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The genus *Meliola* contains about 1,000 species of epiphyllic plant parasitic fungi that produce mycelia with characteristic capitate and mucronate hyphopodia. Growth of these fungi in culture or as artifical inoculations on leaves has never been reported. This paper presents some observations on the morphology of the fungus as seen with the scanning electron microscope (SEM), and some results obtained in culture studies.

INDEX DESCRIPTORS: Electron Microscopy of *Meliola palmicola*, Meliola Electron Microscopy.

The genus *Meliola* contains a large number of species of epiphyllic plant parasites, commonly spoken of as the "black molds." While widely distributed on a world-wide basis, the group reaches its maximum in the tropics. Most species are normally limited to the leaves of their hosts, although young twigs and leaf petioles may also be infected.

Our current knowledge of the genus has been summarized in a monumental monograph by G. C. Hansford (1961), who recognized over 1,000 specific and varietal names for the genus. According to Hansford (1961), most members of the genus are host specific, species identification being dependent on knowing at least the host family.

The general appearance of these fungi on leaves is of scattered, more or less circular, black patches, thin to very dense, and often velvety with setae. Few species are necrotrophic, and the majority apparently have little effect upon their hosts. The thallus consists of a branching mycelium which attaches itself to the leaf by means of characteristic hyphopodia. Reproduction apparently occurs solely through the production of ascospores, which are usually produced two in each ascus. Asexual reproduction is unknown.

According to Hansford (1961), all attempts to germinate the ascospores *in vitro* or on host plants have been unsuccessful. He states that "no worker has yet reported growth of these fungi either in the laboratory or as artificial inoculations on leaves."

This paper will report some observations made with scanning electron microscopy on *Meliola palmicola* Winter, as it occurs on the saw palmetto, *Serenoa repens*. Results obtained in culturing the fungus also will be presented.

Methods and Materials

Leaves of *Serenoa repens* infected by *Meliola palmicola* were collected in Melbourne, Florida, during June, 1972. These were air-dried when collected, and returned within ten days to the University of Rhode Island for study. Identification of the fungus was made by the author, based on the description given by Hansford (1961).

Material for scanning electron microscopy (SEM) was prepared by mounting pieces of leaves bearing mature colonies of the fungus (i.e., with ascocarps) on stubs with Duco cement containing conductive silver paint. These were plated with palladium-gold, and observed with a Cambridge S-4 SEM.

Attempts were made to establish the fungus in culture by transferring expelled ascospores from the leaf surface to plates of corn meal agar. None of the ascospores germinated, but a mycelial fragment carried with one of the ascospores did regenerate, and from the resulting growth, an isolate was established in pure culture.

The fungus was cultured on several common laboratory media, including Difco corn meal agar, potato dextrose agar, rabbit food agar (a decoction of 25 gm of commercial rabbit food pellets), and Blakeslee's malt extract (BME) agar (Raper and Fennell, 1965). Growth experiments were carried out in 125 ml Erlenmeyer flasks, on a rotatory shaker operating at 120 revolutions per minute. Blakeslee's malt extract broth medium was used to determine a growth curve; Lilly and Barnett's (1952) asparagine medium was used for carbon utilization determinations. Three replicates were used for each determination, and growth response was based on the weight of oven-dried mycelium produced. Inoculum consisted of macerated mycelium, which was plated on agar medium at the beginning of each experiment to check for purity.

Inoculation experiments were attempted under greenhouse conditions, using a small banana plant as the experimental host. Macerated mycelium from 30-day-old cultures grown in malt extract broth was spread on the upper surface of the youngest unfurled leaf, and the leaf enclosed in a plastic bag to retain a high humidity. The bag was removed periodically (2-3 times per week) and the leaf surface moistened with water. These experiments were carried out during February and March, when solar intensity was still below its maximum for this area. Temperatures in the greenhouse during the experiment rarely exceeded 80°F.

Results and Observations

SEM observations: Meliola palmicola produces a mycelium bearing both capitate and mucronate hyphopodia, abundant setae, and ascocarps. Figure 1 shows a view of the mycelium taken near the margin of the colony. Limited branching and the predominantly alternate arrangement of the capitate hyphopodia are apparent. An enlargement of a capitate hyphopodium is shown in Figure 2. These hypopodia consist of a "stalk" and a "head" cell; from each "head" cell, a single

