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Mr CARTWRIGHT said: Work on the association of insects and fungi has been confined, up to the present almost entirely, to the entomological side. Many of the details in regard to wood-inhabiting insects have been excellently summarised by Paul Buchner (1) in a recent publication entitled *Holznahrung und Symbiose*, which contains drawings showing the presence of symbiotic organisms. A paper of a more exhaustive character by Breitsprecher (2) has just come out. This is mainly a description of sections cut through the abdomens of species of *Anobiidae*, showing the presence of symbionts.

The classical example of an association between insects and fungi is that of the *Ambrosia* beetles. In this case the fungus is confined to a narrow zone round the galleries of the insect and is definitely transmitted by the ovipositing female; the fungus spores being excreted on to the egg coat. A simpler association was described by Möller in his studies of leaf-cutting ants which cultivate fungi for their food supply, here the fungus, *Roziites gongylophora*, is definitely carried by the queen in a special cavity, to the new colony. Other related ants cultivate a fungus on chewed-up wood.

Much indirect evidence about the subject has been obtained from the practical foresters, architects and others who have so frequently noted that insect and fungus attack appear to be connected, as to suggest that some direct association existed between them. In these notes attention will be confined to the association between wood-feeding insects and fungi. This may be considered under two headings: I. Association of an insect with an internal symbiont, *i.e.* the regular presence in the body of the insect of a foreign organism which is often confined to special glands. II. The regular occurrence of a fungus in the wood or tissues of the host in which the insect is living.

I. It is not intended to discuss at any length the question of internal symbionts, as most of the information so far gained has been on the entomological side. Suffice it to say that in most timber beetles and bark beetles which have been examined, bacteria, yeasts or fungi proper have been found to occur; as an example of these, *Endomyces* may be mentioned.

It has been suggested that symbionts will be found to be present in insects which feed on materials such as wood, which are not readily digestible, whilst these will not occur when the insect lives on substances that are easily absorbed. The conclusion drawn is that these symbionts enable the insects to break up the wood, etc., and make available for their assimilation these foodstuffs. It has been assumed that the insect alone is unable to do this. Chemists who have analysed beetle frass have stated that it appears to be unaltered wood. These results, however, need confirmation as it is difficult to suppose that neither the fungus nor the beetle have in any way altered the wood during its passage through the body of the latter. Such would mean that their relationship was in the nature of a mutual parasitism without any outside source of energy. It would seem probable that such alterations as occur are not detectable by the present methods of wood analysis.

The organisms usually occur either in some part of the gut, into the epithelial layer of which they may work their way, or they may be contained in special glands opening into the vagina, being transmitted to the egg coating either before or during oviposition. The larva in hatching from the egg eats part at least of the egg coating and thus becomes infected.

Unfortunately up to the present no proof, as far as I am aware, has been brought forward that the internal symbionts do really help in the breaking up of the wood and all attempts to obtain these insects free from the organisms have so far failed. Until the technique for achieving this has been worked out, it is by no means certain that the insect really is dependent on its internal symbiont. Should it be found that the insect can live independently of any symbiont it does not rule out the possibility that such insects may not require wood already altered by fungus action or that in some instances the insect larva may be entirely mycophagous.

Although the sterilisation of the insect appears to be necessary to furnish conclusive evidence one way or the other, another line of attack which would afford a certain amount of circumstantial evidence is that of isolating these symbionts from the insects and growing them on artificial media. The actual technique of isolation may prove difficult, but should not be insurmountable. If this could be done, it could at any rate be ascertained whether they were capable of breaking up any of the wood constituents. Up to the present material has not been available on which to attempt this work, but it is hoped to undertake it in the near future.

