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MORPHOLOGICAL AND CYTOLOGICAL
STUDIES IN GNOMONIA FRAGARIAE
KLEBAHN

Thesis for the Degree of M. S.
MICHIGAN STATE COLLEGE

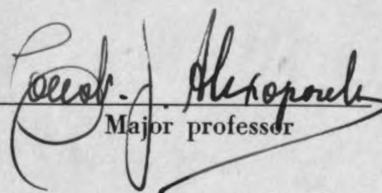
Sung Huang
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MORPHOLOGICAL AND CYTOLOGICAL STUDIES IN
GNOMONIA FRAGARIAE KLEBAHN

By
Sung Huang

A THESIS

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CHAPTER I

INTRODUCTION

Among various isolations from diseased Robinson strawberries collected in Lawrence, Michigan, a few proved of particular interest. Like most of the isolations, they produced pycnidia with conidiophores and spores resembling those of Dendrophoma obscurans, but they developed round, black perithecia with long beaks, typical of Gnomonia fragariae, a fungus hitherto unreported from the Western Hemisphere.

The purpose of this thesis is to study spore germination, pycnidial and perithecial formation, and the cytology of ascus development. Because of time limitation, some phases of the work have not been completed, but the results obtained thus far are of sufficient interest to warrant this paper.

Three isolates designated as DB₁, DL₂ and A₁ were used in this study. DB₁ was isolated from a berry, as a single-pycnospor; DL₂ was isolated from a leaf, also as a single pycnospor; A₁ was derived from a single ascospore isolated from a perithecium of DB₁. Later DB₁ lost its power to produce perithecia; and consequently most of the work was done with DL₂ and A₁. Dendrophoma obscurans cultures were used occasionally for the comparison of the two.

CHAPTER II

REVIEW OF LITERATURE

Gnomonia fragariae was first described by Klebahn in 1918 (37). He noticed the fungus in 1908, while searching for strawberry diseases in Hamburg, Germany. According to Klebahn's description, the fungus produces black round perithecia which are sunk in the tissues with their long beaks protruding. The asci are elongated and spindle-shaped, and the ascus wall is very much thickened at the ^{apex} apices forming a narrow canal. The spores are spindle-shaped, colorless, two-celled. Germination of ascospores takes place easily on agar. Germ tubes are produced after twenty-four hours from one or both cells. The mycelium consists of dense, thick walled, brown colored hyphae, and the aerial mycelium is grayish. Perithecia measure 250 μ to 400 μ in diameter; the beaks are approximately 1.5mm. long; the asci are 50-80 μ X 7-10 μ ; the spores are 2.5-3.5 μ X 13-19 μ .

Klebahn does not mention pycnidia. He probably did not notice them on the host, and in culture the agar used was apparently not favorable for pycnidial formation.

In 1930 G. and M. Arnaud studied strawberry diseases in France (7). On the surfaces of some fruits they found small, light brown protuberances (pycnidia), which oozed spores easily when moist. Beneath the fruit tissue, they

also found young globose perithecia associated with the pycnidia. Artificial cultivation of the fungus on quaker-oat agar, produced pycnidia only. When transferred to plum agar, the fungus produced perithecia with one to four beaks.

According to Arnaud, the pycnidia are light brown and sunken. The conidiophores are needle-shaped and bear at their tips cylindrical, oblong spores with two oil guttules one at each end. The spores are 6-7 μ long and about 2 μ wide.

Perithecia in culture are at first light brown, form one to four beaks, then turn black, carbonaceous. They are globose, 300 to 500 μ in diameter, with one to four beaks, 500-1000 μ in length and 35-50 μ in width. A great many eight-spored, 33 μ long asci are produced. Ascospores are oblong, fusiform, colorless and unicellular, with five to six oil guttules. Some spores are constricted toward the middle and two-celled. They measure 11-13.5 μ X 2-2.5 μ .

Arnaud identified the perfect stage as Gnomonia fragariae Klebahn var. Fructicola G. and M. Arnaud. In spite of the fact that he recognizes the pycnidial nature of the imperfect stage, he classifies it as Gloeosporium fragariae Nob., probably influenced by the fact that most known species of Gnomonia have an acervulus as an imperfect stage.

In 1941 Wormald and Montgomery (56, 57) found a Leaf

Blotch fungus of strawberry leaves in Kent, England. The most important characteristics of the fungus are the small biguttulate spores measuring about $6 \times 2\mu$, borne on long branched conidiophores, and the brownish, waxy, pycnidia. The fungus is associated with large yellowish brown blotches bordered by a dark line on the strawberry leaves. They referred this fungus to Phyllosticta grandimaculans Buback and Krieger, then decided to change to Zythia fragariae Leibach because of the fructifications which are brownish (pale yellow in culture), not black as in a typical Phyllosticta, and the consistency of the fruiting bodies ^{which is} are waxy, not coriaceous or carbonaceous. Perithecia were found in 1944 (58) both in culture and on infected strawberry leaves. They are black spherical bodies with long necks. The ascospores are small, with four oil drops and some of them are two-celled. They assigned the perfect stage to Gnomonia, but did not designate the species. Their description of the perfect and the imperfect stages ~~also~~ resemble those of Gnomonia fragariae as given by Arnaud and Arnaud (7).

Alexopoulos and Cation (1) while investigating various strawberry rots in Lawrence, Michigan in 1947, found a very large percentage (76.3%) of the diseased fruits examined, bearing the pycnidia of a fungus they believed to be Dendrophoma obscurans. Pure cultures were obtained by trans-

transferring pycnosporos or diseased tissue from fruits, calyces, and peduncles of strawberries to agar. Such cultures produced fruiting bodies identical with those produced by isolations from diseased leaves with typical symptoms of Dendrophoma leaf blight. Perithecia were discovered on agar and diseased parts of strawberries associated with the pycnidia. Plantings of ascospores and pycnidiospores both produced perithecia and pycnidia. This proved the relationship between the perfect and the imperfect stages of the fungus. Different media were tried and it was found that no perithecia were produced on potato-dextrose, oat, nutrient, or maltose agar (with two exceptions). In corn meal agar tubes, perithecia were crowded together forming a black band at the base of the slant.

Alexopoulos and Cation identified the perfect stage as a *Gnomonia* and pointed out the similarity between the perithecia of their fungus and those of Wormald and Montgomery found in England. They did not mention Klebahn's fungus in their paper.

The isolates used in the present investigation were isolated by these workers and turned over to the writer for further study. A comparison of the fungus at hand with the descriptions of *Gnomonia fragariae* as given by Klebahn and especially by Aynaud, leave little doubt that the Michigan

fungus is the same species as the European one. The relationship of Gnomonia fragariae to Dendrophoma obscurans, however, needs further investigation.

No other literature on Gnomonia fragariae appears to exist.

CHAPTER III
EXPERIMENTAL METHODS

A. Media Used.

1. Corn meal agar.

It is made by dissolving 19 grams of Difco corn meal agar into 1000 ml. of distilled water. The agar is brought to a boil, filtered through cotton, and sterilized in the autoclave at 15 pounds for 20 minutes.

This medium is very clear, and consequently good for studying the germination of spores and the development of pycnidia.

Perithecia are produced from three weeks to two months with difficulty. Therefore it was not used for studying perithecial development.

2. Malt extract agar.

In order to produce the perithecial stage more rapidly, malt extract agar was tried. It was prepared by mixing the following ingredients:

| | |
|--------------------------|----------|
| Bacto nutrient agar..... | 23 gm. |
| Malt extract | 10 gm. |
| Dextrose | 15 gm. |
| Distilled water | 1000 ml. |

This medium is good for mycelial growth, but is not good for studying formation of fruiting bodies because as soon as the fungus grows, the medium turns black.

