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SYMBIOSIS AND SIRICID WOODWASPS

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The siricid woodwasps are well known as insects of considerable economic importance in forestry, and the habits, development and control of the several species which occur in this country were discussed in detail by Chrystal (1928) and Hanson (1939). These wood-borers have an additional interest for biologists, because they live in symbiosis with certain fungi, which are themselves of economic importance as wood-destroyers. Whereas the fungi in question are of common occurrence apart from the insects, the woodwasps, with the exception of *Xeris spectrum* L. (Francke-Grosmann, 1939), have been found only in close association with the fungi.

The symbiosis was first described by Buchner (1928, 1930) and has since been investigated by Cartwright (1929, 1938), Clark (1933), Müller (1934) and in particular by Francke-Grosmann (1939). As a result of these researches, the history of the fungus has been traced almost completely through the egg, pupal and imaginal stages of the insect, but the occurrence of fungus in the larva, other than in the contents of the alimentary canal, has remained uncertain. The aim of the present work has been to confirm and to extend the observations and conclusions of other workers and, especially, to ascertain whether there is a close association between fungi and siricid larvae. Owing to the war it has not been possible to complete the investigation, but this paper gives an account of the work so far carried out. A note on the most interesting result has already been published (Parkin, 1941).

Buchner (1928, 1930) discovered in the female imagines of *Sirex gigas* L. a pair of small invaginated intersegmental sacs, which projected into the body cavity at the anterior end of the ovipositor and were filled with the oidia of a basidiomycete fungus. He found similar structures in several species of the Siricinae and Xiphydriinae, and the list has been extended by other workers. Various species of basidiomycetes have been isolated from the sacs of some of the Siricinae, but Cartwright (1938) reported that he cultured an ascomycete from *Xiphrydia prolongata* Geoffr. The only exception is *Xeris spectrum* L., which was reported by Francke-Grosmann (1939) to possess very small intersegmental sacs devoid of fungal contents.

My investigations have been confined to *Sirex gigas* L. and *S. cyaneus* F., in which dissection from above reveals the intersegmental sacs as a pair of brownish or pinkish ovoid structures lying on the floor of the abdominal cavity at the anterior end of the ovipositor. Sections show that they are thin-walled and have a chitinous lining which is perforated by the openings of numerous long sinuous ducts originating from unicellular glands in the club-shaped proximal ends of the lateral ovipositor stylets. Fungal oidia, consisting of nearly straight lengths of one to four cells on which clamp connexions are usually prominent, fill the cavity of the structure. It is easy to transfer some of the oidia on the point of a sterilized needle to a malt-agar slope and grow a pure culture of the fungus. Francke-Grosmann (1939) concluded from examination of five species of siricids that the individual

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species are not always associated with the same fungus, although a definite fungal species appears to predominate within a single species of woodwasp. Cartwright (1929, 1938) and myself, however, found in the course of many isolations from *S. gigas* and *S. cyaneus* only one species of fungus, identified by Cartwright as *Stereum sanguinolentum* (A. & S.) Fr. There are slight but distinctive differences in the general appearance of the mycelia grown from the two species of woodwasp, but they are insufficient to be classed as more than varietal differences. Clark (1933) isolated from *Sirex noctilio* F. in New Zealand a fungus which he states is identical in culture with *Stereum sanguinolentum*. Since this species of fungus is a white rot of softwoods, other species must be present in woodwasps which attack hardwoods. No fungus has been detected in adult males, and it is evident that the association is confined to the female wasps.

The intersegmental sacs open into the channel of the ovipositor and not into the oviduct. They have a thin superficial musculature, and during oviposition small amounts of the oidia are extruded into the ovipositor so that each egg becomes infected as it is laid. Cartwright (1938) said that the fungus in *Sirex cyaneus* appears to develop definite mycelial growth on the egg before oviposition, but I have not been able to obtain any fungal growth by incubation on malt agar of eggs of *S. gigas* and *S. cyaneus* dissected out of the ovaries or oviducts. On the other hand, I have made strong, pure cultures of *Stereum* from an egg laid reflexly by a female *Sirex cyaneus* during dissection in physiological salt solution, and from eggs cut out of oviposition tunnels in wood. Cartwright (1929) stated that the fungus can be introduced into sound wood through the oviposition tunnels of *Sirex*, a point of obvious economic importance.

