

The cytological phenomena associated with the development of the sporophyte in Sclerotinia fructicola.

By

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TAXONOMIC NOTE

The following species are cited in this investigation.

Ascodesmis nigricans Tieghem.
Ascobolus citrinus Schweizer.
Ascobolus furfuraceus Pers.
Ascobolus immersus Pers.
Ascobolus magnificus Dodge.
Ascobolus strobilinus Schweizer.
Ascophanus Aurora (Crouan) Boud.
Ascophanus carneus Pers.
Botrytis cinerea Pers.
Ciboria fructicola Winter.
(*Sclerotinia fructicola* (Wint.) Rehm.)
Eremascus albus Eidam.
Erysiphe Polygoni DC. ex S.F.Gray.
Helvella crispa Fries.
Humaria granulata Quel.
Humaria rutilans Fries.
Lachnea scutellata (L. ex Fr.) Gillet.
Lachnea stercorea Pers.
Neurospora sitophila (Mont.) Shear & Dodge.
Neurospora tetrasperma Dodge.
Peziza bolaris Tul.
Peziza vesiculosa Bull.
Phyllactinia Corylea (Pers.) Karst.
Pleuraea anserina (Ces.) Kuntze.
Pustularia bolarioides Ramsb.
Pyronema confluens (Pers.) Tul.
Pyronema confluens var. *irigneum* Brown.
Pyronema domesticum (Sow.) Sacc.
Schizosaccharomyces octosporus Beij.
Sclerotinia Duriaea (Tul.) Rehm.
Sclerotinia fructicola (Wint.) Rehm.
Sclerotinia Fuckeliana (de Bary) Fckl.
Sclerotinia Gladioli (Massey) Drayton.
Sclerotinia sclerotiorum (Lib.) Massey.
Sclerotinia tuberosa (Hedw. ex Fr.) Fckl.
Sordaria coprophila (Fr.) C & DeN.
Sphaerotheca Humuli (DC.) Burrill.

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THE CYTOLOGICAL PHENOMENA ASSOCIATED WITH THE DEVELOPMENT OF THE SPOROPHYTE IN SCLEROTINIA FRUCTICOLA.

INTRODUCTION

It is the purpose of the present investigation to make a cytological study of several phases of the life cycle of Sclerotinia fructicola, especially of the microconidia and apothecia, to obtain data on the nuclear phenomena in the hope of gaining some knowledge as regards its sexuality. No work of this nature has hitherto been reported with this fungus, and there are but two reports in the literature of similar work on other species of Sclerotinia.

The Sclerotinias are of great economic importance as many of them are very destructive plant pathogens, attacking drupaceous fruits and their close relatives. S. fructicola is the causal organism of the brown rot disease of stone fruits, one of our most serious orchard diseases. Except for its sexuality and nuclear phenomena the life cycle has been worked out.

The question of sexuality in Ascomycetous fungi has long been a controversial one. Intensive cytological work has been done by several investigators on a few of the Ascomycetes but in the majority of the genera there is no knowledge of the sexual mechanism involved in the formation of the fruiting bodies, and there is no knowledge, except in a few species, of the nuclear phenomena in the various stages of the life cycle. As there is a distinct alternation of generations, the gametophyte being haploid and the sporophyte diploid, a knowledge of the nuclear phenomena is of fundamental importance for a thoro knowledge of the life cycle of these fungi. It is the hope of the writer that the work presented in this paper will make a contribution on this question.

SEX IN THE ASCOMYCETES

Before reviewing the literature pertaining to the present investigation sexuality as it occurs in the Ascomycetes will be briefly discussed.

The Ascomycetes are one of the sub-divisions of the Eumycetes (true fungi); their distinguishing characteristic being the ascus. The ascus is a sac-like structure in which the spores are produced and is comparable to the spore-mother cell in the higher plants.

The basic sexual organs are the antheridium (male) and the oogonium (female). In general, the antheridium is smaller and more slender than the oogonium. Fertilization is brought about by the passage of the nuclei of the antheridia into the oogonia. After fertilization a number of filaments (ascogenous hyphae) bud out from the oogonia. These receive the oogonial contents and give rise to the asci at their tips. The ascogenous hyphae thus form the sporophyte while the vegetative filaments on which the sexual organs are borne are the gametophyte. There is a well marked alternation of generations that are separated in time but not in space. Various modifications of the sexual organs, and of the sexual process, exist and apogamy (vegetative reproduction) is of wide occurrence. These modifications are listed below:

1. Fusion of pairs of undifferentiated fertile hyphae at their tips. Eremascus albus.
2. Two cells of similar size put out processes which fuse to form a conjugation tube. Schizosaccharomyces octosporus.
3. Differentiation of the sex organs into definite antheridia and oogonia. Sphaerotheca Humuli.

4. Trichogynes are developed on the oogonia and these fuse with the antheridia. Pyronema confluens.
5. The nuclei of the antheridia are not functional. Erysiphe Polygoni.
6. The antheridia are detached small spores known as spermatia. These attach themselves to the trichogynes. Sclerotinia Gladioli.
7. Antheridia are not formed. The nuclei of the oogonia fuse in pairs. Humaria granulata.
8. Both antheridia and oogonia are lacking. Union may take place between the nuclei of the two vegetative cells. Humaria rutilans.

Functional male and female organs are known in comparatively few of the Ascomycetes, and most of those in which they occur are monoecious; only a few species have been reported as being dioecious. The majority of the species are homothallic; few heterothallic species have been reported. Self-sterility and inter-sterility have also been observed.

REVIEW OF THE LITERATURE

The more important literature pertaining to the present investigation will be discussed under the following headings: General, Microconidia, Sexuality, and Other Phases.

General

Most writers credit Peck (42) as being the first to describe the brown rot disease in this country in 1881, but it undoubtedly was known long before. In 1883 Rau, in Pennsylvania, found apothecia growing on a decayed peach. Some of these were sent to Winter (55) who named the fungus Ciboria fructicola. Five years after Rau's discovery Woronin (56) demonstrated the connection between a Monilia (imperfect stage) and a Sclerotinia (apothecial stage) growing on Vaccinium vitis-idaea. In 1902 Norton (41) found apothecia of a species of Sclerotinia developing from peach mummies, and by cultural work he demonstrated beyond any doubt their connection with the common Monilia causing the brown rot disease of peaches. This discovery of Norton's was the most important contribution to our knowledge of the life cycle of S. fructicola. In a recent bulletin Roberts and Dunegan (45) summarize the previous work done on this disease, and its causal organism, and completely present our knowledge regarding it.

Microconidia

In general, microconidia are globose or pyriform spores, 2-4 μ in size, produced successively from small bottle-shaped sterigmata (conidiophores). They may be produced singly, in chains, in heads, and in other groupings.

Microconidia were first described in Discomycetous fungi

by Tulasne (51) in 1851, and in 1865 this author (52) reported the presence of microconidia on the mycelium of Sclerotinia tuberosa, S. Duriaeaana, and Peziza bolaris. In 1881 Brefeld (9) observed them on S. tuberosa and S. sclerotiorum. De Bary (5) reported them on S. sclerotiorum, S. Fuckeliana, and Sordaria coprophila, and Zopf (59) reported on the presence of similar structures on the mycelium of Chaetomium and various species of Sordaria. Between 1888 and 1900 Woronin (56,57,58) well described and figured microconidia on several species of Sclerotinia and he noticed them being produced on sterigmata arising either from the mycelium, the germ tubes of conidia and ascospores, or directly from the spores. He also noticed microconidia being produced directly from the conidia and ascospores. Although microconidia had been known to be present in the Laboulbeniaceae since the work of Karsten (39) in 1869, Thaxter (50) first well described them and discussed their function in 1896. The microconidia of S. fructicola were first described by Humphrey (36) in 1893. Since these early workers microconidia have been reported for a large number of Ascomycetous and other fungi.

Production of microconidia:

In 1870 DeBary and Woronin (6) described the production of microconidia as naked protoplasmic masses oozing out of the tips of the bottle-shaped sterigmata, but in a later paper (56) Woronin described them as being pinched off. However, the pinching off did not occur at the free end of the sterigma but in the base of a very small funnel which originated in such a way that the outer membrane of the sterigma at the top becomes reabsorbed and not somewhat

sunken as Zopf (59) had reported.

The only detailed account of the production of microconidia of S. fructicola is that of Jehle (38) who states: "At first the contents of the sterigmata are homogeneous but soon that of the tip begins to assume a granular appearance, and a little later many small oil drops may be distinguished. These gradually unite until at first a few, and finally only one oil globule is visible. Meanwhile, the tip swells and this globule migrates to the center and finally a cross wall is laid down. The microconidium is then pinched off."

In 1918 Brierley (10) gave a detailed account of the production of microconidia of Botrytis cinerea: "The sterigma becomes swollen at the tip, the cell membrane thinning out very distinctly in the neck region so that the spherical extremity which is surrounded by an extremely thin pellicle appears to be affixed to the end of the tapering sterigma. The thinning of the wall commences abruptly, and just at this point in the neck a transverse septum is formed, separating the spore from the sterigma. This septum splits along the middle lamella liberating the spore. The succeeding spore arises by pushing out the thin pellicle-----. Each spore is connected with those succeeding it by a short neck which represents the part finally cut off from the sterigma, so that although most usually it appears spherical it is in reality slightly pear-shaped."

The question of the mechanics of microconidial production is still an open one.

Structure of microconidia:

Brefeld (9) in 1881 reported the presence in microconidia of a refractive body that had the nature of an oil drop. Since

then, this body has been referred to by various authors as an oil drop, but without giving proof, and by others as a pearly or refractive body.

The first detailed account of the internal structure of microconidia is that of Beauverie and Guilliermond (7) in 1903 for Botrytis cinerea. They reported that the microconidia contain a single nucleus, difficult to distinguish, lying in a layer of peripheral cytoplasm; a large vacuole, occupying the greater part of the volume of the spore, which contains numerous metachromatic granules and abundant glycogen; and one or two metachromatic granules in the peripheral cytoplasm. Brierley (10), working with the same fungus, believed that what Beauverie and Guilliermond described as a vacuole is in reality a granule, more than one of which may be present. Brierley reported that the cytoplasm contains abundant glycogen and a single nucleus which is not easy to distinguish, and that the sterigmata are also uninucleate and contain glycogen. Drayton (21) observed that the microconidia of S. Gladioli contain a single cup-shaped nucleus which occupies about one third of the volume of the spore.

Germination of microconidia:

Few cases of germination of microconidia have been reported. Humphrey (36) and Jehle (38) have reported germination in S. fructicola, Brierley (10) in B. cinerea, Ramsey (44) in S. sclerotiorum, and Dodge (19) in N. sitophila. However, all these cases remain to be satisfactorily explained.

Factors affecting the production of microconidia:

There have been varying reports in the literature, too numerous for mention, as to the factors influencing the

production of microconidia. The most detailed of these reports is that of Brierley (10) who reported that temperature, light, humidity, and nutrition are not determining factors; the age of the culture being the determining factor. The consensus of opinion is that age of the culture and lack of nutrition are the determining factors for microconidial production.

