A preliminary investigation of fungal decay in Jarrah (*E. marginata*).

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A PRELIMINARY INVESTIGATION OF FUNGAL DECAY IN JARRAH (E. MARGINATA).

INTRODUCTION.

Forest pathology in Australia has been developed along taxonomical rather than pathological lines. In cases where fungal attack has been apparent or suspect, it has generally been the practice to search for fruit-bodies of the higher fungi on the affected trees or wood and to identify these as the pathogens rather than to confirm these findings by isolating the causal organism and carrying out pathogenicity tests. Many records of Basidiomycetes occurring on trees and on wood have been made without reference to their pathogenic effects.

McAlpine (1895) compiled a host and habitat index of Australian fungi and made use of works by the following:- Bailey, Berkeley, Cobb, Cooke, Kalchbrenner, Massee, McAlpine, Saccardo, Tepper, Tisdall et alia. Hedgecock (1926) issued a comprehensive list of fungi recorded as occurring on various parts of trees of Eucalyptus spp. in North America and elsewhere. Cleland (1934) in a handbook on the larger fungi of South Australia recorded the hosts and included a number of fungi recorded for the remaining States of Australia. His referances included works by Burt, Cleland, Cheel, Cooke, Cunningham and Wakefield.
At present Brittlebank is preparing an up-to-date host index of Australian fungi.

The mycology and pathogenicity of the wood-destroying fungi appear to have been comparatively neglected. An article in the Australian Forestry Journal (1921) stresses the need for investigatiom into the entomology and mycology of forest diseases in Australia by trained men and suggested co-operation with the Imperial Bureaux of Entomology and Mycology. Samuel (1922) surveyed the forest pathology of pines in South Australia under the following headings:-

(1) general observations on growth, (2) "curly-needle")disease, (3) insect diseases, (4) fungus diseases. Refshauge and Proctor (1935) constructed keys for the identification of fourteen common Australian wood-destroying Basidiomycetes by noting their vegetative characters on various media. At present Refshauge is investigating in a similar manner , an additional number of Basidiomycetes.

The purpose of this investigation is to identify fungi isolated from typical specimens of decay in Jarrah, and to carry out pathogenicity tests on Jarrah blocks.

OUTLINE OF INVESTIGATION.

Specimens of decay in Jarrah were examined for evidence of fungal attack. Isolations were attempted and were successful from the majority of specimens. The fungi isolated were allowed to attack sound true-wood of Jarrah under controlled conditions for periods of time which varied from eight months to twelve months and more. The object of this artificial attack was to establish the identity of the causal fungus for each rot specimen and to test for criteria of decay. According to Koch's Postulates, this necessitated (a) the constant association of the fungus with one type of rot specimen, (b) the isolation and study of the fungus in pure culture, (c) the inoculation of the Jarrah with the fungus, under favourable conditions, and subsequent development of characteristic symptoms of decay, (d) the re-isolation of the fungus and identification as that first isolated.

METHOD.

Specimens of decayed Jarrah were selected by Forester Weston, of the West Australia Forests Department, from Jarrah forests, as examples of the principal types of rot in Jarrah. In many cases, the names used locally by timber workers to distinguish the various types of rot were given. The specimens were designated and forwarded by ship from the Government Stores Department at Fremantle to Melbourne and thence to the Botany School, University were investigations were carried out. At first the specimens were packed simply in wooden crates but later on it was suggested that wrapping specimens in moisture-retaining paper would be advantageous and this was accordingly done. On the arrival of each crate, notes were made on specimens therein.

From the great majority of specimens, attempts were made to obtain a suitable pair of mirror-image surfaces through the middle of the rotted areas in order that the position of removal of inocula from the one image could be recorded on the intact image. Rot specimens were sawn or split with this object in view. From one mirror-image of each rot specimen, a disc approximately one inch thick was cut. Regions were marked off on it from which inocula were to be removed and corresponding marks were made on the intact image.

The disc (after surface-sterilization) was then transferred to the inside of an inoculation chamber where, by the aid of a pair of chisel-forceps, inoculations were made. Small pieces of wood were removed from designated regions of the disc and were sown on to Petri dishes containing malt-extract agar (2.7% malt-extract, 1.5% agar, distilled water) to a depth of one quarter of an inch. This was done aseptically and sterilized medium was used. On an average, fifteen inoculations were made from each disc cut. 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.

Basidiomycetes generally appeared within three weeks and, after vigorous initial growth in the incubator, cultures and sub-cultures were maintained in darkness under room conditions.

Suitable stock cultures were used to inoculate conical beakers containing sterilized malt agar to a depth of one ~~x~~ inch. Beaker cultures were set up in quadruplicate, and in addition, a control, to which no inoculum was added, was used. Beakers were maintained under conditions similar to those used for Petri dishes. When the surface of the medium was covered (usually 3-4 weeks' growth), it presented a suitable sub-stratum for the attack on sound Jarrah blocks, which were of true-wood cut approximately 2" x 1" x 1" from sound Jarrah sticks obtained from a Melbourne timber-yard.

In the earlier part of the investigation, the blocks were saturated, sterilized and added to the beaker cultures. Saturated weights of the blocks before and after fungal attack were recorded. Later on, improvements were effected in that the blocks after being cut were dried to constant weight by heating at 105°C for approximately 45 hours, saturated and sterilized and then added to the beaker cultures. Dry weights of blocks before and after attack were recorded, in these cases.

During the period of attack of fungus on block, which varied from eight months to twelve months or over, observations on fungal growth and apparent effects on blocks were recorded.

After attack, the blocks were examined and changes in appearance and texture were noted. Textural changes were roughly denoted by the insertion of a pen-knife point in various parts of a block and observing the difference in ease of penetration between the sound and apparently decayed parts of the block.

The blocks, after weighing, were next subjected to strength tests carried out with the co-operation of the Division of Forest Products, C. S. I. R. Strips, approximately 2" x ¼" x ¼", were sawn longitudinally from each block to include the surface previously in contact with the mycelial mat on the medium. A strip was supported over a span of constant width (1½") and a weight (1 lbwt.) with constant bearing area (.03 sq.in.) was allowed to fall from a definite height directly above the strip. This height was increased until the falling weight just succeeded in fracturing the strip. When this occurred, the height of weight and type of failure in strip were recorded and used to indicate the strength of the strip and consequently, of the attacked region of the block.

Finally, sections were cut from strips selected at random and from corresponding controls. These were stained by Cartwright's method to demonstrate the presence (or absence) of fungal hyphae.

