COMPARATIVE ONTOGENY OF THELEBOLUS, LASILOBOLUS, AND THECOTHEUS (PEZIZALES, ASCOMYCETES)

By

KENNETH EDWARD CONWAY

A DISSERTATION PRESENTED TO THE GRADUATE COUNCIL OF THE UNIVERSITY OF FLORIDA IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

UNIVERSITY OF FLORIDA
1973
ACKNOWLEDGEMENTS

The guidance, friendship and patience of Dr. James W. Kimbrough throughout this course of study is greatly appreciated. I am also indebted to the entire committee for time spent in critical reading of the dissertation and for their helpful comments and suggestions. Also, I wish to thank my wife, Cynthia, for her love, encouragement, and typing of the dissertation. This dissertation is dedicated to our first child, Kenna Anne, born January 28, 1973.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>ACKNOWLEDGEMENTS</th>
<th>ii</th>
</tr>
</thead>
<tbody>
<tr>
<td>LIST OF FIGURES</td>
<td>v</td>
</tr>
<tr>
<td>ABSTRACT</td>
<td>vi</td>
</tr>
<tr>
<td>INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>MATERIALS AND METHODS</td>
<td>14</td>
</tr>
<tr>
<td>Collection</td>
<td>14</td>
</tr>
<tr>
<td>Spore Shooting</td>
<td>14</td>
</tr>
<tr>
<td>Germination</td>
<td>15</td>
</tr>
<tr>
<td>Single Spore Cultures</td>
<td>16</td>
</tr>
<tr>
<td>Media</td>
<td>16</td>
</tr>
<tr>
<td>Staining</td>
<td>17</td>
</tr>
<tr>
<td>Embedding</td>
<td>19</td>
</tr>
<tr>
<td>Sectioning</td>
<td>22</td>
</tr>
<tr>
<td>THELEBOLUS STERCOREUS</td>
<td>24</td>
</tr>
<tr>
<td>Vegetative Features</td>
<td>24</td>
</tr>
<tr>
<td>Ontogeny</td>
<td>25</td>
</tr>
<tr>
<td>Ascogenous System</td>
<td>26</td>
</tr>
<tr>
<td>Cytology of the Ascus</td>
<td>27</td>
</tr>
<tr>
<td>Discussion</td>
<td>23</td>
</tr>
<tr>
<td>Section</td>
<td>Page</td>
</tr>
<tr>
<td>--------------------------------</td>
<td>------</td>
</tr>
<tr>
<td><strong>Lasiobolus Ciliatus</strong></td>
<td></td>
</tr>
<tr>
<td>Vegetative Features</td>
<td>35</td>
</tr>
<tr>
<td>Ontogeny</td>
<td>36</td>
</tr>
<tr>
<td>Ascogenous System</td>
<td>38</td>
</tr>
<tr>
<td>Cytology of the Ascus</td>
<td>38</td>
</tr>
<tr>
<td>Discussion</td>
<td>40</td>
</tr>
<tr>
<td><strong>Thecotheus</strong></td>
<td></td>
</tr>
<tr>
<td>Thecotheus cinereus</td>
<td>49</td>
</tr>
<tr>
<td>Vegetative Features</td>
<td>49</td>
</tr>
<tr>
<td>Ontogeny</td>
<td>49</td>
</tr>
<tr>
<td>Ascogenous System</td>
<td>50</td>
</tr>
<tr>
<td>Thecotheus pelletieri</td>
<td>51</td>
</tr>
<tr>
<td>Vegetative Features</td>
<td>51</td>
</tr>
<tr>
<td>Imperfect Stage</td>
<td>52</td>
</tr>
<tr>
<td>Discussion</td>
<td>54</td>
</tr>
<tr>
<td><strong>Conclusions</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Literature Cited</strong></td>
<td>91</td>
</tr>
<tr>
<td><strong>Biographical Sketch</strong></td>
<td>97</td>
</tr>
</tbody>
</table>
## LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figures</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-14</td>
<td><em>Thelebolus stercoreus</em></td>
<td>72</td>
</tr>
<tr>
<td>15-25</td>
<td><em>Thelebolus stercoreus</em></td>
<td>74</td>
</tr>
<tr>
<td>26-36</td>
<td><em>Lasiobolus ciliatus</em></td>
<td>76</td>
</tr>
<tr>
<td>37-45</td>
<td><em>Lasiobolus ciliatus</em></td>
<td>78</td>
</tr>
<tr>
<td>46-50</td>
<td><em>Lasiobolus ciliatus</em></td>
<td>80</td>
</tr>
<tr>
<td>57-63</td>
<td><em>Thecotheus cinereus</em></td>
<td>82</td>
</tr>
<tr>
<td>64-69</td>
<td><em>Thecotheus cinereus</em></td>
<td>84</td>
</tr>
<tr>
<td>70-74</td>
<td><em>Thecotheus pelletieri</em></td>
<td>84</td>
</tr>
<tr>
<td>75-84</td>
<td><em>Thecotheus pelletieri</em></td>
<td>86</td>
</tr>
<tr>
<td>85-95</td>
<td><em>Thecotheus pelletieri</em></td>
<td>88</td>
</tr>
<tr>
<td>96</td>
<td>Stalked ascogonium of <em>Coprobia granulata</em> (Kumaria granulata); from Gwynne-Vaughan and Williamson (1930).</td>
<td>90</td>
</tr>
<tr>
<td>97</td>
<td>Stalked ascogonium of <em>Cheilymenia stercorea</em> (Lacnnea stercorea); from Fraser (1907).</td>
<td>90</td>
</tr>
<tr>
<td>98</td>
<td>Diagrammatic cross-section of ascocarp showing the stalked ascogonium of <em>Scutellinia scutellata</em> (Lacnnea scutellata); from Brown (1911).</td>
<td>90</td>
</tr>
<tr>
<td>99</td>
<td>Stalked ascogonium of <em>Lasiobolus monascus</em>; from Kimbrough (1973, in press).</td>
<td>90</td>
</tr>
</tbody>
</table>
Abstract of Dissertation Presented to the Graduate Council of the University of Florida in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy

COMPARATIVE ONTOGENY OF THELEBOLUS, LASIOBOLUS, AND THECOTHEUS (PEZIZALES, ASCOMYCETES)

By

Kenneth Edward Conway

December, 1973

Chairman: James W. Kimbrough
Major Department: Botany

A comparative ontogenetic study was initiated on three genera of coprophilous discomycetes; Thelebolus, Lasiobolus, and Thecotheus. These organisms were chosen because they represented fungi that possess different numbers of asci and ascospores per apothecium. These genera have been placed at one time or another in the family Thelebolaceae. The variability of characteristics for these three genera and others previously reported seems to indicate that the familial limits of Thelebolaceae should be much more restricted.

Thelebolus stercoreus shows an ascocarp ontogeny similar to Trichobolus zukalii. The mycelium of T. stercoreus is uninucleate. Ascogonial initiation begins with an evagination from a parent hypha. No croziers are formed as the single
ascus grows directly from a privileged cell of the ascogonial coil. Nuclear divisions in the ascus have been followed and photographed. _Thelebolus stercoreus_ represents perhaps the highest evolutionary form found in the coprophilous habitat. Its unique structures must limit the family _Thelebolaceae_ to _Thelebolus, Trichobolus_ and perhaps _Ascozonus_ and _Caccobius_. The latter two genera need further study before a familial alliance can be established.

The ontogeny of _Lasiobolus ciliatus_ begins with an evagination from a parent hypha that develops into a multicellular stalked ascgonium. Hyphae from cells below the ascogonium and from surrounding hyphae ensheath the ascogonium. This development is similar to that shown for _Cheilymenia stercorea, Scutellinia scutellata_ and _Coprobia granulata_. Ascogenous hyphal growth is terminated by the development of croziers (pleurorhynque). In the present study ascal cytology has been described and photographed. The mycelium of _L. ciliatus_ is uninucleate. The development of the diagnostic hairs of _Lasiobolus_ is presented. The study of ascocarp ontogeny was facilitated with plastic embedding. It is suggested that _Lasiobolus_ has familial affinities with the _Aleuriaceae_.

Ontogenetic features of _Thecotheus_ were investigated using two species, _Thecotheus cinereus_, an eight-spored species and _Thecotheus pelletieri_, a 32-spored species. The
ascogenous system of *Thecotheus* consists of a series of ascogonial cells similar to that of *Ascobolus citrinus*. The ascogonia give rise to ascogenous hyphae that terminate with the formation of croziers (pleurorhynque). The mycelium of both species is mostly multinucleate. Both species also produce "primordial humps" that consist of bulbous cells that resemble the microconidia of *Ascobolus carbonarius*. This stage precedes ascocarp initiation, and they appear to only function in excipular formation. A sympodial imperfect stage of *T. pelletieri* is described and photographed. Sympodial conidia have also been described for the Aleuriaceae. It is thought until more information is available for *Thecotheus* and other genera, especially in the Aleuriaceae, the best placement is in the Iodophaneae of the Ascobolaceae.
INTRODUCTION

Many mycologists have commented on the "ubiquitous fungi," and this ever-present characteristic is perhaps best illustrated by those fungi that are coprophilous. A world of fascinating and useful organisms is opened for an observer, representing taxa from all major classes of fungi.

Most coprophilous fungi possess a life cycle that includes ingestion by animals. The spores of these fungi are passed through the animal and deposited in the feces. In most cases this passage through the animal seems to be required for spore germination. This habitat specificity and special pre-germination treatment often makes it difficult to germinate, culture and sporulate these organisms in the laboratory.

If one considers the dung substrate as an island in a sea of grass, some problems of dispersal become evident. Many coprophilous organisms have developed certain ecological adaptations which insure the survival of these peculiar organisms. One of these adaptations is the development of a projectile mechanism which hurls the spores from the dung specimen to blades of the surrounding grass where the spores are eaten by grazing herbivores to be "treated" and redispersed.

Another ecological adaptation is the phototropic response. This response is closely correlated with spore
ejection so that maximum distance is obtained by the projectile. Spore ejection usually takes place in the early afternoon coinciding with maximum elevation of the spore-bearing structures.

Perhaps the phycomycetous genus, *Pilobolus*, has been studied the most (Buller, 1934; Ingold, 1965; Page, 1964). It is known that a single multispored sporangium can be projected a considerable distance and with surprising accuracy. This trait is paralleled by other coprophilous forms. Most of these fungi that propel their spores are multispored, a characteristic which better insures the continued survival of the organism since some of the spores discharged from the dung are more likely to reach a suitable substrate.

Other unusual coprophilous forms which appear to have special adaptive features for this habitat include *Sporormia*, *Basidiobolus* and *Sphaerobolus*. In *Sporormia*, a genus of the Sporormiaceae, rather than an increased number of spores per ascus, the eight original spores fragment to produce the multispored ascus. In *Basidiobolus*, a saprobic genus of the Entomophthoraceae, a conidium is propelled by the action of the conidiophore. In culture the projectile mechanism, as well as the phototropic response of the conidiophores, can be observed. The result is a progressive march of the organism across the agar surface as conidia germinate to
produce new conidiophores. Sphaerobolus of the Sphaerobolaceae displays similar tendencies in the Basidiomycetes. The peridiole is surrounded by several layers of cells which reduce desiccation, and each peridiole can be forcibly discharged.

The coprophilous discomycetes show these ecological adaptations plus several additional peculiarities. There is a tendency for the coprophilous forms to have more than eight spores per ascus. Very rarely will one encounter a multi-spored member of the Pezizales outside the coprophilous forms. Ingold (1965) states that the larger spores or spore masses tend to be shot a greater distance and stand a better chance of effective dispersal than smaller ones.

An increased number of spores per ascus is accompanied by decreases in spore size and the number of asci per apothecium. A decrease in the number of asci is also correlated with the angiocarpic development of the ascocarp. Several workers (Kimbrough, 1966b; Kimbrough and Korf, 1967; Eckblad, 1966) have shown that the number of asci per apothecium is variable and is related to ecological conditions, the number of spores per ascus being greater in the uniascal forms. This would seem to indicate that each ascocarp has a certain potential for spore production which may be reached through various combinations in the number of asci and ascospores. Recently, however, cultural work with the genus
Thelebolus (Wicklow and Malloch, 1971) indicates that the reported variability of asci per apothecium is actually the result of several different closely related organisms of the same genus inhabiting the same specimen and that the number of asci per apothecium is not necessarily subject to variation.

