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**POLYPHENOLS AND RESIN IN THE RESISTANCE
MECHANISM OF PINUS RADIATA ATTACKED BY
THE WOOD WASP, SIREX NOCTILIO, AND ITS
ASSOCIATED FUNGUS**

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SUMMARY

A description of the insect's oviposition behaviour and of the action of the symbiotic fungus (*Amylostereum* sp.) is given. From observations on material from trees which were felled at intervals after having been given a light *Sirex* attack (by placing on them female insects with their wings glued), it was possible to describe the various stages in the trees' reactions. The fungus made some growth from the oviposition tunnel in the wood before being sealed off by the tree. Resistance was associated with the appearance of resin in the latewood of the annual rings and the development of polyphenols around infected zones. The fungus remains alive for some time after its growth has been prevented by the tree's reaction. The effect of various treatments (e.g., ringbarking, removal of the tree's crown) on polyphenol production is described. Although very suppressed trees gave anomalous results, the evidence suggests that polyphenols, which appear to protect the cambium from the fungus, are formed most quickly in vigorous trees, presumably from readily available foods. Stored foods can also be used, but concentrations of polyphenols are not formed in fresh logs attacked by *Sirex*.

Trees which die after heavy *Sirex* attack do not form concentrations of polyphenols and the latewood is not normally flooded with resin.

INTRODUCTION

Some of the literature on *Sirex noctilio* and its associated fungus, *Amylostereum* sp., was surveyed in a publication edited by Warcup and Morgan (1963), and again more briefly by Coutts (1965). The female insect alights on the stem of a tree, drills into the wood with her ovipositor, injects fungal arthrospores and sometimes one or more eggs. Hundreds of oviposition tunnels may be made per square foot of bark surface. The arthrospores grow and the fungus sometimes kills the tree. Rawlings (1954) records that in New Zealand, *Sirex* killed thirty per cent. of the trees over some 600,000 acres of radiata pine forest. The greatest proportion of trees killed occurs among the lower crown classes, although dominants may be attacked and killed. However, even under epidemic conditions in New Zealand, seventy per cent of the trees survived. Many of these would have received light attack although some trees survive which receive very large numbers of oviposition tunnels per square foot.

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Rawlings (1948) mentions the capsulation of the fungus by resin, and Jackson (1955) observed that trees which produce long streams of resin from *Sirex* oviposition tunnels are less likely to die than those which produce small beads of resin only. Coutts (1965) began to investigate the mechanism by which trees resist attack, and found that eggs and young larvae are killed by resin, and that resin floods the latewood of the annual rings which have been infected by the fungus. In addition, a red stain was found around the infected zone, which Harris (1964) suggested was a layer containing polyphenols. This was demonstrated by staining with benzidine. The role played in resistance by resin and polyphenols being by no means clear, a more detailed study has been made. Before describing the recent work a brief account of the oviposition behaviour of the insects and the action of the symbiotic fungus will be given.

OVOPOSITION BEHAVIOUR

The ovipositor of the *Sirex* female is a fine tube with sliding valves equipped with "teeth", which enable the insect to drill through the bark of the tree and into the wood. Oviposition tunnels up to 13 mm in length have been found in the wood, so they often pass through several growth rings. The curiously variable oviposition behaviour has been described by Coutts (1965). The female insect will commonly drill one tunnel into the wood, withdraw the ovipositor until its tip is in the bark, and then drill in again at an angle to the first tunnel to give a pair of diverging tunnels in the wood. One or more eggs are commonly laid in the first tunnel of the pair, but the second tunnel normally contains no egg. Recent studies have shown that the fungus is usually injected whether an egg is laid or not, but it does not develop equally from both tunnels (see below). The first of a pair of tunnels (the one with the egg) is frequently shorter than the second, empty one, to make which the insect often drills in right up to the "hilt".

THE ACTION OF THE FUNGUS, AND THE INSECT'S LIFE CYCLE

The fungus develops in the wood, causing it to dry out locally by some unknown process (Coutts and Dolezal, 1965), so eggs hatch, and larvae begin to bore, in comparatively dry wood. The fungus appears to affect the wood in such a way that water is released into the sapstream, as trees which have been coated with grease after oviposition still show typical drying effects subsequently. If the bark, phloem, and cambium are removed before the grease is applied, dried areas still develop. If the fungus continues to grow, the tree dies between one and several months from the time of attack, but the way in which the fungus kills the tree is still uncertain. Measurements made with a dendrometer have shown that diameter growth may be checked in less than two weeks after trees have been attacked by *Sirex*. (This happens even if they resist the attack.) Yellowing of the foliage then occurs and sometimes the needles droop.

The fungus kills the cambium and grows towards the centre of the tree. The water supply to the tree's crown would eventually be cut off, as the wood dried by the action of the fungus is non-conducting. However, the fungus might produce other effects. For example it might supply toxins which affect the foliage, and account in this way for the rapid reaction of the tree to attack.

The egg takes two weeks or more to hatch, depending on temperature. Larvae bore in the wood generally for about a year in Tasmania and pupate in the wood some distance from the outside of the tree. The adult insect bores to the outside by gnawing the wood with its mouth parts, and leaves the tree through a circular exit hole. The purpose of this paper is to consider the processes which take place in trees which resist attack, stop the growth of the fungus, and kill the eggs and larvae of the insect.

