be accorded a man engaged primarily in the field of plant research.

Those interested in Dr. Smith's philosophy of life I can perhaps best refer to his address. Some Thoughts on Old Age, delivered as guest of honor at the annual dinner of the Botanical Society of Washington, in 1924, and epitomized in the following sonnet given at the close:

AT SEVENTY

Backward I look from the summit of the years
At the rugged dusty way of toil and grime,
From level distant plain of boyhood's prime,
-Way strewn with hopes, with triumphs and with tears;
And I am optimist, like him who hears
Clear voices call from higher peaks of Time,
Across the cloudy glens, and turns to climb
What yet remains, with more of hopes than fears.
-I'm but a grain of sand upon Time's shore,
Driven by wind and water evermore!
And millions make but shifting dunes and bars!
Yet I can read in every passy sod,
Divine great thoughts that sweep beyond the stars
And make me one with Him who is our God!

To the world of science and to his friends it will never "be as if he had never been."

BIOLICAL ABSTRACTS,
UNIVERSITY OF PENNSYLVANIA

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BIOLOGIC STUDIES IN THE SPHAERIALES—I

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(With Plates 21 and 22 and 3 Text Figures)

The fungi which constitute the object of this investigation are included in the Sphaeriales by Lindau (16). This order, together with the Perisporiales, Hypocreales, Dothideales, and Laboulbeniales, compose the Pyrenomycetes, an enormous assemblage of forms distinguished by the type of ascal conceptacle which is called the perithecium.

The orders of the Pyrenomycetes are separated by most mycologists in the following manner. The Perisporiales comprise forms in which the perithecia remain closed or have an atypical opening. The Hypocreales have fleshy, bright-colored or colorless perithecia which, though sometimes brown, are never black and hard. In the Dothideales the asci are formed in locules in a stroma, and true perithecia are lacking. In the Sphaeriales plainly differentiated leathery, hard, or carbonaceous perithecia occur with or without an accompanying stroma. Finally, the Laboulbeniales have perithecia, but lack a true mycelium.

The application of these separations has resulted in bringing together clearly unrelated fungi, due in part to a misconception of the fundamental differences between the perithecium and the stroma.

It should be emphasized then that in the Sphaeriales the asci are borne in perithecia, and this order is separated from the Dothideales by the fact that in the latter the asci are located in

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1 Also presented to the Faculty of the Graduate School of Cornell University as a major thesis in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

This investigation was accomplished under the direction of Dr. H. M. Fitzpatrick, to whom the writer wishes to express his appreciation for his suggestions and continued helpful supervision of the problem. The writer is also indebted to Dr. L. Massey for making available the herbarium and the facilities of the laboratory, and to Prof. H. H. Whetzel for much inspiration and encouragement in the prosecution of this investigation.
cavities or locules in a stroma. In reality, this separation has been used only when there are two or more locules or perithecia in the stroma. Species in which only one locule is present have been placed in the Sphaeriales, there being no method known for differentiating an unilocular stroma from a true perithecium. This order, as generally delimited, contains consequently many forms which are properly placed in the Dothideales.

Many modern writers have recognized the presence of these unilocular forms in the Sphaeriales, and some, e.g., von Höhn (10, 11, 12, 13, 14, 15), and Thiers and Sydow (28, 29), have contributed a mass of evidence which will aid in the development of a more natural arrangement. While these investigators have uncovered characters that clearly distinguish unilocular stromata, they have failed to recognize the fundamentally important difference in development between the tissue constituting the boundary of the locule and a true perithecium wall, or the correlated difference in ascigorous development. This has led these investigators, along with Petrak (21) and Geitler (7), into the error of assuming that within any given group of dothideaceous forms a perfect series of transitions into the Sphaeriales can be attained by selecting ones with successively thinner walls. According to them, the unilocular form with an uniformly thin wall, even though the interior of the locule be of the dothideaceous type, falls in the Sphaeriales.

The purpose of this investigation has been to demonstrate differences in development between the Dothideales and the Sphaeriales, which hold for all forms, including those with one locule and those with one perithecium in the stroma; and to show that when the dothideaceous forms are removed from the Sphaeriales the remaining species will constitute a definite series of related forms.

**Explanation of Terms**

**Stroma.** The vegetative matrix called the stroma, which functions in the storage of food for the development of the fructification which later arises in it, is a common and variable structure in the Ascomycetes.

The limits of the term stroma have been defined variously by different investigators. Orton (19) summarizes the concepts of

Persoon (20), Tulanee (30), Fuisting (5, 6), Ruhland (24), and others. Persoon introduced the term in connection with the genus *Sphaeria* and applied it to the structure in which the perithecia are borne. Tulanee employed it for the body in the family Xylariaceae, which forms first conidia, and after further development perithecia.

Fuisting and Ruhland distinguish different types of stromata in those groups of the Sphaeriales in which this structure contains more than one perithecium. The former writer, in the case of *Nuttallaria Bulliardii* Tul., distinguishes a hyaline, pseudo-parenchymatous crust, found in the outer layers of the primary cortex of the host, which produces conidia. He terms this the epistroma, and says that it functions both in rupturing the bark and in producing conidia. Under this crust a hypostroma is produced, in which later perithecia are developed. Ruhland follows the conception of Fuisting, but uses instead the terms euctroma and entostroma, respectively.

Ruhland recognizes further differentiation in the two layers. He designates the ostiole disk by the term placodium and since, in forms like *Diatypa disciformis* Fries, it originates from the entostroma, he characterizes this type as entoplacodial. He then traces transitions from the entoplacodial type to his euctromial type through which the euctroma becomes reduced and the placodium is formed in part at least from the euctroma. Where both euctroma and entostroma are present he terms the body diplostromatic, and where one only is present, haplostromatic. Therefore, with the types with reduced entostroma it is but a step to his haplostromatic type, in which the entostroma disappears entirely, and the perithecial initials develop within and near the base of the euctroma. He says the Xylariaceae belong to this type, and thinks these are the highest forms in the Sphaeriales.

