THE MECHANISM OF PATHOGENICITY OF *SIREX NOCTILIO* ON *PINUS RADIATA*

II.* EFFECTS OF *S. NOCTILIO* MUCUS

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[*Manuscript received October* 28, 1968]

Summary

Effects on *P. radiata* of a mucus secretion from the female wood wasp are described. When injected into the sapwood, this substance induced the rapid physiological changes in the crown which are characteristic of trees attacked by *S. noctilio*. Among these was the accumulation of starch in the leaf and a decrease of starch in the bark of the stem implying that the translocation of photosynthate was inhibited. This was followed by premature senescence and abscission of much of the foliage and increased susceptibility to the fungus, *Amylostereum* sp., which occurs in symbiosis with the insect. Therefore it is concluded that the mucus secretion conditions the tree, which is subsequently killed by the fungus.

Great tree-to-tree variation was observed in the ability to tolerate injections of mucus, and similar variation was found in the susceptibility of detached shoots in mucus solution. Although an enzyme was detected in the mucus, the biological potency of the latter was unaffected by autoclaving.

I. Introduction

The effects of attack on *Pinus radiata* by *Sirex noctilio*, and of inoculation with its fungal symbiont, *Amylostereum* sp. (Thelophoraceae), were described in a previous paper (Coutts 1969). Although the fungus caused the sapwood to dry out locally, and could therefore be expected to restrict sap flow to the crown, symptoms of *S. noctilio* attack such as starch accumulation in the leaf, premature abscission, and yellowing of the foliage could not be accounted for by the action of the fungus alone. Extracts of logs infected with *Amylostereum* sp. had no rapid systemic effects on the tree if the logs were inoculated with a suspension of fungus mycelium, but such effects could be induced by injecting extracts from logs which had been attacked by the insect. This pointed to secretions of the insect as being responsible for the early symptoms of the desease.

In the abdomen of the female insect, paired glands secrete a colourless mucus into a large reservoir connected by a duct to the base of the ovipositor, and Boros (1968) characterized this substance as a protein–mucopolysaccharide complex. This paper describes effects on *P. radiata* of *S. noctilio* mucus, and of injections of mucus+fungus.

* Part I, *Aust. J. biol. Sci.*, 1969, **22**, 915–24.

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II. Methods

Techniques repeatedly used are described in this section, and further details are given before the results of each experiment.

Experimental trees used were in an area of *P. radiata* regrowth 5–7 years old at Pittwater plantation in southern Tasmania.

Leaf starch and leaf dry weight were measured by methods described in part I of this series (Coutts 1969). Starch in the bark was examined in 25-μm radial sections stained in I_2–KI solution.

Solutions of secretions from female insects were made by dissecting out the organs required and dissolving their contents in distilled water. These solutions were then injected into radial boreholes 0·5 mm in diameter and 5 mm deep in branches, and into tree stems in boreholes 3 mm in diameter and 2 cm deep, unless otherwise stated.

Inoculations with *Amylostereum* sp. were made by injecting a suspension, in mucus solution, of arthrospores from the intersegmental sacs of female insects.

The susceptibility of detached shoots to mucus solutions was tested by standing shoots about 12 in. long, and from which the needles had been removed from the lower half, in solutions containing mucus at 50–100 p.p.m. Control shoots were stood in distilled water. Shoots were classed as dead when four-fifths of the needles had turned yellow. Branch whorls are numbered from the top downwards.

III. Results

(a) *Preliminary Tests*

During a histochemical study of the mechanism of resistance to *S. noctilio* attack in the stem, strong polyphenoloxidase activity was detected, by staining with benzidine (Pearse 1961), in the drill holes made by the insect's ovipositor. This activity was traced, not to the arthrospores of *Amylostereum* sp., which are injected into the tree in a mass of mucus, but to the mucus itself. Detection of enzyme activity in the mucus suggested it as potentially active in the physiological processes of the tree, so solutions of mucus were injected into trees and effects were observed.

Mucus reservoirs contain about 10–25 mg of mucus, depending on the size of the insect. As a preliminary test, 0·1 ml of a 1% mucus solution was injected into 10 boreholes in the bases of three branches on each of two trees. One branch in the same whorl on each tree was injected with distilled water only, and two other branches were left as undrilled controls. After 12 days, a slight colour change was noticeable in the leaves of branches injected with mucus, and starch was found to be present in quantity in these leaves, whereas leaves of control branches remained green and contained only traces of starch (Table 1).