METHODS

The method of study was to obtain *Sirex* attack in a number of trees on a certain day, and then fell the trees at intervals to study the development of the fungus, resin, and polyphenols in the wood. Large female insects which emerged from logs from fire-damaged trees were used. The insects were left in a large cage, in full sunlight, containing hundreds of males, so all or nearly all of them were mated. Mating makes them keener to drill oviposition tunnels, and this is important if oviposition is required in a short time. After having their wings glued together the females were put on trees in some nine-year-old regrowth at Pittwater Plantation, and removed the next day. Oviposition holes in the bark were marked, because artificial attack on trees attracts wild attacks, which could easily confuse the issue. Light attack in this paper refers to less than 30 oviposition tunnels per square foot of bark surface, and heavy attack to 300 tunnels or more. The trees were felled at varying times after oviposition, and small wood samples cut out, with a marked oviposition site in the middle of each. The samples were shaved down in cross-section to the level of the oviposition tunnels. Measurements and sketches were made of the tunnels, annual rings, and the extent of the dried areas. The wood was then stained with benzidine and cupric acetate and examined with a binocular microscope. Staining with cupric acetate enables one to see slightly more resin than is visible on unstained wood. It was fully realised that staining with benzidine is a very crude method for the detection of phenolic substances, and at present the identity of substances involved is not known. Concentrations of resin and polyphenols were sketched in on the diagrams. Samples from six to ten oviposition sites on each tree were examined in this way.

Some samples were cut on all faces with a sterilized scalpel, incubated for 24 hours or more at 26° C., and examined for the presence of fungal hyphae before staining. Hyphae grow quickly from the wood and can readily be seen with a microscope if the light is slanted across the wood surface. Hyphae were removed and cultured on agar to check on the identification of the fungus.

Three experiments were done. For the first experiment, the insects were put on seven dominant trees which were then felled at intervals. It showed the approximate time taken for the fungus to develop and for polyphenols to appear and was followed by the second experiment, involving seven dominant and seven suppressed trees. The third experiment comprised a number of trials in which trees were given various treatments in order to learn something about the factors which affect resin and polyphenol production.

THE FIRST EXPERIMENT: SEVEN DOMINANTS

The early stages of the tree's reaction are described for seven young dominant trees attacked on the same day. Observations of later stages were made on a variety of other material, but will be included in this section.

EARLY STAGES IN THE TREES' REACTIONS

The seven young dominant trees were of moderate vigour, about 30 feet tall, and with diameters of about four inches at breast height. The seven trees were felled at 3, 6, 9, 15, 17, 20 and 41 days after light *Sirex* attack in February, 1965. Considerable individual variation between trees was expected, but a clear enough picture emerged for the stages in resistance to be approximately described. For the sake of uniformity, reaction to attack is described for the vicinity of a pair of oviposition tunnels in the first of which an egg was laid, although single and triple tunnels were also encountered.

Three days after oviposition no drying was apparent in the wood radially or tangentially from the oviposition tunnels. The longer (second) tunnel was in every case full, or almost full of resin, most of which had solidified, but the tunnel with the egg was more or less free of resin. It was shown that stimulation of the tree by the fungus is not necessary for the second tunnel to become flooded with resin; holes made by a drill as fine as an ovipositor were all full of resin after three days. Resin probably flows in from broken ducts as soon as a hole is drilled. Tunnels containing eggs have a propensity for keeping out resin. This seems to depend on a lining of mucilage, which may be of great importance to the survival of eggs. If the tree resists attack, resin will eventually get into the tunnel with the egg. In ten trees which resisted attack and which were dissected previously (Coutts, 1965), 54 per cent. of the eggs had failed to hatch. All the larvae which had hatched had died soon afterwards and were found preserved in resin.

One week after oviposition, drying had appeared in the neighbourhood of the second tunnel (Fig. 1 (i)) but not around the tunnel with the egg.

Two weeks after oviposition the drying had spread radially inwards, and outwards towards the cambium, from the second tunnel, and had often begun around the tunnel containing the egg (Fig. 1 (ii)). The fungus grows most quickly longitudinally, more slowly radially, and very slowly indeed tangentially, so it is apparent at this and at later stages that the width of fungus infected wood seen in transverse section depends mainly on the extent to which the oviposition tunnels are angled or curved to one side of the tree's radius. Tunnels drilled on the radius, which are quite uncommon, produce in cross section exceedingly narrow areas of dried wood.

As the drying extends into the latewood of successive growth rings, the latewood floods with resin. This happens up and down the tree to the full extent of the developing lesion, not merely at the level of the oviposition tunnels. Polyphenols produced as a reaction to attack were first found two weeks after oviposition, making their first appearance in the outer-most growth ring.

Three weeks after oviposition (Fig. 1 (iii)), and perhaps at all stages in trees which are resisting attack, the fungus is not present at the outer limits of the dried areas. It appears to grow into wood which dries just ahead of it. From discs of wood, 1 mm samples were cut from just beyond the innermost tip of the dried area, and back towards the cambium. No fungus could be cultured from wood just outside the dried area, nor from inside it within 2 mm. of its inner tip.

The exact position of the fungus in relation to the longitudinal extent of drying is not yet certain, but there are indications that drying of the wood proceeds well ahead of the fungus in this direction also. At the level of the oviposition tunnels, and for one or two centimeters above and below (i.e., in the longitudinal direction), resin spreads into the early wood of the growth ring

Explanation of Figure 1

The stages of the spread of drying caused by the fungus, and the tree's reactions, are shown in transverse sections.

A typical pair of oviposition tunnels is shown, with an egg in the first (short) tunnel, but no egg in the second tunnel, which is 13 mm. long. The relative diameter of the oviposition tunnels has been slightly exaggerated.

A: Soft inner bark and phloem. The part shaded with horizontal lines in (ii), (iii), and (iv), contains dead brown cells killed by the puncture or by the fungus.

B: Latewood of the growth rings. The outer ring has not yet formed latewood.

Grey irregular outline: The limit of the dried area. The fungus lies a short distance behind these limits radially, and perhaps tangentially also.

Heavy vertical dashes: Resin (probably all oleoresin).
Dots: Polyphenols.

(i) One week after oviposition.

