Figure 21.

Germinating wax packets of <u>S. noctilio</u>.

a. Mag. 120x.

b. Fungal threads are growing from the side damaged with a sharp needle. Mag. 650x.

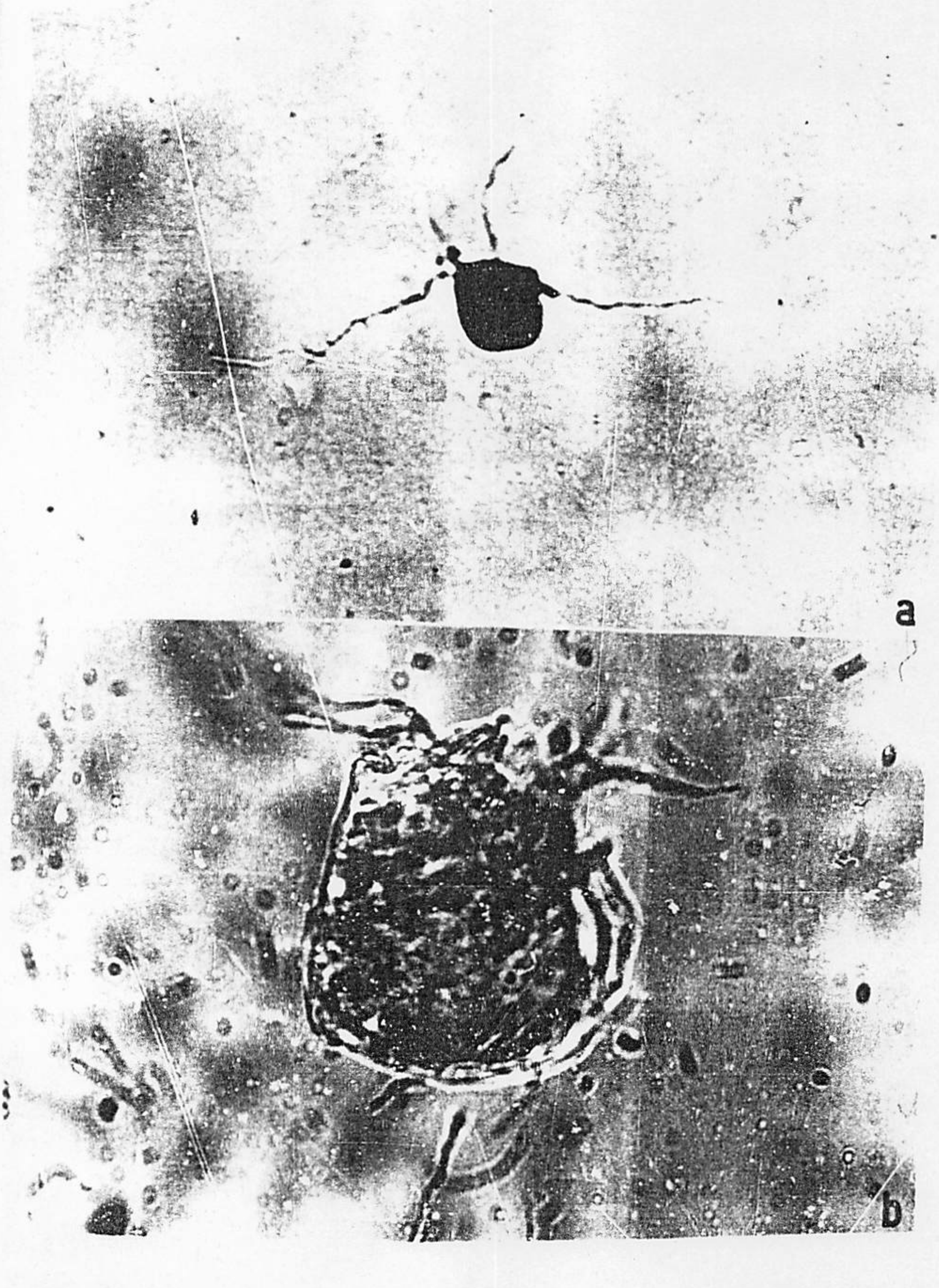

FIG. 2 1

Figure 22a.

Fungus growing on scrapings of wood in pupal chamber.

Figure 22b.

Loose fungal threads found in inter-segmental sacs of adult female which had started boring.