II. Association of insects with fungi living externally in the surrounding material.

From the examination, by means of sectioning, of much wood attacked by beetle, it has been ascertained that wherever larval tunnels are present, there invariably can fungus mycelium be found. Up to the present no case has come under my observation in which this did not prove to be true. In the case of bark beetles for example, the tunnels are often seen lined with fungus which in certain instances is a species of *Endomyces*. Schneider-Orellis found thick-walled fungus spores in the gut of the overwintering female; these germinated readily in the excrement of the insects. During the examination of some ash wood attacked by *Daldinia concentrica*, tunnels of *Hylesinus fraxini* were observed; these were seen to follow closely the black zone lines caused by the fungus. In old beams and furniture there would appear also to exist some connection between beetle attack and the presence of fungi. Furniture which had been in store for about 8 years was found to be attacked by beetle and an examination showed that fungus mycelium was present.

The moisture content in old furniture, beams, etc., may be as low as 7 per cent. and is rarely above 15 per cent. Such low moisture contents would appear to exclude the possibility of finding mycelium in a state of active growth.

The fungi obtained by cultural methods from such old wood, which is often very much broken up by larval galleries, cannot be assumed to be those primarily concerned. It is highly probable that in many cases the fungi perform their work prior to the insect attack and that the beetle larvae feed either on the altered wood or on the dead fungus mycelium or on both.

Many different fungi commonly present in wood, such as *Trichoderma lignorum*, *Torula* sp., *Penicillium* spp., etc., have appeared in such cultures taken from near beetle tunnels. So far my work on the association of timber-destroying beetles and fungi has been confined to the examination of beetle-attacked wood for the presence of fungus and to keeping in culture those fungi which are isolated frequently from cultures taken near the tunnels or from frass.

Only in two instances has a Basidiomycete been obtained. *Salix* wood, containing tunnels of *Xestobium*, was decayed throughout by *Fomes applanatus*, but the beetle attack had obviously come in later and there was no question of the fungus having been introduced by the insect. A *Xestobium* larva has since been placed on a pure culture of this fungus growing on malt agar and it has been alive for about 1 month in the culture, on which it feeds to a certain extent. The second case was also on *Salix*, from which, amongst other fungi, a member of the Cyphellaceae has been obtained. In several beetle-attacked oak samples sent for examination, a fungus with conidia of the *Septocylindrium* type has been found and a species of *Torula* was also present.

These fungi have often been observed in old oak which shows "golden" coloration; from this *Eidamia catenulata* has also been isolated on several occasions. It would be interesting to ascertain whether these richly coloured and much sought after specimens of old oak are more susceptible to insect attack than is the normal, as Prof. Groom (3) and Mrs Williamson (4) have shown that such coloration can be caused by fungus action.

Finally it may be mentioned that a fungus having the β type spore of a *Phomopsis* has been isolated by Mr Day and Mr Nutman from *Lyctus*-attacked wood. This has not yet been identified with certainty and may possibly prove to be a species of *Cytospora*.

To summarise, two explanations of the regular presence of fungus in and around beetle tunnels may be put forward: (1) the fungus already present grows more actively in the region of the beetle tunnels because the aeration is better there and because it derives nourishment from the waste products of the insect which may tend to raise the moisture content of the wood around the tunnel; or (2) the insects make their galleries in that part of the wood where there is most fungus mycelium and also they spread the fungus by infection carried on their bodies.

Probably both explanations hold good to some degree and the fungus once introduced finds the conditions surrounding the tunnel the most favourable for its growth.

We may conclude, tentatively, that beetles always prefer wood which contains fungus, because (1) they are better able to digest wood which has been attacked by the enzymes of a fungus, and (2) they derive part or all of their food supply from the actual mycelium of the fungus, dead or alive. It is not to be expected that any wood-destroying fungi will be found living and growing actively in wood with a moisture content as low as that usually found in old oak beams, etc., as they require in most cases a moisture content about that of fibre saturation point (28 to 30 per cent.). It would be interesting to ascertain the lowest moisture content at which the various fungi isolated from beetle-infested wood could grow, and also whether the moisture content of the wood in the neighbourhood of the galleries was increased by the metabolism of the insect.

Sirex cyaneus *and fungus association.*

The study, of which this is an account, was undertaken on the suggestion of Mr Chrystal after a meeting of this Society on March 23rd of this year, and the work is as yet quite incomplete; thus only tentative conclusions can be drawn as to the results.