(ii) Two weeks after oviposition.

(iii) Three weeks after oviposition.

(iv) Six weeks after oviposition.

TABLE 1

RESULTS OF THE FIRST EXPERIMENT, IN WHICH SEVEN DOMINANT TREES WERE GIVEN LIGHT *Sirex* ATTACK ON 24TH FEBRUARY, 1965

Tree No.	Number of days between oviposition and felling	Mean longitudinal spread of dry streaks in wood (mm)	Mean radial spread of drying beyond inner tip of oviposition tunnel* (mm)	ABOVE TUNNEL		BELOW TUNNEL		Mean number of latewood bands penetrated by dry areat
				5	4	7	4	
1	3		0	0			0	
2	6		1.0	1.0			0	
3	9		2.4	2.4			0.4	
4	15		6.0	6.0			0.8	
5	17		6.1	6.1			1.4	
6	20		8.9	8.9			1.2	
7	41		12.3	12.3			1.8	

* This refers to the oviposition tunnel in which no egg was laid.

† The number of latewood bands penetrated by the dried areas was counted from the inner end of each oviposition tunnel.

(Fig. 2), but beyond one or two centimeters above or below the tunnel, it is found mainly in the latewood. Drying usually spreads further in the latewood than in the early wood.

In this first experiment the average size of the dried areas increased with time but in the tree felled at six weeks the increase may be inflated by variation between trees, since in the more vigorous dominant trees used in the second experiment no increase in the dried areas was found after three weeks.

Polyphenols were found along the radial edges of the dried areas three weeks after oviposition, but not outside them.

Six weeks after oviposition, polyphenols continued to concentrate along but within the edges of the dried area. The last place for polyphenols to become concentrated was at the innermost tip where six weeks after the attack only fairly small concentrations were present.

LATER STAGES IN THE TREES' REACTIONS

Polyphenols are still being concentrated around the edges of the infected wood several months after the fungus has been contained. Living trees felled one or two years after having resisted *Sirex* attack have such concentrations of polyphenols that clear red borders can be seen without staining.

During later stages of the sealing-off process the width of the dried areas increases, creating a border of whitish wood, seldom more than two millimeters wide, between the polyphenols and the adjoining healthy sapwood. It is not known if this is caused by the fungus, although the latter remains alive inside the dried areas for a long time. Lesions in trees which resisted *Sirex* attack years ago are often very resinous, so resin also continues to concentrate for a time after the tree has resisted attack.

When the tree starts to grow again the new tissue produced outside areas of injured cambium secretes resin (traumatic resin) which may begin to creep inwards around the edges of the infected zone. It thickens to something like the consistency of pitch in cavities between the infected growth ring and wood formed subsequently, and may be forced out through the bark to produce hard, amber-coloured blobs. (Fig. 3.) In the wood, in a very viscous state, this traumatic resin seems to remain under pressure. When a tree is cut into discs it will slowly ooze out to form blobs which harden on the surfaces of the discs. Cross sections of trees which have resisted attack, and the resin blobs, if these are broken, have a strong characteristic smell like turpentine.

The above description is of resistance to light *Sirex* attack, when the tree limits each infection to a small area of wood. A tree will sometimes resist heavy attack, in which case only very small wedges of wood remain alive around its circumference. When this happens the fungus probably grows in as far as the pith, or to the heartwood, if there is any, and polyphenols are formed on the borders between the wedges of living wood and the dead wood. This can have curious effects on subsequent heartwood formation as the process by which heartwood is gradually enriched in extractives is blocked by the fungus-infected wood. Blue stain and other fungi sometimes get into the central dead cylinder.

If the cambium is killed over a large area on one side of a tree but the other side resists attack, this may lead to serious deformities like that shown in Fig. 4.