In the oviposition tunnel the oidia on the eggs rapidly develop into hyphae which penetrate the surrounding wood and precede the larva in its boring. It is not yet clear how the larva obtains its nourishment from the decayed wood. Buchner (1928, 1930) supposed that the fungus 'predigested' the wood for the insect. Müller (1934) analysed the food wood and frass of *S. gigas* and *S. phantoma* L., and showed that the frass contained less cellulose and pentosans, but in the absence of detailed knowledge of the effect of the preceding fungal attack the conclusion that woodwasp larvae can themselves digest the cell-wall constituents of the wood is not permissible. Francke-Grosmann (1939) is of the opinion that the larvae live on the fungal mycelium ingested with the wood and, in support of this, has shown that hyphae in sections of attacked wood are rapidly destroyed in vitro by the larval digestive juices. This view is supported by Cartwright's (1929) observations that a newly hatched larva was able to live, and apparently feed, for 3 weeks on a culture of *Stereum sanguinolentum*, and a half-grown larva lived and definitely fed for 3 months. Although the fungus must play an important part in the nutrition of the larvae, its exact role can be determined only after further experiment.

Cartwright (1938) stated that 'sections across late stage pupae also showed fungus to be present in the glands at the base of the ovipositor in the case of female pupae', but Francke-Grosmann (1939) considered that pupae are sterile with regard to fungi and concludes that the fungus must grow into the invaginated intersegmental sacs from the wall of the pupal chamber after the pupal skin is cast and while the immature adult is hardening. She obtained a female *Sirex gigas* free from fungus by removing it from the wood before the pupal skin had been shed; sister woodwasps, emerging normally, were infected. My observations support Francke-Grosmann's conclusions. I have been unable to detect any fungus in sections of female pupae and all attempts to culture fungi from the tissues in the region of the developing sacs have failed. In addition, the fungus could not be obtained in culture from a young female *S. cyaneus* cut out of the wood immediately after eclosion from the pupal skin, but was grown without difficulty from a female captured as it was about to emerge from the log. It appears, therefore, that the fungus enters the intersegmental sacs of the adult female woodwasp after the pupal skin has been cast. It must be assumed that the unicellular glands in the heads of the lateral ovipositor stylets discharge into the lumina of the sacs a substance which acts both as an attractant and a food for the fungus. The hyphae probably grow from the wall of the pupal chamber into the sacs where they proliferate until the nutriment is exhausted and then break up into oidia. On the other hand, although I have successfully cultured *Stereum sanguinolentum* from the intersegmental sacs of newly emerged virgin *Sirex cyaneus* females, other females, extracted from their pupal cells before the exit-holes were large enough to allow emergence and kept without mating for 8 days, possessed tiny sacs containing scarcely any fungus. This observation requires confirmation before it is concluded that secretion of sufficient fluid to enable the fungus to proliferate does not take place until after the stimulation of mating. Francke-Grosmann (1939) raised the interesting point, whether the intersegmental sacs have developed under the influence of symbiosis or whether they were formed to permit a considerable range of movement of the proximal end of the ovipositor and have only secondarily led to the development of a stable symbiosis.

The symbiotic cycle in *Sirex* can thus be completed without reference to the larva. Clark (1933), however, reported the discovery of organs containing fungus in the region of the hindgut of *S. noctilio* larvae and states that they correspond to those found in the adult female of *S. noctilio*, and are carried from the larva through the pupal stage to the adult. From one individual he obtained a culture of *Stereum sanguinolentum*, but his other attempts at isolation were ruined by contaminants. Müller (1934) and Francke-Grosmann (1939), apparently unaware of Clark's work, stated that, apart from mycelial remains in the gut, no fungi could be detected in sections of larvae at different stages of development.

My investigation into the occurrence of fungi in the larvae of *Sirex gigas* and *S. cyaneus* has yielded results which differ completely from the findings of the three workers mentioned above. Careful examination of serial sections of larvae has disclosed the presence of peculiar epidermal structures containing fungal strands. These structures were sought on the living insect and have been found on some, but not all, larvae. There is one on each side of the body, concealed in the deep fold between the first and second abdominal segments, and formed by local modifications of the cuticle and hypodermis on the posterior sides of the hypopleural folds of the first abdominal segment. By reason of their position they will be referred to as the hypopleural organs. The infolding of the skin between the corresponding two segments of larvae not possessing these structures is noticeably shallower. There is evidence for assuming that the organs occur on female larvae only. When part of an infested log was split 30 larvae and 43 pupae and immature *S. cyaneus* wasps were found: of the larvae 18 possessed hypopleural organs, a proportion of 1 to 1·67, and 25 pupae and wasps were females, a proportion of 1 to 1·72. Unfortunately, larvae kept in Petri dishes failed to pupate, and the sexual significance of the presence or absence of hypopleural organs could not be confirmed. I have been unable to detect any similar organs on first-stage larvae examined whole or in sections, but they can be seen in larvae one-quarter to one-third grown and become larger as the larva grows.

