MORPHOLOGY AND LIFE HISTORY OF SOME ASCOMYCETES WITH SPECIAL REFERENCE TO THE PRESENCE AND FUNCTION OF SPERMATIA[1]

B. B. Higgins

The presence of "spermatia," of "microspores," or of a "Phoma" or "Phyllosticta" stage has been mentioned in connection with numerous species in various families of the Ascomycetes; but usually the function or the genetic connection has not been studied, consequently our knowledge of these structures is still very hazy. Do they occur regularly at a definite stage in the development of the fungus, or only under exceptional conditions? Do they function, like ordinary spores, as reproductive bodies for propagating the fungus; or are they sexual elements? If the latter, are they now functional or merely degenerate remnants?

These are questions which have been determined for only a few species outside the Laboulbeniales and the lichens. The spermatia of *Gnomonia erythrostoma* were described by Frank (4) and those of *Polystigma rubrum* by Fisch (3); and in both cases they were thought to be male sexual elements. This view has been sustained by the later studies of Brooks (2), and Blackman and Welsford (1), who considered them to be male sexual elements now functionless through degeneration of the carpogonia. Nienburg (8) considered *P. rubrum* to be a true oogoniate with the spermatia as degenerate functionless elements. Müller (7) found that the spermatia of *Rhytisma acerinum* failed to produce infection on the host plant or to germinate in culture media; and, failing to find carpogonia, he concluded that the spermatia were functionless male elements. The writer has reported the occurrence of spermatia and carpogonia in connection with the young ascocarps of three species of *Coccomyces* (5) and of *Mycosphaerella nigerristigma* (6). In these forms also the spermatia failed to germinate in any culture medium tried.

Further observations have shown that similar structures occur quite commonly in many families of Ascomycetes; and the question as to their possible function has led to a study of the complete life history of several species, some of which have not been previously described. The observations have been confined very largely to the species growing parasitically on the leaves and succulent parts of flowering plants, because of the comparative ease with which such forms may be studied and of the improbability of the association of other species, and also because of the value to plant pathologists of knowing the complete life history of such parasites.

[1] Paper number 14, Journal Series, Georgia Agricultural Experiment Station.

Besides the studies on the life history of these forms, it seems best to report at this time certain observations on the occurrence of spermatia and the development of the ascocarps in several other species. Because of the desirability of recording all observations on the details of morphological development and on parasitism, each species will be considered independently, before taking up a general discussion of the occurrence and function of spermatia.

Sphaerella Bolleana n. sp.

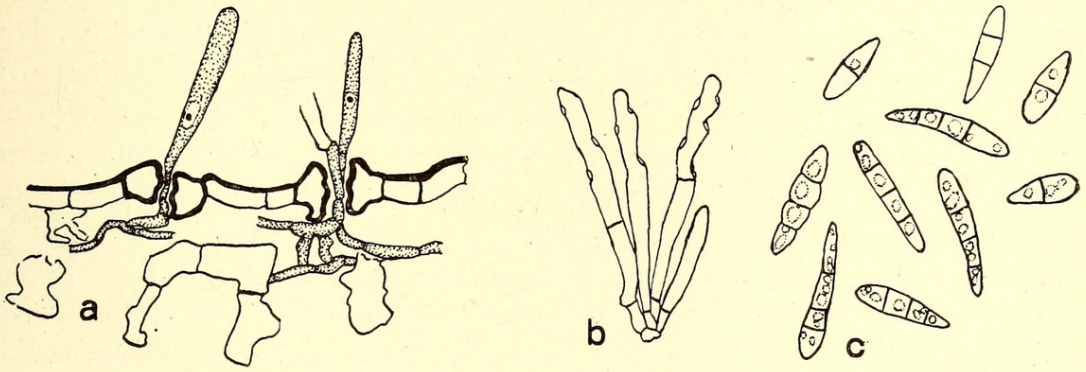
Cercospora Bolleana (Thum.) Speg. is very common and widespread as a parasite on the leaves of the fig, *Ficus carica* L., producing small, irregular brown spots two to five millimeters broad. When infection becomes very abundant distinct spots are not formed, but the whole under surface of the leaf becomes covered with the dark olive-brown conidiophores and the leaf soon drops from the tree.