3. Malt extract and corn meal agar.

2-5% malt extract was added to Difco corn meal agar and was prepared in the usual manner. It is found that 2% malt extract is as efficient as 5% for perithecial production, therefore 2% was used thereafter.

The fungus grows quite well on this medium and forms perithecia in about two weeks. Pycnidia are developed considerably later, so that ^{it} was found to be the best medium for studying perithecial development.

4. Strawberry runners.

Since the fungus is found on strawberries in nature, parts of the plant that can be infected should be a good medium for cultivation. Strawberry runners were cut and put into test tubes with moistened cotton at the bottom. The test tubes were then plugged with cotton and sterilized in the autoclave at 15 pounds for 20 minutes. Spores or mycelium on a bit of agar were inoculated on the runners.

After two days, white mycelium could be seen with the naked eye. Two weeks later yellow pycnidia were found

pushing through the epidermis of runners inoculated with A₁. Perithecia were recognized by their long, black beaks within one month after inoculation with DL₂ and A₁.

Transfers from runners to the plates seem to yield colonies with new strength. This method was used for reviving the fungus.

B. Methods in Studying Spore Germination and Fruiting Body Initials.

A large cover glass was placed in a Petri dish and sterilized in the oven at about 150⁰F for four hours. For studying spore germination and pycnidial formation, corn meal agar was poured on the cover glass so as to form a layer somewhat less than 1mm. in thickness. For perithecial formation, 2% malt extract was added to corn meal agar.

Spore suspensions were made by touching the spore exudates with a sterile needle and transferring into a drop of sterile distilled water. A small loopful of the suspension was then transferred onto the agar over the central part of the cover glass. At the same time a glass ring 40 mm. in diameter, was sealed on a Petri dish with vas^eline, and a little water was poured in the ring. The free edge of the ring was sterilized by wiping with 75% alcohol. Then the

cover glass with agar was cut out of the Petri dish and inverted on the ring forming a Van Tieghem cell.

Spores were examined for germination every four hours.

In studying the development of pycnidia and perithecia, a bit of agar with mycelium was transferred instead of spore suspension. After the fungus grew about 20 mm. from the inoculum, a Van Tieghem cell was made in the manner described. A daily search was made under microscope for perithecial initials. Any structure suspected of being a perithecial initial stage was checked for further development.

C. Staining Methods.

1. Staining paraffin sections.

Bits of agar with various stages of perithecia were cut from the plates and fixed in chrome-acetic acid. Microtome sections were made from 3 μ to 7 μ in thickness, and it is found that 5 μ is the most satisfactory. Heidenhain's iron-alum hematoxylin and triple stains were tried. For studying the structures of the perithecium, that is, the wall, the beak, the cavity and the arrangement of asci, both stains are satisfactory. But for the cytology of asci, this technique was found unsatisfactory.

2. Maceration method.

This method is described (48) as follows:

- 1/ Fix in 3:1 absolute alcohol-propionic acid (45%) solution for 48 hours.
- 2/ Carry through various concentrations of alcohols to water which is slightly acidified with a few drops of propionic acid.
- 3/ Transfer to 4% iron alum for ten minutes.
- 4/ Wash with acidified water overnight with several changes.
- 5/ Stain with propionocarmine.

In following these directions, step 2 was once omitted by accident, and the results were found to be superior to those obtained by following the above method. The procedure which was finally adopted as a result may be outlined as follows:

- 1/ Kill in absolute ethyl alcohol-propionic acid solution for 48 hours.
- 2/ 4% iron-alum 10 minutes.
- 3/ Wash in running water for four hours.
- 4/ Stain on slide with propionocarmine.

The stain was made according to Sass's acetocarmine

formula (48), by substituting propionic acid for the acetic acid. The slide is made as follows:

- 1/ Macerate a perithecium in a drop of the stain on a slide.
- 2/ Drop cover glass over mount and tap gently.
- 3/ Pass slide quickly over an alcohol lamp flame several times.
- 4/ Drain off excess stain, and seal the edges of the cover glass with glycerine jelly.
- 5/ The slides should be aged. They show good results usually after two weeks or longer.

CHAPTER IV

OBSERVATIONS AND EXPERIMENTAL RESULTS

A. Vegetative Hyphae.

The hyphae appear hyaline under ^{the} microscope and white to the naked eye. There are two types of hyphae; one is thin, the other broad (Fig. 1).

The thin hyphae measure from 3.5 μ to 5.25 μ in diameter. These are the young and actively growing hyphae which enter in the formation of fructifications. The cytoplasmic content is dense and granular and exhibits small oil drops. Sometimes the hyphae are highly vacuolated. It has not been possible to stain nuclei with the technique employed. Septa are very few and are far apart, but in a few cases close septations do occur.

The broader hyphae vary from 8.75 μ to 10.5 μ in diameter. They represent the older hyphae. No fruiting bodies have been found in association with this kind of hyphae. The cytoplasm is restricted to a very thin layer. There are large oil drops and vacuoles. The septa are quite close to each other.

Aerial mycelium is produced when the culture is old.

B. Pycnidia and Pycnospores.

Pycnidia are developed beneath the epidermis of various parts of strawberries. As they grow, the epidermis breaks open in a stellate manner, and the short beaks of the pycnidia protrude. In the presence of a considerable amount of moisture, the pycnidium swells and the spores ooze out in droplets of water or in long gelatinous cirrhi through the ostiole (Fig. 3).

In nature the pycnidia are yellowish brown with slightly darker beaks, but in culture they are pale yellow or white. The wall of the pycnidium is made of pseudoparenchyma cells about two to three cells in thickness. The consistency of the wall varies with moisture; it may be carbonous or waxy.

Pycnidia are circular in surface view, ranging from 135 μ to 300 μ in diameter, and are somewhat flattened in side view (Fig. 3). The height of the pycnidia without the beaks varies from 105 μ to 285 μ . The beaks are quite short, about 75 μ to 100 μ in length and 70 μ to 95 μ in width.

Lining the inner wall of the pycnidium are numerous branched conidiophores (Fig. 4). There may be two, three or four branches, the most common ones being two-branched. Pycnospores are borne at the tips of the conidiophores. After successive culturing, the fungus lost its power to

form conidiophores, and only a mass of spores within the thin walled pycnidium could be found.

Most of the spores are bacilloid, with round ends (Fig. 6b). Few of them are ellipsoidal (Fig. 6c) and some are intermediate; that is, one end is round and the other is pointed (Fig. 6a). There are two to three guttules or oil drops in the spores, usually one at each end. They are very conspicuous and are often as large in diameter as the width of the spore. The size of the spores varies from $1.75-2\mu \times 5.25-7.3\mu$.

C. Development of Pycnidia.

Kempton (36) mentioned two types of pycnidial development. If the pycnidium initiates from the interlacing and anastomosing of hyphal branches from different mycelial threads, its development is of the symphogenous type. If the pycnidium arises from the proliferation of one hyphal cell or from branches of the same hypha, its development is of the simple meristogenous or compound meristogenous type respectively. Pycnidia of most fungi develop according to one of the two types. Mercer (42) in his studies of Phoma richardiae found both types of development in that species. This seems to be also true of Gnomonia fragariae.

The symphogenous type of pycnidial development is

found in strain A₁. Pycnidial initials can be found under the microscope four days after inoculation. The first evidence of a pycnidial initial is the growing of several hyphae toward a common point (Fig. 7). The tips of the hyphae then bend and intertwine (Fig. 8). They intersect each other and form the pseudoparenchymatous wall of the pycnidium (Fig. 9, 10). Mature pycnidia (Fig. 2, 3) are found about two weeks after inoculation.