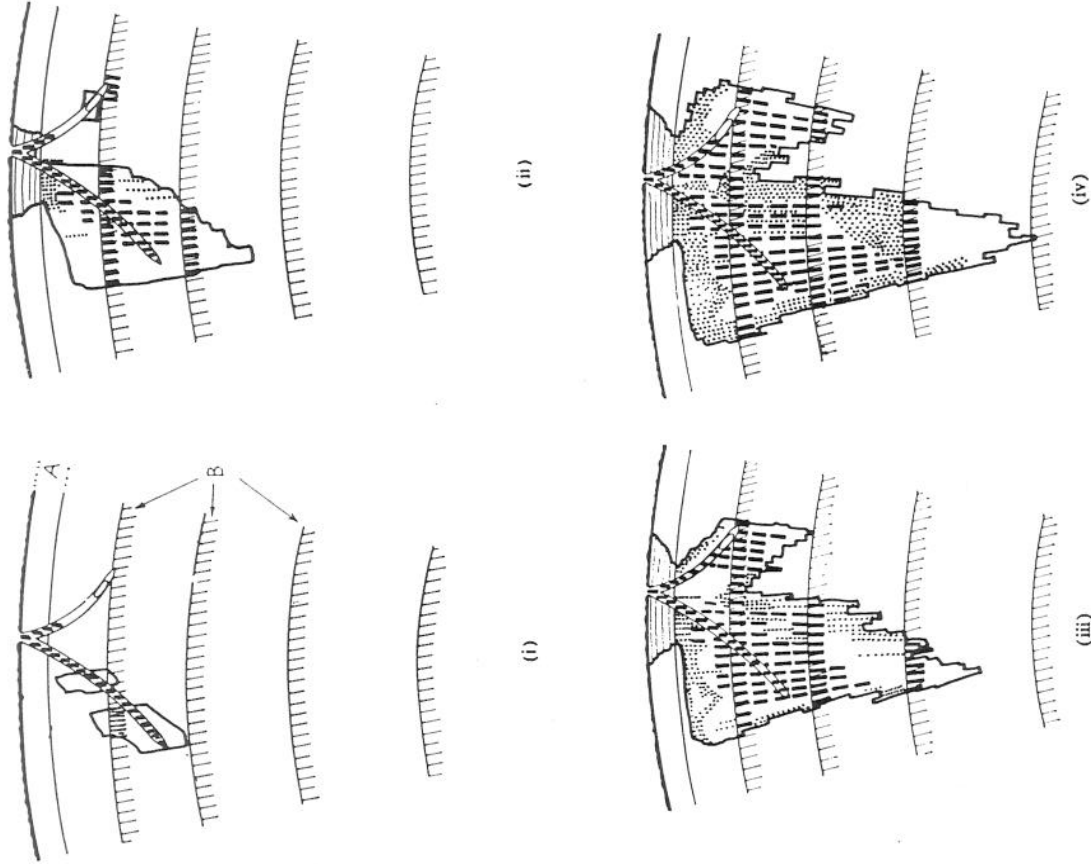


Figure 1: Four stages in resistance to *Sirex* attack, in a young tree. For explanation, see next page.

Figure 2
Diagram of a radial section of a tree which has resisted Sirex attack, about three months after oviposition.
Growth rings, resin, polyphenols, and the outline of the dry area are shown by the same notation as in Figure 1.
Necrotic bark and phloem.
Oviposition tunnel.
Scale: the real length of the oviposition tunnel as seen in this diagram was 1 cm.

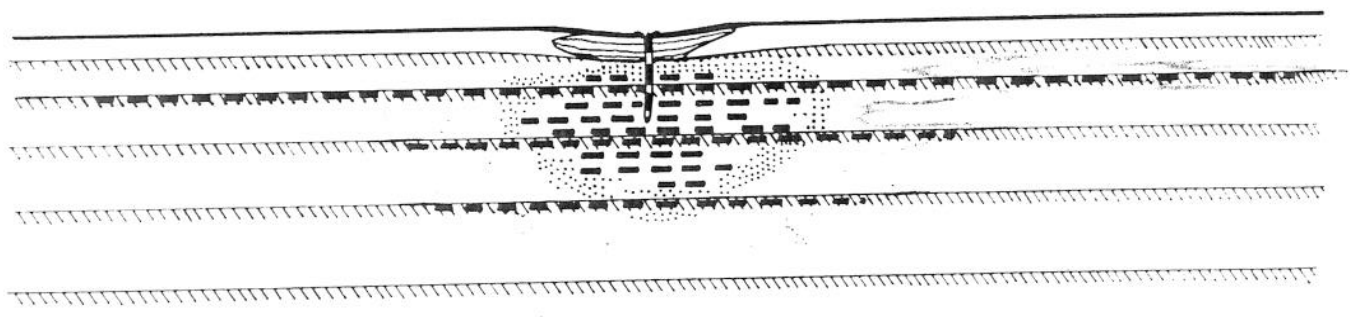




Figure 3.

Cross section of a young tree which resisted quite heavy Sirex attack. At the top a blob of resin, formed after the fungus was sealed off, has been forced out through the bark. The wood has been stained with benzidine, showing polyphenols as a dark border round the pale dried areas. The dark latewood in the dried areas is full of resin.

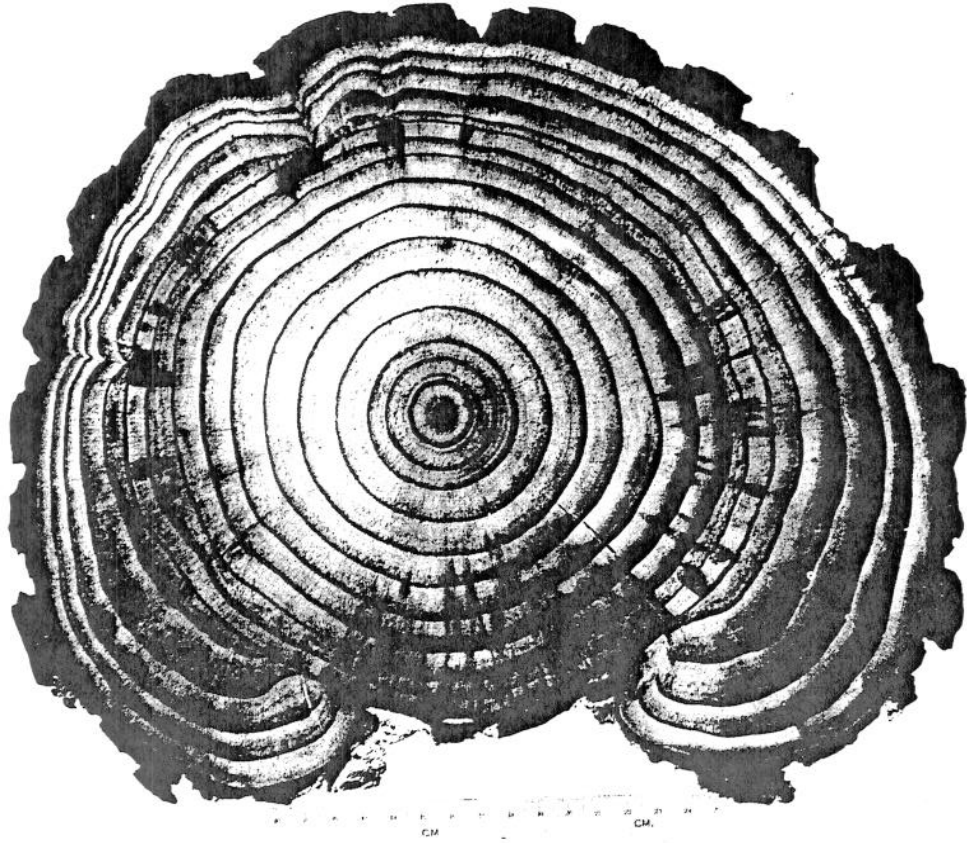


Figure 4.
Cross section of a tree deformed by Sirex attack. The cambium was killed where the attack was heaviest.

