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ULTRASTRUCTURE OF THE MESONOTAL MYCANGIUM OF AN AMBROSIA BEETLE, *XYLEBORUS DISPAR* (F.) (COLEOPTERA: SCOLYTIDAE)*

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Abstract—Fungal symbionts of the ambrosia beetle, *Xyleborus dispar*, are transported within a glandular invagination of the body cuticle, termed the mycangium. The associated secretory cells pass their products into the mycangium via efferent cuticular ductules. Hemidesmosomes on microvilli apparently anchor the end apparatus of each ductule in the compartment. The fungal propagules within the mycangium are of 2 types: a small-celled fungus which is apparently the classical ectosymbiont, *Ambrosiella hartigii*, and a large, multinucleate propagule, which is a yeast-like basidiomycetous derivative.

Index descriptors (in addition to those in title) Symbiosis, mutualism, basidiomycete, exocrine glands, cuticle, insect.

INTRODUCTION

IN MANY OF THE symbiotic associations between insects and fungi, the insect is a vector for the fungal propagule. Often, the fungal ectosymbiont is transported within a mycangium—a glandular cuticular invagination of the insect's body surface (Buchner, 1965; Francke-Grosmann, 1967; and Graham, 1967). To understand these associations better, it is necessary to describe the morphology of the symbiotic partners, to probe their interactions experimentally, and to analyze in some detail the properties of the micro-environment within the mycangium. The glandular nature of the mycangium suggests that secretions of the beetle regulate the species composition of the intramycangial fungal populations and also provide nourishment for proliferating fungal propagules. (Francke-Grosmann, 1967; Schneider and Rudinsky, 1969a, 1969b; Barras and Perry, 1971; Happ *et al.*, 1971).

Two groups of scolytid beetles, differing both in their micro-habitats and taxonomic affinities, are associated with fungal ectosymbionts. The phloem-feeding bark beetles, of which the southern pine beetle (*Dendroctonus frontalis*) is one representative, are associated with blue-stain fungi of the genus *Ceratocystis*. *D. frontalis* transports an imperfect derivative of *Ceratocystis* and a basidiomycete (Barras, 1975; Barras and Perry, 1972; Barras and Taylor, 1973; Happ *et al.*, 1975; Happ *et al.*, 1976). The second group of scolytids, ambrosia beetles, lives primarily in the xylem, and most species are associated with fungi of the genera *Monilia*, *Cephalosporium*, or *Ambrosiella* (Francke-Grosmann, 1967). When growing in the galleries formed by beetles burrowing in the host tree, the symbiotic fungi

* Contribution No. 4 in a series of papers on scolytid-fungal symbiosis.

produce nutrient-rich spores, termed "ambrosia" that are eaten by larvae and adults (French and Roeper, 1975). In the case of the beetle *Xyleborus ferrugineus*, the symbiotic fungus also provides phytosterols which are required for pupation (Norris, 1972). The subject of the present study is a congeneric ambrosia beetle, *Xyleborus dispar*. The fungal ectosymbiont of *X. dispar* has been identified as *Ambrosiella hartigii* (Fungi Imperfecti) (Batra, 1967). *A. hartigii* in pure culture is mycelial (French and Roeper, 1972a). When post-diapause adults of *X. dispar* were introduced into these mycelial cultures, the fungus produced ambrosia (French and Roeper, 1972b).

With the exception of 6 previous papers (Barras, 1975; Happ *et al.*, 1971, 1975, 1976; Whitney and Farris, 1970; Livingston and Berryman, 1972), little is known of the ultrastructure of the mycangia of most insect species or of that of the microsymbionts. In previous papers, we have described the prothoracic mycangium of the southern pine beetle (*D. frontalis*) (Happ *et al.*, 1971) and the morphology and proliferation of 2 species of fungal ectosymbionts transported therein (Happ *et al.*, 1975, 1976). In the study reported below, we investigated the ultrastructure of the mesonotal mycangium in an ambrosia beetle (*Xyleborus dispar*) and of the fungal microflora contained therein during winter diapause.

MATERIALS AND METHODS

Females of *X. dispar* were allowed to enter diapause in the fall, were stored at 0–4°C (Corvallis, Oregon), and were shipped to New York University (in April) for dissection, fixation, and embedding. Further manipulations occurred at Colorado State University.

