

## A PATHOGENIC FUNGUS ASSOCIATED WITH *PLATYPUS* ATTACK ON NEW ZEALAND *NOTHOFAGUS* SPECIES

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### ABSTRACT

The hypothesis that *Platypus*-associated mortality of *Nothofagus* spp. is a direct result of the invasion of the sapwood by a fungal pathogen was tested by drilling holes in living red beech (*Nothofagus fusca* (Hook.f.) Oerst.) trees to simulate *Platypus* tunnels, and inoculating these with the suspected pathogen — a *Sporothrix* species. Other trees were inoculated with sterile distilled water and with a *Platypus* ambrosia fungus, *Endemycopsis platypodis* Baker et Kreger-van Rij. All the *Sporothrix*-inoculated trees and one *E. platypodis*-inoculated tree wilted and died. No trees inoculated with sterile water died. *Sporothrix* sp. was recovered from well above the inoculated zone in all the dead trees, including the *E. platypodis* inoculated tree.

### INTRODUCTION

Mortality in New Zealand beech (*Nothofagus* spp.) was initially suspected to be caused by the beech buprestid *Nascioides enysi* Sharp (Cockayne, 1926; Morgan, 1966). However Dugdale (1965) observed that pinhole borers (species of *Platypus* Herbst) attacked weakened beech 1-2 years before *N. enysi* and suggested that a pathogenic fungus might be introduced by *Platypus*. More recently Milligan (1972) ruled out *N. enysi* as an agency in beech mortality and showed that *Platypus* attack can kill healthy *Nothofagus fusca*. On the basis of his experiments and observations, Milligan (1972; 1974) suggested that a pathogenic sapstain fungus is transmitted by *Platypus* beetles and becomes established initially in the innermost sapwood where the moisture content of the wood is lowest; he stated "*Nothofagus* mortality previously attributed to *N. enysi* is now more convincingly interpreted as a consequence of *Platypus* attack" (Milligan, 1974 p. 35).

The first step in testing these hypotheses and observations was to isolate suspected pathogens. Isolations from adults and tunnels of *Platypus apicalis* White, and from around fresh holes in living trees, included yeasts, a *Ceratocystis* sp., and other fungi (W. Faulds, unpubl.). Tests of the pathogenicity of *Ceratocystis* sp., yeast, *Trichoderma* sp., and a species of bacteria failed to show any such pathogenicity.

Inoculation tests (Faulds, 1973) to determine whether stains associated with *Platypus* attack in *N. fusca* were a response by the tree to mechanical wounds only or a response

to micro-organisms which invade these wounds clearly showed the stains were due to the presence of micro-organisms and were probably a response to toxic substances produced by them. One of the fungi used in these tests was that most frequently isolated from near *Platypus* tunnels in one of Milligan's experimental trees killed by induced *Platypus*-attack. Trees showed greater response around wounds inoculated with this fungus than with any other treatment and the fungus was recovered further from inoculation wounds than other micro-organisms. It appeared to be capable of, and well adapted to, invading live tree tissue. The conidial stage of the fungus has been identified as a *Sporothrix* sp. (P. Gadgil, pers. comm.). This fungus became the primary pathogen suspect and inoculation tests were undertaken to determine its pathogenicity. The locality for these tests was Kaimanawa State Forest Park (N.Z.M.S. 1. Rangitaiki, N103. 700907 (Department of Lands and Survey, 1969)).

### EXPERIMENT 1

#### *Materials and Methods*

Seven apparently healthy *N. fusca*, with no visible signs of *Platypus* attack, were selected for this experiment. These trees were marked A, B . . . G and their diameters were recorded (Table 1). During March and April 1972, holes drilled in A, B, E, F, and G were inoculated with *Sporothrix*, and in C and D with sterile distilled water. The inoculum was prepared and stored as described in an earlier paper (Faulds, 1973).

The experimental treatment for each tree was as follows. A work platform was erected around the tree (Fig. 1) then a band approximately 30 cm wide completely encircling the tree was marked at a height of 1-2 m from the base of the stem (*Platypus*

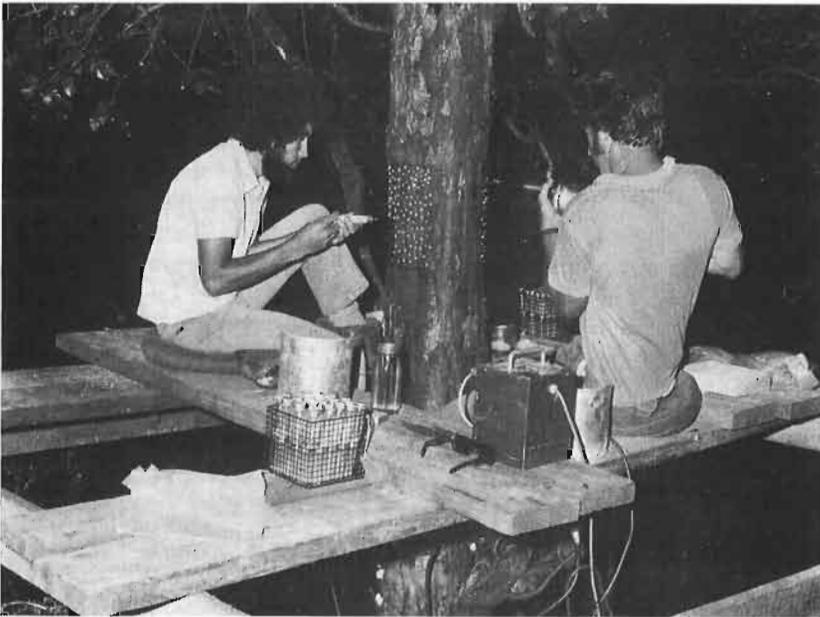


FIG. 1—Drilling and inoculation operation on work platform.