Attention was drawn to the paper of Buchner in which it is stated that oidia of a fungus had been found in a gland or squirt at the base of the ovipositor in *Sirex*

gigas. These glands Buchner figures. They are paired and open out into the vagina, the oidia being extruded on to the eggs after they have left the ovaries. Furthermore, he had observed clamp connections, proving that the fungus belonged to the Basidiomycetes, though in his figures these are by no means convincing, as they are in no case complete, the septa being absent. Certainly one would suspect them of belonging to a Basidiomycete.

Slides prepared at Oxford were sent me for examination in April. These showed oviposition and young larval tunnels, and in every case a mass of mycelium was present in the wood around the vessels. The outer sculptured wall of an ovum was left *in situ* in one of the tunnels and showed hyphae around it. At the same time slips of larch wood with oviposition tunnels and young larval galleries were sent, which, on sectioning, showed mycelium of a similar kind with clamp connections conspicuously present. From an examination of further specimens, both of wood attacked by *S. cyaneus* and of that attacked by *S. gigas*, it would appear that a fungus mycelium belonging to a Basidiomycete is always present in the wood in which these larvae are living. Furthermore, this mycelium in every case seemed to have its focus around the galleries and was definitely attacking the wood, which showed numerous bore-holes. The moisture contents of one sample were determined and found to range between 35 per cent. and 48 per cent., a condition of the wood which would be favourable for the growth of these wood fungi. From the original samples, culture plugs were taken and a fungus which grew readily on a 2 per cent. malt or prune agar, was isolated. This mycelium soon produced numerous clamp connections and was moderately rapid in growth. The culture remained white for some months, but now colour is developing in some of them. They are of a soft downy texture. The same fungus was isolated from several different samples. So far the cultures have shown no sign of fruiting though undifferentiated segments of hyphae separate off and may act as oidia.

The small specimens from which the cultures were taken already showed fungus mycelium throughout, though it was more plentiful in the neighbourhood of the tunnels. One could not feel certain, therefore, that this wood had not contained fungus before the *Sirex* attack. However, in August, material was obtained which contained oviposition tunnels and eggs of about 2 weeks old. These were sectioned and in every case the same type of mycelium was seen having the tunnel as its focus of development, the mycelium rapidly diminishing in amount away from the tunnel. Sections from areas closely adjacent to these tunnels showed no trace of fungus; demonstrating clearly that this really had been introduced by *Sirex* during oviposition.

The extent of development of the fungus after 2 weeks was sufficient to suggest that the 5 weeks' interval between oviposition and the hatching would be enough to allow the fungus to make sufficient growth to assist in the nutrition of the larva.

A few dead female adults and a number of half-grown larvae were obtained in August, the majority of these were fixed and have been embedded for the cutting of serial sections. One of the females was dissected and the eggs removed from the uterus and examined under the microscope. A fine mantle of mycelium was seen in between, and surrounding the eggs closely. No clamp connections were seen in this mycelium.

A few of the eggs were placed in slants of 2 per cent. malt agar: these cultures became contaminated but sufficient could be seen in the mixed culture to show the

presence in one case of a Basidiomycete. Eggs were also extracted from oviposition tunnels made about 2 weeks previously; of these, ten were placed on 2 per cent. prune agar slants and six on malt agar one egg being placed in each tube. Out of the prune tubes three gave cultures of mycelium showing the same characteristics as that of the fungus originally isolated from the wood, and bore clamp connections; four remained sterile, and two gave contaminants. Out of the malt, two gave mycelium with clamps and three remained sterile. From the tenth prune tube and the sixth malt tube the eggs were removed and found still to show the mantle of mycelium as seen round the eggs dissected from a female *Sirex*[1]. No fungus has been found around the eggs in the ovaries; further dissections will have to be made to settle this point definitely. A cursory examination of a few larvae showed mycelium to be present in a disorganised condition as if partly digested, but mycelium which appeared undisorganised was also seen. It is thought probable, therefore, that this may remain in a resting condition through the pupal stage and be found in the adult as described by Buchner.