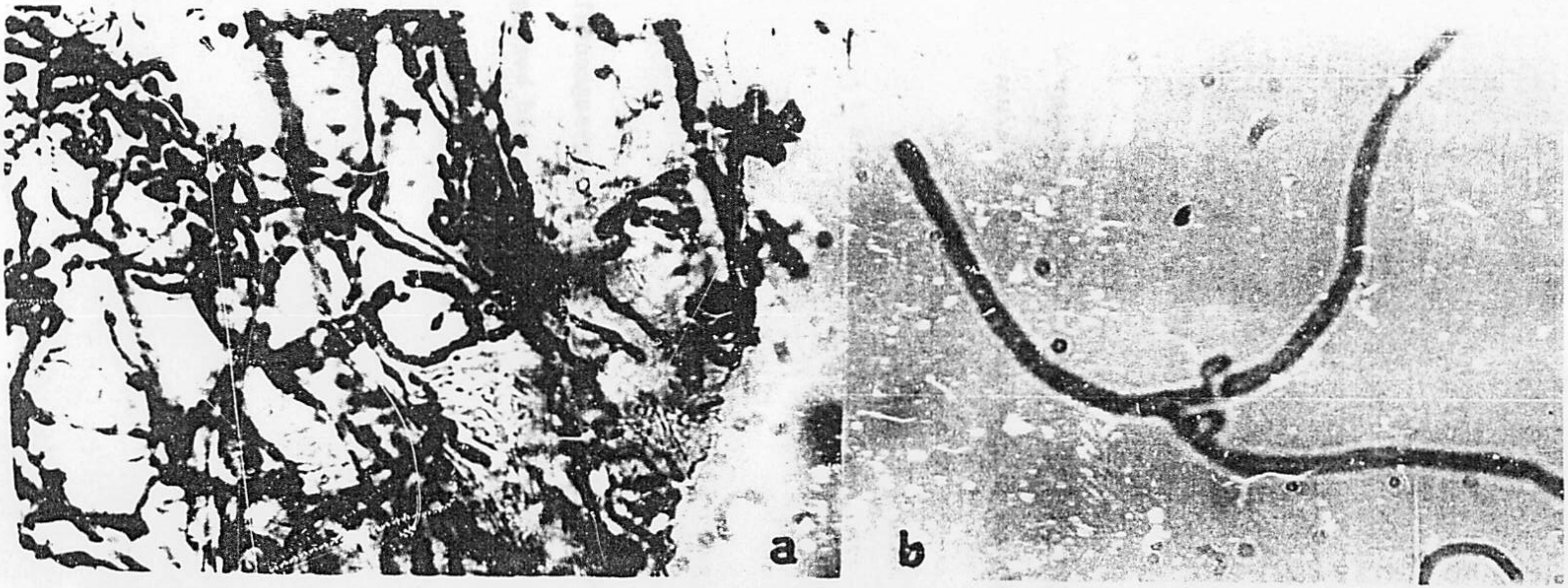

FIG.22

Figure 23.

Experiments to show the effect of the secretions of the female accessory glands on the vegetative growth of the fungus.

a. Corona around isolate after three days on Water Agar.

b & c. Controls.

d. Effect of mucus on the vegetative growth of the fungus.

e. Effect of oil on the vegetative growth of the fungus.

f. Effect of a mixture of oil and mucus on the vegetative growth of the fungus.