The foregoing method was not applied in its entirety to every specimen received. Mirror-image surfaces were not cut from specimens A2, B2, C2, E2, L2, Z2, V2a and C5, but instead, inocula were removed from suitable regions. Specimens R2, V2, S2 and E5 each showed the presence of several rotted areas and attempts were therefore made, in these cases, to cut mirror-image surfaces from each rot. No investigations were made on specimens of sound true-wood and sap-wood. Fungi isolated from specimens Z2, C5 and D5 were allowed to attack the blocks ~~with renewed vigour~~ again after 2nd September, 1937 by transferring the blocks to fresh malt agar in beakers. Experiments on these fungi and blocks were therefore not terminated at time of writing.

OBSERVATIONS ON FUNGI ISOLATED.

In the following account, all specimens are grouped under headings which suggest themselves from the names and descriptions supplied by Weston.

Capitalized colours are from Ridgway's "Colour Standards and Colour Nomenclature".

Type	Specimen	Page	Specimen	Page
1. Heart rot or dry rot.	A2	7-8	F2	9-10
	J2	11	K2	12
	O2	12	P2	13-14
	Q2	14	R2	15-17
	F5	18		
2. Pith or doze.	B2	19-20	C2	21-22
	G2	22a	M2	23
3. Straw rot.	B2	19-20,24	D5	24
4. Decayed included sap.	E2	25-27	S2	27
	E5	28		
5. Yellow-edged pin holes.	V2	29-30		
6. Pencilled Jarrah.	L2	30	Z2	31
	C5	31-32		
7. Sound heartwood ie. truewood.	H2	32	N2	32
	W2	32	X2	32
	X3	32	Y2	32
	G5	32		
8. Sound sapwood.	D2	33	W2	33
	X2	33	X3	33
	Y2	33		
9. Clean included sap.	D2	33		

Type 1. Heart Rot or Dry Rot.

A2. - "Heart rot or dry rot radiating from heart".

Specimen received 19.3.36.

Description of specimen :-

Rot - heart.

Colour - Burnt Sienna.

Texture - powdery.

Cracking - cubical.

Mycelium present - white strands; remnants of white sheets scattered over surface.

Zone lines - absent.

Fungus isolated :- Polyporus eucalyptorum

1st Isolated.	Incubation Period. *	Tot. Inocula.	Effective.	%
25.4.36	15-18 days	30	2	7

P. eucalyptorum in culture :-

Macroscopic Characteristics :-

Colour - Naphthalene Yellow.

Texture - felty; moderately thick.

Exudations- droplet.

Rate of growth - moderate.

Sporophores - not obtained.

Unusual feature - whitish precipitate in medium.

Microscopic Characteristics :-

Clamp connections - absent.

Hyphae ;-

Width - 3-7u.

Appearance - hyaline; vacuolate to granular.

Branching - free; acute-angled.

Chlamydospores - 10-15u; spherical; granular contents; terminal and intercalary.
x 10u

Unusual feature - hyphae sometimes anastomosed.

' Quotations are from names and descriptions as supplied by Weston.

* This refers to period elapsing between sowing of small pieces of wood on medium and first appearance of fungus.

Attack on Jarrah Blocks.

Culture	.247dA/1	.247dA/2	.247dA/3	.247dA/4	Control
Started	24.7.36				
Block added	2.9.36				
" removed	27.7.37				
Period of attack	328 days				
Appearance	Normal				
Texture	Normal to sub-normal; tending to crack.				Normal
Initial wt.	24.25g	24.54	24.25	23.86	24.26
Final wt.	24.25	24.53	24.23	23.81	25.03
% loss	0	0	0.1	0.2	-3.2
Strength test	4,4	5,4	5,3	4,3	9,9
Fungal hyphae	Numerous; crossing fibre-walls, medullary ray cells per bore-holes and pits.				Absent

F2. - "Dry rot; extends through one section of bole but did not touch the heart".

Specimen received 19.3.36.

Description of specimen :-

Rot - heart.

Colour - Hazel, to Cinnamon Rufous.

Texture - powdery.

Cracking - longitudinal; transverse in part.

Mycelium present - none.

Zone lines - present.

Unusual feature - presence of insect tunnels.

Fungi isolated :- Phycomycete I.

Fistulina hepatica.

Fungus	1st Isolated	Incubation Pd.	Tot. Inoc.	Effective	%
P'mycete	30.3.36	7 days	44	19	43
F. hepatica	18.8.36	Under 22 days	22	3	14

Phycomycete I in culture :-

Macroscopic Characteristics :-

Colour - Avellaneous.

Texture - sodden; very thin.

Exudations - absent.

Rate of growth - very rapid.

Unusual features - vigorous submerged growth; regular margin; individual hyphae macroscopically invisible.

Microscopic Characteristics :-

Hyphae :-

Width - 4-7u.

Appearance - hyaline, vacuolate.

Branching - varied.

Chlamydospores - hyaline; spherical, 12u diameter; pleurogenous and terminal; net-veined.

Attack on Jarrah Blocks.

Culture	055/dF/1	055dF/3	055dF/4	Control
Started	5.5.36			
Block added	16.5.36			
" removed	21.4.37			
Period of attack	340 days			
Appearance	Normal			
Texture	Normal			
Initial wt.	Not recorded			
Final "	Not recorded			
% loss				
Strength test	Not tested			
Fungal hyphae	Absent			

F. hepatica in culture :- See pp. 25-26.

Attack on Jarrah Blocks.

Culture	089F/1	089F/2	019F/4	Control
Started	8.9.36	1.9.36		1.9.36
Block added	3.10.36			
" removed	12.8.37			
Period of attack	323 days			
Appearance	Slight brownish discolouration.			Normal
Texture	Normal			
Initial wt.	24.05g	24.08	24.12	24.05
Final "	24.20	24.27	24.20	24.75
% loss	-0.6	-0.8	-0.3	-2.9
Strength test	10,9	9,9	8,7	10,10
Fungal hyphae	Absent			

J2. - "Heart rot".

Specimen received 4.5.36.

Description of Specimen :-

Rot - heart.

Colour - Raw Umber.

Texture - powdery.

Cracking - cubical.

Mycelium present - none.

Unusual feature - much-weathered specimen.

Fungus isolated :- F. hepatica .

1st Isolated	Incubation Period	Tot. Inocula	Effective	%
27.5.36	21 days	12	2	17

F. hepatica in culture :- See pp. 25-26

Attack on Jarrah Blocks.

