PHYSIOLOGIC STUDIES ON SOME ENTOMOGENOUS FUNGI

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I. INTRODUCTION

The initial interest in the subject of this thesis arose from the finding and isolation of an insect parasite which subsequently was identified as a species of Beauveria. Three specimens of the parasitized insects were collected near Mountain Lake, Virginia, in the vicinity of White Pine Lodge. These specimens were taken in the month of August, 1940, during the course of a mycological field trip, which was sponsored by the mycology class of the University of Virginia summer school. Collection of fleshy fungi was the main object of this field trip and the finding of this species of Beauveria was purely accidental and hurried, so that little time was given in checking the extent of the epizootic in the area.

The insect host is an undentified species, probably an heteropterous insect, as tentatively identified by Dr. Underhill of the Virginia Polytechnic Institute Entomology Department. The insects were securely fastened to leaflets of cohosh, Aetaea sp., quite conspicuously with a white encrusted fungous growth on the body. The depletion and drying out of host tissues, as well as the encrusted covering of fungal growth, had nearly destroyed the insect body. The insects were 2-4mm. in length and soft bodied, and a narrow ring of excreta was evident on the leaflet immediately around the dead body.

The plants grew within an area of several square yards in a narrow and shallow ravine. It was only a chance over-turning

of the bushes which revealed their presence. The specimens were carefully collected intact as attached to the leaflets.

Careful examination of the specimens in the laboratory revealed a very interesting and a rather unusual type of fungus growth. The insect body was covered with a white concretion and arising from this surface was some 12 to 40 fruiting structures 1-3mm. in length were clearly visible with 10x magnification. The synnemata were recurved, rather brittle, and the surface of the horns had a nodulated appearance. Enlargements of these structures are shown in Plate I.

With 100x and 400x magnification the myceloid structure of the synnemata was evident. The whole, in cross section, was formed from interwoven strands of a mass of hyphae. The fungus horn tapered uniformly from base to tip and each strand of hyphae produced on its end and at the surface of the horn a cluster or head of conidia. These clusters, which cover the whole surface, account for the nodulated appearance. They were so dense as to prevent any view of the conidiophores or of their structure. The spores were single celled, globose, and hyaline. They were relatively small, having a diameter of 2-3u. The hypha also was of small diameter and hyaline. Plate II is a photomicrograph of hyphae and spores.

Three unsuccessful attempts were made to isolate the fungus by inoculating plates of potato-dextrose agar with conidia from the insects. The fourth attempt, in which plates of egg yolk

culture medium were used, was successful and transfers made therefrom gave pure cultures. Most probably the fungus was merely overlooked in the first attempts at isolation, since it grows readily on potato-dextrose agar as well as on egg yolk medium. The mycelium was white, becoming cream-colored with age. The aerial growth was heaped and slightly overgrew the contaminants on the plate. The conidial heads are produced at intervals along the mycelium. They are so very compact as to obscure the conidiophores, and the spores are very deciduous.

It was very evident, after brief microscopic examination, that the fungus is a species of Beauveria, and belongs in the Fungi Imperfecti. The spore size and appearance, the hyphal characteristics, and the successive stages in conidiophore structure from a simple one to the branched and rebranched phialidae were the characters observed and on the basis of which the identification was made.

II. HISTORY AND REVIEW OF LITERATURE

The fructification of the fungus in the form of synnemata, as observed on the insects and as has been described, is a unique character for Beauveria species. So far as is known this growth character has never before been reported for Beauveria species. The finding of this Beauveria and the occurrence of this unusual expression of fructification naturally raised the question of identity, as to what species it was, or whether it was an undescribed species.

In 1912 Vuillemin (11) described the genus Beauveria in which he placed a number of entomogenous fungi previously distributed among several other genera. This genus belongs to the Fungi Imperfecti, tribe Verticilleae, and the perfect stage is as yet unknown. Since that time several papers have been published dealing with this genus.

At Virginia Polytechnic Institute researches with Beauveria have been carried on by Harrar and Martland (5)(6), and Martland (8). Other work with this genus has been reported by Arnaud (1) in 1927; by Lefebvre (7) in 1931, and by Bartlett and Lefebvre (2) in 1934. Experiments of insect control in the field with Beauveria were reported as partially successful by Beall, et al, in 1937 (3) and 1939 (10).

The entomogenous fungi as a whole have been too inadequately studied to clearly define genera and species, and to establish physiological characteristics which set them apart from other groups

of fungi. Considerable work of a taxonomic nature has been done with the Entomophthorales and the Laboulbeniales, but relatively little is known of those insect parasites found among the Fungi Imperfecti. Research with entomogenous fungi is difficult, embracing as it does the two distantly related biological sciences of mycology and entomology. As a consequence there occurs in literature a confusion of nomenclature, and inadequate descriptions of parasites, their hosts and host ranges, and relatively little work concerning their physiology. Sawyer (9) has reported a series of cultural experiments in which species of Entomophthora were used, and has shown that under optimum conditions these entomogenous fungi are not peculiarly difficult to culture.

After isolating this entomogenous fungus, which according to morphological characteristics was evidently a species of Beauveria, it was desirable to continue the study to settle the question of its specific identity. Particularly in view of the unusual type of fructifications on the parasitized insects, it seemed to be quite probably an undescribed species, or possibly this type of fructification has never been observed before, yet occurring in recognized species of Beauveria.

III. EXPERIMENTAL OBJECTIVES

The first phase of this study, namely, the isolation and preliminary study of this Beauveria, led quite naturally to the second and third phases. A comparative study of host ranges and a comparative study of physiology, of this Beauveria and five other entomogenous fungi, were the next concerns. In these comparative tests of pathogenicity and physiologic characteristics, this Beauveria was compared with a Beauveria isolated from parasitized southern pine bark beetles. The aim was to determine whether these two Beauverias are of the same species or whether they are different species, or different strains of the same species. This attempt was broadened to include four other entomogenous fungi in an effort to determine the physiologic characteristics which set entomogenous fungi apart from other groups of fungi, and to determine whether cultural characteristics of physiology, and pathogenicity have any efficacy in species differentiation.

The classification of fungi to species is a scientific fact only when the description is based on the perfect or sexual stage. Those fungi which are known only in the imperfect stage are classified, on the basis of their morphology in the conidial stage, in the Fungi Imperfecti, a highly artifical and heterogenous group. The imperfect stage is morphologically conservative, shows very few taxonomic features, and any given set of features are not

necessarily coincident with a given set of features of a perfect stage. The true classification of a fungus of which only the conidial stage is known is unpredicable and much less a scientific certainity. Hence, in the differentiation between the two Beauverias, morphology was inadequate.

Where parasites are concerned, pathogenicity may be an aid to taxonomy. Many imperfect fungi bear specific names derived from the host, and separation of species, which morphologically are alike, is recognized when based on parasitism exclusively for different hosts or different groups of hosts. In the case of the two Beauverias, where host range was found to be coextensive and pathogenicity was equal, as will be shown later, parasitism was of no value in differentiating these two fungi.