attacks are concentrated on the lower 6 m of stem; Milligan, 1974). Loose bark was removed from this marked area with a bark scraper. A small area in which two or three holes were to be drilled was swabbed with 95% alcohol. The drill was cleaned with a wire bush, dipped into a jar of 95% alcohol and flamed, and a 2.5-mm hole was drilled until a change in the noise of the drill indicated the heartwood had been reached. A hypodermic syringe, washed with alcohol and rinsed with sterile distilled water, was used to inject about 1 ml of inoculum into the hole. Finally the hole was plugged with a sterile cotton wool bung. After 2-3 holes had been drilled in the swabbed area, a fresh area was swabbed and the process repeated. Holes were drilled randomly within the marked area with a drill powered by a portable generator. Holes of this diameter and depth are similar to those made by *Platypus* beetles (Faulds, 1973). The total number of holes drilled and inoculated in each tree, and the number of holes per 100 cm<sup>2</sup> in the treated area are shown in Table 1.

TABLE 1—Diameter of treated trees, and number of holes drilled

Tree Treatment	Diameter of tree at drilled zone (cm)	Width of treated band (cm)	Total holes drilled	Holes/100 cm <sup>2</sup> of treated band (average)	Time from treatment to complete wilt (months)	Condition of tree at felling
<b>EXPERIMENT 1</b>						
A <b>Sporothrix</b>	31	34	617	18	9	Completely wilted
B <b>Sporothrix</b>	43	34	974	21	9	Completely wilted
C Sterile water	43	36	1069	22	—	Healthy
D Sterile water	40.5	32.5	1226	30	—	Healthy
E <b>Sporothrix</b>	38	32.5	366	9	40	Completely wilted
F <b>Sporothrix</b>	32.5	23	310	13	36	Completely wilted
G <b>Sporothrix</b>	47	28	440	11	13	Completely wilted except for some epicormics
<b>EXPERIMENT 2</b>						
1 Sterile water	41.5	30	664	17	—	Healthy
2 Sterile water	33.5	30	525	17	—	Healthy
3 Sterile water	38.5	30	606	17	—	Healthy
4 Sterile water	35	30	561	17	—	Healthy
5 Sterile water	33	30	523	17	—	Healthy
6 <b>Sporothrix</b>	30.5	30	485	17	4	Completely wilted
7 <b>Sporothrix</b>	35.5	30	588	17	4	Completely wilted
8 <b>Sporothrix</b>	43	30	688	17	9	Completely wilted
9 <b>Sporothrix</b>	31	30	495	17	3	Completely wilted
10 <b>Sporothrix</b>	44.5	30	708	17	2	Completely wilted
11 <b>E. platypodis</b>	27.5	30	438	17	22	Completely wilted
12 <b>E. platypodis</b>	32	30	510	17	—	Healthy
13 <b>E. platypodis</b>	39	30	627	17	—	Healthy
14 <b>E. platypodis</b>	34	30	549	17	—	Healthy
15 <b>E. platypodis</b>	44.5	30	709	17	—	Healthy

The trees were felled between October 1972 and September 1975 (Appendix 1), either after death or when it was considered they would remain alive indefinitely. Micro-organisms were isolated from the stems by cutting discs approximately 15 cm thick from the freshly felled trees from above, below, and within the treated area, e.g., for tree A, 11 discs were taken at distances ranging from 1.2 to 17 m from the base of the stem. Slabs were chopped from the discs and split with secateurs; slivers of wood (called "isolation chips") approximately  $3 \times 3$  mm were then taken from the freshly exposed surface, placed on 3% malt agar slopes, and incubated at 20°C. As the purpose of these isolations was to recover *Sporothrix*, they were not always taken at random, but often from stained areas which, in proportion to clear wood, made up a small part of the total wood in the disc. Altogether 1396 isolations were taken from 59 discs.

### Results

The time from treatment to complete wilt is shown in Table 1. For trees A, B, E, F, and G the time from the first sign of wilt to complete wilt was less than 3 months. Tree G had some epicormics in the lower crown which were still green when it was felled, but all these brown and wilted trees were almost certainly dead (see Experiment 2 — *Results*, trees 6-8). Trees C and D (inoculated with sterile water) had new foliage flushes for three successive seasons after treatment and their foliage was still green and healthy when they were felled. Notable changes in the condition of each tree, as observed on the monthly inspections, are shown in Appendix 1.

*Sporothrix* was recovered from the treated zone to 1.5 m above in tree A, 3.8 m in B, 1.4 m in E, 2.4 m in F, and 5.5 m in G. It was recovered only once from tree C (from the drilled zone) and not at all from D. Most of the isolation chips from which micro-organisms grew were taken from stained or discoloured wood. Clean wood usually proved sterile.

Figure 2 (a, b, c) shows a sample of discs from tree B demonstrating stained wood in discs from which *Sporothrix* was recovered. It was not recovered from the clean disc (Fig. 2d). Stains associated with the fungus-inoculated holes (Fig. 3a) were similar to those recorded by Faulds (1973). Stained wood was uncommon above the treated zone in trees C and D (Fig. 3b).

No new wood was formed after treatment in trees A, B, and G (Fig. 2a). Although all the other trees formed new wood, more wood was formed in trees treated with sterile water (Fig. 3c) than in the fungus-inoculated trees (Fig. 3d).