Occurrence of microconidia in nature:

Although microconidia are common in old cultures on artificial media there have been few reports of their occurrence in nature. Humphrey (36) reported them being produced on mummies placed in damp chambers, and Jehle (37) found them on mummies brought into the laboratory and placed under various conditions. Drayton (21) found that microconidia were produced in sterilized soil cultures and concluded that these spores are probably formed in nature in the soil surrounding diseased plants.

Function of the microconidia:

Since the discovery of microconidia their function has been a matter of great speculation and controversy among mycologists; some considering them as functionless male cells, some as male spores, and others as spores of minute size. Tulasne (51) in 1851 prophesied that "sometime or other it would be demonstrated that there resided in them a certain force or nature like that of pollen." He regarded them as true male cells. In 1869 Karsten (39) described the attachment of microconidia to trichogynes in the Laboulbeniaceae. Working with Collema, a lichen, Stahl (48) in 1877 showed that the microconidia function as fertilizing agents.

De Bary (5) discussed in detail the question of the function of microconidia and he considered them to be male fertilizing organs in all the species which possess an organ (trichogyne or ascogonium) which may be intended to be fertilized. In 1896 Thaxter (50) clearly demonstrated that in the Laboulbeniaceae they are fertilizing agents. On the other hand Brefeld (9), and others, maintained that they were not fertilizing agents.

About 1900 the question of the function of the microconidia evidently lost interest, and it was not until after Craigie (16), in 1927, had demonstrated the sexual role of the pycniospores of the rusts that it was raised again. Whetzel (53), in 1929, advanced the theory that microconidia probably function as do the spermatia (pycniospores) of the rusts, and in 1932 Drayton (20), one of Whetzel's students, clearly demonstrated that the microconidia of S. Gladioli function as male cells. Drayton's paper was quickly followed by those of Ames (1) and Dodge (19) reporting on the sexual function of the microconidia in two other Ascomycetous fungi, Pleuraea anserina and Neurospora sitophila. Thus, it is apparently well established that microconidia function as male sperms and can be considered to be true spermatia.

Sexuality

De Bary (4) was the first to discover sex organs in the Ascomycetes. In 1863 he reported the presence of antheridia and oogonia in Sphaerotheca Humuli, and since then sexual organs in other Ascomycetous fungi have been reported by various investigators. Nuclear fusion, however, was first observed by Dangeard (17), in 1894, in young asci of Peziza

vesiculosa. In longitudinal sections of apothecia he noticed that some of the young asci were binucleate; the two nuclei then fused to form a large fusion nucleus. In tracing the origin of the asci Dangeard observed that they arose from two mycelial filaments, analagous to those of Eremascus and Dipodascus. However, he also observed that frequently the young ascus appeared to have a different origin in which a "crozier" is involved. This crozier is formed in the following manner: A filament (ascogenous hypha) reaches the ascigerous layer and its tip then bends back on itself. The bent portion then increases in length and becomes joined to the other portion. While this is going on the two nuclei in the bent region divide to form four. Septa are laid down between the tip and the stalk so that the bent region is separated. The stalk contains one nucleus, the tip one nucleus, and the bent region two nuclei. The two nuclei in the bent region fuse, and from this fusion the ascus develops. There are various modifications of the manner of crozier formation, as have been shown by Claussen (14) and others.

The year after Dangeard's discovery Harper (31) observed the development of an uninucleate antheridium and an uninucleate oogonium; he also observed the passage of the male nucleus into the oogonium and its fusion with the female nucleus. Ascogenous hyphae developed from the oogonium, and there was a second fusion of the nuclei/ascus hook ^{in the} (crozier). Thus, the fusion nucleus of the ascus should be tetraploid. This phenomenon, a fusion of nuclei in the oogonium followed by a second fusion in the ascus hook, has been observed in other species by various investigators:

Blackman and Fraser (8) for Sphaerotheca; Claussen (13) for Ascodesmis nigricans; Harper (32,33,34) for Erysiphe Polygoni, Pyronema confluens, and Phyllactinia Corylea; Gwynne-Vaughn and Williamson (27,28,29,30) for Pyronema confluens, Ascobolus magnificus, Lachnea scutellata, and Ascophanus Aurora.

Fusion of oogonial nuclei in the absence of functional antheridia has been reported by Fraser (23) for Lachnea stercorea, Fraser and Brooks (25) for Ascobolus furfuraceus, and Gwynne-Vaughn and Williamson (26) for Humaria granulata. In all these cases there is a second fusion in the ascus hook.

Fusion of vegetative nuclei (sex organs not morphologically defined), followed by a second fusion in the ascus hook, has been reported by Fraser (24) for Humaria rutilans and by Carruthers (12) for Helvella crispa.

In 1927 Tandy (49) reported an interesting situation in Pyronema domesticum. Ascogenous hyphae may contain both haploid and diploid nuclei, as some of the male and female nuclei that had not fused in the oogonium pass into the ascogenous hyphae. Thus, after the fusion in the ascus hook the fusion nucleus may be either diploid or tetraploid.

In all of the above cases, with the exception of the diploid nuclei in Pyronema domesticum, a double reduction in the chromosome number should take place in the ascus for the fusion nucleus is tetraploid. This process of double reduction is known as brachymeiosis, the reduction in the chromosome numbers occurring during the first and third divisions in the ascus. Brachymeiosis has been observed in Ascobolus magnificus (28), Ascobolus furfuraceus (25), Ascophanus Aurora (30), Humaria

rutilans (24), Lachnea scutellata (29), Pyronema confluens (27), and Pyronema domesticum (49). In two of the above species, Lachnea scutellata and Ascobolus furfuraceus, the second reduction in the chromosome number occurred during the second division. However, Harper (34) found that the chromosome number remained the same in all three divisions in the ascus in all the Ascomycetes he investigated, and he is of the opinion that the fusion of the nuclei in the ascus hook does not result in doubling the number of chromosomes.

Apogamous development of the sporophytic generation has been reported in several Ascomycetous fungi. In 1907 Dangeard (18), working with Erysiphe Polygoni, found that the male nuclei degenerate in the oogonia, and the nuclei of the oogonia do not fuse before passing into the ascogenous hyphae. The only nuclear fusion is that in the ascus hook. Winge (54) reported on a similar phenomena in Sphaerotheca Humuli. In 1912 Claussen (14) reported that in Pyronema confluens the male and female nuclei pair in the oogonia but do not fuse. They travel up the ascogenous hyphae in pairs and finally fuse in the ascus hook. Other species in which the only nuclear fusion is that in the ascus hook are Ascobolus immersus (43), Ascophanus carneus (43), Pyronema confluens var. irigneum (11), Ascobolus citrinus (46), Pustularia bolar-ioides (2), Ascobolus strobilinus (47), and Neurospora tetrasperma (15). In these species there should be only one reduction division in the ascus as the fusion nucleus is only diploid. This has been found to be the case in four of the above species--Ascobolus immersus, Pyronema confluens var. irigneum, Pustularia bolar-ioides, Neurospora tetrasperma--

in which chromosome counts have been made. The reduction in the chromosome number occurs during the first division.

There have been but two reports in the literature on cytological investigations of species of Sclerotinia. In 1927 Kharbush (40) reported on the development of the ascus, and its nuclear phenomena, of Sclerotinia Fuckeliana. In longitudinal sections of the apothecia he found binucleate cells in the ascigerous layer that arose from two mycelial filaments that had anastomosed at their tips. These binucleate cells were separated from the two mycelial branches by a septum. The nuclei in these binucleate cells fused to form the fusion nucleus of the ascus, and as a result of this fusion the ascus developed. In the three nuclear divisions in the ascus, resulting in the formation of eight ascospores, two chromosomes went to each pole of the spindle. A definite centrosome is present at each pole. After the ascospores are formed the nucleus divides to form binucleate spores. Kharbush states that the diploid chromosome number is 4 and the haploid number 2. Brachymeiosis did not occur.

In a very recent paper Drayton (21) reported on his preliminary cytological work on the nuclear history of the receptive bodies, apothecial fundaments, and apothecia of Sclerotinia Gladioli. The ascogonial coils in spermatized receptive bodies show a definite pairing of the nuclei, and these pass out into the ascogenous hyphae. The ascogenous hyphae are septate and as they develop they branch. In the young ascigerous layer Drayton found young asci with the two nuclei prior to fusion, the fusion nucleus, and the daughter nuclei resulting from the first division. He does not state whether the young

asci arise from croziers or the terminal anastomosis of two filaments, and he does not report on the chromosome numbers. However, he intimates that the asci arise from croziers.

Other Phases

The mycelium has been reported by various workers as being multinucleate and quite vacuolate, and the conidia as being multinucleate. Jehle (38) has reported that the nuclei divide just before the conidia germinate. Woronin (58), working with species of Sclerotinia closely related to S. fructicola, reported that the nuclei divide just before the conidia germinate and that as germination proceeds the nuclei pass out into the germ tube, the conidia becoming quite vacuolated. The ascospores have been described as being binucleate, and they may be septate when mature. The sclerotia have been well described by Honey (35). An excellent anatomical description of the apothecia has been given by Norton (41).

LIFE HISTORY OF SCLEROTINIA FRUCTICOLA

The primary infections in the spring are caused by ascospores discharged from apothecia produced by mummified fruits on the ground, and by conidia produced on the shrivelled fruits that have hung on the trees during the winter. These primary infections occur mainly on the blossoms of drupaceous fruits, such as, peach, plum, prunes and cherries. Twig infection is not common and the leaves are seldom, if ever, attacked. The mycelium of the fungus develops within the infected blossoms and then produces a crop of conidia. These conidia are the cause of secondary infections, principally on young fruits. As secondary infections take place throughout the growing season there is a constant supply of conidia as each new infection produces a crop. The disease is not very destructive on the fruits until they begin to ripen; if it is hot and humid at this time many of the fruits, even in sprayed orchards, become infected. The fruits rot and turn brown as the fungus develops within them, but they do not lose their shape. On the surface of the decaying fruits new crops of conidia are produced. Usually the decayed fruits fall to the ground, but some of them remain attached to the trees. Those that remain on the trees lose their shape and become shrivelled; those that fall to the ground retain their shape somewhat as the fungus develops a sclerotium, a hard rubbery layer of fungus mycelium intermixed with the peach cells. In the late winter and early spring apothecia arise from the under side of these mummies. On the inner surface of the apothecial cups a layer of asci ^{is} ~~are~~ produced and within these ascospores are formed, usually eight to each ascus. These spores are discharged at the

time the trees are blooming.

Microconidia have only been reported on the mummies in the normal life history of this fungus. However, in artificial culture microconidia have been produced on the mycelium, on the ascospores, and on the conidia.

The production of apothecia by over-wintered mummies indicates that a sexual act takes place sometime after the fruits become infected. However, the sex organs have never been found.

MATERIAL AND METHODS

Isolates of S. fructicola were obtained from various regions in Maryland, but as there was a wide variation in the cultural characteristics of the various isolates the type isolate, variety I of Ezekiel (22), was selected for study. This isolate was obtained at Hancock, Md. The cultures were maintained at room temperature in the laboratory on potato dextrose agar. Ascospores were obtained from apothecia developing at College Park, Md.

