms per cubic metre is to be applied, provided which the wood is 'moderately-resistant'.

Causal Organism

Material

The causal organism has been studied in culture. Three types of mycelia were employed, *viz.*, (i) primary mycelium without clamp-connexions, (ii) secondary mycelium with clamp-connexions, and (iii) isolates obtained from rotted wood. Several single-spore-cultures were made following the usual dilution method in which a spore suspension in sterile distilled water was spread on 1% *dextrose-agar* plate, and the superfluous water was carefully drained off. After 12 hours the plates were examined under the microscope, and well, germinated and isolated spores were selected, punched out by means of a sterile cutter and transferred to 2% *malt-agar* slants. In all cases mycelia developed within the tubes, and after careful examinations they were all found without clamp-connexions indicating the fact that all of them were in primary condition. Subsequent mating experiments with these primary mycelia, typical secondary mycelia with clamp-connexions were obtained. The method of isolation of the organism from rotted tissues had already been described.

Spore-germination

The spores were obtained from fresh sporophores collected in the field and also from those produced in wood-block cultures in the laboratory. They were sown aseptically in hanging drops of different media in sterile van Tieghem cells, and incubated in diffused light at 30°C. The media used were as follows : tapwater, distilled water, and distilled water containing traces of alcohol and lactic acid; 1% solutions of malt extract, sucrose, glucose and dextrose; 2% pure agar, 2% *dextrose-agar*, 2% *malt-agar* and *potato-dextrose-agar*. Alcohol and lactic acid were added to distilled water after sterilization. Spore-deposits on perfectly dry and sterilized slides within Petri-dishes were also kept under identical conditions. Observations were made at regular intervals of 3 hours in order to ascertain whether the spores had germinated or not. The results of these observations are given in Table 13. The (+) sign indicates germination of the spores, while the (—) sign shows failure of germination. Very few (10–20%), moderate (50–60%) and profuse germinations (95% or more) are indicated by a single, double or triple (+) signs respectively.

From Table 13 it is evident that the spores fail to germinate even after 24 hours in media like tap water, and distilled water containing alcohol and lactic acid. Very few spores germinate in pure distilled water after 18 hours, but no further growth of the germ-tubes takes place subsequently. In 1% solutions of glucose and dextrose the spores begin to germinate after 12 hours and the rate of germination is moderate (50–60%). The spores

Table 13. *Rate of spore-germination of* Daedalea flavida *in different media in hanging-drop cultures and the time required for it*

Media used	Hours							
	3	6	9	12	15	18	21	24
Tapwater	−	−	−	−	−	−	−	−
Distilled water	−	−	−	−	−	−	−	−
Distilled water containing alcohol	−	−	−	−	−	−	−	−
Distilled water containing lactic acid	−	−	−	−	−	−	−	−
1% malt extract soln.	−	+	++	+++	+++	+++	+++	+++
1% sucrose soln.	−	−	−	+	+	++	++	++
1% glucose soln.	−	−	−	+	+	++	++	++
1% dextrose soln.	−	−	−	+	+	++	++	++
2% pure *agar*	−	−	−	−	−	+	+	+
2% *malt-agar*	−	+	++	+++	+++	+++	+++	+++
2% *dextrose-agar*	−	−	−	−	+	+	++	++
Potato-dextrose-agar	−	−	+	+	++	++	+++	+++
Dry spores	+	*	*	*	*	*	*	*

(−) = No germination
(+) = Very few germination
(++) = Moderate germination
(+++) = Profuse germination
(*) = Further growth stopped.

germinate readily with speed and vigour, within 6 hours in media like 1% malt extract, and 2% *malt-agar*. In both the cases germinations after 12 hours are very profuse (more than 95%) giving rise to branched and septate hyphae. Though germination started within 9 hours in *potato-dextrose-agar*, the rate of germination and further growth in this case are slower in comparison with those on 2% *malt-agar* medium. It is very interesting to note that the spores kept in perfectly dry conditions on slides within Petri dishes, germinate slightly by the formation of short germ-tubes, and this is possibly due to the presence of the 'water-drop' adhering to each spore at its hilum end at the time of spore-discharge. initiating germination of the spore, the 'water-drop' dries up, and as a result no further development of the germ-tube takes place due mainly for want of moisture.