Wehmeyer (31: 579) defines the stroma as "an aggregation of vegetative mycelium not resulting from a sexual stimulus." He excludes the tissue composing the perithecium wall and centrum (ascigorous portion), the tissues of the perithecial, and the purely nutritive mycelium which is neither definitely aggregated nor coalesced. As sclerotia and other sterile masses, not associated with spore-bearing structures, are identical in their histo-
logical nature with the matrices of various compound fruit-bodies, they fall within the limits of the definition. Wehmeier (31: 580) says, further, that he uses the terms ectostroma and entostroma to designate differences in structure and position and not in function, and defines them as follows: "An ectostroma in the Pyrenomycetes is that portion of the stroma which is formed on the surface of the bark, beneath or within the periderm, and which consists typically of fugal tissue only, except that when it is developed within the periderm it may contain the remnants of periderm cells, but never of the bark cortex cells. An entostroma is that portion of the stroma which develops within the cortical or woody tissue of the host or substratum, and is made up of components of both fugal and host tissue or substratum."

The writer feels that these definitions of ectostroma and entostroma are not comprehensive enough to include all Pyrenomycetes, or even all members of the Sphaeriales. Wehmeier says that they designate differences in structure and position, but he uses them to designate differences in position only, i.e. ectostroma in periderm, entostroma in cortex. His definitions are clearly limited to forms in the Allantosphaeriaeaceae and the Diaporthaceae which occur on hosts with periderm, and are not applicable to forms which occur on monocotyledonous hosts, or on decorticated wood or leaves. Furthermore, in the Xylariaeceae, while the entostroma is initiated in the cortex, or woody tissue of the host, it is composed only of fugal tissue alone. This type of entostroma would not come within the scope of his definition.

According to Gwymne-Vaughan and Barnes (8: 1), a stroma is defined as follows: "In most cases the hyphae are richly branched; they elongate by apical growth, and, as a rule, spread loosely throughout the substratum; in some cases, and especially in relation to the fruit bodies of higher forms, they become woven into a dense mass which in section gives the appearance of a tissue, and is therefore described as pseudoparenchymatous; such a mass, when not forming part of a single fructification, is termed a stroma..." This definition is applicable to forms in which there is more than one locule or peritheium in the stroma, and evidently does not include the forms with only one locule or peritheium in the stroma.

In this paper the term stroma will be used to include fugal bodies which are formed of coalesced hyphae, which do not arise as a result of a sexual stimulus. There is apparently a tendency toward the reduction of the sexual apparatus in the Ascomycetes. Nevertheless, the ascospore has always been considered the sexual spore. Its sexual nature has been demonstrated in many cases. Therefore, to the writer, it seems logical to term the body which arises from the archicarp and bears ascospores the sexual fructification, in contrast to the stroma. In the Sphaeriales the body (perthesium) which arises from the archicarp (including wall, ascogenous hyphae, asci, paraphyses and perithecia) is a distinct generation in the life cycle of the fungus, and is just as truly distinct from the other generation (mycelium and stroma) as the sporophyte is distinct from the gametophyte of a higher plant. In Sclerotinia, the sexual fructification (the peritheciun) arises from an archicarp in the sclerotium. The sclerotium is therefore a stroma. In Claviceps the perithecia are the sexual fruit-bodies and the rest of the structure, including the sclerotium, is stromatic. In Rosellinia aquila (Fries) De-Not. (Text Figure 1) an extensive stroma often develops under the

Fig. 1. Rosellinia aquila (Fries) De-Not. Longitudinal section through a mature peritheciun and stroma. This photomicrograph shows a peritheciun in the upper part of an entostroma and oriented under a definite ectostroma, periderm. This ruptures the bark in places, and develops further perpendicularly to the substratum. An archicarp then arises in
the external part and develops into a perithecium. It could not be said that the portion of the stroma that grows out through the ruptured bark, and in which the perithecium is formed, is pushed out as a result of a sexual stimulus. The sexual stimulus is in the archicarp, and the latter arises after the stroma has grown out. In Claviceps, Cordyceps, and certain species of Xylaria, which arise from a sclerotium, the archicarps are found in the periphery of the vertical structure and not in the sclerotium. Therefore, it seems illogical to say that the vertical structure arises as a result of a sexual stimulus. This structure is merely a continuation of the sclerotium and the whole tissue is a stroma.

The terms ectostroma and entostroma are useful in dealing with the Sphaeriales. The former will be limited to the part of the stroma first formed in or on the periderm, or on the wood when the bark has been removed, which functions in rupturing the bark when the latter is present and which usually functions in producing conidia. The term entostroma will be applied to the portion of the stroma which develops under this and bears perithecia in its periphery. The entostroma in most of the forms studied by Wehmeyer consists of stromal elements mixed with wood and, in the sense of a pseudoparenchymatous tissue, is certainly not a true stroma. It often has been termed a valsoid stroma. The writer considers it a primitive type of entostroma. In the Xylariaceae the effused forms, such as Numularia, represent the minimum of entostromatic development, and certain species of Xylaria the maximum. In fact these two genera differ only in the amount of entostroma. The ectostroma is the primary outer layer in both. Also in the Allantosphaeriaceae and Diaporthaceae, as compared with such a form as Hypoxylon concentricum Bull., there is very little entostromatic development.

In the Sphaeriales and Hypocreales, forms such as Rosellinia and Melanospora, in which the perithecia are single, have not been previously considered as stromatic. In the early development the archicarp gives rise to a coiled ball of fine hyphae which forms the perithecial wall, asci, paraphyses, and paraphyses. The surrounding tissue, which is large-celled and pseudoparenchymatous in type, and which may exist later merely in a fragmentary state on the outside of the wall, is certainly stroma. In fact the perithecium in these two orders is, contrary to previous conceptions, apparently always formed within a more or less well-developed stroma.