Since fungi exhibit different needs in nutrition, show differences in their parasitism, and show differential growth rates on common sources of nutrition, it is logical to hypothecate the existance of different physiological characteristics which are the basis of these enumerated nutritional differences. In bacteriology, where morphology characterizes only large groups, and where parasitism is limited, physiology has become the basis of taxonomy. The system is cumbersome but is workable and scientifically sound.

In view of the taxonomic difficulties with the imperfect fungi, in view of difficulties with species identification even where the perfect stage is known, and in view of the usefulness

of cultural characteristics as a basis of taxonomy among bacteria, the projected study of these entomogenous fungi to determine physiologic peculiarities seemed well worth an attempt. The second and third phases of this study were broadened to include more than a concern with the two Beauverias. They included studies with six entomogenous forms with the following three aims: to better differentiate species of fungi, specifically in this case entomogenous forms, by supplementing characteristics of morphology and parasitism with physiology, to characterize entomogenous fungi as a group apart from other fungi in regard to physiologic pecularities, and to correlate specific physiologic types with types or degrees of parasitism.

IV. EXPERIMENTAL RESULTS

Six entomogenous fungi were concerned in these physiologic studies. Included were two species or strains of *Beauveria*, the undetermined mealy bug fungus, and three species of *Entomophthora*. Tentative procedure in these experiments was projected into two phases; insect inoculation experiments to determine host range, and secondly, the study of growth characteristics on culture media. In turn the cultural studies were projected to include the following several phases of physiological tests: growth characteristics on ordinary solid culture media, growth on solid bacteriological differential media, growth in broth of alcohol and acid salts, fermentation of and reaction on sugars, peptinization of albumen and casein, liquefaction of gelatin, hydrogen sulfide production, nitrate reduction, and preliminary studies on oxidation-reduction potentials. Comparison and contrast of physiologic characteristics were to be observed among the species of each group, and between each of the six species and the group as a whole.

Besides the *Beauveria* species isolated from dead insect specimens collected at Mountain Lake, Virginia, another *Beauveria* was available and used in the experiments. The latter was isolated by Martland from the southern pine bark beetle and was the subject of previous research (8)(5)(6). In the course of these experiments it was designated as *Beauveria* A, and the other *Beauveria* was designated as *Beauveria* B. Such designations also

occur in the data which follows. The mealy bug fungus, whose proper taxonomic position is yet unsettled, was tentatively designated as Endosclerotium sp. Three Entomophthora species were available and the species were as follows: Entomophthora apiculata, obtained in culture from the Farlow Herbarium, E. coronata, obtained in culture from the Farlow Herbarium, and E. sphaerosperma which was isolated from the apple leaf hopper and from the pea aphid.

A. Host Range Studies

A number of different species of insects were used in the insect inoculations. In some of the individual experiments only the two Beauveria species were used and compared, while in other experiments all six of the fungi were tested for pathogenicity in a limited comparative host range study. The insects used in the Beauveria inoculations were three species of aphids from willow, the white pine aphid, the common black aphid (from nasturtium) and a species of woolly aphid from the crown of buttercup (Ranunculus sp.) plants. In the more extensive inoculation experiments where all six fungi were concerned the insects used were aphids on wheat plants which were growing in the greenhouse, mealy bugs, larvae of codling moth, larvae of peach moth, and termites.

Moist chambers for these experiments were prepared by the use of filter paper placed in Petri plates. The Petri plates

were first sterilized, then were fitted with three discs of filter paper which were cut so as to exactly fit the bottom plate. The filter paper pad was then moistened and fixed in place on the bottom. On top of this was placed and fitted neatly a single disc of thin black paper. The latter gives a black background and a contrast which permits better observation of the progress of the fungus as it grows out over the insect body.

No attempt was made to maintain sterile conditions in the set up of these moist chambers. The plates were initially sterilized, and as nearly as possible they were protected from contamination during their preparation, during the placement of insects within, and during the duration of the experiment when observations were made or when the plates were moistened. After insects were placed in a Petri plate, and the filter paper pads were properly moistened, they were moistened thereafter at intervals of 48 hours with eight to ten drops of water. The plates were maintained at room temperature, and other than periodic moistening and observation, they needed no attention. Control plates were set up, stocked with insects and given attention parallel to that given the inoculation sets.

1. Inoculations with Beauverias

The six inoculation experiments which were set up to determine hosts of the two Beauverias and to ascertain the existance of any difference in virulence or infectivity of the two

species were started at an adverse season. Six species of aphids were used and they were collected from plants in the field where they occur naturally. The season was late and the insects were rather inactive and were predominantly a mature population. It would have been desirable to make more extensive tests, except for the occurrence of cold temperatures which killed the aphids.

Preliminary attempts at inoculating aphids with Beauveria A and Beauveria B were highly unsatisfactory. The procedure was to place the infested stems or host parts in a clamp with the cut end immersed in water to maintain the host plant in an upright position and in a fresh condition. A similar arrangement was the insertion of the host stem through the hole of a rubber stopper and in turn the insertion of the stopper into a test tube of water to bathe the cut end of the plant. In both cases the insects were dusted with spores by swabbing them with wefts of mycelia. Glass utensils were inverted over the plant sprigs infested with aphids to maintain an elevated humidity.

In every case the aphids soon became restless with the strange conditions indoors and began to wander off the plant. Vaseline proved to be an ineffective barrier. No success was obtained until the insects were collected carefully in position on a portion of the host plant, and the whole carefully placed in the moist chamber whose preparation has been previously described. No attempt was made to collect quantitative data as to

percent of kill. The set up was made to contain about equal numbers of each insect in each of three plates, namely, the control plate, the plate inoculated with Beauveria A, and the plate inoculated with Beauveria B. This set up was used for each of the six aphids in the experiment. The insects were inoculated by swabbing them with wefts of spore-laden mycelia.

The two Beauverias were conclusively pathogenic for only two of the six aphids. They both attacked, with no discernible difference in virulence, the woolly aphid from buttercup and one of the stem aphids of willow. The large black and white aphids collected on white pine were very excitable and soon became entangled in the resin which exuded from the cut pine sprig enclosed with the aphids in the moist chamber. The black aphid collected on leaves of nasturtium did not become infected but gradually died of starvation. The gradual dying off of the population occurred also with two of the three species of aphids from willow. One species was found on willow leaves and appeared to be distinct from the aphids found feeding on the tender portions of willow water shoots. In neither case was a kill obtained, but rather the insect population died gradually, without becoming infected.

On the two aphids which were parasitized, the two Beauverias seemed to attack them with equal virulence and with a very positive and rapid kill. The covering of waxy excretion which occurs on the woolly aphid was too dense to permit

observation of the development of the fungus infection and early external fructification. But fungal growth was easily observed on the aphids infecting the willow. The identification of these two susceptible aphids could not be determined with certainty since the taxonomy of this insect group embraces a knowledge of a full life cycle wherein several complex forms occur. Nor were the four non-susceptible species identified. The susceptible aphids occurred on a low willow about six feet high belonging to an introduced species used in campus plantings. The aphids were clustered solidly on all available space at intervals about one to two feet from the branch tips. They were of medium size and conspicuously a pinkish color. Dr. C. W. Underhill and E. H. Glass of Virginia Polytechnic Institute Experiment Station offered a tentative identification of the species as Melanoxantherum salucus.

