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Title: ASCOCARP DEVELOPMENT IN ANTHRACOBIA MELALOMA

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Cultural and developmental characteristics of a collection of Anthracobia melaloma with a brown hymenium and a barred exterior appearance were examined. It grows well in culture on CM and CMMY agar media and has a growth rate of 17 mm in 18 hours. It is heterothallic and produces asexual multinucleate arthrospores after incubation at 30°C or above for several days in succession. These arthrospores germinate readily after transfer to fresh media.

Antheridial hyphae and archicarps are produced by both mating types although the negative mating type isolates produce more abundant archicarps. Antheridia are indistinguishable from vegetative hyphae until just prior to plasmogamy when they become swollen. Septal pads arise on the septa separating the cells of the trichogyne and ascogonium subsequent to plasmogamy and persist throughout development. The paraphyses, the ectal and medullary excipulum, and the excipular hairs are all derived from the sheathing hyphae. Ascogenous hyphae and asci are derived from the largest cells of the

ascogonium. A haploid chromosome number of four is confirmed for the species.

Exposure to fluorescent light was unnecessary for apothecial induction, but did enhance apothecial maturation and the production of hymenial carotenoid pigments. Constant exposure to light inhibited the production of the brown hair pigments.

# Ascocarp Development in *Anthracobia melaloma*

by

Harold Julius Larsen, Jr.

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# ASCOCARP DEVELOPMENT IN ANTHRACOBIA MELALOMA

## I. INTRODUCTION

Anthracobia melaloma (Albertini & Schweinitz ex Persoon) Boudier is a small pyrophillic fungus (Ascomycetes, Pezizales, Pyronemataceae; Korf, 1972). The slightly cupulate to flattened apothecia characteristically occur on charred wood and/or soil in fire sites, and may be distinguished by their fascicles of brown, round-tipped, excipular hairs and by their brown to reddish-orange to dingy yellow hymenial coloration (Seaver, 1942). The apothecial characteristics overlap those of a second species, A. maurilabra (Cooke) Boudier which is currently recognized by European and other world authorities on discomycetes (Moser, 1963; Dennis, 1968; Rifai, 1968).

Examination of sexuality and ontogeny in some operculate discomycetes has contributed to a better understanding of the organisms involved. Early work by Claussen (1905, 1912), Ramlow (1906, 1914), Dodge (1912, 1920), Gwynne-Vaughan (1913, 1937a, b), Corner (1929, 1930, 1931-32), and Gwynne-Vaughan and Williamson (1930, 1931, 1932, 1934) gave great impetus to these studies. Their observations have been clarified, expanded, and, in some cases, corrected by Bistis (1956, 1957), Rosinski (1956), Gamundi and Ranalli (1963), Moore (1963), Kimbrough (1966), and Jain and

Morgan-Jones (1973). The cytology of ascogenous hyphae and of the ascus has been examined more closely by Olive (1950), Wilson (1952), and Woods (1953). These studies have demonstrated the universality of some aspects of sexuality and ascocarp ontogeny and the variability of others. Occasionally, as with Pyronema omphalodes (Bull. ex St. Amans) Fuckel and P. domesticum (Sow. ex Gray) Sacc., this and cultural information can be useful in distinguishing taxa (Moore, 1963; Moore and Korf, 1963).

A collection was made of an Anthracobia which fit within the narrow European concept of A. maurilabra and within the broad American concept of A. melaloma. An isolate of it readily produced apothecia in culture, and this made possible cultural and developmental studies the results of which could then be compared with those of previous authors (Gwynne-Vaughan, 1937b; Olive, 1950; Rosinski, 1956).

# II. MATERIALS AND METHODS

## Source of Cultures and Isolation Procedures

A collection of Anthracobia melaloma with a fawn-brown colored hymenium was made June 28, 1971 by the author on a forest fire site just east of Glacier National Park, British Columbia, Canada. Cultures were obtained by tissue explants onto sterile 2-1/2% water agar with subsequent transfer to Corn Meal-Malt Extract-Yeast Extract (CMMY) agar. Apothecia were produced within four weeks under room conditions, and subcultures were made by transfer of whole apothecia. Voucher specimens of the original collection and of the culture are deposited in the Oregon State University Herbarium (as OSC 33, 349 and OSC 33, 350) and in the author's personal herbarium (as F-203 and F-203-culture).

An isolate of A. melaloma was obtained from the American Type Culture Collection (ATCC 16141). This isolate was used in the second portion of the mating type studies.

## Culture Media and Techniques

Cultures were maintained on CMMY agar slants and in triple-autoclaved soil tubes as apothecial cultures and as monosporic cultures. Transfers were made at six to nine month intervals. Good viability is maintained for 18 months if the cultures are stored at $10^{\circ}$ - $15^{\circ}$C.

Production of apothecia was facilitated by transferring several 1 cm diameter plugs from five-day old inoculated CMMY or Difco Corn Meal (CM) agar plates to 2-1/2% water agar plates. These water agar plates were then maintained at room temperature and under room lighting for the 14-21 days required for the production of mature apothecia.

Single Spore Isolation Techniques

Two methods were used to obtain monosporic cultures. The first method involved inverting petri dishes containing 2-1/2% water agar over mature apothecia. These plates were rotated periodically to control the spore density at any given spot on a plate. The plates were then incubated at 20°C, 25°C, 30°C, and at 60°C for 30 minutes followed by incubation at room temperature. Spore germination was poor and a second method was used.

The second method used spores that were shot onto the agar surface of the plates where they were produced. The spores did not germinate and were transferred to fresh media two to three weeks later. Distribution was accomplished by sliding the inverted spore-bearing block across the surface of a fresh CM or water agar plate and subsequently removing any mycelial fragments left behind when the block was removed. Germination occurred within 24 hours on both media types. Isolations were made using a microscalpel and a

dissecting microscope with transmitted light. The spores were separated before isolation by using a small agar wedge on the microscalpel tip as a "broom." Single germinating spores were transferred to plates of CM agar for growth and observation. Transfers were subsequently made to 2% Malt agar slants and to soil tubes. Eight single spore isolates were obtained and have been maintained to the present time.