## EXPERIMENT 2

### *Materials and Methods*

Fifteen trees, similar in diameter to those used in Experiment 1, were selected for this experiment (Table 1). During January and February 1974 five trees (No. 1-5) were inoculated with sterile distilled water, five (6-10) with *Sporothrix*, and five (11-15) with *Endomycopsis platypodis* Baker et Kreger-van Rij (Ascomycetes, Endomycetales), the suspected main *Platypus* ambrosia fungus. Seventeen holes per 100 cm<sup>2</sup> were drilled in a band 30 cm wide at a height of approximately 1.5 m from the base of the stem of each tree. The total number of holes per tree is shown in Table 1. Drilling and inoculation techniques were the same as in Experiment 1. These trees were felled between

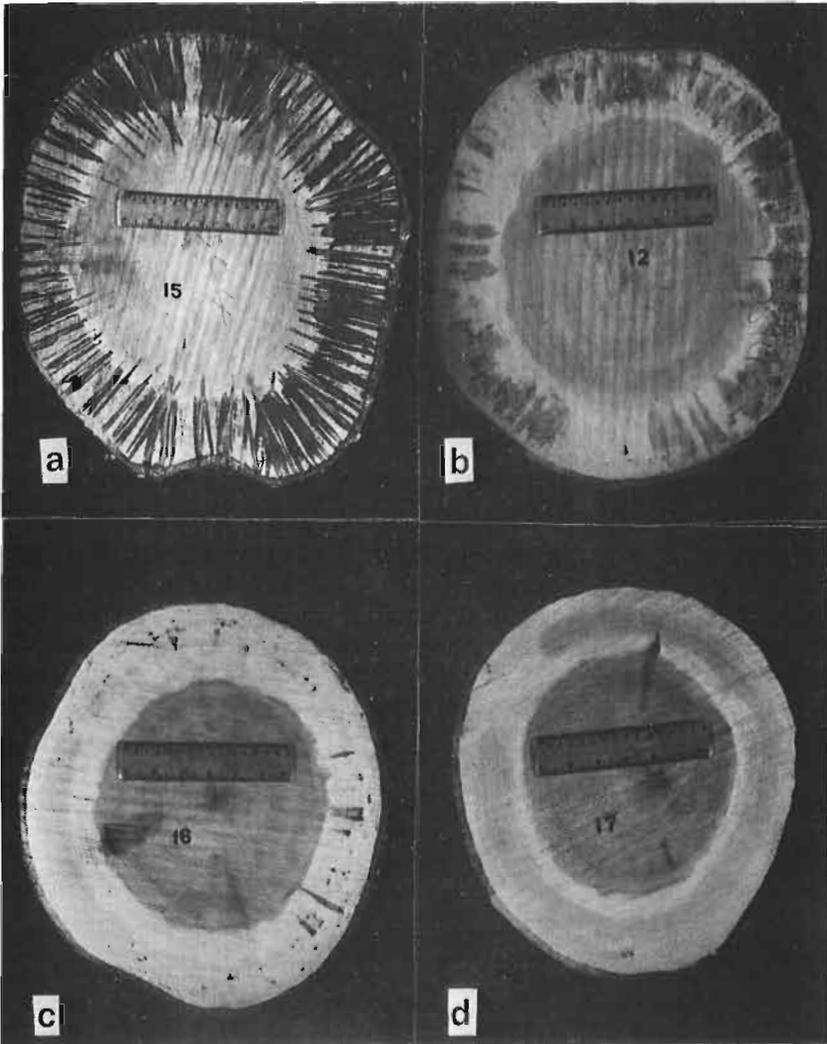
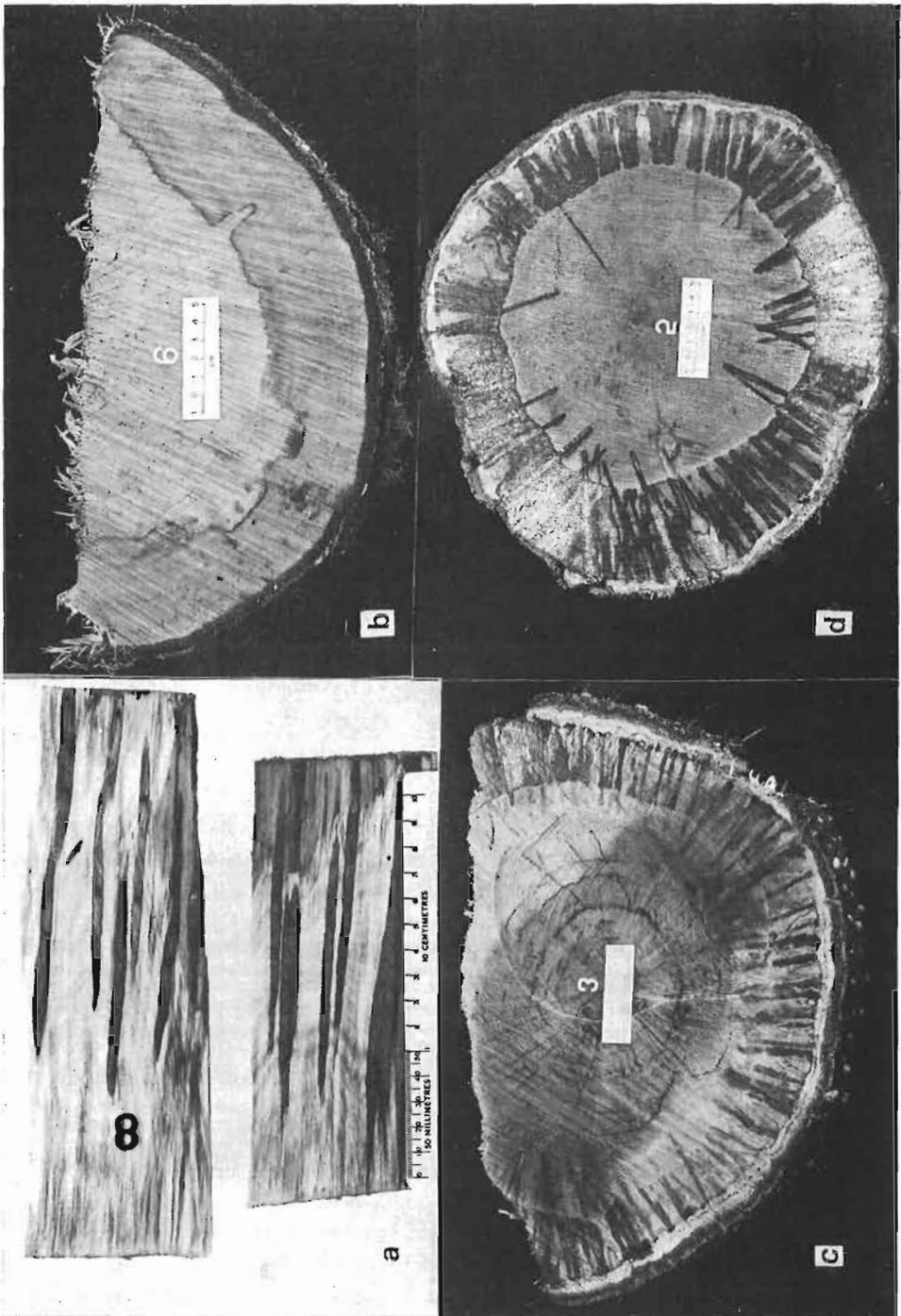