Culture	207J/1	207J/2	207J/3	Control
Started	20.7.36			
Block added	21.8.36			
" removed	2.11.36	28.7.37		
Period of attack	73 days	280 days		
Initial sat. wt.	34g	34	34	33
Final " "	32	31.5	29.5	30
% loss	6	7	13	9
Appearance	Normal			
Texture	Normal to sub-normal			Normal
Strength test		10,10	11,11	9,13
Fungal hyphae	Absent			

K2. - "Dry rot near heart but heart core practically unaffected".

Specimen received 4.5.36.

Description of Specimen :-

Rot - heart.

Colour - Argus Brown.

Texture - powdery.

Cracking - cubical.

Mycelium present - none.

Zone lines - present.

No fungus isolated after 30 inoculations.

02. - "Heart rot".

Specimen received 2.6.36.

Description of Specimen :-

Rot - heart.

Colour - Amber Brown.

Texture - powdery.

Cracking - cubical.

Mycelium present - none.

Zone lines - absent.

No fungus isolated after 35 inoculations.

P2. - "Heart Rot".

Specimen received 2.6.36.

Description of Specimen :-

Rot - heart.

Colour - Argus Brown.

Texture - powdery.

Cracking - tending cubical.

Mycelium present - none.

Zone lines - absent.

Unusual feature - extensively insect-tunnelled, frass deposits.

Fungus isolated :- Basidiomycete VIII.[1]

1st Isolated	Incubation Pd.	Tot. Inocula	Effective	%
6.7.36	19-21 days	18	7	39

Basidiomycete VIII in culture :-

Macroscopic Characteristics :-

Colour - White.

Texture - silk-felty; moderately thick.

Exudations - droplet.

Rate of growth - moderate to slow.

Sporophores - not obtained.

Unusual feature - regular margin.

Microscopic Characteristics :-

Clamp connections - frequent; often producing hyphal branches.

Hyphae :-

Width - 4-6u.

Appearance - hyaline; empty to vacuolate.

Branching - free; acute-angled.

Chlamydospores - in submerged mycelium; intercalary and terminal; sub-cylindrical to sub-spherical; granular contents; 4-10u x 12-18u.

Unusual feature - crystals in medium, rhomboidal, clumped.

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[1] Roman numerals were used temporarily to number the Basidiomycetes as isolated.

Attack on Jarrah Blocks.

Culture	207P/1	207P/2	207P/3	207P/4	Control
Started	20.7.36				
Block added	2.9.36				
" removed	28.7.37				
Period of attack	329 days				
Initial wt.	24.25g	24.49	24.14	24.37	24.11
Final "	23.96	24.21	24.01	24.23	24.47
% loss	1.2	1.1	0.5	0.6	-1.5
Appearance	Normal				
Texture	Brittle to sub-normal; longitudinal cracks.				Normal
Strength test	3	4,3	6,7	4,4	7,7
Fungal hyphae	Not numerous; narrow; crossing fibre walls.				Absent

Q2. - "Heart rot".

Specimen received 2.6.36.

Description of Specimen :-

Rot - heart.

Colour - Chestnut.

Texture - powdery.

Cracking - transverse.

Mycelium present - none.

Zone lines - absent.

Unusual features - insect tunnels containing frass; open Spring wood more decomposed.

No fungus isolated after 30 inoculations.

R2. - "Dry rot and/or heart".

Specimen received 2.6.36. Contains three separate zones of decay.

R2A.

Description of Sub-specimen :-

Rot - heart.

Colour - Burnt Sienna.

Texture - sub-normal

Cracking - longitudinal.

Mycelium present - white, thin, sparse on surface.

Zone lines - absent.

Fungus isolated :- Basidiomycete XII.

1st Isolated	Incubation Period	Tot. Inocula	Effective	%
7.10.36	under 111 days	6	1	17

B'mycete XII in culture :-

Macroscopic Characteristics :-

Colour - white at first, then Cinnamon to Sayal Brown.

Texture - irregular downy; tending to shagginess with stiff hairs; moderately thick.

Exudations - droplet.

Rate of growth - moderately rapid.

Sporophores - not obtained.

Unusual features - very vigorous growth; irregular margin with dense layer of fine hyphae; medium darkened.

Microscopic Characteristics :-

Clamp connections - frequent; occasionally compound and producing hypal branches.

Hyphae :-

Width - 2-8u

Appearance - hyaline, granular to vacuolate.

Branching - free; acute-angled and rectangular.

Chlamydospores - frequent; in submerged mycelium, intercalary and terminal; rare in aerial mycelium; sub-spherical; 8-12u; globular contents.

Unusual feature - crystals frequently present in medium, solitary, also, in clumps of small crystals along submerged hyphae.

Attack on Jarrah Blocks.

Culture	2110Ra/1	2110Ra/2	2110Ra/3	2110Ra/4	Control
Started	21.10.36				
Block added	16.11.36				
" removed	14. 8.37				
Period of attack	271 days				
Appearance	Normal				
Texture	Sub-normal to brittle				Normal
Initial wt.	23.78g	24.84	23.16	23.53	24.05
Final "	23.94	24.98	23.32	23.70	24.35
% loss	-0.7	-0.6	-0.7	-0.7	-1.2
Strength test	8,11	10,11	9,10	10,9	9,10
Fungal hyphae	Not numerous; crossing fibres through pits and bore-holes.				Absent.

R2b.

Description of Sub-specimen :-

Rot - heart.

Colour - Burnt Sienna.

Texture - powdery.

Cracking - tending transverse.

Mycelium present - none.

Zone lines - absent.

Fungus isolated :- Basidiomycete XI.

1st Isolated	Incubation Period	Tot. Inocula	Effective	%
9.9.36	under 20 days	18	2	11

B'mycete XI in culture :- See B'mycete XII, p. 15 and above.

Attack on Jarrah Blocks.

Culture	2610Rb/1	2110Rb/3	2610Rb/3	Control
Started	26/10.36	21.10.36	26.10.36	21.10.36
Block added	19.11.36			
" removed	10. 8.37			
Period of attack	264 days			
Appearance	Brownish discoloration			Normal
Texture	Soft			Sub-normal
Initial wt.	24.35g	20.60	19.39	19.67
Final "	24.65	20.95	19.59	20.09
% loss	-1.2	-1.7	-1.0	-2.1
Strength test	8,9	8,8	8,8	9,9
Fungal hyphae	Not numerous; crossing fibres through pits and bore-holes.			Absent

R2c.

Description of Sub-specimen :-

Rot - heart.

Colour - Chestnut.

Texture - powdery.