Oxidase tests

Following Bavendamm (1928) oxidase tests were performed, in which the primary and the secondary mycelia of the fungus were allowed to grow separately on 2.5% *malt-agar* medium containing 0.5% gallic or tannic

acid in Petri dishes. These were then kept in complete darkness at a room temperature of 30–31°C. and a relative humidity of 62%–82%. Dark brown rings appeared around the inocula in all cases, within 24 hours after inoculation showing that *D. flavida* is a 'white-rot' fungus. It was observed that the intensity of reaction was somewhat greater in gallic acid medium than that in the medium containing tannic acid. The results are summarized in Table 14.

Table 14. *Results of oxidase tests with primary and secondary mycelia of* Daedalea flavida

Types of mycelia	Types of reactions in 2.5% *malt-agar* medium	
	0.5% gallic acid	0.5% tannic acid
Primary mycelium	Very strong (+++++); diameter 20 mm.; diffusion zone very intense, dark brown, opaque, forming a wide corcna around the inoculum; slight growth above the inoculum (Plate VI, fig. 27).	Strong (++++); diameter 15–18 mm.; other characteristics same as in gallic acid medium (Plate V, fig. 29).
Secondary mycelium	Very strong (+++++); diameter 25–28 mm.; diffusion zone very intense, dark brown, opaque, forming a wide corona around the inoculum; no growth of the mat takes place (Plate VI, fig. 28).	Very strong (+++++); diameter 18–20 mm.; other characteristics same as those in gallic acid medium (Plate VI, fig. 30).

Cultural characteristics

For studying the cultural characteristics, the isolates from the rotted wood as well as the secondary mycelium were grown on 2.5% *malt-agar* in Petri dishes. The *p*H of the medium was adjusted and after sterilization was found to be 6. The cultures in replicates of three for each type of mycelium were incubated in darkness under ordinary conditions of temperature (28–32°C.) and humidity (62%–67%) of the laboratory. Cultural characteristics of both the types of mycelia were found to be identical in every respect.

Growth starts with moderate rapidity, appears somewhat silky over the inocula, and entirely appressed and sodden around them. Within the following 3 days the mycelia become more or less raised and each differentiated into three regions : a thin felty zone around the inoculum, a middle broad sub-felty area, and an appressed and sodden zone of advance. Radial striations over the mats at this stage become evident (PLATE VI, FIG. 25). After about 20 days, a few small, hemispherical or irregularly shaped mycelial lumps develop over the mats. In early stages of development they are white in colour, but with age Warm-Buff and Antimony-yellow colour appear. No further development of these lumps takes place and, in no case, they produce fructifications. The average daily increment in diameter of the mat, in both the cases, has been found to be approximately 11 mm.

An attempt was subsequently made to obtain fruit-bodies in cultures from the isolates and secondary mycelium by growing them on sapwood blocks of *Shorea robusta*. The wood-blocks were prepared and kept in identical conditions, as had been done during decay resistance tests. The fructifications began to appear after 30 days and formed fruitbodies with fertile hymenial surface (PLATE VI, FIG. 26) within 60 days, after inoculation. The details of sporophore production has been communicated in a separate paper.

DISCUSSION

The investigation is an attempt to put on record the part played by *Daedalea flavida* in the annual loss of Sāl wood and some aspects of its life-processes, including the etiological complications which have not so far been well understood.

The decay is entirely restricted to the external sapwood portion of the logs rendering the region somewhat soft and 'brashy'. Decay-resistance tests in the laboratory, however, indicate that the fungus is capable of attacking both sapwood and heartwood under favourable conditions of temperature and humidity. The sapwood is 'perishable' to the attack of the fungus, as it undergoes more than 30% loss in dry weight. The heartwood, on the other hand, suffers more than 10% loss in dry weight, and following Findlay (1938), can be classed as 'non-resistant'. This directly points to the fact that field observations of the gross characters of the rot should not be considered as the sole basis in drawing conclusions, regarding the nature of the rot. The loss in weight of the heartwood blocks in the laboratory test can be safely explained, if one remembers the fact that a moisture-content of the wood above the 'fibre-saturation-point' is necessary for active fungal growth. In the timber-yards generally, this point is not reached, due to huge size of the logs and inadequate water supply, while water has been forced to enter the wood-blocks by exposing them under reduced pressure in the laboratory. It has also been found that the nature and extent of fungal decay depend both on the nature of the wood and the types of mycelia attacking them. The secondary mycelium causes greater amount of decay than the primary mycelium irrespective of the types of wood used. The severity of damage is very acute in sapwood, while the heartwood resists decay within certain limits. Kaufert (1936), working on *Pleurotus corticatus*, obtained similar difference in behaviour of the two types of mycelia as regards their decaying capacity. He was of opinion that this differential behaviour of the two types of mycelia is due to the difference in their rate of growth. As in the present investigation the test period has not been extended beyond four months, no statement can be made at present on the validity of Kaufert's comment on the problem.