In the Dothideales the sexual cicatrization consists of the group of asci and the hymenial layer from which they arise. The rest of the tissue making up the structure is clearly stromatic. This has never been a matter of dispute.

The perithecial wall. The Sphaeriales have been considered by all mycologists as having an aecigous cavity bounded by a definite perithecial wall, in contradistinction to the Dothideales, where the cavity lacks the wall. In compound fructifications this difference has been clearly recognized, but the unilocular forms in the Dothideales, and the uniporitellic ones in the Sphaeriales, have resulted in much confusion.

Von Höhnel (10, 11), Theissen and Sydow (29), and others have attempted to consider, together with unilocular forms, those plurilocular forms in which each locule fills an arched-up portion of the stroma resembling externally a perithecium. They segregated these in the Pseudosphaeriaceae v. Höhnel (Pseudosphaeriaceae Th. & Syd.). They adopt as a basis for the separation the character of the interior of the so-called cavity. Neither these writers nor Gaumann (7) take into consideration the ontogeny of a true wall. Gaumann for instance places Botryosphaeria in the family Dothidea of the Myriangiales, and asserts that the asci are arranged in locules in the stroma. Then in regard to the development in this family he says (page 214) that such forms as Botryosphaeria Bokermannii Rohm., B. Queenicum (Schw.) Sacc., and B. Ribis G. & Dug., have attained the highest level, because here the locules appear to be in process of separation, and finally in some cases contain only one locule resting on a stromal base. He then says these latter, isolated forms have their own walls, and so have become perithecia. Clearly, then, his definition of a wall, like that of most mycologists, is based on a gross morphological conception, which does not take into consideration the origin of the wall. The writer has found that in B.
Ribis the size and form of the stroma are dependent on characters of the substratum, such as thickness of bark, firmness, etc. Where the bark is thick the stromata are thick with many locules, and where the bark is very thin the stromata become increasingly thinner with a tendency to be unilocular. Gäumann (7: 284) says, further, that among the higher Scolecosporales of the Hypocreales stromatic forms without perithecial walls arise from stromatic forms with solitary perithecia. This causes him to question the correctness of recognizing the group Dothideales. It would seem from this that he thinks that the presence or absence of a true wall is of no systematic significance. In the Hypocreales the writer has examined no species in which the ascigerous cavity lacks a true wall. The species placed in the genus Ophiocladus of the Dothideales by Saccardo (25: 652), including species of Dothichiza Atk. and Myriogenospora Atk., have distinct perithecial walls, and are related to Balanina, Hypocrella, Claviceps, and Coniocephalus in the Hypocreales. Theissen (28: 187) has called attention to this fact.

To the writer the perithecial wall seems to afford a character which is definitely correlated with characters of the perithecial centrum; and the forms possessing it stand in sharp contrast to the dothideaceous fungi in which it is lacking. The characters that accompany the lack of a perithecial wall are the presence of pseudoparenchyma in the centrum, absence of paraphyses and periphyses, the convex to flat or concave form of the hymenial layer, and the lysigenous type of the opening. When there is a true wall there is no pseudoparenchyma in the centrum, there are true paraphyses and periphyses, concave hymenial layer, and the ostiolum is shizogenous in type. The wall in the Sphaeriales is histologically and ontogenetically different from the tissue of the stroma. The writer will define it as the specialized tissue, which arises from the archicarp, and from the beginning encloses the ascigerous centrum. None of the cells of the wall are derived from those of the stroma other than those of the archicarp. Certain previously published evidence corroborates this view.

Gwynne-Vaughan and Barnes (8: 234, Fig. 186) represent a single hypha, the archicarp, which initiates the whole perithecium in Xylaria polymorpha (Pers.) Grev. They say: "If the stroma

of Xylaria or Hypoxylon is sectioned during the conidial stage, nests of small hyphae are found, and form the first indication of perithecia (Fig. 185). Still earlier a stout hypha with large nuclei, presumably an archicarp, is recognized." The writer will show later that in Hypoxylon the primary coil, or archicarp, arises as a single hypha in the stroma. In its later development its external cells coalesce to form the perithecial wall, while its central cells give rise to asci and paraphyses. Therefore, in a true perithecium, the layer of asci, including the paraphyses, is directly connected with the wall. The writer has often succeeded with macerated stromata in getting the perithecial wall intact and free from stroma. If the wall were only a modified inner layer of the stroma, that would be impossible.

In the Erysiphaceae also the wall arises from the archicarp. Hein (9: 391) says: "The enveloping hyphae arise just below the septum which cuts off the oogone from the basal cell and at a corresponding level from the basal cell of the antheridium.

Also the apothecium of the Discomycetes arises entirely from the archicarp. In regard to Pyronema confluens Tul., De Bary (1: 208) says: "Copiously branched hyphae begin to shoot out from the sterile branches of the archicarp, and from the whole of the rest of the basal region of the rosette to form the envelope portion of the sporocarp."

This explanation of the significance of the wall has been given in detail, because only by having its origin clearly in mind can one easily separate unilocular dothideaceous forms from those with true perithecia.

