A NEW ENTOMOGENOUS FUNGOUS PARASITIZING MEMBERS OF THE ERIOCOCCINAE

By

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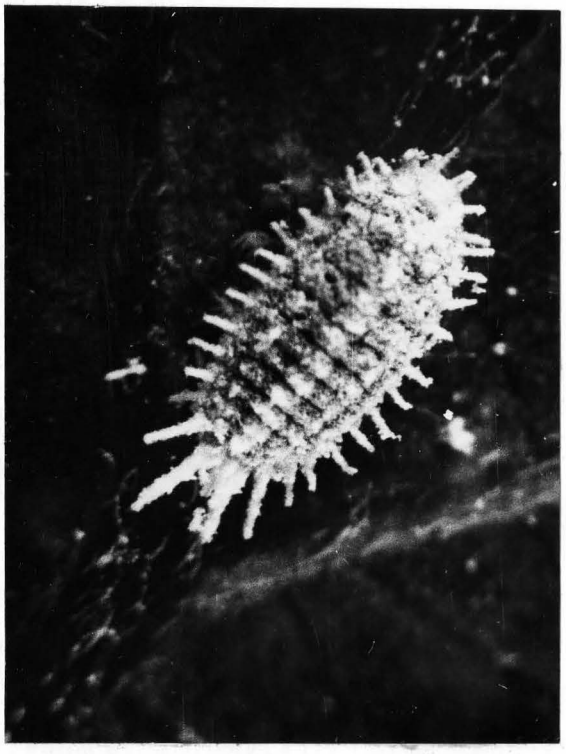

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ACKNOWLEDGMENT

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I. INTRODUCTION

A number of entomogenous fungi attack representatives from practically all orders of insects. The fungi themselves exhibit a high degree of diversification in the hosts that they attack, their methods of penetration, their reaction within the host, and their fruiting structures. Many are Phycomycetous as exemplified by the Entomophthoraceae, many are Ascomycetous among which can be found Cordyceps, Aspergillus and Laboulbenia, a few fall into the class Basidiomycetes such as the Septobasidia, and a large number are Fungi Imperfecti, well illustrated by genera such as Botrytis, Beauveria, Cladosporium, Hirsutella, Sphaerostilbe, and Verticillum.

Perhaps the Homoptera are the victims of the largest number of species of entomogenous fungi. Within this order are some insect pests which are most devastating to plant life. The mealy bugs, the aphids, and the scale insects, all closely related, cause great damage yearly to orchard and green-house crops and require the maintenance of expensive spray programs in order that they be kept in check. In the past many observations have shown entomogenous fungi to be well distributed in environments where abundant host material and favorable weather conditions for fungus development prevailed. That these fungi are a potent force in lowering the population of the insects with which they are concerned is also well known. Only a few investigators, however, have experimented with entomogenous fungi in an effort

to control harmful insects by disseminating these fungi artifically. Forbes (4) in 1895 carried on extensive practical experiments with Sporotrichum globulifera on chinch bugs. He sent insects killed by Sporotrichum to farmers in many parts of the State of Illinois with instructions for using and perpetuating the fungus from these chinch bugs. Unfortunately these experiments were only partially successful. In 1912 Watson (14,15) described methods of inoculating insects with the entomogenous fungi in the citrus groves of Florida. Rolf and Fawcett (10) stressed the effectiveness of these fungi and informed orchardists where they could be procured. Bartlett and Lefebvre (1) did extensive field work to try to check the European corn borer with Beauveria bassiana by spraying spore suspensions, with flour as a carrier, in the cornfields of Massachusettes. They also tried to establish the fungus on plant debris in the field, since it grows saprophytically, but in their report they drew no conclusions about the success of their work. Sweetman (13) cites cases of other investigators who have experimented with artifically disseminating entomogenous fungi but as previously stated few have pursued the subject from this point of view extensively.

This particular study embraces observations on a newly discovered entomogenous fungus in Virginia, as to its physiological and morphological characteristics, its virulence, pathogenicity, and its potentialities as a biological control of insect pests. Studies of the fungus were made in the orchard where it occurred

naturally and in the laboratory and green-house under experimental conditions.

II. REVIEW OF LITERATURE

In 1910 Garrett (5) in a bulletin concerning the sugar cane mealy bug mentioned the genus Aspergillus as being particularly effective in destroying a large percentage of the mealy bugs upon the sugar cane in Louisiana. Speare (11) in 1912 reported an Entomophthora which had parasitized mealy bugs on the sugar cane in Hawaii. This he believed was a new species so he named it Entomophthora pseudococci. Lakon (8), however, in his "Entomophthoraceen Studien" states that this new Entomophthora does not warrent being called a new species. He believes it is identical with Lamia apiculata (Thaxt.). Johnston (7) in a bulletin on the entomogenous fungi of Porto Rico reported that Aspergillus flavus occurred on both young and old mealy bugs, Pseudococcus calceolariae. He stated, moreover, that especially in damp weather this fungus quite materially reduced the mealy bug population. He reported and described in detail Empusa fresenii on mealy bugs and Isaria densa which parasitizes mealy bugs also. This latter fungus, from Johnston's description and drawing, has a similar spore stage to the undetermined fungus on Comstock's mealy bug with which this thesis is concerned. Speare (12) in 1922 discovered Entomophthora fumosa which helped control the citrus mealy bug Pseudococcus citri (Risso) in Florida. Since that time Pospeloff (9) reported Cladosporium on mealy bugs in hothouses near Leningrad in 1935 and Evlakhova (3)

reported a yeast-like fungus pathogenic to Pseudococcus citri (Risso) which she named Blastodendrion pseudococci. In 1940 Charles, Couch, Harrar, and McKelvey (2) reported a fungus, which is unnamed as yet, on Pseudococcus comstocki (Kuw.).

Many cases may be cited from the literature on entomogenous fungi of fungous diseases which have been reported with little or no experimental evidence of parasitism to support them. In the cases of Cladosporium, Aspergillus, Isaria, and Blastodendrion on mealy bugs the author believes that probably toc little critical work has been done to justify accepting them as parasites of mealy bugs.

