[Proc. Roy. Soc. Victoria, 50 (N.S.), Pt. I., 1937.]

Art. VIII.—*A Cultural Study of* Fistulina hepatica (*Huds.*) *Fries, Isolated from Decayed Jarrah* (Eucalyptus marginata *Sm*).

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[Read 10th June, 1937; issued separately 29th December, 1937.]

Introduction.

Fistulina hepatica (Huds.) Fries has a wide distribution being found in Europe, Asia, North America, and Australia. In addition, Saccardo (1891) recorded a species *F. antarctica,* subsequently considered to be a synonym of *F. hepatica* (Saccardo, 1925), from antarctic regions such as South Patagonia. Cooke (1871) reported that it was common in Britain and occurred on the trunks of old oaks. Saccardo (1888) noted its occurrence on trunks of *Quercus, Fagus* spp., and *Castanea* spp. Rea (1922) listed its occurrence in Britain on the trunks of the following trees:—oak, ash, walnut, willow, beech, sweet chestnut, hornbeam and elm. Cartwright and Findlay (1936) stated that the fungus gained entrance through wounds, and probably acted as a mild parasite.

In Australia, Cooke (1892) recorded *F. hepatica* growing on tree trunks in West Australia, while McAlpine (1895) noted its occurrence in Victoria. The fungus was recorded for Tasmania by McAlpine and Rodway (1896), and Cleland (1935) recorded it in South Australia and New South Wales, on the trunks of living Eucalypts, dead stumps, and on fallen logs (Plate IX., figs. 1, 2).

In this study, the writer will be concerned with the relationship existing between Jarrah (*Eucalyptus marginata* Sm.) and *F. hepatica.*

Outline of Investigation.

During a preliminary investigation of fungal rots in Jarrah, one particular fungus was isolated repeatedly from specimens exhibiting various symptoms of rot. This fungus often produced fertile sporophores in culture which exhibited the characteristics of the sub-family Fistulinaceae in having the "hymenium inferior, lining free and separate tubes" (Rea, 1922), (Plate IX., Figs. 3, 4). Their appearance approximated most closely to that suggested by the description of *Fistulina hepatica* Fries by Saccardo (1888).

F. hepatica has been isolated from the following types of rots :— heart rot or dry rot, pith or doze, straw rot, decayed included sap, yellow-edged pin-holes, and pencilled Jarrah. The rot-nomenclature was that used by timber workers to describe the types of rot, based on their macroscopic appearance. Rot specimens were selected by the Senior Timber Inspector of the Forests Department, West Australia, from the Jarrah forests as examples of the principal types of rot in Jarrah.

Jarrah Rot Specimens.		Fungi Isolated.			
Total No.	No. which Yielded Isolations.	Total No.	*F. hepatica.*	Other B'mycetes.	Other Fungi.
24	16*	19*	10	6	3

* Some specimens yielded more than one fungus.

Method.

Suitable discs, 1 inch thick, were cut from selected specimens of rotted Jarrah. Each disc was transferred to the inside of an inoculation chamber where, by the aid of a pair of chisel forceps, small pieces of wood were removed from designated regions of the disc and sown on to Petri dishes containing $\frac{1}{4}''$ depth of 2.7% malt extract agar. The dishes were incubated at 18–22° C., 60–70% relative humidity, in weak light. Later, those inocula which produced fungal mycelia were transferred to malt-extract agar slopes and incubated. Generally *F. hepatica* appeared within three weeks, and, after vigorous initial growth in the incubator, cultures were maintained in darkness under room conditions.

Beaker cultures were started by inoculating conical beakers containing 1 inch depth of 2.7% malt-extract agar with actively-growing mycelia of *F. hepatica,* and were maintained under conditions similar to those used for Petri dishes. When the surface of the medium was covered (usually 3–4 weeks' growth), a suitable sub-stratum was presented for the artificial attack on sound Jarrah blocks. These were of true wood cut approximately 2″ x 1″ x 1″ from sound Jarrah sticks obtained from a Melbourne timber yard.

