

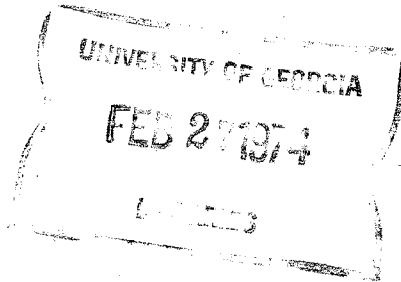
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A comparative study of the phytotoxic effects of siricid woodwasps on conifers

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SUMMARY

The effects of siricid mucus and the symbiotic fungus injected artificially, or by oviposition of caged females on young conifer trees were investigated in a comparative study of the phytotoxicity caused by seven species of siricid woodwasps. Dilute solutions of mucus were bioassayed using detached shoots. Rapid physiological changes in the radial growth of the stem, quantity of starch in the leaves, leaf pressure and colour of the foliage of living trees were induced by only one species, *Sirex noctilio*. Solutions of *S. noctilio* and *S. cyaneus* mucus caused premature senescence of needles on detached shoots. The other species, *Sirex juvencus*, *Urocerus gigas*, *U. augur*, *U. sah* and *Xeris spectrum*, produced no phytotoxic symptoms in living trees and less rapid senescence of needles on detached shoots. These differences are discussed in relation to the ecology of siricids in the northern hemisphere and the successful establishment of *S. noctilio* in pine plantations of Australasia.

INTRODUCTION

The siricid woodwasp, *Sirex noctilio* (F.), is an exotic pest of the pine, *Pinus radiata* D. Don, in New Zealand and Australia where heavy losses of standing timber sometimes occur (Rawlings, 1954). When *S. noctilio* attacks a tree it uses its saw-tipped ovipositor to make a hole into the wood to oviposit. During oviposition arthrospores of a symbiotic fungus, *Amylostereum*, stored in a pair of mycangia formed from intersegmental membranes (Fig. 1), are injected into the tree together with secretions from the mucus gland. Coutts (1969*b*) has established that the mucoid secretion causes rapid physiological changes in the stem and foliage which conditions the host to the fungus. Injection of mucus produces characteristic symptoms but neither mucus nor fungus alone causes the death of a tree, although in combination they are generally lethal (Coutts, 1969*a, b*).

In Europe and North Africa there are eight species of conifer-infesting woodwasps including *S. noctilio*. All species, except *Xeris spectrum* (L.), are associated with a symbiotic fungus similar to or identical with that of *S. noctilio* (Gaut, 1969; R. A. Bedding, personal communication).

This study, made in England, compares the phytotoxic effects of seven species of woodwasps.

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MATERIALS AND METHODS

The woodwasps were reared under outdoor insectary conditions at Silwood Park, Ascot, from infested timber collected throughout Europe, North Africa and Turkey. The species used were *Sirex noctilio*, *S. cyaneus* (F.), *S. juvenis* (L.), *Urocerus gigas* (L.), *U. augur* (Klug.), *U. sah* Mocs and *Xeris spectrum*. *S. noctilio* mucus from Australia was also used. Adults were collected daily and stored at 5–8 °C until required. The paired mycangia at the base of the ovipositor, in which arthrospores of the symbiotic fungus, *Amylostereum* spp., are stored (Fig. 1), were removed not more than

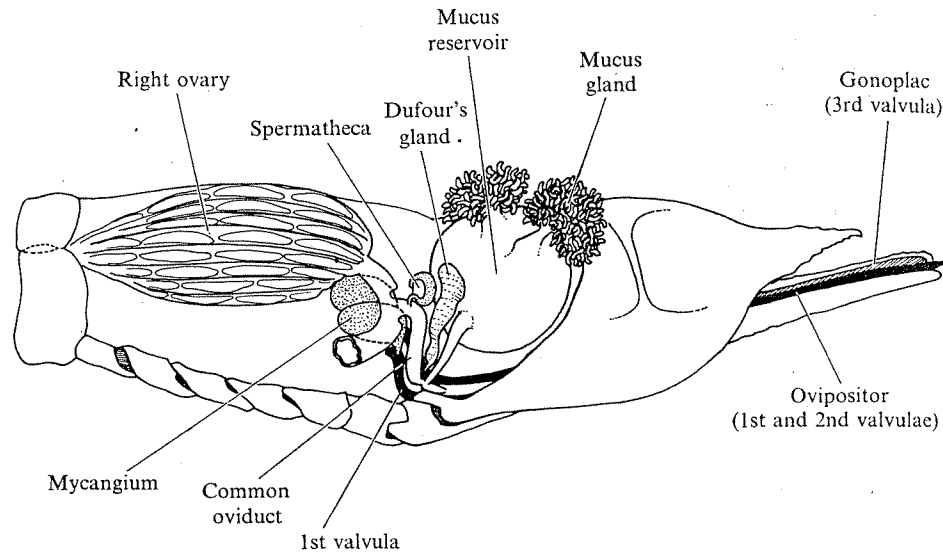


Fig. 1. Reproductive anatomy of *Sirex noctilio* female to show mycangia and mucus glands; left ovary removed, mucus reservoir displaced distally for clarity.

