Prothoracic Mycangium on Pine-infesting *Pityoborus* spp. (Coleoptera: Scolytidae)

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ABSTRACT Fungus spores were abundant in the female-specific pubescent pronotal depressions (mycangia) on four examined *Pityoborus* spp. A culture of the ambrosia fungus associated with the mycangium on *Pityoborus comatus* Zimmermann conforms to *Holtermannia corniformis* Kobayasi. We speculate that spores are raked from galleries by pronotal asperities and deposited in the mycangia. Colonization of small-diameter, nutrient-poor, shaded-out branches of living trees by *Pityoborus* spp. is made possible by their having adopted features of typical ambrosia beetles.

KEY WORDS *Pityoborus* spp., mycangium, Scolytidae

SCOLYTIDS AND their platypodid allies are associated with various fungi. These mutualistic fungi help scolytid colonization by weakening host trees (Berryman 1972), stimulating beetle aggregation (Brand & Barras 1977, Brand et al. 1977), and providing nutrition (Norris & Baker 1967, Kok et al. 1970).

Some scolytids and platypodids carry mutualistic fungi in repositories in their integument (Francke-Grosmann 1963). These repositories may occur on the thorax, head, or elytra of various species. Batra (1963) called them "mycangia" and described them as being of ectodermal origin, found mainly in females, near glandular parts of the body, and occurring in pairs. Barras & Perry (1975) reviewed the literature concerning mycangia.

Farris & Funk (1965) broadened the definition of mycangia to accommodate the unpaired pronotal pits on female *Platypus wilsoni* Swaine, as did Nakashima (1975) for other platypodids. Likewise, Livingston & Berryman (1972) used the term for the numerous cup-shaped pits on the head of *Scolytus ventralis* LeConte. We apply the term "mycangium" to any repository of the insect cuticle that is adapted for the transport of fungus.

Pityoborus spp. infest shaded-out lower branches (<1 to 8 cm diameter) of numerous pine species from Honduras and Belize to North Carolina and Utah. Wood (1958, 1982) described seven species, of which two occur in the United States. *Pityoborus secundus* Blackman infests *Pinus ponderosa* Laws. in Utah and New Mexico, and *Pityoborus comatus* (Zimmermann) infests *Pinus echinata* Mill., *Pinus elliottii* Engelm., *Pinus palustris Mill., and Pinus taeda* L. in the southeastern United States (Blackman 1922, Beal & Massey 1945).

Adult female *Pityoborus* are unique among North American scolytids in having an oval, densely pubescent depression on each side of their pronotum (Fig. 1-4). Because of the affinity of the galleries to those of ambrosia beetles, we sought to determine if the pubescent areas function as mycangia as reported recently by Beaver (1986) for *Hypothenemus curtipennis* (Schedl) from Fiji and Borneo.

Methods

Specimens were examined from the following sources: *Pityoborus comatus*: *Pinus elliottii*, Lake Placid, Florida (M. A. Deyrup, collector); *Pityoborus hirtellus* Wood: *Pinus hartwegii* Lindl., Cerro Tlaloc, Tequesquináhuac, Estado de Mexico (A. Equihua M., collector); *Pityoborus rubentis* Wood: *Pinus lumholtzii* Robins & Fern., La Flor, Durango, Mexico (M. M. Furniss, collector); and *Pityoborus secundus: Pinus cooperi* C. E. Blanco, Guachochi, Chihuahua, Mexico (M. M. Furniss, collector).

Females were cleaned and softened by treatment for 0.5-1 h in a detergent solution in a sonic bath. The pronota were dissected under water, and the pubescent area of each was cross-sectioned with a scalpel. The sections were mounted on edge on double sticky tape attached to stubs, then sputter-coated with gold. The mounted specimens were examined at Washington State University with an ETEC Autoscan electron microscope to study and photograph the mycangium structure and its spores. Film used was Polaroid Type 55.

