FUNGI ASSOCIATED WITH CERTAIN AMBROSIA BEETLES

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INTRODUCTION

The association of certain fungi with the tunnels of ambrosia beetles has been recognized for nearly 100 years (1). These ambrosia fungi apparently are used as food by the beetles, and different fungi are cultivated by different species or groups of species (2). Recently, Leach et al. (3), in a paper on two ambrosia beetles and their associated fungi, summarized the past work on ambrosia fungi, pointing out particularly the scarcity of exact information on this group.

In 1937 the Division of Forest Insect Investigations, Bureau of Entomology and Plant Quarantine, United States Department of Agriculture, started a general study of the biology of ambrosia beetles of the Southern States, and the Division of Forest Pathology cooperated in studying the fungi associated with certain ambrosia beetles of economic importance in the deterioration of green hardwood logs and lumber. The present paper is a report of the mycological findings of this study.

MATERIALS AND METHODS

Wood infested with ambrosia beetles was furnished largely by H. R. Johnston, of the Division of Forest Insect Investigations, Bureau of Entomology and Plant Quarantine, at Saucier, Miss. Some material was gathered at various sawmills in Louisiana. All the beetle material was identified by Johnston.

All cultural studies were made on malt agar (1½ percent agar and 2½ percent malt) at room temperature, approximately 75° to 85° F. Isolations were made by allowing live beetles to walk on the agar surface and also by making transplants from the wood adjacent to tunnels. Some cultures were purified by making single-spore transfers from the original cultures secured from the beetles or from wood. Numerous freehand and microtome sections were cut from wood blocks that contained insect tunnels, for the study of the fungi as they occur in nature.

The species of ambrosia beetles studied were Platypus compositus Say, Pterocyclon mali (Fitch), P. fasciatum (Say), Xyleborus affinis Eich., and X. pecanis Hopkins.

1 Received for publication April 22, 1942. The Southern Forest Experiment Station of the Forest Service, U. S. Department of Agriculture, cooperated in these investigations by providing facilities for field studies at Saucier, Miss.

2 The author is indebted to Ross W. Davidson, of the Division of Forest Pathology, for advice on the taxonomy of the fungi described; and to Edith K. Cash, of the Division of Mycology and Disease Survey, Bureau of Plant Industry, for translating descriptions of species into Latin.

3 Italic numbers in parentheses refer to Literature Cited, p. 144.
FUNGI ISOLATED FROM AMBROSIA BEETLES

WOOD-STAINING FUNGI

Although a great many fungi, bacteria, yeasts, and nematodes were isolated from ambrosia beetles and their tunnels, only the food fungi and general-staining fungi were given special attention. Stain accompanying ambrosia beetle attack is of two distinct types: (1) A restricted darkening adjacent to the tunnels, and (2) general staining of the type common in sapwood without beetle attack.

Restricted blackened areas are common, if not universal, around tunnels of *Platypus compositus*, *Pterocyclon mali*, and *P. fasciatum* in sapwood but absent or indistinct in heartwood. Stain is commonly absent around the tunnels of *Xyleborus affinis*, but occasionally a light-brown stain is found around the tunnels of this species in sweetgum (*Liquidambar styraciflua* L.) and southern sweetbay (*Magnolia virginiana* L.). Around the tunnels of *Xyleborus pecanis* no black stain was observed, although some browning of the adjacent wood cells occurred. Black stain is most pronounced with *Platypus*
compositus (fig. 1, A), extending longitudinally from the tunnel as much as 5 mm, although laterally it spreads but a few cells from the edge of the tunnel. With Pterocyclon (fig. 1, B) the black is just as intense but usually extends less than 1 mm from the tunnel.

Isolations from black stain around tunnels yielded a number of organisms, but the only ones consistently isolated were the ambrosia or food fungi of the beetles concerned; these fungi are described later in this paper. Microscopic examinations of the blackened areas showed the wood cells to be filled with fungus material of a deep-brown color, which in some cases could be identified as an ambrosia fungus by the presence of spores. When the surface of sapwood blocks of sweetgum and oak was sterilized for 30 seconds in boiling water, and the blocks were then inoculated with cultures of the ambrosia fungi, stain was produced. This stain, however, was not as intense as that found around the tunnels of Platypus compositus and Pterocyclon spp. Indications are that the stain around beetle tunnels is caused by the ambrosia fungi, although the color may be intensified by reactions of these fungi with insect secretions or other organisms, such as yeasts and bacteria, which are commonly present.