THE SECOND EXPERIMENT

Seven dominants and seven suppressed trees were given light *Sirex* attack in March, 1965. The times of felling and general results are shown in Table 2. The trees have been numbered D1 to D7 and S1 to S7, in order of felling.

TABLE 2

RESULTS OF THE SECOND EXPERIMENT, IN WHICH SEVEN DOMINANT (D) AND SEVEN SUPPRESSED (S) TREES WERE GIVEN A LIGHT *SIREX* ATTACK IN MARCH

Number of days since oviposition	Tree No.	Polyphenols produced in reaction to attack		Tree No.	Polyphenols produced in reaction to attack		Mean radial spread of drying (mm)*	Mean radial spread of drying (mm)*	Mean No. of late-wood bands penetrated by dried area
		Polyphenols produced in reaction to attack	Tree No.		Polyphenols produced in reaction to attack	Tree No.			
9	D1	nil		S1	nil		3	0.7	0.3
15	D2	plenty		S2	very little		9	1.0	1.1
18	D3	some		S3	nil		8	2.2	0.0
22	D4	some		S4	very little		14	2.3	2.0
26	D5	plenty		S5	very little		11	1.5	0.4
43	D6	plenty		S6	plenty		15	1.8	2.2
69	D7	plenty		S7	plenty		12	1.3	1.3

* "Radial spread of drying" means radial spread beyond the inner tips of the oviposition tunnels, in which eggs were laid. The number of latewood bands penetrated by the dried areas was also counted from the inner ends of the tunnels.

The dominants were more vigorous than those used for the first experiment, all being about six inches in diameter at breast height. The suppressed trees were very suppressed, and only about two and a half inches in diameter.

The processes by which the dominant trees reacted to attack confirmed the observations of the first experiment, although the sealing-off process was completed more quickly. In the dominant felled three weeks after oviposition, the mean radial spread of the dried areas beyond the inner tip of the oviposition tunnels was 14 mm and no significant increase was found in the trees felled subsequently.

Polyphenols were not found in the trees felled nine days after oviposition, but were found in the tree felled 15 days after.

In the suppressed trees the fungus was expected to grow more quickly than in the dominant, but from Table 2 it can be seen that the opposite was the case. In S3, felled 18 days after oviposition, the fungus had had no visible effect on the wood and appeared to have made no growth at all. The outer growth rings of this tree were less than one millimetre wide, and were composed almost entirely

of latewood. In S5, felled nearly four weeks after oviposition, dried areas had spread from only three of the seven oviposition sites examined. In trees S2 and S6 the dried areas had spread about half as far as in the dominants felled at the same time, but the mean number of latewood bands penetrated by the dried areas was similar for the dominant and suppressed trees. In S7 the oviposition tunnels had penetrated to comparatively wide inner rings, and the radial spread of drying was about equal to that of D7. Apart from the anomalous results from trees in which the fungus had scarcely any effect, it was concluded that trees which resist light *Sirex* attack stop the spread of fungus by the time the dried areas have spread radially inwards through one to three growth rings beyond the tip of the oviposition tunnel. Thus, although the size of a dried area depends partly on the length and angle of the oviposition tunnel, both of which are very variable, the areas will generally be smaller if the rings are narrow. In the suppressed trees, the average length of the oviposition tunnels was shorter than in the dominants (Table 3).

TABLE 3

THE MEAN LENGTHS OF OVIPOSITION TUNNELS AND THE NUMBER OF LATEWOOD BANDS THEY PENETRATED IN THE SEVEN DOMINANT AND SEVEN SUPPRESSED TREES

	Single tunnels		Double tunnels	
	Longer of pair	Shorter of pair	Longer of pair	Shorter of pair
Mean lengths of oviposition tunnels (mm) (Measured in wood only)	6.2	3.7	9.8	8.1
Mean number of latewood bands penetrated by oviposition tunnels	2.8	1.4	1.9	3.9
			1.6	3.5

(For the full lengths of the oviposition tunnels, 3 mm should be added for the bark thickness of the dominants, and 1 mm for the suppressed trees.)

The insects were graded before being put on the trees, and those on the suppressed trees were just as big as those on the dominants.

It is not known what stops the fungus after the dried area has penetrated a given number of growth rings from the inner tip of the tunnel, but it might be explained by the flooding of the late wood of the ring with resin. Because the effect of the fungus on the wood runs ahead of it (at least in trees which resist attack) the latewood can flood with resin just before the fungus gets there. Either the barrier formed is not always complete, or some fungus which gets through before it becomes complete may grow radially in a narrower spoke, into the next growth ring, where the process is repeated.

The first five suppressed trees to be examined had produced little or no resin or polyphenols (see Table 2). Tree No. S1 was, of course, examined too early, and in S3 and S5 stimulation due to the growth of the fungus was probably insufficient. Trees S2 and S4 were evidently slower to produce polyphenols than the dominants felled at the same time. The last two suppressed trees to be

examined at six and ten weeks after oviposition, had produced polyphenols in quantities similar to the dominants.

Since *Sirex* has been attacking trees in the pine regrowth at Pittwater for a number of years, very suppressed trees two and a half inches in diameter which show no signs of previous attack are not very common, so the suppressed trees used in this experiment had to be chosen fairly carefully. They may have been quite unattractive to wild *Sirex*, perhaps on account of their extreme suppression. However, when they were cut open, two of them (S4 and S5) were found to have been lightly attacked by *Sirex* in previous years. Typical lesions were found in the wood, with the usual concentrations of resin and polyphenols.