36 h before use. The mucus glands and associated reservoir (Fig. 1) were dissected from females, weighed and stored at –20 °C. Seven- to nine-year-old trees of *Pinus sylvestris* L. and *P. radiata* in Wareham, Dorset, and *Picea sitchensis* Bong. Carr in Windsor Forest were used for injection and oviposition studies and these, together with twenty-nine species of pine from the Bedgebury Pinetum, Sussex, were used for detached shoot bioassay.

For injection and oviposition studies, seventy trees each of *P. radiata* and *P. sylvestris* and fifty trees of *Picea sitchensis* were selected for uniformity of height, stem diameter and foliage. At least 3 weeks before use, the lower branches were cut off to a height of 1 m to enable cages to be fitted. Mucus solutions were made with distilled water so that 25 ml contained 0.1 g of mucus which was injected into two holes 5 mm in diameter and 5 cm deep at the base of each stem. To facilitate uptake of the solution, glass tubes of 5 mm O.D. with 10 ml reservoirs were fitted to a depth of 1–2 cm into the holes.

Inoculations with fungus were made by adding a suspension of spores to the mucus solution at the time of injection. Mycangia from five females were used for each tree. The rate of uptake of the solutions varied and tubes were occasionally blocked with resin. In these cases, fresh holes were drilled and the reservoir tubes transferred. Oviposition by female woodwasps was restricted to approximately 30 cm of stem from the base, using two strips of 10 cm × 10 cm foam rubber. A band of acetate sheeting was fitted round each rubber strip to prevent insects chewing their way out and terylene gauze wrapped round the stem to make a cage. Ten females per tree were used. For each siricid species and treatment, five trees of each tree species were used. Controls included five untreated trees and five trees into which 25 ml of distilled water were injected. Injection and caging of females were done at different times during the summer of 1970 depending on the flight period and availability of insects. There was insufficient *U. augur* material to make experiments on living trees.

To assess physiological changes in the trees the following parameters were measured weekly: radial growth of stem, leaf pressure of needles, degree of yellowing or chlorosis and starch content of needles. Radial growth was measured with a dial-gauge dendrometer. Leaf pressure was measured with a modified Scholander pressure-bomb apparatus incorporating a cylinder of compressed air and pressure gauge (Scholander, Hammel, Hemmingsen & Bradstreet, 1964; Scholander, Hammel, Bradstreet & Hemmingsen, 1965). A needle was inserted into the bomb and pressure applied until sap was expressed from the cut fascicle at which point the pressure (in p.s.i.) was noted. Leaf pressure is equivalent to total water potential which is a criterion of the tree's energy status and is composed of osmotic pressure plus turgor pressure plus matric pressure, the latter being a measure of the capillary force in the cell wall (Scholander *et al.* 1964, 1965). The degree of chlorosis of the needles was scored on a scale of 0-7 to describe the range of symptoms between no visible signs of chlorosis (0) to complete discoloration or needle fall (7). To measure the foliar starch content, ten needles from each tree were pushed into a Polythene tube, cut transversely and dipped into iodine + KI solution to display starch grains in the mesophyll. The density of starch grains was rated on a scale of 0-7 to describe the range from starch absent to starch grains filling the mesophyll cells.

Trees subjected to oviposition were felled after several months and examined for density of oviposition drills, penetration of the wood by hyphae of the symbiotic fungus, and the incidence of resin and polyphenols. Polyphenols and fungal hyphae were displayed by staining wood sections with toluidine and methylene blue respectively.

Bioassay of mucus was made by standing shoots, 15-20 cm long, from which the lower needles had been detached, in 0.1 mg/l (100 ppm) solutions of mucus. Controls were placed in water. Thymol was added to the solutions to prevent moulds. To clear the xylem elements the lower ends of the shoots were trimmed back approximately 5 mm every week. Five shoots were maintained in 200 ml of solution in conical flasks or three shoots in 50 ml in glass tubes. All bioassays were made at a temperature of 25 °C, 70% r.h. and a 16 h photoperiod. To overcome inter- and intra-tree variability, all the shoots for each replicate were derived from the same whorl of one tree.

