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Ascocarp development in selected species of Claviceps and Cordyceps

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ASCOCARP DEVELOPMENT IN SELECTED SPECIES
OF CLAVICEPS AND CORDYCEPS

by

Shirley Ann Nordahl

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INTRODUCTION

Claviceps and Cordyceps belong to a distinct group of fungi, members of which are parasitic on grasses, insects, spiders, or subterranean fungi. They are Pyrenomycetes in which the perithecia lie in a stroma which is either stalked or sessile.

In 1951, Luttrell surveyed the literature and proposed a working hypothesis for a taxonomy of the Pyrenomycetes based on developmental criteria. Since there is very little experimental data relating to development, it is necessary to establish the nature of development in genera and groups of genera of the Pyrenomycetes in order to test the hypothesis. The purpose of this investigation was to establish the pattern of centrum development in Claviceps purpurea (Fr.) Tul., Cordyceps ophioglossoides (Ehr. ex Fr.) Link, and Cordyceps militaris (L. ex St. Amans) Link.
LITERATURE REVIEW

The Pyrenomycetes are a large and heterogeneous group of Ascomycetes in which the ascocarp is a closed structure opening typically by means of an apical ostiole and containing a single layer or cluster of asci and sterile threads or paraphyses in its base (Lindau, 1897). In addition, according to more recent interpretation, only those organisms with a true ascocarp wall which develops in conjunction with the ascogenous hyphal system from the stalk cells of the ascogonium or from neighboring vegetative hyphae are included (Miller, 1928; Miller, 1949; Luttrell, 1951).

As a result, much of the recent work with the Pyrenomycetes has been an attempt to remove from them all those organisms which do not have a true wall and to establish a basis for separation of orders involving characters of fundamental importance (Luttrell, 1951).

Taxonomic units within the Pyrenomycetes have been erected on the basis of gross morphological features such as color, consistency of ascocarp wall and stroma and the position of the ascocarp in relation to the substrate (Saccardo, 1883; Lindau, 1897). These characters may often fluctuate with environmental changes (Miller, 1949), and their use has resulted in artificial groupings.

The classification systems of Saccardo and Lindau are still the two most commonly used. The families of Saccardo correspond in large part to the suborders of Lindau. Since Saccardo's primary separations are based on spore characters, his system has perhaps brought together more closely related genera than Lindau's system (Miller, 1949). Traditionally, however, Lindau's has been followed with a few minor changes.
His suborders have been recognized as orders and the term Pyrenomycete has been retained as a general term without taxonomic rank.

**Claviceps** and **Cordyceps** are two of a group of closely related genera which have been considered rather uniform and distinct. Because of the bright-colored walls of their perithecia, they have been considered members of the family Hypocreaceae (Saccardo, 1883; Ellis and Everhart, 1892) or the suborder Hypocreales (Lindau, 1897).

Seaver (1909) divided the Hypocreaceae of Lindau into two families, the Hypocreaceae with the perithecia immersed in a stroma and the Nectriaceae with the perithecia free and separate or grouped on the surface of a stroma. He used spore characters for generic and specific separations, and included **Claviceps** and **Cordyceps** in the Hypocreaceae. This is again an artificial separation but one which generally was followed (Gwynne-Vaughan and Barnes, 1927; Petch, 1938; Martin, 1945).

Traditionally, the Hypocreales have been separated from the other large order of Pyrenomycetes, the Sphaeriales, simply by color and texture of the perithecial walls. Therefore, some authors have combined them into one large group, the Sphaeriales, with the **Claviceps** group treated as a family in that order (Clements and Shear, 1931; Munk, 1957). Nannfeldt (1932) included all the Hypocreales in the Sphaeriales with the exception of the **Claviceps** group which he raised to the rank of a separate order, the Clavicipitales, largely on the basis of the lack of a true wall.

Miller (1949) included the Clavicipitaceae in the Sphaeriales because of the presence of a true wall and the presence of asci and paraphyses in the perithecium. He retained the other members of the Hypocreales as an order with pseudoparaphyses in the perithecium. Most modern authors
have thought the Claviceps group distinctive enough to be included either as a family in the Hypocreales (Bessey, 1950; Dingley, 1951; Martin, 1961) or as an order (Gäumann, 1952; von Arx and Müller, 1954; Chadefaud, 1960; Alexopoulos, 1962; Dennis, 1968).