The fungus, after infection, ramifies both inter- and intra-cellularly, and easily establishes itself within most of the wood-elements. Enzymic activities of the mycelium undoubtedly render dissolution of some of the components. Eventually, the decay progresses, and cracks appear in the

fibrous tissue. Ultimately, the wood loses its toughness, and becomes somewhat soft and compressible. Groups of relatively unaffected fibres break up and render the wood 'brashy'. The enzymic activity of the fungus causes some chemical changes in the wood. Microchemical studies with partially decayed wood show considerable decrease in the amount of lignin. In later stages of decay, however, lignin destruction is still predominant, but the fungus takes up cellulosic materials also. This, perhaps, is due to the fact that, as decay proceeds, more lignin is utilized by the fungus, and as the amount of lignin falls off, the fungus invades cellulosic materials. which are still in abundance, for nourishment. Microchemical studies of the late stages of decay will probably throw some light on this problem, but the thing is beyond the limit, since wood at this stage cannot withstand sectioning and crumbles into 'brashy' needles.

The decomposition of lignin is essentially a process of oxidation, and secretion of oxidases by a 'white rot' fungus is expected. The strong positive reactions of both primary and secondary mycelia with gallic and tannic acid indicate the lignin decomposing nature of the fungus.

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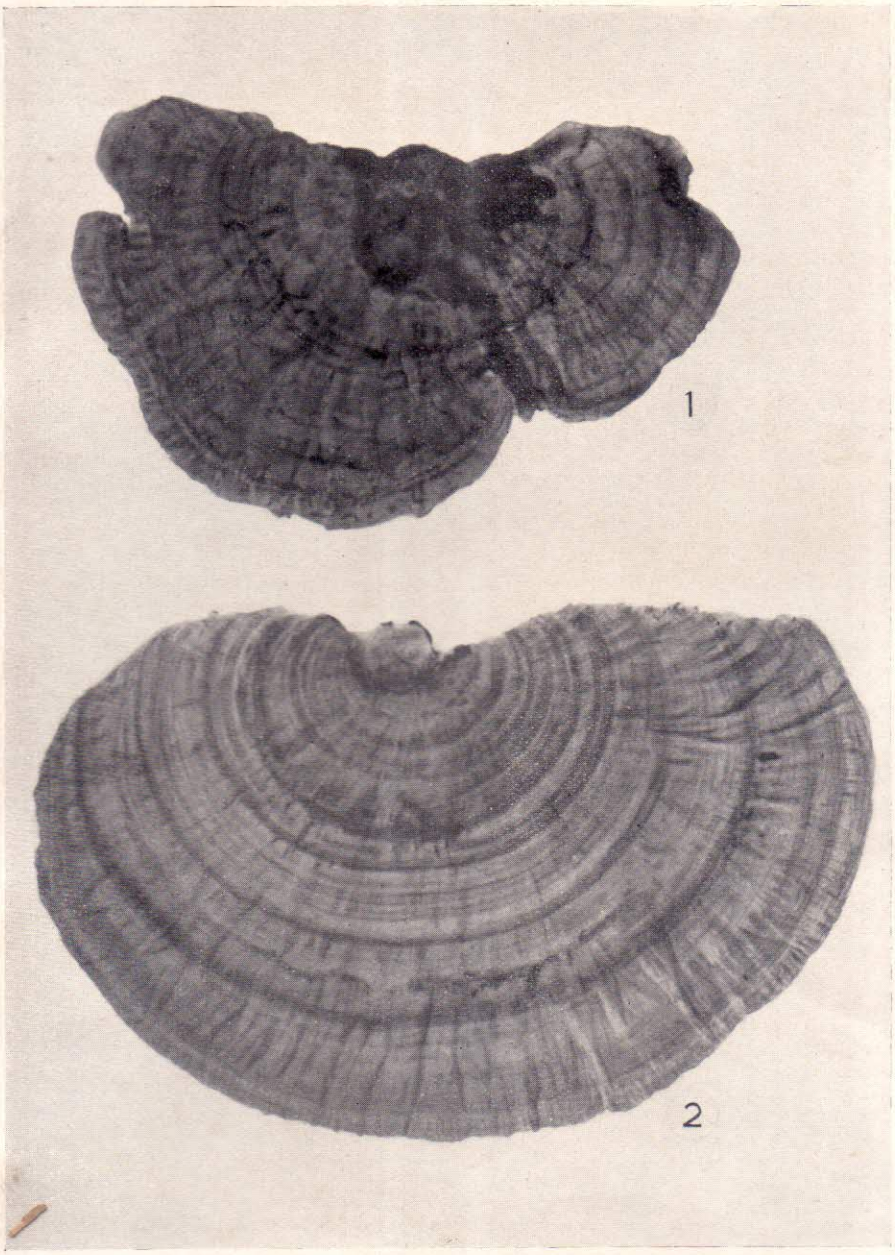