Fungus in Culture.

Macroscopic Appearance.

At first (7–8 days) the centre was white to creamy white in colour, downy in texture and surrounded by an annular region consisting of a flat, sodden, colourless, mycelial mat with an irregular margin. Within two weeks of commencement of growth, the central region of the culture was creamy white, loose-woolly in texture, 3–5 mm. thick, surrounded by the sodden annular region with the typical margin. At the end of the third

week, the central inoculum, which had protruded above the remainder of the culture, began to assume tints of Cartridge Buff, Ivory Yellow to Light Ochraceous Buff (Ridgway, 1912).

Cultures grown in stronger light exhibited less vigorous aerial growth and generally a thinner and more floccose type of growth than the loose-woolly type described. In addition, in some cases, the sodden annular region of growth occurred centripetally as well as centrifugally to the white floccose region. As the mycelium aged, colour changes occurred and the following ranges of tints were exhibited:—Cartridge Buff to Ochraceous Orange and later Ochraceous Tawny to Russet. The texture also changed and became felty to membranous (in part). Watery exudations, which were often reddish in colour, appeared within five to ten weeks of commencement of growth. These either accompanied, or appeared in advance of, the formation of sporophore initials which often arose as round swellings, Cartridge Buff, Marguerite Yellow, or Pale Pinkish Cinnamon in colour, on the surface of the mycelium or block.

The following types of fructification were obtained in culture:—

A. Sterile (*a*) *Ceriomyces*-type (Plate X., fig. 5).
(*b*) *Solenia*-type.

B. Fertile (*c*) *Cyphella*-type (Plate X., fig. 6).
(*d*) Near-typical (Plate X., fig. 7).
(*e*) Typical (Plate IX., fig. 3).

(*a*) These fructifications arose from either medium or block after the seventh week. They varied in shape from round, cylindrical to phalloidal, and in diameter from $\frac{1}{4}''$ to $2''$. Papillations were generally present over the entire surface. Colours were initially Cartridge Buff to Pinkish Buff, giving way later to Cinnamon Buff to Chestnut tints. At this stage, they often split to reveal a striate appearance. On shrivelling they assumed Drab to Olive-Brown tints. It was observed that these fructifications were often produced in response to injury of the mycelium.

Saccardo (1888), in his description of *F. hepatica,* referred to the gasterosporous stage of the fungus as *Ceriomyces hepaticus* Saccardo, although he recognized that *Ceriomyces* Corda was a spurious genus, being a stage in the life history of certain Polyporaceae (1888, p. 385). Saccardo considered that the genus *Ptychogaster* Corda was synonymous with *Ceriomyces* Corda. Lloyd (1909) stated that the conidial stage of *F. hepatica* was the fungus *Ptychogaster hepaticus* which formed a solid compact ball and contained, instead of pores, filaments bearing abundant conidiospores. Davidson (1935) isolated *F. hepatica* from typical specimens of "Brown' Oak and obtained the *Ptychogaster* stage on ordinary culture media such as potato dextrose or malt-extract agar. Examination of a section of a

young sterile fructification cultured from the Jarrah strain of *F. hepatica* showed no conidiospores present but merely a matrix of hyphal elements.

(*b*) *Solenia*-type fructifications obtained in culture were diminutive, narrow cup-shaped and crowded. Initially they appeared as Ivory Yellow papillated patches, on the block or medium after the fifth week and gave rise to a powdery appearance. Some of the papillae remained narrow and cup-shaped while others expanded somewhat at the orifices, which often had fimbriated edges. Hand-sections of the latter fructifications showed the presence of a small number of spores. Final colours varied from Cinnamon Buff to Chestnut.