The development of the spots is very slow and irregular; and this, with the fact that conidiophores may often be found on the lower surface before a distinct spot is discernible above, suggested that the leaf tissue is killed, not by the direct action of the fungus but by the drying of the tissues through the epidermis broken by the emerging conidiophores. In view of the fact that most parasitic species of this genus kill the host tissue rapidly, apparently by means of some poison secreted by the fungous mycelium, some study was made of the development of the spots and of the relation of the mycelium to the leaf tissue.

The results from many artificial inoculations show that about a month is required for a typical leaf spot to form. Infection seems to take place through the stomata, since placing the spores on the upper surface of the leaf failed to produce infection; though the actual entrance of the germ tube has not been observed. The first sign of disease is a browning of the epidermal cells at the point of infection, which begins to appear in from five to ten days. By the end of two to three weeks, tiny brown spots also begin to appear on the upper surface. These gradually enlarge and coalesce until a fully developed spot is formed.

Microscopical examination of sectioned and stained material showed the reason for this peculiar feature. The mycelium grows very slowly and is mostly intracellular. The host cells actually penetrated are killed; but, for a time, the adjoining cells seem to suffer very little injury. Evidently some poisonous substance is produced; but it is of such a nature that it does not diffuse rapidly from cell to cell. Very often the hyphae enter the vascular bundles and spread much more rapidly, sending out branches through the pits in the vessels and killing the surrounding cells. The hyphae are slightly constricted on passing through a cell wall, and do not disintegrate the walls to any appreciable extent.

The conidiophores arise as simple branches which are pushed out through the stomata (text fig. 1, *a*). Later branches arise from the basal cell of the older conidiophore within the stoma, often forming a fascicle of six or eight conidiophores of varying ages. The fascicle may often be scraped off and remain fastened together by this basal cell.



TEXT FIG. 1. Conidiophores and conidia of *Sphaerella Bolleana* from fig. leaf: *a*, section of lower portion of leaf with young conidiophores emerging through stomata; *b*, clump of old conidiophores; *c*, conidia, showing variations in size and shape. *a*, × 580; *b* and *c*, × 300.

Artificial Cultures. On culture media the growth of the mycelium was also very slow. Cultures were obtained by planting the conidia on agar plates and then transferring the conidia, after germination, to plates of sterile agar or to various other media. The germ tubes branch very profusely, forming a small dense colony. The mycelium is at first colorless but after four or five days begins to darken and gradually changes to various shades of olive depending upon the nature of the substratum.

On bean agar and on steamed green bean pods the colonies are small (usually less than a centimeter in diameter), circular in outline, and slightly raised. The base of the colony is slightly stromatic, and composed of black, thick-walled cells. This is covered with a velvety growth of gray to olive-brown hyphae.

On steamed Irish potato plugs the growth is less vigorous than on bean pods; though otherwise it is very similar. Apparently the fungus is not able to assimilate the potato starch.

Steamed sweet potato plugs gave the best growth of any medium tried. The growth was more rapid and the ultimate size of the colony much greater than on the other media. At the end of six weeks the colonies had, in most cases, practically covered the plugs which were one and a half centimeters in diameter by about four centimeters in length. The colony was capitate, raised half a centimeter or more at the center. The base, next the substratum, was composed of large, black filaments which broke up easily into individual cells. The surface growth was composed of more slender hyphae light gray in color with a tinge of pink toward the center of the colony.

No spores of any kind were ever produced in cultures; though a great many media, including those mentioned above, steamed fig leaves, steamed filter paper, steamed corn meal mush, steamed corn grains were tried.