Staining work on microconidia, conidia, ascospores, and germ tubes was carried out by making water mounts on slides having either a thin film of albumin or 2 per cent agar to affix them to the slides. Fleming's weaker solution and Formalin-Acetic-Alcohol were used as killing reagents. Various stains were employed. Germination studies were carried out in liquid mounts on slides in damp chambers at a temperature of 23° C.

Apothecia in various stages of development were collected during the late winter and early spring on over-wintered mummies. They were fixed and killed, under suction, in Fleming's medium solution at various times during the day and night, washed in running water, dehydrated, embedded in paraffin, sectioned, and then stained with iron-alum-haematoxylin. In the early part of the work serial longitudinal sections were cut 10 μ thick but these proved unsatisfactory, so in the later part of the work sections were cut 3-7 μ thick.

Due to the minute size of the structures investigated all detailed microscopic studies were made at a magnification of 1350, using a standard Spencer microscope equipped with a Zeiss 1.30 apochromatic objective and a Zeiss 15x compensating ocular. Artificial light was employed.

EXPERIMENTATION AND RESULTS

MYCELIUM

Vital staining with Neutral Red (1-10,000) showed that the mycelium, with the exception of the growing tips, is very vacuolate. The cytoplasm is scanty and occupies a small space along the walls. The growing tips contain a large amount of dense cytoplasm in which small vacuoles are present. The color reaction (magenta), and the fact that precipitates are formed, indicates that the vacuoles are relatively acid (3).

Staining with iron-alum-haematoxylin demonstrated that the cells are multinucleate. The nuclei are very small and contain a central rounded nucleole surrounded by a band of clear nucleoplasm. The presence of nuclei lying side by side in the growing tips showed that division recently occurred. Although the mycelium was tested at various times during the day and night no nuclei in the process of division were found.

CONIDIA

Vital staining with Neutral Red (1-10,000) demonstrated that the spores are quite vacuolate; one or two large vacuoles may be present or there may be numerous small ones. The reaction of these vacuoles to the stain is similar to that of the vacuoles in the mycelium. As the spores germinate some of the smaller vacuoles pass out into the germ tubes, and as germination proceeds the spores become more and more vacuolate. The germ tube is vacuolate from its inception, several small vacuoles being present in its tip. As the germ tubes increase in length the portion behind the growing points becomes more and more vacuolate.

Sowings of spores were made in drops of the stain and

the cultures were then placed in moist chambers held at a temperature of 23° C. Many of the spores germinated and after 24 hours several germ tubes 90-100 μ long were found. Thus, a study of the vacuoles can be made in stained living germ tubes.

For the study of the nuclei the following method was pursued: Spores were sown in drops of distilled water, tap water, and 5 per cent sucrose solution on slides covered with a thin film of albumin or 2 per cent potato dextrose agar. These slides were then placed in moist chambers and incubated at 23° C. At the desired time the spores and germ tubes were killed, washed, and then stained in iron-alum-haematoxylin, the slides being handled as though they contained paraffin sections. Various killing and fixing solutions of different strengths were tested and the Formalin-Acetic-Alcohol combination (100 cc. 50% alcohol, 10 cc. formalin, and 10 cc. Acetic acid) was found to be the most satisfactory as it caused very little plasmolysis. Killing was done at hour intervals for a period of 24 hours. Several repetitions were made.

The nuclei are very small and contain a central rounded nucleole surrounded by a band of clear nucleoplasm. As high as 16 nuclei have been counted in an ungerminated spore. As the spores germinate some of the nuclei pass out into the germ tubes. No division of the nuclei was observed in the spores or young germ tubes. Three cases of nuclear division were observed in the tips of germ tubes 50 μ long or longer. These cases were on two slides of the same series that had been killed at 3:30 a.m. Each case apparently was an early anaphase. The haploid chromosome number apparently is 4, but accurate counts were impossible due to the minute size of the structures.

ASCOSPORES

Vital staining with Neutral Red (1-10,000) demonstrated the presence of vacuoles in the spores; several large vacuoles may be present or there may be numerous small ones. The germ tubes are vacuolate from their inception, and as they increase in length the portion behind the growing tips becomes more and more vacuolate. The reaction of these vacuoles to the stain is similar to those present in the conidia and their germ tubes and in the mycelium.

The nuclei in the ascospores and germ tubes were stained following the same method used for the conidia. The nuclei are very small, approximately $1\ \mu$ in size, and contain a central round nucleole surrounded by a band of clear nucleoplasm. They are similar to those in the mycelium, the conidia, and the conidial germ tubes.

Mitotic figures were found in material killed at various times during the day and night but they were too minute for study.

SCLEROTIA

The sclerotia are composed of a dense layer of fungus hyphae mingled with the cells of the fruit. On the outside the fungus hyphae are dark colored and thick-walled, while those on the inside are larger, thin-walled, and lighter colored. The hyphae contain numerous oil drops.

MICROCONIDIA

In nature:

Naturally infected mummies of the 1933 peach crop overwintering on the ground in the orchard were brought into the laboratory on January 18, 1934, and immediately examined.

Microconidia were abundant on the surface of these mummies. Further collections were made on February 18 and March 12 and microconidia were found to be abundant on the surface of the mummies.

Factors affecting the production of microconidia in cultures:

(1) A series of plates of 2 per cent potato dextrose agar 1, 5, 10, and 15 weeks old, each adjusted to pH 5.6 before pouring, were inoculated with conidia and then placed at room temperature (23° C.). These were examined daily with a binocular microscope for the presence of microconidia. No microconidia were visible until 12 days after inoculation. At this date they were present in all the cultures. Microconidial masses were visible in all the cultures 25 days after inoculation, being as abundant at the edge of the plates as in the center. Thus, the age of the agar was not the determining factor.

(2) Tubes of freshly made 2 per cent potato dextrose agar, adjusted before sterilization to pH 3.3, 4.1, and 5.5, were inoculated with conidia and then placed at room temperature (23° C.) in the light, in darkness, and in a constant temperature chamber at 26° C. Microconidia appeared at practically the same time in all the cultures. Thus, light and temperature were not determining factors.

(3) Tubes/and 250 cc. flasks of freshly made 2 per cent potato dextrose agar and 2 per cent plain agar, adjusted to pH 4.1, 5.5, and 7.9 before sterilization, were inoculated with conidia of 5 isolates that produced microconidia abundantly. The cultures were placed at room temperature (22° C.) in diffuse light. When the cultures were 3 days old half were placed at 2° C. and left for three days at this temperature. When the cultures

were 6 days old they were examined microscopically. No microconidia were present in any of the flask cultures, in any of the tube cultures of 2 per cent potato dextrose agar kept at room temperature, in any of each agar chilled for 3 days, but were present in considerable numbers in all the tubes of plain agar kept at room temperature.

When the cultures were 21 days old they were examined visually for the presence of microconidial masses. Only 2 of the 15 flasks of potato dextrose agar contained masses, while 9 of the 15 flasks of plain agar had a considerable number of visible masses; only 2 of the 11 tubes of potato dextrose agar had masses, while 6 of the 8 tubes of plain agar contained numerous masses. Of the tubes that were chilled 3 days 6 of the 12 tubes of potato dextrose agar and 9 of the 15 tubes of plain agar had visible masses.

There was no pronounced difference in the 5 strains as regards microconidial production. The production of microconidia was somewhat better at pH 5.5 and 7.9 than at 4.1.

The results of this experiment indicate strongly that lack of nutrition is the determining factor for the production of microconidia.

(4) In drop cultures of conidia in tap water, distilled water, and .5 per cent sucrose solution microconidia were produced earlier, and more abundantly, in tap and distilled water than in the sucrose solution. They appeared in distilled water after 30 hours, in tap water after 40 hours, and after 65 hours in the sucrose solution. Several repetitions were made with similar results.

The results of these experiments show that lack of nutrition is the principal factor influencing microconidial production.

(In the sucrose solution after 48 hours the young hyphae produced clusters of short, thick, pointed side branches . These clusters have been described as "stag horns". They may be the start of sclerotia, or they may be female sex organs.)

Method of production:

Germinating conidia and ascospores in drop cultures were examined at hourly intervals to trace the development of the microconidia. In tap and distilled water microconidia were produced on sterigmata arising from young hyphae, from germ tubes, and directly from the spores; microconidia were produced directly from the spores without a sterigma in some cases.

The formation of the microconidia was observed under oil immersion. The tip of the sterigma swells and a portion of the protoplasm of the sterigma moves along with this swelling. As the tip of the sterigma continues to bulge the neck below it becomes quite narrow. A round refractive body present in the center of the sterigma was observed to move up thru the neck into the young spore. While the protoplasm of the sterigma and spore are still continuous the spore contains a large oil drop and a fairly large crescent-shaped body. From this point on the details of spore formation were obscure as a collar is present at the tip of the sterigma that somewhat obscures the formation of the septum that separates the spore from the sterigma. This septum, however, has been seen and it is in the neck of the sterigma below the collar. Thus, the sterigma is a tube. Succeeding spores are produced in a similar manner, and chains of 10 spores have been seen. The

basal spore in a chain is the youngest.

Sterigmata bearing one spore when stained with iron-alum-haematoxylin showed the following phenomena: A stained mass in the spore, a stained mass just below the neck, a stained mass in the center, and one at the base. Mitotic figures have not been seen.

Structure:

The microconidia of S. fructicola are globose to subpyriform, 2.1-4.6 μ in size, with a flat or slightly concave base where they fit onto the following spore in the chain (Pl. I, Figs. 1-3).

As the unstained spores begin to come into focus (xl350) a pearly spherical spot is seen which appears to be light strongly condensed by the spore or especially the most conspicuous body found in the first clear focus, usually a sphere extending over half the diameter of the spore (Pl. II, Row 5, 1st, 4th, 6th drawings); the rest of the contents appear as a rather hyaline crescent within the rather thick outer wall. With a slight change in focus downward the sphere disappears, or becomes hyaline, and the crescent-shaped body comes into clear focus (Pl. II, Row 5, 7th drawing). This arrangement may be reversed and, of course, variations exist, but when one body is in focus the other is not. Each body in its clearest focus appears to be colored ^{a dilute} yellowish-brown.

Staining with chlor-zinc-iodide, or mounting in India Ink, demonstrates the presence of a wall that is approximately 0.5 μ thick (Pl. II, Row 7). When stained with Sudan III, or Scarlet Red, the sphere, or spheres, takes up the stain and becomes colored orange-red showing that it is an oil or fat globule (Pl. II, Row 6). Three oil drops may be present.

Microchemical tests for fat and oil substantiate the staining reactions. Staining with Neutral Red demonstrates the presence of a large vacuole or 2, 3 or more smaller ones. (Pl. II, Row 4).