Senescence of the needles was determined by measuring leaf pressure and/or rating the degree of chlorosis on a scale of 0-6 to describe the range from no effects to complete yellowing, reddening or shrivelling of leaves.

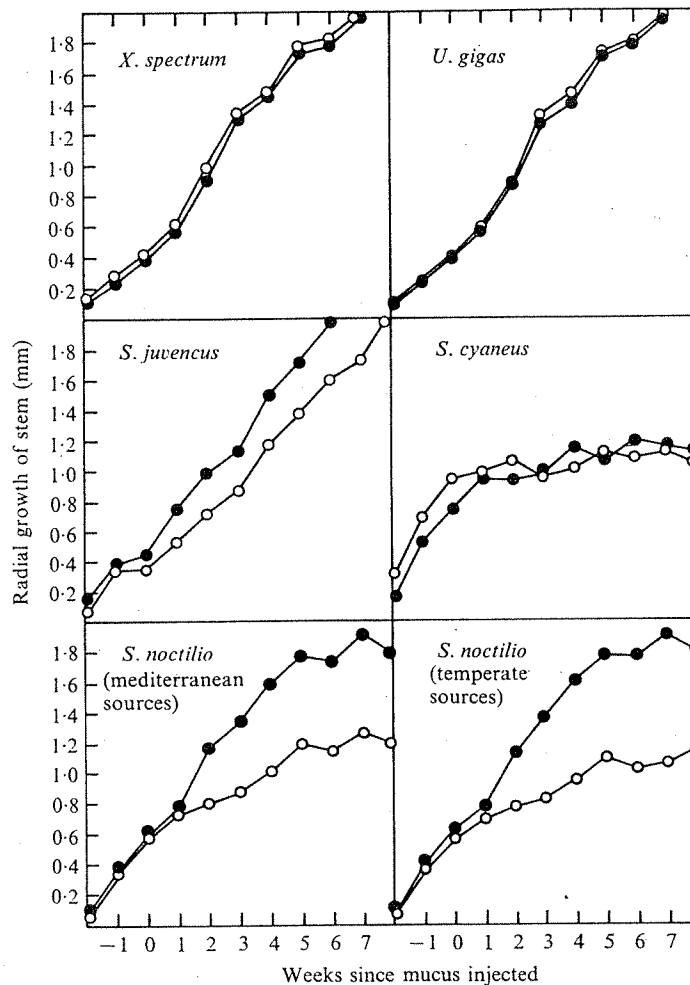


Fig. 2. Radial growth of *Pinus radiata* after injecting mucus only (mean of five trees):
●, controls; ○, mucus injected.

RESULTS

Experiments on living trees

Injections of siricid mucus alone or mucus with a suspension of symbiotic fungal spores showed that only *S. noctilio* caused pathological symptoms in trees. For example, the radial growth rate of the stem was substantially reduced within 2 weeks of *S. noctilio* mucus injections, but the mucus from the other siricid species caused no changes compared to controls (Fig. 2). Chlorosis of needles occurred in most *S. noctilio*-injected trees (Figs. 3-5) but not in the case of other siricids. The accumulation of starch in the needles was only recorded in *S. noctilio* treated trees (Fig. 6).

Marked increases in leaf pressure were recorded in those *S. noctilio* treated trees which showed severe foliar discoloration. The results of treatments on *P. radiata*, *P. sylvestris* and *Picea sitchensis* were similar. No response differences were detected after injection of *S. noctilio* mucus from Australian and Mediterranean and temperate European sources.

