

INTRODUCTION*

It has been known for some time that insect species belonging to many orders are capable of utilizing highly refractive materials in their diet. This ability, in the case of many phytophagous forms, would not be possible without the involvement of microorganisms, viz. fungi, protozoa, bacteria and yeasts. These associations of insects and microorganisms are reciprocal, beneficial relationships termed symbioses, or more precisely, mutualisms. Mutualism is a form of symbiosis in which both parties derive advantage without sustaining injury (Henderson and Henderson 1963).

In the case of lignicolous or wood-feeding insects the involvement of a third organism, the tree, adds a further dimension in which the microorganisms may exert a toxic effect. Graham (1967), reviewing insect-fungus mutualisms in forest trees, concludes that the predominant gains derived from such associations are the maximal conversion of indigestible materials, e.g. celluloses, lignins, etc., into forms readily assimilable by insects, and the provision of otherwise unobtainable vitamins, while the insect acts as a vector in the microorganisms' dispersal.

Buchner (1965), in a treatise devoted to endosymbiosis of animals with plant microorganisms, described many morphologically distinct storage organs within many insect groups, which developed internally (mycetomes) or externally (mycangia) to the symbiont partner. These organs provided storage and sustenance to the microorganism and facilitated dispersal into new host material, usually at the time of oviposition. The contributions by Kok (Chapter 2) and Norris (Chapter 3), in this volume, emphasize the importance and complexities of scolytid symbioses.

SIRICID WOODWASPS AND ASSOCIATED FUNGI

Siricid woodwasps infest a variety of both soft and hard woods in the northern hemisphere (Cameron 1965; Wolf 1967), and they provide an interesting example of a symbiotic association with microorganisms. Most of the insect life cycle is spent in the wood of a tree. The female insect has an ovipositor of a complex structure and 1.0-2.5 cm in length, with which it drills through the bark of the tree stem into the xylem, where eggs are deposited. The larvae feed by gnawing galleries through the wood and eventually pupate inside the tree. The adult insect then emerges and chews its way to the outside.

Buchner (1928) was the first to draw attention to two pear-shaped organs at the proximal ends of the valvulae of the ovipositor of female siricids. These organs, now known as intersegmental organs, were invariably filled with the arthrospores of a basidiomycetous fungus and a mutualistic role

*See also P. H. B. Talbot, 1977. The *Sirex-Amylostereum-Pinus* association. Ann. Rev. Phytopathol. 15: 41-54—Editor.

7
chapterThe Role of Fungi in the Biology
and Ecology of Woodwasps
(Hymenoptera: Siricidae)

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ABSTRACT

A mutualism exists between woodwasps and specific fungi which results in colonization of host trees, insect development and subsequent dispersal of the fungi. Mycangia on female larvae and adults ensure the continuity of the relationship although one siricid genus has evolved from symbiosis to be a parasitic generalist exploiting timbers already infested by other woodwasp species and their fungi.

Woodwasps generally invade moribund or dead trees; however, arthropod mucus and fungus may combine to kill living trees in the *Sirex noctilio-Amylostereum areolatum* association. The ecology of this phenomenon is described from Australian studies on the dynamics of host-tree susceptibility and resistance to attack.

Moisture levels and aeration determine fungal growth and invasion of stems while resistance to attack is by resinosis and the formation of fungistatic polyphenols. Larvae derive their nutrition from the fungus and ultimate size is related to fungal growth.

The symbiotic fungus also operates as a key factor in the detection of hosts by siricid parasitoids and the life cycle of certain entomophagous nematodes.

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was suggested because spores were found to be implanted during oviposition and the mycangia of developing females became recontaminated before the insect emerged from the tree (Cartwright 1929).

Parlin (1942) demonstrated the existence of paired mycangia in larvae and thereby emphasized the close affinity of the insect-fungus association. These organs, known as hypopleural organs, were situated on the lower dorsal surface of the first abdominal segment. The hypopleural organ, which consisted of symmetrically arranged deep crypts embedded in a glandular, secretory hypodermis, maintained arthrospores in a dormant condition by embedding them in brittle wax platelets.