Staining with iron-alum-haematoxylin demonstrates the presence of a body in the spores that has a blackish-blue coloration indicating that it is nuclear material. This body is generally crescent or comma-shaped and is curved against the wall; there is considerable variation in the shape of these nuclear bodies. (Pl. II, Rows 1-2). Fleming's triple stain shows this nuclear material as an oblong or fluke-like body curved against the cell wall, appearing quite like the sperms of some organisms (Pl. II, Row 3)

Germination of microconidia:

Microconidia were sown in drop cultures of various media, such as; distilled water, tap water, potato decoction, peach and plum decoctions, hydrogen peroxide, and various nutrient solutions. The cultures were subjected to temperatures ranging for 2° C. to 38° C., and they were also subjected to light and darkness. Examinations were made at hourly intervals for the first few days and at daily intervals thereafter for two weeks or more.

No germination was observed in any of the cultures.

APOTHECIA

During three seasons--1932, 1933, 1934--apothecia were collected and a study made of their development. The results of these investigations will be recorded under the following three headings: Gross Morphology, General Histology and Cytology, and the Cytology and Development of the Ascus.

Gross Morphology

The first external sign of apothecial development is the presence of numerous minute shining dots, less than half a millimeter in diameter, on the under side of the mummies. These often occur in the orchard as early as the first part of February. These small dots develop into small black horn-like structures, the apothecial fundamentals, out of which the apothecia arise. As development continues the tips of these little horns become light brown in color. This stage is usually reached about about the middle of February. The apothecia continue to develop upward from the under side of the mummies until they reach the light. They now develop a bulb-like head region. This stage usually occurs during the first half of March. However, even before the bulbs develop the young apothecia have a depression in their tips, and it is the region surrounding this depression that develops into the bulb. About the middle of March the apothecia are divided into two parts, a small head region and a slender stipe. After the heads are formed there is usually not much further increase in length of the stipes, the length of the stipes depending on the distance that the young apothecia have to grow to reach the light. The heads increase in size and they become cup-shaped. This stage is usually reached by the first of April. The lips of the cups now bend outward

until finally the heads are shaped like a bell or deep saucer, or sometimes even disc-like. (Frontispiece). The apothecia are now mature and are ready to discharge their spores. This stage is usually reached at the time the trees are in bloom, usually during the first half of April.

The development of the apothecia depends upon suitable moisture and temperature conditions. Thus, the time of development varies from year to year. The first mature apothecia were found on April 6, in 1932, on April 7, in 1933, and on April 13, in 1934. All the apothecia do not develop and mature at the same time, so that each year the period of spore discharge extends over a period of time.

General Histology and Cytology

The apothecial fundaments arise just below the surface of the sclerotium and in the youngest stage studied, the small black shining dot stage, the regions of origin roughly resemble minute bulbs. A detailed search was made for the presence of sexual organs (microconidia, antheridia, and oogonia) in these regions. In only one region was anything resembling a sexual organ found. A cut hypha was found which was larger than the surrounding hyphae and it contained many small nuclei. Lying close to this were cut portions of other hyphae of approximately the same size and these also contained many small nuclei. The general appearance of this particular region was like that of the ascogonial coils in Fig. 4,A, and Plate 7, Fig. D, of Drayton's paper (21).

In the regions of origin nuclei of two distinct types were found. These will be designated as Type 1 and Type 2.

The hyphae containing each type are 3-4 μ wide, septate, branched, and multinucleate, but the nuclei of Type 2 are not as abundant in the hyphal cells.

Type 1: Approximately 1 μ in diameter, containing a large round central nucleole surrounded by clear nucleoplasm (Pl. IV, Fig. 3, left).

(These nuclei are the same as those present in the ascogenous hyphae, mycelium, conidia, and ascospores)

Type 2: Approximately 2 μ in diameter, containing one, two, or three small round nucleoles lying against the nuclear membrane. The nucleoplasm is slightly more dense than in Type 1 (Pl. IV, Fig. 3, right).

(These nuclei are the same as those present in the paraphyses)

Both types of nuclei are found intermixed at the base of the bulb but in the tip region Type 1 is most abundant, only very few of Type 2 being present.

As the tip region of the bulb grows upward a piece of the sclerotial crust often becomes embedded within the growing body. This has been figured by previous investigators. The tips of the young growing apothecial fundaments are always more or less pointed, and the hyphae in this region are smaller than those below. In all regions of the developing apothecial fundaments both types of nuclei were found-- at the tips (Pl. IV, Fig. 1), in the middle region (Pl. IV, Fig. 2), and in the region of origin (Pl. IV, Fig. 3). As the apothecial fundaments continue to increase in size a difference becomes noticeable in the cells. Hyphae containing nuclei of Type 1 are mostly present only in the central region intermixed with hyphae containing nuclei of Type 2. These are surrounded by a sheath of hyphae that have shorter cells with thicker walls. The nuclei in these hyphae are of Type 2.

At this point the apothecial fundaments have the same general structure as the mature apothecia.

Before the apothecia reach any considerable size a depression appears in their tips. The hyphae lining this depression are considerably smaller than the other hyphae, and they have nuclei of Type 2. The depression is closed. As the apothecia continue to grow a head region develops around this depression. The depression remains closed and is almost completely lined by a layer of long narrow hyphae. These are the young paraphyses and they have nuclei of Type 2. Differentiation of the stipe into a sheath of hyphae having small thick-walled cells that ~~s~~^urrounds a central core of hyphae is now complete. Continued growth of the head region causes a rupture, or separation, of the sheath cells at the top of the depression (Pl. V, Fig. 1). At this stage the paraphyses are well developed; they are long and narrow, slightly swollen at their tips, sparsely septate, and they may be branched in their basal regions. They are multinucleate and the nuclei, Type 2, lie in a row.

At this stage, the opening of the head or cup, hyphae having Type 1 nuclei can be seen pushing their way up between the bases of the paraphyses (Pl. V, Figs. 2-3). Their cytoplasm is considerably more dense than that of the paraphyses. These are the ascogenous hyphae. The differences between the nuclei of the paraphyses and the ascogenous hyphae is well shown in Pl. V, Fig. 3, and Pl. IV, Fig. 4. The nuclei of the ascogenous hyphae, Type 1, are 1.0-1.5 μ in size while those of the paraphyses, Type 2, are 1.5-2.0 μ in size. The tip cells of the ascogenous hyphae are binucleate; no pairing of the nuclei is evident.

The attachment of the ascogenous hyphae and paraphyses to hyphae having nuclei of Type 1 and Type 2, respectively, in the tangled mass of hyphae below them was traced. This was quite difficult due to the small size of the intertwined hyphae.

The apothecia have now reached their final stage of development, increase in size of the head and development of the asci, and are divided into several distinct regions. The outer part of the cup and stipe is composed of a sheath of compact hyphae that have short, thick-walled cells. The inner surface of the cup is lined by the paraphyses and young asci, the hymenium. Adjoining the hymenium below is the sub-hymenium; the region where the paraphyses and asci have their origin. Below the sub-hymenium is the hypothecium; the tangled mass of small hyphae. The hypothecium arises from the hyphae in the central region of the stipe; these hyphae have longer, wider cells with thinner walls than the sheath hyphae. This region may be called the core, and it is composed of both ascogenous and vegetative hyphae. Rhizoids may be present on the sheath in the basal part of the stipe.

As the heads develop into the cups the asci push upward between the paraphyses until they reach the same height. Three nuclear divisions now occur in each ascus and, finally, eight spores are formed in each ascus. While the asci are developing the paraphyses apparently disintegrate, for when the apothecia are mature relatively few paraphyses are present compared with the immense numbers that were present before the asci commenced development. The detailed development of the asci from the ascogenous hyphae will be presented below.

Cytology and Development of the Asci

The first indication of ascus development is the appearance of ascogenous hyphae in the sub-hymenium at the time the head is opening. These hyphae are approximately 2 μ wide. The cells are binucleate but the nuclei are not paired, they are separate (Pl. IV, Fig. 4; Pl. V, Figs. 2-3).

From these ascogenous hyphae croziers develop (Pl. VI, Fig. 1; Pl. IX, Figs. 1-7). The actual development of the croziers was not observed, but from their appearance they apparently were formed in the usual manner, which is as follows: The binucleate tip cell of the ascogenous hypha bends over and the two nuclei pass into the bent region and there divide simultaneously to form four nuclei. Two cross walls are then laid down and the crozier is divided into three regions; (1) the tip (ultimate) cell that contains one nucleus, (2) the bent (penultimate) cell that contains two nuclei, and (3) the stalk (antipenultimate) cell that contains one nucleus. The bent (penultimate) cell develops into the ascus. The croziers in S. fructicola are very minute and are thus difficult to distinguish, but hundreds have been observed.

After the croziers have been formed the next step is the fusion of the two nuclei in the bent cell. This fusion was observed, and the various steps in the process are shown in Pl. IX, Figs. 1-9. Figs. 1-2 show the appearance of the crozier just after it is formed, and the two nuclei in the bent cell are distinctly separate. In Fig. 3 the two nuclei have approached one another, and in Fig. 4 they are almost in contact. Fig. 5 shows the two nuclei in contact; the nuclear membranes at the point of contact have disappeared. In

Fig. 6 a slightly later stage is shown where the nucleoles have begun to fuse. In Fig. 7 is shown the large fusion nucleus that results from this nuclear fusion. Fig. 7 also shows that the tip and stalk cells have fused, and that the nucleus of the tip cell apparently has migrated into the stalk cell. After the fusion of the two nuclei the bent cell elongates rapidly and the fusion nucleus increases in size and moves upward in the young ascus. This is shown in Figs. 8-9. The young fusion nucleus is 2.5-3.0 μ in diameter and is characterized by having clear nucleoplasm in which lies a large central nucleole that occupies at least two-thirds of the nuclear area. (Pl. VI, Fig. 2).

Crozier's can fairly easily be demonstrated by smearing a portion of a young hymenium on a slide and staining with .02 per cent Cotton Blue in Amman's solution. Unfortunately, the Cotton Blue does not differentiate the nuclei. By this method, and also in sectioned material, it was observed that occasionally more than one ascus may arise from the same ascogenous hypha. Aceto-carmin was also used for staining crushed mounts but proved unsatisfactory.

The cytoplasm of the young asci is very dense, but as the asci elongate it becomes less and less dense and vacuoles appear (Pl. X, Figs. 10-11). The tips of the young asci at first are rather narrow and pointed but as they elongate they become rounded; finally, the asci are long clavate-cylindrical, tapering gradually in their lower half to their bases. During their elongation the granular cytoplasm tends to become aggregated toward the top, especially as the asci age, and the lower portions become practically devoid of stainable

contents (Pl. VII, Fig. 9; Pl. X, Fig. 18). The asci continue to elongate until they reach the same height as the paraphyses. A pore is present at the apex of the ascus (Pl. VII, Fig. 9; Pl. XI, Figs. 19-22). The fine granular cytoplasm, the sporeplasm, is now congregated in the upper portion and it is rather sharply delimited from the vacuolate region below it, the line of demarcation usually occurring at the region where the asci begin to taper (Pl. VII, Fig. 9; Pl. X, Fig. 18). In some asci the vacuolate region invaginated the sporeplasm for some distance. In many asci deep staining small bodies of unknown nature are present (Pl. X, Figs. 10-11). When the asci have reached their full growth they are approximately 125-175 μ by 7-10 μ .