III. THE INVESTIGATION

A. Importance of the New Mealy Bug Fungus

In August 1939 and also in August 1940 mealy bugs which were diseased were noticed throughout Virginia orchards. In 1940 due to an excessive amount of damp weather, apparently, the ravages the same disease made upon mealy bugs reached the proportions of an epizootic and thus demonstrated potentialities for controlling mealy bugs in Virginia biologically. Following this rainy period approximately 99 per cent of the adult female mealy bugs on the apples and on the apple twigs were observed to be killed by the fungus (Figure 1). In the spring of 1941 a survey of the mealy bug population in orchards at Crozet and at Roanoke, Virginia showed a much lighter infestation from the overwintered egg masses than was the case the year before. Although other factors might be partly responsible for the decrease in number of mealy bugs this spring as compared with last spring, undoubtedly the heavy mortality (due to the fungus) of the adults of the third brood of mealy bugs greatly reduced the number of overwintering eggs. Newly developed sprays and the liberation of parasites against mealy bugs have also aided in lowering their population.

Since this disease was so effective it was thought expedient to determine the causative agent and if it were a fungus to isolate and grow it on artificial media, to study its physiology, to carry on infection experiments so that its

virulence and pathogenicity might be established, and to study its etiology so that its effectiveness as a biological control of mealy bugs might be evaluated. At the present time there is no cheap effective method of controlling mealy bugs by spraying, although soluble kerosene and Loro promise to fulfill the requirements of being cheap and effective.

Nature protects the mealy bug remarkably well. The eggs overwinter underneath the bark of apple trees where sprays seldom reach them and in these places the eggs are further protected by masses of silk. When the eggs hatch and the nymphs crawl from the egg masses to feed on water sprouts of the limbs, they are for a short time vulnerable to sprays. They then migrate to the stem and calyx ends of apples where they are again hard to reach. Fungus spores as well as parasites, however, encounter no difficulties in entering these protective habitats.

If some fungus which when disseminated artifically operated efficiently in controlling mealy bugs, a cheap, easy, and welcome relief from damage done by mealy bugs would be available.

B. Mealy Bug Injury

Comstock's mealy bug was first observed in Virginia on the umbrella catalpa in 1923 (6). Later (1935) mealy bugs became prominent as a pest upon apple trees near Charlottesville, Winchester, and Roanoke in Virginia. The mealy bug injures apple trees both by secreting honey dew on apples and by feeding on the

young apple stems. Upon the honey dew grows a sooty mold which interfers with the absorption of light rays, the coloring of the apples and which makes them generally unmarketable by their sooty appearance. This is the most important damage caused by mealy bugs. However, extremely large numbers of mealy bugs feeding on young apple stems sap strength from the tree.

C. Brief Life History of Mealy Bugs

"Minute yellow eggs are embedded in masses of white cotton-like wax threads and hatch in from eight to three weeks, depending on the time of year" [1]. The nymphs molt in from 22 to 23 days, then undergo another ecdysis nine or ten days later. Up to this point, the third molt, no difference between males and females can be observed. Nine or ten days later male mealy bugs spin a cocoon and female mealy bugs again molt. From this stage on mealy bugs can easily be distinguished from females. After their last molt adult females are fertilized by the winged adult males which have emerged from their cocoon. The adult males live from one to three days only. Females feed for from nine to 12 days, then oviposite for approximately eight days. In about 52 days the mealy bug completes its life cycle. Normally in Virginia the life cycle is completed three times a year. The mature females are oval wingless insects which, although they are soft,

[1] The Biology and Control of Comstock's Mealy Bug on the Umbrella Catalpa. Dr. W. S. Hough, pp.12-16, Va. Agr. Exper. Sta. Tech. Bul. 29: Feb. 1925.

possess a great deal of waxy material about them which gives them a mealy appearance. Females deposit their eggs in a long silky egg sac and upon the completion of oviposition they shrivel and die. The egg sac resembles the male cocoon superficially. Careful examination shows that the female is usually larger than the male and that the female mealy bug is continuous with the egg sac, whereas the male mealy bug develops entirely within the cocoon and can not be seen. Mealy bug eggs can barely be seen with the naked eye, whereas nymphs which are the most active stage in the life cycle can readily be seen. Male mealy bugs, more seldom detected than females and nymphs, are delicate fly-like insects slightly longer than the females. They have white wings, a pink body, and prominent white caudal filaments which are longer than those of the females. Approximately equal numbers of nymphs from each brood develop into male and female adults.

D. Etiology

The fungus produces round spores which germinate into peg-like structures and enter the mealy bugs through the leg joints. The infection tubes penetrate the joints and produce mycelium within the legs of the insect. Hyphae then form and grow through the legs into the body where they further develop to completely replace the body tissues. Then if weather conditions are favorable (if high relative humidity and heavy rains persist) the fungus within the mealy bug will give rise to

conidiophores outside of the body which in turn produce phialides upon which are born spores. If weather conditions do not permit the continued dissemination of the fungus by spore production (if relative humidity is low) the mycelium that occurs in oidial like masses within the insect, shrinks, becomes rearranged and compact into hard olivaceous-green to black sclerotia (Figure 2) which serve as a means of tiding the fungus over periods of extreme heat, dryness, or cold. Mealy bug eggs appear impervious to fungous penetration.

E. Symptoms of Disease

The first symptoms of disease appear approximately two days after inoculation. Mealy bugs manifest difficulty in controlling their appendages, particularly in coordinating the leg joints, and as a result legs become stiff and useless. As fungus growth progresses, decrease in activity, loss of a measure of response to contact stimulation, and general sluggishness ensue. From three to five days after penetration, depending on the age and size of the mealy bug, they die and conidiophores emanate from them. The conidiophores radiate in all directions from the dead bugs and extend outward two or three times the length of an adult bug.

F. Isolation of the Fungus

On August 30, 1940 diseased mealy bugs collected in orchards near Roanoke, Virginia by Dr. J. A. Cox of the Entomology

Figure 1. Dead mealy bugs on apple twigs.

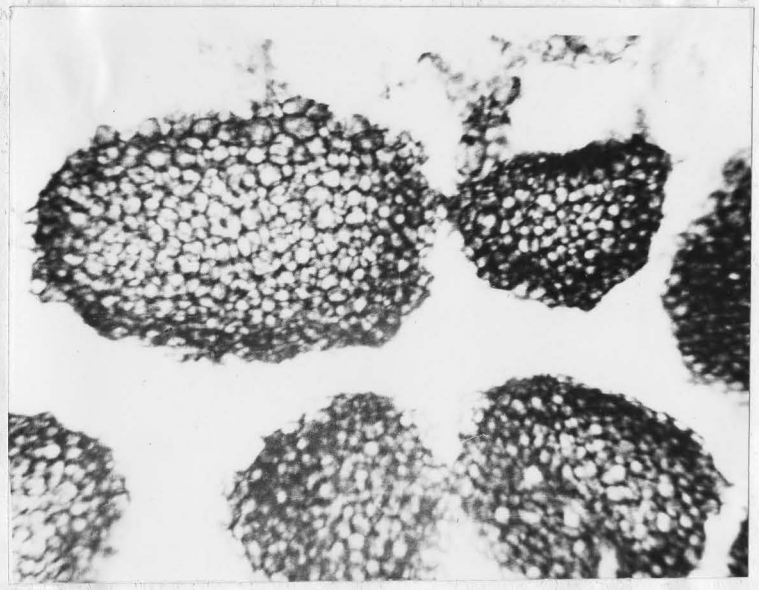

Figure 2. Mature sclerotia.

