DISCOLOURATION ASSOCIATED WITH *PLATYPUS* WOUNDS IN LIVING *NOTHOFAGUS FUSCA*

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ABSTRACT

Wounds similar to those made by **Platypus** beetles were drilled in living red beech (**Nothofagus fusca** (Hook. f.) Oerst.) trees. Sapwood discolouration associated with such wounds was found to be due to the presence of micro-organisms. Results also suggested that this discolouration is a tree response to toxic substances produced by micro-organisms.

INTRODUCTION

The Platypodidae and some Scolytidae are wood borers but not wood feeders: the adult beetles bore into wood and introduce into their tunnels "ambrosia fungi" which grow on the tunnel walls and are the chief source of food for all stages of the insect. The fungi are constantly associated with the insect and are transmitted by the adult, frequently by means of special organs. Such insects are known as ambrosia beetles (Baker, 1963).

Ambrosia beetles usually infest fresh logs or recently dead trees but some species do attack living, apparently healthy trees: Browne (1965) records *Dendroplatypus impar* (Schedl) breeding in living *Shorea* spp., *Trachyostus ghanaensis* Schedl in living *Triplochiton scleroxylon* K. Schum and *Doliopygus dubius* (Samps.) attacking living *Terminalia superba* Engl. and Diels; several North American hardwood species are attacked by *Corthylus columbianus* Hopk. which only attacks living hosts (Giese and McManus, 1965); and *Austroplatypus incompertus (Schedl)* infests living *Eucalyptus* trees in Australia (Browne, 1971). A discolouration in the sapwood is invariably associated with these attacks in living trees.

In New Zealand, living red beech (*Nothofagus fusca* (Hook. f) Oerst.) is attacked by *Platypus apicalis* White, *Platypus caviceps* Broun, and *Platypus gracilis* Broun (New Zealand Forest Service, 1966). Tunnels bored by *Platypus* adults normally extend radially to near the heartwood boundary and then branch tangentially (Milligan, 1972).

A stain (discolouration) is always associated with each *Platypus* wound. At approximately 10 mm into the sapwood, typical stains associated with *P. apicalis* wounds are about 127 mm long and 5 mm wide, tapering gradually toward both ends (New Zealand Forest Service, 1970). Both the length and width of individual stains increase radially toward the heartwood. Similar stains have also been found to be associated with drilled

holes of about the same diameter (2 mm) as *P. apicalis* tunnels (New Zealand Forest Service, 1970).

Inoculation tests were undertaken to determine whether such stains in *N. fusca* are a response to mechanical wounds only, or a response to micro-organisms which invade these wounds. The locality for these tests was Kaimanawa North State Forest.

MATERIALS AND METHODS

Experiment 1

Two species of fungi isolated from *Platypus* tunnels in red beech, a *Ceratocystis* sp. (Ascomycetes, Sphaerales) and *Endomycopsis platypodis* Baker et Kreger-van Rij (Ascomycetes, Endomycetales), were used to inoculate drilled holes in living red beech trees. The fungi were grown in mixed shake culture in six 250 ml flasks at 17°C for 6 days, on a medium consisting of 1.5 g KH_2PO_4, 1.0 g $MgSO_47H_2O$, 25 g glucose, 2 g asparagine, 2 g mycological peptone, and 2 g of yeast extract, dissolved in 1 litre of distilled water. The contents of the flasks were centrifuged, the mycelium washed twice with sterile distilled water, resuspended in sterile distilled water, and stored in sterile McCartney bottles.

During December 1969 80 groups of 3 holes were drilled in 3 trees with diameters at breast height of 325 mm, 350 mm and 400 mm. Groups were drilled randomly over the lower 3 m of stem but were spaced at least 400 mm apart vertically and 50 mm horizontally. Holes within a group were approximately 25 mm apart on the same horizontal plane. All holes were drilled radially to the heartwood using a 165 mm long, 2.5 mm diameter drill powered by a 220 volt portable generator.

After loose bark had been removed with a bark scraper attempts were made to drill the first hole in each group aseptically by swabbing the zone of the whole group with 95% alcohol, flaming the drill, and plugging the hole with sterile cotton wool immediately after drilling. The second and third holes were drilled without aseptic precautions; the second hole was inoculated and plugged and the third hole was left open. A hypodermic syringe, washed with 95% alcohol and rinsed with sterile distilled water, was used to inject about 1 ml of inoculum into the second hole.