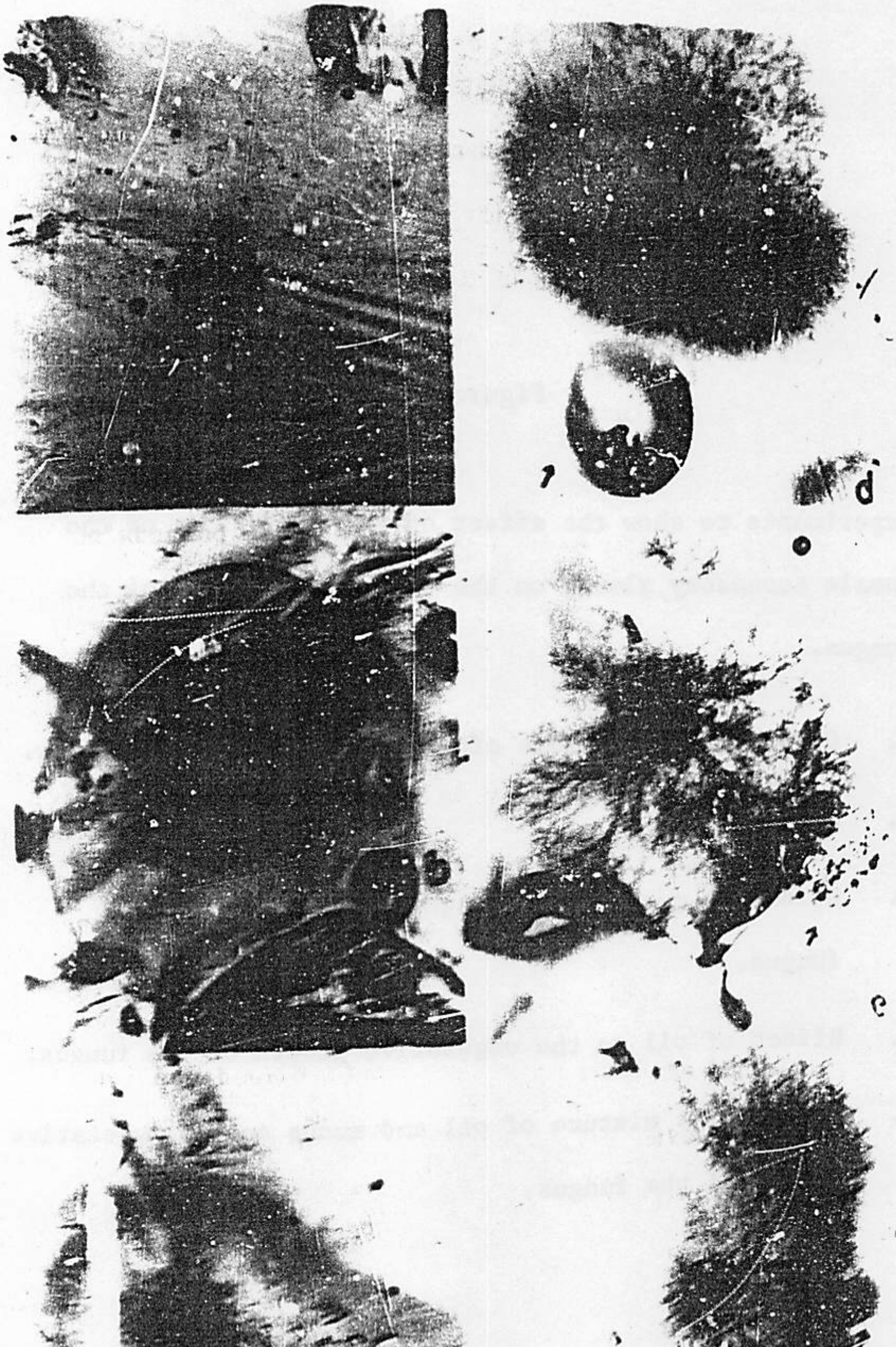

FIG 23

which had emerged, all developed the symbiotic fungus. Unlike the results obtained from the teneral adults, no fungus could be cultured from the surface of head, thorax or abdomen. This suggests that they were tunnelling faster than the fungus was advancing, so reducing the surface contamination.

Females near the bark carried dense balls of fungus in their distended sacs. Only oidia were found in the sacs of emerged females.

(d) Wax packets.

None of the ten undamaged wax packets germinated on plates of agar. A week later, five of these packets were pricked with a needle. Within twenty-four hours they appeared to be germinating. Two days later the mycelium with clamp connexions were clearly evident.

(iv) a) As shown in Fig. 23d, e, f both the mucus secretion and the mixture of oil with mucus caused an increase in the vegetative growth of the fungus. Similar results were obtained using only the oily secretion.

The entire semi-circle of mycelium near the secretion was affected. The increase in vegetative growth was apparent before the growing front of the mycelium reached the mucus secretion. The possibility that volatile components of the mucus were affecting the fungus seems unlikely, even if the volatile substances were being released continuously. During the course of the experiment, the mucus adsorbed water from the agar and became slacker and it seems more likely that some fraction of the mucus was adsorbed onto the agar, causing this reaction.

(b) In one experiment using wax packets obtained from recently shed pre-pupal skins, one packet covered with a mixture of oil and mucus germinated after two days giving a pure culture of the symbiotic fungus. There was no development from the controls which suggests that these secretions may release the fungus from the wax packets.

Figure 24.