A difficulty encountered in examining samples from the suppressed trees was that there were natural dry areas in the growth rings one to two centimetres from the cambium. These probably represented the beginning of heartwood formation. The oviposition tunnels would often penetrate to within a few millimetres of this dry wood. Drying resulting from oviposition would often link up with this and become impossible to define.

Areas of natural drying were present in the dominants also, but, because of the wider growth rings, did not interfere with the study of drying resulting from *Sirex* attack. The mechanism by which water is withdrawn from the heartwood transition zone may even be comparable with that by which wood is dried following *Sirex* attack. In both instances water moves into the surrounding sapwood by some unknown mechanism.

THE THIRD EXPERIMENT

Hillis et al (1962) found that polyphenol formation in the heartwood of *Angophora costata* was related to the amount of carbohydrate available. Hillis and Carle (1960, 1962), and Hillis and Hasegawa (1963) concluded that polyphenols were not transported in the *Eucalyptus* species they studied, but were formed *in situ* from translocated carbohydrates. In our third experiment, nine-year-old dominant trees were subjected to various treatments to disclose factors which might affect polyphenol formation and resistance to *Sirex* attack in *P. radiata*. The treatments, most of which are summarized in Table 4, will be described in turn.

HEAVY AND LIGHT ATTACK

A tree given a light attack (3/1) reacted in the normal way, with plentiful production of polyphenols and resin to seal off the fungus. A tree given a heavy attack (3/2) had concentrations of polyphenols only in the outer growth ring beside the oviposition tunnels. On any cross section, drying caused by ovipositions above or below could be seen on either side of an oviposition site and in this dry wood polyphenols were not found even near the cambium. This suggested that the tree had started to form polyphenols soon after attack, but was overcome by the weight of attack, and could not continue to react. This may have been because there is a limit to the amount of polyphenols a tree can produce or because many inoculations of fungus harm the tree's general health, perhaps by affecting the foliage. The resin barriers were not complete in the latewood of the annual rings.

TABLE 4

THE THIRD EXPERIMENT: TREATMENTS AND RESULTS

Tree No.	Treatment (All trees were dominants)	Date of oviposition, Inter-val*	Tree's reaction to attack in terms of polyphenols (PP) and resin	Mean inward radial spread of fungus (mm) †
3/1	Light attack	16.3 49	PP: present (strongly) round dried areas	7 (Oviposition tunnels mostly short and single)
3/2	Heavy attack (Foliage going yellow at time of felling)	16.3 49	PP: present in outer growth ring close to oviposition tunnels. Little elsewhere	16
3/3	Heavy attack	31.3 99	In most of the tree there were no concentrations of PP's, or resin in the latewood	50 + (Fungus had grown in as far as pith)
3/4	One side debarked after light attack, both sides covered with mastic	23.3 36	PP: scarcely any on debarked side PP: present in normal amounts on opposite side	11
3/5	Treatment as for 3/4 Debarked side	23.3 96	PP: plenty round inner parts of dried areas, very little round outer (cambial) edge PP: plenty all round the dried areas	10
3/6	Opposite side Topped and debranched before light attack	30.3 37	PP: scarcely any (little resin)	10
3/7	Topped and debranched before light attack	30.3 66	PP: plenty (some resin)	9
3/8	Topped and debranched before light attack	30.3 66	PP: plenty (some resin)	10
3/9	Control tree (crown intact)	30.3 66	PP: plenty, (plenty of resin)	20
3/10	Warm on one side and cold on the other. Light attack	1.4 50	Plenty of PP. No. difference between the two sides of the tree	10 (No difference between the 2 sides)

Table 4—continued

Tree No.	Treatment (All trees were dominants)	Date of oviposition, Inter-val*	Tree's reaction to attack in terms of polyphenols (PP) and resin	Mean inward radial spread of fungus (mm) †
3/11	Wet and dry atmospheres on either side of stem. Heavy local attack	23.3 69	Some PP to the outside (i.e. cambial side). No difference between two sides of the tree	About 20
3/12	Pruned to 10 ft., ringbarked at 8 ft. on 5.10.64	15.1 19	Plenty of PP. above girdle, none below	5 above girdle 13 below girdle

* Number of days between oviposition and felling.

† From inner tip of tunnel.

In a tree given an exceptionally heavy attack (3/3) and examined three months later (when it had been dead for some time and the crown was a rusty red colour), no polyphenols were found except near the foot of the tree, where oviposition had taken place only in bark crevices. Below the thick bark, between crevices, slight concentrations were found around little islands of living cambium. This was the only part of the stem where resin had flooded the latewood of the growth rings. The fungus had spread inwards as far as the pith, and the wood felt dry except for some very small brown wet patches. Moisture content samples taken at a height of six feet gave values of 42 to 44 per cent (oven-dry weight) in the outer half-inch of sapwood and for a complete disc of wood respectively. Scarcely any resin had flowed out of the oviposition tunnels on to the bark. No larval tunnels were evident on cross-cut ends of logs from the tree, but when some oviposition tunnels were dissected under the microscope, eleven larvae were found, all apparently healthy, but still only 1.2 to 2.2 mm long.

The general inability of trees to resist heavy attack was demonstrated recently when *Sirex* attack was induced on plots of trees, two of which were kept watered, two left to natural rainfall, and two droughted by preventing rain from reaching the soil with polythene. The amount of attack given was too heavy, and nearly all the trees have died.