FIG. 2—Discs from *Sporothrix*-inoculated tree (a) from treated zone, (b) from 1.4 m above treated zone, (c) from 3.7 m above treated zone, (d) from 6.8 m above treated zone. *Sporothrix* was recovered from stained sapwood in (a), (b), and (c), but not from clean disc (d). Note that in (a) there was no band of new sapwood produced subsequent to inoculation

- FIG. 3 (opposite)—(a) Longitudinal tangential section from sapwood of treated zone of tree B showing stains associated with *Sporothrix*-inoculated holes.
- (b) Disc from 1.7 m above treated zone of tree D inoculated with sterile water, showing clean sapwood compared with stained sapwood in Fig. 2 b, c. The finger-like intrusion of heartwood is a typical sign of earlier *Platypus* attack, i.e., attack during the year corresponding with the annual ring at the apex of the intrusion.
- (c) Disc from treated zone of tree D inoculated with sterile water. Note the wide band of sapwood formed after inoculation compared with the new sapwood formed in the *Sporothrix*-inoculated tree in (d).



(d) Disc from treated zone of *Sporothrix*-inoculated tree F, showing the small amount of new sapwood formed since inoculation. The light areas of sapwood with no new sapwood around their outer edge were dead.

March 1974 and May 1976, and micro-organisms were isolated from them by the methods used in Experiment 1. In all, 1983 isolations were made from 111 discs. In both experiments a sleeve of linen gauze was used to protect the drilled zone of each tree against insect attack, and the basal 5 m of the stem were sprayed with 0.25% dieldrin on the dates shown in Appendices 1 and 2. Also, the condition of the crowns of the trees and any other relevant observations were recorded at approximately monthly intervals.

### Results

The time from treatment to complete wilt is shown in Table 1, and notable changes in the condition of each tree in Appendix 2. Most trees inoculated with *Sporothrix* were completely wilted within 4 months. They were felled only when it was certain they would not flush and recover in the following spring. Trees 1-5 and 12-15 had new foliage flushes for two successive seasons after treatment and their foliage was green and healthy when they were felled.

*Sporothrix* was recovered from the treated zone to 1.2 m above in tree 6, 2.2 m in tree 7, 2.4 m in tree 8, 1.5 m in tree 9, and 3.5 m in tree 10. It was also recovered six, four, and four times from the treated zone only in trees 12, 13, and 14 respectively. In tree 11 it was recovered from up to 1.2 m above the treated zone, but it was not recovered from trees 1-5 or tree 15. The recovery of micro-organisms in relation to wood-staining was the same as for Experiment 1.

After treatment no new wood was formed on trees which wilted and died, but new wood was formed on trees which remained alive.

## DISCUSSION AND CONCLUSIONS

Most ambrosia beetles rear brood only in recently dead woody plants and will not attack living trees. Those which do attack apparently healthy trees include *Dendroplatypus impar* Schedl which attacks the red meranti group of *Shorea* in Malaysia, *Trachyostus ghanaensis* Schedl which attacks *Triplochiton scleroxylon* K. Schum, and *Doliopygus dubius* Samps. on *Terminalia superba* Engl. et Diels (Browne, 1965); *Xyleborus fornicatus fornicator* Eggers which attacks tea bushes in Ceylon (Browne, 1961); *Platypus mutatus* Chapuis which attacks a wide range of hosts (poplar, willow, she-oak, eucalypt, plane, *Ailanthus*, apple, and pear) in Argentina and Brazil (Santoro, 1957; 1962a; b; 1963); *Corthylus columbianus* Hopk. which attacks several North American hardwood species (Giese and McManus, 1965); *Austroplatypus incompertus* Schedl infesting eucalypts in Australia (Browne, 1971); and *Xyleborus truncatus* Erichson which attacks eucalypts (chiefly the branches) in Australia (Moore, 1959; 1962). *Dendroplatypus impar* apparently does no harm to susceptible *Shorea* trees; *T. ghanaensis* attack does not prevent continued growth; *X. fornicatus fornicator* has not been shown to be the vector of any fungal disease to which the tea plant is subject; *P. mutatus* tunnels weaken trees so that they snap off in high winds, but no vector relationship with tree pathogens has been demonstrated. Only *X. truncatus* is known as a disease vector, being associated with a fungus which Stahl (pers. comm. to R. H. Milligan, 1964) has identified as a species of *Ceratocystis* and considers identical with the ambrosia fungus on which broods are reared. Moore (1962) states that the fungus causes brown stains in the sapwood around

*X. truncatus* entries and that attacked trees exhibit terminal dieback and produce epicormic shoots; he also found that, at least in the coastal regions and the highlands of New South Wales, *Eucalyptus saligna* Smith deaths have been widespread.