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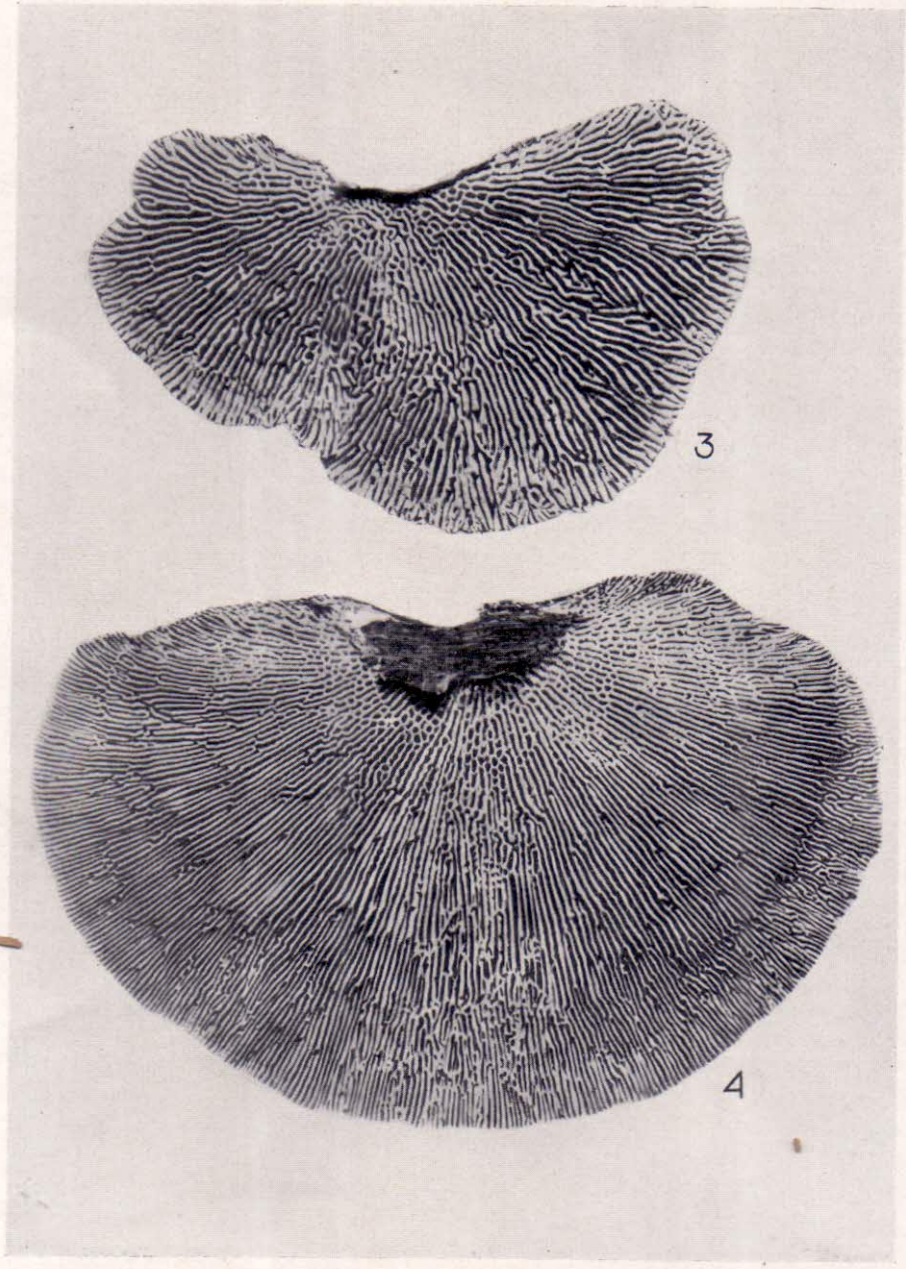