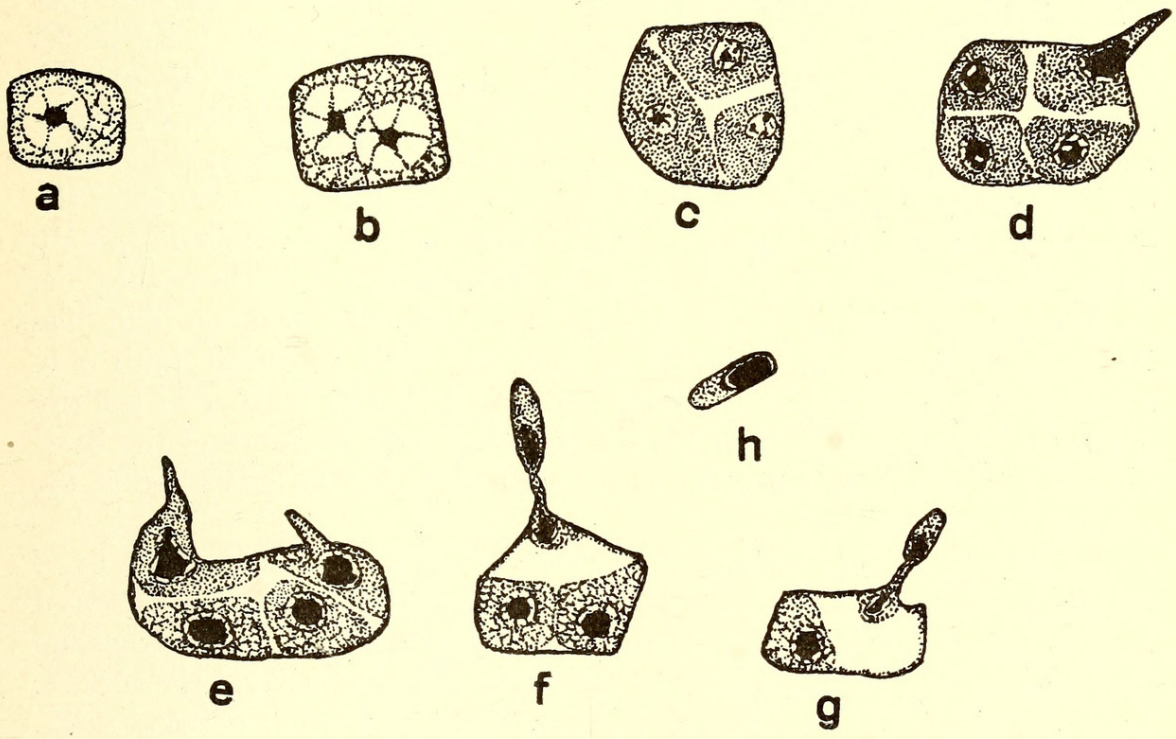
Spermatia. The first spots usually appear on the fig leaves during the month of June; but, because of the slow growth of the fungus, infection is generally not sufficiently abundant to cause leaf casting until the latter part of August. About the time the diseased leaves fall, the fungus shows remarkable activity. Pycnidium-like structures develop very abundantly. These are the spermogonia. The first indication of their development is a profuse branching and coiling of the hyphae at the base of the old conidiophores or at points where new conidiophores are being pushed out (see figs. 2 and 3, Pl. XXX). Such new conidiophores seem to develop abnormally and do not produce conidia. The weft of intertwining hyphae develops into a globose to oval mass which soon breaks the leaf epidermis and pushes out beyond. The cells of the mass enlarge. Those toward the surface coalesce to form the wall of the spermogonium, while toward the center the spermatiferous cells become richer in protoplasm and begin the process of forming spermatia even before the wall of the spermogonium is formed (fig. 4).

