THE DEVELOPMENT OF THE FRUITING BODY OF ARACHNION ALBUM

By Caroline A. Lander

Plates 23–26

The similarity of *Arachnion album* (Schw.) in general appearance and in younger stages of development to the species of *Lycoperdon* seemed of interest and instigated this investigation.

The form is not commonly known. The gleba, according to Coker and Couch (2), is composed of a large number of minute chambers lined by a hymenial layer apparently made up of basidia alone. Between the chambers is an interwoven tissue of delicate hyphae. At maturity the peridioles are freed as granules by the breaking down of the loose tissue. The peridium falls to pieces slowly with no definite pore. No capillitium, or columella, or sterile base is present.

MATERIAL

The material of *Arachnion album* (Schw.) was collected during August at Chapel Hill, North Carolina. For a limited time it was abundant in open places, meadows, edges of flower beds, and paths. The fruiting bodies were killed in formalin-acetic-alcohol, Flemming's medium, or chromo-acetic solutions. Heidenhain's iron-alum haematoxylin with erythrosin or fast-green as a counter-stain gave the best results.

OBSERVATIONS

The younger stages are very similar to those of *Lycoperdon gemmatum* (Lander, 6). The fruiting body arises either terminally or laterally on the rhizomorph as a nodular swelling formed by the increase and growth of the "building hyphae." This primordium of the carpophore is a homogeneous, spherical body composed of closely interwoven, fine hyphae. The hyphae branch frequently, are binucleate, have abundant cross walls with hemispherical pads, and contain fairly dense protoplasm.

With development this tight, compact structure of intertangled and intricately interwoven hyphae expands and becomes loose in arrangement (fig. 14). Growth is general and uniform throughout the whole structure with the peripheral development marked.

The first differentiation is one of arrangement only and takes place at the periphery. Here the hyphae are in a radial direction. Division of the cells is more rapid near the tip, resulting in short cells. Such development originates at the top of the fruiting body primordium; gradually spreading around the central region. The structure is still composed of one type of hyphae and in its center shows the same arrangement as in earlier forms with the exception that the hyphae are more loosely interwoven.

As growth and expansion continue lighter areas become noticeable in the central region (fig. 15). The first ones to appear are in the upper part, while others are soon formed at the sides and lower parts. No doubt the clear portions are due to unequal growth. Thus a stretching, pulling, and a tearing of the hyphae result. There seems to be no uniformity as to size or shape of the spaces. Study of the older stages shows this to be the first step in the differentiation of the cavities.

Small groups of differentiated hyphal tips arise close to the cavity primordium and at scattered points in the immediate region. Such hyphae are clearly distinguishable in an early stage because of the greater affinity for the stain (figs. 1, 16). The hyphae are arranged in intimate groups, intertangled, and twisted into knot-like structures; the tips radiate in all directions. In contrast to the fundamental tissue the cells are much shorter and broader, and branch abundantly in a dichotomous manner. The protoplasm is very dense and cross walls thin. A cluster of several rows of cells is formed by each hypha, the tip cell of which becomes the basidium. Figure 1 illustrates a stage in such formation.

These hyphae, the basidial primordia, radiate from their tangled position toward the opening and become arranged parallel to each other in a palisade-like layer. From the palisade layer the tips project irregularly into the cavity. The cavities rarely have a regular appearance at this stage because of the unequal growth and division of the hyphae (fig. 20). The cavities are not completely lined with the palisade tissue at any one time; instead there are scattered groups around the edge and later basidia are added to complete the hymenial layer.

The expanse of the fundamental tissue enlarges and rounds the opening; while stretching, tearing, and plasmolysis thus produced clears the cavity of all such tissue. Subsequent growth causes the evening up of the basidial tips until the contour of the cavity is regular. With the addition of new basidia and the completion of the formation of the basidial primordia a definite hymenial layer completely surrounds the

rounded cavities (figs. 17, 18). The young carpophore is filled with numerous small rounded cavities in close proximity.

The fundamental tissue between the cavities is relatively sparse and is composed of delicate hyphae varying in diameter and occasionally branching. The cross walls are thick with prominent hemispherical pads on either side. The hyphae are loosely interwoven and tend to circle or to outline the cavities.

Before the cavities are noticeable the peridium is differentiated. It is first distinguishable as a zone of profusely branched hyphae which tend to be more radial. As the cavity primordia first appear a second layer develops just beneath the first (fig. 16). This zone retains the stain more easily than the outer. It is composed of hyphae, apparently of the same type as the first, which may vary slightly in size and shape but which are interwoven more compactly and do not show the radial tendency.