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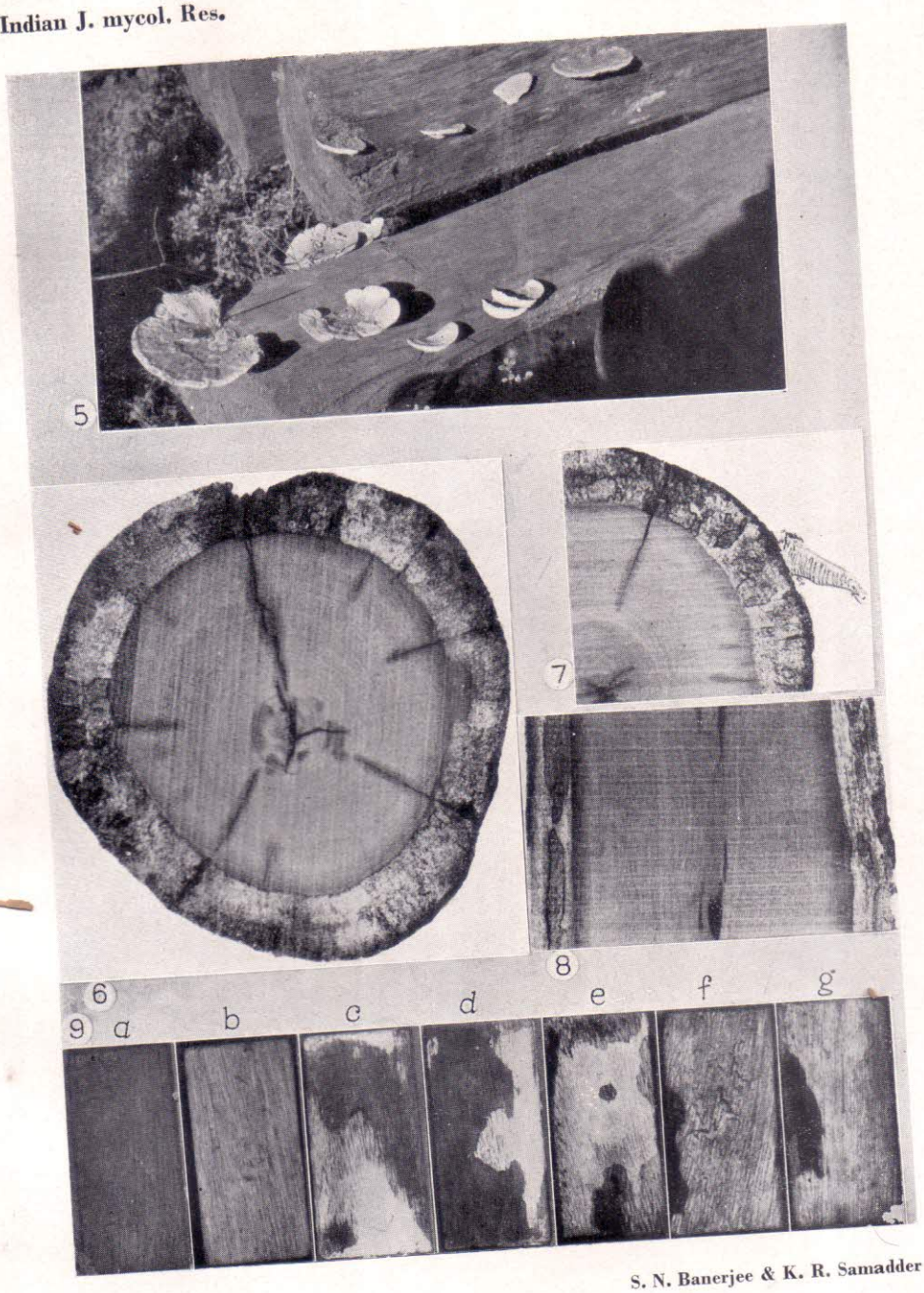