In 1951 Luttrell utilized the literature on developmental studies of the Pyrenomycetes as the basis of a revision of this group based largely upon ascus structure and centrum morphology.

Centrum is a term introduced by Wehmeyer (1926) to replace the term nucleus which had formerly been used (Fries, 1823). In the Pyrenomycetes it includes the ascogenous hyphae, asci, and sterile tissues which develop within the perithecial cavity. The nature of the centrum was overlooked for some time in the Ascomycetes and therefore little developmental information is available. It has been used in definition of a few other groups of Ascomycetes, and was used to delimit orders of the Pyrenomycetes by Miller (1949).

Luttrell (1951) described five types of centrum development in the Pyrenomycetes. He placed Claviceps and Cordyceps in the family Clavicipitaceae in the restricted order Xylariales. This order is distinguished by a Xylaria type centrum in which asci form a single wall layer or rarely a basal cluster, and the centrum is composed of paraphyses and asci. This disposition of the Claviceps group is based upon morphological studies made on Claviceps (Vincens, 1917; Killian, 1919), Cordyceps (Varitchak, 1931; Jenkins, 1934), and Epichloe (Dangeard, 1907; Vincens, 1917; Gäumann, 1927).

Luttrell described a Claviceps type ascus of the unitunicate type characteristic of the true Pyrenomycetes. It is long, cylindrical with
thin lateral walls and has a thickened hemispherical cap penetrated by a fine pore. It contains a fascicle of eight filiform ascospores. Miller (1949) reported this type of ascus in *Claviceps* and *Cordyceps* as well as in other genera of the Clavicipitaceae.
MATERIALS AND METHODS

Collections of *Elymus canadensis* L. bearing sclerotia of *Claviceps purpurea* (Fr.) Tul. were made in July, 1965 along new Highway 30 east of Ames, Iowa and in July, 1967 from a road-side area near the Ledges State Park. Infected *Bromus inermis* Leyss. was collected during July, 1966 at the Iowa Lakeside Laboratory.

The sclerotia were removed from the grass florets and treated using a modification of a method for producing stromata in *Claviceps* (Tiffany, 1946). Each sclerotium was surface sterilized by first wetting it in 95% ethyl alcohol for one minute and then placing it in a 10% solution of commercial sodium hypochlorite for five minutes. The sclerotium was rinsed in three changes of sterile distilled water and placed aseptically on a 1% water agar slant which was tightly plugged with cotton. The slants were placed in 5°, 10°, and 15°C constant temperature chambers for periods of three to six months.

Sclerotia in the 5° chamber had produced only a few stromata at the end of five months, and those in the 15° chamber had produced none. Those in the 10° chamber, however, began to produce stromata after only three months incubation, confirming previous observations of temperature effect on production of stromata (Tiffany, 1946). Approximately 70% to 80% of the sclerotia held at 10° produced from one to many stromata by the end of five months with very little contamination.

During a three year period approximately 600 sclerotia from Canada wild rye and 100 from brome were treated at intervals to allow for reasonably constant production of fresh stromal material.
Cordyceps ophioglossoides (Ehr. ex Fr.) Link was collected in July, 1963 at Ledges State Park and in August, 1966 at Pilot Knob State Park. Cordyceps militaris (L. ex St. Amans) Link was collected in August, 1961 at Woodman Hollow State Preserve and in October, 1966 at Lacey Keosauqua State Park.

Entire stromata of Claviceps purpurea and portions of the stromata of Cordyceps ophioglossoides and Cordyceps militaris were killed and fixed in Graf IV (Sass, 1958). An ethyl alcohol-xylene series was used in dehydration and preparation of the stromata for infiltration and embedding in paraffin.

Sections of the stromata of all species studied were cut at 9-10 μ and stained with iron hematoxylin (Sass, 1958) using a time schedule for all material of 4 hours in mordant and 4 hours in stain. Destaining with half strength mordant produced good results within 1 minute for the Claviceps material while 2-3 minutes were required in the two Cordyceps species. Fast green was used as a counterstain in all cases and only 10-15 seconds was required to give clarity of detail.