The genus Guignardia affords a case in point. This genus has been placed in the family Mycosphaerellaceae of the Sphaeriales, and the perfect fruit-body has been thought to be a true perithecium. Theissen and Sydow (29) noted that the asci arise in a homogeneous pseudoparenchyma in the Mycosphaerellaceae in the same manner as in the Dothideales, and that placed this family in their order Pseudosphaeriales. Von Hönel (12: 629) says: "Die Gehäuse der Sphaerella-arten sind keine Perithecen sondern kleine (meist) einzahige, peritheciennähliche Stromata. Solche Gebilde nenne ich Dothithecen." In regard to the origin of the asci in Guignardia, Reddick (23: 311) says: "Such peri-
Perithecia are surrounded by the usual thick, black, pseudoparenchymatous covering. This pseudoparenchyma becomes thinner walled inwardly, so that the whole interior of the perithecium is filled with it. In the stained sections there are scattered here and there, near or a little below the center of the perithecium, little dots, of much deeper staining quality, which in well bleached preparations are seen to be individual cells when examined with an immersion lens. . . . When activity begins, the ascogenous cell elongates by pushing its way upward, though at the very first it seems to take the path of least resistance and may grow in a longitudinal direction for some distance. He found very young stromata composed of homogeneous pseudoparenchyma, which he termed pycnosclerotia, and he thought they were sporeless pycnidia, which may eventually develop into perithecia. In examining his preparations the writer finds, as stated above, that in the earliest stage seen the so-called perithecium consists of a homogeneous pseudoparenchymatous matrix, in which a little below the center the archicarp arises. At this stage the condition is identical with that shown by the writer for Hypoxylon (PLATE 22, FIG. 7). But in Guignardia (PLATE 21, FIG. 4) as the ascus develops, the tissue directly above disintegrates, and the ascus pushes up between the fragments. In the Sphaeriales (PLATE 22, FIG. 6) the archicarp very early gives rise to a wall, which definitely shuts out all the pseudoparenchyma of the stroma. In Guignardia the archicarp gives rise to no wall, and so has exactly the same type of development found within the Dothideales.

The ostiolum. There seems to be no general agreement among mycologists as to the limits of this term. Some would apply it to any pore of an ascigerous or pycnidial fruiting stage through which spores are liberated. Others apply it to the papilla or neck of the conceptacle.

Weltmeyer (31: 352) says: "The ostioles are merely the erumpent portions of the perithecial necks." Von Höhnel (15: 138), in regard to the Hormosporaceae, characterizes them as having no ostiolum, and places them in the Albatrosphaeriae, all the other members of which have ostiola. Toro (26: 40) created the order Pseudopersporiales on two genera, Perisporium Syd. and Pseudopersorium Toro. He says: "The order differs from the Perisporiales in having perithecia with definite ostiola." If the conception of an ostiolum is of such taxonomic value, it should certainly not mean different things to different mycologists.

De Bary (1: 190), speaking of perithecia, says: "They are bounded on the outside by the wall, which encloses an ascigerous hymenium, and are furnished in the full-grown state with a narrow aperture or ostiule, which is a canal passing through the wall, and serving for the discharge of spores. . . . The ostiule is not formed till the development is more advanced, and it appears as an intercellular passage in the originally closed tissue; it is partly schizogamous by the separation of persistent tissue elements in consequence of unequal growth, partly lysigenetic by the dissolution of a strip of tissue lying originally in the canal."

Most writers seem to agree that the opening in the Dothideales does not constitute an ostiolum. Gaumann (7: 284), in a discussion of this order, says that, from the lack of a special perithecial wall, there follows the lack of an ostiulum, and the summit of the locule is always formed through a definite part of the stroma regardless of the shape of the summit. So he thinks that the lyssigenously formed pore in the summit of the stroma does not constitute an ostiolum. Theissen and Szydow (29), in regard to the Pseudosphaeriaeales, say that an ostiolum is not present. They apply this point of view then to such forms as those in the Cucurbitariaceae, Pleosporaceae, Mycosphaerellaceae, as well as to those in the Dothideales. Blain (2: 17), in his study of the dothideaceous stromata, says: "No definite ostiolum has been found in the study of the fungi involved in this paper except those which obviously belong to the Sphaeriales."

The writer will consider as an ostiolum the canal passing through the papilla, or neck of the perithecium, and terminating in a pore. It is lined with minute periphyses, which are outgrowths of basal cells in the neck wall and have free ends. The pore is formed by the pulling apart of wall tissue at this point due to unequal growth, and the canal is formed by the upward growth of the wall. It is never formed lyssigenously as in the Dothideales. In this conception the term ostiolum is clearly limited to the perithecium of members of the Hypocreales and Sphaeriales.
The latter part of De Bary's definition is applicable to the Dothideales and Pseudosphaerales. In both cases there is a dissolution of a strip of pseudoparenchymatous tissue directly above the asci, which results in an opening, but the tissue lining the canal is histologically stroma, and not wall tissue as in the Sphaeriales. These two methods of forming the canal are so distinct that it would certainly not be accurate to use the term ostiolum for both.

Paraphyses. This term has been applied to sterile threads lying between the asci in a parallel position. However, since such threads are known to arise from different sources, it is necessary to define the term more definitely.

Petrak (21: 67), in discussing the evolution of paraphyses, considers those found in both types of centrum, i.e., the Pseudosphaerales or the Dothideales on the one hand, and the so-called Diaporthean type on the other. The first type of centrum he divides into three categories: (1) Paraphyses completely lacking, examples Mycosphaerella, Sphaeria, and Guignardia. (2) Paraphyses more or less thread-like and cellular, usually atypical, formed from compressed parts of the ground tissue. As examples he cites Wettsteinia, Pseudosphaeria, Butryosphaeria, Dothiota, Pleospora spp., and Lepidosphaeria sp. He calls these paraphysoids, and says they are entirely primitive forms of paraphyses. (3) Paraphyses more or less strongly developed and branched, not at all, or scarcely gelatinizing, grown above to the covering tissue of the perithecial membrane. As examples, he designates Leptosphaeria sp., Melanomura sp., Trematosphaeria sp., Massaria, Massarina, Pichonmassaria, and many others that he considers as belonging to the Sphaeriales, as well as numerous dothideaceous fungi.