Section of Virginia Agricultural Experiment Station, were sterilized in 95 per cent alcohol for one-half a minute then emersed in an aqueous solution (1-10,000) or mercuric chloride for one to five minutes. Mealy bugs so treated were transferred to sterile petri dished containing sterile egg yolk medium. Some mealy bugs were surface sterilized, crushed in sterile water, and then poured over sterile egg yolk medium and potato dextrose medium. Seven days later colonies which seemed to be pure black were formed on the egg yolk medium. These colonies actually consisted of olive-green sclerotia which are characteristic of the fungus in the diseased bugs. On this medium very few conidiophores developed. On potato dextrose agar grayish white colonies were formed and conidiophores were abundant. Although sclerotia also developed the colonies were by no means exclusively sclerotial, as was in the case of egg yolk medium. These colonies were transferred to more egg yolk and potato dextrose media.

Six sterile petri dished with moist filter paper in the bottom served as laboratory infection chambers. Each plate contained fifteen adult female insects. Mealy bugs were allowed to walk over pure cultures of fungus growing on egg yolk medium and then were placed in two of the moist infection chambers. Mealy bugs alloted to the next two plates were rolled over spores of a fungus culture on potato dextrose agar. Mealy bugs destined for the control chambers received no treatment prior to being placed in the petri dishes. Four days later the mealy bugs in the two

plates used as controls showed no signs of infection. Twelve of them in one control plate began laying eggs. Of those 30 mealy bugs inoculated from potato dextrose cultures 25 succumbed to the fungous disease. As yet no fungus appeared on the insects inoculated with sclerotia from egg yolk medium cultures but eight days from the time of inoculation these 30 mealy bugs also died. These dead bugs exhibited typical sclerotia within them and typical conidiophores spreading out from the mummies. The lag in the time of death of the bugs inoculated from fungus cultures on egg yolk medium may be due to the fact that the sclerotia must germinate, to produce conidiophores and spores, before infection can take place. The 30 insects in control plates remained alive and layed eggs. Monosporous cultures from mealy bugs killed in this way have been obtained and these were identical to the original isolates and were used for further infection experiments. Thus has the fungus been isolated and its pathogenicity proven.

G. Cultural and Physiological Characteristics

In order to ascertain the culture medium best suited for prolific spore production, to determine the limits of variability of the fungus, and its versatility of growth on saprophytic media, many media have been tried. On potato dextrose agar abundant conidiophores and spores are produced and growth is fairly rapid. (Figure 3). Mycelial production occurs first at the point of inoculation but following this is

the formation of sclerotia from the mycelium which are relatively abundant on potato dextrose medium.

The fungus grows well on malt extract agar, nutrient agar, and potato slants, with but slight sclerotial development. On corn meal agar, white of egg, macerated grasshopper medium, and Bacto Endos agar, no sclerotia develop. Macerated grasshopper and egg white media do not stimulate conidiophore production very much. It has been difficult to induce any growth on egg white medium although a slow yellow mycelial growth will appear on this medium sometimes and when this growth is transferred to potato dextrose agar typical development follows. Poor mycelial growth and conidiophore development are characteristic on these media. This fungus also develops well in nutrient broth. In broth, although the conidiophores and the sclerotia grow on the surface some mycelium grows in the broth. Sclerotia form most abundantly and exclusively on egg yolk medium on which the fungus also causes revolute growth quite characteristic of the Entomophthoraceae. (Figures 4 and 5). On sterilized egg yolk medium few conidiophores or spores are produced and therefore the fungus appears very dark green to black.

On nitrate agar no sclerotia are formed, the colony appears brown. Few conidiophores arise either, and the fungous growth occurs almost entirely as mycelium within the substratum. Nitrates are apparently not reduced to nitrites for a test for nitrites proved negative. On lead acetate agar rather restricted

growth occurred. A black ring surrounding the culture indicates that the fungus reduced the sulfates and sulfites to hydrogen sulfide.

On purple lactose agar the fungus made the medium more alkaline. This might mean that the fungus after hydrolyzing the sugars, if it did hydrolyze the sugars, also hydrolyzed proteins thereby liberating ammonia which made the medium more alkaline. On 0.1 per cent sodium thioglycolate medium and 0.6 per cent sodium thioglycolate the fungus appeared wrinkled and creamy. Some sclerotia were formed but few conidiophores and spores were evident. Sodium thioglycolate is a highly reduced medium. On this medium the fungus showed slightly different cultural characteristics. However, since no difference in growth occurred in the plates containing 0.1 per cent sodium thioglycolate, and those containing 0.6 per cent sodium thioglycolate no conclusions can be drawn as to how reduced a medium this fungus will grow on and therefore as to the anaerobic qualities of the fungus.

The fact that this fungus has been grown saprophytically for such a short time renders definite conclusions in describing cultural characteristics hazardous and since the fungus ages in culture through successive transfers, it undergoes changes and gives rise to sectors which differ both in appearance and in growth response to the different media upon which it is grown. Four distinctly different sectors which have arisen over a period of six months from the original material isolated from mealy bugs on

potato dextrose agar exhibits different degrees of sclerotial formation, and conidiophore production. Figures 6,7 and 8 represent three of these sectors obtained.

Potato destrose plates, inoculated with the fungus were incubated at temperatures ranging from 10°C. to 45°C. Upon examination of these plates three days later no growth of the fungus was observed at the two extremes but the fungus remained alive at 10°V. and at 41°C. At 37°C. only restricted growth occurred. Optimum temperature appears to be approximately 20°C.