In this process the cell enlarges slowly, while the nucleus grows much more rapidly and soon fills almost the entire cell cavity (text fig. 2, *a*). The nucleoplasm stains very faintly, and the nucleolus at this stage is comparatively small. The cytoplasm is granular or finely alveolar in structure. The nucleus now divides, but no cell division occurs at this time. The two daughter nuclei reorganize rapidly, soon reach approximately the size of the original mother nucleus (text fig. 2, *b*), and both divide again. After this second division the four nuclei remain rather small, and the nucleoli do not reorganize at once (text fig. 2, *c*). Apparently chromatic material is scattered throughout the cytoplasm, which is now very difficult to destain. Following this second nuclear division, the cell divides (by cleavage) into four approximately equal parts without formation of separating walls. Each of the four nuclei with its surrounding cytoplasm ultimately forms a spermatium; and these four daughter cells may, therefore, be termed young spermatia while remaining within the mother cell wall. One or more sterigmata are now pushed out from each spermatial mother cell (text fig. 2, *d*); and the young spermatia pass out, one at a time, each forming a mature spermatium at the apex of the sterigma (text fig. 2, *e–h*).

After the young spermatia have passed out of a mother cell wall, some slightly stainable substance remains. This seems to be a mucilaginous or gelatinous substance that was formed instead of a wall around the young spermatia. It probably plays an important part in creating pressure to force the young spermatia out of the mother cell wall, and also in carrying the mature spermatia out of the spermogonium. The mature spermatia are carried out of the spermogonium and held in a mass at its apex (fig. 5,

Pl. XXX) by some such substance; and, when wet, they spread out over the surface of the leaf.

Spermatia continue to develop until about the middle of December. At this time the spermogonium is a globose or conical structure with a very thin wall composed of two or three cell layers. There is a small pore, but no beak, at the apex. The cells which make up the wall of the spermogonium are thick-walled and brown, and are readily differentiated from the thin-walled and colorless spermatiferous cells.



TEXT FIG. 2. Stages in the development of the spermatiferous cells and the spermatia: *a*, spermatiferous cell just before the first nuclear division; *b*, just before the second nuclear division; *c*, just after the formation of the young spermatia, the fourth spermatium being beneath the three shown; *d*, single sterigma and four young spermatia showing within the mother cell wall; *e*, two sterigmata formed by one mother cell; *f*, *g*, spermatia passing through sterigmata; *h*, mature spermatium, showing large nucleus almost filled by the nucleolus. All drawn with the aid of a camera lucida. × 4200.

Perithecia. The perithecia begin their development coincidentally with that of the spermogonia, or perhaps a few days later; and in the early stages it is not possible to say which are spermogonial and which perithecial primordia. Very soon, however, they may be differentiated by means of the changes which occur in the cells that make up the weft. In the young spermogonium the spermatiferous cells begin to develop; while in the center of the young perithecium the carpogonium, a single filament, becomes very conspicuous in stained material because of its great affinity for protoplasmic stains. Occasionally two carpogonia develop in a single perithecium.

The carpogonium arises from the base of the young perithecium; and

the free end extends up to and often beyond the apex of the mass of hyphae, which by this time has broken through the leaf epidermis (figs. 6, 7). It is very much enlarged and coiled, usually making one complete turn, at the base; but it tapers gradually toward the free end into a very slender trichogyne. The trichogyne is generally erect, extending directly toward the apex of the young perithecium except where it is bent by pressure of the other hyphae. There is no distinct line of demarcation between the enlarged basal portion and the trichogyne. As the terms are used here, the "basal portion" includes only the part that is coiled, and all the erect portion is the "trichogyne." There are two comparatively large nuclei in the basal portion and two much smaller in the trichogyne (fig. 6). In some cases these nuclei are separated by cross walls; but the entire structure, at this stage, takes the stain so evenly that it is extremely difficult to determine details of structure, and it is not possible to say at what stage the cross walls are formed. Observations indicate that they are laid down only after disintegration of the protoplasm has begun at the tip of the trichogyne.

The protoplasm of the trichogyne soon becomes granular and disintegrates, this process beginning at the tip. This continues until only about half the coiled basal portion remains. At this stage two healthy appearing nuclei remain lying close together in the remainder of the basal portion. This cell becomes the ascogonium and later on gives rise to the ascogenous hyphae.