Rawlings (1951) reported that hypopleural organs only occurred in female larvae and Francke-Grosmann (1957) demonstrated that fungal release from the wax platelets only occurred through mechanical dislodgement or shattering at the time of eclosion of the adult when its reflex movements break up the exuviae containing the hypopleural organs. Growth of the fungus at this time resulted in the invasion of the intersegmental sacs. The form of the mycangia varies from species to species. The genus *Xeris* appears to be a degenerate form in which hypopleural organs are wanting and the intersegmental sacs are reduced and contain a variety of wood-inhabiting fungi as contaminants rather than a specific association. Species of this genus occur in woody material already infested with other siricids, so at the expense of the loss of any symbiotic association this genus exploits a wider variety of wood types infested with different species of basidiomycetes, but they forfeit the ability to attack previously uninfested material.

A variety of fungi have been found to be consistently associated with certain species of woodwasps. Cartwright (1938) showed that the mycangia of *Sirex gigas* and the wood infested by this woodwasp contained *Stereum sanguinolentum* Alb. and Schw. ex Fr. A similar but different fungus was found associated within *Sirex cyanus*. Francke-Grosmann (1939) concluded from the examination of five siricid species occurring in both soft and hard woods that a basidiomycete occurred with each species and that the fungus of any one species was closely related but dissimilar to that associated with the other species. *Stereum sanguinolentum* and *Amylostereum chailletii* were identified from different species (Francke-Grosmann 1939, 1957). The majority of these recovery experiments involved sampling the mycangia of adult females and wood infested with siricid larvae. Rawlings (1949) reported a *Stereum sanguinolentum*-like fungus associated with *Sirex noctilio* F. Talbot (1964) and Gaut (1969) concluded respectively that the true generic and species identity was *Amylostereum areolatum* (Fr.) Boidin.

The associated fungi appear to play a complex role in the insects' biology and ecology. Some insight has been obtained from observations on fungus-free females. By removing the exuviae from the pupation chamber it was possible to prevent transfer of the fungus to the adult female (Rawlings

1951; Boros 1968), and Stillwell (1966) employed this method to demonstrate the dependence of larvae on the fungus. In a comparison of the oviposition of fungus-infested and fungus-free *Sirex juvencus* females on *Abies balsamea*, successful development occurred only in those billets in which oviposition had been carried out by fungus-infested females. Rawlings (1951, 1953) reported that the fungus provided a suitable environment for egg incubation and that it regulated moisture content, while Francke-Grosmann (1939) had proposed a nutritive role. In *S. noctilio*, as discussed below, the associated fungus, with a mucus, was also involved in reducing the intensity of the host tree's reaction to attack, which, if not moderated, would be harmful to the insect's developmental stages.

INVESTIGATIONS OF *SIREX NOCTILIO* IN AUSTRALIA

Sirex noctilio was first discovered in Tasmania in 1952 (Gilbert and Miller 1952) and since that time has caused extensive damage to *Pinus radiata* plantations in the states of Victoria and Tasmania. The National Sirex Fund was established in 1960, and full-scale investigations of the insect, the fungus, and the tree commenced; and it is from these studies that further discussion was prepared.

Host trees were attacked during the summer months and successful attack resulted in the death of trees and invasion of the stem with fungus. The number of females attacking an individual tree varied with the size and availability of the field population; thus small trees (less than 2.5 cm d.b.h.) were sometimes attacked and killed by one female whereas dominant trees were observed with as many as 50 females drilling with their ovipositors into their stems. In many instances the attacked tree became chlorotic in the apical region 10-14 days after attack and this chlorosis was progressive throughout the foliage depending on attack intensity and the tree's susceptibility to attack. Irreversible wilting of crown needles was an early indication of successful attack (Coutts 1969a).