All three New Zealand *Platypus* species (*P. apicalis*, *Platypus caviceps* Broun, and *Platypus gracilis* Broun) attack living and apparently healthy *Nothofagus* trees (Milligan, 1974).

The results of these experiments clearly show that *Sporothrix* is a pathogen of *N. fusca*. Even in tree 11 which died after inoculation with *E. platypodis*, *Sporothrix* was recovered from within and well above the treated zone and probably played a role in its death.

Initial establishment of the pathogen in the host tissues probably depends on its introduction into the inner sapwood (Milligan, 1972) and *Platypus* spp. are the only insects boring in larger-diameter living beech trees which make tunnels in this zone. Whether under natural conditions *Platypus* is a vector of the pathogen or whether the pathogen incidentally invades *Platypus* wounds is not known. However, as the fungus was recovered from trees into which it had not been inoculated it can obviously invade *Platypus*-like wounds in the absence of beetles. Also, it is reasonable to assume that *Platypus* adults emerging from infected material would be carrying fragments or spores of the fungus.

Since its original isolation *Sporothrix* has been isolated from other wilting *Platypus*-attacked beech, including *Nothofagus solandri* var. *cliffortioides* (Hook. f.) Poole from Mount Ruapehu and *Nothofagus truncata* (Col.) Ckn. from the Clevedon area (approximately 30 km south-east of Auckland). Milligan (1974) reported that *Platypus* and the associated pathogen were involved in the deaths of mature trees of the other New Zealand *Nothofagus* species, and that although susceptibility to this sort of mortality is not necessarily equal in the various species, none is immune. Nowhere else in the world have species of Platypodidae been implicated as vectors of, or associated with, tree-killing pathogens.

The density of inoculations used in these experiments and the diameter class of experimental trees was based on Milligan's observations of tree mortality after induced *Platypus* attack. In his experiment up to 13 attacks per 100 cm<sup>2</sup> were counted in the most severely attacked parts of the trees which died; but trees smaller than 30 cm diameter did not die even though subject to comparable attack densities. Although some of the drilled trees received a maximum density of drilled holes slightly greater than this, the following differences probably more than offset this. Firstly, many of the real *Platypus* attacks succeeded to the stage where tangential arms off the radial tunnel were constructed (therefore exposing a far greater area of sapwood to infection); secondly, the area of maximum density of attack was larger; thirdly, there were many *Platypus* attacks above and below the area of maximum density of attack.

It is known that many trees survive successive annual *Platypus* attacks (Kershaw, 1969; Litchwark, in prep.) and that much of the stem defect in *Nothofagus* forests arises directly from sublethal *Platypus* attack (Milligan, 1974). In contrast Milligan (1974) found that trees only lightly and abortively attacked succumbed to the fungal pathogen when a drought occurred in the following summer, even without a second attack in the

drought year. Climatic and other conditions affecting tree health obviously play an important role in the successful establishment of the pathogen in the tree. The central plateau of the North Island, including Kaimanawa State Forest Park, suffered from three successive summer droughts in 1971-72, 1972-73, and 1973-74 (New Zealand Meteorological Service, 1972; 1973, 1974).

How did these droughts affect the experiments? One effect might have been to change a possible sublethal inoculation into a lethal inoculation. For example, in comparison with trees A and B which wilted and died rapidly, trees E and F, both of which had approximately half the number of inoculations that A and B had, took a long time to die. These slow-dying trees also produced new wood after treatment. Would they have received only a sublethal inoculation but for the droughts? The fact that trees C and D treated with sterile water survived the drought, in spite of having more holes and a greater density of holes drilled in them than any of the trees inoculated with *Sporothrix*, implies that the drill wounding was not a significant factor in mortality.

In Experiment 2 the *Sporothrix*-inoculated trees all wilted and died quickly. Probably these trees were under severe water stress at the time of inoculation.

Wilting caused by infective agents is thought to involve insufficient water resulting from blockage of the transpiration stream (Smith, 1970). Clearly the experimental trees were killed by interruption of the xylem and hence the water supply to the crown. Death of phloem only would not produce such rapid wilt as occurred in some trees.

The mechanism of wilt has not been studied. Probably the most intensively studied comparable fungal pathogen is Dutch elm disease. Several different possibilities have been suggested as the wilt mechanism for this disease. Many investigators thought plugging of vessels by gums, tyloses, or cytoplasm from parenchyma cells were causes of wilting (Wollenweber, 1927; Broekhuizen, 1929; Clinton and McCormick, 1936; Pope, 1943), and others have postulated systemic toxæmia (Zentmyer, 1942; Zentmyer *et al.*, 1946; Kerling, 1955). On the other hand, Schwarz (1922) stated that degradation of cell walls might be an important factor in the wilt mechanism and Ouellette (1962) said that acute symptoms of the disease could result from the plugging of the smaller vessels by the spores and mycelium of the pathogen alone or in combination with cytoplasm and residues from adjoining cells, and particles arising from the deterioration of cell walls. He also suggested that the modification of vessel walls by fungal action and changes occurring in living parenchyma, which accumulating evidence suggests are involved in translocation (Greenidge, 1955; 1957; Postlethwait and Rogers, 1958), might independently contribute to wilting.

