Emplacement of Fungal Spores by the Woodwasp, Sirex Noctilio, During Oviposition

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Abstract. When ovipositing in *Pinus radiata* D. Don. the female of *Sirex noctilio* F. frequently drills two or more tunnels, which diverge from a common entrance hole in the bark. In these multiple tunnels eggs are normally laid in each tunnel except the final one where, instead, arthrospores of the symbiotic fungus, *Amylostereum areolatum* (Fries) Boidin, are deposited. When arthrospores occur in an egg tunnel this may be due to a "contaminated" ovipositor in which some arthrospores have remained from a previous act of egg and arthrospore deposition. In single tunnels, fungus is generally present but eggs are infrequent. The number of tunnels at one entrance hole appears to be related to the physiological condition of the tree, and single tunnels are relatively more common on healthy than on less vigorous trees. Thus the initial infection of healthy trees tends to be mainly with fungus and an associated secretion, which may weaken and predispose the tree to subsequent multiple tunnel attack when eggs are laid more abundantly. This might partly account for *S. noctilio* being more able than most siricids to attack healthy trees.

Additional key words. *Amylostereum areolatum*, *Pinus radiata*.

WHEN OVIPOSITING in its host tree, which in Australia and New Zealand is chiefly *Pinus radiata* D. Don., *Sirex noctilio* F. drills into the sapwood to a depth of up to 1.2 centimeters (Fig. 1). In the tunnels eggs, certain secretions, and fungal arthrospores are inserted. These arthrospores of the symbiotic fungus, *Amylostereum areolatum* (Fries) Boidin, are carried in the intersegmental sacs at the base of the ovipositor (Buchner 1928), and the fungus that develops from them often contributes to the death of the tree. Indeed the death of the tree, or part of it, is essential if the immature insects are to survive. Their development depends upon the alteration of conditions in the wood by the fungus.

Some aspects of the oviposition behavior of *S. noctilio* have been described previously (Coutts 1965). The ovipositor may be withdrawn from the initial tunnel

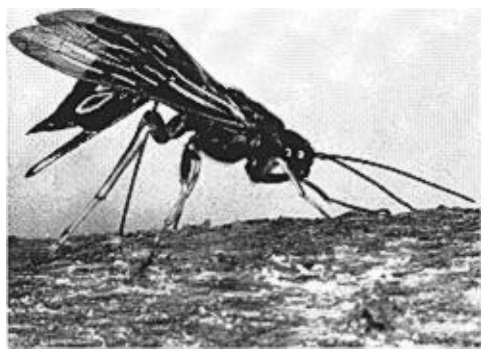

FIGURE 1. *A female* Sirex noctilio *commencing to drill into a tree with its ovipositor.* (*Photo, University of Tasmania.*)

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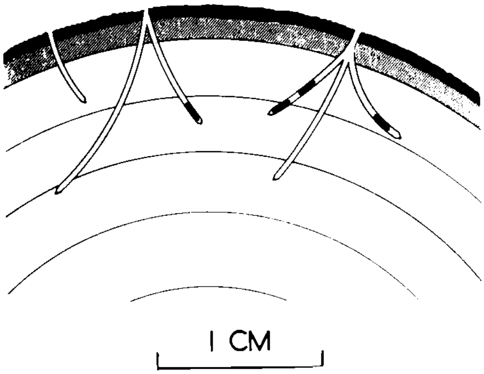

FIGURE 2. *Typical tunnels of* Sirex noctilio, *seen on a transverse section of a tree. The tunnel without an egg is always the last-made tunnel of a set.*

without an egg being laid; the insect then either flies from the tree or walks further on (usually upwards) to drill again. If an egg is laid, the ovipositor is withdrawn to the bark, angled to one side, and a second tunnel drilled into the sapwood. This process may be repeated to produce three, or sometimes more than three, tunnels fanning out laterally from a single hole in the bark (Fig. 2). Before laying an egg there is a pause in the movements of the abdomen, followed by rapid palpitation. When double tunnels are made, one or more eggs are usually laid in the first of the pair but no egg is normally laid in the second tunnel. Behavior is more variable when triple tunnels are made, but nearly always one or more eggs are laid in the first tunnel, less frequently in the second, and rarely in the third. Thus the last tunnel of a group is usually eggless, and this must be considered part of the insect's behavior pattern rather than being due to unfavorable conditions for oviposition. Female *S. noctilio*, which were disturbed after laying an egg in the first tunnel of a pair, exhibited a strong urge to complete the pair by making the second eggless tunnel.