Cracking - transverse.

Mycelium present - none.

Zone lines - absent.

No fungus isolated after 20 inoculations.

F5. - "Dry rot".

Specimen received 29.8.36.

Description of Specimen :-

Rot - heart.

Colour - Auburn.

Texture - powdery.

Mycelium present - thin, white sheet; localized.

Cracking - cubical.

Zone lines - absent.

Unusual feature - tendency to longitudinal cleavage outside advanced stage of decay.

Fungus isolated :- Basidiomycete XIII.

1st Isolated	Incubation Period	Tot. Inocula	Effective	%
21.10.36	7-11 days	18	4	22

B'mycete XIII in culture :- See B'mycete XII, pp. 15-16.

Attack on Jarrah Blocks.

Culture	1211F5/1	1211F5/2	1211F5/3	1211F5/4	Control
Started	12.11.36				
Block added	28.12.36				
" removed	25. 8.37				
Period of attack	240 days				
Appearance	Brownish discolouration				Normal
Texture	Sub-normal				Normal
Strength test	8,6	7,7	4,4	8,7	7,6
Fungal hyphae	Absent				
	Brown rot present initially?		Brown rot present initially?		
Initial wt.	19.72	19.83	19.02	23.50	19.4
Final "	19.62	19.91	18.84	23.44	20.3
% loss	0.5	-0.5	2.0	0.3	-4.6

Type 2. Pith or Doze.

B2. - "Pith or doze (may develop to straw rot) from stump".

Specimen received 19.3.36.

Description of Specimen :-

Rot - heart.

Colour - Vinaceous-Russet.

Texture - stringy.

Cracking - longitudinal.

Mycelium present - thin, frosty appearance, throughout whole decayed region; on smaller block is papery, Yellow Ocher.

Zone lines - absent.

Unusual feature - strong mouldy odour.

Fungi isolated :- Fungus Bns.

F. hepatica

Fungus	1st Isolated	Incub. Period	Tot. Inocula	Effective	%
Bns	25.4.36	15-19 days	48	19	40
F. hepatica	28.4.36	17 days	48	1	3

Fungus Bns. in culture :-

Macroscopic Characteristics :-

Colour - Natal Brown.

Texture - loose-woolly, thin.

Exudations - absent.

Rate of growth - slow.

Sporophores - not obtained.

Unusual features - black, oily sheet in medium; under-surface of medium greyish; margin, dark, regular.

Microscopic Characteristics :-

Clamp connections - absent.

Hyphae :-

Width - 4-6u.

Appearance - hyaline; vacuolate to globular.

Branching - free; rectangular.

Chlamydospores - absent.

Attack on Jarrah Blocks.

Culture	055dB/1	055dBa/1	055dBa/2	Control
Started	5.5.36	5.5.36	. . . . .	
Block added	22.6.36	. . . . .		
" removed	24.6.37	. . . . .		
Period of attack	367 days	. . . . .		
Appearance	Normal	. . . . .		
Texture	Normal	. . . . .		
Initial wt. (sat)	31g	31	31	32
Final " "	30.5	32	32.5	32.5
% loss	1	-3	-4.5	-1
Strength test	8,8	9,9	10,10	10,9
Fungal hyphae	Absent	. . . . .		

F. hepatica in culture :- For description, see pp. 25-26

Attack on Jarrah Blocks.

Culture	125mB/2	125mB/3	125mB/5	125mB/6	Control
Started	12.5.36	. . . . .			
Block added	8.6.36	. . . . .			
" removed	24.6.37	. . . . .			
Period of attack	381 days	. . . . .			
Appearance	Normal	. . . . .			
Texture	Sub-normal	. . . . .			Normal
Initial sat. wt.	32.3g	34	33.4	33.5	32.2
Final " "	31.5	32	32.5	32	33
% loss	2	6	3	5	-2
Strength test	11,10		10,10	10,10	12,13
Fungal hyphae	Absent	. . . . .			

C2. - "Pith or doze from near top of bole".

Specimen received 19.3.36.

Description of Specimen:-

Rot - heart.

Colour - Kaiser Brown.

Texture - soft.

Cracking - absent.

Mycelium present - whitish; in narrow pockets.

Zone lines - absent.

Unusual feature - insect-tunnelled.

Fungi isolated :- F. hepatica .

Basidiomycete IV.

Fungus	1st Isolated	Incub. Period	Tot. Inocula	Effective	%
F. hepatica	3.6.36	16days	17	1	6
B'mycete IV	4.436	10-11 days	53	10	19

F. hepatica in culture :- For description, see pp. 25-26

Attack on Jarrah Blocks.

Culture	226C/1	226C/3	226C/4	Control
Started	22.6.36			
Block added	20.7.36			
" removed	5.7.36			
Period of attack	350 days			
Appearance	Normal			
Texture	Normal			
Initial sat. wt.	32g	32	30	32
Final " "	30.5	31.5	30	31
% loss	5	2	0	3
Strength test	9,10	9,10	9,9	9,11
Fungal hyphae	Absent			

B'mycete IV in culture :-

Macroscopic Characteristics :-

Colour - Orange Pink, Apricot Buff, Onion-skin Pink.

Texture - downy to felty, to ½" thick.

Exudations - absent.

Rate of growth - very rapid.

Sporophores - not obtained.

Unusual features - "ground glass" appearance of young mycelium due to rapid, appressed, hyphal growth initially; whitish precipitate of rhomboidal crystals in medium; medium bleached; irregular margin to mycelium.

Microscopic Characteristics :-

Clamp connections - frequent.

Hyphae :-

Width - 2-6u.

Appearance - hyaline, vacuolate to globular.

Branching - rectangular.

Chlamydospores - in submerged mycelium; intercalary and terminal; globular contents; sub-spherical 5-10 x 10-25u.

Unusual feature - crystals occurring in medium and along hyphae.

Attack on Jarrah Blocks.

Culture	226C/6	226C/7	226C/8	226C/9	22 Control
Started	22.6.36				
Block added	13.7.36				
" removed	5.7.37				
Period of attack	357 days				
Appearance	Irregular Cinnamon streaks; vessels on transverse surfaces empty.				Normal
Texture	Soft and friable				Normal
Initial sat. wt.	33g	34	34	34	34
Final " "	30	30	30.5	30	31
% loss	9	11	10	11	9
Strength test	9,9	12,10	9,9	8,9	13,12
Fungal hyphae	Numerous; broad; crossing fibres and m. ray cells through pits and bore-holes; fibre-cells pushed apart.				Absent

M2. - "Doze. Advanced stage".