The trees were felled in September 1970. Discs approximately 400 mm deep (200 mm each side of the groups) were cut; blocks, each including a single group, were then chopped from the discs and the sapwood split with a chisel into slabs as shown in Fig. 1.

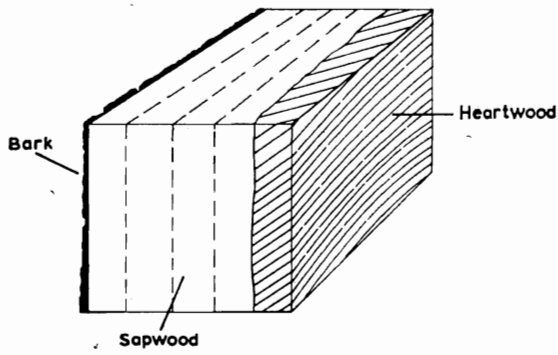

FIG. 1—Dissection of groups into slabs. The broken lines show how the sapwood was split into slabs.

These slabs usually varied from 10-15 mm in thickness and the number of slabs obtained from any group depended on the width of the sapwood band. Sapwood bands measured at 1.5 m from the ground ranged in width from 35-80 mm. Six groups were accidentally destroyed. Discolouration associated with each hole was measured at the different radial depths.

Experiment 2

For Experiment 2, in addition to the two fungal species used in Experiment 1, a non-sporulating fungus (called Fungus A) isolated from near *Platypus* tunnels in a *Platypus*-killed tree was used. The three fungi were grown separately. Drilling procedure was as for Experiment 1.

A total of 139 groups of 5 holes was drilled in 5 trees during February 1971. The diameter at breast height of the trees ranged from 340 mm to 430 mm and the width of the sapwood from 40-65 mm. To facilitate dissection, groups were drilled in bands which consisted of four groups on the same horizontal plane. The bands were spaced approximately 450 mm apart vertically and groups within a band at least 50 mm apart horizontally. Holes within a group were about 25 mm apart on the same horizontal plane. Aseptic techniques were used to drill holes 1-4; hole 1 was not inoculated, hole 2 was inoculated with *Ceratocystis* sp., hole 3 with *E. platypodis,* and hole 4 with Fungus A. These holes were plugged with sterile cotton wool. Hole 5 was drilled without aseptic precautions and left open.

The trees were felled between November 1971 and January 1972, the groups dissected, and the discolouration measured. Seven groups were accidentally destroyed. The slabs were split longitudinally through the hole and discolouration. Slivers of wood (called "isolation chips"), approximately 3 × 3 mm, were taken from the freshly exposed surface and placed onto 3% malt agar slopes. Usually three chips were taken from each discolouration on each slab; one was taken approximately 5 mm from the hole, a second from about half-way between the hole and one extremity of the discolouration, and a third from near where the discolouration ended. A total of 2,903 chips was taken from 57 groups. In both experiments the drilled zone of each tree was protected against insect interference by a sleeve of linen gauze.

The t test for paired plots was used for statistical comparisons.

RESULTS

In Experiment 1 a stain was associated with every wound except three aseptically-drilled holes in one tree. Only a very faint, light brown ring, 2 mm around the hole, was associated with these three holes (Fig. 2).

Stains associated with the aseptically-drilled holes were most frequently the shortest compared with those associated with other holes of the same group (Table 1). Also, at any depth radially toward the heartwood, the length of stains associated with the aseptically-drilled holes was significantly less ($p > 0.1$) than those associated with other holes (Table 2). There was no significant difference between the length of stains associated with the inoculated and open holes.

Individual stains increased in length and width (Table 2), and became more diffuse toward the heartwood.

Although stains were more frequently longer above than below a hole, the difference was usually less than 5 mm and was not statistically significant (Table 3).