Females were fixed in alcoholic Bouin's and embedded in paraffin or in glutaraldehyde (2.5% in 0.05M phosphate buffer pH 7.2) with 0.15M sucrose added, at 0–4°C for 2–6 hr, followed by washing overnight in 4 changes of the same buffer with 0.5M sucrose added (Locke, 1966), with post-fixation for 1 hr in 1% osmium tetroxide buffered with veronal-acetate (pH 7.3) containing 0.4M sucrose. The glutaraldehyde was prepurified by repeated washings through Norit EX charcoal. Tissues were dehydrated in graded alcohols and embedded in Epon 812. Thick sections were stained with toluidine blue. Thin sections were stained routinely for 20 min with saturated uranyl acetate in 50% ethanol followed by lead citrate (Reynolds, 1963) with 1 drop of Triton X-100 added to each 50 ml of lead stain. The electron micrographs were obtained on a RCA EMU 3D or an AE1 EM6B.

OBSERVATIONS AND DISCUSSION

The mycangium of the female *X. dispar*, lies at the forward margin of the mesonotum, (Francke-Grosmann, 1967). The dorsal wall of the mycangium is derived from the recurved scutellum and is lined with tall, secretory cells (Fig. 1).

Each secretory cell is associated with an efferent cuticular ductule (Figs. 3, 4). The secretory unit is similar to that in type 1 cells of the mycangium of *Dendroctonus frontalis* (Happ *et al.*, 1971), and many other insect exocrine glands (Noirot and Quennedey, 1974). Such secretory units constitute another example of an organule (references in Selman and Kafatos, 1975). The secretory cells of the diapausing females appear inactive. The nuclei are large (12–15 μm in diameter), oval, and quite smooth in outline, and they contain numerous nuclear pores (Figs. 2, 6). The chromatin is mostly dispersed; only a few patches of heterochromatin are present along the inner nuclear membrane, but nucleoli are prominent. Mitochondria generally appear to be spherical, small (1.8 μm in diameter), and contain plate-like cristae. Some ribosomes are dotted along irregular cisternae of the endoplasmic reticulum and many others lie free in the intervening cytosol. The intracisternal spaces appear to be devoid of electron dense materials. Small clusters of dense vesicles, which may be Golgi zones are frequently seen (Fig. 6).