Specimen received 2.6.36.

Description of Specimen :-

Rot - heart.

Colour - Cinnamon Rufous.

Texture - sub-normal.

Cracking - absent.

Mycelium present - none.

Zone lines - absent.

Fungus isolated :- Basidiomycete VI.

1st Isolated	Incubation Period	Tot. Inocula	Effective	%
2.6.36	16 days	16	2	12

B'mycete VI in culture :- See B'mycete IV p. 22.

Attack on Jarrah Blocks.

Culture	207M/1	207M/2	207M/3	207M/4	Control
Started	20.7.36				
Block added	2.9.36				
" removed	28.7.37				
Period of attack	329 days				
Appearance	Slight Cinnamon discolouration				Normal
Texture	Soft to sub-normal				Normal
Initial wt.	24.90g	24.20	24.39	23.97	23.72
Final "	24.86	24.16	24.21	23.91	24.15
% loss	0.2	0.2	0.7	0.3	-1.8
Strength test	8,8	9,6	7,9	7,8	9,7
Fungal hyphae	Numerous; broad; crossing fibre walls and medullary ray cells through pits or bore-holes; fibre-cells pushed apart.				Absent

Type 3. Straw Rot.

B2. - "Pith or doze (may develop to straw rot) from stump".

See pp. 19-20.

D5. - "Straw rot in Jarrah".

Specimen received 29.8.36.

Description of Specimen :-

Rot - heart.

Colour - Cinnamon Rufous to Hazel.

Texture - powdery.

Cracking - tending along open Spring wood.

Mycelium present - none.

Zone lines - absent.

Unusual features - insect-tunnelling.

Fungus isolated :- F. hepatica .

1st Isolated	Incubation Period	Tot. Inocula	Effective	%
22.10.36	9-17 days	18	6	33

F. hepatica in culture :- For description, see pp.

Attack on Jarrah Blocks.

Culture	1211D5/1	1211D5/2	1211D5/3	1211D5/4	Control
Started	12.11.36				
Block added	8.12.36				
Initial wt.	20.23g	23.65	19.27	24.62	19.67

Attack still in progress at time of writing.

Type 4. Decayed Included Sap.

E2. - "Cavity forming through decay of included sap".

Specimen received 19.3.36.

Description of Specimen :-

Rot - heart.

Colour - Zinc Orange.

Texture - powdery.

Cracking - absent.

Mycelium present - none.

Zone lines - absent.

Unusual feature - decay apparently preceded by insect-tunnelling.

Fungus isolated :- Fistulina hepatica (Huds.) Fries.

1st Isolated	Incubation Period	Tot. Inocula	Effective	%
21.4.36	18 days	30	3	10

F. hepatica in culture :- Composite Description"

Macroscopic Characteristics :-

Colour - initially white; later Cartridge Buff to Russet.

Texture - loose-woolly, membranous to felty; sub-sulcate; moderately thick; irregular margin.

Exudations - watery or reddish.

Rate of growth - moderate.

Sporophores :-

Stipe :-

Colour - Ivory Yellow, Cartridge Buff to Chestnut.

Shape - round, cylindrical to phalloidal; small, cup-shaped.

Size - to 2cms. in diameter; to 4.5 cms. long.

Occurrence - on mycelium or block.

Exudations - watery or reddish.

" For a more complete description, see the author's "A Cultural Study of Fistulina hepatica (Huds.) Fries, Isolated from Decayed Jarrah (Eucalyptus marginata Sm.)"

Fertile :-

Colour - Cartridge Buff, Pinkish Cinnamon to Wood Brown.

Shape - shelf-like; open cup-shaped.

Size - to 1.5 cms. wide; to 4 cms. long.

Occurrence - on mycelium or block; typical s'phores obtained only on transverse or long. surface of block.

Exudations - watery or reddish.

Hymenium - lining distinct, fistulous Cartridge Buff tubes, 2-5 x 0.2-0.5 mm.; orifices often with fimbriated edges.

Microscopic Characteristics :-

Clamp connections - frequent; simple.

Hyphae :-

Width - 2-8u.

Appearance - hyaline; vacuolate to globular contents (oil globules).

Branching - free; acute-angled and rectangular.

Chlamydospores - frequent; intercalary and terminal; 10-13 x 2-7 u; containing oil; ovoid to sub-cylindrical.

Unusual feature - rhomboidal crystals in medium.

Spores:-

Colour - deposits, initially Empire Yellow, later Antimony Yellow; individual spores hyaline.

Shape - ellipsoidal to sub-spheroidal; apiculated.

Dimensions - 3-5 x 5-8 u.

Attack on Jarrah Blocks.

Culture	045dE/2	045dE/3	045dE/4	155dE/1	Control
Started	4.5.36			15.5.36	4.5.36
Block added	2.6.36			8.6.36	2.6.36
" removed	24.6.37		29.12.36	24.6.37	
Period of attack	386 days		210 days	380 days	386 days
Appearance	Normal				
Texture	Normal				
Initial sat. wt.	32g	32	32	33	31.5
Final " "	33	31.5	35	32.5	33
% loss	-3	2	-9	2	-5
Strength test		10,12		10,10	12,12
Fungal hyphae		Absent		Absent	Absent

S2. - "Included sap about 6½" x ½" across end-section, decayed on one edge, still sound on other edge".

Specimen received 2.6.36.

Description of Specimen :-

Early stage of decay :-

Colour - Vinaceous Cinnamon.

Rot - included sap.

Texture - soft.

Cracking - absent.

Mycelium present - none.

Zone lines - absent.

Late stage of decay :-

Rot - included sap.

Colour - Prout's Brown.

Texture - powdery.

Cracking - cubical.

Mycelium present - none.

Zone lines - present.

Unusual feature - zone lines separate the two stages of decay.

No fungus isolated after 36 isolations.

E5. - "Decayed included sap. (This is from the butt of an old tree and the wood may be over-mature. There is a possibility of other rots being present)".

Specimen received 29.8.36. Consists of a large and small block, both very much weathered and decayed.

Description of Specimen :-

Small block :-

Rot - ?

Colour - Clay.

Texture - powdery.

Cracking - cubical.

Mycelium present - none.

Zone lines - present.

Unusual feature - extensive insect-tunnelling present.

No fungus isolated after 18 inoculations.

Large block :-

Rot - ?

Colour - various including Clay and Chestnut.

Texture - powdery.

Cracking - cubical, in part.

Mycelium present - none.

Zone lines - present.