As the asci elongate the fusion nucleus moves upward in the cytoplasm, and it seems to maintain a central position in the elongating asci both as regards length and width. When the asci have reached their final height the fusion nucleus is either in the center or slightly above the center. While it is moving upward several changes in its appearance occur. At first it just continues to increase in size, but when the asci are approximately half grown a few chromatin strands can be seen attached to the nucleole (Pl. X, Fig. 10). At this stage the asci are 4.5-5.0 μ wide, the fusion nuclei are approximately 4 μ in diameter, and the nucleoles are approximately 2.5 μ in diameter.

When the asci are approximately two-thirds grown the fusion nuclei have a distinct nuclear network (Pl. VI, Fig. 2; Pl. X, Fig. 11). This apparently has the form of a thin thread, or threads, winding within the nuclear cavity. Where portions of the thread, or threads, cross there is a deep

stained bead-like region. In many nuclei it was observed that many of these deeply stained beads were present in segments that did not cross. A definite spireme thread was observed in but few cases (Pl. VI, Fig. 3; Pl. X, Fig. 12). The resting nucleus stage is quite protracted in time as it exists from the time the asci are approximately half grown until they have reached their final height.

The fusion nucleus does not undergo division until the asci have, or almost have, reached the same height as the paraphyses. Only one metaphase stage was seen (Pl. X, Fig. 13). In this, the chromosomes had not yet been completely developed and their double nature is evident. Twelve anaphase stages were found at the following times: 10:15 a.m., 5:00 p.m., 10:00 p.m., 1:00 a.m., and 2:30 a.m. The division figures are very small. A definite spindle, 4-5 μ long, is present. At each end of the spindle is a deep stained centrosome, approximately 1 μ in size (Pl. VI, Figs. 3-5; Pl. X, Figs. 14-15). In one figure astral rays radiated out into the cytoplasm from one of the centrosomes (Pl. X, Fig. 14). The spindles lie diagonally in the asci. Eight chromosomes are present on the spindle, 4 moving toward each pole. The chromosomes are very minute, being smaller than 0.5 μ . No telophase stages were found. The nuclear membrane disappears during division, but the spindle is surrounded by a small clear area. In some of the division figures the nucleole was found to one side of the spindle, but in the majority it could not be identified.

The two daughter nuclei become rather widely separated (Pl. VII, Fig. 6; Pl. X, Fig. 16). At first they are small but they increase in size until they are approximately 4 μ in size. They have the same general structure as the fusion

nucleus. Only 2 asci in the two-nucleate stage were found, and in only one ascus were second division figures found. These figures are similar to those of the first division; the spindles are approximately the same size and they lie diagonally in the ascus. In the anaphase stage 8 chromosomes are present on the spindle, 4 moving toward each pole (Pl. VII, Fig. 7; Pl. X, Fig. 17).

After the second division the asci contain 4 nuclei, and many asci in this stage were seen (Pl. VII, Fig. 8; Pl. X, Fig. 18). The nuclei are not as widely separated as in the two-nucleate stage. They are very small, approximately 2 μ , and have a large central nucleole surrounded by a thin band of clear nucleoplasm. These nuclei have never been seen to contain the chromatin beads present in the one-nucleate and two-nucleate stages.

The 4 nuclei divide simultaneously during the third division. Six asci having third division figures were found at 1:00 a.m., 2:30 a.m., and 10:15 a.m. The figures are the same, in general, as those in the first and second divisions. The spindles are very small, approximately 3-4 μ long, and they generally lie diagonally in the ascus. In the metaphase stage four chromosomes are present. During the anaphase stages 8 chromosomes are present on the spindle, 4 moving toward each pole (Pl. VII, Fig. 9; Pl. VIII, Fig. 10; Pl. XI, Figs. 19-20). The chromosomes are very minute, being approximately 0.3 μ in size, and some of them are hardly distinguishable. No telophase stages were found.

The eight daughter nuclei now present in the asci are approximately 2 μ in size and they lie fairly close together (Pl. VIII, Fig. 11; Pl. XI, Fig. 21). They are similar to the nuclei in the four-nucleate stage.

The process of spore formation could not be clearly seen but the nuclei cut out eight spores by the process of free-cell formation. The first indication of spore development is the presence of an arrowhead-shaped structure near each nucleus. This, in all probability, is the beak of the nucleus but no astral rays could be seen radiating out into the cytoplasm from it. The cytoplasm about each nucleus gradually becomes more dense and then a membrane appears surrounding the mass of cytoplasm. At this stage (Pl. VIII, Fig. 12; Pl. XI, Fig. 22) a deep stained twisted line can be seen running from the membrane toward the nucleus in the spore. This line, apparently, is the remains of the beak of the nucleus.

At first the spores are round to oval-shaped bodies (Pl. XI, Fig. 23) but as they develop they become ellipsoid to ovoid with rounded ends. Finally, the nucleus in each spore divides once so that the mature spores are two-nucleate. This division has not been found. No two-celled spores were found. The mature spores are arranged monostichously in the asci, but just before they are discharged they may become irregularly arranged.

DISCUSSION OF RESULTS

Nuclear Types

The nuclei in the conidia, ascospores, and the mycelium arising from them, have the same structure, and they are similar in structure with the nuclei in the ascogenous hyphae in the apothecia. These nuclei, Type 1, differ widely in structure from those in the paraphyses, Type 2, and in the hyphae from which they arise. The reason for this difference is not known. As the conidia, ascospores, ordinary hyphae, and paraphyses are vegetative structures it would be expected that they would have the same type of nucleus.

The microconidia, which in all probability are spermatia, have nuclei which differ in structure from the above mentioned types. Other workers have observed that the male nuclei in various fungi differ in structure from the female nuclei. In view of this, the nuclei in the ascogenous hyphae were studied in detail to determine if the male, microconidial, nucleus could be identified. However, no differences in the nuclei were observed.

Microconidia

The results of the experiments testing the effect of light, temperature, age of the medium, lack of nutrition, and pH show that the chief determining factor for the production of microconidia is lack of nutrition. In all the experiments they appeared first in the cultures where nutrition was limited. These results are in accord with those of several previous investigators working with microconidia of other fungi. The reaction of the medium has some affect on the abundance of production as microconidia are rather sparsely produced on media having a high acid reaction.

However, microconidia have been produced from pH 3.3 to pH 7.9.

The microconidia apparently are produced on the sterigmata in the same manner as reported by Brierley (10) for Botrytis cinerea. They are not pinched off the end of the sterigma. The sterigmata are open tubes that have a collar at their tips, and below this collar is a narrower neck region. In this neck region a septum is laid down that separates the spore from the rest of the contents of the sterigma; the contents of the spore are continuous with that of the sterigma until this septum is laid down.

The structure of the microconidia is essentially the same as that of the microconidia of B. cinerea as reported by Beauverie and Guilliermond (7). They generally contain a single nucleus, a large vacuole, and a large oil drop. However, there may be several small vacuoles and two or three oil drops present, but there is never more than one nucleus. Brierley (10), working with B. cinerea, believed that the vacuole reported by Beauverie and Guilliermond was in reality a granule, more than one of which may be present. However, the present work clearly shows that a vacuole, or several vacuoles, is present in the microconidia of S. fructicola. The granules reported by Brierley are probably oil drops.

Drayton (21) reported that in S. Gladioli the microconidial nuclei are cup-shaped bodies occupying about one third of the spore volume. The majority of the nuclei of the microconidia of S. fructicola are crescent-shaped or comma-shaped bodies lying against the spore wall, but there are wide variations in the shape of the nuclei in many of the spores.

In all the experiments tried no germination of the microconidia was ever obtained. Humphrey (36) and Jehle (38), working with the same species, reported germination, but their work is open to question as they did not actually observe the process of germination. The majority of investigators that have attempted to germinate microconidia of various species have reported negative results.

The fact that microconidia are present on mummies during the late winter at the time the apothecial fundaments are developing may indicate that they spermatize these structures. Another fact supports this theory and that is that all the apothecial fundaments do not develop into apothecia; as high as 675 apothecial fundaments have been counted on one mummy but rarely over 25 fully developed apothecia ever appear on a mummy.

Sexuality

Drayton (21) found that the nuclei in the ascogenous hyphae of S. Gladioli were paired. In the ascogenous hyphae of S. fruticola the nuclei are not paired.

The origin of the asci of S. fruticola are the croziers formed by the tip cells of the ascogenous hyphae. This is radically different from the situation in S. Fuckeliana reported by Kharbush (40). This author states that the asci developed from binucleate cells that arose from two mycelial filaments that had anastomosed at their tips. However, in view of our present knowledge, especially of the function of the microconidia, Kharbush's conception of the origin of the asci is evidently a misinterpretation.

In the 3 nuclear divisions in the asci 4 chromosomes are present at the metaphase plates in all 3 divisions, and during

the anaphase stages in all 3 divisions 4 chromosomes move to each pole. The haploid chromosome number is 4, and the diploid must be 8. The reduction in the chromosome number must occur during the first division. As there is no double reduction in the chromosome number the fusion of the nuclei in the ascus hook must be the only nuclear fusion.

Kharbush (40) reported the diploid chromosome number to be 4 and the haploid number to be 2 in S. Fuckeliana. There was no double reduction in the chromosome number. Thus, in the only 2 Sclerotinias yet thoroly examined, the fusion of the nuclei in the young ascus is the only nuclear fusion.

The above facts are of great interest for the reason that S. Fuckeliana has a Botrytis as its asexual stage while S. fructicola has a Monilia. The writer has already started work on other species of Sclerotinia to obtain data on the nuclear phenomena, especially the chromosome numbers.

Evidently there is no daily periodicity in nuclear divisions in S. fructicola for meiotic and mitotic figures have been found in apothecia killed at various times during the day and night.

SUMMARY OF RESULTS

1. The microconidia (spermatia?) are globose to subpyriform, 2.1-4.6 μ in size, with a flat or slightly concave base where they fit onto the following spore in the chain. They have a thick wall and contain a single nucleus, generally crescent or comma-shaped, that is curved against the wall, one (or more) vacuole, and one (or more) oil drop.

2. The microconidia are produced in chains on bottle-shaped sterigmata which are tubes that have a collar at their tips below which is a narrower neck region. The microconidia are not pinched off from the tips of the sterigmata but are separated from the sterigmatal contents by a septum that develops in the neck region.

3. The chief determining factor for the production of microconidia is lack of nutrition, but the reaction of the medium has some affect on the abundance of production. Light, temperature, and age of the agar are not determining factors.

4. No germination of the microconidia was obtained.

5. Microconidia are present on the surface of overwintering mummies.

6. Three distinct types of nuclei were found.

- (a) The microconidial type.
- (b) The ascogenous hypha type.
- (c) The paraphysal type.