The chlorotic phenomenon was shown to be due to the effects of a mucus inoculated into the sapstream of the attacked tree by the female wasp during drilling (Coutts 1968; 1969a and b). Spradbery (1973) showed that this response was more likely to occur where mucus of *S. noctilio* was involved rather than with that from other siricid species and that the foliage of *P. radiata* was more susceptible than that of other tree species tested. The emplantment and successful germination of arthrospores resulted in localized drying in the vicinity of the drilling lesion and this drying effect was aided by the presence of mucus (Kile and Turnbull 1974a). The biochemical nature of the mucus was reported by Fong and Crowden (1973).

Individual *P. radiata* trees were rendered attractive to *S. noctilio* by a number of surgical treatments which resulted in different, yet characteristic

patterns of attractiveness (Madden 1971). The basis of this attraction was shown, in a general way, to be the increased rate of release of monoterpene volatiles through the bark following alterations in its permeability by changes in its osmotic and respiratory relationships (Madden 1968a, 1977).

Drilling was accompanied by the deposition of arthrospores and mucus, and the kind of drill made through a single hole in the bark varied from a single up to multiples of five or six. In each instance, the drill made prior to the removal of the ovipositor contained the arthrospores and mucus while the other drills contained eggs (Coutts and Dolezal 1969; Madden 1974; and Table 7.1). Attacked trees with high osmotic pressures of the phloem sap ($> 18.0 \times 10^5$ Pascals) contained only single drills initially, i.e. only mucus and arthrospores were inoculated, and the combined effect of the contents of these drills acting with the initial stress treatment was to progressively reduce osmotic pressure so that increasingly higher proportions of multiple drills were made in time and, in consequence, greater numbers of eggs were deposited. The *S. noctilio* female therefore displayed an economy in egg output by regulating release with respect to host tree physiology.

Coincident with the suppression of host tree physiology by edaphic, climatological or biological factors, was the suppression of its defensive reactions to attack. Coutts and Dolezal (1966) reported that resinosis and polyphenol formation at the drilling lesion site resulted in egg and larval mortality and the containment of the fungus respectively. Coutts and Dolezal (1966) also showed that polyphenol formation in the xylem was reduced by isolating the bark containing drills from translocation. Hillis and Inoue (1968) identified the defensive polyphenols as pinosylvin and pinosylvin monomethyl ether, two of the four naturally occurring heartwood polyphenols of *P. radiata* and the only two possessing appreciable fungistatic activity. Kile and Turnbull (1974b) found that monoterpene volatiles suppress *A. areolatum* growth. The reductions of turgor and

170 INSECT-FUNGUS SYMBIOSIS

translocation by any means suppressed the operation of these resistance mechanisms and hence their effects.

Larvae hatched from eggs in the absence of resistance when the lumen of the drills and adjacent xylem vessels were invaded by fungal hyphae and first and some second stage larvae fed exclusively on the fungus before entering the wood. Later stage larvae fed through fungus-invaded wood and the high nutritive status of the fungus was reflected in the incremental changes in larval gallery diameter relative to the volumes of wood frass excreted within early and late stage instars. The conversion ratio fell by a factor of 20 from the second to the fifth instar (Madden, unpublished data).

The number of instars varied from seven to 12 depending on conditions of aeration and moisture content within the infested log. King (1966) reported that optimal temperature for *A. areolatum* growth was between 20–25°C in cultural studies and Coutts (1965) found that the optimal moisture content of pine sapwood was between 60–70% (oven dry weight). Different-sized adult insects emerged at the same time from infested billets which had different initial moisture contents, and which had been incubated at the same temperature. It was also observed that larvae arising from eggs in the same oviposition drill but, encountering different conditions of wood moisture, and thus fungal growth and activity, were significantly different in size.