The development of infection was rapid and external fungal growth was noted 72 hours after inoculation. The aphids became inactive at this time and with a magnification of 35x the strands of mycelium could be seen at the conjunctivae of the insect body and legs. No spores were observed until about four to five days after inoculation and by the full fifth day, spore heads constructed of phialidae were abundant on the mycelia which occurred in bands or collars at the joints of the body, and more densely at the leg, head and antenna joints. About 50 per cent of the aphid population was dead and the remaining

portion showed signs of in fection and no activity.

The fungus apparently ramifies extensively through the insect body without immediately killing the host. The fungus growth first breaks out of the insect body at the conjunctivae where the exoskeleton is thin. This would seem to indicate mechanical penetration of and exit from the exoskeleton by the fungus. Spores from the dead aphids, and from the dead woolly aphids were inoculated into potato-dextrose agar and gave the typical Beauveria type colony and fructification, thus proving the parasitism of the inoculum from the original culture of each Beauveria. This same set of experiments, involving the inoculation of six species of aphids with the two Beauverias and paralled with control, was repeated. Results obtained and development of parasitism in the duplicate set of experiments were essentially identical to those described for the first set.

2. Inoculations with Six Fungi

In the second series of insect inoculation experiments six fungi were inoculated into five insects. The insects were: codling moth, 10 per plate; peach moth, 10 per plate; mealy bug, 10 per plate; termites, 20 per plate; and wheat aphids, 50 per plate. Seven plates, or Petri plate moist chambers, were prepared with filter paper covered by a disc of black paper, for each insect species. All seven were stocked with the insect, one was held as a control and six were used in which to inoculate the six fungi. All insects were inoculated by dusting them with

fruiting mycelium except in the case of the moth larvae, which were made to crawl over the inoculum.

The moist chambers were moistened at intervals of 24 hours with four to five drops of water, maintained at room temperature, protected as much as possible from contaminants, and periodically observed.

The results in this series were conclusive only in several cases. In Table I which follows, successful parasitism is indicated by the positive sign.

Table I. Insect Inoculation Results.

	Wheat aphid	Mealy bug	Peach moth	Codling moth	Termites
Beauveria A	—	+	++	++	+++
Beauveria B	—	+	++	++	+++
Endosclerotium	—	++	—	—	—
E. apiculata	—	—	—	—	—
E. coronata	—	—	—	+	—
E. sphaerosperma	—	—	—	—	—

The wheat aphids soon left the blades of wheat on which they were collected. Within three days the blades yellowed and became covered with saprophytic fungi. The aphid population gradually died but no evidence of parasitism by any of the six fungi was observed. Results on this species are entirely inconclusive.

None of the Entomophthoras parasitized mealy bugs but

Endosclerotium and the two Beauverias were successful in infection and parasitism. The respective fungi were reisolated from the hosts. Only the two Beauverias successfully parasitized peach moth, codling moth, and termites. Entomophthora coronata however, was observed growing on the termites, and on three of the ten specimens of codling moth. The results with the later fungus are inconclusive and the experiment would have to be repeated in which a large population is used in order to get an accurate percentage of infection.

The peach moth and codling moth larvae were in winter dormancy when secured for the inoculation experiments. They were removed from their cocoon and ten were placed in each plate. Within several hours the larvae became active and had begun to spin new cocoons at intervals around the circumference of the plate in the angle between the side and the bottom. The cocoon was completed in most cases within twelve hours and dormancy was resumed, but at room temperature in this case. The larvae were inoculated in all cases with the six fungi by bodily placement on fruiting cultures of the several fungi, and thus permitting them to crawl about over the mycelium. It was impossible to observe the development of the parasites on the hosts since the larvae were hidden within their cocoons, but at the end of seven days the typical fruiting mycelium of Beauveria had grown through the body wall of the insect and through the web of the cocoon. All plates were inspected at this time for infection by the six

fungi. Besides the two Beauverias only Entomophthora coronata showed infection, and only on three of ten specimens of codling moth. Conidiophores of E. coronata with terminal conidia were arranged palisade fashion on the external body wall of the larvae. They were too short however, to penetrate the thickness of the encasing cocoon.

The most clear cut instance of parasitism and rapid kill was obtained with both Beauverias on termites. The termites, of unidentified species, were gotten from the warmed, south side of a large oak stump during December. In the moist chambers the termites were very active and in some plates were cannibalistic. They were rather hostile to each other and irritable in the light. They cut a few short tunnels in the moistened filter paper, but were more quiet when the plates were darkened with a covering of black paper.

Only four days after inoculation there were evidences of infection and after five days conidia appeared, and all of the insects were dead or unable to move. Each termite became covered with tufts of mycelium growing out at the joints of the body, legs, neck, and antenna in the manner typical of the Beauveria. All of the twenty termites were still living in the control and they were kept alive in the moist chamber for four weeks. Several bodies were found bearing typical Entomophthora conidia in the plate of termites inoculated with E. coronata but the evidence is inconclusive since the growth may not have

been parasitic, but merely a saprophytic growth on several termites killed by cannibalistic combat.

In all of these tests, only Entomophthora coronata of the three Entomophthora species, gave any evidence of parasitism. Endosclerotium gave infection only on the mealy bug, and the two Beauverias were parasitic for but six of the eleven species of insects which were inoculated. In these six cases of infection with Beauveria both attacked all six species, and so far as could be seen, with equal virulence. No distinction between the two species could be made on the basis of differential parasitism.

B. Culturing Experiments

Physiologic studies of the six fungi were made by culturing them on various solid media and broth media. Quantity and quality of growth of each fungus was evaluated on the solid media. These principally contained agar, and included eleven nutrient media and fifteen common bacteriological differential media. In broth cultures a determination was made of the pH reaction of the six fungi on fourteen different sugars, acid salts, alcohols, and simple proteins. Observations were also made on the peptinization of casein, peptone, and albumen, on the liquefaction of gelatin, and on oxidation-reduction potentials as factors in growth rates.

Incidental with these phases of study, tests were made for nitrate reduction and for hydrogen sulphide production.

Observations were made for evidences of mycostatic effects which may have been induced by the differential media, and which may be reflected macroscopically in meager growth, or which may be observed microscopically as a sort of aberrant growth of the mycelium.

1. Solid Culture Media

Table II shows comparative quantitative growth results of the six fungi on eleven solid media, the majority of which are common nutrient media. The positive sign (+) indicated good average quantity of growth of the fungus on any specific medium. The negative sign)(—) indicated less than average quantity of growth, and marked stimulation of growth is indicated by a double plus (+ +).

In evaluating these observations concerning the quantity of growth, potato-dextrose agar was used as a standard for the six fungi as a whole. This medium gave the best culturing results of all solid media when quantity of both the mycelium and conidia, and rapidity of growth were considered collectively. Each fungus shows growth characteristics differing slightly or grossly from any of the other five on any one medium and comparison of growth in both Tables II and II is not a comparison among the six fungi on any one medium. It is a comparison of the quantitative growth of a fungus on a specific medium with the quantity of growth of the same fungus on potato-dextrose. The symbols indicate three degrees of growth, namely, less than

Table II. Growth on Culture Media.