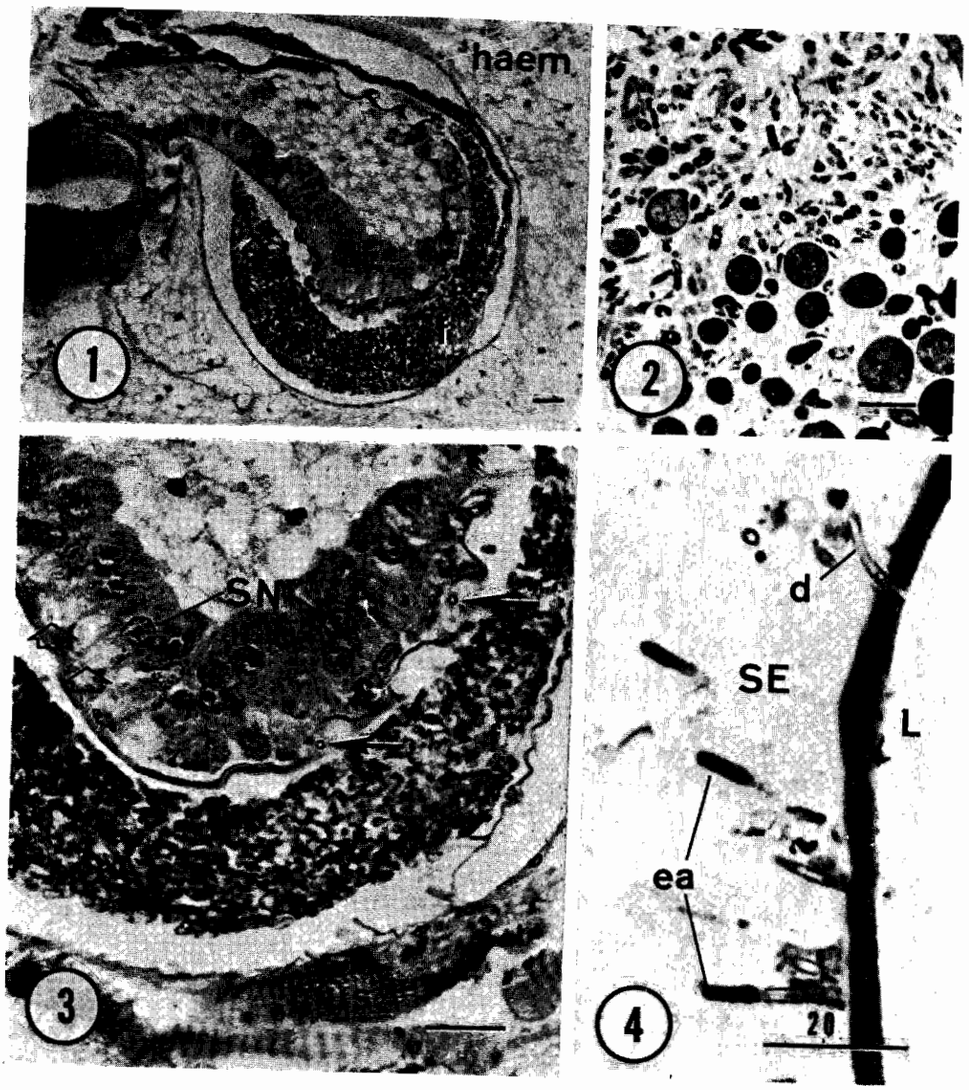


PLATE 1.

FIG. 1. Mycangium of *X. dispar* filled with fungal propagules. f, fungal propagules; haem, haemocoel; mus, dorsal longitudinal flight muscle; SE, Secretory Epithelium. Alcoholic Bouin's, Delafield's haematoxylin. ×175

FIG. 2. Two populations of fungi. Larger multi-nucleate propagules are in lower portion of micrograph and smaller propagules in upper portion. Glutaraldehyde, osmium tetroxide, toluidine blue. ×400

FIG. 3. Secretory cells line thick, uneven ventral cuticle of the mycangial wall. Ductules can be seen within cuticle at right (horizontal arrows) and cavities enclosed by secretory cells are indicated by hollow arrows at left. Muscles (mus) are attached to thin flexible cuticle opposite secretory epithelium. f, fungal propagules; SN, Secretory Cell Nuclei. Alcoholic Bouin's, Delafield's haematoxylin. ×550

FIG. 4. Cuticular ductule (d) runs from end apparatus (ea) through mycangial wall and into lumen (L). SE, Secretory Epithelium. Glutaraldehyde, osmium tetroxide, toluidine blue. ×970