7. The apothecial fundaments originate just below the surface of the sclerotia.

8. The asci develop from croziers formed by the tip cells of the ascogenous hyphae.

9. There is no double reduction in the chromosome number in the three divisions in the asci; the only reduction occurs

during the first division. Thus, the only nuclear fusion is that of the two nuclei in the croziers.

10. The haploid chromosome number is 4.

11. There is no daily periodicity in the nuclear divisions in the asci.

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EXPLANATION OF PLATES

All drawings, with the exception of those in Plate I, were made with the aid of an Abbe camera lucida and were drawn under a Zeiss 1.30 apochromatic objective with a Zeiss 15x compensating ocular. Magnification approximately 2300 diameters. The photomicrographs were taken at various magnifications.

Plate I

Figs. 1-2. Attachment of microconidia in chains. Stained to show the thick cell wall. x9000.

Fig. 3. Bases of microconidia showing their slightly concave nature. x9000.

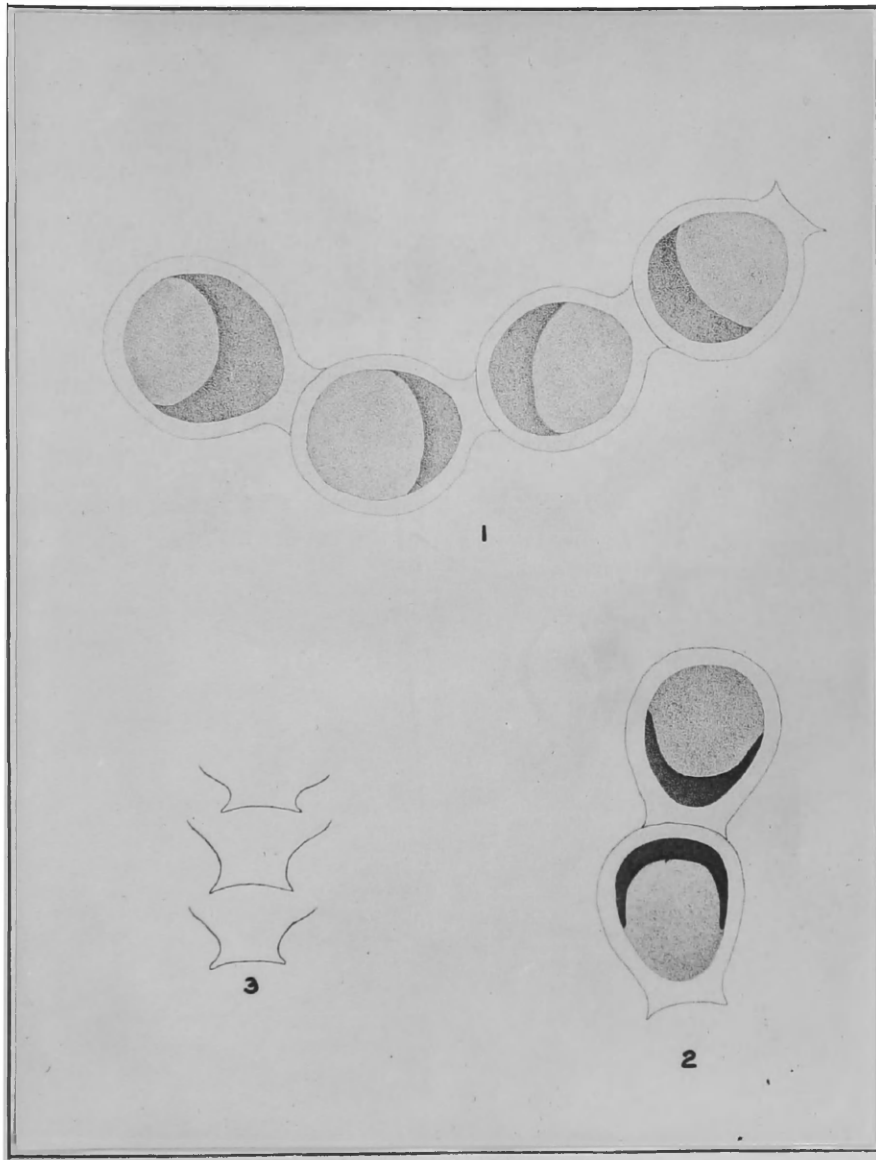


Plate II

Rows 1-2. Nuclear structure of microconidia. Stained with iron-alum-haematoxylin. The nuclei in the 3rd and 7th drawings in Row 1 are the most typical. x1725.

Row 3. Nuclear structure of microconidia. Stained with Fleming's triple stain. The 1st, 4th, 5th, and 6th drawings show the most typical nuclei. x1725.

Row 4. Vacuoles of microconidia. Stained with Neutral Red 1-10,000. x1725.

Row 5. Unstained microconidia. Oil drops are shown in the first 5 drawings. The last 2 drawings show the oil drops out of focus with the other spore contents in focus. x1725.

Row 6. Oil drops in microconidia. Stained with Sudan III. x1725.

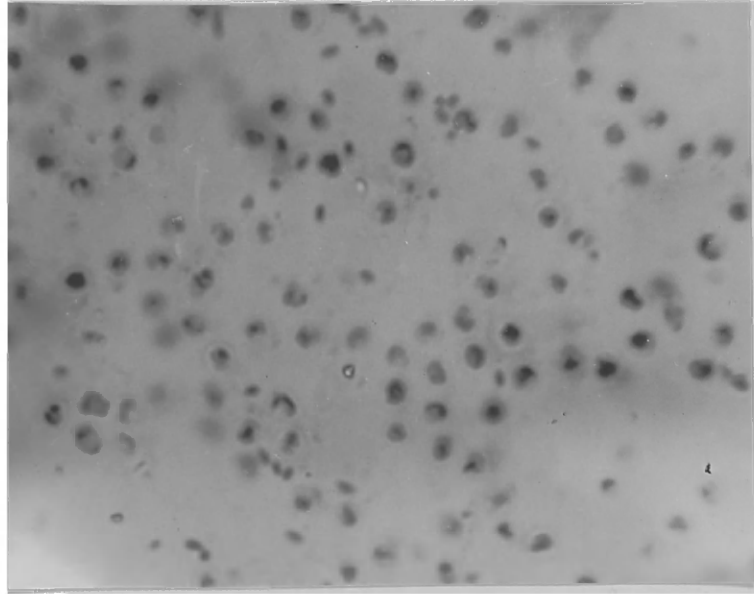
Row 7. Wall structure of microconidia. Stained with chlor-zinc-iodide. x1725.

Plate III

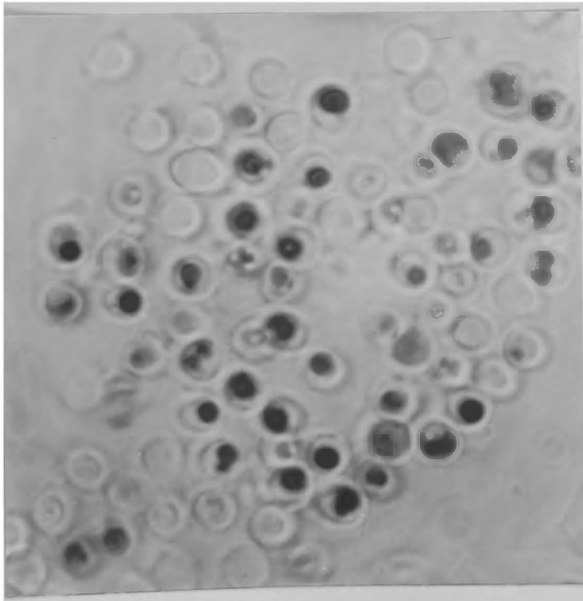
Fig. 1 Nuclei of microconidia. Stained with iron-alum-haematoxylin. x1400.

Fig. 2. Oil drops in microconidia. Stained with Sudan III. x1550.

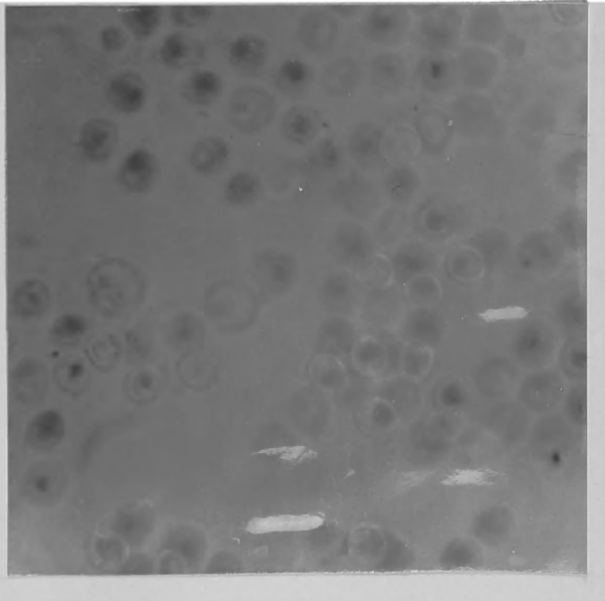
Fig. 3. Vacuoles in microconidia. Stained with Neutral Red 1-10,000. x1550.



1



2



3

Plate IV

Fig. 1. Types of nuclei in hyphae at the tip of an apothecial fundament. Left, ascogenous hypha; right, paraphysal hypha. x1725.

Fig. 2. Types of nuclei in hyphae in the middle region of an apothecial fundament. Left, ascogenous hypha; right, paraphysal hypha. x1725.

Fig. 3. Types of nuclei in hyphae at the point of origin of an apothecial fundament. Left, ascogenous hypha; right, paraphysal hypha. x1725.

Fig. 4. Types of nuclei in hyphae in the sub-hymenium. Left, ascogenous hypha; right, paraphysal hypha. x1725.

Fig. 5. Types of nuclei in hyphae in the hypothecium. Left, ascogenous hypha; right, paraphysal hypha. x1725.

Fig. 6. Types of nuclei in hyphae at the tip of a young apothecium. Left, ascogenous hypha; right, paraphysal hypha. x1725.

