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THE DEVELOPMENT OF PLEUROTUS ULMARIUS FR. GROWN IN PURE CULTURE

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INTRODUCTION

Developmental studies of various fleshy fungi have been made by various workers, viz. Atkinson (1,2,3), Beer (4), Blizzard (5), de Bary (6), Douglas (7,8), Fayod (9), Fischer (10), Hartig (12), Hein (13), Johnson (14), Levine (17,18), Sawyer (19), Swartz (21,22), Walker (25), Zeller (26), et al. Ordinarily such studies have been carried out upon fruit bodies collected in their natural habitats. Few, if any, studies of this kind have been made on fruit bodies produced on laboratory media.

MATERIALS AND METHODS

In the fall of 1960, tissue cultures of **Pleurotus ulmarius** Fr. were obtained from a young specimen collected from a dead elm tree near Fayetteville, Arkansas. Tramal tissue was removed aseptically and placed in flasks of malt extract agar* and allowed to incubate until a relatively large mycelical mat had appeared on the surface of the agar, and fruit body initials began to appear. After inoculation the cultures were kept in a coldroom at 4.4° C. for 24-48 hours prior to removal to the laboratory at room temperature. Cultures placed in the light in a laboratory window showed great acceleration in fruit body formation.**

Fruit bodies at representative stages of development were killed in Flemming's strong solution, washed, dehydrated, and embedded in paraffin in the usual way. Sections were made 7-10 microns in thickness and stained in Heidenhain's Iron-Alum Haematoxylin. (In order to be sure that our specimens were typical, comparative studies were made using sterile segments removed from dead elm branches. Fruit bodies studied from their natural habitat as checks showed no significant variation microscopically or macroscopically.)

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*Formula for malt extract agar:
25 gms. malt extract (Difco); 0.5 gms. $MgSO_4$; 0.5 gms. $Ca(NO_3)_2$; 0.25 gms. KH_2PO_4; 0.1 gm. peptone; 25 gms. Agar Agar; 1000 ml. distilled water.

**More than 500 mature fruit bodies were produced in cultures between October 1960 and May 1961.

OBSERVATIONS

Fruit bodies (Figs. 1,2,3) showing no external differentiation were selected. The smallest ones studied measured 560 microns by 294 microns wide. In median longitudinal section a mass of interweaving, uniform hyphae was seen. The hyphae exhibited no specific directional growth and stained uniformly.

In slightly older fruit bodies, darkly staining elements scattered randomly through the fruit body were visible (Fig. 3). These darkly staining elements vary in length from 1 to 100 microns, but the average is about 25 microns. They have a uniform thickness, averaging 5 microns. They present a striking contrast with the surrounding mass of interweaving hyphae. These interesting elements disappear as the fruit bodies approach maturity. Similar structures have been reported by Sawyer (19).

Appearance of Primordia of the Stipe and Pileus

As development proceeds the young fruit body gradually becomes somewhat barrel-shaped. Differentiation of the stipe and of the pileus is first noticeable when the hyphae near the apex bend outward and curve downward, thus forming a shallow furrow. At this stage the primordium is an elongate structure having an enlarged base and a narrow blunt apex (Fig. 4). The hyphae usually grow in a direction parallel to the long axis. The staining is relatively uniform at this time, yet the previously mentioned dark staining hyphae are very conspicuous. Marginal hyphae are greatly curved. Despite the characteristic bending outward and downward by the hyphae described above, primordia are not sharply delimited, and the primordium of the pileus is simply an extension to that of the stipe. At this time the appearance of the fruit body is very similar to that described by Douglas (8) in **Mycena subalcalina Atkinson, Hygrophorus miniatus Fr., H. nitidus B & C, H. borealis Peck, Entoloma flavifolium** Pk., **E. grayanum** Pk., and **E. cuspidatum** Pk., by Walker (23) in **Pleurotus admirabilis** Pk., by Blizzard in **Omphalia chrysophila** Fr., **Clitocybe adirondackensis** Pk., **C. cerussata** Fr., and **Clitopilus novaboracensis** Pk.

Origin of Hymenial Primordium

The hymenial primordium arises in the furrow which was formed by the epinastic growth of the marginal hyphae of the pileus primordium. This area is composed of characteristically crowded hyphae which stain more deeply than the surrounding tissue. The initial cells which compose this layer have acuminate apices and are uneven (Fig. 5). New elements of the hymenial primordia originate near the margin of the pileus.

