I. THE ROLE OF TOXIC EXTRACTIVES IN THE RESISTANCE OF TALLOWWOOD (EUCALYPTUS MICROCORYS F. MUELL.) TO DECAY

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[Manuscript received July 30, 1957]

Summary

The hypothesis that the natural resistance of certain timbers to decay is due to the deposition in the heartwood of materials toxic to fungi is discussed, and criteria for assessing the role of these materials are outlined. These criteria have been applied in a study of the decay resistance of the highly durable Australian timber tallowwood (*Eucalyptus microcorys* F. Muell.).

Sawdusts from the outer heartwood of four trees of tallowwood were extracted successively with ether, methanol, acetone, water, and dilute alkali. Unextracted and ether-extracted sawdusts were highly resistant to decay by *Trametes lilacino*gilva Berk., *Coniophora cerebella* Pers., *Coriolus versicolor* (Fr.) Quel., and *Fomes durus* (Jungh.) G. H. Cunn. The methanol extraction greatly reduced decay resistance for all trees and all fungi; the extractions with acetone, water, and dilute alkali had no consistent effect.

The material removed by each solvent was incorporated into heartwood sawdust from the decay-susceptible mountain ash (*E. regnans* F. Muell.) in concentrations (w/w) equal to those in tallowwood. Decay tests showed the methanol extract (12–15 per cent. of oven dry weight) to be highly toxic to all test fungi (as was confirmed by toxicity tests in agar), whereas other extracts showed only slight indications of toxicity.

It is concluded that the high resistance of tallowwood to decay is due almost entirely to the presence of a toxic methanol-soluble material (or materials) and further work to isolate this material is in progress.

I. INTRODUCTION

Although it has been long recognized that some timbers are much less readily attacked than others by such biological agents as wood-rotting fungi, termites, wood-boring insects, and marine organisms, very little is known of the chemical and physical properties of the wood which are responsible for these differences. The high resistance of some timbers to decay is, however, generally ascribed to the deposition in the heartwood of materials toxic to fungi. Though this theory has been held for a long time, little experimental work was done until comparatively recent years, but a number of investigations have now been made and have been partly reviewed by Erdtman (1952, 1955) and by Findlay (1957).

Consideration of the results of these investigations shows that several substances toxic to wood-destroying fungi have been isolated from the heartwoods of durable timber species. In some cases, variations in concentration of the toxic extractives have been shown to be correlated with measured variations in decay

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resistance, but it has not been proved conclusively that the toxic materials isolated give a complete explanation of the decay resistance of the timber. For conclusive proof, it would be desirable to show, firstly, that the extractive concerned will inhibit decay, secondly, that their complete removal from a durable timber renders it highly susceptible to decay, and thirdly, that the decay resistance of individual samples of the timber can be quantitatively related to their content of the specific extractives.

Since our present investigations are concerned with explaining the variations in durability of timber, it was decided to commence with a detailed investigation of the role of various extractives in the decay resistance of one highly durable timber. Tallowwood is one of the most important commercially, and most durable, Australian timbers and is obtained from one clearly defined botanical species, *Eucalyptus microcorys* F. Muell. Laboratory tests had confirmed its reputedly high resistance to decay and had suggested that this resistance was due to toxic extractives. The chemistry of tallowwood has not previously been studied from this aspect, although Cox, King, and King (1956) have isolated from the heartwood a petroleum ethersoluble material which could be saponified to give a steroid which they named *cyclo*eucalenol.

II. MATERIALS AND METHODS

(a) Selection and Preparation of Wood Samples

Material for this investigation was obtained from four large mature trees of tallowwood, each of which came from a different district. A billet, 5 by 5 in. in cross section, was taken from the outer heartwood near the butt of each tree and air dried. A sample representing the entire cross section of the billet was reduced to fine sawdust in a Wiley mill fitted with a 2-mm screen. Decay-susceptible sawdust, into which the tallowwood extracts could be incorporated to study their inhibitory effect on wood-destroying fungi, was similarly prepared from the outer heartwood of mountain ash, E. regnans F. Muell., which has a relatively low resistance to decay and is moderately absorbent.