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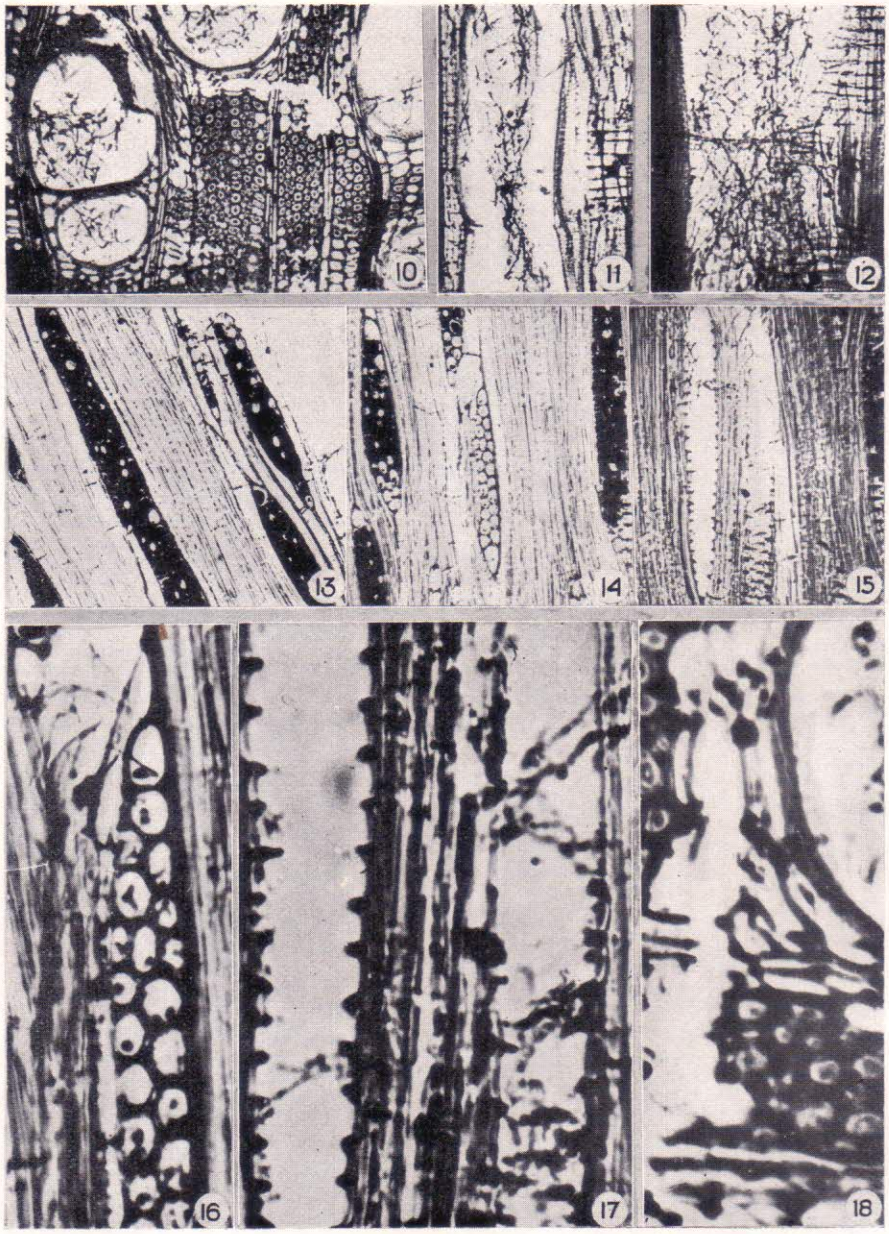