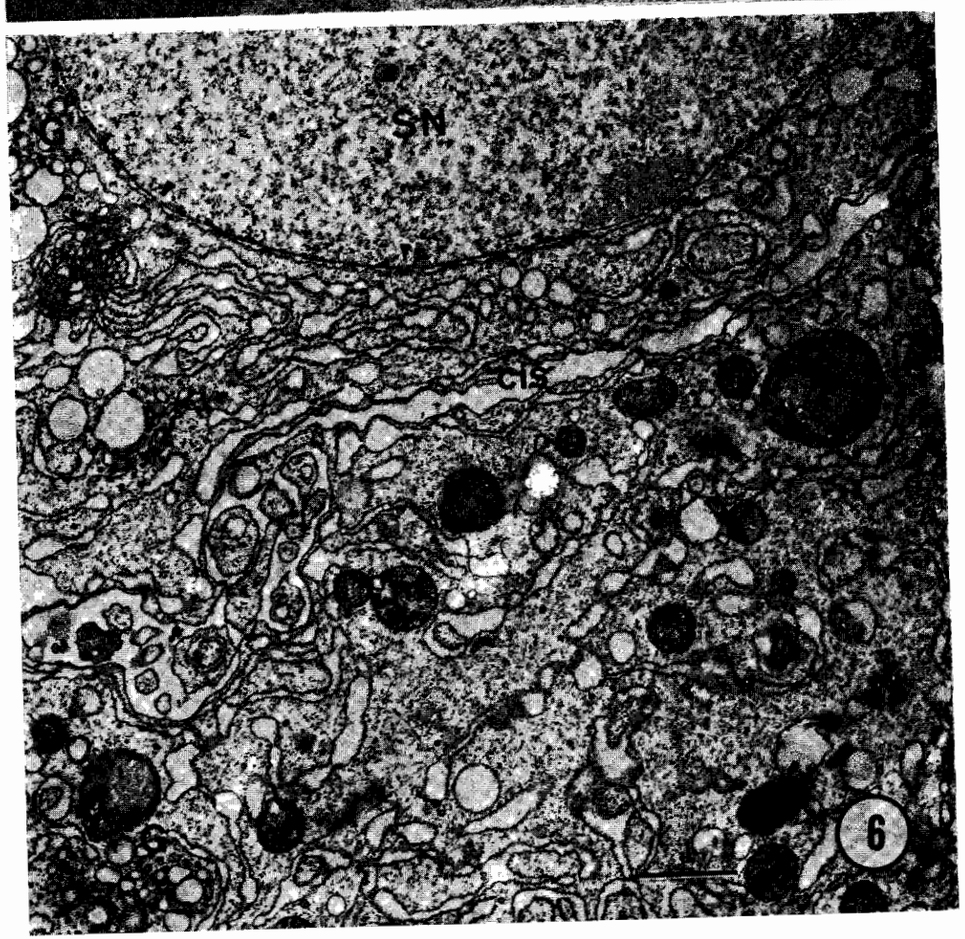
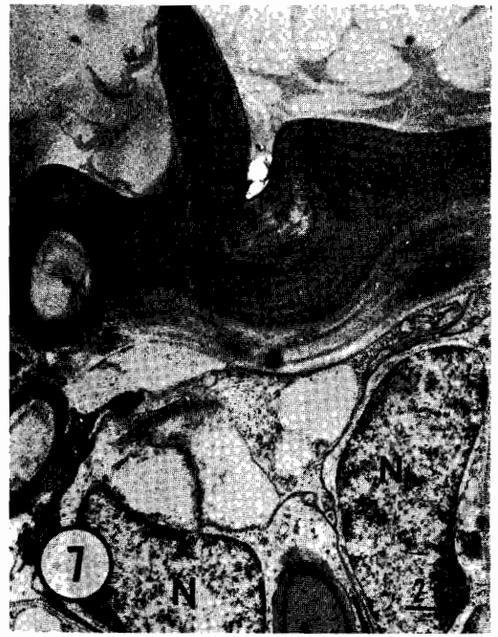


PLATE 2.

FIG. 5. Superficial layers of non-secretory cuticle. A granular inner epicuticle (1E) underlies a discontinuous trilaminate outer epicuticle (between arrows). Scale = 0.1 μm. ×100,000

FIG. 6. Secretory cell cytoplasm contains inflated, electron transparent cisternae (cis), Golgi zones consisting of a variety of vesicles (G), and scattered mitochondria (m). Cytoplasmic ground substance is of low density. Within nucleus (SN) chromatin is dispersed. Nuclear pores (arrow-



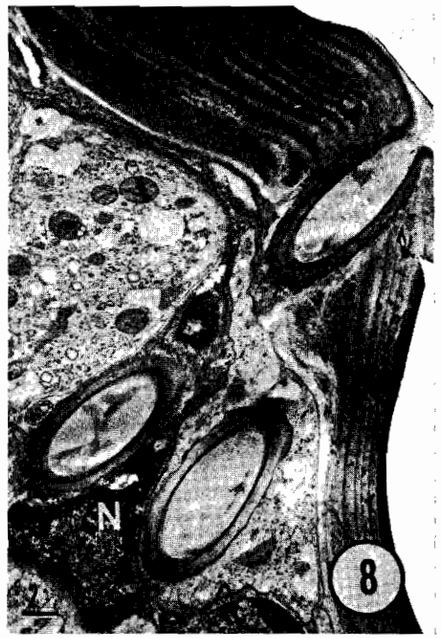
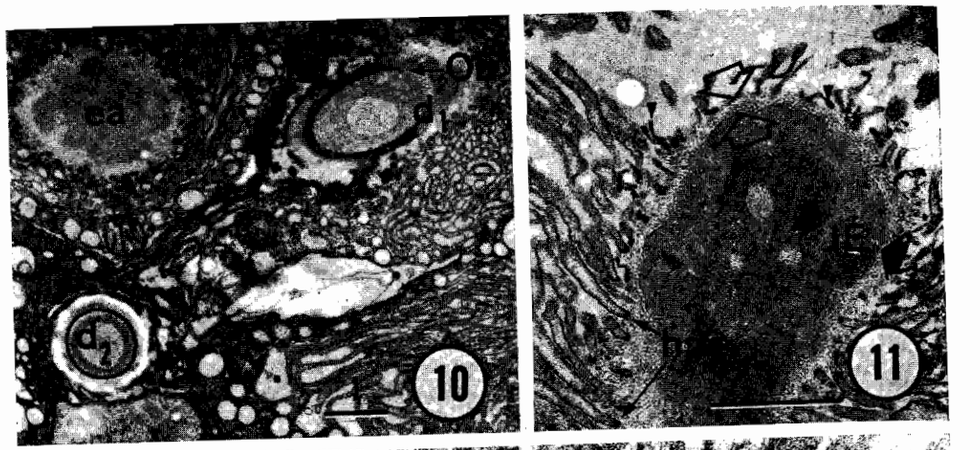