Additional pronota were dehydrated in tertiary butyl alcohol, embedded in paraffin, and 15-μ-thick cross sections obtained with a rotary microtome (Johansen 1940). The sections were stained by the

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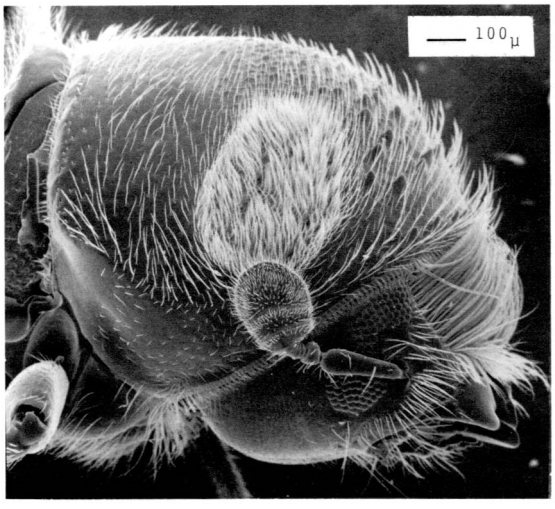


Fig. 1. Right side of prothorax and head of *P. rubentis* showing the oval, pubescent mycangium that characterizes the genus.

modified Gram-Weigert method (Leach 1940) to confirm further that the inclusions were spores.

Pronota of reared *P. comatus* females were surface-sterilized by the method of Norris & Baker (1967). Pieces of mycangium were transferred aseptically to petri dishes containing PDA and malt agar. Dishes were incubated at 20°C and examined periodically. After 3 wk, temporary slide mounts in lactophenol–cotton blue (Sass 1958) were examined microscopically to classify the fungus as ambrosial, using the generic key of Batra (1967). Subsequently, test tube cultures on PDA were sent to L. R. Batra (ARS–USDA, Beltsville, Md.) for specific determination.

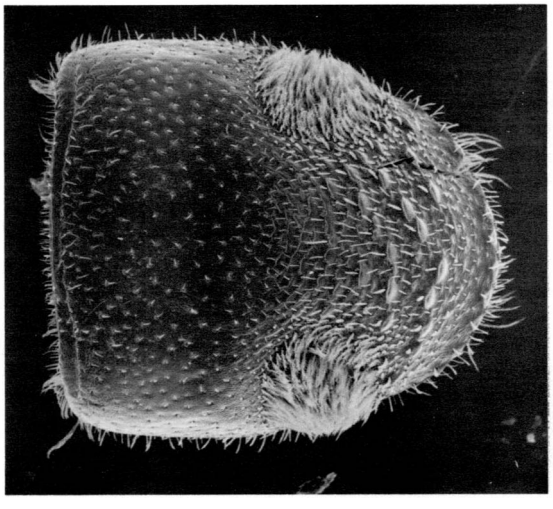


Fig. 2. Dorsal aspect of *P. comatus* pronotum with the lateral, pubescent mycangia and asperites anterior to the mycangia.

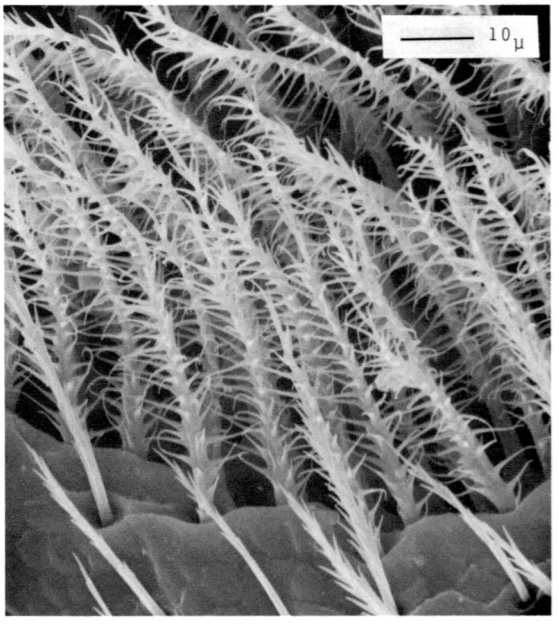


Fig. 3. Lower edge of the mycangium on *secundus* showing the dense, branched setae that occupy the mycangial depression.