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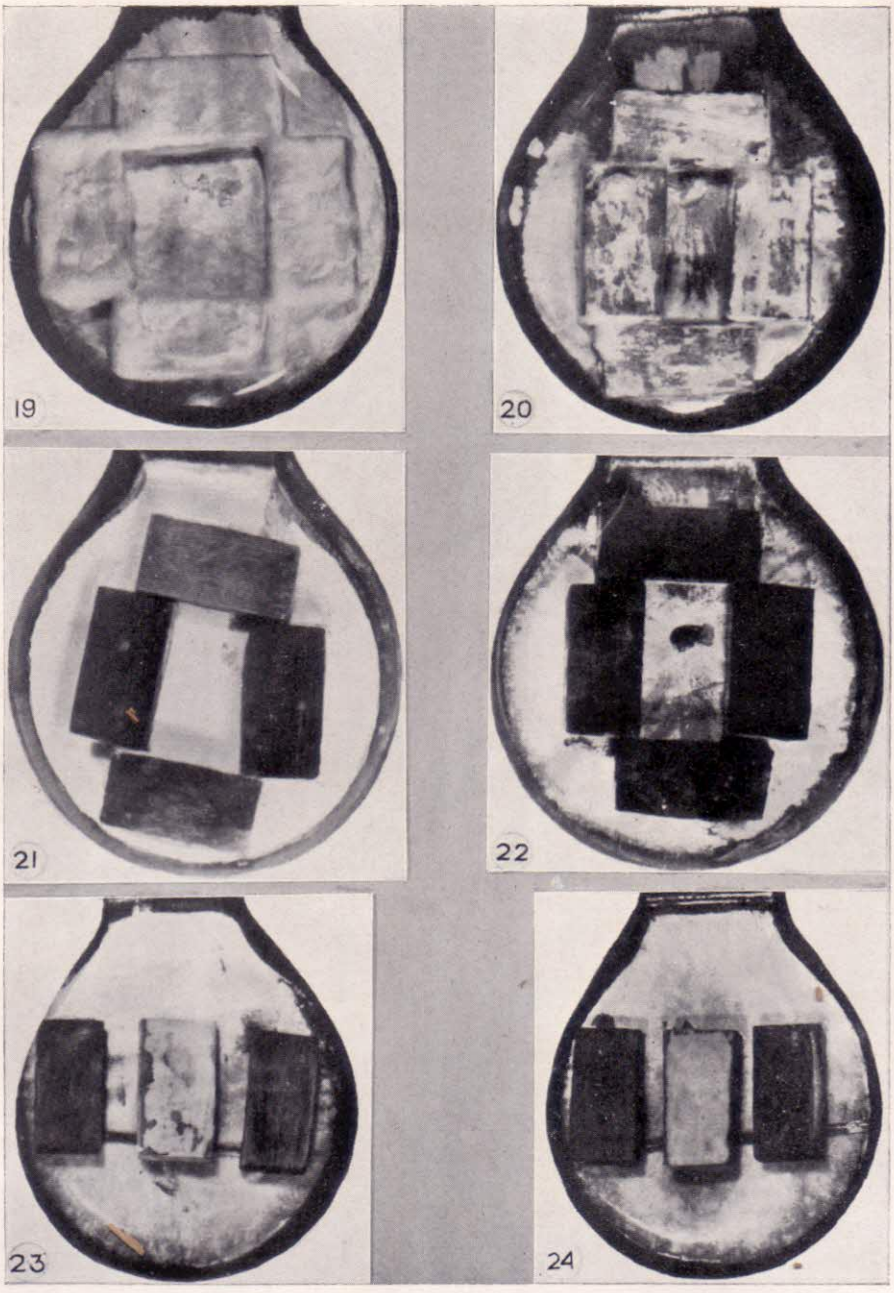

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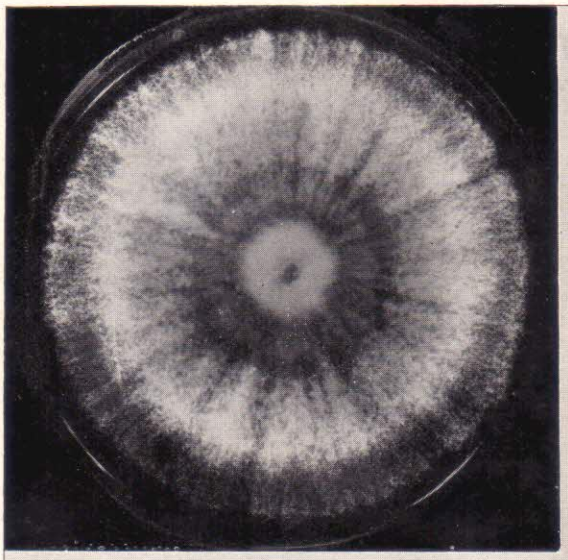

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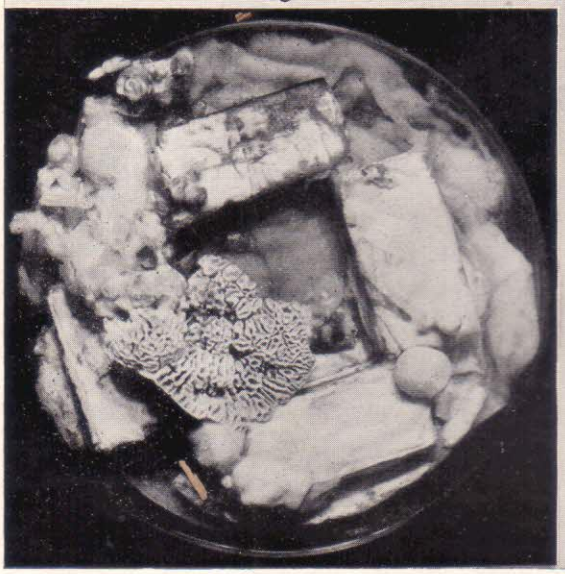

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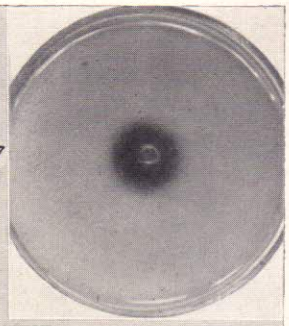