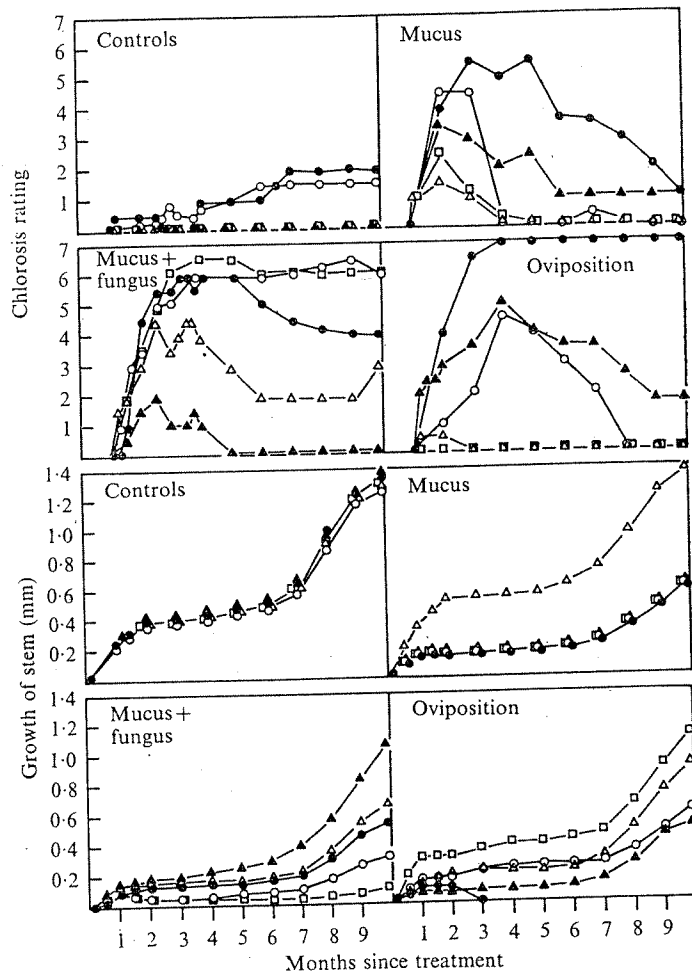


Fig. 3. Effects of *Sirex noctilio* treatments on needle necrosis and stem growth in *Pinus radiata*; each symbol denotes an individual tree.

Following induced oviposition by siricid females, only trees subjected to *S. noctilio* attack exhibited pathological symptoms. Dissection of trees confirmed that all species drilled into the stems of living trees. Oviposition density by *S. noctilio* was generally higher than the other species (Table 1), although there was considerable between-tree variation. Oviposition density by *S. noctilio* into *P. sitchensis* was less than in both *Pinus* species and penetration beyond the cambium was rarely recorded.

One tree each of *P. radiata* and *Picea sitchensis* died after *S. noctilio* oviposition, and one *P. sylvestris* tree died after injection of *S. noctilio* mucus and fungus (see Figs. 3-5). The sequence of physiological changes in the *P. radiata* tree which died is given in Fig. 7. The first detectable change was a check to growth rate of the stem 1 week after oviposition, followed by the yellowing of the older needles within 4 weeks. At this time, most of the younger needles suddenly wilted, bending in the region of

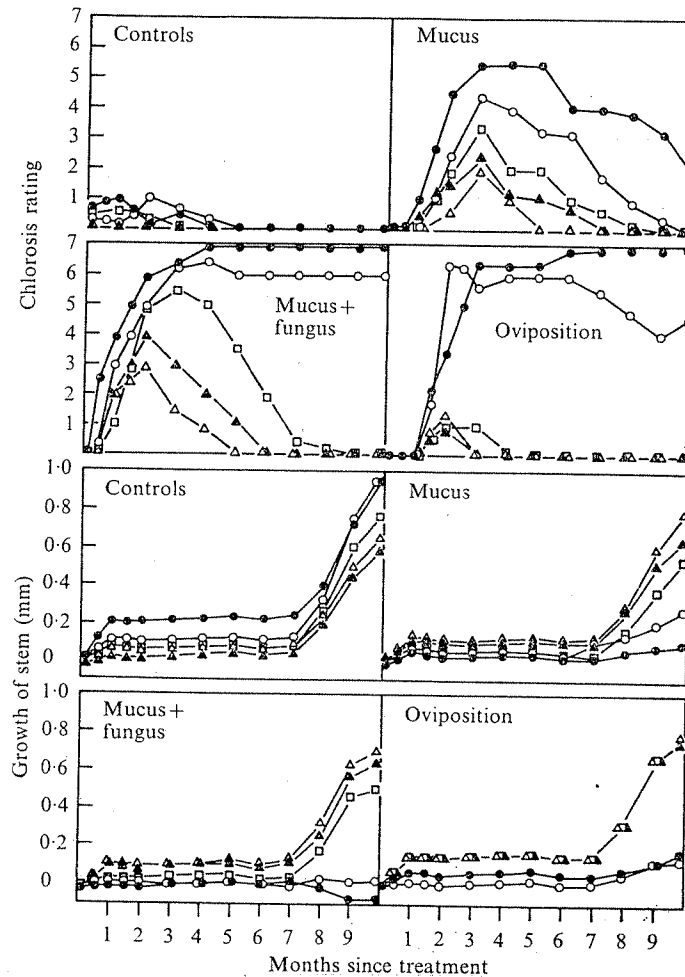


Fig. 4. Effects of *Sirex noctilio* treatments on needle necrosis and stem growth in *Pinus sylvestris*; each symbol denotes an individual tree.

the fascicle sheath. Chlorosis of the foliage was accompanied by an accumulation of foliar starch reaching a peak 6 weeks after oviposition. After 1 month, the diameter of the stem decreased rapidly, reflecting a breakdown in the water relations of the tree with consequent shrinkage. The leaf pressure increased when the needles became desiccated and their starch content decreased. After 3 months, most of the foliage had fallen and the bark of the stem was splitting as the tree dried out.