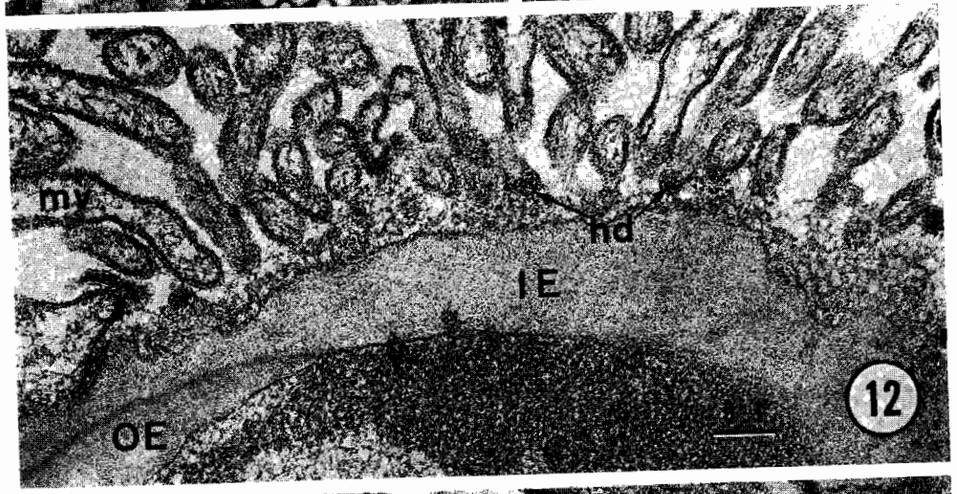
PLATE 3.

FIG. 7. Lamellate cuticle (LC) of above the secretory epithelium of mycangium is thrown up into spine-like processes (asterisk). Two nuclei (N) of ductule-carrying cells are visible. ×1800

FIG. 8. A ductule running through lamellate cuticle (LC) and opening into the lumen. ×2300

FIG. 9. End apparatus (ea) of ductule lies in a secretory cavity. Lamellate cuticle is at upper left. SC, Secretory Cell; DC, Ductule-carrying cells. Scale bar = 2 μm. ×2800





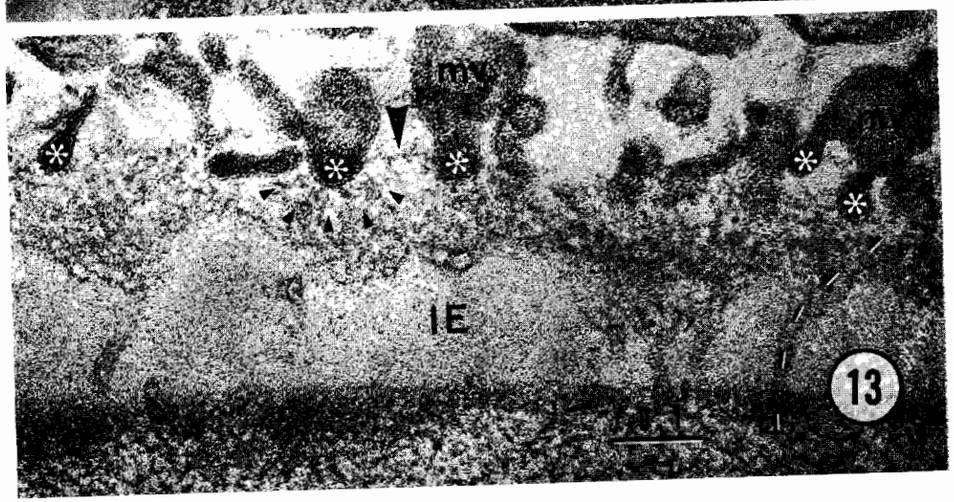


PLATE 4.