The compound meristogenous type is found in strain DL₂. Instead of several hyphae intertwining, there is usually one hypha branching (Fig. 11) and forming knots (Fig. 12). These then proliferate into a young pycnidium.

The young pycnidium is distinguished from the young perithecium in that the former is more or less irregular in shape and the latter is spherical and more compact (Fig. 24) even in an early stage of development.

The origin and development of conidiophores and spores have not been studied.

D. Pycnospore Germination.

Hanging drops of corn meal agar were used for the study of pycnospore germination. No more than two or three per cent of the spores germinate within twenty-four hours.

Forty eight hours after sowing, more spores are found to be germinating and the germ tubes of some are already forming branches. No further germination has been observed after forty eight hours. Total germination reaches about 15%.

At the first stage of germination, the spore swells, usually enlarging in width rather than in length. A little protuberance now buds out from the spore (Fig. 5a) and in some cases one of the guttules moves to the edge, at the initiation of the bud (Fig. 5e). Germination usually occurs on the side of a pycnospore, near the center (Fig. 5a) or to one side (Fig. 5g). Germ tubes have been found pushing out from the ends of spores (Fig. 5f) , but this type of germination is very rare. Figures 5a to 5d show different stages in spore germination. Branches are formed very soon after the germ tubes have elongated, but the septa are formed considerably later. Profusely branched mycelium can be seen within the third day of germination.

E. Perithecia, Asci and Ascospores.

In nature perithecia are recognized by their black, long, bristle like beaks which protrude from the strawberry tissues in which the perithecial bodies are deeply buried. Normally each perithecium has one beak. In culture, however, from one to four beaks are usually produced. Sometimes groups of six to ten beaks were found on a single perithecium,

but such perithecia, when crushed, contained no ascospores. Figure 48 shows some of the various types of perithecia that have been observed. The wall of the perithecium is shining and leathery, and consists of four to seven layers of pseudoparenchymatous cells (Fig. 47). The wall is black when examined with the naked eye or hand lens, but dark brown when observed under ^{the} microscope.

Within the perithecium are numerous eight-spored asci. They are characterized by the thick apices provided with a canal (Fig. 53, 54). The ascus wall disintegrates before the ascospores are released from the perithecium. Therefore when a very mature perithecium is crushed, there are no asci, but a mass of ascospores held together in groups of eight.

The ascospores are hyaline, spindle-shaped, and two-celled. When the spores are young, they are one-celled with six to seven oil droplets (Fig. 49, 53). As they become mature, a septum develops, dividing the spore into two cells. The two cells are slightly unequal, each of them usually contains three oil droplets (Fig. 50, 54).

Average measurements are as follows: Perithecia 250-550 μ in diameter, with beaks 550-1500 μ in length and 45-60 μ in width; asci 55-85 μ X 7-11 μ ; ascospores 7-12 μ X 2-3 μ .

F. Perithecial Development.

1. The production of perithecia.

Various methods have been tried in efforts to promote the production of perithecia in different fungi. The effects of thiamin, biotin, and different sources of sugars have been studied by many workers (11, 12, 29, 30, 31, 32, 38, 39). The influence of chemical stimulation and extracts from other fungi are also emphasized (9, 34, 40, 54). Furthermore, ultra-violet irradiation was found to be very useful in some fungi (49, 50).

Gnomonia fragariae will form perithecia on corn meal agar from two weeks to two months irregularly. To study the initial stages of the development is quite a problem; when too much agar is poured on the plate, it is impossible to see the structures with the aid of the microscope, and when a thin layer of agar is poured, it will dry out before the initials are formed. With 2-5% malt extract in the corn meal agar, plexi of hyphae were found within four days, black young perithecia were obtained within seven days and long-beaked mature perithecia occurred about two weeks from the date of inoculation. This result was quite satisfactory, therefore other methods were not tried for the production of perithecia.

2. Perithecial initials.

Perithecial initials could not be determined with certainty. There were several interesting branches and coils growing out the hyphae (Fig. 13 - 21) about the third day of inoculation. They were suspected to be the perithecial initials, but further development could not be traced. Some of the advanced stages (Fig. 23, 24) were found, but their origin was unknown.

No spermagonia have been found during the development of perithecia. The branches shown in figures 15, 20, 21 may represent antheridia. Since fertilization has not been studied, the above statement is subject to further investigation.

G. Cytology and Development of Asci.

When a young perithecium with an already differentiated beak about 20 μ long (Fig. 43), is crushed and stained, a group of ascogenous cells (Fig. 25) come out. Most of them are binucleate, few are uninucleate. Occasionally croziers are found among them. When the beak reaches its full length, but with the ostiole not yet open (Fig. 44), various stages of the asci can be found within the perithecium.

Croziers are initiated by the binucleate ascogenous

cells. A hook-like structure is produced from the ascogenous cell (Fig. 26). One of the two nuclei moves to the hook and the other remains in the cell (Fig. 27). Then those two nuclei divide simultaneously giving four nuclei (Fig. 28). Septa are developed so as to separate the four nuclei into three cells; one in the hook, two in the penultimate cell and one in the basal cell (Fig. 29). Nuclear fusion takes place in the penultimate cell (Fig. 30, 31) immediately after the septa are laid down. No division figures have been observed in croziers.

The ascus originates from the elongation of the penultimate cell after nuclear fusion. Figure 32 which shows the long big fused nucleolus with chromosomes, probably represents the early prophase I.

At metaphase I chromosomes are arranged on the equatorial plate (Fig. 33). The propiono-carmin does not have affinity for spindle fibers, so those structures are not shown. The nuclear vacuole at this stage is spindle shaped. At anaphase I the chromosomes are reduced very much in size and the homologues are separated into two groups (Fig. 34). The individual chromosomes are very indistinctⁿ in telophase I (Fig. 35).

Prophase II is apparently of very short duration, for

this stage was missing in all preparations. V-shaped chromosomes (Fig. 36) which are the sister chromatids that attach to each other by means of centromeres, are the characteristics of the Metaphase II. At anaphase II the sister chromatids become separated to opposite poles (Fig. 37). Chromosomes of those two stages are fairly distinct so the basic haploid chromosome number is determined as eight. The two nuclei at that time may lie side by side (Fig. 36), or separate (Fig. 37), each of them containing sixteen chromatids.

A very interesting figure was observed in metaphase III. One of the asci had two nuclei with chromosomes arranged on equatorial plates, and the other two nuclei with chromosomes arranged in rings (Fig. 38). The ring arrangement is typical of meiotic division. If this is the case, a brachymeiosis as proposed by Gwynne-Vaughan and Williamson (27) for Ascobolus magnificus may ^{be} taking place here. The other figures of metaphase III, anaphase III (Fig. 39) and telophase III (Fig. 40) are normal mitotic divisions. The chromosome numbers here are eight in each nucleus.

The octonucleate stage of asci with large nucleoli have been found (Fig. 42). While the walls of the ascospores are formed (Fig. 41), numerous oil droplets present, interfere with the staining of the nuclei. At this time the wall of the ascus begins to thicken at the apex and the canal is

formed.

When the spores are mature, they are two-celled. Repeated attempts to stain their nuclei with propiono-carmin, have failed.