	Beauveria A	Beauveria B	Endo-sclerotium	E. apiculata	coronata	E. sphaerosperma
Egg Yolk	+	+	+	++	++	++
Egg White	−	−	−	−	−	−
Cheese	o	o	o	o	o	o
Butter Agar	−	−	−	−	−	−
Potato-dextrose Agar	+	+	+	+	+	+
Nutrient Agar	−	−	−	−	−	−
Cornmeal Agar	−	−	−	−	−	−
Yeast Extract Agar	−	−	−	−	−	−
Malt Extract Agar	−	−	−	−	−	−
Carrot Agar	+	++	+	−	−	−
Nitrate Agar	−	−	−	−	−	−

average, average, and above average or marked stimulation, where comparison is made to the quantity of growth on potato-dextrose agar.

A number of the agar media were prepared by adding distilled water to the commercially prepared powder form and autoclaving, as specified by the manufacturer. These included potato-dextrose agar, nutrient agar, cornmeal agar, malt extract agar, and nitrate agar. Yeast extract agar was prepared by adding 1.5 per cent plain agar to the yeast extract solution made up of six parts per thousand of distilled water. Butter agar was prepared by adding one gram of butter to 100 cc. of 1.8 per cent plain agar, autoclaving and shaking vigorously immediately before pouring in plates. Carrot agar was prepared by steaming 125 grams of ground carrots in 250 cc. of distilled water for 30 minutes, filtering, adding distilled water to make up to 250 cc. and adding in turn 250 cc. of 3.6 per cent plain agar. These agar media were autoclaved at 15 pounds pressure for 15 minutes and poured in Petri plates.

Cheese used in these experiments was used both plain and sterilized. A small slab was placed in each sterile Petri plate. Egg yolk medium was prepared by homogenizing the yolks for 10 - 15 minutes, pouring in Petri plates, then sterilizing. Sterilization was accomplished by three steam treatments in an Arnold sterilizer at 12 to 16 hour intervals. Each time the temperature was raised initially to 40°C. and then raised 10°C.

per 10 minute intervals until an 80°C. temperature was reached and maintained for 20 minutes. Egg white was prepared by the same procedure used for egg yolk.

In Table II it will be noted that all media except potato-dextrose, egg yolk, and carrot agar gave less than average growth, and no growth occurred on cheese. Growth on potato-dextrose is taken as a standard of average, good growth. The Entomophthora species showed markedly better growth on the egg yolk and for the species of this genus, as a group, this media gave best growth though not the best spore production. Carrot agar gave average growth or more for the two Beauverias and for Endosclerotium.

The quality of growth of each of these fungi on potato-dextrose agar differs from the others and is characteristic in a general way of the fungus on all of the solid media. That is, each fungus has a general growth habit which is characteristic of it on all media and variation therefrom is one of degree rather than kind. The descriptions which follow of each fungus, are more or less characteristic for each on all media.

On potato-dextrose, Beauveria A grows readily, its aerial mycelium is dense and fills about half of the height to the top of the Petri plate, the colony is a beautiful white, and conidia production is abundant. After two weeks the colony dries down to a mass of spores of a dirty white color, occassionally a spot of secondary growth occurs, zonation and guttation are

conspicuous. Rather distinctly, Beauveria B differs in that aerial mycelium is usually not dense and fills the Petri plate to the top. Lobing, and overhanging tufts of mycelium are characteristic, and the colony does not readily dry down but retains its heaped type of growth and its white color. Conidia production is good but less abundant than Beauveria A.

Endosclerotium makes a slow growth on potato-dextrose and all media. The rate of colony spread is least of any of the six fungi. Growth is mainly surface and sub-surface of a rather opaque color in a narrow band around the edge. Small sclerotia are formed progressively outward from the center, and give the characteristic black color to the central portion of the colony. Aerial mycelium is sparse, usually is a mere weft over the surface, and is a dirty gray color.

Entomophthora apiculata spreads rapidly over the surface of the whole plate within three to four days and within the same time abundant conidial production begins. No aerial mycelium is produced. Conidia are shot upward against the Petri plate top, and soon the whole top is covered with conidia. In time the agar surface is covered with a smooth dusty film of spores and hyphal bodies. Entomophthora coronata likewise spreads rapidly, makes no aerial growth, and produces conidia abundantly. Shooting of spores is meager, the colony growth is more "spotted" and conidial production less abundant. Entomophthora sphaerosperma grows only half as rapidly as E. apiculata

and E. coronata, usually shows an opaque thin pad-like growth which is radially fissured. Conidia production is scant, no shooting occurs and after a week or more azygospore production occurs very abundantly.

These characteristic types of growth noted on potato-dextrose agar occurred rather consistently on all media listed in Table II and Table III. The Entomophthoras proved to be most conservative in growth variation and Endosclerotium most diverse. No growth of any fungus occurred on cheese. Butter agar produced in all six fungi only a mere web of growth. Egg white was likewise very unsuitable media and the fungi grew in mere pin-point colonies where the medium was inoculated. According to increasing order of abundance of growth the following six media gave rather typical types, but very meager growth. They are, cornmeal, malt extract, nitrate, yeast extract, carrot and nutrient agars.

On egg yolk plates the Entomophthoras make the best growth, Endosclerotium gives its maximum sclerotia formation and the two Beauverias showed a marked contrast. Beauveria A and B show slow initial growth. Indication of growth by Beauveria B is observed first as a reddish brown coloring which develops at the point of inoculation, then shortly the sparse mycelium appears. Growth is low, spreading at first, and always with the dark narrow zone in advance of the aerial mycelium of the colony. When the plate is practically covered, a heaped aerial growth rapidly

develops, fills the whole plate but bears very few conidia. The reddish brown color fades as the colony spreads and the media surface is thrown into folds which split along their crests. Beauveria A does not show the early initial browning of the media, but develops it later and the color persists. Aerial mycelium is never heavy and dense, and fruiting is sparse. The medium becomes folded and splits occur along the crest of the folds. As the medium dries out, a nodulated cleavage occurs deep in the substrate in the case of both fungi.

The Entomophthora spread at a moderate rate over the medium surface. Furrows develop and progressive wasting away of the egg produces deep folds in the medium. Conidia production is sparse and no evidence of spore shooting were seen. Entomophthora apiculata grew in deep, coarse folds of primary and secondary overhanging ridges. Few conidia were produced and no mycelial overgrowth occurred. E. coronata gave light brown growth, coarsely furrowed, bearing no conidia, and followed by slight mycelial overgrowth. E. sphaerosperma gave a light chalky growth, very finely folded, with no mycelial growth, no conidia, but abundant azygospore production.

Endosclerotium grew rather slowly but produced numerous large, discreet, black sclerotia. Aerial mycelium was very sparse.

2. Differential Media

In Table III is summarized the quantitative growth on fifteen differential media. The quality of growth was generally

Table III. Growth On Differential Media.