He follows this with his Diaporthean type. In this type of centrum there is no pseudoparenchyma. He divides this into three groups: (1) Pseudopara paraphyses completely lacking. Here he puts most of the genera of von Höhnel's (13: 631) Diaportheaeae, also Valsa, and Melanomura sp., "but no single Dothideales form." (2) Pseudopara paraphyses rather numerous, cellular, relative broad, usually delicate, and at early stages strongly gelatinizing. He gives, as examples of this type, many species of Melanomura and Pseudosphaeria. (3) Pseudopara paraphyses more or less, often very numerous, not distinctly cellular, threadlike, not easily gelatinizing, free above. As examples displaying this last type of pseudopara paraphyses he cites Herospora, Rosellina, Hypoxylon, and Xylaria. He calls these pseudopara paraphyses of his third type paraphyses.

In his first division he has a series of transitions from no paraphyses to paraphyses, to so-called genuine paraphyses. However, as he says, all of these are connected to the perithecial membrane above the asci. In his second type, the Diaporthean, all arise from the ascal layer, and are free above.

Guignardia Bidwellii (Ellis) Viala & Rava (PLATE 21, FIG. 4) represents Petrak's first type: also Dothiota collae (Schw.) Ellis (TEXT FIGURE 2). The tissue above the asci is stroma in process of dissolving, and there are no paraphyses. The condition in Chaetosphaeria gheosweria (Mont.) Sacc. (PLATE 21, FIGS. 2, 3) and also Dibotryon mucedo (Schw.) Th. & Syd. (PLATE 21, FIG. 6) corresponds to his second and third types. The strips of tissue are connected at the top and are plainly to be seen as only strips of dissolving pseudoparenchyma. Hypoxylon Hoehnei (Ellis) Peck. (PLATE 22, FIG. 5) is of the Diaporthean type, and has what Petrak calls metaparasites.

It is evident that Petrak has not considered the origin of his so-called dothideaceous type of paraphyses. He says (21: 67) that pseudoparasites have free ends, while genuine paraphyses are grown above to the perithecial cover. The illustrations cited above show plainly that these strands are only stromal remnants. Moreover, no part of the stroma, which is external to the ascogenous layer, could by any series of transitions become genuine paraphyses. In the Deconcomozales true paraphyses are never found growing down from the top of the peritheium. There is no way that one can reconcile these stromal parts with paraphyses. Petrak is merely attempting here to show by means of the pseudoparenchymatous threads hanging from the top of the centrum that the Pseudosphaerales grade into the Sphaeriales. The separation between paraphyses in the Sphaeriales and
stromal remnants in the Dothideales is just as exact as between
the wall in one case and the stroma in the other.

True paraphyses, then, are sterile hyphae, which arise in the
ascogenous layer, and have free ends converging towards the
ostiolum. They appear before the asci, and usually gelatinize
at maturity.

Comparison of Development of the Dothideales
and the Sphaeriales

The illustrations of Chaetosphaeria phasestruma (Mont.) Sacc.
(PLATE 21, FIGS. 1, 2, 3) show a development typical of the
Pseudosphaeriales of Theissen and Sydow (29). First a pseudo-
parenchymatous stroma develops; then an archicarp appears
slightly below its center. As this grows the ascogenous hyphae
and asci appear, and the latter grow upward in the pseudo-
parenchyma. This tissue appears to undergo a chemical disso-
lution as the asci increase in size. Its cell walls become thinner,
and the black coloration in them dissolves. Apparently, this
part of the stroma acts as nurse tissue in the same manner as do
tapetal layers surrounding pollen grains in the Spermatophyta.
The formation of the opening is accomplished by the same type
of chemical dissolution. The dark-walled external cells directly
above the developing archicarp become hyaline and thin-walled
and gradually break apart. The actual break is probably caused
by the expanding hymenial layer. With the low power of
the microscope the canal appears as a typical ostiolum, but when
examined with the high power it is seen to contain, instead of
peraphyses, merely cell fragments.

The method of development typical of the Dothideales is to be
seen in the longitudinal section through the stroma of Dothidea
collecta (Text Figure 2). The asci develop from a convex
placenta, in the same manner as in Mycosphaerella Fragarieae
(Schw.) Lind., growing upward in the stroma, and at maturity
the stroma disintegrates to form an irregular opening through
which the spores escape. In Guignardia Bidwellii (PLATE 21,
FIG. 4), the same type of development as that in Dothidea colletta
is shown, except in that the ascogenous layer is flat instead of
convex. In all cases the stroma develops first, and the archicarp
later. There is no indication of a perithecial wall, the centrum
being solidly pseudoparenchymatous.

![Fig. 2. Dothidea colletta (Schw.) Ellis & Ev. Longitudinal section through
a mature stroma showing groups of asci in locules.](image)

Blain (2:17) says in regard to the Dothideales: "Nearly all of
the fungi examined possessed a concentric layer of thin, hyaline
compressed cells around the periphery of the locule, the lining." This
is not comparable to a wall in any sense. It is due to the
dissolution process, and to pressure from the growing asci, to
which these cells are subjected.

This dothideaceous type of development is also seen in Dibo-
tryon morbosum (PLATE 21, FIG. 6), and in Leptosphaeria Doliosil
(Pers.) Wint. (PLATE 21, FIG. 5), the threads simulating paraphys-
es being stromal remnants as previously explained.

Nichols (20:316) says in regard to the development of
Trichospora obducens (Fries) Fuckel and Trichospora sporadica
Atk.: "A single cell of the mycelium by successive divisions and
growth forms a solid sphere of parenchymatous tissue. Certain
of the interior cells of this tissue become enlarged and differen-
tiated into asci." This is typical of dothideaceous develop-
ment. The cell that initiates the stroma, in locular forms, cannot
possibly be considered the archicarp. That organ arises later.