Fresh asci of Claviceps purpurea were studied in squash preparations of perithecia stained using a combination of Wittman's acetoiron-hematoxylin with Hoyer's mounting medium (Bowen, 1963). Fresh material of the two species of Cordyceps was not available for this type of study.
RESULTS

Centrum Development in Claviceps purpurea

Stromata of Claviceps purpurea are stalked globose structures varying in color from white to pink to lavender, which arise from black spurred-shaped sclerotia formed in the florets of various grasses (Fig. 1). One to many stromata may arise from each sclerotium (Fig. 2). Perithecia are formed just beneath the surface of the globose head and at maturity are noticeable by their erumpent nature (Fig. 3). The heads average 1-2 mm in diameter and the length of the stalks varies greatly.

The stalk is composed of closely compacted, longitudinally parallel hyphae (Figs. 4 and 10) which are septate and multinucleate. The interior of the head is composed of pseudoparenchyma, while the entire surface of the head consists of a peculiar palisade layer of hyphae (Figs. 4 and 10). This palisade consists of parallel hyphae, each of which branches dichotomously at least twice from the region at which the parallel orientation becomes apparent to the outside of the head (Figs. 5 and 6). The cells of these hyphae appear to be mostly binucleate. As the fungus develops, scattered enlarged multinucleate cells are produced in a narrow band beneath the palisade layer in the region of pseudoparenchyma. They cover the entire head (Figs. 7, 8, and 9), and were interpreted as a type of ascogonial cell. Branches of neighboring hyphae become oriented around the ascogenous cells and the ascogenous hyphae, and opposing hyphal elements develop above the expanding mass of ascogenous cells (Figs. 9, 10, 11, and 12).

As the perithecium increases in size, asci arise in a basal cluster
Figs. 1-2. Sclerotia and stromata of *Claviceps purpurea*

Fig. 1. *Bromus inermis* Leyss. bearing sclerotia

Fig. 2. Sclerotium bearing several stromata
Figs. 3-4. Stroma of *Claviceps purpurea*

Fig. 3. Close-up of the head of a stroma showing the erumpent perithecia (X35)

Fig. 4. Longitudinal section of a young stroma (X78)
Figs. 5-6. Palisade layer of hyphae from *Claviceps purpurea* stroma

Fig. 5. A portion of the layer of palisade cells (X1550)

Fig. 6. Palisade cells separated (X1550)
Figs. 7-9. Perithecial initiation in stromata of *Claviceps purpurea*

Fig. 7. Stroma showing palisade layer and region of ascogonial formation (X580)

Fig. 8. Multinucleate ascogonium (X1100)

Fig. 9. Ascogonium with stromal hyphae beginning to become oriented around it (X1100)
Figs. 10-12. Perithecial initiation in stromata of *Claviceps purpurea*

Fig. 10. Longitudinal section of a stroma showing the structure of the stalk and head and the arrangement of the perithecial initials (X70)

Fig. 11. Perithecial initials with increased development of the stromal hyphae (X900)

Fig. 12. Perithecial initials with developing lateral paraphyses, ascogenous hyphae and stromal hyphae oriented around the ascogenous hyphae (X900)
by means of croziers. The opposed hyphal tips in the upper area of the perithecium appear to push apart due to their growth or are pulled apart by the tangential growth of the wall hyphae. They line the upper one-third to one-half of the developing inner perithecial wall and appear to be the same type of hyphal material as the periphyses which will eventually line the neck of the perithecium (Figs. 13, 14, and 15).

Regardless of the mechanism involved, space is formed within the perithecium as the asci expand to full length (Figs. 16 and 17). A thin but definite wall forms most noticeably first in the upper region of the perithecium (Fig. 13) and finally at maturity is conspicuously present in the basal region also (Figs. 16 and 17). A pad of sterile hyphae forms in the base of the perithecium beneath the ascogenous cells (Figs. 16 and 17). The asci are not all the same age as they are being formed continuously from the ascogenous hyphae.

As the ascocarp increases in size, meristematic activity in a region near its apex causes rapid growth of the wall toward the surface of the stroma (Figs. 13, 14, and 15). This growth results in the formation of an ostiolar region which eventually opens to the outside and is lined by periphyses (Fig. 17).