General staining of the type common in sapwood without beetle attack is not universal with ambrosa beetle attack. However, past tests and observations have shown that severe staining of logs is not prevented by chemical sprays when ambrosia beetle attack is heavy (5). The role of ambrosia beetles in disseminating general wood-staining fungi has been discussed more fully in another paper (7).

**AMBROSIA OR FOOD FUNGI**

**AMBROSIA** Fungus of *Platypus compositus*

The only fungus consistently observed in the tunnels of *Platypus compositus* or consistently isolated from the adults or wood adjacent to the tunnels of this insect was a species of *Endomyces* Rees. A review of the literature disclosed no described species having the spore, asci, and hyphae measurements of this ambrosia fungus or any species of *Endomyces* with only two-spored asci. Therefore, the fungus is here described as a new species:

*Endomyces bispora* sp. nov.

On malt agar, colonies (fig. 2, C) are very slow growing, reaching 5–8 mm in radius in 6 days at room temperature (75° to 85° F.). At first the colony is hyaline, then turns a light yellowish brown, although under the microscope the hyphae and spores appear hyaline. The edge of the colony is distinctly mycelial with many conidia formed singly or in heads on short recumbent conidiophores or laterally on the hyphae (fig. 2, A and B). The cells of the conidiophores often disjoint and act as conidia, and buds form from the conidia. Most of the colony is composed of a slimy mass of conidia and ascospores, which give it a yeastlike appearance. Practically no aerial mycelium is formed.

Conidia commonly germinate on malt agar by producing buds, forming a yeast-like colony from the margin of which hyphae eventually protrude. No germination of ascospores was observed.

Conidia, including disjointed conidiophores and buds, are hyaline, unicellular, and of various shapes and sizes: 2.5μ–12.5μ×2.2μ–5.0μ, averaging 6.3μ×3.8μ, usually elongate and somewhat pear-shaped. Asci are hyaline, slightly pear-shaped, and are usually formed terminally in small groups of two to six. Asci are occasionally formed singly at the tip or nodes of the asci-bearing hyphae (fig. 2, A) or in compound heads on short branches. Asci are 7.5μ–11.2μ×6.2μ–10.0μ, averaging 7.4μ wide and 8.6μ long. Asci-bearing hyphae range from 1.8μ to 3.3μ in diameter, averaging 2.5μ. Ascospores (fig. 2, A) are hyaline, hat-shaped, 6.2μ–9.8μ, averaging 7.2μ, across the brim, and 1.8μ–2.2μ, averaging 2.4μ.
are usually aligned within the ascus with the brims extending from the base to the
in width perpendicular to the brim. The brim surface is concave. The spores

Figure 2.—Endomyces bispora on malt agar: A, Asci, ascospores, and conidia; 
B, margin of a culture showing hyphae and conidia production, × 150; C, cul-
ture 15 days old grown at about 80° F., × 1.

tip of the ascus and nearly filling the ascus. Only two spores were observed in
each ascus.
Isolated from adults of *Platypus compositus* Say and from wood adjacent to their tunnels. Observed in pecan (*Carya* sp.), sweetgum (*Liquidambar styraciflua* L.), swamp tupelo (*Nyssa biflora* Walter), and locust (*Gleditsia* sp.).

Cultureae in agaro maltoso lente crescentes, primum hyalinae, dein pallide flavo-brunnescentes, margine distincte myceliali; conidia numerosa, singula vel in capitulis in conidiophoris brevibus recumbentibis vel in lateribus hypharum formata; cellulae conidiophorum sese disjungentes et ut conidia agerentes; gemmæ e conidiis formatae; conidia conidiophoris et gemmis inclusis hyalina, unicellularia, 2.5 μ-12.5 μ longa, 2.2 μ-5.0 μ lata, plerumque elongata et paulo pyriformia; asci hyalini, paulo pyriformes, plerumque terminales et 2-6 caespitosi, interdum ad apicem vel nodos ascophorarum singuli, vel in capitulis compositis in ramos brevibus nati, 7.5 μ-11.2 μ longi, 6.2 μ-10.0 μ lati, bispori; ascoporiae hyalinae, pileiformes costulatae, costula inculsa 6.2 μ-9.8 μ in diam., ad angulum rectum 1.8 μ-3.2 μ in diam., vulgo costulatis e basi ad apicem extensis dispositae et ascum fere implentes.