Desiccation is another factor which affects the survival of the multispored discomycetes. Single spores in species with eight-spored asci often possess thick pigmented walls which help prevent harmful effects of sunlight and desiccation. The multispored forms eject their spores in a mucilaginous mass. These spores are usually smaller and possess thin walls. The multispored feature may insure the survival of many spores since the inner core of spores is surrounded with an expendable, dead outer layer. The thinner walls of the surviving spores may require less treatment than the thicker walled spores and, therefore, germinate more readily on a suitable substrate.

As these unusual forms were studied and more information gathered by research, mycologists turned to the task of classifying the various coprophilous fungi. These discomycetes were originally placed in the Ascobolaceae. Boudier (1669) saw differences in spore color and using this criterion separated the Ascobolaceae into two groups: Ascobolei Spurii, for the hyaline spored species, and
Ascobolei Genuini for the dark spored forms. Into the Ascobolei Spurii he placed three genera, separating each according to the number of spores per ascus; Ascophanus with eight or sixteen spores, and Ryparobius and Thecotheus, both multispored. Thecotheus possessed thicker walled spores, more cylindrical asci, more elongate paraphyses and larger apothecia than Ryparobius.

Saccardo (1884) recognized differences in apothecial characteristics of Ascophanus and erected the genus Lasiobolus for the setose species.

New insight into the coprophilous discomycetes was offered by Chenantais (1918) when he examined specimens of Ascophanus cinereus (Cr. & Cr.) Boud. and determined that the affinities of this eight-spored species lay within the multispored genus Thecotheus. Until this time the artificial classification, using spore number per ascus established by Boudier, had been used to separate genera. This contribution of Chenantais was later reinforced by the work of Kimbrough (1966b) and Kimbrough and Korf (1967).

Kimbrough and Korf (1967) erected the tribe Theleboleae of the Pezizaceae to include those genera with hyaline, eight- to multispored asci which are non-amyloid, operculate or irregularly dehiscing. Their major emphasis was on the nature of the ascus, ascospores, ascocarps and cultural characteristics. They rejected the taxonomic importance of
the spore number for separation of genera. They maintained that there is close correlation between these emphasized features within each group found in the tribe Theleboleae. The Pseudoascoboleae was abandoned because it was not based on the name of a genus.

Van Brummelen (1967) elevated the tribe Theleboleae to a subfamily Theleboloideae of the Ascobolaceae. He excluded several species included by Kimbrough and Korf in their tribe Theleboleae. Later, Eckblad (1968) and Rifai (1968) raised the subfamily to familial status, Thelebolaceae. Eckblad extended Van Brummelen's characterization of the Theleboloideae to include the inoperculate genera of Kimbrough and Korf (1967) and Thecotheus. His reason for familial status was that a taxon containing both operculate and inoperculate forms should be separated from other exclusively operculate taxa at the familial level.

The investigations of Kimbrough (1966b) and Kimbrough and Korf (1967) have greatly aided the characterization of genera in the Thelebolaceae. Thus far, the main emphasis has been morphology of apothecia, asci and ascospores. Little emphasis has been placed on cytological, developmental and cultural studies.

Recently, the work of Wicklow and Malloch (1971) has helped to explain the many forms of Thelebolus. By culturing various forms of Thelebolus they found that the number of spores
per ascus and the number of asci per apothecium is consistent at the species level. Previous to this, variable numbers of spores and asci had been reported by different authors (Kamlow, 1906; Massee and Salmon, 1901; Kimbrough and Korf, 1967).

At the generic level, however, the phenomena of increased spores per ascus and reduced number of asci per apothecium is very evident in the coprophilous Pezizales. This tendency is present in seven genera of the Thelebolaceae and in one genus of the Pezizaceae (Kimbrough, 1972a). Very little is known of the cytological events that determine whether asci will be eight-spored or multisспорed, and whether the apothecium becomes uniascal or multiascal.

Recently there has been increasing evidence that the Thelebolaceae may not be a natural group due to the heterogeneity of many characteristics (Arpin, 1968; Berthet, 1964a; Kish, 1971; Kimbrough and Korf, 1967; Kimbrough, 1970; Milam, 1971).

The mycelium of the coprophilous discomycetes possesses a variety of nuclear conditions. Berthet (1964a) stated that all Pezizales studied possess a coenocytic mycelium. However, there are some organisms such as Trichobolus (Kimbrough, 1966a) and Coprotus (Kish, 1971) that have been shown to be uninucleate. The excipulum also possesses varying numbers of nuclei. Milam (1971) has shown that the
excipular cells and mycelium of *Loedophanus granulipolaris* Kimbr. are coenocytic. Kimbrough (1966a) has shown that the excipulum of *Trichobolus zukalii* Kimbr. is uninucleate.

The diversity of the coprophilous discomycetes is also illustrated by the morphology of their ascogonia, crozier systems and ascocarp ontogeny. Types of plasmogamy in the coprophilous discomycetes vary greatly. In genera such as *Thelebolus* and *Trichobolus* compatible nuclei are already in the system. In *Lasiobolus*, hyphal fusion occurs, whereas, in *Coprotus lacteus* fusion is the result of an ascogonium and a trichogyne. Ascogonia vary in form from a coil of ascogonial cells such as in *Trichobolus zukalii* to a well formed *Pyronema*-like ascogonium complete with trichogyne as in *Coprotus lacteus* (Ck. & Phill.) Kimbr., Luck-Allen and Cain.

Chadefaud (1943) described several types of systems, including acrorhynque, pleurorhynque, aporhynque, and compose. The acrorhynque consists of a terminal uninucleate cell with a binucleate subterminal cell. The pleurorhynque has the greatest variability. Essentially, it is the typical crozier. The variability depends on whether the terminal cell fuses with the uninucleate cell beneath the subterminal cell or whether it remains free. The aporhynque is a simplification of the pleurorhynque type. In the aporhynque type two nuclei undergo parallel mitoses and two different daughter nuclei
are included in the terminal cell through the formation of a cross wall. No lateral hook is formed. In the compose type a series of binucleate cells is delimited; each cell may be fertile and produce an ascus.

Many difficulties are encountered in the study of the ascogenous systems and are summarized by Berthet (1964a) who outlined types of ascogenous systems known for various ascomycete taxa. From his summary and research it appears that a majority of ascomycetes possess the pleurorhynque system. The next most frequent ascogenous system is the aporhynque type.

The Ascobolaceae, Humariaceae, and Aleuriaceae have representative genera of both the pleurorhynque and aporhynque types. Variability is the rule even in ascogenous systems. Certain species, such as Galactinia ampelina, possess both pleurorhynque and aporhynque systems within the same apothecium.

There are several known examples of ascogenous systems in the Thelebolaceae. Tricnobolus zukalii possesses no ascogenous hypnae or croziers (Kimbrough, 1966a). However, it could be classified as acrorhynque because the ascal mother cell in T. zukalii is produced by septation of the parent hypha resulting in the inclusion of two compatible nuclei into one non-terminal cell. Kish (1971) has shown that Coprotus lacteus possesses a compose type, and Milam (1971)
has shown that *Lodophanus granulipolaris* possesses a true crozier system of the pleurorhynque type. *Lasiobolus ciliatus* is reported by Berthet (1964a) as possessing an aporhynque ascogenous system.

Berthet also reports that in the Ascobolaceae, *Ascobolus carbonarius* has an aporhynque system, while *Pyronema omphalodes* has a fused pleurorhynque system. Genera of the Humariaceae possessing the pleurorhynque system include: *Anthracobia melaloma*, *Cheilymenia aurea*, and *Scutellinia sp.*

Van Brummelen (1967) outlined a system of ascocarp ontogeny according to the maturation and exposure of the hymenium. The heterogeneity of the coprophilous forms is again evident. The hymenium of organisms such as *Thelebolus stercoreus* and *Trichobolus zukalii* is closed throughout its development. Ascocarps of other genera open at various times exposing their hymenia at different stages of development.

Berthet (1964b) and Eckblad (1968) summarized the imperfect stages of the discomycetes. The majority of the genera listed possess an *Oedocephalum* imperfect stage. *Oedocephalum* forms blastoconidia. Operculate genera listed as possessing an imperfect stage were found in the families Pezizaceae, Pyronemaceae and Aleuriaceae.
Paden (1972) has reclassified many of the imperfect stages to fit within the current taxonomy for imperfects. The Pezizaceae now contains imperfect stages that form blastoconidia and alopeconidia. The Pyronemaceae form oidia. The family Otideaceae possesses botrytis-like imperfect stages which are blastoconidia. The imperfects of the Aleuriaceae form sympoduloconidia. Oidia occur in the Ascobolaceae. There are no reports of imperfect stages in the Thelebolaceae.

The presence of an imperfect stage in the coprophilous forms has been considered, until recently, of little importance. The only forms that have been linked to an imperfect stage are _locopanhus carneus_ (Pers. _per_ Pers.) Korf and two species of _Ascobolus_. Korf (1956) and Gamundi and Ranalli (1964) reported an Oedocephalum imperfect stage for _L. carneus_, and Greene (1931) has shown oidia in _Ascobolus_.

The emphasis placed on the systematics of the discomycetes in 1967 (Van Brummelen), 1968 (Eckblad and Hifal) and 1970 (Kimbrough) was re-emphasized at a worldwide symposium in Exeter, England.

In his summary at the symposium Korf (1972b) listed the current methods of study of the discomycetes. Included in the discussion were: microanatomical studies of the apothecia, cultural studies, studies of nuclear numbers, ontogenetic studies of ascocarp development, ascal wall characteristics, and chemotaxonomic and physiotaxonomic studies.
The organisms that I have chosen to investigate show a marked variation in spore number per ascus and in the number of asci per apothecium. From such variations, evolutionary tendencies perhaps can be postulated. *Lasiobolus ciliatus* (Kunze and Schmidt) Boud. represents one end of a line in which eight spores are formed per ascus and a multiascal apothecium is characteristic. *Theoctheus pelletieri* (Cr. & Cr.) Boud. represents an intermediate condition, having a 32-spored ascus with fewer asci per apothecium. *Thelebolus stercoreus* represents perhaps the most advanced coprophilous form, for it has a multispored ascus and a uniascal apothecium.

With regard to current emphasis in discomycete studies, the following information will be included wherever possible: vegetative features, ontogeny of initials, ascogenous system and cytology of the ascus. Information concerning the vegetative features includes: determination of the nuclear condition of the mycelium, mycelial structures such as coils and chlamydospores, rate of growth in cultures, and occurrence of inter-hyphal growth. Ontogeny includes types of plasmogamy, coiling of hyphal initials (archicarp), and significant changes occurring during ascocarp ontogeny. Observations are included concerning the nuclear condition of the ascogonia and the presence, absence, and type of crozier system. Other observations concern the cytology of
the ascus, showing the nuclear divisions leading to spore formation and the nuclear conditions of the spores.

It now appears that the family Thelebolaceae is a very heterogeneous group of organisms with their occurrence on dung being perhaps the only common bond which unites them as a family. Therefore, the purpose of this investigation is to describe the significant features of three species that have been placed, at one time, in the family. The criteria that are developed will then be used to place these species into more natural familial alliances.
MATERIALS AND METHODS

Collection

Collections of the dung of various animals in the Gainesville area were made beginning in January, 1970. These collections were placed in moist chambers to encourage the growth of fungi. Mature apothecia appeared within 1-2 weeks and were picked off the dung. Care was taken to avoid contamination from bacteria and other fungi which are abundant on dung.