Rawlings (1948) noted that arthrospores were inserted into tunnels whether an egg was laid or not. When *A. areolatum* grows in the tree, it causes a rapid decrease in the moisture content of the sapwood (Coutts 1965, Coutts and Dolezal 1965). The dry wood is visible as a light-colored streak a few days after attack. When wood was sectioned, it was often noted that the dry streak extended from only one tunnel of a pair, and dissection revealed that this was the tunnel without an egg. It thus seemed possible that arthrospores were deposited mainly in the second tunnel of a pair. In later studies (Coutts and Dolezal 1966b), the fungus sometimes grew from tunnels containing eggs. Experiments were therefore undertaken to investigate the relation between oviposition and the emplacement of the fungal arthrospores.

Experiment 1—Incubation of Double Tunnels

Two female *S. noctilio* were placed on each of seven logs in the laboratory, and, when they made double tunnels, the position of the first tunnel was marked. The bark was removed, and a block of wood containing both tunnels was cut out and shaved down in transverse section until both tunnels could be seen through about 0.5 mm of wood. The block was then cut to separate the two tunnels, and both halves were surface sterilized and incubated on 2-percent malt agar. From the 45 double oviposition sites, 90 tunnels were examined for the presence of eggs and fungus.

Most of the incubated double tunnels conformed to the type shown in Figure 2, with an egg in the first tunnel but not in the second. The data below show that in 76 percent of the 45 double tunnels fungus was present in the second but not in the first tunnel of the pair. In only 11 percent was fungus present in the first tunnel also.

	No. of occurrences of eggs	*No. of occurrences of fungus*
In first tunnel only	43	0
In second tunnel only	0	34
In both tunnels	2	5
In neither tunnel	0	6

Six pairs contained no fungus at all; these were all from one log and may have been made by a single insect. The fungus is transferred to the adult female before she leaves the pupal chamber, and is moved from the cast larval skin up the ovipositor to the intersegmental sacs (Francke-Grosmann 1957). The transfer of the fungus may have failed in this instance.

The body of a female woodwasp contains a large sac of mucus connected by a duct to the ovipositor. Some of this mucus was found in both tunnels, but it was more abundant in association with the fungus in the second tunnel of a pair. The pairs of tunnels cited above as containing no fungus also contained no mucus. A different type of substance is present in the egg tunnel. It stains strongly with cotton blue and dries with a crazed appearance like that in the tunnels of *Urocercas gigas* L. figured by Chrystal (1928).

Experiment 2—Microscopic and Histochemical Examination of Tunnels

Sirex females with their wings glued together were placed on five trees and left long enough to give such a light attack that the trees were certain to survive. Blocks of wood that included the *Sirex* tunnels were chiselled out after various intervals, sectioned, and stained with cotton blue to show the presence of the fungus. Twenty-one such blocks were dissected, and the presence or absence of eggs anywhere along the length of the tunnels was recorded. In the blocks were 2 triple tunnels, 18 double tunnels, and 1 single tunnel, making a total of 43 tunnels.

Whereas fungus was found in only 3 of the 20 egg-containing tunnels, it was present in all 23 tunnels with no eggs. In 25μ sections cut from some of the latter on the day of attack large numbers of arthrospores were readily seen. Germination was rapid, and hyphae were found in the wood around the fungus tunnels within 24 hours.

All 11 of the egg-containing tunnels examined within 2 weeks of oviposition contained no fungus, and there were no hyphae connecting with them. In blocks from one tree, hyphae were present around three of the egg tunnels examined between 2 and 4 weeks after oviposition; the mycelium appeared to have grown across to two of these tunnels from the associated fungus tunnels. The third egg tunnel with hyphae around it appeared to have had arthrospores inserted together with the egg—the mycelium was not continuous around the associated fungus tunnel. In all sections examined 2 to 4 weeks after attack, the fungus-infected zones were bordered by polyphenols, which appeared to prevent any further growth of the mycelium.

Experiment 3—Examination of Severed Ovipositors

The ovipositors of 13 females at various stages of oviposition were severed at the base, dissected out of the wood, and broken up on slides in lactophenol cotton-blue; and arthrospores were counted.

All ovipositors severed in the first tunnel immediately after palpitation contained eggs but, with one exception, had few or no arthrospores (Table 1). There was, of course, no way of telling whether the insects would have proceeded to make a second or third tunnel if left unmolested, since eggs are sometimes laid when only a single tunnel is drilled. Ovipositors severed while in the second tunnel contained no eggs but thousands of arthrospores, which on the slides could be seen issuing from the ovipositors in a cloud of mucus.

