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# JARRAH AND WANDOO ROTS INVESTIGATION

## General Report

of

Work from August, 1935, to November, 1936.

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C O N T E N T S

# JARRAH AND WANDOO ROTS INVESTIGATION

## General Report on Work from Aug. 1935 - Nov. 1936

### General field work

Work was commenced in August 1935 when an intensive search for sporophores was made in the mixed Jarrah and Marri forest areas around Mundaring.

All sporophores apparently associated with definite rot of standing timber or fallen logs were collected, identified as far as possible and cultured. Cultures were made on 2 % malt (Kepler's) agar, from context or stipe transplants.

The following sporophores were collected and cultured in August to September, 1935.

Fistulina hepatica
Trametes lilacino-gilva
Trametes cinnabarina
Polystictus versicolor (never found on Jarrah)
Polyporus eucalyptorum
Polyporus pelles (syn. atrohispidus)
Polyporus gilvus
Fomes sp.
Stereum hirsutum (not found on Jarrah)

Following this preliminary field work, samples were received through the Forests' Department of the various rots of Jarrah as recognised by their inspectors. The first collection of rots was received early in Dec., 1935, when laboratory and cultural work on the rots commenced.

It was soon apparent that considerable confusion existed as to the identity of the different rots. Various names were used in different localities, sometimes with quite erroneous application.

During the summer of 1935-36 further field work was carried out in the Mundaring district and the causal fungus of a "Xylostroma heart rot" discovered. This species of Polypore belongs to the Ovinus group and forms a relatively enormous sclerotium buried to some depth in the soil, under or close

beside the rotten log. Many fruiting specimens of this fungus were found following bush-fires in February. Its constant association with rotten Jarrah logs was noticed and cultures of the fungus from spores, context and stipe were made. Cultures were found to correspond in every detail with those of the fungus previously isolated from the "Xylostroma heart rot".

As the fungus was suspected of being a new species, specimens were sent to Dr. J.B. Cleland of the Adelaide University, who confirmed my opinion.

The complete proof of the relationship between this fungus and the "Xylostroma heart rot" has since been established.

In May, 1936, a tour through over 500 miles of the South-West in the Busselton, Collie, Narrogin, Kirrup areas, was made, when practically every condition of rot and abnormality in Jarrah was studied in the living tree, in cut sleepers, mill logs and stacked timber.

Sporophores were also search for, the following being found on living Jarrah -

Polyporus eucalyptorum
Polyporus pelles
Fistulina hepatica

While in the Narrogin district opportunity was taken to study the rots of Wandoo and specimens of these, as well as all sporophores noticed, were collected.

The following fungi were found fruiting on Wandoo -

Polyporus australiensis
Fomes rimosus
Trametes cinnabarina
Stereum hirsutum

Of these fungi, Fomes rimosus has been since proved responsible for the Honey-comb rot of Wandoo - a white pocket rot causing considerable cull in this timber.

During the whole of last winter field work, occupying approximately 2 days per week was carried out in the Jarrah belt in the Darling Ranges.

In August, 1936, several days were spent in the Dwellingup

district when valuable observations were made of the relative importance of the various rots of Jarrah in this area and of their location and progress in the living tree and in felled timber.

A previously unrecognised root and butt rot of Jarrah was found to be common at Dwellingup, being particularly frequent in certain regrowth stands on regenerated areas.

A number of sporophores were collected including the species now suspected, from cultural comparison, of causing the above mentioned root and butt rot. This species has been tentatively identified and specimens sent to Dr. Cleland for confirmation.

General Laboratory Work

1. Rots and other conditions of abnormality investigated in Jarrah

   1. Xylostroma heart rot
   2. Polyporus eucalyptorum heart rot
   3. Straw rot, Doze or Pith
   4. Black Straw Rot or Wet Rot
   5. A white pocket butt and root rot
   6. Dry Rot
   7. Speckled heart (Not reported on)
   8. Included sap
   9. Pencilled Jarrah

2. Isolation of the Fungus.

In general the following procedure has been adopted. Transplants from the typical and incipient areas of the rots were made on 2 % malt agar, the resultant growths being purified if necessary.

In this way, by making numerous isolations from several different specimens all the causal fungi have been isolated in pure culture - except in cases where viability appears to have been permanently lost.

Cultures from wood were then compared with authentic sporophore cultures and with published descriptions of cultural

characteristics. In some cases cultures have fruited readily in vitro which has materially assisted in making comparisons.

Several media were tried but 2 % malt agar has remained the standard medium, with the proviso that acidification is sometimes beneficial in securing the initial isolation. (e.g. Polyporus eucalyptorum, Black Straw Rot.)

Optimum temperature work was carried out in April, using several fungi isolated from Jarrah rots. Three cultures of each fungus were kept at each of four different temperatures ( 20 °, 25° , 30° , 35°C), and daily measurements of colony diameter were made. During the second week a downward shift of the optimum was noticeable, particularly with the Xylostroma heart rot fungus.

Careful examination of all fungi isolated has been made, camera lucida drawings of chlamydospores, vesicles, clamps etc. prepared, and photographs of growth characteristics at different temperatures and of fructification in vitro, taken.

Description of cultural characters has not yet been completed owing to the fact that no copy of Ridgway's "Colour Standards" is as yet available.

3. Examination of rotted woods

Anatomical investigations have been practically completed for most of the above rots and other conditions of abnormality and photomicrographs taken of outstanding anatomical features.

Various stains and stain combinations have been used both for general staining and for hyphal differentiation. For the latter, three stains have been tried - Cartwright's picro-aniline blue, Hubert's methyl violet - bismark brown, and the cotton blue - lactic acid stain. Generally speaking Cartwright's stain has proved most satisfactory especially when using aniline blue (Certification Number NK 3) supplied by the National Aniline and Chemical Co., New York.

Apart from general histological stains, microchemical indicators have been used in tracing the progress and type of

attack - the following having proved satisfactory - chlor-zinc iodide, iodine and $CaCl_2$, iodine and Conc. $H_2SO_4$, and phloro-glucin-HCl.

Wood sections have been cut with a sliding microtome. At first attempts were made to soften hard wood by boiling, by use of hydrofluoric acid etc., but these aids to sectioning were found quite unnecessary following the use of the steam jet method due to Kisser. Transverse sections as thin as 5μ have been cut without embedding or previously treating the wood by use of this technique.

Apart from conventional microscopic work, surface examination of wood, using a dissecting microscope for lower powers and an ordinary microscope for higher powers, (up to a 6 mm. objective and x 10 eye-piece) was found particularly helpful in some cases. By this means perithecia of a Plectomycete were readily located in the vessels of specimens of "Included Sap" of Jarrah. Their presence was suspected from the occasional appearance of asci in microtome sections.

4. Artificial Production of the Rots in Vitro

In January 1936 attempts were made to devise a suitable scheme for artificial production of wood rots, using pure cultures of the suspected fungus and sterilised Jarrah blocks.

Boiling tubes 6" x 1" were used, a single well saturated block being placed on a thick pad of wet cotton wool at the base of the tube. After sterilisation the fungus was inoculated about half way up the block.

All fungi which had been isolated at that date and also a number of sporophore cultures were inoculated onto blocks, and kept at room temperature.

The experiment was not completely satisfactory owing to the subsequent rapid drying out of the blocks.

However, the blocks inoculated with the Xylostroma heart rot fungus gave positive results, the typical stage of the rot being produced in six months.

Blocks inoculated at the same date (Jan. 1936) with the straw-rot fungus and with Polyporus eucalyptorum showed signs of incipient decay, but were not sufficiently attacked to render comparison positive.

A much more satisfactory type of experiment was commenced in April when wide mouth Erlenmeyer flasks were used. Flasks contained about 100 ccs. of 2 % malt agar with pieces of glass tubing placed in the bottom and not quite covered by the agar. The flasks were then inoculated and incubated at 25 °C till the fungus had almost covered the surface of the medium. Three or four sterilised, wet Jarrah blocks were then introduced and arranged on the mycelial mat so that the glass tubing prevented the blocks from actually touching the surface of the medium.

Prior to sterilisation blocks were numbered, dried for a standard time at a standard temperature, cooled in a $CaCl_2$ desiccator and weighed. Controls were run in every case.

Positive results are confidently expected from these experiments.

5. Photographs

Numerous photographs have been taken to illustrate the macroscopic features of the various rots of Jarrah and Wandoo. Photomicrographs of wood sections of Pencilled Jarrah, P. eucalyptorum heart rot, Xylostroma heart rot, Dry rot and Black Straw rot have also been taken.