Spore Shooting

The apothecia were fastened on the lid of a petri plate with petroleum jelly and inverted over dung agar. The lid was rotated four times at 30-minute intervals, resulting in four groups of discharged spores. Another technique for obtaining pure cultures was to pick an apothecium off the dung sample and put it into a petri plate. A sterile Van Tieghem ring was placed around the apothecium and a sterile coverslip placed on the ring. Released spores were impacted on the coverslip. Agar blocks or coverslips with spores were used in germination and cultural studies.
Germination

In most cases spores, upon germination, were picked from the agar and transferred to dung oatmeal agar plates. Sometimes, however, as in the case of Thecotheus cinereus, the spores would not germinate and additional treatment was required. This consisted of cutting out blocks of agar containing the spores and placing them on sterile slides enclosed in petri plate moist chambers (Riddell, 1950). The blocks were treated with a drop of 2% KOH and covered with a sterile coverslip. Spores thus treated usually germinated within two days. If this treatment was unsuccessful, the KOH treatment was modified by placing the moist chamber in a 60 C oven for a few hours. Blocks with germinated spores were then cut into quarters and transferred to dung oatmeal agar plates for further growth. Slide cultures also provided an excellent opportunity to study spore germination, primordial development and to check for conidial stages.

In mature colonies of Thelebolus stercoreus and Lasiobolus ciliatus, apothecia appeared in 1-2 weeks. Thecotheus required a longer period of time for development in deep dish agar cultures. Slant cultures were kept on dung oatmeal agar.
Single Spore Cultures

Cultures used in determination of mating compatibility were obtained in several ways. One method that was similar to the germination technique was to shoot spores onto dung agar, cut out blocks of agar and place them on a sterile slide. The slide was placed in a moist chamber and the spores allowed to incubate overnight at room temperature. Germinating spores were picked off the block with a fine needle under low power (40X) of a microscope. Macerating a few apothecia in a drop of water and using a sterile loop to streak the drop over the surface of a dung agar plate, was also employed. Again, incubation was overnight at room temperature. The germinating spores were picked from the plate with a fine needle under low power of the microscope. Single spore cultures were then transferred to dung oatmeal slants and plates.

Media

Dung agar was used for primary isolation because it is a better growth medium for coprophilous organisms. It was prepared by soaking approximately 12.5 gm. of rabbit, cow or horse dung overnight in 100 ml. of water. For full extraction the dung was macerated into small pieces and this mixture was filtered through several layers of cheese-cloth. The supernatant was refiltered through a Buchner funnel with the aid of a vacuum pump. The volume was
brought to 500 ml. with the addition of distilled water and Difco agar was added to give a 2% medium. Kanamycin, an antibiotic, was added at first (1:10,000) to reduce bacterial contamination. However, this later was found to be unnecessary.

The growth medium was a dung oatmeal agar, prepared by boiling 10 gm. of oatmeal in 500 ml. of distilled water for five minutes. This preparation was filtered through several layers of cheesecloth and several changes of filter paper. A vacuum pump was again used to accelerate the process because of the stickiness of the oatmeal which clogged the filter paper and reduced the flow of the supernatant. The supernatant was mixed with 500 ml. of dung media to produce a 2% agar. Plates and slants were stored in a refrigerator without apparent loss of nutrient value.

Staining

Several stain techniques were used with varying success. The problems encountered with staining were inability to obtain penetration of the ascus and stain the chromatin material, and the overstaining of the ascus cytoplasm to partially obscure the chromatin.

A modification of the technique used by Tu, Roberts and Kimbrough (1969) to stain Rhizoctonia worked well for staining hyphal nuclei of the organisms under investigation. Material to be stained was fixed in Carnoy's solution for
18-24 hours. This was followed by a washing in 50% acetic acid, hydrolysis in 1N HCl at 65 C for 20-30 minutes and rinsing in acetic acid. The procedure used was to stain with 2% aceto-orcein in 50% acetic acid for 6-8 hours.

Aceto-orcein and aceto-carmine were not successful in staining the ascal nuclei. Other stains employed included: lacto-phenol cotton blue, pentacyl blue-black, Giemsa stain (Furtado, 1970), basic and acid fuchsin and methyl blue in Hoyer's solution.

The stains giving the best results were 2% methyl blue in Hoyer's solution and 1% pentacyl blue-black in water. With both preparations the nucleolus stained intensely and after several minutes the nuclear envelope also began to stain. There was no staining of chromatin material. Lacto-phenol cotton blue stained the nucleolus but to a lesser degree and did not stain the halo around the nucleolus. The aceto-orcein and aceto-carmine stains could be enhanced by soaking blocks of material in FeCl₃ solution.

Fixation overnight with Carnoy's solution was also tried. The longer the fixation time, the better the results. The specimen was hydrated in an alcohol series (95-70-50-30-10-distilled water). Hydrolysis was in 1N HCl for 70 minutes at 60 C followed by a rinse in distilled water for 10 minutes. A mordant was applied containing 4% iron alum for 45 minutes at 60 C, followed by a brief rinse in
distilled water. An apothecium was then crushed and pinched apart on a slide and a drop of aceto-carmine was applied. A coverslip was immediately placed over the apothecium and pressure was applied to further spread the material. The intensity of the stain increased with time. Material thus fixed produced good results. Hot lacto-phenol cotton blue stained the apiculi of Thcotheus cinereus.

**Embedding**

Most staining was used in conjunction with squash mounts. Other techniques also used were sectioning with the cryostat and plastic embedding.

Feder and O'Brien (1968) had much success with plastic embedding using glycol methacrylate with certain higher plant material. This plastic was obtained from Polyscience. The plastic kit from this supplier included a suggested embedding schedule. Blocks of agar containing mature apothecia were fixed overnight with 3% glutaraldehyde in 0.1M veronal buffer at 0°C.

The blocks were transferred without a water rinse to methyl cellosolve. These were kept in a refrigerator and changed twice in 24 hours. They were then transferred to 100% ethanol (4-24 hours), to n-propanol (4-24 hours) and finally to n-butanol. The blocks could then be stored for four weeks.
A new plastic mixture was tried similar to Moore's (1963) plastic used in his work with Ascodesmis. He used a plastic consisting of butyl methacrylate, ethyl methacrylate, and 1.5% Luperco. Ethyl methacrylate was not available; therefore, another plastic was substituted, methyl methacrylate. This is similar to ethyl methacrylate in both molecular weight and density. It is also very slightly soluble in water but soluble in alcohol. The proportions for this plastic were 3 parts butyl methacrylate, 2 parts methyl methacrylate and 1.5% Luperco. This plastic was clear and of a good consistency for sectioning. Initial trials were unsuccessful for embedding due to the addition of a cross linking monomer, ethylene glycol dimethacrylate. The monomer was added to prevent swelling of tissues during the staining process. When this was discontinued, the blocks were very acceptable. Polymerization was carried out in a 50 C oven overnight. The ratio of butyl methacrylate to methyl methacrylate was later changed to 4:2 to obtain a softer block.

Blocks with apothecia were dehydrated in a graded alcohol series and embedded in the final plastic mixture using vacuum infiltration. To accomplish this the blocks were placed in disposable 50 ml. beakers and 15 ml. of solution was used in each change. It was found that 15 ml.
of the final embedding plastic gave an acceptable block. For the final embedding the squares of apothecia would be oriented to any position desired and then placed in the embedding oven. The plastic blocks were popped out of the beaker and small sections were cut out which contained apothecia. These were then glued to a plastic dowel using epoxy glue. The glue was hardened in the 50°C oven. After the glue hardened, the sections were "faced off" using razor blades. The blocks were then ready for sectioning.

There were problems initially in using the Polyscience plastic embedding kit. The difficulties centered around: plastics used, inadequate instructions, and finding a suitable means of hardening the plastic.

Early embedding problems traced their origin to errors in the Polyscience data sheet. Upon a re-examination of the original work of Leduc and Bernhard (1967), we discovered that several errors existed in the data sheet. The original procedure included three embedding mixtures. The initial embedding solution consisted of 60% glycol methacrylate and 20% distilled water. The next change was 97% glycol methacrylate and 3% water. The third mixture was inaccurate, and according to Leduc and Bernhard should have consisted of 7 parts of 97% glycol methacrylate and 3% distilled water plus 3 parts butyl methacrylate with 2% Luperco added. Clouding of the plastic resulted from the inability of Luperco to go into solution. The correct procedure to insure its solubility in the final plastic mixture is to add the
distilled water to the glycol methacrylate, and separately add the Luperco to the butyl methacrylate. Both solutions can then be mixed together. This procedure is necessary as Luperco is only slightly soluble in water.

It was also found that without a U.V. light source at 0°C the prepolymer would not harden. However, the plastic would eventually harden if placed in a 45-50°C oven. If the plastic was poured too thin, bubbles would distort the block. The plastic could be sectioned at 2μ with little damage to the razor blade.

**Sectioning**

Sectioning was accomplished on a manual rotary microtome. The need for ultrathin sections was not anticipated for light microscopy. A razor blade attachment proved unsuccessful as blades rapidly became dull. Disposable blades for the rotary microtome were employed with better success. Sections of 1μ thickness would be obtained and continuous sectioning achieved due to the larger cutting surface. Sectioning was facilitated with a water boat made from paraffin. As the sections were cut they would float on the surface of the water without curling. These sections could then be picked up with an eyelash or camel hair brush and placed on a microscope slide. A drop of water on the slide facilitated the removal of the section from the brush.
Sectioning with the cryostat was done at -20 C. Several settings for section thickness were used ranging from 1-5 μ. Cryostat sectioning was not as successful as had been expected. The mounting medium interfered with staining and sections had a tendency to fall apart.
THELEBOLUS STERCOREUS

Thelebolus stercoreus Tode per Fr. occurs on most types of dung. It was first isolated from rabbit dung. The apothecia appear white on the dung and are immersed to semi-immersed. The spores germinated readily on dung oatmeal agar.

Vegetative Features

Pure colonies grew quite rapidly, expanding to a diameter of 2.5 cm. in five days. Mature apothecia were present on the plates within two weeks. Fruiting occurred synchronously around the margin of growth, producing consecutive rings of apothecia.

Apothecia occurring in culture were more orange colored than that observed on dung. Apothecial size was similar to that occurring naturally, 100-250 μ in diameter. Apothecia remained closed until spore ejection at which time the top of the ascocarp was ripped open by the expanding ascus.

Single haploid spore cultures were easily obtained by picking single spores from a mass of spores streaked over the surface of the agar. Spores would germinate within 24-48 hours. Fruiting occurred within two weeks. The formation of mature apothecia indicated that T. stercoreus is homothallic. This supports the findings of Cooke and Barr (1964).
Olive cultures were used to study vegetative and reproductive features. The vegetative mycelium of *T. stercoreus* is uninucleate (Fig. 1). Ramlow (1906) reported and illustrated (Fig. 33, l.c.) uninucleate hyphae in *T. stercoreus*. My own observations of the vegetative mycelium in this species confirm his findings. The vegetative nuclei stained best with aceto-orcein. Hyphal coils (Fig. 2) were prevalent on the surface of the agar. Anastomoses between the hyphae of the coil were frequently seen, allowing perhaps for the exchange of nuclei. Vegetative sphaeroid terminal swellings of the hyphae were frequently observed (Figs. 3, 4) similar to those found by Berthet (1966) in *Trichophaea* sp. Their function in *Thelebolus* is unknown. The swellings measured 9-10 μ in diameter, were hyaline, and contained many oil droplets.

**Ontogeny**

Ascocarp initiation begins when a hypha gives rise to a side branch. This branch is very slender at first (1-2 μ) but enlarges in diameter to produce a swollen blunt tip 5-6 μ in diameter. The branch may reach a length of 30-40 μ, the tip becoming circinate (Fig. 5). This apical curving usually ensnares another hypha (Fig. 6). The original hypha now becomes septate and each cell becomes swollen. These cells continue to divide to form a mass of cells surrounding
the inner hypha (Fig. 7) and eventually form the sterile excipulum in which the cells are of a textura angularis (Fig. 8).

The sheathing cells continue to divide to form a rounded mass of cells. Many hyphae radiate from the developing ascocarp on the surface of the agar (Fig. 9). A closer inspection of these hyphae (Fig. 10) shows that they fuse to the outer layer of cells of the ascocarp. Anastomoses are frequently seen between these hyphae.