H. Germination of Ascospores.

The ascospores as described before are two-celled. The two cells are unequal in size. The germ tube usually come out from the larger cell of the ascospore (Fig. 52a, b, c, d, f). Germ tubes emerging from both cells (Fig. 52e) or from the tips of the smaller cells (Fig. 52g) have been found, but such cases are very rare. Ascospores usually germinate within twenty four to forty eight hours.

CHAPTER V

DISCUSSION

A. Pycnidial Development.

Kempton (36) who made extensive studies of pycnidial development in several of the Sphaeropsidales, classified the types of development into three groups: 1. the simple meristogenous type; that is, development of a pycnidium by the proliferation of one hypha, 2. the compound meristogenous type in which a pycnidium is developed from the intertwining of branches from the same hypha; and 3. the symphogenous type in which the interlacing of different hyphae takes place. He found that some fungi such as Phoma herbarum, Septoria helianthi, and Sphaeronemella fragariae developed their pycnidia entirely by the first of these methods, that the pycnidia of Sphaeropsis malorum, and of Hendersonia opuntiae according to the second method, and that of Diplodia manilliana, Sphaerella nigerristigma and Cicinnobolus cesatii developed their pycnidia according to the third method. On the other hand he also noted that a number of fungi may employ two or even all three methods in developing their pycnidia. Among them are: Phoma destructiva, Sphaeropsis citricola, Septoria polygonum etc. In Gnomonia fragariae, the pycnidia have been found to develop both by the compound meristogenous and the symphogenous methods. The first of these was found to prevail in strain A₁, and

the second in strain DL₂. In its pycnidial development G. fragariae resembles most Phoma richardiae found by Mercer (42) to form its pycnidia according to both the meristogenous and the symphogenous types of development.

B. Sex Organs and Perithecial Development.

Nichols (44) found two kinds of perithecial initials in Pyrenomycetes. The first type of initials are developed from swollen cells in the mycelium. A single cell divides to form a solid sphere of pseudoparenchymatous tissue, the asci arising from the differentiation of interior cells of this tissue. The second type of initials are curved branches or several coiled structures which develop into perithecia. Arnold (8) who studied Aporonia leporia Nicaal found^{that} the perithecia originate as single swollen cells, each of which contains one nucleus. Such types of cells are not found in Gnomonia fragariae. In Melanospora zamiae (18), Neurospora tetrasperma Dodge (17), Ceratostomella fimbriata (5, 10, 28), and Ceratostomella multiannulata (4), etc. perithecia are found to develop from either recurved branches or coiled structures. Such kind of structures have been found in Gnomonia fragariae but actual perithecial development has not been traced directly to them.

Antheridia were found in Ceratostomella fimbriata (10), Ceratostomella multiannulata and Mycosphaerella melonis

(17). Elliott even saw a single nucleus from the antheridium pass through the trichogyne into ^{the} oogonium in Ceratostomella fimbriata. Gwynne-Vaughan and Broadhead (27) studied the same fungus, about ten years after, but failed to find antheridia. Although Andrus (4) found antheridia in Ceratostomella multiannulata, he could not trace their function. Most of the workers (14, 18, 19, 43, 45, 52) did not find antheridia. In Gnomonia fragariae, the presence of antheridia is uncertain. Figures 15, 20 and 21 show structures which somewhat resemble antheridia, but their function is unknown. Spermatogonia were found in Gnomonia erythrostoma (14) and Polystigma rubrum (13), but were regarded as functionless. Trichogynes were found in Gnomonia erythrostoma (14) and Polystigma rubrum (13), but the connection with the ascogonium was uncertain. Brooks considered them as respiratory and Blackman and Wellsford said that they were vegetative in function. Trichogynes have not been found in Gnomonia fragariae.

Blackman and Wellsford (13) considered the ascogenous hyphae to develop later from purely vegetative cells within the perithecium. Nuclear fusion takes place in the ascus and sometimes in the ascogenous hyphae. Caley (16) reached the same conclusion in Nectria galligena. McIntosh (41) considered the hyphal outgrowth from ascogonia of Nectria mammoidea to be abortive structures. Cookson (19) believed

the central cord of Melanospora zamiae Corda to become differentiated into fertile and sterile elements. The fragmentation of the fertile portion gives rise to ascogenous cells. The crozier type of cell division among the ascogenous cells give origin to the asci. This is the case in Gnomonia fragariae.

C. Crozier Development and Ascus Formation.

Croziers were first found by Dangeard (21), as hook-like structures arising from the ascogenous hyphae. Asci developed from the penultimate cells of the crozier. Sometimes the binucleate ascogenous cells give rise to asci directly without the formation of crozier, such as in the case of Gnomonia erythrostoma (14), Ophiobolus graminis (35) etc.

Backus and Keitt studied Venturia inaequalis (10), and found the primary ascus nucleus to be large and to show prominent chromatin beads. Three successive nuclear divisions in the ascus result in the formation of eight nuclei. The number of chromosomes is questionable, the haploid number being somewhere between four and six. The method of formation of the eight spores in the ascus seems to be more or less uniform in most cases, but the number of chromosomes and the process of meiosis are difficult to determine and are therefore uncertain. Colson found that the haploid

number of chromosomes is six and that reduction takes place in the first ascal division in Neurospora tetrasperma (18). In Glomerella (53) the number of chromosomes was determined as four in some of the pseudoparenchyma cells surrounding the ascogenous hyphae. In Gnomonia fragariae, the haploid number of chromosomes is eight, but it was not possible to determine with certainty where reduction takes place.

CHAPTER VI

SUMMARY AND CONCLUSIONS

1. The mycelium of Gnomonia fragariae consists of two types of hyphae, the thin type and the broad type. Fruiting bodies are found to be derived from the thin type hyphae.
2. Pycnidial formation is both symphogenous and compound meristogenous.
3. The pycnospores germinate within twenty four to forty eight hours. Germ tubes may originate at any part of the spore, but usually emerge from the sides.
4. Initial stages of perithecial formation could not be determined with certainty. It is believed that the recurved or coiled structures are the most probable initial stages.
5. Croziers are formed from the binucleate ascogenous cells.
6. Asci are developed from the penultimate cells of croziers, after nuclear fusion.
7. Three successive divisions of the nucleus in an ascus form eight spores. Each spore forms a septum at maturity, so as to become two-celled.
8. Germ tubes of germinating ascospores are usually found on the side of the large cell. Sometimes both cells

germinate.

9. A comparison is made between Gnomonia fragariae and other Pyrenomycetes.

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PLATE I

Fig. 1. ---- Vegetative hyphae, showing thin and broad types; X 1300.

Fig. 2. ---- Mature pycnidium; X 270.

Fig. 3. ---- Spores ooze through ostiole of the pycnidium in long gelatinous cirrhi; X 120.