Figure 1

Figure 2

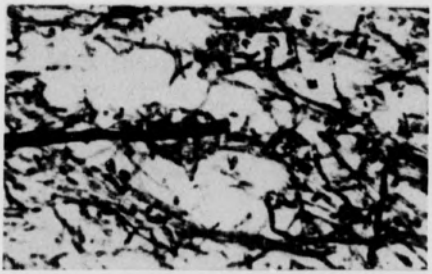

Figure 3

Figure 4

Figure 5

Figure 6

Figure 7

Figure 8

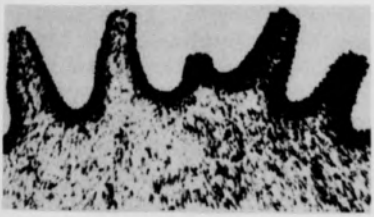

Figure 9

Figure 10

As a result the hymenial layer nearest the stipe may be almost mature while those near the margin of the pileus may be in very early stages of development. A cross section through the uppermost portion of the stipe shows the hymenial primordium to be a very darkly staining area around the periphery (Fig. 6). In a median longitudinal section the hymenial primordium is visible in the annular furrow, and extends below the annular furrow on the stipe for varying distances in different fruit bodies.

Prior to the expansion of the pileus there are hyphae which traverse the hymenial primordium, and resemble a partial veil. These hyphae originate in the margin of the pileus and in the periphery of the stipe, just below the hymenial primordium. The relative position of these hyphae varies greatly in different fruit bodies. These hyphae are very variable in length, and interweave conspicuously at the point they come together. These hyphae are loosely connected, appear to be quite fragile, and form a poorly defined thin layer upon the hymenial primordium.

Development of 'Palisade Layer'

It was stated above that the primordial stage of the hymenium consists of an area of densely interwoven, dark-staining hyphae. In this area the hyphal ends appear at different levels on the surface, thus causing a ragged, extremely irregular appearance. During the maturation of the hymenium the free hyphal ends change from acuminate to clavate. Accompanying this change there is a continued projection outward of these component hyphae resulting in the development of a relatively smooth periphery. This growth is the result, in part, of frequent branching and the extension of the branches to the periphery. In addition to the increase in number there is also an increase in diameter. The foregoing happenings result in the formation of a differentiated palisade layer which is made up of relatively even, darkly-staining blunt hyphae. This layer rapidly becomes oriented to a position perpendicular to the long axis of the stipe. Continued centrifugal growth takes place due to the formation of new elements. As this takes place there is less uniformity in the palisade layer. New elements continue to form at the margin of the pileus while gills are forming in the furrow. (Similar development has been reported in a number of agarics: Atkinson (1,2,3), Beer (4), Blizzard (5), Douglas (7,8).

Gill Development

The earliest evidence of gill formation is shown in Fig. 6. In the foregoing section it was mentioned that the palisade lay-

er becomes extremely crowded because of the active addition of new elements. The relatively even palisade layer bulges to form radial ridges which are the first indications of the gills. These ridges appear first near the apex of the stipe. Subsequent differentiation results in the extension of the gills to the margin of the pileus. The hyphae which are adjacent to these radial folds extend downward and give rise to the trama of the gills. Tramal hyphae are easily distinguishable because of their failure to take as deep a stain as the surrounding hyphae (Figs. 7, 8,9). Additional hymenial elements result from continued growth and branching of the tramal hyphae. The change which results during the development of a compact gill layer from the looser tissue composed of newly formed gills is visible in Figs. 8 and 10.

Secondary gills arise frequently between previously formed (primary) gills Figs. 7 and 9. These gills arise apparently in the same manner as the primary ones. Gill development in this species is essentially the same as that reported by other workers for various species.

Formation of Spores

As the 'palisade layer' matures, certain hyphae project outward one to four microns from the surface (Fig. 8). These hyphae are easily identifiable because of their larger size and average 4.5 microns in diameter. The apices become blunt and expanded and give rise to basidia. Four spores are characteristically borne on relatively short sterigmata. No cystidia or setae were observed at any time in the hymenium. Further, no clamp connections were observed during the course of these studies.

DISCUSSION

The Agaricales are divided into six families on the basis of the morphological characters of the hymenophore (15). The present study was concerned with one species of the Agaricaceae, in which the hymenophore is in the form of gills. Again, the Agaricaceae are divided morphologically into two groups depending on the method of gill formation, since the gills may arise either exogenously or endogenously, depending on the species.

The endogenous forms may be divided into two groups depending on the order of differentiation of the pileus and hymenial primordia, i. e. those forms in which the primordium of the hymenium is the first part to appear, and those in which the pileus is differentiated first. The initiation of gills in the endogenous forms are two types, viz. (1) the 'Agaricus' type, in which the gills arise by downward growing radial salients of the hymenophore, accompanied or preceded by a more or less well developed annular prelamellar cavity, and (2) the 'Am-

anita' type in which there is no general annular prelamellar cavity, and the origin of the lamellae is a series of trabeculae extending from the pileus fundament to the stem, and attached to both.

Although the exogenous forms lack many structures found in the endogenous forms, the development is more uniform as a group. In general the following sequences of development occur: the primordium of the fruit body is characterized by a homogenous weft of intertwined hyphae which elongate and the pileus and stipe primordia are initiated by the epinastic growth of the hyphae at or near the apex. The hymenium may be initiated simultaneously as reported by Douglas (8) in **Hygrophorus miniatus, H. nitidus,** and **H. borealis,** or the hymenial primordium may take place after that of the pileus and the stipe. The latter type of development is seen in **Clitocybe laccatus** Scop. (4), **C. adirondackensis** (5), **C. cerussata** (5), **Clitopilus novaboracensis** (5), and **Omphalia chrysophylla.** (5). Douglas also reported the latter type of development for three species of Entoloma.