(b) Test Fungi

The four wood-destroying fungi used for decay tests were:

- (i) Trametes lilacino-gilva Berk., strain DFP 1109, isolated 1944 from sporophore on Eucalyptus sp. An Australasian brown-rot fungus occurring mainly on fallen eucalypt timber; a common cause of destruction of eucalypt timber in service.
- (ii) Coniophora cerebella Pers., strain DFP 1779, isolated 1938 from decay in E. marginata Sm. A cosmopolitan brown-rot fungus, causing both heart rot in standing trees and decay of timber in service; extremely destructive to any kind of timber.
- (iii) Coriolus versicolor (Fr.) Quel., strain DFP 2666, isolated 1950 from sporophore. A cosmopolitan white-rot fungus, commonly attacking the sapwood of fallen eucalypt logs, but not regarded as capable of severe attack on eucalypt heartwood.

(iv) Fomes durus (Jungh.) G. H. Cunn., strain DFP 3882, isolated 1953 from sporophore on Sterculia laurifolia. A white-rot fungus, recorded in Australia on rain-forest species in tropical areas; probable effect on eucalypt heartwood not known.

(c) Preparation of Extracts

A 60-g sample of air dried sawdust (of known moisture content) from each tree was taken, weighed into a Soxhlet thimble, and extracted with ether until no more material was being dissolved. The sawdust was then air dried and weighed and one-quarter set aside for decay tests. The remainder was placed in a Soxhlet thimble for extraction with methanol. The ether, methanol-extracted sawdust was air dried and weighed and one-third set aside for decay tests, the remainder being used for acetone extraction. After this stage, a water extraction was carried out on half the remaining sawdust. In this way, approximately equal amounts of etherextracted sawdust, ether and methanol-extracted sawdust, and so on, were obtained for decay tests.

A known fraction of each extract was set aside for removal of solvent followed by weighing to enable estimation of the concentration (w/w) of each extract in tallowwood on an oven-dry basis. The remainder of the extract was concentrated to a small volume (2n ml) and added to a predetermined weight (n g) of air dried mountain ash sawdust and thoroughly incorporated therein. The impregnated sawdust was then dried slowly at room temperature, being stirred periodically to prevent any concentration of solute at the surface of the sawdust. The weight of mountain ash sawdust impregnated was calculated so that the final concentration of the specific extract (w/w) in the impregnated mountain ash was the same as that in the untreated tallowwood.

The general scheme of successive extractions, impregnations, and selection of samples for decay tests is set out diagrammatically in Table 1.

A final extraction with 0.01N NaOH (200 ml/10 g sawdust) was carried out on a separately prepared sample of ether, methanol, acetone, and water-extracted tallowwood (of known weight and moisture content) in a boiling water-bath. After 2 hr the alkali-extracted sawdust was filtered off in a sintered-glass crucible, washed, and suspended in distilled water. The pH was adjusted to $5 \cdot 4 - 5 \cdot 6$ with hydrochloric acid and the sawdust filtered, dried to constant weight, and a moisture content determination made, thus enabling the percentage of alkali-soluble material to be determined by the loss in weight. The pH of the extract was adjusted to $5 \cdot 0$ with hydrochloric acid; the extract was then concentrated under vacuum and impregnated into the predetermined amount of mountain ash. The amount of alkali used was kept to a low level because the presence of any appreciable amount of salt in the neutralized alkali extract would tend to inhibit the growth of the test fungi. In the case of tree MI7, the amount of alkali used may possibly have been insufficient to remove all the alkali-soluble material present.

The extracted tallowwood and impregnated mountain ash sawdusts were air dried and stored at room temperature pending decay tests. Preliminary tests on mountain ash sawdust had failed to show any toxic effect from residual solvent with