Microorganisms other than the fungus recovered from the midgut of *S. noctilio* larvae and larval frass included *Saccharomyces*, *Flavobacterium*, *Azotobacter* and *Acetobacter* (Madden 1975). The nitrogen content of larval frass in the galleries of large, actively feeding larvae was six times greater than that of the surrounding generally fungus-infested wood (0.03% Kjeldahl) and its moisture content was greater by 1.5–2.0-fold. Although nitrogen fixation could occur, the results suggested that the grazing activity of the larva within the gallery resulted in a net migration of both water and nitrogen via the fungus to the larval space for the ninhydrin positive materials contained in alcohol extracts of frass were comparable to those in extracts of the fungus. The differentials in moisture and nitrogen contents between frass and wood in wet or dry logs containing slowly developing larvae were either poorly represented or absent (Madden, unpublished data). Adults emerge through the bark in the summer following pupation in late spring.

Madden (1977, and Fig. 7.1) has proposed that damage to trees resulted in an initial loss of water through the stomata and increased water tension of the tree system. As a result of these changes, translocation was inhibited and a respiratory stress within the phloem was created with a reduction of both the osmotic and turgor status of the tree. Rupture of the xylem vessels by the ovipositor resulted in the invasion of these elements by air and the creation of optimal conditions for fungal growth and invasiveness and subsequent development of the siricid larvae.

Table 7.1 Frequency of Eggs in Single and Multiple Drills of *S. noctilio*.

| | Single drills | Double drills | Treble drills | Quadruple drills | Total Total |
|------------------------------|---------------|---------------|---------------|------------------|-------------|
| Total drills dissected | 749 | 392 | 172 | 55 | 1368 |
| Number with no eggs | 718 | 152 | 27 | 2 | 899 |
| Number with one egg | 31 | 208 | 56 | 7 | 302 |
| Number with two eggs | 0 | 32 | 70 | 27 | 129 |
| Number with three eggs | 0 | 0 | 18 | 15 | 33 |
| Number with four eggs | 0 | 0 | 1 | 4 | 5 |
| Proportion with eggs | 0.042 | 0.610 | 0.784 | 0.965 | |
| Mean No. per group with eggs | 1.00 | 1.14 | 1.92 | 2.30 | |
| Mean No. eggs per group | 0.042 | 0.684 | 1.550 | 2.220 | |

The interdependence of the insect-fungus relationship was also reflected in the behavior and biology of certain natural enemies of *S. noctilio* and other siricids. Madden (1968b) and Spradbery (1970a and b) found that the cynipid, *Ibalia leucospoides* Hochenw. and the ichneumonids, *Rhyssa persuasoria* (L.) and *Megarhyssa nortoni nortoni* Cressou, important parasitoids of siricids, exploit materials of fungal origin in their location of hosts. In the former instance, volatiles produced during fungal establishment resulted in the detection of drills containing mature eggs or early instar larvae while materials associated with mature fungal growth, and particularly concentrated in fresh, wet frass, resulted in the exploitation of larval hosts deep in the wood by the latter group of parasitoids during the spring prior to pupation.

Bedding (1967, 1968, 1972) reported on a unique instance of female dimorphism in the entomophagous nematode, *Deladenus siricidicola* Bedding. The free-living form was mycetophagous and fed specifically on *Amylostereum areolatum* whereas in the presence of the siricid host and its frass a non-mycetophagous, infective female was formed which invaded the larva and resulted in sterilization of the adult female. The mycetophagous form possessed the diagnostic features of the Neotylenchidae while the entomophagous form compared to those of the Allotonematidae.