PARTIALLY-DEBARKED TREES

Two trees (3/4 and 3/5) were given a light attack on the lower eight feet of their stems. The following day the bark, phloem, and cambium were removed from a four foot length of one side of the stem of each. This was immediately coated with grafting mastic and the side with bark intact was also coated. Five weeks after oviposition one tree was felled. Dried areas had formed evenly on both sides, but scarcely any polyphenols were found on the debarked side, although

they were present in normal quantities on the side with the bark retained. The second tree was felled fourteen weeks after oviposition. On the debarked side polyphenols had still failed to form in the outermost layers of wood, where they are normally strongest, but were present on the radial edges of dried areas where these extended into the inner growth rings. On the opposite side they were present in normal amounts.

TREES WITH ALL BRANCHES REMOVED BEFORE ATTACK

These trees were also topped, leaving a pole with roots, the day before *Sirex* were put on them. Five weeks after attack one of them (3/6) was examined, and scarcely any sign of polyphenols was found, although resin had flooded the latewood of the annual rings. Two others (3/7 and 3/8) were examined nine to ten weeks after oviposition, together with a control tree (3/9) attacked at the same time. All had produced polyphenols in amounts characteristic of unpruned dominants. A tree must be able to mobilize food reserves for polyphenol production. Removal of the entire crown may have retarded the spread of drying in these debranched trees by reducing the water stress in the xylem.

SIREX ATTACK ON FRESH LOGS

In various experiments female *Sirex* have been induced to attack fresh logs the ends of which have been greased to prevent drying. Although the fungus grows but slowly in such logs, concentrations of polyphenols have never been found around the dried areas.

TEMPERATURE AND HUMIDITY

The effect of temperature was studied by keeping one side of a tree (3/10) warm (about 78° F.) and one side cool (50° F.) after light *Sirex* attack. Temperature differences were maintained by insulated water baths, elongated and curved down one side to fit around the stem of the tree. One was heated by a small gas flame, and the other kept cool by refrigerated blocks. Temperature control was very crude, but thermometers in the wood and bark showed that the system was producing the required differences. Seven weeks after attack the tree was felled and examined. No difference was found in polyphenol or resin concentrations on the two sides, and the fungus had spread inwards evenly.

On another tree (3/11) humidity was varied by the use of long polythene cages running up either side, one containing water, and the other dried silica gel, which was changed from time to time. *Sirex* put into the cages heavily attacked both sides of the tree. When it was felled ten weeks later, there was no apparent differential effect on resin and polyphenol production and the radial spread of drying by the treatments.

RINGBARKING AND BARK CINCTURING

The term "cincturing" is used to describe the cutting off of an area of bark from food supplies from the crown by removing a strip of bark and phloem around it. In this case a rectangular panel of bark was left on one side of the tree.

The resistance mechanism was not studied thoroughly in ring barked or cinctured trees, but examination of a small number of samples from trees treated some months before oviposition suggests that polyphenols form more quickly than usual above the girdle in the former, and on the side of a tree with the bark connected to the crown in the latter, and more slowly below a girdle or on the starved side. However, the strong preference of the insects for the starved part of the stem (Coutts and Dolezal, 1966) resulted in a very uneven attack. Until oviposition is carefully controlled above and below a girdle conclusions must be regarded as indefinite.

THE DEVELOPMENT OF POLYPHENOLS IN THE GROWTH RING

In a tree which had high and low humidities on different sides, drying had spread inwards through four growth rings. The distribution of polyphenols in the rings was determined and probably shows how they develop as the fungus spreads slowly inwards from ring to ring. In the fourth ring from the cambium, polyphenols were found in the medullary rays only; in the third ring, they were found in medullary rays and in tracheids on either side of the rays; in the second ring, polyphenols were present in radial streaks several tracheids wide; and in the outer annual ring patches of polyphenols covered wide areas of wood which stained very strongly with benzidine. There were concentrations of polyphenols in tracheids around longitudinal resin ducts in the two outer rings, but this has also been seen in the healthy sapwood.

MECHANICAL DAMAGE

Farkas and Kiraly (1962) have discussed the effect of mechanical damage on polyphenol production by plants. Effects similar to those caused by a pathogen are sometimes produced, but polyphenols appear more slowly and may differ chemically from those produced in reaction to fungal attack.

Trees in which drill holes were made for resin pressure manometers a year previously were found to have produced polyphenols in a band less than half a millimetre wide in the wood bordering the holes. Drilling was then tried as a method of testing trees for polyphenol production but no appreciable amounts of polyphenols were found after two months.

Two trees which, two years previously, had had their stems scorched by fire on one side had polyphenols (visible as a red stain before using benzidine) between living and dead wood in the outer growth ring. It seemed possible that heat might be more effective than drilling for stimulating a tree, so red hot nails were hammered into healthy trees but no polyphenols could be detected five weeks later.

DEVELOPMENT OF THE FUNGUS IN LOGS CUT FROM TREES FELLED AT VARYING TIMES AFTER OVIPOSITION

In the experiments described above only parts of the trees were cut up for examination. During the first sampling of the trees immediately after felling the spread of the fungus had been noted and measured. Additional logs from

some trees were kept long enough to see if the fungus would continue to develop, or whether it had been completely "fixed" by the trees, and also whether there was further damage to the cambium. These logs were pruned and their ends and branch stubs were greased.

Some of the sampling was done too soon; it was not certain whether the fungus had grown or not. It is necessary to keep the logs for two months or more to be sure. Some of the work was spoilt by blue stain fungi. The findings from logs which gave a clear result are summarised in Table 5.