	Beauveria A	Beauveria B	Endo-sclerotium	E. apiculata	E. coronata	E. sphaerosperma
Sodium Thioglycollate Agar .1%	++	++	++	++	++	++
" " " .2%	++	++	++	++	++	++
" " " .6%	++	++	++	++	++	++
" " " .9%	++	++	++	++	++	++
Endo's Agar	+	+	−	+	+	+
Purple Lactose Agar	+	+	−	−	−	−
Green Bile Agar	+	+	−	−	−	−
Kliger's Iron Agar	++	++	−	−	−	−
Tryptone Glucose Extract Agar	−	−	−	−	−	−
Eosin-Methylene Blue Agar	−	−	−	−	−	−
Bismuth Sulfite Agar	−	−	+	−	−	−
Triple Sugar Agar	++	++	++	−	−	−
McConkey's Agar	−	−	−	−	−	−
Violet Bile Agar	−	−	−	−	−	−
Lead Acetate Agar	−	−	−	−	−	−

much the same for each fungus as described for each on potato-dextrose agar. Beauveria A produced less heaped aerial growth but more conidia than Beauveria B. Endosclerotium was consistently slow growing. E. apiculata and E. coronata spread rapidly, but spore shooting was not in evidence. E. sphaerosperma grew much slower than the other two species of Entomophthora.

Some notable features, however, were seen which bear comment. The first was that observed on sodium thioglycollate agar, and concerns the marked stimulation of growth which resulted. A commercially prepared broth medium, which contains oxidation-reduction indicators, was used in the specified concentrations to which was added 1.5 per cent plain agar to make a solid medium for pouring plates. The concentration of sodium thioglycollate was .1 per cent. Other media were made in the same manner except that additions of sodium thioglycollate powder were taken to give respectively .2 per cent, .6 per cent, and .9 per cent concentrations. Sodium thioglycollate is a reduced substance and additions of it to media gives a lower O - R potential in the product. Media of this sort are used to culture certain fastidious bacteria.

The results from this series of media having different concentrations of sodium thioglycollate was one of pronounced stimulation for all six fungi. Colony spread was more rapid, growth more abundant, and generally conidia production was much more abundant. At the concentrations of .6 per cent and .9 per

cent the degree of stimulation was less as compared to media containing .1 per cent and .2 per cent concentration of sodium thioglycollate. In the four concentrations there was a stimulation of growth, but no sharper qualitative differences between the six species of entomogenous fungi.

Since this thesis was an attempt to study the unique features of a physiologic nature which may distinguish entomogenous fungi from other fungi, it was of interest to select several representative species of plant parasites and saprophytes and to determine whether they also would exhibit a stimulation of growth on sodium thioglycollate agar. In this comparison, sodium thioglycollate was added to potato-dextrose agar in concentrations of 0.5 per cent, 1.0 per cent, 1.5 per cent and 2.0 per cent and checked with growth on plain potato-dextrose. The media were tubed and inoculated with eleven organisms. These were Beauveria B, Endosclerotium, E. apiculata, Aspergillus clavatus, Fusarium lini, Pleospora sp., Pleurotus corticatus, Pythium sp., Phytophthora cactorum, Sclerotinia libertiana, and the bacterium Phytomonas tumefaciens. The quantitative growth results are indicated in Table IV.

The fungi and the bacterium did not show consistently different growth rates by groups when entomogenous forms are compared with other fungi and the bacterium. The general response by all eleven organisms was a decrease in growth or absence of growth as the concentration of sodium thioglycollate

Table IV. Differential Growth Results On Sodium Thioglycollate Media.

	Potato-dextrose agar	Potato-dextrose agar plus 0.5 % sodium thio-glycollate	Potato-dextrose agar plus 1.0 % sodium thio-glycollate	Potato-dextrose agar plus 1.5 % sodium thio-glycollate	Potato-dextrose agar plus 2.0 % sodium thio-glycollate
Beauveria B	+++ +	+++	++	+	—
Endosclerotium	+ +	+	—	—	—
Entomophthora apiculata	+ + + +	+++ ++	+ + +	++	++
Aspergillus clavatus	+ + + + +	+++ +	+++	++	++
Fusarium lini	+ + + ++	++ ++	+++	+++	++
Pleospora sp.	+ + + +	+++	+++	++	+
Pleurotus corticatus	+ +	+	—	—	—
Pythium sp.	++ + +	+ + +++	—	—	—
Phytophthora cactorum	+++	—	—	—	—
Sclerotinia libertiana	+++	++	+	—	—
Phytomonas tumefaciens	++	+	—	—	—

increased. Only E. apiculata of the insect parasites, and Aspergillus clavatus, Fusarium lini, and Pleospora sp., were able to make growth at the maximum concentration of sodium thioglycollate. All organisms except E. apiculata and Phythium made better growth on the plain potato-dextrose than with any addition of sodium thioglycollate. Possibly clearer species distinction could be gotten to use cultures where the percentage of sodium thioglycollate is increased in a series of but 0.1 per cent interval.

The use of Endos agar gave the growth quantities indicated. Only Endosclerotium made less than average growth. The agar initially was faintly pink but all organisms induced a change to deep crimson, an indication of the formation of acetaldehyde. The fungus mycelium, particularly of the Beauverias, was stained red. Quality of growth of each was typical as previously described.

Only the Beauverias showed growth stimulation on purple lactose agar. Growth types were typical for the three Entomophthora species, and no change was noted in the original purple color of the agar. Endosclerotium produced only aerial mycelium, no sclerotia, and induced no pH reaction. Beauveria B induced but slight lowering of pH and made a symmetrical heaped growth flat on top and deeply sunken in the center. Beauveria A induced a lowering of pH of 1.4 units and the aerial growth soon dried down to a browned mass of spores and mycelium.

Green bile agar was satisfactorily prepared for pouring by adding to the bile medium 1.2 per cent plain agar. The Beauverias made average growth, and each according to type. Endosclerotium scarcely developed and formed a rugose pad-like growth. The Entomophthora species made sparse growth, which appeared to be the usual macroscopic type. But microscopically it was noted, that possibly in the nature of a mycostatic effect, the mycelium did not break apart into hyphal bodies although separation was several fold more frequent, and protoplasm was limited to the tips of hyphae. Progressively the hyphal segments became clear and void.

Kliger's iron agar became reddened, indicating fermentation of dextrose preferentially over lactose, in all cases except with Beauveria species. Beauveria A gave a yellow color and low aerial brownish growth with darkened centers. Beauveria B seemed to attack dextrose preferentially to lactose as indicated by the reddened rather than yellowed medium. Growth was heaped, filled the whole plate and was a cream-white color. Only Endosclerotium produced hydrogen sulphide, for which this medium is a test, as is indicated by a blackened area around the colony.

Growth was less than average but generally typical on tryptone-glucose extract agar. Endosclerotium produced no sclerotia, but gave a pad-like surface growth.

Less than average growth occurred on eosin methylene blue agar, quality was typical for each, and all plates changed

from orange to bluish color, indicating a more oxidized condition than found in the freshly prepared medium.

On triple sugar agar the Entomophthoras gave less than average but typical growth and an alkaline reaction. Endosclerotium was greatly stimulated in growth, both sclerotia and aerial mycelium were abundant and the reaction was alkaline. Beauveria B filled the whole plate, conidia production was sparse and the aerial growth produced a crude type of fasicle in spots against the upper plate. This feature was suggestive of the synnemata on the original insects. Time did not permit the repetition of culturing on this medium to settle this point. The reaction was alkaline. In contrast Beauveria A made a flat compact growth, more brownish colored, and reaction was more acid as indicated by a change of the medium to yellow color. This again would indicate a preferential attack on dextrose by Beauveria B over saccharose or lactose. The reverse as indicated as true of Beauveria A.