Most trees exposed to *S. noctilio* treatment exhibited symptoms similar to those of trees which died. However, after several months, recovery was accompanied by a resumption of normal growth and decrease in chlorotic rating with the abscission of chlorotic needles and growth of new foliage in the following year (Figs. 3-5).

Examination of stems in the vicinity of oviposition drills showed that only in *S. noctilio* treatments had the fungus invaded the wood surrounding the drill shafts. In the *P. radiata* tree which was killed, the fungal hyphae had completely permeated

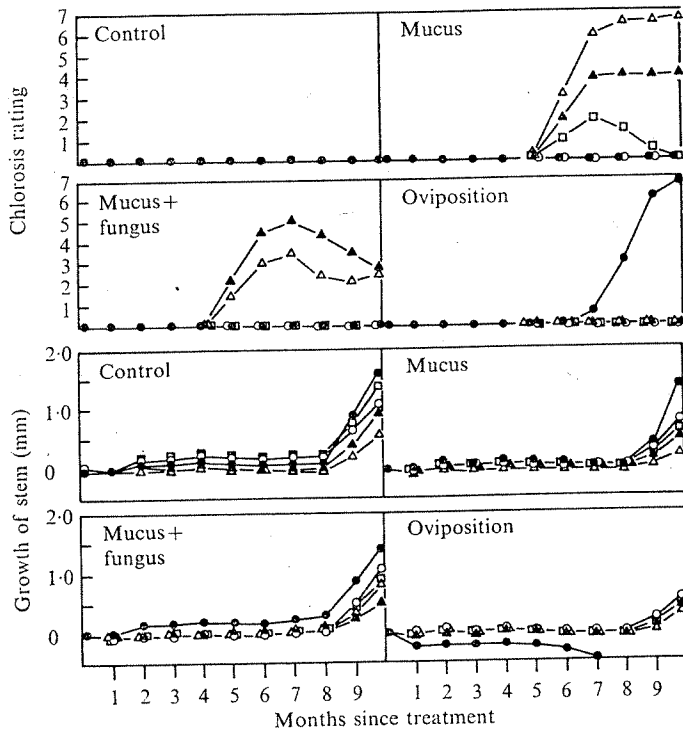


Fig. 5. Effects of *Sirex noctilio* treatments on needle necrosis and stem growth in *Picea sitchensis*; each symbol denotes an individual tree.

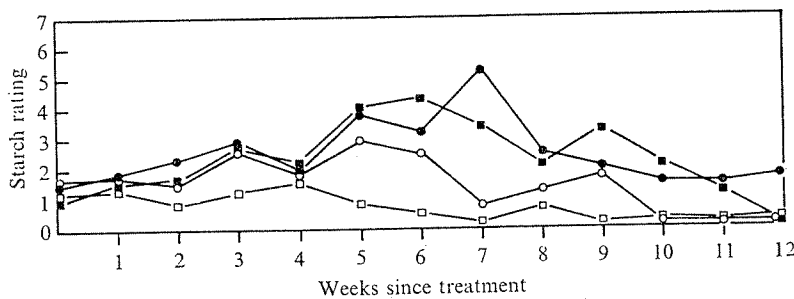


Fig. 6. Differences in the density of starch grains in the leaves of *Pinus radiata* after *Sirex noctilio* treatments (mean of five trees per treatment): ●, Mucus and fungus; ○, Oviposition; ■, Mucus only; □, Controls.

Table 1. *The effects of siricid oviposition on living trees**

Siricid species	Tree species	No. of† oviposition drills	Growth of fungus (cm)
<i>S. noctilio</i>	<i>Pinus radiata</i>	340-500	1-5
	<i>Pinus sylvestris</i>	207-500	1-5
	<i>Picea sitchensis</i>	15-203	0.5-5
<i>S. cyaneus</i>	<i>P. radiata</i>	84-120	0-0.2
	<i>P. sylvestris</i>	71-94	0-0.1
	<i>P. sitchensis</i>	12-38	0-0.1
<i>S. juvencus</i>	<i>P. radiata</i>	41-180	0-0.1
	<i>P. sylvestris</i>	64-207	0-0.2
	<i>P. sitchensis</i>	83-241	0-0.2
<i>U. gigas</i>	<i>P. radiata</i>	80-500	0-0.2
	<i>P. sylvestris</i>	74-500	0-0.1
	<i>P. sitchensis</i>	62-386	0-0.2
<i>X. spectrum</i>	<i>P. radiata</i>	2-12	—
	<i>P. sylvestris</i>	1-10	—
	<i>P. sitchensis</i>	1-14	—

* Five trees per treatment.