Mating Type Studies

Eight single spore isolates were used in a study of mating types. Each of the monosporic isolates was grown singly and crossed with the other monosporic isolates to determine its mating type. Additionally, each monosporic isolate was crossed with the ATCC isolate of A. melaloma (ATCC 16141). The procedures outlined previously for apothecial production were followed with these exceptions: 1) 60 x 15 mm plastic petri dishes and 5 mm diameter inoculation plugs were used; 2) the inoculated plates were maintained under constant fluorescent light; and 3) the plates were examined for apothecial production 14 days after inoculation.

Photoeffect Studies

Developing cultures were subjected to six combinations of light and darkness to determine the effects of light on apothecial production.

Three plates were used for each lighting combination, and cultures were grown on 2-1/2% water agar plates. Total darkness was provided by double wrapping plates in aluminum foil. Lighted conditions were provided by placing non-wrapped plates in transparent crisper pans and positioning these crispers under constant fluorescent light. The cultures were maintained at room temperature under the following lighting conditions: 1) 18 days of darkness; 2) 18 days of light; 3) six days of darkness followed by 12 days of light; 4) six days of light followed by 12 days of darkness; 5) 12 days of darkness followed by six days of light; and 6) 12 days of light followed by six days of darkness. All plates were examined at the conclusion of 18 days, and observations of apothecial production and morphological characteristics were made.

Microtechnical Procedures and Materials

Whole mounts of ascogonia were mounted directly in 0.5% Trypan Blue in lactophenol (Boedijn, 1956). Temporary mounts of ascogonia stained to show the nuclei were made by fixing pieces of agar bearing ascogonia or apothecial initials in Carnoy's fluid for ten minutes, rinsing in 45% acetic acid for five minutes, staining in 2% aceto-orcein in 45% acetic acid for 20-30 minutes, rinsing and destaining in 45% acetic acid for 5-10 minutes followed by 50% glycerine for 5 minutes, and mounting in non-acidified 50% glycerine.

The aceto-orcein was made by dissolving 1 g orcein in 50 ml of 45% acetic acid, boiling and refluxing the solution for two hours, and subsequently cooling and filtering the solution (Conn et al., 1960). The mounts were gently warmed over an alcohol lamp to just melt the agar and a slight pressure was applied to flatten them. These temporary mounts, ringed with porcelain glaze, kept for several months with little evidence of deterioration.

Apothecia for permanent mounts were fixed daily from the third to the thirtieth day following inoculation. Weak chrome-acetic acid, FAA, and Newaschin (Craf) type II fixatives were used (Johansen, 1940; Sass, 1958). Johansen's (1940) tertiary butyl alcohol dehydration schedule was followed, and the apothecia were embedded in Paraplast. Apothecia fixed in the weak chrome-acetic acid fixative were found to be satisfactory for both morphological and cytological details, and most sectioning was done using apothecia fixed in this fixative. Sectioning was done on a Spencer rotary microtome at a thickness of 10 μ. Haidenhains iron hematoxylin was used for cytoplasmic and nuclear staining, and ferric chloride was used as the mordant. The cell walls were lightly counter-stained with a dilute solution of fast green. The stained sections were mounted in Balsam.

Photography and Microscopy

Photomicrographs were taken with a Honeywell Pentax Spotmatic camera mounted on a Leitz Ortholux microscope with Leitz fluorite objectives of 40X and 100X oil immersion and with a Zeiss 10X phase objective. Plus-X panchromatic film was used, and an external shutter minimized vibration from the camera mirror slap.

# III. RESULTS

## Ascospore Germination

Spore shootings onto water agar plates gave poor germination ratios of 0 to 10 spores per plate with an estimated 500-600 spores at 20°C, 25°C, 30°C, and 60°C. It was subsequently found that two to four week old spores which had aged under the influence of the mycelium and which were then transferred to and distributed on CM or water agar plates had germination ratios of 100 to 200 per plate with 500-600 spores. This increased germination ratio finally facilitated isolation of sufficient numbers of monosporic cultures for subsequent studies on mating types.

Germinating spores produce one to three germ tubes with varying orientation from the spore. Single germ tubes arise from the sides or the ends of the spores (Figure 1), while double germ tubes usually arise from opposite ends of the spores (Figure 2). The production of triple germ tubes is extremely rare and was observed only once. The germ tube and the spore cytoplasm is multinucleate during and following germination. By the time the first septum is formed, the spore and germ tubes contain from 20 to 60 nuclei. Early mycelial growth also produces highly multinucleate mycelial cells.

Figures 1 and 2. Temporary mounts of germinating ascospores stained with trypan blue in lactophenol. 825X.

Figure 1. Germinating ascospore with one germ tube.

Figure 2. Germinating ascospore with two germ tubes.

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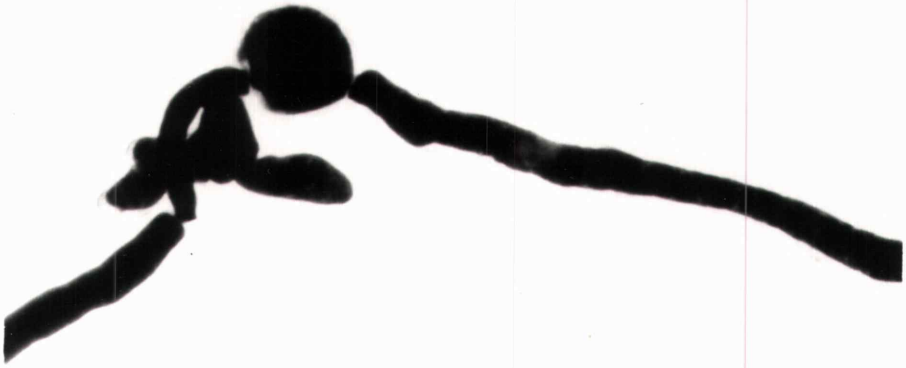

Cultural Characteristics

The isolates grow rapidly and cover a 60 x 15 mm petri dish within 48 hours after inoculation. Abundant aerial mycelium is produced within four days on CM and CMMY agar. Growth was excellent between 20° and 25°C (17 mm in 18 hours), and cultures were routinely maintained at 20°C. Asexual spores are produced by fragmentation of the marginal and aerial mycelium in cultures exposed to temperatures above 30°C for several days in succession (Figures 3 and 4). These were called oidia by Gwynne-Vaughan (1937b) and by Olive (1950), but are currently termed arthrospores (Barron, 1968). Arthrospores transferred to fresh media germinated within 24 hours (Figure 5).