(*c*) *Cyphella*-type fructifications were observed only on malt-agar slopes and were larger (up to 3 mm. in diameter), more expanded and somewhat less crowded than the *Solenia*-type fructifications. Colours were generally Tilleul Buff to Chestnut. Basidiospores were observed in hand sections, but spore deposits were not evident. Transition stages between *Solenia*- and *Cyphella*-type fructifications were observed.

(*d*) Near-typical fructifications were obtained from both media and blocks. These represented a more advanced stage in the evolution of the typical fructification, in that the open cup-shaped forms (*Cyphella*-type) gave way to tubes 2–5 x 0.2–0.5 mm. These tubes were disposed equilaterally, and sections showed the presence of basidiospores. No spore deposits were obtained. The development of these fructifications was similar to that for typical fructifications.

(*e*) The early development and coloration of typical fructifications were similar to that for the *Ceriomyces*-type already described, but the former soon became more shelf-like and developed comparatively short stipes after the fifteenth week. Immediately prior to hymenium formation, the papillated surface underneath the pileus became Pale Pinkish Cinnamon and the pileus began to flatten, and to expand laterally, while assuming Auburn to Wood Brown tints. This papillated surface developed into free, vertical cylindrical tubes Cartridge Buff in colour, 2–5 x 0.3–0.5 mm., and orifices often with fimbriated edges. The region of the fructification adjacent to the tubes was papillated, and graded into the tubes. The lower parts of the fructification were also papillated.

While tube-differentiation proceeded, the pileus rapidly expanded, flattened, and spore-discharge began. Spore-discharge took place over a period of about ten days, and resulted in the appearance of deposits at first Empire Yellow in colour, but which later darkened to Antimony Yellow. Spore deposits resembling miniature stalagmitic processes were obtained providing the cultures were not moved during spore-discharge. Typical

fructifications have, as yet, been obtained in culture only from the surfaces of Jarrah blocks artificially inoculated by *F. hepatica.* The transverse surface seems to supply a more favorable sub-stratum for their production than the longitudinal.

Lloyd (1908) noticed that the normally white flesh of a young sporophore of *F. hepatica* quickly turned red on exposure to air. Buller (1931) observed that excretions of drops of water from the pileus of *F. hepatica* under moist conditions were coloured red. Injury to a sporophore of the Jarrah strain of *F. hepatica,* or its maturation, resulted in the production of a reddish colouration of the matrix, which was usually white. Further, reddish exudations have also been observed in cultures (as previously mentioned). These observations suggest the presence of an oxidase enzyme in the fungus.

Microscopic Appearance.

The submerged hyphae were 2–8μ wide, hyaline but often granular to vacuolate and contained numerous oil globules. Clamp connexions were abundant, simple, present on both narrow and wide hyphae. Branching was free and either acute-angled