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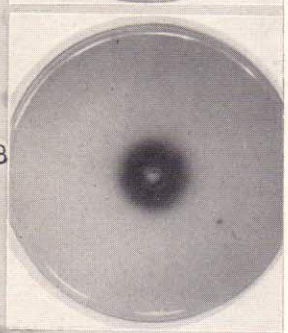

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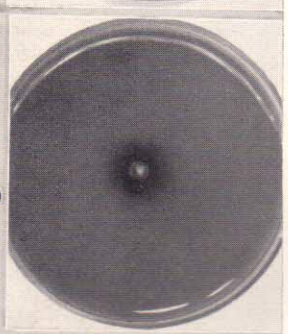

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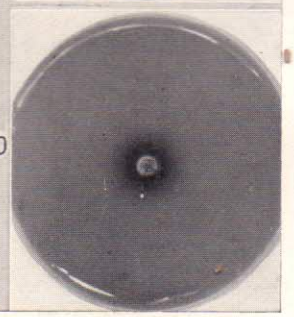

30

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EXPLANATION OF PLATES

Plate I

Figs. 1 & 2. Fructifications of *Daedalea flavida* Lév., showing the upper surface with characteristic zonations.

Plate II

Figs. 3 & 4. Fructifications of *D. flavida*, showing the hymenial surface with characteristic poroid, labyrinthiform or partly lamellate pore-mouths.

Plate III

Fig. 5. Fructifications of *D. flavida* growing both on the cross-section-end and side of Sāl log stacked in a timber-yard.
Fig. 6. Cross section of a log of *Shorea robusta* with sap rot caused by *D. flavida;* the heart-wood portion appears unaffected but with fissures caused due to shrinkage.
Fig. 7. Same type of sap rot with a portion of the basidiocarp of *D. flavida* in contact with the log.
Fig. 8. Part of a longitudinal section of an infected log of *Shorea robusta* showing saprot due to *D. flavida*.
Fig. 9. Test-blocks of sapwood of *Shorea robusta* showing various degrees of bleaching (b–g) due to attack of *D. flavida* after a period of four months' decay-resistance tests; the control test-block (a) does not show such bleaching.

Plate IV

Fig. 10. Part of transverse section of sapwood of *Shorea robusta* in an advanced stage of decay with lumina of the vessels filled with the mycelium of *D. flavida;* other elements of the wood are in various stages of disintegration (×80).
Figs. 11 & 12. Portions of radial-longitudinal sections of sapwood showing distribution of the hyphae within the wood-elements (×80).
Figs. 13–15. Portions of tangential-longitudinal sections of sapwood showing gradual bleaching and disintegration of the ray cells; hyphal elements can be recognised within the lumina of the ray cells at the centre; dark-coloured gummy materials of the ray cells being gradually used up and finally removed (×80).
Figs. 16 & 17. Portions of tangential-longitudinal sections of sapwood showing disintegration of the ray cells; hyphal elements are noticeable within lumina of the ray cells, and complete disintegration is evident (×1200).
Fig. 18. Part of a transverse section of sapwood showing dissolutions of parenchyma cells and eventual crack in the woody tissues (×1200).

Plate V

Figs. 19 & 20. Wood-block cultures of primary (fig. 19) and secondary (fig. 20) mycelia of *D. flavida* growing on sapwood test-pieces; photographed after 30 days.
Figs. 21 & 22. Wood-block cultures of primary (fig. 21) and secondary (fig. 22) mycelia of *D. flavida* growing on heart-wood test-pieces photographed after 30 days.
Figs. 23 & 24. Toxicity test with zinc chloride (fig. 23) and ascu 'A' (fig. 24) respectively in Kolle flasks after a period of four months' test; untreated control test-block in the middle is heavily infected with mycelium, while the treated blocks placed on the two sides of the control remain absolutely free from infection.

Plate VI

Fig. 25. 7-days'-old culture of secondary mycelium of *D. flavida* on 2.5% *malt-agar*.
Fig. 26. Fructification of *D. flavida* formed by an isolate in wood-block culture; the wood-blocks with the fructifications have been carefully taken out from the Kolle flasks and kept on cotton wool in a Petri dish.
Figs. 27 & 28. Oxidase rings around the inocula of primary (fig. 27) and secondary (fig. 28) mycelia of *D. flavida* growing on 2.5% *malt-agar* containing 0.5% gallic acid.
Figs. 29 & 30. Oxidase rings around the inocula of primary (fig. 29) and secondary (fig. 30) mycelia of *D. flavida* growing on 2.5% *malt-agar* containing 0.5% tannic acid.