Endosclerotium grew very little on MacConkey's agar, sclerotia were produced but aerial mycelium was sparse. Beauveria A exhibited a pink zone around the colony and this would be correlated with a more vigorous attack on lactose, than would be indicated of Beauveria B which lacked this pink zone. Both made a rather low, dense type of growth. The Entomophthoras grew slowly but typically.

Only Endosclerotium indicated hydrogen sulphide pro-

duction on the lead acetate agar, just as this fungus was also the only one which produced hydrogen sulphide on Kliger's iron agar.

Bismuth sulfite and violet bile agars gave less than average growth but growth characteristics were typical.

All of the agar media tabulated in Table III were prepared from the commercial, dehydrated product, according to the directions by the manufacturer. Several slight variations in the procedure of preparing the media have been indicated.

Within the few preceeding pages a brief summary of the six entomogenous fungi has been given as an elaboration upon the qualitative growth of each on the several solid media of two types which are listed in Tables II and III. The data given in the tables are merely of a quantitative nature and were inadequate to set forth the clearest distinction between the six species.

A review of the characteristics recalls a few significant points. The three genera of fungi represented, namely, Beauveria, Endosclerotium, and Entomophthora, showed in their respective members certain growth types which remained essentially the same on all media. The Entomophthoras showed considerable variation in amount of growth on the several media but they showed the least variation in type of growth from medium to medium. E. apiculata and E. coronata are closely similar in their type of growth, and differ appreciably from the growth type exhibited

by E. sphaerosperma. Beauveria A and Beauveria B showed consistent differences in all media. These were differences in mycelium production, conidia production, sugar utilization, and in reaction. Endosclerotium gave the greatest variation in growth type, from medium to medium, of any.

All of the fungi were stimulated greatly by the sodium thioglycollate. None of the fungi gave a nitrate reduction test. Only Endosclerotium produced hydrogen sulphide. A myoostatic effect upon the Entomophthoras was noted with green bile agar.

3. Broth Cultures of Sugars

The cultural studies with the six fungi on broth media of 14 different kinds of sugars were not particularly significant in the case of the Entomophthoras and Endosclerotium, but marked differences were noted between the two Beauverias. In Table V the reaction of the fungi on these media is indicated, as drawn from experiments made in three replications. Endosclerotium and the three species of Entomophthora gave in general reactions at the upper pH range of the indicators used. Since the cultures were initially adjusted to the upper pH value of the indicator range, no change in reaction was observed. Beauveria A and Beauveria B seem to be acid producers on sugars, and acid production by the two is of a differential nature which becomes more marked with time.

The media were prepared in 250 cc. flasks, which had

Table V. Acid Production On Sugars.

	Beauveria A	Beauveria B	Endo-sclerotium	E. apiculata	E. coronata	E. sphaerosperma
Adonitol	++	+	—	—	—	—
Arabinose	++	+	+	—	—	—
Dulcitol	++	+	—	—	—	—
Inositol	—	—	—	—	—	—
Inulin	—	—	—	—	—	—
Lactose	++	+	—	—	—	—
Maltose	++	++	—	—	—	—
Mannitol	++	+	—	—	—	—
Mannose	++	++	—	—	—	++
Rhamnose	—	—	—	—	—	—
Salicin	++	++	—	—	—	—
Sorbitol	++	+	—	—	—	—
Sucrose	++	+	—	—	—	+
Xylose	++	+	++	—	—	—

been cleaned by autoclaving after being filled approximately one-third full of distilled water. Seventy-five hundredth grams of each sugar was added to 150 cc. of distilled water to make a 0.5 per cent sugar broth. Yeast extract was added in 1.0 per cent concentration to supply growth promoting substances. The extract used was decanted from a suspension of one gram of dry yeast in 50 cc. of distilled water, which has been autoclaved three times. In the first replication of broth cultures Brom Cresol Purple was used as the indicator. The range of the indicator, yellow-purple, is from pH 5.2 to pH 6.8. Each medium was tubed in twelve 3/4 x 6 inch tubes, plugged and autoclaved, the final pH was recorded, and the fungi were inoculated in duplicate on the twelve tubes.

The second replication was prepared in the same procedure except that two indicators were used. Six tubes of each media contained brom cresol purple indicator, and six tubes of each contained brom thymol blue, and the inoculations of fungi in each medium and indicator was done singly.

The data in Table V is recorded from results obtained on the broth cultures which consisted of 14 sugars. The object was to determine whether the six fungi might be designated rather clearly as acid producers or non-acid producers on sugars, and to determine whether the fungi of the same genus show differential acid production on a series of sugars. It was the application of bacteriological methods to mycology.

These data, as previously stated, are not particularly significant for all of the fungi inoculated in the 14 sugars. They indicate acid production by the two Beauverias, acid production in a few cases by Endosclerotium and only erratic cases of acid production by the Entomophthora species. The pH initially was adjusted very close to the upper range of the indicators used, so any shift to alkaline conditions, as was probably the case to a greater or less extent with the Entomophthora species, could not be ascertained. To determine the reactions of the non-acid producers, a series of inoculations would have to be made with the pH adjusted initially low, or with an indicator having a higher range.

The data of Table V present the salient results from the culturing on sugars, but thay bear interpretation and elaboration since they have been tabulated not on the basis of one reading but on the basis of two or three readings taken at intervals, and wherein discrepancies exist between consecutive readings. As a legend for Table V, the minus (—) sign indicates no acid production or a change of pH less than 0.2 from the initial pH. The positive (+) sign indicates mild acid production where the initial pH has been lowered from 0.4 to 0.8 units. Double plus (++) indicates acid production to the extent of more than one unit lowering in pH, and in maximum cases indicates a change spreading over the entire indicator range. The table is in no way an indication of the quantity of

mycelium which grew in the broth cultures. All fungi grew readily in the sugars. In most cases the fungi grew as a pad on the surface of the broth and fruited normally. Any exceptions, where the mycelium occurred at the bottom of the tubes or grew suspended in the broth, seemed to have no particular significance since the mycelium in those cases may also occur as a pad at the surface, and microscopically growth was typical except that no fruiting occurred on sub-surface growth.

It will be noted that only E. sphaerosperma of the three Entomophthora species showed any acid production on the 14 sugars. On sucrose the results with this fungus were inconsistent in the three replications. Twice the change in pH was insignificant but in one replication, which appeared to be free of any contamination, the reading was positive. On mannose results were also inconsistent among the three replications. The preferable evidence would indicate that it is a strong acid producer on mannose.

If the two recorded cases of acid production by E. sphaerosperma are correct, a point of significance is the fact that the species in this experiment behaved consistently in that this one species gave results differing from the reactions of E. apiculata and E. coronata. Throughout the experimental work of this thesis, observations have consistently shown E. apiculata and E. coronata to be very similar in physiologic behavior, and as a pair differ from E. sphaerosperma.