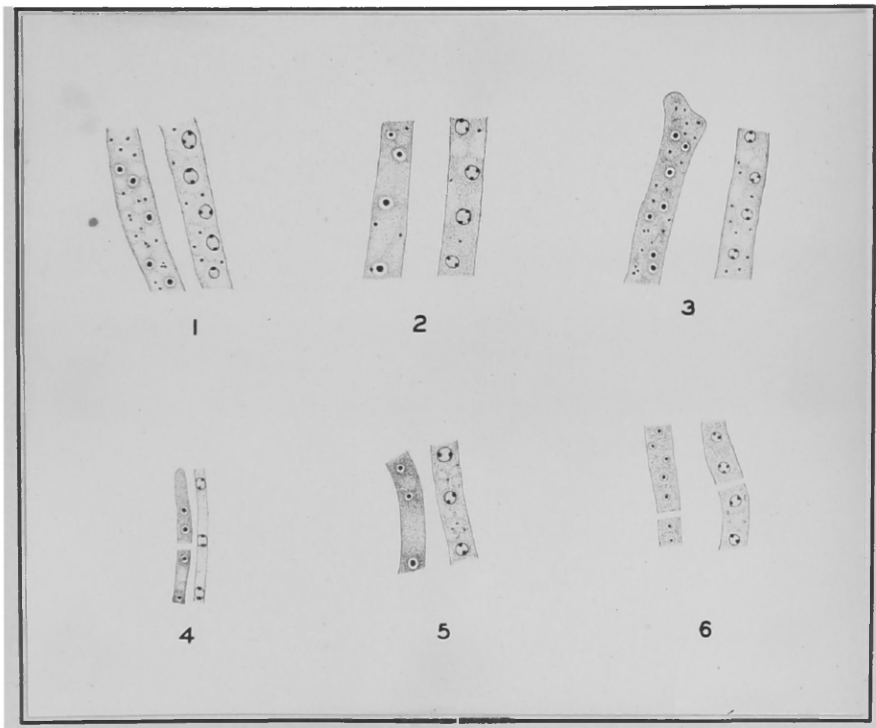


Plate V

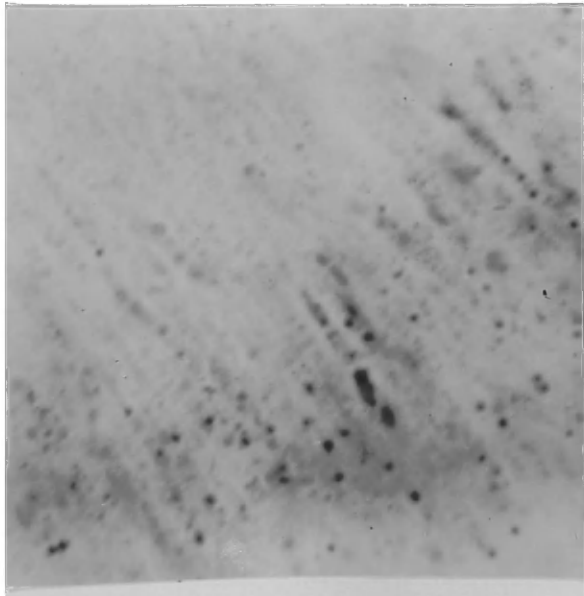
Fig. 1. Longitudinal section of an apothecial cup just opening. The darker line at the base of the paraphyses is formed by the more deeply stained ascogenous hyphae. x900.

Fig. 2. Ascogenous hyphae in the sub-hymenium of the apothecial cup shown in Fig. 1. The binucleate condition, especially of the tip cells, is evident. x1400.

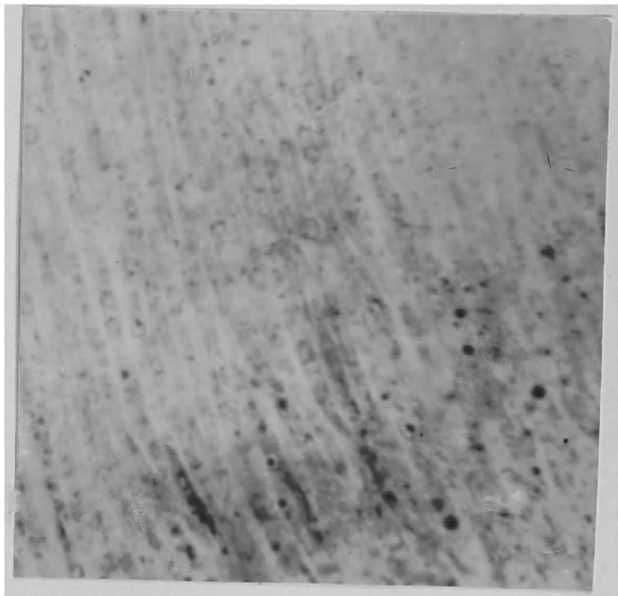
Fig. 3. A section of the sub-hymenium of the apothecial cup shown in Fig. 1. The difference in structure between the nuclei of the ascogenous hyphae and the paraphyses is shown. x1400.



1



2



3

Plate VI

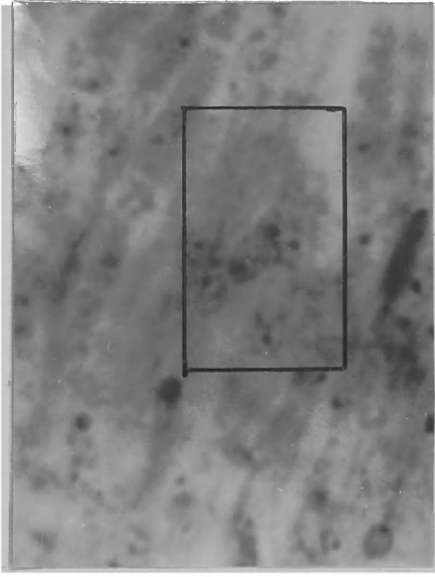
Fig. 1. A crozier before the fusion of the nuclei. x2200.

Fig. 2. Lower right, fusion nucleus in a young ascus. Center, fusion nucleus showing chromatin network. x1400.

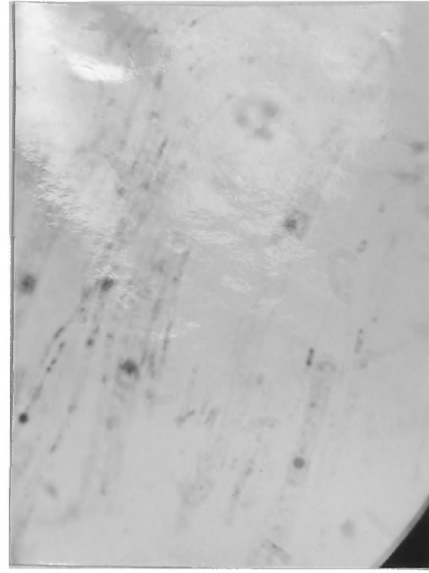
Fig. 3. Right, spireme of fusion nucleus. Upper left, early anaphase of first division. Six of the 8 chromosomes, and the centrosomes, are clearly shown. x1400.

Fig. 4. Upper left, anaphase of the first division. Six of the 8 chromosomes, and the centrosomes, are shown. x1400.

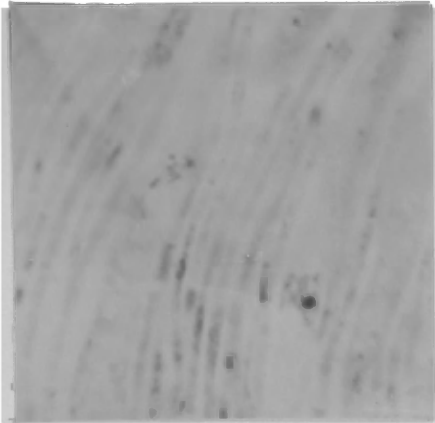
Fig. 5. Early anaphase of the first division. The chromosomes are just separating. The spindle fibres can be seen, and the centrosomes are very distinct. x1400.



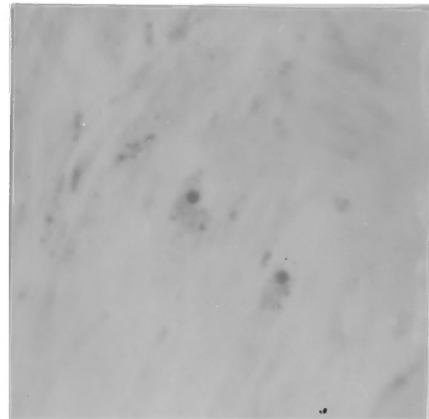
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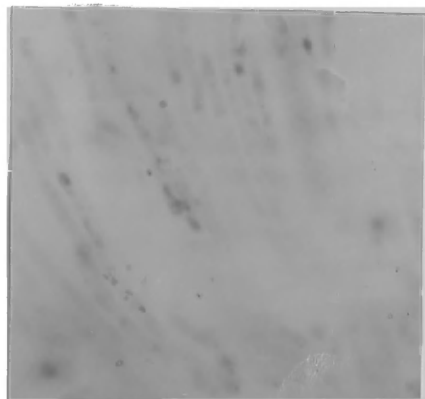


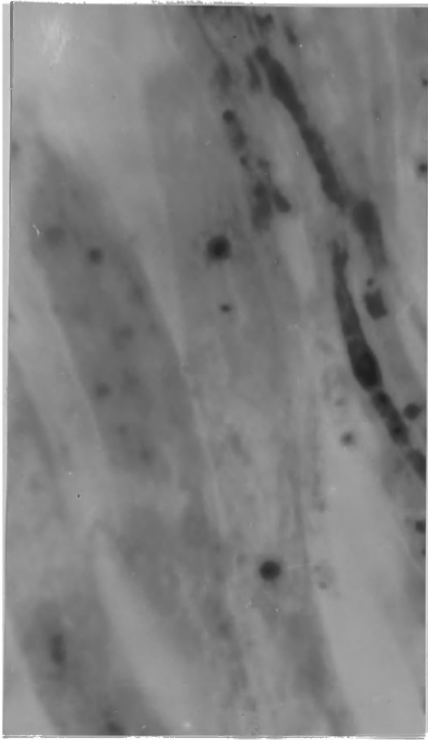
Plate VII

Fig. 6. Two-nucleate ascus. x2500.

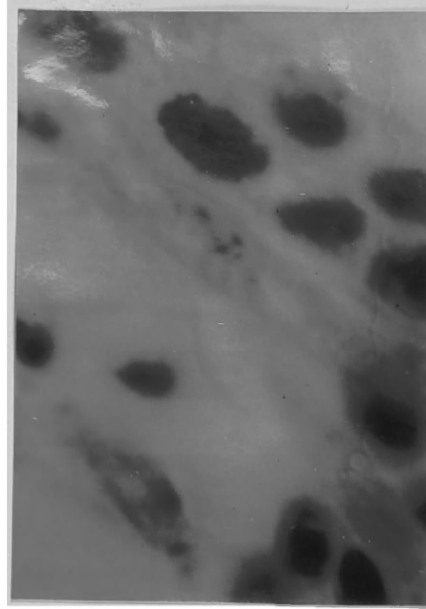
Fig. 7. Anaphase, second division. x2500.

Fig. 8. Four-nucleate ascus. x2500.

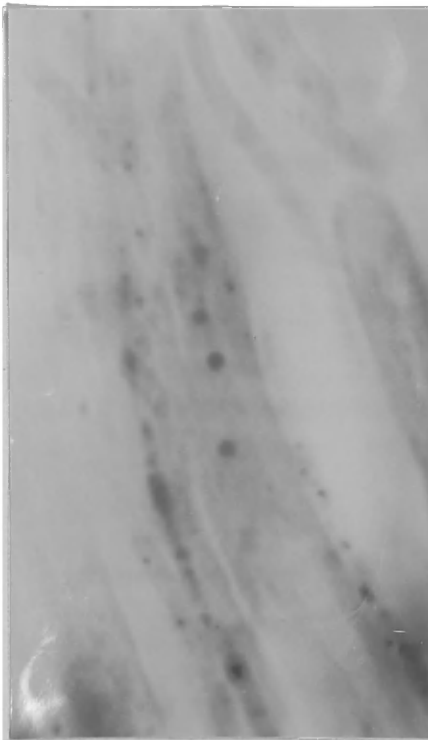
Fig. 9. Anaphase, third division. x2500.