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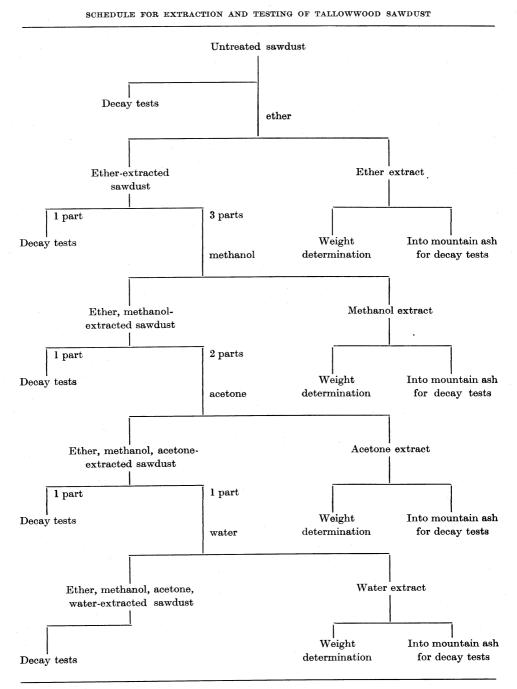


TABLE 1

this method, but some anomalous results in the main tests suggested such an effect. Part of the test was therefore repeated on sawdusts from the same sources which had been vacuum dried at 40° C for 48 hr; no anomalous results were found in the repeated tests.

The pH of sawdust from each of the four trees of tallowwood was determined by adding distilled water (50 ml/g) and recording the equilibrium figure reached after cessation of stirring. Determinations were carried out in a like manner on ether, methanol-extracted and on ether, methanol, acetone, water-extracted tallowwood, and on the aqueous suspensions of the methanol extracts (1 g/50 ml water).

(d) Decay Tests

The relative susceptibility to decay of the various types of sawdust was assessed by the percentage loss in oven dry weight obtained when small samples of each sawdust in perforated aluminium dishes were incubated for several weeks in close contact with the test fungi in pure culture. The test fungi were grown on nutrified "feeder strips" resting on moist soil in glass jars, using a technique similar to the "soil block" technique developed by Leutritz (1946) for measuring decay resistance of wood blocks.

The culture jars each contained approximately 100 g of a clay loam soil of exceptionally high organic matter content and water-holding capacity, with a moisture content of 60 per cent., below which capillary movement of water is negligible with this soil. The feeder strips were $1\frac{1}{2}$ -in. squares of $\frac{1}{16}$ -in. coachwood (Ceratopetalum apetalum D. Don) veneer, soaked in a nutrient solution based on that of Jennison, Newcomb, and Henderson (1955), with the composition: casein hydrolysate, 0.08 per cent.; KH₂PO₄, 0.15 per cent.; MgSO₄.7H₂O, 0.05 per cent.; thiamin hydrochloride, 0.001 per cent.; sucrose, 2 per cent. The jars were autoclaved for 2 hr at 15 lb/sq. in., inoculated with the test fungi, and incubated at 25°C and 95-99 per cent. R.H. until the mycelia had covered the feeder strips, when a sterile dish of sawdust was placed in each jar. The dishes containing the sawdust samples were $1\frac{1}{2}$ in. in diameter, $\frac{3}{16}$ in. deep, of 0.003 in. thick aluminium foil, the bottom being perforated by numerous holes (0.001-0.010 in. in diameter) to allow the fungus to grow up into the sawdust (Plate 1, Fig. 1). (These dishes may readily be prepared by smoothing and perforating clean, unlacquered caps from milk bottles.) Each dish was labelled in waterproof Indian ink and tared and approximately 1 g of air dry sawdust (of known moisture content) was weighed out into it (Plate 1, Fig. 2). As it was considered that the composition and toxicity of the wood extractives might be affected by high temperatures, the dishes of sawdust were sterilized at room temperature by fumigation with propylene oxide, as suggested by Hansen and Snyder (1947). The dishes were placed in 4-in. petri dishes and held overnight in a gas-tight vessel containing propylene oxide (4 ml per litre of air space). They were stored for 3 days to allow any residual propylene oxide to evaporate before being placed in the culture jars (Plate 1, Fig. 3), which were then incubated at 25°C and 95–99 per cent. R.H.

Under these conditions, hyphae of the test fungi penetrated throughout the sawdust in the dish in a few days and decay proceeded rapidly in susceptible material,

even though no water or nutrient had been added to the sawdust. Apparently the fungus was able to obtain sufficient water for its needs, since the final moisture content was often as high as 80 per cent. even in relatively unattacked sawdust. The incubation period was 7 weeks for *Coniophora cerebella*, 9 weeks for *Trametes lilacino-gilva*, and 14 weeks for *Coriolus versicolor* and *Fomes durus*.