The type of development found in the Sphaeriales produces
Petra's "Diaporthe centrum." The archicarp always
develops in a stroma, whether there be a single peritheceum or many.
The wall forms early, shutting out all of the stromal parenchyma.

In Text Figure 1, Rosellinia aquila (Fries) De-Not. is pictured
to provide an example of a single peritheceum enclosed in a stroma.
This form has not been considered as one of the stromatic Sphaeriales. The stromal part, which encloses the perithecidium, has been thought as a part of the perithecidial wall. This, however, is ectostroma, under which the perithecidium develops in the same manner as in Hypoxylon. The stroma is hard and carbonaceous, but the perithecidial wall is not. The illustrations of perithecidium of Neurospora by Shear and Dodge (27: Pl. 4, A, B, C) show typical perithecidia, in which there is a definite wall, and also a thin pseudoparenchymatous layer on the outside, just as in species of Rosellinia. This pseudoparenchyma is stroma. Histologically it is the same as the stroma in such compound fructifications as in Hypoxylon. All the tissue that does not arise from the original coil is stroma. Apparently all the so-called nonstromatic forms in the Sphaeriales are in reality stromatic.

The development of Hypoxylon Howeiwnum Peck. is illustrated on Plate 22. A coiled archicarp arises in the periphery of the ectostroma (Figs. 4 and 7). By further growth this gives rise to a ball of hyphae. The outer hyphae of this knot are smaller in diameter than those on the inside. Next, the outer threads coalesce to form a gibbose wall. Inside of this wall are loosely coiled segments of large diameter, which later give rise to ascogenous hyphae. These have been called "Woronin hyphae." The wall definitely shuts out the stroma. The fertile segments gradually settle out, forming a peripheral layer lining the wall. At this stage the apical region of the wall begins to grow upward, cone-like. This gradually grows out through the ectostroma (Fig. 3) and forms the ostiolum in the manner described above under the explanation of ostiolum. The mature perithecidial centrum is then surrounded by a wall (Fig. 5), the sides and base of which are lined with asci and paraphyses, and the ostiolar canal is lined with periphyses. This fully equals Petrak's Diaporthean type of centrum as described previously.

This method of development corresponds closely with that of Xylaria polymorpha (Pers.) Grev., as described by De Bary (1: 216): "The primordium of the perithecidia make their appearance in the form of small spherical bodies, which lie in the medulla close beneath the black rind, and are at once distinguished from the medullary tissue by containing no air and therefore being transparent. They are formed of a closely woven mass of slender hyphae, which are much thinner than the hyphae of the original tissue and must therefore be a new formation in it. In somewhat older specimens an irregular large-celled coil of tissue is found lying in the middle of the sphere. The spheres now increase in size in the direction of the medulla, the shape, structure and position remaining the same. Then a dense tuft of straight hyphae, in the shape of a broad truncated cone, shoots forth from the part which abuts on the rind and elongates in the direction of the rind, which is first bulged out a little and then gradually pierced through, so that the extremitities of the hyphal project above the surface. The young perithecidium has meantime become egg-shaped, its broader portion lying in the medulla being the future basal part, while the narrow end which is wedged into the rind is the future neck with ostiolum."

Modern investigators have added to our knowledge of the early stages of development within the centrum.

Brown (3: 4) says of Xylaria, "Soon a definite perithecidial wall is to be seen (Fig. 10). As this grows it seems to spread so as to make more space within; the Woronin hyphae appear to lie loosely within the space enclosed. As the segments enlarge the ends tend to become rounded so that the connection between segments is very slight, and they finally separate completely. Each seems to be an independent structure. The hyphae during the stages figured in 10 and 11 seem to be loosely coiled in the large space in the center of the developing perithecidium but a little later they come to lie near the perithecidial wall. . . . Later some of them send out branches and these branches give rise to ascogenous hyphae (p. 5). Following the stage shown by Figures 23 and 24, with the enlargement of the perithecidium, there is an increased growth of threads from the inner portion of the perithecidial wall. They extend from the wall and gradually fill the space within. These ingrowing hyphae form the paraphyses and paraphyses (p. 7)."

Lupo (17: 494), in regard to the development of Hypoxylon cocccinum Bull., says: "The formation of the perithecidium is initiated by the massing of the hyphae into a circular knot, within the center of which the Woronin hyphae differentiate."
Dawson (4: 255), in regard to the development of *Poronia pannata* Fries, says: "The mature peritheium consists of a very definite wall of closely interwoven hyphae lined with a smaller-celled hyphal layer, whence arise the very numerous club-shaped asci, intermingled with numerous paraphyses. The somewhat long neck which opens by an ostiole to the exterior, is lined by delicate periphyses, which more or less completely fill the cavity leading into the perithegium."

The fundamental point of difference then between the Sphaeriales and the Dothideales is not the presence of pseudoparenchyma in the centrum in the latter, but the presence of the wall in the former, which determines that stromal tissue may not be enclosed in the centrum. If one could eliminate the stroma, it could be said in truth that in the Dothideales the ascal layer is gymnocarp from the beginning, and in the Sphaeriales angiocarp.