6. Rots investigated in Wandoo

Only two rots have been studied in detail

1. Honey-comb rot of Wandoo
2. Straw-rot of Wandoo

The same procedure has been followed in investigating these rots as was previously outlined for Jarrah.

The fungi causing both rots have been isolated and the causal species of the honey-comb rot identified. Erlenmeyer flask cultures have been made only for the honey-comb rot fungus,

(Fomes rimosus), which has made very virorous growth over the Wandoo blocks.

7. Included Sap of Karri

Specimens of "Included Sap" in Karri were examined in May, 1936, in order to compare this condition with sap-inclusion in Jarrah.

A report of this examination was forwarded in October, since when no further work has been done.

## Specific Investigations

The following account of specific work on the various Jarrah rots etc. is of necessity very brief and only the more important and characteristic features are recorded. The complete account will be published in the thesis.

1. Xylostroma heart-rot

(a) General - Xylostroma heart rot is one of the brown, somewhat cubical heart rots of Jarrah. It appears to be most common towards the northern fringe of the Jarrah belt, especially round Mundaring, is probably rare in the Dwellingup areas, but undoubtedly occurs further south, as a sporophore and sclerotium were obtained from the Karri-Marri forest near Boranup.

(b) Causal fungus - This extremely interesting rot of Jarrah is caused by a sclerotium forming Polypore of the Ovinus group, somewhat like Polyporus tumulosus, but differing chiefly in size and thickness of the sporophore, in spore measurements and in size and type of the sclerotium. As far as is known this fungus is a new species and will be named and described as soon as possible.

The life history is somewhat as follows. Spores finding a suitable germination court in fire scars, wounds, dead limbs, etc., produce a germ tube and the mycelium penetrates to the heart of the living tree, where the vegetative stage commences. Later, through windthrow, fire, progress of the rot, etc., the tree is brought down. Vegetative activity continues until the rotten

area makes contact with the ground. The fungus then passes into the soil forming a large false sclerotium of mycelium - impregnated soil. Several such sclerotia are frequently formed from one log and may be of considerable size - individual sclerotia weighing up to 25 lbs. having been found.

A resting stage probably then occurs, and later, a stimulus, usually indirectly due to bush fires, causes the sclerotium to fruit. Sporophores collected during the summer of 1935 -36 were almost invariably on country which had been burned, and were always in close connection with rotted logs, or along the track of a burnt log.

The causal fungus was first isolated from badly rotted fallen logs, but has since been isolated from the living tree.

Some months after the initial isolations, sporophores were collected and cultures obtained from spore plates and context transplants. The comparison between wood and sporophore cultures was immediately noticed and the causal relation of the fungus to the rot was undeniably proved by this and by numerous field observations.

(c) Cultural Characters

The fungus exhibits some characteristic peculiarities in culture. The hyphae in the wood and in the mycelial sheets bear no clamps, but these immediately arise in culture, possibly due to nutritive stimulus. Clamps are of a large simple type and occur both on the aerial and submerged mycelium. After a short period in culture the fungus commences to produce long aerial hyphae which are completely devoid of clamps and grow upwards to produce a dense white mycelial mat. On subculturing clamps again appear.

On the first formed submerged mycelium bearing clamps, numerous very characteristic chlamydospores are formed. These chlamydospores have a very typical symmetry and possess an unusually thick wall. Probably owing to the fact that they are

Fig. 1. Xylostroma heart rot of Jarrah in the typical stage of the rot, showing the white mycelial plates. The rot is very similar, macroscopically, to the heart rot of Jarrah caused by Polyporus eucalyptorum.

Fig. 2. Photomicrograph of a transverse section of Jarrah showing the characteristic appearance of the fibres in the typical stage of the Xylostroma heart rot. The almost complete disappearance of the middle lamella and the presence of hyphal tracks readily distinguish Xylostroma heart rot from the heart rot caused by Polyporus eucalyptorum.

Fig. 3. A sclerotium and sporophore of the new species of Polyporus causing the Xylostroma heart rot.

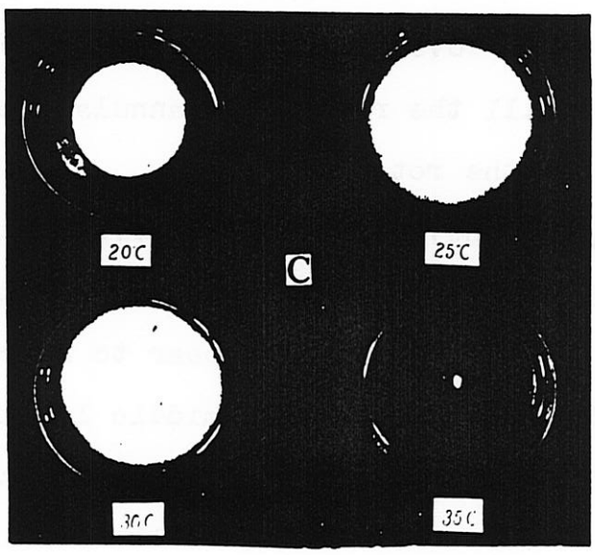


Fig. 4. Cultures of the Xylostroma heart rot fungus on 2 % malt agar incubated at different temperatures for 11 days.

frequently formed with a clamp at the base they are readily separated from the parent hypha.

In culture the fungus has a temperature optimum at 30° C during the first week and shifts back sharply to an optimum of 25° C during the second week. The daily growth increment at optimum temperatures is about 9 mms. on 2 % malt agar.

(d) Artificial Production of the Rot - The typical stage of the rot was produced in vitro on sterilised Jarrah blocks six months after inoculation. The fungus was isolated readily and in an uncontaminated state, from an "artificially" rotted block.

Compared with other rot-fungi tested to date, the Xylostroma fungus appears to be very vigorous and to produce an unusually rapid rot increment.

(e) Macroscopic Features of the Rot - The fungus attacks the heart and the true wood, producing a brown rot which tends to become cubical on drying out. The rot is very similar in appearance to that caused by Polyporus eucalyptorum, except that the rotted wood is harder, less powdery and more brittle. The white mycelial sheets are thinner and less floccose and appear almost to possess a pellicle due to the close woven matted hyphae at the edges. These mycelial sheets fill the radial and annular shrinkage cracks in the typical stage of the rot.

(f) Microscopic Features - Transverse sections reveal what appear to be very considerable chemical changes which may be described somewhat as follows - the fibre walls appear to have partly liquified and then flowed together, the middle lamella often completely disappearing in the process. Hyphal tracks are frequently observed. The colour of the cell wall instead of being translucent becomes an opaque muddy red.

2. Polyporus eucalyptorum heart rot

(a) General - Polyporus eucalyptorum causes a brown trunk rot of Jarrah and is one of the most serious cull-producing rots of this timber. It is widely distributed throughout the whole Jarrah

forest and appears to be particularly common in mature and over-mature stands.

Field observations indicate that the rot column works down the tree and may extend almost out to the sap except at the lower end where it is roughly conical. When a sporophore appears it invariably (?) indicates the lower limit of the rot which normally extends only a few feet below the fructification. This fact may have some economic significance in marking of trees for felling, and does not seem to have been previously noted - at least locally.

(b) Causal fungus - The fungus, Polyporus eucalyptorum, is sometimes difficult to isolate from the wood or mycelial plates, owing to contamination, and frequently gains in vigour after several subcultures. This does not appear to be due entirely to purification.

Apart from the fact that sporophores have been found frequently in obvious association with the rot, the usual culture-comparison methods and wood-block inoculations have been used to demonstrate the causal relationship between fungus and rot.

(c) Cultural Characteristics - Two strains of P. eucalyptorum (strains A & B) have been obtained in culture, differing chiefly in vegetative vigour - particularly in the production of aerial mycelium, and in growth rate and colouration.

Thin walled chlamydospores or vesicles occur on the submerged mycelium within a week or ten days of culturing. No clamps have ever been observed either in cultures, in wood or in sporophore context.

In culture, aerial mycelium is at first white and fluffy, but later tends to become more matted and may develop a plug at the upper end of the slope, often completely filling the test tube at this region. Further down the slope less vigorous aerial growth is made. (The same type of plug at the thin end on the slope has been noticed in the Xylostroma heart rot fungus.)