Plate I

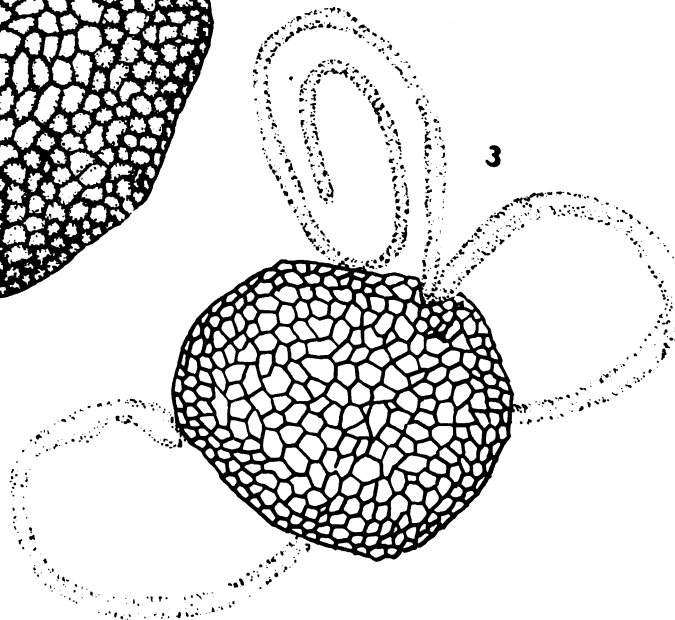
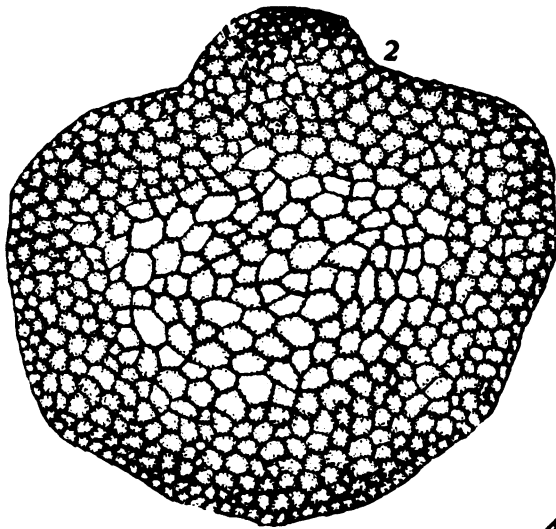
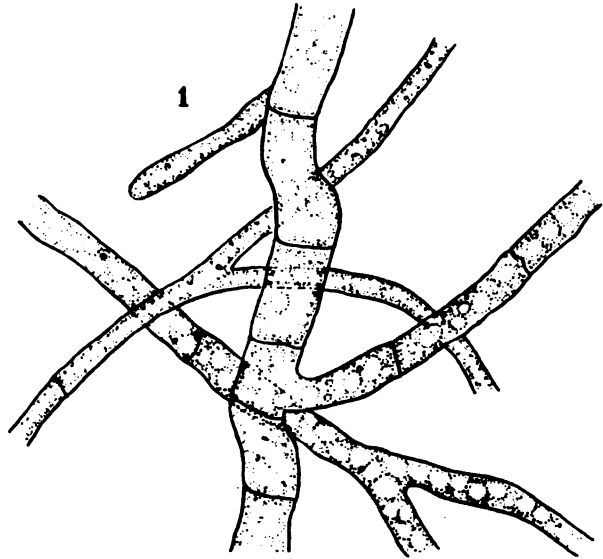


PLATE II

Fig. 4. ---- Branched conidiophores; X 1300.

Fig. 5. ---- Germination of pycnospores; X 1800.

a-d. ---- Different stages of pycnospore
germination from the side of
the pycnospore.

e. ----- The guttule initiates the germ tube.

f. ----- Germ tubes emerging from the tip
of the pycnospores.

g. ----- Germ tube emerging from the side of
the pycnospores near the tip.

Fig. 6. ---- Different pycnospores:

a. ----- Pycnospore with one pointed end
and one round end.

b. ----- Pycnospore with round ends.

c. ----- Pycnospore with pointed ends.

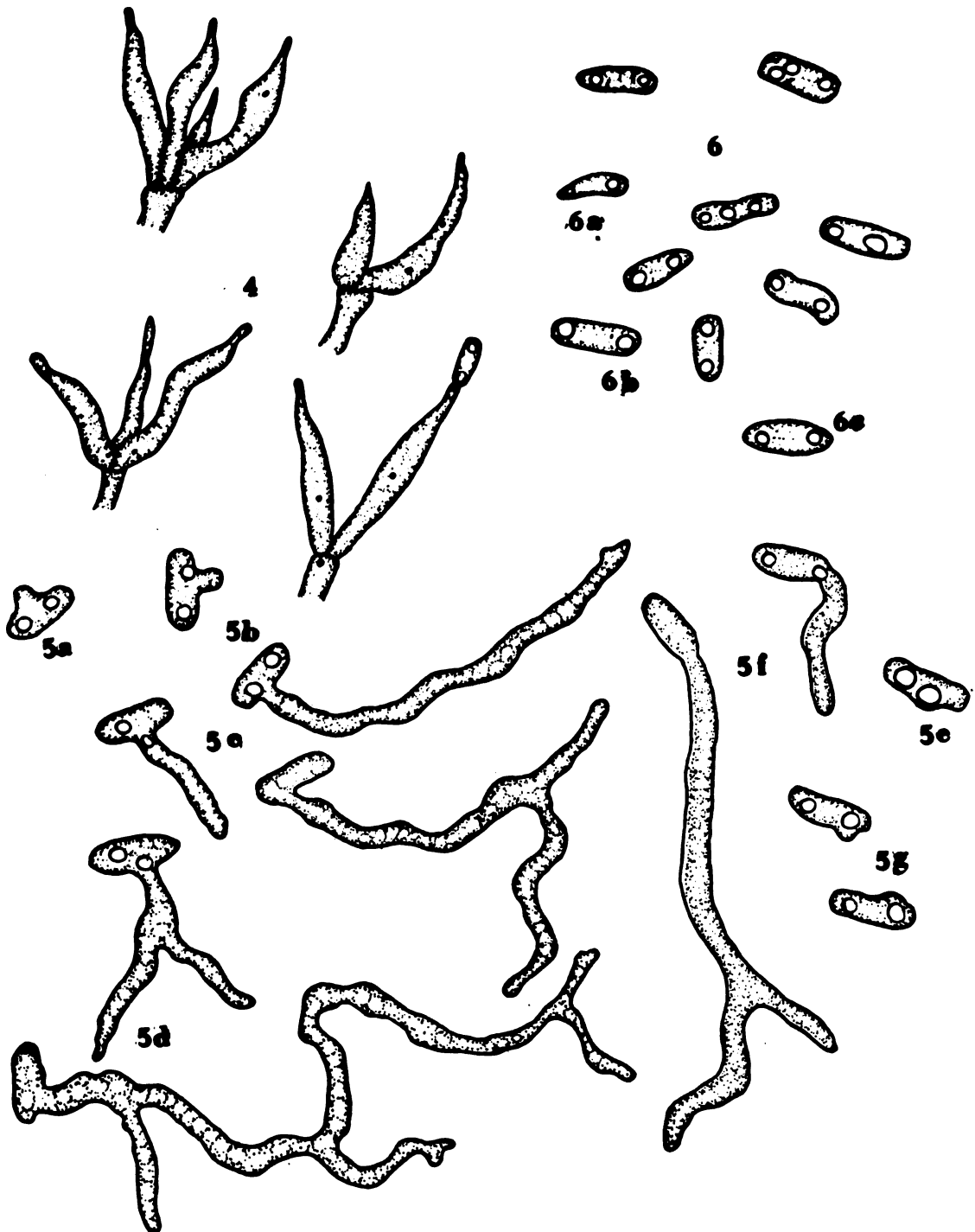


PLATE III

Figs. 7 - 10.----Symphogenous type of pycnidial development:

Fig. 7. ----Hyphae growing toward a common point; X 950.

Fig. 8. ----Hyphae intertwine; X 950.

Fig. 9. ----Hyphae intersect; X 1500.

Fig. 10. ---Young pycnidium with pseudoparenchymatous wall; X 1500.

Figs 11-12.-----Compound meristogenous type.

Fig. 11. ---Branching of hypha; X 1800.

Fig. 12 ---Hyphae forming knots; X 1500.