CONCLUSIONS

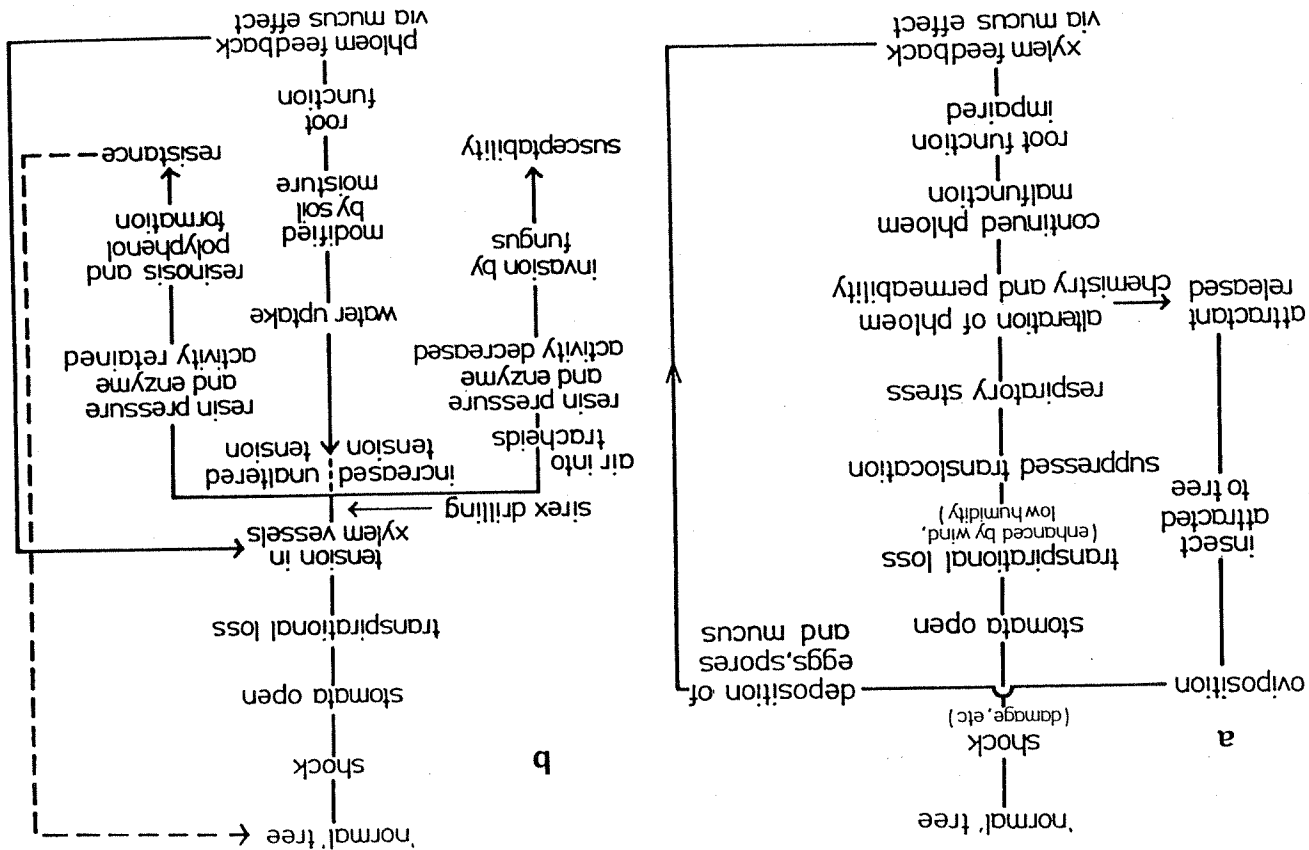
The association of *A. areolatum* with *S. noctilio* has been found to be not only essential to a continued existence of the insect, through its debilitating effects on the host tree and the provision of larval food, and the fungus, through its active transport to new host trees, but also to the existence and success of siricid parasitoids and nematodes. In the former case the fungus provided olfactory uses which resulted in the detection of hosts and in the latter, the *S. noctilio* female was the vehicle of dispersal for the nematode and the fungus upon which the nematode fed.

Research on *S. noctilio* has provided much information on the distribution of siricids, their host trees and natural enemies and the basis for definitive studies on the association of different fungi with the different siricids and their role in larval nutrition.

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Figure 7.1. Possible sequence of events occurring within (a) the phloem and (b) the xylem of *P. radica* which may influence the establishment, or otherwise, of *A. areolatum* and its symbiont partner, *S. noctilio*.



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- 174 INSECT-FUNGUS SYMBIOSIS
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