The fungus has as yet never fruited in vitro, but in older cultures the canary yellow hymenial colour develops on the surface of the aerial mat.

(d) Artificial Production of the Rot - Boiling tube and Erlenmeyer flask experiments have been used in an attempt to produce the rot artificially.

In the boiling tube experiments, inoculated in January, 1936, the fungus made a somewhat sparse growth over the lower end of the blocks. One tube was opened after six months, and though the typical rot had not been produced the wood was somewhat softer. The remaining tubes have as yet not been opened.

The Erlenmeyer flask experiments were set up in September, 1936, using acidified 2 % malt agar, which appears to support a better aerial growth.

Both strains isolated, (strains A & B) were used, and very vigorous growth has been made, strain A having completely embedded the lower blocks in a dense mycelial mat.

Flasks will be opened in February or March.

(e) Macroscopic features of rot - Affected wood becomes darker in colour, loses lustre, and in the typical stage of the rot, is soft, very brash, and easily crumbled. In this stage, especially when dried out, the wood has a tendency to crack cubically.

In the tree the wood is traversed by radial and annular shrinkage cracks, filled by sheets of white mycelium which are often large and thick, individual sheets up to ¾" in thickness having been found.

Unfortunately, the two brown heart rots caused by "Xylostroma" and by P. eucalyptorum are difficult to separate on macroscopic features alone.

(f) Microscopic Features - Microscopically this rot can be readily distinguished from Xylostroma heart rot by the mode of attack on the fibres, resulting in a completely different microscopic appearance in the typical stage.

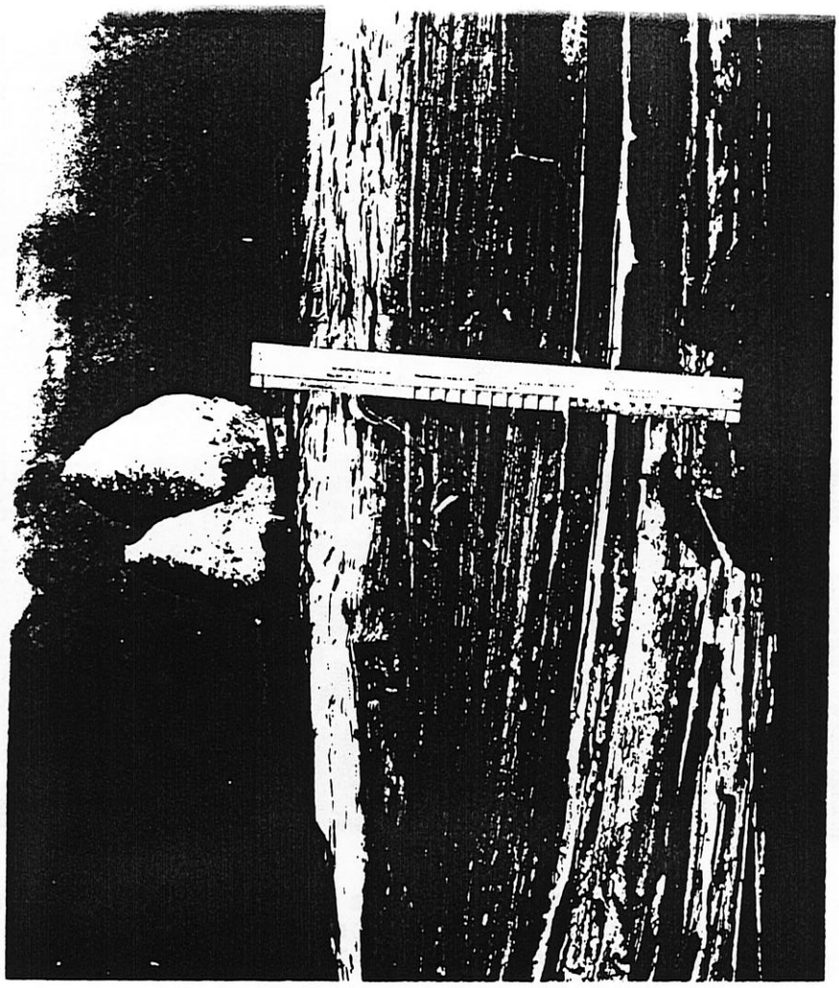

Fig. 5. The heart rot of Jarrah caused by Polyporus eucalyptorum showing the sporophores of the fungus and the type of rot produced.

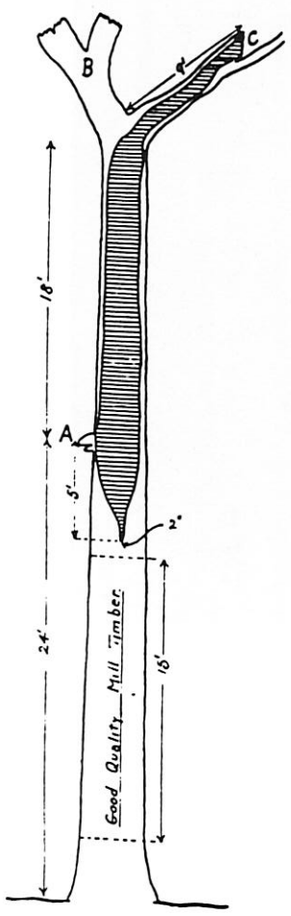


Fig. 7. Diagram showing the heart rot of Jarrah caused by Polyporus eucalyptorum. The rot column which is moving downward is shown hatched. Note the position of the sporophore at (A) relative to the lower limit of the rot.

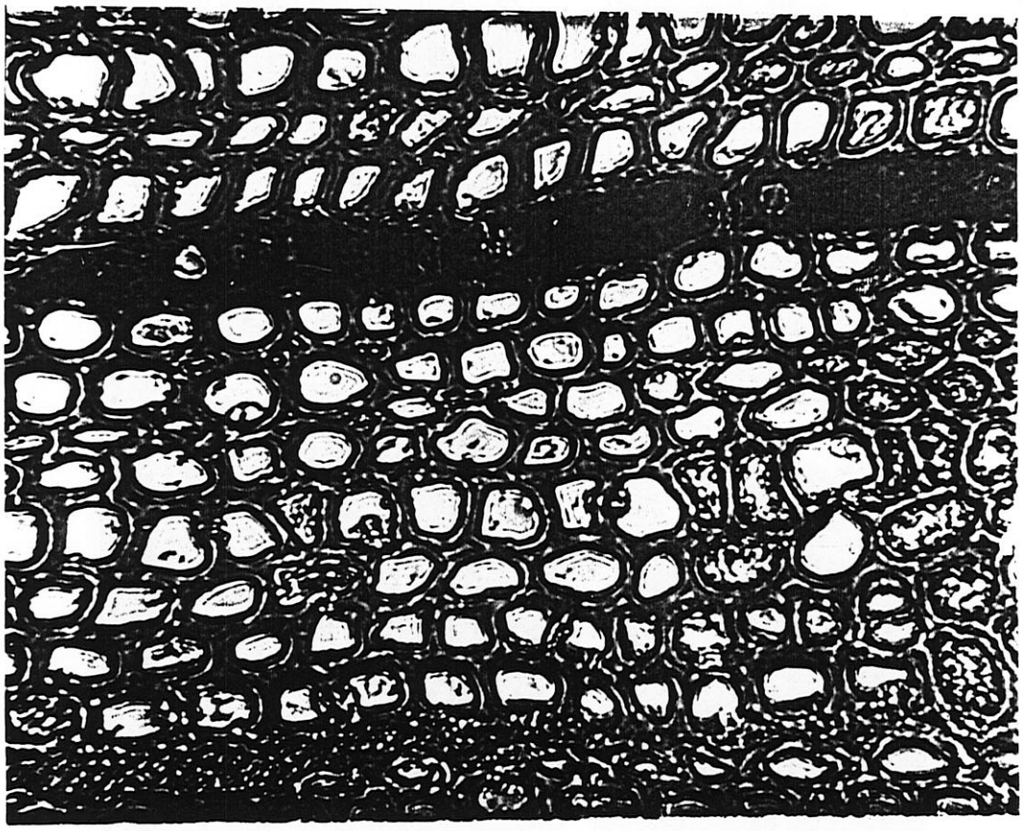

Fig. 6. Photomicrograph of a transverse section of Jarrah in the late typical stage of the rot produced by Polyporus eucalyptorum.