Endosclerotium gave reactions which are inconclusive regarding acid production since in this case also the replications and consecutive readings were inconsistent. Moderate acid production occurred on arabinose, and strong acid production sufficient to cover the entire indicator range was observed on xylose. On lactose one replication indicated acid production but more probably the results are negative as shown. On maltose the initial reaction was acid after ten days, but after 20 days and 40 days a shift to alkaline conditions was noted, and alkalinity exceeding the initial pH was indicated in the inoculations where brom thymol blue was the indicator. As a generalization Endosclerotium is a non-acid producer on sugars.

The two Beauverias gave consistent results as acid producers on sugars, and consistently Beauveria A was a stronger acid producer than Beauveria B. Neither induced any color change on inositol, inulin, and rhamnose. With an appropriate indicator range they may be found to induce an alkaline reaction on these sugars, and perhaps to a differential degree. Both were strong acid producers on mannose, maltose, and salicin. It is interesting to note however, that both showed acid production at the first, second, and third readings on maltose, and salicin, but on mannose Beauveria B was negative at the first reading, then more slowly than Beauveria A it became strongly and equally positive.

Both species were negative at the first observation,

after ten days, on adonitol, arabinose, dulcitol, and sorbitol. But after 30 days on these sugars both gave acid reactions in which Beauveria A gave strong reaction and Beauveria B gave feeble reactions. On lactose, mannitol, sucrose, and xylose, Beauveria A was distinctly a stronger acid producer, and was positive with the first reading. Upon second and third reading it had shifted the pH to the lower range of the indicator, and Beauveria B had become positive, although it was negative on the first reading.

From the results of culturing these fungi on sugars, it is clearly possible to separate species or strains by physiologic characteristics where two or more organisms are morphologically and pathologically similar. This observation is true in particular of the acid producing Beauverias and probably would be equally true of the three Entomophthora species if the experiments were designed to gauge differential shift to alkaline conditions in the broth cultures. The results with sugars are essentially consistent with the observations described on solid media.

4. Miscellaneous Broth Cultures

A miscellaneous group of cultural experiments are reported in Table VI. They include the reactions of the six fungi when grown on broth cultures of acid salts, reactions when cultured on alcohol, glycerol, and starch broth media, reactions on and peptinization of protein broth cultures, and liquefaction

Table VI. Growth Reactions on Miscellaneous Broth Cultures.

		Beauveria A	Beauveria B	Endo-sclerotium	E. apiculata	E. coronata	E. sphaerosperma
Reaction On Acid Salts	Citric Acid	−	−	−	−	−	−
	Lactic Acid	−	−	−	−	−	−
	Tartaric Acid	−	−	−	−	−	−
	Uric Acid	+	+	−	−	−	−
Reaction On Miscellaneous Substances	Alcohol	+	+	−	−	−	−
	Glycerol	++	+	−	+	+	+
	Starch	++	+	−	−	−	+
Reaction On Proteins	Albumen	+	++	−	−	−	+
	Casein	−	−	−	−	−	+
	Peptone	+	−	−	−	−	+
Peptinization Of Proteins	Albumen	−	−	−	+	+	−
	Casein	−	−	−	+	+	−
	Peptone	−	−	−	+	+	−
Protein Liquefaction	Gelatin	−	−	−	+++	+++	++

of gelatin in plates. All of these broth media were prepared in the manner and concentrations described in detail for the sugars. That is, each medium was a 0.5 per cent broth to which yeast extract was added in 1.0 per cent concentration, and brom cresol purple and brom thymol blue were used as indicators. Only one replication was made with the acid salts and two replications were made with the other broth cultures. Gelatin was made up in 15 per cent concentration, and neutralized with N/10 NaOH. The resultant medium, of 12 per cent concentration was poured in plates.

It is of interest to note that all fungi grew readily on the acid salts and on alcohol, glycerol, and starch. It would seem that these representative members of entomogenous fungi are rather non-specific in their culture requirements, and are omnivorous saprophytes although in some cases are rather host specific parasites. Growth on these media, consisting of organic acids, alcohols, and a polysaccharide, demonstrate that entomogenous fungi are not limited to vegetation on animal proteins as a characteristic peculiar to them.

The acid salts media were prepared by neutralizing the organic acids with N/100 NaOH to a pH which ranged from 5.4 in the case of lactic acid to 6.8 and 7.4 in the case of the other acids. No change in pH was observed in any case, either more acid or more alkaline, except that the two Beauverias gave a very slight acid reaction on uric acid, but to an equal extent.

Growth on these media was slow and meager in all cases.

Reactions on ethyl alcohol was negative, except that the two Beauverias, consistent as acid producers, gave a very slight lowering of pH. Where brom thymol blue was used, it seems that the Entomophthora species gave an equally slight alkaline reaction on alcohol but the results are inconclusive. On glycerol and starch, the acid producing nature of Beauveria was pronounced, and again with consistency, Beauveria A gave a stronger acid reaction than Beauveria B. The Entomophthora species, equally, gave a very slight acid reaction on glycerol. Only E. sphaerosperma produced acid on starch and but slightly. Except in the case of the two Beauverias the acid reactions were not of a degree to be conclusive nor significant.

The use of proteins in broth culture was of interest to study the metabolism of these entomogenous fungi on substances of the same class as that of the tissues of the host. The results are not all shown in Table VI since with brom thymol blue indicator, it seems sufficiently clear that in several cases the reaction was alkaline. These protein broths were difficult to prepare, especially albumin and casein, even though the soluble dehydrated materials were used. The media were cloudy at the time of inoculation and the positive peptinization reaction was indicated by a clearing of the broth.

The Beauverias both produced acid on albumin, but in this single exceptional case, Beauveria B was the heavier acid

producer. E. sphaerosperma also produced acid, and again behaved differently from E. apiculata and E. coronata, both of which gave alkaline reactions on albumin. Only E. sphaerosperma induced an acid reaction on casein but an alkaline reaction was observed from the other five fungi, with the exception of Endosclerotium. On peptone E. sphaerosperma was the most prominent acid producer of all and Beauveria A gave a slight acid change. The others showed rather slight changes to alkalinity, but the evidence is not conclusive.

The results with the protein cultures are difficult to interpret, and the experiments should be repeated to be conclusive. The difficulty of dissolving the protein powders, and the masking of the indicator colors which comes from the cloudy nature of the media may have led to faulty observations. It is notable that E. sphaerosperma was consistently an acid producer of moderate degree on the proteins and that the other two Entomophthora species were slightly alkaline in reaction. The Beauveria species gave a reversal in the usual order of differential acid production. As a generalization it can be said that these fungi tend to give alkaline reactions on protein broth cultures. Possibly the later observation could be explained as a case of preferential utilization of the acid radical where amino-acid substances are the cultural media.

Peptinization is a phenomenon which occurs when a cloudy protein solution is made clear as a result of organic

or enzymatic action. This phenomenon occurred in all three protein broths, as indicated in Table VI, which were inoculated with E. apiculata and E. coronata, but none of the other four fungi gave this reaction. Initially the broth cultures were cloudy or milky and those remained so which were inoculated with organisms giving negative peptinization results. But within three days of inoculation the solution was noticably clearing, and had reached the maximum translucency by the seventh day, in the case of E. apiculata and E. coronata. Again, in this instance thay behaved differently from E. sphaerosperma, and they seem to produce rather powerful and rapid acting enzymes which solublize the protein media.