The plasma membrane of each secretory cell is deeply invaginated to enclose an irregular compartment that is truly extracellular. This extracellular compartment is not continuous with the hemocoel which surrounds the cell, for the narrow neck of the compartment is tightly plugged with a cuticular ductule ensheathed in a process of ductule-carrying cell (Fig. 9). The end apparatus of the cuticular ductule lies within the cavity. The cuticular wall of the end apparatus is perforated by fine tortuous channels, each about 150 Å in diameter. The channels are bounded by a denser zone, *ca.* 30 Å wide, which is apparently continuous with a similar dense zone on the outside surface of the wall (Fig. 13). In contrast to similar cells which we have described in the mycangium of *D. frontalis*, secretory product was absent within these fine channels and also absent from the surrounding extracellular compartment. Dense granular and fibrous material lies in the lumen of the end apparatus (Figs. 9, 12, 13). As suggested for the type 1 cells of *D. frontalis* (Happ *et al.*, 1971), this filamentous material may be a permanent structural feature rather than merely secretion in transit.

The absence of secretory products around the end apparatus of these diapausing beetles facilitated our examination of its outer surface. The outer surface is coated with a reticular filamentous coat, 30–100 μm thick, and analagous in its position to subcuticle. Microvillar evaginations of the plasma membrane impinge upon the filamentous coat. The microvilli are not packed with parallel microfilaments, as are those in the spermathecal accessory gland of *Tenebrio* (Happ and Happ, 1970) nor do they contain smooth endoplasmic reticulum, as do those in the type 1 cells in the defensive glands of *Eleodes* (Eisner *et al.*, 1964). Rather, the inner surface of the plasma membrane is associated with an undercoat of fine filaments which often surrounds a clear central channel, free of well-defined structures, that runs down the center of the microvillus (Fig. 12). Within the tip of each microvillus is a dense plaque that resembles a hemidesmosome (Figs. 11, 12). Where microvilli are densely packed around the end apparatus, these dense plaques are evaginated and can be seen closely appressed to and sunken into the filamentous reticular coat of the end apparatus. Strands of material appear to radiate from each microvillus into the surrounding reticulum (Fig. 13). Presumably, the massed microvilli anchor the end apparatus in place.

In cross sections through the end apparatus, the precise boundary between the wall and the contents is sometimes difficult to distinguish (Figs. 10, 11). However, as the ductule

FIG. 10. A dense outer epicuticule (OE), absent from end apparatus (ea), is acquired as the end apparatus joins the efferent ductule (d_1). It is clearly seen as a cross-section through the efferent ductule (d_2). × 8900

FIG. 11. Inner epicuticle (IE, between solid arrows) forms the wall of end apparatus. It is coated with a layer of fine filaments (between the hollow arrows). Tips of microvilli which are embedded in this filamentous layer contain dense hemidesmosomes (hd). A number of vermiform electron-dense anchoring processes are indicated by small arrowheads. ×16,700

FIG. 12. An oblique section through junction between end apparatus (at right) and efferent cuticular ductule (at left). Inner epicuticle (IE) of efferent ductule is continuous with that of end apparatus whereas outer epicuticle (OE) is restricted to efferent ductule. Loose fibrous material lies within microvilli, and dense materials (hemidesmosomes, hd) are found at their tips. ×83,000

FIG. 13. Fine channels (dashed line) traverse inner epicuticle (IE) of end apparatus. Electron-dense anchoring processes, indicated by white asterisks, are often attached to larger microvilli (mv). Outer surface of these processes is covered with a fuzzy glycocalyx. Individual processes may be connected to one another (large arrowhead) or glycocalyx may radiate out into filamentous coat around the end apparatus (semi-circle of small arrowheads). ×120,000. Scale lines are in microns.

leaves the cavity and becomes ensheathed in a ductule-carrying cell, an electron-dense outer epicuticle appears and the fine perforations disappear (Figs. 9, 10, 12). The efferent ductule runs into and through the layers of lamellate cuticle which form the dorsal wall of the mycangium (Figs. 7, 8). The lamellate cuticle is thrown up into a variety of ridges and projections which probably assist in retention of the mass of fungal propagules (Figs. 7, 8). The configuration of ductule openings is rather like that in the pit glands of *Scolytus* sp. (Livingston and Berryman, 1972).