TABLE 5
THE DEVELOPMENT OF *Sirex* FUNGUS IN LOGS CUT FROM TREES WHICH WERE
FELLED AT DIFFERENT TIMES AFTER OVIPOSITION

Tree No.	Tree type, etc.	Number of days between oviposition and felling	Number of days between felling and sampling of logs	Damage to cambium	Inward radial spread of fungus	Tangential spread of fungus
1	Dominant	3	25	—/	—/	—/
S1	Suppressed	9	128	—/	—/	—/
D3	Dominant	19	116	0	—/	(slight)
S4	Suppressed	23	134	0	—/	—/
YC1	Dominant (topped and debranched; scarcely any polyphenols formed)	37	83	—/	—/	—/
7	Dominant	41	85	0	—/	—/
S6	Suppressed	43	94	0	—/	(slight)
D6	Dominant	43	44	0	—/	(slight)
H1	Dominant (Heat and cold treatment, but this log was taken from below the treated length)	50	55	0	(slight) Fungus had "escaped" from only 2 out of 8 samples examined	(slight) 0
D7	Dominant	69	76	0	0	0

Note: Trees 1 and 7 were from the First Experiment.
Trees S1, D3, S4, S6, D6 and D7 were from the Second Experiment.
Trees YC1 and H1 were from the Third Experiment.

It will be seen that in logs cut from trees which had been left standing, and had resisted the attack for three weeks or more, the fungus had done no further

damage to the cambium or phloem whilst the logs were stored. There was one exception to this. The tree which had had all branches removed just before it was attacked, and which had produced scarcely any polyphenols, showed extensive damage to the cambium and phloem, i.e., big brown patches had developed from the oviposition sites.

In only two of the logs examined had inward and tangential spread of the fungus been completely stopped. One of these was from tree H1, which had been given the ineffectual heat and cold treatment, and which was felled seven weeks after oviposition. The stored log had been cut from below the treated length of the stem so it probably represented normal conditions. In two out of the eight samples which were incubated the fungus was found in the wood a short distance beyond the inner tips of the dried areas, but it had only grown a few millimeters so it must have had difficulty in passing the barrier laid down in the tree. There was no further damage to the cambium.

In the log kept from tree number D7 (the last vigorous dominant to be felled and sampled in the second experiment), the fungus had failed to make any further growth in any direction. It had been completely contained by the tree.

Although growth of the fungus had been checked in the tree with the heat and cold treatment, and in one of the dominant trees, it was still viable within the lesions. It grew up vigorously from the end grain of the incubated wood samples, spreading out over their cut surfaces.

Concentrations of polyphenols never form in cut logs as a result of *Sirex* attack although the parenchyma cells remain alive for months if the ends of a log are greased. One might expect sufficient food to be present for polyphenols to be formed when only a few isolated oviposition tunnels are present; some movement of the sap stream may be necessary for food to be transported to the infected zones. If *Sirex* are induced to attack a fresh log, the wood dries out as the fungus develops, and this drying appears to be due to evaporation accelerated by increased permeability of the wood and bark. But as the fungus grows inwards, evaporation has to take place through an increasing thickness of wood, and the radial spread of drying becomes extremely slow. Under these conditions the fungus spreads very slowly ahead of the drying, causing a brown discoloration of the wood. This brown-stained wood is much more permeable than normal sapwood, and when samples were dipped in benzidine it was at first thought that polyphenols were concentrated in this layer, but the colouration was merely due to increased absorption of the stain.

DISCUSSION

The mechanism of resistance to *Amylotereum* has been shown to be associated with both resin and polyphenol formation. Resin of low viscosity under high pressure would be expected to fulfil the needs of the mechanism most rapidly. Production, viscosity, and pressure of resin are all known to be under a measure of genetic control in various species of pines. Little is known of genetic variation in polyphenol production within a species. Resin production is strongly influenced by phenotypic characters, such as the size and vigour of the tree; the dependence of the tree on the availability of carbohydrates for polyphenol production may provide a further link between vigour and resistance to *Sirex* attack, although this is complicated by the selective behaviour of the insects.

In the case of both resin and polyphenols we do not know the relative importance of amounts present in the tree before it is attacked by *Sirex* and the amounts produced in reaction to attack. The first resin to appear in oviposition

tunnels and in the latewood was assumed to be oleoresin, which is present in the tree at the time of attack and bursts out of the resin ducts. The tree may depend on being able to manufacture more resin, especially to cope with a heavy attack. Our study has shown that resin appears before polyphenols in the infected areas, polyphenols being concentrated in appreciable quantities about two weeks after oviposition. However, more precise cytological methods may show that phenolic substances begin to form earlier than two weeks after attack, the technique used in these studies having been too crude to detect them. An advantage of the method used has been to allow examination of enough trees to study the mechanism in fairly broad terms and to give an idea of the variation to be expected. We do not know what phenolic compounds are involved, so identification and testing for toxic or inhibiting effects on the fungus are required.

The dependence of trees on carbohydrates for polyphenol production has been stressed by Hillis (1962). Our observations suggest that the rapid concentration of polyphenols in the outer growth ring protects the cambium, and that these polyphenols are produced from foods translocated from the crown, but the debarking and debranching experiments suggest that stored foods can also be used. The establishment of the fungus in the sapwood would block translocation of foods from the phloem to the innermost edge of the infection, so polyphenols formed at the inner edge are presumably derived from sugars in the sapstream. Available sugars may be increased by mobilization of local stores; or distant supplies may be tapped—attack evidently affects growth of the tree as a whole.

Titze (1965) has suggested that suppressed trees have a greater ratio of fats to carbohydrates in their stored foods than dominants, with the implication that suppressed trees are less able to manufacture phenolic substances.

One factor which affects the growth rate of the fungus in logs is wood moisture content (Coutts and Dolezal, 1965). The fungus grows very slowly radially in logs with moisture contents of 70 per cent. of saturation or more. Its growth rate relative to the rate of production of substances involved in the resistance mechanism may be important. However, no positive connection has been established between wood moisture content and resistance to attack in living trees.

The resistance mechanism as described in this paper takes into account what happens in the stem alone. Besides local damage to the stem, heavy *Sirex* attack often causes rapid death of the foliage, and certain factors of resistance should perhaps be sought in the crown of the tree.

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