† Average area of bark exposed to siricids was 900 cm² (1 ft²).

the stem after nine months. In those trees which resisted *S. noctilio* attack, the fungus invaded the wood 1-5 cm from the drill shaft.

Concentrations of polyphenols and resin were found in the vicinity of oviposition shafts and drill holes of all pines which resisted treatments. In the two pines which died, no polyphenols were demonstrated although resin deposits were found in many of the oviposition shafts and the drill holes. In trees which resisted attack, varying amounts of traumatic (= necrotic) tissue were found, although new wood was laid down, frequently growing over the oviposition shafts (Fig. 8). No polyphenols were demonstrated in *Picea sitchensis*, but the quantity and distribution of resin was similar to that in *Pinus* species.

Experiments using detached shoots

The reactions of detached shoots in dilute solutions of mucus were a more sensitive indicator of phytotoxicity. All siricids' mucus caused premature senescence of needles compared with controls (Fig. 9). Increases in the degree of chlorosis of needles were accompanied by equivalent increases in leaf pressure readings. However, the *Sirex* species caused the most rapid chlorosis of needles. *S. noctilio* and *S. cyaneus* mucus had similar effects on detached shoots and caused complete chlorosis within 25 days. After rapid chlorosis of needles during the first 40 days, the remaining species' mucus caused a comparatively slower decline thereafter. *X. spectrum* mucus was the least phytotoxic. Bioassay of *P. radiata*, *P. sylvestris* and *Picea sitchensis* gave identical results although the time required to cause complete chlorosis was least in *P. radiata* and longest in *P. sitchensis*. When young shoots of *P. sitchensis* were bioassayed, only *S. noctilio* and *S. cyaneus* mucus suppressed the opening of the terminal leaf buds.

Comparison of the effects of *S. noctilio* mucus solution on twenty-nine species of pines showed a wide range of tolerance (Table 2).

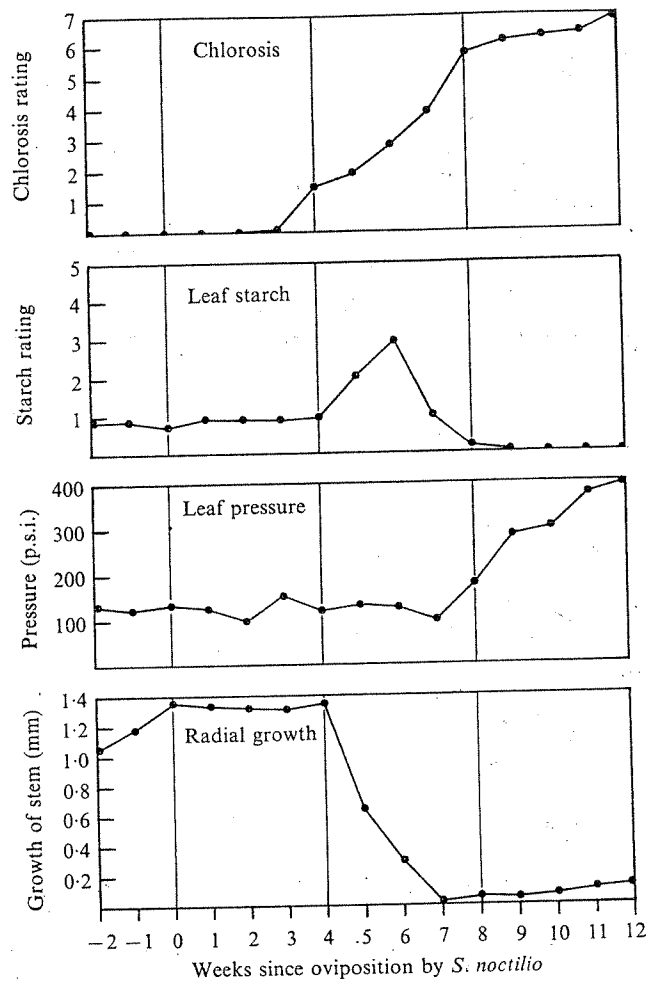


Fig. 7. Physiological changes in a *Pinus radiata* tree which died following oviposition by *Sirex noctilio*.