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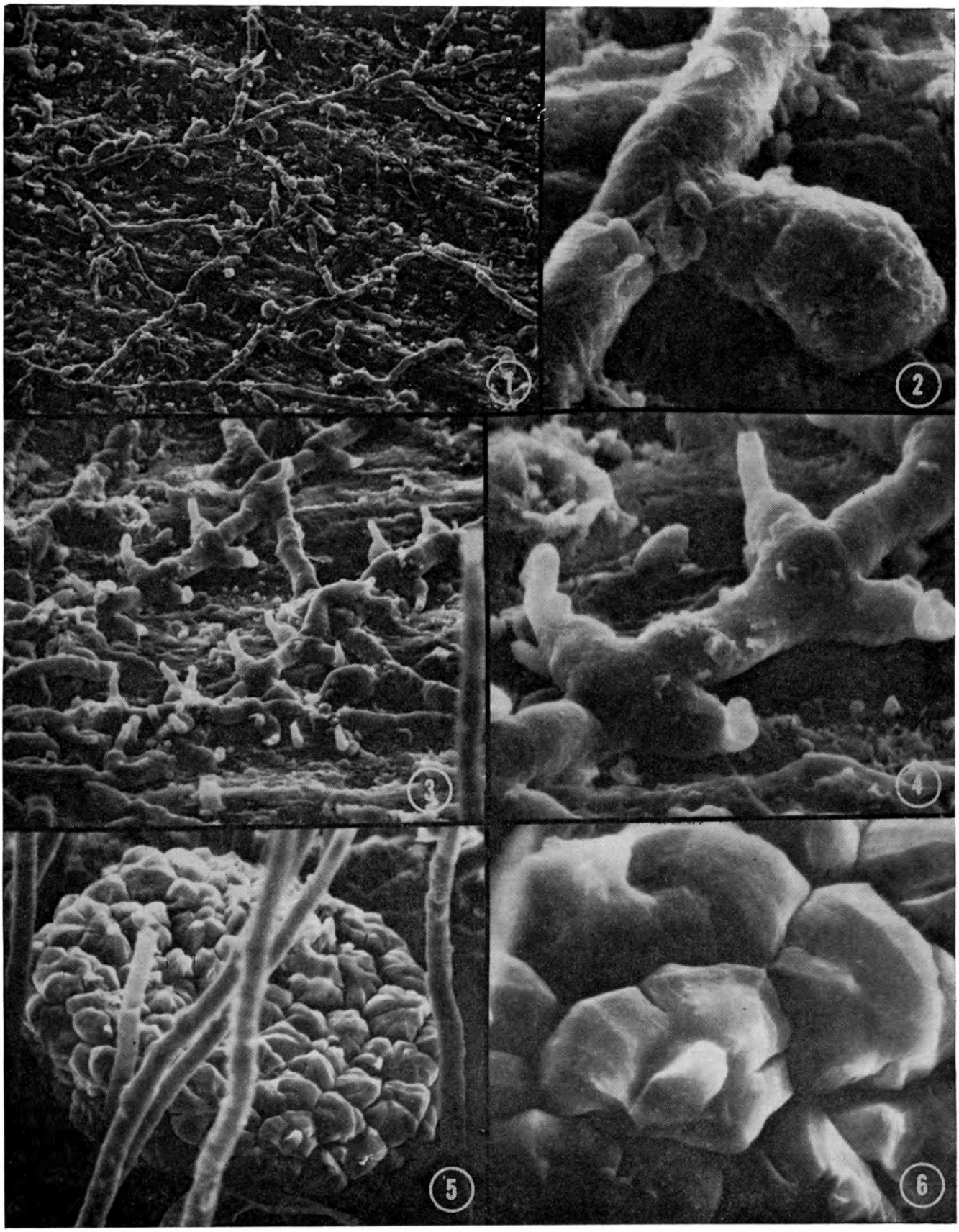
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Figure 1. View of the margin of a colony on the host plant. Figure 2. Capitate hyphopodium. X 1900. Figure 3. Mycelium bearing mucronate hyphopodia. X 500. Figure 4. Mucronate hyphopodia. X 1500. Figure 5. Ascocarp surrounded by setae. X 500. Figure 6. Ascocarp surface. X 2000.