Unusual features - specimen very much decayed; extensive insect-tunnelling, discoloured wood round pin-holes.

No investigations carried out in the pathology of large block.

Type 5. Yellow-edged Pin-holes.

V2. - "Pin-holes surrounded by discoloured wood ".

Specimen received 2.6.36.

Description of Specimen :-

V2a - wood immediately associated with borer tunnels.

Rot - heart.

Colour - Sayal Brown.

Texture - soft to powdery.

Cracking - absent.

Mycelium present - none.

Zone lines - absent.

Unusual feature - interior of tunnel, Bay to Black.

No fungus isolated after 30 inoculations.

V2b - wood showing general rot.

Rot - heart.

Colour - Tawny to Cinnamon Rufous.

Texture - powdery.

Cracking - absent.

Mycelium present - none.

Zone lines - absent.

Numerous insect-tunnels present.

Fungus isolated :- F. hepatica.

nlst Isolated	Incubation Period	Tot. Inocula	Effective	%
6.7.36	18 days	18	8	43

Attack on Sound Jarrah Blocks.

Culture	207Vb/1	207Vb/2	207Vb/3	207Vb/4	Control
Started	20.7.36				
Block added	21.8.36				
" removed	5.8.37				
Period of attack	349 days				
Appearance	Brownish discolouration				Normal
Texture	Sub-normal to normal.				Normal
Initial sat. wt.	34g	35	33	32	34
Final " "	32.5	34	33	31	30.5
% loss	4	3	0	3	10
Strength test			8,7	7,10	10,9
Fungal hyphae			Absent		

Type 6. Pencilled Jarrah.

L2. - "Pencilled Jarrah .... "

Specimen received 2.6.36

Description of Specimen :-

Lines - dark; occurring in true-wood; extending to 2" radially transverse, to ¼" radial-longitudinally.

Colour of surrounding wood - Kaiser Brown.

Texture - normal.

Cracking - absent.

Mycelium present - none.

Zone lines - absent.

No fungus, except a sporulating one (suspected of being a contaminant), isolated after 17 inoculations.

Z2. - "Clean pencilled Jarrah from an over-mature tree".

Specimen received 20.6.36.

Description of Specimen :-

Lines - dark; occurring in both true-wood and sap-wood; extending to 2" radially transverse and to ½" radial-longitudinally.

Colour of surrounding wood - Cacao Brown, Cinnamon Rufous.

Texture - normal.

Cracking - absent.

Mycelium present - none.

Zone lines - absent.

Unusual feature - zones of softer, lighter-coloured wood - probably incipient decay.

Fungus isolated :- F. Hepatica .

1st Isolated	Incubation Period	Tot. Inocula	Effective	%
27.10.36	21 days	9	3	33

F. hepatica in culture :- See pp. 25-26.

Attack on Jarrah Blocks.

Culture	1211Z2/1	1211Z2/2	1211Z2/3	1211Z2/4	Control
Started	12..11.36				
Block added	8.12.36				
Initial wt.	20.23g	23.65	19.27	24.62	19.67

Attack still in progress at time of writing.

C5. - "Pencilled Jarrah".

Specimen received 29.8.36.

Description of Specimen :-

Lines - dark; occurring in true-wood only; extending to 1" radially, transverse.

Colour of surrounding wood - Vinaceous Russet.

Texture - normal.

Cracking - absent.

Mycelium present - none.

Zone lines - absent.

Fungus isolated :- F. hepatica .

1st. Isolated	Incubation Period	Tot. Inocula	Effective	%
7.11.36	31 days	9	3	33

F. hepatica in culture :- See pp. 25-26.

	Attack on Jarrah Blocks.		
Culture	17205/2	17205/3	17205/4
Started	17.2.37		
Block added			
Initial wt.	23.51g	23.62	23.87

Attack still in progress at time of writing.

Type 7. Sound Heart-wood (i.e. true-wood)

H2. - "Heart nominally sound".

Specimen received 4.5.36. No investigations made.

N2. - "Heart".

Specimen received 2.6.36. No investigations made.

W2. - "Clean true-wood and sap."

Specimen received 20.6.36. No investigations made.

X2. - "Clean true-wood and sap".

Specimen received 20.6.36. No investigations made.

X3. - "Clean true-wood and sap".

Specimen received 20.6.36. No investigations made.

Y2. - "Clean true-wood and sap".

Specimen received 20.6.36. No investigations made.

G5. - "Clean heart from sound tree".

Specimen received 29.8.36 No investigations made.

Type 8. Sound Sap-wood.

D2. - "Clean included sap".

Specimen received 19.3.36.

No included sap-wood present, according to Cummins' method of identification(1936). No further investigations made.

W2, X2, X3, Y2. - See above. p. 32.

Type 9. Clean Included Sap.

D2.- See above.

NOTES ON MACROSCOPIC CHARACTERISTICS OF DECAYED WOOD.

The principal difficulty encountered in the investigation was the complete absence of history concerning each specimen. This defect introduced a number of varying factors, many of which may have operated to conceal the primary effects of the attack by wood-destroying fungi. These factors included maturity of wood and age of specimen, type and amount of weathering, environment, presence of insects and secondary fungi. The fact that decay of wood is in itself a slow and gradual process ensures the progressive change of the symptoms of rot with time.

Some of the difficulties encountered in the description of specimens were as follows:-

Rot:- some specimens described as sap-roots and containing coloured zones indicative of sap-wood actually contained no sap-wood on examination. (Cummins, 1936).

Colour:- this was probably dependent to a great extent on type and amount of weathering and on secondary attack by fungi and insects; in addition, sound Jarrah itself was often variable in colour.

Texture:- at times this was difficult to guage and was probably influenced by weathering. Cracking and mycelium present were probably also influenced by weathering. In addition, the presence of insect funnels with frass deposits and discoloration of wood, the manifestation of wound reactions, such as gum veins, included sap-wood as well as the presence of other saprophytes all added to the difficulties of recognition of the primary symptoms of decay.

IDENTIFICATION OF FUNGI ISOLATED.

The fungi isolated may be divided into three groups as follows :- (1) F. hepatica .

(2) Basidiomycetes other than F. hepatica .

(3) Fungi other than Basidiomycetes.

(1) Fistulina hepatica (Huds.) Fries.