Although systemic toxæmia might be a factor in the wilt mechanism of Dutch elm disease, it seems unlikely to be involved in the wilt of *Platypus*-attacked *Nothofagus*. Infection of healthy elms is usually caused by bark beetles feeding in crotches of twigs and eating out a small tunnel or groove, thus exposing the xylem to the fungal spores carried internally and on the bodies of the beetles, i.e., infection occurs near the foliage (Forestry Commission, 1958). In contrast there is no such feeding by *Platypus* adults, so the point of infection must be the *Platypus* tunnels in the main stem — well away from the foliage. The wilt mechanism is more likely to involve one or more of the other factors mentioned.

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## REFERENCES

- BROEKHUIZEN, S. 1929: Wondreacties van hout; het ontstaan van thyllen en wondgom in het bijzonder in verband met de iepenziekte 80 P. Leiden.  
[Translation by E. W. J. Reyers (typescript), In Plant Pathology Library, Cornell University, Ithaca, New York.]
- BROWNE, F. G. 1961: The biology of Malayan Scolytidae and Platypodidae. **Malay. For. Rec. 22.**
- 1965: Types of ambrosia beetle attack on living trees in tropical forests. XIIth International Congress of Entomology: 680.
- 1971: *Austroplatypus*, a new genus of the Platypodidae (Coleoptera), infesting living *Eucalyptus* trees in Australia. **Commonw. For. Rev. 50(1), No. 143:** 49-50.
- CLINTON, A. P. and McCORMICK, F. 1936: Dutch elm disease, *Graphium ulmi*. **Connecticut Agric. Exp. Sta. Bull. 389:** 701-52.
- COCKAYNE, L. 1926: Monograph on the New Zealand Beech Forests, Part 1. **New Zealand State Forest Service, Bull. 4.**
- DEPARTMENT OF LANDS AND SURVEY, 1969: New Zealand Mapping Series 1, N103. Government Printer, Wellington.
- DUGDALE, J. S. 1965: Damaging insects of beech and possible control measures. In "Beech Forestry in New Zealand," **N.Z. For. Serv., For. Res. Inst., Symposium 5. Vol. 2:** 72-78.
- FAULDS, W. 1973: Discolouration associated with *Platypus* wounds in living *Nothofagus fusca*. **N.Z. J. For. Sci. 3(3):** 331-41.
- FORESTRY COMMISSION, 1958: Elm Disease *Ceratostomella ulmi*. **For. Comm. Leaflet 19.**
- GIESE, R. L. and McMANUS, M. L. 1965: The relationship of ambrosia beetles and their microsymbionts to sapwood staining in hardwood hosts. **Proceedings North Central Branch Entomological Society of America 20:** 135-6.
- GREENIDGE, K. N. H. 1955: Studies in the physiology of forest trees. III. The effect of drastic interruption of conducting tissues on moisture movement. **Amer. J. Bot. 42:** 582-7.
- 1957: Ascent of sap. **Annual Review of Plant Physiology 8:** 237-56.
- KERLING, L. C. P. 1955: Reactions of elm wood to attack of *Ophiostoma ulmi*. **Acta bot. neerland. 4(3):** 398-403.
- KERSHAW, D. J. 1969: Incidence of *Platypus* spp. in living silver beech at Rowallan Forest, Southland. **N.Z. For. Serv., For. Res. Inst., Ent. Rep. No. 24.**
- LITCHWARK, H. S. Insect and fungal defects in red and silver beech (in prep.).
- MILLIGAN, R. H. 1972: A review of beech forest pathology. **N.Z. J. For. 17(2):** 201-11.
- 1974: Insects damaging beech (*Nothofagus*) forests. **Proc. N.Z. Ecol. Soc. 21:** 32-40.
- MOORE, K. M. 1959: Observations on some Australian forest insects. 4. *Xyleborus truncatus* Erichson 1842 (Coleoptera : Scolytidae) associated with dying *Eucalyptus saligna* Smith (Sydney blue gum). **Proc. Linn. Soc. N.S.W. 84(2):** 186-92.
- 1962: Entomological research on the cause of mortalities of *Eucalyptus saligna* Smith (Sydney blue gum). **For. Comm. N.S.W. Res. Note No. 11.**
- MORGAN, F. D. 1966: The biology and behaviour of the beech buprestid *Nascioides enysi* (Sharp) (Coleoptera : Buprestidae) with notes on its ecology and possibilities for its control. **Trans. Roy. Soc. N.Z. (Zool.) 7:** 159-70.
- NEW ZEALAND METEOROLOGICAL SERVICE, 1972: Rainfall observations for 1972. **N.Z. Met. Serv. Misc. Pub. 110 (1972).**
- NEW ZEALAND METEOROLOGICAL SERVICE, 1973: Rainfall observations for 1973. **N.Z. Met. Serv. Misc. Pub. 110 (1973).**
- 1974: Rainfall observations for 1974. **N.Z. Met. Serv. Misc. Pub. 110 (1974).**