A series of plates of potato dextrose agar made up in quadruplicate were adjusted by a glass electrode potentiometer to pH values ranging from pH 4.5 to pH 10 with 0.5 pH intervals. Only slight variations of growth of the fungus over the entire range could be observed although the lower pH values 4.5, 5, and 5.5 gave restricted growth. Fewer sclerotia appeared at the higher pH values and at pH 5 and 5.5 sectors were formed (Figure 9). This may mean that an acid medium stimulates formation of sectors. No experiments have yet been conducted to determine any difference in pathogenicity of these sectors, or of those sectors which have resulted from long time artificial culturing. Growth at lower pH values favored increased sclerotial formation. Optimum pH centered around 6.5 although the pH range in general of this fungus was very extensive.

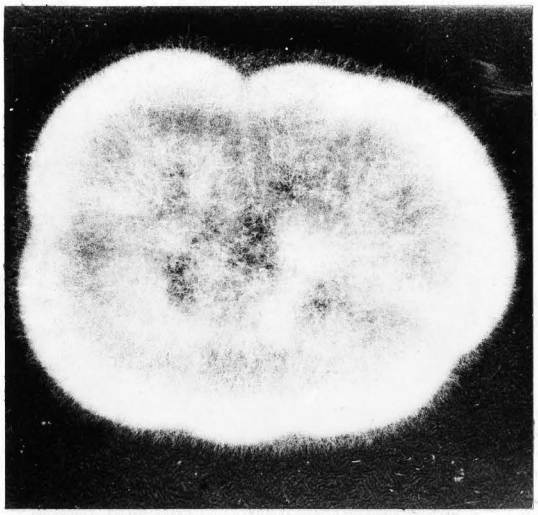

Figure 3. Typical growth on potato dextrose agar

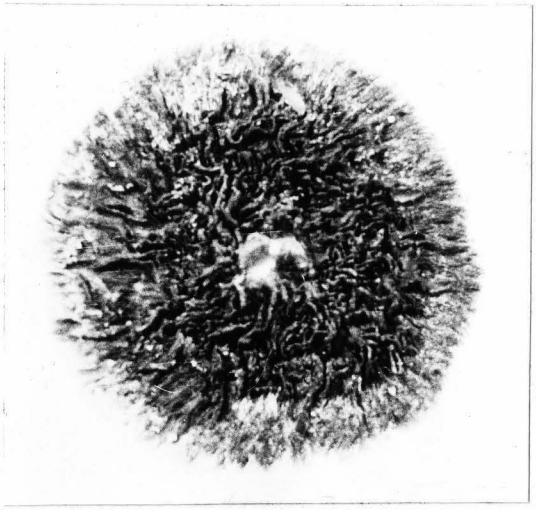

Figure 4. Colony on egg yolk medium

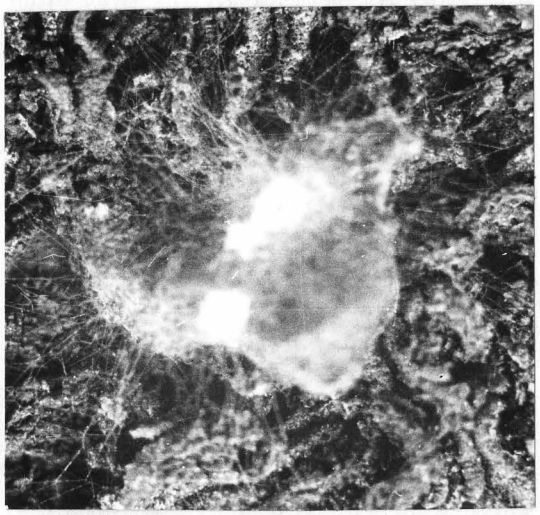

Figure 5. Colony on egg yolk medium (enlarged)

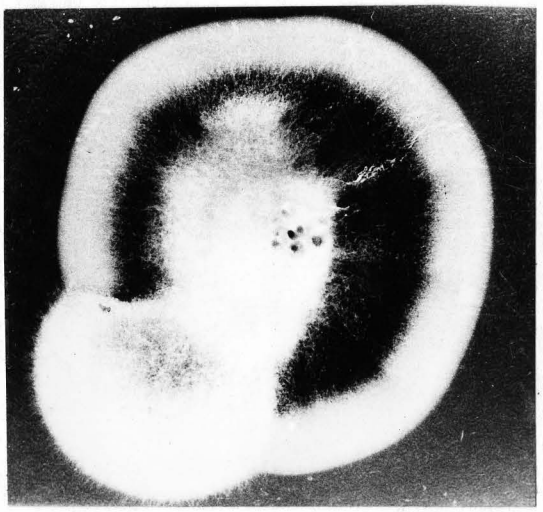

Figure 6. Sector on potato dextrose agar

H. Morphological Characteristics

Conidiophores and mycelium are septate (Figure 10) and measure 4u in diameter. Spores are spherical and range from 4u to 6u in diameter. Phialides which are bottle shaped measure from 13u to 34u in length, averaging 20 u long, and 4u wide (Figure 11). Sclerotia are extremely irregular (Figure 12) but probably average 150u by 400u in size. Spores, phialides, conidiophores, and mycelium are hyaline, sclerotia are olive-green to black. Phialides typically bear one sterigma upon which is one spore (Figure 13), but frequently in old cultures phialides bear secondary sterigmata and become septate and branched (Figures 14, 15, and 16). Phialides arise mostly from one side of the conidiophore, but two, and occasionally three in a whorl have been observed. Sclerotia form into hyphae (Figure 17) into compact irregular masses which shrink and become hard as the mycelium divided into smaller much thicker walled cells. The sclerotia produced are typical of the fungus, beginning as hyphal aggregates, they eventually appear as thick walled cells often containing cavities (Figure 18 and 19).

Sclerotia which have been stored at 5°C. for eight months retain their viability and may be germinated for use in inoculation experiments. Spores germinate within 24 hours by sending out one or more germ tubes which develop into conidiophores. Spores may also germinate to form secondary conidia or to form a phialide and secondary conidium (Figure 20). The spores are borne singly but are mucilaginous and consequently often appear to be paired.