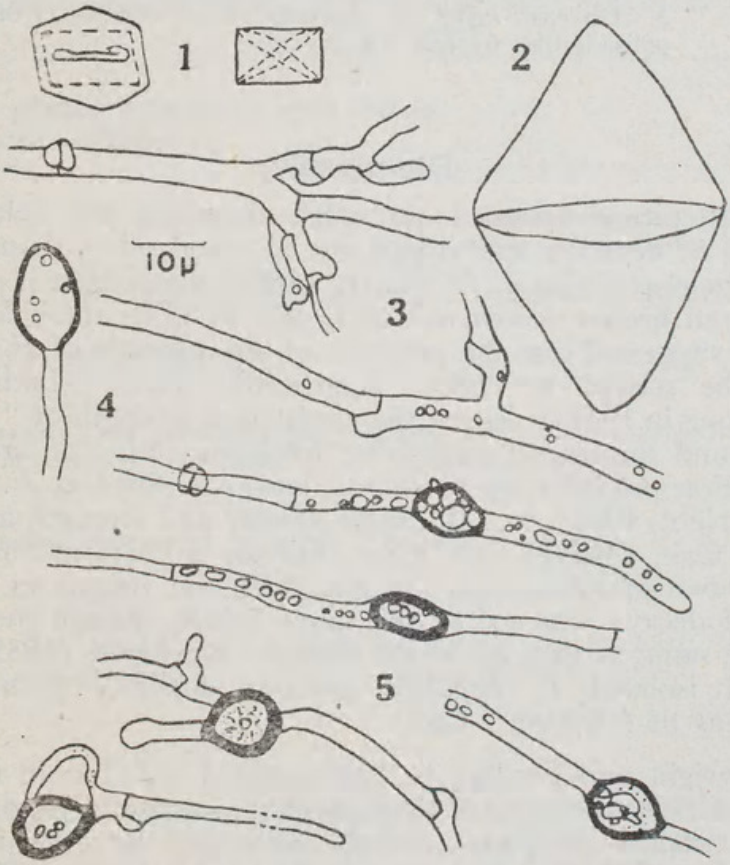

Fig. 1.—1. Crystals from young culture. 2. Crystal from old culture. 3. Clamp connections. 4. Terminal chlamydospore. 5. Intercalary chlamydospores.

or rectangular. Intercalary and terminal chlamydospores contained oil globules, and occurred in the submerged mycelium. They varied in shape from ovoid to sub-cylindrical and measured 2–7 x 10–13μ. Rhomboidal crystals of various sizes occurred in the medium. The aerial mycelium exhibited a similar microscopic appearance to that of the submerged mycelium (crystals being absent). Basidiospores produced on typical sporophores were hyaline, apiculated, varied in shape from ellipsoidal to subspheroidal, and measured 3–5 x 5–8μ. The external layer of hymenial tubes was composed of palisade-like hyphae.

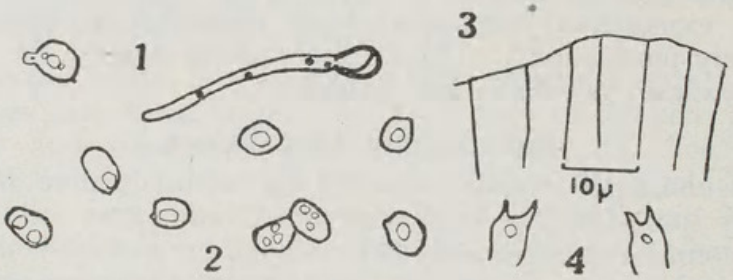

Fig. 2.—1. Germinating basidiospores. 2. Basidiospores. 3. External layer of hymenial tube, composed of palisade-like hyphae. 4. Basidia.

Discussion.

The literature revealed no work done on the relationship between *F. hepatica* and fungal rot in wood other than English Oak (*Quercus Robur* L.). Hartig (1894) stated that it produced a deep red brown decomposition in the wood of the oak. Braid in 1924 suggested that the presence of sporophores of *F. hepatica* might be related to hollow stag-headed Oaks. Latham and Armstrong in 1934 in testing the mechanical strength of "Brown" Oak found that wood containing irregular stains of brown discolouration produced by a fungus, later identified as *F. hepatica* (Cartwright, 1936), had the same density and strength as that of normal Oak, whereas Oak wood showing a uniform intensity of dark brown discolouration (produced by the fungus in the later stage of decay) was softer and more brittle, though the density was the same as that of sound timber. Davidson (1935) stated he had isolated *F. hepatica* without difficulty from typical specimens of "Brown" Oak.

Cartwright and Findlay in 1936 pointed out that in the early stages of infection of Oak by *F. hepatica,* a brown discolouration of heartwood is produced, and the colour of the wood improved so that it commands a higher price than normal Oak. This is the "Brown" Oak of the furniture trade. However, in the later stages of infection, the wood becomes deep reddish-brown.

exhibits cubical cracking and gives the general indications of a heart rot. Davidson (1935), on the other hand, stated that in no instance did he find *F. hepatica* associated with any apparent disintegration of the wood which remained hard and heavy. From the foregoing, it appears that the fungus *F. hepatica* has little wood destroying properties in the early stages of infection, but in the later stages it brings about a definite heart rot in Oak.