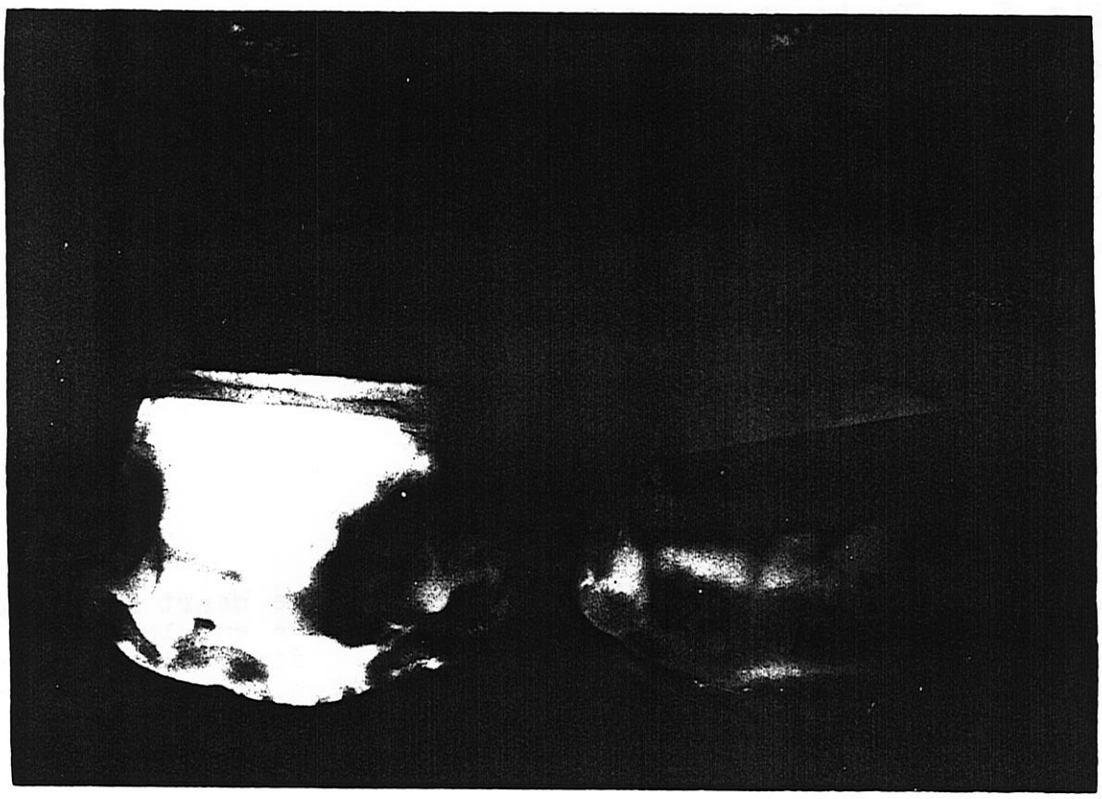

Fig. 8. Artificial production of rot . Erlenmeyer flask experiments showing wood blocks covered by vigorous mycelial growth.
Left - Xylostroma heart rot fungus completely covering three Jarrah blocks after four months growth
Right - Polyporus eucalyptorum about one month after the introduction of the sterilised blocks.

The differences may be described somewhat as follows. In the Xylostroma heart rot profound chemical change has occurred in the fibre elements causing the individual cells to lose their identity. The secondary walls are not generally absorbed and the lumina though often very irregular are usually not unduly enlarged.

In P. eucalyptorum heart rot the fibres have been markedly thinned resulting in greatly enlarged often irregular lumina filled with a somewhat granular kino. The middle lamella is quite distinct except in the late typical stage when the affected fibres have however a characteristic skeletonal appearance, little more than the primary wall remaining.

3. Yellow Straw Rot

(a) General - This rot is most commonly found as a butt rot, but occasionally occurs at the top of the bole, and was once found as a general trunk rot extending up for 30 feet. It appears to be widely distributed through the Jarrah belt, except perhaps in the Northern Fringe, where I have never found a specimen, despite very thorough search.

(b) Causal Fungus - The causal fungus was first isolated in Dec., 1935, and has since been obtained from numerous specimens.

Although sporophores have been searched for during two winters over all except the southern areas of the Jarrah belt, fructifications have never been found associated with this rot. During last winter, sporophore collection was extended to other timber species (Marri, Banksia, etc.) but no fungus as yet collected and cultured is in any way comparable to the fungus isolated from the wood.

In order to test the viability of the fungus in the wood, transplants were made from two specimens which had been stored under dry laboratory conditions for seven and four months respectively. In the older specimen 100 % of the transplants grew the causal fungus, while in the other specimen 80 % viability was recorded.

(c) Cultural Characteristics - The fungus isolated is almost certainly the causal species, having been obtained from every specimen of Straw rot collected. It is a typical Polypore with few to numerous clamps, and fruits readily in culture, often forming fertile pore surfaces after the second week. No conidia, vesicles or chlamydospores are formed in culture.

The fungus has a temperature optimum at about 30° C, with a daily growth increment on 2 % malt agar of 9 mms. at this temperature. Growth is completely inhibited at 35° C.

In culture the fungus is characterised by a very long, sparse advancing zone of closely appressed hyphae. Very little aerial growth is made for 4 - 5 days when a sparse buff to pinkish-buff downy mat develops. Later further aerial growth may be made, which however tends to be patchy. The colour in older cultures varies somewhat but is generally some shade between pinkish-buff and buff-brown.

The medium is never stained, but tends to be decolourised in older cultures.

(Accurate colour descriptions cannot be made until a Ridgway is obtained.)

(d) Artificial Production of Rot - Boiling tube and Erlenmeyer flask experiments were set up in January and May respectively.

The blocks in the boiling tubes are now showing yellowish discoloured pockets on the surface and on removing and sectioning one the typical blue cellulose reaction was obtained with chlor-zinc iodide. It thus appears that the incipient stage of the rot has been produced, but it is hoped to obtain more positive evidence from the flask experiments.

(e) Macroscopic Features - Some confusion exists among foresters in the identification of this rot, and three distinct rots are often not separated.

Straw rot, in the sense referred to here, is a typical straw coloured, stringy, heart rot, with a somewhat streaked or

mottled advancing zone, due to delignification commencing normally in pockets. Later in the typical stage, delignification is general and the wood becomes more or less uniformly bleached to a deep straw colour characterised by its stringy nature.

(f) Microscopic Features

Microscopically the rot is quite typical, especially in transverse section where the medullary rays run like dark bands through the bleached and thinned fibre elements, giving a very characteristic appearance to the section.

The middle lamella is definitely attacked and the fibres readily separate, thus rendering sectioning somewhat difficult.

Delignification first occurs in pockets, the fibres being badly attacked at these areas even in the incipient stage.

4. Black Straw Rot

(a) General - This rot is more common than was originally suspected by Forestry inspectors, undoubtedly because it is very frequently confused with yellow straw rot. This confusion is due to the fact that until dried out the two rots are often very similar macroscopically. After drying however the differences are quite marked, but probably on account of initial confusion, have not been clearly recognised.

It has been found typically as a trunk rot in the living tree and field observations at Dwellingup have indicated that the rot may continue in fallen timber as old reject logs were found practically rotted away.

(b) Causal fungus - The successful isolation of this fungus has proved unusually difficult. Over 100 transplants were made from several different specimens before a Hymenomycete was isolated. Various contaminants were at times suspected, but the real causal fungus was eventually isolated in August, 1936.

Transplants from the extreme edge of the advancing zone were relatively free from contamination and consistently grew the same fungus, which on account of its numerous clamps was

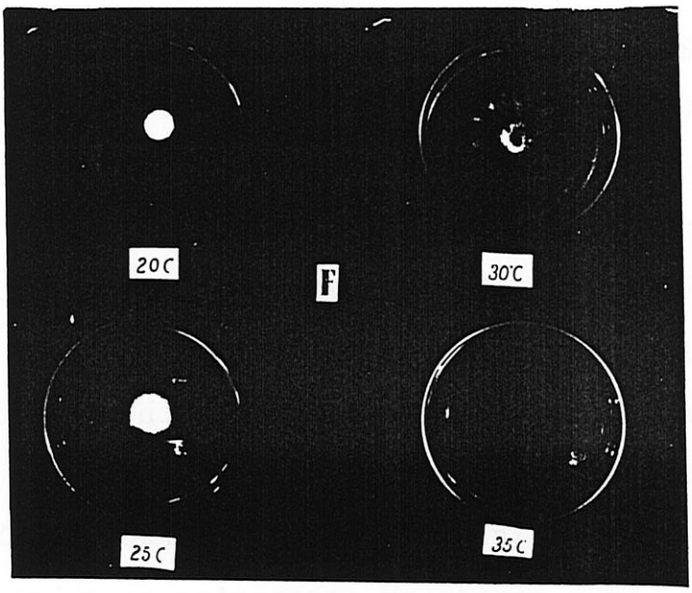


Fig. 10. Cultures of the Straw rot fungus on 2 % malt agar incubated at different temperatures for 11 days.