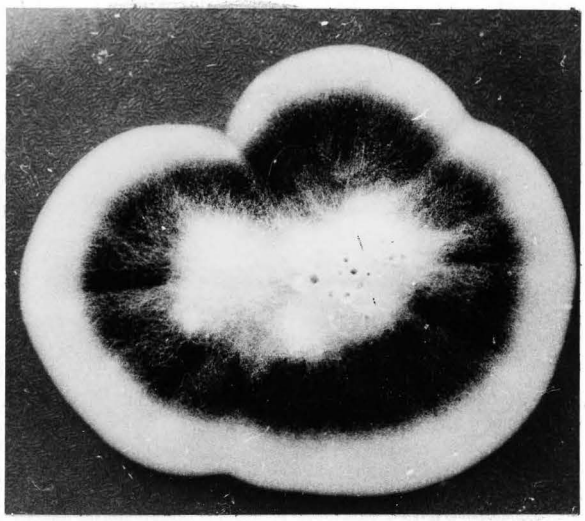

Figure 7. Sector on potato dextrose agar

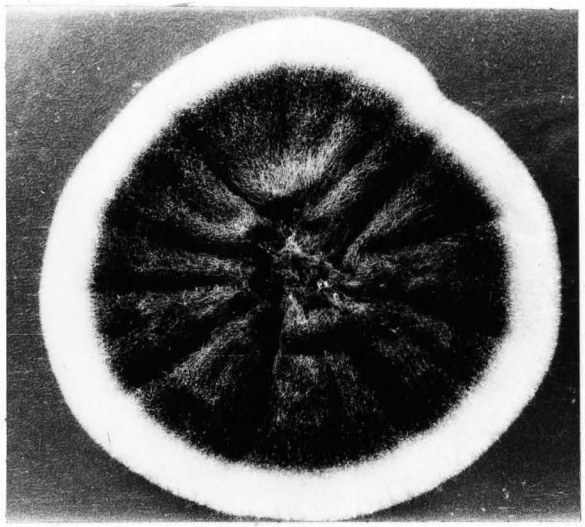

Figure 8. Sector on potato dextrose agar

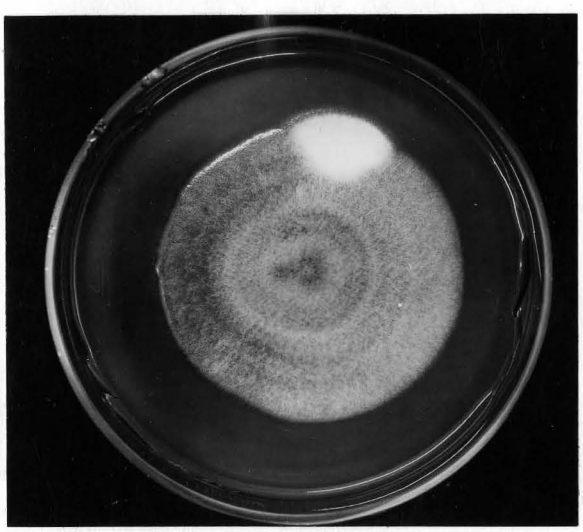

Figure 9. Sector formed at pH 5.5 on potato dextrose agar

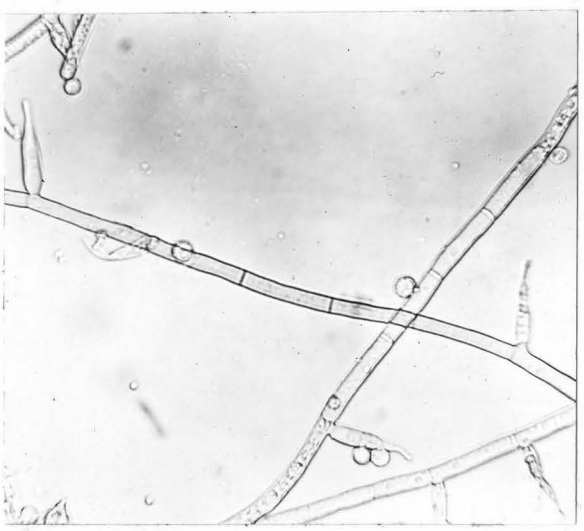

Figure 10. Septate conidiophores x320

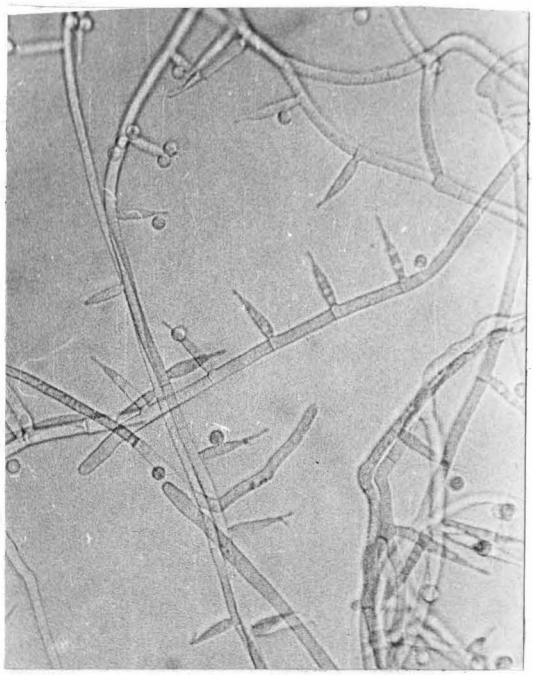

Figure 11. Phialides x320

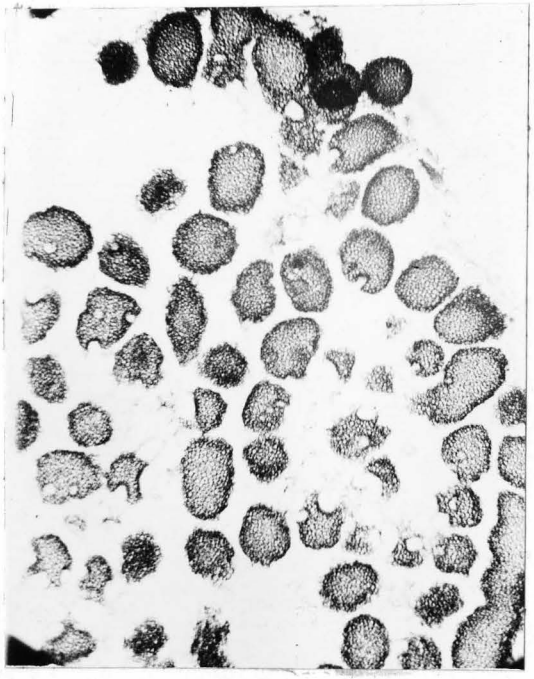

Figure 12. Sclerotia in mealy bugs

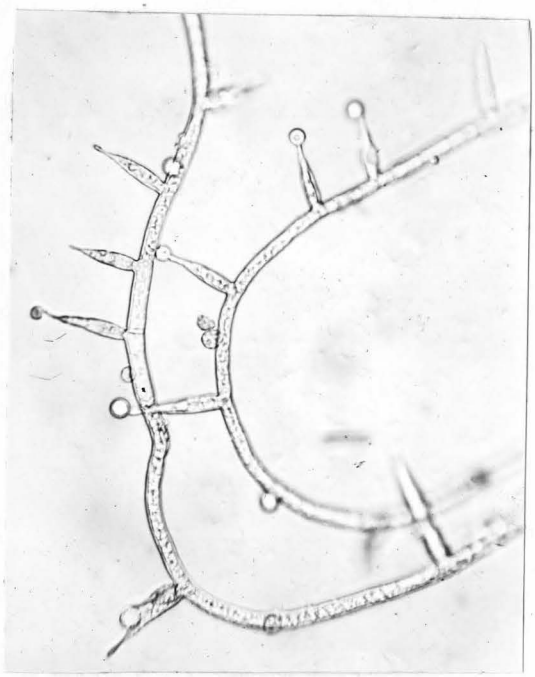

Figure 13. Typical phialides x320

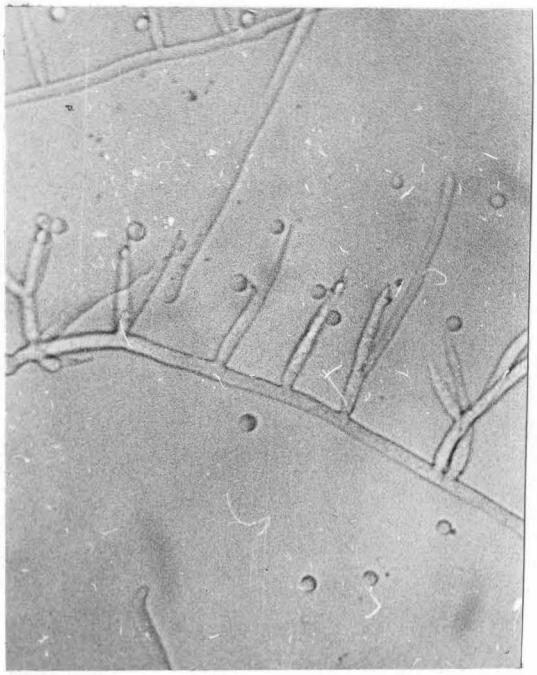

Figure 14. Branched phialides

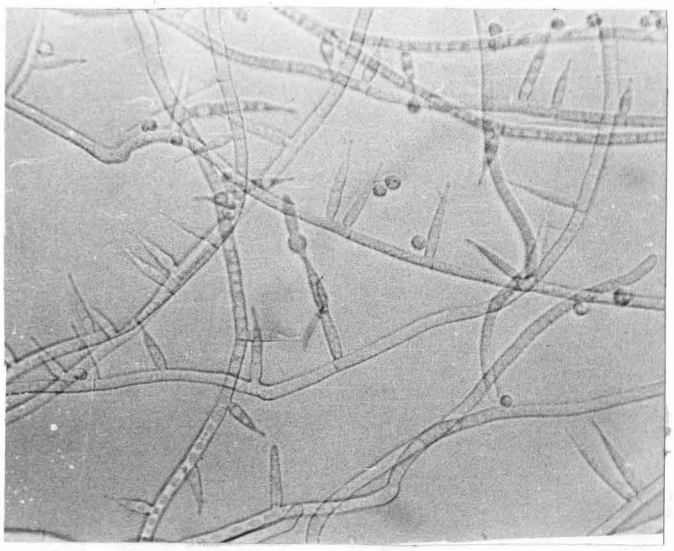

Figure 15. Atypical phialides x320

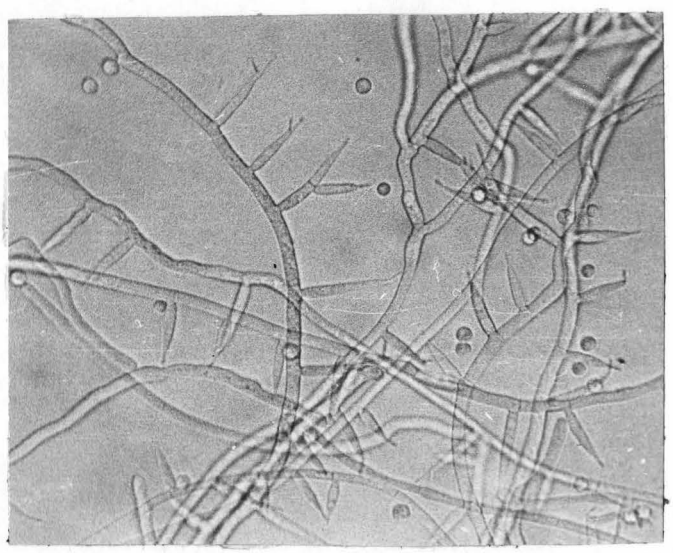

Figure 16. Branched and septate phialides x320

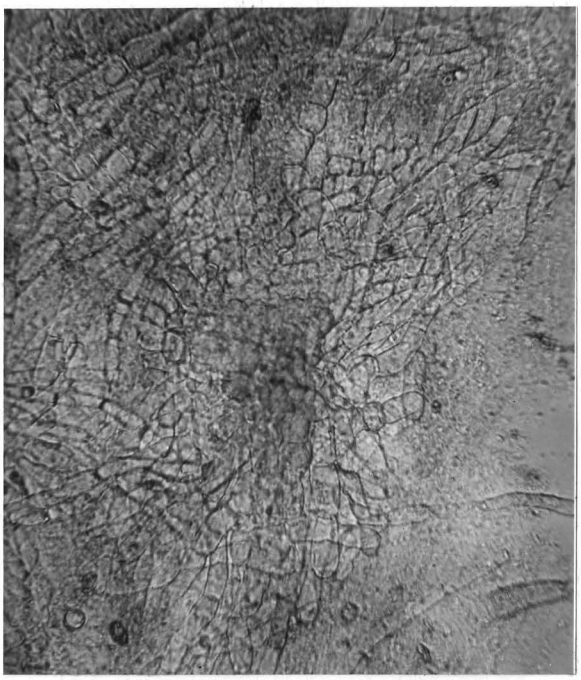

Figure 17. Pro-sclerotium x320

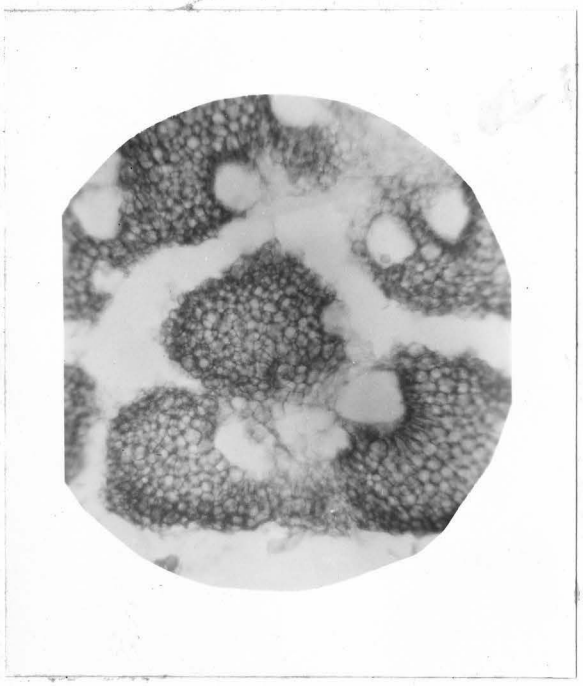

Figure 18. Sclerotia x320

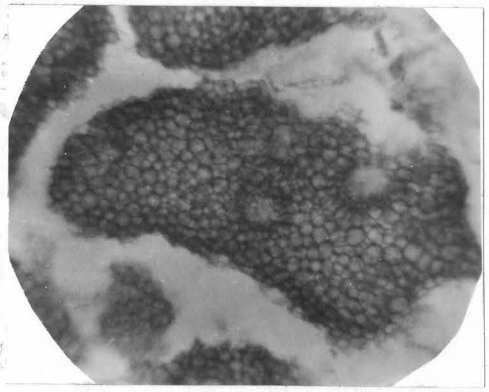

Figure 19. Sclerotia x320

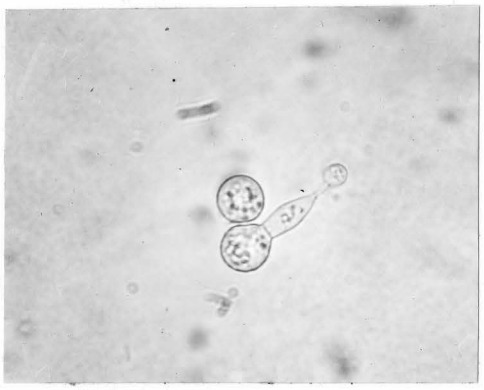

Figure 20. Phialide and secondary spore x900

I. Host Range

Infection experiments with codling moths, oriental fruit moths, and termites were negative. Neither could aphids which are closely related to mealy bugs be infected. In these experiments the procedure was identical to that used in obtaining infection in healthy mealy bugs. When the pathogen was tested against Pseudococcus comsrocki (Kuw.), Pseudococcus citri (Risso)n and Phenococcus colemani it was found to equally effective in the destruction of all three species. It is possible that all species of the true mealy bug are susceptible to attack by this parasite.

It has been suggested that the ,ealy bug fungus might also be the cause of a storage rot of apples and while this seemed a remote possibility, it was decided to check this hypothesis. Using the needle puncture technique apples were inoculated with the mealy bug fungus. No infection was obtained in this experiment nor was it ever possible to secure infection of apples with this organism. It is thought that enough data have been collected to demonstrate conclusively that the mealy bug fungus is not a parasite of the apple fruit.