Apothecia are produced during the third or fourth week on undisturbed CM or CMMY agar plates and are mature by the sixth week. Sexual reproduction may be hastened by artificially starving the mycelium. This was accomplished by the transfer of nutrient agar plugs from inoculated CM or CMMY plates to water agar plates. Initiation of ascogonial production occurs under these conditions within five days, and mature apothecia are produced within 16-21 days. Mating type studies show the organism is heterothallic.

The apothecia are 3-5 mm in diameter and slightly cupulate to flattened. The hymenium is pale Cream Color (19'f) to Cinnamon-Buff

Figures 3-5. Temporary mounts of arthrospores stained with aceto-orcein and mounted in 50% glycerol.

Figure 3. Arthrospores with typical simple cylindrical shape. Note the several nuclei (arrows) per cell. 2,050X.

Figure 4. Arthrospore with variant shape. Note the several nuclei (arrows) per cell. 2,050X.

Figure 5. Germinating arthrospore (arrow). 825X.

3

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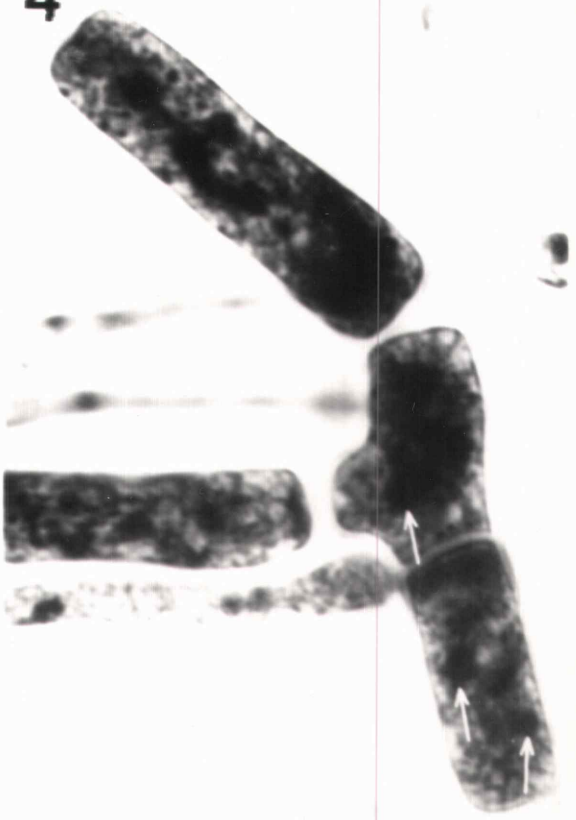

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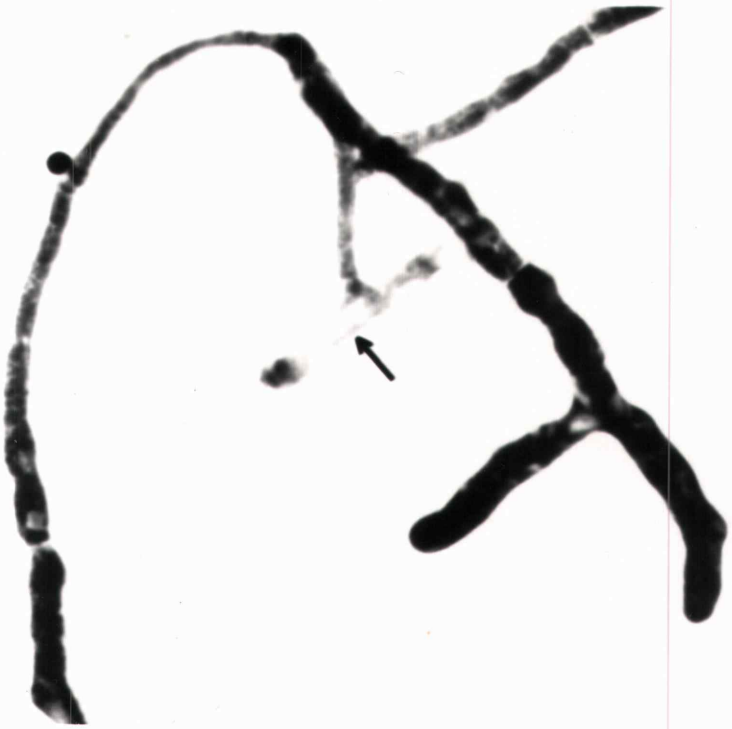

(17''b) (Ridgway, 1912), and the exterior is concolorous. The outer surface and margin are clothed with short, blunt, stiff, dark brown, septate hairs (60-95 x 6-10 μ) which occur in vertically oriented fascicles that give the exterior a barred appearance. The ectal excipulum consists of textura angularis-textura globosa, grading into textura prismatica around the upper margin (Starback, 1895; Korf, 1951). The medullary excipulum consists of textura intricata.

The asci are operculate, cylindrical, iodine negative, eight spored, and 180-200 x 13-15 μ. The ascospores are ellipsoidal, smooth, biguttulate, and 18-21 x 8-10 μ. The paraphyses are slender with the slightly clavate tips measuring 4-5 μ.

Ascogonia, Trichogynes, and Antheridia

The production of ascogonia is the first visible evidence that ascocarp formation has begun. The ascogonial initial is a lateral, recurved, and thickened branch which when first recognizable contains four to eight nuclei. The archicarp initials continue to elongate, form a coil consisting of one to several loops (Figure 6), and finally produce the trichogyne. Septa are formed in the multinucleate initial when the trichogyne develops, and the "cells" formed contain four to eight nuclei. The basal two or three cells of the archicarp become the stalk region and remain approximately the same size. The middle five to eight cells increase in girth after septum formation but remain

Figures 6-8. Temporary mounts, shown 825X. Figure 6 stained with trypan blue in lactophenol. Figures 7 and 8 stained with aceto-orcein and mounted in 50% glycerol.

Figure 6. Ascogonial coil.

Figure 7. Trichogyne and antheridial hypha prior to plasmogamy. Note the slight swelling of the antheridial cell (Ac) and the initiation of a lateral anastamosis (arrow) in the trichogyne.