By the time disintegration of the trichogyne is completed, the cells of the surrounding hyphae composing the young perithecium have coalesced to form an almost solid mass of pseudoparenchyma; but the perithecium continues to enlarge, apparently by formation of new cells over the surface. The perithecium soon reaches its full size. The outer cells develop thick, brown cell walls and become the permanent wall of the perithecium. The cells in the center remain thin-walled and colorless and are broken down by the later development of the asci (figs. 8, 9).

Both the question as to the origin of the two nuclei in the ascogonium and the question as to their fusion must remain unsettled for the present.

After a short rest the ascogonium becomes rapidly multinucleate. The nuclei are paired in the ascogonium and pass out in pairs into the ascogenous hyphae (fig. 8). Here they divide, one pair of the four resulting nuclei in each ascogenous hypha passing to the tip of the hypha, the other pair remaining in the base. A cross wall is now formed separating the two pairs of nuclei. The terminal cell enlarges and becomes the ascus. The two nuclei in the ascus soon fuse. The ascogenous hyphae apparently do not branch; and there is no crozier formation. Nearly all the cytoplasm of the ascogonium passes with the nuclei into the ascogenous hyphae, leaving the ascogonium almost empty and very difficult to distinguish; but in some cases its wall may yet be seen when the asci are nearly mature.

This stage in the development of the young asci is reached early in the

winter, before the end of December. They then seem to pass through a necessary resting stage. All attempts to hasten the maturity of the perithecia and the formation of ascospores by placing the leaves bearing them in a moist chamber in the laboratory at this period have resulted in failure; although, after about the first of February, mature ascospores may be obtained by this same method in from one to four days.

The nuclear divisions in the ascus have not been studied carefully. They seem to occur at irregular intervals during the spring and winter. This irregularity, together with the difficulty of proper killing and fixing of material, makes such a study very tedious. The colorless pseudoparenchyma in the interior of the perithecium is of such a nature as to prevent penetration of Flemming's solution and other killing agents of the chromic acid group, which give very good results at all other stages. The only solution which has proved at all satisfactory is one made by dissolving $1\frac{1}{2}$ grams of picric acid in 100 cc. of 70 percent alcohol and then conducting into this solution the fumes from 1 gram of $NaSO_3$ treated with a few cubic centimeters of sulphuric acid. After these colorless cells had been crushed by the growth of the asci, that is, when the asci were nearly mature, Flemming's solution gave excellent preparations. Asci containing four nuclei have been found during January, and by the first of March the young ascospores are beginning to form; but the spores do not mature until the early part of May.

The spores are imperfectly biseriate in the ascus. They are at first continuous; but when mature they are septate, each spore being constricted into two slightly unequal cells. The smaller cell, toward the base of the ascus, is pointed at the end; the other cell is thicker and rounded at the apex. The ascus is slightly thickened at the apex but does not open by a pore.

The maturity of the perithecia is remarkably uniform. Spore discharge continues during only a few days, if the leaves remain damp.

The ascospores are apparently the only means by which the fungus is carried through the winter and are probably responsible for all spring infection of the host plant. No conidia of any sort have ever been found during the winter and spring. The conidiophores appear to be dead soon after the leaves fall. The first spots begin to appear on the older leaves of the host about a month after the ascospores mature.

Genetic Relationship of the Spore Forms. The relation of the spermogonia and of the perithecia to the Cercospora stage is shown by their development at the base of, and direct connection with, the conidiophores. Very often remnants of these old conidiophores may be seen on the perithecial walls when the ascospores are mature in the spring. Frequently also a spermogonium and a perithecium develop side by side with a single wall between the two cavities (see fig. 8). This observed connection was also corroborated for the conidial and ascigerous stages by comparison in cultures and by inoculations. All attempts to germinate the spermatia failed.