At the end of the incubation period, the dishes of sawdust were oven dried for 48 hr at 105°C and reweighed. The percentage decrease in oven dry weight of the sawdust was calculated for each sample, and the results statistically analysed to determine the significance of the differences observed. Each type of sawdust for each tree was tested in duplicate against each test fungus, except that unextracted tallowwood and untreated mountain ash were tested in quadruplicate.

	Tree MI7	Tree TW10	Tree TW9	Tree QMI25
		-		
Successive extractives as percentage of oven-				
dry weight:				
Ether	$2 \cdot 82$	2.52	$2 \cdot 92$	$2 \cdot 43$
Methanol	$15 \cdot 05$	13.80	12.77	$12 \cdot 21$
Acetone	0.10	0.07	0.04	0.10
Water	0.81	1.20	1.06	0.56
Alkali	$5 \cdot 71$	4.97*	0.70	0.44
Total, excluding alkali extractives	18.78	17.59	16.79	$15 \cdot 30$
Total, including alkali extractives	$24 \cdot 49$	$22 \cdot 56*$	17.49	15.74
pH of sawdusts:				
Unextracted	$3 \cdot 8$	3.8	3.7	3.6
Ether and methanol-extracted	$4 \cdot 7$	4.7	4.6	$4 \cdot 6$
Ether, methanol, acetone, and water-		1.0		
extracted	$4 \cdot 8$	$5 \cdot 1$	$5 \cdot 1$	$5 \cdot 1$
pH of methanol extract in water:	3.6	3.6	3.6	3.4

		т	ABLI	c 2		
EXTRACTIVE	CONTENT	AND	\mathbf{pH}	OF	TALLOWWOOD	SAWDUSTS

* 0.1N alkali (other results for 0.01N alkali).

(e) Dilution Tests with Methanol Extract in Sawdust

Preliminary work had indicated that the material responsible for the decay resistance of tallowwood would probably be located in the methanol extract. Accordingly, this extract was investigated more intensively and was tested at a wide range of concentrations in mountain ash sawdust to determine the degree of its toxicity. These concentrations are expressed as percentages of the concentration (w/w) present in the relevant untreated tallowwood.

(f) Dilution Tests with Methanol Extract in Agar

Agar toxicity tests were carried out using the methanol extract in concentrations (w/w) corresponding to those in sawdust. The test was carried out in small glass phials each containing a total of 2 g of solid medium (Plate 1, Fig. 4). The medium chosen for this purpose contained 2 per cent. agar, 4 per cent. carboxymethylcellulose, and 2 per cent. malt extract, in tap water. The methanol extract, agar, and carboxymethylcellulose were mixed in the solid state as fine powders in the phial, the correct volume of 2 per cent. malt extract solution in tap water added, and the mixture stirred. After steaming for 25 min to dissolve the agar, the viscous material was stirred, and the phial capped and laid flat so that the agar solidified as a layer along one side. The phials were then sterilized with propylene oxide in the same manner as the sawdust dishes used in the decay tests. The phials were inoculated at one end of the agar strip, and incubated at 25° C and 95-99 per cent. R.H. for several weeks. Once growth had commenced, the position of the advancing front of mycelium growing along the phial was recorded at 2–3 day intervals and the average rate of linear growth calculated as a percentage of control growth.

III. RESULTS

(a) Chemical Results

Tallowwood is so named because of its greasy nature and it is the material responsible for this which is extracted by ether. The product, a yellow viscous oil, was readily extracted and was present in 2-3 per cent. yield, based on the oven dry weight (Table 2). This probably included the steroid from which cycloeucalenol was obtained (Cox, King, and King 1956). Concentration of the methanol extract from the ether-extracted tallowwood readily gave a brown solid. The yield from different trees varied between 12 and 15 per cent. Ether, methanol-extracted tallowwood had lost much of its original lustre, but the original colour variations between trees could still be noticed. Acetone extraction of ether, methanol-extracted tallowwood removed a very small amount of a grey-brown solid which was, in all probability, related to the methanol extract. 'Water extraction of ether, methanol, acetone-extracted tallowwood also removed a comparatively small amount of greybrown solid. At this stage it was still possible to discern colour differences between trees MI7 and QMI25, on the one hand, and trees TW10 and TW9 on the other. Colour differences between MI7 and QMI25, or between TW10 and TW9, had disappeared, the two former were buff and the two latter grey.