Figure 8. Trichogyne and antheridial hypha at plasmogamy. Note the increased swelling of the antheridial cell (Ac).

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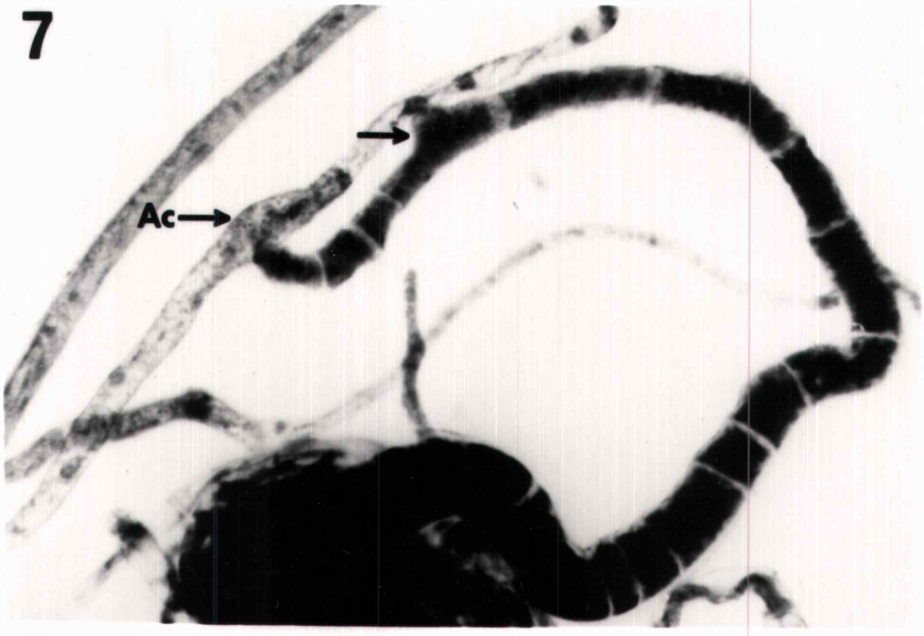


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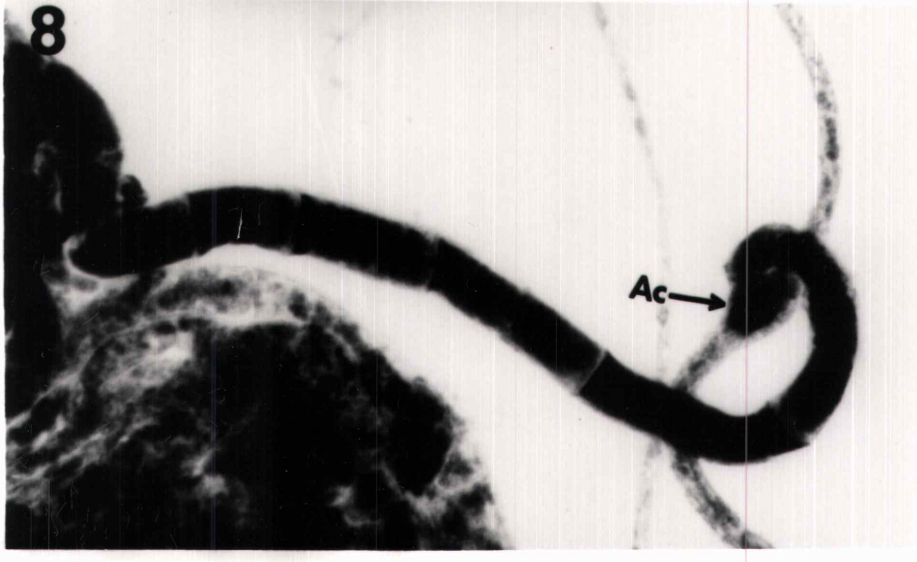


constricted at the septa. There is a gradual diminution in size from the largest cell in this middle section back toward the base and forward to the apex of the trichogyne. The trichogyne commonly branches once to several times toward the tip and is frequently irregular or crooked. All cells of the archicarp have dense cytoplasm and are multinucleate.

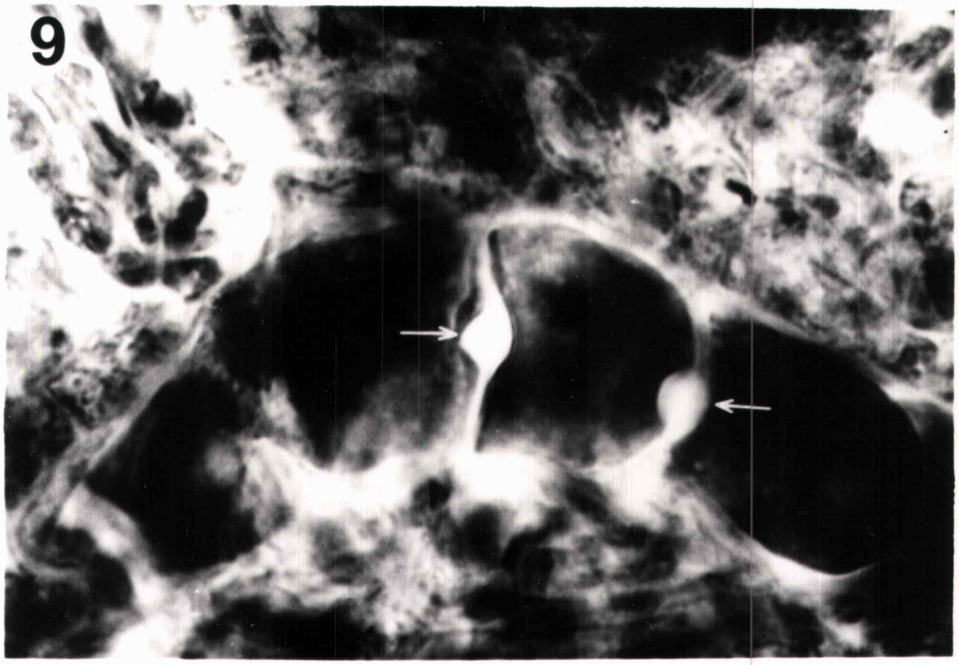
Antheridial hyphae could not be differentiated from vegetative hyphae. Swelling of the cells that fuse with the trichogyne was observed (Figures 7 and 8). Anastomosis between the trichogyne and the antheridial filament was observed in 12 instances (Figures 7 and 8). Shortly after anastomosis, the septa between the cells in the ascogonium and lower trichogyne region develop a thickened pad-like structure (Figures 9 and 10). These pads remain throughout ascocarp development and aid in identification of the archicarp in the ascocarp sections.