Cartwright and Findlay, in their publication of 1936 also give some characteristics of the Oak fungus in culture. These are briefly compared with those of *F. hepatica* isolated from specimens of rot in Jarrah.

Oak Strain.	Jarrah Strain.
On 5 per cent. malt agar, forms soft woolly mat, at first creamy-white, and develops series of tints from Pale Pinkish Cinnamon to Straw Yellow, later Light Vinaceous Brown or Russet	On 2·7 per cent. malt agar, forms loose woolly mat, at first white to creamy-white, and develops series of tints from Cartridge Buff to Ochraceous Orange, later Ochraceous Tawny to Russet
Abnormal, phalloid fruit-bodies often formed, apex papillated	Ditto
	Typical fruit-bodies occasionally formed on surfaces of artifically inoculated blocks
Hyphae very variable in diameter	Ditto
Clamp connexions not numerous, particularly in submerged mycelium	Clamp connexions numerous, also in submerged mycelium
Prolific conidial production in some cultures	Conidia not observed
Intercalary and terminal chlamydospores occasionally seen	Intercalary and terminal chlamydospores frequently seen

Cartwright, in a private communication, writes that he has in press a paper to appear in the Transactions of the British Mycological Society on a re-investigation into "Brown" Oak caused by *F. hepatica* and to include a more detailed study of some aspects of its physiology.

It is probable, in view of the repeated isolation of *F. hepatica* from specimens of rot in Jarrah, that the fungus may be considered responsible for a heart rot in Jarrah. At present, experiments are in train in an attempt to ascertain the amount of decay produced in Jarrah blocks artificially inoculated with *F. hepatica.*

Summary.

1. The method used for isolation and culture of *F. hepatica* from specimens of rotted Jarrah as well as the artificial inoculation of sound Jarrah by *F. hepatica* is described.

2. Observations on the macroscopic and microscopic characteristics of *F. hepatica* are recorded. Types of fructifications obtained in culture are figured and described.

3. A comparison of the strain of *F. hepatica* from Oak and of *F. hepatica* from Jarrah is attempted.

4. The view is expressed that *F. hepatica* is responsible for a heart rot of Jarrah.

Acknowledgments.

The writer wishes to thank Associate-Professor E. I. McLennan of the Botanical Department, University of Melbourne, for her help and guidance throughout, and Professor S. M. Wadham of the School of Agriculture, University of Melbourne, for his many helpful suggestions.

Thanks are also due to Mr. I. H. Boas, of the Division of Forest Products of the Council for Scientific and Industrial Research, by whose suggestion this investigation was begun, and to Mr. S. L. Kessell of the West Australian Forests Department for having made available a grant, as well as specimens of rot in Jarrah.

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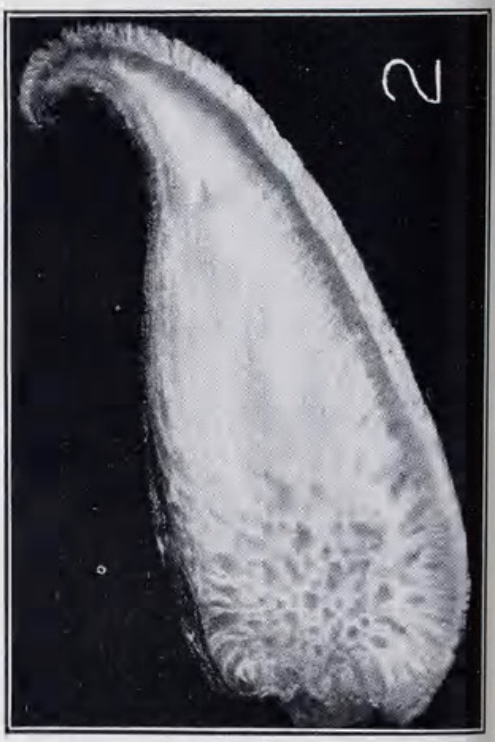