Cultures obtained from single ascospores were grown on steamed green bean pods, bean agar, steamed Irish potato plugs, steamed sweet potato plugs, and corn meal mush, in comparison with cultures obtained from the Cercospora conidia. The resultant colonies were similar in every particular of shape, size, coloration, and general development, and neither produced spores of any sort.

The facts that no spores were obtained in cultures and that the old mycelium from cultures failed to produce infection made inoculation with pure cultures very difficult. Infection with the production of spots and Cercospora spores was obtained several times by crushing perithecia, containing mature ascospores, in a drop of water which was placed on the under surfaces of fig leaves in the greenhouse, the plants being kept under bell jars for a few days; but one could not be positive with this method that some conidia had not been included with the perithecia. It was therefore necessary to devise some method for inoculating with ascospores of known purity. For this purpose the idea of isolating in agar single spores or single asci containing germinating spores was hit upon.

On March 16, 1916, perithecia containing mature ascospores were crushed and plated out in agar. The next day ascospores and asci containing spores were located with a microscope and their position was marked. The spore or ascus, with a surrounding block of agar, was then lifted out with a sterile scalpel and transferred to the surface of sterile agar in another plate. These transferred blocks were examined under the microscope at intervals for two days, and only those blocks which showed no contaminating organism were used in making inoculations. Eight such blocks were then transferred to the under surfaces of leaves of fig plants grown in the greenhouse from potted roots. Two typical spots resulted, and at the end of two months Cercospora spores were abundant on these spots. The experiment was repeated on April 10, and four spots developed in a similar manner.

While the percentage of infection was small, there can be little doubt that the infections were produced by the ascospores, since there was no possibility of Cercospora spores being present on the leaves and the checks receiving blocks of sterile agar developed no spots at all. The agar probably held the spores so far from the leaf surface that the germ tubes had in many cases lost their power of entering the tissue before reaching the leaf surface. Abundant infection always resulted when the ascospores were placed directly on the leaf surface.

Systematic. The structure of the perithecia, together with the eight-spored asci lacking the apical pore and the two-celled, hyaline spores, places the fungus unquestionably in the genus *Sphaerella* Ces. et de Not. So far as the writer has been able to find, no similar *Ascomycete* has been described as occurring on the leaves of the fig; and since—from the nature and sequence of development of the stages of the fungus—it is not likely to occur except on leaves parasitized by the conidial or *Cercospora* stage, describing it as a new species seems justified.

In order to facilitate the association of the new name with the well known disease of the fig, the specific name, *Bolleana*, applied to the conidial stage is used; and the name *Sphaerella Bolleana* is suggested with the following diagnosis.

Mycosphaerella Bolleana[2] n. sp. Perithecia mostly hypophyllous, partly embedded in the leaf tissue, erumpent, 60–105 × 55–95 μ, black; ostiolum papillate; asci cylindrical to club-shaped, almost sessile, eight-spored; spores club-shaped to cylindrical, two-celled, 17–20 × 3.5–5.5 μ, hyaline.

Spermogonia produced in the autumn, hypophyllous, embedded in the leaf tissue, with only the ostiolum emergent, ovate, 40–90 × 30–75 μ; spermatia small, rod-shaped, 2–3 × 1 μ, hyaline.

Conidial stage: Spots ferrugineous to olive-brown, irregular, 2–5 mm. in diameter; conidiophores hypophyllous, simple, solitary or fasciculate, slightly geniculate, brownish olive, continuous or sparingly septate, 50–90 × 5–6 μ; conidia olive-brown, clavate to fusoid, 32–53 × 6–8 μ; 1- – 5-septate.

Conidial stage parasitic on the leaves of *Ficus carica* L.

Perithecia and spermogonia on the dead fallen leaves.

Peritheciis hypophyllis, semiimersis, sparsis, ovatis, 60–105 × 55–95 μ; ostiolis prominulis; ascis cylindricis vel clavatis, brevissime stipitatis, aparaphysitis, 35–40 × 11 μ, octosporis; sporidiis hyalinis, clavatis, 17–20 × 3.5–5.5 μ, 1-septatis.