Liquefaction of gelatin is a familar bacteriological test of organic metabolism for the production of protein enzymes, specifically gelatinase. In Table VI it is indicated that the three Entomophthora species all induced very rapid liquefaction of gelatin in plates. Liquefaction proceeds ahead of the progressive spread of the fungus colony. In three days from the time of inoculation, it was evident, and by seven days the whole disc of gelatin was liquid, with the exception that the action is somewhat slower in the case of E. sphaerosperma. The two Beauverias and Endosclerotium, grew on the surface of the solid gelatin just as readily, but at parallel intervals of time after inoculation and even after three weeks the medium was quite firm.

The data presented in this thesis have been twice presented in part before scientific bodies, but this is the full body of data and analysis of the data taken in the experiments. The first partial presentation of this work was a demonstration, which in part consisted of a display of the plate cultures herein described, before the Plant Pathology Section of the American Association for the Advancement of Science at Philadelphia on December 29, 1940. The second partial presentation of these data was given before the Virginia Academy of Science in Richmond, May 2, 1941.

V. SUMMARY AND CONCLUSION

In view of the objects set forth in this thesis, and with analysis of the data obtained, a few points in conclusion may be taken as being reasonably true and evident.

1. On the basis of consistently different and respectively characteristic physiologic traits it appears that Beauveria B is distinct from Beauveria A, even though they are essentially similar im morphology and pathogenicity. Whether they are distinct species, or are strains of the same species is unsettled.

2. Physiologic characteristics peculiar to different species of closely related fungi may be used to supplement morphology and pathogenicity as criteria of classification and reidentification.

3. In the limited host range studies, the Beauveria species were most cosmopolitan in parasitism. The Entomophthora species were largely uninfective for the insects used and under the conditions obtained, possibly having been in culture so long as to loose virulence. Endosclerotium was highly specific for a single host, the mealy bug.

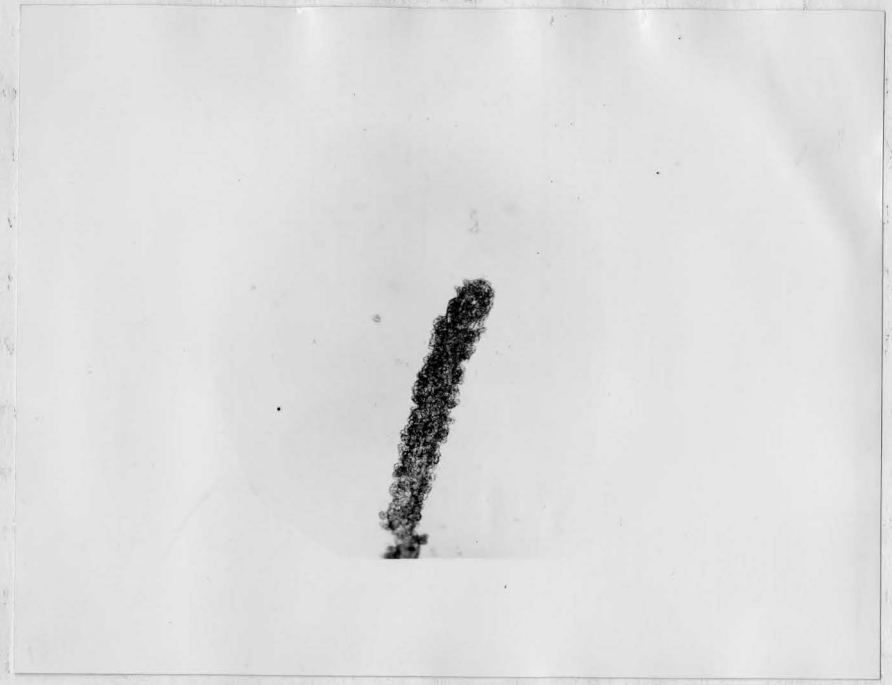
4. In this study of entomogenous fungi, embracing members of several representative groups, it does not appear that they can be characterized as a group apart from other fungi on the basis of physiology. They grow saprophytically on a wide range of some 50 different proteins, sugars, nutrient solid media, and organic acids.

5. In contrasting the physiological characteristics of the slow growing E. sphaerosperma with the other two species of Entomophthora, it can be stated that consistently different physiologic results were correlated with the distinctly different morphology. Further, the near identical physiological behavior by E. apiculata and E. sphaerosperma, paralleled with essentially similar morphology, may prove that these two are one and the same species.

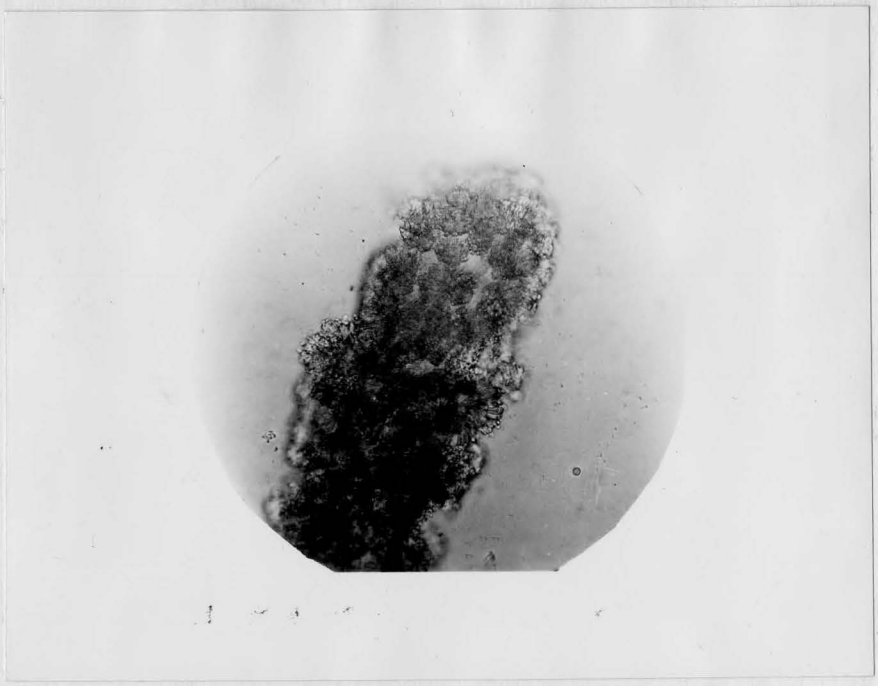
6. In the case of Endosclerotium, it may be observed that slow growth and cultural inflexibility seem to be correlated with extreme host specificity.

7. The liquefaction of gelatin and the peptinization of proteins indicate that the Entomophthora species produce proteinase enzymes in abundance.

PLATE I



Synnema from parasitized insect from which Beauveria B was isolated. x100.



Same as above, showing the conidial clusters at the periphery of the synnema. x400.

PLATE II

Photomicrograph of typical fruiting mycelium of Beauveria

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