As the asci increase in length, the lateral hyphae become less conspicuous and at maturity the cavity is completely filled with asci (Figs. 16 and 17).

The asci are formed from typical croziers which develop from the ascogenous hyphae (Figs. 14 and 18). Fusion of nuclei takes place early in the young ascus and the ascus elongates to its full length with the fusion nucleus about mid-way in the ascus. What appear to be a large
Figs. 13-15. Development of young perithecia of *Claviceps purpurea*

Fig. 13. Young perithecia with a mass of asco-genous tissue at the base and lateral paraphyses (X500)

Fig. 14. Young perithecia in which ascogenous hyphae are beginning to form croziers, lateral paraphyses are developed and ostiole formation is beginning (X1070)

Fig. 15. Young perithecium (X1070)
Figs. 16-17. Mature stroma and perithecia of *Claviceps purpurea*

Fig. 16. Mature stroma showing dark staining palisade region and the crowded arrangement of mature perithecia (X150)

Fig. 17. Mature peritheciun with definite wall layer, basal pad of sterile tissue, and periphysate ostiole opening to the outside through which asci extend (X400)
nucleolus and chromatin material are present. There is no visible differ-
entiation in the ascus tip at this age (Fig. 19). Meiosis occurs result-
ing in a binucleate (Fig. 20) and then a four-nucleate ascus (Fig. 21).
A clear apical cap is evident by the end of the first division of meiosis.
This apical cap remains clear when asci are stained with iron-hematoxylin
and has a fine pore through its center (Fig. 22). Following the second
division of meiosis and the subsequent mitotic divisions of each of the
four meiotic nuclei, an eight-nucleate ascus is formed. Cytoplasm con-
denses around each nucleus and the next visible event is the appearance
of a fascicle of eight scolecospores (Figs. 22 and 23). Nuclear divisions
occur in each spore resulting in a multinucleate, aseptate spore (Fig. 24).
Mature asci in water mounts or stain preparations break very easily
at their basal ends and the apical caps are often absent. In sectioned
material the asci are often observed protruding from the ostiole of the
perithecium (Fig. 17).
All the perithecia within any one stroma were of similar age and
were spaced evenly, if a little crowded, over the surface of the stroma
(Figs. 10 and 16).
Centrum Development in *Cordyceps ophioglossoides*

Stromata of *Cordyceps ophioglossoides* are formed singly or in groups
from ascocarps of parasitized *Elaphomyces* species (Fig. 25). They are
upright, club-shaped, olive-green structures. Each stromata consists of
a sterile stalk portion and an elongate apical fertile portion in which
the perithecia are formed.
In the fertile portion, three more or less distinct regions can be
Figs. 18-22. Ascus development in *Claviceps purpurea*

Fig. 18. Crozier with budding ascus (X1120)

Fig. 19. One-nucleate ascus (X1120)

Fig. 20. Two-nucleate ascus with well developed ascus tip (X1120)

Fig. 21. Four-nucleate ascus (X1120)

Fig. 22. Mature ascus with filiform spores. Ascus tip with pore visible (X1120)
Figs. 23-24. Mature ascus and spores of *Claviceps purpurea*

Fig. 23. Mature ascus with filiform spores (X900)

Fig. 24. Multinucleate, aseptate scolecospores (X400)
Figs. 25-26. Cordyceps sp. stromata and host

Fig. 25. *Cordyceps ophioglossoides* parasitizing ascocarps of *Elaphomyces* sp.

Fig. 26. *Cordyceps militaris* stromata growing from insects
seen. There is a central core of pseudoparenchymatous hyphae, an outer rind-like area of very densely staining hyphae, and between these a region of rather loosely packed hyphal branches (Figs. 27 and 30). It is in this latter region that perithecia are formed. The first evidence of ascocarp formation seen in this study was ascogenous material already encircled by several layers of neighboring hyphae (Fig. 27). The growth of the wall is rapid and soon a very thick wall is formed around the complete circumference of the young ascocarp (Fig. 27). The ascogenous tissue located in the basal portion of the ascocarp enlarges and above it overlapping lateral hyphae push or are pulled apart as the cavity becomes larger (Figs. 28 and 29). Active growth of wall cells in the upper region of the ascocarp results in the formation of a schizogenous ostiole which eventually opens through the surface of the stroma (Fig. 29). The neck of the perithecium is lined with periphyses (Figs. 29 and 30). As the size of the perithecium increases, opposed lateral hyphae line the upper one-third of the perithecium (Fig. 29).