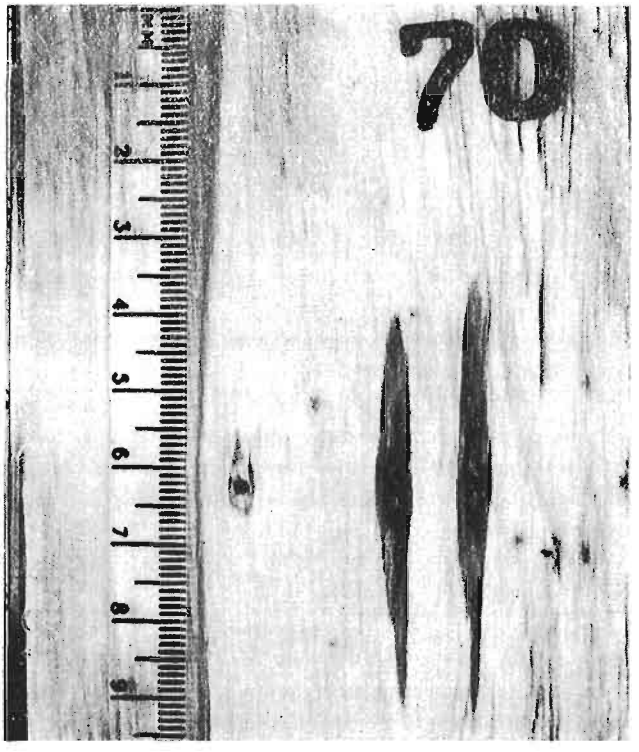

FIG. 2—A group in Experiment 1 at 18 mm into the sapwood, showing faint stain around the aseptically-drilled hole. Left to right—aseptically-drilled hole, inoculated hole, open hole.

TABLE 1—Frequency (percent) when each stain was shortest relative to others in the same group (Expt. 1)

Depth toward heartwood at which stain was measured	Treatment		
	"Sterile"	Inoculated	Open
First depth (5-15 mm)	67	10	23
Second depth (16-30 mm)	71	12	17
Third depth (31-50 mm)*	64	10	26
Fourth depth (40-70 mm)*	80	3	17

* The overlap in the range of depths was due to fewer slabs being split from some blocks with narrower sapwood bands

TABLE 2—Mean length and width of stains associated with each treatment (Expt. 1)

Depth toward heartwood at which stain was measured	Treatment					
	"Sterile"		Inoculated		Open	
	Length (mm)	Width (mm)	Length (mm)	Width (mm)	Length (mm)	Width (mm)
First depth (5-15 mm)	38	4.1	54	4.4	65	4.4
Second depth (16-30 mm)	54	4.4	73	4.7	78	4.7
Third depth (31-50 mm)	69	4.9	92	5.3	94	5.3
Fourth depth (40-70 mm)	102	6.2	124	6.5	122	6.3

At all depths the length of the "sterile treatment stain" was significantly different from others at the 0.1% level. The widths of stains at any two consecutive depths were significantly different at the 1% level.

TABLE 3—Mean length of stains above and below holes (Expt. 1)

Treatment	Depth toward heartwood at which stain was measured (see Expt. 1 method)	Mean length of stain	
		Above (mm)	Below (mm)
"Sterile"	First Depth	19	19
	Second depth	27	26
	Third depth	36	34
Inoculated	First Depth	28	27
	Second depth	38	36
	Third depth	47	45
Open	First Depth	33	32
	Second depth	39	39
	Third depth	48	48

Stains were associated with every wound in Experiment 2 with the exception of three aseptically-drilled holes in one tree and one in a second tree. Only a faint brown ring was associated with these holes (Fig. 3). Excluding *Penicillium* spp., which were probably contaminants (*see* below), micro-organisms were recovered from only one of the 24 isolation chips extracted from near two of these holes. No isolation chips were taken from near the other two holes as the cut slabs were left in the laboratory for several days and became contaminated. A third tree had very faint stains associated with two aseptically-drilled holes, and micro-organisms were recovered from three of the 26 isolation chips taken: bacteria were recovered from one of the 14 chips taken from near one hole, and yeast and bacteria from two of the 12 chips from near the other. Brown stains at least 35 mm long were associated with all other holes in all trees.

As in Experiment 1, the stains associated with aseptically-drilled holes in Experiment 2 were most frequently the shortest relative to stains with other holes of the same group, and were significantly shorter ($p > 0.001$) than those associated with inoculated holes.