The mucus solution was soon absorbed by the xylem when injected into the boreholes, but the cut surfaces of phloem and cambium were also wetted. To avoid direct contact of mucus with the outer tissues, 50 ml of 0·1% mucus solution were fed into each of three 10-ft-tall trees, from a reservoir attached to a pipe hammered 1 cm into a 6-mm-diameter hole, drilled 3 cm radially into the xylem at a height of 6 in. Three control trees were supplied with distilled water by the same method. The liquids were absorbed over a period of 2 days. After 2 weeks, the mucus-treated trees were found to have accumulated large quantities of starch in their foliage. This was accompanied by yellowing of their 2- and 3-year-old needles. Control trees were not affected.

As enzyme activity in the mucus was suspected of causing these effects, a further experiment was carried out to compare the effects of injecting trees with fresh and with boiled mucus; and, to provide information on the role of the fungus, some trees were injected with mucus solution containing a suspension of *Amylostereum* sp. arthrospores.

(*b*) *Injections of Mucus and Mucus plus Fungus*

Twenty trees, 8–12 ft tall, were pruned to a height of 3 ft, and four were allotted to each of five treatments, being injected with the following solutions:

(1) fresh mucus;
(2) mucus which had been boiled for 30 sec;
(3) mucus which had been boiled for 2 hr;
(4) fresh mucus containing a suspension of arthrospores;
(5) distilled water.

A 1% solution of 100 mg of mucus (the equivalent of four large insects) was injected into two vertical files of seven boreholes in the lower 3 ft of the stem of each tree in treatments 1–4. Changes in leaf water deficit were measured on 1-year-old needles from the topmost whorl of branches.

Table 1

EFFECT OF INJECTING MUCUS INTO BRANCHES ON THE AMOUNT OF STARCH IN THE FOLIAGE

Starch content measured at 0 and 12 days after injection of trees, on a 0–6 scale as described by Coutts (1969): 1, trace of starch; 6, mesophyll cells packed with starch

Treatment	Starch Grade of Leaves in Tree No.:			
	1 (0 days)	1 (12 days)	2 (0 days)	2 (12 days)
Mucus injection				
Branch 1	1·0	6·0	1·5	4·5
Branch 2	1·0	5·0	1·0	5·0
Branch 3	0·0	6·0	1·0	5·0
Water injection				
Branch 4	1·0	0·0	1·0	1·0
No treatment				
Branch 5	0·0	1·0	1·0	1·0
Branch 6	0·0	1·0	1·0	1·5

Visible changes in the foliage induced by injections of mucus+arthrospores were the same as those which occur after heavy *S. noctilio* attack. Most of the crown changed to an olive green colour during the second week after inoculation, and this was accompanied by yellowing of the oldest needles. Yellowing then progressed to the younger needles, but the foliage in the upper parts of the trees shrivelled and died without turning conspicuously yellow. The four trees died 3–4 weeks after being inoculated.

All trees injected with mucus alone survived. There were no clear differences between trees injected with fresh mucus, and those injected with mucus boiled for 30 sec or 2 hr. All the trees reacted at first like those injected with mucus+arthrospores, but after the oldest needles had turned yellow, certain differences became apparent. Some trees showed no further change and recovered from the treatment, whereas on others the yellowing progressed through the crown leading to severe defoliation of all branches except the top branch whorl and leading shoot. There was no shrivelling of young needles while still in the green state, as occurred with the mucus+fungus treatment. Only one tree wilted in the manner described for *S. noctilio* attack (Coutts 1969), but the experiment was done in early winter when the needles of most trees did not seem to be growing.

The appearance of control trees was unaffected by the distilled water injection.

The dry weight of the leaves of most trees decreased during the 2 days prior to the injections, and this may have been a result of pruning. Characteristically, thereafter leaf dry weight of mucus-treated trees (with or without fungus) increased fairly uniformly at a rate of about 1·4% per day, for a period of up to 2 weeks, and this was in some trees followed by a decrease, and in others by a slower rate of accumulation resulting a few weeks after injection in an increase in leaf dry weight of as much as 40% of the original value (Fig. 1).