The ventral mycangial wall, derived from invaginated inter-segmental membrane, is not lamellate, and appears to lack a procuticle altogether. The inner layer is 4–6 μm thick and contains loosely sweeping fine filaments, 20–30 Å in diameter. The outer epicuticle is granular, 130–140 μm thick, and appears to be coated with a waxy, trilaminate, and discontinuous superficial epicuticle, *ca.* 100 Å thick (Fig. 5). This non-glandular anterior wall is associated with muscle (Fig. 3).

The mycangial fungi

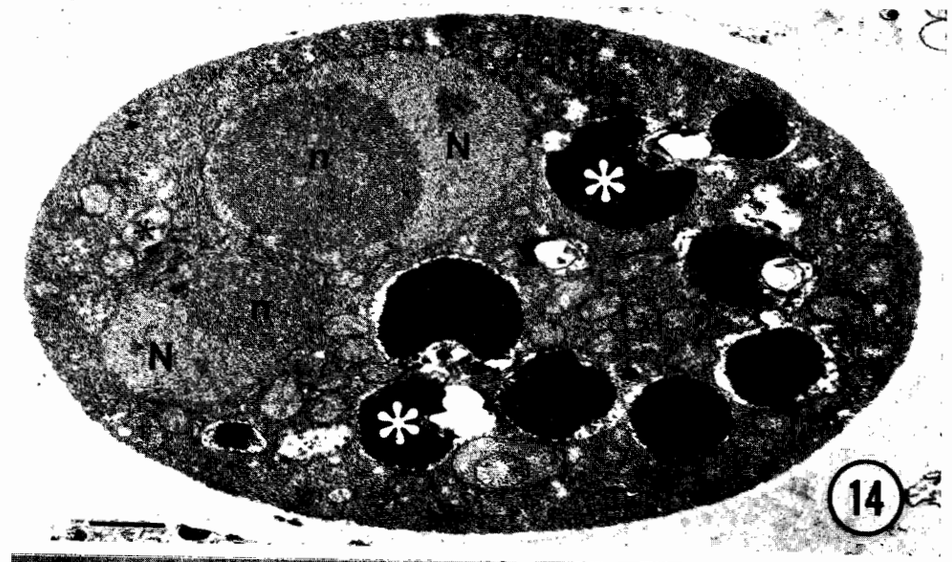
The propagules in the mycangium fall into 2 distinct size classes (Fig. 2): the larger propagules (Figs. 2, 14, 15) are multinucleate and contain distinct storage vacuoles and well-defined mitochondria. Their lamellate cell walls are characteristic of basidiomycetous yeasts (Kreger-Van Rij and Veenhuis, 1971). Except for the slightly lower cytoplasmic density, these propagules are very similar to the basidiomycetous yeast in the mycangium of the southern pine beetle (Happ *et al.*, 1976).

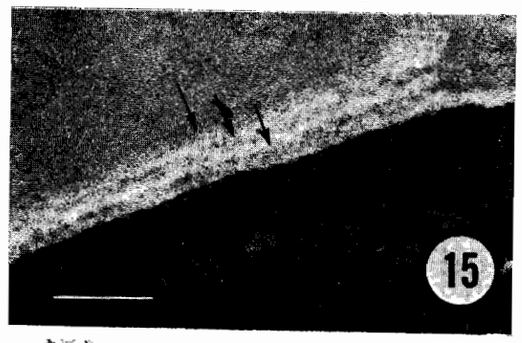
The more numerous smaller propagules are uninucleate and closely resemble *Ceratocystis* derivatives (Happ *et al.*, 1975), and probably are *Ambrosiella hartigii* which has been isolated previously from the mycangium of *Xyleborus dispar*. Their cell walls are not lamellate (Figs. 16, 17), and their septa are of the ascomycete type (Fig. 18).