- OUELLETTE, G. B. 1962: Studies on the infection process of *Ceratocystis ulmi* (Buis.) C. Moreau in American elm trees. **Canad. J. Bot.** **40**: 1567-75.
- POPE, S. E. 1943: Some studies on the Dutch elm disease and the causal organism. Ph.D. Thesis, Cornell University, Ithaca, New York.
- POSTLETHWAIT, S. N. and ROGERS, B. 1958: Tracing the path of the transpiration stream in trees by the use of radioactive isotopes. **Amer. J. Bot.** **45**: 753-7.
- SCHWARZ, M. B. 1922: Das Zweigsterben der Ulmen, Trauerweiden und Pflirsichbäume Mededeel. Phytopathology Lab. "Willie Commelin Scholten" Baarn. 5: 7-32. [Transl. Kelsey, Lillian D., **Bartlett Res. Lab. Bull. No. 1**, (1928): 5-25.]
- SMITH, William H. 1970: "Tree Pathology. A Short Introduction." Academic Press, New York and London, 309p.
- SANTORO, F. H. 1957: Contribucion al conocimiento de la biologia de *Platypus sulcatus* Chapuis. **Rev. investig. for.** **1(3)**: 7-19.
- 1962a: Fundamentos para el control manual de *Platypus sulcatus* (Col., Platypodidae). **Rev. investig. for.** **3(1)**: 17-23.
- 1962b: La copula en *Platypus sulcatus* (Col., Platypodidae). **Rev. investig. for.** **3(1)**: 25-27.
- 1963: Bioecologia de *Platypus sulcatus* Chapuis (Col., Platypodidae). **Rev. investig. for.** **4(1)**: 47-79.
- WOLLENWEBER, H. W. 1927: Das Ulmensterben und sein Erreger, *Graphium ulmi*, Schwarz. Sonderabdruck aus dem Nachrichtenblatt für den deutschen Pflanzenschutzdienst 10. [Transl. Kelsey, Lillian D., **Bartlett Res. Lab. Bull. No. 1**, (1923): 26-31].
- ZENTMYER, G. A. 1942: Toxin formation by *Cerostomella ulmi*. **Science** **95**: 512-3.
- ZENTMYER, G. A., HORSFALL, J. G. ad WALLACE, P. P. 1946: Dutch elm disease and its chemotherapy. **Connecticut Agric. Exp. Sta. Bull. No. 498**: 1-70.

## APPENDIX 1—Notable changes and observations in Experiment 1

	<b>Tree A</b>		<b>Tree E</b>
March 1972	Inoculated with <b>Sporothrix</b> ; crown healthy until 30 August 1972.	April 1972	Inoculated with <b>Sporothrix</b> .
30 Aug 1972	Lower branches wilting; upper crown healthy.	27 Nov 1972	New foliage flushed.
16 Sept 1972	Lower branches to approximately 10 m dead or wilting; terminals in mid crown wilting; upper crown wilting.	15 Feb 1973	Some <b>Platytypus</b> attack.
29 Sept 1972	Lower crown wilted except for some epicormics with green foliage; mid crown wilting but still a little yellow and green foliage; upper crown wilted.	27 Feb 1973	A few dead branches present; some <b>Psepholax</b> attack; stem to 5 m sprayed with 0.25% dieldrin.
27 Nov 1972	Completely wilted—felled.	28 Nov 1973	New foliage flushed.
		27 Mar 1974	Quite a few dead branches; stem to 5 m sprayed with 0.25% dieldrin.
	<b>Tree B</b>	6 Dec 1974	New foliage flushed.
March 1972	Inoculated with <b>Sporothrix</b> ; crown healthy until 30 August 1972.	5 May 1975	Lots of dead branches in upper crown, mid, and lower crown, with wilted foliage on some branches.
30 Aug 1972	Lots of foliage with a brown appearance.	27 Jun 1975	Lower crown wilted and dead; still some green leaves on upper crown.
29 Sept 1972	Whole crown wilted or wilting except for a few green branches in mid crown.	14 July 1975	Lower crown wilted; upper crown foliage going yellow.
27 Nov 1972	Completely wilted.	18 Aug 1975	Completely wilted—felled.
13 Dec 1972	Felled.		<b>Tree F</b>
	<b>Tree C</b>	May 1972	Inoculated with <b>Sporothrix</b> .
March 1972	Inoculated with sterile distilled water; some dead branches; foliage generally sparse.	27 Nov 1972	New foliage flushed.
27 Nov 1972	New foliage flushed.	22 Feb 1973	<b>Platytypus</b> attack.
22 Feb 1973	Some <b>Platytypus</b> attack.	27 Feb 1973	Stem to 5 m sprayed with 0.25% dieldrin; <b>Psepholax</b> attack.
27 Feb 1973	Stem to 5 m sprayed with 0.25% dieldrin.	15 Nov 1973	New foliage flushed.
15 Nov 1973	New foliage flushed.	27 Mar 1974	Stem to 5 m sprayed with 0.25% dieldrin.
27 Mar 1974	Stem to 5 m sprayed with 0.25% dieldrin.	6 Dec 1974	New foliage flushed.
10 Apr 1974	Some <b>Psepholax</b> (Curculionidae) attack.	16 Jan 1975	Small branches in upper crown with very few leaves; rest of crown healthy.
14 Nov 1974	New foliage flushed.	7 Apr 1975	Foliage yellow and wilting, only a few green leaves left.
28 Jan 1975	Crown healthy—felled.	5 May 1975	Completely wilted—felled.
	<b>Tree D</b>		<b>Tree G</b>
April 1972	Inoculated with sterile distilled water.	May 1972	Inoculated with <b>Sporothrix</b> .
27 Nov 1972	New foliage flushed.	27 Nov 1972	Crown healthy but new foliage not yet flushed.
7 Feb 1973	Some gum bleed from drilled holes.	18 Jan 1973	Lots of dead branches and terminals in upper and mid crown; lower crown green.
22 Feb 1973	A few <b>Platytypus</b> attacks.	25 Jan 1973	Many dead branches and terminals; few remaining leaves, some of these yellow.
27 Feb 1973	Stem to 5 m sprayed with 0.25% dieldrin.	1 Feb 1973	Over 50% of foliage in mid and upper crown gone.
15 Nov 1973	New foliage flushed; a few dead branches present.	7 Feb 1973	Some <b>Platytypus</b> attack.
27 Mar 1974	Stem to 5 m sprayed with 0.25% dieldrin.	15 Feb 1973	Some foliage wilting in upper crown.
6 Dec 1974	New foliage flushed.	27 Feb 1973	Stem to 5 m sprayed with 0.25% dieldrin; most of upper crown without foliage; a little green foliage on small branches.
7 Apr 1975	Crown healthy—felled.	7 May 1973	Upper crown dead; epicormics in lower crown still green.
		23 May 1973	Felled.