Microscopic examination of pieces of larval skin, excised so as to include a hypopleural organ, shows (Fig. 1) that in surface view the general outline of the structure is fusiform, the long axis being slightly curved so that the concavity faces the middle line of the insect. The cuticle is pitted in a characteristic fashion, and the size and number of the pits increase as the larva grows. Measurements of the hypopleural organs of nearly fully grown larvae are as follows:

Species	Length	Max. width
S. cyaneus	1·22 mm.	0·25 mm.
S. gigas	0·94 mm.	0·31 mm.

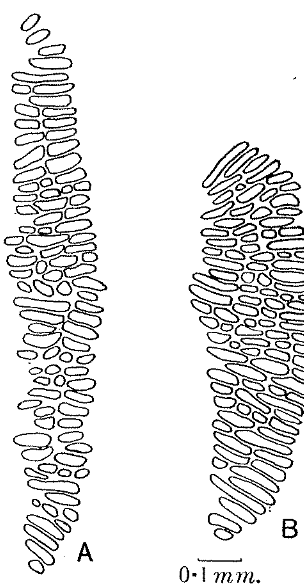

Fig. 1. Surface view of larval hypopleural organ, showing arrangement of pits. A, *Sirex cyaneus*; B, *S. gigas*.

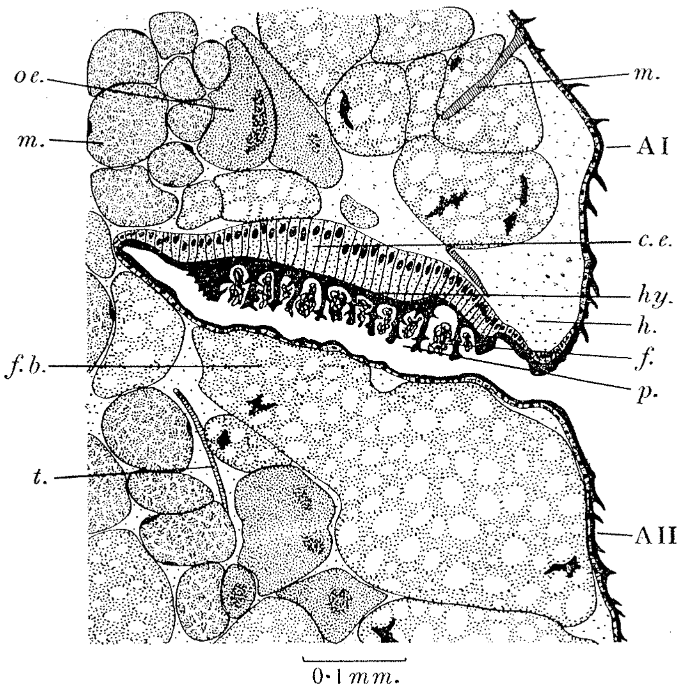

Fig. 2. Part of longitudinal section through *Sirex cyaneus* larva in region of hypopleural organ. A I, A II, first and second abdominal segments; *c.e.* columnar epithelium; *f.* fungal threads; *f.b.* fat body; *h.* haemocoele; *hy.* hypopleural organ; *m.* muscle; *oe.* oenocyte; *p.* pit in cuticle; *t.* tracheole.

The different proportions of length to width makes it possible to distinguish the hypopleural organs of the two species. Examination of a siricid larva of unknown species, found in a log of softwood imported from Poland, showed that, although the organ was of similar shape to that of *S. gigas*, the individual pits were very small and much more numerous in each transverse row. It is believed that the hypopleural organs may be used not only to indicate the sex of siricid larvae, but also to identify the various species.

In section (Fig. 2), the organ appears as a series of deep recesses in the thickened cuticle, the walls of the recesses being furnished with spines. These spines serve to retain within each pit a mass of fungal threads so closely intertwined that it has not been possible to determine whether the fungus is present as hyphal filaments or oidia. Clamp connexions have not been observed, but this may be a fault of the staining technique which was directed to differentiation of the insect tissues. The underlying hypodermal cells are greatly enlarged to form a columnar epithelium which, no doubt, has a special secretory function. It is not known whether this hypertrophy is connected with secretion of the complex cuticular structure at each moult or whether the cells provide a nutrient material for the fungus.

In order to discover the identity of the fungus, larvae of *S. gigas* and *S. cyaneus* were immersed for a few seconds in 95 % alcohol and dried on a filter paper previously sterilized in alcohol. A small piece of skin including a hypopleural organ was dissected off, freed from adhering muscles, fat and tracheae, and planted on a slope of malt agar in a test-tube: all instruments used in these operations were sterilized in a flame or in alcohol. The tubes were maintained at 22° C. and, after a few days, a strong pure growth of *Stereum sanguinolentum* was observed in the majority: the identity of the fungus was confirmed by Cartwright. In the remaining tubes growth of this fungus was often visible before it was swamped by contaminants. Isolation of *Stereum* was particularly difficult when larvae were cut from wood heavily infected with *Trichoderma lignorum* (Tode) Harz.