J. Method of Penetration

Large numbers of mealy bug eggs were placed on fungus cultures and did not become infected but hatched normally into nymphs. These first nymphal stages were picked from the cultures at intervals for observation of penetration. Mode of entry appears

confined to penetration through the leg joints, particularly at the joint between the tarsal segments and the tibia. Infection may possibly take place also at the base of the setae where the exoskeleton is thin and vulnerable. Spores frequently lodge here and in germination their contents may dissolve through the setal membrane. Experiments are now in progress to settle this point, as observations to date are inconclusive. Direct penetration through the cuticula has not been observed and it is doubtful if this fungus produces the necessary enzymes to dissolve the mealy bug exoskeleton where the cuticula is thick. Observations on penetration were made by studying young nymphs directly under a microscope in vivo. Adult insects were sectioned and stained with Delafields haemotoxylin using fast green or eosin as counter stains. Both procedures resulted in good differentiation of tissue. In stained sections mycelium and sclerotia could be detected but the insects which were sectioned had passed the stage where fungus penetration could be observed. It is hoped that histological experiments which are now in progress may more clearly demonstrate penetration.

K. Dissemination

Laboratory experiments and observations in orchards have established the fact that this fungus disease is disseminated by wind or by water. Both spores and sclerotia can be disseminated and used as inoculum but spores act as inoculum more often

than sclerotia. Mealy bugs themselves may act as disseminating agents and by crawling over uninfected bugs, transmit the disease. It is possible that parasites and predators of mealy bugs transmit the disease also but no experimental evidence has been adduced to support this hypothesis.

L. Infection Experiments