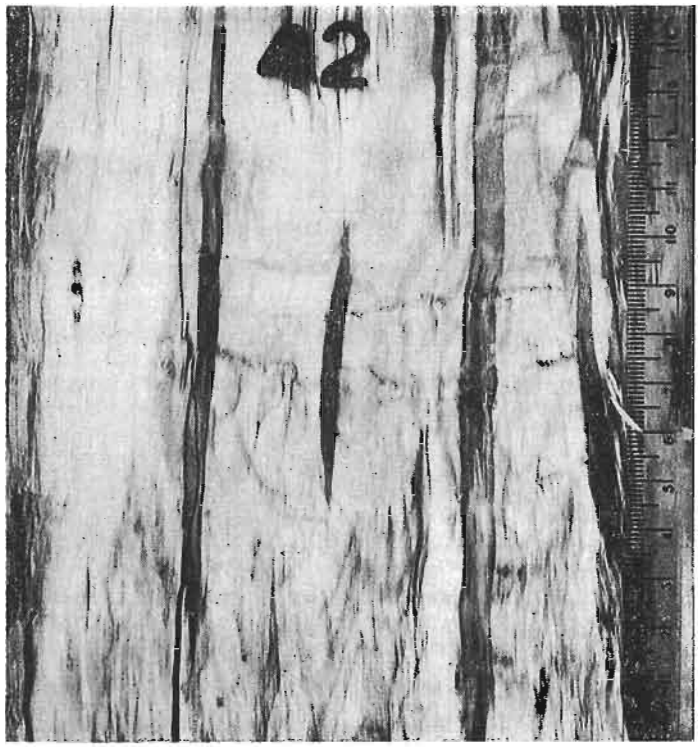

FIG. 3—A group in Experiment 2 at 10 mm into the sapwood, showing faint stain around the aseptically-drilled hole. Left to right—aseptically-drilled hole, **Ceratocystis**-inoculated hole, **E. platypodis**-inoculated hole, Fungus A-inoculated hole, open hole. Note that the stain below the open hole is partly obscured by shadow and the stain below the hole inoculated with Fungus A runs off the slab.

The mean lengths of the stains associated with each treatment are shown in Table 4.

Comparison between stain lengths of the three inoculated and open holes showed that:

1. Stains with holes inoculated with *E. platypodis* were not significantly longer than those with open holes, but were shorter ($p > 0.001$) than those associated with other inoculated holes.

TABLE 4—Mean length of stains associated with each treatment (Expt. 2)

Depth toward heartwood at which stain was measured	Treatment				
	"Sterile" (mm)	**Ceratocystis** (mm)	**E. platypodis** (mm)	Fungus A (mm)	Open (mm)
First depth (9-15 mm)	67	192	86	264	78
Second depth (20-30mm)	81	—	93	—	87

Stains with holes inoculated with **Ceratocystis** and Fungus A were longer than the dissection blocks at the second depth and could not be measured.

2. Stains with holes inoculated with *Ceratocystis* sp. at a depth of 9-15 mm into the sapwood were longer than those with other treatments ($p > 0.001$), except the stains with wounds inoculated with Fungus A.
3. Stains with wounds inoculated with Fungus A at a depth of 9-15 mm into the sapwood were longer than those with any other treatment ($p > 0.001$).

In many groups, stains associated with wounds inoculated with both *Ceratocystis* sp. and Fungus A were longer than the sawn blocks and could not be compared statistically.

Staining occurred well beyond the tissues from which micro-organisms were recovered. The recovery of micro-organisms in relation to the distance from the wound at which isolation chips were taken is shown in Table 5.

TABLE 5—Recovery of micro-organisms in relation to the distance from the wound at which isolation chips were taken.

Distance from hole (see Expt. 2 method)	Number of isolations	
	Positive	Negative
First distance	365	32
Second distance	173	224
Third distance	35	362

N.B.—Only isolations taken within 2 days of dissection are included.

The three fungi used as inocula were recovered from stains associated with all treatments (Table 6). *Ceratocystis* sp. and Fungus A were most frequently isolated from stains with holes into which they had been inoculated, and "yeasts" most frequently from stains with holes inoculated with *E. platypodis*. There were probably several species included in yeasts and no attempt was made to separate them; however, as yeasts were recovered from more than twice as many stains with *E. platypodis*-inoculated holes than from stains with any other treatment, most were probably *E. platypodis*.