## APPENDIX 2—Notable changes and observations in Experiment 2.

<b>Trees 1-5</b>		<b>Tree 9</b>	
Jan 1974	Inoculated with sterile distilled water.	Jan 1974	Inoculated with <b>Sporothrix</b> .
22 Mar 1974	A few <b>Platypus</b> attacks on Tree 2.	22 Mar 1974	Some foliage on lower branches going brown; ten <b>Platypus</b> attacks and two <b>Psepholax</b> attacks seen.
27 Mar 1974	Stem to 5 m sprayed with 0.25% dieldrin.	27 Mar 1974	Stem to 5 m sprayed with 0.25% dieldrin.
24 Oct 1974	Stem to 5 m sprayed with 0.25% dieldrin.	10 Apr 1974	Most of foliage wilted; only a few small branches with green leaves.
6 Dec 1974	New foliage flushed.	23 Apr 1974	Completely wilted.
6 Nov 1975	Stem to 5 m sprayed with 0.25% dieldrin.	30 Apr 1974	Felled.
Dec 1975	New foliage flushed.	<b>Tree 10</b>	
16 Jan 1976	Tree 3 felled — healthy when felled.	Jan 1974	Inoculated with <b>Sporothrix</b> .
27 Jan 1976	Tree 1 felled — healthy when felled.	23 Mar 1974	Crown yellowing and some foliage wilting; foliage sparse; a few <b>Platypus</b> attacks.
23 Feb 1976	Tree 2 felled — healthy when felled.	27 Mar 1974	Stem to 5 m sprayed with 0.25% dieldrin.
1 Mar 1976	Tree 4 felled — healthy when felled.	10 Apr 1974	Completely wilted.
8 Mar 1976	Tree 5 felled — healthy when felled.	30 Apr 1974	Felled.
<b>Tree 6</b>		<b>Tree 11</b>	
Jan 1974	Inoculated with <b>Sporothrix</b> .	Feb 1974	Inoculated with <b>E. platypodis</b> .
22 Mar 1974	Lower branches wilted; foliage in upper crown yellowish; some <b>Platypus</b> and <b>Psepholax</b> attack.	22 Mar 1974	Some <b>Platypus</b> and <b>Psepholax</b> attack.
27 Mar 1974	Stem to 5 m sprayed with 0.25% dieldrin.	27 Mar 1974	Stem to 5 m sprayed with 0.25% dieldrin.
10 Apr 1974	Foliage wilting or yellow; only a few green leaves left in upper crown.	24 Oct 1974	Stem to 5 m sprayed with 0.25% dieldrin.
23 Apr 1974	Crown wilted except for a few leaves in upper crown.	20 Oct 1975	Lots of yellow foliage throughout crown and some small branches in lower crown wilted.
17 May 1974	Completely wilted.	3 Nov 1975	Lower branches wilted; mid crown yellow or wilted.
26 Nov 1974	Felled.	24 Nov 1975	Completely wilted.
<b>Tree 7</b>		5 Jan 1976	Felled.
Jan 1974	Inoculated with <b>Sporothrix</b> .	<b>Trees 12-15</b>	
22 Mar 1974	Foliage in lower crown wilting; upper crown looking unhealthy; some <b>Platypus</b> and <b>Psepholax</b> attack.	Feb 1974	Inoculated with <b>E. platypodis</b> .
27 Mar 1974	Stem to 5 m sprayed with 0.25% dieldrin.	22 Mar 1974	Three to four <b>Platypus</b> attacks in Trees 12, 13, and 14.
10 Apr 1974	Foliage wilted or wilting except for patches of green leaves.	27 Mar 1974	Stem to 5 m sprayed with 0.25% dieldrin.
23 Apr 1974	Foliage wilted except for a few green leaves.	24 Oct 1974	Stem to 5 m sprayed with 0.25% dieldrin.
17 May 1974	Completely wilted.	Dec 1974	New foliage flushed.
12 Dec 1974	Felled.	6 Nov 1975	Stem to 5 m sprayed with 0.25% dieldrin.
<b>Tree 8</b>		Dec 1975	New foliage flushed.
Jan 1974	Inoculated with <b>Sporothrix</b> .	12 Jan 1976	Tree 12 felled — healthy when felled.
22 Mar 1974	Some wilting foliage in lower crown; a few <b>Platypus</b> attacks.	22 Mar 1976	Tree 13 felled — healthy when felled.
27 Mar 1974	Stem to 5 m sprayed with 0.25% dieldrin.	29 Mar 1976	Tree 14 felled — healthy when felled.
10 Apr 1974	Lots of wilted foliage and yellow leaves throughout crown; a few branches still with green foliage.	5 Apr 1976	Tree 15 felled — healthy when felled.
23 Apr 1974	Crown wilted except for some green and yellow leaves on one side of tree and epicormics in lower crown.		
17 May 1974	Completely wilted except for epicormics.		
24 Jul 1974	Epicormics in lower crown starting to wilt.		
24 Oct 1974	Completely wilted.		
6 Dec 1974	Felled.		