Discussion of the Pseudosphaeriaceae Question

Von Höhnell, Theissen, and Sydow were the first mycologists to recognize that there are unicellular forms in the Sphaeriales with a different type of development from the remainder. Von Höhnell (10, 11) founded the family Pseudosphaeriaceae on the genera *Weitsteinia* and *Pseudosphaeria*, and separated it as follows: stromata small, sunken, peritheciun-like, with several locules standing near one another, each of which contains a single ascus. Theissen and Sydow (29) raised this family to ordinal rank. They say this order is recognized for sphaeraceous fungi whose asci are separated by thin pseudoparenchymatous strands; and each ascus cavity is accordingly demonstrated to be a "monaster Lokulus," and the entire visible conceptacle as a stroma with many locules. They found many forms in the Sphaeriales that had this pseudosphaeraceous centrum. The condition was considered to be related to that in *Myriangium* and *Plectodiscella*, where each ascus rests in its own locule. Also the Dothideales, *senis stricta*, were seen to have this type of centrum. These writers, therefore, created the group Dothidiineae to comprise the three orders, 1. Myriangiales, 2. Dothideales, and 3. Pseudosphaeriaceae. They (29: 5) say that these orders are united by the common basic character of one-ascus locules. The last two orders were separated as being stromatic (Compositae) and simple (simplices), respectively. In the Pseudosphaeriaceae they placed the following families: Epipoleiaceae, Parodiellaceae, Pleosporaceae, Cucurbitariaceae, Botryosphaeriaceae, Pseudosphaeriaceae, and the Sphaeriaceae.

Von Höhnell (11: 634) considered the Pseudosphaeriaceae as being a connecting link between the Sphaeriaceae and the Dothideales, but says they remind one of the Myriangiales, due to the fact that the locules contain single asci.

Petra (21) made a comparative study of a great many forms that von Höhnell or Theissen and Sydow had placed in the Pseudosphaeriaceae von Höhnell (or Pseudosphaeriaceae Th. & Syd.), and says that neither von Höhnell nor Theissen and Sydow grasped their true meaning. After studying the species in the genera of the family Pleosporaceae, he says (21: 48) that with the species of the genera *Pleospora*, *Pyrenospora*, and *Leptosphaeria* the development of the peritheium of the typical Sphaeriales from a dothideaceous stroma can be followed very beautifully. The gradual formation of the ostiolum goes hand in hand with the changing of the stromatically formed wall into a perithecial membrane typical of the Sphaeriales, and with the increase in the number of asci there follows the development of typical paraphyses from the pseudoparenchymatous centrum tissue of the dothideaceous stroma. Further (21: 64), he says that von Höhnell's Pseudosphaeriaceae are nothing but the primitive forms from which the Sphaeriaceae have developed, and that they bind the Sphaeriaceae directly with the Dothideales.

He brings this transition about through four developmental stages as follows:

1. Ostiolum still to be considered as a small, papillate extrusion of the conceptacle. Conceptacle wall very thickly pseudoparenchymatous, of rather homogeneous structure, differentiated into a dark colored outside crust, and a hyaline pseudoparenchymatous ground tissue. Asci not numerous, but very thick. Ground tissue still at maturity very plainly pseudoparenchymatous. . . . *Weitsteinia, Pseudosphaeria*.
2. Ostiolum distinct, however atypically developed; that is, remaining closed, but at maturity breaking out more or less.
Perithecial membrane strongly differentiated. Asci somewhat numerous, elongate, or thick clavate. Centrum tissue at maturity more or less threadlike, not plainly cellular. ... Pyrenophora phaseolina, P. trichostoma and Pleospora herbarum.

3. Ostiolum entirely typical; that is, at first completely closed, opening late, through partial gelatinous absorption of its tissue. Conceptacle wall still rather thick, more or less plainly differentiated in two layers. Asci numerous, slender. Ground tissue in the mature condition scarcely to be distinguished from typical paraphyses. ... Leptosphaeria Dolichium.

4. Ostiolum typical, opening through a more or less round pore. Perithecial membrane composed of a few to numerous layers of bright colored to dark colored, more or less compressed cells, membranaceous, leathery, or carbonaceous, never sclerotial. Asci very numerous, slender, centrum tissue composed of typical, more or less branched, robust, usually numerous paraphyses.

It is impossible for the gap between the Dothideales and the Sphaeriales to be bridged over in this manner. The fact that forms can be found with consecutively thinner walls does not mean that a membrane which is histologically stroma can ever be a true wall, nor can an ostiolum in the sense of Petrak by any series of transformations ever become a genuine ostiolum. The latter could exist only in the apex of a wall formed from the archicarp in the manner previously described in this paper for Hypoxylon Haeckelianum. Interethecial tissue (stromal remnants) is found in all of Petrak's pseudosphaeraceous fungi, and that very fact shows that there is no continuous wall as in the Sphaeriales. Any species which lacks a true wall necessarily has stromal tissue above the ascal layer, and so definitely belongs in the Dothideales.

Von Höhnel considered that a fruit-body with a thin wall and an apical papillum constituted a genuine peritheium. He separated the genus Leptosphaeria, all of whose species have the dothideaceous type of centrum, into three genera. He (14: 133) first divided the genus into a dothideaceous Leptosphaeria Ces. & De Not., based on the type, and a sphaeraceous Leptosphaeria, which he put in Nodulosphaeria Rab. Later (15: 158), he distinguished a pseudosphaeraceous Leptosphaeria, which he named

Sclerosphaera. He also failed to take into consideration the primary separation between the Dothideales and the Sphaeriales; i.e. the wall character.

It is now evident that von Höhnel, Theissen and Sydow, and Petrak, while recognizing in part the characters of the unilocular forms, fail to agree on the limits of the Pseudosphaeriales, on the species and genera they include in the group, and in their conception of the morphology and homology of certain characters, e.g. wall, ostiolum, and paraphyses, which are of fundamental importance. Neither the taxonomic position of these forms nor their morphologic characters have been satisfactorily treated by any of these investigators. The unsatisfactory character of their work on the Pseudosphaeriales has been due to their failing to distinguish between pendant strips of partially dissolved stromatic pseudoparenchyma, and to their confusing the perithecial wall with the stroma.

It is impossible to make these forms transitions between the Dothideales and the Sphaeriales. None of them have a true wall, nor true paraphyses, and all have stromal parenchyma in the centrum. All of the characters found in the Pseudosphaeriales are common to members of the Dothideales. Therefore, they should be placed in the Dothideales, and it would seem that their position there should be determined by characters found in the centrum, such as characters of the asci and ascospore and characters of the ascal hymenium. Such characters as the thickness or thinness of the stromatic wall or of the number of locules in the stroma apparently have no value.