With further differentiation the hyphae of the inner zone become irregular in size and shape and likewise irregular in arrangement. In some portions they fuse together and undergo other changes resulting in the formation of a pseudoparenchymatous layer. Increase in growth from the interior increases this zone. The hyphae of the outer layer expand and spread apart farther and farther, sloughing off very early (fig. 17).

The outer zone continues to slough off and the inner to increase in width so that the single peridium of the mature fruiting body is composed almost entirely of pseudoparenchymatous tissue.

The basidia completely surrounding the cavity are crowded together. They are narrowly clavate when young and broaden at the tip as they mature. Each basidium produces four sterigmata at its tip (figs. 10, 11). The sterigmata are extremely long, narrow, and thin-walled; some extending to the center of the cavity (fig. 13). They vary greatly in length in the same cavity or in different cavities. In general it would seem that the earliest formed are longer and the last quite short. The sterigmata of the same basidium will vary in length. In some cases apparently the sterigmata attain their full length before the initiation of the spore; while others grow considerably after the spore is formed, even after it has attained full size. At a definite stage the cavity is filled by a tangled mass of the long sterigmata with or without spores.

Few cases of all four spores of the basidium maturing were found though on the majority of the sterigmata spores, at least in the initial stage, were seen. The spores attain their full size before the sterigmata

break. Frequently, however, the wall of the spore is still thin and the nucleus has not divided. The mass of mature spores and broken pieces of sterigmata completely fill the cavity (figs. 13, 21).

Throughout the carpophore from its earliest stages the cells are binucleate. The two nuclei in the basidium fuse. The fusion nucleus in the apical portion of the basidium enlarges (fig. 3). The spindle of the first division is transverse to the longitudinal axis of the basidium (fig. 4). The poles taper toward the walls of the basidium and there is present a dark stained body at each pole suggestive of a centrosome. The chromatic mass divides and the halves separate and move toward the poles (fig. 4). Two daughter nuclei are organized (fig. 5). The rarity of the two-nucleate as compared with the four-nucleate and the one-nucleate stages suggests that interkinesis is of short duration.

The second division, occurring simultaneously in both daughter nuclei, takes place rapidly. The spindles of this like that of the first division lie in the apical portion of the basidium, transverse to its longitudinal axis; they make a right or oblique angle with each other (figs. 6, 7, 8). The spindles are in such close proximity that it is often difficult to distinguish one from the other. This division, so far as observed, is similar to the first, and results in the organization of four nuclei (fig. 9).

The four nuclei move to the central or basal portion of the basidium where they remain for some time. As soon as the sterigmata begin to protrude a large vacuole is formed below the nuclei; the increase in size of this vacuole seems to push the nuclei to the apical region of the basidium. When the nuclei come to lie close to the apical wall, they become fusiform. Their membranes become indistinguishable, and oftentimes it is difficult to distinguish the nuclei from one another. A nucleolus is conspicuous at this time in each nucleus; it is surrounded by a homogeneous group of dark granules.

Each nucleus moves toward the corresponding sterigma, becomes greatly elongated, and passes through the sterigma into the enlarged distal end (fig. 10). In moving through the sterigma the nucleus is stretched to a threadlike structure; as soon as it reaches the distal portion it begins to thicken. Sometimes in passing through the extremely long sterigma the nuclear material may be in a continuous fine thread, while at other times it is broken. A typical nucleus is evident soon after the complete passage of the nuclear material into the distal end. As the spore matures, either before or after breaking from the sterigma, the nucleus divides (fig. 11).

At the time of the initiation of the sterigmata the first signs of the

breaking of the fundamental tissue are noticeable. There is a definite cleavage or splitting, forming cracks or fissures between the cavities (figs. 17, 18). This tearing progresses, outlining and cutting out the cavities with their surrounding tissues from each other. The torn hyphae plasmolyze, collapse, and finally disintegrate. Surrounding the hymenial layer four or five cells in thickness, the remaining living fundamental tissue encircles the cavity in a compact zone.

When the spores have matured the disintegration has progressed to within two or three cells of the basidia. The basidia broaden and become flattened against each other; some walls fuse so that a definite layer is formed. Further changes occur until the basidial region becomes pseudoparenchymatous. A cross section of a portion of the hymenial layer illustrates this fact (fig. 12). The last cells to plasmolyze and die, collapse against the basidial layer; the walls harden and they tend to remain there for some time, strengthening the peridiole protective layer.

The mature carpophore is filled with peridioles crowded with spores, which are surrounded by protective layers of basidial origin, and separated one from the other by space (fig. 21). As the peridium crumbles away the "peridioles" are freed.