Discussion

In spite of recent advances in knowledge of the symbiosis between siricid woodwasps and wood-destroying fungi, there are a number of puzzling features requiring further investigation. There is still doubt as to whether each species of woodwasp is associated with a particular species or variety of fungus. Francke-Grosmann (1939) considered that the relation is not so specialized, but Cartwright (1929, 1938) and myself have never isolated from *Sirex gigas* or *S. cyaneus* any other fungus than *Stereum sanguinolentum*, except as a contaminant. We have independently isolated this fungus on many occasions and at considerable intervals of time from adults, eggs, oviposition tunnels, and larval galleries. In addition, I have regularly isolated the same species from the hypopleural organs of half to fully grown larvae. It therefore seems that this species of fungus is the only one in this country associated with the two woodwasps mentioned: in this connexion Clark's (1933) isolation of the same fungus from *Sirex noctilio* in New Zealand is of particular interest. Finally, the case for some degree of specificity is supported by Hanson's (1939) observation that *Sirex* larvae never burrow in wood penetrated by *Fomes annosus* Cooke and often die when tunnelling through wood decayed by *Armillaria mellea* Vahl., although trees killed by either fungus are attractive to adult wasps for oviposition.

Buchner (1928, 1930) emphasized the close association between woodwasps and fungus, which he supposed had resulted in the development in the adult female wasp of special organs for storage of the fungus and for its transmission to the eggs during oviposition. Francke-Grosmann (1939) suggested that the intersegmental sacs were originally developed as lubricating organs for the basal parts of the ovipositor and that they have become regularly invaded by fungal hyphae growing from the wall of the pupal chamber during the quiescent period between the casting of the pupal skin and the emergence of the hardened wasp. Neither view, however, seems to offer any explanation of why the fungus should occur in special organs on a proportion of the larvae. Nothing is yet known about the fate of the larval hypopleural organs during moulting, but, as that part of each organ which actually contains the fungus is a cuticular structure, the association must presumably be broken each time the skin is cast. If this is so, it must be assumed that the hypopleural organs of the newly moulted larva are reinfected by hyphae growing in from the wall of the tunnel in the wood. One may well ask, what attracts the fungus to grow into the organs and what can be the value of the fungus to the larva when the association is broken and must be re-established at each ecdysis? Also, why does the fungus occur only in some larvae, probably the females? Since the remaining larvae exist without fungus in bodily association, the contents of the hypopleural organs can apparently have no direct influence upon growth or metamorphosis, especially as they are virtually external to the body of the larva.

In the absence of fungus from the pupa, as shown by the observations of Francke-Grosmann (1939) and myself, it becomes all the more difficult to understand why female larvae should develop a pair of special structures in which the hyphae are intermittently stored. The fungus seems to be unnecessary to the larva except in the wood surrounding the gallery, where its presence has, no doubt, a nutritional significance. The mycelium may itself be the chief food of the larva or it may render the wood more available or more easily digestible for the insect. The larval gut, however, is much simpler than the types generally found in larvae digesting wood and Cartwright (1929) and Francke-Grosmann (1939) produced evidence to support the view that siricid larvae are mycetophagous. In either case, why should only *Stereum sanguinolentum* be associated with *Sirex gigas* and *S. cyaneus* (and perhaps *S. noctilio*), when one might think that any other fungus of similar type attacking softwoods would serve the insect equally well? The answers to these questions may be forthcoming when the investigation can be resumed.

Summary

A reinvestigation of the association between fungi and the woodwasps, *Sirex gigas* L. and *S. cyaneus* F., showed that a single species of fungus, *Stereum sanguinolentum* (A. & S.) Fr. is present in the intersegmental sacs situated at the anterior end of the ovipositor of adult females. The egg becomes infected with fungal oidia at the start of its passage down the ovipositor. When the egg has been deposited in timber, mycelial growth commences and the fungus subsequently precedes the larva in its boring. The larva is probably, at least in part, mycetophagous. Modifications of the larval integument on the posterior aspect of the first abdominal segment have been discovered. The structures so formed, termed hypopleural organs, contain fungus which has been cultured and identified as *Stereum sanguinolentum*. The organs are found in a proportion of the larvae, which suggests that they occur in female larvae only. The fate of the larval hypopleural organs during ecdysis or at pupation is unknown, nor can the importance of these organs in the symbiotic cycle be assessed. No fungus could be detected in pupae and it is thought that the fungus must grow from the walls of the pupal chamber into the intersegmental sacs of the immature female immediately after emergence from the pupal skin.

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