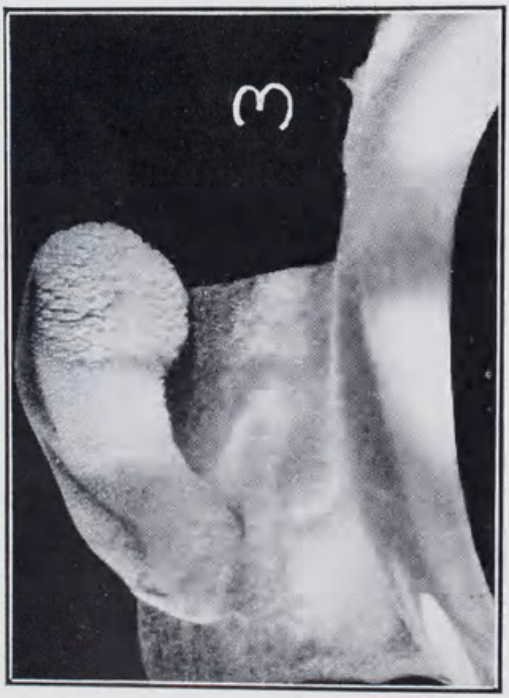

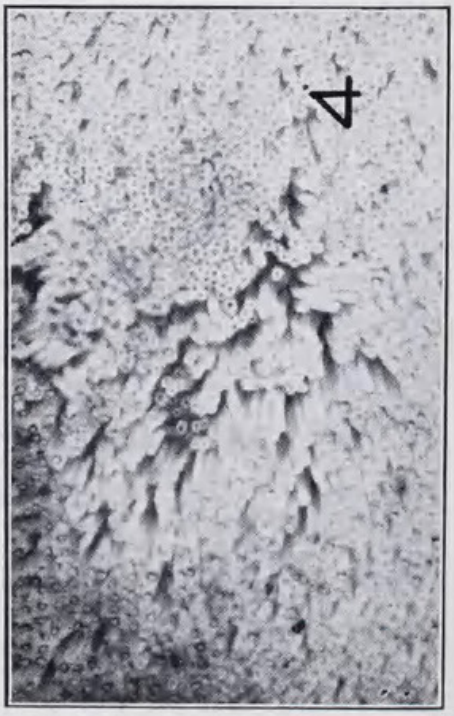

Fistulina hepatica.

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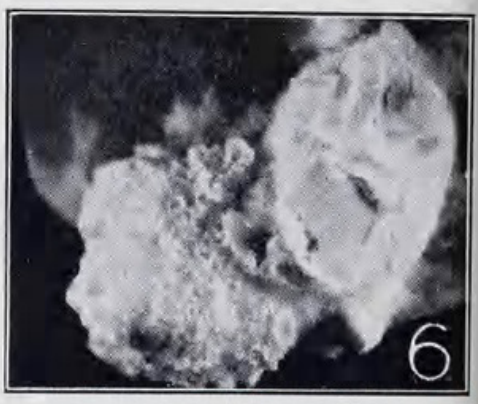