Whilst it is unlikely that ether or methanol extraction of tallowwood had much effect on the wood structure, hot water extraction would cause a small amount of hydrolysis, which may have slightly lowered the resistance of the wood to decay. The final extraction with weak alkali would unquestionably have affected the cell wall as well as the extraneous materials present and probably predisposed the wood to fungal attack.

Tallowwood had a pH of $3 \cdot 6 - 3 \cdot 8$ and gave a methanol extract with a pH of $3 \cdot 4 - 3 \cdot 8$ in aqueous medium, the ether, methanol-extracted tallowwood having a pH of $4 \cdot 6 - 4 \cdot 7$. After the aqueous extraction the pH rose to $4 \cdot 9 - 5 \cdot 1$.

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TABLE	

PERCENTAGE WEIGHT LOSSES OF TALLOWWOOD SAWDUSTS

Values given are mean weight losses for two replicate specimens, except values for untreated tallowwood and untreated mountain ash which are means of four replicates

			Tallowwood		our repucates Sawdust after Successive Extractions with:	ive Extraction	is with:	Mountain A	Mountain Ash Controls
Test Fungus	Tree No.	Untreated Tallowwood Sawdust	Ether	(1) followed by	(1), (2)followed by	(1), (2), (3) followed by	(1), (2), (3), (4) followed by	Untreated	Alkali- extracted
	-		(1)		Acetone (3)	(4)	(5)	oawaust	oawausu
Trametes lilacino-gilva	LIM	0.7	0.2	11.5	23.4	29.0	19.8		
DFP 1109	TW10	0.8	$0 \cdot 0$	14.4	17.3	30.0	34 4		
	1W9	0.5	0.4	9.4	29.4	30.8	35.4		
	QM125	$9 \cdot 0$	$0 \cdot 0$	$24 \cdot 8$	33 · 0	34.8	25.4	÷.,	
Mean*		$=$ $2 \cdot 0$	- 0 • 3	15.0	< 25.8	31.1	28.8	43.8	44 · 8
Coniophora cerebella	MI7	. 0.0	0.0	44.4	40.0	41.6	42.6		
DFP 1779	TW10	-0.6	0.2	47.2	44 · 1	49.4	57.4		
	6MT	-1.2	-0.2	44.6	45.0	46.2	52.2		
	QMI25	-1.4	0.2	47.5	47.4	49.6	50.8	-	
Mean		-0·8 -0.8	0.1	46.0	44.1	46.7	20.8	50.6	60.8
Coriolus versicolor	MI7	1.0	-0.4	22.0	15.6	29.8	$26 \cdot 2$		
DFP 2666	TW10	-0.2	0.0	19.8	27.0	22.3	32.2		
	TW9	-0.4	2.8	21 · 4	28.9	20.0	32.6		
	QMI25	-1.0	1.0	25.0	22.0	$24 \cdot 0$	29.0		
Mean		-0.2	6.0 =	22.0	- 23.4	24 · 0	30.0	25.8	40.4
Fomes durus	MI7	0.5	-2.7	15.5	20.0	18-4	16.7		
DFP 3882	TW10	-4.4	-3.9	16.6	11.4	20.0	23.4		
	TW9	$6 \cdot 0 - 0$	-1-4	20.7	15.2	4.8	18.2		
	QM125	-1.2	-3.8	23.4	10.3	17.8	12.8		
Mean		-1.5 =	-3.0	■ 19·1 =	14.2	- 15.2	17.8	39.2	26.0
* Means linked by the		signs = do not differ significantly,		< differ significantly at 5 per cent. level, and	antly at 5 per	· cent. level, a		\ll at 1 per cent. level.	