TABLE 6—Frequency (percent) of recovery of micro-organisms from isolation chips (Expt. 2)

Treatment	Total no. of isolation chips taken	**Ceratocystis**	Yeasts	Fungus A	**Penicillium**	Bacteria	Isolation chips with no micro-organism
"Sterile"	572	11.2	5.4	1.4	12.9	15.9	41.1
Ceratocystis	558	26.3	4.3	2.1	15.9	10.2	37.1
E. platypodis	558	7.9	21.5	7.0	13.8	10.4	35.5
Fungus A	564	3.2	3.0	52.5	14.9	9.0	21.8
Open	542	21.8	3.9	7.6	17.2	8.7	35.2

Micro-organisms were sometimes recovered as a mixed culture.

Fungus A appears to be capable of, and well adapted to, invading live tree tissue. It was recovered more frequently from its associated stains than other inocula were from their associated stains (Table 7) and was isolated at a maximum of 120 mm from wounds, compared with a maximum of 75 mm for *Ceratocystis* sp. and 70 mm for yeasts.

Also, Fungus A was recovered further than 60 mm from wounds in 25 groups, while *Ceratocystis* sp. was recovered further than 60 mm in three groups, and yeasts in one group only. In many groups, the stain with the hole inoculated with Fungus A had coalesced with either or both adjacent stains, i.e., stains with the *E. platypodis*-inoculated and the open holes. Fungus A was recovered more frequently from these adjacent stains than from stains further away (Table 7).

TABLE 7—Frequency (percent) of recovery of micro-organism from stains (Expt. 2)

Treatment	Total no. of stains isolations taken from	Micro-organism recovered				
		Ceratocystis	Yeasts	Fungus A	**Penicillium**	Bacteria
"Sterile"	57	32	35	5	40	67
Ceratocystis	57	68	26	11	46	44
E. platypodis	57	26	84	28	40	61
Fungus A	57	18	25	97	42	42
Open	55*	49	25	27	53	49

* Two stains were destroyed during dissection.

Most *Penicillium* spp. had obviously contaminated the wood between the time the groups were dissected and the time the isolation chips were taken. Of the 324 times *Penicillium* spp. were isolated from stains with plugged holes, 94% were from 22 groups dissected and left 3 or more days before isolation chips were taken. The remaining 6% were from 35 groups from which isolation chips were taken within two days of dissection.

Bacteria were recovered from stains with all treatments. Other micro-organisms recovered were *Nodulosporium* sp., fungi provisionally determined as *Ustulina* sp. and *Aposphaeria* sp., and several unidentified fungi.

DISCUSSION AND CONCLUSIONS

The role of micro-organisms in hardwood staining is controversial. Some investigators do not attribute discolouration in hardwoods to micro-organisms (Lorenz, 1944; Zycha, 1948; Giese and McManus, 1965; Shigo, 1967); others do (Good *et al.*, 1955). The absence of associated stains with seven aseptically-drilled holes, compared with other treatments where stains were associated with all holes, demonstrates that stains associated with drilled *Platypus*-like wounds were due to the presence of micro-organisms. This conclusion is reinforced by the length of stain varying with the micro-organism; if stains were a response solely to a mechanical injury, stains with all treatments would have been similar. Also, because the isolation results showed that staining occurred well beyond the infected tissues, the stain effect is presumably a tree response to toxic substances produced by the micro-organism. The toxic substances permeate beyond the infected tissue equally both above and below the tunnel. Although this evidence needs to be substantiated by examination of stained wood for fungal hyphae, similar conclusions were reached by Good *et al.* (1955) with *Acer* spp. Giese and McManus (1965) drilled and inoculated holes in silver maple to simulate *Corthylus columbianus* Hopk. wounds but found no difference between the stains associated with the inoculated wounds and

those with the aseptically-drilled holes. The difference between their results and those in this study might be attributed to differences in the trees studied, or possibly to contamination of their aseptically-drilled holes. The difficulty of drilling holes aseptically is shown by the results of this study. Furthermore, the streptomycin sulphate they filled the holes with to preclude naturally occurring microflora in the wood might have produced a stain.