Fig. 9. The Straw rot fungus fruiting in vitro on a 2 % malt agar slope.

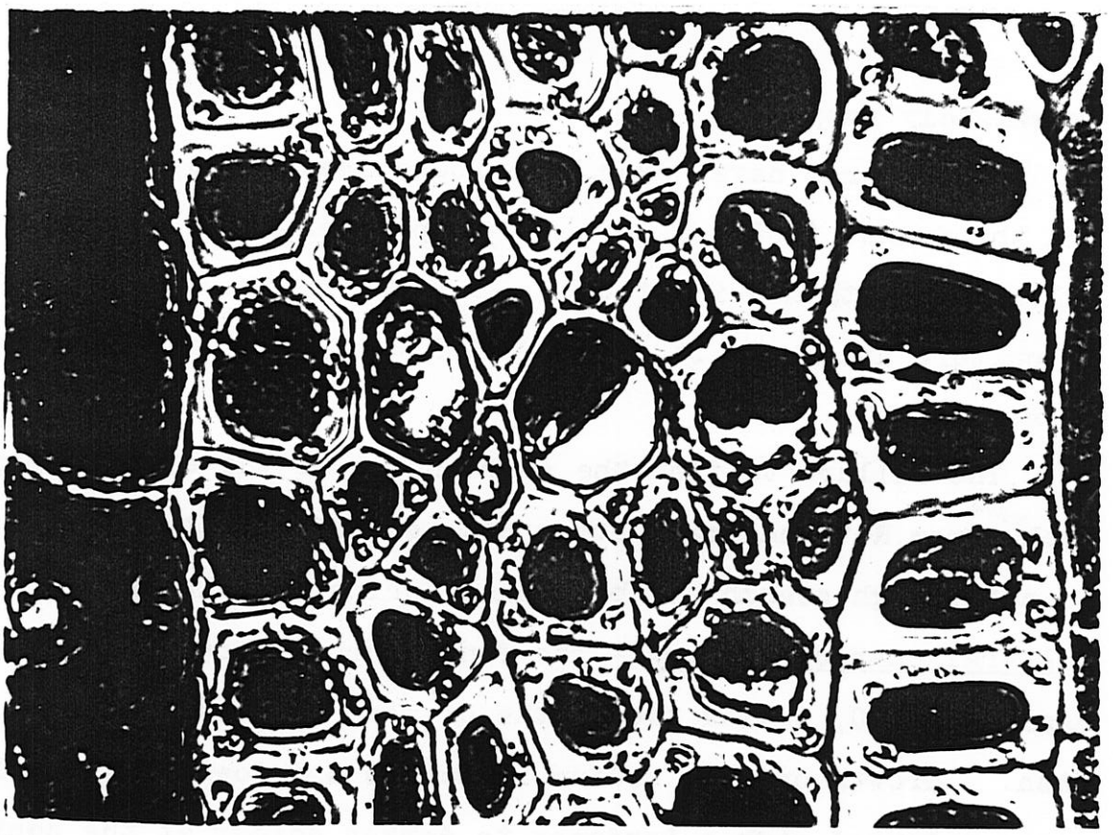

Fig. 11. Photomicrograph of a transverse section of Jarrah attacked by the Black Straw rot fungus. The bore holes in the fibre walls can be clearly seen. This type of longitudinal boring represents a very unusual method of hyphal penetration.

Fig. 12. A photograph showing the "White pocket" butt rot of Jarrah in the butt of a sapling.

immediately suspected.

Sporophores have never been found associated with the rot, and the fungus isolated does not compare with any sporophore cultures maintained in stock. Also the fungus has never fruited in culture and hence it is not known what type of Hymenomycete is responsible.

The failure to find the sporophores of the causal fungi of both Yellow Straw Rot and Black Straw rot is difficult to account for as a thorough search has been made during two winters.

(c) Cultural Characteristics - The fungus grows slowly on 2 % malt agar at 25° C, taking two months or more to cover a 10 cm. Petri dish. Growth on this medium is sparse and downy, typically cream coloured with a tendency to darker colour at the centre of the colony.

Numerous large terminal and intercalary chlamydospores occur both on the submerged and on the aerial mycelium. Clamps are abundant and of the simple type. Many of the aerial hyphae possess a rather swollen terminal cell which is covered with a brown granular deposit.

(d) Artificial Production of the Rot - Blocks have as yet not been inoculated with the suspected fungus. Experiments will, however be commenced shortly.

(e) Macroscopic Features - This rot is most unusual in appearance as the wood becomes blackened in the typical stage, and is especially characteristic in dried out specimens. A good deal of variation occurs, some over-typical specimens being almost dull black, while others are only slightly darker than normal. In general however the usual effect is a pronounced darkening of the wood with a very stringy typical stage.

A pinkish discoloration sometimes accompanies the areas of incipient rot.

(f) Microscopic Features - This rot possesses one probably unique microscopic feature - transverse sections always reveal numerous

bore holes in the fibre walls running parallel to the long axes of the fibres. In the typical stage of the rot the fibre walls are almost completely perforated by these numerous longitudinal, almost contiguous bore holes. This phenomenon of longitudinal hyphal penetration appears to be very rare, if not unique. Mr. Findlay, of Princes Risborough, in a recent letter, stated he had never seen boring of this type in any rots known to him.

5. A White Pocket Butt and Root Rot

(a) General - While at Dwellingup in August, 1936, a white pocket root and butt rot was found rather commonly in certain regrowth sapling stands. The same type of rot was later found in a similar position in old dead stumps.

This rot had not been previously recognised as distinct, and apparently when occasionally noticed was considered as an early stage of straw rot.

The rot is probably confined to the butt and roots though it was traced up through the heart for four feet, in a sapling with a 10 inch butt. In many cases the rot was principally in the heart, but in one specimen obtained the true wood was rotted almost out to the sap.

(b) Causal Fungus - The fungus was quite readily isolated from fresh specimens provided transplants were made from the extreme edge of the rot.

Sporophores collected at Dwellingup and believed to be associated with the rot were also cultured and in culture proved very similar to the fungus isolated from the wood. The sporophores have been tentatively identified, but I am at present awaiting confirmation from Dr. Cleland before specifying the causal fungus.

A certain amount of cultural variation has occurred both in different isolations and in different subcultures from a single isolation. Also sporophore cultures vary somewhat. At present nine cultures from different sources (wood and sporophores) are

being compared to obtain the range of variation between the extremes.

(c) Cultural Characteristics - The fungus has a relatively slow growth rate of about 3 mms. per day at 25° C.

The mycelium is at first white and appressed, spreading out regularly from the inoculum.

Within a few days or sometimes not till the second week the mycelial mat begins to turn brown and becomes closely felted. Cultures often fruit in about three weeks, the fertile area being somewhat irpiciform in appearance, and bearing normal basidia and basidiospores on the peg-like projections.

Discoloration of the medium occurs, varying somewhat in degree between different isolations.

No clamps or chlamydospores have been observed.

(d) Artificial Production of the Rot - The suspected fungus will be inoculated on to sapwood and true wood blocks as soon as possible.

(e) Macroscopic features - Small white pockets about ¼" long, or long irregular white streaks are typical features of this rot. The delignified areas are not sharply marked and the pockets and streaks are often irregular and without definite boundaries giving a totally different appearance from the white pocket rot of Wandoo where the pockets are large regular and sharply defined.

In the typical stage the pockets are often filled with brown mycelium giving the area a decidely brown appearance and sometimes masking the white colour of the rotted wood.

Less characteristic forms of this rot have been found where delignification has not produced typical pockets but has given the rotted wood the appearance of having numerous fine white threads running longitudinally through it. Such wood has a tendency to split into thin annular plates, due to the somewhat ring-scaled type of attack.

(f) Microscopic Features - The microscopic features are generally similar to those described for Straw Rot, but the macroscopic

appearance is sufficiently different to render confusion impossible.