The ambrosia in the insect tunnel consists of conidia and asci, at first hyaline, but, as larval activity increases, changing into a slimy yellowish coating on the tunnel wall. The adjoining wood-cell lumina become filled with hyphae and conidia, which turn to deep brown, causing an intense black stain of the wood adjacent to the tunnel. The reason for the darkening of hyphae and conidia in the wood was not determined, although inoculations showed that the color change occurred.

**AMBROSIA FUNGUS OF XYLEBORUS AFFinis**

An imperfect fungus, which apparently is the ambrosia of *Xyleborus affinis*, was observed in association with this beetle and was consistently isolated from tunnels and from adults. The fungus is apparently an undescribed species of *Cephalosporium* Corda.

*Cephalosporium pallidum* sp. nov.

On malt agar, colonies (fig. 3, C) are moderately slow growing, reaching 9 to 14 mm. in radius in 6 days at room temperature (75° to 85° F.). The margins are usually appressed and hyaline while the rest of the colony is covered with a thin layer of hyaline, fluffy aerial mycelium which often becomes appressed with age except for isolated tufts. Aerial mycelium may be entirely lacking. Occasionally a slight brownish tinge develops in parts of old cultures. Yellowish, yeasty mounds develop in aging cultures. In the yeasty mounds mycelium may be limited largely to pointed, short-celled hyphae (fig. 3, A) projecting but shortly from the yeasty mass of conidia and monilioid cells. Compact helicoid hyphal formations were commonly observed in the filamentous mycelium. Conidia germinate on malt agar by forming monilioid chains of cells which finally give rise to hyphae (fig. 3, A). Spore heads are formed relatively soon after germination.

In culture typical fruiting consists of cephalosporic heads of conidia protruding but slightly above the agar (fig. 3, A) on erect or decumbent conidiophores. Conidiophores are usually unbranched and hyaline and terminate in 1 to 10 or more hyaline, unicellular conidia which are nearly spherical to slightly pear-shaped, 7.6 μ-14.4 μ long and 7.9 μ-14.0 μ wide, averaging 10.8 μ×10.4 μ. When appreciable aerial mycelium occurs, conidiophores elongate and branch (fig. 3, A). Sometimes conidiophores are composed partly or totally of moniliiform cells, particularly in the yeasty mounds. Occasionally buds were observed forming laterally on hyphae (fig. 3, A), and monilioid chains of spores of irregular sizes and shapes were observed in the agar or protruding above it (fig. 3, A).

Isolated from adults of *Xyleborus affinis* Eich. and from the wood adjacent to their tunnels. Observed in sweetgum (*Liquidambar styraciflua* L.), swamp tupelo (*Nyssa biflora* Walter), and southern sweetbay (*Magnolia virginiana* L.).

Coloniae in agaro maltoso lente crescentes; mycelium plerumque apressum et hyalimum, in culturis vetustis brunneotinctum; conidiophorae plerumque non ramosa, hyalina, in conidiis 1–10 vel pluribus, hyalinis, unicellularibus, fere sphericis vel leniter pyriformibus, 7.6 μ-14.4 μ longis, 7.9 μ-14.0 μ
latis terminata, partim vel omnino e cellulis moniliformibus composita; gemmae interdum in lateribus hypharum formatae; catenulae monilioidcae sporarum magnitudinis formaque variabilium praeentes; conidia in natura singula vel 2-4 in capitulis, rare in catenulis brevibus nata.

The ambrosia (fig. 3, B) in the tunnels of *Xyleborus affinis* consists of conidia, mostly solitary, sometimes in heads of 2 to 4 spores and occasionally in short monilioid chains. In new tunnels single conidium-bearing conidiophores (12μ–38μ long) are easily seen, but later only a jumbled mass of cells is visible. The fungus mostly remains hyaline in the tunnel and adjacent wood cells, and does not produce stain around the tunnel except slightly in sweetgum and southern sweetbay.