Shigo (1965) stated that following injury in many plants the cellular phenols are oxidised and subsequently polymerised to polyphenols. He believed the function of this response in some trees is to wall off the affected area. Both Zycha (1948) and Jorgensen (1962) thought some of these materials cause a protective effect. According to Zaprometov (1970) phenolic compounds and the products of their oxidation exert a fungicidal or fungistatic effect and the majority of phytoalexins are phenolic compounds. In the present study, although the length of stains increased toward the heartwood their colour became less intense. Shigo (1966) also found that discolouration columns usually advance fastest near the pith and suggested that aeration may be a factor affecting the process. Possibly the increase in length and decrease in intensity of stains toward the heartwood can be explained by the outline of the process of stain formation given by Milligan (1972). He described the process as having two phases, firstly, formation of a stain by the production of gums intracellularly in the outer sapwood and secondly, the inclusion of this stain in ray-like extensions of the transition zone. Irrespective of whether the stains in these investigations were formed as Milligan described or whether the gradient of intensity was, in fact, a gradient of gum concentration, the observations of both Milligan and myself suggest that gum appears more concentrated in the outer sapwood. If these gums are protective in nature it seems the outer sapwood may have greater protection against invasion by micro-organisms than the inner sapwood. The formation of gum barriers, a high moisture content, and the secretion of coloured substances which are possibly fungicidal are listed by Milligan as factors making invasion of beech sapwood from the outside by pathogens an improbable event.

If stained areas do function as protective barriers how long do such barriers persist, and is the barrier effect peculiar to functional tissues and effective only against certain micro-organisms? Discolourations resulting from injuries are widely considered to be pathological heartwood (Shigo, 1967). Although Jorgensen (1962) stated that position in the tree is the only difference between heartwood and pathological wood, Shigo found that certain organisms can, and do, infect pathological heartwood. Milligan (1972) pointed out that pathological wood in red beech appeared to lack the extractives which make heartwood resistant to rot fungi. He found that in freshly cut samples sapstain fungi invade this pathological wood in preference to other woody tissue, and heartrot fungi preferentially destroy it in living trees. I have observed decay of stained wood in living trees where no heartwood decay was seen. Also, in this study, several days after discs were cut the stained areas became superficially covered with fungal hyphae. In contrast, no hyphae were evident on the heartwood and unstained sapwood. *Penicillium* spp. were regularly isolated from stains in slabs left for more than three days before isolation chips were taken.

Water conduction in trees is upward. Because stains were about the same length below wounds as above, the upward movement of water does not seem to have influenced

the vertical extent of stain formation above the wound differently to that below the wound. However, this does not mean that staining will not affect water conduction. I observed that discs dried out on the cut surface of the stained sapwood and heartwood areas one to two days after being cut, while unstained sapwood remained wet for much longer. Heartwood drying was superficial and was apparently due to water being unable to move out of the tissues to the cut surface. Unstained sapwood obviously remained wet on the cut surface because water could move out of the tissues. Because the moisture contents of both stained and unstained wood from freshly cut discs were approximately the same, it appears that stained sapwood resembled heartwood in that water movement to the cut surface was impeded and the rapid surface drying was superficial.

The frequency with which *Ceratocystis* sp. was isolated from stains associated with open holes was expected. A *Ceratocystis* sp. has been isolated from *Platypus* tunnels and *Ceratocystis piceae* (Münch) Bakshi was recorded by Butcher (1968) as the major cause of sapstain in red beech.

Bacteria were isolated more often from stains associated with aseptically-drilled holes than from other stains; the opposite applied to fungi. This suggests that fungal competition inhibited bacterial growth, either in the wood or in culture, as the inoculated wounds and the aseptically-drilled holes should have been equally susceptible to bacterial infection, and the open wounds more susceptible.

Whenever *Platypus* attack occurs on living red beech there is interaction between at least three living organisms—the tree, the beetle, and one or more species of micro-organism. This study has shown that materials are produced by the tree in response to the presence of micro-organisms. Many questions, however, remain unanswered, among which the chemistry of the response materials, their specificity to and effectiveness against a particular micro-organism, and their effect on the tree itself must rank high. Also, the phenology of stain formation needs investigating because it affects the interpretation of the results obtained in these studies.

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