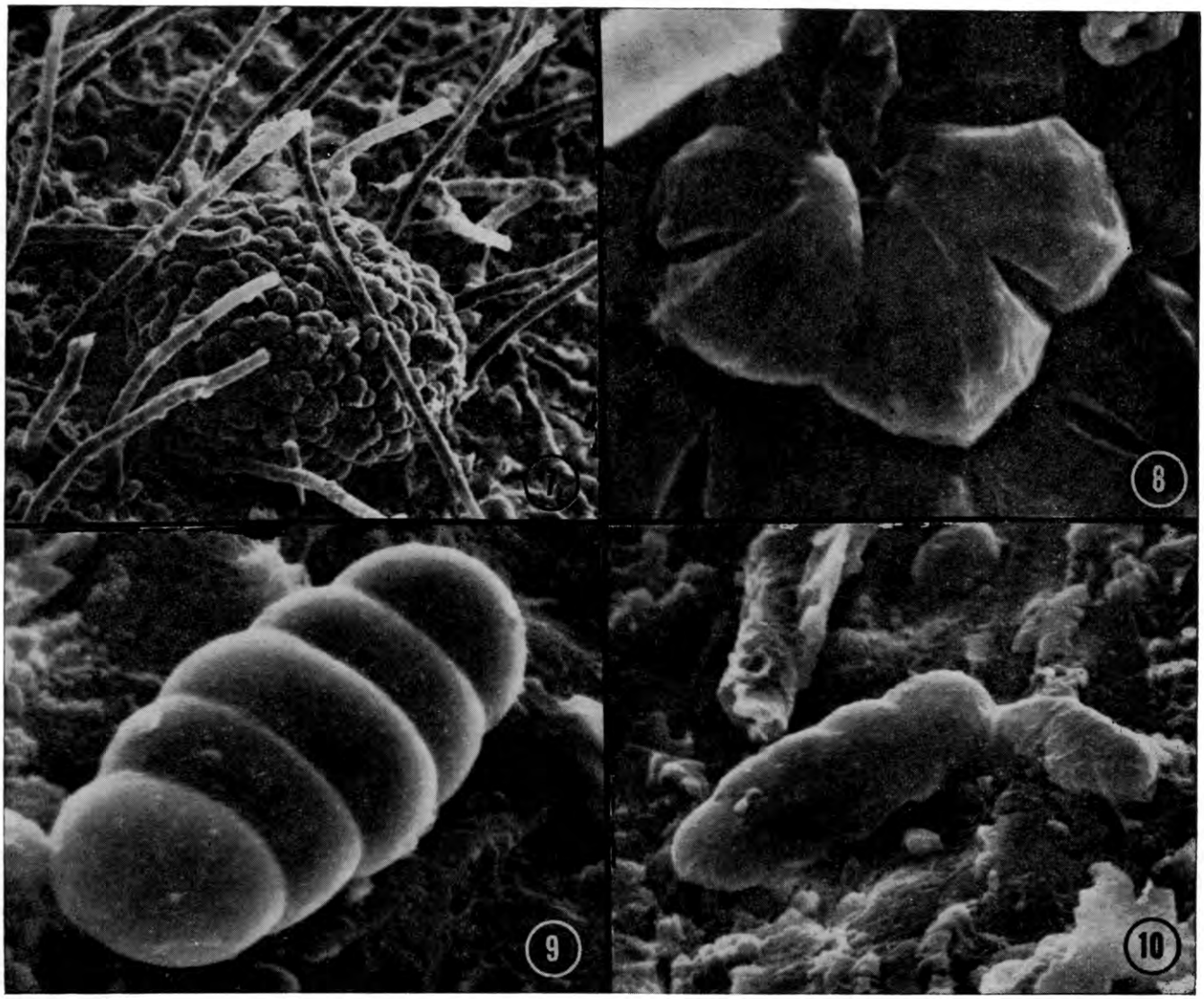

Figure 7. Ascocarp. X 273. Figure 8. Ascocarp surface. X 2100. Figure 9. Expelled ascospore on leaf surface. X 2050. Figure 10. Germinated ascospore bearing a single capitate hyphopodium. X 1040.

haustorium is produced (Hansford, 1961). Nearer the center of the colony, hyphae bearing mucronate hyphopodia are found (Figure 3). These hyphopodia are characteristically produced opposite one another, although this is not always the case in *M. palmicola*. A mucronate hyphopodium consists of a single cell, usually directed away from the leaf surface. The function of the mucronate hyphopodia has never been determined. From the shape of the attenuated tip (Figure 4), it has been speculated that these structures may function in the production of spermatia or conidia, but no evidence for this exists. Observations made during this study indicate these cells to be closed at the tip.

According to Hansford (1961), the outer surface of the ascocarps of most species is roughened (verrucose). SEM observations indicate that in *M. palmicola* the surface of the ascocarp is composed of overlapping scale-like structures (Figures 5-8), suggestive of a pine cone. The ascospores are smooth-walled, and markedly constricted at the septa (Figure 9). On germination, the ascospore gives rise to a short germ tube with a capitate hyphopodium (Figure 10). Hans-

ford states that "apparently it is an invariable rule that the germinating spore first forms a capitate hyphopodium."

Culture studies: In culture, *M. palmicola* grew slowly on all agar media employed, as a dark, nonsporulating mycelium, producing neither setae, hyphopodia, nor ascocarps. Growth was limited, and on corn meal agar reached a width of 1.0 cm from streaked inoculum after 16 days. Growth was similar on all media tested, variations occurring mainly as differing rates of growth and in density of the colony.

Relatively rapid growth was obtained in shaken broth cultures inoculated with macerated mycelium. Results of a 14-day growth experiment using BME broth are shown graphically in Figure 11, from which it is evident that a substantial weight increase occurred during this incubation period.