The ambrosia fungus of *Xyleborus affinis* is apparently related to
that of *X. dispar*. The fungus associated with *X. dispar* was originally described by Hartig as *Monilia candida* (1). Neger (4) expressed the opinion that it may be an endomycete, although no asci or ascospores have been observed. Schneider-Orelli (6), in describing and illustrating the ambrosia fungus of *X. dispar*, shows it to be similar to that associated with *X. affinis* except that no mention is made of yellowish yeasty mounds or cephalosporic heads such as occur with the ambrosia fungus of *X. affinis*, although the author does illustrate a conidiophore bearing a single conidium similar to those in yeasty mounds of *Cephalosporium pallidum*. Furthermore, black stain occurs around the tunnels of *X. dispar* but is absent or not so pronounced around those of *X. affinis*.

**Ambrosia Fungus of Xyleborus pecanis**

The ambrosia fungus associated with the tunnels of *Xyleborus pecanis* is apparently an undescribed species of *Cephalosporium* Corda.

![Fig. 4.—Cephalosporium luteum: A, Young ambrosia in insect tunnel, × 99; B, culture on malt agar, 15 days old, grown at about 80° F., × 0.8.](image)

**Cephalosporium luteum** sp. nov.

On malt agar, colonies (fig. 4, B) are fast growing, reaching 60-70 mm. in radius in 6 days at 75° to 85° F. They are at first hyaline but soon become sulfur yellow to light brown. The agar is stained a deep brown. Aerial mycelium is at first fluffy but becomes appressed with age. No tendency for budding or yeasty growth was observed as with other ambrosia fungi encountered in this study. The margin of the colony is decidedly filamentous.

Fruiting on malt agar is sparse, and spores are often difficult to find. When a block of mycelium-bearing agar is removed from a culture and placed in a moist chamber, a few spores usually can be found along the cut edge on protruding conidiophores within 24 hours. Spores are formed on simple or branched conidiophores, mostly singly, sometimes in heads of two or three spores, are hyaline, nearly spherical, unicellular, and average 6.5μ in diameter. In nature spores are larger, 5.3μ–12.5μ wide and 6.1μ–15.0μ long, averaging 8.8μ×10.1μ.

Observed in association with *Xyleborus pecanis* Hopkins in swamp tupelo (*Nyssa biflora* Walter) and southern sweetbay (*Magnolia virginiana* L.).

Colonies in agar maltosso rapide crescentes, primum hyalinae, dein e sulphuris brunneolae; mycelium aereum primum floccosum, vetustum appressum; fructificatio in culturis sparsa; conidiophora plerunque non ramosa et brevia; sporae
singulae vel 2–3 in capitulis, hyalinae, fere sphericæ, unicellulares, in culturis
6.5µ in diam., in natura 5.3µ–12.5µ latae, 6.1µ–15.0µ longae, plerumque singillatim
interdum 2–3 in capitulis natae.

The ambrosia in the beetle gallery is at first hyaline but later forms
a yellowish to brownish lining to the brood chamber. It consists of
mycelium and conidia (fig. 4, A), mostly borne singly on short, simple
or branched conidiophores, or in heads of two to three spores. The
conidia remain hyaline. The wood around the chamber is stained
a light brown.

Young ambrosia is similar to that in the tunnels of Xyleborus affinis.
Later, however, the ambrosia of X. pecanis turns a sulfur yellow and
does not become yeasty, as does that of X. affinis. The conidia of
the ambrosia fungus of X. pecanis are, in general, similar to some of
the conidia of the ambrosia fungi of X. affinis and of X. dispar, but
no monilioid chains, such as occur in the last two, were observed. The
ambrosia fungus of X. pecanis is apparently the same as that of X.
xylographus Say or is closely related to it, as illustrated and described
by Hubbard (2).

**Figure 5.** Monilia brunnea: A, Ambrosia in insect tunnel, × 84; B, culture
15 days old grown on malt agar at about 80° F., × 0.7.

AMBROSIA FUNGUS OF PTEROCYCLON MALI AND P. FASCIATUM

The ambrosia fungus of *Pterocyclon mali* is apparently the same as
the fungus associated with *P. fasciatum* and appears to be a new
species of *Monilia* Pers.