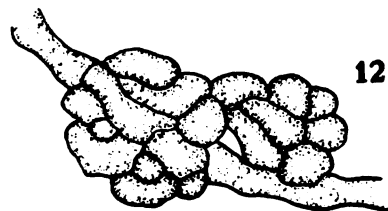
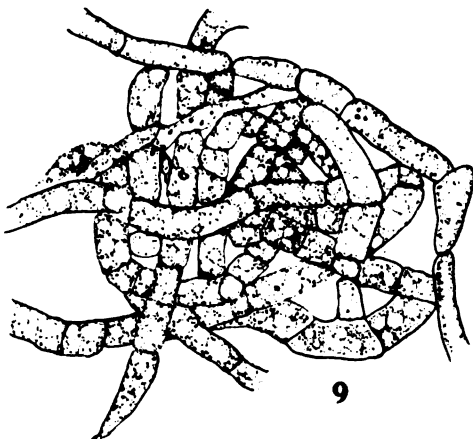
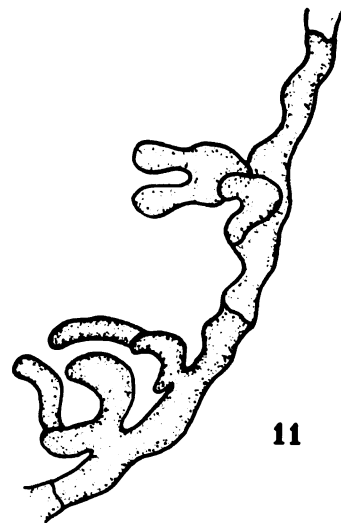
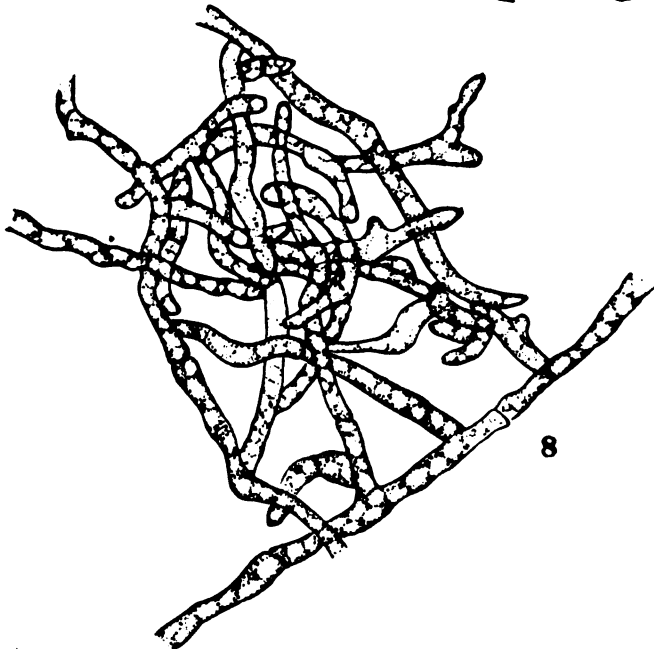
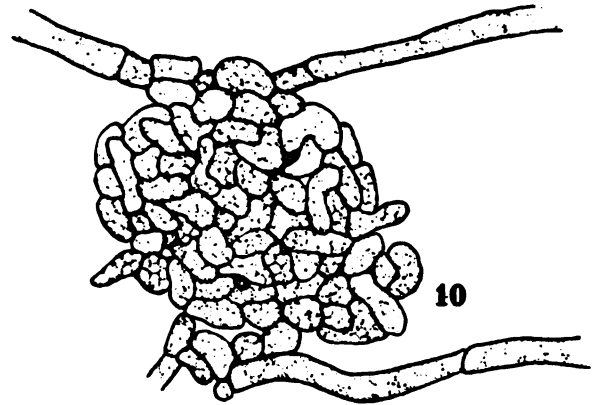
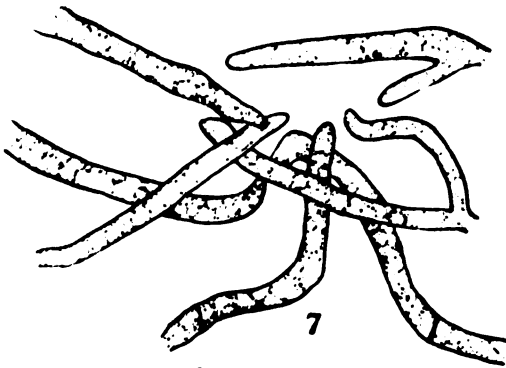


PLATE IV

Figs. 13-22 ---- Probable perithecial initials; branches coming from other hypha shown in figs. 15, 20 and 21 may represent antheridia; X 1800.

Figs. 23-24 --- Advanced stages in perithecial development; X 950.

Plate IV.

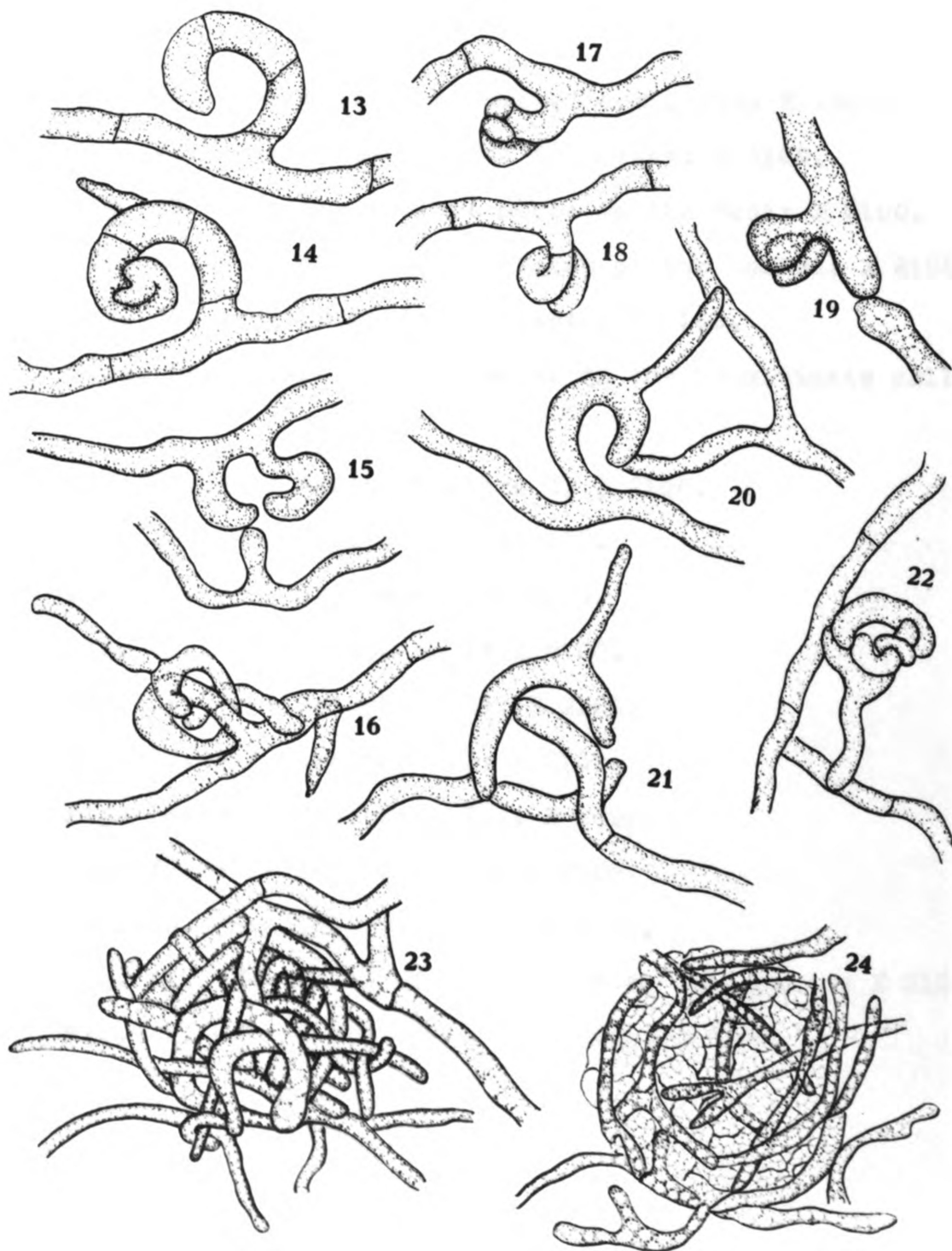


PLATE V

Fig. 25. ---- A group of ascogenous cells; X 1800.