Side view of the abdomen of a female pupa of *S. noctilio* showing the exuvial cap on the tip of the ovipositor.

VII. INFECTIVE BEHAVIOUR

The success of a mutualistic relationship depends on mechanisms ensuring the continuity of the association. The mechanisms which achieve the transfer of the microorganisms to the next generation may be fortuitous but inevitable, or there may be special adaptations of the insects' behaviour.

Observations upon a teneral adult female of S. noctilio indicate that the infection of the inter-segmental sacs may result from specialised activity occurring before boring commences.

Soon after the final moult, when the body was soft, and the antennae hung down beside the legs, the abdomen was rotated vigorously. Later the darkening valvulae could be seen sliding back and forth within the pale sheath, while the sub-genital plate was turned down to expose the inter-segmental sacs and the bases of the valvulae, which receive the ducts of the oil and mucous glands.

When the third pair of legs had strengthened, the female was able to brace herself while deflecting the last three segments of the abdomen upwards through an angle of 30°. While the abdomen was held momentarily in this attitude, the sub-genital plate was flicked away from the ovipositor, then snapped down again. The movement of the valvulae continued throughout this time. With alternating periods of rest, this behaviour was repeated intermittently during the two hours the female was under observation.

How the larval hypo-pleural organ becomes infected after each moult is still open to speculation. The suggestions of Parkin and Morgan have been mentioned in the introduction.

DISCUSSION

Two significant facts revealed by the dissections of larvae are the form of the salivary glands, which are extensively branched with large reservoirs, and the fluid condition of the contents of the gut. These observations, in conjunction with the repeated failure of attempts to culture the fungus from the larval gut, prompted Morgan's suggestion (1966) that the fungus is digested extra-intestinally by saliva discharged into the cupped mandibles. Further evidence to support this theory, and the associated view that wood scrapings do not pass through the gut, might be gained from watching feeding larvae to see whether droplets of saliva are discharged and sucked back again.

The woodwasps occurring in Australasia have been identified from living and dead specimens at all stages of development, whereas Maxwell (1955) obtained many of her specimens from museums. It is reasonable to attribute the anatomical differences between the larvae of S. noctilio obtained from Tasmania and those from the N. Hemisphere described by Maxwell (1955), to the fact that her specimens were not correctly identified.

The studies on the development of the hypo-pleural organ have answered many of the questions raised by Parkin (1942) concerning the changes which take place during moulting. Now that hypo-pleural organs have been found in second instar larvae, sections of first instar larvae and late embryos are required to complete the series, with the possibility of showing how the pits are formed in the cuticle.

The anatomical studies on pupal and adult females not only revealed the existence of the median oil sac, the paired lateral pouches covered with white patches of glandular cells, and the remains of spermatophores in the spermatheca, but they also showed details of the structure of the club gland, and the inter-segmental sacs.

The stimulating effect of the oil and mucus on the vegetative growth of fungal cultures has provided experimental evidence in support of suggestions that insect secretions might be involved in the symbiotic relationship. These experiments were crude, though effective. Quantitative estimates of the limiting dose of secretion required to stimulate the vegetative growth of the fungus, could be assessed from the increase in dry weight of liquid cultures to which graduated doses of the secretions had been added.

The provisional identification of the chemical composition of the mucus of the adult females as an acid mucopolysaccharide-protein complex, and the oil as a mixture of at least five fatty acids, has led to further speculation on the role of these secretions in the symbiotic relationship, which must be considered in conjunction with the common host of insect and fungus - the pine tree.

Krainsk (1966) identified the contents of the first oviduct gland of Cynips foliae as acid mucopolysaccharide-protein globules associated with mucopolysaccharides-phospholipid-protein globules. She maintains that this secretion, which is smeared over the surface of the egg before it is laid, functions together with the chitinous egg envelope to protect the embryo from the "noxious action of the vegetal medium". Possibly the mucus surrounding the siricid egg serves this function, as well as providing the arthrospores with a rich source of nourishment.

While it has been demonstrated that the oily secretion prevents the mucus from sticking to glass and, therefore, might act as a non-stick lubricant for the shafts of the ovipositor, the presence of at least fine fatty acids for this one function is unlikely. The stimulating effect of the oil on the vegetative growth of the fungus indicates that it must also be important for fungal nutrition.