Experiment 4—Presence of Fungus in Single Tunnels

At various times freshly drilled single tunnels have been tested for the presence of *Amylostereum* by staining with lacto-

TABLE 1. Presence of eggs and arthrospores in the ovipositors of Sirex noctilio *at various stages of oviposition.*

Specimen no.	Presence (+) or absence (−) of eggs in ovipositor	No. of arthrospores in ovipositor
Insect about to lay an egg in first tunnel		
1	+	200
2	+	30
3	+	1
4	+	0
5	+	0
6	+	0
7	+	0
Insect starting to drill second tunnel		
8	−	0
Second tunnel completed and ovipositor still inserted		
9	−	>1,000
10	−	>1,000
11	−	>1,000
Ovipositor almost withdrawn from second tunnel		
12	−	50
13	−	0

phenol cotton-blue or by incubation on agar plates.

In the staining tests 12 out of 15 tunnels were positive for the presence of fungus, whereas 8 out of 9 excised tunnels plated on surface-sterilized agar produced cultures of *Amylostereum areolatum*, and the ninth was contaminated by another fungus. Thus of the 24 single tunnels tested 20 contained the fungus; it could have been missed in some of the others by deficiencies of technique when testing at an early stage.

Discussion

The infrequent occurrence of arthrospores in the egg tunnels and then their comparatively small numbers suggest that their presence is due to contamination from the inside of the ovipositor rather than to intentional insertion. Considering the very large number of spores deposited in fungus tunnels, it is surprising that they are not always present in the egg tunnels. It may be that the ovipositor is normally flushed out with mucus after the release of arthrospores into the fungus tunnel.

Uroceras gigas L. and *Sirex cyaneus* F are recorded as depositing both fungus and eggs in single tunnels (Chrystal 1930), and *S. augur* Kig. deposits eggs and fungus in a mass of mucilage in single tunnels (Francke-Grosmann 1939). Thus by usually placing eggs and fungus in separate tunnels *S. noctilio* differs from other siricids that have been described.

Although initially the fungus is placed some distance from the egg, it is thought to be essential for the developing larva. In successfully attacked trees the fungus is present in the egg tunnel by the time of hatching, but its stimulus is not always essential for the egg to develop and hatch—larvae were found in two egg tunnels which had been separated from their associated fungus tunnels. The egg tunnel could be infected by hyphae growing along the fungus tunnel to its intersection with the egg tunnel, but this seems unlikely in view of the frequently observed failure of the fungus to reach the egg tunnel because of polyphenol production. More probably it grows tangentially through the wood to reach the egg tunnel in approximately the same horizontal plane, although the tunnel may already have been infected from other tunnels above or below it. Heavily attacked trees may have more than 500 tunnels per square foot of bark surface, and the fungus grows more quickly in the direction of the grain than it does tangentially.

One possible advantage of the eggs and fungus being placed in separate tunnels is that the chances of the egg being flooded by resin are reduced. Histochemical studies have shown that resin appears in fungus tunnels and in the wood above and below them far more often and in greater quantities than in egg tunnels. It is not clear whether the additional substance in the egg tunnel helps to keep out resin; but inoculation experiments (Titze and Stahl 1969) have shown that the presence of *Amylostereum* definitely stimulates resin flow. By the time the fungus reaches the egg tunnel, resin synthesis would probably be impaired because attack by *S. noctilio* has a systemic effect on the tree (Coutts 1968).

Earlier work on logs (Coutts 1965) showed that 67 percent of single tunnels, 56 percent of double tunnels, and 29 percent of triple tunnels contained no eggs. Recent unpublished work on insects induced to attack healthy trees showed that 94 percent of single tunnels and 45 percent of double tunnels were eggless. In the material examined in Experiment 4, over 80 percent of single tunnels contained fungus; this supports the common observation that single tunnels generally show the streaks of drying characteristic of fungus tunnels. The other results reported in this paper indicate that the fewer the tunnels at any oviposition site the greater the proportion likely to contain fungus.

In Coutts (1965) changes in the "oviposition pattern," i.e. the number of tunnels at one site, were ascribed to variation in wood moisture content. More recently (Coutts and Dolezal 1966a) it was found that a cincturing treatment, which reduced the vigor on one side of a tree without much affecting moisture content, rendered that side highly attractive to ovipositing *S. noctilio* and induced a high proportion of multiple tunnels, whereas the vigorous side of the tree was only lightly attacked and all the tunnels were single ones. Thus healthy trees or sites are probably infected mainly with fungus and mucus placed in single tunnels, most of which will be without eggs. In time, however, the injection of fungus and mucus may reduce the vigor of the tree and make it attractive for multiple-tunnel attack when a higher proportion of the tunnels will contain eggs. This may be the mechanism by which *S. noctilio* has been able to extend its host range into healthier trees than are available to other siricids.

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