Asci, formed from croziers, grow up into the cavity (Fig. 29). From sectioned material it was possible to determine that the asci reached almost mature length before meiosis took place. Details of ascus development were not observed. At maturity the ascus has a well formed cap with a fine pore through it, and contains a fascicle of eight spores which become multiseptate within the ascus (Figs. 30 and 31). Each part-spore contains one nucleus. Mature asci were observed protruding through the ostioles of the perithecia.
Figs. 27-29. Perithecial development of *Cordyceps ophioglossoides*

Fig. 27. Perithecial initial with an ascogonial mass within an already thick wall (X1200)

Fig. 28. Young perithecium with a mass of ascogenous tissue at the base, a heavy wall and overlapping lateral paraphyses (X710)

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Fig. 30. Mature stroma showing nature of the stroma, arrangement of the perithecia, and a wall, basal pad of tissue, asci and periphysate ostiole in the central perithecium (X170)

Fig. 31. Mature perithecium with wall, periphysate ostiole and multiseptate ascospores in the asci (X445)
Centrum Development in *Cordyceps militaris*

Stromata of *Cordyceps militaris*, formed from hyphal masses in the parasitized bodies of insect hosts, are upright, club-shaped, orange structures (Fig. 26). They have a sterile stalk-like portion and an elongate fertile head from which the perithecia noticeably protrude.

Internally the stroma is uniform with an outer portion of slightly smaller and more compacted hyphal cells (Fig. 35).

Ascocarps are formed over the entire fertile head just beneath the surface (Fig. 35). In the earliest stage of development observed, the ascogenous hyphal system and ascocarp wall were well established. The basal pad of sterile tissue was also apparent (Fig. 32).

Asci arise in an aparaphysate basal clump by typical croziers formed from the ascogenous hyphae (Figs. 33 and 34).

Lateral opposing hyphae are present but not conspicuous (Figs. 33 and 34). Growth of the perithecium creates a cavity into which the asci grow (Figs. 33 and 34). The asci reach almost full length before meiosis occurs, and at maturity they fill the cavity (Figs. 35 and 36). Growth takes place rapidly in the upper region of the wall to form a periphysate ostiole (Figs. 33, 34, and 36).

Mature asci have an ascus cap with a central pore and contain a fascicle of eight scolecospores which become multiseptate within the ascus. Each ascospore cell is uninucleate. Asci protrude through the ostioles of the perithecia (Fig. 36).
Figs. 32-34. Perithecial development of *Cordyceps militaris*

Fig. 32. Perithecial initial with a mass of ascogenous hyphae, a well developed wall, and paraphyses (X530)

Fig. 33. Young perithecium with croziers and developing asci (X480)

Fig. 34. Young perithecium with developing asci filling most of the centrum (X480)
Figs. 35-36. Mature perithecia of *Cordyceps militaris*

Fig. 35. Mature stroma showing the nature of the stroma and the position of the mature perithecia (X90)

Fig. 36. Mature perithecium with definite wall, septate ascospores, basal pad of tissue and remnants of lateral paraphyses (X185)
Centrum development in *Claviceps purpurea*, *Cordyceps ophioglossoides*, and *Cordyceps militaris* appears to be of a similar pattern. In *Claviceps*, a multinucleate ascogonial cell arises in the outer region of the stroma and stimulates the growth of stromal hyphae to form a wall around it. It was not ascertained whether any of the wall material was initiated from the stalk cell of the ascogonium itself. The production of wall hyphae from the ascogonial stalk was a basic feature in Miller's (1949) definition of a true wall in the Pyrenomycetes. Luttrell (1951) extended the concept of a true wall to include any wall hyphae stimulated to develop by the presence of an ascogonium.