As the trichogyne differentiates and elongates, the stalk cells and lower cells of the ascogonium produce lateral outgrowths which, with outgrowths from neighboring vegetative hyphae, envelop the archicarp (Figures 11, 12, and 13). The hyphal sheath soon becomes optically dense, and subsequent development was seen only in sectioned and stained material.

Figures 9 and 10. Septal pads as seen in young ascocarps.

Figure 9. Septal pads (arrows) in a squash mount of an enclosed archicarp stained with aceto-orcein and mounted in 50% glycerol. 2,050X.

Figure 10. Septal pads (arrows) in a young apothecial section stained with haematoxylin and fast green and mounted in balsam. 825X.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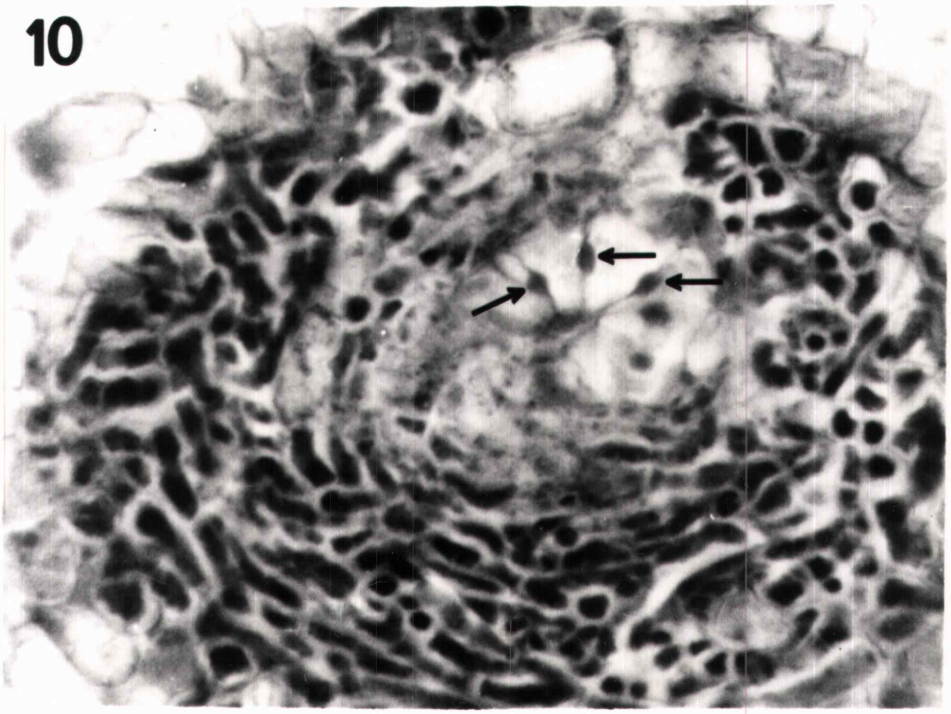
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Figures 11 and 12. Temporary mounts of archicarps with sheathing hyphae. Stained with trypan blue in lactophenol. 825X.

Figure 11. Archicarp with sheathing hyphae (arrows) arising from the stalk region.

Figure 12. Archicarp and sheathing hyphae (arrows) arising from the vegetative cells of the supporting hypha.

11

12

## Ascocarp Development

Ascocarp development involves the production and the differentiation of two types of tissues, fertile tissues and sterile tissues. The fertile tissues are those which ultimately give rise to the ascus and ascospores. The sterile tissues are those which are not involved in ascospore formation, but which provide support and protection for the fertile elements. They include the sheathing hyphae of the ascocarp initial, the paraphyses, the excipular tissue, and the excipular hairs.

The fertile tissues are derived from the largest two, three, or four cells of the ascogonium. Ascogenous hyphae first appear as large, thin-walled outgrowths from these ascogonial cells and frequently arise adjacent to the cross-walls of the ascogonial cells. They may emerge as simple protrusions or may branch profusely.

The ascogenous hyphae rapidly elongate and produce septa. Consequently very few intermediate stages between protrusion and the formation of croziers were seen. In the few seen, septation appeared to occur at random with reference to the numbers of nuclei enclosed. The nuclear numbers within the resultant cells varied from one to eight. Those cells with only one to three nuclei were seldom involved in the formation of asci.

Crozier formation is the next major change that occurs within the ascogenous hyphae. Croziers may develop initially from the

terminal cells in short ascogenous hyphae. Usually, however, the primary ascogenous hyphae grow out to the margin of the apothecium and produce lateral outgrowths along their length. Primary ascogenous hyphal cells contain from two to eight nuclei and produce only one lateral or secondary ascogenous hypha. No nuclear divisions were seen in the ascogenous hyphae, but conjugate division is assumed to occur because of the subsequent crozier development and proliferation.

Growth of the ascogenous hyphal system subsequently is dependent upon the young secondary ascogenous hyphae. The older primary ascogenous hyphae become increasingly vacuolate and eventually lose their contents entirely. Continued growth occurs in the apical portions of the secondary ascogenous hyphae by crozier proliferation of the penultimate cells or by branching and crozier proliferation from the antepenultimate cells. Proliferation of the secondary ascogenous hyphae continues while some lateral or terminal cell nuclei fuse and initiate ascus formation. Thus, croziers and asci of various developmental stages occur throughout the hymenium of an ascocarp which is actively expanding.

A cytological study of nuclear behavior during ascus development was not undertaken. The elongation of the ascus initial begins before meiosis and continues through the third division and maturation. During maturation the density of the cytoplasm decreases with the outer distal portions becoming disorganized. Spore walls cleave out

cytoplasm surrounding the eight daughter nuclei and become progressively thickened. The spores shift from their original diagonally adjacent positions to a strictly linear alignment in the upper portion of the ascus. The quality of fixation and staining in the spores decreases with the maturation of the spore wall. The mature spores appear to be uninucleate.