The Relationship of the Myriangiaceae to the Dothideales and Pseudosphaeriales

According to Theissen and Sydow (29) the Myriangiaceae, Dothideales and Pseudosphaeriales are united by the common, basic character of the uniascal locule. Petrak (21: 61) points out that this is true in a strict sense for Myriangium, but not for members of the other two orders. However, he derives the latter from simple Myriangiaceae by increasing the number of ascii from one to many, increasing the ascus plectenchyma in a horizontal direction in the stroma, and decreasing the thickness
The writer has sectioned material of *Phaeoporia* hebelorwm (Pers.) Rabl, and finds the situation not at all comparable to Giumann's figure (7. Fg. 145) of this fungus. In his diagram, the ascospores are shown at the top and bottom of the locale, apparently putting each ascus at the tip of each stalk. The writer finds the asci of *Phleboporia* to be continuous, and that Giumann's figures show the ascospores developing in the stroma, continuous with the ascospores of the stroma, and then giving rise to the ascospores of the ascus. The writer considers that Giumann's figures are misleading, and that his interpretation of the ascospores is not correct. The writer proposes a new interpretation of the ascospores, and suggests that the ascospores develop in the stroma, and then give rise to the ascospores of the ascus. The writer also discusses the development of the ascospores, and suggests that the ascospores develop from a common archicarp, which may give rise to the ascospores of the ascus, and then give rise to the ascospores of the stroma.
Leptosphaeria Dolichum and Leptosphaeria acuta (Moug. & Nestl.) Wint. represent the beginning and end points of a series which leads from the genuine Pseudosphaeriales to the genuine Sphaeriales. Then further on (7: 253), he says that the Sphaeriales were erected as a parallel line to the Hypocreales from which they differ through their dark colored, leathery, hard, or carbonaceous perithecium, but recent examinations have shown him that this holds for only a part of the order, while another part of the developmental series is an offshoot from the Hypocreales. From this he thinks that the Sphaeriales as at present grouped have no right to exist. Then he enumerates the Diaporthales type and the Pseudosphaeriales type as the two types in the Sphaeriales. In other words, he admits that if the fungi of the Pseudosphaeriales type were taken out, then the Sphaeriales would be of one type, and the centrum would then correspond to that found in the Hypocreales.

The mistakes made by the above investigators in the treatment of the Myriangiiales were due to a lack of knowledge of the origin of the essential structures in the locule and in the perithecium. The Myriangiiales may have arisen, as pointed out, by the reduction of the number of the asci in the locule to one, or these unisaccate locular forms may represent the primitive ancestral types of all forms in which the ascus pleurot symphyly develops in a stroma and is not surrounded by a special wall, such as found in the Phaeidales, Hysteriales, Hemisphaeriales, Peripsoriales, Coryneliae, Dothidea and Pseudosphaeriales.

Ordinal Separations in the Pyrenomycetes

In the Pyrenomycetes, the Hypocreales, Sphaeriales, Laboulbeniales, and the Erysiphaceae contain asci in true perithecium. The first two groups are closely related. Both have the Diaporthean type of centrum, true perithecial walls and ostioli. The separation of these two orders in the first place was due to a misconception of the "wall" in the so-called Sphaeriales. The black, carbonaceous "wall" in the Sphaeriales is a reality stroma. In all the members of the Sphaeriales the true wall is thin, membranaceous, and quite variable in color. Moreover, the stroma is always fleshy when young, becoming woody, leathery, or carbonaceous when old. Whenever a fungus of the type of Roseliniia is found with a relatively thin stromatic layer on the outside of the wall, it is impossible in the present system of classification to know whether to place it in Roseliniia, Sordaria, or Medicospora. In the texture of the stroma Balasnia, Myriogenespora, Ophiocolea, Hypocrella and Claviceps, all in the Hypocreales, exhibit a series that definitely connect the Hypocreales with the Sphaeriales. All have the same type of ascus and ascospore, all have true perithecial wall, but the texture of the stroma varies from hard and carbonaceous to fleshy, and from black on the outside to colored. In view of these it seems that the color and texture of the stroma and perithecial walls are of doubtful value in separating these two orders. Therefore, it is suggested that the two be merged as one order characterized by the possession of a true perithecial wall and ostiolum.

The Erypfigaceae have a true perithecial wall, but the lack of an ostiolum should be sufficient to place them in a distinct order. The membrane in the Laboulbeniales is apparently a true perithecial wall, but the lack of a well-developed mycelium is sufficient to maintain them in a distinct order.

The Myriangiiales, Pseudosphaeriales, Peripsoriales, Coryneliae, and Dothidea all belong in one group distinguished by the absence of a true perithecial wall and by the asci being borne in locules in the stroma. They cannot be said to have a perithecium and are, therefore, not Pyrenomycetes.

The Erysiphaceae, Hypocreales, Sphaeriales and Laboulbeniales are sharply separated from the above group by the asci being borne in perithecia, and so are true Pyrenomycetes.

Literature Cited

EXPLANATION OF PLATE 21

Development of *Hyposphorium* Fossacephalus

1. Longitudinal section through initial stroma showing the ruptured test and the ectostroma being pushed upward by the developing entostroma.
2. Longitudinal section through a very young perithecium showing the beginning of arical growth of the wall to form the ostiolar canal.
3. Longitudinal section through a young perithecium showing the manner in which the perithecial neck penetrates through the periphery of the entostroma.
4. Longitudinal section through a mature perithecium, with the neck protruding fully beyond the entostroma.
5. Longitudinal section of the neck region showing only perithecial segments.
6. Longitudinal section through the entostroma, including the ostiolar canal.