The origin of the fruiting body of *Arachnion album* and its early stages of development correspond very closely to those of certain species of *Lycoperdon* (Cunningham, 3; Lander, 6; Rehsteiner, 9; and Swartz, 10). The origin of the cavities through mechanical splitting of the hyphae; the irregular differentiation of groups of basidia primordia; and early development of the palisade tissue around the cavities are likewise similar.

However, *Arachnion* lacks the progressive splitting, enlargement, and fusions of the cavities. In this form, instead of large irregular labyrinthine cavities, numerous small rounded cavities are present.

Although the formation of the palisade tissue around the cavities is more uniform in development in *Arachnion* than in species of *Lycoperdon*, the formation of the basidia is essentially the same (Lander, 6).

The outstanding difference in the younger stages is the breaking of the fundamental tissue between the cavities in *Arachnion*. Later the formation of a pseudoparenchymatous layer by the basidia around the cavity which is filled with spores results in the formation of the peridioles. At maturity the fruiting body composed of granular peridioles, with no columella or tissue between them, and a single-layered peridium lacking the pore is easily distinguishable from *Lycoperdon*.

SUMMARY

The fruiting body originates as in species of *Lycoperdon* as a nodular swelling on the rhizomorph laterally or terminally, and formed by the "building hyphae."

The earliest development consists only of general growth, expanse and loosening of the hyphal arrangement.

The peridium is the first to be differentiated, the hyphae of which exhibit a more radial direction.

The peridium in young stages is composed of two zones: an outer tissue of loosely interwoven hyphae with radial tendency; and an inner of intricately, closely intertangled hyphae with greater affinity for the stain.

The inner zone becomes pseudoparenchymatous and the outer sloughs off as the fruiting body matures. The single peridium at maturity is without a pore and consists chiefly of pseudoparenchymatous tissue.

Corresponding to the early stages in gleba differentiation in *Lycoperdon*, the cavities arise by an irregular mechanical splitting of the tissue; basidial primordia develop in close knot-like groups irregularly arranged in close proximity to the cavities; and gradually the palisade tissue surrounding the cavities is formed.

The progressive splitting, enlargement, fusion, and formation of irregular labyrinthine cavities in species of *Lycoperdon* is lacking in *Arachnion*. The mature gleba is composed of numerous rounded, regular cavities crowded close together.

At the time the basidia are formed the fundamental tissue splits between the cavities and progressively plasmolysis and disintegration of the tissue follow.

After the sterigmata and spores have been formed the basidia form a compact pseudoparenchymatous layer around the cavity.

The sterigmata are exceedingly long, four are produced on each basidium, and each produces one spore; though many do not mature.

Throughout the fruiting body the cells are binucleate, abundantly septate, and contain prominent hemispherical pads.

The fusion of the two nuclei in the basidium primordium is followed by an enlargement of the basidium and of the fusion nucleus. The spindle lies in the apical portion of the basidium; transverse to the longitudinal axis of the basidium. Dark stained granules are present at the poles suggestive of centrosomes. The chromatin mass becomes stretched out along the spindle, divides, and reaches the poles.

The binucleate stage is of short duration. The spindles of the second

division are likewise transverse and are at right angles or oblique to each other.

The four nuclei remain for a short time in the lower part of the basidium; later are pushed against the apical wall by a large vacuole. The nuclei become elongated; stain only as dark irregular masses; and move toward the sterigma.

The nuclear material streams in a threadlike manner through the sterigma into the distal end. The nucleus resumes its characteristic form in the spore and later divides.

This work was carried on while the writer was the holder of the Sarah Berliner Research and Lecture Fellowship given by the American Association of University Women.

The writer wishes to express her appreciation for the use of the botanical laboratory at the University of North Carolina, and to Dr. J. N. Couch for help in the collection and identification of the material; and to Dr. E. M. Gilbert for helpful suggestions throughout the investigation.

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EXPLANATION OF PLATES

All drawings were made with an Abbe camera lucida at table level. A Spencer 1.5 mm. achromatic objective, N.A. 1.25, and a Leitz 15× ocular were used, giving a magnification of about 3000 times. Plate reduced one-fourth.

PLATE 23

Fig. 1. Stage in formation of basidial primordia.
Fig. 2. Young basidium before the fusion of nuclei.
Fig. 3. Basidium with large fusion nucleus.
Fig. 4. Basidia showing various stages of first division; dark stained granules at poles.
Fig. 5. Two-nucleate basidium.
Figs. 6, 7, 8. Stages in second division.
Fig. 9. Four-nucleate basidium.
Fig. 10. nuclei in process of passing through sterigmata.
Fig. 11. Basidium with extremely long sterigmata; nucleus in one spore in stage of division. (Note: The number for this figure was lost from the plate.)
Fig. 12. Cross section of pseudoparenchymatous layer around peridiole.
Fig. 13. Section of peridiole.