Fig. 26. ---- Early crozier formation; X 2100.

Fig. 27. ---- One nucleus moves to the hook; X 2100.

Fig. 28. ---- Conjugate division of two nuclei; X 2100.

Fig. 30. ---- Formation of septa; X 2100.

Figs. 29, 31.-Fusion of nuclei in the penultimate cell;
X 2100.

Fig. 32. ---- Early prophase I; X 2100.

Fig. 33. ---- Metaphase I; X 2100.

Fig. 34. ---- Anaphase I; X 2100.

Fig. 35. ---- Telophase I; X 2100.

Fig. 36. ---- Metaphase II; X 2100.

Fig. 37. ---- Anaphase II; X 2100.

Fig. 38. ---- Metaphase III; X 2100.

Fig. 39. ---- Anaphase III; X 2100.

Fig. 40. ---- Telophase III; X 2100.

Fig. 41. ---- Ascus with eight young ascospores; X 2100.

Fig. 42. ---- Eight nucleated stage; X 2100.

Plate V

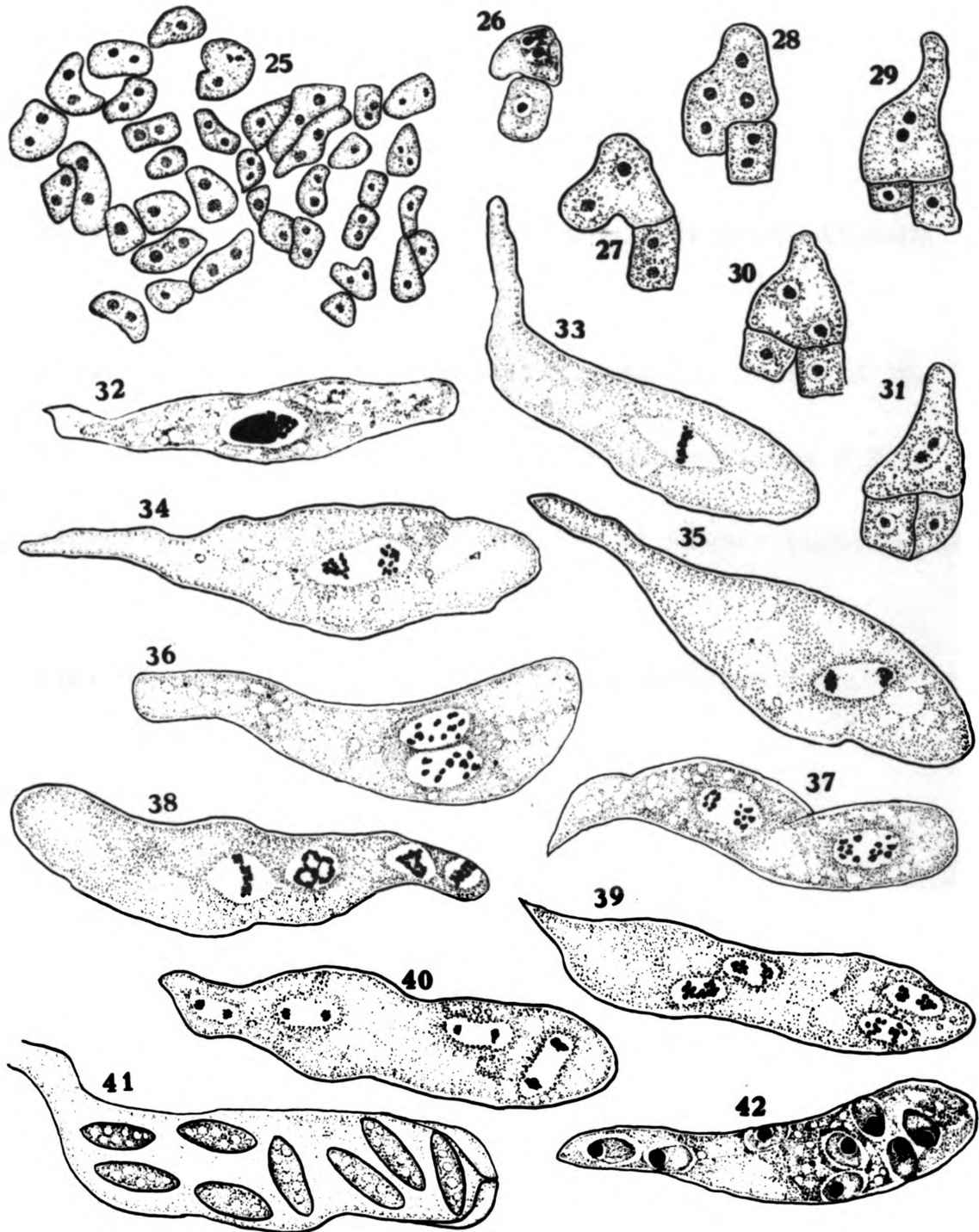


PLATE VI

Fig. 43. ---- Young perithecium; X 80.

Fig. 44. ---- Beak of perithecium with ostiole closed;
X 290.

Fig. 45. ---- Mature perithecium with two beaks; X 72.

Fig. 46. ---- Mature perithecium with one beak; X 72.

Fig. 47. ---- Longitudinal section of mature perithecium;
X 120.

Fig. 48. ---- Different types of perithecia; X 21.