If this fungus is to be used as a biological control of mealy bugs it is necessary to fine the range of environmental conditions under which it is able to induce a high mortality among mealy bug populations. Evidently such conditions do occur as witnessed by sporatic epizootics of the mealy bug disease which have been observed.

In order to determine the minimum conditions for infection, constant temperature cabinets were used in infection experiments. The first cabinet was regulated to a mean temperature of approximately 72°F. and a mean relative humidity of 77 per cent. Coleus plants, maple leaved viburnum, and lantana plants which were heavily infested with mealy bugs were placed in this cabinet and were subsequently sprayed with an aqueous spore suspension of the fungus. Although over a period of three weeks numerous dead bugs could be seen, the percentage killed was not great enough to materially reduce the mealy bug population on the leaves and stems of the plants on which adult females continually laid eggs. (Figure 21). However by the end of five weeks nearly 100 per cent

of the insects were killed. In the second constant temperature and humidity experiments the humidity was raised to nearly saturation. Similar plants, heavily infested with mealy bugs were placed in this cabinet and were kept well watered. Within six days after inoculation of these mealy bugs by spore suspension sprays dead mealy bugs, mostly young ones, could be found (Figure 22). Twenty-one days from the time of inoculation nearly 100 per cent of the mealy bugs had died and new green leaves free from mealy bugs sprouted from the plants. The incidence of disease in this case correlated well with the percentage of adult females killed in orchards, in the vicinity of Roanoke, Virginia in August 1940, by the fungus after a week of damp weather. As previously stated, ninety-nine per cent of the adult mealy bugs were estimated as having been infected by this fungus at that time. Mealy bugs of all ages were included in these experiments, hence, many of them matured to lay eggs (Figure 23) before they becoame infected and many of the eggs already laid hatched since the fungus does not penetrate the egg.