No evidence of antheridial elements or fusion with any type of spermatia or spores was observed. Killian (1919) had reported a multinucleate antheridium which branched from a multinucleate ascogonium and fused with it. Vincens (1917) described the ascogonium as a deeply staining filament and found no antheridia in *Claviceps microcephalia* (Wallr.) Tul.

Jenkins (1934) found septate ascogonia which coiled in *Cordyceps agariciformia* (Bolt.) Seaver. Certain ascogonial cells became large and multinucleate and gave rise to ascogenous hyphae. In *Cordyceps militaris*, Varitchak (1931) found multinucleate, aseptate ascogonia which gave rise to ascogenous hyphae. Both Jenkins and Varitchak stated that the nuclei in the ascogonial cells were products of mitotic divisions of the original nuclei and neither found any evidence of antheridial elements.
Increase in size of the perithecium in all three species appeared to be due to the growth of wall hyphae and also to space making mechanisms within the perithecium. These mechanisms were the growth of the ascogenous hyphal mass in the base of the perithecium and the growth of the laterally opposed, free hyphal elements which developed from the inner surface of the wall in the upper half of the perithecium. Vincens (1917) indicated that the ascogonial cell mass played a part in the expansion of the perithecium. Jenkins (1934) reported in *Cordyceps agariciformia* that pressure exerted by the growth of opposed hyphae is responsible for the creation of the perithecial cavity. Although not specifically reported, the drawings of Vincens (1917), Killian (1919) and Varitchak (1931) all indicate the function of these paraphyses as space making devices. The majority of authors, including Killian (1919) attribute the expansion of the perithecium largely to tangential growth of the wall hyphae with paraphyses growing out into the already formed cavity.

The wall is simple, being composed of one layer of pseudoparenchymal cells slightly more compressed than the cells of the rest of the stroma. It is relatively thin in *Claviceps purpurea* and *Cordyceps militaris* and much thicker, but still homogeneous, in *Cordyceps ophioglossoides*. The wall develops evenly in both species of *Cordyceps* but in *Claviceps purpurea* it develops more slowly in the basal region of the perithecium.

The asci arise from typical croziers in *Claviceps purpurea*. This is in accordance with the findings of Killian (1919). Vincens (1917) reported that asci arise directly from the ascogenous hyphae in *Claviceps microcephalia*. Detailed ascus studies of the *Cordyceps* species were not possible, but ascus formation appeared to be typical.
Varitchak (1931) reported crozier formation in *Cordyceps militaris* and Jenkins (1934) found most asci to originate by typical crozier formation in *Cordyceps agariciformia*.

Asci, characterized by thin lateral walls and a distinctive thickened tip with a fine pore, are similar in all three species. De Bary (1887) described the tip as having the appearance of a cork set on the tube formed by the thin lateral walls. He suggested that the apical thickening is reserve wall material which permits the extension of the ascus through the ostiole.

Munk (1957) described an ascus tip in *Barya* which he places in the *Claviceps* group, as a simple thickened apical cap with a flat bottom and a central pore. He believed that this is typical of the Clavicipitaceae. Chadefaud (1960) described similar ascus tips in *Cordyceps* and *Epichloe*. Doguet (1960) described a slightly different ascus tip in *Epichloe typhina* (Pers. ex Fr.) Tul. which he said was near the double ring Diaporteen type of Chadefaud.

It is interesting to note that the *Claviceps* type of ascus is found in one order of Discomycetes, the Ostropales (Nannfeldt, 1932). The occurrence of this type of ascus, plus the fact that he regarded the wall of the perithecium as a nest of paraphyses, led Chadefaud (1960) to postulate that the Clavicipitales fell phylogenetically somewhere between the Pyrenomycetes and the Discomycetes.

Spore formation was not studied in detail in *Claviceps purpurea*. Eight-nucleate asci with areas of cytoplasm condensed around the nuclei were present and also asci with fascicles of spores. Apparently a very short time elapses between these two stages. Ascospores quickly become
multisepitate in the two species of *Cordyceps*.