DISCUSSION

In southern Australia and New Zealand, the dominant conifer species is *P. radiata*, an exotic pine which is commercially grown in large monocultures. During the past two decades, the introduced *S. noctilio* has spread throughout much of the New Zealand and Tasmanian plantations and a substantial area of Victoria. The importance of *S. noctilio* as a primary pest of pine in Australasia contrasts with its relatively insignificant pest status in the northern hemisphere. Those factors which have contributed to the success of *S. noctilio* in Australasia undoubtedly include the availability of host material, its climatic compatibility with the pine areas, an absence of natural enemies and its capacity to render living trees suitable for larval development. Of the seven species of woodwasp studied here, only *S. noctilio* caused pathological symptoms

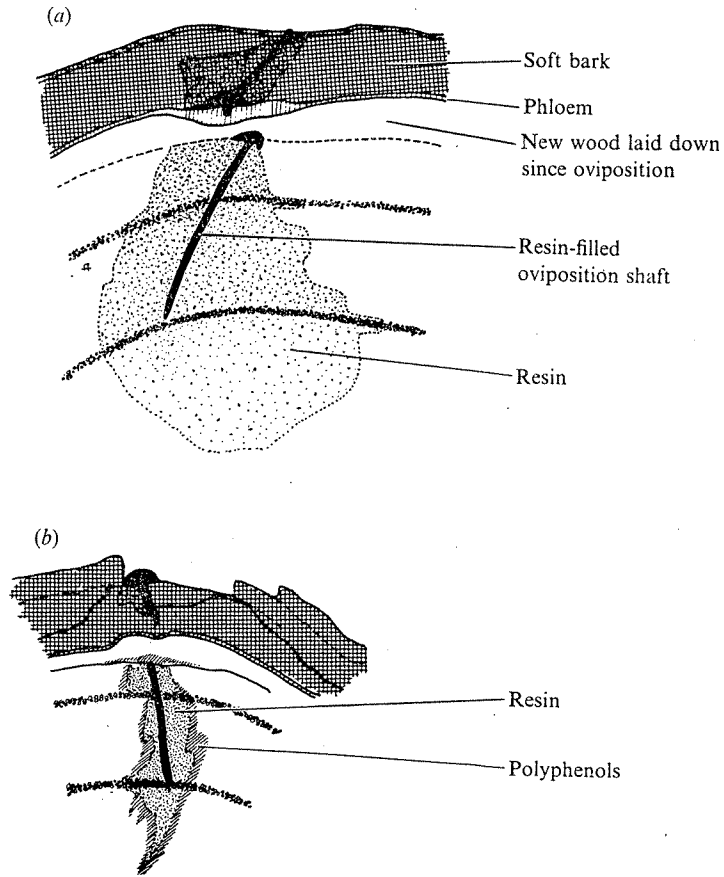


Fig. 8. Details of traumatic tissue in (a) *Picea sitchensis* and (b) *Pinus sylvestris* stems.

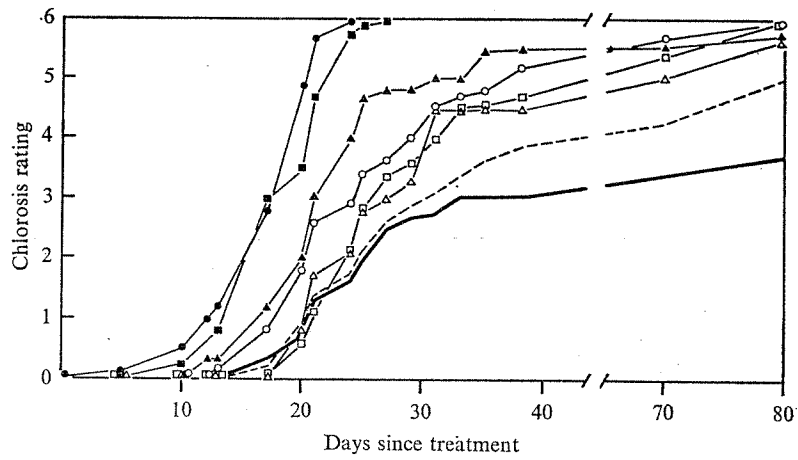


Fig. 9. Effects of dilute mucus solutions (0.1 mg/l) on detached shoots of *Pinus radiata* (mean of five replicates): ●, *Sirex noctilio*; ■, *S. cyaneus*; ▲, *S. juvencus*; ○, *Urocerus gigas*; □, *U. sah*; △, *U. augur*; ---, *Xeris spectrum*; —, Controls.

in young conifers and *S. noctilio* and *S. cyaneus* mucus caused very premature senescence of needles of detached shoots. The mucus of the *Sirex* species was more phytotoxic than that of *Urocerus* species. That of *X. spectrum*, which has no symbiotic fungus, was the least phytotoxic. The response of the different tree species was similar although there was considerable variation in the response time of individual trees. One tree each of *P. radiata*, *P. sylvestris* and *Picea sitchensis* were killed after exposure to *S. noctilio* oviposition or injection with mucus and fungal arthrospores. These results confirm the findings of Coutts (1969*b*) who showed that while neither the mucus nor the fungus of *S. noctilio* alone causes the death of a tree they are frequently lethal in combination.