The sheathing hyphae are the first sterile tissues produced and arise as lateral hyphae from the proximal vegetative hyphae and from the stalk cells of the archicarp (Figures 11 and 12). These multinucleate hyphae arise prior to plasmogamy and rapidly form a closely packed sheath around the archicarp by repeatedly branching and intertwining (Figure 13).

After formation of the closed hyphal sheath, further growth occurs and differentiation of paraphyses, excipular tissue, and hairs begins. The upper portion of the sheath produces hyphae which elongate parallel to each other and branch toward their bases. Terminal growth in these hyphal elements ceases when they reach a length approximately 150 μ. These parallel hyphae recurve slightly toward the center at their tips and become the first paraphyses and marginal excipulum. The initial recurving of the tips lessens and disappears with apothecial expansion. Subsequent paraphyses form from branches arising from the bases of the first paraphyses. The mature paraphyses are branched near the base, narrow for most of

Figures 13 and 14. Temporary and permanent mounts showing the sterile tissue structure.

Figure 13. Temporary mount of an archicarp in which the sheathing hyphae have enclosed the ascogonial region. Stained with aceto-orcein and mounted in 50% glycerol. 825X.

Figure 14. Permanent mount of a slightly immature apothecial section. Note the developing ectal (Ee) and medullary (Me) excipulum and the excipular hairs (arrow). Stained with haematoxylin and fast green and mounted in balsam. 205X.

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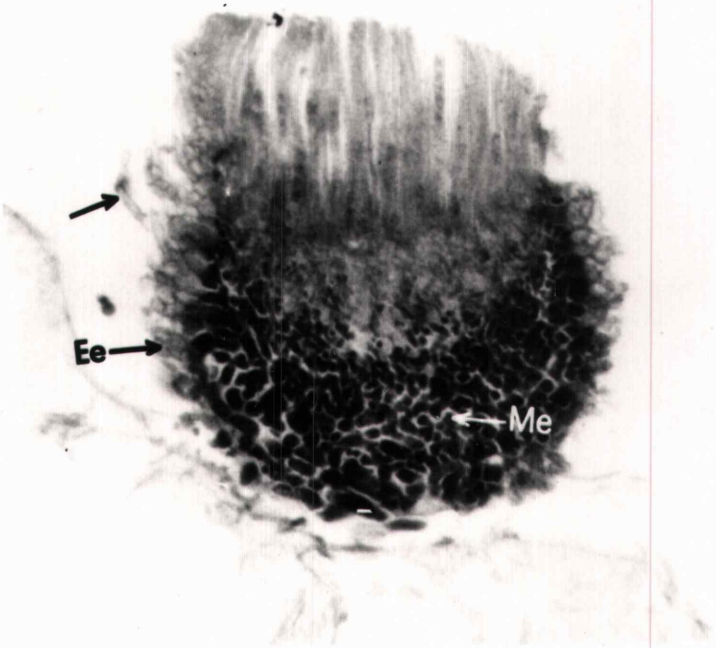

their length (1.5-2.0 μ in diameter), and are slightly enlarged to 3.5 to 5.0 μ in diameter at the apex. The septa are thin and widely spaced, and each cell is multinucleate. Nuclear division was not observed in the paraphyses.

Excipular tissue is produced by formation of chains of pseudoparenchymatous cells arising from the lower sheath region (Figure 14). These continue to proliferate and intertwine to produce the basal and outer excipular tissue classified as textura globosa-textura angularis (Starback, 1895; Korf, 1951). The inner excipulum also arises from the sheath hyphae and consists of textura intricata. The outer excipulum, above the region where the hairs are formed, consists of broad, linearly arranged hyphae forming a tissue classified as textura prismatica.

The excipular and marginal hairs arise as outgrowths from the outer excipulum. These outgrowths have thicker, more pigmented cell walls and occur in tufts. They commonly twist or curve gently and soon are devoid of protoplasm. The tips of these hairs are blunt or rounded and occasionally swollen. Coloration is dependent upon exposure to light. Those produced under greatly reduced light or in darkness are hyaline, while prolonged exposure to light produces brown excipular hairs.

Apothecial expansion occurs by the proliferation of paraphyses and ascogenous hyphae and by the subsequent enlargement of these

elements. Expansion of the hymenium changes the shape of the ascocarp from narrowly-obpyriform with a central cavity to cupulate, and finally, to a flattened or even slightly reflexed disc. As expansion occurs, the distance between the fascicles of excipular hairs increases and gives the outer excipulum a barred appearance. Development of the hymenial pigments is influenced by light and occurs concomitantly with apothecial expansion and proliferation of the paraphyses.

Ascospore dissemination begins within 18 days and may continue up to 33 days after inoculation of the water agar plates. It is affected by temperature, light, humidity, and mechanical vibration. Higher rates of spore release were observed under increased temperature, light, or mechanical vibration and under decreased humidity.

## Mating Type Studies

Anthracobia melaloma is heterothallic. Apothecia were produced only in plates crossing two of the monosporic isolates with each of the other six. The six isolates of identical mating type are designated as positive in this study, and the other two isolates are designated as negative. Abundant archicarps are produced by the negative isolates while the positive isolates produce few or none.

The ATCC isolate of A. melaloma is positive in the above terminology. Apothecia occurred only in plates in which it was

crossed with the negative isolates. It produces no archicarps or apothecia when grown singly, but does produce arthrospores.

Photoeffect Studies

The production of apothecia and apothecial pigments was enhanced by exposure to fluorescent light. The plates kept in constant darkness had few apothecia, all of which were hyaline and immature. Some of the apothecia had excipular hairs which had a light brownish tinge, but most were hyaline. The plates given 12 days of constant darkness followed by six days of constant light had very few apothecia, and these were also poorly developed. The hymenial coloration was pale Light Buff (17'f), while most of the hairs were Tawny-Olive (17''i) (Ridgway, 1912). The plates given six days of light followed by 12 days of darkness had many well-developed, mature apothecia. Their hymenial coloration ranged from Cinnamon Buff (17'b) to Pale Pinkish Buff (17''f) and the hair coloration ranged from hyaline to pale Tawny-Olive (17''i) with the darkest hairs occurring on apothecia in the bottom plate of the stack which received the least light. These plates and those kept in continuous light produced the most apothecia.