A newly hatched larva, removed from its tunnel, was placed on a culture of the fungus where it lived for 3 weeks apparently feeding on the fungus. One of the half-grown larvae lived for 3 months on cultures of this fungus. It was transferred from time to time to fresh cultures which it could be definitely seen to eat.

Identification of the fungus.

The culture of the fungus from *Sirex cyaneus* agrees with none of the fairly numerous type cultures which I keep for comparative purposes. No clue has so far been vouchsafed either in the finding of sporophores on the trees or in the type of rot produced. It is probable that identification will be secured when more comparative cultures are obtained.

In regard to *Sirex gigas*, the species with which Buchner chiefly deals, I have only had a few specimens from some larch props sent for another purpose.

From an examination of these samples which contained pupae and from which adults hatched out in the laboratory, it would appear that a similar association exists here also.

Conclusions.

To sum up, it appears that sufficient evidence of a circumstantial nature has been brought forward to show that:

1. A Basidiomycete fungus is always present in wood-containing *Sirex cyaneus*.
2. This fungus has proved so far to be identical in every case examined.
3. That the fungus is introduced with the egg during oviposition. In the cases examined this has been in the form of a fine mycelium which, being in the primary condition at the time of oviposition, has no clamp connections. It develops these, however, both in the wood and in artificial media.
4. The fungus causes a rot of the wood in which bore-holes are made.
5. It can advance sufficiently in the time elapsing between oviposition and the hatching out of the young larva either to have formed food for the larva itself or to have acted upon the wood to a sufficient extent to make this available as food.

[1] Some of the cultures which had remained sterile are now showing fungus development.

6. The larva can live and definitely grow on a pure culture of the fungus for a period of at least 3 months, showing that it can derive some nourishment at any rate from eating fungus alone. Further information is needed about the methods whereby the fungus is transmitted from the larval through the pupal stage to the adult, and at what stage in the life-history of the insect the special glands containing the fungus are formed.

Probably some stimulus due either to fertilisation or gland secretion, when the resting oidia are extruded from the gland into the vagina, starts growth.

In the case of *Sirex gigas* the fungus may also be introduced into the wood as oidia: details of this have still to be worked out. It is hoped next season to obtain cultures from eggs directly after oviposition and possibly even to induce egg-laying directly into a medium. Material was not available for this during last summer.

Finally, I wish to acknowledge the debt I owe to the previous speaker who started me on this work and who, with the skillful help of his assistant, has provided me with such excellent material.

REFERENCES.

(1) BUCHNER, PAUL. (1928). *Holznahrung und Symbiose.* Berlin: Julius Springer.

(2) BREITSPRECHER, E. (1928). Beiträge zur Kenntnis der Anobiiden symbiose. *Zeitschrift für Morphologie und Okologie*, Band II, Heft 34, August.

(3) GROOM, PERCY (1915). "Brown Oak" and its Origin. *Ann. Bot.* XXIX, No. 115, July.

(4) WILLIAMSON, HELEN S. (1923). The Origin of "Golden Oak." *Ann. Bot.* XXXVII, No. 147, July.

The following letter has been received by the Editors of the *Annals of Applied Biology*:

ABOL RESEARCH LABORATORIES,
PADDOCK WOOD,
KENT.
October 1928.

DEAR SIRS,

With reference to the article on "The use of Tetrachlorethane for Commercial Glasshouse Fumigation," which was published under my name in the May issue of your *Journal*, I regret that I failed to acknowledge that I was indebted for a considerable part of my information to Messrs Murphy and Son, Ltd., as a result of investigations which I made for them while engaged by them. I omitted to mention in the article that the introduction of tetrachlorethane into Commercial Horticultural practice by the writer in 1920 was in fact effected through Messrs Murphy and Son, Ltd., and was as a result of the investigations referred to above.

Yours faithfully,
(*Signed*) THEODORE PARKER.

THE EDITORS,
The Annals of Applied Biology,
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