This fungus was isolated from specimens of "heart rot or dry rot", "pith or doze", "straw rot", "decayed included sap", "yellow-edged pinholes" and "pencilled Jarrah". Typical diminutive fertile sporophores, obtained in culture, exhibited the characteristics of the sub-family Fistulinaceae in the "hymenium inferior, lining free and separate tubes". (Rea, 1922). The description of Fistulina hepatica Fries by Saccardo (1888) agreed closely with the appearance of these sporophores in culture. Additional proof of the identity of the fungus was given by setting up comparative cultures against F. hepatica obtained from sporophore tissue of a fruit-body growing on felled Mountain Ash (E. regnans) timber at Healesville, Victoria. The fungus was recorded by Cooke (1892) for W. Australia. Tamblyn (1937) isolated the fungus from pencilled Jarrah and stated that its "causal relationship to pencilling has been reasonably established". He does appear, however, to have isolated the fungus from other types of specimens although the common occurrence of the fungus on Jarrah trunks is noted.

(2) Basidiomycetes other than F. hepatica.

Polyporus eucalyptorum Fries. This fungus was isolated from specimensA2 ("heart rot or dry rot") and comparison showed it to be identical with cultures of P. eucalyptorum obtained from sporophore tissue of a fruit-body growing on felled Mountain Ash timber at Healesville, Victoria. This fungus was recorded for W. Australia by Cooke (1892). Tamblyn (1937) isolated this fungus from a specimen-type he refers to as "brown trunk rot" which, in its late stage, appears to be similar in description to specimen A2.

Basidiomycetes XI, XII and XIII isolated from "dry rot and/or heart rot" and "dry rot" specimens showed a close similarity to one another, in culture.

Basidiomycetes IV and VI isolated from "heart rot or dry rot" and "pith and doze" specimens, were identical

These Basidiomycetes, as well as Basidiomycete VIII isolated from a "heart rot or dry rot" specimen, were not identified by the writer and their comparison with the key constructed by Refshauge and Proctor(1935) yielded no positive results.

(3) Fungi other than Basidiomycetes.

The Phycomycete isolated from specimen F2 as wellas fungus B n s isolated from specimen B2, were considered unimportant as wood-destroying fungi. This view was borne out by the greater frequency of their isolation compared with other fungi and the absence of any change in Jarrah blocks following on "attack" by these fungi. Consequently, no attempts were made at identification.

NOTE. Two Basidiomycetes, F. hepatica and Basidiomycete IV, were isolated from specimen C2 ("pith or doze").

ARTIFICIAL ATTACK ON JARRAH BLOCKS.

Certain difficulties were encountered in the interpretation of results of pathogenicity tests by fungi on Jarrah blocks. In the mechanical test, these were due to (a) the fact that matching of blocks prior to inauguration of attack had not been resorted to and that consequently there existed, in all probability, initial differences in strength between the blocks attacked by any one fungus and (b) over-drying of blocks before and/or after attack for 45 hours at 105° C produced varying degrees of brittleness and afforded no reliable opportunity for comparison of fracture. Apparent inconsistencies in losses in weight of blocks after fungal attack were due primarily to the uncertainty of fungal attack in culture during the relatively short periods of attack.

F. hepatica. This fungus was allowed to attack Jarrah blocks under controlled conditions for periods of time ranging from 73 to 386 days. Insignificant changes in weight, appearance, texture and strength test of the blocks as well as the absence of fungal hyphae in wood sections all indicated negative evidence for pathogenicity based on artificial inoculation under the conditions of this investigation.

This may have been due to a number of causes (a) shortness of period of attack - an effort to diminish the effect of this cause is being made by continuing the attack with certain cultures (see pp. 24, 31, 32) - further, it is of interest to note that Cartwright (1937) stated that F. hepatica caused no appreciable loss in strength in the early stages (presumably on living Oak) but later, considerable softening took place and the timber became somewhat brittle; (b) unsuitability of the medium and/or controlled conditions - the specialized metabolism of the fungus, possibly with regard to tannin (Cartwright 1937) may have been responsible here; (c) the possibility, that the fungus uses sapwood as a base for attack on true-wood in the living tree, must not be overlooked, in which case artificial culture on Jarrah

sapwood blocks would probably throw more light on this aspect.

P. eucalyptorum. Jarrah blocks were attacked by this fungus for 328 days. Although no significant changes in appearance or weight were obtained, the slight but definite deterioration in te testure and the very definite reduction in strength supported by the presence of numerous fungal hyphae crossing wood fibres and medullary ray cells by means of bordered pits and boreholes, indicated positive pathogenicity under the conditions used.

Basidiomycete Xl, Xll, Xlll. This fungus attacked Jarrah blocks for periods of time from 240 to 271 days. Attacked blocks tended towards a brownish discoloration and this was associated with a degradation of texture but no apparent loss in strength was recorded. No consistent losses in weight were obtained as a result of fungal attack but, to the contrary, increases in weight were obtained in a number of blocks. Fungal hyphae occurred infrequently in wood sections and were observed crossing wood fibres through bordered pits and bore-holes. Further experiments will be necessary to establish proof of pathogenicity of this fungus.

Basidiomycete lV, Vl. Jarrah blocks were attacked for periods of 257 to 329 days by this active fungus (Basidiomycete lV was considerably more vigorous). Cinnamon-coloured streaks on wood previously in contact with the fungal mycelium, associated with a marked softening in texture and a lowered strength test gave definite signs of the pathogenicity of the fungus. In addition, numerous hyphae were observed in longitudinal sections crossing wood fibres and medullary ray cells through bordered pits and bore-holes. A photograph shows the transverse surface of a block which was attacked and in which the wood vessels were emptied as a result of the presence of the fungus.

Basidiomycete Vlll. This fungus, after attacking blocks for 329 days, produced no change in appearance and very little change in weight. However, deterioration

in texture and in strength occurred, and fungal hyphae were observed crossing wood-fibres. These observations pointed to the fungus being pathogenic.

Koch's Postulates were not completely satisfied in this investigation - (a) the number of specimens examined was too small to prove the constant association of a fungus with one type of rot specimen, (b) fungi were isolated and studied in pure culture, (c) sound Jarrah was inoculated but failed to reproduce the characteristic symptoms of decay, possibly due to a number of causes referred to on p.37, (d) re-isolation of fungi from blocks subjected to artificial attack was considered unnecessary since the attacking cultures resembled their parent cultures throughout the whole period of attack and controls remained sterile.

GENERAL DISCUSSION.

The rot terminology for specimens, as used by the West Australia Forests Department, was inaccurate and unstandardized, and, as was subsequently proved, gave little or no indication of the identity of the fungus present. A revised system of rot nomenclature based on accurate macroscopic characteristics seems therefore desirable. Investigations on a large number of specimens would then assist in the problem of recognizing the causal fungus from an accurate description of the rot. Finally, an opinion on the rate of development of the rot in the living tree or in converted timber based on this accurate description of the rot could be given with some degree of certainty.