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(b) Decay Resistance of Untreated Tallowwood

Even when reduced to fine sawdust, tallowwood proved very resistant to decay, virtually no loss in weight being caused by any of the four test fungi (Table 3). Other tests were made with longer incubation periods, but even after 30 weeks'

TABLE 4

PERCENTAGE WEIGHT LOSSES OF MOUNTAIN ASH SAWDUSTS CONTAINING TALLOWWOOD EXTRACTIVES

Values given are mean weight losses for two replicate specimens, except values for untreated controls which are means of four replicates

			Source	of Extra	actives		
Test Fungus	Tree No.	Ether Extract	Methanol Extract	Acetone Extract	Water Extract	Alkali Extract	Untreated Control
m , 1'1 ' '1	MTS	40.0		90.0	40.0	0.0	
Trametes lilacino-gilva DFP 1109	MI7 TW10	$40 \cdot 0$ $45 \cdot 5$	$1 \cdot 4 \\ 1 \cdot 0$	$38 \cdot 6$ $26 \cdot 2$	$40 \cdot 2$ $42 \cdot 8$	$9 \cdot 2$	
DFP 1109	TW10 TW9	$45 \cdot 5$ $45 \cdot 2$	$1 \cdot 0$ $2 \cdot 0$	$33 \cdot 4$	$\begin{array}{c} 42 \cdot 8 \\ 41 \cdot 0 \end{array}$	35.4	
	QM125	$43 \cdot 2$ $43 \cdot 3$	2·0 1·6	$\begin{array}{c} 33 \cdot 4 \\ 42 \cdot 6 \end{array}$	$\begin{array}{c} 41 \cdot 0 \\ 32 \cdot 0 \end{array}$	$35 \cdot 4$ $26 \cdot 6$	
Mean	 	44.0†	1.5**	3 5 · 2*	39.0*	23.8**	43.8
Coniophora cerebella	MI7	49.2	1.1	51.8	51.5	$52 \cdot 0$	
DFP 1779	TW10	$48 \cdot 3$	$0 \cdot 9$	$48 \cdot 0$	47 · 4		
	TW9	$50 \cdot 2$	1.1	$50 \cdot 2$	$47 \cdot 2$	48.4	
	QMI25	48 · 4	0.4	$52 \cdot 8$	47.8	49 ·0	
Mean		4 9 · 0†	0.9**	50·7†	4 8 · 5†	4 9 · 8†	50·6
Coriolus versicolor	MI7	41.9	1.0	31 · 9	31.8	35.4	
DFP 2666	TW10	$40 \cdot 0$	0.3	$36 \cdot 7$	$20 \cdot 2$		
	TW9	$30 \cdot 4$	-0.4	$44 \cdot 2$	$37 \cdot 0$	$35 \cdot 6$	
	QMI25	26.8	0.6	$38 \cdot 2$	26 · 1	38.0	
Mean		34 ·7†	0.4**	37 · 7*	28.8†	36.3*	$25 \cdot 8$
Fomes durus	MI7	33 · 2	-1.4	42.8	22.6	3 0 · 1	
DFP 3882	TW10	$40 \cdot 2$	-3.6	$37 \cdot 6$	$25 \cdot 6$		
	TW9	$39 \cdot 2$	-1.4	40.0	$21 \cdot 2$		
	QM125	$15 \cdot 8$	-0.4	$39 \cdot 3$	14 · 4	36 · 1	
Mean		32.1†	-1.7**	40 · 0†	21.0**	33 · 1†	39 · 2

* Differs significantly from control at 5 per cent. level.

** Differs significantly from control at 1 per cent. level.

† Does not differ significantly from control.

incubation, weight losses were negligible except with *Fomes durus*, which produced weight losses of up to 25 per cent. There was no appreciable variation among trees in regard to decay resistance, either in the main test or in the longer tests.