These factors seriously complicated any attempts to keep an accurate count of the diseased and the healthy mealy bugs throughout the experiment. Random counts and estimations were the closest approximations to the actual numbers of diseased mealy bugs which could be obtained. On this basis over 20,000 mealy bugs are estimated as having been used in these experiments of which 50 per cent in one cabinet and 95 to 99 per cent in the

Figure 21. Female mealy bugs showing egg sacs

Figure 22. Young diseased mealy bugs on coleus leaf

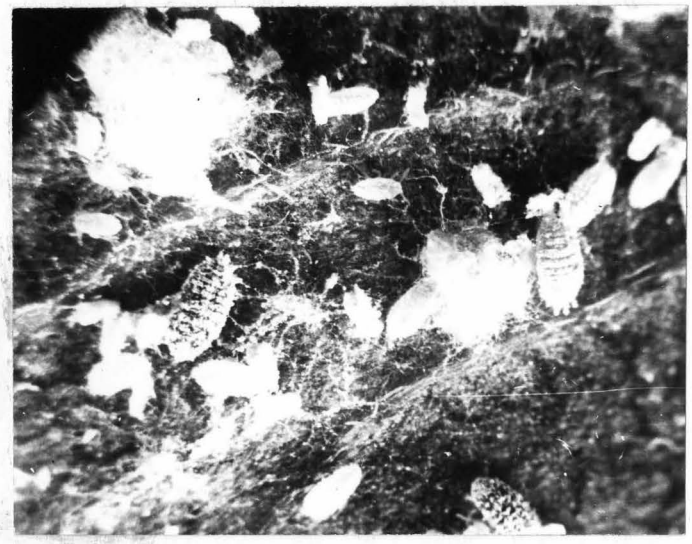

Figure 23. Diseased mealy bugs in different instars

second cabinet are estimated to have been killed within three weeks.

Plants infested with mealy bugs were placed in the constant temperature and humidity cabinet which was used for the other infection experiments but this time they were not sprayed for it was deemed advisable to discover if there was enough inoculum from the previous experiments to cause infection. Nine days later several dead mealy bugs containing sclerotia within them were found on the leaves of the plants. The experiment was continued until it became evident that natural infection had occurred and that another local epizootic was in progress.

Efforts to duplicate these experiments in the local green-houses proved largely unsuccessful due mainly to the difficulty of maintaining a high relative humidity in these houses.

IV. SUMMARY

1. An unidentified fungus which occurs naturally in Virginia and which is parasitic in mealy bugs has been isolated and its pathogenicity proven by Koch's postulates.

2. The physiological, cultural, and morphological characteristics of the fungus have been studied in an effort to identify the pathogen, to propagate it for future control work and to determine its nutritive requirements.

3. Preliminary host range studies indicate that the pathogen may be specific for mealy bugs, although further experiments with such insects and aphids are deemed advisable and are in progress.

4. Inoculation experiments under controlled conditions have been performed. They indicate that the fungus causes infection most readily at a high relative humidity (approximately between 75 per cent and saturation) and at temperatures of 70°F. Once infection has occurred death invariably results but subsequent infections of healthy hosts is largely dependent upon favorable humidity and temperature.

5. The etiology and symptoms of the disease which the fungus causes have been thoroughly investigated, compared with other entomogenous fungi and evaluated in considering the use of this fungus for controlling mealy bugs.

6. Mycological literature has been extensively reviewed in an

effort to classify this fungus. Dr. Vera K. Charles of the Bureau of Mycology in the United States Department of Agriculture and Dr. J. N. Couch of North Carolina University are collaborating in a study of this fungus at the present time.

V. CONCLUSIONS

This fungus possesses a restant resting stage, the sclerotium, which is present not only in the winter time but normally develops in all seasons of the year coincident with the mealy bug. It grows and germinated well under wide temperature ranges, tolerates a wide pH range, and utilizes various sources of proteins, carbohydrates, and fats or other substances which it needs. As a result it offers promise as a biological control of the mealy bug which becomes each year an increasing menance to apple growers in Virginia. Since the fungus shows little or no preference for species of mealy bugs it might well be disseminated artifically to control other mealy bugs, particularly those which thrive in green-houses and in tropical or sub-tropical countries. The ease with which the fungus can be isolated and grown in pure culture make the problem of procuring enough inoculum at the proper time for artificial dissemination a simple one.

How toxic spray materials are to the fungus and the part that predators and parasites play in disseminating the fungus remain as yet only partially investigated since experiments concerning these points have been only recently started. That sprays decrease the efficacy of the fungus seems quite probablo for this fungus has been observed to cause the most damage to mealy bugs late in the summer when most of the spray-

ing is over even though favorable weather conditions for development of an epizootic often appear to exist in the spring as well as in the late summer.

Two characteristics of this undetermined fungus may hinder its use in orchards. For infection to take place high relative humidity is required as in the case with most entomogenous fungi but if the spores were disseminated artifically before a heavy rain and period of high relative humidity, infection should take place immediately, and once the fungus penetrated the mealy bug the humidity factor probably is of little importance. However, for production of spores and penetration, high relative humidity is essential. The second characteristic of this fungus that is disadvantageous for efficiency in its operation as a biological control is that penetration of the host by the fungus appears limited to the joints of the insect. This is less effecient than if the spores could penetrate the exoskeleton at any point as do many entomogenous fungi, either by chemical or mechanical means.

Although in sectioned insects a number of stages of fungus development within the mealy bug have been observed, the exact order in which the tissues are invaded has not been completely established. Eventually the fungus completely replaces all insect tissues and the ultimate stage consists merely of the insect exoskeleton, which itself is partly replaced by the fungus and is filled with the fungus and the chalky meal left on the

exterior. Further study upon the order in which the insect tissues are invaded would undoubtedly reveal the food best suited for the fungus at each particular phase of its life cycle. This in turn would probably help explain sclerotial formation which depends upon more than the temperature and humidity factors.

As to the systematic placement of the fungus all that can be said is that to the present time no sexual fruiting structures have been observed. This necessarily places this fungus among the Fungi Imperfecti. No species or genus within the Fungi Imperfecti corresponds to the characteristics of this fungus, apparently, and it therefore seems likely that a new genus must be set up to receive this fungus. The name Endosclerotium entomogenum is being considered for the designation of this pathogen.

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