**Monilia brunnea** sp. nov.

On malt agar, colonies are slow growing, reaching 8–10 mm. in radius in 6 days
at 75° to 80° F. They are appressed, and at first are nearly hyaline but with
age become dark brown (fig. 5, B). Original isolates were quite yeasty in general
appearance and consisted largely of monilioid chains of rounded cells that budded
in situ, similar to the growth described and illustrated by Leach et al. (3) for
the ambrosia fungus of *Trypodendron betulae* Sw. and *T. retusum* (Leb.). After
repeated subculturing, more filamentous mycelium was formed, although the
monilioid type of growth persisted in parts of the culture. When filamentous
mycelium was formed the surface of the culture became finely tomentose, the
amount of aerial mycelium being limited. In old cultures there was a tendency
for small mounds to form, consisting mostly of monilioid cells. In one isolate short, pointed hyphae were formed, similar in shape to those described for the ambrosia of Xyleborus affinis but dark brown in color.

Monilioid cells are mostly hyaline, although in old cultures and old tunnels they may be distinctly brown, and are borne in simple or branched chains. Cells contain large granular particles. The terminal one to three cells are usually nearly spherical while the other cells are oblong or rectangular to spherical. Mature cells are 7.5\(\mu\)-11.5\(\mu\) long by 5.2\(\mu\)-10.0\(\mu\) wide, averaging 8.5\(\mu\) in each direction. In some isolates there was a tendency in culture for conidia to collect in heads as well as in chains.

Spores germinate on malt agar by forming monilioid groups of cells that eventually give rise to hyphae.

Observed in association with Pterocyclon mali (Fitch) in sweetgum (Liquidambar styraciflua L.), swamp tupelo (Nyssa biflora Walter), maple (Acer sp.), and oak (Quercus sp.), and with P. fasciatum (Say) in oak (Quercus sp.).

Culturae in agaro maltoso lente crescentes vetustae fuscescentes; mycelium plerumque appressum; sporae interdum gemmantes; hyphae in articulos oidiiformes secendentes; sporae plerumque hyalinae, interdum in culturis vetustis et in viis insetorum brunnea, in catenulis simplicibus vel ramosis natae; sporae terminales 1–3 fere sphericae, alterae ex oblongis vel rectangularibus sphericae, 7.5\(\mu\)-11.5\(\mu\) longae, 5.2\(\mu\)-10.0\(\mu\) latae.

The ambrosia in the tunnels of the beetle consists of masses of monilioid cells (fig. 5, A) with little filamentous mycelium. Freshly formed ambrosia is hyaline, but in older tunnels it may turn dark brown. Ambrosia forms in both the main tunnel and the larval cradles. Wood cells adjacent to the tunnels become filled with hyphae and monilioid cells that soon turn brown, causing an intense black stain around the tunnel. Stain occurred in the wood of each species in which the beetles were found.

The ambrosia fungus of Pterocyclon apparently is closely related to that of Trypodendron. With both of these genera larvae are reared in separate cradles in contrast to the other genera encountered in this study, in which the larvae are free in the egg-laying tunnel. The ambrosia fungus associated with Pterocyclon mali and P. fasciatum apparently is a species of Monilia Pers. However, it is not identical with Monilia candida Hartig, associated with Xyleborus dispar (6), or the fungus described by Leach et al. (3) as the ambrosia of Trypodendron betulae and T. retusum and considered by those authors as a possible strain of M. candida.

**SUMMARY**

Four fungi commonly associated with southern species of ambrosia beetles are described as new species: Endomyces bispora, associated with Platypus compositus; Cephalosporium pallidum, with Xyleborus affinis; C. luteum, with X. pecanis; and Monilia brunnea, with Pterocyclon mali and P. fasciatum.

These fungi apparently are used as food by the ambrosia beetles with which they are associated and probably cause the restricted black or brown stain adjacent to the beetle tunnels.
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(1) Hartig, T.

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1940. OBSERVATIONS ON TWO AMBROSIA BEETLES AND THEIR ASSOCIATED FUNGI. Phytopathology 30: 227-236, illus.

(4) Neger, F. W.

(5) Scheffer, T. C., and Lindgren, R. M.

(6) Schneider-Orelli, O.

(7) Verrall, A. F.
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