TABLE 1. CARBON UTILIZATION BY *Meliola palmicola*

Carbon Source	*Mg Dry Weight**
Glucose	39.4
Fructose	35.9
Galactose	55.7
Lactose	35.1
Maltose	38.5
Sucrose	31.6
Starch	40.2
Cellobiose	22.3
None (Control)	20.2
Blakeslee's Malt Extract	113.3

* Based on three replicates; 12 days incubation.

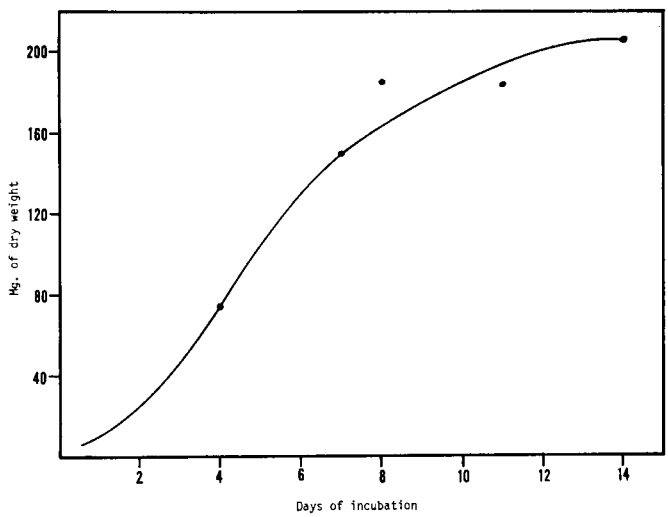

Figure 11. Growth of *Meliola palmicola* in shaken broth medium.

Inasmuch as *M. palmicola* lives as a parasitic fungus, it was of interest to determine its ability to utilize various types of carbon substrates. Results obtained with several carbon sources, with asparagine serving as the nitrogen source, are shown in Table 1. While this medium did not support the amount of growth obtained on BME broth, the results do appear to be a valid indication of the carbon utilization pattern of this fungus. Essentially equal amounts of growth were obtained on glucose, fructose, lactose, maltose, and starch. Galactose supported more growth than any of the other hexose sugars, and maltose appeared to be more readily utilized than sucrose. Starch (Difco soluble starch) was as readily utilized as glucose, whereas cellobiose did not support growth. The latter result indicates that this fungus probably does not attack cellulose.

Inoculation studies: Fragmented mycelium, when inoculated onto the surface of banana leaves, produced some linear growth. Thirty days after inoculation, the fungus had extended up to one centimeter over the leaf surface from the point of inoculation. There was no evidence that a parasitic relationship was established, and hyphopodia did not develop. Growth of the fungus ceased after the initial extension, even though the experiment was continued for about sixty days. Hyphopodia, setae, and ascocarps failed to develop, although short broad hyphae, suggestive of rudimentary setae, were present. Failure to obtain the characteristic growth of *M. palmicola* on the banana under the conditions employed is not surprising, however, since the conditions were far from ideal and because of the supposedly high degree of host specificity among these fungi (Hansford, 1961). According to Hansford (1961), one would not expect *M. palmicola* to develop on the banana under field conditions, and it is questionable whether host specificity would be overcome under greenhouse conditions. The results do suggest, however, that with a suitable host and controlled environmental conditions, successful inoculations may be obtainable.

DISCUSSION

Two observations made with SEM appear to merit special comment. First, no evidence was found during this study to explain the function of the mucronate hyphopodia. Close examination of the tips of several of these cells indicate that they are closed at the tip; no evidence of conidium or spermatium production from these cells was observed. It would be of interest to examine other species in the genus for further evidence on this question. The second point concerns the scale-like nature of the surfaces of the ascocarp. To my knowledge, this type of surface characteristic has not been shown in any other ascomycete.

Results obtained in the carbon utilization studies appear to be consistent with the role of this fungus in nature. The ability to utilize simpler organic compounds, without the ability to degrade more complex compounds, is generally considered characteristic of specialized plant parasitic fungi. The inability of *M. palmicola* to grow on cellobiose indicates that it probably cannot degrade cellulose and therefore is poorly equipped for saprobic existence.

The culture and greenhouse studies of *M. palmicola* reported here must be considered preliminary and inconclusive, but the results do indicate that some members of the genus can be cultured apart from their hosts, and suggest that experimental inoculations on the appropriate host may be accomplished under suitable environmental conditions.

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REFERENCES CITED

HANSFORD, C. G. 1961. The Meliolineae: A monograph. *Sydowia* II: 1-806.

LILLY, V. G., and H. L. BARNETT. 1951. Physiology of the fungi. McGraw-Hill Book Co., New York.

RAPER, K. B., and D. I. FENNELL. 1965. The genus *Aspergillus*. The Williams and Wilkins Co., Baltimore.