**Ascogenous System**

As the ascocarp enlarges the centrum becomes very active as is evident by the concentration of nuclei in this region (Fig. 11). Sections through the middle of the ascocarp show an ascogenous system surrounded by the exciuplar cells. One of the cells of the ascogenous system contains compatible nuclei (Fig. 12). This cell is always intercalary. No croziers are formed. Kimbrough (1966a) has shown the manner of attachment of the ascus of *Trichobolus* where there are two small cells at the bottom of the ascus which would indicate that the privileged cell would be the subterminal cell of the ascogonium. The same system is also present in *Thelebolus*. The privileged cell begins to enlarge (Fig. 13) and stains in methyl blue in Hoyer's solution. When the ascocarp is sectioned (Fig. 14),
it appears that the layer of cells adjacent to the ascus adheres and conforms to its expanding shape. Many cells are crushed in the process of ascus enlargement. However, this may be the stage of initiation of paraphyses. As the ascus enlarges, the peripheral cells continue to add cells to the outside. The ascus may now occupy one-third of the entire mass.

**Cytology of the Ascus**

When the ascus is carefully squeezed out from the ascocarp at this point, the $2N$ nucleus becomes evident (Fig. 15). The nucleus is approximately $5 \mu$ in diameter with a large prominent nucleolus.

During the first division of meiosis the nucleolus disappears. In the diplotene-diakinesis stage there appear to be six bivalents (Fig. 16). Division I takes place on an axis perpendicular to the long axis of the ascus (Fig. 17).

Division II is accomplished almost synchronously and parallel to the long axis of the ascus (Figs. 18, 19). After division II the four nuclei lie in the center of the ascus (Fig. 20). Subsequent divisions produce the many spores typical of *Thelebolus* (Figs. 21, 22). It has been estimated that as many as 1000-2500 spores will finally fill the ascus (Cooke and Barr, 1964; Kimbrough, 1966b).

By this time the ascus has increased in size filling most of the cleistothecium-like apothecium (Fig. 23), the
ascus approaching the point of spore discharge. It ruptures the cleistothecium-like apothecium (Fig. 24) and the ascal tip becomes somewhat extruded due to the thinner wall in that region. Kimbrough (1966b) attributes this thinner wall to a discontinuing and abrupt thickening of the next to the outside wall layer (Fig. 24). The layering of the ascal walls is very prominent at higher magnifications. The spores are then released as pressure from below the ascus forces the spore mass to split or tear the ascal tip. When the ascus is young and has not fully expanded, paraphyses are present (Fig. 25). These paraphyses are very slender, measuring approximately 2 µ in diameter. They may be branched and are usually held in close contact to the ascus.

Discussion

Thelebolus stercoreus is an excellent organism to work with. It is easily grown in culture and fruits prolifically. Cultures for this investigation were grown on dung oatmeal agar. However, the species has been reported to grow on potato dextrose agar and to fruit on a modified Leonian's agar (Cooke and Barr, 1964).

The vegetative mycelium of T. stercoreus presents many structures that need explanation. It is known that T. stercoreus possesses the compatible nuclei necessary to fruit in a single spore culture (Kimbrough, 1966b). If one
looks at the vegetative mycelium and its structures in light of nuclear migration and compatibility, several possibilities appear. One of the most prevalent superficial structures present were hyphal coils. Anastomoses occurred between cells of different layers of the coil and could provide the opportunity needed for exchange of compatible nuclei.

The swellings found in the vegetative phase of \textit{T. stercoreus} appear similar to structures found by Berthet (1960) for \textit{Trichophaea confusa} (Cooke) Berthet (Pezizeae). Berthet refers to these structures as apparent conidia and has seen them germinate. No germination of these structures was observed in the present study.

Gordon's (1964) work on the centrum development of \textit{Diporotheca} has some interesting implications when applied to \textit{Thelebolus}. Gordon found that there was no crozier system formed in \textit{Diporotheca}, but that the penultimate cell of an ascogonial initial divides and surrounds the terminal cell. These cells then send out trichogynes or receptive hypnae which fuse with other vegetative hyphae. Gordon then contends that compatible nuclei migrate through these receptive hyphae to form the heterokaryotic condition in the lower part of the centrum, from which the asci ultimately develop.
In this study of *Thelebolus* one of the striking features of the developing ascocarp was the radiating of the hyphae from the peripheral cells. It was often apparent that these radiating hyphae anastomosed. However, their role as receptive hyphae is very doubtful as the compatible nuclei are already paired in the ascogenous system before the hyphae begin to radiate. Rather than radiating, they appear to add new hyphal elements to the developing excipulum.

Gordon also mentioned that the developing ascus may fuse with the inner cells of the excipulum and incorporate their material into the ascus. The formation of the ascus in *Thelebolus* would make a good electron microscope study to determine if the young ascus crushes or envelops the excipular cells and paraphyses.

A transfer of compatible nuclei may also occur when a hypha branches from the vegetative mycelium and curves and coils itself around another hypha. The curved hypha becomes septate and ensheaths the second hypha with cells. Compatible nuclei may pass into the ascogonium (inner hypha) by fusion with these peripheral cells. However, this stage may be analogous to the stage in *Trichobolus* (Kimbrough, 1966a) when hyphae from the parent hypha ensheath the ascogonium without fusion occurring. In *Thelebolus*, as in *Trichobolus*, the compatible nuclei appear to be present in
the parent hypha and to flow into the ascogonium prior to septation of the latter. No ascogenous hyphae or croziers are formed. However, the ascogenous system could be classified as acrorhynque in Chadeau's (1943) system.

The development of ascogonia in T. stercorarius is similar to that shown by Schweizer (1923) for Ascobolus citrinus ( = Ascobolus michaudii Boud., Van Brummen, 1967). The ascogonium is a privileged cell that has developed from an evagination of the parent hypha.

The exact phylogenetic position of Thelebolus has long been a controversy. Thelebolus was first erected as a genus by Tode (1790) and was originally placed in the Gasteromycetes (Order Angiogastres). Fuckel (1870) was the first to consider Thelebolus as an Ascomycete, placing it in the Perisporiaceae of the Pyrenomycetes. Saccardo (1888) transferred it to the Nidulariaceae. Earlier Zukal (1886), having studied the development of T. stercorarius, placed it close to Podosphaera in the Erysiphaceae.

Boudier (1869) recognized multispored taxa in the Ascobolii spurii, but did not consider Thelebolus. Heimerl (1889) expanded Boudier's concept, however, to include Thelebolus. He was the first mycologist to consider Thelebolus a discomycete. Brefeld (1891) studied the entire life cycle and regarded Thelebolus as a single genus in the
Thelebolaceae of the Carpohehmiacei. Saccardo (1892) later established two infrageneric categories, Eu-Thelebolus and Tricnobolus, which were reaffirmed by Kimbrough (1966b). Schroeter (1893) considered Thelebolus a member of the Ascoboleae, family Pezizaceae.

Cooke and Barr (1964) in their comparative study of Thelebolus used Saccobolus depauperatus (Berk. & Br.) Rehm and the mature structures of Podosphaera and Sphaerotheca of the Erysiphaceae. They concluded that Thelebolus possessed a greater number of characteristics similar to the Erysiphaceae and recommended that Thelebolus be placed back into the Erysiphaceae.

In a more recent work (Dennis, 1968) Thelebolus is treated in the Pseudoascoboleae. Kimbrough and Korf (1967) in their work concluded that the name Pseudoascoboleae was invalid and proposed a name change to Theleboleae based on the genus Thelebolus.

The question of the final disposition of Thelebolus seems to revolve around several important points. Included among these are the nature of the ascocarp and the morphology of certain parts of the ascocarp. According to Cooke and Barr, T. stercoreus possesses a completely enclosed perithecium and lacks paraphyses. Kimbrough (1966b) rejects these points by concluding that T. stercoreus is actually a reduced discomycete and does contain paraphyses. His
discussion (1966a) of the disposition of *Trichobolus* would also apply to *Thelebolus*. Kimbrough points out that if *Thelebolus* is considered in light of other multispored genera, its characteristics are more truly aligned with Pseudoascoboleae and indicate that it is a reduced discomycete.

It has been shown in the present study that the development of *Thelebolus* is similar to the development of *Trichobolus* and corresponds to Hamlow's (1906) earlier work with a few exceptions. The ascogenous system is similar to that of the Erysiphaceae in which a privileged cell becomes the ascal mother cell. No ascogenous hyphae and croziers are formed. Therefore, according to Cooke and Barr one might deduce the same conclusion. However, if as Kimbrough (1966b) suggests, one compares this development to species of *Ryparobius* Boud. and other multispored forms of the Pseudoascoboleae, one must conclude that *Thelebolus* is in the discomycetes. He also points out the difference in ascal structure between *Thelebolus* and the Erysiphaceae. Also, if one considers the nature of growth (parasitic vs. saprobic), spore morphology, spore release, and possession of paraphyses, it can be concluded that *Thelebolus* should indeed remain in the discomycetes. That the unique ascal feature of *Thelebolus stercoreus* is found in several taxa
with variable number of asci per ascocarp and spores per ascus further reinforces this argument. If Ingold's (1965) hypothesis is correct that there is a selection toward larger projectiles in spore release for coprophilous fungi, then *T. stercoreus* would represent the end-point of evolution in this genus.

Kimbrough and Korf (1967) validated the tribe Theleboleae based on the criteria that the genera possessed iodine negative asci, elliptical spores and showed a tendency to be both eight-spored and multispored. *Thelebolus* is the type genus for the family. Most work has concerned the uniascal, multispored species *T. stercoreus*. However, other species do exist that show multiascal tendencies with lesser spore numbers. If *T. stercoreus* is the end of the evolutionary line on the dung substrate, its family alliance should be based on characteristics of the eight-spored species. The ascal structure for all the species of *Thelebolus* is remarkably stable. However, other features like pigment and shape of paraphyses may differ in the other forms that are evolutionarily closer to their terrestrial or lignicolous counterpart. Based on the material presented here and the work of Kimbrough (1966a) for *Trichobolus*, it appears that the family Thelebolaceae must be much more restricted.
Lasiobolus ciliatus (Kunze and Schmidt per Pers.) Boud. is found on a variety of dung in the Gainesville area, including that of cow, horse and rabbit. Cultures used in this investigation were isolated from rabbit dung occurring in the northwest area of Gainesville.

Excellent growth of Lasiobolus was achieved on dung oatmeal agar. Fruiting occurred within two weeks from the time of initial isolation. Colony growth was rapid, covering a diameter of 6 cm. in five days. Fruiting was abundant, with approximately 9,600 apothecia produced per plate. Lasiobolus is an excellent organism to work with due to its rapid growth and fruiting ability.

Vegetative Features

Most of the vegetative features occurred below the surface of the agar and as the isolate became older, subsequent transfers resulted in the development of the apothecia below the surface. Lasiobolus ciliatus is an eight-spored species having an operculum at the top of the ascus. The spores are inconsistently uniseriate in the ascus. Single haploid spore cultures developed apothecia, which proved the species is homothallic.
The spores of *Lasiobolus* in culture are similar in size to those occurring in nature: 15-25 x 10-12 μ. The spore germinates at or near one end (Fig. 26). The germ tube branches almost immediately following its exit from the spore. Further branching of the tubes occurs after a period of growth. A cross wall delimits the germ tube from the spore.

The mycelium is multicellular and each cell is uninucleate (Fig. 27). Another aspect of the vegetative mycelium is the occurrence of interhyphal growth (Fig. 28).

**Ontogeny**

Ascogonial initiation is peculiar to *Lasiobolus*. Activity begins when cells in a hyphal tip swell. Protuberances evaginate from each cell (Fig. 29). These elongate into a hook so that the protuberance from the terminal cell grows toward the protuberance from the subterminal cell (Fig. 30). Nuclei can be seen near the tips of the protuberances. Fusion of these protuberances was observed (Fig. 29). As fusion continues the ascogonia take on a highly coiled appearance (Fig. 31). This process seems to attract other hyphae which grow toward the coil and entwine themselves around it (Fig. 32). Other ensheathing hyphae may grow from the same parent hyphae (Fig. 33). The ensheathing hyphae then divide and produce a mass of cells around the ascogonial initials.
After a period of growth an expanding ascogonium appears within the mass of cells (Fig. 34). Additional ascogonia are produced by other cells of the parent hyphae developing protuberances and fusing with adjacent cells. Usually three ascogonial cells can be observed within the expanding mass of cells (Fig. 35).