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(c) Decay Resistance of Extracted Tallowwood

The amounts of decay occurring in unextracted tallowwood and in tallowwood subjected to the various extraction procedures are set out in Tables 3 and 5 and the amounts of decay occurring in untreated mountain ash and in alkali-extracted mountain ash are also given for comparison. It will be seen that in all cases the methanol extraction produced a highly significant loss of decay resistance, whereas no other extraction procedure had any consistent effect. Even after extraction with ether, methanol, acetone, and water, the tallowwood sawdust was significantly (1 per cent. level) more resistant than unextracted mountain ash sawdust to attack by *Trametes lilacino-gilva* and *Fomes durus*, though not to attack by *Coniophora*

TABLE 5

PERCENTAGE WEIGHT LOSSES OF TALLOWWOOD SAWDUSTS AND OF MOUNTAIN ASH SAWDUSTS CONTAINING TALLOWWOOD EXTRACTIVES AFTER VACUUM DRYING Values given are mean weight losses for two replicate specimens

		Sawdust afte	Mountain Ash Sawdust		
Tree No.	Ether followed by Methanol (1)	(1) followed by Acetone (2)	(1), (2) followed by Water (3)	Untreated	Containing Acetone Extract
	-				
MI7	$25 \cdot 4$	$25 \cdot 6$	$29 \cdot 8$		$43 \cdot 2$
TW10	$29 \cdot 0$	$29 \cdot 8$	$36 \cdot 8$		$45 \cdot 9$
TW9	$29 \cdot 6$	33.8	$35 \cdot 0$		$44 \cdot 2$
QMI25	29.8	$32 \cdot 8$	$39 \cdot 5$		41.8
······	28.4 =	= 30.5 <	< 35.3 <		= 43.8
	MI7 TW10 TW9	Tree No. Ether followed by Methanol (1) MI7 25 · 4 TW10 29 · 0 TW9 29 · 6 QMI25 29 · 8	Tree No.Ether followed by Methanol (1)(1) followed by Acetone (2)MI7 TW10 TW9 QNI25 $25 \cdot 4$ $29 \cdot 6$ $33 \cdot 8$ $32 \cdot 8$ $25 \cdot 6$ $29 \cdot 8$ $32 \cdot 8$	MI7 25.4 25.6 29.8 TW10 29.0 29.8 36.8 TW9 29.6 33.8 35.0 QMI25 29.8 32.8 39.5	Tree No.Ether followed by Methanol (1)(1) followed by Acetone (2)(1)(1), (2) followed by Water (3)UntreatedMI7

* Means linked by the sign = do not differ significantly, < differ significantly at 5 per cent. level, \ll differ significantly at 1 per cent. level.

cerebella and Coriolus versicolor. After further extraction with dilute alkali, the sawdust was still significantly more resistant than alkali-extracted mountain ash, except in the case of *Fomes durus*, but owing to the small number of alkali-extracted mountain ash controls involved in this comparison, too much importance should not be attached to this difference.

(d) Toxicity of Tallowwood Extractives

The effects of the various tallowwood extracts in protecting mountain ash sawdust against decay are shown in Tables 4 and 5. These show that the methanol extract was highly toxic in all cases, and that no other extract produced a consistently toxic effect, although some showed slight toxicity to one or more fungi.

The relative effectiveness of different concentrations of this toxic methanol extract in mountain ash sawdust is shown in Figure 1. Dosage-response curves for three of the fungi are closely similar, substantially complete inhibition being obtained only when the concentration approaches that in the original tallowwood, but *Coriolus versicolor* was strongly inhibited by low concentrations of the extract.

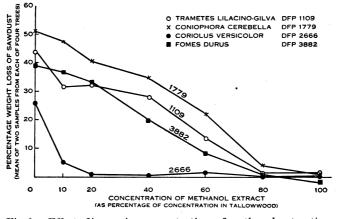


Fig. 1.—Effect of increasing concentrations of methanol extractives on decay of mountain ash sawdust.

The toxicity of the methanol extract in agar is shown in Figure 2. (The growth of *Fomes durus* on agar was extremely erratic and no reproducible results were obtained with this fungus.) Results are reasonably comparable with those of the sawdust bioassay, but the methanol extract is apparently less toxic in agar than in wood.

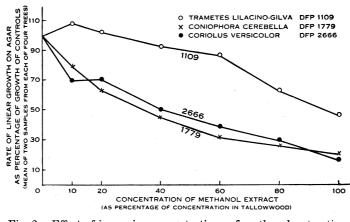


Fig. 2.—Effect of increasing concentrations of methanol extractives on growth of test fungi on agar.