6. Dry Rot or Pocket Rot , etc.

Ideas are confused regarding this rot, and any brown cubical rot is likely to be referred to as dry rot, pocket rot, pockets of dry rot, cubical rot etc., without any real discrimination.

So far time has not permitted of a thorough investigation of this condition, but a number of specimens from living trees, dead logs, cut timber, etc., have been collected and cultures attempted.

A number of spore-forming saprophytes have been isolated, generally characterised by a sodden appressed type of growth, but no fungus has been found to be constantly associated with the rot.

In wood sections Basidiomycete mycelium has been occasionally found, but all attempts to obtain such a fungus in culture have failed.

Microscopic examination of wood sections reveals much the same appearance as is typical of the late stage of Polyporus eucalyptorum heart rot.

This type of Pocket rot has often been found in old dead logs probably 2o - 30 years after felling, where the pockets are quite similar in size and appearance to those found in the living tree and show no signs of having increased in size since felling.

It is generally believed however that many of these pocket-infections continue in fallen and in cut timber, but no authentic case has yet been investigated.

While it is not suggested that all cases of cubical Pocket rot in Jarrah are similarly caused, I am very much of the opinion, however, that many such pockets found in living trees represent infections which have failed.

Field observations, microscopic examination and the negative culture results obtained tend to support this view. The reported development of cubical rot in Jarrah sleepers has not yet been investigated.

7. Pencilled Jarrah

(a) General - This abnormal condition which is particularly common in some areas and is widely distributed, is of very considerable interest both academically and economically.

It had been suggested in the past that this condition was one of incipient decay, but field and laboratory work have tended to disprove this idea completely.

Pencilling has been found to occur anywhere in the true wood from the heart to the sap and occasionally extends into the sap-wood. A pencilled root was found at Dwellingup which indicates that it may be quite common in roots as well as in the trunk.

The black appearance of the pencilled areas is solely due to a superabundance of kino, which in this region clogs the vessels and fibre lumina in a quite abnormal manner.

Fungal hyphae are constantly associated with pencilled areas and can be readily found by surface microscopic examination.

Field observations tended to indicate that a rather unusually high percentage of trees affected with Yellow Straw Rot were also showing pencilling, suggesting that Pencilled wood might be more susceptible to this particular rot. This would of course need wider confirmation to be established.

(b) Cultural Work - A number of samples of Pencilled Jarrah have been collected and numerous transplants made both from the discoloured streaks and from the normal adjacent wood.

A green Penicillium was the only fungus obtained and as this species often grew from the majority of transplants it was at first thought that it might be a saprophyte attracted to the pencilled areas by the abundant kino.

This Penicillium was proved quite tolerant to kino by the following experiment.

Blocks of Jarrah and Wandoo were autoclaved in water for ½ hour until the aqueous extract appeared very concentrated. The Penicillium grew quite vigorously on the surface of the

liquid. In the case of Jarrah the kino is only sparingly soluble under these conditions, but is much more readily leached out from Wandoo.

As the Penicillium grew so readily on what was expected to be a relatively toxic extract, a comparison was made to determine the percentage of Pencillia in normal Jarrah compared with Pencilled Jarrah.

Extreme precautions were taken to prevent any outside contamination and using three different specimens of pencilled wood and three of normal Jarrah, the following results were obtained.

Pencilled Jarrah - 15 transplants - 1 grew Penicillium - 14 sterile

Normal Jarrah - 15 transplants - 2 grew Penicillium - 13 sterile

Apart from the fact that the percentage of Pencilled Jarrah transplants which grew this fungus, was much lower than previously obtained, the experiment indicated that the specimens here tested were quite normal.

As a result of culture work to date, it is obvious that if the associated fungus is not a Penicillium, then viability has been completely lost in all specimens tested.

As the fungus is obviously viable at some period it is intended to culture from fresh specimens in which pencilling has extended into the living sap-wood.

(c) Macroscopic Features - Pencilling does not occur throughout the whole of the trunk, but is confined to a sector which may extend for only a few feet or for several feet.

On a transverse face the discolorations appear like dark pencil lines radiating outwards.

Each "pencil line" starts abruptly, runs radially outward for any distance up to 2" or so, and then finishes abruptly. The lines are usually less than 1 mm. thick. In radial longitudinal face the pencilled areas appear like irregular diffuse smudges,

while on the tangential face they occur as elongate pockets averaging about 1 mm. in width and 5 mms. in length.

Such wood is generally stated to be very free splitting in a radial direction.

(d) Microscopic Features - Microscopic examination showed that the discoloration was due to abnormal secretion of very dark kino, resulting in the complete clogging of the ray cells and fibre lumina in the pencilled areas.

Where the discolored streak extended into the sap-wood it was found that though tylosis and kino secretion had not commenced in the surrounding wood elements, the ray cells and fibre lumina in the pencilled area were already completely clogged.

Sections cut through mature true wood showed that apart from the greater abundance of kino the colour of the deposit was much darker than normal.

Although a fungus is invariably (?) present the very regular marking of the wood suggested that certain cell groups in the cambium were being affected resulting in the production, for a more or less protracted period, of xylem elements with an abnormal tendency to early and heavy kino infiltration.

Transverse sections were therefore cut as follows -

(1) Inwards from the cambium to the beginning of a pencilled streak.

(2) Outwards from the true wood towards the sap, following a pencilled streak.

The following facts were observed.-

(1) That though a fungus is present in the vessels of the pencilled area, and also extends into the adjacent normal wood, no evidence of attack on the wood elements could be found.

(2) That the amount of mycelium becomes progressively less towards the peripheral end of the streak.

(3) That no sign of mycelium could be found in advance of the pencilled area.

~~(4) Various histological~~

(4) Various histological stains gave no indication of differences between the normal sap-wood elements and those immediately in advance of a pencilled streak.

(5) Pencilling often stops abruptly at the end of a growth ring in the sap.

(6) That the hyphae present appeared to be all of the same type, septate and without clamps.

The following tentative explanation is offered to account for the regular radial arrangement of the pencilling and for the presence of the fungus.

When fungal infection first occurs - presumably in the sap wood and perhaps at only one point, the products of fungal metabolism diffuse, or are transported through the medullary rays, towards the cambium. Developing xylem elements immediately in front of the infection become stimulated to early and heavy secretion of kino, resulting in a "streak" developing in advance of the fungus.

The complete clogging of the ray cells prevents further transport of the stimulating products and hence eventually the excessive kino secretion stops. The fungus then grows out into the normal sap-wood surrounding the streak and here again causes excessive kino production in the wood elements immediately in advance, resulting in another streak. This continues perhaps for a century until a sector-like area of pencilled wood results.

8. Included Sap of Jarrah

(a) General - "Included sap" is a term used locally to describe the inclusion of an area of sap-wood at some depth in the normal true wood. Such areas may be entirely free from rot, when the term "clean included sap" is applied, or may be associated with areas of rot, when the term "sap rot" is used.

This condition is frequently met with in Jarrah, and in this timber appears to be invariably associated with an old wound such as a logging scar or a fire scar etc., and is particularly