As the archicarp develops, other hyphae from the parent hyphae grow out and fuse with the mass of cells (Fig. 36). The apothecium has long non-septate setae (Fig. 37) which arise from the developing apothecium when the mass of cells is only 140 μ in diameter (Fig. 38). They arise from the base of the mass of cells (Fig. 39).

As the apothecium continues to increase in size, more setae arise near the initial growth (Fig. 40). The setae double their length rapidly (Fig. 41). At maturity the setae have the characteristic appearance typical of Lasiobolus. The non-septate setae root in the ectal excipulum and possess a bulbous base (Fig. 42). Concurrently numerous hyphae radiate from the base to form the rhizoidal hyphae that anchor the apothecium to the substrate. The difference between rhizoidal hyphae and setae is in the thickness of the cell walls. At maturity the base of the apothecium possesses dozens of "rooting" hyphae (Fig. 43).
Plastic embedding was effective in the study of ascocarp development. Plastic sections showed that the excipulum encloses the ascogenous system until sporogenesis is completed (Fig. 44). Lasiobolus develops angiocarpically or is cleistohymenial, opening in the late mesohymenial phase (Van Brummelen, 1967). Paraphyses outgrow the developing asci and converge to an area at the top of the ascocarp; at this point the ascocarp will eventually separate to expose the asci (Fig. 45). Stages of meiosis in the asci (Fig. 46) were observed in several sections.

**Ascogenous System**

The ascogonia within the young apothecium continue to develop and the multinucleate condition becomes more evident, especially when the ascogonia are teased from the mass of surrounding cells (Fig. 47). Ascogenous hyphae begin to grow out from the ascogonia. The ascogenous hyphae become highly branched as croziers are formed, terminating growth (Fig. 48). Numerous observations of croziers were made (Figs. 48, 49).

**Cytology of the Ascus**

After the croziers are formed and the nuclei of the penultimate cell unite, the 2N nucleus migrates into the expanding ascus. The nucleus is rather large, approximately 6 μ in diameter, with a centrally prominent nucleolus (Fig. 50). The 2N nucleus undergoes meiosis. The first division
takes place parallel to the long axis of the ascus (Fig. 51). The nucleolus disappears during each division, but reappears again. The second division is asynchronous; one nucleus divides and the daughter nuclei take an extreme position in the ascus through a division parallel to the long axis of the ascus. Nuclei now occupy the top and bottom positions in the ascus.

The second nucleus now divides in a plane more or less perpendicular to the long axis. At this stage there is a pause in division and the four nucleoli reappear (Fig. 52). Division begins again asynchronously and occurs first in the daughter nuclei in the upper end of the ascus (Fig. 53). Finally all nuclei divide and the resultant haploid nuclei move to the positions along the length of the ascus (Fig. 54). The young ascus is usually thicker above and narrower below, so that the majority of the nuclei reside in the upper portion of the ascus. Sometimes inconsistencies occur in spore cleavage and two nuclei are incorporated into one spore (Fig. 55).

The immature spore wall is thick, but becomes thinner with the age of the spore. The wall prevents the entrance of stain into the spore, and it is, therefore, necessary to break the exospore to stain the single nucleus (Fig. 56).
Discussion

*Lasiobolus ciliatus* is a useful organism in several aspects: cultural, ontogenetical and cytological. The organism is easily cultured and fruits abundantly in younger cultures. As the isolate grew older, subsequent transfers to new media fruited less. It is not known whether this phenomenon is a result of some intrinsic factor in the fungus or perhaps reflects a nutrient deficiency of dung oatmeal agar. Studies of coprophilous fungi would benefit by the establishment of a defined growth medium.

Plastic sections were most useful in the study of ascocarp development. Plastic embedding retains the shape of the ascocarp and its vegetative features much better than free hand or cryostat sections. It was desirous that fixation and embedding of these fungi for light microscopy could be extended to electron microscopy. *Lasiobolus* appears to be more suitable to plastic embedding than *Thelebolus*, because of the better penetration of the plastic into the ascus.

*Lasiobolus ciliatus* possesses a uninucleate mycelium and has a unique ascogonial ontogeny. In the genus *Thelebolus*, compatible nuclei are already in the evagination of the parent hypha. However, in *Lasiobolus* a mechanism is necessary in order for compatible nuclei to pair.
Several cells of the parent hypha swell and protuberances are formed which grow out from each cell and fuse with the adjacent cell. Proliferation of the protuberances and the fusion of additional vegetative hyphae soon ensheath the developing ascogonia. Kimbrough (personal communication) has also found this same type of development in a recently discovered species of Lasiobolus: *Lasiobolus monascus* Kimbr. (1972b) (Fig. 99).

The ascogonial development of *L. ciliatus* is similar to the type shown by Blackman and Fraser (1906), and Gwynne-Vaughan and Williamson (1930) for *Humaria granulata* (Fig. 96) which is now classified as *Coprobvia granulata* (Bull. ex Fr.) Boud. The development of the ascogonia in *C. granulata* is characterized by the development of a stalked ascogonium which is multinucleate. The ascogonium is seen being enveloped by hyphae which originate from sub-terminal cells. A similar sheathing of the ascogonia occurs in *L. ciliatus*; however, in the latter species, several ascogonial cells develop rather than one.

Fraser (1907) has shown a stalked ascogonial ontogeny in *Lachnea stercorea* Pers. (= *Cheilymenia stercorea* Pers.) (Fig. 97). The terminal cell becomes the ascogonium and a wide trichogyne is formed. Fraser mentions an antheridium which had the appearance of a sac filled with nuclei.
However, its functional role is questioned as ascogonous hyphae are formed before fusion of the trichogyne with the antheridium.

A similar development was observed by Brown (1911) in Lachnea scutellata (=Scutellinia scutellata) (Fig. 98). The ascogonium was produced on a 7-9 celled stalk. However, no trichogyne or antheridium was observed.

Fraser (1913) studied Lachnea creta (=Tricharia creta (Cke) Eckblad) and illustrated an ontogeny similar to that shown by Milam (1971) for Iodophanus granulipolaris. The ascogonium is a series of bulbous cells with a long trichogyne produced terminally. No fusion with an antheridium was observed. This type of development with a long trichogyne is found in Anthracobia melaloma by Cwynne-Vaughan (1937) (as Lachnea melaloma) and Rosinski (1956). It is also typical of many species of Ascobolus.

Recently, Durand (1970) studied the development of a species of Lasiobolus identified as Lasiobolus equinus (Mull.) Karst., which may be synonymous with L. ciliatus. The development of the ascogonia is similar to that observed in the present investigation. However, the initial stages as described by Durand are similar to Ascodesmis nigricans (Gäumann and Dodge, 1928). It is most difficult to interpret the beginning stages of ascogonial initiation, but
Durand's drawings can also be interpreted as showing the same type of development as that found in the current investigation. Durand could not determine definitely the species of the fungus he used. Therefore, two conclusions can be drawn from his work. If the species is the same as *L. ciliatus*, the development corresponds closely. A more important aspect, however, is that if his fungus represents another species of *Lasiobolus*, then this would indicate that the development of the ascogonia is consistent in at least three species of the genus.

Recently, related genera have been studied developmentally. Milam (1971) studied *Iodophanus* (Pezizaceae) and has shown a multinucleate ascogonial coil which is ensheathed by hyphae from the parent hypha. This development is similar to *Ascobolus carbonarius* (Dodge, 1912) and has some similarities to the development of *C. granulata*. Eckblad (1966) states that *Coprobia* and *Iodophanus* have close affinities and includes both in his family Pyronemaceae.

Kish (1971) followed the development of *Coprotus lacteus* (Ck. & Phill.) Kimbr., a species with trichogynous ascogonia and described the ascogenous system as similar to that of *Pyronema*. He proposed transferring *Coprotus* to the Pyronemaceae.

Berthet (1964a) in his summary of the ascogenous systems of the discomycetes lists *Lasiobolus ciliatus* as
possessing the aporhynque type of ascogenous hyphae. This is in contradiction to the results presented in this study. *Lasiobolus ciliatus* has true croziers and the ascogenous hyphae are of the pleurorhynque type.

*Lasiobolus* differs slightly from *Thelebolus stercoreus* in ascocarp development. *Lasiobolus*, a multiascal species, opens in the late mesohymenial phase as spores mature. *Thelebolus*, the uniascal form, opens in the telohymenial phase; during spore liberation. *L. ciliatus* also differs developmentally from *T. stercoreus* by the presence of croziers. *Lasiobolus ciliatus* also has an operculum at the tip of the ascus, and several ascogonial cells per apothecium.

Species of *Lasiobolus* were classified as members of the genus *Ascophanus* by Boudier (1869) when he split the genera of the Ascobolaceae into the Ascobolei Genuini and Ascobolei Spurii. Included in the latter hyaline-spored group were: *Ascophanus* with eight-spored asci, *Thecotheus* and *Ryparobius* with multispored asci.

Saccardo (1884) separated *Lasiobolus* from *Ascophanus* by virtue of its setose apothecia. Kimbrough and Korf (1967) further delimited *Lasiobolus* by emphasizing the non-septate nature of the setae. This was done to differentiate it from *Trichobolus*, a multisporied, uniascal form with septate setae.
The nature of the ascus is also stressed, Lasiobolus possessing cylindric, operculate asci, while Trichobolus possesses an ovate irregularly dehiscing ascus. Kimbrough (1972a) has shown that basic ascal wall structure differs also in these taxa even though in both genera there is tremendous variation in the size of the ascus and number of spores.

Although the taxonomy of the genus Lasiobolus has been relatively free of controversy, the opposite is true of the species under investigation. The controversy centers around four species: L. papillatus (Pers. per Pers.) Sacc., L. equinus (Müll. per Pers.) Karst., L. pilosus Fr., and L. ciliatus (Schmidt ex Fr.) Boud. All have been placed, at one time or another, in synonymy under each other. No comparative study has determined if they are the same fungus. However, a search of the literature reveals the following facts.

The name Lasiobolus papillatus (Pers. per Pers.) Sacc. is used by Seaver (1928) who states in his book that L. papillatus is the type for the genus. However, the holotype no longer exists. In the description of the fungus by Boudier (1869) it was noted that his specimen had septate hairs. His concept of L. papillatus must, therefore, be rejected, in keeping with the current description of Lasiobolus, which has non-septate hairs. L. papillatus sensu Boudier may well represent an eight-spored Trichobolus.
The name *Lasiobolus equinus* (Müll. *per* Pers.) Karst. is used by Kimbrough and Korf (1967) and Seaver (1928). Seaver places *L. ciliatus* in synonymy under *L. equinus*. The name *L. equinus* was not validated by Fries but by Sowerby (1821) in Gray's *Natural Arrangement of British Plants* as *Peziza equina*. This name would not have priority over either of the two remaining names.

The name *Lasiobolus pilosus* Fr. was validated by Fries (Systema Mycologicum volume 2 on page 164). This name has been used by Van Brummelen (1967) and Eckblad (1968). Eckblad correctly places *L. equinus* in synonymy under *L. pilosus* because of priority. He makes no mention of *L. ciliatus*. Van Brummelen states that *L. pilosus* is a highly variable species.

The name *Lasiobolus ciliatus* (Schmidt *ex* Fr.) Boud. has been used by Dennis (1968) and Rifai (1968). Van Brummelen (1967) discussed the validity of the names; however, because he had not seen *L. ciliatus*, he did not include it in synonymy under *L. pilosus*. Rifai in his discussion of the species states that Persoon (1822) revalidated the name as *Ascobolus ciliatus* before Fries (1822) validated *Ascobolus pilosus*. The point being; what is the correct starting point for nomenclature? Is it the date January 1, 1821 or is it names validated by Fries?
If one assumes that the date is the starting point, Persoon's name is valid; however, if it is Fries' publication, then *L. ciliatus* would be rejected.