The vitality of the fungi isolated is probably an indication of the possibility of decay occurring in converted timber. F.hepatica retained its vitality in felled Jarrah for at least 22 weeks (after which period no further isolations were attempted). This observation is in contrast to F.hepatica in felled English Oak, according to Cartwright (1937), who stated, "the fungus soon loses its vitality in felled timber."

Of the remaining fungi isolated from Jarrah, Basidiomycetes XI, XII, XIII remained viable for at least 20 weeks, P.eucalyptorum 6 weeks, and Basidiomycetes IV, VI and VIII 4 weeks.

The isolation of two different Basidiomycetes from one specimen (p.21) is in agreement with the view often expressed that for the complete disintegration of wood, more than one fungus is necessary (Robertson, 1934) . Experiments to study this point of view would necessitate the attack on wood with one fungus and then with another fungus, and vice versa.

Field work was not carried out by the writer in this investigation on decay in Jarrah. It is probable that field work may have been useful in suggesting various methods of attack and in the further interpretation of the results of laboratory work.

The inevitable artificiality of all cultural work is a great handicap to the interpretation of results. Cultural work does not necessarily indicate the relative, and consequently, economic importance of attack by various fungi. Similarly, artificial penetration of wood cells by a fungus differs from natural attack. In the case of rot in a living tree, complete proof of the identity of the causal fungus demands, in addition to cultural work, the production of symptoms of decay in an attack on a sound tree.

CONCLUSIONS.

Fungi isolated from a number of typical specimens of decay in Jarrah were identified as F. hepatica, P. eucalyptorum and three other Basidiomycetes.

F. hepatica revealed little or no pathogenic properties on Jarrah, in culture, but the writer, in view of the repeated isolations from decayed Jarrah and undoubted pathogenicity on English Oak of the same fungus (Cartwright and Findlay, 1936; Cartwright, 1937), considers the fungus may be pathogenic on Jarrah. In the case of P. eucalyptorum, Cunningham (1927) stated that the fungus was the cause of a serious heart rot of beech (Nothofagus spp.). Cleland (1935) stated that the fungus was unquestionably destructive. Pathogenicity on Jarrah was demonstrated in this investigation.

Of the remaining Basidiomycetes, one (XI, XII, XIII) did not produce conclusive pathogenicity while the other two proved to be pathogenic, one in particular (IV, VI), being very vigorous.

SUMMARY.

1. A number of Basidiomycetes were isolated from typical specimens of decay in Jarrah and the majority were identified.

2. Three of these Basidiomycetes, namely Polyporus eucalyptorum Fries and two others which were not identified (Basidiomycetes IV, VI and VIII), demonstrated pathegenicity on sound Jarrah. Fistulina hepatica (Huds.) Fries was frequently isolated from specimens but did not demonstrate pathogenicity in culture. Nevertheless, reasons are given for expressing the view that the fungus is pathogenic. Another fungus Basidiomycete XI, XII, XIII in culture, gave inconclusive proof of pathogenicity.

3. The viability of the fungi isolated varied from 4 to 22 weeks and this fact suggests the possibility of their attack in converted timmber.

4. The isolation of two diff rent Basidiomycetes from one specimen of rot in Jarrah is not unique but emphasizes the complexity of wood decay.

5. The rot-nomenclature at present used by the West Australia Forests Departmentnis inaccurate and unstandardized and as such is of doubtful value for the purpose of recognizing the causal fungi of different rots in Jarrah.

6. The absence of field work increased the difficulties of the investigation.

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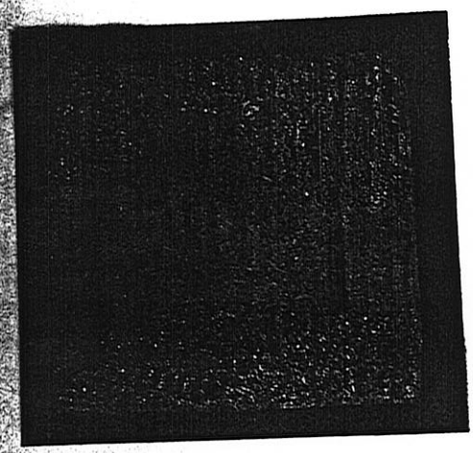
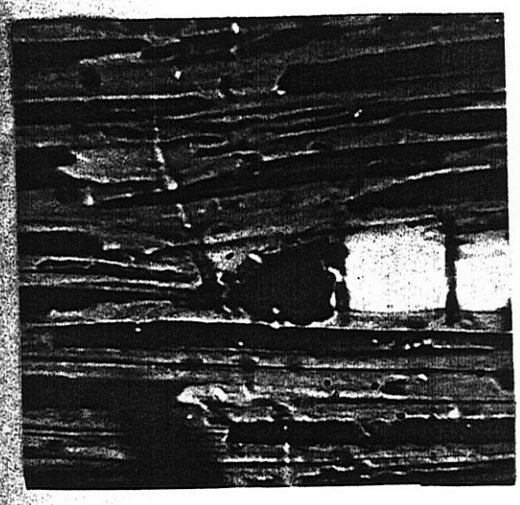
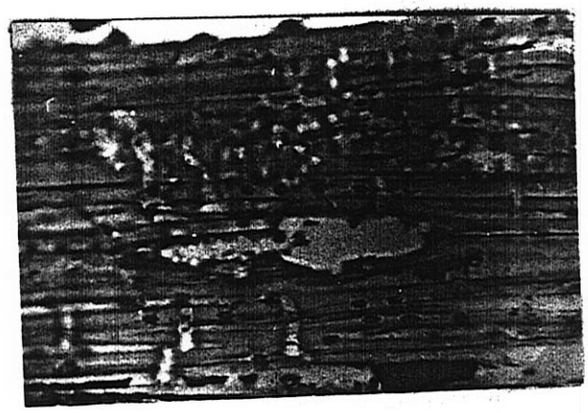


Transverse surface of Jarrah block after 12 months' attack by Basidiomycete IV. Upper zone - sound; middle - incipient attack; lower zone - empty vessels following on fungal attack. x 2.

Pileus of naturally-occurring sporophore of F. hepatica obtained from E. Segnana. x 1/3.



Hyphal penetration of sound Jarrah produced by artificial attack. x 500.



Hyphal proliferation within sound Jarrah produced by artificial attack. x 500.