The plates given six days of darkness and 12 days of light developed some mature apothecia and many immature apothecia. The mature apothecia had a hymenial coloration ranging from Ochraceous

Orange (15') to Zinc Orange (13'). These apothecia had the darkest hairs, Dresden Brown (17'k), and the brightest hymenial coloration of any of the treatments.

The plates given 12 days of light followed by six days of darkness also had several mature apothecia accompanied by many immature apothecia. The hymenial coloration was the darkest observed in the experiment, Clay Color (17'') to Cinnamon Buff (17''b), and the excipular hairs were also quite brown, Buckthorn Brown (17'i).

The plates exposed to constant light produced numerous apothecia ranging from mature through very immature. The hymenial coloration was quite bright, Antimony Yellow (17'b), in all but the youngest apothecia. Only the apothecia in the bottom plate which received a reduced light intensity exhibited any pigmentation in their excipular hairs. These were Buckthorn Brown (17'i).

Informal tests were run shortly after the culture was first obtained checking for color variation between apothecia grown in constant darkness and those grown in diurnal room light. In these trials, the apothecia grown in constant darkness were pale Cream Color (19'f) to pale Cartridge Buff (19''f) and had no hair pigmentation. Those grown under diurnal lighting had a Yellow Ocher (17') hymenium and dark brown hairs. A second Anthracobia species which was tested simultaneously exhibited the same type of variation.

# IV. DISCUSSION

## Spore Germination and Cultural Characteristics

The difficulty in obtaining good spore germination was not anticipated. Gwynne-Vaughan (1937b) and Rosinski (1956) both report that ascospores of A. melaloma germinate readily. One difference between their studies and mine was that they used fresh field collections for their spore isolations while I used apothecia produced in culture for my isolations. The percentage of germinating spores appears to decrease with succeeding crops of apothecia. However, spores which age under the influence of the mycelium after release from the ascus have higher germination rates.

Some cultural characteristics of A. melaloma have been reported previously by Gwynne-Vaughan (1937b), Olive (1950), and Rosinski (1956) and my results agree with theirs. A. melaloma grows well on CM, Malt, and CMMY agar media. Growth rates, not measured by previous investigators, were quite rapid in this study. Asexual reproduction by the fragmentation of hyphae to produce arthrospores (oidia) was previously reported by Gwynne-Vaughan (1937b) and Olive (1950) and was observed in this study. Rosinski (1956), however, failed to find arthrospores. In the present study, the production of arthrospores was induced by exposures to temperatures of 30-35$^{o}$C during the first two to three days following

inoculation. No information concerning arthrospore germination or relating environmental conditions to arthrospore production was given by the previous authors.

## Ascogonia, Trichogynes, and Antheridia

I found differentiated ascogonia and functional trichogynes. Anastamosis between a trichogyne and an antheridial hypha was observed 12 times. This disagrees with Gwynne-Vaughan's (1937b) conclusion that the trichogyne is non-functional and supports Rosinski's (1956) observations. The antheridial hyphae are morphologically indistinct from vegetative hyphae except that the cell which fuses with the trichogyne often becomes swollen (Figures 7 and 8). This agrees with Rosinski's observations.

Differentiated antheridia and antheridial hyphae have been reported for other operculate discomycetes. Cylindrical, peg-like antheridia to which the ascogonial trichogynes are attracted are reported for Pyronema confluens (Pers.) Tul. (= P. omphalodes [Bull. ex St. Amans] Fuckel) by Gwynne-Vaughan and Williamson (1931), for P. domesticum (Sow. ex Gray) Sacc. by Moore (1963), and for Ascobolus magnificus Dodge (= A. scatigenus [Berk.] Brumm.) by Dodge (1920) and Gwynne-Vaughan and Williamson (1932). Ascobolus stercorarius (Bull. ex St. Amans) Schroet. (= A. furfuraceous Pers. ex Hook.) produces oidia which function as the

antheridial elements (Bistis, 1956). Bistis found that a sexual hormone is involved in the attraction of the trichogyne to the oidium.

Septal pads (Figures 9 and 10) form in the archicarp following plasmogamy. These pads, and the septa on which they formed, persist throughout subsequent development of the ascogonium. The presence of these pads has been previously reported (Gwynne-Vaughan, 1913; Fitzpatrick, 1918; Rosinski, 1956), and they were thought to precede breakdown of ascogonial septa. Since the pads and septa persist, it is not presently clear how antheridial nuclei migrate from the trichogyne to the ascogonium.

## Ascocarp Development

Multinucleate ascogenous hyphae are produced by two to four multinucleate ascogonial cells and, in turn, produce lateral binucleate outgrowths as they grow out toward the margin. These secondary ascogenous hyphae grow sympodially by the proliferation of croziers and eventually produce asci. This sequence agrees with that recorded by Gwynne-Vaughan (1937b) and Rosinski (1956). Similar observations have been made for Pyronema confluens (Pers.) Tul. (= P. omphalodes [Bull. ex St. Amans] Fuckel) by Gwynne-Vaughan and Williamson (1931), P. domesticum (Sow. ex Gray) Sacc. by Moore (1963), Ascobolus magnificus Dodge (= A. scatigenus [Berk.] Brumm.) by

Gwynne-Vaughan and Williamson (1932), and A. stercorarius (Bull.) Schroet. by Gamundi and Ranalli (1963).

The cytology of the ascus was not studied in detail, but the few observations made concerning ascus development and ascospore production parallel those made by Olive (1950) and Rosinski (1956). In both cases, nuclear fusion followed by meiosis and a mitotic third division was reported to occur within the ascus. A haploid chromosome number of four reported by both authors was confirmed during the present study.

Observations of the development of sterile tissues recorded by Gwynne-Vaughan (1937b) and by Rosinski (1956) were duplicated in the present study. Sheathing hyphae were derived from the stalk cells of the archicarp, but in the present study additional sheathing hyphae were observed to arise from neighboring vegetative hyphae. Similar observations that sheathing hyphae may develop from neighboring vegetative hyphae have been reported for Lachnea cretea (Cke.) Phill. (=Trichophaea abundans [Karst.] Boud.) by Gwynne-Vaughan (1913) and for Hypomyces aurantius (Pers. ex Fr.) Tul., a perithecial ascomycete, by Samuels (1973).