One point overlooked is that Fries also validated *Ascobolus* (*Lasiobolus*) *ciliatus* in his work in 1822 (also in volume 2, page 164). *Ascobolus ciliatus* appears before *A. pilosus* and if both are the same fungus, then the name *Lasiobolus ciliatus* (Schmidt *ex* Fr.) Pers. has priority.

The differences that exist between *Thelebolus* and *Lasiobolus* make it necessary to remove *Lasiobolus* from the *Thelebolaceae*. Although *Lasiobolus* shows the same evolutionary trends as does *Thelebolus* on dung (Kimbrough, 1972a) with uniascal and multispored forms, its development and ascal structure take priority. The ascogonial development of *Lasiobolus ciliatus* and *L. monascus* may give clues to their taxonomic disposition. *Lasiobolus* may represent an evolutionary excursion onto dung from terrestrial species similar to *Scutellinia*, *Cheilymenia*, *Coprobia* or some intermediate genus. It is felt that *L. ciliatus* would be best placed in the *Aleuriaceae* (Arpin, 1968). Arpin's criterion for inclusion into this family was the possession of both gamma and beta carotenoid pigments. *Lasiobolus* has not been shown to possess carotenoid pigments; however, as Nannfeldt (1972) has suggested, can we be sure that the
species is really devoid of carotenoids? Is it not possible to assume that these are present in the shape of colorless precursors or colorless derivatives?

_Lasiobolus ciliatus_ could also be placed in Rifai's family _Humariaceae_ tribe _Ciliarieae_ along with _Scutellinia_, _Cheilymenia_ and _Coprobia_ according to ontogeny. _Anthracobia_ of the _Aleurieae_ and _Tricharia_ of the _Lachneae_ have an ontogeny similar to _Iodophanus_. 
Theotheus cinereus (Cr. & Cr.) Chenantais

Vegetative Features

Cultures of *T. cinereus* were received from Dr. J. W. Paden, who had isolated the fungus from dung in British Columbia. The cultures were not derived from single spores and after a month of growth in deep dish cultures, several fruit bodies were discovered. The early stages of fruiting were sectioned and they consisted of a mat of bulbous cells (Fig. 57). These patches of cells were designated as "primordial humps" as they may function as part of an ascogonial-antheridial apparatus.

The mycelium is mostly multinucleate, although many individual cells may be uninucleate. Intehyphal growth occurred in culture. The first stage in fruiting is an intertwining of the vegetative mycelium. Coiling continues as a mass of intertwining hyphae is produced. This coiling takes place underneath the primordial humps.

Ontogeny

Within this mass certain cells begin to swell and form ascogonial cells (Fig. 58). Compatible nuclei were assumed to enter the ascus mother cells before septation. A branching ascogenous system then develops from the ascogonia. *Theotheus* may have several ascogonial cells per apothecium.
**Ascogenous System**

As the ascogenous system is developing, the cell layers surrounding the centrum continue to proliferate forming a well defined medullary and ectal excipulum (Fig. 59). The ectal excipulum is composed of bulbous cells (Fig. 60). These bulbous cells appear to be the same as those of the primordial humps. The emerging ascogenous system pushes the cells upward and to the side where they form the excipulum. These bulbous cells are uninucleate (Fig. 61); however, the hyphae that join the cells to the base of the apothecium are multinucleate (Fig. 62).

The centrum, meanwhile, becomes densely nucleated as the branching ascogenous system pushes upward through the paraphyses (Fig. 63). Finally, after a period of longitudinal growth, croziers are formed (Fig. 64), and the penultimate cell enlarges forming the ascus. Compatible nuclei fuse and a large diploid nucleus (diameter 10-11 μ) is formed (Fig. 65). The nuclear envelope is observable as a halo surrounding the densely stained nucleolus.

Meiosis and ascosporogenesis were not observed due to the scarcity of material. Mature ascospores were present, and it was observed that the wall is rather thick and smooth at first (Fig. 66), and apiculi develop at the ends of the spores (Fig. 67). Also the spores may become ornamented
(Fig. 60). The apiculi stain best with hot lacto-phenol cotton blue and this improves the longer the specimen is in the stain. The spores of *T. cinereus* are uninucleate and are irregularly uniseriate in the ascus (Fig. 69).

**Thecotheus pelletieri (Cr. & Cr.) Boud.**

**Vegetative Features**

A single haploid spore culture of *T. pelletieri* was obtained by methods previously described. Actively growing cultures were maintained for a month or more in deep dish petri plates. The culture formed primordial humps. Bulbous cells similar to those found in *T. cinereus* were present (Fig. 70); however, apothecia failed to develop.

Interhyphal growth was evident in cultures of *T. pelletieri* (Fig. 72). Also present were vegetative mycelial loops (Fig. 71). The mycelium is septate, approximately 3-3.5 μ in diameter and is mostly multinucleate (Fig. 71). Nuclei divided in the hyphae in the loop and also in the hyphae entering the loop. The hyphae radiating from the loop produced small side branches (Fig. 73). These small branches were similar to conidiophore initials. Once the loops were formed, the main hypha from the loop gave rise to several radiating hyphae. These hyphae radiated rapidly and branched. Some of the branches appeared to fuse with other hyphae (Fig. 74). The function of the loops is unknown.
Fresh material from dung showed that the diploid nucleus of *T. pelletieri* is relatively large (10 x 11 μ), and occupies the central portion of the ascus (Fig. 75). The nucleolus is also large (5-6 μ), and stains deeply with methyl blue in Hoyer's solution. The nucleus is evident only as an envelope surrounding the nucleolus. The ascus enlarges to almost its full size while containing the diploid nucleus.

Due to the lack of fruiting in culture, cytological stages of meiosis and ascosporogenesis could not be obtained. Asci from dung specimens were seen that contained several nuclei (Fig. 76), but no division stages were observed. After the last division a spore wall is formed around each nucleus. The wall is initially very thick (Fig. 77). The spores are uninucleate (Fig. 78). The ascus is broadly clavate and the spores are ejected in a gelatinous mass (Fig. 79). The spores germinate from one end after swelling to approximately twice their normal size (Fig. 80). The ascocarp develops angiocarpically; however, the phase in which the hymenium is exposed could not be determined.

**Imperfect Stage**

Both *T. pelletieri* and *T. cinereus* produce an imperfect stage in culture, conidial production being more abundant in *T. pelletieri*. A few weeks after transferring *T. pelletieri* to dung oatmeal agar, conidia were observed. A similar imperfect stage was also discovered in cultures and slants of *T. cinereus*. 
The conidia of *T. pelletieri* are hyaline and borne on short side branches, 15-22 μ long (Figs. 85-95). The hyaline conidiophores measure 4 μ wide at their base and taper to a width of 2 μ at their tip. The conidiophores begin as small evaginations on a hypha (Fig. 81). The small protuberance elongates and a side branch may bud out from it (Fig. 82) later developing a hooked appendage (Fig. 83). In many cases the conidiophore develops this hooked appendage from its base. The function of the hook is unknown. The mycelium is superficial.

The conidia are 3-4 x 6.5-7 μ. They appear to be uninucleate (Fig. 90). Division of a nucleus takes place near the tip of the conidiophore (Fig. 84), and the upper nucleus migrates into the conidium. The primary conidium seems to be blown out from the tip of the conidiophore giving the conidium an abovate appearance (Figs. 85-87). A cross wall delimits the conidium from the conidiophore (Fig. 88). The tip of the conidiophore appears to be "meristematic" and continues to increase in length just to the side of the primary conidium (Fig. 89). A secondary conidium is then blown out (Figs. 90-93), and becomes delimited (Fig. 94). This process continues so that finally as many as ten conidia are produced per conidiophore (Fig. 95).
During the period of conidial formation, the conidiophore increases in length. The distance between succeeding conidia, however, is very short and gives the mature conidiophore a swollen appearance.

Discussion

Although a thorough investigation of the two species of \textit{Thecotheus} was not possible due to their slow and scanty growth in culture, several contributions to our understanding of their ontogeny can be made. Overton (1906) was unable to germinate the spores of \textit{T. pelletieri} and relied on an abundant supply of natural material for his research. He noted that \textit{T. pelletieri} was a late fruiting organism in regard to its succession on dung. His source of material was old and partly dried-up cultures of horse dung. In the present investigation the dung specimen was almost four weeks old, and this may be one reason that this organism is not encountered more frequently.

\textit{Thecotheus pelletieri} was obtained in a single spore culture during this study. Although several attempts were made to induce apothecia to form, none were successful. It is possible that \textit{T. pelletieri} is heterothallic, the absence of compatible nuclei making fruiting impossible.

\textit{Thecotheus cinereus} did fruit sparsely during the course of this study. However, this was not a single spore
culture. Dr. Paden simply poured agar over the dung and isolated the fungus as it grew up through the agar, but could not obtain fruiting in culture.

The primordial humps may represent the end of the vegetative phase of the fungus, fruiting being delayed only until karyogamy of compatible nuclei. Good evidence is presented that these primordial humps form a part of the ectal excipulum of the Thecotheus apothecium. Kimbrough (1969) describes and illustrates the bulbous cyanophilous cells of the excipulum in Thecotheus. In his developmental study, all species of Thecotheus show angiocarpic development on dung. This supports the assumption that the primordial humps are the early developmental stage of the apothecium and excipulum of Thecotheus, and that the emerging ascogenous system pushes up through these humps.

Bulbous cells that function in excipular formation may be found in other genera. Squash mounts of apothecia of other genera may obliterate or alter their presence. This may have occurred in Ascobolus albidus (Van Brummelen, 1967, Plate 5, Fig. F-1), a species that is shown to possess paraphyses with subglobular elements.

The bulbous cells of the primordial humps are also very similar to the microconidia found in Ascobolus carbonarius (Dodge, 1912). In A. carbonarius a conidium fuses with the
trichogyne and provides the ascogonium with a compatible male nucleus. The role of the bulbous cells in *Thecotheus*, however, was not determined.

The presence of primordial humps in both species of *Thecotheus* suggests a close relationship between the two. It may also indicate a similar ascogonial ontogeny for both species. However, this must be confirmed by further studies. There appears to be little difference between the development of the two species, except for the additional mitoses in order to produce 32 spores in *T. pelletieri*.

The mycelial loops found on the surface of the agar may act as a mechanism that initiates the conidial system since branches from the loop appear similar to the conidiophore initials.

The imperfect stages not only possess a similar morphology but also a similar ontogeny. These similarities confirm a close relationship between the perfect stages of the eight- or 32-spored species. However, this may not be as factual as it appears because other perfect stages as those in *Nectria* may have several different imperfect stages. Also, Kimbrough and Korf (1967), in listing characteristics of importance that should be considered in distinguishing genera of the Theleboleae, place the use of imperfect stages on a low priority. However, as more imperfect stages are induced or discovered this feature may be of great use in establishing natural relationships.
The type of conidial ontogeny found is very similar to Hughes' Section II (1953) which he labels "terminis spore." The formation of the initial conidium itself is a blasto-conidial type. More recently Kenarick and Cole (1968) have described this type of development as a sympodial conidium. Barron (1968) in his book on soil hyphomycetes would classify this specimen in his section sympodulospore. The imperfect stage seems to have affinities with Sporothrix (Hekten and Perkins) Barron and Rhinotrichella Arnaud. In Sporothrix the conidia are borne on denticles in an acropetal succession. They have no septation and are truncate at the attachment to the conidiophore. The head of the conidiophore, however, appears broader than the imperfect stage of Thecotheus.

Oberwinkler, Casagrande and Müller (1967) have found an imperfect stage of Ascocorticium anomalum (Ell. & Harkn.) Earle which is morphologically similar to that of Thecotheus. The conidiophore has a hook which closely resembles the hook in the imperfect stage of Thecotheus. They describe the conidial ontogeny as sympodial with the conidiophore elongating to form a zig-zag appearance. The base of the conidiophore is brown with the conidiogenous portion hyaline. The conidia are ellipsoid to round (1.5-2.5 u). In discussing the classification of the imperfect stage, Oberwinkler
et al. say that it resembles Tsubaki's (1958) *Chlorochium minutum*, but that since Hughes (1958) places *C. minutum* in synonymy under *Bisporomyces*, it cannot be placed there. Instead, they suggest a close relationship with *Rhinotrichella* Arnaud (1953). However, they would not name the species until more work has been done on the imperfect stage. Likewise, until more work is accomplished with this imperfect stage of *T. pelletieri*, a genus cannot be assigned.