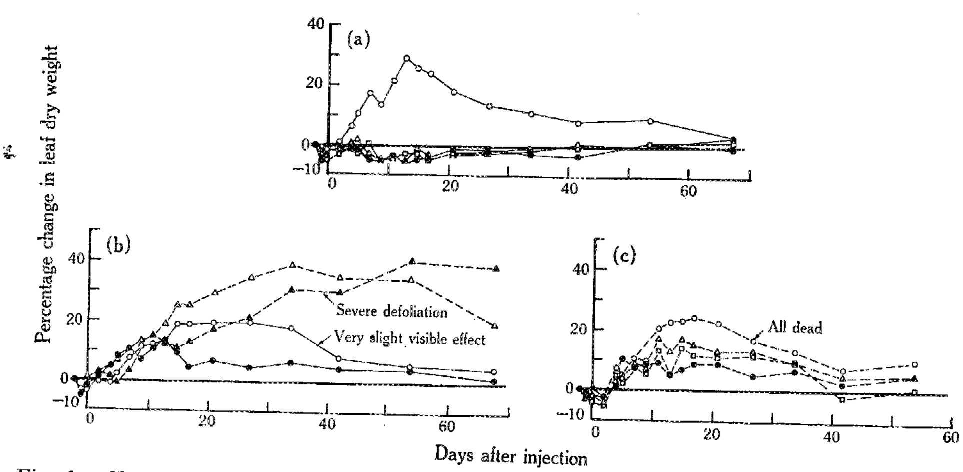

Fig. 1.—Change in dry weight of 1-year-old needles after injecting trees with distilled water (*a*), mucus (*b*), or mucus plus fungus (*c*). Each line represents results for one tree.

Fresh and heat-treated mucus caused similar increases in leaf dry weight, the variation encountered being due to differences between individual trees. The length of the period over which leaf dry weight increased was related to the occurrence of visible symptoms in the foliage. Trees whose leaves increased in dry weight for only 2–3 weeks after the injections showed slight visible symptoms, whereas those whose leaves continued to increase in dry weight for 7–8 weeks were badly defoliated.

The dry weight of leaves of three of the four control trees changed very little from their original values during the period of the experiment. But in the fourth control tree, leaf dry weight increased slightly faster for 2 weeks than in any other tree in the experiment, and then began to decrease [Fig. 1(*a*)]. No other untreated tree behaved in this way during the course of this study.

It appeared as though the fungus was causing only the later symptoms of the disease, such as shrivelling of young needles in a more-or-less green condition. Early symptoms (accumulation of substance in the leaf, senescence, and premature abscission of the older suites of needles) could be induced by injecting mucus alone, although some trees could tolerate relatively large amounts of it. Another experiment was done to confirm this result and to examine further the effects of heat treatment on the biological properties of the mucus.

(*c*) *Injections of Autoclaved Mucus*

Some trees were injected with fresh mucus and some with a mucus solution which had been autoclaved at a pressure of 15 lb/in^2 for 1 hr. None of the trees was pruned, and as a further control on the effects of injury measurements were made on trees which had not been injured in any way, as well as on those which had been drilled and injected with distilled water. Twenty-two trees were allotted to four treatments as follows:

Treatment	Substance Injected	Amount	No. of Trees
A	Fresh mucus	10 ml of 1% solution	7
B	Autoclaved mucus	10 ml of 1% solution	5
C	Distilled water	10 ml	5
D	Undrilled control	—	5

The trees were bigger than those used in the first experiment, being 17–22 ft tall. They were allotted to the various treatments as evenly as possible on a basis of size and relative vigour. The dry weight of 2-year-old needles on a branch whorl at a height of 5–7 ft was measured.

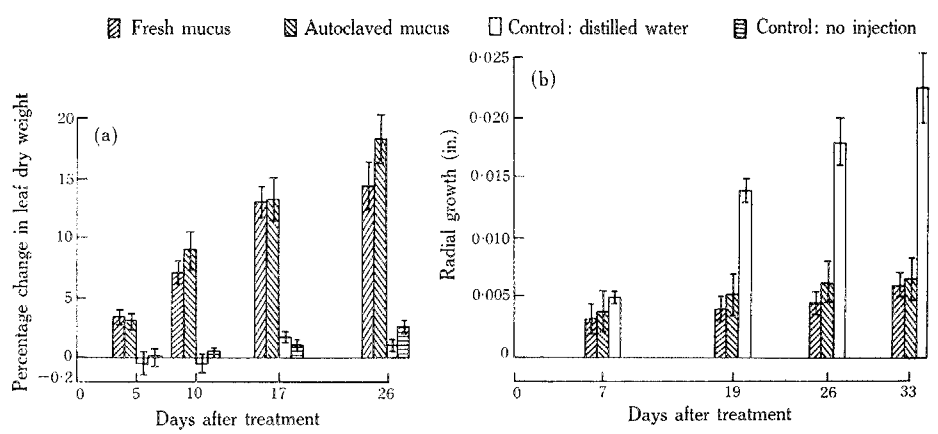

Fig. 2.—Effect of *S. noctilio* mucus injections on dry weight of 2-year-old needles (*a*), and on cumulative radial growth of the stem (*b*). Means of five trees per treatment are shown, except for fresh mucus injections (seven trees). Standard errors of the means are indicated by vertical bars.