Paraphyses are commonly derived either from sheathing hyphae or from the archicarp stalk cells in Ascomycetes (Gwynne-Vaughan, 1913, 1937b; Gwynne-Vaughan and Williamson, 1930, 1931, 1932, 1934; Rosinski, 1956; Gamundi and Ranalli, 1963; Moore, 1963;

Kimbrough, 1966). An exception is the report that paraphyses arise from ascogenous hyphae in Mycoarctium ciliatum (Jain and Morgan-Jones, 1973). I did not observe paraphyses arising from ascogenous hyphae.

Excipular tissues are also usually derived from the sheathing hyphae or ascogonial stalk cells (Gwynne-Vaughan, 1913, 1937b; Gwynne-Vaughan and Williamson, 1930, 1931, 1932, 1934; Rosinski, 1956; Gamundi and Ranalli, 1963; Moore, 1963; Kimbrough, 1966; Jain and Morgan-Jones, 1973; Samuels, 1973). They are produced by proliferation and differentiation of the sheathing hyphae in A. melaloma. The production of the excipular hairs from the outer cells of the ectal excipulum seen in this study supports the observations of Gwynne-Vaughan (1937b) and Rosinski (1956). Similar origins for excipular hairs have been observed in Lachnea cretea (=Trichophaea abundans) by Gwynne-Vaughan (1913), in Trichobolus zukalii (Heim.) Kimbrough by Kimbrough (1966), and in Mycoarctium ciliatum by Jain and Morgan-Jones (1973).

Apothecial expansion involves the proliferation and development of paraphyses and ascogenous hyphae. This supports observations made by Gwynne-Vaughan (1937b) and Rosinski (1956) and agrees with the ideas presented by Corner (1929). Development of pigmentation in the hairs and hymenium occurs simultaneously with expansion provided sufficient amounts of light are present to stimulate pigment production.

During this period the cupulate apothecia become flattened and eventually may become convex. Following release of the spores, the apothecia return to a cupulate shape which also supports Corner's (1929) statements.

Photoeffect Studies

Several workers have examined the effects of light upon discomycete sporocarp production, but few studies have been concerned with effects of light on sporocarp morphology. Many workers have observed that discomycetes fail to fruit in the absence of light (Robinson, 1926; Carlile and Friend, 1956; Hawker, 1957; Kimbrough, 1966; Brummelen, 1967). Anthracobia meulleri (J. E. Roxon and L. R. Batra, pers. comm.) also requires light for apothecial formation. However, Rosinski (1956) reported that A. melaloma produced normal apothecia when incubated in constant darkness. My studies indicate that A. melaloma does not require light for apothecial production, but exposure to light affects apothecial morphology and pigmentation.

Photoinduction of pigmentation has been demonstrated by several workers for fungi other than discomycetes. Zaloker (1955) studied the biosynthesis of carotenoids in Neurospora and reported that photoinduction of carotenoid pigment synthesis involved the blue region of the spectrum. Rau (1967) determined that exposure to

wavelengths less than 520 nm was necessary for carotenoid synthesis in a species of Fusarium. Carlile (1956) presents a survey of information known about photobiology to that time. Since then Leach (1967) reported that radiation from both near ultraviolet ("NUV /360") and daylight fluorescent lamps will induce pigmentation in some fungi imperfecti. However, Brandt (1964) has shown that ultraviolet radiation (360 nm region) suppresses production of the dark pigment melanin in Verticillium.

My results indicate that exposure to light is required for production of the carotenoid pigments (Arpin, 1968) which are localized in the paraphyses. Furthermore, constant high level fluorescent illumination produced only carotenoid pigments while reduced intensity and duration of exposure allowed the development of darker pigments in the hairs and hymenial tissues.

## Mating Type Studies

All workers agree that A. melaloma is heterothallic (Gwynne-Vaughan, 1937b; Olive, 1950; Rosinski, 1956). The present study reaffirms this fact and demonstrates that the ATCC isolate of A. melaloma (ATCC 16141) is a monosporic isolate of the positive mating type as defined in this study.

# V. SUMMARY AND CONCLUSIONS

Anthracobia melaloma is a small operculate discomycete which commonly occurs on charcoal and soil in fire sites. It grows and fruits well in pure culture. Asexual reproduction in the species is accomplished by the production of arthrospores, most of which germinate within 24 hours after transfer to fresh media. The species is heterothallic, and archicarps are produced more abundantly by the negative mating type isolates when grown separately than by the positive mating type isolates. Antheridial elements, except for the swollen cell in contact with the trichogyne, are indistinguishable from vegetative hyphae. Plasmogamy occurs by anastomosis between the swollen antheridial cell and the trichogyne.

The archicarp is composed of three regions prior to the production of the ascogenous hyphae. The tapering portion is the trichogyne, the enlarged middle four or five cells compose the ascogonium, and the basal two to four cells are the stalk portion. The fertile tissues of the ascocarp are derived from the ascogonial cells while the sterile tissues are produced from the stalk cells and neighboring vegetative hyphae.

Multinucleate ascogenous hyphae are produced by the largest two, three, or four ascogonial cells. These grow rapidly throughout the young ascocarp and become septate. Secondary ascogenous hyphae

arise as lateral branches from the cells of the primary ascogenous hyphae and two nuclei migrate into each. The branches then grow sympodially by the proliferation of croziers to produce an extensive ascogenous system before the asci are formed.

The sterile tissues of the apothecium develop from hyphae produced from the stalk cells of the archicarp and from neighboring hyphae. These are initially the sheathing hyphae, and they undergo sympodial growth to produce the excipular tissue and the paraphyses. The terminal excipular cells which become the hairs elongate and darken, and subsequently produce thick walls.

Inoculation studies with the ATCC isolate of A. melaloma (ATCC 16141) indicate that it is a monosporic isolate of the positive mating type as defined in the present study. It produces arthrospores but no archicarps in single culture.

Exposure to fluorescent light was found to be unnecessary for apothecial induction, but apothecial maturation and the production of apothecial pigments were affected by varying exposures to constant fluorescent light. Exposure to light enhanced apothecial maturation and the development of the hymenial carotenoid pigments. However, lower light intensity or shorter exposure to light was more effective in the production of the brown hair pigments, and constant high intensity exposure to light was found to inhibit the production of these brown pigments.

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