Spermogoniis autumno, hypophyllis, immersis, emergentibus, punctiformibus, nigris, ovatis, 40–90 × 30–70 μ; spermatiis minutis, cylindricis, 2–3 × 1 μ, hyalinis.

Hab. in foliis dejectis *Fici caricae.*

Status conidicus: Maculis brunneis vel olivaceo-fuscis, irregularibus, 2–5 mm. lat., interdum subeffusis; hyphis hypophyllis, solitariis aut fasciculatis, apice geniculatis, continuis aut septatis, 50–90 × 5–6 μ; conidiis clavatis vel tereti-fusoideis, 32–53 × 6–8 μ, apice obtusioribus, chlorino-olivaceis, 1- – 5-septatis.

Hab. in foliis vivis *Fici caricae.*

REFERENCES

1. **Blackman, V. H., and Welsford, E. J.** The development of the perithecium of *Polystigma rubrum*, DC. Annals of Botany **26**: 761–767. Pls. 70, 71. 1912.
2. **Brooks, F. T.** The development of *Gnomonia erythrostoma*, Pers. The cherry-leaf-scorch disease. Annals of Botany **24**: 585–605. Pls. 48, 49. 1910.
3. **Fisch, C.** Beiträge zur Entwickelungsgeschichte einiger Ascomyceten. Bot. Zeit. **40**: 851–870, 875–897, 899–905. Pls. 10, 11. 1882.

[2] The technical description bears the generic name Mycosphaerella Johanson, since this name is used in many recent systematic works; though it seems desirable that Sphaerella Ces. et de Not. may be one of the *genera conservanda* in the report of the committee appointed by the Botanical Congress at Vienna in 1910.

4. **Frank, B.** Ueber *Gnomonia erythrostoma*, die Ursache einer jetzt herrschenden Blattkrankheit der Süsskirchen im Altenlande, nebst Bemerkungen über Infection bei blattbewohnenden Ascomyceten der Bäume überhaupt. Ber. Deutsch. Bot. Ges. 4: 200–205. 1886.
5. **Higgins, B. B.** Contribution to the life history and physiology of Cylindrosporium on stone fruits. Amer. Journ. Bot. 1: 145–173. Pls. 13–16. 1914.
6. ——. Life history of a new species of Sphaerella. Mycol. Centralbl. 4: 187–193. 2 figs. 1914.
7. **Müller, K.** Zur Biologie der Schwartzfleckenkrankheit der Ahornbäume, hervorgerufen durch den Pilz *Rhytisma acerinum*. Centralbl. Bakt., Parasitenk. u. Infectionsk. II, 36: 76–98, 4 figs. and 4 pls. 1913.
8. **Nienburg, W.** Zur Entwicklungsgeschichte von *Polystigma rubrum* DC. Zeitschr. Bot. 6: 369–400. 17 figs. 1914.

EXPLANATION OF PLATE XXX

(All figures × 580)

FIG. 2. A young spermogonium (or perithecium) in the lower side of a fig leaf, developing at the base of conidiophores; from material killed October 16, 1915.

FIG. 3. Young spermogonium (or perithecium) breaking the host epidermis.

FIG. 4. Young spermogonium in which spermatia are beginning to form. The wall not yet differentiated.