PLATE 24

Fig. 14. Section of undifferentiated fruiting body. × 150.
Fig. 15. Section of young carpophore showing early peridium differentiation and origin of cavities. × 150.
Fig. 16. Stage with zones of peridium shown; basidial primordia around cavities present. × 150.
Fig. 17. Section of older stage; outer zone of peridium sloughed off, inner pseudoparenchymatous; cavities lined with basidia; fundamental tissue split between the cavities. × 150.

PLATE 25

Fig. 18. Cavity of the above enlarged. × 650.
Fig. 19. Cavity with early sterigmata and cavity formation. × 650.

PLATE 26

Fig. 20. Enlarged portion of gleba of stage as fig. 16. × 800.
Fig. 21. Peridioles with pseudoparenchymatous covering. × 650.

PLATE 23

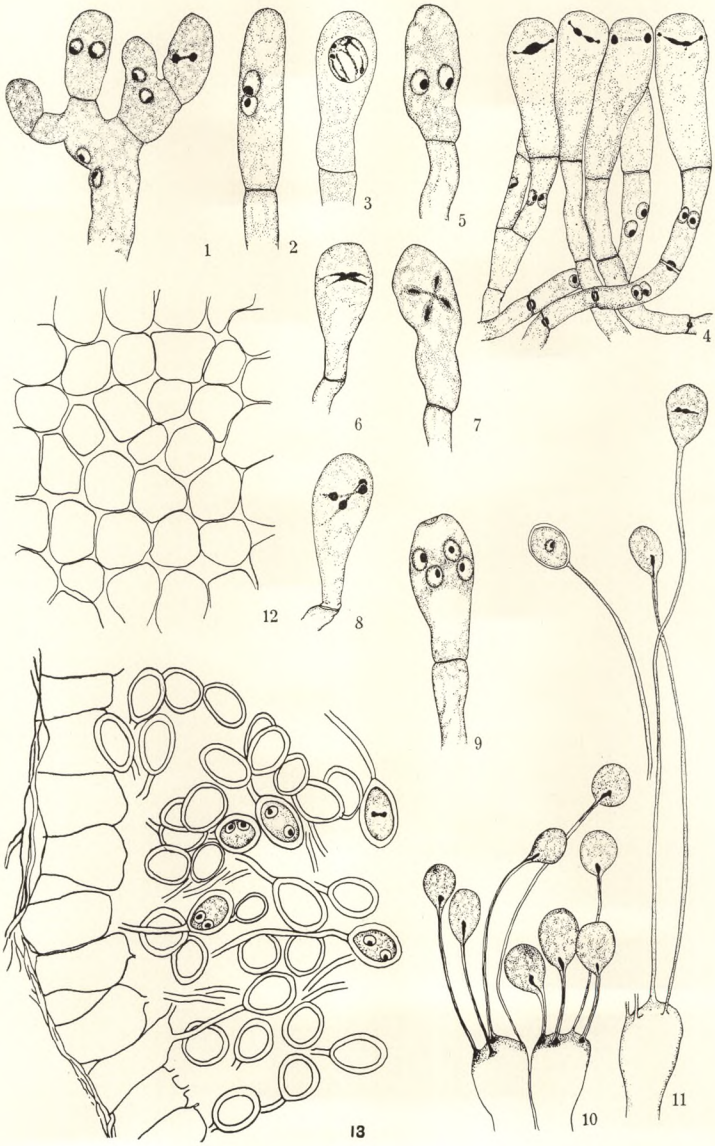

PLATE 24

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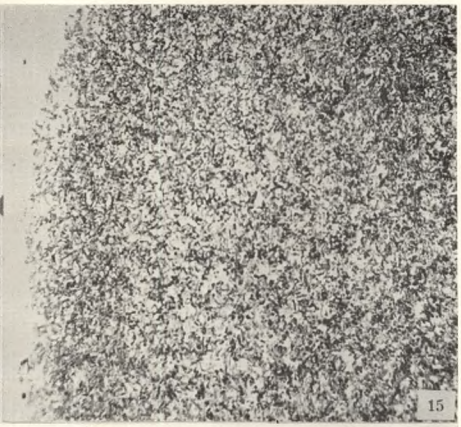
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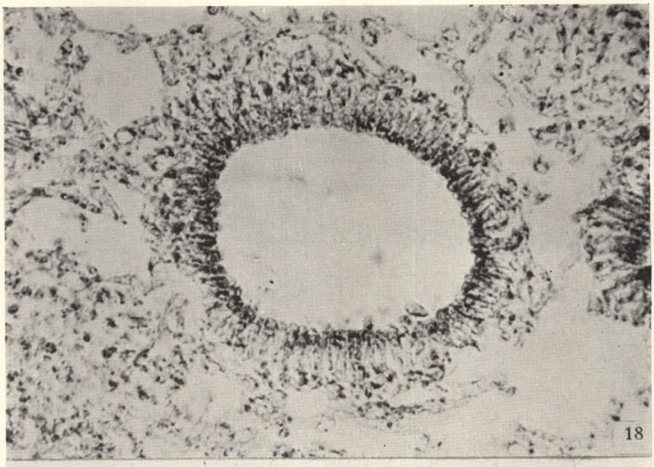
PLATE 25



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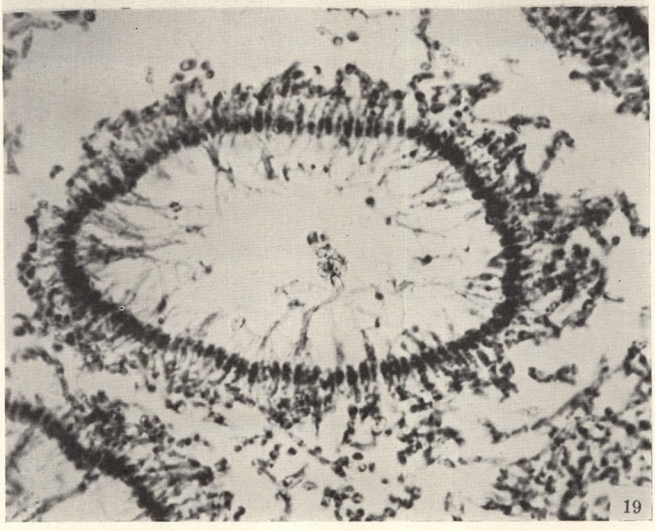

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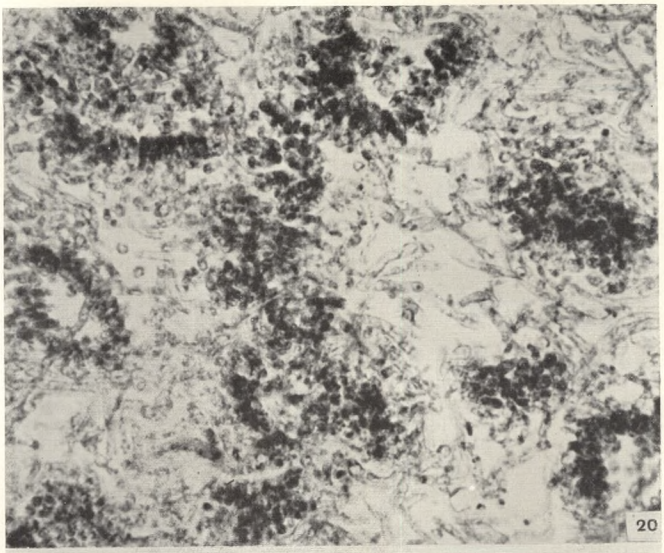
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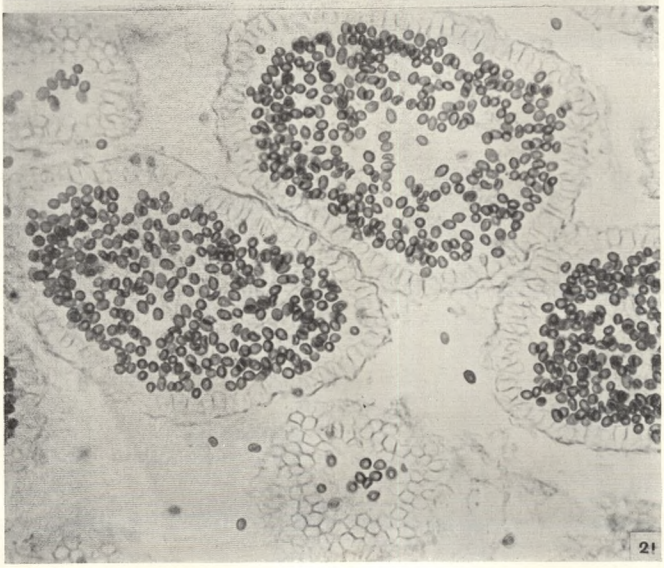
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