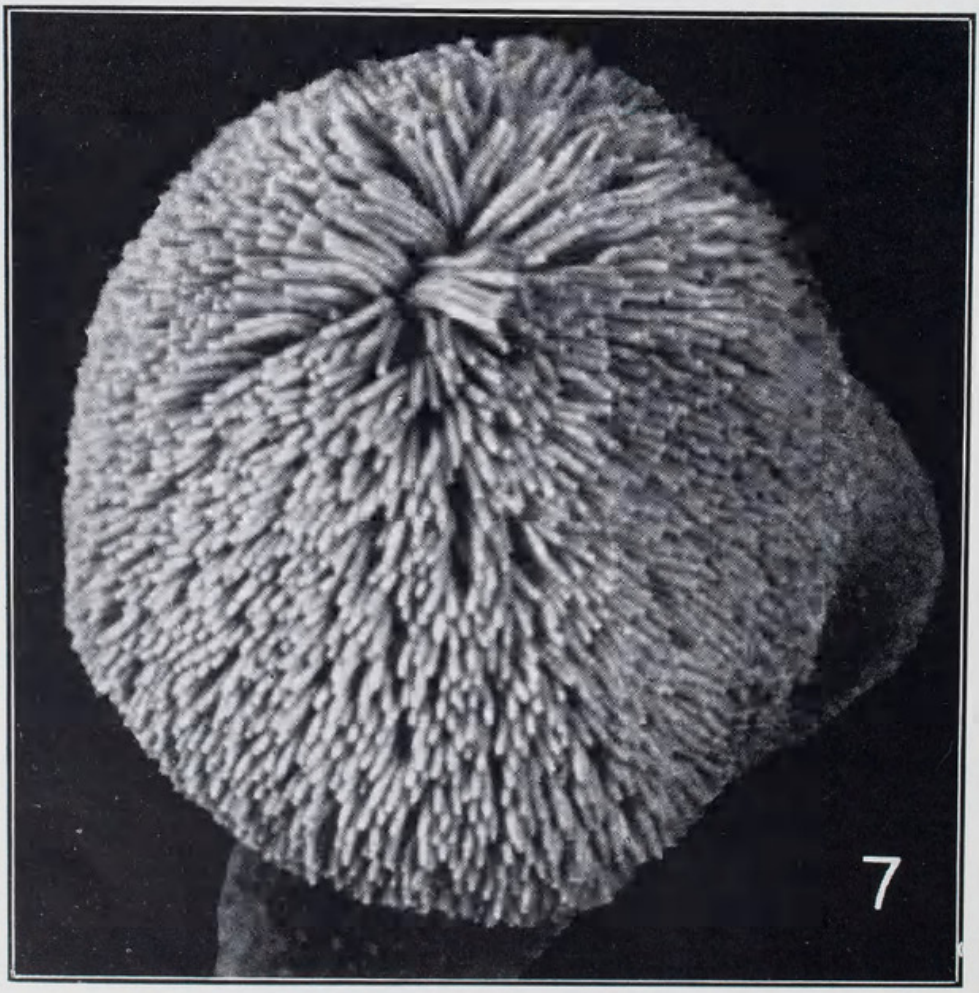

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Explanation of Plates.

Plate IX.

Fig. 1.—*F. hepatica* growing on a woody root of a living *Eucalyptus* sp. at Healesville, Victoria. × $\frac{1}{6}$.

Fig. 2.—Vertical section of *F. hepatica* (in fig. 1) showing hymenial layer composed of separate tubes. × 7/10.

Fig. 3.—Typical fructification of *F. hepatica* in artificial culture. Spore deposits. Culture 5 months old. × 1 7/10.

Fig. 4.—Hymenial surface of *F. hepatica* (in fig. 1) showing separate tubes. × $3\frac{1}{3}$.

Plate X.

Fig. 5.—*Ceriomyces*-type fructifications of *F. hepatica* in artificial culture. The striate interior and papillations may be observed in one fructification. Exudations present on the mycelium. Culture 3 months old. × $1\frac{1}{3}$.

Fig. 6.—*Cyphella*-type fructifications of *F. hepatica* on malt extract agar slope. Culture $7\frac{1}{2}$ months old. × 7.

Fig. 7.—Near typical fructification of *F. hepatica* cultured on sound Jarrah on malt extract agar. Free and separate hymenial tubes are present. Culture $4\frac{1}{2}$ months old. × $6\frac{1}{2}$.