Several other researchers have discovered imperfect stages in allied families. Greene (1931), working with the *Ascobolaceae*, observed oidia in cultures of *Ascobolus*. The oidia were formed by the separation of rectangular cells of the aerial mycelium. These oidia were abundant in cultures that failed to produce apothecia. This was also observed in *Thecotheus* as conidial production was greater in *T. pelletieri* which did not fruit. The oidia of *Ascobolus* germinated but produced no fertile apothecia.

Paden (1967) found a conidial stage of *Peziza brunneacatra* Desm. Conidia of *P. brunneacatra* are similar to the bulbous cells found in the primordial humps of *Thecotheus* (Figs. 1, 2). The bulbous cells measured 7-9 x 13-16 u, approximately the same size as the conidial stage of *P. brunneacatra*. Germination of these bulbous cells was not observed and their function seems limited to excipular formation.
Tubaki (1950) described an Odeocephalum imperfect stage for Iodophanus testaceus (Moug. in Fr.) Korf. This is similar to the imperfect stage of Thecotheus except for a more inflated apical apex of the conidiophore. An Odeocephalum conidial apparatus was reported for Pyronema omphalodes (Bull.) Fuckel (Hughes, 1953). Odeocephalum is placed in Hughes' Section Ib.

The occurrence of a sympodial conidial type in Thecotheus is significant. A review of the literature dealing with imperfect stages (Berthet, 1964b; Eckblad, 1968), indicates that the occurrence of the sympodial conidial type is rare.

Most of the imperfect stages listed occur in the form genus Odeocephalum. This genus is included in Section Ib of Hughes' (1953) classification, characterized by blastoconidia. This imperfect stage is found in three operculate families: Pezizaceae, Pyronemaceae and Aleuriaceae.

Oidia are found frequently. Hughes placed in Section VII those fungi that produce oidia. Oidia are now designated by other workers (Barron, 1968; Tubaki, 1958) as arthroconidia. Oidia are found in three operculate families: Ascobolaceae, Pezizaceae and Pyronemaceae.

No imperfect stage has been found in either the Otideaceae or the Rhizinaceae.
Sympodial conidia have been found in two families: Morchellaceae and Sarcoscyphaceae. These conidia are found in Section II of Hughes.

There are thirty-two species of operculate discomycetes known to possess an imperfect stage, of these twenty-six possess either an Oedocephalum or oidial conidia. Two genera have a Botrytis imperfect stage (Section Ib) and another possesses an Ostracoderma imperfect stage (Section Ib). Therefore, only three genera possess a sympodial conidia imperfect stage.

In a more recent work, Paden (1972) has re-evaluated conidial formation of the imperfect state of the Pezizales. Four families, Sarcoscyphaceae, Sarcosomataceae, Aleuriaceae and the Morchellaceae possess sympoduloconidia. Cookina sulcipes, C. tricholoma, Pithya cupressina and Sarcoscypha coccinea (Sarcoscyphaceae) have sympoduloconidia that could not be placed into a known genus. Desmazierella acicola (Sarcosomataceae) has a Verticicladium imperfect stage and Urnula craterium, Plectania nannfeldtii, and Sarcosoma latahensis have Conoplea imperfect stages. Two species of Geopyxis (Aleuriaceae) have Nodulisporium imperfect stages and Caloscypha fulgens has a sympoduloconidial stage that could not be placed into a known genus. Morchella (Morchellaceae) has a Costantinella imperfect stage.
The Pezizaceae are reported to possess imperfect stages representing three different genera. Peziza petersii and Iodophanus are associated with an Odeocephalum stage, four species are associated with an Ostracoderma stage and three species possess aleuricioconidia. Botrytis-like conidia are found in the Otideaceae.

The Ascobolaceae possess oidia and a Papulaspora stage. No conidia are reported for the Thelebolaceae.

Other imperfect stages have been found in inoperculate families including, Orbiliaceae, Hyaloscyphaceae, Helotiaceae, Geoglossaceae and Sclerotiniaceae.

Overton's (1906) observation of ascogonia was confirmed. The ascogonial cells of T. cinereus observed were like those found in Ascobolus citrinus (Schweizer, 1923) as Overton illustrated for T. pelletieri. Ascus formation is initiated by crozier formation.

Thecotheus was first described in the Ascobolaceae by Boudier (1869) as a hyaline, multisспорed species of the Ascobolei Spurii. It was separated from Ryparobius, the other multisспорed form, by its larger thick walled spores, and its larger fruiting body. Chenantais (1918) later transferred the eight-spored Ascobolus cinereus Cr. & Cr. to Thecotheus.
Kimbrough and Korf (1967) removed *Thecotheus* and *Iodophanus* from the Thelebolaceae (Pseudoascoboleae) and placed both in the tribe Pezizeae of the Pezizaceae. Both genera were considered similar due to the diffuse amyloid reaction of their asci and their callose-pectic marking on the spores. Eckblad (1968) retained *Thecotheus* in the *Thelebolaceae* because of its simpler excipular structure, its multisспорed tendency, and its protruding asci. He placed *Iodophanus* in the more primitive family Pyronemaceae. Eckblad based this decision on the presence of carotenoid paraphyses and simpler excipular structure. Kimbrough (1969) further typified the entire genus with a morphological and cytochemical study of four species of *Thecotheus*.

Milam (1971) studied *Iodophanus carneus* (Pers. ex Fr.) Korf both developmentally and structurally. Results from his research and from the present study point to some of the differences between the two genera. *Iodophanus* has coenocytic excipular cells and an _Oedocephalum_ imperfect stage. *Thecotheus* has a sympodial conidial type and uninucleate excipular cells. Milam also showed that the ontogeny of *I. granulipolaris* consisted of a multicellular archicarp with a long trichogyne. This type of development has also been reported by Fraser (1913) for *Lachnea creta* (=*Anthracobia* sp). *Anthracobia* is now placed in the Aleuriaceae by Arpin (1968).
Pfister (1972) has recently described several members of the genus *Thecotheus* that occur on wood. A correlation of their ontogeny, cytology and their possible imperfect stages would be most significant. The finding of these terrestrial species reinforces the theory that the coprophilous species represent an evolutionary and ecological excursion from some terrestrial species. Many features of *Thecotheus* appear similar to members of the Ascobolaceae: diffusely amyloid asci, thick-walled ascospores, bulbous excipular cells (at least in *Ascobolus albidus*), and a perisporic sheath that encrusts the mature spore. In *Ascobolus* a characteristic pigment is obvious in its outer spore wall. However, Kimbrough (personal communication) has noted that when the spores of *Ascobolus* are bleached, there are cyanophilic markings on the inner walls of the spore similar to *Thecotheus*. Another feature in common with the Ascobolaceae is the similarity of the bulbous cells in the primordial humps of *Thecotheus* and the microconidia of *Ascobolus carbonarius*.

Korf (1972a) has placed both *Iodophanus* and *Thecotheus* in the Iodophageae of the Ascobolaceae. For the moment this appear to be the best placement for *Thecotheus*. However, as more is learned about the ontogeny and excipular cells of *Thecotheus* a relation with the Aleuriaeae may be possible
due to the possession of sympodial conidia. It is recommended that other mycologists working with genera in the Aleuriaceae note such features as bulbous cells in the excipulum and also the presence of imperfect stages which may show relationships to Thecotheus. Until these studies are performed, a conclusive family alliance is not possible.
CONCLUSIONS

It has long been recognized by many authorities (Kimbrough, 1966b; Kimbrough and Korf, 1967; Eckblad, 1968; Rifai, 1968) that the family Thelebolaceae represents a unique and diversified group of fungi. The purpose of this research was to compare the ontogeny of representatives of three genera that have been at one time classified as members of this group. These genera were chosen because they represented fungi with varying number of asci per apothecium and varying number of spores per ascus.

Contrary to reports by Berthet (1964a) and Eckblad (1968) that the mycelium of the Pezizales is coenocytic, that of Lasiobolus ciliatus and Thelebolus stercoraceus is uninucleate. Thecotheus pelletieri and T. cinereus are highly variable. The mycelium of each is mostly coenocytic; however, the large cyanophilous bulbous cells that comprise the excipulum of both genera are uninucleate.

The initiation of the ascogonia differs in all three genera. Thelebolus stercoraceus has been shown to have an ontogeny similar to Trichobolus zukalii. Ascogonia are initiated by evaginations from the parent hypha which contains the compatible nuclei.
Lasiobolus ciliatus possesses an ontogeny similar to that found in Coprobia granulata (Blackman and Fraser, 1906; Gwynne-Vaughan and Williamson, 1930); a stalked ascogonium. Essentially, the parent hypha swells and evaginations grow from these swollen cells. These evaginations fuse, and it is thought that through these fusions compatible nuclei are introduced into the same cells. Ensheathing hypha then grow from the parent hypha, and also from other vegetative hyphae and enclose the ascogonia.

Lasiobolus ciliatus has an ontogeny similar to Coprobia. Pigment analyses may show a relationship between these fungi and other related genera such as Scutellinia. The finding of a multispored, uniascal species of Lasiobolus has completed the evolutionary line within this genus. However, the major comparison with the genus Lasiobolus to terrestrial species will have to be determined by L. ciliatus. This, according to Ingold (1965), would represent more closely the ancestral type, as the uniascal form would possess the more highly evolved mechanisms associated with a coprophilous environment.

The presence of ectal hairs and an ontogeny similar to Coprobia granulata indicate a close relationship to the Aleuriaceae (Arpin, 1968). Analysis of carotenoid pigments will be necessary to determine if L. ciliatus has the same biochemistry as Coprobia, Scutellinia and Cheilymenia.
These genera possess \( \gamma \)-carotene as their major pigment. Rifai (1960) also recognizes these genera as being closely related and includes them in the tribe Ciliareae of the Humariaceae. A further correlation is that they, along with \( L. \) ciliatus, possess stalked ascogonia. Rifai also erected the tribes Lachneae, Aleurieae, and Otideae. It is known that Tricharia (Lachneae) and Anthracobia (Aleurieae) possess ascogonia similar to Iodophanus (Milan, 1971) and Ascobolus (Dodge, 1912). Ascogonial formation in the Otideae has not been studied. Therefore, the closest relationship appears to exist between \( L. \) ciliatus and the genera of the Ciliareae.

Thecotheus cinereus has an ontogeny that appears similar to Ascobolus citrinus. This species possesses a group of inflated ascogonial cells which are soon enclosed by enveloping hyphae. The occurrence of bulbous cells in primordial anemata raises the possibility that these cells may play a role in the passage of compatible nuclei by fusion with a trichogyne or some similar structure. It has been shown that these bulbous cells are uninucleate and ultimately form part of the ectal excipulum.

An imperfect stage has been found in association with Thecotheus pelletieri and T. cinereus. The imperfect stage is classified as a sympodial conidium resembling Rhinotrichella and Sporothrix. Conidial stages have been found in association with only two other closely related
operculate genera that occur on dung, *Iodophanus* and *Ascobolus*. The sympodial imperfect stage of *Thecotheus* differs developmentally from the other genera, which are arthroconidial or blastoconidial. Sympodial conidia have been found only in the Morchellaceae, Aleuriaceae, Sarcosomataceae and Sarcosyphaceae of the Pezizales, and are, therefore, unique for this group. The presence of imperfect stages may play an important role as more work is done in this area.

The use of plastic sections has been useful in following ascocarp development. *Thelebolus stercoreus* is cleistohymenial because the hymenium is not exposed until spore release. *Lasiobolus ciliatus* is angiocarpic with the hymenium opening in the late mesohymenial stage, as the spores mature. *Thecotheus cinereus* is also angiocarpic; however, it could not be determined when the hymenium becomes exposed.