Results and Discussion

We found sporelike inclusions in the pubescent areas on the pronota of the four *Pityoborus* species of which we had specimens (Fig. 5–11). The setae composing the pubescent areas are generally bare near their bases but plumose above. The spores were wrinkled and spheroidal (except elongate on *P. rubentis*, Fig. 11) and were concentrated among the basal stems of setae, where they loosely fitted the interspaces.

The spheroidal objects in *P. comatus* mycangia stained blue, confirming that they were spores (Fig.

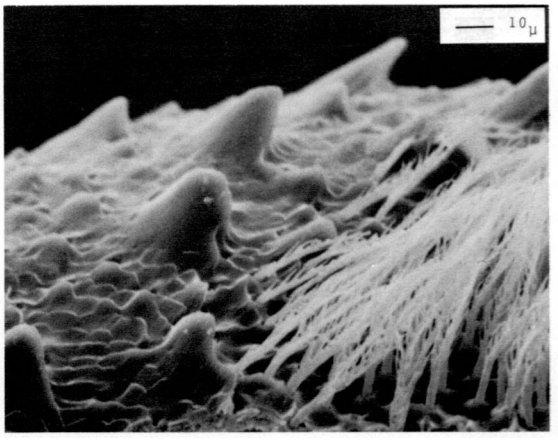


Fig. 4. Low-angle, upward perspective of an excised lateral section of the left mycangium of *P. comatus*. The asperites (left) are thought to rake spores from the gallery wall into the mycangia behind them.

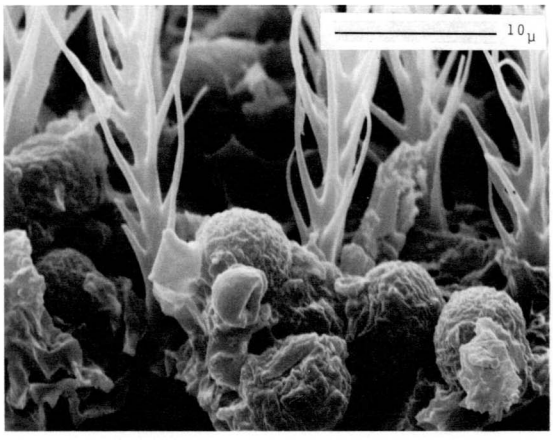


Fig. 5. Spores and associated waxlike substance among setae on the floor of the mycangium on *P. comatus*.

6). When cultured, the fungus sporulated and did not differ from the description of *Holtermannia corniformis* Kobayasi, although no standard culture was available for comparison. *H. corniformis* is also an associate of *Dendroctonus frontalis* Zimmermann (L. R. Batra, personal communication). The fungus culture has been deposited in the mycological collection (USDA Forest Products Laboratory, Madison, Wis.) under accession numbers "Batra 4482 to 4485."

We did not study the histology of mycangia. The inner setae of *P. secundus*, however, have unique, raised sockets open on one side (Fig. 9). Furthermore, the mycangial setae on some specimens of all four species were mired in a substance that may be a secretory product (Fig. 5, 10, and 11). This substance withstood thorough sonic cleaning in warm detergent solution.