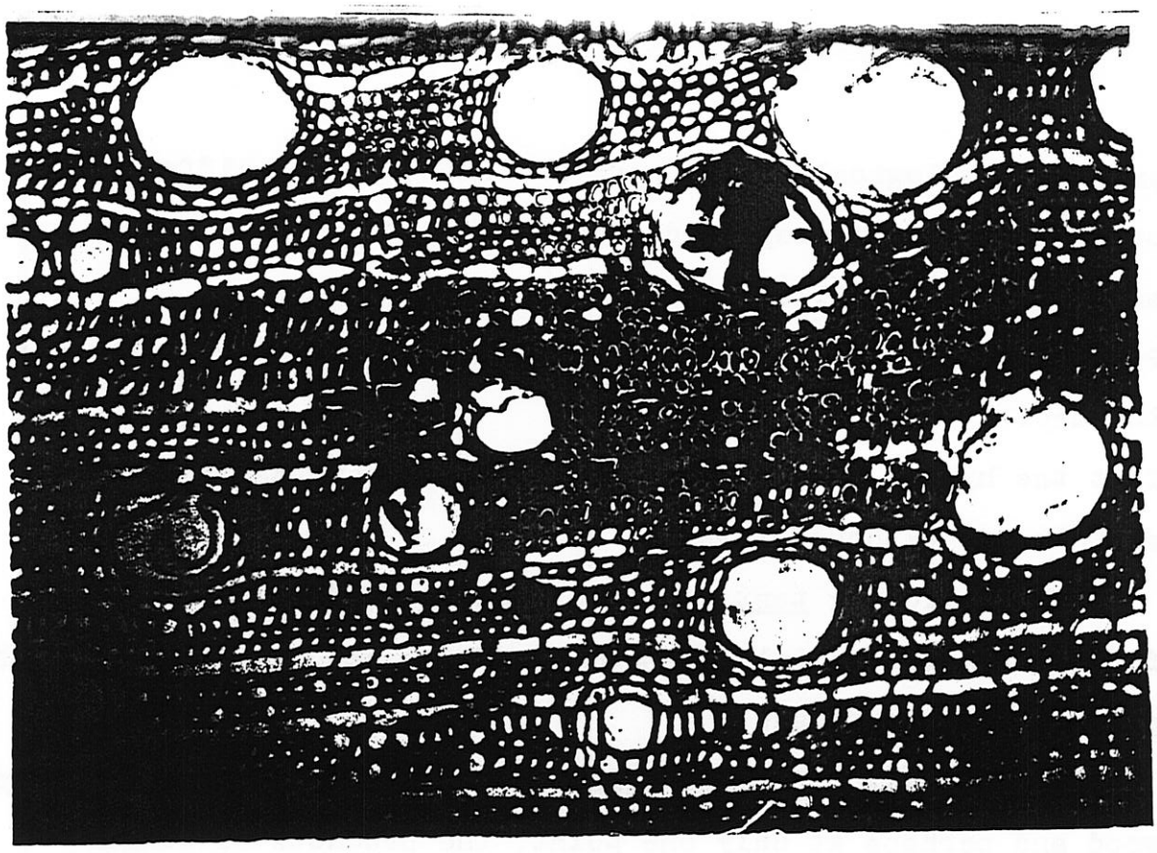

Fig. 13. Photomicrograph of a transverse section of Pencilled Jarrah showing the end of a "pencil streak" in the sap wood.

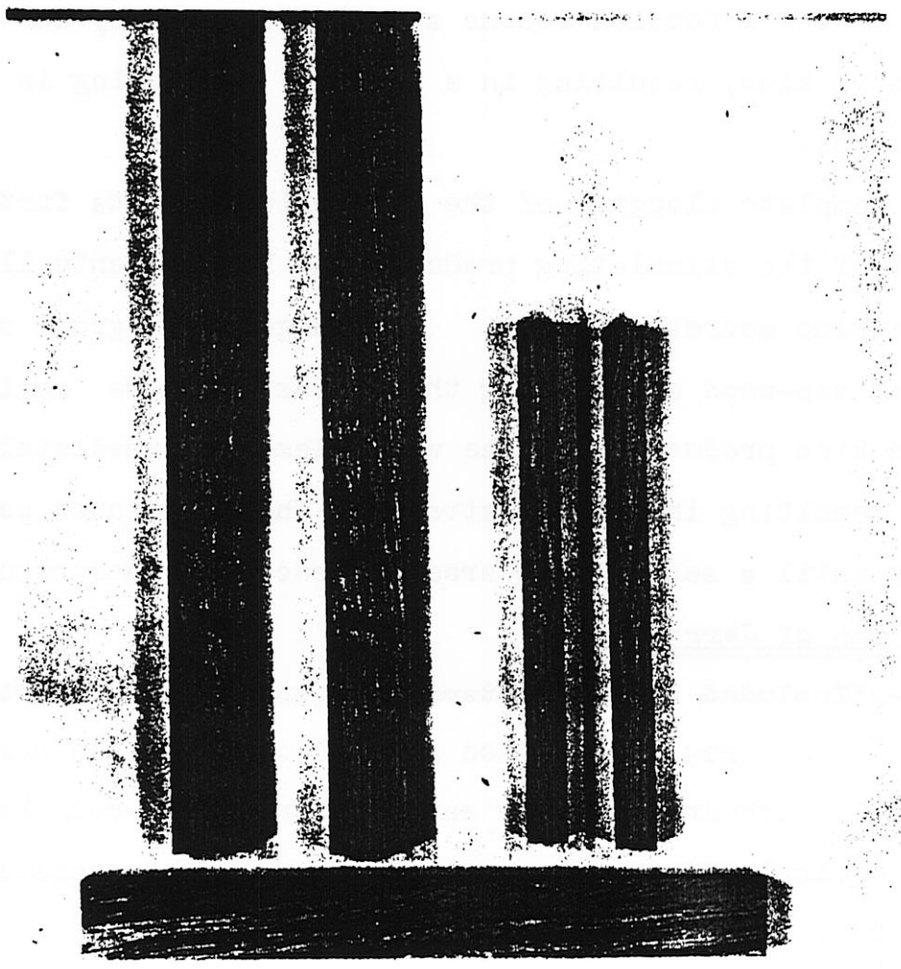

Fig. 14. Pencilled Jarrah showing the appearance of the wood on radial and tangential face. A normal Jarrah block is shown at the bottom.

common in old bush where careless felling or frequent fires have previously injured the growing stand.

On account of the porous nature of the included sap areas, timber showing this defect is unsuitable for pipes or "tight" cooperage. Also such areas cannot be expected to be very durable in service and eventually rot away leaving a cavity. Generally speaking the rot does not appear to extend into the surrounding true wood.

) Examination for a Fungus - Microscopic examination revealed the frequent presence of fungal hyphae, both in clean included sap and in areas of sap rot. It was quite obvious from detailed examination of hyphae and their mode of penetration that several fungi may be present and in areas of clean included sap are generally of the "sap stain" type.

Microtome sections occasionally showed scattered asci of a plectomycete and on surface examination of the wood, the perithecia were readily located using a x 21 magnification. These appeared as minute yellow balls in the vessels and could be removed quite easily with a needle.

Examination of these perithecia places the fungus in the Aspergillaceae and it was believed to be either a Eurotium or one of the Penicillia of the P. luteum type, where the asci are surrounded only by a loose mass of hyphae.

Several specimens of included sap from different localities were examined and perithecia frequently found both in sap rot and clean included sap areas.

Specimens showing perithecia in situ have been sent to an American authority for comment.

(c) Cultural Work - Transplants were made from rotted and normal specimens of included sap and in every case, all transplants grew a brown penicillium. No other fungus has been isolated.

This fungus was inoculated on to sterilised normal sap-wood blocks with the object of determining whether it would grow and

penetrate the wood and perhaps eventually produce perithecia. Vigorous mycelial growth was made over the surface of the wood blocks which however have not yet been examined.

Areas of rot are commonly found associated with included sap in the living tree, but as a number of common fungi ( e.g. Fistulina hepatica, Polyporus pelles etc.) are certainly capable of causing a sap rot, no comprehensive attempts have been made to isolate wood destroying fungi from the rotted areas.

(d) Microscopic features - Sections show some variation in the amount of kino and in the degree of tylosis in included sap areas. In general the ray cells are blocked with kino, and a certain amount of vessel tylosis has occurred, but the fibres while often almost normally thickened are completely free from kino-infiltration.

Fungal hyphae are commonly present and a certain amount of transverse penetration occurs, sometimes a single thread penetrating across several fibres. These hyphae are very constricted where they pass through the bore-holes in the fibre walls but thicken again in the lumina

In general however the wood elements are not materially affected, which supports the opinion that the hyphae present are those of "sap stain" fungi.

Whether the failure of the wood to mature normally is due to the effect of the wound or whether it is due to the entrance of a fungus is not clear at this stage.

Fig. 15. Photograph of a Jarrah mill log showing a cavity caused by the rotting of an area of "Included sap". Note the overgrowth which marks the site of an old wound, immediately above the cavity.