Much of the recent research by Wicklow and Malloch (1971), Durand (1970), Kish (1971), Milam (1971) and now this study points to the great diversity of features found in the coprophilous discomycetes. Kish has suggested that the affinities of *Coprotus* may lie with the Pyronemaceae. *Iodophanus* has been placed in the Pezizaceae by Kimbrough and Korf (1967) and more recently Korf (1972a) has erected the tribe Iodophaneae to include *Iodophanus*, *Thecotheus*, *Boudiera* and *Sphaerosoma* in the Ascobolaceae.
The phylogenetic proximity of Thecotheus and Iodophanus is now in doubt due to the vegetative features of both. Until further studies have been undertaken on the terrestrial species of Thecotheus, final disposition to a family cannot be made because of the variability of characters when compared to established families. For instance, the ascal structure found in Thecotheus is also found in *Ascobolus immersus* (Van Brummelen, 1967). Also, subglobular elements are found in *Ascobolus albidus* that are similar to the bulbous cells of Thecotheus. The terrestrial species of Thecotheus were identified previously as Peziza. The imperfect stage of Thecotheus is closer morphologically to the Aleuriaceae than the Ascobolaceae. Finally, the ascogonia of Thecotheus are similar to the ascogonia of Ascobolus. Until more characteristics of other families are established, such as ontogeny, imperfect stages and excipular features, Thecotheus seems best placed in the Iodophaneae of the Ascobolaceae.

*Thelebolus stercoreus* represents the highest evolutionary level in the genus *Thelebolus*. The uniqueness of its structures may exclude most other genera from the family Thelebolaceae. It now appears that the Thelebolaceae may be restricted to *Thelebolus*, *Trichobolus*, and perhaps *Ascozonus* and *Caccobius*. The latter two genera have not
been studied enough to determine family placement. Kimbrough (personal communication) suggests that the pore mechanism in the ascus of Caccobius may indicate an excursion onto dung from an inoperculate genus. It may well be that the coprophilous discomycetes represent excursions onto the dung habitat by several families that have through parallel evolution evolved many similar features. These features would include an increased number of spores per ascus, a decreased number of asci per apothecium, and various modifications in the ascus wall itself. The irony of Thelebolus stercoreus may be that this organism that was once considered to be primitive, and even classified in families outside the Ascomycetes, may well be among the most advanced fungi in its habitat and morphology.
Figs. 1 - 25. *Thelebolus stercoreus*.

Fig. 1. Nuclear stain of vegetative hypha showing uninucleate (arrows) condition. X 1250.

Fig. 2. Superficial mycelial loop on agar surface. X 1000.

Figs. 3 - 4. Bulbous cells of the vegetative mycelium. X 1000.

Figs. 5 - 6. Early hyphal coiling. X 1000.

Fig. 7. Proliferation of cells surrounding ascogonial initial. X 1000.

Fig. 8. Excipular cells of textura angularis. X 1000.

Fig. 9. Developing ascocarp with radiating hyphae. X 160.

Fig. 10. Fusion of hyphae to the developing ascocarp. Note anastomosis of these hyphae (arrow). X 1000.

Fig. 11. Increased nuclear activity in the expanding ascocarp. X 1000.

Fig. 12. Plastic section showing ascogonial cells. X 1000.

Fig. 13. Young ascus in the cleistothecium-like apothecium. X 400.

Fig. 14. Plastic section showing the layer of cells surrounding the ascus. X 400.
Fig. 15. Nuclear stain showing a large diploid nucleus (arrow) in the ascus. X 1000.

Fig. 16. Nuclear stain showing Prophase I (arrow). X 1000.

Fig. 17. Ascus containing two nuclei (arrows). X 400.

Fig. 18. The start of Division II. Note the appearance of a spindle (arrow). X 400.

Fig. 19. Higher magnification of spindle. X 1250.

Fig. 20. Four nuclei (N) present in the ascus. X 1000.

Figs. 21 - 22. Plastic sections of young asci showing the prominent nucleoli (NU). X 1000.

Fig. 23. A fully expanded ascus. X 400.

Fig. 24. Ripe ascus pushing through the excipulum. Note the characteristic ascal tip. X 400.

Fig. 25. Ascus surrounded by closely adhering paraphyses. X 400.
Figs. 26 - 36. *Lasiobolus ciliatus*.

Fig. 26. Germinating ascospores. X 400.

Fig. 27. Nuclear stain of vegetative mycelium showing uninucleate condition (arrows). X 1000.

Fig. 28. Internyphal growth in culture. X 1000.

Fig. 29. Developing ascogonium. Note evaginations from the parent cells. X 1000.

Fig. 30. Developing ascogonium showing growth of evaginations. Note nuclei in protuberance. X 1000.

Fig. 31. Highly coiled ascogonium due to proliferation of surrounding cells. X 1250.

Fig. 32. Ascogonium surrounded by cells. X 400.

Fig. 33. Ensheathing hypha growing from the parent hypnæ. X 1000.

Fig. 34. Enlarged ascogonium surrounded by a layer of cells. X 400.

Fig. 35. Three ascogonia in a developing ascocarp. X 1000.

Fig. 36. Fusion of hyphae from the parent hypha to the ascocarp. X 1250.
Fig. 37. Ascocarp on dung showing characteristic hairs. X 40.

Fig. 38. The beginning of the development of a hair (arrow). X 400.

Figs. 39 - 41. Elongation of the hair (arrow). X 400.

Fig. 42. Higher magnification of the base of a hair showing its attachment in the excipulum. X 1250.

Fig. 43. Rhizodial hyphae at the base of the ascocarp. X 100.

Fig. 44. Plastic section showing the enclosed hymenium. X 400.

Fig. 45. Plastic section showing paraphyses growing to a point above the asci where the ascocarp will eventually rupture. The result of nuclear divisions can be seen in the asci. X 600.
Fig. 46. Plastic section showing nuclei (N) present in an ascus. X 1000.

Fig. 47. Squash mount of an ascocarp with three multinucleate ascogonia. X 1000.

Fig. 48. Ascogenous system showing asci in various stages of development. A crozier (C) is present. X 400.

Fig. 49. A crozier. X 400.

Fig. 50. An ascus containing the large diploid nucleus. X 1000.

Fig. 51. Ascus after Division I. X 1250.

Fig. 52. Ascus with four nuclei (N). X 400.

Fig. 53. Ascus with four nuclei. Two (arrows) are beginning to undergo further division. X 1250.

Fig. 54. Ascus containing eight nuclei (!) near the start of spore cleavage. X 1000.

Fig. 55. Ascus after spore cleavage. Note that there has been an inconsistency in cleavage with one spore receiving two nuclei (arrow). X 1000.

Fig. 56. A uninucleate ascospore. X 1250.
Figs. 57 - 63. *Thecotheus cinereus*.

Fig. 57. A "primordial hump" showing the bulbous cells (arrows). X 200.

Fig. 58. Plastic section showing a series of ascogonia. X 1000.

Fig. 59. A frozen section using the cryostat showing the centrum and excipulum. X 100.

Fig. 60. Uninucleate bulbous cells near the edge of a primordial hump. X 400.

Fig. 61. Uninucleate bulbous cells forming the excipulum. X 1250.

Fig. 62. Nuclear stain showing the multinucleate hyphae beneath the bulbous cells. X 1250.

Fig. 63. Plastic section showing ascogenous hyphae (arrows) pushing through the paraphyses. X 1000.
Fig. 64. Section showing a crozier (C). X 1250.

Fig. 65. Nuclear stain showing an ascus containing a diploid nucleus (arrow). X 1000.

Fig. 66. A young ascospore with thick, smooth spore walls. X 1250.

Fig. 67. A young ascospore with apiculi. X 2000.

Fig. 68. Mature ascospore with apiculi and ornamented walls. X 1250.

Fig. 69. An ascus with irregularly uniseriate ascospores. X 440.

Figs. 70 - 95. *Thecotheus pelletieri*.

Fig. 70. Bulbous cells and filaments of the "primordial nump". X 1000.

Fig. 71. Nuclear stain of a mycelial loop to show the multinucleate condition. X 600.

Fig. 72. Interhyphal growth in culture. X 1000.

Fig. 73. Mycelial loop with evaginations from the loop similar in shape to conidiophore initials (CI). X 1000.

Fig. 74. Mycelial loop with radiating hyphae. Note the fusion of hyphae (arrows). X 100.
Fig. 75. Nuclear stain showing the large diploid nucleus (arrow) in the ascus. X 1000.

Fig. 76. An ascus with four nuclei (N). X 1000.

Fig. 77. An ascus with young thick walled ascospores. Note the ascal tip. X 1250.

Fig. 78. Uninucleate ascospore. X 1250.

Fig. 79. Ascospores ejected from an ascus showing that they are released in one large clump. X 440.

Fig. 80. Germinating ascospores. Note large vacuoles in the spore and that the ascospores have swollen to twice their original size. X 440.

Fig. 81. A conidiophore initial. X 1000.

Fig. 82. The initial has increased in length and a hooked appendage has begun to form. X 1000.

Fig. 83. The conidiophore and hook have continued to increase in length. X 1000.

Fig. 84. Nuclear division in the conidiophore tip. X 1000.
Fig. 85. The primary conidium is blown out. X 1000.

Fig. 86. The conidium elongates. X 400.

Fig. 87. The conidium continues to elongate. Note hooked appendage at the base of the conidiophore. X 1000.

Fig. 88. A septum is formed between the conidium and the conidiophore. X 1000.

Fig. 89. The tip of the conidiophore becomes meristematic and begins to elongate to one side of the primary conidium. X 1000.

Figs. 90 - 93. A secondary conidium is blown out and elongates. The conidium is uninucleate. X 1000.

Fig. 94. The secondary conidium is delimited from the conidiophore by a septum. X 1000.

Fig. 95. A conidiophore with a head of conidia. X 1000.
Fig. 96. Stalked ascogonium of *Coprotia granulata* (*Numaria granulata*); from Gwynne-Vaughan and Williamson (1930).

Fig. 97. Stalked ascogonium of *Cheilymenia stercorea* (*Lachnea stercorea*); from Fraser (1907).

Fig. 98. Diagrammatic cross-section of ascocarp showing the stalked ascogonium of *Scutellinia scutellata* (*Lachnea scutellata*); from Brown (1911).

Fig. 99. Stalked ascogonium of *Lasiobolus monascus*; from Kimbrough (1973, in press).
LITERATURE CITED


Lasibolus ronas.  In press.


---. 1888. Sylloge fungorum. 7:1-882 & 1lix.

---. 1892. Sylloge fungorum omnium hucusque cognitorum. 10.


BIOGRAPHICAL SKETCH

Kenneth Edward Conway was born June 7, 1943 in Philadelphia, Pennsylvania. In June, 1961 he was graduated from Cazenovia Central High School in Cazenovia, New York. He received the Bachelor of Arts with a major in Secondary Science Education from the State University of New York College of Education at Potsdam, New York in June, 1966. In January, 1966 he enrolled in the Graduate School of the State University of New York College of Forestry at Syracuse University, Syracuse, New York. He worked as a graduate assistant until June, 1966 when he received the degree of Master of Science with a major in Botany. From September, 1966 until the present time he has pursued his work toward the degree of Doctor of Philosophy. He is the author of two research articles.

Kenneth Edward Conway is married to the former Cynthia Anne Gary, and is the father of a daughter, Kenna Anne. He is a member of the Mycological Society of America, the Society of Sigma Xi, the Florida Academy of Science, the National Education Association, the Florida Education Association, the Alachua County Education Association, and is an appointed member of the Gainesville City Beautification Board.
I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

James W. Kimbrough, Chairman
Associate Professor of Botany

Leland Shanor
Professor & Chairman of Botany

Henry C. Aldrich
Associate Professor of Botany

Dena G. Griffin, III
Assistant Professor of Botany
I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Robert A. Schmidt
Assistant Professor of Forestry

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Francis W. Zettler
Assistant Professor of Plant Pathology

This dissertation was submitted to the Dean of the College of Agriculture and to the Graduate Council, and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

December, 1973

Dean, College of Agriculture

Dean, Graduate School