In *Cordyceps* the spindles of mitotic divisions have been reported to be parallel to or slightly oblique to the long axis of the ascus (Jenkins, 1934; Varitchak, 1931). Such an orientation results in a linear arrangement of nuclei. Jenkins (1934) states that spore initials were first delimited by condensation of the cytoplasm around the nuclei. These spore initials grew rapidly in length and septations did not occur until the spore initial had grown considerably in length (Jenkins, 1934; Varitchak, 1931). At maturity 35-50 septa are found in each spore and each cell is uninucleate (Jenkins, 1934).

Spores of *Claviceps* have been reported as one continuous cell (Tulasne, 1853), as tri-septate by Gussou (1914) and Tiffany (1948) and as consisting of about 64 cells by Freeman (1905).

In the present observations the spores of *Claviceps* were unicellular in the ascus. They may become multicellular after being released from the ascus. Tiffany (1948) reported septate spores with 8-16-20 septa which may send out many germ tubes which readily ramify. She observed no set pattern of germination or any fragmentation of spores.

No studies were conducted as to the mechanism and manner of spore dispersal. De Bary (1887) accounts for dispersal in *Cordyceps militaris* by saying that each ascus emerges through the ostiole and expels its spores singly and in quick succession. Jenkins (1934) observed in *Cordyceps agariciformia* that often the spores fragment into part-spores before being released from the ascus.

Mature asci of *Claviceps* were so frequently found with the basal ends broken, the apical caps absent, and each fascicle of spores essentially
free and unwinding within the ascus walls, that it might be tentatively suggested that the spores are released from the ascus when the ascus ruptures, and need not necessarily be liberated through the apical pore. This is supported by Dingley's (1951) observation that the contents of the mature ascus force off a definite cap or apical segment similar to the operculum in the Pezizales.

The preceding information and the fact that in this study mature asci were often observed protruding through the ostioles of the perithecia make it probable that entire asci, not single spores, are ejaculated from the perithecia.

The type of centrum development found in all three species is of the special type of centrum development in the Xylaria centrum type which Luttrell described for the Claviceps group. It differs from the typical Xylaria type centrum by the presence of evanescent lateral paraphyses and the basal aparaphysate cluster of asci. The typical Xylaria centrum has a basal hymenium of paraphyses and asci which covers the base and often the sides of the perithecium.

Luttrell established this group as the Clavicipitaceae within the Xylariales because of the presence of paraphyses within the perithecial wall, but he suggested that the fasciculate arrangement of asci may be sufficient cause for establishing an order Clavicipitales. He follows Miller (1949) in this interpretation.

In the most recent study in the Claviceps group by Doguet (1960) on Epichloe typhina, the same type of centrum development is described as was found in this study. He concluded that Epichloe should be in the Xylariaceae of the old Sphaeriales or the Xylariales as defined by Luttrell.
Until enough information has been collected to evaluate the uniformity of the characters proposed by Luttrell, and until trends can be interpreted throughout the Pyrenomycetes, the degree of importance that should be assigned to particular characters is difficult to ascertain. It is not possible on the basis of our present information to know whether the developmental features observed make the *Claviceps* group distinctly different at the familial or at the ordinal level.
SUMMARY

Claviceps purpurea, Cordyceps ophioglossoides, and Cordyceps militaris were studied to determine the nature of the centrum in each. The pattern of centrum development was found to be similar in all three species and is of the special type within the Xylaria type centrum which Luttrell described for the family Clavicipitaceae. This type of centrum has evanescent lateral paraphyses and at maturity consists of a basal aparaphysate cluster of scolecosporous asci of the Claviceps type, seated on a basal pad of tissue.

The asci are similar in all three species and are the Claviceps type of unitunicate ascus described by Luttrell. This type of ascus is long and cylindrical with thin lateral walls and a thickened hemispherical cap, which is penetrated by a fine pore. A fascicle of eight scolecospores is formed within each ascus. The spores of Claviceps purpurea remain unicellular in the ascus whereas the spores of the two Cordyceps species become multicellular quite soon after spore formation.

On the basis of this developmental and morphological information these three species should be included in the family Clavicipitaceae in the order Xylariales, as suggested by Luttrell, or in a separate order, the Clavicipitales as suggested by Luttrell (1951) and Miller (1949).


Saccardo, P.A. 1883. Syllogae fungorum omnium hucusque cognitorum. Sumptibus auctoris, Patavii, Italy.


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