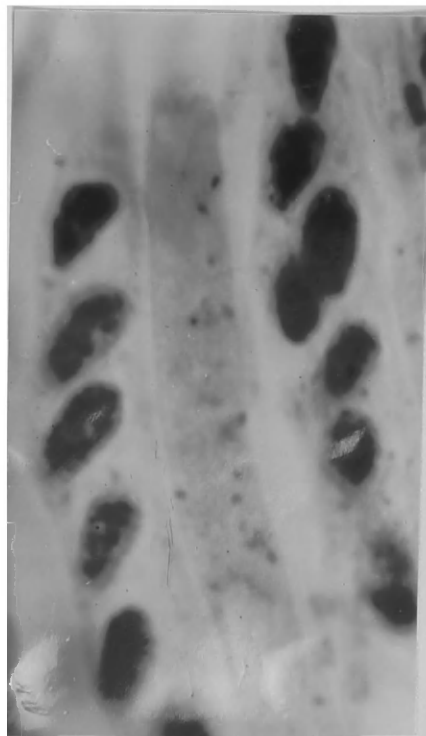
6



7



8



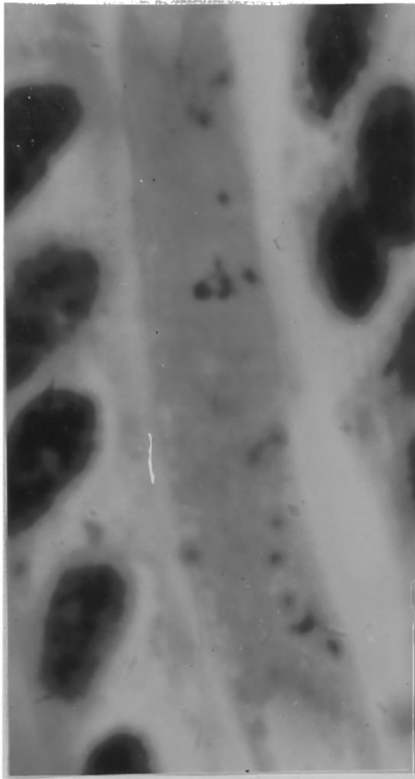
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Plate VIII

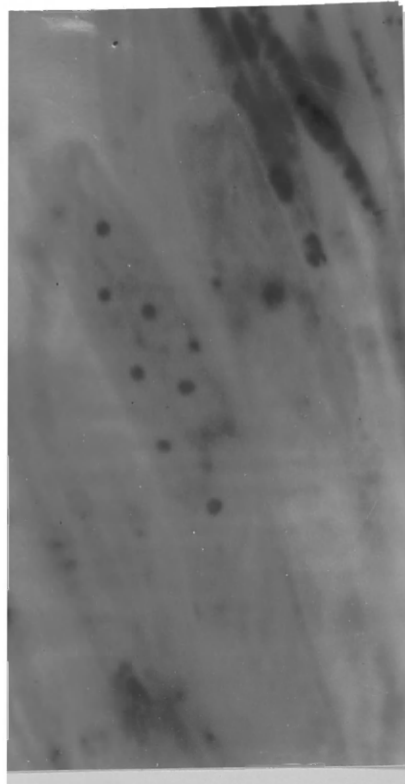
Fig. 10. Anaphase, third division. Two of the nuclei are out of focus. x3700.

Fig. 11. Eight-nucleate ascus. x2500.

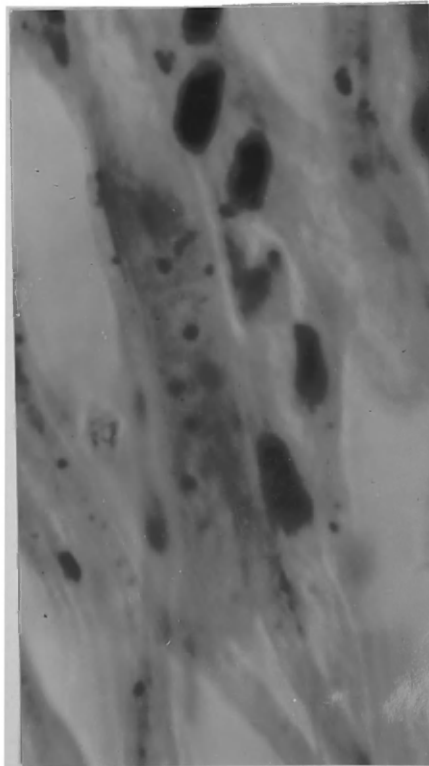
Fig. 12. Delimitation of the spores. The spores are one-nucleate. x2500.



10



11



12

Plate IX

Figs. 1-2. Typical croziers before nuclear fusion. x1380.

Fig. 3. Nuclei approaching each other. x1380.

Fig. 4. Nuclei almost in contact. x1380.

Fig. 5. Nuclei in contact. x1380.

Fig. 6. Nucleoles beginning to fuse. x1380.

Fig. 7. Nuclear fusion complete. x1380.

Fig. 8. Ascus elongating. x1380.

Fig. 9. Fusion nucleus moving upward in the elongating ascus. x1380.

PLATE IX

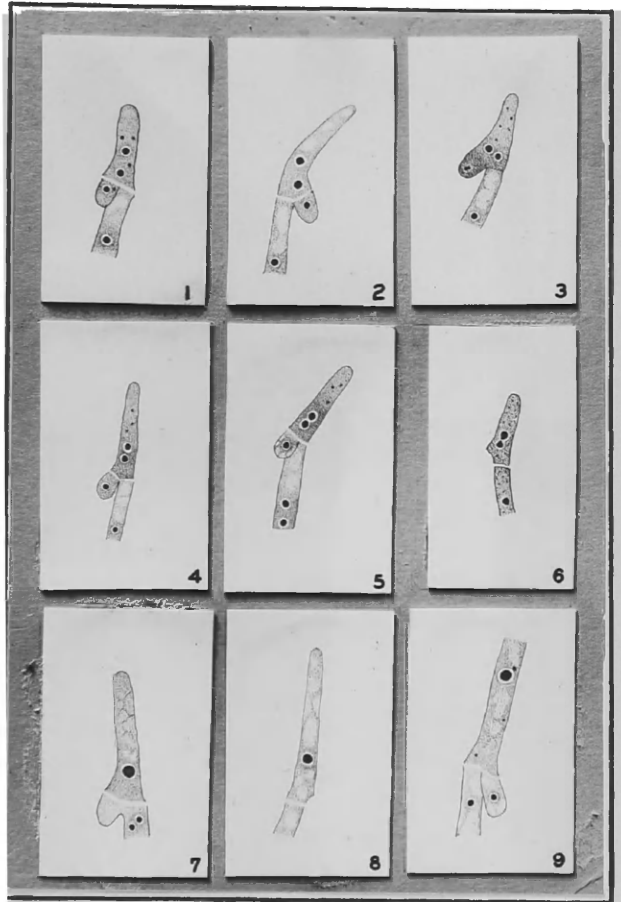


Plate X

Fig. 10. Fusion nucleus showing chromatin strands. x1380.

Fig. 11. Fusion nucleus showing chromatin network. x1380.

Fig. 12. Fusion nucleus showing portions of the spireme thread. x1380.

Fig. 13. Early metaphase plate stage, first division. x1380.

Fig. 14. Early anaphase, first division. Astral rays are present on one of the centrosomes. x1380.

Fig. 15. Anaphase, first division. x1380.

Fig. 16. Two-nucleate ascus. x1380.

Fig. 17. Anaphase of one nucleus, second division. x1380.

Fig. 18. Four-nucleate ascus. x1380.

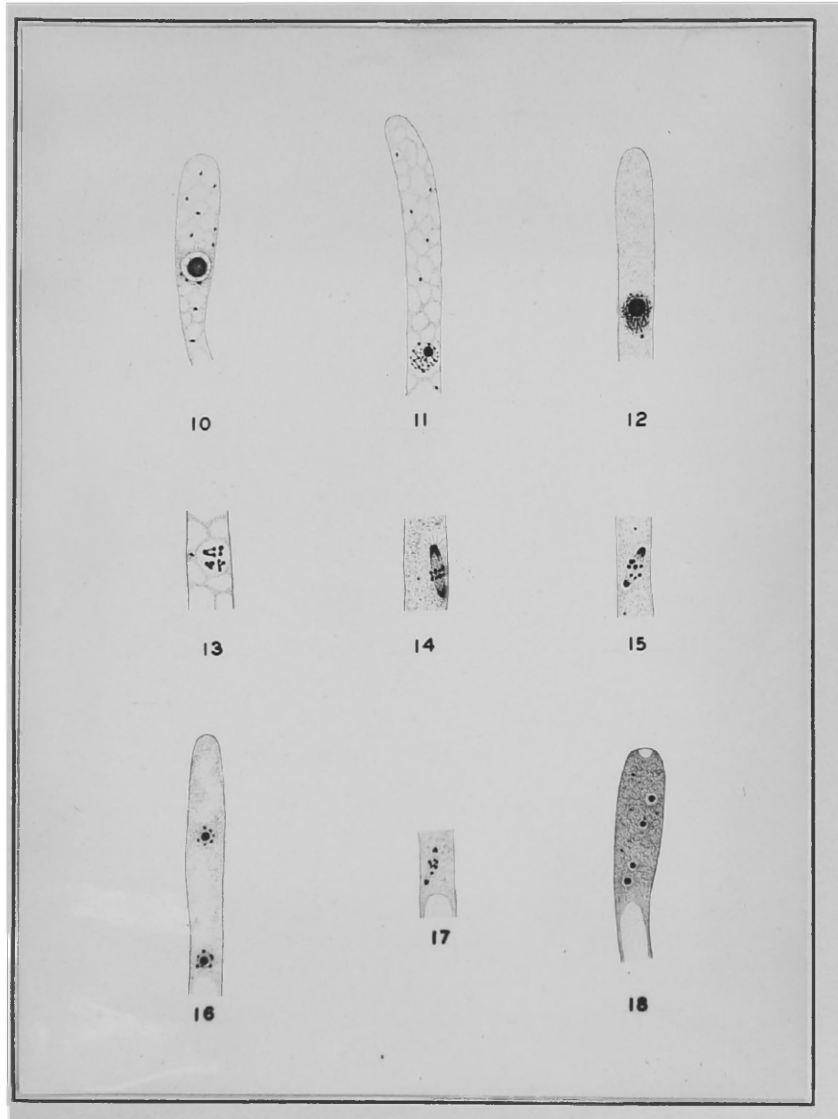


Plate XI

Fig. 19. Third division. One nucleus in anaphase and 3 in metaphase. x2080.

Fig. 20. Third division. All 4 nuclei are in anaphase. x2080.

Fig. 21. Eight-nucleate ascus. x2080.

Fig. 22. Delimitation of the spores. x2080.

Fig. 23. Delimited one-nucleate spores. x2080.