Fresh mucus and autoclaved mucus caused similar increases in leaf dry weight, whereas that of all control trees remained normal [Fig. 2(*a*)]. Visible foliar effects were like those on mucus-injected and control trees in the previous experiment.

Experimentally induced *S. noctilio* attack has been found to cause a check to diameter growth after about 9 days (Coutts and Dolezal 1966*a*). Growth of trees which resisted attack was resumed after several weeks, but the growth of shoots was sometimes affected for 2–3 years, having a dwarfing effect on the tree. To find whether the mucus was responsible for the growth check, radial growth of the trees in treatments A, B, and C was measured with a dial-gauge dendrometer. The growth rate of mucus-treated trees did not differ significantly from the controls 8 days after injection, but a distinct growth check was recorded at the time of the measurement made at 19 days [Fig. 2(*b*)].

Four weeks after the mucus was injected, starch was present in the bark of all control trees, and was generally abundant (Table 2). Most of the trees which had been injected with mucus had much less starch than controls, and in seven of them no starch at all was detected in the bark.

Table 2

Starch in stem bark 4 weeks after mucus injection

Treatment	No. of Trees	No. of Trees in which Starch was Assessed as: Abundant	Intermediate	Scarce	Absent
Fresh mucus injected	7	1	1	2	3
Autoclaved mucus injected	5	—	—	1	4
Distilled water injected	5	3	2	—	—
Untreated controls	5	4	1	—	—

(*d*) *Susceptibility of Foliage on Detached Shoots in* S. noctilio *Mucus Solution*

Detached shoots were stood in mucus solutions to investigate the variable resistance of the foliage. Preliminary tests showed that discoloration of the foliage occurred after about 1 or 2 weeks, at mucus concentrations of 100 and 50 p.p.m. respectively, whereas control shoots in water survived for more than 2 months. In the experiments described below, mucus was used at a concentration of 80 p.p.m.

Susceptibility of leaves of different ages was investigated on 20 trees, using two shoots per tree from the second branch whorl, and bearing 1- and 2-year-old needles. One detached shoot from each tree was stood in mucus solution, and the control shoots stood in water. Two-year-old needles of shoots in mucus began to turn yellow after 1 week, and died much more quickly than 1-year-old needles, the mean survival times being $2\cdot5\pm0\cdot5$ and $4\cdot6\pm1\cdot6$ weeks respectively (significantly different at the 1% level). Control shoots survived during the 7-week period of observation.

To study the susceptibility of foliage of the same age from different parts of the tree, the leading shoot and shoots bearing 1-year-old needles from the first, second, and third branch whorls from each of 10 trees were stood in mucus solution. Leading shoots were more resistant than lateral branches, and shoots from upper branch whorls were more resistant than shoots from lower whorls, as the following tabulation shows:

	Leading Shoot	Top Whorl	Second Whorl	Third Whorl
Mean survival time (weeks)	6·8	4·9	3·9	3·6
Means differing significantly				
At the 1% level	└────	────┘		
At the 5% level	└────	────┘ └────	────	────┘

Apparent differences in the susceptibility of individual trees were investigated in the next experiment.

Five trees of very similar size and appearance were selected. Six shoots were taken from the top branch whorl of each tree, five being stood in mucus, and one in distilled water. Variation between trees was obvious, the twigs from individual trees dying by groups. Results are summarized in the following tabulation:

Tree No.	1	5	3	4	2
Mean survival time (weeks) (five shoots per tree)	3·4	3·5	5·2	7·2	8·8
Means differing significantly at the 1% level		└────	────┘ └────	────┘ └────	────┘

Control shoots remained alive after all shoots in mucus had died.

Because bacteria and fungi will grow in mucus solutions, the experiment last described was repeated with shoots from a further five trees, but with the addition of streptomycin (30 p.p.m.) and captan (80 p.p.m.) to the mucus solution, and to the distilled water of the controls. All shoots were transferred to freshly made solutions at weekly intervals. The experiment was not accurately scored on a weekly basis, but it was observed that shoots in mucus+antibiotics died over a 3–7-week period (i.e. similar to that of the previous experiment), whereas all controls survived for 8 weeks.

(e) *Effects of Mucus on Growth of the Fungus*

Boros (1968) found that the addition of *S. noctilio* mucus to water agar stimulated the growth of *Amylostereum* sp., and concluded that the function of the mucus is to give the fungus an initial boost when it is injected into the tree.

Because of the probable importance of fungal growth rate in the disease, tests were made to assess the effects of the mucus under more natural conditions.