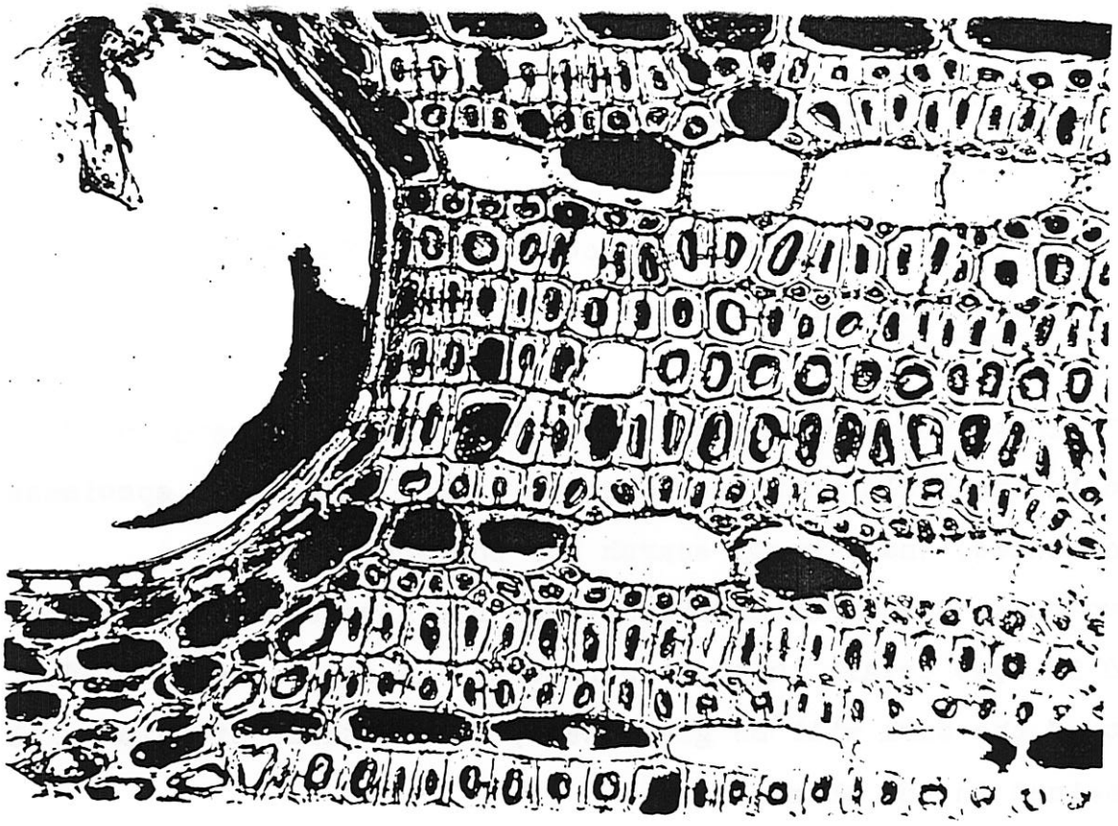

Fig. 16. Photomicrograph of a transverse section of normal Jarrah true wood.

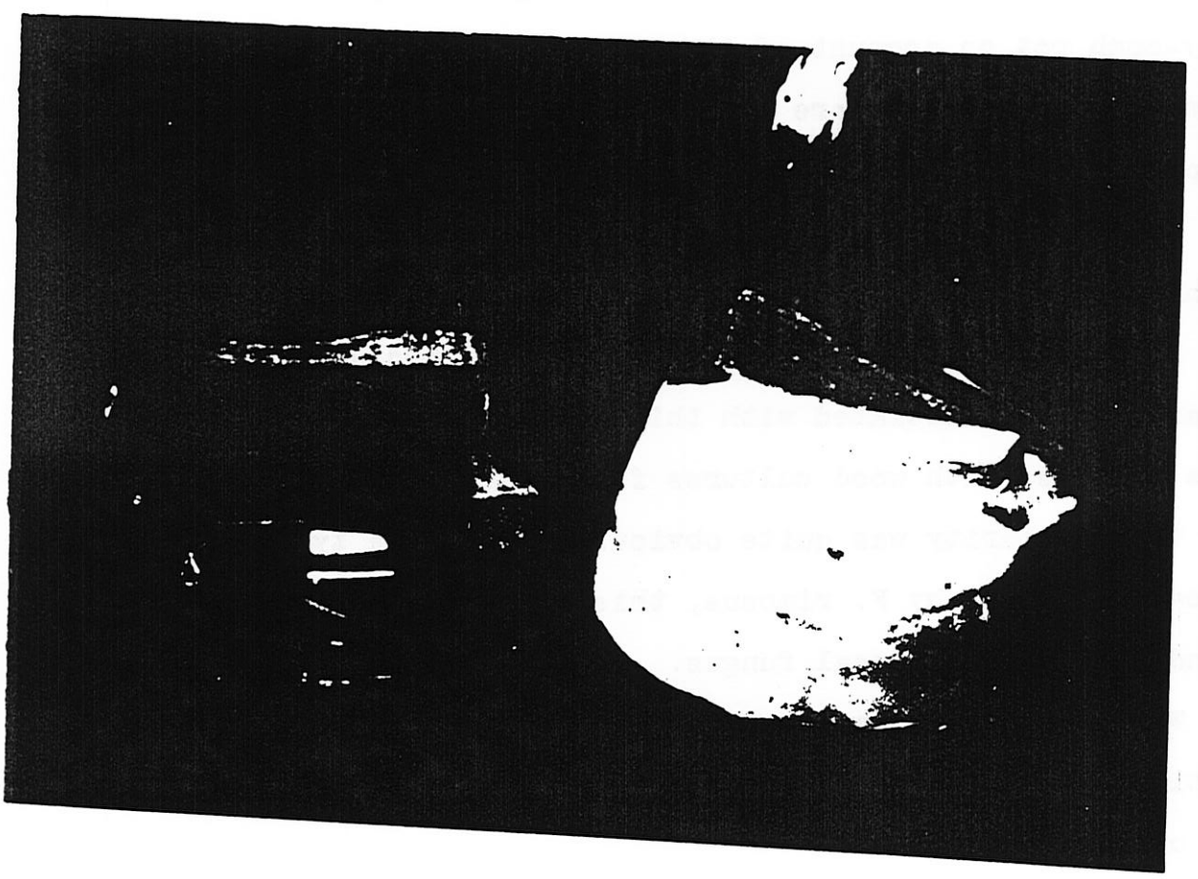

Fig. 17. Artificial production of rot. Erlenmeyer flask experiment showing.

Left - Control flask containing sterile wood blocks, raised above the surface of medium by glass tubing.

Right - Wandoo blocks well covered by the vigorous mycelial growth of Fomes rimosus, the causal fungus of the honey-comb-rot of Wandoo

## ROTS INVESTIGATED IN WANDOO

The three rots most commonly occurring in Wandoo are -

1. Honey comb rot
2. Straw rot
3. Cubical rot

All three are trunk rots of the living tree and were briefly studied in the field while at Narrogin in May 1936, when specimens were collected, and a hasty search for sporophores made.

Two rots, Straw rot and Honey-comb rot have been since investigated in some detail in the laboratory, but only a very brief account of the work will be given here, full details of which will be contained in the thesis.

Specific Investigations

1) Honey comb rot of Wandoo

This white pocket rot of Wandoo is generally referred to as honey-comb rot on account of its appearance in the late typical stage when the pockets are filled with brown mycelium giving an appearance suggestive of honey comb.

The rot is often associated with the insertion of branches but has been found completely rotting the butt of a living tree.

Sporophores of Fomes rimosus were collected at Narrogin and appeared to be associated with this rot. On comparing sporophore cultures with wood cultures from several different specimens the similarity was quite obvious, and as the type of rot is typical of attack by F. rimosus, this species was considered to be undoubtedly the causal fungus.

Wandoo blocks were introduced into Erlenmeyer flask cultures of this fungus in Sept. 1936 and very vigorous mycelial growth has been made over the wood.

In order to prove beyond doubt that both fungi are identical, flasks were inoculated both from sporophore cultures and from cultures obtained from rotted wood.

As a point of interest Jarrah blocks were also inoculated

with F. rimosus as a rather similar type of honey comb rot has been found to occur occasionally in Jarrah - though as yet it has not been investigated.

Straw rot of Wandoo

This rot is considered to be the commonest rot of Wandoo, but is believed by inspectors not to continue in cut timber.

The rot occurs as a yellow, more or less spongy heart rot, with a narrow, often discoloured advancing zone. It is not macroscopically similar to yellow straw rot in Jarrah, and is caused by a different fungus.

The causal fungus, which has been only recently isolated (Oct. and Nov.) is a typical Polypore, and fruits readily in culture, usually during the third week.

So far, its identity has not been established as it does not compare with any sporophore cultures in stock.

It is hoped however to discover the sporophores of this fungus when time permits of further field work.

As the fungus isolated is almost certainly the causal species, it will be inoculated onto Wandoo blocks as soon as possible.

Fig. 18 Photograph showing the appearance of Straw rot in Wandoo.