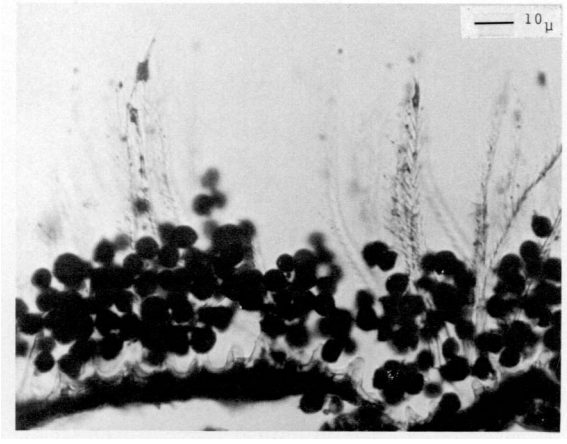
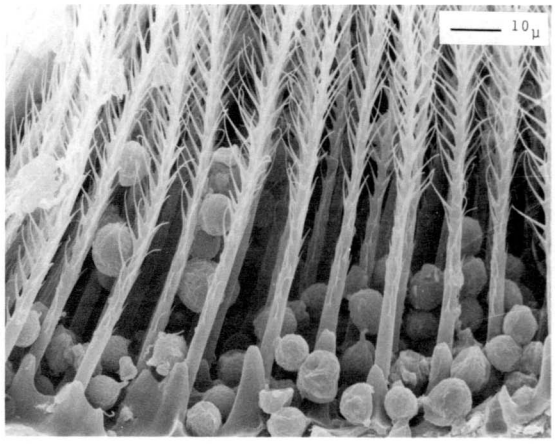
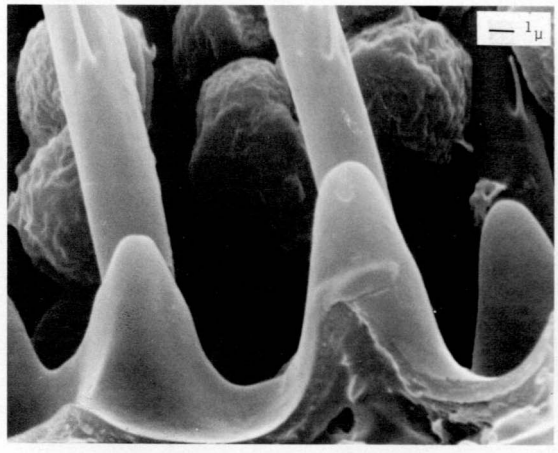


Fig. 6. Microtome section of the mycangium on *P. comatus* showing the dark-stained spores, branched setae, and raised setal sockets on the mycangial floor.





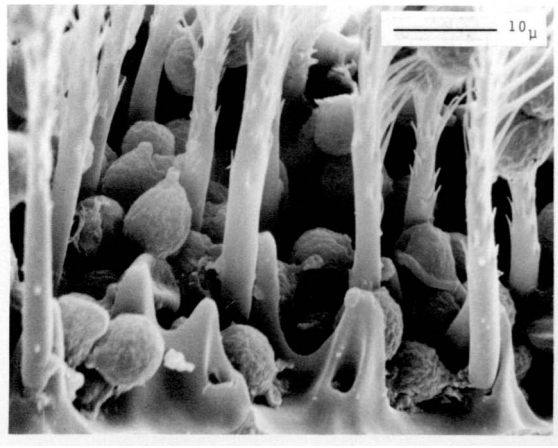
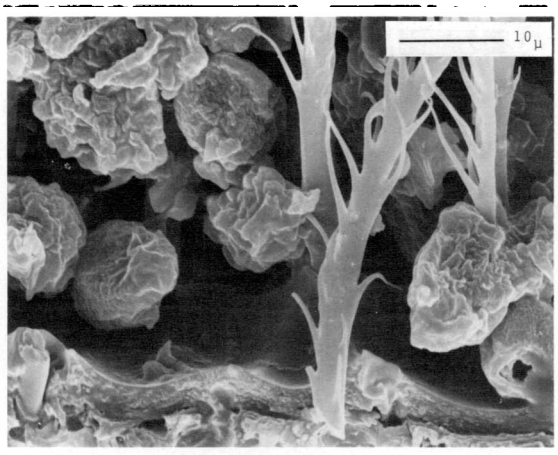


Fig. 7–9. Spores, setae, and the setal sockets that open to one side (*P. secundus*). Setae of the three empty sockets (9) were lost during sectioning of the specimen.