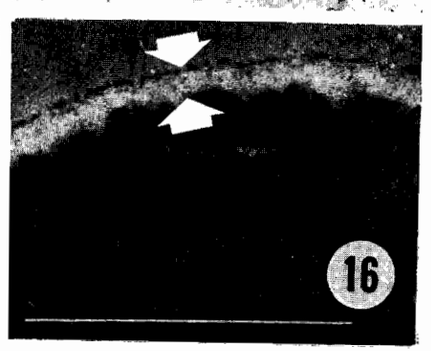
Ectosymbionts have been reported in a variety of insect–fungal associations; Attine ants and macrotermitine termites culture basidiomycetes (Wilson, 1971). The fungal genus *Septobasidium* is found on living plants in mutualistic association with scale insects (Couch, 1938). Perhaps the best-known example in a non-social insect is the multi-nucleate arthrospore of the fungus *Amylostereum areolatum* carried by the wood wasp *Sirex noctilio* (Gant, 1969).

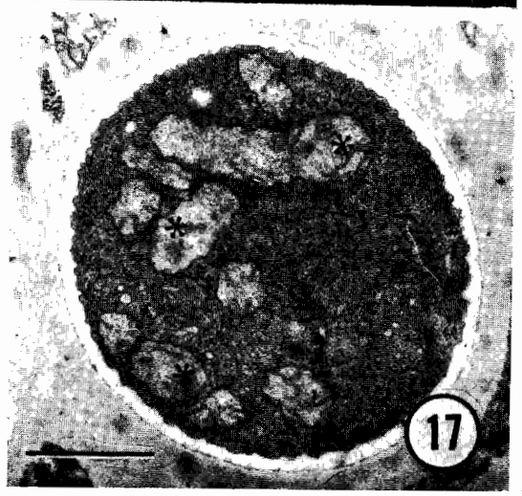
Basidiomycetes have been found in trees infested with *Dendroctonus* (Bramble and Host, 1940; Borden and McClaren, 1970, 1971). More recently, basidiomycetes have been isolated from the mycangia of 2 species of phloem-feeding bark beetles, *Dendroctonus frontalis* and *Dendroctonus brevicomis* (Barras and Perry, 1972; Whitney and Cobb, 1972). At least in *D. frontalis*, the mycangial growth stage is multinucleate (Happ *et al.*, 1976). In the present report, we show that the mycangia of an ambrosia beetle harbor multinucleate basidiomycetous propagules. To our knowledge, these few cases are the only known multinucleate basidiomycetous yeasts in these scolytid beetles.

Apparently, the more obvious blue-stain fungi, which are faster growing in culture and more readily isolated, caused the basidiomycetous microsymbionts of *Dendroctonus* and *Xyleborus* to be overlooked in the past. Investigation of phylogenetic relationships among the basidiomycetous ectosymbionts of the two genera of scolytid beetles, of termites, of scale insects, of attine ants, and of *Sirex* offers an appealing set of problems for mycologists. The interactions among the imperfect basidiomycetes, the ascomycetes, and the insect suggest new challenges to microbial-ecologists. The involvement of exocrine secretions as mediators of the association adds fascination. Since the biological success of many of these









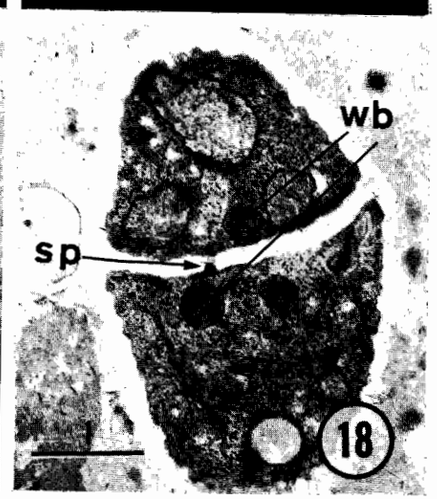


PLATE 5.

FIG. 14. A large propagule. Two nuclei (N) and nucleoli (n) are in plane of section, in addition to numerous storage vacuoles (white asterisks) and mitochondria (black asterisks). This particular cell is rather more elongate than most. ×11,520

FIG. 15. Cell wall of larger propagule is lamellate, a characteristic of basidiomycetous yeasts. Outer 2 lamellae have been lost at the upper right. ×13,600

FIG. 16. Cell wall (between arrows) of the smaller class of propagules is not lamellate and has an electron-dense outer margin. ×45,200

FIG. 17. A small propagule containing several mitochondria (asterisks). ×17,400

FIG. 18. Short chains of cells, of small size class, are separated from one another by an ascomycete-like septum, with a septal plug (sp) and Woronin bodies (wb). Scale bar = 1 μm. ×15,800

symbiotic associations causes significant financial loss, their further investigation is certainly warranted on economic as well as scientific grounds.

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