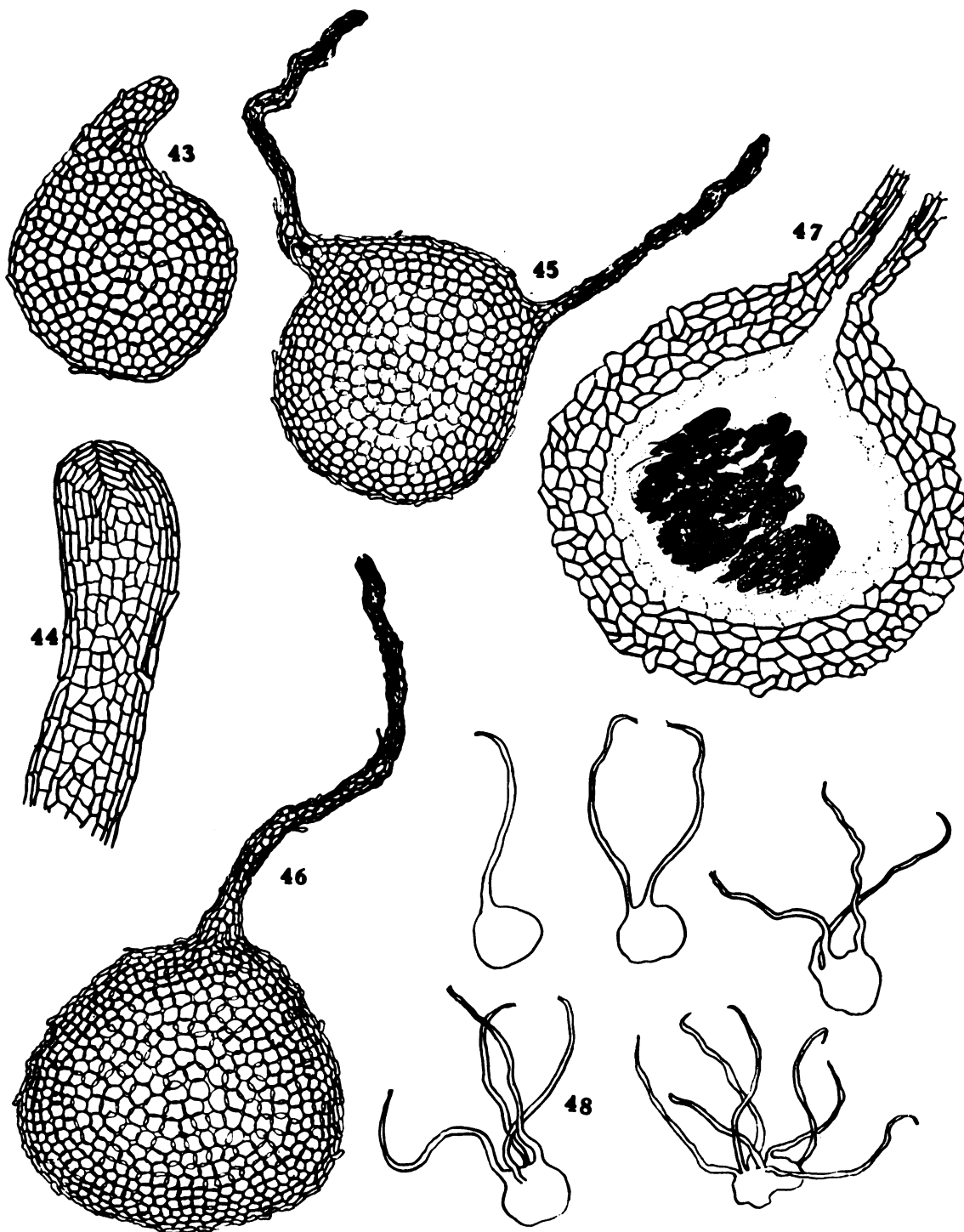


PLATE VII

Fig. 49. ---- Young ascospores; X 1800.

Fig. 50. ---- Mature ascospores; X 1800.

Fig. 51. ---- Mature ascospores when stained with propiono-carmines; X 1800.

Fig. 52. ---- Ascospore germination; X 1500.

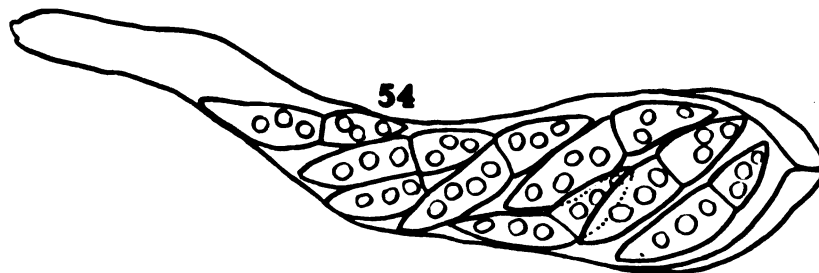
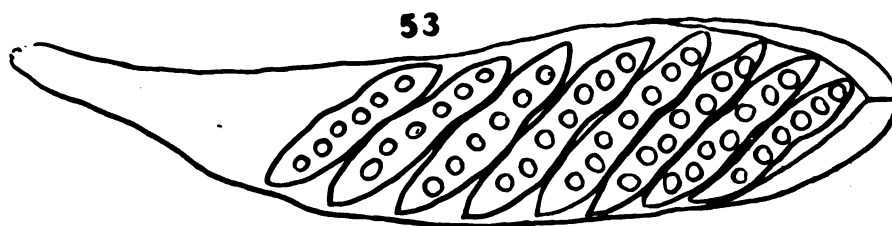
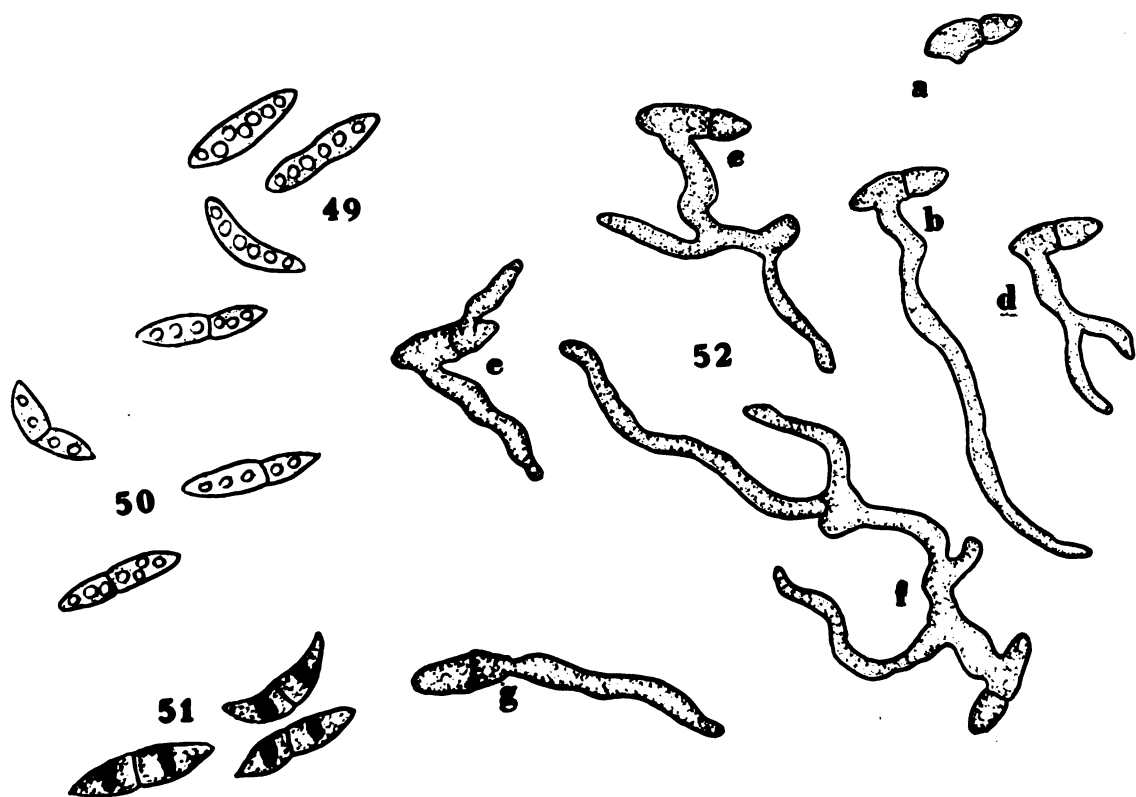
a,b,c,d and f ----germ tubes emerging from
the large cell.

e. -----germ tube emerging from
both cells.

g. -----germ tube emerging from
the tip of the small
cell.

Fig. 53. ---- Ascus with young ascospores; X 3000.

Fig. 54. ---- Ascus with mature ascospores; X 3000.



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