The information revealed by experiments with these two secretions emphasizes the need for similar work on the secretions of the club glands which are closely associated with the fungus. Presumably they are concerned with the establishment of the fungus and its development within the sacs which culminates in the formation of arthrospores. Stillwell (1966) succeeded in infecting the inter-segmental sacs with fungus from the hypo-pleural organ. This suggests that the secretions of larval and adult mycangia are similar both chemically and in their effect on the fungus.

Mechanisms of Infection.

In support of Francke-Grosmann's (1957) hypothesis, wax packets of S. noctilio which had been damaged deliberately, germinated within twenty-four hours, whereas undamaged packets did not germinate. Contrary to Francke-Grosmann's claim, no trace of wax packets could be detected in inter-segmental sacs of females, although stained squashes of sacs were examined from females at all stages of development. It seems reasonable to assume that wax packets can be passed along the ovipositor only where the first and second pair of valvulae slide against one another, and that they will be stranded near the base where the valvulae diverge. It is at this point that the ducts of the oil sac and mucous glands open into the ovipositor. The oil and mucus have been shown to stimulate the vegetative growth of cultures of <u>Amylostereum</u>. In one instance a pure culture of the symbiotic fungus was obtained from a wax packet which had been covered with a mixture of oil and mucus. This evidence suggests that wax packets which have reached the base of the ovipositor might be provided with a growth medium, and possibly a chemical release mechanism, enabling <u>Amylostereum</u> to grow the short distance to the inter-segmental sacs.

Francke-Grosmann (1957) maintains that the waxy coating around the fungal packets preserves the fungus when low moisture content within the wood prevents fungal growth and explains the successful inoculation of the i.s. sacs of adult females which emerge from packing cases and other examples of dry wood.

It is possible that attempts to explain the infection of the inter-segmental sacs in terms of experiments carried out on newly moulted, firm wax packets deals with an unnatural situation. In a few cases, cultures of the symbiotic fungus have been obtained from the amorphous, crumbling remains of wax packets on old exuviae. If this waxy coat normally deteriorates with time, it will not always "preserve" the fungus in wood with a low moisture content. In these situations the stimulating effect of the secretions of the accessory glands and possibly the club gland of the teneral adult will assume greater significance. Even when low moisture content prevents fungal growth, the fungus will not be killed. The symbiotic fungus has been cultured many times from the minute wood scrapiugs scattered over the surface of the pupa and pupal chamber. These scrapings must also be considered as a possible source of fungus for the infection of the inter-segmental sacs. Recent attempts at culturing the fungus from the ovipositor of females at different stages of development indicated that there might be a progressive growth of the fungus from the tip, along the shafts, to the sacs. Perhaps the fungus grows away from the ruptured or crumbling packets at the tip rather than the base of the ovipositor.

Recent observations suggest that the teneral adult female exhibits infective behaviour when the sub-genital plate is flicked open simultaneously with deflection the abdomen upwards.

One can only speculate whether the action of the sub-genital plate moves wax packets from the base of the ovipositor into the sacs, or whether fungal fragments are scraped off the walls of the pupal chamber in this way. Büchner (1965) claims that the sacs can be infected by fungus growing in from the pupal chamber. In either case, the oil and mucus would provide a rich growth medium.

Apart from Parkin (1942) speculation that fungus grows into the newly formed hypo-pleural organ of the female larva after each moult, Morgan (1966a) placed glass sheets over cut pine logs to observe larvae tunnelling. He observed that when the body is stretched out, the hypo-pleural organ becomes exposed. Having seen scraps of fungus caught on cuticular spines, he suggests that fungus could be scraped from the walls of the larval tunnel, so infecting the organ.

Permanent mounts of larval cuticle made during this study show that the cuticular spines are finer and smaller in the inter-segmental fold than on the remainder of the cuticle. The thickened rim around the openings of the major pits appear to be devoid of spines, see Figs. 3b, 3c and 7b. Possibly secretions from the hypo-pleural organs direct the growth of fungal fragments caught on larger spines towards the pits.

The role of the fungus in the modification of the micro-environment of egg and larva, and in larval nutrition, has been reviewed in the introduction. It has been assumed that the fungus derives benefits through being dispersed by the insect and placed directly into the wood of a suitable host without having to penetrate any protective tissues. The present series of experiments has demonstrated that the oil and mucous secretions of the adult female woodwasp stimulate the vegetative growth of the fungus. It is possible that these secretions may be anti-bacterial and toxic to other wood-rotting fungi and it seems that this line of investigation could be pursued further.

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