Table 2. The effects of *Sirex noctilio* mucus on *Pinus* species

<i>Pinus</i> species	No. of days before leaf pressure reached 300 p.s.i.	<i>Pinus</i> species	No. of days before leaf pressure reached 300 p.s.i.
<i>P. arista</i>	32	<i>P. nigra caramanica</i>	10
<i>P. attenuata</i>	32	<i>P. nigra cebennensis</i>	10
<i>P. ayacahuite</i>	21	<i>P. nigra maritima</i>	17
<i>P. banksiana</i>	46	<i>P. parviflora</i>	21
<i>P. contorta</i>	32	<i>P. peuce</i>	28
<i>P. cembra</i>	32	<i>P. pinaster</i>	32
<i>P. echinata</i> × <i>rigida</i>	17	<i>P. ponderosa</i>	28
<i>P. gregii</i>	21	<i>P. radiata</i>	28
<i>P. griffithii</i>	12	<i>P. resinosa</i>	32
<i>P. jeffreyi</i>	28	<i>P. rigida</i>	14
<i>P. leucodermis</i>	32	<i>P. sabiniana</i>	21
<i>P. mugo</i>	12	<i>P. strobus</i>	21
<i>P. mugo</i> × <i>rostrata</i>	32	<i>P. sylvestris</i>	46
<i>P. muricata</i>	28	<i>P. virginiana</i>	32
<i>P. nigra nigra</i>	28		

The symbiotic fungus, *Amylostereum areolatum* (Fr.) Boidin, is associated with *S. noctilio*, *S. juvencus* and occasionally *S. cyaneus*, and *A. chailettii* (Pers. ex Fr.) Boidin is associated with *S. cyaneus* and the *Urocerus* species (Gaut, 1969; R. A. Bedding, personal communication). It would appear, therefore, that the species of symbiont does not play a significant role in tree pathology. Injection of *S. noctilio* mucus with arthrospores of *A. chailettii* was not possible due to lack of siricid material at the appropriate time. *X. spectrum*, which emerges early in the season, oviposits in trees previously attacked by other siricid species (unpublished observations).

Although the *S. cyaneus* treatments reported here had no effect on living trees, experiments with *S. cyaneus* from North American sources suggest that this species can cause a decrease in radial growth rate and chlorosis of needles (unpublished results). A possible explanation for the lack of response to *S. cyaneus* mucus is the timing of treatments, for injections were made in early autumn when the growth rate of trees had declined (Fig. 2). Coutts (1969*a*) has noted that wilting of *P. radiata* needles following *S. noctilio* attack was rare when meristems were inactive in late autumn.

In Europe, North Africa and Turkey, *S. noctilio* is the only siricid species known to attack living trees (A. A. Kirk, personal communication, and my own observations)

although Chrystal (1928) reported *S. cyaneus* attacking live but unhealthy conifers. *S. noctilio* has only been recorded from *Pinus* species hosts and is the only siricid species occurring in the Mediterranean bioclimatic area (Kirk, 1973). The quantity of mucus relative to the live weight of *S. noctilio* females was considerably greater than in the other species (unpublished results).

The flight period of *S. noctilio* in Europe occurs during September and October when the rate of tree metabolism (as measured by radial growth and meristem activity) has declined to its lowest level of the year. Emergence of *S. noctilio* in Australia begins in late summer with a major peak in early autumn (March and April) (Taylor, 1969) which coincides with a period of increase in growth and development of *P. radiata* (Fielding, 1966). In Australia, injections of *S. noctilio* mucus and fungus caused the death of all treated trees after 3-4 wk (Coutts, 1969*b*) but only one *P. radiata* tree was killed during my experiments in England. The rate of response by trees in England was generally slower; for example, the peak in the accumulation of starch in needles was achieved 6 wk in England, compared to 2 wk in Australia (Coutts, 1968). Differences in the cycles of insect emergence and tree growth between the northern and southern hemispheres could be a major factor contributing to the pest status of *S. noctilio* in Australasia.

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