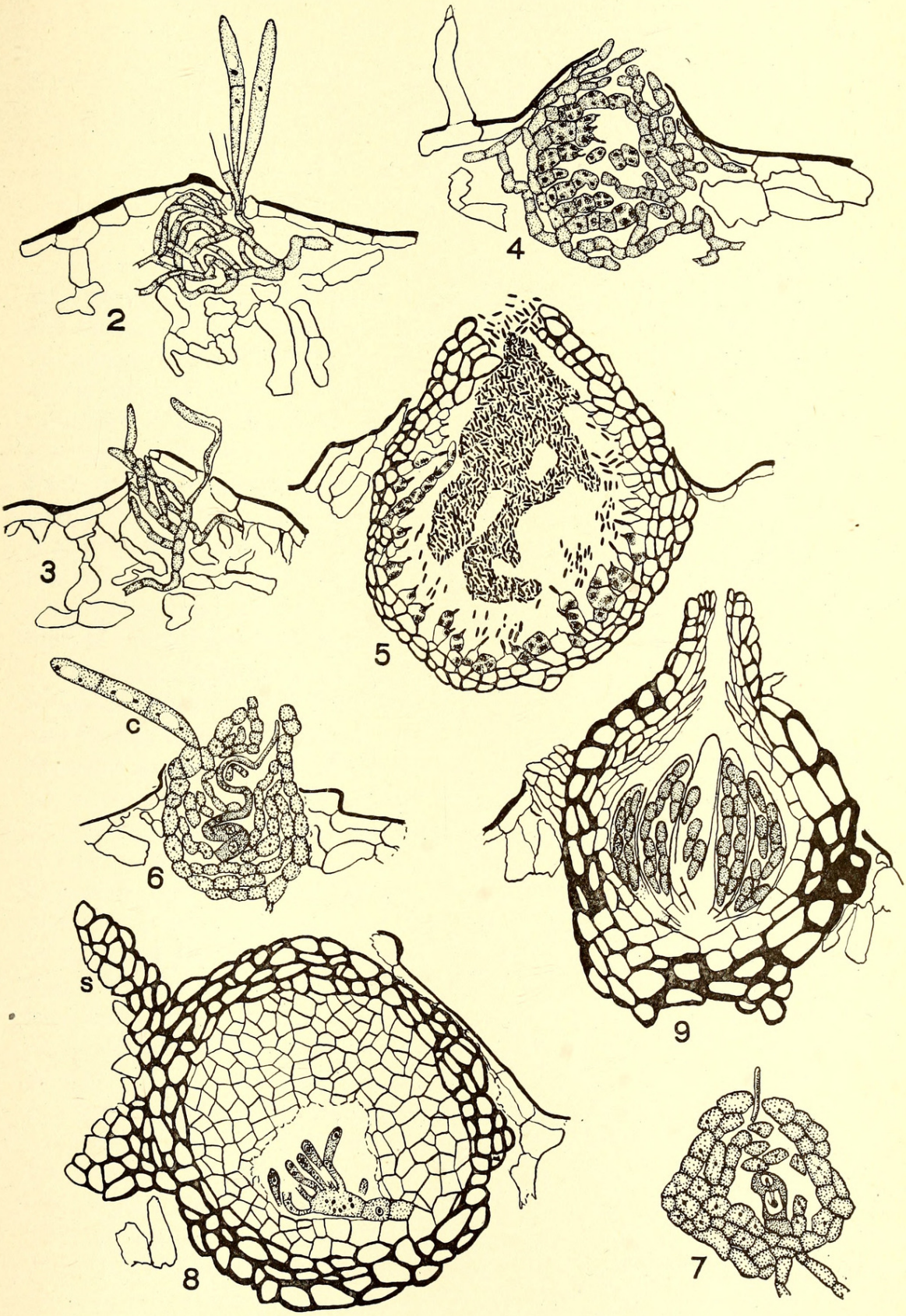
FIG. 5. Mature spermogonium with spermatia being pushed out in a mucilaginous mass.

FIG. 6. Young perithecium with four-nucleate carpogonium. *c*, an abnormal conidiophore.

FIG. 7. Young perithecium with binucleate ascogonium; portion of dead trichogyne still persistent at the apex.

FIG. 8. Slightly excentric section of perithecium, with ascogenous hyphae and young asci. *s*, portion of the wall of an old, empty spermagonium.

FIG. 9. Section of mature perithecium.



HIGGINS: FUNCTION OF SPERMATIA IN ASCOMYCETES.