Further, development of all exogenous forms show striking similarity. The hymenial primordium undergoes maturation and the palisade layer results. Gills are initiated first in or near the annular furrow and extend to the margin of the pileus. The growth of the hymenial primordium, palisade layer and gills is centrifugal.

Pleurotus ulmarius has the exogenous type of development and follows closely the usual sequence in exogenous forms. However two minor deviations were found: (1) Dark staining hyphae are present in the young fruit bodies of this species, but have not been reported elsewhere. Sawyer (19) reports similar hyphae in Pholiota, and states that they were present in all stages of development. In our study we found that these darkly staining hyphae disappeared with the approach of maturity. The significance of these structures and this behaviour has not been determined. (2) Hyphae were found which traversed the hymenial primordium prior to the expansion of the pileus. These hyphae originated in the margin of the pileus, and on the periphery of the stipe below the hymenial primordium. Four different conditions were found in reference to the hyphae which traverse the hymenial primordium: (1) Elements originate in the margin of the pileus and extend down the hymenial primoridum. (2) Elements from the pileus margin and stipe traverse the hymenial primordium and intermingle at the point of contact. (3) Elements originate on the stipe and extend upward traversing the hymenial primordium. (4) Elements from both pileus margin and stipe are absent.

In the fruit bodies studied, the development of these hyphae was not sufficient to be called a partial veil. The situa-

tion encountered here is not unlike that reported by Walker (23) in **Pluteus admirabilis** Pk. Here the epinastic growth at the pileus margin "causes the margin of the pileus to roll inward so closely that the edge of the pileus becomes pressed against the loosely interwoven ends of the filaments covering the stem." Hartig (12) reported that in **Armillaria mellea** Fr. the hymenophore developed exogenously at first, and at a later stage the pileus margin became incurved, and hyphae from the margin of the pileus interwove with the hyphae from the stem covering the annular furrow with a hyphal layer, the veil. Fischer (10) reported in **Armillaria mucida** Fr. that the hymenophore does not develop exogenously, "the marginal veil is not an aftergrowth, but is formed by the neutral tissue which is present at the beginning." Beer (4) substantiated the work of Fischer in finding that in **A. mellea** the "hymenium is never exposed in an open furrow. On the contrary, the marginal veil is present from the first, and is never an aftergrowth as Hartig supposed." Fayod (9) studied a great number of agarics and stated that all of them develop endogenously. In **P. ulmarius** there are many degrees of hyphal development covering the hymenial area. It is possible that the extreme variability found here may be present in other species. If variability is so wide spread it could possibly account for some of the conflicting descriptions of some species.

SUMMARY

1. Fruit bodies of **Pleurotus ulmarius** were collected and tissue cultures were prepared on sterile malt extract agar.
2. Fruit bodies were produced in culture in such quantity that all stages of development were readily available for study.
3. Fruit bodies produced on a sterile elm stick proved to be identical in development to those grown on agar.
4. All fruit bodies were killed in Fleming's strong solution, washed in tap water, dehydrated in alcohol, transferred to xylene and imbedded in paraffin in the usual way.
5. Sections were cut 7-10 microns in thickness and stained with Heidenhain's iron-alum haematoxylin.
6. The developmental morphology has been studied in a species for which studies had not been made. The sequence of development is very similar to other agarics whose hymenium develops exogenously.
7. The undifferentiated fruit body primordium consists of a mass of generally uniform hyphae which form a conical or hemispherical structure.
8. The first differentiation occurs as dark-staining hyphae which appear scattered at randon throughout the young fruit body.

9. The fruit body primordium elongates into a barrel-shaped structure and hyphae at the margin of the apex bend out and downward forming an annular furrow.
10. This epinastic growth marks the first differentiation between pileus and stipe primordia.
11. At a slightly later stage the hymenial primordium appears in the region of the annular furrow at the apex of the stipe. This area is differentiated by the darker staining, pointed hyphae which form a compact, uneven layer.
12. By centrifugal growth the hymenial primordium is extended to the pileus margin.
13. Maturation of the hymenial primordium gives rise to a layer of compact, relatively even hyphae with blunt apices: the palisade layer.
14. Like the hymenial primordium, development of the palisade layer is centrifugal, and the order of its development is from the apex of the stipe to the pileus margin.
15. Gills are initiated as radial folds of the palisade layer at the apex of the stipe and progressive development is centrifugal.
16. Secondary gills arise in spaces between the primary gills.
17. Spore development is normal; each basidium bearing four spores on short sterigmata.
18. Cystidia, setae and clamp connections were not found in any of the fruit bodies studied.

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