All species of *Pityoborus* have asperites on their pronota, located forward of the mycangia (Fig. 1, 2, and 4). Numerous other members of the Scolytidae possess crenulations and asperites on their



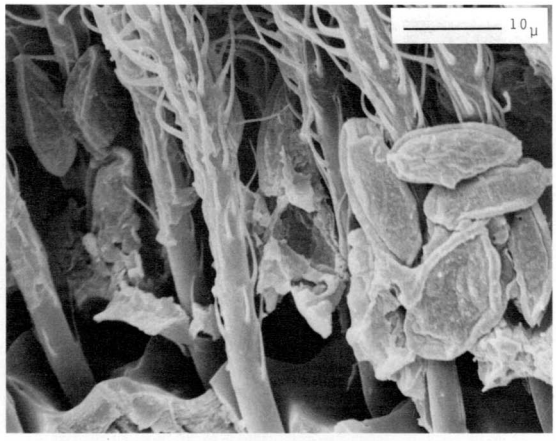


Fig. 10 and 11. Spores and associated wax-like substance on *P. hirtellus* (10) and *P. rubentis* (11).

pronota. These projections may aid the activities of beetles in their galleries, especially by providing an anchor during boring.

In the case of *Pityoborus* spp., however, the asperites may have an added function. The location, size, and shape of the asperites suggest to us that they may serve also as rakes to loosen spores from the gallery wall and to direct them backward into the mycangia. Examples of fungi sporulating on the walls of beetle galleries were illustrated by Leech et al. (1940), Funk (1965), and Barras & Perry (1972).

A similar spore-gathering function was attributed by Farris (1965) to a comb on the coxae of *Monarthrum scutellare* LeConte. Also, Strohmeyer (1911) in Leach et al. (1940) noted that females of some tropical ambrosia beetles have chitinous hooks or brushes on the front of the head, on which ambrosial spores and mycelia are always found. Additionally, Beeson (1917) found brushes on the head of the platypodid, *Diapus furtivus* Sampson, that carry spores (cited in Farris & Funk [1965]).

We speculate that the movement of adult female *Pityoborus* spp. in the confinement of their galleries causes their mycangial setae to brush back and forth, allowing loosened spores to filter onto the mycangial floor. The shape and size of spores permit them to enter between the setae, but bark fragments are excluded.

The factors and mechanisms involved with the release of fungi from scolytid mycangia in newly colonized trees are virtually unstudied. However, the release site of spores from the southern pine beetle, *Dendroctonus frontalis* Zimmermann, was illustrated by Barras (1975). We theorize that conditions in the new galleries of *Pityoborus* spp. favor spore germination in the mycangia, and that the escape of fungus thereafter is a function of growth of the fungus (Farris & Funk 1965), coupled with the mechanical movement of the host beetle during gallery construction. Alternatively, or in concert, volatile chemicals in the host tree at time of attack may act as solvents and lubricants in freeing spores from the waxlike substance within shallow mycangia such as those possessed by *Pityoborus*.

Identification of the ambrosia fungus isolated from the mycangium of female *P. comatus* strengthens the affinity of *Pityoborus* spp. with xylomycetophagous scolytids. Other factors in common include production of few eggs and development of larvae to maturity in short larval cradles, due apparently to dependency upon ambrosia fungi for nutrition.

The behavior of *Pityoborus* spp. may result from their adapting to an unoccupied niche presented by small-diameter, shaded-out branches of living trees. By tunneling in the branchwood, sometimes only on the underside (*P. comatus*), and evidently feeding on ambrosia fungi, these beetles escape the adverse effects of drying and competition while gaining sustenance in dead branches, which may otherwise be nutrient-poor.

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