The mucus reservoir and its two associated glands were removed from some *S. noctilio* females through an incision in the abdomen, which was then sealed. These insects, with clipped wings, were put on a standing tree in the plantation, and normal insects were put on a similar tree. The insects were removed after 10–15 ovipositions per tree had been made. In addition, treated and normal insects were induced to drill, in the laboratory, two logs cut from a single tree. The distance the fungus had spread from the insect drill holes in each tree was measured in 25-μm tangential sections, from 10 oviposition sites; and in the logs 10 streaks of fungal drying were measured. The results are presented below:

	Mucus Present	Mucus Absent
Fungal growth (mm) after 8 days	$2 \cdot 5 \pm 0 \cdot 3$	$2 \cdot 1 \pm 0 \cdot 2$
Dry zones (mm) after 14 days	$86 \pm 5 \cdot 1$	$85 \pm 7 \cdot 2$

The small differences between treatments in the presence and absence of mucus were not significant.

Although the results must be considered as preliminary, it was observed that the addition of mucus to 2% malt agar on which *Amylostereum* sp. was growing stimulated growth less than the addition of small surface-sterilized blocks of *P. radiata* sapwood, and J. F. Titze and C. R. A. Turnbull (personal communication) found that the addition of mucus to a sawdust medium caused no increase in the respiration rate of *Amylostereum* sp.

IV. Discussion

Effects on *P. radiata* of *S. noctilio* mucus and of *Amylostereum* sp., as described in this paper and in Part I of this series (Coutts 1969), may be summarized as follows.

Neither the fungus alone nor mucus alone were normally capable of killing trees, but they were lethal in combination. Mucus caused the accumulation of starch in the foliage, and sometimes a check to growth. These changes were reversible. In some trees, mucus caused the continued accumulation of photosynthate in the leaf, and chlorophyll began to disappear. Old needles turned yellow before there was any change in their leaf water deficit, and yellowing progressed to the younger needles. Growth was checked and abscission resulted in severe defoliation. Starch disappeared from the stem bark.

Thus most of the early changes observed after *S. noctilio* attack can be attributed to the mucus, and no evidence has so far come to light to indicate that the fungus produces toxins having any systemic effect on the crown.

Amylostereum sp. caused the sapwood to dry out locally, but well in advance of the mycelium. When sufficient wood was colonized, the coalescence of dry zones cut off the sap supply to the crown and, in contrast with the effect of the mucus, death of the tree was preceded by a sudden increase in leaf water deficit.

Little can yet be deduced about the mechanism by which the mucus affects the tree. When injected into *Populus* seedlings and *Eucalyptus* saplings it had no effect on leaf starch and produced no visible symptoms in the foliage (unpublished data), so it may be effective only in certain conifers of which *S. noctilio* attacks a number of species.

The increase in starch in the leaf is due to an accumulation of current photosynthate, rather than to movement of substances out of the stem, because the increase does not occur in shaded branches of *S. noctilio*-attacked trees (Coutts 1969). The decrease in starch in the stem bark may be attributed not only to reduced translocation from the leaf, but also to greatly increased respiration of the stem, which has been detected after injection of only 1 mg of *S. noctilio* mucus (J. Madden, personal communication). It is not clear whether decreased translocation from the leaf is brought about by mucus in the leaf, or by an effect on the sink for photosynthate in the stem. Accelerated senescence can be induced in detached needles by standing them in mucus solution (unpublished data), so the phytotoxic effect is presumably brought about by mucus which reaches the leaf.

Senescence of leaves on detached shoots in mucus solution was similar to that on standing trees attacked by *S. noctilio* or injected with mucus. Young needles were much more resistant than old needles, and the resistance of young needles increased with their nearness to buds in the upper part of the tree, so some growth substance such as auxin, which is produced both by buds and young needles, but to a much smaller extent by old needles (Onaka 1950), is probably involved in the resistance of the leaf.

An important aspect to be clarified is the way in which the mucus reduces the resistance of the sapwood to the fungus. In trees which resist the fungus, borders of polyphenols develop around the infected zones in the sapwood, resin appears within those zones, and growth of the fungus stops after 3 weeks (Coutts and Dolezal 1966*b*). Such tissue reaction is absent or greatly reduced in trees which die of *S. noctilio* attack.

Although the mucus has some phytotoxic effects, it is chiefly a conditioning agent. *S. noctilio* normally attacks suppressed or subnormal trees, but *Amylostereum* sp. is a weak parasite, and such trees must be reduced to a more susceptible state by the action of mucus, if the fungus is to invade the sapwood and kill the tree by restricting sap flow.

V. References

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