IV. DISCUSSION

The results obtained show that tallowwood is highly resistant to decay when tested in the form of fine sawdust, but that this resistance is largely destroyed by

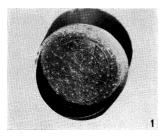
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the removal, during the methanol extraction stage of the extraction procedure used, of a substance (or substances) which is highly toxic to all four test fungi used. It is of interest that this methanol extract is much more toxic to *Coriolus versicolor*, which was known to find difficulty in attacking eucalypt heartwood, than to *Coniophora cerebella* and *Trametes lilacino-gilva*, which were known to attack even durable eucalypts. The data in Figure 1 suggests that tallowwood contains barely enough toxic material to protect it against these fungi, but as the distribution of the material in the impregnated mountain ash sawdust will be different from that in the tallowwood, the "margin of safety" may be greater than these data suggest.

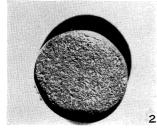
The relatively slight difference between the decay resistance of tallowwood extracted successively with ether, methanol, acetone, and water and that of the non-durable mountain ash indicates that only a small proportion of the decay resistance of tallowwood can be due to the chemical composition or fine structure of its cell walls. The extent to which the decay resistance of tallowwood is enhanced by its anatomical structure, and particularly by its low void volume, cannot be clearly evaluated here since the effect of these factors was partly, but not entirely, removed by converting the heartwood to fine sawdust. The fact that this fine sawdust was still highly resistant to decay suggests that the effect of gross anatomical structure is not very important in this species.

There are some indications in Tables 3 and 4 that extractives other than those removed by the methanol play a minor part. However, close inspection of the detailed results, particularly those for tallowwood exposed to *Trametes lilacinogilva* after methanol and after acetone extraction, suggested that some of these indications might be due to retention of toxic amounts of solvent in the sawdust. A portion of the test was therefore repeated for this fungus, the procedure being as before except that all sawdust was vacuum dried. The results, as shown in Table 5, differ from those of the main test in that the acetone extract showed no toxicity and the effect of extraction with acetone was not significant. It seems likely, therefore, that effects connected with the acetone extraction may be due to retention of acetone by the sawdust in amounts large enough to affect the growth of some fungi. Some organic solvents are very strongly retained by sawdust and cellulose even at temperatures well above their boiling point, e.g. methanol, acetone, ethanolbenzene (Wiertelak and Garbaczowna 1935; Anderson 1946).

It might be inferred from Tables 3 and 4 that the toxic extractives of tallowwood are readily soluble in methanol but not in ether. Some caution in accepting this is indicated by Erdtman's "membrane substances" (Erdtman 1943; Kondo and Kitamura 1955*a*, 1955*b*; Kondo, Koyama, and Tanaka 1955) which prevent the extraction with ether of the ether-soluble pinosylvins in pine heartwood; both membrane substances and pinosylvin are readily extracted with methanol or acetone. Work here has shown that a similar situation occurs with some of the ether solubles in tallowwood. Chromatographic fractionation of the methanol extract is now being carried out, together with estimation of the toxicity of the various fractions.



1.5 IN.





3 IN.

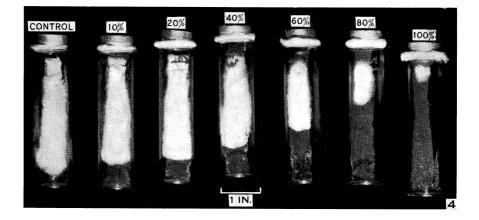


Fig. 1.—Perforated aluminium dish, empty.

Fig. 2.—Perforated aluminium dish, with 1 g sawdust.

Fig. 3.—Culture jar, with dish of sawdust, after incubation.

Fig. 4.—-Effect of increasing concentrations of methanol extract in nutrient agar on growth of test fungus.

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V. ACKNOWLEDGMENTS

The authors are indebted to Miss J. O. Hrapko and Mrs. N. Rakowski for assistance in carrying out these investigations and to Miss N. Ditchburne for statistical analysis of the data.

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